Twelfth EuroBlight workshop
Arras (France), 3-6 May 2010

In vitro evaluation of difenoconazole and chlorothalonil on conidial germination and mycelial growth of *Alternaria alternata* and *A. solani* causal agent of early blight in Algeria

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SUMMARY
Diseases are still the main cause of reduction of yield in potato crops in Algeria. After late blight which is the most destructive disease, early blight is also an important foliar disease, reported to be caused by *A. alternata* and *A. solani,* responsable of yield losses in our Algerian climatic conditions. This research was initiated to examine in laboratory conditions the efficacy of two fungicides used in Algeria. The results showed that Difenoconazole had a better effectiveness than Chlorothalonil in inhibition of mycelial growth and conidial germination of *A. solani and A. alternata*. *A.solani* showed also a higher sensitivity than *A. alternata* to the two tested fungicides.

KEY WORDS
Early blight, potato, difenoconazole, chlorothalonil, fungicides effectiveness.

INTRODUCTION

Potato (*Solanum tuberosum*) is traditionally one of the most cultivated crops in Algeria. Among biotic stresses, early blight is an important foliar disease reported to be caused by *Alternaria alternata* and *A. solani* responsable of yield losses under our climatic conditions. Control of these two pathogens can be accomplished through various means: use of resistant potato cultivars, appropriate farming techniques such as careful tillage, crop rotation, etc., as well as fungicide application that may directly affect the growth of fungi.

The present research was conducted to evaluate the efficacy of the two fungicides chlorothalonil and difenoconazole used in Algeria towards *Alternaria solani* and *A. alternata*.

In-vitro experiments were conducted on mycelial growth and conidial germination of the early blight causal agents, using two fungicides available on the Algerian market.

MATERIALS AND METHODS

Fungicides

The tests were performed *in vitro* to evaluate the effectiveness of two fungicides: difenoconazole (250 g i.a./l) and chlorothalonil (720 g i.a. /l), on conidial germination and mycelial growth of *A. solani* and *A. alternata*, and to compare them with the concentrations used in field. Concentrations of difenoconazole and chlorothalonil were then calculated from dose used in field treatments as shown in Table 1.

Fungal material, and estimation of mycelial growth and conidial germination

Isolates of *A. solani* and *A. alternata* (Fig.1a and 1b) were obtained from leaves of potatoes showing characteristic symptoms of early blight (Fig. 2a and 2b) collected in Algeria.
The tests were carried out in Petri dishes for mycelial growth and on slides for conidial germination

Fig. 1a. *Symptoms of A. alternata*

Fig. 1b. *Symptoms of A. solani*

Fig. 2a . *Conidia of A. alternata*

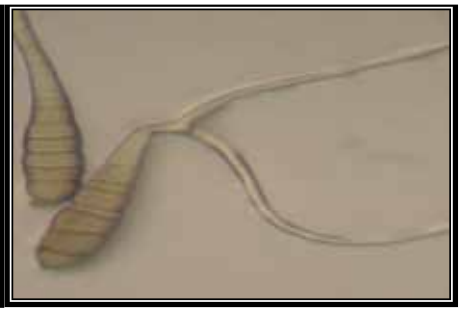

Fig. 2b. *Conidia of A. solani*

For mycelial growth, the tests were conducted on malt agar medium and diameter of the colonies was measured after 7 days of incubation at 18-22°C.
Tests were performed with twelve cultures (3 x 4 repetitions), per fungicide concentration and per isolate.

The inhibition of mycelial growth (CI50) was evaluated by:

$$Im\ \% = \frac{V0-V}{V0} .100$$

V0 mycelium growth on medium without fungicide and V growth on medium with fungicide.

For conidial germination, a drop of conidial suspension of each fungicide concentration was deposited on glass slide recovered by agar. Conidial germination was observed under microscope,

after 24 hours of incubation at 18-22 °C. One hundred conidia were observed per isolate and fungicide concentration.

The inhibition of conidial germination was evaluated by: $Ic\% = \frac{Q - Q0}{100 - Q0} .100$

Q0 was the number of germinated conidia on medium without fungicide and Q, the number of germinated conidia on medium with fungicide

Table 1. *Doses used for mycelial growth and conidial germination test*

active substance	Chemical group	Species of fungi	Doses used for mycelial growth test		Doses used for conidial germination test	
			ppm	µl i.a. /l	ppm	µl i.a. /l
difenoconazole	triazoles	*A. solani* *A. alternata*	250 125 62,5 31,25 15,62 7,81 3,90 1,95 0,97 0,48 0,24 0,122 0,061 0,030 0	1000 500 250 125 62,5 31,25 15,62 7,81 3,90 1,95 0,97 0,48 0,24 0,12 0	0 1.95 0.48 0.122 0.030	0 7,81 1,95 0,48 0,12
chlorothalonil	chloronitriles	*A. solani* *A. alternata*	2880 1440 720 360 180 90 45 22,5 0	4000 2000 1000 500 250 125 62.5 31,25 0	0 2880 720 180 45	0 4000 1000 250 62,5

RESULTS

Effectiveness of fungicides on development of mycelial growth

The results obtained (Fig. 4 a,b,c and d) showed that difénoconazole had a strong inhibition effect on *A. solani* (89%) with a concentration of 0,97 ppm (or 7.81 µl i.a. /l) , whereas it was only 56% with the same concentration (Fig.) for *A. alternata*. With this level of inhibition, the mycelial growth speed was only respectively 0,33 mm/j and 1,5 mm/j for *A. solani* and *A. alternata*, whereas the control mycelial growth speed was 3.25 mm/j for *A. solani* and 3.45 mm/j for *A. alternata*.

On the other hand, chlorothalonil (Fig. 3 a,b,c and d) showed a lower effectiveness with always a difference between the two pathogens. Thus, *A. solani* was inhibited at 89% with a concentration of

1440 ppm (or 2000µl i.a. /l), and *A. alternata* was inhibited at 57% for the same concentration. These inhibitions had an effect on the mycelial growth speed of both pathogens *A. solani* and *A. alternata*, which were respectively 0.33 mm/j and 1.46 mm/j. This difference showed that this product is more effective on *A. solani* than on *A. alternata*.
The curves of regression (y=ax +b) for each fungicide, obtained by transformation of the percentages of inhibition into probits (Finney, 1952), allowed to determine the CMI and the CI50 for the two products. It was noted that the CI50 of difenoconazole was weaker than that of chlorothalonil, with 0.446µl i.a. /l and 44.59 µl i.a. /l respectively for *A. solani* and 2.720 µl i.a. /l and 970.9 µl i.a. /l respectively for *A. alternata*.

Effectiveness of fungicides on conidial germination
Our results (Table. 2) clearly showed that the percentages of inhibition of conidial germination were strongly related with doses used. We noted that difenoconazole strongly reduced the conidial germination of *A. solani* and reached 92% at 1.95 ppm (7.81µl i.a. /l), whereas the conidial germination of *A. alternata* was only reduced to 65% for the same concentration

Table 2. *Effectiveness of fungicides on conidial germination: Percentage of inhibition and probits.*

Active substance	Species	Doses (**µl i.a/l**)	Log (10xC)	% Inhibition	Probits
Difenoconazole	*A.solani*	7,81	1,89	92	6,41
		1,95	1,29	71	5,55
		0,48	0,68	60	5,25
		0,12	0,08	38,4	4,69
	A.alternata	7,81	1,89	65	5,39
		1,95	1,29	41	4,77
		0,48	0,68	34	4,59
		0,12	0,08	20	4,16
Chlorothalonil	*A.solani*	4000	3,60	78	5,77
		1000	3 ,00	69	5,50
		250	2,40	31	4,50
		62,5	1,80	23	5,26
	A.alternata	4000	3,60	53	5,08
		1000	3 ,00	34	4,59
		250	2,40	26	4,36
		62,5	1,80	16	4,01

From results obtained with chlorothalonil, we noticed that the reduction of conidial germination was less important for *A. solani* than for *A. alternata*. It was respectively 78% at 720 ppm (1000 µl i.a./l) for *A. solani*, whereas it was 53% at the same concentration for *A. alternata* (table 2).
Inhibition was determined for the two products by the CI 50 which is the minimal concentration which inhibits 50% of conidial germination. Results are represented in Table 3.

Table 3. *CI 50 of difenoconazole and chlorothalonil for conidial germination of A. solani and A. alternata*

	Active substance	
pathogens	difenoconazole CI50 en µl i.a./l	chlorothalonil CI50 en µl i.a./l
Alternaria solani	0.30	490,68
Alternaria alternata	2.60	3600,41

DISCUSSION AND CONCLUSION

The results obtained showed that the two fungicides tested, difenoconazole and chlorothalonil, had an effect *in vitro* on mycelial growth and spore germination of both *Alternaria* species. Furthermore, *A. solani* was more sensitive than *A. alternata* in regard to the two products, whose IC50 allowed to rank the two fungicides as follows : difenoconazole>chlorothalonil. Then, our results showed that difenoconazole had a better effectiveness than chlorothalonil in inhibition of mycelial growth and conidial germination of *A. solani* and *A. alternata*.

In previous works, Tofoli *et al* (2003) also showed efficacy of chlorothalonil against *A. alternata* and Badoc (2005) obtained efficacy of azoxystrobin on germination and mycelial growth of *A. alternata*, the causal agent of fruit storage rots. In fields, more recent report (MacDonald *et al*., 2007) showed efficacy against *A. solani* of other active ingredients belonging to the same family of strobilurin (azoxystrobin, pyraclostrobin). *In vitro* results do not always reflect what happens in the field. This study should be complemented by field trials to prove or disprove the effectiveness of these products on *Alternaria* inoculum on the plant, and to compare them to new fungicides.

ACKNOWLEDGEMENTS

We are grateful to Syngenta (Algeria) to provide the opportunity to Z. Bouznad, with a financial support, to present this work in the Euroblight meeting.

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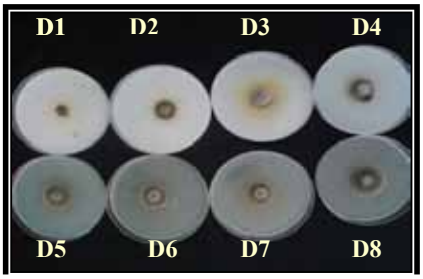

Fig. 3a. *Effect of Chlorothalonil against A. solani.*

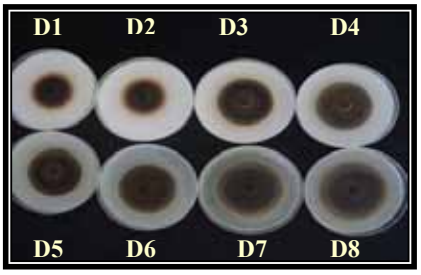

Fig. 3b. *Effect of Chlorothalonil against A. alternata*

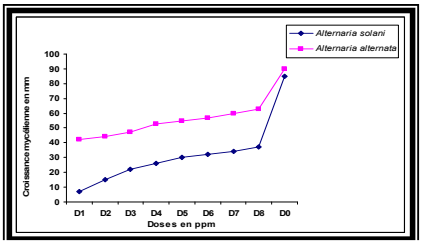

Fig. 3c. *Effect of chlorothalonil on diametral growth of mycelium*

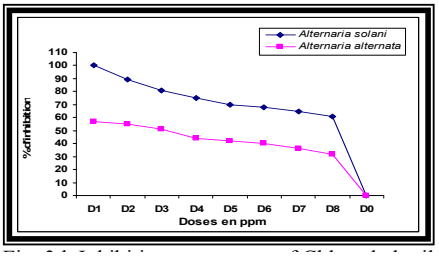

Fig. 3d. *Inhibition percentage of Chlorothalonil on diametral growth of mycelium*

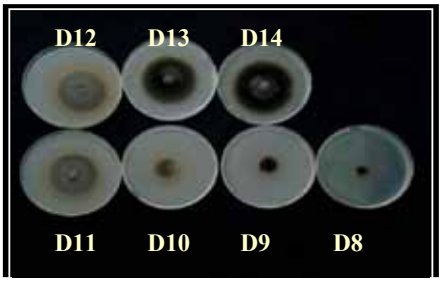

Fig. 4a. *Effect of difenoconazole against A. solani.*

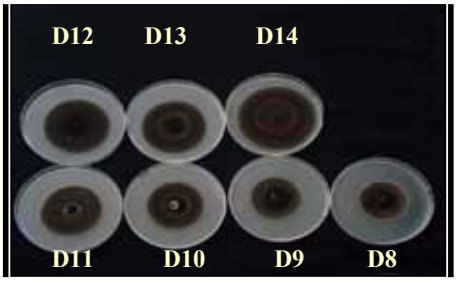

Fig.4b. *Effect of Difenoconazole against A. alternata*

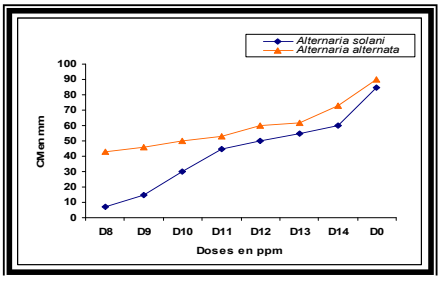

Fig. 4c. *Effect of difenoconazole on diametral growth of mycelia*

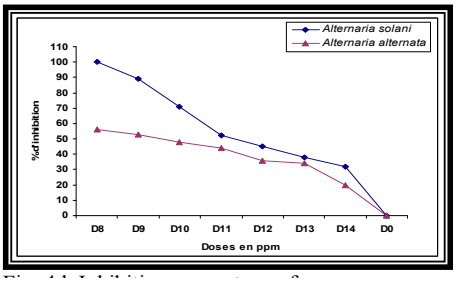

Fig. 4d. *Inhibition percentage of difenoconazole on diametral growth of mycelia*