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In vivo effect of some fungicides on the development of *Pyricularia grisea* and *Helminthosporium oryzae*

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Summary. The effect of various fungicide treatments of seeds and leaves of rice on the development of the pathogenic fungi *Pyricularia grisea* and *Helminthosporium oryzae* was studied. When applied to seeds, tricyclazole, mancozeb and the carboxin+thiram combination were the only fungicides that reduced all the symptoms induced by both pathogens without damaging the development of young plants. Tricyclazole and the tricyclazole+mancozeb protected rice plants against *P. grisea* and *H. oryzae* respectively throughout the vegetative phase. To ensure protection during the entire vegetative phase, pyrazophos should be applied every two weeks while the carboxin+thiram combination, thiabendazole and mancozeb should be applied every week.

Key words: rice, *Oryza sativa*, rice blast, brown spot disease, fungicides.

Introduction

Rice is the most cultivated cereal in the world and a staple food for the majority of the world's inhabitants. In Morocco, rice is grown in the Gharb and Larache regions where 20,000 ha are cultivated, but where it is subject to some serious diseases, of which rice blast caused by *Pyricularia grisea* is the most devastating. Under favourable environmental conditions, particularly temperature and humidity, rice blast can even cause total crop loss (Okeke *et al.*, 1992). The second most serious disease of rice is considered, worldwide, to be brown spot caused by *Helminthosporium oryzae* (Vidhyasekaran *et al.*, 1986). When the disease is severe, losses can reach 75% (Kohls *et al.*, 1987).

Pyricularia grisea and *H. oryzae* attack the aerial parts of rice, including the seeds, in which mycelia and conidia can survive for several years (Manandhar, 1996). For this reason, fungicide treatment of seeds is a common practice to ensure seed health. Ideally, these fungicides should eliminate the pathogen without causing any negative effects on the germination of seeds or on the young plants.

The objectives of this work were to test *in vivo* the effect of some fungicides applied at various concentrations to rice seeds on seed germination and the development of necrotic lesions with or without the presence of pathogenic agents. A further aim was to devise a treatment program against rice blast and brown spot that will protect rice plants throughout the vegetative phase. Each fungicide was also tested to determine its optimum dose and its persistence.

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Materials and methods

Plant material, pathogens and inoculum preparation

The rice variety Triomphe was used since it is the most sensitive to isolate FK_1 of *Pyricularia grisea* and isolate Hot of *Helminthosporium oryzae*. These isolates are also considered the most pathogenic on rice (Ouazzani Touhami *et al.*, 2000), and they were obtained from leaf lesions of the varieties Kenz and Triomphe respectively. Isolates were cultivated in a ground-rice medium consisting of 14 g of ground rice, 4 g of yeast extract and 15 g of agar l^{-1} distilled water. *P. grisea* cultures were incubated for 10 days at 28°C in the dark, and those of *H. oryzae* under continuous light for 15 days. Sporulating cultures were scraped with a metal spatula under sterile conditions and the mixture of conidia and mycelium was stirred for one minute in sterile distilled water. The resulting suspension was filtered through muslin to remove the mycelium (Xiao *et al.*, 1991). Spore concentration was adjusted with water containing 0.05% of Tween and 0.5% of gelatine so as to give a final concentration of 10^5 spores ml^{-1}

Fungicides

The fungicides tested were tricyclazole (Beam 75%), thiabendazole (Thiabendazole 100%), benomyl (Benlate 50%), thiophanate methyl (Pelt 70%), mancozeb (Dithane 48%) and the combination carboxin+thiram (Vitavax 198 g l^{-1} of carboxin and 198 g l^{-1} of thiram), pyrazophos (Afugan; a.i.: 295 g l^{-1}).

Treatment and inoculation of rice seeds and plantlets

Seeds were soaked and stirred in 100 ml suspensions of each fungicide for 30 min (Dharam *et al.,* 1971). Fungicides were applied at rates of 1, 2, and 3 g kg^{-1} of rice seeds, except for the carboxin+thiram combination, which was used at rates of 1, 2, and 3 ml kg^{-1} of seeds. Control seeds were soaked and stirred in distilled water. Treated seeds were dried for 24 hours on sterile filter paper at room temperature and then divided into two lots.

The first lot was inoculated with spore suspensions of either *P. grisea* or *H. oryzae*. Control seeds were soaked in distilled water containing 0.05% of Tween and 0.5% of gelatine. Inoculated control seeds were first soaked in distilled water containing 0.05% of Tween and 0.5% of gelatine and then inoculated with the pathogens. All the seeds, inoculated or not, were put into Petri dishes containing cotton soaked in distilled water and were incubated at 28°C for 3 days in the dark, and then under continuous light for 5 days. Eight days after inoculation, percent germination was calculated, and coleoptile length and necrotic lesions were measured. Percent inhibition of necrotic lesions on the coleoptiles was calculated.

The second lot of seeds was pre-germinated in Petri dishes containing cotton soaked with sterile distilled water and incubated for 75 hours at 28°C in the dark. They were then transplanted to jars, transferred to a greenhouse and watered at regular intervals. The jars were divided into four sub-lots. Two weeks after transplanting, the first sub-lot was inoculated by spraying with 60 ml of spore suspensions of the pathogenic fungi. This operation was repeated the following week on another sub-lot until all sub-lots had been inoculated for different lengths of time. Control plants, grown from seeds treated with distilled water containing 0.05% of Tween and 0.5% of gelatine, were sprayed with 60 ml of spore suspensions of the fungi.

Seven days after inoculation of each sub-lot, the severity (S) of infection on the leaf surface was scored according to Barrault's scale (1989) for *H. oryzae* and the scale of Nottenghem *et al.* (1980) for *P. grisea*. From these scores, the percent reduction caused by the pathogen was calculated. A reduction was considered significant if it exceeded 50%.

Treatment and inoculation of young plants

Seven fungicides were used in this test: tricyclazole, pyrazophos, mancozeb, thiabendazole, benomyl, thiophanate-methyl and carboxin+thiram combination. Each fungicide was tested at concentrations of 300, 750, 1000, 1500 and 2000 ppm.

Pre-germinated seeds were transplanted to jars, watered until the 3–4-leaf stage (4–5-week-old plants) and divided into two lots.

Plants of the first lot were sprayed with 60 ml of fungicide solution at different concentrations and inoculated 24 hours later by spraying with 60 ml of spore suspensions of either of the two pathogens. Control plants, not fungicide-sprayed, were inoculated only with the pathogens.

All the plants, inoculated, sprayed and control, were kept in the laboratory for 48 hours under black plastic sheeting to maintain a relative humidity of about 100%. Thereafter they were kept in the greenhouse. After 7 days from inoculation, disease incidence (I= number of leaves infected) and disease severity (S) were determined. From these data, the infection coefficient CI=I × S was calculated (Loegering, 1959) and the percent reduction of disease (%R) was determined.

The second lot of young plants was sprayed with those fungicides that had been most effective in the first experiment. For each fungicide, the lowest dose giving maximum inhibition was now used. These plants were likewise divided into four sub-lots. Twenty-four hours after fungicide spraying, the first sub-lot was inoculated with spore suspensions of the fungi; the second sub-lot was inoculated one week later and so on. Control plants were inoculated only with the pathogens and were of the same age as the sprayed plants. After each inoculation, the plants were kept in the laboratory for 48 hours under black plastic sheeting before they were returned to the greenhouse. Percent disease reduction was assessed one week after each inoculation.

Statistical analysis

Each treatment was replicated 15 times. The results were tested for statistical significance using the Newman and Keuls test with the significance level set at 0.05.

Results

Treatment and inoculation of rice seeds

Inoculation of rice seeds with *P. grisea* and *H. oryzae* induced necrotic lesions on the coleoptiles, a reduction in percentage seed germination and a decrease in coleoptile length (Tables 1 and 2). The fungicides protected the seeds against both pathogens. The degree of protection depended on the nature of the pathogen, the fungicide, and the fungicide-application rate.

Table 1. Percent seed germination, coleoptile length, coleoptile lesion length and percent inhibition of coleoptile lesions. Seeds were treated with fungicides and inoculated 24 hours later with *Pyricularia grisea*.

Treatment	Fungicide dose (g kg^{-1} seeds)	% G[a]	Coleoptile length (mm)	Lesion length (mm)	% Inhibition[b]
Non inoculated control seeds	-	83.3 a[c]	35 a	0 f	-
Inoculated control seeds	-	46.6 j	15 h	10 a	-
Tricyclazole	1	66.7 e	25 d	0 f	100 a
	2	80 b	33 ab	0 f	100 a
	3	83.3 a	32 b	0 f	100 a
Carboxin+thiram[d]	1	56.7 h	18 efg	0 f	100 a
	2	63.3 f	26 d	0 f	100 a
	3	73.3 c	27 d	0 f	100 a
Mancozeb	1	60 g	20 e	2.5 e	75 c
	2	70 d	25 d	1.1 f	87 b
	3	83.3 a	30 c	1 f	89 b
Benomyl	1	66.7 e	25 d	8 bc	20 g
	2	53.3 i	21 be	4.5 d	55 d
	3	46.7 j	17 fgh	4.5 d	55 d
Thiabendazole	1	60 g	20 e	7.6 c	24 f
	2	53.3 i	18 efg	5.5 d	45 e
	3	53.3 i	15 h	5 d	50 d
Thiophanate-methyl	1	60 g	16 gh	10 a	0 i
	2	63.3 f	19 ef	9 ab	10 h
	3	63.3 f	20 e	8.2 bc	18 g

[a] Percent seed germination.
[b] Percent inhibition of necrotic lesions on coleoptiles.
[c] Means with the same letters in the same column are not significantly different at 5% (Newman and Keuls test).
[d] ml kg^{-1} seeds.

Tricyclazole and the carboxin+thiram combination completely prevented necrotic lesions on the coleoptiles induced by *P. grisea* (Table 1). Mancozeb gave the greatest inhibition of *H. oryzae* (Table 2).

Moreover (Table 3) it is seen that tricyclazole did not damage the grains, and mancozeb, and the carboxin+thiram combination, actually stimulated seed germination and growth of coleoptiles.

Benomyl at 2 g kg^{-1} and thiabendazole at 3 g kg^{-1} inhibited, weakly but significantly, the development of lesions on coleoptiles, but those doses were toxic to seeds (Table 3). Thiophanate-methyl was not toxic to seeds (Table 3), but it did not significantly reduce coleoptile lesions caused by the two pathogens (Tables 1 and 2).

Treatment and inoculation of plantlets

Only those fungicides that increased in seed germination and coleoptile length and decreased the number of lesions on coleoptiles were used in this experiment.

Treatment of rice seeds with mancozeb, a contact fungicide, or with carboxin+thiram caused a slight reduction in disease severity (Tables 4 and 5). It is not clear if this inhibition was due to the action of these fungicides on the pathogens, or to the added vigour given to the plants (see Table 3). However, although carboxin is a systemic agent, it did not protect plants for more than two weeks.

The treatment of rice seeds with tricyclazole at 3 g kg^{-1} of seeds continued to protect plantlets against *P. grisea* 5 weeks after treatment (39.7% disease reduction). For plantlets inoculated with *H. oryzae*, tricyclazole at 3 g kg^{-1} of seeds still reduced disease by 42% 4 weeks after treatment. After 5 weeks, however, inhibition was no longer significant.

Tricyclazole mixed with mancozeb produced a synergistic effect. Percent disease reduction was better and the effect was more prolonged than when these fungicides were used separately: 100% of reduction of the disease against *P. grisea* and 84.3% against *H. oryzae* with a dose of 1.5 g of tricyclazole + 1.5 g of mancozeb kg^{-1} of seeds in the 5th week.

Table 2. Percentage seed germination, coleoptile length, coleoptile lesion length and percent inhibition of lesions on coleoptiles. Seeds were treated with fungicides and inoculated 24 hours later with *Helminthosporium oryzae*.

Treatment	Fungicide dose (g kg^{-1} seeds)	% G[a]	Coleoptile length (mm)	Lesion length (mm)	% Inhibition[b]
Non inoculated control seeds	-	83.3 b[c]	35 bc	0 i	-
Inoculated control seeds	-	66.7 d	25 gh	18 a	-
Tricyclazole	1	80 b	33 c	6.8 f	66.6 e
	2	86.7 a	35 bc	6 fg	66.6 e
	3	80 b	38 a	3.5 h	80.5 b
Carboxin+thiram[d]	1	66.7 d	24 hi	8 ef	55.6 g
	2	73.3 c	29 def	6.4 fg	64.4 e
	3	80 b	36 b	5 gh	72.2 d
Mancozeb	1	70 d	29 def	5 gh	72.2 d
	2	73.3 c	30 de	4 h	77.7 c
	3	83.3 b	34 bc	1 i	94.4 a
Benomyl	1	66.7 d	27 fg	10 d	44.4 i
	2	50 f	22 i	7 f	61.1 f
	3	36.7 g	19 j	7 f	61.1 f
Thiabendazole	1	60 e	23 hi	9 de	50 h
	2	50 f	19 j	8 ef	55.6 g
	3	33.3 h	15 k	7.8 ef	56.7 g
Thiophanate-methyl	1	73.3 c	28 ef	18 a	0 l
	2	80 b	31 d	15 b	16.7 k
	3	83.3 b	35 bc	13 c	27.8 j

[a], [b], [c] See Table 1.
[d] ml kg^{-1} seeds.

Treatment and inoculation of young plants

The degree to which the infection coefficient was reduced depended on the concentration of the fungicide and on the pathogen (Tables 6 and 7). Compared to the control plants, none of the fungicides at any of the concentrations caused any foliar damage, except the carboxin+thiram combination, which was toxic at concentrations greater than 1000 ppm. Complete inhibition of disease with this combination was however achieved at levels from 300 ppm against *P. grisea* and from 750 ppm against *H. oryzae*. Pyrazophos had practically no effect on *H. oryzae*. Thiabendazole at 1500 ppm was the most effective against *P. grisea* and *H. oryzae,* and this dose is about equal to the recommended dose for this fungicide (1600 ppm). For thiophanate-methyl however this dose is not feasible because, although it completely inhibits the disease induced by *P. grisea,* the recommended dose for this product is only 1000 ppm (Hassikou, 2000).

In the following tests, the only fungicides considered were those with minimal doses lower than or equal to the recommended doses, and which gave complete protection against *P. grisea* (carboxin+thiram combination, mancozeb, pyrazophos and thiabendazole) and against *H. oryzae* (carboxin+thiram combination, thiabendazole and tricyclazole+mancozeb combination).

Disease severity was determined on four lots of young plants inoculated 8, 15, 22 and 29 days after treatment with all these fungicides. Control plants were not treated with any fungicides but were simultaneously inoculated with the pathogens. Disease severity and percent disease reduction were determined. Results are shown in Figure 1 for inoculation with *P. grisea* and in Figure 2 for inoculation with *H. oryzae*. It will be seen that all the fungicides completely protected the plants against both pathogens for 8 days.

Protection of plants with the carboxin+thiram combination was reduced by the 15th day with 64.8% disease reduction against *P. grisea* and 59% against *H. oryzae*. By day 22, plants were not protected any more (6.2% disease reduction against *P. grisea* and 1.4% against *H. oryzae)*. This shows that, for complete protection with carboxin+thiram,

Table 3. Percent germination of rice seeds treated with fungicides, and coleoptiles length of the plants grown from such seeds.

Treatment	Fungicide dose (g kg^{-1} seeds)	% G[a]	Coleoptile length (mm)
Control seeds	-	83.3 d[b]	35 ef
Tricyclazole	1	90 b	40 c
	2	100 a	40 c
	3	86.7 c	35 ef
Carboxin+thiram[c]	1	86.7 c	37 de
	2	100 a	38 cd
	3	100 a	43 b
Mancozeb	1	100 a	40 c
	2	100 a	40 c
	3	100 a	45 a
Benomyl	1	76.6 e	33 f
	2	66.7 g	27 h
	3	56.7 h	22 j
Thiabendazole	1	60 h	31 g
	2	60 h	25 i
	3	50 i	22 j
Thiophanate-methyl	1	83.3 d	36 de
	2	80 d	35 ef
	3	73 f	35 ef

[a] See Table 1.
[b] Means with the same letters in the same column are not significantly different at 5% (Newman end Keuls test).
[c] ml kg^{-1} seeds.

Table 4. Disease severity (S), and percent disease reduction (R), due to fungicides, in rice plantlets infected with *Pyricularia grisea*. Seeds were treated with fungicides and inoculation was done on two-, three-, four- and five-week-old plantlets. Disease severity was recorded 7 days after inoculation.

Treatment	Fungicide dose (g kg^{-1} seeds)□	Age of plantlets (weeks) [a]							
		2		3		4		5	
		S	R	S	R	S	R	S	R
Control plant	-	8.9		7.5		7.2		6.3	
Mancozeb 1	8.9	0 v [a]	7.3	2.7 t	7.2	0 v	6.3	0 v	
	2	8.1	9 q	6.9	8 qr	6.5	9.7 q	6	4.8 s
	3	6.5	27 lm	5.8	22.7 n	5.5	23.6 mn	4.2	33.3 jk
Carboxin+thiram[b]	1	6.5	27 lm	7.4	1.3 u	7.2	0 v	6.2	1.6 u
	2	5.7	36 hij	7	6.7 rs	7	2.8 t	6.2	1.6 u
	3	5.5	38.2 ghi	6.5	13.3 p	6	16.7 o	5.2	17.5 o
Tricyclazole	1	0.6	93.3 b	1.6	81.3 d	5.1	29.2 kl	6.1	3.2 t
	2	0	100 a	0.8	89.3 c	4.2	41.7 g	4.5	28.6 kl
	3	0	100 a	0	100 a	1.6	77.8 e	3.8	39.7 gh
Tricyclazole+mancozeb	0.5+0.5	0	100 a	1.3	82.7 d	3.5	51.4 f	4.1	34.9 ij
	1+1	0	100 a	0	100 a	0	100 a	1.3	79.4 e
	1.5+1.5	0	100 a	0	100 a	0	100 a	0	100 a

[a] See [b] in Table 3.
[b] ml kg^{-1} seeds.

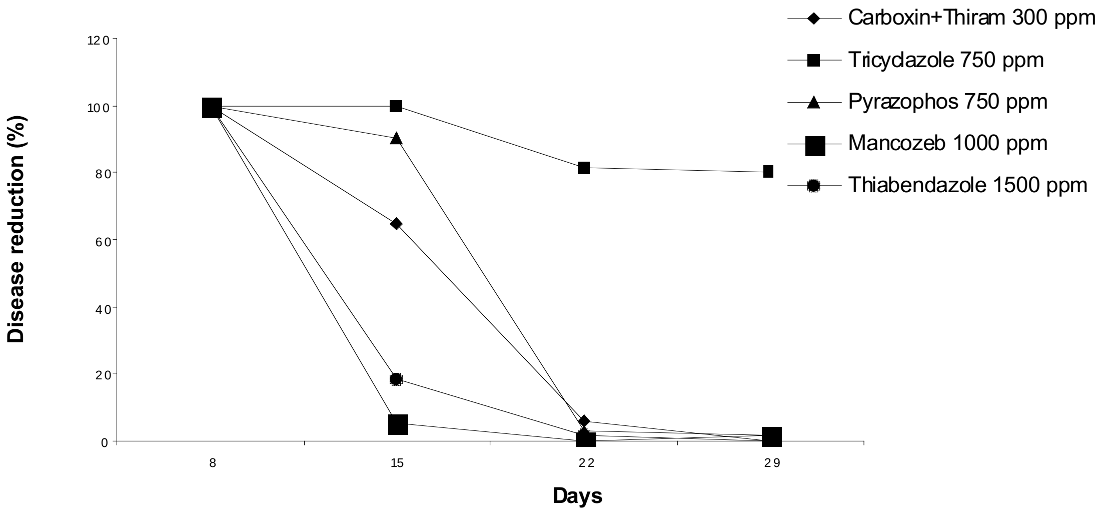

Fig. 1. Percent reduction of blast disease on rice plants inoculated with *Pyricularia grisea* 8, 15, 22 and 29 days after treatment with fungicides.

plants must be treated every week.

Pyrazophos still protected plants on the 15th day (%R=90.1) but after 22 days, protection was no longer ensured, with a reduction of only 3.1%. Consequently, this product should be applied every 15 days.

After two weeks, plants were no longer protected by mancozeb, with only 5.6% disease reduction against *P. grisea* and 20.5% against *H. oryzae*. Thiabendazole also failed to protect plants after two weeks, when it achieved a reduction of 18.3% against *P. grisea* and 15.4% against *H.*

Table 5. Disease severity (S), and percent disease reduction (R), due to fungicides, in rice plantlets infected with *Helminthosporium oryzae*. Seeds were treated with fungicides and inoculation was done on two-, three-, four- and five- week old plantlets. Disease severity was recorded 7 days after inoculation.

Treatment	Fungicide dose (g kg^{-1} seeds)	Age of plantlets (weeks) [a]							
		2		3		4		5	
		S	R	S	R	S	R	S	R
Control plant	-	9		8.2		8.1		7	
Mancozeb	1	9	0 r	8	2.4 q	8.1	0 r	6.9	1.4 q
	2	4	55.6 f	4.3	47.6 g	5.5	32.1 i	7	0 r
	3	2	77.8 c	2.4	70.7 d	4.7	42 h	5.3	24.3 q
Carboxin+thiram[b]	1	8.8	2.2 q	8.2	0 r	7.9	2.5 q	7	0 r
	2	8.5	5.6 p	8.2	0 r	8	1.2 q	6.8	2.9 q
	3	7.4	17.8 lmn	6.9	15.6 mn	7.2	11.1 o	5.7	18.6 lmn
Tricyclazole	1	7	22.2 kl	8.2	0 r	8	1.2 q	7	0 r
	2	6.5	27.8 j	7.6	7.3 p	7.4	8.6 op	7	0 r
	3	6	33.3 i	7	14.6 n	6.8	16.1 mn	5.9	15.7 mn
Tricyclazole+mancozeb	0.5+0.5	1.1	87.8 b	3	63.4 e	4.9	39.5 h	5.6	20 klm
	1+1	0	100 a	0	100 a	1	87.7 b	2.4	65.7 de
	1.5+1.5	0	100 a	0	100 a	0	100 a	1.1	84.3 b

[a] See [b] in Table 3.
[b] ml kg^{-1} seeds.

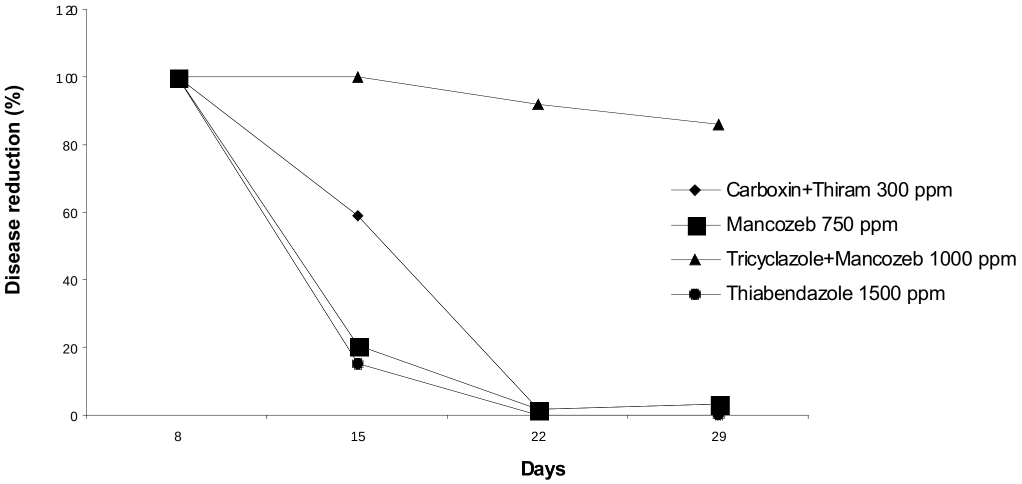

Fig. 2. Percent reduction of brown spot on rice plants inoculated with *Helminthosporium oryzae* 8, 15, 22 and 29 days after treatment with fungicides.

oryzae. Thus, the effect of mancozeb and thiabendazole against *P. grisea* and *H. oryzae* lasted only a week and these products must be applied every 8 days.

By contrast, tricyclazole and the tricyclazole+ mancozeb combination applied to rice plants supplied excellent protection against *P. grisea* and *H. oryzae* respectively, even until the 4th week after the application, with a reduction of 80% against *P. grisea* and 86% against *H. oryzae*. These fungicides will ensure protection of plants until the emergence of the panicles.

Table 6. Disease incidence (I), severity (S) and infection coefficient (CI) induced by *Pyricularia grisea* on young rice plants treated with various doses of fungicides and percent disease reduction (R).

Treatment	Fungicide dose (ppm)	Disease development			
		I	S	CI	R
Control plant	-	45	7	315	-
Carboxin+thiram	300	0	0	0	100 a[a]
	750	0	0	0	100 a
	1000	T	T	T	T
	1500	T	T	T	T
	2000	T	T	T	T
Tricyclazole	300	31.5	2.5	78.7	75 d
	750	0	0	0	100 a
	1000	0	0	0	100 a
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Pyrazophos	300	29.1	2.9	84.4	73.2 d
	750	0	0	0	100 a
	1000	0	0	0	100 a
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Mancozeb	300	21.5	2.2	47.3	85 c
	750	24.3	1	24.3	92.3 b
	1000	0	0	0	100 a
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Thiabendazole	300	44.1	7	308.7	2 i
	750	42	5.1	214.2	32 g
	1000	33.4	3.3	110.2	65 e
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Benomyl	300	43.6	7	305.2	3 hi
	750	44	5.1	299.2	5 h
	1000	38.8	3.3	151.3	52 f
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Thiophanate-methyl	300	44.3	7	310.1	1.5 i
	750	44	7	308	2.2 i
	1000	44.6	6.7	298.8	5.1 h
	1500	0	0	0	100 a
	2000	0	0	0	100 a

[a] See [b] in Table 3.
T, toxic dose.

Discussion

It was shown that *P. grisea* and *H. oryzae*, inoculated on rice seeds by spore suspensions, reduced germination and coleoptile length and produced necrotic lesions on the coleoptiles of young plants growing from pre-germinated seeds (Tables 1 and 2).

Pyricularia grisea was radically inhibited by tricyclazole applied at 1 g kg^{-1} grains while *H. oryzae* was 80% inhibited with 3 g of tricyclazole kg^{-1} grains (Tables 4 and 5). Young plants were still well protected against *P. grisea* five weeks after tricycla-

Table 7.Disease incidence (I), severity (S) and infection coefficient (CI) induced by *Helminthosporium oryzae* on young rice plants treated with various doses of fungicides and percent disease reduction (R).

Treatment	Fungicide dose (ppm)	Disease development			
		I	S	CI	R
Control plant	-	45	7	315	-
Carboxin+thiram	300	29.8	1.2	35.8	90.4 c[a]
	750	0	0	0	100 a
	1000	T	T	T	T
	1500	T	T	T	T
	2000	T	T	T	T
Tricyclazole	300	40.8	6.5	265.2	29 i
	750	43.2	5.1	220.3	41 h
	1000	32.2	2.9	93.4	75 e
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Pyrazophos	300	44.9	8.1	363.7	2.6 mn
	750	44.3	8	354.4	5.1 l
	1000	44	7.8	343.2	8.1 k
	1500	42.1	7.1	298.9	20 j
	2000	40.2	6.5	261.3	30 i
Mancozeb	300	38.6	4.7	181.4	51.4 g
	750	36.4	2	72.8	80.5 d
	1000	9.7	0.5	4.9	98.7 b
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Thiabendazole	300	44.4	8.3	368.5	1.3 n
	750	40.8	6.2	253	32.3 i
	1000	36.9	4.1	151.3	59.6 f
	1500	0	0	0	100 a
	2000	0	0	0	100 a
Benomyl	300	45	8.4	378	0 o
	750	44.4	8.2	364.1	2.5 mn
	1000	40.9	7.3	298.6	20.1 j
	1500	31.1	2.4	74.6	80 d
	2000	0	0	0	100 a
Thiophanate-methyl	300	44.6	8.1	361.3	3.3 lm
	750	44.8	7.9	353.9	5.2 l
	1000	43.1	7.8	336.2	10 k
	1500	40.8	6.4	261.1	30 i
	2000	0	0	0	100 a
Tricyclazole+mancozeb	300	26.1	1.5	39.2	89.5 c
	750	0	0	0	100 a
	1000	0	0	0	100 a
	1500	0	0	0	100 a
	2000	0	0	0	100 a

[a] See [b] in Table 3.
T, toxic dose.

zole was applied at 3 g kg^{-1} grains. At that dose, tricyclazole also significantly inhibited *H. oryzae*, though at lower percentages, four weeks after application. These results are in agreement with Froyd *et al.* (1976) who found that tricyclazole protected rice plants against *P. grisea* 5 weeks after treatment, but at a dose of 1 g kg^{-1} grains. According to Variar *et al.* (1993), treatment of sensitive field-grown rice varieties with tricyclazole (4 g kg^{-1}) followed by spraying iprophenphos at 1 l ha^{-1} reduced rice blast incidence and increased yield. A study realized in field trials in Senegal by Mbodj *et al.* (1987) showed that tricyclazole at 300 and 600 g ha^{-1} reduced infection with *P. grisea*. According to Gouraminis (1996), this systemic fungicide is very active specifically against *P. grisea*. Tricyclazole protects undamaged plant parts against *P. grisea* but not those already damaged. This active ingredient does not affect the germination, growth or sporulation of *P. grisea*, but acts by inhibiting the synthesis of a precursor of melanin, with consequent loss of pathogenic power. Woloshuk *et al.* (1983) suggested that the failure of appressoria to penetrate the cuticle was due to a loss of their rigidity because they were not permeated with melanin. Lazarovits *et al.* (1989) stated that tricyclazole inhibited the synthesis of polyketides by the pathogen. Wheeler and Klich (1995) found that tricyclazole was among the best inhibitors of pigmentation of the conidia of *Penicillium* and *Aspergillus* fungi except *Aspergillus flavus* and *A. parasiticus*.

Our findings on mancozeb are consistent with Dev (1980) who reported that mancozeb is effective against *H. oryzae* at 2 kg ha^{-1}. According to Johnson and Percich (1992), mancozeb applied at 1.12 g ha^{-1} at 7-day intervals, or after treatment with propiconazole protects rice plants from natural infection with *H. oryzae*. According to Geetha and Sivaprakasam (1993), treatment of rice seeds by mancozeb (0.4%) inhibits *P. grisea* and *H. oryzae,* and plants growing from grains so treated are more vigorous. Mancozeb also favours the germination of rice seeds. Our study on the protectant effect of mancozeb on rice leaves showed that this effect lasted only for 8 days, so that it is necessary to spray weekly. By contrast, the tricyclazole+mancozeb combination protected rice plants until panicle emergence. These results are in agreement with Chhetry (1993).

The carboxin+thiram combination radically inhibited *P. grisea* at 1 ml kg^{-1} seeds and gave 72% inhibition of *H. oryzae* at 3 ml kg^{-1}. This effect was not persistent so that the combination had to be applied weekly until the end of the vegetative phase.

Leaf spraying with pyrazophos appeared effective at 750 ppm against *P. grisea* but was ineffective against *H. oryzae*. This compound was not toxic to plants even at the highest doses, confirming the results of Helyer and Ledieu (1985). Zeun *et al.* (1992) found that a combination of pyrazophos and propiconazole reduced the growth of *Pyrenophora teres* synergistically on barley. Lastly, the study showed that for good protection against *P. grisea*, it is necessary to treat the rice plants every two weeks throughout the vegetative phase.

The use of fungicides against pathogenic fungi is the traditional method and remains the most effective short-term approach. However, it sometimes has its limits. Diekman (1994) found that in the field it was difficult or impossible to achieve 100% disease control with fungicides because some grains received too little of the compound while other received too much in respect of the recommended dose. With good equipment to coat the grains, this problem can be minimised.

On the basis of this study, we propose a program of treatment during the vegetative phase using the more effective fungicides and taking account of the persistence of their effect against *P. grisea* and *H. oryzae*:

- treatment of seeds with tricyclazole+mancozeb combination (1.5 g+1.5 g kg^{-1} grains)
- treatment of leaves from the 6th week with:
  - one application of tricyclazole (750 ppm) against *P. grisea* or tricyclazole+ mancozeb combination (750 ppm) against *H. oryzae*;
  - one application, every 15 days, of pyrazophos (750 ppm) against *P. grisea*;
  - one application every week of mancozeb (1000 ppm) against *P. grisea* and *H. oryzae*.

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