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Original article

Resistance of Telluric Fungi to Chemical Fungicides

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Abstract

Our work focuses on the identification of resistance of telluric fungi to commonly used fungicidesin chemical control. We investigate the resistance of telluric fungi (*Fusarium* sp., *Chalara* sp., *Sporonema* sp., *Stiebum* sp., *Didymabotrium* sp., *Dothichiza* sp. and *Sclerotopsis* sp.) from cereal fields to four fungicides (Propicone, Vapcotop, Curitine V and Kazir). This study was based on direct contact of the telluric isolates with fungicides on Petri dishes. For the four tested fungicides different rates of resistance and susceptibility were observed according to the isolate and the used concentration. One of the isolates showed a high resistance to Vapcotop with 500 mg/l. Generally the statistical analysis revealed that there are no significant differences in the effect of fungicides concentrations against isolates, while significant differences are observed between isolates.

Keywords: Fungicide, Resistance, Sensitivity, Telluric fungi.

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INTRODUCTION

Plant protection products used in agriculture limit the development of organisms that can affect crops. Chemicals have been widely used against phytopathogenic microorganisms and insect pests. The chemical control of phytopathogenic agents mainly concerns fungi responsible for fungal diseases of plants. There is currently a wide range of fungicides to control most diseases caused by phytopathogenic fungi, the main problem being to delay the development of resistance.

Most fungicides directly affect essential functions such as respiration, sterol biosynthesis or cell division. This may be harmful for humans and non-target organisms and, on the other hand, can lead to the development of resistant fungal strains (Leroux, 2003).

Among the fungicides tested, Propicone a benzimidazole and Vapcotop a Thiophanate have similar modes of action at the level of the fungal cell (FRAC, 2013). These fungicides act on microtubules, which are the major constituents of the cytoskeleton and the achromatic spindle. These fungicides bind to β-tubulin of many Ascomycetes (*Fusarium* sp.) and Basidiomycetes, but their interaction is weak with Oomycetes (Leroux, 2003). Compounds which interfere with the formation and/or the functioning of these microtubules blocks cell division and elongation of mycelial hyphae (FRAC, 2013). The agricultural use of benzimidazoles has been strongly affected by the selection of resistant strains in many phytopathogenic fungi (Leroux, 2003).

In contrast, multi-site fungicides (Kazir and Curatine V) affect respiratory processes, and all are characterized by strong preventive activity. These fungicides have a relatively low persistence requiring frequent treatments, renewal in case of rain and high doses (more than 1 to 2 kg of active ingredient per hectare). On the other hand, the virtual absence of acquired resistance in certain phytopathogenic fungi, particularly the downy mildew, allows these multi-site fungicides to be important partners in the fight against these fungi (Leroux, 2003). This would partly explain the significant resistance of the fungi studied. However, marketed fungicides are effective only on fungi that remain on the surface of the plant or penetrate very little. This sometimes gives farmers an irrational use of these compounds.

The objective of this work is to look for possible resistance in telluric phytopathogenic fungi from cereal fields in the region of Setif with respect to the four fungicides mentioned and which are widely used by farmers.

Material and Methods

Biological material

Phytopathogenic fungi

The fungi were previously isolated by Belatrous (2011) and Sayah (2011), from potato and wheat fields in Setif (east of Algeria), and identified by Taoui (2011) as shown in Table 1.

Table 1. Fungi used for the resistance tests

Fungi	Attributed numbers
Fusarium sp.	2
Fusarium sp.	5
Chalara sp.	8
Fusarium sp.	9
Nd	10
Sporonema sp.	12
Nd	14
Stiebum sp.	15
Didymabotrium sp.	18
Dothichiza sp.	20
Sclerotopsis sp.	28
Fusarium sp.	29

Nd: not determined

Preparation of fungicide solutions

Stock solutions of fungicides (Table 2) are prepared in sterile distilled water: Propicone 10 g/l, Vapcotop 10 g/l, Curitine V 20 g/l and Kazir 1 g/l. After stirring and sterilization by filtration, a series of half dilution is performed.

Preparation and seeding of media containing fungicides

For each fungicide tested, a determined concentration is added to PDA (45-50°C), before the canning of the medium. Using a cookie cutter, discs are cut in a week-long mycelial culture. Three or five disks are equidistantly deposited (Magnien, 2012).

Table 2. Tested fungicides, chemical family, formulation and used concentrations (Index of plant protection products for agricultural use, 2011)

Active material	Trade name	AM%	Chemical family	Formulation	SCS	FC
Propinebe	Propicone	70	Benzimidazoles	WP*	10g/l	300mg/l
Mancozebe	Kazir	80	Dithiocarbamate	WP*	10g/l	100mg/l
Mancozèbe + cymoxanil	Curatine V	46.5+4	Dithiocarbamate + Acetamides	WP*	20g/l	100mg/l
Methyl – Thiophanate	Vapcotop	70	Thiophanate	WP*	1g/l	5mg/l

AM%: Active material percentage, SCS: Stock concentration solution, FC: final concentrations, WP*:Wettable powder

Effectiveness of fungicides

Resistance to increasing concentrations of fungicide was evaluated. Two repetitions have been performed (Three to five discs are deposited per box). The inoculated dishes are then randomized and incubated at room temperature for six days in the dark. The growth diameters are then measured and compared to the control without fungicide. The sensitivity (efficacy) of the fungicide to mycelial growth was determined by calculating the percent inhibition compared to controls without fungicide, according to the formula:

$$\% I = (C\text{-}Ct) / C$$

Where:% I is the percentage of inhibition. C the diameter of the control colony (control) and Ct the diameter of the colony in the medium with fungicidal treatment (Trivedi, 2008).

Statistical analysis

Data were analyzed by the one way analysis of variance (ANOVA) using Student-Newman-Keuls Method, and the test with $P<0.01$ was considered as statistically significant.

Results

Effect of Propicone (Propinebe) on mycelial growth

With propicone, the fungus 2 showed the highest resistance with a concentration of 500 mg/l (IC50 460 mg/l), while the fungus 28 showed the highest sensitivity with a concentration of 300 mg/l (IC50 360 mg/l; Fig. 1).

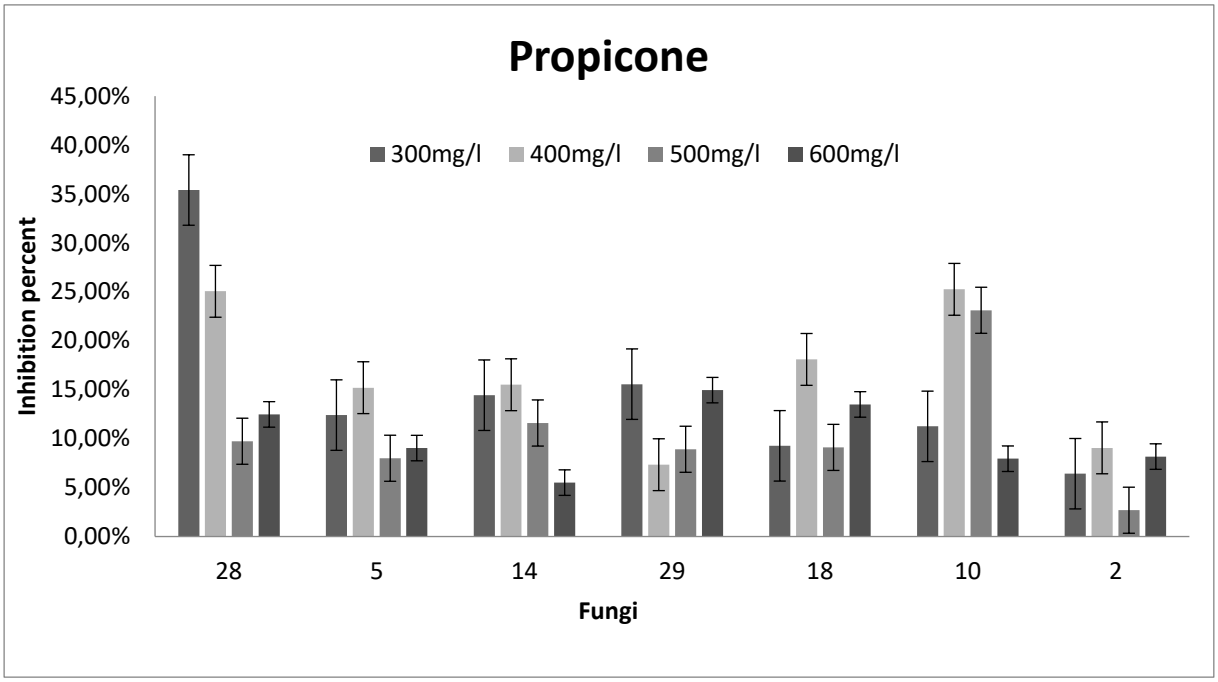

Figure 1. Fungal inhibitions according to increasing concentrations of Propicone

28, 5… 2: tested fungi. Statistically no significant differences were observed between stains or fungicide concentrations.

Effect of Vapcotop (methyl-Thiophanate) on mycelial growth

With methyl-thiophanate, the fungus 10 showed the highest resistance with a concentration of 500 mg/l and an IC50 of 490 mg/l, while the fungus 5 showed the highest sensitivity with a concentration of 100 mg/l (Fig. 2).

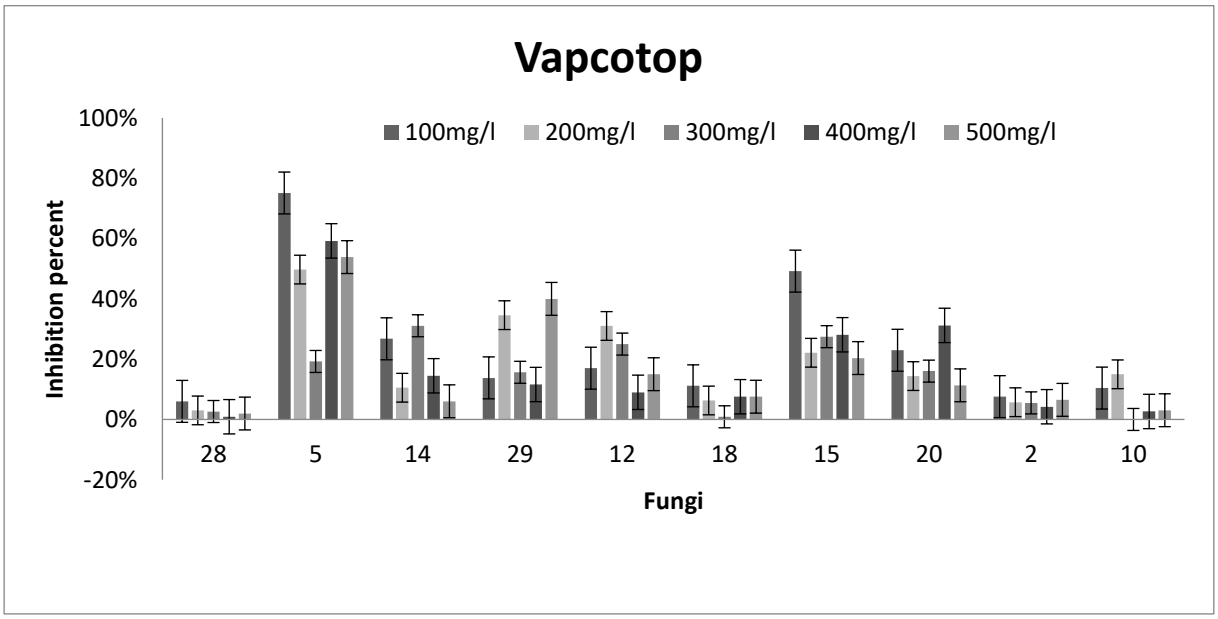

Figure 2. Fungal inhibitions according to increasing concentrations of Vapcotop

28, 5,… 10 : tested fungi. Statistically there is a significant difference between stains,
but no significant differences among fungicide concentrations.

Effect of Curitine V (cymoxanyl-makozeb) on mycelial growth

Curitine V showed with the fungus 28 the highest resistance with a concentration of 500 mg/l, whereas the fungus 15 showed the highest sensitivity with a concentration of 400 mg/l (Fig. 3) and an IC 50 of 322 mg/l for the fungi 15.

Effect of Kazir (makozeb) on mycelial growth

With the Kazir the fungus 20 showed the highest resistance with a concentration of 30 mg/l, whereas the fungus 14 showed the highest sensitivity with a concentration of 20 mg/l (Fig. 4), with IC 50 of 40 and 28 mg/l respectively.

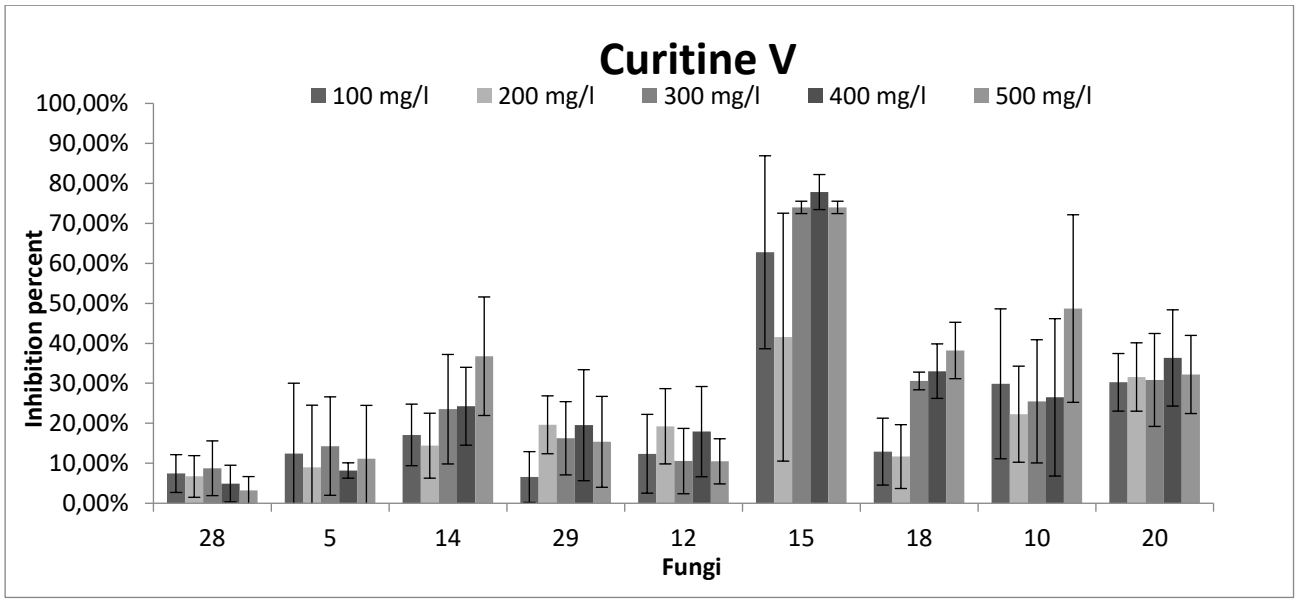

Figure 3. Fungal inhibitions according to increasing concentrations of Curatine V

28, 5,…20 : tested fungi**.** Statistically there is a significant difference between stains,

but no significant differences among fungicide concentrations

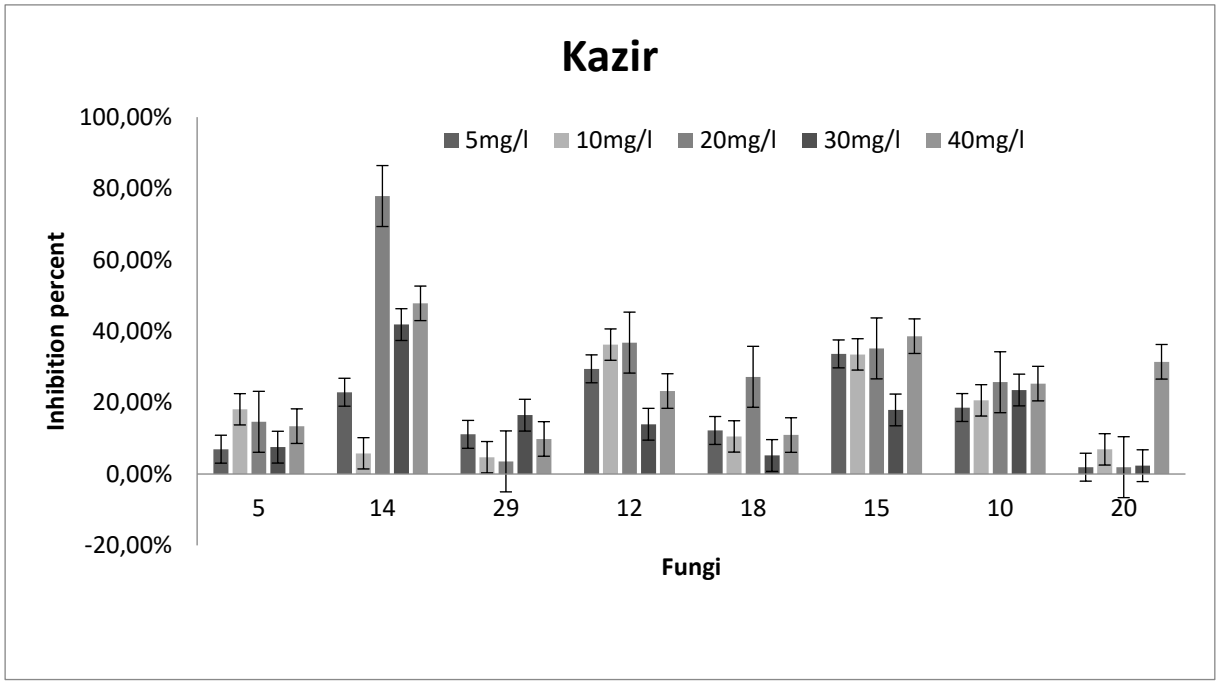

Figure 4. Fungal inhibitions according to increasing concentrations of Kazir

5, 14,… 20 : tested fungi. Statistically there is a significant difference between stains, but no significant differences among fungicide concentrations.

Discussion

Fungicide resistance has been defined by Leroux (1985) as: (i) physiological resistance, and (ii) resistance in practice. Practical resistance is observed following a loss of effectiveness of the fungicide applied by the appearance of resistant individuals. Resistant individuals increase such resistance may have a variable impact on practical efficacy in the field. Thus, any resistance detected at the individual level does not necessarily induce loss of efficacy of the concerned fungicide (field resistance; Walker et al., 2012).

Statistically the resistance of the fungal isolates to the tested fungicides are significantly different. This may be the fact that the tested fungi belong to different genera. Isolate 2 (*Fusarium* sp.), showed the highest resistance to Propicone with an IC50 of 460 mg/l followed by isolate 28 (*Sclerotopsis* sp.) with 360 mg/l. Herein, the statistical analysis revealed that no significant difference was observed between the isolates.

It should be noted that *F. culmorum*, *F. graminearum*, and *F. avenaceum* species are naturally less sensitive to benzimidazoles than *F. nivale*. In addition, the acquired resistance of these *Fusarium* remains exceptional. On the other hand, many cases of resistance to benzimidazoles have been reported for *F. nivale* (Maufras et al., 2002). This acquired resistance is related to the low affinity of these fungicides toβ-tubulin, which results from mutations in codons 198 or 200. However when it occurs,

this β-tubulin mutation at 198 shows increased sensitivity to phenylcarbamates (Leroux, 2003). In contrast, for Vapcotop the isolate 10 was resistant with an IC50 higher than 500mg/l, and the isolate 5 (*Fusarium* sp.) with an IC50 of 490 mg/l.

Isolate 20 (*Dothichiza* sp.) in the presence of Kazir a dithiocarbamate, exhibited the highest resistance with an IC50 of 40 mg/l, while the highest sensitivity is observed with the fungus 14 which has an IC 50 of 28 mg/l. Dithiocarbamates are considered as a low-risk group with multi-site anti-energy activity (Leroux et al., 2006). These fungicides have a wide range of activity, including Ascomycetes and Basidiomycetes, by inhibition of spore germination; or Oomycetes by immobilization of zoospores (Leroux, 2003). On the other hand, isolate 28 showed resistance to Curitine V (combination of Dithiocarbamate and Acetamides) with an IC 50 greater than the highest concentration tested (500 mg / l). In contrast, the most sensitive is the fungus 15 (*Stiebum* sp.) with an IC50 of 322 mg/l. Acetamides are fungicides that act on the cell wall. However, according to FRAC (2013) the precise site of action is poorly known. Further studies are needed to determine the mode of resistance, and therefore avoid the use of fungicides that are ineffective and harmful to the health of humans and animals.

Conclusion

Fungicides are an important means of controlling phytopathogenic fungi. The appearance of resistance may be more or less rapid depending on the chemical family and the pathogen concerned. The results of our work show the presence of resistance in telluric fungi (phytopathogenic or not) from cereal fields in the region of Sétif. However, this resistance varied from one fungus to another and from one compound to another. This resistance appears to affect low-risk products such as Kazir, and fungi that are not targeted by treatments such as *Sclerotopsis* sp., which is not a pathogen responsible for economic losses. This again explains the misuse of these fungicides, high cost, sometimes useless.

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