REVIEW ARTICLE

CHEMICAL CONTROL OF SOME STRAWBERRIES FUNGAL PATHOGENS BY FOLIAR FUNGICIDES UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

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ABSTRACT

Ten formulations belonging to 8 chemical families approved on Strawberry plants in Morocco were tested *in vitro* and *in vivo*. Cyprodinil + fludioxonil induced a significant inhibition of mycelial growth of the *Botrytis cinerea* and *Pestalotia longisetula* isolates reaching 90.5% at low dose (93.7 ppm). The inhibition percentages of *Bartalinia laurina* and *Colletotrichum gloeosporioides* were equal to 68.3 and 59% at 375 ppm. Complete inhibition of germination of 7 isolates was noted at 93.7 ppm. In comparison, mepanipyrim and pyrimethanil were less active on mycelial growth, its inhibition percentages ranged from 5.2% to 88.4% at low concentrations and 24.3% to 93.2% at 800 ppm. Inhibition percentages of the conidia production ranged from 23.2% to 98.8%, for those against the germination has reached 100% especially in the presence of mepanipyrim. Thiram was more effective than mancozeb, it reduced by 79% to 100% mycelial growth of seven isolates respectively at 500 ppm and 2000 ppm and completely inhibited sporulation and germination of seven isolates. The effect of pyraclostrobin + boscalid combination was enhanced against *C. gloeosporioides* ranging from 91% to 100%. Facing fenhexamid, isolates of *B. cinerea* were more sensitive with percentages of inhibition of 85.3% to 100%, while those relating to chlorothalonil ranged between 34% and 92%. As for procymidone, its action is more significant on the growth of *B. cinerea* isolates and *P. longisetula* with a percentage exceeding 80% inhibition at 500 ppm.

In vivo, Cyprodinil + fludioxonil combination was the most powerful with inhibition percentage adjusted to 100% against *B. cinerea* and *C. gleoesporioides*. Fenhexamid provided similar protection against *B. cinerea* 3 days after inoculation followed by pyrimethanil (70%) and procymidone (66.6%) and chlorothalonil (42.6%). The preventive effect of thiram and pyraclostrobin + boscalid combination was more apparent against *C. gloeosporioides*, rots inhibition percentages were 83.6% and 57% respectively.

INTRODUCTION

Strawberry plants and fruits suffer from the yield losses during the culture, at the time of harvest to consumption. The main factors restricting strawberry production in many countries is yield losses up to 15-92% - or even whole plantation- due to fungal diseases (Kapytowski and Bojarska, 2005).

Indeed, the growing season is characterized by long periods of leaf wetness, periodic rains, and mild temperatures, which are conducive to the diseases development caused by numerous fungi. Among these microorganisms, *B. cinerea* is a common disease of strawberry. The primary inoculum for *Botrytis* fruit rot epidemics is conidia produced on dead strawberry foliage in the field (Braun and Sutton, 1987). Symptoms might be developed on fruit either in the field or postharvest. Although symptoms are expressed on mature fruit, most Botrytis fruit rot infections occur at the flower stage of development (Powelson, 1960; Mertely *et al*., 2002). Mature fruit can also be infected by *B. cinerea* through wounds or by direct contact with a diseased fruit (Jarvis, 1962; Blacharski *et al*., 2001). This mechanism of infection may be important during storage and transport. In experimental trials, preharvest losses to Botrytis fruit rot ranged from 0.5 to 13% in a standard Captan-Rovral treatment, and up to 35% in untreated plots (Legard and Chandler, 1998).

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Postharvest losses also can be significant (Legard *et al.*, 1997). Strawberry fruit have a very short postharvest life particularly that is extremely perishable, more likely susceptible to decay, softening and water loss (Riad and Brecht, 2005). Postharvest decay of strawberries (*Fragaria ×ananassa* Duch.) causes major crop losses worldwide (Ceponis *et al.*, 1987). In tropical Africa and India, the losses were found to be 30 % (Wilson and Pusey, 1985) compared with 28 % as the average of strawberry postharvest losses in Kurdistan province in Iran (Salami *et al.*, 2010). Additionally, the anthracnose caused by several species of the *Colletotrichum* genus (Freeman and Katan, 1997), is counted among the major diseases of the strawberry plant. Under the appropriate environmental and cultural conditions, considerable yield loss can be inflicted by the pathogen (Maas, 1998) up to 50 % in the zones of production where control measures are applied (Howard *et al.*, 1992). Other fungal infections can be expected on strawberries, it is *Pestalotia longisetula* that shows virulence during the fresh seasons under the conditions of high humidity and low temperature of the field (Embaby, 2007). Severe damage was brought in commercial plantations of strawberry plant produced by *Pestalotia longisetula* according to Howard and Albregts (1973) who isolated it in winter from strawberries.

In front of these pathogens those can infect the various parts of plant and more specifically the flower to reach the fruit and cause significant economic damage, the adoption of the control strategies by the fungicides application to reduce the initial inoculum of these fungi and to prevent the infection of flowers turns out to be necessary (Holtz *et al.*, 2003; Zitter and Wilcox, 2006).

Several groups of fungicides were previously used such as Benzimidazoles, Dicarboximides and Phenylcarbamates, but their massive use has been ended in a strong selection of the resistant populations in several countries. Numerous cases of *B. cinerea* resistance to Anilinopyrimidines, Benzimidazoles, and to Dicarboximides were reported (Beever *et al.*, 1989; Latorre *et al.*, 1994; Leroux *et al.*, 1999; Yourman and Jeffers, 1999; My and Michailides, 2005; Esterio *et al.*, 2007; Banno *et al.*, 2008). Also, the isolates of *C. gloeosporioides* have presented a moderated resistance to the methyl-thiophanate and elevated towards the Mancozèbe (Kumar *et al.*, 2007).

Furthermore, the tolerance to residues fixed by importers countries limit their use in several cultures (Latorre *et al.*, 2002a; Sallato *et al.*, 2006).

So, new fungicidal treatments with a low toxicological risk are required (Förster *et al.*, 2007) such as the boscalid, the fludioxonil combined in the cyprodinil, fenhexamid and pyraclostrobin which contributed effectively to the control of *B. cinerea* and *R. stolonifer* (Latorre *et al.*, 2002b; Sallato *et al.*, 2007). Also, in Morocco, these antifungal products were introduced as treatments against several strawberry plants diseases (Ezzahiri *et al.*, 2014).

Surveys carried out in 2010 in two strawberry farms in Moulay Bousselham (Northwest Morocco) showed the existence of numerous symptoms (gray mold, anthracnose) on the strawberry plants of three varieties (Camarossa, Festival and Splendor). The Strawberry plants examined harbored a diverse pathogenic fungi, antagonist and saprophyte which can give indication about the health of the plants growing in this region and diseases likely to emerge (Mouden *et al.*, 2013).

In this study, the spectrum action of some foliar fungicides registered for use on strawberries in Morocco was screened and their effects were evaluated *in vitro* and *in vivo* towards fungal species responsible for the most important decays of the strawberry culture.

MATERIALS AND METHODS

In vitro fungicides essays

Fungal material

Seven isolates of four species isolated from strawberry plants belonging to the Laboratory of Botany, Biotechnology and Plant Protection, *Botrytis cinerea* (SC, BCV and BC), *Colletotrichum gloeosporioides* (Cg), *Bartalinia laurina* (BL), *Pestalotia longisetula* (Pd and PF) grown on PSA medium (potato: 200 g, sucrose: 20 g, Agar-agar: 15 g, sterile distilled water 1000 mL) and incubated at 22°C in the dark for 2-4 days.

Table 1 The characteristics of the fungicides according to the Pest Phytosanitary Index of Morocco (Ezzahiri *et al.*, 2014).

Trade name	Chemical group	Active ingredient (a.i)	Concentration of a.i	Recommended Dose
Pyrus 400 sc	Anilinopyrimidines	Pyrimethanil	400 g.L^{-1}	800 ppm
Switch 62,5 wg	Anilinopyrimidines Phenylpyrroles	Cyprodinil+ Fludioxonil	37.5% 25%	375 ppm
Frupica 50 wp	Anilinopyrimidines	Mepanipyrim	50%	400 ppm
Sumisclex 50% wp	Dicarboximides	Procymidone	50%	500 ppm
Basultra	Dithiocarbamates	Thiram	80%	2000 ppm
Dithan m45	Dithiocarbamates	Mancozeb	80%	1600 ppm
Signum wg	Strobilurins Carboxamides	Pyraclostrobin Boscalid	26.7% 6.7%	501 ppm
Teldor 50 wg	Hydroxyanilides	Fenhexamid	50%	750 ppm
Clortosip	Chloronitriles	Chlorothalonil	75%	1500 ppm
Ortiva 25 sc	Strobilurins	Azoxystrobin	250 g.L^{-1}	2000 ppm

Tested fungicides

Ten fungicides were screened belonging to eight chemical groups (Table 1) and registered for use in Morocco against fungal diseases of strawberry (Ezzahiri *et al.*, 2014).

For each fungicide, a range of concentrations was made by adding stock fungicide solution prepared as above, to molten (50°C) Water agar medium 1.5% Agar-agar. Fifteen mL aliquots were poured into Petri dishes and inoculated within 2–4 h after pouring. For the inoculation, conidia were gathered in sterile distilled water from colonies cultivated on PSA. The conidial concentration was adjusted to 10^3 conidia.mL^{-1} before spreading 0.2 mL aliquots onto plates of fungicide-amended agar. Three replicate plates were inoculated for each of the same sevens isolates as before for all fungicide concentrations. Inoculated plates were incubated at 25°C for 24 h in dark. The frequency of germination (germ tube length was greater than the conidia length) in a sample of 200 conidia per plate was determined by the microscopic examination.

In vivo fungicides essays

Plant material

Strawberries of Festival variety at green and red stages were disinfected by soaking them in a solution of Hypochlorite Sodium 5%, washed with sterile distilled water, dried under a laminar flow and each filed a plastic box containing two slices of sterile filter paper humidified with sterile distilled water.

Strawberries Treatment

Strawberries were artificially injured using a punch of 5 mm in diameter creating 2 mm deep in the middle of the fleshy part and sprayed with the fungicides solutions selected and based on their *in vitro* efficacy adjusted to a recommend dose. The control strawberries were sprayed with sterile distilled water. Strawberries were dried for 24 hours at 25°C in the dark.

Strawberries inoculation

Strawberries were inoculated at a cutting injury with 5 mm mycelial disk preleaved from the actively growing front of 1 week old colonies of the isolates BCV of *B. cinerea* and Cg of *C. gloeosporioides* and were placed in the same conditions as above. 6 replicates were performed for each isolate and each of the treatments.

After three days of incubation, the perpendicular rot diameters were measured by a double decimeter on processed strawberries. Uninfected strawberries were sprayed a second time with a fungicides solutions tested as above and incubated for 4 days at 25°C in the dark. The percentages of rot inhibition on strawberries (IRs%) relative to control were calculated for each time. After ten days of incubation at 25°C in the dark, a fragment of 1 cm^2 was cut from the surface of the strawberry fleshy portion and placed in a tube containing 3 mL of distilled water with one Tween drop (0.5%). After one minute stirring vortex, the conidia were counted with a Malassez slide at the rate of three per suspension. The inhibition percentages of the conidia production on strawberries (ICPs%) relative to the control were calculated for each time.

Statistical analysis

The *in vitro* inhibition means percentages of mycelial growth (IMG%), conidia production (ICP%) and germination (ICG%) were calculated for each fungicide relative to controls. Means were plotted against $\log_{10}$ values of the fungicide concentrations. Probit analysis was used to fit curves and to calculate the EC_{50} values (concentrations of the fungicides which reduced mycelial growth, production of conidia or germination of conidia by 50%). Data were analyzed by one-way analysis of variance (ANOVA) and LSD test at 5% level.

RESULTS

The inhibition percentage of the various isolates colony was variable. Among the tested Anilinopyrimidines, the pyriméthanil was very effective against the isolates *P. longisetula* (PD and PF) whose inhibition was about 80% even in small doses, of an efficiency moderated towards the isolate Cg of *C. gloeosporioides* who recorded a great inhibition varying from 49 to 84.2% and being thought of 14 to 65.6% as the isolate BL of *B. laurina*. Its action was limited on the isolates of *B. cinerea* keeping the low percentage not exceeding 44%. At 200 ppm, a 50 and 56.8% inhibition of the conidia production concerned respectively BCV and BC. From 266 ppm, the inhibition exceeded 50% for Cg and PD, below 44% in case of BL and SC whose inhibition reached approximately 54% at 400 ppm. At the recommended dose (800 ppm), the inhibition reached values going from 78.8 to 95.6%. At 800 ppm, the pyrimethanil succeeded in inhibiting the conidial germination of BCV, BC and SC in the respective percentages of 48.9 %, 52.4 % and 60 %. The pyrimethanil succeeded in inhibiting the conidial germination of the 7 isolates in the percentages ranged from 69.8 to 100% at 800 ppm (Table 2).

Mepanipyrim also applied a visible action on the mycelial growth. Two tested isolates of *B. cinerea* (BCV and SC) recorded respective percentages of inhibition of 73.8 % and 79.0% at 100 ppm. That of PD is being inhibited by 61.7% of its growth. Lower values varying from 18.0 to 29.5% and 28.7 to 47.7% were respectively obtained by BL and PF, whereas *C. gloeosporioides* registered the lowest values of the order of 5.2% at 100 ppm and of 24% at 400 ppm. Concerning its action on the conidia production, a lesser efficiency was noted at 100 ppm with percentages of inhibition going from 23.2 to 37% against 59.1 to 98.8% at 400 ppm. The germination of conidia of all the isolates was totally inhibited by the tested concentrations of mepanipyrim (Table 2).

Cyprodinil + fludioxonil combination has proved very effective, by leading an important inhibition of the mycelial growth of five studied isolates calculated by percentages of at least 90.5% in small doses and 99.6% to increased dose. BL and Cg was averagely inhibited at 93.7 ppm in particular *C. gloeosporioides* who registered only 34.5%, improved in the presence of 375 ppm by reaching 59%. Also, this fungicide inhibited completely the production of conidia by BCV, SC, BC, PD and PF under the effect of the low concentrations

which did not manage to repress effectively the production of the conidia of BL and Cg which the percentages of inhibition were of about 30.7 and 37.6% respectively and from 93.5% for BL to 87.8% for Cg in 375 ppm. The conidia germination of the species tested was completely prevented by Cyprodinil + fludioxonil combination (Table 2).

spore germination from the ¼ of the recommended dose equal to 500 ppm (Table 3). In front of mancozeb, the isolates PD, PF and BL (76-93%) are more sensitive than BCV, SC, Cg and BC (21-82%) who present more tolerance than the latter.

Table 2 Comparison of the inhibition percentage means of the three life stages (mycelial growth [MG], conidia production [CP] and germination [CG]) of fungal species isolated from strawberry plants in presence of different fungicides of Annilinopyrimidines family

Doses of the active ingredient in ppm		Mepanipyrim					Pyrimethanil					Cyprodinil+Fludioxonil				
		100	133	200	300	400	200	266	400	600	800	93,7	125	187.5	281	375
%IMG	PD	61.7b	64.0b	66.2b	70.3c	71.6a	84.8a	88.4a	90.0a	91.5a	92.5a	92.0ab	93.8b	95 b	97.5a	99.0ab
	PF	28.7d	36.6c	38.5d	44.2d	47.7c	80.6a	84.3a	91.0a	91.0a	93.2a	94.0a	95.1a	96.8a	97.9a	99.6a
	BL	18.0e	20.7d	23.2e	26.4e	29.5d	14.0d	25.0c	44.0c	44.0c	65.6c	52.4c	59.2d	64.0d	66.6d	68.3d
	BCV	73.8a	78.1a	81.8a	86.1a	87.0a	23.0c	27.2c	30.0c	41.0cd	44.0d	90.5b	91.5c	93.0c	94.2c	96.6c
	BC	38.3c	39.3c	43.7c	44.6d	49.6c	8.60b	26.0c	34.0c	37.0e	39.6e	92.1ab	93.8b	94.8c	95.7b	98.0bc
	SC	79.0a	79.6a	81.0a	83.7b	85.5a	23.4c	27.0c	30.5e	38.0de	43.7d	92.2ab	94.7a	96.3a	97.0a	98.0ab
	Cg	5.20f	9.40e	17.3f	19.8f	24.3e	49.0b	60.3b	81.3b	81.0b	84.2b	34.5d	40.4e	44.2e	50.7e	59.0e
%ICP	PD	34.0ab	60.7a	75.6a	92.5a	98.8a	30.9b	50.0bc	76.9b	89.7a	95.6a	100a	100a	100a	100a	100a
	PF	33.0ab	47.8b	67.1b	84.0b	91b	23.4b	44bcd	55.2e	65.7e	82.4d	100a	100a	100a	100a	100a
	BL	28.0ab	44.0bc	56.4c	68.8c	78.3c	25.1b	40.0d	64.1d	72cd	84.0cd	30,7b	52 ab	76.7b	86.4b	93.5b
	BCV	37.0a	55.0ab	61bc	69.0c	85.2c	50.0a	65.7a	81.9a	86.8a	90.4b	97,2a	98,7a	100a	100a	100a
	BC	35.0ab	51.0ab	63.8b	81.2b	93.4b	56.8a	63.0a	70.6c	81.1b	88.0bc	100a	100a	100a	100a	100a
	SC	23.2b	34.0c	41.5d	46.7d	59.1e	26.4b	42.2cd	54.2e	66 de	78.8d	100a	100a	100a	100a	100a
	Cg	28.4ab	46.5b	66.2b	81.5b	89.9bc	25.1b	52.6b	67cd	76bc	81.3d	37.6b	49.6b	59.6c	73.6c	87.8c
%ICG	PD	100a	100a	100a	100a	100a	84.4a	92.3a	96.7a	100a	100a	100a	100a	100a	100a	100a
	PF	100a	100a	100a	100a	100a	90.8a	96.4a	100a	100a	100a	100a	100a	100a	100a	100a
	BL	100a	100a	100a	100a	100a	84.8a	87.3b	89.4b	96.1b	100a	100a	100a	100a	100a	100a
	BCV	100a	100a	100a	100a	100a	48.9cd	51.2e	60.4e	70.0e	83.6c	100a	100a	100a	100a	100a
	BC	100a	100a	100a	100a	100a	52.4c	63.5d	73.1d	83.8d	93.7b	100a	100a	100a	100a	100a
	SC	100a	100a	100a	100a	100a	60.0b	73.9c	80.8c	90.1c	94.8b	100a	100a	100a	100a	100a
	Cg	100a	100a	100a	100a	100a	43.0d	51.2e	57.5e	59.2f	69.8d	100a	100a	100a	100a	100a

Botrytis cinerea isolates: BCV, BC, SC; *Colletotrichum gloeosporioides* isolate: Cg; *Pestalotia longisetula* isolates: PD and PF; *Bartalinia laurina* isolate: BL.
Two results for the same fungicide and the same concentration affected by the same letter are not significantly different at the 5% level (PPDS Test).

Table 3 Comparison of the inhibition percentage means of the three life cycles (mycelial growth [MG], conidia production [CP] and germination [CG]) of fungal species isolated from strawberry plants in presence of different fungicides of Dithiocarbamates family.

Doses of the active ingredient in ppm		Thiram					Mancozeb				
		500	666	1000	1500	2000	400	533	800	1200	1600
%IMG	PD	90.8a	92.1b	93.8b	100a	100a	84.0a	87.9b	89.4b	89.8b	92.0ab
	PF	91.9a	91.9b	92.4c	100a	100a	87.0a	91.0a	91.6a	92.3a	93.0a
	BL	86.9b	88.3c	90.8d	94.6b	100a	76.0b	78.0c	83.0c	84.3b	90.0b
	BCV	91.2a	93.9a	96.6a	100a	100a	22.0e	24.5g	27.2g	37.2e	52.0d
	BC	87.6b	89,3c	91.2c	93.7b	100a	53.0c	57.7d	64.0d	71.0c	82.0c
	SC	89.8a	91.2b	93.1b	100a	100a	30.0d	34.6e	38.8e	43.6d	54.0d
	Cg	79.0c	79.0d	83.0d	84.7c	87.0b	21.0e	29.0f	35.0f	37.0e	39.0e
%ICP	PD	100a	100a	100a	100a	100a	44.0ab	62b	72.6b	83.0b	97.0a
	PF	100a	100a	100a	100a	100a	49.0a	69.4a	84.2a	93.0a	98.0a
	BL	100a	100a	100a	100a	100a	35.0bc	44d	63.3b	67.0c	88.0b
	BCV	98.3a	99.0a	100a	100a	100a	28.5c	40.7d	45.6c	53,8c	54.0d
	BC	100a	100a	100a	100a	100a	17.4d	21.0e	26.0d	31,4d	43.0d
	SC	100a	100a	100a	100a	100a	39abc	45.5d	51.0c	57.0c	66.0c
	Cg	96.0b	98.1a	100a	100a	100a	16.8d	25.3e	31.3d	34.0d	46.0d
%ICG	PD	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	PF	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	BL	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	BCV	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	BC	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	SC	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
	Cg	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

Botrytis cinerea isolates: BCV, BC, SC; *Colletotrichum gloeosporioides* isolate: Cg; *Pestalotia longisetula* isolates: PD and PF; *Bartalinia laurina* isolate: BL.
Two results for the same fungicide and the same concentration affected by the same letter are not significantly different at the 5% level (PPDS Test).

On the other hand, thiram has acts effectively against seven tested isolates by presenting a strong inhibition on the mycelial growth of which the percentages oscillate between 79 % and 91.9 % and a total inhibition of the conidia production and

The conidia production turns out less inhibited than the mycelial growth with percentages of less than 50 % were registered at 400 ppm. However, conidia of the four species could not germinate in the presences of the presence of the

different concentrations tested (Table 3). Pyraclostrobin + boscalid combination was marked in this case by a reduced inhibitive action of the mycelial growth and the conidia production of BCV and BC registering an inhibition of less than 50% and 55-87% of conidia production not comparable to the strong inhibition obtained for SC, 69% of growth and 100% of conidia production with elevated concentration of 375 ppm.

stages development particularly regarding isolates of *B. cinerea* and *C. gloeosporioides* (Table 4). At 750 ppm of fenhexamid, a total inhibition of the mycelial growth distinguished the isolates of *B. cinerea* with a sensibility raised to the low doses which exceed that of the PD and PF but exceeding widely that of *C. gloeosporioides* and BL for who the relative percentages of inhibition vary respectively from 15.7 to 38.7% and 24 to 37%.

Table 4 Comparison of the inhibition percentage means of the three life cycles (mycelial growth [MG], conidia production [CP] and germination [CG]) of fungal species isolated from strawberry plants in presence of different fungicides of only Strobulirines family or combined with Carboxamides

Doses of the active ingredient in ppm		Pyraclostrobin+Boscalid					Azoxystrobin				
		93.7	125	187	281	375	500	660.6	1000	1500	2000
%IMG	PD	77.0c	82.0c	85.0c	79.7c	81.6b	17.7c	22.4c	25.0c	29.9d	33.0c
	PF	65.0d	69.0d	70.8d	73.0d	75.5c	18.5c	31.5b	49.0b	56.0b	59.4b
	BL	95.0a	96.7a	100a	100a	100a	68.6a	73.0a	79.0a	81.0a	82.6a
	BCV	36,3f	37.6f	40.7f	42.4f	45.0e	0d	0d	0e	0f	18.0e
	BC	17.2g	20.0g	24.6g	29.4g	33.0f	0d	0d	0e	0f	0f
	SC	53.0e	55.0e	61.0e	65.6e	69.0d	0d	0d	12.9d	14.4e	24.8d
	Cg	91.0b	93.0b	95.6b	98.0b	100a	31.0b	35.6b	45.5b	53c	60.5b
%ICP	PD	24.0d	42.0d	54.0c	63.0d	71.0d	29.7b	34.2c	39.3c	49.7b	53.0c
	PF	30.0c	45.0d	58.0c	67.5c	81.0c	30.2b	40.0b	45.7b	50.6b	59.0b
	BL	98.0a	98.0a	98.0a	100a	100a	70.1a	78.5a	84.5a	89.7a	92.0a
	BCV	26.6c	37.6e	44.0d	51.0e	55.8e	0d	0e	0e	0d	0e
	BC	43.0b	56.0c	71.6b	82.0b	87.0b	0d	0e	0e	0d	0e
	SC	96.0a	97.0b	98.0a	100a	100a	0d	0e	0e	0d	0e
	Cg	96.0a	96.0ab	97.0a	100a	100a	22.0c	25.2d	28.0d	31.9c	38.0d
%ICG	PD	100a	100a	100a	100a	100a	32.5b	35.3b	42.2b	44.2b	52.9b
	PF	100a	100a	100a	100a	100a	28.0b	30.0b	34.7c	40.7c	45.7b
	BL	100a	100a	100a	100a	100a	42.6a	46.3a	54.0a	59.6a	63.2a
	BCV	100a	100a	100a	100a	100a	9.50c	11.3c	15.6d	20.7d	23.3c
	BC	100a	100a	100a	100a	100a	6.80d	9.20c	12.5d	14.6d	18.4d
	SC	100a	100a	100a	100a	100a	10.4c	12.7c	16.2d	22.6d	25.0c
	Cg	100a	100a	100a	100a	100a	28.1b	35.3b	40.7b	45.2b	50.5b

Botrytis cinerea isolates : BCV, BC, SC ; *Colletotrichum gloeosporioides* isolate : Cg ; *Pestalotia longisetula* isolates : PD and PF ; *Bartalinia laurina* isolate BL.
Two results for the same fungicide and the same concentration affected by the same letter are not significantly different at the 5% level (PPDS Test).

Table 5 Comparison of the inhibition percentage means of the three life cycles (mycelial growth [MG], conidia production [CP] and germination [CG]) of fungal species isolated from strawberry plants in presence of different fungicides of Hydroxyanilides, Chloronitriles and Dicarboximides family

Doses of the active ingredient in ppm		Fenhexamid					Chlorothalonil					Procymidone				
		187	250	375	562	750	375	500	750	1125	1500	125	166	250	375	500
%IMG	PD	61.7d	67.5e	68.7d	70.6c	75.5c	70.1b	81.3a	83.8a	86.1a	87.0b	77.8a	81.6a	83.2a	84.0a	85.0a
	PF	66.5c	69.0d	70.5d	73.5c	78.5c	74.6a	78.5b	81.5b	84.8a	87.0b	69.0bc	72.0cd	74.0bc	76.0cd	80.0b
	BL	15.7f	24.3g	27.7f	32.8d	38.7d	40.6e	43.0 e	47.0e	49.4d	55.0e	68.0bc	70.0d	73.0c	76bcd	81.0b
	BCV	87.6b	89.2b	90.4b	94.3b	100a	34.0f	36.4f	42.4f	44.8e	52.0e	72.3b	74.8b	76.4b	78.0bc	81.0b
	BC	85.3b	86.9c	88.9c	94.1b	100a	75.6a	80.0 ab	83.6ab	84.7a	92.0a	72.0b	73.0bc	75.0bc	78.0bc	79.0b
	SC	90.4a	91.7a	95.4a	98.0a	100a	61.0c	64 c	65.8b	68.7c		65.0c	67.0e	68.0d	74.6d	77.0c
	Cg	24.0e	28.5f	30.4e	33.7d	37.0d	50.0 d	54.6d	58d	60.0c	62.0d	35.0d	42.0f	46.0e	52.0e	57.0d
%ICP	PD	39b	53.6b	60.2bc	71.5b	81.3b	37.2a	44.9a	59.4a	67.6a	74.0b	41abc	71.0a	78.0a	86.0ab	91.0b
	PF	41b	56.5b	64.8b	71.3b	81.5b	12.6b	33.6b	42.8b	62.4a	84.0a	47.0ab	57ab	69.0b	80.0b	93.0b
	BL	20.8c	29.2d	43.2d	44.0d	69.7c	30.8a	52.4a	63.4a	71.5a	83.1a	48.0a	64.7a	73.0ab	89.2a	98.0a
	BCV	97.7a	0.98a	100a	100a	100a	11.0b	27.8b	39.6b	49.3b	68.0b	9.0d	19.4d	34.8e	46.0e	57.0e
	BC	100a	100a	100a	100a	100a	28.1a	28.2b	30.5cd	50.0b	52.0cd	31.0c	39.0c	51.3d	59.7d	68.0d
	SC	100a	100a	100a	100a	100a	27.4a	32.5b	37.1bc	48.0b	58.3c	35.0bc	45bc	53.0cd	63.0cd	72.0d
	Cg	32.6bc	47.2c	54.8c	60.4c	64.3d	6.7b	17.5c	28.5d	37.7c	49.0d	34.7c	45bc	59.7c	70.0c	83.0c
%ICG	PD	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	54.0a	58.0b	69.0a	74.0b	82.0b
	PF	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	42.2b	51.7c	55.9b	70.0b	81.0b
	BL	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	55.3a	65.7a	73.4a	82.3a	100a
	BCV	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	37.0bc	41.0d	49.0bc	53.0c	60.0c
	BC	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	29.4d	38.5d	44.0c	49.0c	51.0d
	SC	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	33.0cd	38.4d	45.3c	53.4c	60.0c
	Cg	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	24.5d	29.0e	33.0d	39.0d	43.0e

Botrytis cinerea isolates: BCV, BC, SC; *Colletotrichum gloeosporioides* isolate: Cg; *Pestalotia longisetula* isolates: PD and PF; *Bartalinia laurina* isolate: BL.
Two results for the same fungicide and the same concentration affected by the same letter are not significantly different at the 5% level (PPDS Test).

Its action with low concentration was very considerable towards the mycelial growth and the sporulation of Cg and BL having very high percentages (100%) with regard to those of PD et PF varying between 71 and 81%. With the exception of BL, the efficiency of the azoxystrobin was reduced on three

With regard to the production of the various isolates conidia, the percentages of inhibition showed a notable increase (81-100%) particularly of those of BL and *C. gloeosporioides* (69.7-64.3%) at strong dose (Table 5).

The reduction of both development stages reached during theapplication of Chlorothalonil, was apparently variable, on the mycelial growth, the respective percentages of 75.6 and 56.9 % were obtained in small doses by BC and SC against only 34 % by BCV and 40.6 % by BL. Chlorothalonil has proved effective on Cg with a percentage of the order of 50% clearly lower than that of the PD (70.1%) or of PF (74.6%). On the other hand, the production of conidia was weakly influenced by 375 ppm of this fungicide; the highest inhibition was of the order of 37.2% and the lowest about 6.7% (Table 5). The inhibitions given by procymidone show a high sensibility of the various isolates. The inhibition of more than 50 % of the mycelial growth is obtained at 125 ppm with the exception of *C. gloeosporioides* who required a higher dose adjusted into 375 ppm. The efficiency of this active ingredient on conidia production was less important, clearly lower percentages were obtained at 125 ppm. The inhibition of this stadium of life becomes more stressed in the presence of the high doses. Fenhexamid and chlorothalonil inhibited totally conidia germination species from the lowest tested dose. The obtained inhibitions of germination in the presence of 500 ppm of procymidone were able to reach percentages going from 51 to 60 % of *B. cinerea* isolates, 43 % of Cg and 54 %, 81-82% of isolates of *P. longisetula* and 100% of BL (Table 5).

Table 6 Inhibitory concentrations (EC_{50}) of the fungicides tested inhibiting the three stages of the life cycle of four strawberry pathogens at 50%.

	PD	PF	BL	BCV	BC	SC	Cg
Mycelial growth							
Pyrimethanil	3.8	24.5	588.8	703	955	1321.3	139
Azoxystrobin	>2000	1294	82,8	>2000	-	>2000	1452
Procymidone	59.4	14.4	12.9	1.5	6.1	32.7	318
Mancozeb	7.1	1.1	66.8	1849	384	1462	2924
Mepanipyrim	23.6	469.8	3412	15.8	502	1.8	1043
Cyprodinil+Fludioxonil	12.4	9.3	65	2	2.9	6.4	245
Fenhexamid	47.2	36	3810	34.4	575	47.3	3311
Chlorothalonil	64.5	45.7	1057	1448	83.2	148.6	543
Pyraclostrobin+Boscalid	8.1	26.8	9.6	1039	1671	102.5	26.8
Thiram	104	149.6	128.5	97.3	66.4	128	17.7
Conidia production							
Pyrimethanil	244.3	363	338	168.6	182.8	365.6	17.5
Azoxystrobin	1667	1312	203.7	-	-	-	>2000
Procymidone	125.6	142.8	139	400	251	216	88
Mancozeb	471	408.3	66	1099	2558	727	2055
Mepanipyrim	124.7	140	172	138	139	149.6	297
Cyprodinil+Fludioxonil	<93.7	<93.7	124.7	4	<93.7	<93.7	4
Fenhexamid	254.7	231	501	2	3.1	3.5	347
Chlorothalonil	196.8	268.5	187.5	338	482	471	502
Pyraclostrobin+Boscalid	246	207.5	1.7	359	144.5	1.1	2.5
Thiram				<500			
Conidia germination							
Pyrimethanil	83	38	87	242	198	150	280
Azoxystrobin	1849	>2000	811	>2000	>2000	>2000	1931
Procymidone	106	170	133	278	398	313	781
Mancozeb				<400			
Mepanipyrim				<100			
Cyprodinil+Fludioxonil				<93,7			
Fenhexamid				<187			
Chlorothalonil				<375			
Pyraclostrobin+Boscalid				<93,7			
Thiram				<500			

Botrytis cinerea isolates: BCV, BC, SC; *Colletotrichum gloeosporioides* isolate: Cg; *Pestalotia longisetula* isolates: PD and PF; *Bartalinia laurina* isolate BL.

The observed EC_{50} remain widely lower than the lowest tested doses at the stage of growth, conidia production or germination for the most part of the studied active ingredients. Nevertheless, greater cases of resistance observed in the presence of the mancozeb, the concentrations inhibiting 50% of growth of BCV and Cg and conidia production of BC and Cg where superior to recommended dose (Table 6). The levels of protection provided by the fungicides sprayed on the non-inoculated fruits were significantly greater; they reached 100% upon application of the recommended dose except for mepanipyrim which has managed to inhibit 84% of the rot (Figure 1A). However, mepanipyrim and thiram failed to control infections occurring during the second period of incubation (Figure 1B).
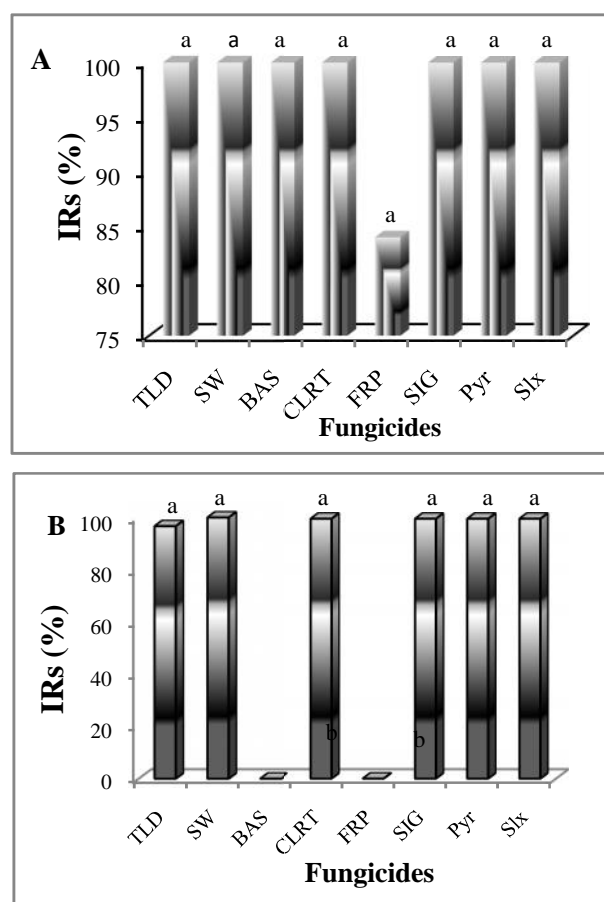

Figure 1 Efficacy of the fungicides tested on the development of strawberries rot at green stage uninoculated and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

By comparing the effect of fungicides treatment after the third day of inoculation by *B. cinerea*, only cyprodinil + fludioxonil and fenhexamid protected green strawberries at 100%. Pyrimethanil appeared moderately effective in inhibiting 70% of rot caused by *B. cinerea* and 66.7% by the procymidone (Figure 2A). After seven days of inoculation, green strawberries inoculated with *B. cinerea* showed more apparent decay following the two treatments containing different

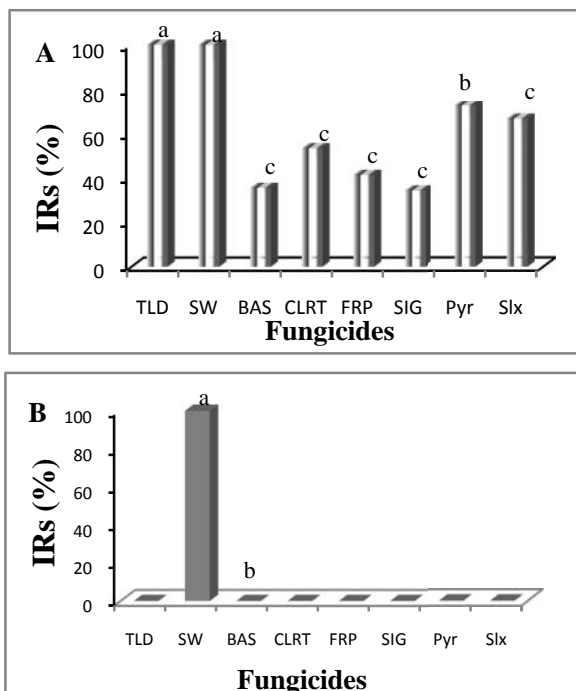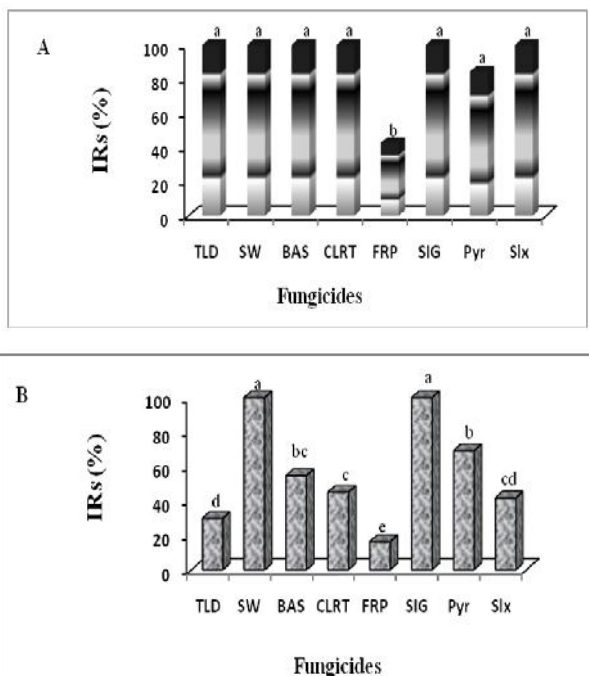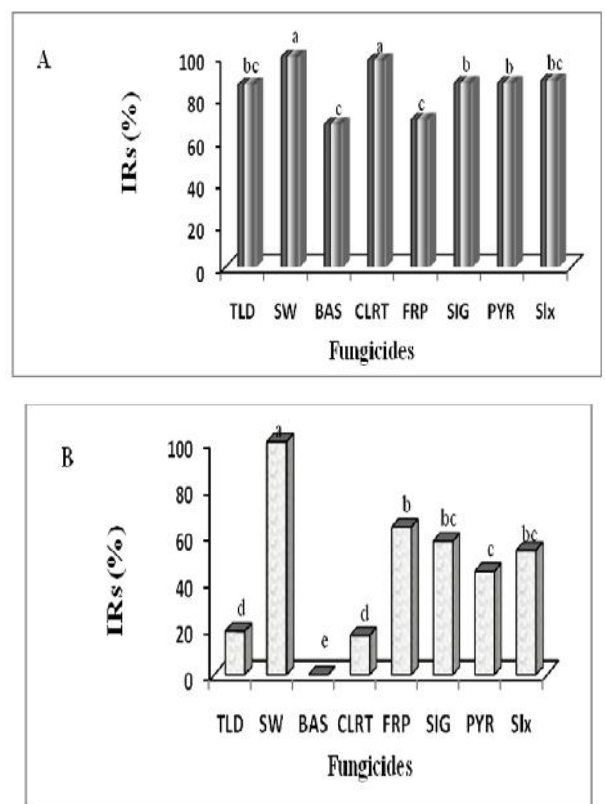fungicides except cyprodinil + fludioxonil that inhibited rot at 100% (Figure 2B).

Figure 2 Efficacy of the fungicides tested on the development of strawberries rot at green stage inoculated with *B. cinerea* and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram ; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil ; **Slx:** Procymidone

Figure 3 Efficacy of the fungicides tested on the development of strawberries rot at green stage inoculated with *C. gloeosporioides* and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

The anthracnose developed on the green fruit with various degrees was more inhibited in the presence of the fungicides tested except mepanipyrim which has inhibited 42.6% of the rot caused by *C. gloeosporioides* (Figure 3A). The applied second treatment has allowed only limited protection by fenhexamid and mepanipyrim to moderate reaching around the inhibition percentages of 69, 54.66, 45 and 41.66% respectively for pyrimethanil, thiram, Chlorothalonil and procymidone. Pyraclostrobin+ boscalid and cyprodinil + fludioxonil have kept their maximum efficiency (Figure 3B).

Without artificial inoculation, red strawberries were strongly protected by tested fungicides. The Chlorothalonil and cyprodinil + fludioxonil showed a very important efficiency reached 98.3% (Figure 4A). During the second period of incubation, the ability of fungicides to control infections may arise declined considerably in the presence of thiram, Chlorothalonil and fenhexamid (Figure 4B).

Figure 4 Efficacy of the fungicides tested on the development of strawberries rot at red stage uninoculated and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

Fungicides conferred a partial protection on red strawberries until the third day after the artificial inoculation by *B. cinerea* or *C. gloeosporioides*. Only cyprodinil + fludioxonil protected of 100% strawberries against gray mold followed by Pyrimethanil, fenhexamid and mepanipyrim with the almost identical percentages (Figure 5A). In spite of a second treatment, the pre-treated strawberries developed an extended gray rot gaining the whole of strawberries sprayed with fungicides solutions except for cyprodinil + fludioxonil (Figure 5B).
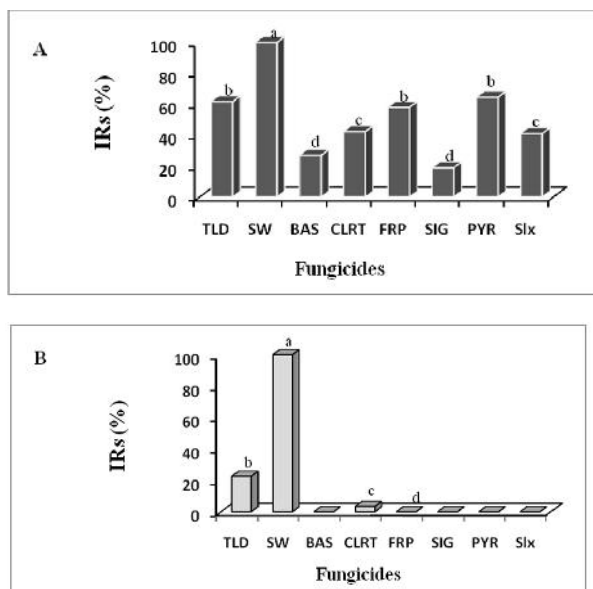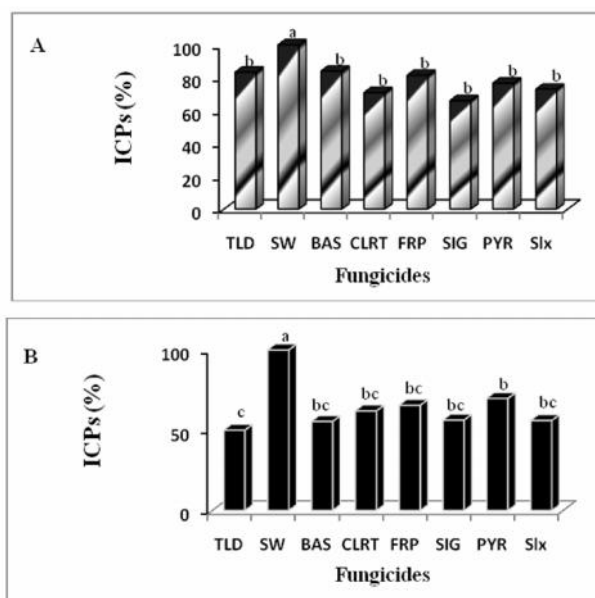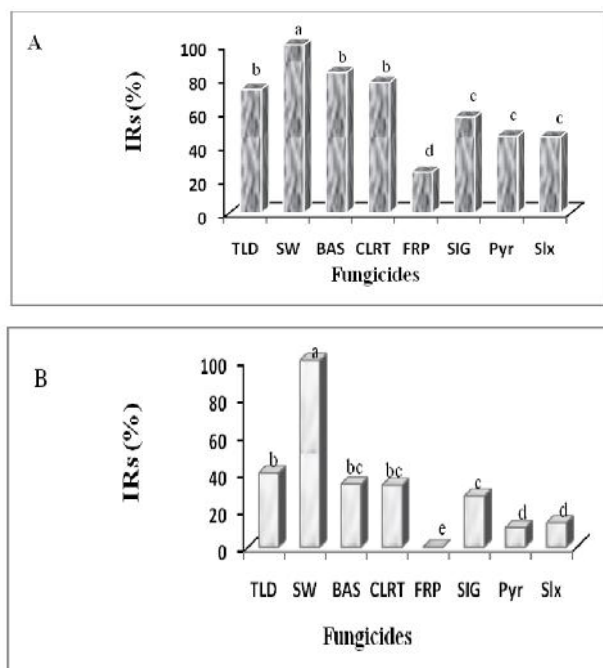
Figure 5 Efficacy of the fungicides tested on the development of strawberries rot at red stage inoculated with *B. cinerea* and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone.

Figure 6 Efficacy of the fungicides tested on the development of strawberries rot at red stage inoculated with *C. gloeosporioides* and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone.

Figure 7 Efficacy of the fungicides tested on inhibition of *B. cinerea* conidia production on strawberries at green stage uninoculated and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

Figure 8 Efficacy of the fungicides tested on inhibition of conidia production of *B. cinerea* inoculated on green strawberries and incubated for 3 days (A) and 7 days (B).

Values followed by the same letter do not differ significantly at 5%.

TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

Three days after inoculation, thiram, chlorothalonil and fenhexamid have managed to bloke the development of rot caused by *C. gloeosporioides* on red strawberries by over 83, 77 and 73% against 100% by cyprodinil + fludioxonil (Figure 6A).

After 7 days of incubation, only cyprodinil + fludioxonil had continued to protect red strawberries (Figure 6 B). However, a weak protective activity was observed by means of fenhexamid, thiram, Chlorothalonil and pyraclostrobin + boscalid ((Figure 6B).

7A) and not more than 69.8% after 7 days of incubation (Figure 7B).
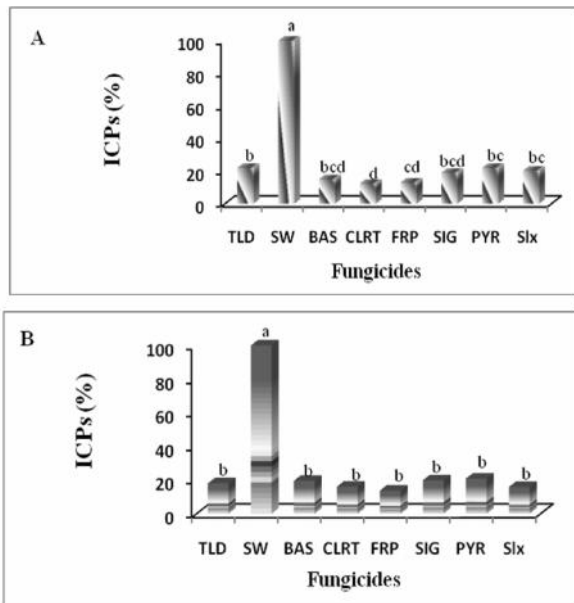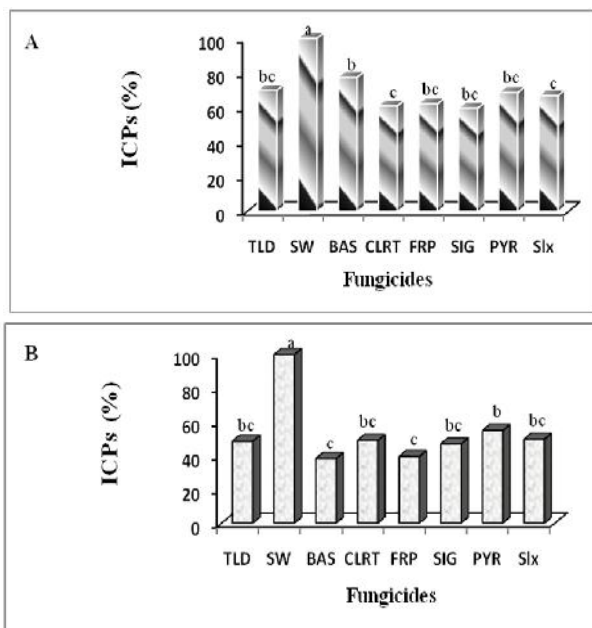

Figure 9 Efficacy of the fungicides tested on inhibition of conidia production of *C. gloeosporioides* inoculated on strawberries at green stage and incubated for 3 days (A) and 7 days (B).
Values followed by the same letter do not differ significantly at 5%.
TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

Figure 10 Efficacy of the fungicides tested on inhibition of *B. cinerea* conidia production on strawberries at red strawberries uninoculated and incubated for 3 days (A) and 7 days (B).
Values followed by the same letter do not differ significantly at 5%.
TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone

Cyprodinil + fludioxonil combination has completely inhibited conidial ''fruiting- bodies'' on non-inoculated green and red strawberries and those inoculated with *B. cinerea* and *C. gloeosporioides* as the action of other fungicides applied is reduced (Figures 7 to 12). *B. cinerea* conidial production on uninoculated green strawberries was inhibited by the fungicides tested with percentages ranging from 66.6 to 84.1% (Figure
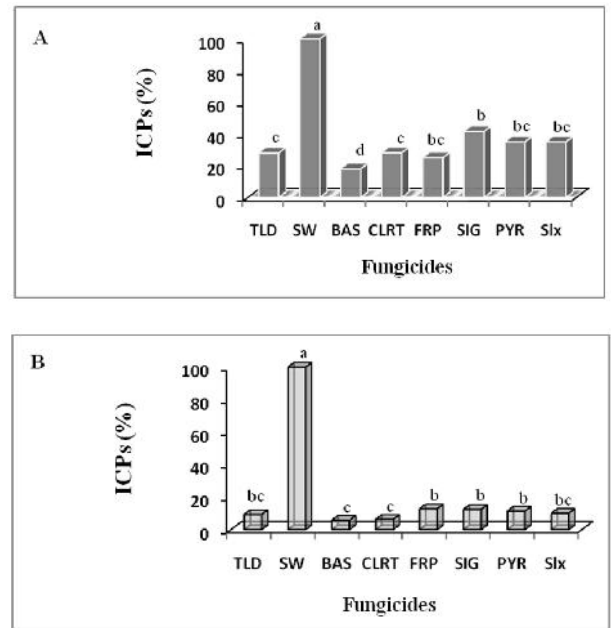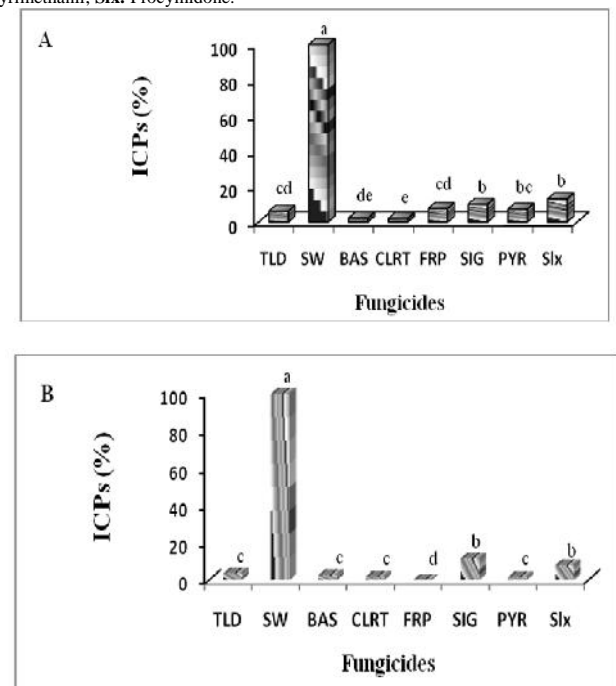

Figure 11 Efficacy of the fungicides tested on inhibition of conidia production of *B. cinerea* inoculated on red strawberries and incubated for 3 days (A) and 7 days (B).
Values followed by the same letter do not differ significantly at 5%.
TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone.

Figure 12 Efficacy of the fungicides tested on inhibition of conidia production of *C. gloeosporioides* inoculated on red strawberries and incubated for 3 days (A) and 7 days (B).
Values followed by the same letter do not differ significantly at 5%.
TLD: Fenhexamid; **SW:** Cyprodinil+fludioxonil; **BAS:** Thiram; **CLRT:** Chlorothalonil; **FRP:** Mepanipyrim; **SIG:** Pyraclostrobin+boscalid; **Pyr:** Pyrimethanil; **Slx:** Procymidone.

Reductions of *B. cinerea* conidia production on inoculated green strawberries were less important and ranged from 35.3 to 69.8% (Figure 8A). The second applied treatment limited only

very slightly fructification (Figure 8B). The inhibition percentages of the conidia production of *C. gloeosporioides* vary from 36 to 56% (Figure 9A) and less than or equal to 22% after various treatments of the second sprays (Figure 9B).

An incomplete inhibition of conidia production was observed on uninoculated red strawberries subjected to the first treatment. Thus, higher percentages of inhibition reaching 77.5 and 71% were obtained by applying the recommended doses of thiram and fenhexamid, and varying from 59.7 to 68.7% for the other fungicides (Figure 10A) but were attenuated after the second treatment (Figure 10B).

The production of *B. cinerea* and *C. gloeosporioides* conidia was abundant on the surface of red strawberries after the first and the second sprays of different fungicides. A non-significant difference in the inhibitory effect of *B. cinerea* conidia production was observed in pyraclostrobin+ boscalid, pyrimethanil, procymidone and mepanipyrim, not exceeding 41.5% after the first treatment (Figure 11A) and decreases to 12.7% during the second application (Figure 11B). Faced to *C. gloeosporioides* conidia production, both treatments had a low to null efficiency (Figure 12A and B).

DISCUSSION AND CONCLUSION

The effectiveness of various fungicides approved on strawberry crop in Morocco was evaluated for the first time against *B. cinerea* and *C. gloeosporioides*, the most dreaded fungal agents of this culture, the new colonizers fruit and vegetative parts of the plant named *Pestalotia longisetula* and *Bartalinia laurina*.
The analysis of the results allowed distinguishing thiram, cyprodinil + fludioxonil and fenhexamid as being the most effective active ingredients on the three stages of the life cycle. The Switch formulation combines two active ingredients, Cyprodinil and Fludioxonil belonging respectively to the chemical families of Annilinopyrimidines and Phenylpyrroles. According to Mercier *et al*. (2010), a frequency of 29% of *B. cinerea* isolates showing resistance to 50 ppm of cyprodinil + fludioxonil. Forster *et al*. (2007) reported that the high efficacy of fludioxonil against *Monilinia fructicola* (G. Wint.) and *B. cinerea* was substantiated by low effective concentrations necessary (0.063 mg/liter) for 50% inhibition of mycelia growth *in vitro* which is well below those of different isolates tested including that of *C. gleoesporioides* weakly inhibited at 93.7 ppm. Works undertaken by Lee (2005) showed the inefficiency of fludioxonil towards *C. gleoesporioides*. On the contrary, Sjulin (2008) affirms that the association of cyprodinil to fludioxonil is active on *B. cinerea* or *Colletotrichum* spp.

The activity of Basultra containing 80% of thiram was appreciated by Gullino *et al*. (1985) against *C. gloeosporioides* of which the inhibition of 50% of its mycelial growth required only 2 ppm towards 17.7 ppm reaches by the tested Cg isolate. The resulting high action of inhibition of thiram on spore germination and sporulation of various isolates approves that given by Hmouni *et al*. (2003) and Kenny *et al*. (2012). Compared to thiram, Mancozeb was less active on the three stages of life of the isolates of the two species.

Relating to Kumar *et al*. (2012) works, various inhibition rates had identified *C. gloeosporioides* isolates highly resistant, resistant and highly sensitive to mancozeb with inhibition rate greater than 90%.

The other tested commercial formulation was Signum combining pyraclostrobin and boscalid. Reduced susceptibility occurred in two isolates of *B. cinerea* opposing the very appreciable fungicidal activity found in other isolates. However, this resistance shown by an EC_{50} value greater than 1000 ppm is consistent with suspected resistance reported by other authors (Smilanck *et al*., 2010; Kim and Xiao, 2010). Indeed, other pathogens have developed resistance to boscalid such as *Didymella bryoniae* (Keinath, 2012), *Corynespora cassiicola* isolated from cucumber (Miyamoto *et al*., 2009) and *Alternaria solani* (Fairchild, 2013).

Indeed, the pyraclostrobin is part of fungicides group called quinone outside inhibitors (QoIs). The fungicidal activity of this group relies on their ability to inhibit mitochondrial respiration. This inhibition blocks the transfer of electrons between the cyrochrome b and cytochrome c1 and stopping the synthesis of ATP. The active ingredient boscalid inhibits succinate dehydrogenase of the mitochondrial respiratory chain. According to Fernández-Ortuño *et al*. (2008), the primary molecular mechanism conferring resistance to QoIs (Pyraclostrobin) is target site based, involving mutations in the mitochondrial cytochrome b gene and resulting in peptide sequence changes as the substitution of glycine with alanine at position 143.

The low sensitivity to Teldor compared to that of Switch joined the results obtained by (Köycü *et al*. 2012). The fenhexamid as an active ingredient of this product is a botryticide reducing ergosterol synthesis by inhibiting 3-ketoreductase (Debieu *et al*., 2001). It has effectively limited the mycelial growth, sporulation and germination of *B. cinerea* isolates. However, repeated cases of resistance have been reported (Baroffio *et al*., 2003; Esterio *et al*., 2011; Kretschmer and Hahn, 2008; Topolovec-Pintaric, 2008).

Chlorothalonil performance against the tested isolates was generally reduced. The mycelial inhibition of *C. gloeosporioides* obtained at 500 ppm is still low compared to that reported by Ferreira *et al*. (2009). Different degrees of sensitivity were noted in 34 isolates of *C. gloeosporioides* isolated from *Euonymus fortunei* (Lamondia, 2001) and in *Sclerotinia homoeocarpa* (Burpee, 1997). Its effect on *B. cinerea* remains controversial after Trolinger and Strider (1984) and Wearing *et al*. (1995), but its effect on germination is similar (R'Houma *et al*., 1998).

The deficiency of Frupica to completely stop the mycelial growth of *B. cinerea* is consistent with the results of the work of Miura *et al*. (1994a) and those rated on *Fomitiporia mediterranea* and *Phaeomoniella chlamydospora* by Platzer and Schweigkofler (2009). Unlike the effect of this fungicide and Switch, the pyrimethanil is less effective although further work approve his performance in *B. cinerea* (Leroux and Credet 1995; Mouden *et al*., 2010). Apparently, the

pyrimethanil was faced with the problem of resistance in all isolates except those of *P. longisetula*. Its inefficiency towards *C. gloeosporioides* joined results tests of Everett *et al*. (2005). This fungicide group acts on a specific site of the target fungus with the risk of resistance development produced by mutation of a gene (Beresford *et al*., 1999).

Against *B. cinerea* isolates, the procymidone has good efficiency also reported by Hmouni *et al*. (2003), while many studies have raised significant reductions in sensitivity and high frequencies of resistant isolates in fields of several cultures (Pappas, 1997; Fourie and Holz, 1998; Myresiotis *et al*., 2007; Weber, 2011). This decrease was due to strategies for the reduction of the frequency of antifungal treatment effect by season and reduced survival capacity of resistant isolates in the absence of selection pressure (Moorman and Lease, 1992; Raposo *et al*., 2000). However, towards *C. gloeosporioides*, the iprodione and vinchlozolin of the Dicarboximides family have lower efficiency (Gullino *et al*., 1985).

As for azoxystrobin, its action was less importance. Other studies have supported the implementation of this fungicide which opposes the mycelial development of *A. alternata*, *A. brassicae* and *A. dauci* isolates (Survilinné and Dambrauskieni, 2006), as in *C. gloeoesporioides* (Sundravadana *et al*., 2006; 2007; Filoda, 2008), and against post avocado crop pathogens (Everett *et al*., 2005). However, the development of resistance to this fungicide class was reported (Avila-Adame *et al*., 2003; Vega and Dewdney, 2012; Asadollahi, 2013). Banno *et al*. (2009) were able to identify two types of mitochondrial cytochrome b genes conferring resistance to phytopathogenic fungi.

In vivo, it is important to note the susceptibility of red strawberries to more infection than immature fruit which agrees with the results of Wilson *et al*. (1990). Switch proved most effective. This product provides effective protection against *B. cinerea* and *Rhizopus stolonifer* (Sallato *et al*. 2007), the good control of strawberries from anthracnose at pre-harvest period (Ivanovic *et al*., 2007) and reduce the mortality of strawberry plants caused by *C. gloeosporioides* (Mackenzie *et al*., 2009). Fludioxonil is able to control the brown and gray mold (Förster *et al*., 2007), reduce the sclerotia formation rate of *Sclerotinia sclerotiurum* (Mueller *et al*., 1999) and a lettuce disease (Matheron and Porchas, 2004). According to Xiao and Boal (2009), the pyrimethanil as fludioxonil has some systemic activity. Knauf-Beiter *et al*. (1995) demonstrated the protective and curative activity attributed to cyprodinil.

Concerning Signum, its protective activity is prevalent on *C. gloeosporioides*. Indeed, the pyraclostrobin/boscalid combination ensured a significant reduction of the anthracnose incidence on sweet pepper fruits (Ivey *et al*., 2004). Pyraclostrobine is active on *C. acutatum* (Turechek *et al*. (2006), early blight of tomato (Ganeshan and Chethana, 2009) The failure of Signum to suppress the proliferation of *B. cinerea* and latent infections caused by the fungus is consistent with the results of Kim and Xiao (2010) who attributed first to the more frequent occurrence of isolates with a double resistance to both active ingredients and secondly to the low amount of boscalid residues.

The preventive action of Teldor already announced by Cavalieri (2000) is also appreciated by Smilanick *et al*. (2010), Legard *et al*. (2005), Köycü *et al*. (2012) and Tanovic *et al*. (2012). Combined with Captan, fenhexamid effectively limits as well the development of *Gnomonia comari* as anthracnose and strawberries gray mold (Wedge *et al*., 2007).

The chlorothalonil showed a less effectiveness than that of fenhexamid and cyprodinil+fludioxonil. However, its effect is satisfactory on *C. acutatum* (Daugovish *et al*., 2009) and on *Colletotrichum capsici* (Shukla *et al*., 2010).
In vivo, thiram is less effective. This disparity of activity would be due to the interactions between the pathogenic ones and the environmental conditions existing *in vivo*. On the other hand, its effect is appreciable in controlling the gray rot (Stall, 1964) and the invasion of *C. gloeosporioides* (MacKenzie *et al*., 2009). According to Thomas and Sweetingham (2003), this product presents no systemic activity.

Two divergent actions marked the class of Annilinopyrimidines represented by pyrimethanil and mepanipyrim. This last showed less effectiveness towards *C. gloeosporioides* like that observed between *B. cinerea* and *C. lagenarium* or of *Cochliobolus miyabeanus* (Miura and Maeno, 2007). These same authors attribute this difference *in vivo* activity to the inhibition of the pectinase secretion at pathogenic sensitive compared to those the least sensitive. According to Cremers (1994), this class of fungicide provides a better control of apple scab on leaves than on fruits (Kunz *et al*., 1998).

Compared to Mepanipyrim, Pyrimethanil is more active. According to Wedge *et al*. (2007), this fungicide reduces more the incidence of the gray rot of strawberries than the diseases resulting from *Colletorichum* spp. and *Gnomonia comari*. Work of Li and Xiao (2008), reported its ability to also control blue mold of apples.

The results demonstrate the highly significant inhibitory effect and persistent cyprodinil + fludioxonil on the development and dissemination of these two fungi. The preventive action relating to pyrimethanil, mepanipyrim, procymidone and pyraclostrobin+boscalid remains acceptable in front of the curative action which partially agrees with protective and curative properties asserted in the class of Annilinopyrimidines (Daniels *et al*., 1994; Knauf-Beiter *et al*., 1995). The latter are suspected by their interference with the methionine biosynthesis and inhibition of hydrolytic enzymes secretion (Masner *et al*., 1994; Miura *et al*., 1994b). According to Kline *et al*. (1957), in the event of reducing the rate of methionine, these fungicides could result in inhibition of under cuticular growth of pathogens and the undifferentiation of conidiophores.

Based on the presented results, the tested molecules showed varied potentialities towards the three stages of pathogenic life studied that their levels of sensitivities are high even with low dose. In front of *B. cinerea*, the thiram, Cyprodinil + fludioxonil, fenhexamid and mepanipyrim fungicides are most active although they reflect a variability of sensitivity dependent on the isolate. Pyraclostrobin + boscalid and Chlorothalonil were effective against *C. gloeosporioirdes*. As

for procymidone and pyrimethanil, they preserved more ability to repress the growth than the conidia production and the germination of *B. cinerea* isolates. However, mancozeb and azoxystrobin showed a moderate efficacy with regard to *C. gloeosporioides* which exceeded that of procymidone.

A maximum protection was delivered by the cyprodinil + fludioxonil formulation following preventive or curative treatment. For other fungicides, the possibilities of curative action were limited.

This study showed that cyprodinil + fludioxonil and fenhexamid with a weak risk of resistance besides their favorable toxicological and environmental profile have completed a significant pathogen control. Consequently, their use on pre-harvest chemical control of pathogenic fungi of strawberries would be beneficial.

The study of these fungicides effect on the development of the four fungi expands the range of pathogens to include in the spectrum of action of these active substances acting on a definite mode of action or interfere with another which is a possible advantage to reduce the risk of resistance development and the emergence of fungal diseases.

Acknowledgments

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