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In vitro Study of the Compatibility of Three Fungicides with Biocontrol Agents *Trichoderma asperellum* and *Pseudomonas protegens*

Benedicto Martínez Coca[1] and Edwin Ronnie Gakegne[2*]

[1]*Centro Nacional de Sanidad Agropecuaria (CENSA), Apartado 10, San José de las Lajas, Mayabeque, CP 32700, Cuba*
[2]*Instituto de Investigação Agronômica (IIA), CP 405, Chianga, Huambo, Angola*

**Corresponding author*

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A B S T R A C T

The objective of this work was to establish the compatibility under laboratory conditions, between three fungicides (Cuproflow 37.7 % SC, Yaba M GD 66 and Mancozeb 80 % WP) approved and regularly used to control early blight in Cuba and the biocontrol agents *Trichoderma asperellum* (strain *Ta.* 85) and *Pseudomonas protegens* (Pf-5). The effect of the concentrations of the chemical products, on the mycelial growth, sporulation of *T. asperellum* and the number of colonies of *P. protegens* was evaluated by the poisoned culture medium technique. The compatibility was classified according to the T value. The results showed that the three fungicides affected the mycelial growth and the sporulation capacity of the strain of *T. asperellum.* The colonies of the strain of *P. protegens* were also affected by the recommended field dose for all tested fungicides. Cuproflow excelled for presenting the smallest affectation in all the performed tests, besides, it was also classified as compatible. The results bring out the probability of incorporating the biological control agents under study and Cuproflow 37.7 % SCat low concentration in the integrated management of the *Alternaria solani*, causing early blight of potato. These results are novel and important for the application of microbial consortiums with synthetic fungicides. With the application of low dosages of fungicides, the appearance of resistant and aggressive pathogens can be limited. These will contribute to the preservation of environmental health.

Introduction

In general, early blight is considered one of the fungal diseases that cause the greatest economic losses on potato crop worldwide (Agrios, 2005; Mamgain*et al.*, 2013; CABI, 2018). This disease attacks practically all the organs of the plant with a short-term harmfulness, capable of destroying the crop if not taking account of an efficient phytosanitary management (Hernández *et al.*, 2016).

Various tactics are incorporated in the management of early blight: Cultural tactics, which provide the removal of stubble from the previous crop and weeds control, the use of healthy seed, the selection of tubers, the elimination of focal point of infections and the sanitation of affected leaves and stems,

the non-adjacent with related crop areas such as tomato or the same crop with significant phenological differences (Castro and Contreras, 2011; Pérez and Forbes, 2011; MINAG, 2016).

Genetics, that suggests the use of resistant cultivars (Barna) or moderately resistant (Atlas, Ajiba, Romano and among others). The Chemicals, recommends the use of fungicides with a protective effect from the initial phenological stages of the crop, alternated with those of systemic action and curative, to avoid the appearance of fungi-resistance (Wharton and Wood, 2013; MINAG, 2016).

The Biological ones, those show satisfactory results of the applications of biological products from the antagonistic microorganisms (fungi and bacteria) for the control of phytopathogen. Different strains of *Trichoderma* have shown a good antagonistic effect *in vitro*, against various *Alternaria* species (Murtaza *et al.,* 2012; Khaliq *et al.,* 2017; Ramakrishna *et al.,* 2017). In the same way, Magesh *et al.,* (2018), reported maximum growth reduction of *A. solani in vitro*, with *Pseudomonas fluorescens* strains. Gakegne and Martínez (2019) determined the efficacy of the biological product based on *Pseudomonas protegens* in the control of early blight of potato crop under field conditions.Regardless of these results, there are no yet promising antagonist strains for the control of early blight under field conditions.

However, the implementation of such an approach strongly relies on the compatibility between the biocontrol agents and the conventional synthetic fungicides. This study aimed to assess *in vitro* the compatibility between the *Ta.* 85 strain of *T. asperellum* and the Pf-5 strain of *P. protegens* with three fungicides frequently used in the control of *A. solani* in potato cultivation.

Materials and Methods

Origin of microbial cultures and inoculum production

The work was carried out in the Laboratory of Plant Mycology and Plant Bacteriology of the National Center for Animal and Plant Health (CENSA) (Latitude 22.991867; Longitude -82.153892), Mayabeque Province, Cuba.

As microbial agents, *T. asperellum* strain *Ta.*85 belonging to the culture collection of the Laboratory of Plant Mycology and *P. protegens* strain Pf-5, from the culture collection of the Laboratory of Microbial Cultures of the Faculty of Biology of the University of Havana, were used. These strains were selected for their parasitic effect against *A. solani* in previous studies (Gakegne *et al.,* 2017; Gakegne and Martínez 2018).

The inoculum of *T. asperellum*, was prepared by passing of discs of 5 mm diameter of the strain into Petri plates (90 mm Ø) with Malta Agar medium (MA) (BIOCEN) and incubated for 72 h at 30 ± 1°C in dark.

For the bacterial inoculum, the strain was prepared in Petri plates containing King B medium (BIOCEN) and incubated for 24 h at 28°C. Subsequently, they were poured in test tubes containing 5 ml of Nutrient Broth (NB) medium (BIOCEN) and incubated in agitation for 48 h in brand shaker (Thys 2) at 28°C and 150 rpm. The final cell suspension was prepared at a concentration of 10^8 CFU.

Three fungicides: Cuproflow 37.7% SC, Yaba M GD 66 and Mancozeb 80% WP, used for the control of early blight in potato crop under the conditions of Cuba (MINAG, 2016), were tested for compatibility *in vitro* by poisoned food technique at three different concentrations 100, 80 and 60% of the recommended dosage (MINAG, 2016) as shown in table 1. The fungicide solutions

were prepared in flasks with 25 ml of distilled water previously sterilized for 15 min at 121°C, and allowed to cool down to 40°C and each fungicide was added aseptically at the desired concentration and homogenized manually for 30 s.

Effect of fungicides on the mycelial growth of *T. asperellum*

For this experiment, 25 ml of the solution of fungicides were poured separately into the flask with 25 ml medium Potato Dextrose Agar (PDA) (BIOCEN) pH 5.6 at 40°C, and stirred manually for 30 s, then poured into Petri plates (90 mm Ø). After solidification, the plates were inoculated centrally with 5 mm disc of mycelium of 72 h hold *T. asperellum* taken from the periphery of actively growing colony. PDA Petri plates without the poisoned compounds were inoculated centrally with the antagonistic fungus served as controls. Three replicates (Petri plate) were used for each concentration of every tested fungicide. Petri plates were sealed with parafilm and incubated at 30 ± 1°C in dark. The mycelial growth of *T. asperellum* was measured every 24 h, upto 72 h. To determine the effect of the fungicide on the mycelial growth of *T. asperellum*, the mycelial growth of the control plates was taken as reference. The percentage of radial growth inhibition was calculated according to the formula suggested by Abbott *et al.,* (Ciba-Geigy, 1981).

Effect of fungicides on the sporulation of *T. asperellum*

The spores' suspension was obtaining by using the colony sweeping method. 7 days old colonies of antagonist, grown on PDA medium poisoned with the fungicides were used. To this end, 30 ml of sterilized distilled water was added fractionally on the plate. The obtained suspension was placed in 160 x 20 mm test tubes, stirred for 30 s in a Vortex tube agitator. The counting of spores per quadrant was performed using the Neubauver chamber. With the collected data, the sporulation of the fungal growth area was calculated based on $\pi * \gamma 2$ and the percentage of inhibition of sporulation by the formula of Abbott *et al.*, (CIBA-GEIGY, 1981).

The compatibility of each fungicide with the antagonistic fungus was calculated according to the T value proposed by Alves *et al.*, (1998), and the two indicators evaluated above were used: percentage of inhibition of the mycelial growth and the effect on sporulative capacity through the formula:

T = [20*CV + 80*[ESP] / 100

where: T: Corrected value for the classification of the product
CV: Percentage of vegetative growth in relation to the control
ESP: Percentage of sporulation in relation to the control.

The T values were classified according to the scale established by Alves *et al.*,

0 a 30	Very toxic
31 a 45	Toxic
46 a 60	Moderately toxic
> 60	Compatible

Effect of fungicides on the Pf-5 strain of *P. protegens*

For this experiment, the culture media were prepared in a similar way to that described above. In Petri plates containing PDA, 10 µl of the previously obtained bacterial suspension were inoculated, and spread uniformly, with a Drigalsky spatula. Control plates without the poisoning solution of fungicides were inoculated with the bacterial suspension using the same technique as the

treatment plates. All plates were incubated at 30 ± 1°C in dark. Three replicates were used per variant. The number of colonies was evaluated every 24 h, upto 72 h. Inhibition percentages were calculated according to the formula suggested by Abbott *et al.,* (CIBA-GEIGY, 1981) and processed according to the proportional comparison test.

Statistical analysis

The data collected for all the experiments were tabulated in Microsoft Excel and processed by simple analysis of variance. Mean comparisons were conducted according to Tukey's multiple range comparison test for a significance level of 95%. The statistical package Info Stat / Professional version 1.1 (Di Rienzo *et al.,* 2016) was used. Percentage data were analyzed using the system for comparison of multiple proportions (COMPAPROP) (Castillo and Miranda, 2014). A completely randomized design was used for all experiments.

Results and Discussion

Effect of fungicides on the mycelial growth of *T. asperellum*

The three fungicides showed an inhibitory effect against the *T. asperellum* strain *Ta.* 85, at the tested concentrations with significant differences ($p \leq 0.05$) (Table 2). Although no total fungicidal effect on this strain was observed in any of the variants, Mancozeb at the highest concentration inhibited strongly and progressively, up to 98.09% of the growth in the control.

Cuproflow 37.7% SC presented the lowest inhibitory effect of the mycelial growth of the antagonistic fungus at all concentrations, especially at the concentration of 60%, that had significant differences with the remaining treatments, during the evaluation period. Mancozeb 80% PH, regardless of the concentration, presented the greatest inhibitory effect on the growth of *T. asperellum* in the three moments of the evaluation (Fig. 1).

The differential response of *T. asperellum* strain *Ta.* 85 against fungicides in the present study may be due to its tolerance to these fungicides and/or its metabolic capacity to degrade different substrates, among them. Durán *et al.,* (2007) observed *in vitro* tests that copper oxychloride, metalaxyl and dimetomorf were compatible with different species of *Trichoderma*; while zineb, mancozeb and tiram showed slight toxicity and benomil behaved as toxic (Muiño *et al.,* 2006). However, *Trichoderma* was reported to be capable of degrading organochlorines, chlorophenols, and insecticides such as dichloride diphenyl trichloroetane (DDT), endosulfan, pentachloronitrobenzene, aldrin, and dihedron, and herbicides such as trifluralin and glyphosate (Espósito and Dasilva, 1998). The effects of pesticides are also observed on the cultural characteristics of *T. asperellum*, with variations in the edges, coloration of the colonies and texture of the mycelium (Martínez *et al.,* 2013).

The results evidenced the need to evaluate the compatibility of each new promising strain of antagonists with the agrochemicals used in the common application scenarios. From a practical point of view, this is of great importance in determining the timing of the application of the antagonist or of the agrochemical in crop management.

The results of the present investigation have a similar tendency to those obtained by Terrero *et al.,* (2018), who observed that copper hydroxide had the lowest inhibitory effect, at low concentration against *Trichoderma ovalisporum* Samuels & Schroers. The results of this work did not coincide with those of Shashikumar *et al.,* (2019), nor with those of Nandini *et al.,* (2018), who reported a 6.67%

and 19.75% inhibitory effect of the mycelial growth of *Trichoderma harzianum* Rifaiand *Trichoderma viride* Pers ex S. F Gray, respectively, in the test with Mancozeb 75%WP, while in this present work the inhibition of the growth of *T. asperellum* ranged between 94.93 and 98.13% with Mancozeb 80% WP. From these results, it is inferred that, from a practical point of view, the applications of Mancozeb 80% WP have to be done 7 days before the application of biocontroller, since the residual time of the fungicide in the culture can be up to seven days.

Effect of fungicides on the sporulation of *T. asperellum*

The three tested fungicides caused sporulation inhibition of the *Ta*. 85 strain (Fig. 2) in comparison to the control. The fungicide Cuproflow 37.7% SC presented the lowest inhibitory effect of the sporulation of the *Ta.* 85 strain. Regardless of concentrations, Mancozeb 80% WP completely inhibited the sporulation of the antagonistic fungus. These results are in agreement with those reported by Terrero *et al.,* (2018), who informed that *Trichoderma*is tolerant to a wide range of pesticides, and also Harman *et al.,* (2004) expressed that this tolerance is an innate characteristic for this genus of fungus.

These results showed that the effect of fungicides is greater on sporulation, in relation to the effect on the mycelial growth of the *Ta.* 85 strain. These results are similar to those obtained by Guerrero and Arias (2012) who found that copper oxide did not inhibit the production of spores of *Trichoderma koningiopsis* Samuels. However, the inhibition of sporulation by fungicides influences the spread of the biological agent in the soil, which limits its controlling action.

According to the compatibility classification proposed by Alves *et al.,* (1998), the fungicides Yaba and Mancozeb at all concentrations and Cuproflow at the concentration of 100% showed the high level of toxicity. Cuproflow at 60% showed the highest compatibility with 68.40% (Table 3). These results are similar to those obtained by Terrero *et al.,* (2018) who observed that low concentration copper hydroxide showed compatibility with *T. ovalisporum* and *Trichoderma stromaticum* Samuels & Pardo-Schulth. However, they do not match with the results of Nandini *et al.,* (2018) who found compatibility between Mancozeb 75% WP and *T. viride*. However; this fungicide was moderately toxic for A-34 strain of *Trichoderma harzianum* Rifai (Castellanos *et al.,* 2015). This difference is due, either to the species of *Trichoderma* and / or to the strain.

There are investigations that suggest the compatibility or not of *Trichoderma* with cupric fungicides (Terrero *et al.,* 2018). The direct effect on the growth of *Trichoderma,* may be associated with metal ions, such as copper, having an impact on the development of this type of fungi, particularly on the mycelial growth [Harman *et al.,* (2004); Martínez *et al.,* (2013)].

Effect of fungicides on the Pf-5 strain of *P. protegens*

The tested fungicides inhibited the number of colonies of the *P. protegens* strain Pf-5, with significant differences ($p \leq 0.05$) compared to the control (Table 4), but none of them showed bactericidal effect. The lowest inhibitory effect was observed on Cuproflow 37.7% SC and the biggest on Mancozeb 80% WP. Cuproflow, as in the previous experiment, is the product that showed the highest compatibility with *P. protegens* compared to the remaining fungicides (Fig. 3).

The results with Mancozeb coincide with those obtained by Mohiddin and Khan (2013) who did not observe compatibility between *Pseudomonas fluorescens* Migula and this fungicide. Although, Nandini *et al.,* (2018) found compatibility between Mancozeb 75% WP and *P. fluorescens*; however, they correspond to the results with the Cu base fungicide, finding compatibility between them.

In general, fungicide treatments, although they show significant differences among them, they showed that these antagonists have very few tolerances to the tested fungicides. These results prove that BCAs could be used with these products at low concentrations, which would reduce environmental contamination. Despite the fact that no fungicidal effect or bactericidal effect was found in any of the treatments, it was statistically observed that *P. protegens* proved to be more tolerant than *T. asperellum* to the tested fungicides, which confirms the said by Aislabie and Jones (1995) who demonstrated that *Pseudomonas* is more tolerant to fungicides than fungi, and that this could be because some bacteria can use pesticides as a source of nutrients and, therefore, can tolerate high concentrations of chemicals.

Table.1 Fungicides used in the test

Fungicides	Active ingredient	Field dosage
Cuproflow 37.7 % SC	Copper Oxychloride	2.5 L (PC).ha^{-1}
Yaba M GD 66	Valifenalate+mancozeb	2.5 kg (PC).ha^{-1}
Mancozeb 80 % WP	Manozeb	2.0 kg (i.a).ha^{-1}

Table.2 Inhibitory effect of different concentrations of fungicides on the mycelial growth of *T. asperellum*

Treatments (*Ta.*85)	Percentage of inhibition of the mycelial growth		
	24h	48h	72h
Cuproflow 100%	91.53 bc	84.88 cd	75.82 c
Cuproflow 80%	87.36 b	71.69 b	68.19 b
Cuproflow 60%	57.36 a	53.66 a	41.91 a
Yaba 100%	93.61 bc	91.45 def	85.27 d
Yaba 80%	89.31 bc	88.72 cde	81.47 cd
Yaba 60%	85.14 b	81.09 c	80.14 cd
Mancozeb 100%	96.81 c	98.13 f	98.09 e
Mancozeb 80%	96.81 c	96.69 ef	95.53 e
Mancozeb 60%	96.81 c	95.24 ef	94.93 e
ESx	0.84	1.87	2.79

Means with different letters, in the same column differ significantly ($p \leq 0.05$, according to Tukey's multiple range test).

Table.3 Classification of the toxicity of fungicides on *T. asperellum*

Fungicides / Concentration	Compatibility (Alves *et al*.,)	
	T value	Classification
Cuproflow 100%	23.13	Very toxic
Cuproflow 80%	46.65	Moderately toxic
Cuproflow 60%	68.40	Compatible
Yaba 100%	13.44	Very toxic
Yaba 80%	18.40	Very toxic
Yaba 60%	22.28	Very toxic
Mancozeb 100%	0.38	Very toxic
Mancozeb 80%	0.89	Very toxic
Mancozeb 60%	1.01	Very toxic

Table.4 Inhibitory effect of different concentrations of fungicides on the colonies number of *P. protegens*

Treatments (Pf-5)	*Percentage of inhibition of number of the colonies *P. protegens*		
	24h	48h	72h
Cuproflow 100%	74.23 c	70.29 c	59.04 c
Cuproflow 80%	61.50 b	59.50 b	48.42 b
Cuproflow 60%	34.57 a	42.75 a	30.22 a
Yaba 100%	94.98 ef	90.14 ef	81.00 f
Yaba 80%	90.40 e	87.35 e	76.28 e
Yaba 60%	82.66 d	80.74 d	67.79 d
Mancozeb 100%	98.48 f	96.45 f	94.20 g
Mancozeb 80%	95.74 ef	94.67 f	91.27 g
Mancozeb 60%	95.37 ef	87.89 e	85.74 f
ESx	4.96	7.01	7.29

Means with different letters, in the same column differ significantly ($p \leq 0.05$, according to Tukey's multiple range test).

Figure.1 Mycelial growth (mm) of *T. asperellum* in culture medium with fungicides at different Concentrations at 7 days. A: Cuproflow 37,7 % SC: a) 60%, b) 80%, c) 100%. B: Yaba M GD 66: a) 60%, b) 80%, c) 100%. C: Mancozeb 80 % WP: a) 60%, b) 80%, c) 100%. D: Control without fungicides

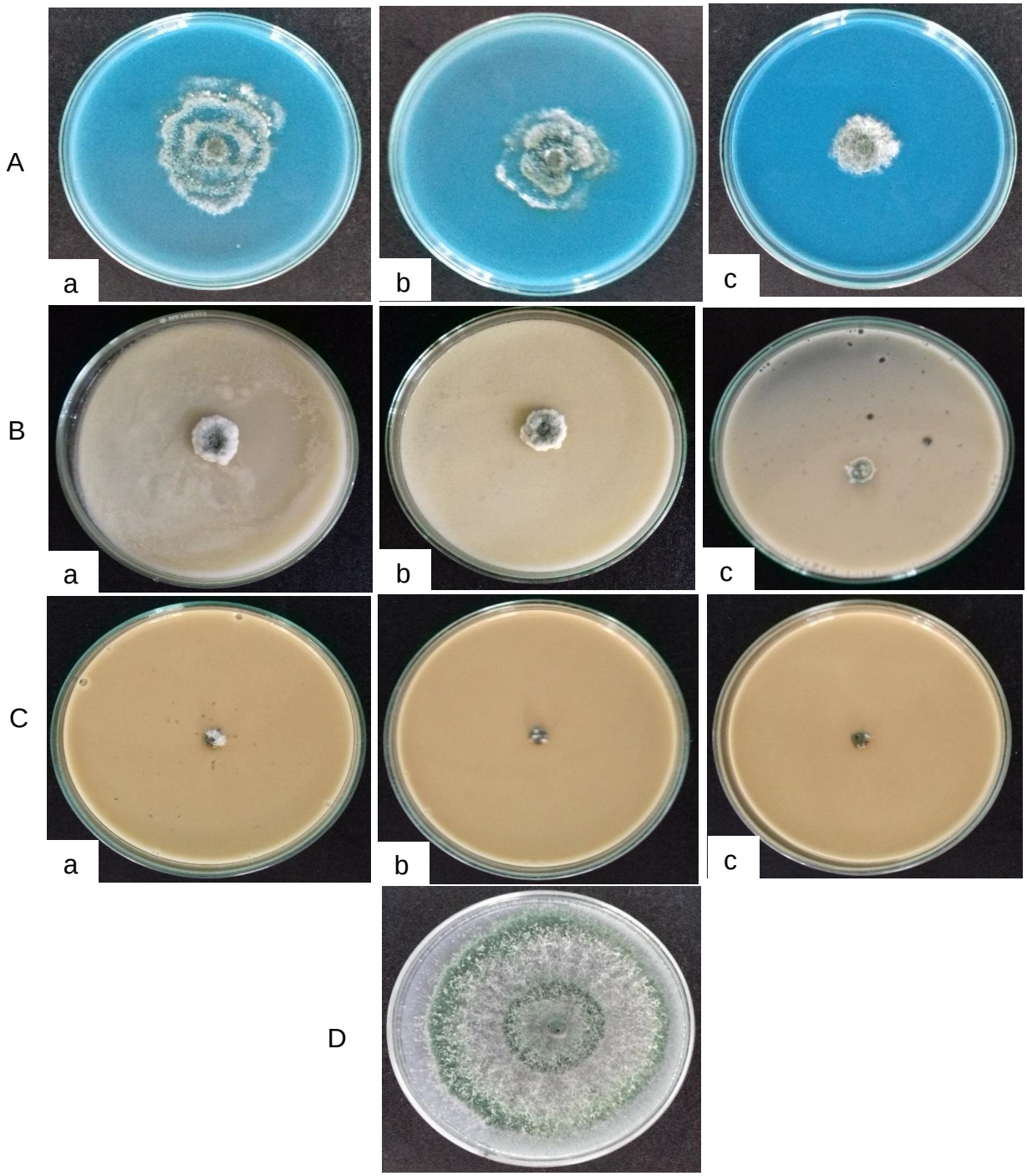


Figure.2 Inhibitory effect of fungicides at different concentrations on the sporulation of *T. asperellum* at 7 days

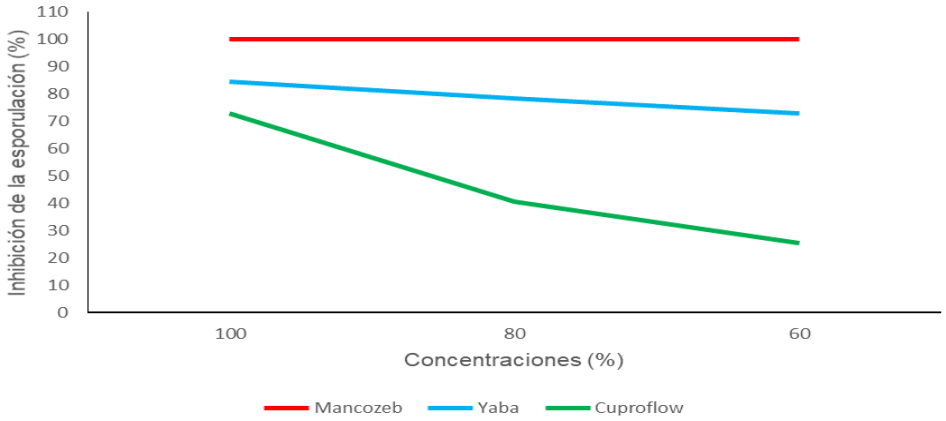
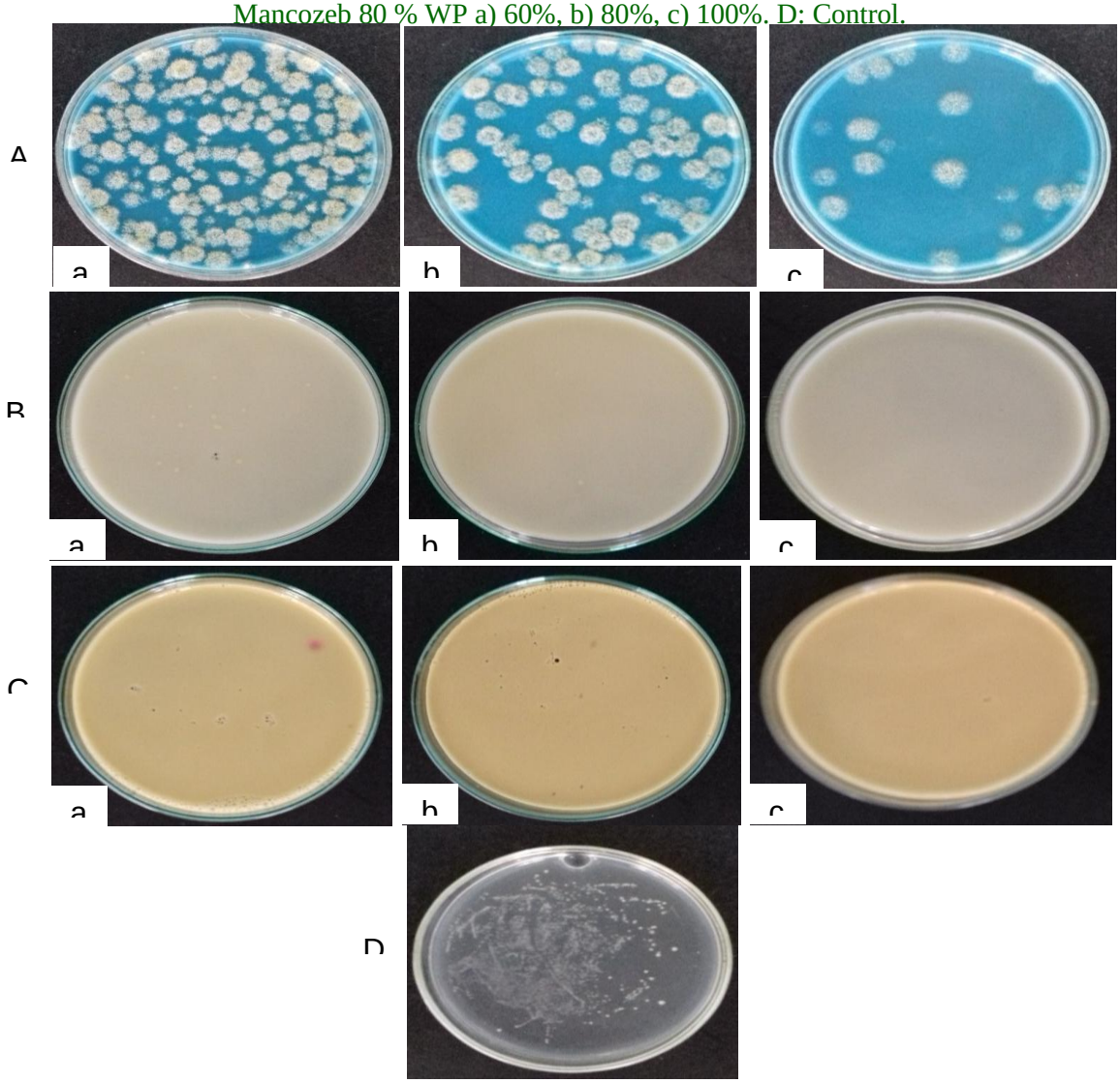


Figure.3 Effect of different concentrations of fungicides on number of the colonies of *P. protegens* A: Cuproflow 37,7 % SC: a) 60%, b) 80%, c) 100%. B: Yaba M GD 66: a) 60%, b) 80%, c) 100%. C: Mancozeb 80 % WP a) 60%, b) 80%, c) 100%. D: Control.



To develop an effective disease management programme, the compatibility of potential bioagents with fungicides is essential. Combining antagonists with fungicides may eliminate the chance of resistance development and reduces the fungicide application.

The results obtained in this work leave open the possibility of integrating the BCAs under study and the fungicide Cuproflow 37.7% SC, at low concentration in the integrated management of *A. solani* in the potato crop, which confirms the said by Erayya *et al.,* (2018) who indicated that the integration of bio-agents with lower concentration or sub-lethal doses of fungicides is becoming an acceptable technological-approach in sustainable agriculture. This must be verified in the field conditions with the aim to enhance crop health and productivity as stated by Kumar *et al.,* (2019) the integration of compatible bioagent with fungicides, may enhance the effectiveness of disease control and provide better management of diseases. Hence, the combination of biological control agents with fungicides would provide similar disease suppression as achieved with higher fungicide use.

In conclusions, organic measures to combat plants disease are the need of the hour and highest priority has been given for this. However, bio-agents have not yet attained efficiencies matching those of currently available fungicides. Integration of fungicides with bio-control agents will be a better option for improving the efficiency of bio-control agents. Combined application of chemical and bio- agents will help in outspreading the period of active disease control as well as reducing the cost of crop protection. Cuproflow fungicide 37.7% SC at low concentration showed high compatibility with the *Ta.* 85 strain of *T. asperellum* and Pf-5 of *P. protegens* under *in vitro* conditions.

References

Agrios, G.N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, San Diego, USA. Pp. 922.

Aislabie, J., and Jones, G. 1995. A review of bacterial degradation of pesticides. Aust. J. Soil Res. 33: 925-942.

Alves, S. B., and Moino, J. E. M. 1998. Almeida: Produtos fitossanitários entomopatógenos In: Alves, S. B.(ed.).Controle microbiano de insetos, FEALQ, Piracicaba, Brasil.; pp. 217-238.

Castellanos, G. L., Lorenzo M. E., Muiño, B. L., Hernández, P. R., Guillen, S. D. 2015. *In vitro* effect of commercial pesticides on *Trichoderma harzianum* strain A- 34. Rev. FCA UNCUYO. 47(2): 185-196.

Castillo, Y., and Miranda, I. 2014. COMPAPROP: Sistema para comparación de proporciones múltiples. Rev. Protección Veg.; 29(3): 231-234.

Castro, I., and Contreras A. 2011. Manejo de plagas y enfermedades en el cultivo de la papa. Imprenta Austral, Valdivia-Chile. 1-72.

Centre for Agriculture and Biosciences International (CABI). 2018. *Alternaria solani* (early blight of potato and tomato). Invasive Species Compendium. En: https://www.cabi.org/isc/datasheet/4528#totaxonomicTree

CIBA-GEIGY. 1981. Manual para ensayos de campo en protección vegetal. Segunda edición revisada y ampliada. Editado y publicado por Werner Püntener, División Agricultura, CIBA-GEIGY S.A. Basilea, Suiza. Pp. 33-35.

Di Rienzo, J., Balzarini, M., Casanoves, F., González, L., Tablada, M., Walter, R. 2016. InfoStat versión 1.1. Grupo InfoStat, FCA, Universidad Nacional

de Córdoba, Argentina.

Durán, E., De Romero, Y., Romero, M., Ramallo, J. *In vitro* sensitivity of *Trichoderma* strains isolated from soybean seeds against the MAXIM® XL fungicide. Mycological Bulletin. 2007; 22: 51-54

Erayya, S. N., Balodi, R., Tewari, A.K., Kumar, J. 2018. Compatibility of *Trichoderma* with Copper-Chitosan Graded Combinations, Int. J. Pure App. Biosci. SPI: 6(3): 631-634.

Espósito, E., andDasilva, M. 1998. Systematics and environmental application of the genus *Trichoderma*. Microbiology. 24:89-98.

Gakegne, E. R., and Martínez, C. B. 2018. Antibiosis y efecto de pH-temperaturasobre el antagonismo de cepas de *Trichoderma asperellum* frente a *Alternaria solani* Rev. Protección Veg. 33(2):1-9.

Gakegne, E. R., Plasencia, O., Hernández-Rodríguez, A., de La Fe, Y., Martínez C. B. 2017. Actividad antagónica de *Pseudomonas* spp. fluorescentes ante *Alternaria solani* Sorauer, patógeno de la papa (*Solanum tuberosum* L.). Rev. Protección Veg.; 32(3): 1-11.

Guerrero, R., and Arias, D. 2012. Evaluación del efecto de fungicidas sobre el desarrollo de dos especies de *Trichoderma* (Fungi: Ascomycota: Hypocreaceae) utilizadas en el biocontrol de hongos patógenos de cacao.Agroecología.; 1(1): 1-8.

Harman, G., Viterbo, A., Chet, I., Lorito, M. 2004. *Trichoderma* species--opportunistic, avirulent plant symbionts. Nature Reviews. Microbiology. 2(1): 43-56.

Hernández, O., Castillo, D., Herrera, R., Fuentes, E., Drouaillet, E., Santillán, A. 2016. Actividad antagónica de *Trichoderma* spp. sobre *Rhizoctonia solaniin vitro*. Investigación y Ciencia. 24(67): 5-11.

Joseph, A., Best-Igbinosa, O., Tope-Alori, E., Ademiluyi, O., Aluko, A. 2017. Effectiveness of *Pseudomonas* species in the management of tomato early blight pathogen *Alternaria solani*. African journal of microbiology research. 11(23): 972-976.

Khaliq, A., Khan, H., Khan, N., Zada, L., Ali, M., Zahoor. M., *et al.*, 2017. *In vitro* efficacy of *Trichoderma harzianum* Rifai against various isolates of *Alternaria* sp. Journal of Entomology and Zoology Studies. 5(1): 442-445.

Kumar, A., Bansal, R. D., Chelak, K. Y. 2019. Compatibility of *Trichoderma viride* with Fungicides for Plant Disease Management. Int. J. Pure App. Biosci. 7 (3): 44-51.

Magesh, M., Sudha, R., Ahila-devil, P. 2018. Impact of rhizosphere *Pseudomonas fluorescence* against *Alternaria solani* in tomato. J.Bio.Innov. 7(2): 242-251.

Mamgain, A., Roychowdhury, R., Tah, J. 2013. *Alternaria* pathogenicity and its strategic controls. Res. J. Biol. 1: 1-9.

Martínez, C. B., Infante, D., Reyes, Y. 2013. *Trichoderma* spp. y su función en el control de plagas en los cultivos. Rev. Protección Veg. 28(1): 1-11.

Ministerio de la Agricultura (MINAG). 2016. Instructivo Técnico para la producción de papa en Cuba. Dirección de Agricultura. Editorial Ministerio de Agricultura. La Habana, Cuba. 62.

Mohiddin, A., and Khan, R. 2013. Tolerance of fungal and bacterial biocontrol agents to six pesticides commonly used in the control of soil borne plant pathogens. Glob. J. Pest Dis. Crop Prot. 1(1): 1-4.

Muiño, B., Sáenz, M., Stefanova, M., Pomas, A., Díaz, I. 2006. Compatibilidad de *Trichoderma* spp. con plaguicidas y fertilizantes en el cultivo del tabaco (*N. tabacum* L.). Fitosanidad.

10(2):153.

Murtaza, A., Shafique, S., Anjum, T., Shafique, S. 2012. *In vitro* control of *Alternaria citri* using antifungal potentials of *Trichoderma* species. African Journal of Biotechnology. 11(42): 9985-9992.

Nandini, M., Ruth, C., Gopal, K. 2018. Compatibility of biocontrol agents with fungicides used in turmeric cultivation under *in vitro* conditions. Res. J. Chem. Env. Sci.; 6(4): 19-25.

Pérez, W., and Forbes, G. 2011. Guía de identificación de plagas que afectan a la papa en la zona andina. Centro Internacional de la Papa (CIP). 1-48.

Ramakrishna, A., Desai, S., Uma, G., Uma, M. T. 2017. Efficacy of different isolates of *Trichoderma*against early blight of tomato. Journal of Pharmacognosy and Phytochemistry. 6(5): 1060-1062.

Shashikumar, M., Sumangala, K., Navyashree, E. 2019. Compatibility of *Trichoderma viride* and *Trichoderm aharzianum* with Fungicides against Soil Borne Diseases of Tomato and Cabbage. Int. J. Curr. Microbiol. App. Sci. 8(4): 1920-1928.

Terrero, P., Peñaherrera, S., Solís, Z., Vera, D., Navarrete, J., Herrera, M. 2018. Compatibilidad *in vitro* de *Trichoderma* spp. con fungicidas de uso común en cacao (*Theobroma cacao* L.). Investig. Agrar.; 20(2):146-151.

Wharton, P., and Wood, E. 2013. Early blight biology and control in potatoes. University of Idaho. College of Agriculture and Life Sciences. 1-5.

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