"IN VITRO" EFFECT OF FUNGICIDES ON MYCELIAL GROWTH OF *Bipolaris maydis*

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ABSTRACT

Leaf spot is a disease provoked by the fungus Bipolaris maydis, that it causes great damages for annual crops, as the corn, the wheat and the oats. Tests "in vitro" were accomplished seeking to evaluate the efficiency in the inhibition of the mycelial growth of that patogenic fungus through five fungicides: tetraconazol, tebuconazole, azoxystrobin + cyproconazole, trifloxystrobin + propiconazole and trifloxystrobin + cyproconazole were tested fungicides. For the determination of the efficiency of the products, the daily growth of the fungus was evaluated, being compared with the control. It was determined the percentage of growth inhibition. All of the treatments were effective in the inhibition of the growth of this fungus.
Keywords: Fungus, inhibition, disease, chemical control.

RESUMEN

Efecto "*in vitro*" de los fungicidas en el crecimiento mycelial de *Bipolaris maydis*

El mancha de la hoja es una enfermedad causada por el hongo Bipolaris maydis, que causa grandes daños a los cultivos anuales de gramíneas como el maíz, el trigo y la avena. Se realizaron pruebas "in vitro" para evaluar la eficacia de la inhibición de crecimiento mycelial de hongos fitopatógenos através de cinco fungicidas. Los fungicidas probados fueron tetraconazol, tebuconazole, azoxystrobin + cyproconazole, trifloxystrobin + propiconazole and

trifloxystrobin + cyproconazole. Para determinar la eficacia de los productos, evaluó el crecimiento diario del hongo, en comparación con el control. Se determinó el porcentaje de inhibición de crecimiento. Todos los tratamientos fueron eficaces en la inhibición del crecimiento del patógeno.
Palabras clave: Hongos, inhibición, enfermedad, control quimico.

INTRODUCTION

Bipolaris maydis is a plant pathogenic fungus that causes leaf spot or "helmintosporiose" and usually infects corn, wheat, barley, oats, triticale and rye (Antoniazzi & Deschamps, 2007). In Brazil, the leaf spot occurrence is frequent, having reports of its incidence in all production regions of the aforementioned crops, mainly corn (Fernandes & Oliveira, 2000). Fungal infections occur at any stage of development and often involve the roots, crown, stem, leaves and seeds.

The attack on the leaves can cause significant reduction in production due to destruction of photosynthetic active tissues, as well as influencing these tissues more susceptible to attack by other pathogens (Kimati et al., 2005).

Despite advances in breeding, seeking the tolerance of cultivars of different species to attack by diseases such as leaf spots (Pinto et al., 2004), and studies related to biology pathogen (Bach & Kimati, 2004), studies have evaluated the fungicidal effect of control over these diseases are constantly exceeded (Ottoni, et al., 2000; Tavares & Souza, 2005) due to the steady influx of new active ingredients on the market.

According to Barlett et al. (2002) and Pinto et al. (2004), the evaluation of fungicides "in vitro" is a technique often used to evaluate the behavior of certain pathogens when subjected to the action of various fungitoxic products under field conditions.

The objective of this study was to evaluate the effectiveness of five fungicides with different active ingredients on the mycelial growth of *B. maydis*.

MATERIAL AND METHODS

The isolate of *B. maydis* was obtained from corn leaves with typical symptoms of the disease, collected in the region of Cuiabá-MT, Brazil. The isolate was cultivated in Petri dishes containing culture medium (potato dextrose agar) for 4 days in growth cameras (BOD) at 25 °C and 12 hours of light. Then the colonies were subcultured by

scraping the mycelial and incubated under a photoperiod of 12 hours and temperature of 25 °C for 72 hours in BOD.

Disks 0.5 cm of diameter of the isolates were removed from the boards of colonies with seven days of age and transferred to the center of Petri dishes (9.0 cm of diameter) containing PDA + fungicides in the respective doses. The fungicides and their doses are described in Table 1. Each of the products were incorporated into the culture medium melting (45-47 °C) by means of dilutions and preparation of stock solutions. After plating, the plates were incubated under a photoperiod of 12 hours at 25 °C for a period of 12 days, at which time the colony took all the petri dish in control (without added fungicide).

Table 1. General characterization of fungicides evaluated for control of mycelial growth of *Bipolaris maydis*.

Active Ingredient	**Commercial name**	**Chemistry group**	**Mode of action**	**% a.i.[1]**
Tetraconazole	Domark	Triazole	Sistemic	0,250
Tebuconazole	Folicur	Triazole	Sistemic	0,325
Azoxystrobin + Cyproconazole	Priori Xtra	Strobirulin + Triazole	Mesostemic + Sistemic	0,150
Trifloxystrobin + Propiconazole	Stratego	Strobirulin + Triazole	Mesostemic + Sistemic	0,200
Trifloxystrobin + Cyproconazole	Sphere	Strobirulin + Triazole	Mesostemic + Sistemic	0,100

[1] % a.i: percentage of active ingredient.

The experimental design was completely randomized design with 5 repetitions; each repetition was represented by a Petri dish.

The evaluations were accomplished by measuring colony growth in two directions perpendicular to each other, taking the value of growth as the average of two measures. These data were transformed into the arc sin √ x/100 and submitted to analysis of variance with the averages subsequently compared by Tukey test at 5% probability.

Of the mycelial growth, the index of mycelial growth speed (IMGS), was made calculations according to the formula described by Oliveira (1991):

$$IMGS = \sum \frac{(D - Da)}{N}$$

Were:
IMGS = index of mycelial growth speed
D = actual diameter of colony
Da = diameter of colony on previous day
N = number of days after of inoculation

RESULTS AND DISCUSSION

The growth of ***B. maydis*** was influenced by fungicides in all evaluations ($p < 0.05$).

During evaluations, the mycelial growth showed significant increase in treatment without fungicide (Figure 1) while the other treatments did not accompany such growth. It was observed that the mycelial growth stood just on the surface of the disc placed in each Petri dish.

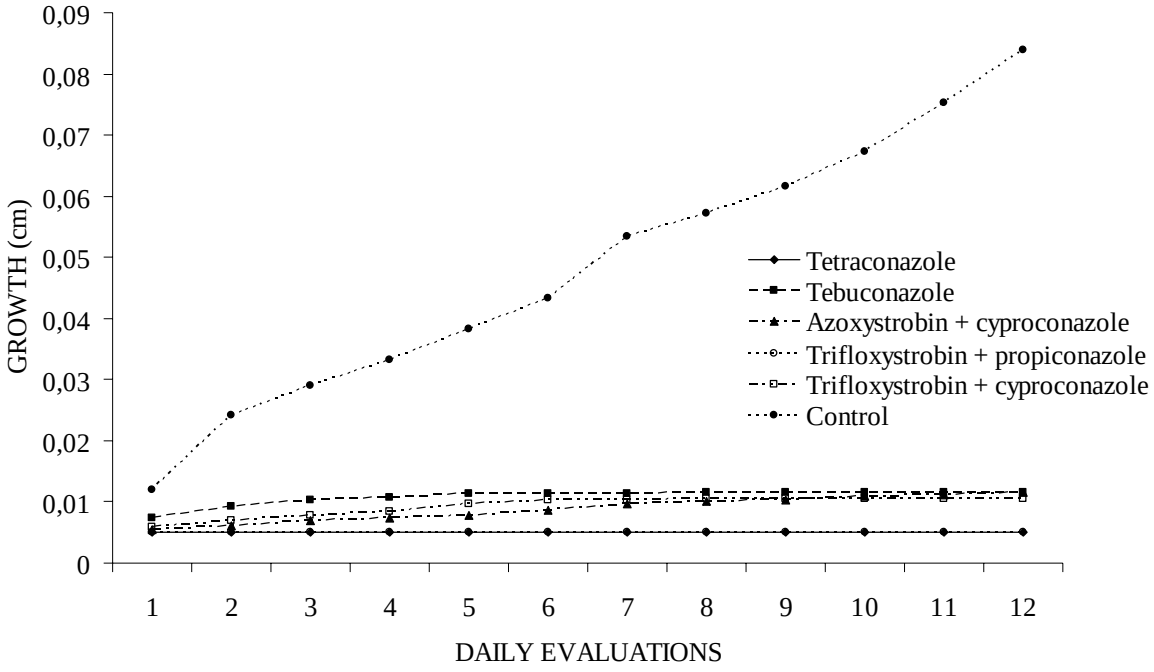

Figure 1. Mycelial growth of ***Bipolaris maydis*** submitted to treatment "in vitro" with fungicides.Tetraconazone: 0,250% i.a.; Tebuconazole: 0,325% i.a.; Azoxystrobin + Cyproconazole: 0,150% i.a.; Trifloxystrobin + Propiconazone: 0,200% i.a.; Trifloxystrobin + Cyproconazole: 0,100 %i.a. Average fungal growth over a 12 day trial.

In the first three evaluations, the highest growth (except for control) was observed in treatment with tebuconazole, which did not differ statistically trifloxystrobin + cyproconazole. Despite these treatments showed values of mycelial growth larger than the others, when compared with control, both tebuconazole as trifloxystrobin + cyproconazole recorded growth well below the control (36% and 27% respectively). From the evaluation performed after three days of incubation here were more differences among fungicides.

The difference in the control of different fungicides on mycelial growth may be related to greater or lesser sensitivity of this isolate to the fungicides tested. Fungicides can act as protective or curative. As protectors, the toxicity of the product is put on the germination of spores, and dressings, is inhibiting the development of the haustorium, or mycelium growth by the fungicide (Silva et al., 2006). The fungicides tested were effective in inhibiting the mycelial growth of isolate of *B. maydis*.

At the last evaluation was done at 12 days after incubation, differences were observed between the treatment, demonstrating again values of pathogen growth when subjected to higher growth in culture medium with tebuconazole. Did not differ with treatments trifloxystrobin + cyproconazole and azoxystrobin + cyproconazole. Although the results were different between the fungicide treatments, it is evident that even those who had higher levels of mycelial growth, the difference with the control reached 86% for tebuconazole.

The rate of mycelial growth rates (IMGS) was highly significant, characterizing the fungus control in some treatments (Table 2).

Table 2. Index of mycelial growth speed (IMGS) of ***Bipolaris maydis*** submitted to treatment "in vitro" with fungicides.

TREATMENT	MEDIUM
Tetraconazole	0,000 A
Tebuconazole	0,866 B
Azoxystrobin + Cyproconazole	0,806 B
Trifloxystrobin + Propiconazole	0,000 A
Trifloxystrobin + Cyproconazole	0,814 B
Control	2,206 C
C.V.	32,9%

Means followed by same letter within each column do not differ by Tukey test at 5% probability.

For the treatments with tebuconazole and trifloxystrobin + propiconazole, we obtained zero value for IMGS, reinforcing previous data that both fungicides were highly effective to control the fungus, preventing their growth.

The mean IMGS of tebuconazole and trifloxystrobin + propiconazole differ from the tebuconazole, azoxystrobin + cyproconazole and trifloxystrobin + cyproconazole values ranged from 0.806 to 0.866 which did not differ among themselves. These values, however, were significantly lower than the average witness IMGS (2.206). These results allow

us to infer the lower capacity of these fungicides on the mycelial growth of ***B. maydis*** the experimental conditions.

Despite the control of mycelial growth, to protect the plant by the application of fungicides before the occurrence of the pathogen or in low disease severity, and delay the onset of the epidemic, reducing the chance of significant damage in promoting this culture. The use of fungicides before penetration and colonization of fungal etiology of diseases such as ***B. maydis***, in areas with high inoculum pressure and environmental conditions conducive to the development of epidemics, can reduce the disease's progress. The leaf spot is influenced by high temperatures and high humidity, so the application of preventive products suitable in these environmental conditions, may be an important tool in managing the disease.

Although effective in controlling the development of ***B. maydis***, the choice and application of fungicides should be preceded by appropriate management. The cultivation in areas with no history of epidemics caused by pathogenic fungi, under unfavorable conditions for its development are measures that can optimize the management of this disease.

Opting for fungicide spraying, you should prioritize which can be used simultaneously to control other diseases. Moreover, these are classified according to the ability of the pathogen to develop resistance to the active principle.

The fungicides studied were classified according to the biochemical action of the product as high risk (strobilurin) and moderate risk (triazole), induction of resistant populations of fungi (Brent & Hollomon, 1998; Gisi et al., 2000).

The risk of induction of resistant populations of pathogenic fungi is much higher when using fungicides whose mode of action is restricted to a specific site in the pathogen (strobilurin and benzimidazole), than others who work at multiple sites (triazole and dithiocarbamate) (Delen & Tosun, 2003). In such cases, the alternation of fungicides with different modes of action or mixtures of two active ingredients with synergistic effect, is a way of managing potential resistance of ***B. maydis*** to chemical treatment.

CONCLUSIONS

The fungicides that showed greater efficiency in controlling the mycelial growth in vitro of ***Bipolaris maydis*** were tetraconazole and trifloxystrobin + propiconazole, featuring the capability to use chemical control in the field.

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