



# In vitro Evaluation of Difenconazole and Chlorothalonil on conidia 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. Diseases are still the main cause of reduction of yield in this crop. After late blight which is the most damageable, early blight is also an important foliar disease reported to be caused by *Alternaria alternata* and *A. solani* causing yield losses in our conditions.

This research was initiated to examine in laboratory conditions the efficacy of two fungicides used in Algeria. The results showed Difenconazole has a better effectiveness than Chlorothalonil in inhibition of mycelial growth and conidial germination of *A. solani* and *A. alternata*. *A. solani* showed also a best sensitivity than *A. alternata* to the two tested fungicides

## 1. 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* causing yield losses under our conditions. The control of these two pathogens can be accomplished through various means: the use of resistant varieties, of appropriate farming techniques such as careful tillage, crop rotation, etc. ..., as well as fungicide application that may directly affect the growth of fungi.

**Objectifs:** The present research was conducted to evaluate the efficiency of the two fungicides Chlorothalonil and Difenconazole 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.

## 2. MATERIAL AND METHODS

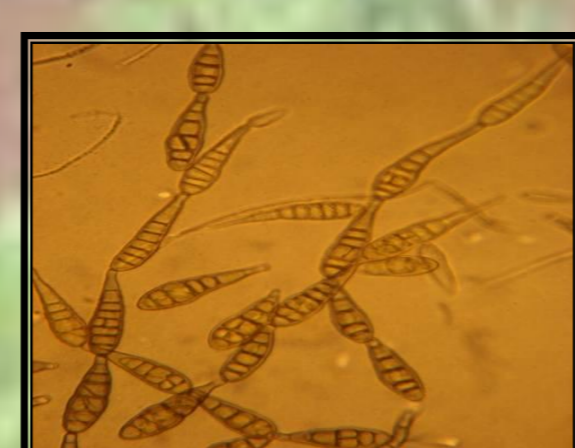
**2.1. Fungicides:** the test are realised to evaluate *in vitro* the effectiveness of two fungicides: Difenconazole (250 g/l of i.a.) and the chlorothalonil (720 g/l of i.a.), on germination conidia and mycelial growth of *A. solani* and *A. alternata*, and to compare them with the usually concentrations used in field.

### 2.2. Fungal material

Isolates of *A. solani* and *A. alternata* are obtained from leaves of potatoes showing characteristic symptoms of early blight collected in Algeria



Symptoms of *A. alternata*



Conidia of *A. alternata*



Symptoms of *A. solani*



Conidia of *A. solani*



Tests in Petri dishes for mycelial growth



Tests on slides for germination of conidia

### 2.4. Doses used for mycelial growth test

active substance	Chemical group	Species of fungi	Doses used for mycelial growth test	
			ppm	µl/l
Difenconazole	triazoles	<i>Alternaria solani</i>	250	1000
			125	500
			62,5	250
			31,25	125
			15,62	62,5
			7,81	31,25
			3,90	15,62
		<i>Alternaria alternata</i>	1,95	7,81
			0,97	3,90
			0,48	1,95
			0,24	0,97
			0,122	0,48
			0,061	0,24
			0,030	0,12
0	0			
Chlorothalonil	chloronitriles	<i>Alternaria solani</i>	2880	4000
			1440	2000
			720	1000
			360	500
			180	250
		<i>Alternaria alternata</i>	90	125
			45	62,5
			22,5	31,25
			0	0
			0	0

### 2.5. Doses used for conidia germination test

active substance	Chemical group	Species of fungi	Doses used for conidia germination	
			ppm	µl/l
Difenconazole	Triazoles	<i>Alternaria solani</i> <i>Alternaria alternata</i>	0	0
			1,95	7,81
			0,48	1,95
			0,122	0,48
			0,030	0,12
Chlorothalonil	chloronitriles	<i>Alternaria solani</i> <i>Alternaria alternata</i>	0	0
			2880	4000
			720	1000
			180	250
			45	62,5

### 2.3. Fungicides used

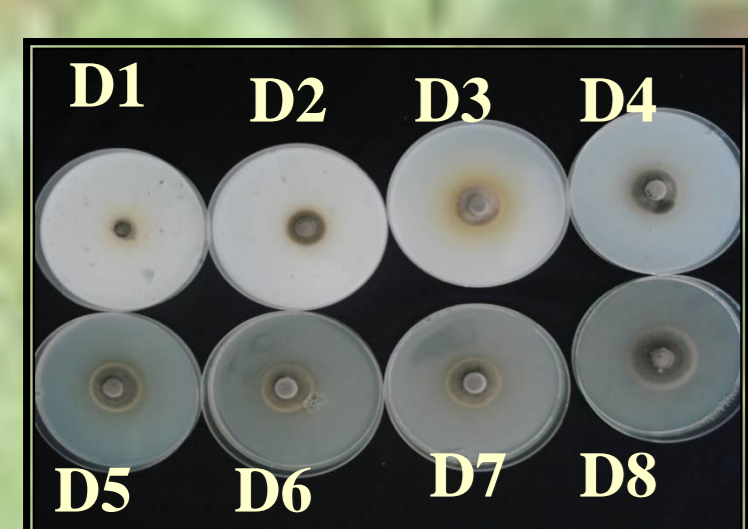
Concentrations of Difenconazole and chlorothalonil, are calculated from dose used in field treatments as shown in table 2.4. The tests are carried out in Petri dishes for mycelial growth and on slides for germination of conidia

% inhibition of conidia is evaluated by:  $\frac{Q - Q_0}{100 - Q_0} \cdot 100$

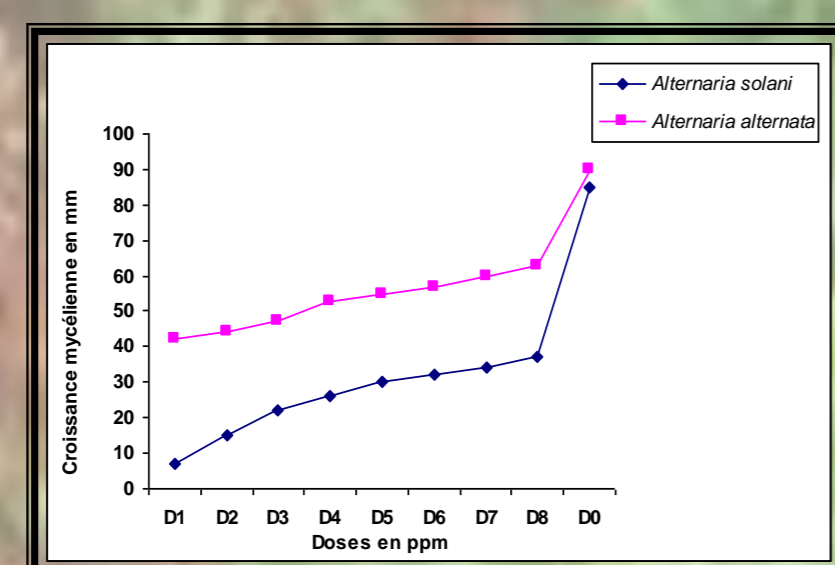
Inhibition of mycelial growth (CI50).  
1% =  $\frac{V_0 - V}{V_0} \times 100$

## 3. RESULTS

### 3.1. Effectiveness of fungicides on development of mycelial growth



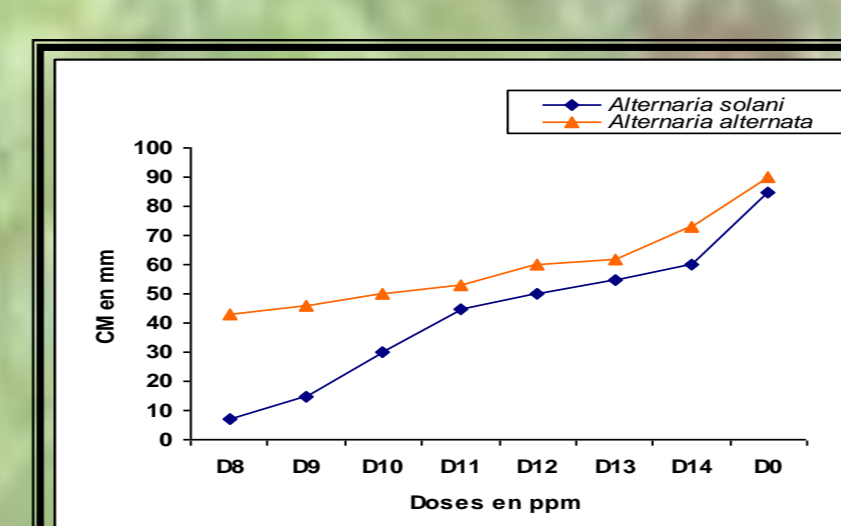
Effect of Chlorothalonil against *A. solani*.



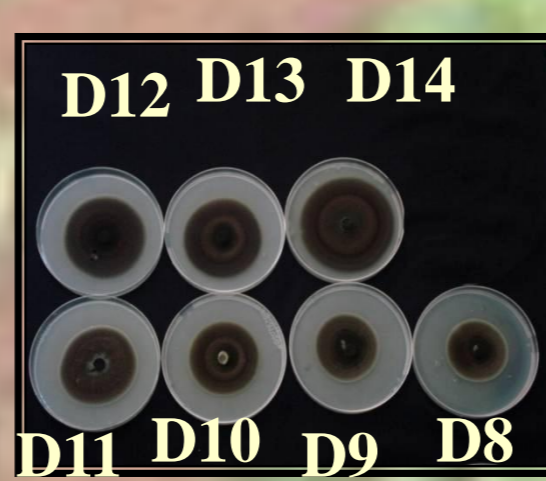
Effect of Chlorothalonil on diametral growth of mycelium



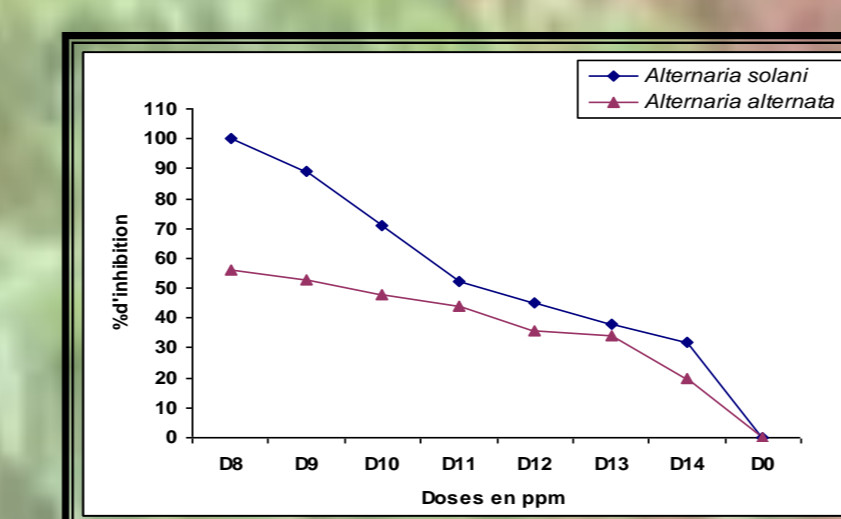
Effect of Difenconazole against *A. solani*.



Effect of Difenconazole on diametral growth of mycelium

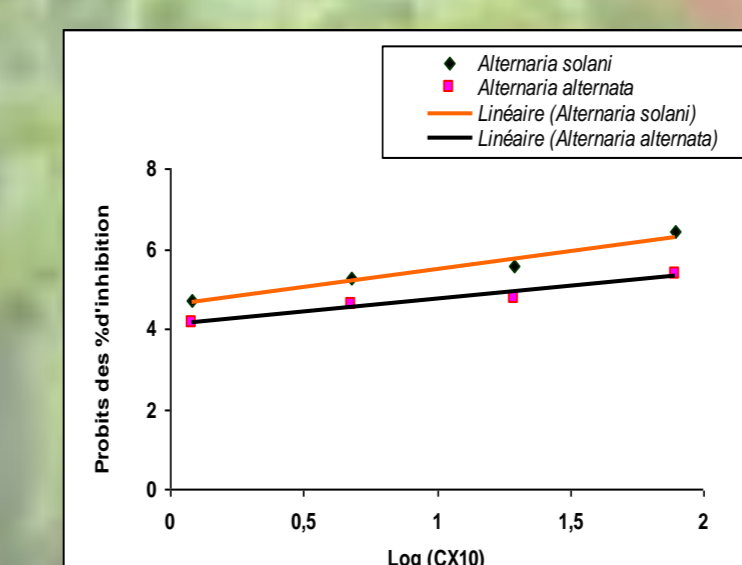


Effect of Difenconazole against *A. alternata*

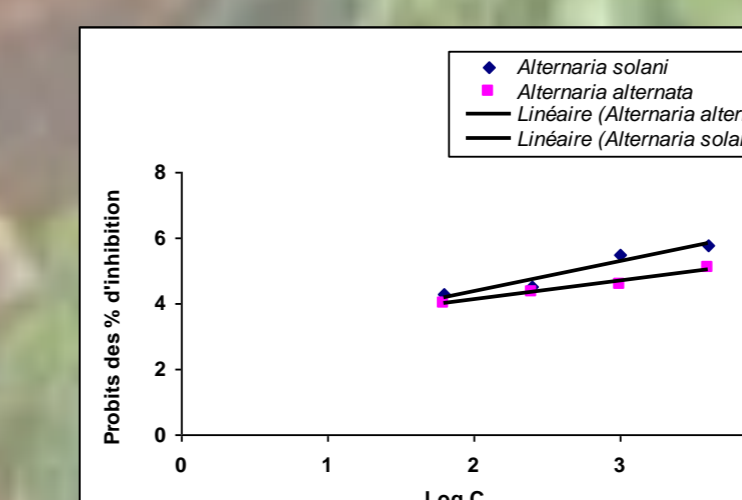


Percentage inhibition of Difenconazole on diametral growth of mycelia

### 3.2. Effectiveness of fungicides on germination of conidia



Regression curve ax + b) Of Difenconazole



Regression curve (ax + b) Of Chlorothalonil.

Active substance	Isolates	Doses (µl/l)	Log (10xC)	% inhibition	Probits
Difenconazole	<i>Alternaria solani</i>	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
	<i>Alternaria alternata</i>	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	<i>Alternaria solani</i>	4000	3,60	78	5,77
		1000	3,00	69	5,50
		250	2,40	31	4,50
		62,5	1,80	23	4,26
	<i>Alternaria alternata</i>	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

## 4. CONCLUSION AND DISCUSSION

The results obtained showed that the two fungicides tested, Difenconazole and Chlorothalonil had an effect *in vitro* on the 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: Difenconazole > Chlorothalonil.

In previous works, Tofoli et al (2003) showed also efficiency of Chlorothalonil against *Alternaria alternata* and Badoc (2005) obtained efficiency of Azoxystrobin on germination and mycelial growth of *Alternaria alternata*, the causal agent of fruit storage rots

While more recent report (MacDonald et al., 2007) showed efficacy in the field of other active ingredients belonging to the same family of strobilurin (azoxystrobin, pyraclostrobin) against *Alternaria solani*. *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 the plant, and to compare them to new fungicides.

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