

Efficiency of some fungicides on blast and blight of the rice leaves

Abstract. The effect of pathogens on grains is closely related to the severity of the disease on plants. Similarly, infection of leaves is very important in epidemic blast and blight. The objective of this study is to provide a treatment program suitable to different fungicides to protect the leaves of rice plants throughout the vegetative phase against blast and leaf blight, by determining the required dose and the duration of protection for each fungicide.

The results showed that *Pyricularia oryzae* is drastically inhibited, on the leaves of rice at a 300 ppm dose of the Carboxin + Thiram combination, whereas *Helminthosporium oryzae* is not completely inhibited at a dose of 750 ppm. However, this product causes a total leaf yellowing. So this is not recommended, especially since it requires repeated applications.

Furthermore, Pyrazophos has no effect on *Helminthosporium oryzae* even at very high doses. However, this product completely inhibits *Pyricularia oryzae* at a dose of 750 ppm. To protect against rice leaf blast throughout the vegetative phase, the product should be applied every two weeks.

On the other hand, the 750 ppm Tricyclazole totally inhibits the severity of *Pyricularia oryzae* on rice leaves, whereas, a dose of 1500 ppm of the product is required to achieve the same level of protection against *Helminthosporium oryzae*. If this product is combined with Mancozeb, the *H. oryzae* is completely inhibited at 750 ppm.

Furthermore, Mancozeb inhibits radically both pathogens at a dose of 1000 ppm. However, if the Mancozeb must be applied each week to protect rice leaves until the end of the vegetative phase, a single application of Tricyclazole or a combination Tricyclazole + Mancozeb continues to protect rice leaves respectively against *P. oryzae* and *H. oryzae* until the emergence of panicles.

A treatment program with the most effective fungicides is then established taking account into the duration of rice plants protection throughout the vegetative phase against blast and leaf blight. This calendar is distributed as follows:

Foliar from the sixth week either by: One treatment with Tricyclazole 750 ppm against *P. oryzae* or Tricyclazole + Mancozeb to 750 ppm related to *H. oryzae*; One application every 15 days by the Pyrazophos 750 ppm against *P. oryzae*;

Introduction.

The fungal diseases, such as the blast caused by *Pyricularia oryzae* and the blight caused by *Helminthosporium oryzae* are among the most danger for the rice culture in the world, including Morocco. Indeed, in this country, there are many studies and works which were

35 interested to the rice foliar diseases (Ennaffah, 1999; Hassikou, 2000; Tajani, 2000, Tajani *et*
36 *al.*, 2001; Serghat *et al.*, 2002; 2005a; 2005b; Bahous *et al.*, 2008).

37 As many of the other fungal diseases, the control of these two fungi is classically established
38 by fungicides (Lawrence *et al.*, 2001; Serghat *et al.*, 2004; Bahous, 2006, Suzuki *et al.*, 2010;
39 Kunova *et al.*, 2014). Since the incidence of both pathogens on grains is associated to the
40 severity of the disease on plants (Nyvall *et al.*, 1995), the infection of leaves is very important
41 in the epidemics of the blast and leaf blight (long *et al.*, 2001). Consequently, it is important
42 to treat the leaves of rice against these two pathogens to reduce the quantity of aerial
43 inoculum resulting from necrosed and sporulated lesions or to prevent the grains from being
44 infected if there is transmission from panicles to grains in a systematic way (Manandhar *et*
45 *al.*, 1998a, 1998b).

46 The objective of this study is to determinate the required dose and duration of protection for
47 each fungicide in the aim to realize a program of treatment by various fungicides able to
48 protect the rice leaves, during all the vegetative stages against the blast and leaf blight.

49 **Materials and methods**

50 **1-Fungal material.**

51 The isolates FK1 of *Pyricularia oryzae* and Hot of *Helminthosporium oryzae*, isolated
52 respectively from the foliar lesion of the varieties *Leona* and *Triumph* of rice were cultivated
53 on a grain rice flour medium during 15 days.

54 When the cultures cover the Petri dishes, the surface that was full with spores was scraped by
55 a metallic spatula. The mycelium was put in sterile distilled water, then filtered through
56 muslin tissue. The concentration of the obtained sporale suspension was adjusted, with the
57 distilled water containing 0.02 % of Tween 20 and 0.5 % of gelatin, to 10^5 spores / mL.

58 **2-Treatment of rice leaves**

59 The rice grains of the *Triumph* variety, very sensitive to the pathogens *P. oryzae* and *H.*
60 *oryzae* (Ennaffah *et al.*, 1999; Ouazzani Touhami *et al.*, 2000) were disinfected by soaking in
61 the 0.6 % of sodium hypochlorite during ten minutes, then rinsed strictly in the sterile
62 distilled water. After 24h of drying, grains were placed in Petri dishes containing some sterile
63 cotton imbibed with distilled water. After 75h of incubation in 28°C in the darkness, the
64 obtained seedlings were planted in pots containing the soil of the forest Mamou. Then, they
65 were watered with the tap water until the required stage for the inoculation (plant with 3 or 4
66 leaves).

67 **2.1-Fungicides and used doses**

68 Seven fungicides were used in this test (Tricyclazole, Carboxine + Thirame, Pyrazophos,
69 Mancozèbe, Thiabendazole, Benomyl and Methylthiophanate). Every fungicide was tested in
70 the concentrations of 300, 750, 1000, 1500 and 2000 ppm.

71 2. 2- Plants treatment

72 These types of treatments were conducted and each treatment was the subject of 15
73 repetitions:

74 *Plants inoculated with the sporale suspension, (10^5 spores / mL), of each pathogen. They
75 constitute the positive control (diseased).

76 * Plants, treated only by water, constitute the negative control (healthy).

77 * Plants treated by fungicides were then inoculated, 24 hours later, by the sporale suspension
78 (10^5 spores/mL) of the tested pathogens.

79 All the plants were placed in the conditions of the laboratory, during 48 hours, under black
80 plastic covers allowing to maintain a relative humidity in the order of 100 %, Then, they were
81 taken in the greenhouse.

82 The severity of the disease was estimated, seven days after every inoculation by the pathogen,
83 by using the scale of Barrault (1989) for *H. oryzae* and that of Notteghem *et al.* (1980) for
84 *P. oryzae*.

85 The infection coefficients were then calculated by multiplying the incidence by the severity.
86 Then, the reduction's percentage of the disease was deducted comparing to the controls. A
87 reduction has been considered as significant, if it was superior to 50 %.
88 Each treatment was performed with two repetitions of five pots..

89 2. 3- Persistence's estimation of the rice plants protection by various fungicides.

90 A plots of plants were treated, only once at the stage of 3 to 4 leaves, by the most effective
91 fungicides retained in the previous experience. For every fungicide, the minimum dose which
92 gave the maximum of inhibition was used.

93 24 hours after the treatment, one of the plots was inoculated with sporale suspensions at 10^5
94 spores / mL of the pathogens. The reduction's percentage of the disease was noted one week
95 later. At this time, the second plot was inoculated with the pathogens to see if the protective
96 effect of the tested fungicides persisted one week after treatment.

97 Every week, the same operation was repeated to see the effect of fungicides, after 15, 22 and
98 29 days. So, according to the obtained results, a program of treatment by each fungicide
99 along the vegetative stage of the rice cycle development could be considered.
100 Controls, inoculated only with pathogens, have the same age to those of treated with
101 fungicides. After every inoculation with the pathogens, all the plants were placed, during 48
102 hours under black plastic covers in the conditions of the laboratory. Then, they were placed
103 a greenhouse. In all these works, every treatment was the object of 15 repetitions.

104 Results

Effect of tested fungicides on the severity and the incidence of the blast. The examination of the table 1 showed that the percentage of the incidence reduction and the severity of the blight on the leaves of the rice plants and consequently its infection coefficient, by the various fungicides, depends on the quantity to which the latter was applied. Indeed, the disease inhibition in the presence of Carboxine + Thirame at 300 ppm was complete. In the same quantity, Tricyclazole and Pyrazophos inhibit considerably the blast. The inhibition's percentages were respectively in the order of 75 and 73.2 %. The inhibition was total from 750 ppm.

Mancozebe was very effective against *P. oryzae* at the doses of 300 and 750 ppm, with inhibition's percentages which were respectively 85 and 92.3 %. A dose of 1000 ppm of this fungicide was necessary to reach 100 % of disease's reduction. Thiabendazole, Benomyl and Methylthiophanate required to be brought at 1500 ppm to inhibit completely the pathogen. Thiabendazole and Benomyl inhibited, significantly, the disease at 1000 ppm, the percentages of inhibition were higher than 50 %. At this dose, the inhibition by Methylthiophanate was practically inexistant.

No damage was brought to the rice leaves after their treatment with fungicides at all the tested doses in comparison with the healthy control, With the exception of the Carboxine + Thirame combination, which was found to be toxic to the plant from 1000 ppm.

2. 2-Effect of fungicides tested on the severity and the incidence of the blight.

The noted results in the table 2 showed that *H. oryzae* was completely eliminated at a dose of 750 ppm in the combination Carboxine + Thirame while a dose of 300 ppm inhibited it with 90.4 %.

Tricyclazole could protect totally the rice plant against *H. oryzae* only if it was applied in 1500 ppm. The total inhibition of the blight was reached by applying Mancozebe and Thiabendazole with the same doses from which we obtain a total elimination of *P. oryzae*. These doses were respectively 1000 and 1500 ppm.

Pyrazophos which were very effective against *P. oryzae*, practically had no effect on *H. oryzae* even if it was applied at the highest dose (2000 ppm). Indeed, in this dose the inhibition's percentage of the disease was only 30 %. Benomyl and Methylthiophanate applied at 1500 ppm, inhibited *P. oryzae* in 100 %. These products were effective in the same degree, against *H. oryzae* when they were applied at 2000 ppm.

2. 3-Estimation of the protection's persistence of the rice plants by various fungicides

The results which appear in tables 3 and 4 show that the association (Carboxine + Thirame) protected completely plants against *P. oryzae* and *H. oryzae* during one week. In 15th day, this protection was reduced (64.8 % against *P. oryzae* and 59 % against *H. oryzae*). The next week, the plants were not protected compared to control plants those showed that to have a protection, plants had to be almost treated every week. After the application of Pyrazophos

against *P. oryzae*, the protection of plants in the 15th day, was the highest (in the order of 90.1 %). It seemed that to protect plants, this product had to be applied every 15 days.

Besides, Mancozebe could not protect plants against *P. oryzae* and *H. oryzae* more than one week also for Thiabendazole, applied so well against *P. oryzae* that against *H. oryzae*. In the 15th day the protective effect of these products did not persist anymore. These products had to be thus applied every week.

Concerning Tricyclazole and Tricyclazole + Mancozebe, applied to the rice plants aged to 3 weeks, they continued to supply an excellent protection in these plants respectively against *P. oryzae* and *H. oryzae*, even 4 weeks after treatment. These fungicides could thus protect the plants against these two pathogens until the emergence of panicles.

Discussion and conclusion

The results showed that *P. oryzae* was radically inhibited, on the leaves of rice plants, at a dose of 300 ppm of the association Carboxine + Thirame, while *H. oryzae* was completely inhibited only at the dose of 750 ppm of this product. However, this product provoked a total yellowing of leaves. Thus, this product was not recommended, especially as it requires repeated applications.

Besides, Pyrazophos has no effect on *H. oryzae* at very high doses. However, this product inhibits totally *P. oryzae* at a dose of 750 ppm. To protect the leaves of rice plant against the blast during all the vegetative phase, this product must be quite applied during two weeks. On the other hand, Tricyclazole at 750 ppm inhibited totally the severity of *P. oryzae* on the leaves of rice. While against *H. Oryzae*, a dose of 1500 ppm of the product was required to reach the same level of protection. when this product was combined to Mancozebe, *H. oryzae* was completely inhibited in 750 ppm.

Besides, Mancozebe inhibited radically both pathogens at the dose of 1000 ppm. However, when Mancozebe had to be applied every week to protect the leaves of rice plants till the end of the vegetative phase, a single application of Tricyclazole or combination Tricyclazole + Mancozebe continued to protect the leaves of rice respectively against *P. oryzae* and *H. oryzae* until the emergence of panicles.

A study, carried in field in height, by Mbodj *et al.*, (1987) showed that in Senegal, Tricyclazole reduces the infection of the rice by *P. oryzae* in the same obtained degree by using resistant varieties. These authors tested doses 300 and 600 g / ha, which showed an inhibition considerable and better than at the dose of 150 g / ha. This product was also used to differentiate the sensibility of the varieties in the leaves blast of the panicles.

Gouraminis (1996) also reported that this systemic fungicide is specifically very active towards *P. oryzae*. Also, this fungicide showed itself the most effective *in vitro* and *in vivo* against *Curvularia lunata* considered as a pathogen of rice plants; (Hassikou *et al.*, 2002). Sowa *et al.* (2014), formulated a mixture of fungicides to control the most rice fungal

180 pathogens of which *P. oryzae* and *H. oryzae*. Among the components of this mixture there
 181 were Tricyclazole, Mancozebe and Pyrazophos. Mustafa *et al.* (2013) showed that
 182 Tricyclazole applied on leaves was effective on the yield. Kurahashi (2001) reported that
 183 Tricyclazole was an inhibitor of the melanin's synthesis in the appressoria of *P. oryzae*
 184 preventing the penetration in the rice also by inhibiting the polyhydroxynaphtaline reductase.

185 The efficiency of the mancozebe was indicated by several authors. Indeed, Dev (1980)
 186 reported that Mancozebe, inhibits *H. oryzae* but at a dose of 2 kg/ha. Besides, Percich (1989
 187) and Johnson and Percich (1992) showed that this product, applied only, in 7 days apart
 188 either further to an application of Propiconazole protects the rice plants of the natural
 189 infection by *H. oryzae* and so increased the yield (38-120 %) in height fields. Besides, Kohls
 190 *et al.* (1987) maintained control pots not affected naturally by *H. oryzae*, along the vegetative
 191 cycle of the rice, by a treatment (processing) with 2,24 kg / ha of Mancozebe.

192 In the term of this study, a program of treatment (processing) by the most effective fungicides
 193 has to be established while taking into account their rice plants protection's duration during
 194 all the vegetative phase against the blast and the blight. This calendar was distributed as
 195 follows:

196 The foliar treatment of the sixth week is as follows:

- 197 - A single treatment by Tricyclazole in 750 ppm against *P. oryzae* or Tricyclazole +
- 198 Mancozebe in 750 ppm towards *H. oryzae*;
- 199 - An application everything 15 days by Pyrazophos in 750 ppm against *P. oryzae*;
- 200 - An application every week by Mancozebe in 1000 ppm against *P. oryzae* and *H. oryzae*.

201 Table-1 : Effect of the tested fungicides on reducing the severity and incidence of blast on
 202 rice leaves.

Fungicide		Dose (ppm)				
		300	750	1000	1500	2000
control	I	45	45	45	45	45
	S	7	7	7	7	7
	IC	3.5	3.5	3.5	3.5	3.5
Carboxine+Thirame	I	0	0	T	T	T
	S	0	0	T	T	T
	IC	0	0	T	T	T
	R (%)	100 a	100 a	T	T	T
Tricyclazole	I	31.5	0	0	0	0
	S	2.5	0	0	0	0
	IC	78.7	0	0	0	0
	R (%)	75 d	100 a	100 a	100 a	100 a
Pyrazophos	I	29.1	0	0	0	0
	S	2.9	0	0	0	0

	IC	84.4	0	0	0	0
	R (%)	73.2 d	100 a	100 a	100 a	100 a
Mancozebe	I	21,5	24,3	0	0	0
	S	2,2	1	0	0	0
	IC	47.3	24.3	0	0	0
	R (%)	85 c	92.3 b	100 a	100 a	100 a
Thiabendazole	I	44.1	42	33.4	0	0
	S	7	5.1	3.3	0	0
	IC	308.7	214.2	110.2	0	0
	R (%)	2 i	32 g	65 e	100 a	100 a
Benomyl	I	43.6	44	38.8	0	0
	S	7	5.1	3.3	0	0
	IC	305.2	299.2	151.3	0	0
	R (%)	3 hi	5 h	52 f	100 a	100 a
Methyl-thiophanate	I	44.3	44	44.6	0	0
	S	7	7	6.7	0	0
	IC	310.1	308	298.8	0	0
	R (%)	1.5 i	2.2 i	5.1 h	100 a	100 a

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204 I: Incidence ; S: Severity; IC : Infection's Coefficient; R(%) : Reduction's Percentage, T :
 205 Toxic Dose.

206 Two results affected by the same letter did not differ significantly, at the 5% threshold
 207 (Newman and Keuls test).

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210 **Table-2** : Effect of the tested fungicides on the reduction of the severity and incidence of
211 helminthosporiasis on rice leaves.

Fungicide	Dose (ppm)					
		300	750	1000	1500	2000
Control	I	45	45	45	45	45
	S	7	7	7	7	7
	IC	315	315	315	315	315
Carboxine+Thirame	I	29.8	0	T	T	T
	S	1.20	0	T	T	T
	IC	35.8	0	T	T	T
	R (%)	90.4 c	100 a	T	T	T
Tricyclazole	I	40.8	43.2	32.2	0	0
	S	6.5	5.1	2.9	0	0
	IC	265.2	220.3	93.4	0	0
	R (%)	29 i	41 h	75 e	100 a	100 a
Pyrazophos	I	44.9	44.3	44	42.1	40.2
	S	8.1	8	7.8	7.1	6.5
	IC	363.7	354.4	343.2	298.9	261.3
	R (%)	2.6 mn	5.1 l	8.1 k	20 j	30 i
Mancozebe	I	38.6	36.4	9.7	0	0
	S	4.7	2	0.5	0	0
	IC	181.4	72.8	4.85	0	0
	R (%)	51.4 g	80.5 d	98.7 b	100 a	100 a
Thiabendazole	I	44.4	40.8	36.9	0	0
	S	8.3	6.2	4.1	0	0
	IC	368.5	253	151.3	0	0
	R (%)	1.3 n	32.3 i	59.6 f	100 a	100 a
Bénomyl	I	45	44.4	40.9	31.1	0
	S	8.4	8.2	7.3	2.4	0
	IC	378	364.1	298.6	74.6	0
	R (%)	0 o	2.5 mn	20.1 j	80 d	100 a
Méthyl-thiophanate	I	44.6	44.8	43.1	40.8	0
	S	8.1	7.9	7.8	6.4	0
	IC	361.3	353.9	336.2	261.1	0
	R (%)	3.3 lm	5.2 l	10 k	30 i	100 a
Tricyclazole + Mancozebe	I	26.1	0	0	0	0
	S	1.5	0	0	0	0
	CI	39.2	0	0	0	0
	R (%)	89.5 c	100 a	100 a	100 a	100 a

212 I: Incidence ; S : Severity ; IC : Infection's Coefficient; R(%) : Reduction's Percentage, T :
213 Toxic Dose.

214  results affected by the same letter did not differ significantly, at the 5% threshold
215 (Newman and Keuls test).

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218 **Table -3 :** Persistence of the fungicides effect on the reduction of blast, following a single treatment
219 of rice plants in the 3-4 leaves stage.

Fongicide	Dose (ppm)		8 days	15 days	22 days	29 days
Control	0	S	7.2	7.1	6.5	5
Carboxine+Thiram e	300	S	0	2.5	6.1	5.2
		R (%)	100 a	64.8 d	6.2 f	0 h
Tricyclazole	750	S	0	0	1,2	1
		R (%)	100 a	100 a	81.5 c	80 c
Pyrazophos	750	S	0	0.7	6.3	4.9
		R (%)	100 a	90.1 b	3.1 g	2 g
Mancozebe	1000	S	0	6.7	6.6	4.9
		R (%)	100 a	5.6 f	0 h	2 g
Thiabendazole	1500	S	0	5.8	6,4	5.2
		R (%)	100 a	18.3 e	1.5 g	0 h

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221 Two results affected by the same letter did not differ significantly, at the 5% threshold
222 (Newman and Keuls test).

223 **Table -4 :** Persistence of the fungicides effect on the reduction of blight, following a single treatment
224 of rice plants in the 3-4 leaves stage.

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Fungicide	Dose (ppm)		8 days	15 days	22 days	29 days
Témoin	0	S	8	7.8	7.3	5.7
Carboxine+Thirame	300	S	0	3.2	7.2	5.5
		R (%)	100 a	59 d	1.4 h	3.5 g
Mancozèbe	750	S	0	6.2	7.2	5.5
		R (%)	100 a	20.5 e	1.4 h	3.5 g
Tricyclazole+Mancozèbe	1000	S	0	0	0.6	0.8
		R (%)	100 a	100 a	91.8 b	86 c
Thiabendazole	1500	S	1.5	3	7.1	5.8
		R (%)	100 a	15.4 f	0 i	0 i

226 S : Severity ; R(%): Réduction percentage.

227 Two results affected by the same letter did not differ significantly, at the 5% threshold
228 (Newman and Keuls test).

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