Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (10):157-162
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation *in vitro* about the compatibility between *Trichoderma citrinoviride* spp. strain 19 and chemical, biological and organic fungicides to control *Botrytis cinerea*

Diego Patricio Núñez Ocaña[1]*, E. Urbano[1], Carlos Chiriboga[1], Darwin Rueda[1], Bangeppagari Manjunatha[1], Sikandar I. Mulla[2] and Mariadoss Selvanayagam[3,4]

[1]*Department of Life Sciences, Universidad de las Fuerzas Armadas-ESPE, Sangolquí, Quito, Ecuador, South América*
[2]*Department of Biochemistry, Karnatak University, Dharwad, Karnataka, India*
[3]*Department of Environmental Engineering, Universidad Estatal Amazonica, Puyo, Ecuador, South America*
[4]*Loyola - ICAM College of Engineering and Technology (LICET), Loyola Campus, Chennai, India*

ABSTRACT

This study was carried out to determine the compatibility of Trichoderma citrinoviride spp. Strain 19 on different kinds of fungicides like: organics, chemicals and biological, are all used to control gray mold in roses. The preliminary selection was conducted to select 32 active ingredients (AI), and were excluded the following AI:ascorbic acid, citric acid, lactic acid, a citrus extract, prochloraz and also the mixtures with ISR's. Later in compatibility assays were evaluated based on the interaction between the antagonistic fungus vs two doses of each active ingredient. After 15th day of incubation, it was evaluated for conidiative capacity of the antagonistic fungus at the first generation and the germination capacity of those conidia. Finally evaluated the best mixtures aimed to control Botrytis cinerea in vitro. The compatible ingredients to be used in mixtures the antagonistic fungus was: ISR's made of copper, manganese, zinc, specific organic acids, fosfonates, cupric sulphate, ammonium ions, extracts of Azadirachta indica, and others. The results conclude that all of this mixtureis compatible and once checked the results in the field, is possible to reduce the use of synthetic Agrochemicals, preserve natural predators of diseases and pests, lower the atmospheric pollution and reduce the possibility to cause resistance in the disease/plague.

Keywords: *Botrytis cinerea*, *Trichoderma citrinoviride*, Interaction, Mixture.

INTRODUCTION

Cut flower has been a major boom for Ecuador flower growers and exporters while the rose has been the main leader. The major advantages springing from climatic, geographical, technological strengths are coupled with infrastructure factors making Ecuador's cut flower industry one of the strongest in the world market place. Thus, the cut flower export has become one of the sustainanle livelihood and employment for many Ecuadarins specially in the Sierra región. One of the disadvantage of ornamental crops is it ssusceptibility to root diseases. Until 2004 Methyl bromide was considered for soil treatment to prevent soil diseases. Methyl bromide is banned in many developed countries for their known effects as GHG (greenhouse gas) and arises the need to find new alternatives to the use of Methyl bromide in Ecuador. *Trichoderma* spp. since two decades ago has been considered as an

alternative to control diseases in various crops [1, 2, 3, 4]. It is well known that microorganisms play an important role as biological control agent of plagues in agriculture and has been proved effective in many countries [5].

There are strategies to control diseases in which it is used biocontrolling fungi with sublethal doses of insecticides or botanical extracts to improve efficiency and accelerate the mortality of the pests [6]. The need to reduce the use of synthetic chemicals in agriculture has attracted some concern in the use of essential oils for the control of plant pathogens. Among other compounds and microorganisms [7].

In Ecuador it is frequently used microorganisms, especially in the flower industry as organic management strategy, it is applied to crops or soil through the irrigation system, drench or spray applications for crops. Intensive agriculture is known that the use of biological control cannot replace the need to quickly suppress an epidemic [6].
In flower growth *Trichoderma* spp. is mixed with synthetic chemical fungicides prior spraying trying to reduce the use of chemicals and indirect production costs; however it is done empirically because it has not been scientifically proven that these mixtures are effective and efficient for disease control, also it has not been shown that the interaction between Trichoderma spp. and agrochemicals are harmless to the fungus. And the development of "synthetic fungicides compatible" strains of *Trichoderma* spp. should be a priority for the future system integrated pest management [8]; because is it considered that during the field application of the biocontrol fungi, these are easily affected by environmental condition and biotic factors as the interaction with other antagonistic microorganisms [6]. Plant breeding companies like De Ruiter, Rosen Tantau, EB & B, Plantec, Brown Breeding among other produce commercial and attractive rose varieties for selling to international markets, this varieties have good agronomic performance, good disease resistance and specially adapted to the environmental conditions of our country. Those breeders sometimes tend to release commercial varieties with moderate to high susceptibility to diseases such Botrytis cinerea, which affects the vase life of cut flowers that are not properly marketed.

And considering that synthetics and entomopathogenic fungi work synergistically, in this way permits the use of lower doses of synthetic insecticides thereby preserving the natural enemies of certain pests, reduces the pollution to the environment and the ability to cause resistance in the insect [6].

MATERIALS AND METHODS

Preliminary selection compatible with active ingredients *Trichoderma citrinoviride*
To determine pre compatibility *Trichoderma citrinoviride* and 32 active ingredients (control mechanisms), subjected to a selection phase in which the compatibility of the antagonist was evaluated considering a single dose of the active ingredient and a single standard concentration of conidiaml $^{-1}$.

Conidia suspension was dispensed into each test tube, each active ingredient for the corresponding dose was added and vortexed for 30 seconds to homogenize the mixture and immediately 10µl of the suspension was dispensed in the center was prepared PDA medium [6]. With alcohol burner and was flamed boxes sealed with parafilm. She entered the scheduled incubator at 25°C and the growth area 2 and 3 day incubation was assessed.

Calculation of the percentage of area growth inhibition of *Trichoderma citrinoviride*
These evaluations to the second and third day of incubation, each treatment was performed and consisted of the following stages:

Preparation of the conidia suspension of *Trichoderma citrinoviride*
In laminar flow chamber substrate 75 grams of hydrated wheat previously mixed in a carrot broth, said substrate was massively colonized by conidia Trichodermacitrinovirideand with 100ml of sterile distilled water was collected. With the aid of sterile gauze separated solid fraction and in a beaker of sterile 250ml conidial suspension (approximately 80ml) which was maintained under constant agitation until the suspension was prepared for each treatment was collected.

Preparation of stock solutions
Immediately after harvest conidia concentration of conidia ml^{-1} stock solution to what their respective dilutions was conducted and counted four times in the Neubauer chamber to determine the concentration of conidia suspension, it was determined the volume of the solution water + 0.1% tween 80 added that this way was determined to get to the

following dilutions: $1x10^5$, $1x10^7$, $1x10^9$ conidia ml^{-1}. The dilutions were performed in sterile Erlenmeyer flasks of 250ml volume.

Mixture of active ingredients with the conidia suspension of *Trichoderma citrinoviride*
In sterile test tubes made dilutions (5 to 6ml depending on the active ingredient) and the respective sterile micropipette was dispensed (depending on the volume to be dispensed) the active ingredient is dosed into each tube. Each treatment, according to the planning of the trial was identified.

Treatments planting Middle PDA
Mixtures of each test tube were homogenized for 20seconds and finally dissolving the active ingredient in the suspension of conidia. Using a sterile micropipette 10ul of the mixture was dispensed in the center of the Petri dish, allowed to stand 24 hours sufficient time in which the mixture is absorbed onto the PDA, Petri dishes were flared and sealed with parafilm and immediately were admitted to the incubator was set at 25°C and established the "day 0" is the time and day when planting was done.

Evaluation of *Trichoderma citrinoviride* growth area to day 2 and 3 from sowing
Due to the rapid growth of *Trichoderma citrinoviride* was assessed at day 2 and 3 and that in all treatments 4th antagonist hyphae reached the edge of the box. After completing the treatment, a photographic record with a professional digital camera in connection with the ImageJ software that you scanned the surface of the Petri dish where the growth area of the antagonist was determined using the software was performed.

Measuring the capacity conidiativaof *Trichoderma citrinoviride*
The technique proposed by [9] was used in this trial, in which the fifteenth day of incubation, the Petri plates from the incubator and each experimental unit was removed using a # 2 a disk punch of 5 mm in diameter perimeter of the Petri dish was removed. This disc was placed inside a sterile test tube and 2ml of isotonic solution consisted of sterile distilled water with sodium chloride 0.5% and 0.1% tween 80 was added. It was stirred for 15 seconds in the vortex and a 1 was performed: 100 in a new sterile test tube and again vortexes and determined under the microscope and camera neubauer the number of conidia ml^{-1} each experimental unit.

Calculation of the percentage of viable conidia *Trichoderma citrinoviride* in the first generation
After the interaction with chemical, biological or organic product, for each treatment of conidia suspension of *Trichoderma citrinoviride* was taken and a sterile micropipette dispensed and held 10µl of the suspension at the center of the petri dishes containing medium TSM. This medium allows the germination of conidias [10]. All seeded houses were sealed with parafilm and incubated in a 25°C controlled environment. Colony counting was performed at the sixth day of incubation [5].

Determination of diameter growth of *Botrytis cinerea*
Botrytis spores were isolated from infected rose buttons with the disease and the pathogen was purified on Petri dishes with PDA medium, the eighth day of growth discs of 5 mm in diameter with a punch # 2 were extracted. In Petri dishes with PDA medium 10 ul of each treatment, it dispensed with the handle and dispersed over the entire surface of PDA medium. *Botrytis cinerea* disc was then placed in the center of the petri dish and flamed and sealed with parafilm. It was left to incubate in a controlled environment at 22°C on the eighth day of incubation the diameter growth of the pathogen was measured.

RESULTS AND DISCUSSION

Active Ingredient selection based on area growth *Trichoderma*at 48 and 72 hours of incubation
It was determined that the active ingredients: ascorbic acid, citric acid, lactic acid, prochloraz and interactions with the same, completely inhibited the growth of *Trichoderma citrinoviride* strain 19 at 48 hours and 72 hours of incubation.

Compatibility tests
Trials for evaluation are grouped, selecting all relevant treatments were within the range that means was done: $\chi + 2\sigma$ and a score of 10 was assigned to 1 from best to worst on. The results are presented below:

Table 1: Test No. 1

	AREA 72 HRS	SCORE #1	GERM.	SCORE #2	TOTAL
Dose C3 1.5 cc / l of copper ions, zinc, manganese	4840.75	9	143.00	5	14
Dose C2 1.35 g / l Calcium sulphate	4961.91	10	41.67		10
Dose C3 0.5 cc / l of potassium, calcium, folic acid, organic acids specific	3704.80		246.33	10	10
C3 Dose 3 cc / l of copper ions, zinc, manganese	4765.16	7	112.00	2	9
Dose C3 1.5 cc / l potassium phosphonate, phosphorus oxide (V) oxide, potassium	3813.63		211.00	9	9
C3 Dose 2 g / l ammonium ion	4784.85	8	98.00		8
C1 Dose 3 cc / l of copper ions, zinc, manganese	3390.72		189.33	8	8
Dose C3 1cc / l of potassium, calcium, folic acid, organic acids specific	3253.08		157.67	7	7
C2 Dose 2 g / l ammonium ion	4733.30	6	25.33		6
C1 Dose 1 cc / l of potassium phosphite	3492.87		152.33	6	6
C3 dose 4 g / l of ammonium ion	4702.98	5	12.00		5
C2 dose 4 g / l of ammonium ion	4692.98	4	46.67		4
Dose C1 0.5 cc / l of potassium phosphite	3386.54		118.33	4	4
C3 Dosage 2.7 g / l of calcium sulphate	4686.27	3	32.00		3
C3 Dose 6 cc / l of potassium phosphite, magnesium phosphite, calcium phosphite	4675.92	2	107.33	1	3
Dose C1 2 g / l ammonium ion	3294.27		116.33	3	3
Dose C2 1.5 cc / l of copper ions, zinc, manganese	4672.41	1	62.67		1

Table 2: Test No. 2

TREATMENTS	AREA 72 HRS	SCORE#1	GERM.	SCORE #2	TOTAL
Dose C2 2.5 cc/l of Cubiet	5406.60	8	513.00	10	18
C2 Dose 5 cc/l of Cubiet	5603.19	10	275.00		10
Dose C3 0.75 cc/l of copper sulfate	5232.23		427.67	9	9
Dose C2 0.75 cc/l of copper sulfate	5477.40	9	183.00		9
Dose C2 1.5 cc/l of copper sulfate	5338.22		340.00	8	8
C1 Dose 5 cc/l of Cubiet	4752.91		324.50	7	7
Dose C3 2.5 cc/l of Cubiet	5401.77	7	59.00		7

Table 3: Test No. 3

TREATMENTS	ÁREA 72 HRS	SCORE #1	GERM.	SCORE #2	TOTAL
Dose C2 12.5 cc/l of extract from *Azadirachta indica*, Alliaceae, and potassium salts Solanaceae	4757.59	9	329.33	9	18
Dose C2 0.25 g/l of Polioxin	3534.48		546.33	10	10
Dose C2 6.25 cc/l of extract from *Azadirachta indica*, Alliaceae, and potassium salts Solanaceae	4847.22	10	316.00		10
C3 Dosage 0.5 g/l of Polioxin	4461.34		328.33	8	8
C3 Dosage 0.5 g/l of boscalid	4673.81	8	192.00		8
C2 Dosage 0.5 g/l of Polioxin	3757.97		322.00	7	7
Dose C3 12.5 cc/l of extract from *Azadirachta indica*, Alliaceae, and potassium salts Solanaceae	4554.99	7	192.00		7

Table 4: Test No. 4

LABELS	ÁREA 72 HRS	SCORE#1	GERM.	SCORE #2	TOTAL
C3 Dose 1 ml/l of extract *Reynoutria sachalinensis*	5043.25		1873.33	10	10
C3 Dose 2 cc/l Camomile extract	5348.27	10	93.33		10
C1 Dose 2 cc/l of extract *Reynoutria sachalinensis*	4941.22		1236.67	9	9
C3 Dose 1 cc/l Camomile extract	5297.76	9	73.33		9
C1 Dose 1 cc/l Camomile extract	4615.99		1153.33	8	8
C2 Dose 2 cc/l Camomile extract	5277.89	8	63.33		8
C3 Dose 3 cc/l of extract from *Azadirachta indica*	4922.31		883.33	7	7
C3 Dose 5 cc/l of extract of thyme	5116.59	7	140.00		7
Dose C1 2 ml/l Camomile extract	4615.13		803.33	6	6
C2 Dose 1 cc/l Camomile extract	5099.44	6	86.67		6
C1 Dose 1 ml/l of extract *Reynoutria sachalinensis*	4599.89		666.67	5	5
C2 Dose 3 cc / l of extract from *Azadirachta indica*	5083.97	5	50.00		5

Table 5: Test No. 5

TREATMENTS	AREA 72 HRS	SCORE#1	GERM.	SCORE #2	TOTAL
C3 Dose 0.4 cc/l *Bacillu ssubtilis* GBO3	4501.51	5	153.33	10	15
C3 Dose 0.8 cc/l *Bacillus subtilis* GBO3	4697.21	10	20.00		10
C1 Dose 4 cc/l *Bacillus pumillus*	2626.87		100.00	9	9
C3 Dose 2.8 cc/l *Gliocladium* spp.	4639.18	9	30.00		9
C1 Dose 2 cc/l *Bacillus pumillus*	2602.01		96.67	8	8
C3 Dose 2.4 cc/l *Bacillus subtilis* QST713	4587.03	8	36.67		8
C3 Dose 4.8 cc/l *Bacillus subtilis* QST713	4264.68		73.33	7	7
C3 Dose 1.2 cc/l *Bacillus* spp.	4578.89	7	43.33		7
C2 Dose 1.2 cc/l *Bacillus* spp.	3805.08		63.33	6	6
C3 Dose 0.6 cc/l de *Bacillus* spp.	4512.22	6	5.00		6
C3 Dose 4 cc/l *Bacillus pumillus*	4342.74		56.67	5	5
C2 Dose 2.8 cc/l *Gliocladium* spp.	3972.16		50.00	4	4
C3 Dose 2 cc/l *Bacillus pumillus*	4437.80	4	16.67		4

Table 6: Test No. 6

TREATMENTS	ÁREA 72 HRS	SCORE #1	GER M.	SCORE #2	TOTA L
C3 Dose 1 ml/l of active ingredient	4449.11	9	96.67	8	17
C2 Dosage 0.5 g/l of Polioxin + 1 cc/l of potassium, calcium, folic acid, organic acids specific	4429.07	8	106.67	9	17
Dose C2 0.25 g/l of Polioxin + 0.5 cc/l of potassium, calcium, folic acid, organic acids specific	4469.30	10	0.00		10
C3 Dosage 0.5 g/l of Polioxin + 1 cc/l of potassium, calcium, folic acid, organic acids specific	4227.01		163.33	10	10
C3 Dose 2 cc/l Active ingredient	4407.93	7	36.67		7
C3 Dosage 0.5 g/l of Polioxin + 2 cc/l Active ingredient	4238.84		86.67	7	7
C2 Dosage 0.5 g/l of boscalid + 1 cc/l Active ingredient	4353.13	6	16.67		6
C1 Dose 1 ml/l of active ingredient	3131.26		86.67	6	6
Dose C2 0.25 g/l of Polioxin + 1 cc / l Active ingredient	4348.27	5	20.00		5
C1 Dosage 0.5 g/l of Polioxin + 1 cc / l of potassium, calcium, folic acid, organic acids specific	3371.26		83.33	5	5
Dose C3 0.25 g/l of Polioxin + 0.5 cc/l of potassium, calcium, folic acid, organic acids specific	4303.24	4	6.67		4
C2 Dose 1 g/l of boscalid + 1 cc/l of potassium, calcium, folic acid, organic acids specific	4047.39		80.00	4	4

For the final evaluation all treatments had one score were selected.

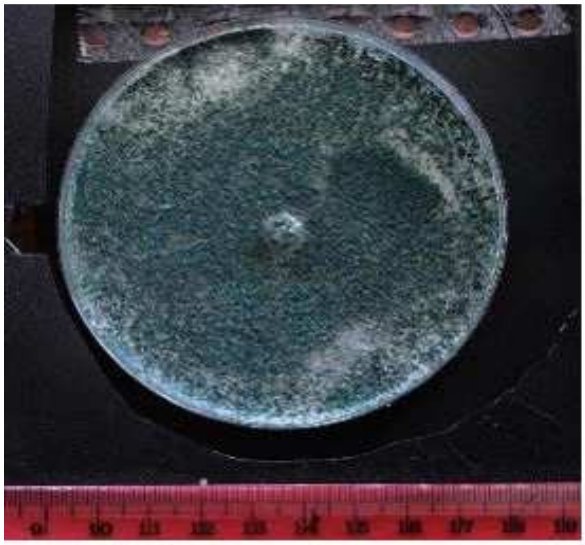

Figure1: Dual antagonism test *Trichoderma* and *Botrytis*. Total *Trichoderma* on *Botrytis* colonization is observed

Final Test-Results in vitro confrontation with *Botrytis cinerea*

It was observed that the growth of the pathogenic fungus *Botrytis cinerea* in all treatments was zero except the control, provided that the pathogen was limited by the aggressive colonization of antagonistic *Trichoderma citrinoviride* strain 19 preventing the development under these conditions the development of infective structures *Botrytis cinérea* (Figure 1).

CONCLUSION

The active ingredients which inhibit the normal development of *Trichoderma citrinoviride* strain 19 are:Procloraz, Ascorbic acid, citric acid, lactic acid, Citrus Extract, Procloraz + active ingredient, Prochloraz + potassium, calcium, folic acid, organic acids specific. *Trichoderma citrinoviride* in concentration $1x10^9$ conidia ml^{-1} X is compatible with copper ions, calcium, folic acid, specific organic acids, zinc, magnesium, potassium phosphonate, phosphorus oxide, potassium oxide, ammonium. calcium sulfate, manganese, copper sulfate, cubiet, Polixin, boscalid, *Trichoderma citrinoviride* in concentration $1x10^9$conidia ml^{-1} X extarcts supports of: *Azadirachta indica*, Alliaceae, Solanaceae, *Reynoutria sachalinensis*, *Matricaria chamonilla*, *Azadirachta indica*, *Thymus vulgaris*, *Reynoutria sachalinensis*. *Trichoderma citrinoviride* in concentration $1x10^9$ conidia ml^{-1} X is compatible with the active ingredients of: *Bacillus subtilis* QST713 and GBO3, *Gliocladium* spp, *Bacillus pumillus*. *Trichoderma citrinoviride* 100% inhibits the growth of *Botrytis cinerea* when the antagonist compatible with the active ingredients is mixed.

Recommendations

Do not mix *Trichoderma citrinoviride* Cepa 19 active ingredients: Prochloraz, ascorbic acid, citric acid, lactic acid and citrus extract; because it is not compatible with the antagonist because it inhibits mycelial growth of the fungus.

REFERENCES

[1] M. Ahmad, Najah National University (Nablus, Palestine, **2006**).
[2] K. Brunner, S. Zeilinger, R. Ciliento, S. Woo, M. Lorito, C. Kubicek, R. Mach, *Applied And Environmental Microbiology*, **2005**, 71, 7, 3959–3965.
[3] P. Chaurasia, S. Joshi, *Nepal Agric. Res. Journal*, **2000**, 4 & 5.
[4] S. Fowler, M. Jaspers, M.Y. Walter, A. Stewart, *Proc. 52nd N.Z. Plant Protection Conf*. **1999**, 141-147.
[5] L. Castellanos, B. Muiño, M. Lawrence, A. Rodriguez, M. Gomez, *Plant Health*, **2011**, 15, 1, 31-38.
[6] M. Ramzan Asi, M. Hamid, M. Afzal, M. Ashfaq, S. Talib, *Pak. J. Bot*, **2010**, 42 (6), 4207-4214.
[7] L. Barrera, L. Garcia, Antifungal activity of essential oils and their compounds on the growth of Fusarium sp. Isolated papaya (*Carica papaya*). Product Development Center Biotic. National Polytechnic Institute. Mexico, **2008**.
[8] S. Gowdar, H. Ramesh, V. Nargund, M. Krishnappa, *Agric. Sci. Digest*, **2006**, 26 (4), 279-281.
[9] L. Castellanos, M. Lawrence, B. Muiño, A. Rodriguez, *Plant Health*, **2008**, 12, 1, 45-50.
[10] Y. Elad, L. Chet, *Phytoparasitica*, **1983**, 11(1), 55-58.