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Vol. 11(19), pp. 1699-1711, 12 May, 2016
DOI: 10.5897/AJAR2015.10727
Article Number: 025BE4858446
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

In vitro susceptibility of *Corynespora cassiicola* isolate from Brazil fields to fungicide

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Received 11 December, 2015; Accepted 10 March, 2016

Corynespora cassiicola **which cause the target spot in soybeans can lead to significant reductions in grain yield. Chemical control mechanisms recommended for disease control was performed with low efficacy in the field due to loss of the pathogen sensitivity to fungicides. This study evaluated the effect of fungicides in inhibiting *C. cassiicola* using *in vitro* test. Four isolates from different regions of Rio Verde - GO were used. The experimental design was completely randomized with nine treatments and five doses (0.0, 0.1, 1.0, 10 and 100 mg). The fungicides, in the various concentrations, were added in PDA medium and poured into Petri dishes, 80 mm in diameter. Then 5 mm discs, containing fungy mycelia, were transferred to the center of the plate and incubated in growth chamber at 25°C with photoperiod of 12 h. The mycelial growth in colony diameter was measured every 24 h. The inhibition percentage of each fungicide on various isolates of fungi was determined, by observing area under the curve of mycelial progress (AUCMP) and by determining the mycelial growth speed rate (MGSR) was determined. All treatments showed a decrease in SRMG with increased applied dose, the fungicide fluazinam had the best performance, with 100% mycelial growth inhibition at all dose tested and in both areas in which the isolate was obtained. The choice of product and dose to be applied directly will be helpful in the chemical control programs ensuring higher yields at the end of the crop cycle.**

Key words: Target spot, *Corynespora cassiicola*, *in vitro,* test.

INTRODUCTION

The fungus, *Corynespora cassiicola* (Berk. & MA Curtis) CT Wei, causal agent of the target spot, is associated with wide range of host species (Silva et al., 1995). In Brazil, the target spot has existed in soybeans since 1976 (Almeida et al., 1976), and as a result of higher susceptible seeding, its incidence has increased in recent seasons, being found in almost all soybean production regions in Brazil (Godoy et al., 2012). In soybean, the losses in yield is up to 20 - 50% (Silva et al., 2008). Control strategies recommended for the disease is the use of resistant cultivars, seed treatment, the rotation/succession of culture with corn and grass species and chemical control (Almeida et al., 2005; Silva et al., 2008).

Fungicides described for complex late season diseases (CLSD) are the same recommended for target spot

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Table 1. Fungicides and doses used in the experiment to evaluate the sensitivity of *Corynespora cassicola* isolates.

Fungicides (active ingredient)	Concentration gi.a. L^{-1} ou Kg^{-1}	Chemical group
Picoxystrobin + Cyproconazole	200 + 80	Strobilurin + Triazol
Pyraclostrobin + Epoxiconazole	133 + 50	Strobilurin + Triazol
Azoxystrobin + Cyproconazole	200 + 80	Strobilurin + Triazol
Pyraclostrobin + Fluxapyroxad	333 + 167	Strobilurin + Carboxamide
Trifloxystrobin + Prothioconazole	150 + 175	Strobilurin + Triazolinthione
Procymidone	500	Dicaboximida
Fluazinam	500	Fenilpiridinilamina
Carbendazim	500	Benzimidazol
Methyl thiophanate	500	Thiophanate
Control treatment (without fungicide)	--	---

control in the shoot of soybean culture, being: azoxystrobin, azoxystrobin + cyproconazole, carbendazim, difenoconazole, flutriafol, pyraclostrobin + epoxiconazole, tebuconazole, methyl thiophanate, methyl thiophanate + flutriafol, trifloxystrobin + cyproconazole, trifloxystrobin + propiconazole (Embrapa, 2007). However, there are concerns about chemical control options, as fungicides from benzimidazole, triazole and strobilurin groups recommended for the control of this disease have presented low efficacy in the field (Godoy et al., 2012).

After these reports on the difficulty in the chemical control of the disease in recent harvests in the Midwest region of Brasil, some studies have shown a variability between populations of *C. cassiicola* and consequently the reduction or loss of the pathogen sensitivity to fungicides (Avozani et al., 2014; Teramoto et al., 2012; Soares et al., 2012). This response has occurred when successive applications of the same product are done in association with improper application conditions (eradicant applications, subdoses and inadequate technology) (Reis et al., 2010).

However, studies that characterize isolates from different regions are scarce and little is known about the variability of the same, making it an obstacle for genetic improvement programs and also to evaluate the efficacy of chemical control due to possible variability of these pathogens.

Considering the difficulties in the control strategy of fungi that cause the target spot and the need for studies on sensitive populations to fungicides, this study aimed at evaluating the sensitivity of *C. cassiicola* isolated from experimental areas of the city of Rio Verde.

MATERIALS AND METHODS

Assay

The experiment was carried out in Phytopathology Laboratory at the University of Rio Verde – UniRV – 2014/2015. The experimental design was a completely randomization with six replicates, using nine fungicides in four doses of active ingredient (AI) [100 ppm (20 mg), 10 ppm (2 mg), 1 ppm (0.2 mg) and 0.1 ppm (0.02 mg)] obtained from the stock solution (Table 1). Four isolates of *C. cassiicola* from different locations in the city of Rio Verde, where field trials (efficacy test to products) had already been done were used in sensitivity tests (Table 2). For each treatment, a control was added without fungicides application.

Isolation and *in vitro* test

For isolation, the trefoils of three plants were selected in the plots with disease symptoms. The material was taken to the Phytopathology Laboratory and the fragments plant tissues were disinfected in a solution of sodium hypochlorite 1% by three minutes. Later, the fragments were washed with distilled water to remove excess. Then, the peace of fragments were distributed into acrylic boxes gerbox (11 x 11 x 3.5 cm) containing a nylon foam and two overlapping sheets of filter paper, moistened with sterile distilled water and kept in chamber growth at 25°C ± 2:12 photoperiod. After fungal growth, the colonies visually recognised were transferred to another dish containing medium of potato dextrose agar (PDA), later kept in chamber growth at 25ºC ± 2:12 photoperiod.

For the *in vitro* tests, the different doses of fungicides were prepared by dissolving the commercial fungicide formulation in sterile deionized water (SDW) until use. They were then further diluted to obtain the desired concentration and poured into plastic Petri dishes (80 mm diameter) and added at the time of PDA culture medium preparation and after been poured into Petri dishes of 80 mm.

The day after culture medium preparation, 6 mm-diameter mycelial plugs of each isolate, taken from seven-day-old colonies, were placed on the center of each dish. The plates were sealed with PVC plastic film and incubated in a growth chamber at 25 ± 2°C and 12 h photoperiod provided by three fluorescent 40 W lamps placed at 50 cm above the plates. When the colony in the control treatment reached the edge of the plates, the diameter of all colonies was measured with a digital calliper as described by Avozani et al. (2014).

Evaluations

The first evaluation took place after 48 h of the experiment. The diameter of each colony was measured in two directions

Table 2. Sites of *Corynespora cassicola isolates*, in the Rio Verde city, used in the *in vitro* sensibility tests.

Characterisation	Sites	Altitude (m)	Coordinates
Isolated A	Agricultural Research Center– CPA	731	S: 17°47'05.0" O: 50°59'47.0"
Isolated B	Rio Doce Farm	751	S: 17°36'10.0" O: 51°32'54.0"
Isolated C	Laje Farm	712	S: 17°40'23.0" O: 50°49'46.0"
Isolated D	São Tomaz Rio do Peixe Farm	689	S: 18°02'30.0" O: 51°02'19.0"

(represented the total growth percentage), at 48 h intervals from the time of inoculation up to the end of the experiment. After measurements, the percentages of inhibition in fungal growth were determined in each treatment, calculating the mycelial growth speed rate (MGSR), used to calculate the inhibition of mycelial growth. This performed MGSR was calculated based on equation MGSR= Σ[(D-Da)/N] (Dias et al., 2005). Where: D = current average diameter of the colony; Da = the average diameter of the colony in the previous day; N = number of hours or days after inoculation.

A completely randomized experimental design using four replicates was adopted. A Petri dish was used as an experimental unit. Data on fungal colony diameter were transformed into growth percentage. The inhibitory concentration (IC_{50}) able to inhibit 50% of mycelial growth for evaluated fungicides and each isolate was calculated from the generated equation.

Classification of isolates based on fungicides sensitivity used was performed according to the criteria proposed by Edginton et al. (1971), in which chemical compounds with IC_{50} less than 1 mg/L was considered highly fungitoxic, with IC_{50} between 1 and 50 mg/L are moderately fungitoxic and IC_{50} higher than 50 mg/L are not fungitoxic. A useful tool to quantify the shift in sensitivity to a fungicide in a fungus is the sensitivity reduction factor (SRF) (Kunz et al., 1998), which is calculated by dividing the IC50 of the fungal strain suspected of having reduced/lost its sensitivity by the IC50 of the sensitive strain. SRF value of 1 means no change in sensitivity, while values > 1 indicate the shift for sensitivity reduction (Reis et al., 2010; Russel, 2004).

Data analysis

All the assays were repeated twice using a completely randomised experimental design with four replicates per treatment. Data were subjected to Shapiro-Wilkand Bartlett tests (significance level, P>0.05) for normality and homoscedasticity, respectively. Distribution of isolates (% inhibition colonisation) was subjected to one-way ANOVA, and means were compared using Scott-Knott tests (P< 0.05) (Scott and Knott, 1974). The regression model was fit to the quantitative variables as log transformation using the Sigma Plot 11.0 program.

RESULTS

Evaluation of mycelial growth speed rate (MGSR) of *C. cassicola* isolates by different fungicides doses

After the calculation of the MGSR and in accordance with the regression analysis for each variable, it was observed that generally all fungicides produced decrease in growth of fungal mycelia with increasing dose. However, the picoxystrobin + cyproconazole treatments (Figure 1), pyraclostrobin + epoxyconazole (Figure 2), azoxystrobin + cyproconazole (Figure 3), pyraclostrobin + epoxiconazole + fluxapyroxad (Figure 4) and procymidone (Figure 5) showed significant reduction ($p < 0.05$) in mycelial growth with increased rates of fungicides for all isolates.

For the treatment containing the fungicide trifloxystrobin + prothioconazole (Figure 6) according to regression analysis to MGSR, the isolates from CPA, Rio Doce Farm and São Tomaz Rio do Peixe Farm showed a significant reduction in mycelial growth of *C. cassicola*. However, for the isolate from Laje farm, there was no dose effect in reducing growth in the studied treatment.

Treatment containing the fungicide carbendazim (Figure 7) and methyl thiophanate (Figure 8) showed a significant reduction in mycelial growth of *C. cassicola* in isolates from CPA, Laje Farm and São Tomaz Rio do Peixe Farm observed by regression analysis of MGSR. However, isolates from Rio Doce Farm showed no dose effect in reducing the growth.

Inhibition percentage of *C. cassicola* isolate by different doses of fungicides

In the inhibition evaluations, a partial or total inhibition of *C. cassicola* was observed. The control (0.0 mg) had mycelial growth of 100% in all evaluated replications. For isolates from CPA, there was 100% of inhibition at doses of 100 mg in all the treatments, except for treatment with methyl thiophanate where inhibition was 68.54% (Table 3). The treatment containing trifloxystrobin + prothioconazole inhibited 100% of the growth of *C. cassicola* at doses of 10 and 100 mg from São Tomaz Rio do Peixe Farm. On the same property, it was noted that the pyraclostrobin + epoxiconazole + fluxapyroxad, trifloxystrobin + prothioconazole, procymidone, fluazinam and carbendazim treatments, inhibited 100% of mycelial growth of *C. cassicola* in doses of 10 and 100 mg, with a

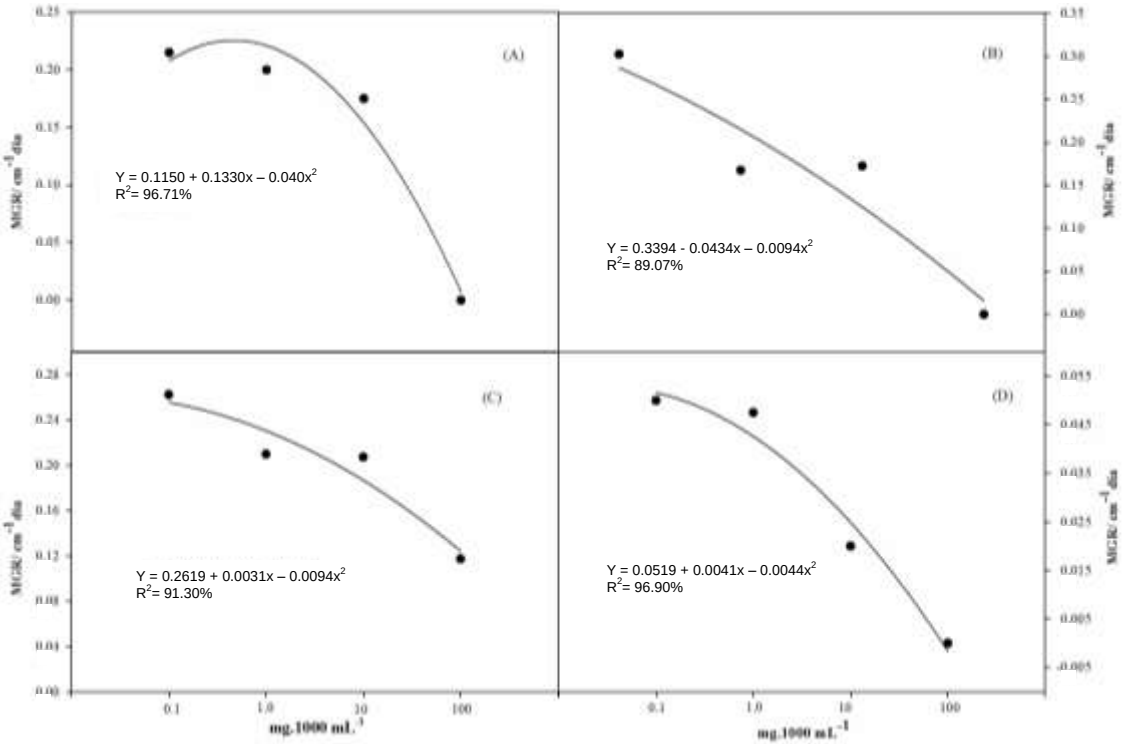

Figure 1. Mycelial growth rate (MGR - cm/day) of isolates: A (São Tomaz); B (CPA); C (Rio Doce Farm); D (Laje Farm) after treatment with fungicide picoxystrobin + cyproconazole, for used doses.

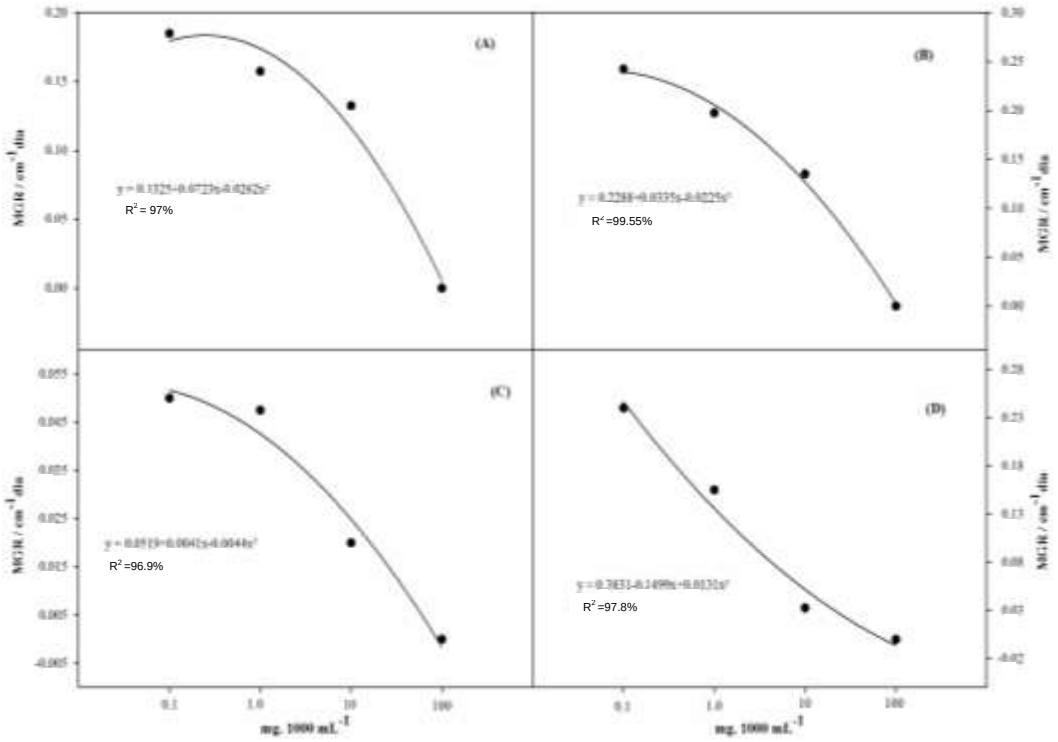

Figure 2. Mycelial growth rate (MGR - cm/day) of isolates: A (CPA); B (Rio Doce Farm); C (Laje Farm); D (São Tomaz Farm) after treatment with fungicide pyraclostrobin + epoxyconazole, in function of used doses.

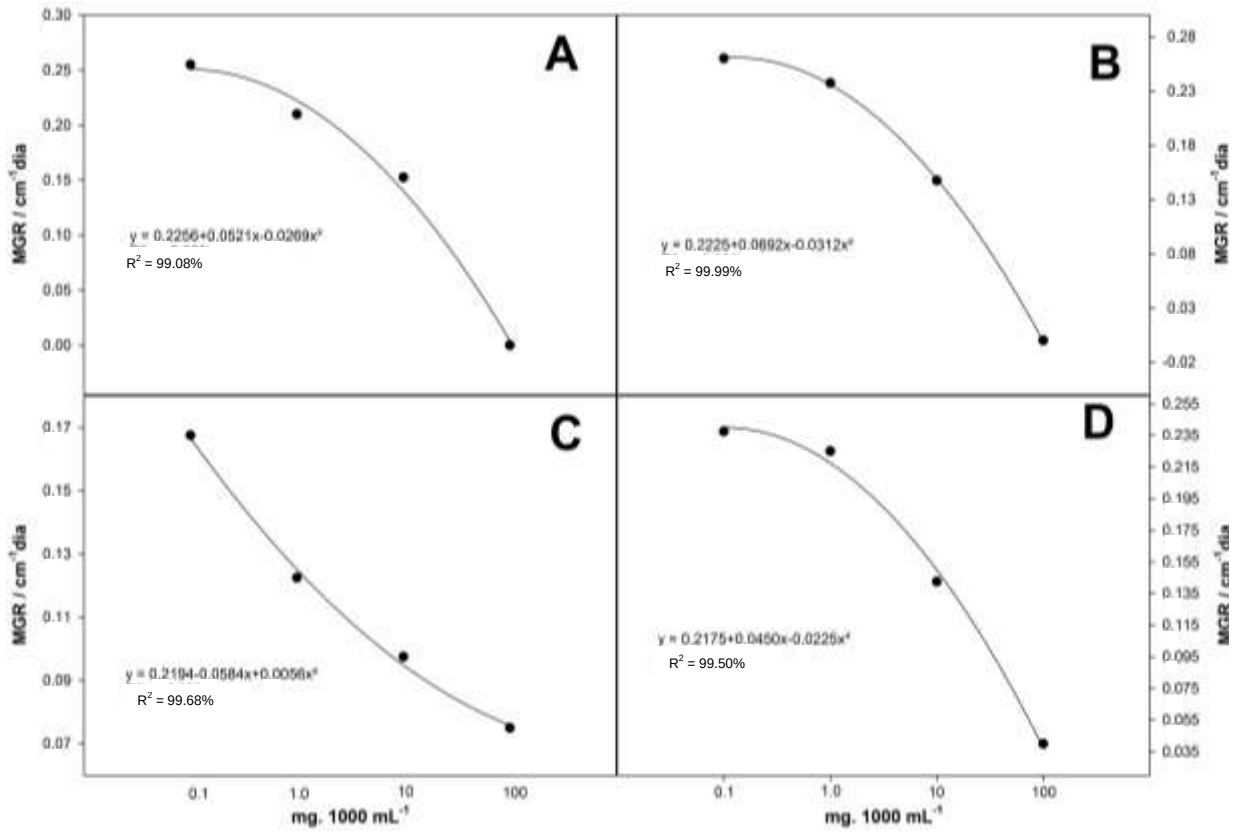

Figure 3. Mycelial growth rate (MGR - cm/day) of isolates: A (CPA); B (Rio Doce Farm); C (Laje Farm); D (São Tomaz Farm) after treatment with the fungicide azoxystrobin + cyproconazole, in function of used doses.

significant difference when compared with the doses of 1.0 and 0.1 mg. For azoxystrobin + cyproconazole treatments and methyl thiophanate in the dose of 100 mg, 79.22 and 55.52% of inhibition respectively were observed, which were lower percentages than other treatments that reached 100% of inhibition when 100 mg of active ingredient was used (Table 3).

In assessing the isolates from Rio Doce Farm, treatments that stood out with 100% of mycelial growth inhibition of *C. cassicola* were fluazinam and methyl thiophanate in four doses: 0.1, 1.0, 10 and 100 mg. On the other hand, picoxystrobin + cyproconazole and procymidone treatments achieved the maximum inhibition of 66.72 and 80.97%, respectively (Table 3). The treatment containing fluazinam had the best result in the mycelial growth inhibition, similar in the four doses (0.1, 1.0, 10 and 100 mg), inhibiting 100% of the growth in all isolates of the study areas (Table 3).

Evaluation of the inhibitory concentration of the *C. cassicola* isolates

Low concentrations of the fungicide picoxystrobin + cyproconazole reduced growth of isolates from CPA and Sao Tomaz farm at IC_{50} fungal growth, showing significant difference considering the other isolates. The IC_{50} for picoxystrobin + cyproconazole fungicide for isolate from Rio Doce and Laje Farms was not significant.

Treatment with pyraclostrobin + epoxiconazole in both study areas with their isolates was significant at the level of 0.01%, so the IC_{50} had significant effect on this active ingredient, being classified as highly fungitoxic (Table 4). In most cases, azoxystrobin + cyproconazole when compared with the other treatments applied in the Laje Farm, showed no significant effect. In the other areas of study, with the exception of Laje Farm, the IC_{50} demonstrated that azoxystrobin + cyproconazole has fungicidal action. Pyraclostrobin + epoxiconazole + fluxapyroxad had similar effect on other treatments for its areas, where the IC_{50} indicated high fungitoxic action of the active ingredient used.

The trifloxystrobin + prothioconazole treatment showed significant effect on the four study areas, where the IC_{50} showed high fungicidal activity of its active ingredient. Procymidone (Table 4) also showed IC_{50} with high fungicidal action in all the studied areas. Treatment with fluazinam at IC_{50} showed no significant effects. The carbendazim treatment showed no significant difference for the isolates from Rio Doce and Laje Farms.

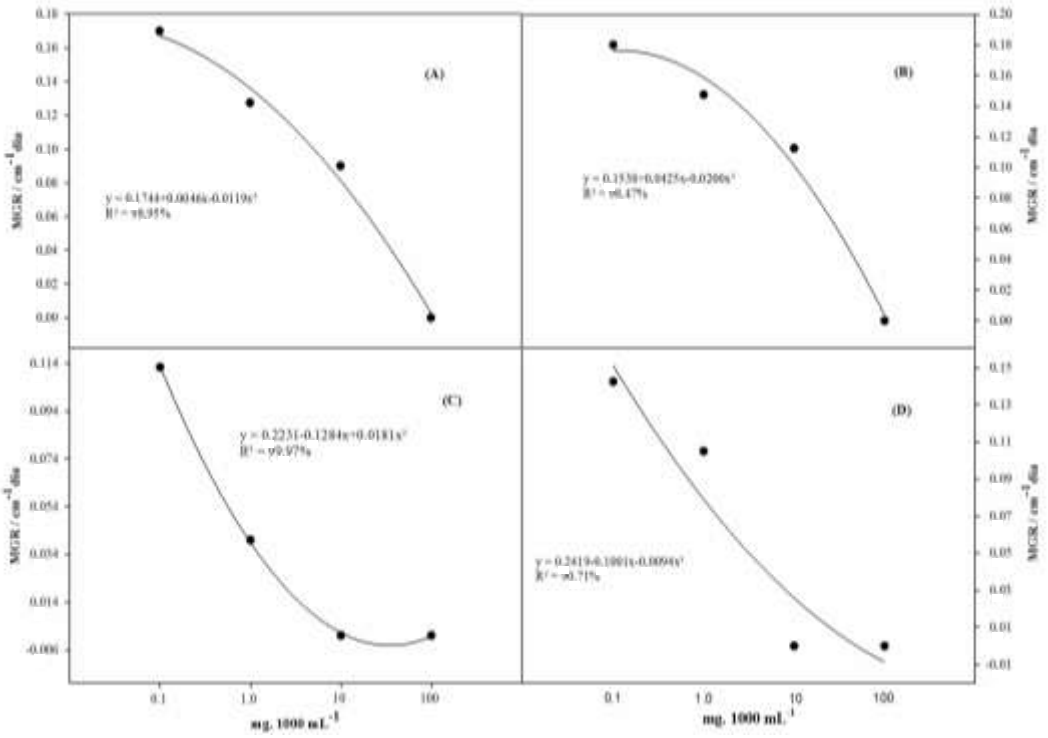

Figure 4. Mycelial growth rate (MGR- cm/day) of isolates: A (CPA); B (Rio Doce Farm); C (Laje Farm); D (São Tomaz Farm) after treatment with the fungicide fluxapyroxad + pyraclostrobin + epoxyconazole, in function of the used doses.

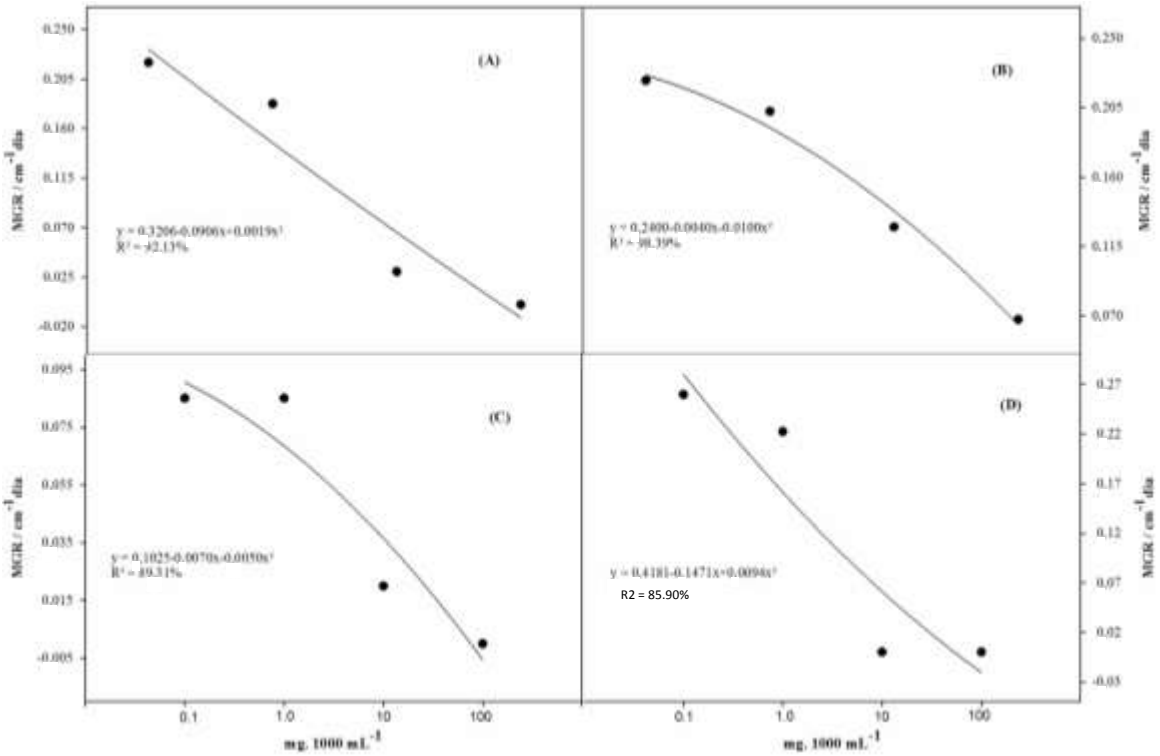

Figure 5. Mycelial growth rate (MGR- cm/day) of isolates: A (CPA); B (Rio Doce Farm); C (Laje Farm); D (São Tomaz Farm) after treatment with the fungicideprocymidone, in the function of used doses.

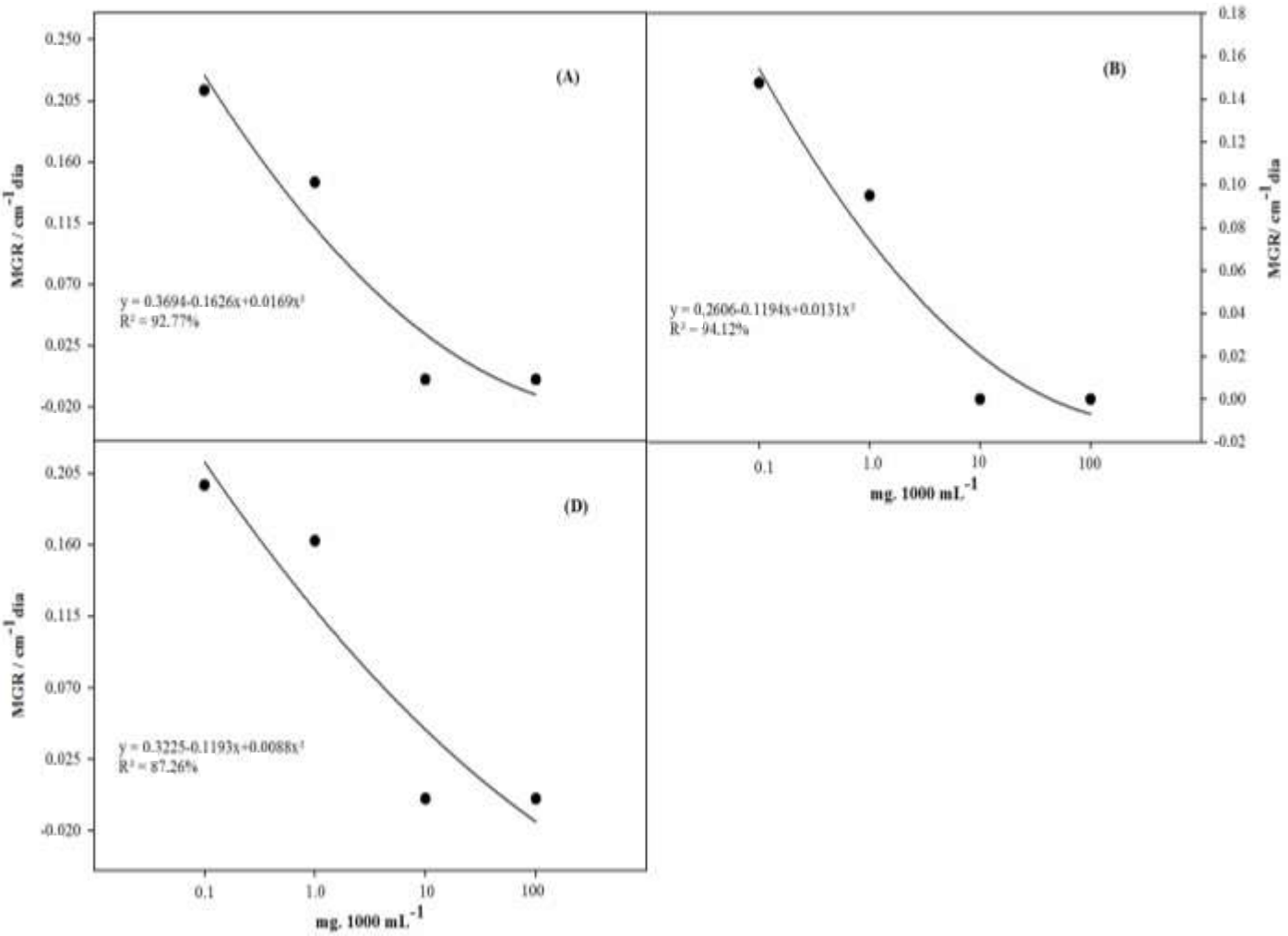

Figure 6. Mycelial growth rate (MGR- cm/day) of isolates: A (CPA); B (Rio Doce Farm); and D (São Tomaz Farm) after treatment with the fungicide trifloxystrobin + prothioconazole, in the function of used doses.

Methyl thiophanate demonstrated significance level of 0.01% for isolates from CPA and Laje Farm, as for isolates from Rio Doce and São Tomaz farms, there was no significant difference.

The fungicide pyraclostrobin + epoxiconazole was highly toxic for isolates from CPA and Laje Farm, however, to isolates from São Tomaz and Rio Doce farms it was moderately toxic. The fungicide azoxystrobin + cyproconazole was highly toxic to isolates from CPA and moderately toxic for isolated from São Tomaz and Rio Doce farms. However, to isolate from Laje Farm, the cyproconazole + azoxystrobin fungicide was not toxic. The pyraclostrobin + epoxiconazole + fluxapyroxad fungicide was highly toxic for all isolates tested. The trifloxystrobin + prothioconazole fungicide was moderately toxic for isolates from São Tomaz Farm and, the other isolates were highly fungitoxic. Procymidone was moderately toxic to isolate from São Tomaz Farmand to the others, it was highly toxic.

Fluazinam was highly toxic to all isolates used. Carbendazim was also highly toxic to isolates from CPA and São Tomaz Farm. For the isolate from Laje Farm, the fungicide carbendazim was moderately toxic and showed no antifungal effect on isolate from Rio Doce Farm. For the fungicide, methyl thiophanate was slightly toxic to isolates from CPA and Laje Farm, were slightly toxic and showed no fungitoxicity for all other isolates.

DISCUSSION

The differences in the behavior of the isolates from different areas indicate the possible change of genetic variability of these isolates causing low sensitivity to

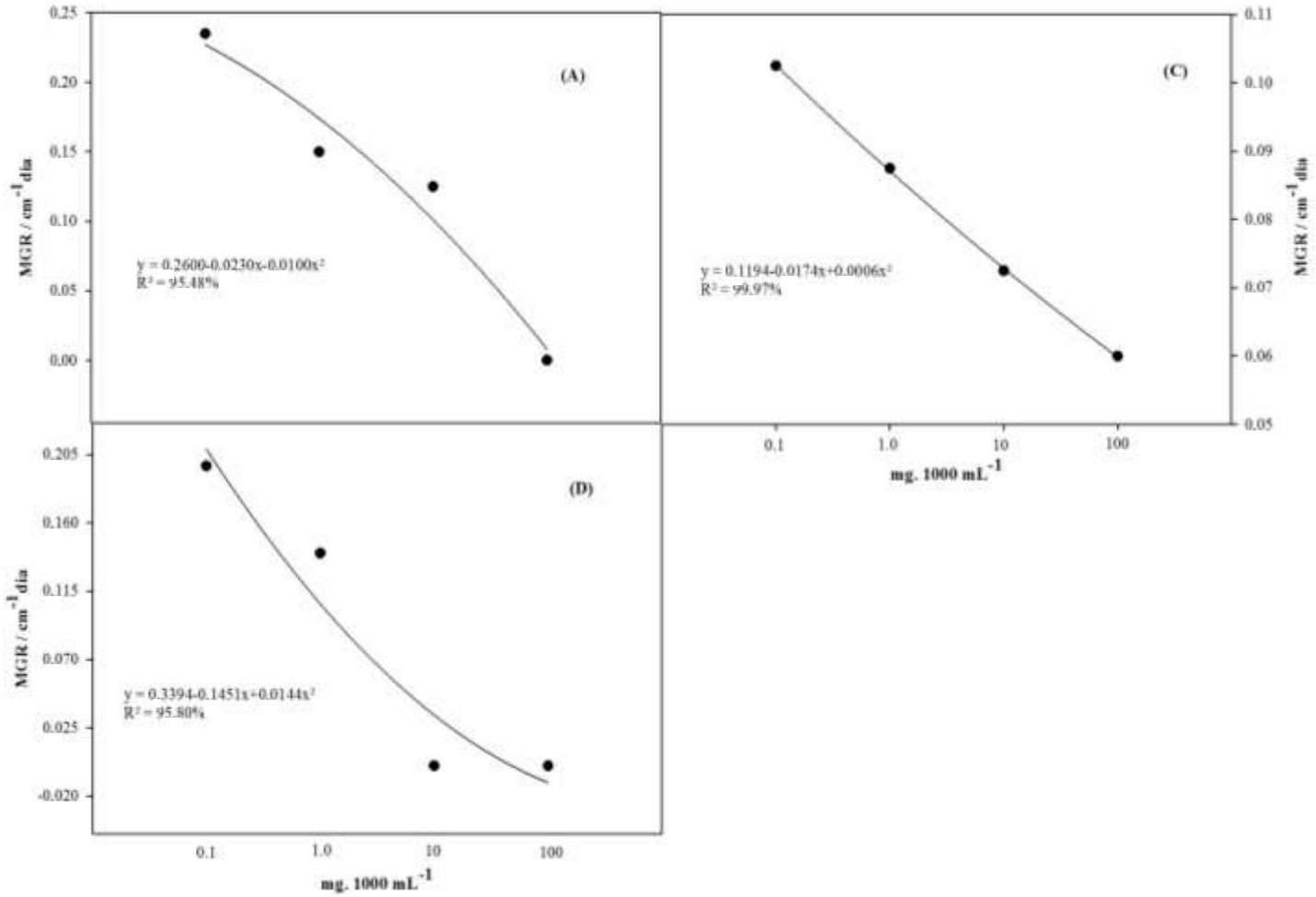

Figure 7. Mycelial growth rate (MGR - cm/day) of isolates: A (CPA); C (Laje Farm) and D (São Tomaz Farm), after treatment with the fungicide carbendazim, in the function of used doses.

fungicides. It is known that the abuse of systemic molecules to control pathogens causes reduction in the sensitivity to products (Reis et al., 2010). In some studies, the fungicide carbendazim had low efficiency in controlling the target spot, which could have been as a result of the resistance to this chemical group on the pathogen (Teramoto et al., 2013; Avozani et al., 2014). However, in this work, carbendazim fungicide did not appear to be inefficient in its toxicity. On the other hand, there was a highlight for methyl thiophanate considering its percentage inhibition of mycelial growth, which was less efficient in almost all locations.

The fungicides belonging to the chemical group of benzimidazoles act on fungi by inhibiting α and β tubulin specific proteins (Coutinho et al., 2006). The affinity of benzimidazole with tubulin is the main factor that determines the fungicidal activity in organisms. The higher the affinity, the more sensitive the organism to the fungicide.

Probably due to various selection factors, a mutation occurred to β-tubulin protein gene leading to formation of β-tubulin protein that has reduced binding affinity with benzimidazole leading to a new generation of resistant population (Brent, 1995; Hewitt, 1998). Therefore, the high selection pressure caused by intensive use of fungicides such as benzimidazoles, may result in the selection of resistant fungus at a short period of time (Parreira et al., 2009), explaining the difference of inhibition displayed by methyl thiophanate.

According to Deising et al. (2008), the resistance acquired by the pathogen population to the product is directly proportional to applied doses, frequency of application, degree of coverage, persistence in culture or in soil and the size of the treated area. This justifies the lower results observed for azoxystrobin + cyproconazole treatment at the highest dose when compared with the other treatments (Table 3). In the evaluations performed in this research, the product Fluazinam showed 100% efficient in the percentage inhibition of mycelial growth in all doses and in both areas in which they were obtained.

In previous work carried out by Töfoli et al. (2003), the fungicide fluazinam was also responsible for higher levels

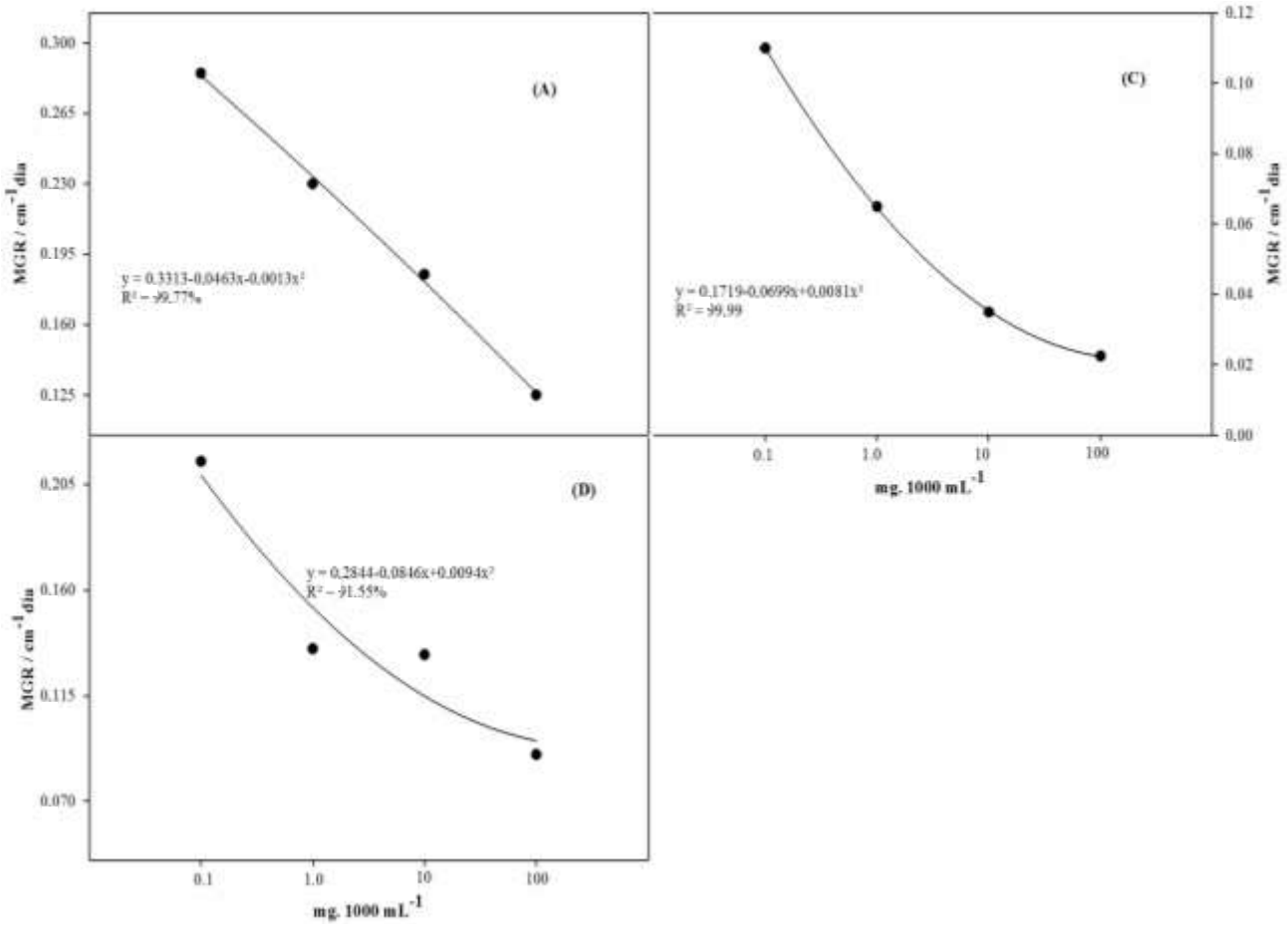

Figure 8. Mycelial growth rate (MGR-cm/day) of isolates: A (CPA); C (Laje Farm) and D (São Tomaz Farm), for the treatment containing the fungicide thiophanate methyl, in the function of used doses.

of mycelial growth inhibition in isolates of *Alternaria solani* and noted that the action of this fungicide showed complete inhibition of spore germination of *A. solanis* from doses of 1 µg.mL^{-1}. In other studies using the same product, fluazinam, Guimarães et al. (2008) found efficiency in the control of *Monosporascus cannonballus* at different doses.

The inhibitory concentration (IC_{50}) studies for different fungicides and specific to *C. cassiicola* in soybean are scarce, yet it is very useful in carrying out research and sensitivity monitoring, especially in areas where the control of this disease is not being efficient (Avozani et al., 2014). The isolates from the Laje Farm treated with azoxystrobin + cyproconazole showed no significant effect on the IC_{50} values but had high IC_{50} value.

This effect may be due to high-pressure selectivity for this area specifically, or the inappropriate use of the product in the past situations, which may, according to the obtained data, have selected individuals resistant to the products. The high value of IC_{50} (Table 4) clearly shows that this area of study (Laje Farm), the respective active component has low fungicide action. Since treatment with fluazinam at the IC_{50} showed no significant results, however, was highly fungitoxic for all used isolates. In this study, thecarbendazim treatment showed no significant difference for the isolate from Rio Doce and Laje farms, and for the Laje Farm, according to the IC_{50}, this fungicide can be classified as moderately fungitoxic, according to the criteria proposed by Edgington et al. (1971). Furthermore, Avozani et al. (2014) found that the isolates of *C. cassiicola* showed less sensitivity to the carbendazim active ingredient and the cyproconazole active ingredient presented best valore of IC50. In studying the sensitivity of isolates submitted to the treatments, it was noted that the increased resistance of the isolates from the Laje Farm for some treatments, should necessitate the investigation of the previous management methods in this region that could have contribute to the multiplication of resistant populations.

Conclusion

Generally, fungicides used showed good control levels

Table 3. Inhibition percentage of *Corynespora cassicola* from Rio Verde towns, after different fungicides doses.

Active ingredient	Sampling places	Inhibition (%)				CV
		0.1 mg	1.0 mg	10 mg	100 mg	
Picoxistrobina + Ciproconazol	CPA	23.72^{c}	58.05^{b}	57.32^{b}	100.00^{a}	9.04
Piraclostrobina + Epoxiconazol		60.77^{b}	53.09^{c}	66.50^{b}	100.00^{a}	
Azoxistrobina + Ciproconazol		36.09^{d}	46.78^{c}	61.58^{b}	100.00^{a}	
Piraclostrobina + Epoxiconazol + Fluxapyroxad		58.00^{b}	67.44^{c}	77.20^{b}	100.00^{a}	
Trifloxistrobina + Protioconazol		46.08^{c}	62.89^{b}	100.00^{a}	100.00^{a}	
Procimidona		44.73^{d}	54.54^{c}	92.55^{b}	100.00^{a}	
Fluazinam		100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	
Carbendazim		40.61^{c}	62.90^{b}	67.71^{b}	100.00^{a}	
Tiofanato Metílico		28.61^{b}	41.25^{c}	53.49^{b}	68.54^{a}	
Picoxistrobina + Ciproconazol	Fazenda Laje	8.77^{c}	15.66^{b}	25.37^{b}	100.00^{a}	20.08
Piraclostrobina + Epoxiconazol		16.84^{d}	33.33^{c}	79.78^{b}	100.00^{a}	
Azoxistrobina + Ciproconazol		14.44^{c}	19.33^{c}	40.34^{b}	79.21^{a}	
Piraclostrobina + Epoxiconazol + Fluxapyroxad		43.69^{b}	45.66^{b}	100.00^{a}	100.00^{a}	
Trifloxistrobina + Protioconazol		31.37^{b}	37.38^{b}	100.00^{a}	100.00^{a}	
Procimidona		10.13^{c}	21.70^{b}	100.00^{a}	100.00^{a}	
Fluazinam		100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	
Carbendazim		25.31^{c}	45.73^{b}	100.00^{a}	100.00^{a}	
Tiofanato Metílico		24.57^{c}	33.39^{b}	46.97^{a}	55.52^{a}	
Picoxistrobina + Ciproconazol	São Tomaz Rio do Peixe	8.78^{c}	15.66^{b}	23.37^{b}	100.00^{a}	20.08
Piraclostrobina + Epoxiconazol		16.84^{d}	33.33^{c}	79.78^{b}	100.00^{a}	
Azoxistrobina + Ciproconazol		14.45^{c}	19.33^{c}	40.34^{b}	79.22^{a}	
Piraclostrobina + Epoxiconazol + Fluxapyroxad		43.70^{b}	45.66^{b}	100.00^{a}	100.00^{a}	
Trifloxistrobina + Protioconazol		31.38^{b}	37.38^{b}	100.00^{a}	100.00^{a}	
Procimidona		10.13^{c}	21.70^{b}	100.00^{a}	100.00^{a}	
Fluazinam		100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	
Carbendazim		25.31^{c}	45.73^{b}	100.00^{a}	100.00^{a}	
Tiofanato Metílico		24.57^{c}	33.39^{b}	46.97^{a}	55.52^{a}	
Picoxistrobina + Ciproconazol	Fazenda Rio Doce	27.13^{c}	41.35^{b}	42.26^{b}	66.72^{a}	8.10
Piraclostrobina + Epoxiconazol		32.75^{d}	45.15^{c}	61.92^{b}	100.00^{a}	
Azoxistrobina + Ciproconazol		27.97^{c}	33.42^{c}	59.08^{b}	100.00^{a}	
Piraclostrobina + Epoxiconazol + Fluxapyroxad		50.57^{d}	59.10^{c}	68.14^{b}	100.00^{a}	
Trifloxistrobina + Protioconazol		59.08^{c}	73.28^{b}	100.00^{a}	100.00^{a}	

Table 3. Cont'd.

Procimidona	37.28[c]	43.48[c]	63.82[b]	80.97[a]
Fluazinam	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Carbendazim	69.03[b]	100.00[a]	100.00[a]	100.00[a]
Tiofanato Metílico	100.00[a]	100.00[a]	100.00[a]	100.00[a]

Analysis by one way ANOVA. Means followed by same letter in the column are not significantly different according to the Scott and Knott's test at 5% probability.

Table 4. Inhibitory concentration at 50% and the sensitivity reduction factor (SRF) from different isolates of *Corynespora cassicola* for fungicides.

Sampling places – product	Equation*	Inhibition (%)			
		R^2	*P*	IC_{50}**	SRF
Picoxistrobina+ciproconazol					
A- CPA	Y= 9.91 Ln(x) + 48.36	88.89	< 0.01	1.18	1.18
B- Fazenda Rio Doce	Y= 5.20 Ln(x) + 38.38	88.37	***n.s.	9.34	9.34
C- Fazenda Laje	Y= 4.71 Ln(x) + 72.20	92.51	***n.s.	0.009	0.009
D- Fazenda São Tomaz	Y= 12.31 Ln(x) + 23.25	74.98	< 0.01	8.78	8.78
Piraclostrobina+epoxiconazol					
A- CPA	Y= 6.36 Ln(x) + 62.76	83.56	< 0.01	0.13	0.13
B- Fazenda Rio Doce	Y= 9.49 Ln(x) + 49.03	93.02	< 0.01	1.11	1.11
C- Fazenda Laje	Y= 8.24 Ln(x) + 72.09	79.90	< 0.01	0.07	0.07
D- Fazenda São Tomaz	Y= 12.31 Ln(x) + 42.69	96.45	<0.01	1.81	1.81
Azoxistrobina+ciproconazol					
A- CPA	Y= 8.97 Ln(x) + 50.78	90.99	< 0.01	0.92	-
B- Fazenda Rio Doce	Y= 10.50 Ln(x) + 49.03	90.25	< 0.01	1.10	-
C- Fazenda Laje	Y= 7.33 Ln(x) + 20.22	99.48	***n.s.	58.13	-
D- Fazenda São Tomaz	Y= 9.35 Ln(x) + 27.57	88.92	< 0.01	11.01	-

distinguishing between places where the experiments were carried out. All treatments caused an increase in productivity as compared to the control treatment. The fluazinam fungicide was better among the other fungicides with 100% of mycelial growth inhibition in all doses and in all areas in which the isolate was obtained so it is considered highly fungitoxic. The low sensitivity of these pathogens to some molecules can guide the development of management strategies reducing the loss in yield and quality of crops around the world.

Table 4. Cont'd.

Piraclostrobina+epoxiconazol+fluxapyroxad					
A- CPA	Y= 5.89 Ln(x) + 68.87	94.59	< 0.01	0.04	0.04
B- Fazenda Rio Doce	Y= 6.83 Ln(x) + 61.59	88.49	< 0.01	0.18	0.18
C- Fazenda Laje	Y= 10.44 Ln(x) + 63.31	85.95	< 0.01	0.28	0.28
D- Fazenda São Tomaz	Y= 9.69 Ln(x) + 61.18	81.38	< 0.01	0.31	0.31
Trifloxistrobina+protioconazol					
A- CPA	Y= 8.64 Ln(x) + 67.30	89.36	< 0.01	0.13	0.13
B- Fazenda Rio Doce	Y= 6.49 Ln(x) + 75.62	89.76	< 0.01	0.02	0.02
C- Fazenda Laje	Y= 6.06 Ln(x) + 81.40	60.00	< 0.01	0.006	0.006
D- Fazenda São Tomaz	Y= 11.66 Ln(x) + 53.76	83.35	< 0.01	1.38	1.38
Procimidona					
A- CPA	Y= 8.85 Ln(x) + 62.77	92.26	< 0.01	0.24	0.24
B- Fazenda Rio Doce	Y= 6.57 Ln(x) + 48.82	96.22	< 0.01	1.97	1.97
C- Fazenda Laje	Y= 10.05 Ln(x) + 54.53	91.69	< 0.01	0.64	0.64
D- Fazenda São Tomaz	Y= 15.11 Ln(x) + 40.56	84.79	< 0.01	1.87	1.87
Carbendazim					
A- CPA	Y= 7.95 Ln(x) + 58.65	93.00	< 0.01	0.34	0.34
B- Fazenda Rio Doce	Y= 100.00	-	***n.s.	-	-
C- Fazenda Laje	Y= 3.22 Ln(x) + 42.46	93.07	***n.s.	10.40	10.40
D- Fazenda São Tomaz	Y= 12.09 Ln(x) + 53.84	88.72	< 0.01	0.73	0.73
Tiofanato metílico					
A- CPA	Y= 5.73 Ln(x) + 41.37	99.78	< 0.01	4.51	4.51
B- Fazenda Rio Doce	Y= 100.00	-	***n.s.	-	-
C- Fazenda Laje	Y= 7.96 Ln(x) + 49.46	97.45	< 0.01	1.07	1.07
D- Fazenda São Tomaz	Y= 3.44 Ln(x) + 36.15	55.01	***n.s.	56.04	56.04

*y = Percentage of mycelial growth inhibition, x = concentration of the fungicide; ** calculated by the equation concentration (mg / L); *** n.s. = non significant.

Conflict of Interests

The authors have not declared any conflict of interests.

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