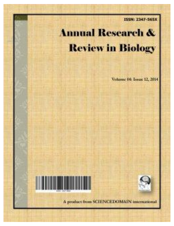

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# Integrated Control against Rice Blast and Leaf Spot by *Trichoderma harzianum* and Two Fungicides

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Integrated pest management is performed at intervals of 10 days, during all the season of rice cultivation, by the application of *Trichoderma harzianum* at a concentration of $10^8$ spores/ml, in alternation with the mancozeb at 1000 ppm against rice blast and rice leaf spot and the pyrazophos at 750 ppm against blast. The assessment of symptoms is performed at the beginning of the panicles appearance, by estimating the incidence of the disease and the symptoms severity.

Thus, at the end of treatment programs, the alternation of pyrazophos and *T. harzianum* reduced blast at a rate similar to that noted when pyrazophos is used alone (i.e. respectively 90.5 and 89.1%). This percentage is better than that recorded following treatment by *T. harzianum* alone (78.4%). Mancozeb alternated with *T. harzianum* reduced blast at a rate of (83.49%) compared with the fungicide or the antagonist alone (77 and 78.4%).

The application of mancozeb alone reduced the leaf spot at a rate similar to that noted following its alternation with *T. harzianum* (79.2 and 75.64%) and better than that obtained after treatment with *T. harzianum* alone (69.5%).

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*Keywords:* *Rice; rice blast; leaf spot; Trichoderma harzianum; mancozeb; pyrazophos; integrated pest management.*

## 1. INTRODUCTION

Fungal diseases such as blast due to *Pyricularia oryzae* and the leaf spot caused by *Helminthosporium oryzae* are among the most imminent threats for the cultivation of rice in the world. Indeed, in Morocco, several studies have focused on foliar diseases of rice [1,2,3,4,5,6,7,8].

Like all other fungal diseases, the fight against these two fungi is classically based on the use of fungicides [9,10,11,12,13]. The application of biological techniques through the use of *Trichoderma* could be a promising approach, but its effectiveness remains, however, lower than that of chemical products. Thus, it is necessary to define the various factors involved in antagonism processes, which optimize the effectiveness of biological products. Researchers have been interested in integrating several techniques of control, particularly the biological control [14,15].

The objective of this work is to establish a control approach which aims to integrate the use of *Trichoderma* with certain fungicides, against rice blast and rice leaf spot.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The grains of rice Triomphe variety, highly susceptible to *Pyricularia oryzae* and *Helminthosporium oryzae* [16,17], were disinfected by soaking in the sodium hypochlorite at 0.6% for ten minutes and then rinsed thoroughly with sterile distilled water. After 24 hours of drying, the grains are pre-germinated in Petri dishes containing sterile cotton moistened with distilled water. After 75 hours of incubation in the dark at a temperature of 28°C, the resulting seedlings are transplanted into pots containing soil of Mamora. Then, they are watered with tap water until the stage required for inoculation (4 to 6 leaves).

### 2.2 Fungal Material

The antagonistic strain (*Trichoderma harzianum*) obtained from the 'Mycology collection' of the laboratory of Botany and Plant Protection of the Sciences Faculty of Kenitra (L.B.P.P.F.S.K) is cultivated on medium PDA and incubated in darkness for 15 days.

The isolates FK1 of *Pyricularia oryzae* and Hot of *Helminthosporium oryzae*, isolated respectively from leaf lesions of rice varieties Kenz and Triomphe were cultivated on rice flour medium for 15 days.

The spore's suspensions were prepared from these cultures with distilled water containing 0.02% of Tween 20 and 0.5% of gelatin, and adjusted at $10^8$ spores/ml for *Trichoderma* and $10^5$ spores/ml for the pathogens.

### 2.3 Fungicides and Selected Concentrations

Fungicides have proved very effective against *P. oryzae* and *H. oryzae*, requiring repeated applications to protect all the vegetative phase of rice [18]. The fungicides retained were mancozeb at 1000 ppm, against *P. oryzae* and *H. oryzae* and the pyrazophos at 750 ppm against *P. oryzae*.

### 2.4 Treatment Programs

The evolution of symptoms was followed on the leaves to assess the effectiveness of different treatment. Thus, plants were treated every ten days by:

- *T. harzianum* alone (program 1);
- One of the fungicides only (program 2);
- The alternation of the fungicide with the antagonist *T. harzianum* (program 3).

### 2.5 Inoculation Method

During the entire duration of the experience, the artificial inoculation of rice plants was carried out by spore suspensions of the pathogens at an interval of 7 days and a rate of l, 5 ml/plant.

The infection of plants is favoured by spraying the plants with water, every two days. Similarly, the surrounding space of treated plants is moistened.

The experiment is carried out in the greenhouse. The control batches of each treatment program are sprayed by the same amount of water instead of fungicides or antagonist.

### 2.6 Reading Results

Assessment of symptoms is performed at the end of treatment programs by estimating the incidence of the disease and the severity of the blast symptoms in the scale of Notteghem et al. [19] and the leaf spot in the scale of Barrault [20] below. The coefficients of infection are then calculated by multiplying the incidence by the severity. Then, the percentage of the disease reduction is reduced compared to the control. A reduction is considered as significant if it is greater than 50%. Each treatment is made with two repetitions of five pots each.

## 3. RESULTS

The results of Table 1 showed that 90 leaves of control plants, out of a total of 150, were affected by the blast. The average index of the disease severity was in the order of 3.5. Therefore, the infection coefficient is 315. All treatment programs significantly reduced these parameters. Thus, the alternation of pyrazophos and *T. harzianum* reduced blast at a rate similar to that noted when pyrazophos was used alone (90.5%). This percentage was better than that recorded during the treatment with *t. harzianum* alone (78.4%).

For mancozeb, its alternation with *T. harzianum* led to a reduction of the disease of 83.49%. The percentages of reduction resulting from single treatments are weak and similar (77 and 78.4%).

On the other hand, *H. oryzae* (Table 2) inoculated alone to plants, has generated a coefficient of infection in the order of 488. Mancozeb reduced disease to 79.02%. This percentage is slightly better than that noted by alternating this product with *T. harzianum*. While the inhibition rate recorded when plants were treated only with *T. harzianum* is about 69.5%.

## 4. DISCUSSION

Treatments by fungicides have presented good results in the short term, their side effects in long-term on environment become worrying particularly through their involvement in the surface water and groundwater pollution [21].

**Table 1. Scale of Notteghem et al. [19]**

| Note | Deseased leaf area (%) |
|---|---|
| 0 | 0 |
| 1 | 0,05 |
| 2 | 0,5 |
| 3 | 1,5 |
| 4 | 3,5 |
| 5 | 7,5 |
| 6 | 17,5 |
| 7 | 37,5 |
| 8 | 62,5 |
| 9 | 87,5 |

Moreover, even though the massive use of fungicides is becoming more and more rational [22], the fact remains that a certain number of toxicological problems are of human concern, related firstly to the presence of residues in food [23] and also to the inherent risks in the use of these products [24]. Similarly, some fungicides may increase the incidence of the disease by removal of natural antagonists of the pathogens [25].

Also, following the intensification of chemical control, the development of resistant strains has come to destroy the potentialities initially expressed. As *P. oryzae* is equipped with large

**Table 2. Scale of Barrault [20]**

| Infested leaf area (%) | Class | Description of symptoms |
|---|---|---|
| 0 | 0 | No observed leaf infection. |
| 0 – 2,5<br>2,5 – 5<br>5 – 10 | 1<br>2<br>3 | Small lesions: tiny necrotic areas that may or not be accompanied by chlorosis of surrounding tissues. |
| 10 – 20<br>20 – 30<br>30 – 40<br>40 – 50 | 4<br>5<br>6<br>7 | Small to medium-sized lesions: Medium-sized necrotic areas surrounded by chlorosis zones. |
| 50 – 75<br>75 - 100 | 8<br>9 | Medium to large lesions: leaves intensively infected with wide necrotic areas surrounded by areas of chlorosis = dead leaves. |

**Table 3. Reduction of the blast on rice plants aged 55 days, following various treatment programs that plants undergo since the age of 3 weeks**

| Treatment programs | Severity | Incidence | Infection coefficient | Reduction Percentage (%) |
|---|---|---|---|---|
| Control (PKI) | 3,5 | 90 | 315 | - |
| *T. harzianum* | 1,7 | 40 | 68 | 78,4 c |
| Pyrazophos | 1 | 30 | 30 | 90,5 a |
| Pyrazophos/*T. harzianum* | 0,8 | 43 | 34,4 | 89,1 a |
| Mancozebe | 1,6 | 45 | 72 | 77,14 c |
| Mancozebe/ *T. harzianum* | 1,2 | 43,3 | 52 | 83,49 b |

*The control one was treated every 7 days by P. oryzae only.*
*The other plants are treated along the experiment every 10 days with the fungicide, the antagonist or the alternation of the two, and they are inoculated by spraying by P. oryzae every 7 days.*
*Two results on the same column differ significantly at the 5% threshold, if they are assigned to any joint letter (Newman and Keuls test).*

**Table 4. Reduction of leaf spot on 55-day-old rice plants, following various plant treatment programs since the age of 3 weeks**

| Treatment programs | Severity | Incidence | Infection coefficient | Reduction Percentage (%) |
|---|---|---|---|---|
| Control (Hot) | 4 | 122 | 488 | - |
| *T. harzianum* | 2,1 | 88,02 | 148,84 | 69,5 b |
| Mancozebe | 1,2 | 85,3 | 102,36 | 79,02 a |
| Mancozebe /*T. harzianum* | 1,5 | 80,8 | 121,2 | 75,64 a |

*The control one was treated every 7 days by P. oryzae only.*
*The other plants are treated along the experiment every 10 days with the fungicide, the antagonist or the alternation of the two, and they are inoculated by spraying by P. oryzae every 7 days.*
*The results of the same column followed by different letters differ significantly at 5%*

variability, it developed resistance to several fungicides following their intensive applications. Indeed, the resistance of *P. oryzae* to pyrazophos has been invoked by several authors [26,27]. Sen Gupta and Das [28] have obtained *in vitro* a stems from *H. oryzae* resistant to mancozeb. Leroux [27] reported that negative cross resistance was observed in *P. oryzae*, between phosphorothiolates (PBI, edifenphos) and phosphoramidates.

The purpose of this study was to reduce the use of fungicides during rice cultivation season. For this reason, the application of fungicides is alternated with *T. harzianum*. The level of control of both diseases by the fungicides tested was similar, sometimes better than that obtained following fungicide treatments alone. Therefore, IPM approach has achieved a double effect. First, there are fewer fungicide residues in the vicinity of the cropping system, and secondly, pathogenic populations are less exposed to fungicides and therefore less likely to develop resistance to these fungicides.

As an alternative to chemical control, against *Botrytis cinerea*, which leads to the appearance of resistant pathotypes [29,30,31], several works have successfully integrated, strain T39 of *T. harzianum* to the chemical control by different benzimidazoles against this pathogen [32,33,34,35].

## 5. CONCLUSION

In view of the results obtained, the treatment of rice plants with pyrazophos alone or with mancozebe alone, respectively against *P. oryzae* and *H. oryzae*, reduced the two pathogens at similar rates as those obtained with the two fungicides alternated with *T. harzianum*. While the combination of mancozebe and *T. harzianum* reduced blast rice more strongly than the application of mancozebe alone. Therefore, for to rational use of fungicides, the introduction of *T. harzianum* in rice blast and leaf spot control systems is a promising alternative.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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