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CHEMICAL CONTROL OF ALTERNARIA BROWN SPOT ON MANDARINS CULTIVARS IN TUNISIA

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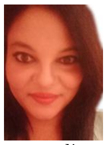

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ABSTRACT

Three field experiments were conducted to evaluate the efficiency of seven active ingredients (Tryfloxystrobin, Iprodione, Procymidone, Mancozebe, Propinebe, Tebuconazole and Coppersulfate), against Alternaria brown spot (ABS) caused by Alternaria alternata on Minneola, Fortune, Nova and Tangerine mandarins citrus cultivars in Tunisia. Over the three trials, citrus trees received 6, 10 and 16 fungicides sprays. The results of the present investigation demonstrated the prevention ability of Mancozebe, Iprodione and Copper sulfate according to disease incidence, fruit infection and losses caused by ABS. Furthermore, the applications of those three fungicides used in mixture reduce the ABS development. In fact, disease incidence was reduced better after an application of a mixture of Mancozebe and Iprodione compared to application of Mancozebe or Iprodione alone and after an application of an alternation of Mancozebe and Iprodione with copper sulfate. From this study, it revealed that the incidence of ABS, an emerging fungi disease on Citrus in Tunisia, could be reduced by using chemical products.

Contribution/Originality: This study documents the evaluation of the efficiency of seven active ingredients against citrus Alternaria brown spot caused by Alternaria alternata on Minneola, Fortune, Nova and Tangerine mandarin cultivars in Tunisia.

1. INTRODUCTION

Alternaria brown spot (ABS), caused by *Alternaria alternata* (Fr.) Keissl., is a serious citrus disease, especially to mandarin cultivar (*Citrus reticulata*) [1]. Symptoms of citrus ABS are presented as irregular brown necrotic areas with yellow halos, vein darkening and necrosis on young leaves and a defoliation of infected trees [2]. First ABS symptoms were detected in 1903 in Emperor Mandarin cultivar in Queensland, Australia [3]. Currently, the disease is widespread in many humid citrus-growing areas worldwide and causes serious epidemics in semi-arid areas [4-7]. In Tunisia, ABS was first detected in 2008 on tangerine cultivar and their hybrids [8].

Citrus fruits are susceptible to ABS infection until about mid-summer in many growing areas [9] and may be susceptible for much longer in cooler climates [10, 11]. On young fruits, ABS appear as necrotic slightly depressed spots to circular and dark brown spots on the external surface, and often associated with premature abscission and substantial yield losses [12]. On mature fruits, ABS necrotic spots became between 1 and 10 mm in diameter and affect the fruit quality [13, 14].

A. alternata survives in winter on infected leaves and stems [15]. *A. alternata* conidia are disseminated by air and their infection are activated with the presence of susceptible tissues and favorable climatic conditions such as temperatures and wetness [9]. Optimum conditions of ABS infection are temperatures from 23 to 27°C and 8 to 12 h of continuous leaf wetness [14]. According to Haddad, et al. [8], ABS infection is accentuated from late April to October, but infections occur mainly in spring and autumn due to the rainfall and also induced in summer in the presence of dew. *A. alternata* incubation period is extremely short and lesions appear within 2 days after infection due to the rapid effects of host-specific toxin ACT-toxin [14].

As the fungus only multiplies in dead tissue, it is recommended the execution of a cleaning, pruning, eliminating the remains of the orchard and pruning in winter to improve aeration plant [4]. ABS cause severe epidemics in semi-arid citrus growing regions due to its environmental flexibility [12]. Currently, ABS control is primarily based on chemical control that must be scheduled to protect susceptible organ during the critical periods of infection [16]. For chemical treatment efficiency, a system called alter-rater was developed in Florida based on the relationships between environmental factors and ABS severity [9]. This model was effective to determine the appropriate period for fungicide sprays in Wang, et al. [7]. One or two sprays fungicides applications are generally needed to protect spring flush foliage and to reduce defoliation and prevent inoculums build-up [2]. For this, many fungicides applications (between four and ten fungicides sprays) are required to reduce the disease severity [2, 4, 17]. Thereafter, all sprays are applied solely for the control of fruits infection.

However, regardless of the disease, the total number of sprays and the disease control level achieved depend also on the spray frequency during the infection periods [2]. These authors reported that too long application intervals result in severe losses, and too short intervals generate unnecessary sprays application. In fact, in Spain, growers are advised to spray every 10 to 15 days during the infection periods and treatment should be repeated immediately after a rainfall [2]. Fungicides treatments to control the disease became imperative. The disease was initially controlled with the Dicarboximide and Iprodione fungicides [18]. However, the development and spread of *A. alternata* strains resistant to these two fungicides had significantly reduced their effectiveness [19-21].

In the Tunisian conditions, the search of effective alternatives is necessary. Thus, this investigation was undertaken to evaluate the effect of seven fungicides against ABS disease and to determine the best timing of fungicides sprays to reduce fruit lesions under field conditions.

2. MATERIAL AND METHODS

2.1. Fungicides Used

Seven fungicides were used in this study Table 1. These fungicides were applied in citrus trees in orchards at Beni-Khalled region from the area of Cap Bon, North-East Tunisia. The orchards used were severely affected by ABS in previous years and contained the most susceptible citrus cultivars to this disease. This locality is belonging to a semi-arid bioclimatic stage and the experiments were conducted on two seasons from May 2010 to December 2011 and from March 2013 to January 2015. The daily maximum temperature in this area was 15-27°C in the spring (March to May) and 24°C in autumn (October to November). The average annual rainfall for the same period was 470mm.

2.1.1. Experiment 1

A curative trial against late infections of citrus ABS was carried out after a severe attack of the disease in May 2010. Fungicides were applied in citrus orchards cultivated with Fortune, Minneola, Nova (14 years-old) and Tangerine mandarin (40-year-old) grafted onto sour orange (*Citrus aurantium*) rootstock. Spacing between citrus trees was 4-5. Sixteen foliar sprays of each fungicide were made at 2-week intervals, beginning from May to December 2010 when the experiment was achieved and the fruits survey was conducted.

Table-1. List of fungicides used in the experiments.

Chemical family	Active ingredient (a.i.)	Commercial names	Concentration of product in spray	Dose
Strobilurins	Tryfloxystrobin	Zato	50%	0.2 g/L
Dicarboximides	Iprodione	Rovral	50%	1 g/L
Dicarboximides	Procymidone	Sumixlex	50%	1 g/L
Dithiocarbamates	Mancozebe	Caïman	80%	2.5 g/L
Dithiocarbamates	Propinebe +Cymoxamil	Antracol combi	70%+6%	2.5 g/L
Conazoles	Tebuconazole	Horizon	250g/l	1 cc/L
Copper-based	Coppersulfate	Bouillie bordelaise	20%	1.5g/L

2.1.2. Experiment 2

The second trial was preventive treatment and carried out in spring 2011 (from March to December) in the same citrus orchards of the experiment 1. A total of ten sprays per treatment. The experimental fungicides were applied in March (before spring growth of foliage) to June with five sprays, followed by two summer sprays and three autumn treatments applied at 2-week intervals from the beginning of September. Fruits survey was established in December 2011.

2.1.3. Experiment 3

This experiment was conducted from March 2013 to January 2015 in a citrus orchard at Technical Center of Citrus in Beni-Khalled region, North-East of Tunisia. Cultivars Fortune and Minneola were tested and treated with fungicides, in order to compare Iprodione and Mancozebe efficacy as the main applied fungicides, with copper sulfate used in alternation in the third and fifth sprays. In addition, a mixture of Iprodione and Mancozebe was assessed. In total, six foliar sprays were made, the first application was performed in 15 March at bud break to protect the spring flush of growth in order to reduce initial *A. alternata* inoculums. Application was applied in 2 weekly intervals. Fruits were controlled in December 2013 and January 2015.

2.2. Experimental Design and Disease Evaluation

Plots were arranged in a completely randomized design. An untreated block was included as control (no sprays). Each plot contained eight to ten trees in a row, and data were obtained from six central trees. For each experiment, fruits survey was recorded from fruits arbitrarily selected from each of the eastern and western sides of the tree at commercial harvest time. A total of 50 fruits per tree were picked randomly from each plot. The fruits disease severity index was rated according to the following scale (0–3): 0= no lesions; 1= 1–5 lesions; 2= 6–15 lesions; 3= 16 or more lesions [22].

The incidence was measured and expressed as percentage of affected fruits in each category. To evaluate the commercial impact of disease incidence, fruits were classified into three classes: class I which provides the highest income and ranked in disease incidence scale 0, class II can be sold in the local market and classified in the scale 1 and 2 and class III form unmarketable fruit belong to the scale 3 [22].

2.3. Data Analysis

The mean disease incidence was calculated for each treatment and citrus cultivar. Standard errors of the means deviation were calculated. Disease severity index, disease incidence and percentages of infested fruits were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). All the variables were compared by analysis of variance (ANOVA) and means of the values were separated with Duncan test at $P < 0.05$.

3. RESULTS

3.1. Experiment 1

Disease pressure was prominent especially in spring causing severe fruits damage. On average, the ABS incidence as significantly reduced by a level of 25 to 65% among all fungicides compared to untreated control. Using Tryfloxystrobin and Tebuconazole treatments, all cultivars showed similarity in comparison with the untreated control Table 2.

For Minneola mandarin, the most effective fungicides were Iprodione (44%), then Mancozebe (34%). No significant difference was found between Procymidone, Tryfloxystrobin and Tebuconazole compared with the untreated control which reached 80% of disease incidence.

In this experiment, the use of Iprodione, Copper sulfate and Mancozebe generated a yielding between 32 and 25% of fruits in class I compared with 6% in the untreated control. Tryfloxystrobin and Tebuconazole were not efficient for this disease management and 42% of fruits belong to class III. For Fortune cultivar, disease was extremely severe with premature leaf drop, and yield was reduced to zero in the unsprayed control Table 2.

Table-2. Effects of curative treatments of seven fungicides and canopy aspect on the incidence of citrus brown spot of cv. Fortune, Minneola, Tangerine and Nova mandarin fruit.

Active ingredient and content (a.i.)	Minneola	Fortune	Tangerine	Nova
Tryfloxystrobin 0.2g/L	89.53±0.43^{f*}	79.09±0.12^{e}	86.9±0.56^{g}	57.46±0.13^{e}
Copper sulfate1.5g/L	44.67±0.57^{b}	35.91±0.10^{a}	26.36±0.55^{c}	32.08±0.07^{b}
Iprodione 0.5g/L	42.03±0.65^{b}	40.59±0.11^{b}	10.56±0.25^{a}	36.13±0.12^{c}
Procymidone 1g/L	67.36±0.53^{d}	49.57±0.11^{c}	29.32±0.44^{d}	36.64±0.08^{c}
Mancozebe 1.25g/L	33.12±0.3^{a}	39.15±0.09^{b}	21.34±0.37^{b}	21.34±0,.08^{a}
Propinebe 2.5g/L	61.39±0.41^{c}	71.50±0.09^{d}	80.51±0.47^{f}	49.37±0.10^{d}
Tebuconazole 1cc/L	79.48±0.44^{e}	83.04±0.10^{f}	75.95±0.34^{e}	62.61±0.12^{f}
Control	100.00±00^{g}	100.00±00^{g}	100.00±00.00^{f}	77.12±0.13^{g}

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

Fortune cv. presented a percentage of fruit class I between 0% and 47% when, Tryfloxystrobin and copper sulfate were used, respectively. For the same cultivar, 6 and 41% of unmarketable fruits (class III) were obtained from trees treated with copper sulfate and Tebuconazole, respectively. Iprodione and Mancozebe fungicides were the most effective product with the Tangerine cultivar with a percent of healthy fruits of 72% and 78% respectively and these fruits belong to in class I Table 3.

Procymidone and Iprodione were also very effective, with 69% of fruits in class I. The lowest percentages of fruits were registered for trees treated by Tryfloxystrobin (8%) and Tebuconazole (9%). For Nova cultivar, the incidence of brown spot was significantly reduced by Copper sulfate (32%) and Iprodione (36%). In this experiment, the mixture of Iprodione and Mancozebe generated 1% and 2% of fruits in class III (compared with 21% in the untreated control), respectively.

3.2. Experiment 2

The outputs of this experiment demonstrated that all the tested products reduced significantly the disease incidence and increased the percentage of marketable fruits Tables 4 and 5. In fact, no fruit drop occurred in any of the treatments. The incidence of brown spot on Minneola cultivar were significantly reduced by 87 and 68%, by Mancozebe and Copper sulfate, respectively Table 4. Both Iprodione and Mancozebe generated 45% of fruits in class I and 0.3% in class III, compared with 26% (class III) for the untreated control.

Table-3. Evaluation of fungicides applied for control of Alternaria brown spot in a Fortune, Minneola, Tangerine and Nova orchards.

Active ingredient (a.i.) and content	Minneola				Fortune				Tangerine				Nova			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Tryfloxystrobin 50%	6±0.06^{a*}	7±0.11^{a}	45±0.10^{e}	42±0.11^{e}	9±0.07^{c}	3±0.06^{b}	51±0.07^{h}	37±0.07^{e}	8±0.06^{b}	12±0.06^{a}	50±0.07^{g}	30±0.08^{e}	20±0.07^{h}	23±0.06^{d}	28±0.08f	29±0.08c
Cupper sulfate 20%	25±0.09^{d}	15±0.13^{c}	33±0.08^{bc}	27±0.09^{b}	47±0.13^{h}	31±0.09^{f}	16±0.08^{c}	6±0.06^{b}	60±0.11^{d}	21±0.06^{d}	6±0.06^{b}	13±0.7^{c}	52±0.07^{c}	20±0.07^{c}	19±0.09d	9±0.05e
Iprodione 50%	32±0.07^{e}	38±0.13^{g}	20±0.06^{a}	10±0.07^{a}	34±0.09^{f}	36±0.07^{g}	22±0.07^{d}	8±0.06^{c}	72±0.08^{f}	25±0.07^{e}	3±0.07^{a}	0±0.00^{a}	56±0.08^{a}	40±0.08^{f}	3±0.07 a	1±0.05f
Procymidone 50%	12±0.07^{b}	16±0.13^{cd}	43±0.08^{d}	29±0.16^{c}	21±0.07^{e}	13±0.06^{d}	34±0.09^{e}	32±0.07^{d}	69±0.08^{e}	19±0.06^{e}	10±0.06^{c}	2±0.05^{b}	52±0.07^{d}	18±0.06b	17±0.06c	13±0.05e
Mancozebe 80%	21±0.08^{c}	17±0.12^{d}	50±0.08^{f}	12±0.08^{a}	41±0.08^{g}	39±0.09^{h}	12±0.07^{b}	8±0.06^{c}	78±0.09^{g}	16±0.07^{b}	5±0.06^{b}	1±0.04^{b}	74±0.07^{b}	19±0.06b	5±0.06 b	2±0.05g
Propinébe 70%	13±0.07^{b}	21±0.10^{e}	32±0.08^{b}	34±0.08^{d}	13±0.08^{d}	15±0.08^{e}	40±0.07^{f}	32±0.07^{d}	13±0.06^{c}	15±0.08^{b}	45±0.8^{f}	27±0.08d	23±0.06e	17±0.06a	43±0.07h	17±0.08d
Tebuconazole (250g/l)	6a 6±0.06 ^{a}	10±0.09^{b}	42±0.07^{d}	42±0.08^{e}	6±0.07^{b}	8±0.05^{c}	45±0.09^{g}	41±0.07^{h}	9±0.05^{b}	12±0.06^{a}	44±0.09^{e}	35±0.07^{f}	18±0.08^{g}	26±0.07e	32±0.08g	24±0.06b
Control	6±0.07^{a}	24±0.15^{f}	34±0.09^{c}	36±0.11^{d}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	5±0.05^{a}	12±0.06^{a}	37±0.08^{d}	46±0.08^{g}	12±0.06^{f}	42±0.06g	25±0.08e	21±0.07a

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

The low yielding of marketable fruits was registered with Tryfloxystrobin (2%). All the fungicides reduced significantly the ABS incidence for Fortune cultivar with Procymidone and Iprodione. Fruits infection was significantly decreased with Copper sulfate (22%). Iprodione, Mancozebe and Copper sulfate significantly reduced fruits infection with 48 and 39% of fruits in class I, respectively. For Tangerine, Tryfloxystrobin and Tebuconazole revealed to be unable to reduce this disease for which the incidence reached 72% Table 4.

Table-4. Effects of preventive treatment of seven fungicides and canopy aspect on the incidence of citrus brown spot of cv. Fortune, Minneola, Tangerine and Nova mandarin fruit.

Active ingredient (a.i.) and content	Minneola	Fortune	Tangerine	Nova
Tryfloxystrobin 0.4g/L	74.89±0.53^{f*}	48.47±0.63^{d}	70.03±0.08^{f}	53.24±0.16^{d}
Copper sulfate 3g/L	32.87±0.37^{b}	19.02±0.11^{b}	15.41±0.08^{d}	11.83±0.06^{a}
Iprodione 1g/L	17.36±0.41^{a}	11.07±0.09^{a}	6.87±0.08^{a}	10.83±0.06a
Procymidone 1g/L	40.14±0.7^{c}	31.97±0.10^{c}	12.89±0.06^{c}	16.66±0.13^{b}
Mancozebe 2.5g/L	13.95±0.32^{a}	21.57±0.12^{b}	10.56±0.06^{b}	12.52±0.06^{a}
Propinebe+Cymoxamil2.5g/L	55.05±0.51^{d}	53.09±0.13^{d}	43.62±0.13^{e}	46.06±0.14^{c}
Tebuconazole 2cc/L	66.19±0.72^{e}	62.48±0.13^{e}	71.02±0.08^{e}	60.73±0.15^{f}
Control	100±00^{g}	100±00^{f}	100±00^{g}	70.35±0.15^{g}

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

The ABS incidence with Iprodione and Mancozebe treatments showed a significant decrease less than 10%. The incidence of the infection in the untreated control plots was so high that only 3% of the fruits were in class I. The best disease control was obtained with Mancozebe (70%) and Iprodione (69%) which exhibited high levels of fruits in class I of 70 and 69%, respectively. Treatments with procymidone and Copper sulfate significantly reduced fruits infection with 49-48% in the class I Table 5.

3.3. Experiment 3

Seven sprays of Iprodione or Mancozebe were effective to reduce fruit lesions. Two copper sulfate sprays additional to the third and fifth sprays followed by a Mancozebe and Iprodione spray generated significant disease incidence and fruits lesions decreases Table 6.

For Minneola, the use of Copper sulfate added to Iprodione was very effective in controlling fruits lesions. The disease incidence reduction was of 18% and the proportion of fruits belonging to class I was 52%, compared to 5% for the untreated control. In the same sens, additional applications of Iprodione with Mancozebe generated a low disease incidence rate (12%), and also a high percent of fruits in class I (55%). However, alternation of Iprodione and Mancozebe with Copper sulfate did not differ statistically from the mixture of Iprodione and Mancozebe. Adding Copper sulfate to Mancozebe was effective in controlling fruit lesions; the proportion of fruits in class I was 55%, compared to 6% in the untreated control. Mixing Iprodione with Mancozebe, the disease incidence of ABS was 12% in both citrus cultivars. Also, this mixture was able to reduce the infection rate of fruits which are mainly included class I (69%) Table 7.

4. DISCUSSION

Alternaria Brown Spot was reported in Tunisia, for the first time, in 2008 [8] and becomes a serious disease on citrus varieties such as tangerine and their hybrids. Fungicides applications are essential to reduce ABS incidence and to produce a high quality of marketable fruits. Nova and tangerine are the most appreciated cultivars by the consumers due to their good quality (caliber, color and juice…). However, these citrus cultivars are known to be very susceptible to ABS [8]. For this reason, blemishes are important to reduce and fungicides application should be sprayed in early spring to protect spring flush and reduce yield losses using a model for timing of fungicide sprays, and cultural measures could be associated to the best brown spot management.

Table-5. Evaluation of fungicides applied for control of Alternaria brown spot in a Fortune, Minneola, Tangerine and Nova orchards.

Active ingredient (a.i.) and content	Minneola				Fortune				Tangerine				Nova			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Tryfloxystrobin 50%	2±0.05^{a}*	10±0.06^{a}	40±0.07^{g}	48±0.08^{g}	9±0.06^{b}	21±0.06^{c}	40±0.07^{f}	30±0.08^{g}	5±0.08^{b}	8±0.05^{a}	42±0.08^{g}	45±0.09^{e}	11±0.07a	26±0.06+d	52±0.08g	11±0.06^{d}
Coppersulfate 20%	25±0.07^{e}	30±0.07^{e}	23±0.08^{c}	22±0.07^{d}	39±0.07^{f}	30±0.07^{e}	21±0.07^{b}	10±0.06^{c}	48±0.09^{e}	31±0.07^{f}	15±0.05^{d}	6±0.06^{c}	52±0.1e	39±0.09f	6±0.06a	3±0.07^{b}
Iprodione 50%	45±0.08^{g}	40±0.08^{g}	15±0.08^{a}	0±0.00^{a}	48±0.09^{h}	38±0.07^{f}	14±0.08^{a}	0±0.00^{a}	69±0.09^{f}	23±0.09^{e}	8±0.09^{a}	0±0.00^{a}	61±0.1f	28±0.07e	11±0.06c	0±0.0a
Procymidone 50%	37±0.06^{f}	38±0.09^{f}	20±0.06^{b}	5±0.05^{c}	28±0.10^{e}	30±0.08^{e}	22±0.08^{b}	20±0.07^{d}	49±0.1^{e}	39±0.09^{h}	12±0.07^{c}	0±0.00^{a}	47±0.07d	42±0.08g	11±0.07c	0±0.0a
Mancozebe 80%	45±0.07^{h}	38±0.09^{f}	14±0.07^{a}	3±0.06^{b}	41±0.08^{g}	29±0.07^{e}	21±0.07^{b}	9±0.06^{b}	70±0.07^{g}	19±0.06^{d}	10±0.06^{b}	1±0.05^{a}	69±0.06g	22±0.08b	9±0.07b	0±0.0a
Propinébe 70% ; Cymoxamil 6%	10±0.07^{d}	27±0.06^{d}	29±0.09^{d}	25±0.05^{e}	20±0.06^{d}	14±0.09^{a}	34±0.08^{d}	32±0.07^{h}	39±0.08^{d}	37±0.10^{g}	20±0.06^{e}	4±0.06^{b}	40±0.08c	38±0.1f	17±0.07d	5±0.07c
Tebuconazole (250g/l)	17±0.08^{c}	18±0.07^{c}	30±0.06^{e}	25±0.06^{e}	18±0.07^{c}	19±0.09^{b}	30±0.07^{c}	23±0.06^{e}	10±0.07^{c}	16±0.08^{c}	45±0.07^{h}	29±0.1^{d}	21±0.07b	24±0.08c	26±0.07e	29±0.08e
Control	5±0.06^{b}	15±0.07^{b}	34±0.08^{f}	26±0.07^{f}	4±0.06^{a}	22±0.08^{d}	37±0.07^{e}	27±0.08^{f}	3±0.06^{a}	10±0.07^{b}	37±0.07^{f}	50±0.00^{f}	12±0.07a	15±0.07a	44±0.07g	29±0.08e

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

Table-6. Effects of chemical and canopy aspect on the incidence of citrus brown spot of cv. Fortune, Minneola mandarin fruit.

Active ingredient (a.i.) and content	Minneola	Fortune
Iprodione	32.30±0.33^{d*}	35.30±0.52^{e}
Iprodione+copper sulfate	17.80±0.44^{b}	18.10±0.24^{c}
Mancozebe	38.60±0.53^{c}	24.50±0.23^{d}
Mancozebe+copper sulfate	20.00±0.42^{b}	17.40±0.33^{b}
Iprodione +Mancozebe	14.50±0.40^{a}	14.80±0.40^{a}
Control	89.90±0.33^{e}	88.80±0.49^{f}

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at P < 0.05).

Table-7. Evaluation of fungicides applied for control of Alternaria Brown Spot in a Fortune, Minneola, Tangerine and Nova orchards.

Active ingredient (a.i.)	Minneola				Fortune			
	0	1	2	3	0	1	2	3
Iprodione	35.00±0.14^{b*}	31.00±0.15^{b}	22.00±0.11^{e}	12.00±0.10^{e}	55.00±0.11^{c}	41.00±0.12^{f}	0.00±0.00^{a}	4.00±0.07^{c}
Iprodione+cupper sulfate	52.00±0.10^{e}	39±0.12^{c}	9.00±0.09^{b}	0.00±0.00^{a}	49.00±0.11^{b}	39.00±0.12^{e}	12.00±0.10^{c}	0.00±0.00^{a}
Mancozebe	54.00±0.09^{f}	41.00±0.12^{c}	4.00±0.09^{a}	1.00±0.06^{b}	60.00±0.18^{d}	20.00±0.09^{b}	14.00±0.09^{d}	6.00±0.08^{d}
Mancozebe+cupper sulfate	40.00±0.09^{c}	33.00±0.13^{b}	17.00±0.09^{d}	10.00±0.10^{d}	55.00±0.11^{c}	29.00±0.09^{d}	15.00±0.10^{d}	1.00±0.06^{b}
Iprodione +Mancozebe	55.00±0.11^{d}	40.00±0.14^{d}	5.00±0.10^{c}	0.00±0.09^{c}	69.00±0.13^{e}	25.00±0.09^{c}	6.00±0.10^{b}	0.00±0.00^{a}
Control	5.00±0.09^{a}	10.00±0.10^{a}	47.00±0.15^{f}	38.00±0.13	6.00±0.08^{a}	12.00±0.10^{a}	42.00±0.09^{e}	40.00±0.10^{e}

Note: *Numbers within columns followed by the same letter are not significantly different (LSD at *P* < 0.05).

Field trials carried out from 2010 to 2014, in citrus growing areas in Tunisia, demonstrated the effectiveness of the majority of the treatments in controlling Alternaria brown spot on fruits by decreasing the disease incidence, the incidence and the percent of affected fruits for different cultivars of mandarins. The results of the present investigation revealed that three compounds active ingredient (Iprodione, Mancozebe and Copper sulfate) were effective in reducing fruit ABS infection. In each of the two consecutive seasons, spray programs of Tryfloxystrobin was statistically ineffective compared to Iprodione or Copper sulfate fungicides f. Our findings are in agreement with those of Miles, et al. [23] reporting that Tryfloxystrobin applied at low concentration was ineffective for controlling brown spot.

Moreover, Raid and Timmer [24] reported that Tryfloxystrobin was less effective for controlling ABS than the other strobulirin fungicides. Our results showed that Tryfloxystrobin was less effective at a low rate or doubling the rate of product from 0.2 to 0.4a.i g/L. In other experiments, Miles, et al. [25] proved that Tryfloxystrobin (0.07 a.i g/L) was effective against citrus black spot as well as Azoxystrobin (0.4 a.i g/L). In our study, Tryfloxystrobin reduced slightly the disease incidence on Fortune fruits when sprayed early in March. In fact, the strobilurin fungicides have post-infection al activity and reduce inoculums production.

The results of our study generated also that five sprays of Copper sulfate applied from early March performed well in field in the 2011 experiment. These findings are in concordance with those of Solel, et al. [22] and affirmed that an early season treatment with copper fungicide could reduce the inoculums of pathogen. Moreover, Bhatia, et al. [26] and Timmer and Zitko [27] asserted that copper fungicides are highly effective to control brown spot and the Alter-Rater model was validated using these products. However, Peres and Timmer [28] demonstrated that it is impossible to ovoid g fruits phytotoxicity so copper products may be used only early in the season for control of ABS in some citrus areas in Brazil. The findings of the present investigation confirmed that five applications of Mancozebe proceeded by two copper sulfate treatments after first flush in spring showed a very good control against *A. alternata* and they deduced that Mancozebe was the main contributor to disease control. These results are in agreement with those of Schutte and Beeton [29]. In the same sense, we demonstrated the effectiveness of Iprodione and confirmed the results of Schutte, et al. [30] and Solel, et al. [21]; Solel, et al. [22].

Tebuconazole was ineffective to control brown spot as also reported by many authors [22, 27]. However, these results are in contrast with Schutte, et al. [30] who reported the effectiveness of this product in South-Africa. Despite the importance of preventing infection of spring flush growth from fungal pathogens of citrus, the fungicides evaluated in this study were effective for only a short time after application. Resistance of *A. alternata* to Iprodione was observed in Murcott (Tangor) mandarin plot in Australia [19], where disease management was based on iprodione. Likewise, Iprodione resistance was reported in various pathovars of *A. alternate [31]*. In the second experiment of our investigation, fungicides applied exhibited a complete coverage of fruits. To reduce the risk of development of disease resistance to Iprodione, two modalities were assessed on using fungicides either as mixture or in alternation.

After determining the efficacy of each fungicide alone Iprodione or Mancozebe, or a mixture of both at regular rates performed good control ability. Solel, et al. [22] demonstrated the efficacy of a mixture of Iprodione with meritram that were more effective for the control of ABS and also proved that Iprodione mixed to copper hydroxide was not effective to reduce the degree of disease. In our study when Iprodione was in alternation with Copper sulfate, the disease incidence was acceptable, and the percentage of healthy fruits was high in class I. However, Solel, et al. [21] reported that when Iprodione was mixed with Copper hydroxide the degree of disease was high.

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