Use of Three Synthetic Fungicides to Reduce the Incidence of Ascochyta Blight (*Ascochyta rabiei*) in Chickpea (*Cicer arietinum* L.): A Susceptible Cultivars Case

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**ABSTRACT**

This study examined the effect of three synthetic fungicide, maneb “Manebe808” chlorothalonil “Bravo80” and azoxystrobin “Oraivia80” on the incidence of ascochyta blight (AB) of chickpea caused by *Ascochyta rabiei* using three chickpea germplasm (ILC482, ILC484 and Flip 1025). The results, statistically reliable (C.V.< 20%), indicated the in vitro test of chemical control has significant effect at P < 0.01 on the mycelial growth of pathogen. All three fungicides caused important MGI% (Mycelial growth inhibition rate), which varied between 30 and 66%. There was a significant action induced by chlorothalonil fungicide (54 – 65%), followed closely by azoxystrobin (46 – 63%) and maneb (30 – 65%). In the in vivo test of chemical control for AB incidence by detached leaves showed a remarkable percentage of reduction in the severity of ascochyta blight varied between 20 and 80%. We noticed that the systemic fungicide like azoxystrobin can reduce the ascochyta blight severity (RDS%), with ranging between 71 and 80%, for other two contact fungicides (chlorothalonil and maneb), the mycelia growth inhibition rate was close to 50% (from 20 to 47%). These results indicated that the systemic fungicides, like azoxystrobin, have a significantly reduced the incidence and development of ascochyta blight disease in the susceptible cultivars (ILC1929, ILC263 and ILC484).

**Key words:** Ascochyta rabiei, Chemical control, Cicer arietinum, Disease incidence, Fungicides.

**INTRODUCTION**

Chickpea (*Cicer arietinum* L.), is an important edible leguminous crop which represents a significant source of dietary protein in many parts of central Asia and Africa (Gan et al. 2006; Kanouni et al. 2011). Chickpea is the third most important pulse crop, after bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.), in the world, with a total production of 8.8 million tonnes and cultivated in an area of 9.6 million ha with an average yield of 920 kg ha⁻¹ (FAOSTAT 2018).

Ascochyta blight, a disease caused by *Ascochyta rabiei* (Pass.) Labr. is a major constraint, limiting chickpea productivity worldwide (Chongo et al. 2003; Bayraktar et al. 2016). The disease is devastating in areas where cool (15-25°C) and humid weather (>150 mm rainfall) prevails during the crop season (Pande et al. 2005). The disease reduces grain yields and quality significantly and in some circumstances yield losses for susceptible cultivars are as high as 100%. In Algeria, among the diseases observed on chickpea, ascochyta blight is the most frequently encountered disease and the one that causes the most damage (Zikara-Zine and Bouznad 2007; Benzohra et al. 2015; Zikara-Zine et al. 2015). Data from several years of surveys have shown its presence with yield reductions of up to 100% (Bouznad et al. 1996). Mabsoute et al. (1998) reported that in Algeria as well as in other Maghreb countries, ascochyta blight remained the major constraint of chickpea cultivation.

Cool and humid climates are most favourable factors for its spreading (Bhardwaj et al. 2010; Imtiaz et al. 2011) and there are limited chickpea germplasm which have excellent resistant to the pathogen (Ahmad et al. 2013; Duzdemir et al. 2014; Jabbar et al. 2014; Baite et al. 2016). But this genetic control had limits to reduce the damage caused by this disease (Gan et al. 2006), there was several resistant chickpea germplasm, gave no stable level of resistance *A. rabiei* (ICARDA, 2003), for several years, there...
were chickpea cultivars considered as resistant to *A. rabiei* for several years like ICC 3996, ILC 72, Flop 88-85 and ICC 12-004, which currently are sensitive. Therefore chemical control intervention during the risky seasons is needed when the disease becomes aggressive (Imtiyaz et al. 2011), especially in the event of sudden epidemics when climatic and cultural conditions are favorable for the development of this disease (Imtiyaz et al. 2011; Kosiada and Irykowska 2013; Amin and Melkamu 2014).

In this study, the *in vitro* evaluation of fungicides like Maneb “Maneb 80®”, Chlorothalonil “Bravo®” and Azoxystrobin “Ortiva®” was done for their inhibitory effect, on the mycelial growth of *A. rabiei* isolates originating from the province of Mascara and the *in vivo* chemical control test on the ascochyta blight incidence using detached leaves screening technique.

**MATERIALS AND METHODS**

**Fungal material**

*Ascochyta* blight infected chickpea plant samples were collected from chickpea growing provinces of Northern west of Algeria namely, Relizane, Mascara, Ain Temouchent, Sidi Bel Abbés, Tlemcen and isolated 20 isolates of *A. rabiei* from the samples on chickpea dextrose agar (CDA) medium (Chickpea Seed-Meal dextrose Agar). The associated fungus, *A. rabiei* was isolated from stem and pods of chickpea on CDA medium following standard tissue isolation procedures (Table 1).

**Synthetic fungicides**

In order to study the action of certain synthetic active ingredients on the mycelial growth of *A. rabiei* and the disease incidence, three fungicides were used: Manebe 80® (maneb), Bravo® (chlorothalonil) and Ortiva® (azoxystrobin) (Table 2).

**Purification and conservation of isolates**

The isolates were purified by single spore isolation. Throughout the study, the isolates were maintained by transferring periodically on CDA slants and stored at 4±1°C for further use (Chen et al. 2017).

**Test in vitro of mycelial growth inhibition**

Three synthetic fungicides were incorporated aseptically at the registered dose (2.5 g / l for Manebe, 0.6 g / l for azoxystrobin and 0.5 g / l for chlorothalonil) (Hibar et al., 2007), in the CDA culture medium which was maintained at a temperature of 20-25°C (Gautam et al. 2013; Gursoy et al. 2019). After flow and solidification of the mixture (culture medium and fungicide), agar explants 5 mm in diameter, carrying the pathogen, were placed in the center of Petri dishes. The petri plates were incubated in a BOD incubator at a temperature of 20 ± 2°C, corresponding to the optimum growth of mycelium *Ascochyta rabiei* (Kumar et al. 2013).

**Evaluation of mycelial growth inhibition (MGI%)**

To estimate the mycelia growth, the technique used was that indicated by Kuçük and Kivanç (2003). This method initially consists of measuring the mycelial growth linear day until the seventh day, according to the following formula:

\[
L = \frac{(D - d)}{2} \times n \times (\frac{L}{D})
\]

C: Average of mycelial growth (mm/day);
D: Mycelial growths on the day *i*;
D: Colony diameter (mm);
L: Mycelial growth (mm);
d: Explant diameter (=5mm),
n: Days number.

The mycelia growth inhibition rate (MGI%), was obtained using the formula:

\[
\text{GRI} = \frac{(C1 - C2) \times 100}{C2}
\]

**Table 1: Ascochyta rabiei isolates with its origin.**

| Isolate name | Province      | Year of isolation |
|--------------|---------------|-------------------|
| AR1          | Relizane      | 2011              |
| AR2          | Relizane      | 2011              |
| AR3          | Relizane      | 2011              |
| AR4          | Relizane      | 2012              |
| AR5          | Relizane      | 2012              |
| AR6          | Mascara       | 2011              |
| AR7          | Mascara       | 2011              |
| AR8          | Mascara       | 2011              |
| AR9          | Mascara       | 2012              |
| AR10         | Mascara       | 2012              |
| AR11         | Sidi Bel Abbès | 2010              |
| AR12         | Sidi Bel Abbès | 2010              |
| AR13         | Sidi Bel Abbès | 2011              |
| AR14         | Sidi Bel Abbès | 2011              |
| AR15         | Sidi Bel Abbès | 2012              |
| AR16         | Tlemcen       | 2010              |
| AR17         | Tlemcen       | 2010              |
| AR18         | Ain Temouchent| 2012              |
| AR19         | Ain Temouchent| 2012              |
| AR20         | Ain Temouchent| 2012              |

**Table 2: Synthetic fungicides used for tests with its proprieties.**

| Commercial name | Molecule | Concentration | Chemical class | Formule chimique | Action type |
|-----------------|----------|---------------|----------------|------------------|-------------|
| Manebe80®      | manebl   | 500g/l        | Dithiocarbamats | C<sub>2</sub>H<sub>4</sub>MnS<sub>2</sub>N<sub>2</sub> | Contact     |
| Ortiva<sup>®</sup> | azoxystrobin | 125g/l | Strobilurins | C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub> | Systemic    |
| Bravo<sup>®</sup> | chlorothalonil | 500g/l | Chloronitrils | C<sub>2</sub>ClN<sub>2</sub> | Contact     |
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Where
GRI% represents growth inhibition rate, C1 means mycelia growth of A. rabiei in presence of the fungicide and C2 means mycelial growth of control (Kuçuk and Kınanç 2003). The averages of colony diameter were calculated by two perpendicularly diameters after 10 days of incubation.

Statistical analysis
All the experiments schemes were randomized complete. Three replicates per treatment were performed and each whole experiment was repeated independently three times. The collected data were submitted to one way analysis of variance (ANOVA) by one factor and was performed for the data on antibiosis action was represented by AFS’s inhibitions rates of mycelial growth. The F-values were calculated at \( p<0.05 \). Standard errors has been calculated and marked in tables. The significance of differences among treatments was recorded at \( p<0.05 \) by the experimental statement named global randomized with one studied factor (inhibition rates) and multiple comparisons of the means were conducted according to the Newman–Keuls test at \( p<0.05 \) (Kuçuk et al. 2007).

In vivo chemical control
The fungicides namely were evaluated using detached leaves inoculation technique (Benzohra et al. 2011) at the registered dose (2.5 g / l for Maneb, 0.6 g / l for azoxystrobin and 0.5 g / l for chlorothalonil) (Hibar et al. 2007), colonies of isolates are immersed in sterile distilled water and then scraped by a sterile glass spatula (Benzohra et al. 2011). The suspension is adjusted to \( 5 \times 10^5 \) conidia/ml using a Malassez cell, this suspension contains the inoculum with sterile distilled water for the control and the inoculum is mixed with the fungicide in the sterile distilled water for treated (Hibar et al. 2007).

The chickpea seeds used were sterilized with sodium hypochlorite (2%) for 3 minutes and then rinsed 3 times with sterile distilled water. Then, they were sown in pots of 10 cm × 6 cm, containing a sterile peat, at the rate of 2 seeds / pot. After 15 days, leaflets can be removed for the inoculation test with 3 leaflets for each treatment (Hibar et al. 2007).

Disease severity assessment
Ascochyta blight symptoms are assessed 15 days after inoculation of chickpea leaflets. The severity of the disease is noted according to the rating scale of Dolar (1997), (1, 3, 5 and 7), which represent respectively 0, 25, 50 and 100% of the surface of the necrosis). This scale is based on the percentage of the surface area of infection relative to the entire area of the leaflet.

Chickpea lines ranked from 1.0 to 4.9 were considered resistant and those coded from 5 to 7 are susceptible (Dolar 1997).

Based on the symptom rating, the incidence of ascochyta blight disease percentage (ID%) is calculated using the following formula:

\[
\text{ID\%} = \frac{\sum (\text{Notations} \times \text{Nb infected leaves})}{\text{Bigest notation} \times \text{global Nb leaves}} \times 100
\]

By using these percentages, we can calculate the disease severity reduction (DSR%) using this formula:

\[
\text{DSR\%} = \frac{\text{IRR\% control} - \text{IRR\% tested}}{\text{IRR\% control}} \times 100
\]

RESULTS AND DISCUSSION
In vitro test on mycelial growth
The mycelial growth of the isolates tested is lower compared to the control (Fig 1).

In fact, the inhibition rate (MGI%) is very important on the mycelial growth of the isolates, it varies between 30 and 66% (Fig 1), with a significant inhibition for the fungicide ‘Chlorothalonil’ which allows an inhibition of 53 at 66% depending on the isolate.

The results obtained showed the reliability of the chemical control test (CV <20%) and the analysis of variance (ANOVA) revealed a highly significant interaction (\( P<0.01 \),

Myceial growth inhibition (MGI%)

Fig 1: Growth mycelial inhibition rate (MGI%) of A. rabiei isolates by synthetic.
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**Disease severity reduction (DRS%)**

*Fig 2:* Symptoms severities of ascochyta blight on detached leaves of chickpea by synthetic fungicides effect.

**Table 3:** Effect of three fungicides on mycelial growth of 20 isolates of *Ascochyta rabiei*.

| Isolates       | Statistical analysis results | Fungicides | Maneb x ± δ | Azoxyostrob $x ± δ$ | Chlorothalonil $x ± δ$ |
|----------------|-----------------------------|------------|-------------|---------------------|------------------------|
|                |                             |            | Treated     | Control             | Treated               | Control               |
| AR6, 7, 8, 9, 10 | LSD value                   | Maneb      | 56.88**     | 96**                | 96.94**               |
|                | C.V.                        | Azoxyostrob| 18.18%      | 12.83%              | 11.68%                |
| AR16, AR17     | LSD value                   | Chlorothalonil| 18.66%     | 8.59%               | 12.34%                |
| AR11, 12, 13, 14, 15 | LSD value  | Maneb      | 105.3**     | 123.85**            | 147**                 |
|                | C.V.                        | Azoxyostrob| 10.95%      | 11.68%              | 8.06%                 |
| AR1, 2, 3, 4, 5 | LSD value                   | Chlorothalonil| 151.73**   | 123.85**            | 147**                 |
|                | C.V.                        | Maneb      | 9.75%       | 11.68%              | 8.6%                  |

$x$: Averages of mycelial growth; $δ$: standard deviation; C.V.: Coefficient of variation; $*$: significant effect at $P_{0.05}$; $**$: significant effect at $P_{0.01}$; LSD: values of F significance according to Newmann-Keuls test.

between the tested products and the isolates of *A. rabiei* used in this study (Table 3).

**Disease severity reduction incidence of ascochyta blight (DSR%)**

The fungicides tested showed efficacy in reducing the severity of anthracnose disease compared to controls (Fig 2), with a reduction percentage of up to 80%. It was noted that the systemic fungicide ‘Azoxyostrob’ was able to reduce the severity of anthracnose between 71 and 80% compared to the other two products (maneb and chlorothalonil), whose action could not exceed 47% inhibition (average less than 40%) (Fig 3, Table 4).

From these results, it could be confirmed that the systemic fungicides have the capacity to reduce the growth of plant pathogenic diseases greater than the contact fungicides.

The efficacy of three synthetic fungicides, maneb “Manebe80®”, chlorothalonil “Bravo®” and azoxyostrob “Ortiva®” on mycelial growth, has been reported on several pathogens like *Fusarium roseum*, agent of dry rot of potato (*Solanum tuberosum*) and *Fusarium solani*, agent of sudden death of soybean (*Glycine maxima*), (Mclean and Lawrence 1994). By studying the effect of other fungicides of the same chemical family on the mycelial growth of *Ascochyta rabiei*, Chang *et al.* (2007) showed that Dithiocarbamates group (such as maneb) and strobilurins (such as azoxyostrob) are effective on *A. rabiei* mycelial growth. Bahous *et al.* (2005) reported that azoxyostrob has 70% of inhibition rate on *Helminthosporium oryzae*, agent Helmintosporiosis of rice (*Oryza sativa*). Hibar *et al.* (2007) also found maneb effective against *Phytophthora infestans*, potato late blight agent, *Erysiphe graminis* f. sp. *tritici*, powdery mildew agent of cereals and *Alternaria alternata*, agent of alternariose of tomato. Wise *et al.* (2008) reported that the inhibition rate of azoxyostrob ranging from 30 to 45% on the mycelial growth of *Alternaria solani*, agent of earlier blight of tomato (*Lycopersicum esculentum*) and *Uncinula necator*, agent of powdery mildew of vine (*Vitis vinifera*). In this study, it was observed that a significant inhibition rate of mycelial growth (30-66%) on *A. rabiei* isolates by azoxyostrob fungicide effect, which was more marked than that reported by Wise *et al.* (2008).

The inhibition efficacy of Chlorothalonil fungicide on mycelial growth has been reported for *Phytophthora*...
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**Table 4:** Disease severity reduction (DSR%), on three chickpea germplasm by using of three synthetic fungicides.

| Isolats          | Maneb | Chlorothalonil | Azoxystrobin |
|------------------|-------|----------------|--------------|
|                  | ILC482 | ILC484 | FLIP1025 | ILC482 | ILC484 | FLIP1025 | ILC482 | ILC484 | FLIP1025 |
| AR6, 7, 8, 9, 10 | 43     | 43    | 24       | 43     | 24    | 24       | 78     | 75     | 71       |
| AR18, 19, 20     | 43     | 43    | 20       | 41     | 41    | 41       | 80     | 80     | 80       |
| AR16, AR17       | 43     | 47    | 24       | 43     | 43    | 43       | 80     | 80     | 80       |
| AR11, 12, 13, 14, 15 | 43 | 43 | 29       | 43     | 43    | 43       | 75     | 75     | 80       |
| AR1, 2, 3, 4, 5  | 43     | 41    | 30       | 41     | 43    | 43       | 78     | 78     | 73       |
| Total averages of RDI (%) | 39 | 38 | 77 |

Fig 3: Averages of diseases severities reductions rates (DSR%) by synthetic fungicides action.

*infestans*, agent late blight in potato, with 70% of inhibition rate (Daayf and Platt 2002). This inhibition was obtained in our *A. rabiei* assay with MGI% arrived to 66%. This study revealed that the three synthetic fungicides (maneb, azoxystrobin and chlorothalonil) were effective on mycelial growth of *A. rabiei*.

Also the effectiveness of these three synthetic fungicides (Maneb, Chlorothalonil and Azoxystrobin), on the incidence of disease severity has been reported on several pathogens such as the parasites *Venturia inaequalis*, agent of apple scab (*Malus sylvestris* L.), *Venturia pirina*, pear scab agent (*Pyrus communis* L.), *Phytophthora palmivora*, agent of brown rot of cocoa pods (*Theobroma cacao*) and *Rhizoctonia solani*, agent of rice leaf blight (*Oryza sativa*) (Obanor et al. 2005).

The systemic fungicide Azoxystrobin showed a significant reduction (70%) in *Sclerotium rolfsii*, a causal agent of stem wilt of tomato (*Lycopersicum esculentum* L.), *Fusarium graminearum*, wheat wilt agent (*Triticum durum*) and *Phytophthora cactorum*, root rot of apple (*Malus sylvestris* L.) (Matheron and Porchas 2000). Similarity of these results with 80% reduction of symptoms have been found in present study.

Maneb fungicide was able to reduce the development of cucumber mildew (*Cucumis sativus* L.) caused by *Pseudoperonospora cubensis* (Chaudhry et al. 2009) by reduction up to 48% and up to 60% development of downy mildew in chilli pepper (*Capsicum annuum*), caused by *Phytophthora capsici*. However in the present study, it was found less effective. For *Uromyces phaseoli*, a bean rust agent (*Phaseolus vulgaris* L.) (Shinde and Hunje, 2019), 25% of DSR (?) for *Pseudopeziza medicaginis*, common spot agent of alfalfa (*Medicago sativa*) and *Colletotrichum trifolii*, agent of anthracnose disease on alfalfa (Kour et al. 2013). These results were similar to the findings of this study.

**CONCLUSION**

It has been noticed that the systemic fungicides such as azoxystrobin may provide good chemical control and may be used as for integrated disease management program when the climatic and agronomical factors are favorable for *Ascochyta rabiei* development.
Based on these results, a significant chemical control solution against ascochyta blight of chickpea can be provided for important but susceptible cultivars namely: ICC3996, ILC72, Flip 88-85 and ICC 12-004) (ICARDA 2003).

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