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# Field Evaluation of the Efficacy of Fungicides and Rhizosphere Bacterial Antagonists against Rice Stem Rot Disease

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***Authors' contributions***

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

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## ABSTRACT

Rice stem rot disease in paddy cultivation leads to reduction of yield. Management of this disease is difficult as it is soil borne. In the present study, field trials were conducted during *Rabi* 2016-17 and *early Kharif* 2017-18 to assess the effectiveness of twelve fungicides and four rhizosphere bacterial antagonistic isolates (RRB-1 to RRB-4) against rice stem rot disease. Disease incidence was recorded from the date of initial incidence of the disease *i.e*., once in 15 days from maximum tillering to panicle emergence. Pooled analysis reveals that Hexaconazole recorded lowest per cent disease index (32.22) with highest grain yield and straw yield of 7.43 t/ha and 7.22 t/ha respectively, followed by Difenoconazole with grain yield and straw yield of 7.29 t/ha and 6.88 t/ha

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respectively. On the other hand, bacterial antagonists using root dipping and foliar spray with RRB-4 showed less PDI of 32.92 with grain yield of 6.97 t/ha and straw yield of 6.61 t/ha. With this study, hexaconazole (0.2%) and bacterial antagonists RRB-4 using root dipping and foliar spray ($10^5$ to $10^6$ CFU/ml) can be effectively managed the rice stem rot.

*Keywords: Fungicides; bacterial antagonists; stem rot; rice.*

## 1. INTRODUCTION

Rice serves as the main source of millions of livelihoods and staple food. The production oiif rice was affected by pest diseases significantly [1]. Stem rot disease due to *Sclerotium oryzae* Catt. (*Magnaporthe salvinii* (Catt.) is one of the major soil borne diseases of rice crop and the diseases damages the crop from forty five days after sowing to harvest of the crop. Earlier was considered as a minor disease but in recently it has been emerged as a major disease and it lead to reduction of rice yield by 5 - 80 % [1-3]. The stem rot decease initially appears during the tillering stage of the crop. *Sclerotium oryzae* produces irregular jet black lesions on the leaf sheath just above the water line or basal portion of the stem. As the disease advances, jet black irregular lesions advances in the upward direction which leads to death of the infected sheath. The infection is deep seated, pathogen not only affect the leaf sheath and also penetrate the culm thereby reduces the panicle size and grain quality [1,2]. When the pathogen attacks early in the season, either tillers are lodged or killed. Severe cases of infection lead to the formation of sterile panicles. During off season the pathogen survives by producing hard, black resting structures (i.e., sclerotia) in the soil and in plant debris. Stem rot limits rice (*Oryza sativa*) production in when there are high inoculum levels in the soil [4]. Management of *Sclerotium oryzae* is very difficult as it produces sclerotial bodies in soil. Although cultural, physical control methods are available but they are in limited use especially under epidemic conditions. Chemical and bio control methods are found to be reliable and effective [5,6]. In the present study an investigation has been conducted to evaluate the comparative efficacy of different chemical fungicides and antagonistic bacteria against rice stem rot disease.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Bacterial Antagonists

In the present study soil samples were collected from rice field rhizosphere and these samples were dried at room temperature. Bacterial antagonists were isolated from the collected soil by following serial dilution technique [7] and were purified using streak plate method. Isolated bacterial antagonists as *Pseudomonas* were identified based on gram reaction, colony morphology, fluorescence and cell shape [8]. Based on the dual culture technique [9] and by using the following formula by Vincent [10].

$$I = \frac{C - T}{C} X100$$

I= Inhibition percentage, C= test pathogen growth in control, T= test pathogen growth in treatment

Four effective antagonistic *Pseudomonas* isolates (named as RRB-1 to RRB-4) against *S. oryzae* were chosen in field evaluation.

### 2.2 Evaluation of Selected Fungicides and Bacterial Antagonists against *Sclerotium oryzae* under Field Conditions

Field studies were conducted during *Rabi* 2016-17 and *Early Kharif* 2017-18 under natural disease condition to evaluate the efficacy of fungicides and antagonistic *Pseudomonas* isolates (Table 1). The compatibility between antagonistic *Pseudomonas* isolates and fungicides were carried out through spectrophotometric method *in vitro* [11]. Field studies were carried out in a randomized block design (RBD) using MTU-1010 with a spacing of 15*15 cm gross plot size of 13.455 sq m. with three replications, the ten diseased plants/ samples were selected randomly in respective treated plot and observed for percent disease index (PDI). Suspension of *Pseudomonas* isolates were used for root dipping of seedlings at possible highest concentrations ($10^5$ to $10^6$ CFU /ml of suspension) before transplanting and foliar application on diseased plants after transplanting. The treatments (fungicides and antagonistic isolates) were tested *in vitro* for their efficacy and standardised their respective

**Table 1. Treatments implemented in the field study during *Rabi* 2016-17 and *Early Kharif* 2017-18**

| S. No. | Treatments | Dosage |
|---|---|---|
| $T_1$ | Carbendazim | 0.1% |
| $T_2$ | Propiconazole | 0.1% |
| $T_3$ | Hexaconazole | 0.2% |
| $T_4$ | Difenoconazole | 0.2% |
| $T_5$ | Tebuconazole | 0.15% |
| $T_6$ | Trifloxystrobin+ Tebuconazole | 0.08% |
| $T_7$ | Azoxystrobin | 0.1% |
| $T_8$ | Krysoxymethyl | 0.1% |
| $T_9$ | Isoprothiolane | 0.15% |
| $T_{10}$ | Mancozeb + Carbendazim | 0.2% |
| $T_{11}$ | Thiophanate methyl | 0.1% |
| $T_{12}$ | Benomyl | 0.1% |
| $T_{13}$ | Root dipping and foliar spray with RRB-1 | $10^5$ to $10^6$ CFU/ml of suspension |
| $T_{14}$ | Root dipping and foliar spray with RRB-2 | |
| $T_{15}$ | Root dipping and foliar spray with RRB-3 | |
| $T_{16}$ | Root dipping and foliar spray with RRB-4 | |
| $T_{17}$ | Control | |

dosage by poisoned food technique [12] by using the following formula [10].

$$I = \frac{C - T}{C} X100$$

I= Inhibition percentage, C= test pathogen growth in control, T= test pathogen growth in treatment

**Table 2. Description of stem rot disease scale (0-9 scale of SES for rice)**

| Scale | Percentage of infected tillers |
|---|---|
| 0 | No disease observed |
| 1 | Less than 1% |
| 3 | 1-5% |
| 5 | 6-25% |
| 7 | 26-50% |
| 9 | 51-100% |

## 2.3 Observations

Disease incidence and severity of stem rot before each spray once in 15 days from maximum tillering to panicle emergence was recorded. Appropriate disease scores (Table 2) (0-9 scale of SES, IRRI, 2013) or percentages for disease incidence (PDI) was used. In this study percentages for disease incidence, Percentage of disease severity (PDS), Grain yield and straw yield were recorded. The percentages for disease incidence and PDS were calculated using equ 1 and 2 respectively.

(a) Percentage of disease incidence =

$$\frac{\text{Number of infected tillers}}{\text{Total number of tillers per hill}} \times 100 \quad (1)$$

(b) Percentage of disease severity (PDS) =

$$\frac{\text{Number of individual ratings}}{\text{Number of plants assessed}} \times \frac{100}{\text{Maximum scale}} \quad (2)$$

## 3. RESULTS AND DISCUSSION

In this study an attempt is made to evaluate the chemicals and *Pseudomonas* isolates to reduce chemical usage in order to manage the rice stem rot. The investigation was conducted to evaluate different fungicides (12) and rhizosphere *Pseudomonas* isolates (4) against stem rot of rice. The data on stem rot disease (PDI) was recorded from the initial incidence of the disease at two times i.e before first spray and second spray of treatments at fifteen days interval. Among the tested treatments during *Rabi* 2016-17, $T_1$ showed the least PDI with 32.82 followed by $T_{11}$, $T_3$, T, $T_7$ and $T_8$. Highest PDI 48.01 was recorded in $T_{14}$. In untreated control, percent disease index was 54.67 at fifteen days after first spray. The percent disease index was recorded after second spray of all treatments at fifteen interval reveals that, treatment $T_3$ with least PDI with 36.79 followed by $T_1$, $T_7$, $T_4$, $T_8$ was observed. In untreated control, the Percent disease index of 64.29 was recorded Fig. 1. In this present study, treatment $T_1$ showed lowest PDI at first spray of treatments but after second spray $T_3$ controlled the disease effectively. At initial stages of infection, $T_1$ showed good control over the disease, as the disease advances the efficacy was slightly reduced. The treatment $T_3$ (Hexaconazole) performed effectively in controlling the disease at maximum infection.

During *Early Kharif* 2017-18, the efficacy of fungicides and four rhizosphere *Pseudomonas* isolates (Table 1, Fig. 2) was studied. Among all the treatments, $T_{16}$ showed least PDI of 25.00 followed by $T_3$, $T_{15}$ and $T_4$. Least PDI of 32.99 was observed in $T_3$. The PDI of stem rot in treatments $T_2$, $T_5$, $T_{11}$, $T_4$, $T_{10}$, $T_6$, $T_1$, $T_{16}$, $T_9$, $T_8$, $T_{12}$, $T_{14}$, $T_7$ and $T_{13}$ treatments ranged from 40.32 to 53.00 were on par among themselves at fifteen days after the second spray. The disease in untreated control ($T_{17}$) with 62.00 PDI. During initial stages of infection the treatment $T_{16}$ was effective but the as the infection becoming worse, the efficacy of $T_{16}$ was not up to the mark in disease control where as the treatment $T_3$ found to be effective and stable in managing the disease.

Results from the two crop seasons was subjected to Pooled analysis (Table 3) showed that least PDI of 32.22 was recorded in $T_3$ followed by $T_{16}$, $T_4$, $T_{11}$, $T_1$ and $T_5$ which were at par with each other. Treatment $T_{14}$ (Root dipping and foliar spray with RRB-2; 47.34 PDI) showed maximum PDI next to untreated control ($T_{17}$) followed by $T_9$ (Isoprothiolane; 46.32 PDI), $T_{13}$ (Root dipping and foliar spray with RRB-1; 44.13 PDI), $T_{12}$ (Benomyl; 43.93 PDI) and $T_6$ (Trifloxystrobin + Tebuconazole; 41.35 PDI) and all these treatments were at par with each other at 15 days after the first spray. Data recorded at second spray showed that least PDI in $T_3$ which was statistically significant with all other treatments. While among other treatments, (other than untreated control) maximum PDI was recorded in $T_{15}$ followed by $T_{14}$, $T_{13}$ and $T_9$ which were on par with each other.

## 3.1 Grain and Straw Yield

The stem rot disease also effects the grain yield in paddy. Among the treatments, maximum grain yield was observed in Hexaconazole ($T_3$) followed by $T_4$, $T_5$, $T_{16}$, $T_1$ and $T_{10}$ (Table 4). Lowest grain yield was observed in $T_{15}$. Straw yield also recorded in the treated plots. Among them, maximum straw yield was observed in $T_3$ followed by $T_4$ and $T_2$.

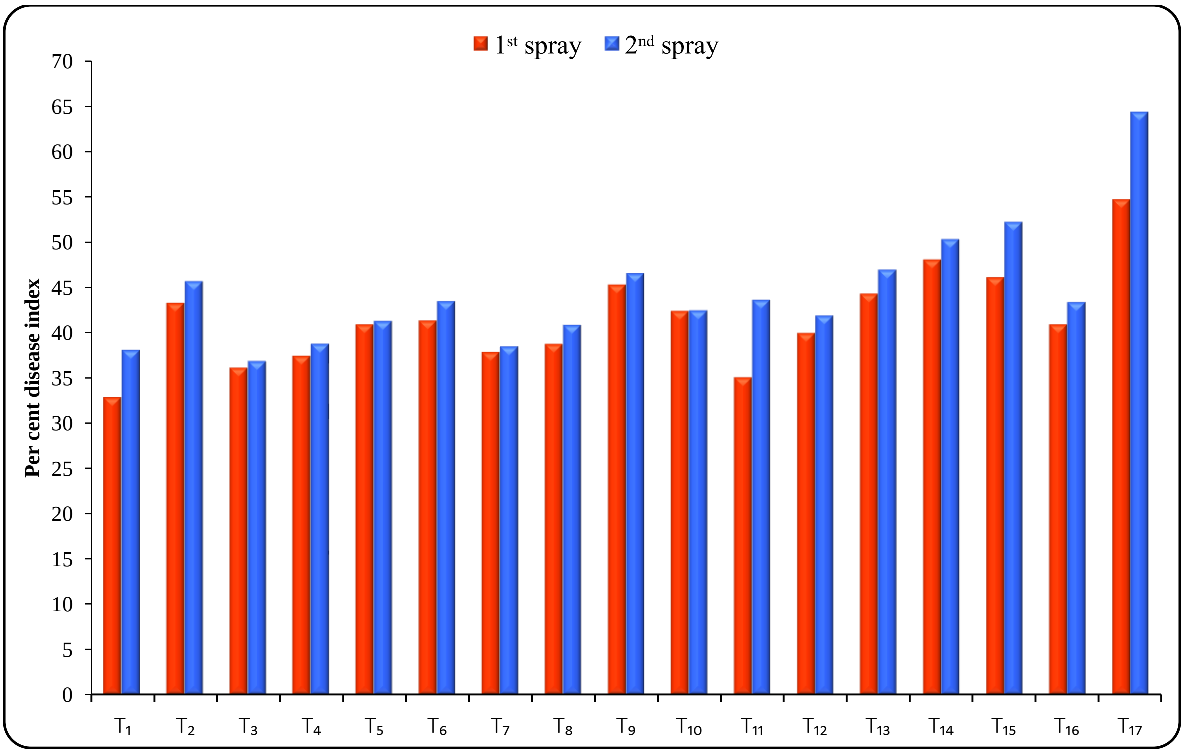


**Fig. 1. Efficacy of fungicides and bio control agents at 15 days interval on stem rot per cent disease index during *rabi* 2016-17**

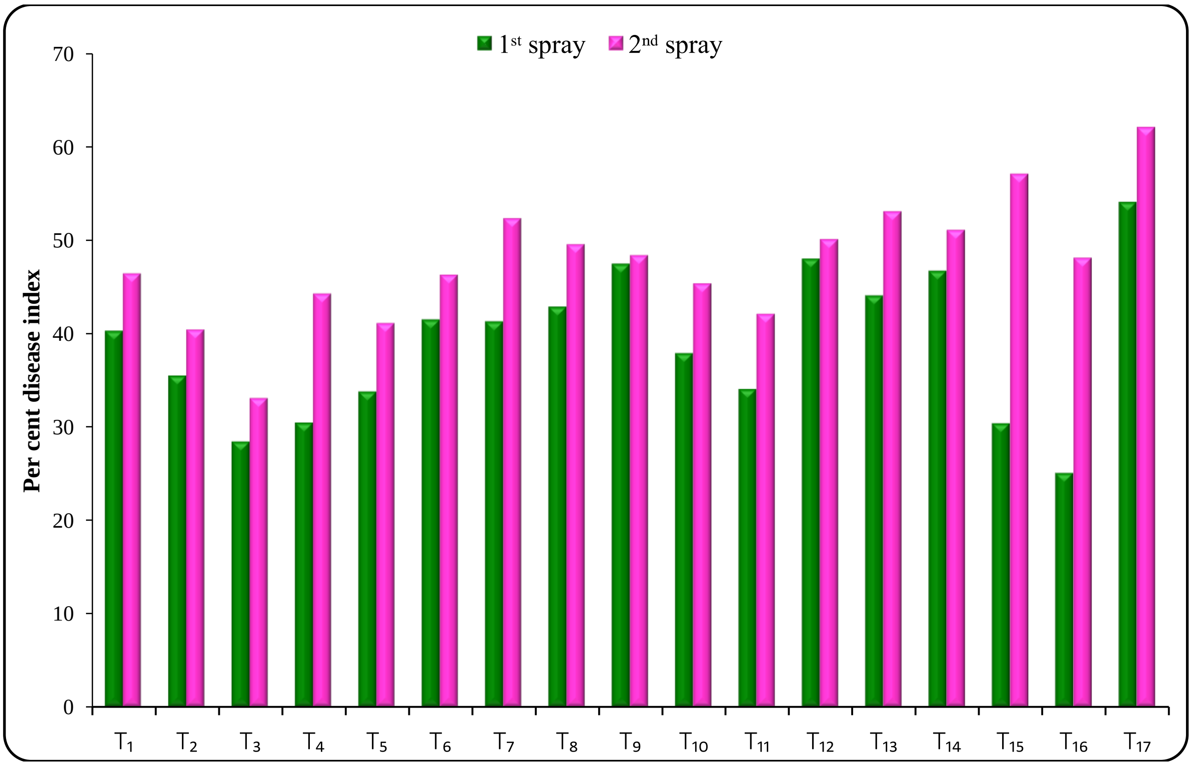


**Fig. 2. Efficacy of fungicides and bio control agents at 15 days interval on stem rot per cent disease index during *early kharif* 2017-18**

**Table 3. Field evaluation of the selected fungicides and antagonistic bacterial isolates on stem rot of rice-pooled data**

| S. No. | Treatments | Percent disease index (PDI) | |
|---|---|---|---|
| | | 15 days after 1st spray | 15 days after 2nd spray |
| $T_1$ | Carbendazim | 36.53 (37.16)$^{efghi}$ | 43.51 (41.25)$^{e}$ |
| $T_2$ | Propiconazole | 39.34 (38.83)$^{defg}$ | 44.30 (41.72)$^{de}$ |
| $T_3$ | Hexaconazole | 32.22 (34.55)$^{i}$ | 34.89 (36.18)$^{f}$ |
| $T_4$ | Difenoconazole | 33.90 (35.58)$^{ghi}$ | 41.44 (40.06)$^{e}$ |
| $T_5$ | Tebuconazole | 37.29 (37.62)$^{dfghi}$ | 41.11 (39.87)$^{e}$ |
| $T_6$ | Trifloxystrobin + Tebuconazole | 41.35 (40.01)$^{bcde}$ | 44.80 (42.01)$^{cde}$ |
| $T_7$ | Azoxystrobin | 39.53 (38.95)$^{defg}$ | 45.33 (42.31)$^{cde}$ |
| $T_8$ | Krysoxymethyl | 40.75 (39.65)$^{cde}$ | 45.12 (42.19)$^{cde}$ |
| $T_9$ | Isoprothiolane | 46.32 (42.88)$^{bc}$ | 47.39 (43.50)$^{bcde}$ |
| $T_{10}$ | Mancozeb + Carbendazim | 40.41 (39.27)$^{def}$ | 43.50 (41.25)$^{e}$ |
| $T_{11}$ | Thiophanate methyl | 35.84 (35.96)$^{fghi}$ | 42.77 (40.83)$^{e}$ |
| $T_{12}$ | Benomyl | 43.93 (41.51)$^{bcd}$ | 45.90 (42.64)$^{cde}$ |
| $T_{13}$ | Root dipping and foliar spray with RRB-1 | 44.13 (41.63)$^{bcd}$ | 49.93 (44.96)$^{bcd}$ |
| $T_{14}$ | Root dipping and foliar spray with RRB-2 | 47.34 (43.47)$^{b}$ | 50.62 (45.35)$^{bc}$ |
| $T_{15}$ | Root dipping and foliar spray with RRB-3 | 38.20 (38.17)$^{defgh}$ | 53.57 (47.05)$^{b}$ |
| $T_{16}$ | Root dipping and foliar spray with RRB-4 | 32.92 (35.00)$^{hi}$ | 45.65 (42.50)$^{cde}$ |
| $T_{17}$ | Control | 54.33 (47.49)$^{a}$ | 63.14 (52.62)$^{a}$ |
| | CD (0.05) | 3.575 | 3.627 |
| | CV | 5.472 | 5.104 |

**Table 4. Effect of fungicides and antagonistic rice rhizosphere bacterial isolates on grain and straw yield of rice against stem rot**

| S. No. | Treatments | Yield (t/ha) | Straw yield (t/ha) |
|---|---|---|---|
| $T_1$ | Carbendazim | $6.91^{abcde}$ | $6.14^{d}$ |
| $T_2$ | Propiconazole | $6.79^{bcde}$ | $6.84^{abc}$ |
| $T_3$ | Hexaconazole | $7.43^{a}$ | $7.22^{a}$ |
| $T_4$ | Difenoconazole | $7.29^{ab}$ | $6.88^{ab}$ |
| $T_5$ | Tebuconazole | $7.19^{abc}$ | $6.35^{cd}$ |
| $T_6$ | Trifloxystrobin+ Tebuconazole | $6.42^{defg}$ | $6.46^{bcd}$ |
| $T_7$ | Azoxystrobin | $6.52^{defg}$ | $6.42^{bcd}$ |
| $T_8$ | Krysoxymethyl | $6.30^{efgh}$ | $6.54^{bcd}$ |
| $T_9$ | Isoprothiolane | $6.52^{defg}$ | $6.12^{d}$ |
| $T_{10}$ | Mancozeb + Carbendazim | $6.89^{abcde}$ | $6.58^{bcd}$ |
| $T_{11}$ | Thiophanate methyl | $6.45^{defg}$ | $6.27^{cd}$ |
| $T_{12}$ | Benomyl | $6.65^{def}$ | $6.56^{bcd}$ |
| $T_{13}$ | Root dipping and foliar spray with RRB-1 | $6.06^{fgh}$ | $6.31^{bcd}$ |
| $T_{14}$ | Root dipping and foliar spray with RRB-2 | $5.93^{gh}$ | $6.22^{d}$ |
| $T_{15}$ | Root dipping and foliar spray with RRB-3 | $5.74^{hi}$ | $6.11^{d}$ |
| $T_{16}$ | Root dipping and foliar spray with RRB-4 | $6.97^{abcd}$ | $6.61^{bcd}$ |
| $T_{17}$ | Control | $5.22^{i}$ | $5.47^{e}$ |
| | CD (0.05) | 0.61 | 0.592 |
| | CV | 5.599 | 5.548 |

The present findings were in confirmatory with Kumar et al. [13] has been reported that propiconazole was very effective fungicide in reducing stem rot disease followed by isoprothiolone. Gopika and Jagadeeshwar [14] also reported that spraying with propiconazole (100 ppm) prominently reduced the disease (1.2 disease index) over the other treatments. Pramesh et al. [5] tested new combination of chemical Captan 70% + Hexaconazole 5%) with dosage of 750 g/ha which was showed promising effects in controlling stem rot disease. Gowdar et al. [3] conducted field efficacy studies with different chemicals against rice stem rot. Thiophanate methyl 70% WP @ 1gm/l found to be effective followed by hexaconazole (0.1%) over the disease. Prameela et al. [15] conducted *invitro* studies to evaluate the efficacy of fungicides against *S.oryzae.* Among the tested chemical's, propiconazole (100ppm) and hexaconazole (200ppm) found to be very effective. Ratnakar et al. [6] evaluated different chemicals at 5 different locations against rice stem rot. They reported that spraying of hexaconazole @2ml/lit twice at maximum tillering and panicle initiation gave significant control over the disease. The present investigation revealed that, hexaconazole gave good control over the stem rot and also resulted the maximum yields. Bacterial bioagent (*Pseudomonas*) ($T_{16}$) also performed better in controlling the disease with good productivity of crop. With these results, farmers can use both chemicals and bioagents for the management of rice stem rot. Chemical usage or number of chemical sprays reduces thus least residual effect.

## 4. CONCLUSIONS

The present investigation revealed that the Hexaconazole recorded lowest PDI (34.89), highest grain yield and straw yield. Chemical control with hexaconazole (0.2%) found to be very effective against the disease. In this investigation, we also tested the bacterial bio agents, among them treatment-T16 also resulted better control against disease (45.65 PDI). Hence, this field study enabled that stem rot of rice can be managed either with bioagents or fungicides or alternate sprays of fungicides with bioagents to avoid pathogen resistance to chmeicals.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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