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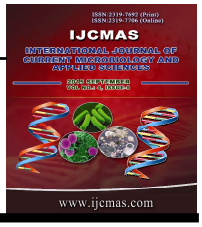

Original Research Article

Soil Fungicide Application in Combination with Grafting for the Control of Monosporascus Root Rot and Vine Decline on Cucurbits

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Keywords

Chemical control, Grafting, *Monosporascus cannonballus*, *M. eutypoides*, EC50, Soil drenching

A B S T R A C T

Nine fungicides (eight chemical groups) were evaluated for *in vitro* mycelial inhibition of *Monosporascus cannonballus* and *M. eutypoides*, obtained from cucurbits roots affected by Monosporascus Root Rot and Vine Decline in Tunisia. Results showed that (Propiconazole + cyproconazole), tebuconazole and fludioxonil were the most effective in reducing mycelial growth of the two species. EC_{50} values ranged from 0.31 to 3.33 and 0.55 to 2.82 mg a.i.l^{-1}, respectively. These fungicides were evaluated on three cucurbit crops (muskmelon cv. Galia, watermelon cv. Dumara, and watermelon cv. Dumara grafted on rootstock cv. TZ-148) in field. Each crop was subjected to nine different applications during the growing season. The disease incidence was determined at the end of the growing season as the mean percentage of symptomatic plants in each crop and treatment with *M. cannonballus* isolation percent (%) and fruit yield (Kg/plant). Applications of Alto Super and Horizon using drenching soil to grafted watermelon at 19, 40 and 59DAP have recorded the lowest isolation percentage with 19.40 and 14.3%, respectively. Similarly, for disease incidence with 40% (grafted watermelon treated with Alto at 19, 40 and 59DAP) and a highest fruit yield showed in grafted plant treated with Horizon at 19, 59 and 73DAP (2.89Kg/plant).

Introduction

Monosporascus root rot and vine decline (MRRVD) caused by the soil-borne ascomycetes *Monosporascus cannonballus* Pollack & Uecker (1974) and *M. eutypoides* (Petrak) von Arx (28) is an important cucurbits disease worldwide (Ben Salem *et al.*, 2013; Cohen *et al.*, 2012; Martyn and Miller, 1996). Symptoms of MRRVD are characteristic; the older crown leaves start to turn yellow and senesce within 2 to 3 weeks of harvest and the whole canopy collapses, exposing the fruits to the sun's solar radiation. Belowground symptoms may include necrotic root lesions, root rot and loss of secondary and tertiary feeder roots (Martyn and Miller, 1996). *Monosporascus cannonballus* and *M. eutypoides* produce black, smooth perithecia in the diseased

roots of the affected hosts, in which asci containing large spherical ascospores are produced (Pollack and Uecker, 1974; Sivanesan, 1991a). These species were considered conspecific, because of similar morphological features, and both species have been reported on similar hosts causing root rot in almost identical climatic habitats (Ben Salem *et al.,* 2013; Lovic *et al.,* 1995; Martyn and Miller, 1996; Sivanesan, 1991a, b). Only recently, the employment of the internal transcribed spacer (ITS) of nuclear ribosomal DNA, the elongation factor 1-α (*EF*-1α), and the β-tubulin (β-*tub*) gene sequence diversity analyses, and the resulting phylogenies, identified a level of polymorphism that enabled separation of *M. cannonballus* and *M. eutypoides*, demonstrating that they are distinct species (Ben Salem *et al.,* 2013). Consequently, *M. cannonballus* is a well characterized species but, unfortunately, little information exists regarding *M. eutypoides,* which is crucial to implement effective disease management strategies in cucurbit growing areas such as Tunisia, in which both *Monosporascus* spp. have been reported, being MRRVD an important disease of muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) crops (Armengol *et al.,* 2011; Boughalleb *et al.,* 2010; Ben Salem *et al.,* 2013). Different techniques such as soil solarization, soil fumigation alone or in combination with solarization, post-planting fungicide application, postharvest plant destruction, grafting on *Cucurbita* rootstocks, biological control, and host resistance and breeding, have been evaluated to control MRRVD, with varying degrees of success (Cohen *et al.,* 2012). An integrated approach, combining several of these techniques, appears to be the most successful strategy (Cohen *et al.,* 2000). In Israel, chemical control via chemigation (fungicides applied through the drip irrigation system) (Pivonia *et al.,* 2010) and grafting (Cohen *et al.,* 2007) have proven useful and effective in managing MRRVD.

Fungicides can be applied to the soil during the cucurbit growing season, with the aim of controlling ascospore germination and penetration on cucurbit roots (Cohen *et al.,* 2012). This is considered as an effective, easy and inexpensive method for the control of MRRVD. *In vitro* experiments conducted by Cohen *et al.* (2007) demonstrated that the fungicides fluazinam and kresoxim-methyl completely inhibited the growth of this pathogen; in addition, the efficacy of fluazinam was also reported in the field, although disease control ranged from 87% to only 32%. In subsequent research, Azoxystrobin, Prochloraz, and Pyraclostrobin+boscalid exhibited high and similar efficacies in the control of MRRVD (Pivonia *et al.,* 2010). In Egypt, Mennatoullah *et al.* (2010) evaluated the fungicides thiophanate-methyl, tolclofos-methyl+thiram and carboxin+thiram, both *in vitro* and *in vivo*, being thiophanate-methyl the most effective against *M. cannonballus*. The aim of this study is, to determine the *in vitro* and in field efficacy of selected fungicides against Monosporascus root rot and vine decline (MRRVD).

Materials and Methods

In vitro evaluation of fungicides

In this study, nine commercial formulations of fungicides, representing eight chemical groups (Table 1), were evaluated for *in vitro* mycelial growth inhibitory effect of three *M. cannonballus* isolates (MT67, MT68 and MT72), and three *M. eutypoides* isolates (MT45, MT47 and MT54), obtained from roots of cucurbits affected by MRRVD in Tunisia. All isolates were hyphal-tipped and stored at 25°C in darkness in plastic vials

containing sterilized peat (Gramoflor GmbH & Co., Vechta, Germany). Prior to use, a small portion of the colonized peat from each plastic vial was transferred to potato dextrose agar (PDA) (Biokar-Diagnostics, Zac de Ther, France).

Determination of EC_{50} values

Appropriate volumes of each fungicide were added to molten potato dextrose agar (PDA) at approximately 50°C in order to obtain a final concentration of 100, 10, 1 and 0.1 mg of active ingredient per liter ($a.i.l^{-1}$). Mycelial plugs (4 mm in diameter), obtained from the margins of 10-days old actively growing cultures, were transferred to fungicide-amended plates. Control PDA plates were prepared similarly but adding sterile distilled water (SDW) instead of the fungicide solution.

There were four replicates of each fungicide concentration, and the experiment was repeated twice in time. The plates were incubated for 20 days at 25°C in the dark, and the diameter of each colony was measured twice perpendicularly. Measurements were made at the same time and averaged. The inhibition percent was calculated in relation to the no-fungicide amended controls for each isolate. Inhibition percent of mycelial growth for each isolate at each concentration was calculated as a percentage with respect to the control treatment, following the formula: Percentage inhibition = (C-T) x 100/ C: Where, C = colony diameter (mm) of the control, T = colony diameter (mm) of the test plate. Percentages of mycelial growth inhibitions were converted to probits and plotted against log10 values of the fungicide concentration. Probit regression analysis was used to calculate the effective dose to reduce growth by 50% (EC_{50} value) for all the fungicides.

Field trial

The trial was performed in an experimental plot with a total area of $1834m^2$ located in a field in The High Institute of Agronomy Chott Meriem (Sousse, Tunisia). This plot had been used for cucurbits cultivation in previous years and had a history of MRRVD caused by both *M. cannonballus* and *M. eutypoides*. In this study, seedlings of three cucurbit crops (muskmelon cv. Galia, watermelon cv. Dumara, and watermelon cv. Dumara grafted on *Cucurbita maxima* × *C. moschata* rootstock cv. TZ-148); and three selected fungicides: Propiconazole + Cyproconazole (Alto Super 330, 180/50 $g.l^{-1}$ EC, Bioprotection), Fludioxonil (Maxim, 100 $g.l^{-1}$ FS, Bioprotection) and Tebuconazol (Horizon, 250 $g.l^{-1}$ EW, Promochimie) were used. The field trial was conducted in summer 2012 the crops were planted in June 05. Each crop was subjected to nine different control strategies during the growing season (Table 2). Each plant was treated by root dipping at transplanting time by immersion of the roots of each seedling in 50 ml of the corresponding fungicide solution. Then, the subsequent fungicide applications were performed with a portable sprayer and each plant received 250 ml of the tested fungicide solution. Treatments consisted of: (T1) Alto Super 330 EC applied at 19, 40 and 59 days after planting; (T2) Alto Super 330 EC applied at 19, 59 and 73 days after planting; (T3) Alto Super 330 EC applied at 19, 40, 59 and 73 days after planting; (T4) Maxim 100 FS applied 19, 40 and 59 days after planting; (T5) Maxim 100 FS applied at 19, 59 and 73 days after planting; (T6) Maxim 100 FS applied at 19, 40, 59 and 73 days after planting; (T7) Horizon EW applied at 19, 40 and 59 days after planting; (T8) Horizon EW applied at 19, 59 and 73 days after planting; (T9) Horizon EW at 19, 40, 59 and 73 days after planting. T10 consisted of control plants

without any fungicides application. This experiment had a split plot design with three blocks (replicates). Each block was divided into three sub-plots (crops), with ten randomized experimental plots (treatments) each. Each subplot had an area of 6.4 m^2, and included two rows of 5 plants each. The plants were transplanted onto raised beds spaced 1.6 m from center to center, with an in-row spacing of 0.80 m.

Management assessment

Disease incidence (DI) and percentage of *M. cannonballus* isolation

In each experimental sub-plot, plants located in the first row were used for disease assessment. The incidence of MRRVD was determined at the end of the growing season of each experiment as the mean percentage of symptomatic plants for each crop and treatment. Moreover, at this moment one plant per sub-plot was collected arbitrarily from the second row of plants. Roots of each plant were exposed by carefully washing the soil away. Roots were inspected visually for presence of root necrosis, and the observation of roots bearing perithecia of *Monosporascus* spp. In addition, for isolation, small root fragments were surface sterilized for 1 min in sodium hypochlorite solution (1.5% active chlorine) and washed twice with sterile water. Root fragments from necrotic areas of tissue were transferred onto potato dextrose agar (PDA) (Biokar-Diagnostics, Zac de Ther, France) containing streptomycin sulfate (Sigma-Aldrich, Madrid, Spain) (PDAS) at 0.5 mg ml^{-1} and incubated in darkness at 25 ºC. In all, 21 root fragments per plant (3 Petri dishes containing 7 root fragments each) were prepared. Plates were examined daily for fungal growth during 7 days, and hyphal tips from all colonies were transferred to PDA and V8-juice agar for subsequent growth and sporulation. In addition, for molecular confirmation of *Monosporascus* species, fungal mycelium was obtained from pure cultures grown on potato dextrose broth (PDB) (Sigma-Aldrich, Steinheim, Germany) for three weeks at 25°C in the dark. Total DNA was extracted using the EZNA Plant Miniprep Kit (Omega Bio-tek, Norcross, GA).The ITS nrDNA region of the isolates was amplified using the universal primers ITS1F and ITS4. The PCR reaction mix was adjusted to a final volume of 25 µl with water (Chromasolv Plus, Sigma-Aldrich). PCR products were purified and sequenced in both directions by the DNA Sequencing Service Macrogen Inc., Sequencing Center (Seoul, South Korea). Sequences were edited using the Sequencher software (Version 5.0, Gene Codes Corporation, Ann Arbor, MI) and subjected to a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/).

Fruit yield (Kg/plant)

At the end of the growing season of each cucurbit, fruit yield (Kg/plant) was collected from each subplot with 10 fruits per repetition (three repetitions).

Data analysis

Probit regression analysis was used to calculate the effective concentration values that inhibited mycelial growth and conidial germination by 50% (EC_{50} values). EC_{50} values were analyzed by an analysis of variance (ANOVA) performed with the General Linear Model (GLM) of STATIX 9.0. Factors considered in the model were: experiment (performed twice), fungicide, pathogen (*M. cannonballus* and *M. eutypoides*) and isolate nested in pathogen (three isolates for each pathogen). F tests for each term of the model were derived from the expected mean squares obtained by

application of the rule for finding expected mean squares. Means were compared using the least significant difference (LSD) value at $p<0.05$. Statistical analysis of the experimental results was carried out using a two-way ANOVA, with crops and treatments as independent variables and the dependent variable was Disease Incidence (DI) (%), the percentage of isolation of *M. cannonballus* and fruit yield. The overall means of each crop were compared using LSD values at $p<0.05$. Analyses were performed using SPSS.12 for Windows (SPSS Inc., Chicago, IL, USA), the significant interaction between crops and treatments was performed using STATIX 9.0 for each parameter.

Results and Discussion

In vitro evaluation of fungicides

Analysis of variance showed that there were no differences in the inhibition of mycelial growth between the two conducted experiments, but the effect of fungicide, pathogen and their interaction were all significant ($p<0.05$) (Table 3). There were also significant differences between pathogen isolate and the effect of the interaction between fungicide and pathogen (isolates) ($p< 0.05$) (Table 3).

Mean EC_{50} values for reduction in mycelial growth of *Monosporascus cannonballus* and *M. eutypoides* are given in table 4. Propiconazole + cyproconazole, Tebuconazole and Fludioxonil were the most effective fungicides in reducing mycelial growth of the two species; EC_{50} values were comprised between 0.31 and 3.33 mg a.i. l^{-1} for *M. cannonballus* and between 0.55 and 2.82 mg a.i. l^{-1} for *M. eutypoides*. The other fungicides were less effective in inhibiting mycelial growth, presenting EC_{50} values between 11.53 (Chlorothalonil) and 66.9 (Copper oxychloride) mg a.i. l^{-1} for *M. cannonballus*; and EC_{50} values ranging from 16.82 (Azoxystrobin) to 81.97 (Copper oxychloride) mg a.i. l^{-1} for *M. eutypoides* (Table 4).

Field trial

Percentage of *M. cannonballus* isolation

Monosporascus cannonballus was identified morphologically by the formation of perithecia containing only one large (rarely two), spherical ascospore per ascus that did not germinate on PDA and V8-juice agar and was confirmed by molecular identification. The different crops and treatments, and their interaction had a significant effect on the percentage of *M. cannonballus* isolation ($p<0.05$). In the subplots in which melon was cultivated, all applications of the three fungicides (Super Alto, Maxim and Horizon) have resulted in a relatively low value of *M. cannonballus* isolation compared to control ($p <0.05$).

The effective treatment was T7 (Horizon applied at 19, 40 and 59DAP) and T3 (Alto Super applied at 19, 40, 59 and 73JAP) with a respective values of 32.63 and 33.97%, whereas, T4 (Maxim applied at 19, 40 and 59DAP) recorded a high value with 37.1% (control = 44.9%). LSD test has identified four homogenous groups ($p <0.05$) within watermelon plant treated with the different fungicide, T4 has scored the lowest percentage with 32.93%. Grafted watermelon plants showed a reduced value of percentage isolation ($p <0.05$). Both treatments T5 (Maxim applied at 19, 59 and 73DAP) and T1 (Alto Super applied at 19, 40 and 59DAP) presented a respective moderate percentage with 16 and 14.3%. T8 (Horizon applied at 19; 59 and 73DAP) has recorded relatively a low value with 14.87% (Table 6).

Table.1 Fungicides selected for in vitro sensitivity testing

Chemical Group	Fungicide	Trade name	Manufacturer	Formulation	Registred concentration in Tunisia
Methoxy-Acrylates	Azoxystrobin	Ortiva	Agriprotec	250g/L SC	100cc/hl
Phthalimide	Captan	Akotan	Agriprotec	83% WP	0.2kg/hl
Benzimidazole	Carbendazim	Prodazim	Protagri	50% WP	50g/hl
Inorganic	Copper oxychloride	Kocide 2000	Agriprotec	35% WG	150– 200 g/hl
Chloronitrile (Phthalonitrile)	Chlorothalonil	Bravo	Agriprotec	720g/L SC	250cc/hl
Phenylpyrrole	Fludioxonil	Maxim	Bioprotection	100g/L FS	200cc/T
Dicarboximide	Iprodione	Rovral	SEPCM	50% WP	150g/hl
Triazole	Tebuconazole	Horizon	Promochimie	250g/L EW	1 L/ha
	75% Propiconazole +25% Cyproconazole	Alto Super	Bioprotection	330 g/L EC	0.5 L/ha

WP, wettablepowder; WG, water dispersible granule; EC, emulsifiableconcentrate; SC, suspension concentrate; EW, emulsionoil in water; SL, soluble concentrate

Table.2 Experimental design of field experiments conducted during 2012 to study the effect of different fungicides alone, based on three or four applications to control Monosporascus vine decline and root rot

Treatments	Fungicides	Application dates			
		05/June	16/July	06/August	20/August
T1	Alto Super 330 EC[a]	+	+	+	-
T2	Alto Super 330 EC	+	-	+	+
T3	Alto Super 330 EC	+	+	+	+
T4	Maxim 100FS	+	+	+	-
T5	Maxim 100FS	+	-	+	+
T6	Maxim 100FS	+	+	+	+
T7	Horizon EW	+	+	+	-
T8	Horizon EW	+	-	+	+
T9	Horizon EW	+	+	+	+
T10	Control	-	-	-	-

[a] Fungicide rate in ml/ha or g/ha of commercial product.

Table.3 Analysis of variance for the effects of experiment, fungicide, pathogen and isolate (pathogen) on mycelial growth of Monosporascus cannonballus and M. eutypoides

	df[a]	EC50	
		MS[b]	P <F[c]
Experiment	1	1569.909	0,989
Fungicide	8	18252.363	<0,05
Pathogen	1	2407.045	0,0158
Isolate (pathogen)	2	667.056	0,0249
Experiment x fungicide	8	234.784	0,892
Experiment x pathogen	1	60.914	0,738
Experiment x isolate (pathogen)	2	803.198	0,0536
Fungicides x pathogen	8	893.862	0,0236
Fungicides x isolate (pathogen)	16	1071.164	0,0137
Residual	42	536.712	-

a Degrees of freedom.
b Mean square.
c Probabilities associated with individual F tests.

Figure.1 Significant interaction carried out by STATIX 9.0 for DI (%); A: sampling moments*crops, B: applications* crops. Crops: watermelon, grafted watermelon and melon; Applications: control, C2: at 19, 40 and 59DAP; C3: at 19, 59 et 73DAP et C4: at 19, 40, 59 et 73DAP

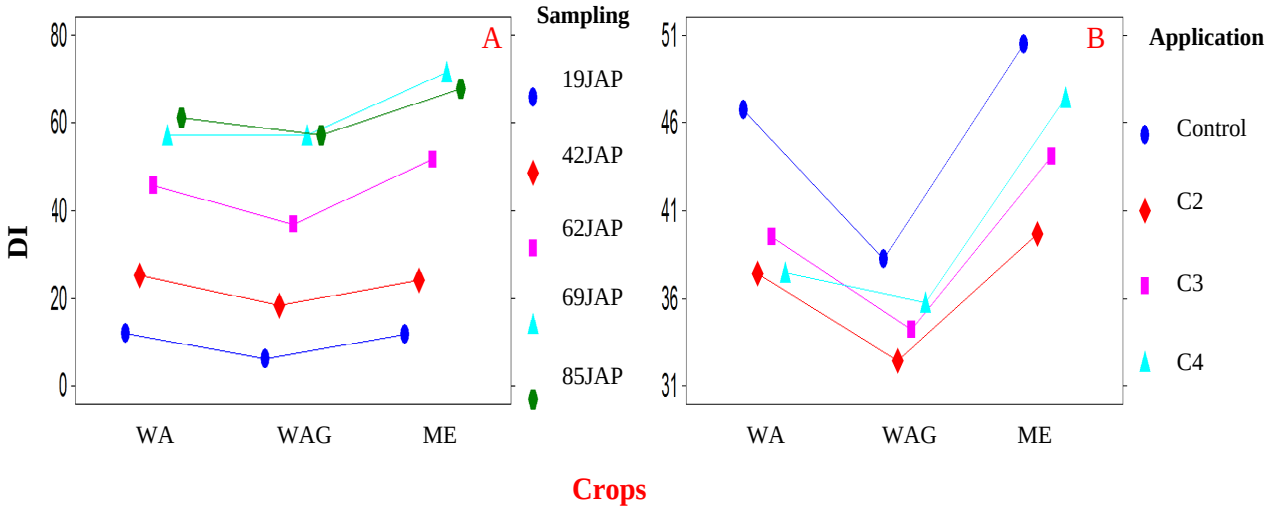

Table.4 EC50 values for inhibiting in vitro mycelial growth of *Monosporascus cannonballus* and *M. eutypoides* by fungicides representing different chemical classes

Fungicides	Mycelial growth[a]						
	Monosporascus cannonballus			*Monosporascus eutypoides*			
	MT67	MT68	MT72	MT45	MT47	MT54	LSD 0.05[c]
Azoxystrobin	45.55ab[b]A[c]	14.47c D	34.72abc ABC	37.78a AB	16.82c CD	25.29b BCD	2.1
Captan	25.18cd A	15.16c A	25cd A	17.92b A	19.51c A	20.19b A	nd
Carbendazim	18.13de B	16.59bc B	48.94a A	48.94a A	27.01c B	31.99b AB	3.77
Copper oxychloride	57.75a AB	66.9a AB	42.52ab B	40.75a B	61.33b AB	81.97a A	2.85
Chlorothalonil	18.31de B	15.72c B	11.53de B	48.11a A	18.42c B	21.46b B	1.5
Fludioxonil	2.24e AB	1.78d AB	1.75e AB	2.66b A	1.52d B	2.48c A	3.42
Iprodione	37.38bc BC	22.67b C	27.58bcd C	50.77a B	77.19a A	24.56b C	1.55
Tebuconazole	1.63e C	2.49d ABC	3.33e A	2.68b AB	1.99d BC	2.82c AB	3.06
Propiconazole + Cyproconazole	0.47 e AB	0.31d B	0.39e AB	0.55b AB	0.56d AB	0.67c A	2.31
LSD 0.05[b]	1.32	1.22	2.45	1.64	2.45	1.38	

nd=not determined.
[a] EC50 values (mg a.i. l^{-1}).
Least significant difference: means followed by the same letter do not differ significantly ($P< 0.05$).
Capital letters are for comparison of means in the same row.
Small letters are for comparison of means in the same column.
[b] LSD0.05 is for comparison of means among pathogens with the same fungicide on mycelial growth.
[c] LSD0.05 is for comparison of means among fungicides in the same pathogen on mycelial growth.

Table.1 Comparison of percentage of *M. cannonballus* isolation at the end of trial field, with muskmelon cv. Galia, watermelon cv. Dumara, watermelon cv. Dumara grafted onto *Cucurbita maxima* × *C. moschata* rootstock cv. TZ-148 and nine applications (drenching)

Fungicides	**Treatements**	**Percentage of *M. cannonballus* isolation (%)[a*]**		
		Watermelon	**Melon**	**Grafted watermelon**
Alto Super 330 EC	T1	35.03±0.64 b	35.87±0.4 c	14.3±0.20 c
	T2	34.5±0.10 b	35.57±0.15cd	15.83±0.97 b
	T3	34.4±0.23b	33.97±0.68 e	15.07±0.67 bc
Maxim 100 FS	T4	32.93±0.21c	37.1±0.45b	15.6±0.85 bc
	T5	34±0.56 bc	34.43±0.25 de	16±0.45 b
	T6	34.7±0.53 b	34.63±0.51de	14.57±0.5 bc
Horizon EW	T7	33.73±0.21bc	32.63±0.23f	15.4±0.46 bc
	T8	33.87±0.55 bc	34.67±0.15 cde	14.87±0.64bc
	T9	34.53±0.32 b	34.53±0.32 de	15.53±0.71bc
Témoin	T10	44.97±0.74 a	44.9±0.79 a	24.43±0.20 a
***P valeurs*[b]**		0.000	0.000	0.000

[a] Percentage of M. cannonballus isolation. Mean of three repetitions with 7 fragments each.

[b]ANOVA. Means in a column followed by the same letter are not significantly different according to Students least significant difference test at P<0.05.

Table.2 Analysis of variance for the effects of Crop, fungicide, sampling moments and applications on disease incidence (DI)

	df[a]	Disease incidence (%)	
		MS[b]	P <F[c]
Crops	2	4745.02	0.000
Fungicides	2	90.4550	0.2026
Sampling moments	4	59589.11	0.000
Applications	3	1750.122	0.000
Crops*Fungicides	4	14.5277	0.9052
Crops*sampling moments	8	426.0339	0.000
Crops*Applications	6	181.9716	0.0041
Fungicides*sampling moments	8	37.0124	0.7308
Sampling moments* Applications	12	0.0943	0.753
Fungicides*Applications	6	22.6738	0.8781

[a] Degrees of freedom, [b] Mean square, [c] Probabilities associated with individual F tests.

Table.3 Comparison of Monosporascus root rot and vine decline incidence at the end of chemical control field trial, with muskmelon cv. Galia, watermelon cv. Dumara, watermelon cv. Dumara grafted onto *Cucurbita maxima* × *C. moschata* rootstock cv. TZ-148 and nine applications (drenching)

Fungicides	Treatments	Disease incidence (%)[a]		
		Watermelon	Melon	Grafted watermelon
Alto Super 330 EC	T1	52.77±0.42 cd[b]	63.47±1.45 b	40±0.10 c
	T2	58.61±1.05 bc	61.60±1.59 b	56.67±0.35 ab
	T3	49.84±0.59 d	65.65±1.08 b	60±0.10 a
Maxim 100 FS	T4	56.97±0.97 bc	61.52±1.42 b	60±0.91 a
	T5	61.39±1.1 b	66.67±1.77 b	53.33±0.56 ab
	T6	57.22±0.64 bc	67.85±1.36 ab	60±0.96 a
Horizon EW	T7	59.64±0.69 bc	63.33±0.18 b	46.67±1.55 bc
	T8	55.52±0.26 bcd	66.67±0.56 b	56.67±0.03ab
	T9	52.53±0.05 cd	68.69±0.22 ab	60±0.19 a
Control	T10	76.67±1.26 a	79.24±0.63 a	63.33±1.26 a
P values		0.000	0.0304	0.0117

[a]Percentage of plants showing symptoms of Monosporascus root rot and vine decline. Mean of three repetitions of 5 plants each.
[b] ANOVA. Means in a column followed by the same letter are not significantly different according to Students least significant difference test at P<0.05.

Table.4 Comparison of fruit yield (kg/plant) at the end of the trial field, with muskmelon cv. Galia, watermelon cv. Dumara, watermelon cv. Dumara grafted onto *Cucurbita maxima* × *C. moschata* rootstock cv. TZ-148 and nine applications (drenching)

Fungicides	**Treatements**	**Fuit yield (kg/plant)**[a]		
		Melon	**Watermelon**	**Grafted watermelon**
Alto Super 330 EC	T1	2.14±0.59	2.16±0.12	2.3±0.46
	T2	2.45±0.04	2.19±0.18	2.1±0.68
	T3	2.84±0.55	2.23±0.28	2.45±0.43
Maxim 100 FS	T4	2.37±0.01	2.29±0.13	2.51±0.63
	T5	2.35±0.19	2.51±0.38	2.45±0.45
	T6	2.37±0.11	2.44±0.09	2.66±0.16
Horizon EW	T7	2.30±0.12	2.27±0.11	2.51±0.46
	T8	2.24±0.04	2.56±0.29	2.89±0.34
	T9	2.31±0.14	2.35±0.08	2.66±0.45
Témoin	T10	2.56±0.00	2.46±0.16	2.89±0.34
P valeurs[b]		0.2478	0.2710	0.5864

[a]Fruit yield (kg/plant). Mean of three repetitions of 10 plants each. [b]ANOVA. Means in a column followed by the same letter are not significantly different according to Students least significant difference test at P<0.05.

Disease incidence (DI)

Analysis of variance showed that there were no differences in the disease incidence (DI) between the three fungicides, but the effect of crops, sampling moments, applications and the interaction between crops*sampling moments and crops*applications were significant (P <0.05) (Table 6).

According to the interaction between application * culture, all the treated plants at 19, 40 and 59 DAP showed a lowest disease incidence (Figure 1B). Cumulative frequency of fungicides created a difference in efficacy of fungicides, particularly at the last two sampling moments (69 and 85 DAP) (Figure 1A).

The MRRVD incidence values for each crop and treatments are shown in table 5. In watermelon, all treatments resulted in significant reduction in DI compared with control plants ($p<0.05$), and the best treatments were: T3, T9 and T1 with 49.84, 52.53 and 52.77%, respectively. For melon, a significant differences between treatments were found ($p=0.0304$), and the DI recorded was comprised between 61.52 (T4) and 61.60 (T2). In general, grafted watermelon plants were the least affected by MRRVD, and presented low DI values ($p=0.0117$), presenting 40 for treatments T1 and 46.67% for treatment T7 (Table 7).

Fruit field (Kg/plant)

Despite, the obtained results did spotted any significant difference among the three cucurbits, the best treatments have been T3 and T8 recording 2.84 kg/plant (melon) and 2.56 kg/plant (watermelon), respectively. Grafted watermelon plant have recorded 2.89 kg/plant (T8) (Table 8).

Plant disease control is an important way for maintaining yield potential of arable crops. At present, when the economic efficiency is of prime importance, the disease control in grain crops by fungicides should be biologically substantiated for getting the economic benefit. The efficiency of chemical disease control depends, on the one hand, on a fungicide and its mode of action, on the other hand, on the fungi that caused the disease and peculiarities of its action. Plant growth stage during fungicide application, the development rate of diseases and fungicide application timing influenced the efficacy of the fungicide and the duration of its protective action. Compared with fumigation, the use of fungicides in the soil is usually less expensive. In addition, fungicide chemistry and application is generally more specifically targeted and is likely to have less adverse effects on soil microbial populations and diversity. Fungicide application to crops for the management of soilborne pathogens is mainly practiced with seedling disease pathogens such as *Pythium* and *Rhizoctonia*, since the plants need only short-term protection.

This approach is not used with soilborne pathogens such as *Fusarium*, *Verticillium*, and *Monosporascus*, which cause diseases in mature plants (Erwin, 1981; Shnha *et al.*, 1988). Fungicide efficacy in soil also depends on the physical, chemical, and biological properties of both the soil and the fungicide used. Processes such as sorption, degradation, mobility, penetration into the host tissue, and translocation within the plant determine the activity of a compound (Helling *et al.*, 1974). This work was conducted to investigate the potential of fungicide application in combination with grafting for the management of MRRVD by testing the effect of different active ingredients on *M. cannonballus* and *M. eutypoides in vitro* and on *M. cannonballus* in a field trial. Several research reports have shown that the use of fungicides is a key

measure to manage MRRVD of cucurbits (Pivonia *et al.*, 2010). The sensitivity of *M. eutypoides* and *M. cannonballus* towards all tested fungicides was quite varied. Our results *in vitro* showed that Propiconazole + Cyproconazole, Fludioxonil and Tebuconazole were the most effective fungicides for the inhibition of mycelial growth of both *M. cannonballus* and *M. eutypoides*; while, Azoxytrobin, Chlorothalonil and Copper oxychloride were less effective. Some fungicides tested had similar and/or different results to those reported by other research. Cohen *et al.* (1999) reported that Fluazinam, Kreoxim-methyl, Pyraclostrosbim + Methiram, and Propiconazole have completely inhibited the mycelial growth of *M. cannonballus* at low concentrations. These authors reported that Carbendazim has decreased the mycelial growth with 95.7% at 10ppm and Iprodione with 66.5%. Medeiros *et al.* (2006c) indicated that chlorothalonil has reduced the mycelial growth with 69% at 1ppm, unlike our results showing that this fungicide has resulted a significant inhibition percentage against *M. cannonballus* at 100ppm with 78.26 (MT68) to 80.23% (MT67) and for *M. eutypoides* with 19.34 (MT45) to 76.12 % (MT47) at 10 ppm and with 65.71 (MT54) to 67.23% (MT45) at 100ppm. Cohen *et al.* (1999), Guimaraes *et al.* (2008) and Medeiros *et al.* (2006a; 2006b) in search of fungicides against *M. cannonballus* concluded that Propiconazole had a potential for use in control, taking order to inhibit mycelial growth *in vitro* of that pathogen, as a percentage of 68%. The more efficient active ingredient included Propiconazole, as the mycelial growth was 100% inhibited in the lowest concentration, demonstrating the potential of this active ingredient to control *M. cannonballus*. In our study, Propiconazole+ Cyproconazole were highly effective; these results are in agreement with Medeiros *et al.* (2006c). Pivonia *et al.* (2010) have tested *in vitro* 12 fungicides and showed that eight of them have completely suppressed mycelia growth of *M. cannonballus* mycelial growth in culture like Fludioxonil and Azoxytrobin at 0.1 µg a.i ml^{-1}. Cannonball (Fludioxonil) is a commercial fungicide registered by Syngenta Crop Protection (2005) against *M. cannonballus* in California, Arizona and Texas, as either treatment befor and during the growing season by applying through the drip irrigation system (Miller & Amador, 2001). Also, Tebuconazole and Carbendazim were effective at 1-10 µg a.i ml^{-1}. However, our obtained results showed that Azoxytrobin and Carbendazim were less effective in reduction of linear growth of both species of *Monosporascus* sp. Our results also illustrate the huge variation in fungicides sensitivity not only between species, but within species. Further research is needed to investigate this effect. At the subplots cultivated in melon, watermelon and grafted watermelon, all the three fungicides applications (Super Alto, Maxim and Horizon) have resulted in a significantly lower disease incidence compared to control. The best treatments are T2 and T8 recording 52.08 and 56.55%, respectively. Watermelon is the only crop which the highest (66.21%) were recorded regardless all treatments. The diagnosis of the root system at the end of the trial revealed the presence of perithecia on melon and watermelon roots recording the highest frequencies. The highest fruit yield was recorded in subplots cultivated in grafted watermelon treated by Horizon at 19; 59 and 73DAP (C3) with 2.89 kg / plant. Generally, in the field trial, application of Alto Super (Propiconazole + cyproconazole) at 40 and 59 days after planting, and also at 40, 59 and 73 days after planting was the most effective strategy to reduce the incidence of MRRVD. Helmy (2003) and Mostafa and Eltoony (2004) have reported an improving

efficiency in fruit yield after applying fungicide in Egypt. The interaction between the application of fungicides and bio-fungicides showed a significant improvement on fruit characteristics (Mennatoullah *et al.,* 2010). The progression of MRRVD was relatively attenuated in the three cucurbits treated versus controls. It is the consequence of improper installation of the pathogen in roots showing the efficacy of chemical control combined with grafted plant against this disease. We can say that the absorption of the three fungicides within the root tissues of the three cucurbits begins to reduce the damage of the disease not to mention that the plants treated by submersion. While at the end of culture, percentages of disease incidence and isolation recorded were important, showing a relatively low efficiency this can be explained by the significant infiltration and evaporation of fungicide in soil. The application at 19; 40 and 59DAP (C2) of the three fungicides is the most effective recording the reduced disease incidence with 35.08 (Alto Super), 36.55 (Horizon) and 37.9% (Maxim). Both Alto Super and Horizon have been effective against *M. cannonballus*. Pivonia *et al.* (2002; 2010) indicated that fungicide applications in a 3-week interval are needed for effective control of the disease during the short summer growing season, when disease progress is very rapid. Unlike preplanting soil fumigation, which aims to kill the fungal survival structures (ascospores) in the soil before planting, Pivonia *et al.* (2010) hypothesized that *Monosporascus* spp., could be controlled during its biologically active stages. Ascospores of *Monosporascus* sp., might germinate and penetrate into cucurbits roots throughout the season according to the environmental conditions and fungus-root interactions. The longer a fungicide persists in the soil, the fewer number of applications would be needed to obtain significant disease control. Fungicides efficacy in suppression of sudden wilt was confirmed in the field, but results were variable. Variation in control may also result from differences in the level of inoculum in the soil, growing conditions, and temperature regimes that prevail during the season (Pivonia *et al.,* 2010). Alternation of fungicides during a season would enable long-term use of this control measure, as it might reduce the risks of development for resistant pathogen biotypes and, or the development microbial populations in the soil that possibly could accelerate degradation of the fungicides (Pivonia *et al.,* 2010). Application of fungicides to the soil is a possible solution, but application prior to planting (as soil disinfestation) is more common and successful than application of fungicides during the growing season (Cohen *et al.,* 1999; Cohen *et al.,* 2000), that was in concordance with our findings about efficient treatments in field. Infection with *Monosporascus* spp. occurs at all stages of cucurbit growing season. Consequently, more emphasis should be placed on finding more effective chemical or biological control agents for these pathogens. Therefore, the use of an integrated management program that includes other control measures, such as biological treatments should be evaluated for effectiveness at reducing *Monosporascus cannonballus* and *M. eutypoides* infection. In addition, treatments based on three or four applications of conventional protective fungicides alone such as Fludioxonil or Tebuconazol could effectively reduce the incidence of MRRVD. These treatments could be recommended in order to avoid risk related to resistance development. Resistant cultivars and grafting may also contribute to the integrated pest management. Grafted plants used alone or combined with soil treatment may be enough to reduce the attack of *M. cannonballus*. Any agricultural

practice improving the capacity of the plant to overcome the disease may be an important component of the integrated pest management. Some authors studying the impact of the irrigation system on the decline caused by *M. cannonballus* have found that an irrigation system that contribute to disease reduction without reducing the yield (Pivonia *et al.,* 2004). A suitable combination of control methods could lead to a better and wider range of pathogens with mastery and efficiency in the long run concurrent to a reduction in pesticide use (Katan, 1996). This study aimed to ascertain the effectiveness of fungicides in the control of *Monosporascus cannonballus* and *M. eutypoides* and combined with grafting against MRRVD. Thus, it appears that the tested active ingredients can be recommended at its lowest dose to control the disease. Considering that, until this moment there is no product registered in Tunisia for the watermelon and melon that has effectively against *Monosporascus* sp. This information can be valuable to implement soil drenching programs considering the optimum number of applications and fungicides selection in order to achieve both satisfactory control of the disease and reduced risk of resistance.

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