EVALUATION OF THE ASSOCIATION OF 8-HYDROXYQUINOLINE WITH FUNGICIDES AGAINST PHYTOPATOGENIC FUNGI *Fusarium meridionale* and *Fusarium graminearum*

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INTRODUCTION

The use of fungicides is one of the most important aspects in agriculture to protect rice seeds during storage, as well as in the field, preventing the growth of fungi that can produce toxins (FATMA et al., 2018). The application of fungicides helps to reduce the incidence of *Fusarium* infection, also reducing the levels of mycotoxins in commercial grains (SHISHATSKAYA et al., 2018). Considering the importance of the economic impact caused by the contamination of grains by phytopathogenic fungi, and also the use of agricultural fungicides, our group evaluated the efficacy of Mancozeb (MZ), Difenoconazole (Score®) and 8-Hydroxyquinoline (8-HQ) individually and in association, against *Fusarium graminearum* and *Fusarium meridionale*.

Thus, this work presents the evaluation of the minimum inhibitory concentration, double and three-dimensional synergism and inhibition of mycelial growth of *Fusarium* spp. treats with agricultural fungicides Mancozeb and Difenoconazole, in addition of the antifungal 8-hydroxyquinoline.

MATERIAL AND METHODS

Antifungal susceptibility test through determination of minimum inhibitory concentration (MIC): MIC of Mancozeb (MZ), Difenoconazole (Score®) and 8-hydroxyquinoline (8-HQ) were determined by the broth microdilution method, according to the M38-A2 protocol (CLSI, 2008), where conidia inocula (1.10^3 to 3.10^3 UFC.mL^{-1}) was prepared in cultures obtained on potato dextrose agar (PDA) at 32 °C. Assays were conducted with RPMI medium, containing L-glutamine (without sodium bicarbonate), buffered to pH 7 with 0.165 mol^{-1} MOPS (3[N-Morpholino] propanesulfonic acid). The MIC values were defined as, the lowest concentration of antifungals, and 8-OH in which the *Fusarium* species did not demonstrate visible growth within 48 h.

Checkerboard test: the interaction of 8-HQ with MZ and Score® was evaluated for all strains using the chess method, where the requirements tested vary in MIC/8, MIC/4, MIC/2, MIC, MICx2, MICx4 and MICx8 for each agent. Considering that the assays, were performed in triplicate and incubated at 32 °C for 48 h, the interaction effect was defined quantitatively as an index of fractional inhibitory concentration (IFI), for knowledge, synergism was defined when FICI≤0.5, antagonism when FICI>4 and indifference when 0.5<FICI≤4 (DA COSTA et al., 2020).

Three-dimensional checkerboard: the three-dimensional gridded assay combining MZ, Score® and 8-HQ was performed according to Stein et al. (2015), with slight modifications for each strain and agent, as selected concentration ranges depended on pre-determined in the MIC, then MIC/8,

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MIC/4, MIC/2, MIC and MICx2 rules were used. As microplates were filled with 100 µL of a solution of the three agents at different application levels from suppliers with 100 µL of the fungal suspensions, the microplates were incubated for 48 h at 32 °C, and the experiment was carried out in duplicate and for the sake of knowledge, the synergism was defined when FICI≤0.75, antagonism when FICI>4 and indifference when 0.75<FICI≤4, and the fractional inhibitory concentration index (FICI) for triple antifungal combination (DA COSTA et al., 2020).

Evaluation of mycelial growth inhibition in a petri dish: fungal colonies of isolates of *F. graminearum* (09TR107) and *F. meridionale* (09MI21), were cultivated in the Applied Mycology Research Laboratory - Faculty of Pharmacy, Federal University of Rio Grande do Sul, in potato dextrose agar (BDA) for 7 days to remove discs. Mycelium with 6 mm in diameter, which were transferred to the center of new petri dishes with PDA medium containing different concentrations of the agents (25, 50, 100, 200, 200 and 800 $\mu g.mL^{-1}$), in addition to the control, without treatment. These were kept at 25±2 °C in continuous dark. Colony diameter was measured after 6 days, in two perpendicular directions, and three replicates per strain were used (BECHER et al., 2010; SPOLTI et al., 2012).

RESULTS AND DISCUSSION

All agents tested were able to inhibit the growth of *F. graminearum*, *F. meridionale* and *F. asiaticum*. MIC values ranged from 0.25 $\mu g.mL^{-1}$ to 8 $\mu g.mL^{-1}$ (Table 1).

Table 1. Values of minimum inhibitory concentrations (MICs) for fungicides

Strains	MZ	Score®	8-HQ
		$\mu g.mL^{-1}$	
09MI21	4.00 ± 0.00	4.00 ± 0.94	0.50 ± 0.00
09TR107	8.00 ± 1.88	4.00 ± 1.88	0.50 ± 0.12
9AR04	0.25 ± 0.12	8.00 ± 1.88	2.00 ± 0.47

Values expressed as mean ± standard deviation. 09MI21: *F. meridionale*; 09TR107: *F. graminearum*; 09AR04: *F. asiaticum*. MZ: Mancozeb; 8-HQ: 8-hydroxyquinoline.

In a double association test, the fungicide MZ + 8-HQ presented synergism for *Fusarium meridionale* and *Fusarium graminearum*. The association between Score® + 8-HQ showed synergism only for *Fusarium meridionale*. As for *Fusarium asiaticum*, both associations did not show synergism (table 2).

Table 2. MIC values for fungicides

Strains	MZ + 8-HQ	ICIF	Score® + 8-HQ	ICIF
		$\mu g.mL^{-1}$		
09MI21	1.00 ± 0.06	0.31*	2.00 ± 0.50	0.50*
09TR107	0.50 ± 0.06	0.28*	1.00 ± 0.06	1.00
09AR04	0.50 ± 0.12	1.12	2.00 ± 0.06	1.25

Values expressed as mean ± standard deviation. *: Synergism; 09MI21: *F. meridionale*; 09TR107: *F. graminearum*; 09AR04: *F. asiaticum;* MZ: Mancozeb; 8-HQ: 8-hydroxyquinoline.

In triple association, both fungicides, MZ, Score® and 8-HQ presented synergism against *Fusarium meridionale* (09MI21) and *Fusarium graminearum* (09TR107) (Table 3).

Table 3. Checkerboard values in triple association of Mancozeb, Score® and 8-Hydroxyquinoline fungicides against *Fusarium meridionale* (09MI21) and *Fusarium graminearum* (09TR107)

Strains	MZ	Score®	8-HQ	FICI
		µg.mL^{-1}		
09MI21	0.25 ± 0.11	0.25 ± 0.11	0.50 ± 0.17	0.50*
09TR107	0.25 ± 0.00	0.25 ± 0.05	0.50 ± 0.11	0.50*

Values expressed as mean ± standard deviation. *: Synergism; 09MI21: *F. meridionale*; 09TR107: *F. graminearum;* MZ: Mancozeb; 8-HQ: 8-hydroxyquinoline.

The concentration reduction of these fungicides was taken from the *in vitro* test performed, where comparing the individual MIC and the MIC of the double association, a reduction of 4 to 8 times was obtained, for MZ and 8-HQ, respectively. In the mycelial growth assay, the fungicide MHQ was tested, where we can observe that, for *Fusarium meridionale* (09MI21), there was a small growth observed up to the concentration of 50 µg.mL^{-1} (0.3 X 0.4 X 0 0.3 X 0.2 mm) after this concentration, no growth was observed. As for *Fusarium graminearum*, there was only growth in the control, with no growth being observed in any of the concentrations tested (Figure 1)

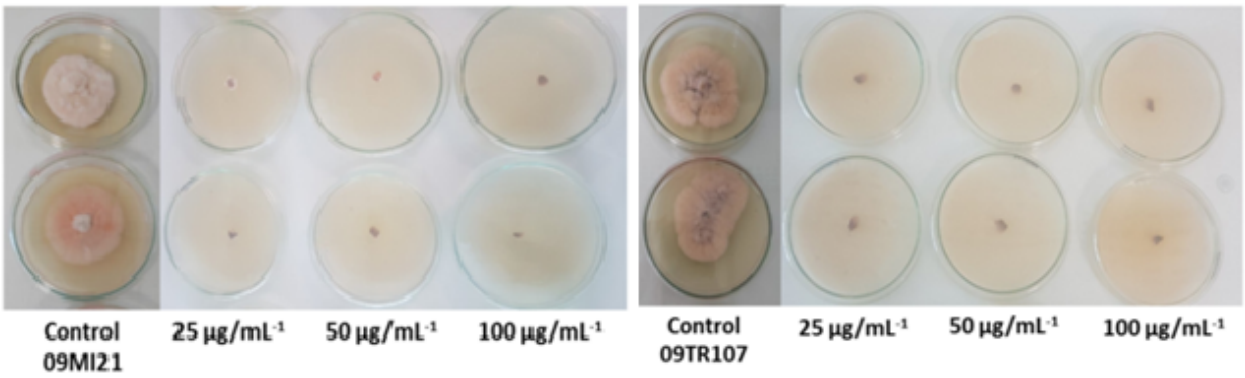

Figure 1. Mycelial growth assay
09MI21: *Fusarium meridionale*; 09TR107: *Fusarium graminearum.* Concentrations of 25, 50 and 100 µg.mL^{-1}.

Evaluating the mycelial growth, it was possible to verify the effectiveness of our association, considering that the mycelial growth, in addition to being observed only at low concentrations, evaluated in terms of growth measures, was also reduced, compared to our control (*Fusarium meridionale*), and for *Fusarium graminearum* there was no growth.

There is no comparison in the literature between these three tested fungicides. As synthetic fungicides are more exclusive and can have dangerous effects on human health and the environment, different fungicides were evaluated to try to control the growth of *Fusarium graminearum* and *Fusarium meridionale* in rice. Jatoi et al. (2018) tested MZ against *Helminthosporium oryzae*, responsible for brown spot in rice, which exhibited excellent control. Serghat et al. (2002) evaluated fungicides against *H. oryzae* and found that tricyclazole and the combinations tricyclazole + MZ, carboxin + thiram, thiabendazole and MZ were highly effective against the tested fungus.

CONCLUSION

All tests carried out demonstrated the effectiveness of the combination of fungicides in combating *Fusarium graminearum* and *Fusarium meridionale*, which cause infections in rice crops

and seeds.

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