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RESEARCH ARTICLE

COMPATIBILITY OF *TRICHODERMA* SPP. WITH SOME FUNGICIDES UNDER *IN VITRO* CONDITIONS

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ABSTRACT

The compatibility between strains of *Trichoderma harzianum* (Tcomp, TH1 and TH3) and *T. viride* (TV1) and different active ingredients used as fungicides against seeds borne and foliar diseases of some cultures were tested under *in vitro* conditions. The activities of the formulations, pyraclostrobin + boscalid, cyprodinil + fludioxanil, fenhexamid and mepanipyrim were null to low on the mycelial growth of the strains tested. The compatibility percentages were low to moderate the first week, respectively 35.9, 10.0, 28.7 and 34.4% for TH3 and 59.1, 32.8, 61.9 and 84.1% for Tcomp at the recommended doses 501, 375, 750 and 400 ppm. They become high during the second week respectively 88.1, 75.4 and 100%.

Seven days after incubation, the compatibility of Tcomp and TH3 was moderate (51.4%) at 200 ppm of pyrimethanil and become important after 16 days, respectively 65.0 and 78.7%. 32 days after incubation, this fungicide showed a moderate activity at the recommended dose (800 ppm), the compatibility percentages varied between 33.3 and 68.5%.

After the second week, the chlorothalonil at 500 ppm showed good compatibility with Tcomp and TV1 (51.9 and 48.2%) and all the strains tested except TH1 become moderately compatible (43.5 – 72.2%) with the different concentrations after 24 days of incubation. However, thiram was able to completely inhibit mycelial growth of the *Trichoderma* stains during 4 weeks. No compatibility was observed between them.

After one month, the *Trichoderma* strains produced conidia in the presence of low concentrations of chlorothalonil and in the recommended dose of fenhexamid, showing compatibility respectively ranging from 50.5 to 89.7% and 51.5 to 97.1%. The conidia production of the tested strains was inhibited at the recommended doses of the other fungicides tested. However, thiram showed good compatibility at 500 and 666.6 ppm ranging from 45.5 to 84.9%. Similary, boscalid + Pyraclostrobin at 125.5 ppm and cyprodinil + fludioxanil at 93.7 ppm were compatible respectively with TH3 and TV1 (50.6-56.1%) and TH1-TH3 (90.4-79.3%).

After 24 hours of incubation, the *Trichoderma* conidia could germinate in the presence of different concentrations of mepanipyrim and fenhexamid showing compatibility respectively ranging from 45.0 to 98.0% and 37.5 to 97.1%. The Tcomp strain showed good ability to germinate in presence of low doses of thiram (500 ppm), chlorothalonil (375 ppm), boscalid + pyraclostrobin (125.2 ppm) and cyprodinil + fludioxonil (93.7) with respective compatibility percentages of 54.3, 54.2, 75.5 and 57.2%.

INTRODUCTION

Biological control involves the use of beneficial microorganisms to attack and control plant pathogens, and the diseases they cause. It is an environmentally acceptable approach to disease management. Among the fungal biocontrol agents *Trichoderma* spp. have acquired much importance (Papavizas, 1985; Sreenivasaprasad et Manibhushanrao, 1990). *Trichoderma* spp. are fungi that occur worldwide. Recent studies show that they are not only parasites of fungal plant pathogens but also can produce antibiotics. In addition, certain strains can induce systemic and localized resistance to several plant pathogens. Moreover, some strains may enhance plant growth and development (Ha, 2010).

Trichoderma spp. has received the most attention for control soil borne pathogens. *Trichoderma harzianum* is a fungal

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biocontrol agent that attacks a range of pathogenic fungi. It can be used either alone or in combination with other *Trichoderma* species in biological control of several plant diseases (Papavizas, 1985; Chet, 1987; Samuels, 1996).

Although not normally associated with foliar surfaces, *Trichoderma* spp. have been widely investigated also for the control of foliar diseases, particularly gray mould caused by *Botrytis cinerea* on Lettuce (Card, 2005), snapbeans (Nelson and Powelson, 1988), strawberry, blackberry and grape (Archbold *et al*., 1997), cucumber (Lee *et al*., 2006), tomato (Hmouni *et al*., 2006; Lisboa *et al*., 2007), *Eucalyptus globulus* (Zaldúa and Sanfuentes, 2010) and Faba bean (Bendahmane *et al*., 2012).

Trichoderma has been successful applied to aerial plant parts for the biocontrol of decay fungi in wounds on shrubs and trees (Papavizas, 1985), brown blotch caused by *Colletotrichum truncatum* on cowpea (Bankole and Adebanjo, 1996), anthracnose induced on strawberry by *Colletotrichum acutatum* (Freeman *et al*., 2004), *Helminthosporium oryzae* causal agent of Brown spot of rice (Mouria *et al*., 2003), four species of *Bipolaris* (*B. maydis*, *B. sorokiniana*, *B. sorghicola* and *B. tetramera*) on Sorghum (Berber *et al*., 2009). Actually, among the aspects that future research should focus on to make better use of *Trichoderma* as a biocontrol agent for the management of crop diseases, the first is the ability of *Trichoderma* for control of foliar pathogens / air (Ramanujam *et al*., 2010).

Although use of biocontrol agents could reduce chemical application to a limited extent, it is less reliable and less efficient (Monte, 2001). One of the most promising possibilities for the application of biocontrol *Trichoderma* strains is within the frames of a complex integrated plant protection, which is based on the combined application of physical, chemical and biological means of control. In the case of the application of a complex integrated strategy when *Trichoderma* strains are combined with chemical pesticides, therefore it is important to collect information about the effects of pesticides on the biocontrol agent (Kredics *et al*., 2003).

The combined use of biocontrol agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (Locke *et al*., 1985). The effect of certain fungicides and herbicides on *Trichoderma* spp. was reported earlier with an emphasis on practical applications (Kredics *et al*., 2003).

The objective of the present study was to check the compatibility of some strains of *Trichoderma* spp. with different fungicides actually used against seed borne diseases (sugar beet, wheat, corn and pulses) and foliar diseases (peach leaf curl, *Monilia* brown rot and scab leaves of pear, peach, apple, almond; grey mould and/or anthracnose of strawberry, tomato, melon, grape vine, lettuce, green beans).

MATERIALS AND METHODS

Trichoderma spp. strains

Three strains of *Trichoderma harzianum* (Tcomp, TH1 and TH3) and a strain of *Trichoderma viride* (TV1) belonging to the culture collection of the Laboratory of Botany, Biotechnology and Plant Protection (Morocco) grown on PSA medium (potato 200 g , Sucrose 20 g, Agar-agar: 15 g, distilled water 1000 ml) and incubated at 28 ± 1°C in the dark.

Tested fungicides

Seven fungicides were tested viz. BASULTRA (Thiram 80% WP), CLORTOSIP (Chlorothalonil 75% WP), FRUPICA 50 WP (Mepanipyrim 50% WP), PYRUS 400 SC (Pyrimethanil 400 g/L), SIGNUM WG (Boscalid 26.7% + Pyraclostrobin 6.7% WG), SWITCH 62.5 WG (Cyprodinil 37.5% + Fludioxonil 25% WG), TELDOR 50 WG (Fenhexamid 50% WG) (ONSSA, 2015).

Compatibility estimation of Trichoderma spp. *with fungicides*

Stock solutions of agrochemicals were prepared by dissolving the required quantities of each into sterile distilled water. Increasing concentrations (¼ (R.D.), ⅓ (R.D.), ½ (R.D.), ¾ (R.D.) and Recommended dose) were then prepared and incorporated into the PSA culture medium kept molten at 50°C and were mixed thoroughly by gentle shaking. The mixture was then poured into Petri plates. PSA plates without any added compounds served as controls.

After solidification, the plates were inoculated with 5-mm discs of 7 days-old *Trichoderma* spp. *strains* cultures. Three replicates were used for each concentration of every tested fungicide. The inoculated plates were incubated at 28 ± 1°C in the darkness and radial colony diameter was recorded every eight days after inoculation for one month. To determine the number of the conidia produced, four 5-mm discs were taken from one month old cultures on PSA for each treatment, put in a test tube containing 1 ml of sterile distilled water and shaken for 5 min using an orbital shaker at 30 rpm. The conidia concentration was measured using a Malassez slide. Each experiment was replicated ten times. To determine the percentage germination of conidia, 0.2 mL of a conidial suspension (10^3 conidia / mL) obtained using 7 days old cultures grown on PSA was spread on the surface of Petri plates containing Water agar Medium (Agar-agar: 15 g, Distilled water 1000 ml) supplemented with different concentrations of the tested fungicides. The plates were then incubated for 24 h at 28°C in the dark. At the end of the incubation period, the frequency of germinated conidia (germ tube length greater than the length of the conidia) in a sample of 200 conidia per plate was determined by microscopic examination. Three replicates were used for each concentration of every tested fungicide. The mean percentages of inhibition of mycelial radial growth, conidia production and conidia germination relative to controls, were calculated for each fungicide using the following formula (Vincent, 1947) and converted into percent compatibility (100- Percent Inhibition):

Percent Inhibition = ((X – Y)/X) × 100
Where
X = control
Y = treatment

Data were analyzed by one-way analysis of variance (ANOVA) and LSD test at 5% level.

Table 1 Observations of the compatibility (%) of four *Trichoderma* spp. with the fungicides Balustra, Chlortosip and Frupica estimated on the mycelial radial growth.

Trichoderma strains	Time (days)	Thiram concentrations (ppm)					Chlorothalonil concentrations (ppm)					Mepanipyrim concentrations (ppm)				
		500	666,6	1000	1500	2000	375	500	750	1125	1500	100	133,3	200	300	400
Tcomp	8	11.1**c**	0**c**	0	0	0	35.2**c**	34.3**c**	28.7**d**	16.7**d**	18.1**d**	59.3**b**	46.3**b**	44.5**b**	41.5**b**	34.1**b**
	16	34.8**b**	16.7**b**	10.0	10.6	0	49.3**b**	51.9**b**	42.6**c**	40.8**c**	40.8**c**	100**a**	100**a**	100**a**	100**a**	100**a**
	24	70.4**a**	44.1**a**	22.2	15.6	0	62.1**ab**	55.6**b**	54.6**b**	55.6**b**	50.9**b**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	74.9**a**	52.8**a**	34.3	20.4	0	79.6**a**	74.1**a**	68.5**a**	64.8**a**	62.1**a**	100**a**	100**a**	100**a**	100**a**	100**a**
TH1	8	06.7**d**	0**b**	0	0	0	12.9	09.3	0**b**	0**b**	0**b**	36.1**b**	28.7**c**	26.8**c**	26.8**c**	14.8**c**
	16	22.2**c**	0**b**	0	0	0	24.1	18.5	16.1**a**	16.7**a**	14.8**a**	100**a**	83.3**b**	68.5**b**	51.5**b**	30.4**b**
	24	40.0**b**	11.5**a**	0	0	0	24.1	18.5	16.1**a**	16.7**a**	14.8**a**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	50.0**a**	21.3**a**	07.4	0	0	24.1	18.5	16.1**a**	16.7**a**	14.8**a**	100**a**	100**a**	100**a**	100**a**	100**a**
TH3	8	0**c**	0**c**	0	0	0	29.6**b**	29.1**b**	19.6**c**	19.5**c**	17.8**c**	51.9**b**	41.7**b**	37.1**b**	37.1**b**	34.4**b**
	16	28.2**b**	12.2**b**	2.6	0	0	40.8**b**	37.9**b**	33.3**b**	31.5**b**	27.8**b**	100**a**	100**a**	100**a**	100**a**	100**a**
	24	43.7**ab**	21.3**a**	12.4	11.9	0	61.1**a**	57.4**a**	55.6**a**	53.7**a**	43.5**a**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	57.4**a**	28.9**a**	15.9	12.8	0	61.1**a**	57.4**a**	55.6**a**	53.7**a**	43.5**a**	100**a**	100**a**	100**a**	100**a**	100**a**
TV1	8	11.1**c**	0**d**	0	0**b**	0	35.5**c**	34.7**c**	34.7**c**	20.4**c**	20.7**c**	52.8**b**	46.3**b**	44.5**b**	37.9**b**	32.4**b**
	16	35.9**b**	27.8**c**	10.0	10.4**b**	0	49.1**b**	48.2**b**	44.5**b**	38.9**b**	38.9**b**	100**a**	100**a**	100**a**	100**a**	100**a**
	24	72.8**a**	50.0**b**	32.2	31.7**ab**	0	57.4**b**	53.7**b**	53.7**b**	53.7**a**	50.0**ab**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	74.1**a**	61.1**a**	44.4	45.2**a**	0	72.2**a**	68.5**a**	68.5**a**	64.8**a**	59.3**a**	100**a**	100**a**	100**a**	100**a**	100**a**

Two results of the same concentration and the same strain affected by the same letter show no significant difference at 5%

Table 2 Observations of the compatibility (%) of four *Trichoderma* spp. with the fungicides Pyrus, Signum, Switch and Teldor estimated on the mycelial radial growth.

Trichoderma strains	Time (days)	Pyrimethanil concentrations (ppm)					Pyraclodtrobine concentrations (ppm) + Boscalid					Cyprodinil + Fludioxonil concentrations (ppm)					Fenhexamid concentrations (ppm)				
		200	222,6	400	600	800	125,2	167	250,5	375,7	501	93,7	125	187,5	281,2	375	187,5	250	375	562,5	750
Tcomp	8	54.1**b**	42.4**b**	26.5**b**	16.3**b**	11.1**c**	63.9**b**	63.5**b**	63.0**b**	59.2**b**	59.1**b**	45.9**b**	45.6**b**	45.6**b**	40.8**b**	32.8**b**	100	100	66.7	62.9**b**	61.9**b**
	16	85.0**a**	69.4**a**	46.5**a**	26.1**b**	25.9**bc**	100**a**	97.2**a**	96.3**a**	91.6**a**	88.9**a**	89.6a	88.9**b**	86.9**a**	70.1**a**	75.4**a**	100	100	100	100**a**	100**a**
	24	87.0**a**	70.8**a**	52.6**a**	35.4**ab**	33.3**b**	100**a**	100**a**	100**a**	100**a**	99.0**a**	100**a**	96.3**a**	96.3**a**	96.3**a**	96.3**a**	100	100	100	100**a**	100**a**
	32	97.0**a**	72.2**a**	60.2**a**	55.6**a**	52.4**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100	100	100	100**a**	100**a**
TH1	8	30.8**c**	29.1**c**	30.5**b**	19.4**c**	19.3**b**	36.3**c**	35.9**d**	34.3**c**	34.6**c**	34.6**c**	19.9**c**	16.3**c**	15.4**c**	14.4**c**	12.4**c**	51.9**b**	45.6**b**	53.7**b**	53.7**b**	42.6**b**
	16	49.5**b**	51.9**b**	46.9**b**	40.8**b**	34.3**ab**	82.6**b**	68.5**c**	67.0**b**	67.0**b**	51.8**bc**	68.3**b**	68.7**b**	69.8**b**	59.3**b**	55.6**b**	100**a**	100**a**	100**a**	100**a**	100**a**
	24	79.1**a**	71.9**a**	70.5**a**	67.8**a**	38.9**a**	100**a**	87.1**b**	77.7**b**	74.0**b**	73.1**ab**	100**a**	96.3**a**	96.3**a**	88.9**a**	75.1**a**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	79.6**a**	77.8**a**	74.3**a**	70.8**a**	44,8**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	89.8**a**	100**a**	100**a**	100**a**	100**a**	100**a**
TH3	8	54.1**c**	42.2**b**	22.2**c**	12.8**b**	0**c**	39.3c	39.2**c**	38.9**c**	39.1**b**	35.9**b**	30.0**b**	20.0**b**	16.6**a**	09.3**b**	10.0**b**	64.8**b**	26.9**b**	27.8**b**	27.8**b**	28.7**b**
	16	78.7**b**	75.0**a**	42.0**b**	32.6**b**	24.3**b**	54.6**bc**	52.4**b**	52.2**b**	50.4**ab**	46.5**ab**	91.7**a**	82.5**a**	74.5**a**	75.4**a**	54.6**ab**	100**a**	100**a**	100**a**	100**a**	100**a**
	24	79.1**a**	80.9**a**	72.0**a**	64.3**a**	60.2**a**	67.6**ab**	63.9**a**	62.0**a**	59.8**a**	53.7**a**	94.5**a**	88.9**a**	88.9**a**	88.9**a**	88.9**a**	100**a**	100**a**	100**a**	100**a**	100**a**
	32	79.6**a**	80.9**a**	75.7**a**	70.9**a**	68.5**a**	70.3**a**	70.3**a**	67.6a	62.6**a**	55.0**a**	100**a**	94.4**a**	100**a**	91.1**b**	89.7**a**	100**a**	100**a**	100**a**	100**a**	100**a**
TV1	8	38.9**c**	37.9	17.0**c**	17.0**c**	16.3	68.2**b**	50.1**b**	49.3**b**	49.3**c**	47.9**c**	16.7**c**	14.8**c**	13.9**b**	12.4**b**	11.9**c**	100	40.8**b**	40,8**b**	40,8**b**	40,8**b**
	16	50.0**bc**	50.4	28.9**b**	24.6**b**	23.7	100**a**	100**a**	90.8**a**	89.8**b**	88.9**b**	50.6**b**	46.9**b**	36.3**b**	30.4**b**	23.9**c**	100	100**a**	100**a**	100**a**	100**a**
	24	60.0**ab**	58.9	35.4**a**	34.3**a**	28.2	100**a**	100**a**	100**a**	96.3**ab**	92.6**b**	100**a**	96.3**a**	86.7**a**	85.2**a**	65.9**b**	100	100**a**	100**a**	100**a**	100**a**
	32	65.7**a**	62.6	41.3**a**	37.1**a**	33.3	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	100**a**	92.6**a**	92.6**a**	92.6**a**	100	100**a**	100**a**	100**a**	100**a**

Two results of the same concentration and the same strain affected by the same letter show no significant difference at 5%

Table 3 Observations of the compatibility (%) of four *Trichoderma* spp. with the fungicides Balustra, Chlortosip and Frupica estimated on the conidia production (Cp) and germination (G).

Trichoderma strains	parameters	Thiram concentrations (ppm)					Chlorothalonil concentrations (ppm)					Mepanipyrim concentrations (ppm)				
		500	**666,6**	**1000**	**1500**	**2000**	**375**	**500**	**750**	**1125**	**1500**	**100**	**133,3**	**200**	**300**	**400**
Tcomp	**Cp**	55.6**a**	45.5**b**	20.7**c**	18.8**c**	0**d**	71.7**a**	52.2**b**	50.5**b**	40.6**b**	39.8b	1.6**a**	1.1**ab**	0.9**bc**	0.6**bc**	0.4**c**
	G	54.3**a**	48.7**a**	43.0**ab**	40.8**ab**	31.3**c**	54.2**a**	46.2**b**	40.2**bc**	38.3**c**	36.2**c**	92.0**a**	90.8**a**	86.8**ab**	81.0**b**	48.5**c**
TH1	**Cp**	69.5**a**	53.9**b**	47.6**b**	38.0**c**	0**d**	63.2	63.9	62.6	62.0	61.5	2.9**a**	2.8**a**	2.4**ab**	1.4**b**	0**c**
	G	40.2**a**	40.0**a**	35.8**ab**	32.2**bc**	28.7**c**	18.5**a**	16.8**ab**	15.2**bc**	13.3**bc**	11.8**c**	55.5**a**	50.8**ab**	48.7**ab**	47.7**ab**	45.0**b**
TH3	**Cp**	84.8**a**	84.9**a**	52.7**b**	49.2**b**	0**c**	85.6**a**	74.6**b**	69.3**bc**	65.5**c**	56.5**d**	6.7**a**	5.9**a**	5.0**ab**	3.9**b**	0**a**
	G	37.3**a**	37.7**a**	37.6**a**	32.8**ab**	26.8**b**	20.3**ab**	20.2**ab**	21.2**a**	18.0**bc**	17.7**c**	65.3**a**	58.5**b**	49.8**c**	47.7**c**	45.7**c**
TV1	**Cp**	71.9**a**	61.4**a**	35.2**b**	24.9**b**	0**c**	89.7**a**	84.4**a**	50.9**b**	37.7**c**	28.3**c**	7.0	3.5	6.4	1.7	0
	G	43.8**a**	42.0**ab**	38.2**ab**	34.2**ab**	32.3**b**	25.2	23.5	23.2	21.7	21.7	58.7**a**	53.3**b**	51.7**b**	50.0**ab**	46.8**b**

Two results of the same fungicide in the same line affected by the same letter show no significant difference at 5%

Table 4 Observations of the compatibility (%) of four *Trichoderma* spp. with the fungicides Pyrus, Signum, Switch and Teldor estimated on the conidia production (Cp) and germination (G).

Trichoderma strains	parameters	Pyrimethanil concentrations (ppm)					Boscalid + Pyraclodtrobine concentrations (ppm)					Cyprodinil + Fludioxonil concentrations (ppm)					Fenhexamid concentrations (ppm)				
		200	**222,6**	**400**	**600**	**800**	**125,2**	**167**	**250,5**	**375,7**	**501**	**93,7**	**125**	**187,5**	**281,2**	**375**	**187,5**	**250**	**375**	**562,5**	**750**
Tcomp	**Cp**	1.2**a**	0.9**ab**	0.4**bc**	0.4**bc**	0.3**c**	21.8**a**	14.6**ab**	12.9**b**	12.3**b**	12.2**b**	34.9**a**	18.1**b**	12.8**bc**	10.7**cd**	5.1**d**	63.6**a**	55.9**b**	44.5**c**	38.7**c**	22.5**d**
	G	10.0	3.5	18.8	1.2	0	75.5**a**	72.8**a**	61.3**ab**	57.2**ab**	49.0**b**	57.2**a**	38.0**ab**	19.8**b**	19.0**b**	16.0**b**	95.2**a**	93.3**a**	82.8**ab**	84.2**ab**	60.7**b**
TH1	**Cp**	3.1**a**	1.8**ab**	1.5**bc**	1.4**bc**	1.0**c**	15.3**a**	7.7**ab**	3.4**b**	2.5**b**	2.1**b**	96.4**a**	78.0**a**	65.6**ab**	53.6**bc**	32.9**c**	82.8**a**	79.5**ab**	71.9**ab**	63.9**bc**	51.5**c**
	G	0	0	0	0	0	30.3**a**	24.7**a**	8.0**b**	6.8**b**	6.3**b**	22.5**a**	13.7**a**	1.7**b**	0.5**b**	0**b**	51.3	52.5	51.2	50.3	45.2
TH3	**Cp**	19.8**a**	4.6**b**	1.7**b**	1.4**b**	0.8**b**	50.6**a**	28.5**ab**	23.1**b**	9.9**c**	8.6**c**	79.3**a**	33.2**b**	29.6**b**	24.9**b**	18.1**b**	78.7	73.3	55.1	66.0	60.8
	G	0.8	0.17	0	0	0	66.5**a**	55.7**a**	38.5**b**	33.2**b**	10.2**c**	40.5**a**	21.2**a**	18.3**b**	0.8**c**	0**c**	46.8**a**	43.5**a**	44.0**a**	38.0**b**	37.5**b**
TV1	**Cp**	6.3**a**	2.2**ab**	1.7**b**	0.8**b**	0.8**b**	56.1**a**	46.8**ab**	37.1**bc**	25.9**c**	20.2**c**	7.5**a**	4.8**ab**	3.0**ab**	2.6**ab**	1.2**b**	97.1**a**	89.8**a**	57.5**b**	58.7**b**	56.9**b**
	G	5.5	2.0	21.6	0.7	0	67.2**a**	64.5**a**	63.8**a**	60.5**a**	46.0**b**	30.3**a**	30.3**a**	4.7**b**	4.5**b**	4.3**b**	45.4	44.0	42.3	43.3	39.2

Two results of the same fungicide in the same line affected by the same letter show no significant difference at 5%

RESULTS

The compatibility of some active ingredients was tested *in vitro* on mycelia radial growth, conidia production and germination of three strains of *Trichoderma harzianum* and one strain of *T. viride*.

After one week, the mycelial growth of *Trichoderma* spp. strains was totally inhibited on PSA media amended with the concentrations tested of thiram, the compatibility was null. This absence of compatibility persists after 4 weeks of incubation at the recommended dose (2000 ppm). At the lowest dose, the strain of *T. harzianum* (Tcomp) and *T. viride* (TV1) showed a good compatibility equal to 70.4 and 72.8% three weeks after incubation. In the presence of chlorothalonil, mycelial growth of Tcomp, TH3 and TV1 was low after the first week of incubation. The compatibility was moderate to high in the third week. TH1 strain showed a low compatibility during the four weeks. The four strains compatibility was low to moderate with all the mepanipyrim concentrations in the first week and high (100%) in the second week (Table 1).

Trichoderma harzianum strains (Tcomp and TH3) showed compatibility of 54% at 200 ppm of pyrimethanil after 8 days and reached 97 and 79% respectively after 32 days of incubation. This compatibility was low to medium at 800 ppm. In the presence of the recommended doses of boscalid + pyraclostrobine; cyprodinil + fludioxonil and fenhexamid, Tcomp showed a moderate compatibility in the first week. This compatibility has become very important for all strains in the second week (Table 2).

After one month of incubation, thiram and mepanipyrim prevented conidia production by different strains of *Trichoderma* spp. at the different tested concentrations except at 500 and 666.6 ppm of thiram concentrations, which allowed a compatibility ranging from 55.6 to 84.9%. In the presence of chlorothalonil, compatibility was low to medium at 1500 ppm and increases gradually as the fungicide concentration decreases (Table 3).

No compatibility was observed between the conidia production of *Trichoderma* spp. strains and pyrimethanil, boscalid + pyraclostrobin and cyprodinil + fludioxonil. However, the strains TH3 and TV1 exhibited moderate compatibility in the presence of 125.2 ppm of boscalid + pyraclostrobin. Indeed, TH1 and TH3 strains showed a significant compatibility in the presence of 93.7 ppm of Cyprodinil + fludioxonil. Only TH3 strain showed moderate compatibility with the other concentrations of cyprodinil + fludioxonil. The Fenhexamid was compatible with the conidia production of the four strains of *Trichoderma* spp. It has been moderate for Tcomp and important for the other strains (Table 4).

A moderate to low compatibility was detected between the conidia germination of the *Trichoderma* spp. strains and thiram after increasing its concentrations. This compatibility was moderate at 375 ppm of Chlorothalonil only with Tcomp (54.2%). The mepanipyrim was highly (92 to 81%) to moderately (48.5%) compatible with the conidia germination of Tcomp as increasing concentrations and moderate with the other strains (Table 3).

The pyrimethanil was not compatible with the conidia germination of the four tested strains. The boscalid + pyraclostrobin showed a great to a medium compatibility with Tcomp strain in parallel to the increase of the fungicide concentrations. Low compatibility was remarked between the association cyprodinil + fludioxonil and conidia germination of tested *Trichoderma* spp. strains except Tcomp which showed a compatibility of 57.2% with 93.7 ppm of the fungicide. This same strain of *T. harzianum* showed high compatibility with fenhexamid varied from 95.2 to 60.7% at progressively increasing concentrations. However, this compatibility is moderate for the other strains (Table 4).

DISCUSSION AND CONCLUSION

Fungicides tested *in vitro* showed variable activity on the three life cycle stages of the *Trichoderma harzianum* and *T. viride* strains.

The compatibility estimated on the mycelial radial growth was moderate between the combination pyraclostrobin + boscalid and *Trichoderma* spp. in the first week and become very important from a second week. This compatibility was moderate with conidia germination and low with the conidia production. Pyraclostrobin and boscalid belong respectively to the strobilurins and carboxamides chemical groups. The fungitoxic activity of strobilurins is exercised at the reduction site ubihydroquinone Qo, by blocking electron transfer between cytochrome b and c1 of the mitochondrial respiratory chain, by inhibiting the production of ATP (Becker *et al*., 1981; Von Jagow et Link, 1986) and therefore the fungus growth (Zheng *et al*. 2000; Bartlett *et al*., 2002; Bahous *et al*., 2005). carboxamides are inhibitors of complex II (Leroux, 2003).

Divya *et al*. (2011) showed that the wild strain of *T. harzianum* was insensitive to the half of the pyraclostrobin recommended dose. Conidia germination of *T. atroviride* was highly sensitive to Signum but *T. asperellum* was not sensitive to low concentrations of this fungicide (Shovan, 2012). The synergism mechanism of *T. harzianum* combined with boscalid to *Botrytis cinerea* determined by microscopy method, mycelium growth rate and disc filter method was showed by the mycelium partial dissolution of *B. cinerea*, the beneficial to *T. harzianum* to occupy nutrition and space and enhanced the antifungal activity of volatile compounds of *T. harzianum* to *B. cinerea* (Fangsheng *et al*., 2013).

The combination cyprodinil + fludioxanil had moderate effect on mycelial growth of *Trichoderma* strains tested but showed good compatibility from the second week. However, only the TH1 strain gives a moderate compatibility on the conidia production whose germination was completely inhibited. The dual action of cyprodinil and fludioxonil is due to its containing active ingredients of two different families, anilinopyrimidine (cyprodinil) (Heye *et al*., 1994; Hilber and Hilber-Bodmer 1998) and phenylpyrrole (fludioxonil) (Gehmann *et al*., 1990; Nyfeler and Ackermann, 1992). The first inhibits the biological synthesis of methionine, one of the principal components of the fungus protein synthesis (Masner, 1994; Leroux 1996), while fludioxonil stimulates intracellular glycerol accumulation, which blocks the cell growth in the fungus (Leroux 1996; Pillonel and Meyer, 1997). *Botrytis*

control by a mixture of cyprodinil with fludioxonil remained excellent (Forster and Staub, 1996) and the three fungicides, fludioxonil, cyprodinil, and a mixture of fludioxonil and cyprodinil, were equally effective against thiabendazole-sensitive and -resistant isolates of *P. expansum* (Errampalli and Crnko, 2004). The conidia germination of *T. atroviride* and *T. asperellum* were moderately sensitive to Switch (Shovan, 2012).

The pyrimetanil inhibited moderately mycelial growth and strongly production of conidia and germination of all strains of *Trichoderma* spp. tested. Yuan *et al.* (2007) reported that the strain of *Trichoderma* T-21 has shown pyrimethanil resistance and that the growth and the conidia production of the strain were good. Two mutants tolerant to pyrimethanil were obtained by UV-light induction on PDA amended with pyrimethaniol from a wild *Trichoderma* strain. Tolerance level was observed 20 times in comparison with that of mother strain. The mutants could maintain their tolerance after 8 times of transfers on the fungicide free PDA. Two tolerant strains kept the ability of antagonism against *Botrytis cinerea in vitro* and *in vivo* (Hongman *et al*., 2005). The study conducted by Gabriolotto *et al.* (2009) on effectiveness of control strategies against *Botrytis cinerea* in vineyard and evaluation of the residual fungicide concentrations showed that the application of three treatments of a *Trichoderma* spp. was really ineffective against grey mold, even though in association with pyrimethanil.

Pyrimethanil, whose mode of action is not fully understood, interfere at the amino acid level and mainly at the methionine biosynthesis (Heye *et al*., 1994). Polygalacturonase, cellulase, proteinase and laccase activities were all decreased in the medium of three day-old cultures grown of *Botrytis cinerea* in the presence of pyrimethanil. No significant growth inhibition was observed at the pyrimethanil concentrations tested. Pyrimethanil did not inhibit the enzymes directly, nor did it inhibit the synthesis of cytosolic proteins. Therefore, it was proposed that the fungicide inhibits protein secretion at a post-translational stage in the secretory pathway. It appears that pyrimethanil is most active in media where the fungus has to utilize extracellular enzymes to mobilize the nutrients it requires for growth (Milling and Ridchardson, 1995). Biochemical studies indicate that the anilinopyrimidines inhibit the biosynthesis of methionin by blocking cystathionine-*β*-lyase (Rosslenbroich and Stuebler, 2000).

Strains of *Trichoderma* spp. grew well in the presence of mepanipyrim, conidia germination and production were respectively moderately and completely reduced. Thus, this strain showed good compatibility with the mepanipyrim. This fungicide showed excellent activity on *Botrytis cinerea* and no significant phytotoxicity (Nagata *et al*., 2004). It controls *Venturia* spp. on grapes, vegetables, apples and pears, and *Monilinia fructicola* on Fisheries (Maeno *et al*., 1990). The mepanipyrim is 2-anilino-4-methyl-6- (1-propynyl) pyrimidine affect the intracellular transport of secreted proteins process (Miura *et al*., 1994) This fungicide tended to affect both mycelial growth and pectinase secretion of younger *B. cinerea* mycelia more strongly than older cultures, which is suggestive of its mode of action. Mepanipyrim was more effective in inhibiting pectinase secretion and in disease control activity against *B. cinerea* in the early stages of growth and infection (Miura and Maeno, 2007**).**

Trichoderma strains tested showed significant compatibility in the presence of Fenhexamid. Caron *et al.* (1994) have established the compatibility chart of *Trichoderma* MAUL-20 with the most commonly used pesticides in greenhouses. They showed that the Fenhexamid is among the active ingredients compatible with Trichoderma MAUL-20. Fenhexamid, hydroxyanilide, is an inhibitor of sterol biosynthesis. When the fungus *Botrytis cinerea* was grown in the presence of fenhexamid, the ergosterol content was reduced, and three 3-keto compounds, 4α-methylfecosterone, fecosterone and episterone, accumulated, suggesting an inhibition of the 3-keto reductase involved in C-4 demethylation (Debieu *et al*., 2001). Bagwan (2010) showed that the Thiram (0.2%) was compatible with *Trichoderma harzianum* and *T. viride*. However, strains of *Trichoderma* spp. tested could not grow in the presence of Thiram. The results are confirmed by the work of Mclean *et al.* (2001), which showed by the test spore germination *in vitro* that *T. harzianum* is very sensitive to this fungicide. Roberti *et al.* (2006) also assess the *in vitro* sensitivity of *T. atroviride*, *T. harzianum*, *T. reesei* and *T. viride* to thiram. The active ingredient thiram belongs to the family of dithiocarbamates that are effective on many pathogenic fungi including *B. cinerea* (Hmouni *et al*., 2003). dithiocarbamates inhibit a variety of enzymes, such as those of glycolysis (Ragsdale et Sisler, 1991).

As for chlorothalonil, *Trichoderma* strains (TH1 and TV1) have appeared very sensitive and the two others (Tcomp and TH3) have proved moderate resistance to this active ingredient. Bagwan (2010) showed that *Trichoderma* was very sensitive to chlorothalonil, other researchers found that some wild strains of *Trichoderma* are tolerant to high concentrations of chlorothalonil during germination (Abd-El Moity *et al*., 1982).

The results obtained made it possible to demonstrate the compatibility of the *Trichoderma* strains with the tested fungicides. Thus, Tcomp *Trichoderma harzianum* strain showed high compatibility both for the mycelial growth and for the germination of the conidia in the presence of most active ingredients. These data can be used as part of an integrated strategy against *Botrytis cinerea* by *T. harzianum* with compatible fungicides.

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