

1 **Efficiency of selected fungicides on blast and blight of rice leaves**

3 **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 of blast and blight. The objective of this study is to develop 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.

4 The results showed that *Pyricularia oryzae* is drastically inhibited on the leaves of rice at a
5 300 ppm combined dose of Carboxin + Thiram, whereas *Helminthosporium oryzae* was completely inhibited at a dose of 750 ppm with the same fungicide combination. However, this product caused a total leaf yellowing and requires repeated applications So it is not recommended.

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

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

9 Furthermore, Mancozeb inhibited both pathogens at a dose of 1000 ppm. However, when Mancozeb was applied each week to protect rice leaves until the end of the vegetative phase, a single application of Tricyclazole or a combination Tricyclazole + Mancozeb continued to protect rice leaves against *P. oryzae* and *H. oryzae* until the emergence of panicles.

10 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. The appropriate time for application for effective results is shown as follows :

11 Foliar treatment from the sixth week (from the day of planting) either by :

12 - One treatment with Tricyclazole at 750 ppm against *P.oryzae* or Tricyclazole + Mancozeb at 750 ppm related to *H. oryzae*;

13 - One application every 15 days by the Pyrazophos at 750 ppm against *P. oryzae*;

14 -One application every week by the Mancozeb at 1000 ppm against both pathogens.

15 **Keywords** : rice leaves, *P. oryzae*, *H. oryzae*, treatment program, Carboxin + Thiram combination, Pyrazophos, Tricyclazole, mancozeb, Tricyclazole + Mancozeb combination, vegetative phase.

17 Introduction.

18 The fungal diseases, such as the blast caused by *Pyricularia oryzae* and the blight caused by
19 *Helminthosporium oryzae* are among the most limiting factors towards rice cultivation and
production in the world, including Morocco. Indeed, in this country, many studies and
research have been focused on the rice foliar diseases due its importance (Ennaffah, 1999;
Hassikou, 2000; Tajani, 2000, Tajani *et al.*, 2001; Serghat *et al.*, 2002; 2005a; 2005b; Bahous
et al., 2008).

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

46 The objective of this study was to determinate the required dose and duration of protection for
each fungicide with the aim of developing a treatment program for various fungicides which
are able to protect rice leaves from the fungal pathogens during the vegetative stages against
blast and blight diseases of rice.

47 Materials and methods

48 1-Fungal material.

49 The isolates FK1 of *Pyricularia oryzae* and Hot that of *Helminthosporium oryzae*,
isolated respectively from the foliar lesion of the varietiesKenz and Triumph of rice were
cultivated on a grain rice flour medium for 15 days.

50 When the cultures colonize the whole surface of twenty Petri dishes, spores and mycelia were
scraped by a metallic spatula from these fungal colonies. This suspension was put in sterile
distilled water then filtered through muslin tissue in the aim to obtain suspension that contain
only spores. The concentration of the obtained spore's suspension was adjusted, with
distilled water containing 0.02 % Tween 20 and 0.5 % gelatin, to 10⁵ spores / mL.

51 2-Treatment of rice leaves

52 Rice grains of the Triumph variety, very sensitive to the pathogens *P. oryzae* and *H.*
53 *oryzae* (Ennaffah *et al.*, 1999; Ouazzani Touhami *et al.*, 2000) were disinfected by soaking in
54 0.6 % Of sodium hypochlorite for ten minutes, then rinsed quickly with sterile
distilled water. After 24h of drying, grains were placed in Petri dishes containing sterile cotton
imbibed with distilled water. After 75h of incubation in 28°C in darkness, the seedlings
were planted in pots containing disinfected soil of the forest Mamora. Then, they were watered
with tap water until the required stage for the inoculation (plant with 3 or 4 leaves).

55 2.1-Fungicides and used doses

56 Seven fungicides were used in this test (Tricyclazole, Carboxine + Thirame, Pyrazophos,

57 Mancozeb, Thiabendazole, Benomyl and Methylthiophanate). Every fungicide was tested at concentrations of 300, 750, 1000, 1500 and 2000 ppm.

58 2. 2- Plants treatment

59 Three lots of treatments were conducted and each treatment was performed with two repetitions of 15 pot plants :

60 a- Plants inoculated with the spore suspension, (10^5 spores / mL), of each pathogen. They constitute the positive control (diseased).

61 b- Plants, treated only with water, constitute the negative control (healthy).

62 c- Plants treated with fungicides cited in the used fungicide part were then inoculated, 24 hours later, by the spore suspension (10^5 spores/mL) of the tested pathogens.

63 All the pot plants were placed in the laboratory for 48 hours, under black plastic covers to maintain a relative humidity of 100 %, then, they were taken into greenhouse of the University.

64 The disease incidence (number of infected leaves per treatment) is calculated and the symptom's severity is estimated, seven days after every inoculation with the pathogen, by using the scale of Barrault (1989) for *H. oryzae* and that of Notteghem *et al.* (1980) for *P. oryzae*.

65 The infection coefficients were then calculated by multiplying the incidence by the severity.

66 Then, the reduction's percentage of the disease was deducted comparing to the controls.

67 The most effective fungicides were used for the next experience, with the minimum dose which gave the maximum of inhibition, for each fungicide.

68 2. 3- Persistence's estimation of the rice plants protection by various fungicides.

69 Each plot containing the various treatments were treated only once at the stage of 3 to 4 leaves per plant by the most effective fungicides retained in the previous experience. For every fungicide, the minimum dose which gave the maximum of inhibition was used.

70 Twenty four (24) hours after various treatment applications, one of the plots was inoculated with spore suspensions at 10^5 spores / ml of the pathogens. The reduction's percentage of the disease was noted one week later. At this time, the second plot was inoculated with the pathogens to see if the protective effect of the tested fungicides persisted one week after treatment.

71 Every week, the same operation was repeated to see the effect of fungicides, after 15, 22 and

72 29 days. So, according to the obtained results, a program of treatment by each fungicide along the vegetative stage of the growth cycle development could be considered and developed.

73 Controls, inoculated only with pathogens, have the same age to those treated with fungicides. After every inoculation with the pathogens, all the plants were placed, 48 hours under black plastic covers in the conditions of the laboratory. Then, they were placed in a greenhouse. In all these works, every treatment was the object of 15 repetitions.

74 Statistical Analysis

75 The result's statistical treatment was focused on the variance analysis with a single classification criterion according to (Newman and Keuls test).

76 Results

77 1. Effect of tested fungicides on the severity and the incidence of the blast.

78 The results in table 1 showed that the percentage of the incidence reduction and the severity of
the blast on the leaves of the rice plants and consequently its infection coefficient, by the
various fungicides, depend 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.

113 Mancozebe was very effective against *P. oryzae* at the doses of 300 and 750 ppm, with
114 inhibition's percentages which were respectively 85 and 92.3 %. A dose of 1000ppm of this
115 fungicide was necessary to reach 100 % of disease's reduction. Thiabendazole, Benomyl and
116 Methylthiophanate required to be brought at 1500 ppm to inhibit completely the pathogen.
117 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 non existant.

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

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

122 The noted results in the table 2 showed that *H. oryzae* was completely eliminated at a dose of
123 750 ppm in the combination Carboxine + Thirame while a dose of 300 ppm inhibited it with
90.4 %.

127 Tricyclazole could protect totally the rice plant against *H. oryzae* only if it was applied in
128 1500 ppm. The total inhibition of the blight was reached by applying Mancozebe and
129 Thiabendazole with the same doses from which we obtain a total elimination of *P. oryzae*.
130 These doses were respectively 1000 and 1500 ppm.

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

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

138 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
139 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

140 against *P. oryzae*, the protection of plants in the 15th day, was the highest (in the order of 90.1
141 %). It seemed that to protect plants, this product had to be applied every 15 days.

142 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.

143 Concerning Tricyclazole and Tricyclazole + Mancozebe, applied to the rice plants aged to 3
144 weeks, they continued to supply an excellent protection in these plants respectively against
145 *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.

146 Discussion and conclusion

147 The results showed that *P. oryzae* was radically inhibited, on the leaves of rice plants, at a
148 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.

149 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.

150 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.*
151 *oryzae* was completely inhibited at 750 ppm.

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

157 A study, carried in field, 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. Among all treatments, tested by Vinod Kumar
Nirmalkar et al 2017, Tricyclazole 75% WP showed to be the best fungicides in terms of minimum
disease incidence in both stages of blast (leaf and neck blast) 16.3 and 21.22 %, with maximum
disease reduction of about 66.61 and 43.15%,

158 Gouraminis (1996) also reported that this systemic fungicide is specifically very active towards
P. oryzae. The same efficiency was reported by Pandey (2016) when he studied in a field
experiment the efficacy of 11 foliar fungicides against leaf blast pathogen *Magnaporthe grisea* in
susceptible rice variety and proved that the treatments tricyclazole at 0.6 g/l/kg, was found
significantly superior in controlling the disease severity. 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).

- 159 Sowa *et al.* (2014), formulated a mixture of fungicides to control the most rice fungal
pathogens of which *P. oryzae* and *H. oryzae*. Among the components of this mixture there
160 were Tricyclazole, Mancozebe and Pyrazophos. Mustafa *et al.* (2013) showed that
161 Tricyclazole applied on leaves was effective on the yield. Kurahashi (2001) reported that
162 Tricyclazole was an inhibitor of the melanin's synthesis in the appressoria of *P. oryzae*
preventing the penetration in the rice also by inhibiting the polyhydroxynaphthalene reductase.
Chen Yan *et al.* 2016 concluded in his study of the relationship between Tricyclazole and
associated genes of rice resistance that the prevention of rice blast by tricyclazole is not only by
inhibiting the synthesis of melanin but the results show that tricyclazole can trigger rice
resistance by inducing expression of key genes in the jasmonic acid pathway.
- 163 | The efficiency of chemicals as the mancozebe ; carbendazim, propiconazole and
Azoxystrobin was indicated by several authors (Hegde, 2015 ; Vinod Kumar Nirmalkar *et al.*
2017; Dolar Pak *et al.* 2017). Indeed, Dev (1980) reported that Mancozebe, inhibits *H. oryzae*
but at a dose of 2 kg/ha. Besides, Percich (1989) and Johnson and Percich (1992) showed that
this product, applied alone every seven days or after an application of Propiconazole protects
the rice plants of the natural infection by *H. oryzae* and so increased the yield (38-120 %) in
height fields. Besides, Kohls *et al.* (1987) maintained control pots not affected naturally by *H.*
oryzae, along the vegetative cycle of the rice, by a treatment (processing) with 2,24 kg / ha of
Mancozebe.
- 164 In the term of this study, a program of treatment+ (processing) by the most effective fungicides
has to be established while taking into account their rice plants protection's duration during all
the vegetative phase against the blast and the blight. This calendar was distributed as follows:
- 165 Foliar treatment from the sixth week either by:
- 166 - A single treatment by Tricyclazole in 750 ppm against *P. oryzae* or Tricyclazole +
167 Mancozebe at 750 ppm towards *H. oryzae*;
 - 168 - An application every 15 days by Pyrazophos in 750 ppm against *P. oryzae*;
 - 169 - An application every week by Mancozeb at 1000 ppm against *P. oryzae* and *H. oryzae*.

Table 1. Effect of the tested fungicides on reducing the severity and incidence of blast 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	3.5	3.5	3.5	3.5	3.5
Carboxine+Thirame	I	0	0	L	L	L
	S	0	0	L	L	L
	IC	0	0	L	L	L
	R (%)	100 a	100 a	L	L	L
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

I: Incidence ; S: Severity; IC : Infection's Coefficient; R(%) : Reduction's Percentage, L : Lethal dose.

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

178

179 **Table 2.** Effect of the tested fungicides on the reduction of the severity and **incidence** of
blight on rice leaves.

Fungicide		Dose (ppm)				
		300	750	1000	1500	2000
	I	45	45	45	45	45
Control	S	7	7	7	7	7
	IC	315	315	315	315	315
	I	29.8	0	L	L	L
	S	1.20	0	L	L	L
Carboxine+Thiram	IC	35.8	0	L	L	L
	R (%)	90.4 c	100 a	L	L	L
	I	40.8	43.2	32.2	0	0
	S	6.5	5.1	2.9	0	0
Tricyclazole	IC	265.2	220.3	93.4	0	0
	R (%)	29 i	41 h	75 e	100 a	100 a
	I	44.9	44.3	44	42.1	40.2
	S	8.1	8	7.8	7.1	6.5
Pyrazophos	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
	I	44.4	40.8	36.9	0	0
	S	8.3	6.2	4.1	0	0
Thiabendazole	IC	368.5	253	151.3	0	0
	R (%)	1.3 n	32.3 i	59.6 f	100 a	100 a
	I	45	44.4	40.9	31.1	0
	S	8.4	8.2	7.3	2.4	0
Bénomyl	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
	I	26.1	0	0	0	0
Tricyclazole +	S	1.5	0	0	0	0
Mancozebe	CI	39.2	0	0	0	0
	R (%)	89.5 c	100 a	100 a	100 a	100 a

180 I: Incidence ; S : Severity ; IC : Infection's Coefficient; R(%) : Reduction's Percentage, L :
181 Lethal dose.

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

Table 3. Persistence of the fungicides effect on the reduction of blast, following a single treatment 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

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

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

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
Mancozeb	750	S	0	6.2	7.2	5.5
		R (%)	100 a	20.5 e	1.4 h	3.5 g
Tricyclazole+Mancozeb	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

S : Severity ; R(%): Reduction's percentage.

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

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