Original Research Article

Sustainable application of NATIVO 300 SC fungicide (Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) in the control of brown spot (*Bipolarisoryzae*) in strict rainfed rice cultivation in Côte d'Ivoire

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ABSTRACT

Rice brown spot is a devastating disease caused by *Bipolaris oryzae*. Various fungicide formulations are used against this phytomycosis, which occurs in all ecologies in Côte d'Ivoire. This this study aimed to rationalize the application of a fungicide effective against brown spot in strict rainfed rice cultivation.The experimental design was a Fisher block with three replications. It was carried out in strict rainfed ecology at Biankouma between July and November 2023. The fungicide used was NATIVO 300 SC (Tebuconazole 200 g/L + Trifloxstrobin 100 g/ L). Five different treatments were tested (T0: no fungicide treatment; T1: fungicide treatment every 10 days from the date of appearance of the first symptoms of brown spot until 50% of rice panicle heading; T2 : fungicide treatment at seedling (4 to 5 leaves), booting and complete panicle heading; T3: fungicide treatment at seedling (4 to 5 leaves), maximum tillering and complete heading; T4: fungicide treatment at early tillering (2 to 3 visible stems), maximum tillering and complete heading.) The main data collected were leaf severity indices and agronomic data. Statistical analyses were performed using SAS 9.4 software. As results, treatments T1 and T4 proved more effective, with leaf severity indices of 2.0±0.98 and 3.52±0.78 versus 6.52±0.66 for the control, and yield gains of 67.69 and 60.09% respectively. In conclusion, application of NATIVO 300 SC fungicide at a dose of 350 ml/ha can be recommended at the early tillering, maximum tillering and complete heading stages of rice for effective management of brown spot and environmental protection.

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Keywords: Chemical management, fungal disease, rice, phenological stages, environmental protection

1. INTRODUCTION

Rice is a cereal that is consumed as a staple food in Côte d'Ivoire. In fact, the national average consumption of rice is estimated at 70 kg of milled rice per person, per year [1]), while other staple foods are 17 kg per capita per year for wheat and 40 kg per capita per year for maize [2]. However, this cereal is insufficiently produced in Côte d'Ivoire. This gap in local production has been growing since the dissolution of SODERIZ at the end of the 1970s and will reach 50% by 2022. Faced with this situation of food insecurity, the government has been implementing a National Rice Development Strategy (SNDR) since 2012, with the short-term goal of achieving self-sufficiency by 2025 [2]. To achieve this, it will be necessary, among other things, to overcome the major biotic and abiotic constraints at the root of crop losses [3]. Among biotic constraints, fungal and viral diseases of rice reduce yields and increase production costs [4]. Among fungal diseases, brown spot due to the fungus *Bipolarisoryzae* has been one of the most damaging to rice cultivation for about fifteen years [3]; [5]; [6]; [7[8]. Paddy losses due to brown spot have also been reported by several authors [10]; [11]; [12]. The occurrence of rice brown spot in almost all rice crops in Côte d'Ivoire threatens food security [8]. In fact, this disease was the cause of the Bengal famine in India in 1942, which resulted in the deaths of more than two million people [9]. Various phytosanitary products have been tested in several rice-producing countries to control rice brown spot [5] [6];[8]; [10]; [11]; [12]; [13]. Some of these formulations have been shown to be effective against rice brown spot in Côte d'Ivoire. These are the fungicides NATIVO 300 SC (tebuconazole 200 g/L +trifloxystrobin 100 g/L) and ANTRACOL 70 WP (propineb 70%); [5]; [8]. Although these products are effective, the financial implications of their use and their potential impact on the environment require intelligent approaches or recourse to other control methods. Moreover, the systematic use of chemical molecules exposes us to the risk of the emergence of resistant strains of pathogens [14]; [15]. The aim of this study was to

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rationalize the use of a fungicide effective against brown spot in a strict rainfed rice production system.

2. MATERIAL AND METHODS

2.1. MATERIAL

The plant material used in this study is a widely cultivated local traditional cultivar named "*Gbéklélé*", with a medium cycle (105 days) and susceptible to brown spot.

2.2. METHODS

2.2.1. Study site

The trial was carried out in strict rainfed ecology at Biankouma (Côte d'Ivoire) July to November 2023. This locality has been endemic for brown spot in recent decades [5]; [6]. Geographically, the study site was referenced 07°40'36.2"N longitude, 007°36'25.9"W latitude at an altitude of 536 m.

2.2.2. Experimental design

A Fisher block design with three (03) repetitions was used. The fungicide NATIVO 300 SC (a.i: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) was used at a dose of 350 ml/ha for its efficacy against *Bipolarisoryzae*, the fungus responsible of rice brown spot according to the work of Bouet et al. [6].

The factor studied was "the NATIVO 300 SC fungicide application method" at five (05) levels as follows:

- **T0**: no fungicide treatment;

- **T1**: NATIVO 300 SC at a dose of 350 ml.ha^{-1}, every 10 days interval from the initial appearance of brown spot symptoms until 50% heading stage;

- **T2**: treatment with NATIVO 300 SC at a dose of 350 ml.ha^{-1}, at the seedling (4 to 5 leaves), booting and complete heading stages;

- **T3**: treatment with NATIVO 300 SC at a dose of 350 ml.ha^{-1}, at seedling (4 to 5 leaves), maximum tillering and complete heading stages;

- **T4**: treatment with NATIVO 300 SC at a dose of 350 ml.ha^{-1}, at early tillering (2 to 3 stems visible), maximum tillering and complete heading stages.

The surface area of each elementary plot was 12 m^2 (4 m x 3 m). The elementary plots were 2 m apart. The same distance was observed between blocks.

2.2.3. Conduct of the trial

Setting up the trial began with two main operations to prepare the land: clearing biomass removal and shallow ploughing. Four hundred (400) m² of plots were prepared. Sowing was then carried out in aligned stacks spaced 0.20 m apart. A single-plant removal was carried out 10 days after emergence in moist soil. Two manual weeding operations were carried out to maintain the individual plots. The first was carried out at 20 days after sowing (DAS), at the early tillering stage. The second weeding was carried out at maximum tillering at 45 JAS. Fungicide treatments were applied to these elementary plots according to the above fungicide application rates. Protection against aphids was applied from the milky grain stage. The crop was harvested at technological maturity (paddy with 18% moisture content).

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2.2.4. Collected data

Phytosanitary and agronomic data were collected during this study. Phytosanitary data concerned leaf severity indices and brown spot incidence. These data were scored according to IRRI's standard scoring scale[16]. Ratings were made every ten days from the early tillering stage when the first symptoms of brown spot were observed. Agronomic parameters (fertile tillers, non-fertile tillers, plant height 1000-grain weight and paddy weight) were recorded at technological maturity (paddy at 18% moisture). The paddy weights per elementary plot were determined after drying and winnowing. In addition, certain data were estimated. These are:

- AUBSPC (area under brown spot progression curve) using the formula:

AUBSPC=$\sum_{n=i}^{n}(Y_{n+1}+Y_i)0,5(T_{n+1}+T_i)$ (where: Yi = brown spot severity at ith date; Ti = date on which the Yi severity scoring was obtained; n= number of times where observations were made) ;

- Yield gain, calculated according to the formula below:

$$\boldsymbol{Yield\ gain}\ (\%) = \frac{\boldsymbol{Yield\ Tf - Yield\ T0}}{\boldsymbol{Yield\ T0}} * \mathbf{100}$$

Where Yield **Tf**= Yield of the fungicide treatment (T1, T2, T3 or T4) plots and Yield **T0**= Yield of control plots (T0)

2.2.3. Statistical analysis

Data were analyzed using SAS 9.4 software[17]. Brown spot severity indices data and area under brown spot progression curve (AUBSPC) values, analysis of variances were performed using a general linear model (GLM), while for agronomic data, it was performed using one-factor ANOVA. The Newman-Keuls test (probability threshold p=0.05) was used for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1. Effect of treatments on brown spot severity at different stages of rice development

At the early tillering stage (20 days after sowing), the first symptoms of the disease were observed on all plots before fungicide application, the severity index of brown spot was (1) for all treatments (Fig. 1).

At the maximum tillering stage, i.e. 45 days after sowing, the average severity index of brown spot in plots under treatment T0 was 2.33. For plots in treatment T1, the severity index of the disease was (1). For plots under treatments T2 and T3, the average severity index of brown spot was the same, i.e. (1.67). For plots under treatment T4, the average brown spot severity index was 1.33 (Fig. 1).

At the booting stage (75 days after sowing), the brown spot severity index for plots treated with T0 was 5.67. In plots receiving treatment T1, the disease severity index was 1.67. In plots receiving treatments T2 and T3, the same severity index of brown spot was observed with an average value of 3.00. For treatment T4, the average severity index of brown spot at the bolting stage was 2.33 (Fig. 1).

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At the heading stage (90 days after sowing), plots receiving treatment T0 showed an average severity index of 6.67. For plots under treatment T1, brown spot severity index was 2.33. On plots receiving treatments T2 and T3, the disease severity index was the same 3.67. For treatment T4, brown spot severity index was 3.33 (Fig. 1).

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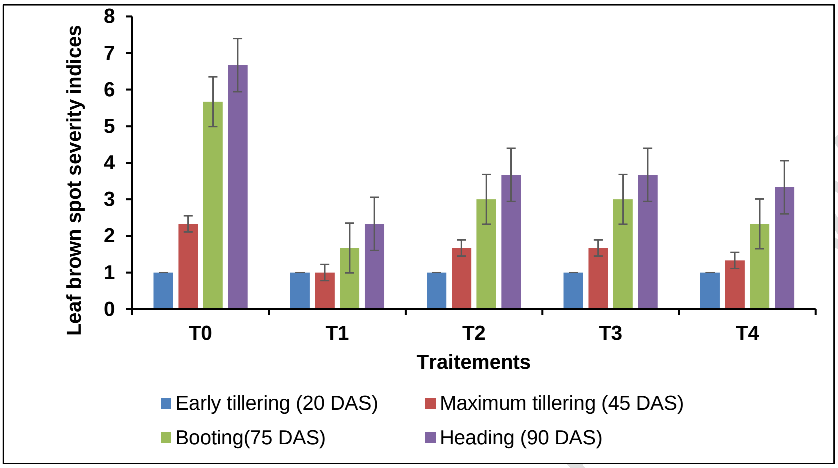

Fig.1: Brown spot severity index progression according to rice development stage and the treatments

3.2. Effect of NATIVO 300 SC on the severity of rice brown spot according to application methods

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According to the application modality of NATIVO 300 SC (ma: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L), the severity of brown spot recorded at rice maturity was 2.09±0.98 for plots under treatment T1. For plots receiving treatment T2, the severity index of brown spot was 3.78±1.45. In plots receiving treatment T3, the average disease severity index was 3.52±0.78. In the plots under treatment T0 a high evolution of the disease was recorded, with an average severity index of 6.54±0.66 (Table 1). The mean values of the area under the brown spot progression curve (AUBSPC) for the fungicide treatments ranged from 93.57±1.5 for treatment (T1) to 155.52±0.48 for (T3), compared with a value of 270.5±0.00 for the control (T0).

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Statistical analysis of severity indices data and AUBSPC values recorded at the maturity stage showed a significant difference (p=0.0001) between fungicides treatments applied in this trial. Treatment (T1) was fund to be more effective, with brown spot severity index of 2.09±0.98 and AUBSPC mean value of 93.57±1.5. This treatment was followed by the fungicide treatment (T4), which showed a mean severity index of 3.52±0.78 and AUBSPC mean value of 129.20±0.83. Treatments T2 and T3 had roughly the same effects, but weaker than those of treatments T1 and T4 (Table 1).

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Table 1: Brown spot severity according to NATIVO 300 SC (Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) fungicide application methods

Treatment	Leaf severity index	AUBSPC
T0	6.54±0.66a	270.07±0.00a
T1	2.09±0.98d	93.75±1.50d
T2	3.78±1.45b	155.27±1.27b
T3	3.85±0.60b	155.52±0.48b
T4	3.52±0.78c	129.20±0.83c
Overall mean	3.96±0.56	159.26±5.90
Probability (p)	0.0001	0.0001
Effect	S	S
CV(%)	18.14	0.612

AUBSPC: area under brown spot progression curve; CV (%): coefficient of variation; s: significant (p<0.05). The values given in the table are the mean±standard deviation for each parameter. Means followed by the same letter are not significantly different at the probability threshold (p= 0.05) according to the Newman-Keuls test.

3.3. Effect of treatments on rice agronomic parameters

3.3.1. Effect of treatments on the number of total tillers

In terms of the results obtained, the number of total tillers varied from one treatment to another. The average number of total tillers recorded in fungicide-treated plots ranged from 7.04±1.26 (T1) to 4.83±1.80 (T4). The overall average number of total tillers was 6.5, with a coefficient of variation 19.42%. A significant difference (p=0.0063) was recorded between the fungicide treatments and the control, where the average number of total tillers was 7.52±1.57 (Table 2).

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3.3.2. Effect of treatments on the number of fertile tillers

The average number of fertile tillers observed in plots treated with NATIVO 300 SC fungicide (a.i: Tebuconazole 200 g/L + Trifloxystrobin 100 g/L) ranged from 6.5 (T1) to 3.70±1.72 (T4). In the control plots (T0), the average number of fertile tillers recorded was 6.59±1.49. The overall mean number of fertile tillers was 5.64±1.47, with a coefficient of variation 24.78%. There was a significant difference (p=0.034) between the different treatments applied (Table 2).

3.3.3. Effect of treatments on rice plant height

Average rice plant height ranged from 102.69±7.47 cm (T3) to 109.22±6.02 cm (T4) on plots treated with NATIVO 300 SC fungicide (a.i: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L). For the control (T0), average height of rice plants was 102.80±5.28 cm. The overall mean of rice plants height was 106.09±6.90 cm, with a coefficient of variation 7.84%. The statistical analysis results showed a significant difference (p=0.0001) between the treatments applied (Table 2).

Table 2: Agronomic parameters (numbers of total tillers, fertile tillers and rice plants height) severity according to NATIVO 300 SC (Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) fungicide application methods

Treatment	Total tillers	Fertile tillers	Heigh (cm)
T0	7.52±1.40a	6.59±1.49a	102.80±5.28b
T1	7.04±1.26ab	6.50±0.99a	107.30±5.07a
T2	6.66±1.94ab	6.03±1.87ab	108.58±8.26a
T3	6.48±1.12ab	5.37±1.26ab	102.69±7.47b
T4	4.83±1.80b	3.70±1.72b	109.22±6.02a
Overall mean	6.50±1.57	5.64±1.47	106.091±6.90
Probability (p)	0.0063	0.034	0.0001
Effect	S	S	S
C V (%)	19.42	24.78	7.84

AUBSPC: area under brown spot progression curve; CV (%): coefficient of variation; s: significant ($p<0.05$). The values given in the table are the mean±standard deviation for each parameter. Means followed by the same letter or group of letters are not significantly different at the probability threshold ($p= 0.05$) according to the Newman-Keuls test.

3.3.4. Effect of treatments on yield and yield gain

Average paddy yields ranged from 0.63±0.16 $t.ha^{-1}$ for the control (T0) to 1.95±0.25 $t.ha^{-1}$ for the fungicide treatment (T1). The overall average yield for the trial was 1.43±0.48 $t.ha^{-1}$ with coefficient of variation 15.26%. Statistical analysis showed a significant difference ($p=0.0003$) between the yields of the fungicide-treated plots and the control plots (T0). No significant difference was observed between fungicides treatments for the yields. However, a yield gain of 67.69% was obtained with treatment (T1) followed by treatment (T4), which showed a yield gain of 60.37%. For treatments T2 and T3, the yield gains observed were 58.82% and 58% respectively (Table 3).

Table 3: Rice yields severity according to NATIVO 300 SC (Tebuconazole 200 g/L +Trifloxystrobin 100 g/L)on Rice yields fungicide application methods

Treatment	Yield ($t.ha^{-1}$)	Yield gain (%)
T0	0.63±0.16b	-
T1	1.95±0.25a	67.69
T2	1.53±0.33a	58.82
T3	1.50±0.14a	58
T4	1.59±0.13a	60.37
Overall mean	1.43±0.48	
Probability (p)	0.0003	
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CV(%)	15.26	

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AUBSPC: area under brown spot progression curve; CV (%): coefficient of variation; s: significant (p=0.05). The values given in the table are the mean±standard deviation for each parameter. Means followed by the same letter or group of letters are not significantly different at the probability threshold (p= 0.05) according to the Newman & Keuls test

3.4. Regression between AUBSPC and performance

A negative correlation was noted between yield and AUBSPC (R^2 = 0.8447). AUBSPC contributed 84.47% to yield variation, while other factors contributed for 15.53%. According to the linear regression equation for one unit of AUBSPC, yield is reduced by 0.0073 tonnes or 7.3 kg (Figure 2).

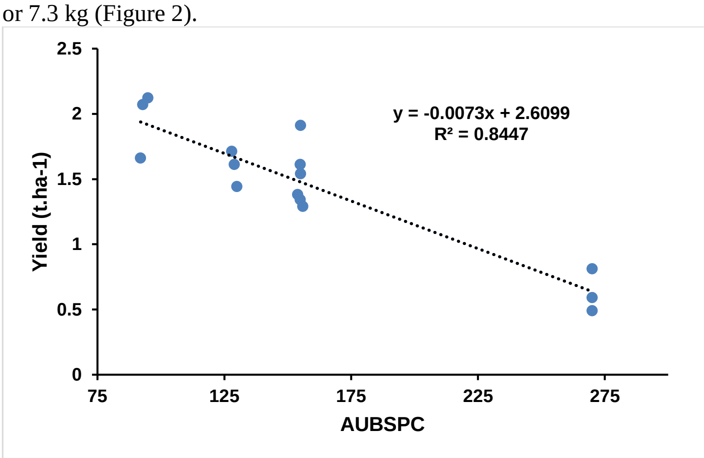

Fig. 2: Linear regression curve between yield and area under brown spot progression curve (AUBSPC) values

4. DISCUSSION

Application of NATIVO 300 SC fungicide at the dose of 350 ml/ha at different phenological stages of the rice crop, induced a significant reduction of brown spot leaf severity. Indeed, for all plots that received the fungicide treatments (T1, T2, T3 and T4), the brown spot leaf severity was reduced. In the case of fungicide treatment (T1), application of NATIVO 300 SC at a frequency of ten days from the observation of the first symptoms of brown spot at the early tillering stage (20 days after sowing), reduced the brown spot leaf severity in rice to an average index of 2.09 and AUBSPC average value of 95.75, resulting in a yield gain of 67.69% compared with the control. Furthermore, when the fungicide treatments were applied at specific phenological stages, in the case of treatments T2, T3 and T4, a significant reduction of brown spot leaf severity was also observed. However, treatment T4 (application of the fungicide NATIVO 300 SC (a.i: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) at a dose of 350 ml/ha at the early tillering (2 to 3 stems visible), maximum tillering and complete panicle heading stages) was more effective than treatments (T2 and T3). Treatment T4 reduced the disease severity index to 3.52, with AUBSPC average value of 129.20 and a yield gain of 60.37% compared to treatments T2 and T3, for which the observed leaf severity indices were 3.78 and 3.85, and AUBSPCs average values of 155.27 and 155.52 respectively. Average yield gains for these treatments were 58.82 and 58% respectively. For treatment T1, NATIVO fungicide was applied eight times from observation of the first symptoms to 50% heading. For the other treatments (T2, T3 and T4), the fungicide was applied three times.

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The work of Bouet et al [6] had shown that the fungicide NATIVO 300 SC (a. I: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) is effective at the dose of 350 ml/ha in reducing brown spot leaf severity. Treatment T1 was more effective, as it appears to prevent the complete development cycle of the fungal agent *Bipolaris oryzae* responsible of rice brown spot. In fact, the period during which this study was carried out was one of high humidity (75-80%), with an average temperature varying between 24° and 28°C, and therefore favorable to the development of the disease.

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According to Datnoff & Lentini[18], *Bipolaris oryzae* infection can occur between 20° and 26°C, and is favored by high relative humidity (86 248 to 100%) and the presence of free water on the plant surface. *Bipolarisoryzae* conidia germinate by producing a germ tube which penetrates the leaf through the epidermis or stomata. In the case of treatments (T2, T3 and T4) where the duration between two successive applications exceeded 25 days at least, the pathogen was able to establish itself adequately, as this duration allows *Bipolaris oryzae* to complete its life cycle. It has been found that rice leaf brown spot mainly attacks the late vegetative stages and becomes severe from the reproductive stage through to maturity, affecting photosynthetic activity where maximum translocation of sugars occurs from the leaves to the grains, which is responsible for the formation of light, shrivelled and straw-like grains and reduced yield [19]. Also, during rice growth, fungus spores are formed on leaf spots and can subsequently infect other leaves and panicles [20]. Severely infected leaves may die before maturity and these plants will produce light or empty grains [21], [20]. The low yield recorded in the control plots is therefore due to the reduction in the leaf area of rice plants. In the case of treatment T4, despite the low average number of fertile tillers compared with the other treatments, yield was high, as was yield gain. This could be explained by good grain filling in plots receiving this treatment.

Kohls et al. [22] reported that yield losses specific to each growth stage and indicated that rice brown spot (*B. oryzae*) caused the greatest yield reductions when epidemics were triggered at the germination stage, and successively decreased when triggered late at the panicle initiation, heading and grain filling stages. Furthermore, Percich et al [23] demonstrated that the highest rates of infection by *B. oryzae* occurred at temperatures of 25 and 30°C and generally increased with periods of continuous humidity of 16, 18, 24 and 28 hours. Shafeeqa & Nandini, [24] reported that fungicide treatments increased the number of tillers and the height of tillers compared with the absolute control. Furthermore, they also showed that NATIVO 75 WP fungicide at 70 ppm could extend the grain filling period by up to 6 days compared to the absolute control, maximizing grain yield. This could be due to ethylene production, which has the ability to decrease leaf and panicle senescence [24]. Balgude&Gaikwad, [11] also showed that spraying a fungicide combination of trifloxystrobin 25% + tebuconazole 50% at a dose of 0.04% at 15-day intervals, starting the first spray immediately after disease onset, proved highly effective against brown spot, leaf blight, neck blight, knot blight, sheath rot and leaf scald, resulting in improved paddy grain yields. To ensure the safe application of trifloxystrobin and tebuconazole, Wang et al. [25] conducted a study on their dissipation, terminal residues, distribution and associated risk factors. The results of their study indicated that trifloxystrobin and tebuconazole dissipate rapidly in rice, with an estimated half-life of between 4.1 and 7.7 days. The T4 treatment could therefore be recommended for rational use of the fungicide NATIVO 300 SC (a. i: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) at a dose of 350 ml/ha in the control of rice brown spot, in order to prevent the risk of environmental pollution and ensure good rice quality.

5. CONCLUSION

This study evaluated the effect of different application methods of the fungicide NATIVO 300 SC (ma: Tebuconazole 200 g/L +Trifloxystrobin 100 g/L) at a dose of 350 ml/ha on helminthosporiosis of rice. All fungicide treatments **tested** significantly reduced

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disease severity. However, treatments T1 and T4 were the most effective, with yield gains of 67.69% and 60.09% respectively. Phenological stages that proved favorable for effective control of brown spot were early tillering, maximum tillering and heading. Application of the fungicide NATIVO 300 SC (Tebuconazole 200 g/L + Trifloxystrobin 100 g/L) according to treatment T4 can be recommended to rice growers at these phenological stages, as it not only reduces production costs but also less fungicide is used, thus preserving the environment. Looking ahead, a study needs to be carried out on the biochemical and organoleptic quality of rice produced under these conditions to confirm the absence of fungicide residues.

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