Morphological and Cultural Variability among Rhizoctonia solani Kuhn isolates and Management of Banded Leaf and Sheath Blight disease in Kodo Millet

التعليق [DS21]: When many isolates were studied

التعليق [DS22]: How many isolates were studied?

Abstract

The current study was undertaken to study the morphological and cultural characterisation of *R. solani* causing banded leaf and sheath blight in kodo millet which were obtained from different millet growing regions of India. *In vitro* studies were conducted in order to study the variability on six different culture media and notable variations were observed in colony growth, time taken for sclerotial initiation, pattern of arrangement of sclerotia. Oat Meal Agar showed highest growth rate and PDA exhibited maximum sclerotial number and weight. Whereas, on the other hand a field experiment was conducted using eight treatments. The fungicide Trifloxystrobin 25% + Tebuconazole 50 % WG @ 0.05% was found to be effective with low disease incidence of 14.08 %and high grain yield of 3094.44 kg ha⁻¹ and fodder yield of 70338.39 kg ha⁻¹ and greater B:C ratio.

Keywords- Media, Millet, Growth rate, *R. solani*

1. Introduction

Millets are termed as "yesterday's coarse grains and today's nutri-cereals." These are considered to be "future crops" as they are resistant to most of the pests and diseases and adapt well to the harsh environment of the arid and semi-arid regions of Asia and Africa (Gowda

et al., 2022). They are categorized as two main groups viz., major millets (sorghum and pearl millet) and minor/small millets based on grain size and area of cultivation. Kodo millet (Paspalum scrobiculatum) is one of the hardiest crops grown in Madhya Pradesh, Maharashtra, and Uttar Pradesh and various other parts of India (Patro et al., 2018). It is locally known as arika, koden, kodra, varagu, harka, kodua in different parts of the country. It is an annual grain that is grown in primarily in India, but also in the Philippines, Indonesia, Vietnam, Thailand, and in West Africa where it originates. It is grown as a minor crop in most of these areas, with the exception of the Deccan plateau in India where it is grown as a major food source and is also known as cow grass, rice grass, ditch millet, native paspalum or Indian crown grass (Patro et al., 2017). Banded blight of kodo millet incitedcaused by

التعليق [DS23]: Please mention the Scientific name

Rhizoctonia solani (Kuhn.) (Basidial stage: Thanatephorus cucumeris) is one of the emerging maladydisease in its successful cultivation. The pathogen overwinters as soilborne sclerotia and mycelium in plant debris; these constitute the primary inoculums. The disease is characterized by oval to irregular, light grey to dark brown lesions on the lower leaf sheath. In early stages, the lesions expand rapidly and coalesce to cover large area of the sheath and leaf lamina. At this stage, the disease symptom is look like a series of copper or brown color bands across the leaves giving a very characteristic banded appearance (Patro et al., 2020). The evaluation of the disease's variety is one of the requirements for designing disease management programmes to control sheath blight (Desvani et al. 2018). There have been numerous attempts to group R. solani isolates based on their morphology (Francis et al., 2023; Lal and Kandhari 2009; Susheela and Reddy 2013; Mishra et al., 2014; Debbarma and Dutta 2015; Rajput and Harlapur 2016; Gopireddy et al., 2017; Singh et al., 2018). The present study was undertaken to study morpho-cultural variability of R. solani isolates, causing banded leaf and sheath blight disease in kodo millet, on a single isolate which was tested on six different media - Oat Meal Agar (OMA), Potato Dextrose Agar (PDA), Richard's Synthetic Agar (RSA), Czapek's Dox Agar (CDA), Corn Meal Agar (CMA) and Water Agar (WA). Management of banded blight was done under field conditions.

2. Mathodology Methodology

Morphological variability of R. solani on six different media

Six culture media *viz.*, Potato dextrose agar (Peeled and sliced potato 200 g, Dextrose 20 g, Agar 20 g and Distilled water 1000 ml), Oat Meal Agar (Oat Meal Agar, 60 g, Agar – Agar 20 g, and Distilled water 1000 ml), Corn meal agar (Cornmeal, 20.0 g, Glucose 20.0 g, Agar 20.0 g and Distilled Water 1000 ml), Czapek's agar (Sucrose 30 g, Sodium nitrate 20 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g, Agar 20 g and Distilled Water 1000 ml), Richard's Synthetic Agar (Sucrose, 50g, Potassium Dihydrogen Phosphate, 5 g, Potassium nitrate 10 g, Magnesium sulphate 2.5 g, Ferrous chloride, 0.02, Agar - Agar 20 g, and Distilled Water 1000 ml), Water agar (Agar 20 g and Distilled Water 1000 ml) were used to compare the variability of *R. solani*. The culture media were prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for 15 minutes. Mycelial discs of the seven days old culture of *R. solani* (5 mm diameter) were placed on the middle of each pre poured medium and incubated at 25±1 °C.

التعليق [DS24]: In Materials and methods not in the introduction

التعليق [DS25]: How were the isolates obtained?
How many isolates were used in this study?

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التعليق [DS27]: How many replication for each isolate and medium?

التعليق [DS28]: What parameters ar morphological characteristics were stud and how?

Efficacy of different treatments on disease severity

The field experiments were laid in Randomized Block Design (RBD) with three replications maintaining spacing of 22.5 x 10cm during kharif, 2024 using susceptible variety (Co-2). Total of eight treatments as mentioned were adopted. Two fungicides viz., Propiconazole (0.1%) and Tebuconazole + Trifloxystrobin (0.05%) and three potential biocontrol such agents as Bacillus subtilis, Pseudomonas fluorescens and Trichoderma asperellum and SiO2 at 0.15 % were used to evaluate their efficacy against banded leaf and sheath blight disease. The bioagents viz., T. asperellum, P. fluorescens and B. subtilis in talc formulation were precolonized on 90 kg FYM and 8 kg Neem cake incubated for 15 days and were applied in the soil before sowing. Similarly, seeds were treated with the Propiconazole (0.1%), Tebuconazole + Trifloxystrobin (0.05%), viz., T. asperellum P. fluorescens and B. subtilis and SiO₂ was applied in the form of foliar spray. After treating with fungicides, seeds were allowed to air dry before sowing. Unamended plots served as check. The fungi toxicants (Propiconazole- 1 g lit⁻¹ Tebuconazole+Trifloxistrobin- 0.5 g lit⁻¹, B. subtilis @ 10 g lit⁻¹) were applied in the form of spray on the above ground parts of plants. Spray of only water served as check. First foliar sprays were done just at the appearance of the disease i.e., at 30 days after sowing (DAS) whereas second foliar of Propiconazole 0.1% was given at 15 days interval from the first spray i.e., at 45 DAS. Observations on disease severity (PDI) were recorded twice at 15 days interval, once at 37 DAS and the other at 52 DAS after imposition of foliar sprays of the respective treatments during kharif, 2024.

3. Results

The cultural traits and mycelial growth of *R. solani* were examined onagainst six different solid media. The colour of the colony, growth patterns and various sclerotial features like sclerotial weight, formation of sclerotia, number of sclerotia were varied significantly among different media tested. *R_hizoctonia solani* growth on the six culture media at different time intervals *i.e.*, 3 DAI, 4 DAI and 5 DAI was observed. The fastest mycelial growth was recorded on Oat Meal Agar (OMA) (5.63 cm) followed by Czapek Dox Agar (CDA) (4.67 cm) at 3 DAI, which was on par with Potato Dextrose Agar (PDA) (4.47 cm). However, the least mycelial growth was observed in Corn Meal Agar (CMA) (3.00 cm) medium at 3 DAI. At 5 DAI *R. solani* cultures in all the media tested attained full mycelial growth of 9.00 cm except CMA medium (6.23 cm) (Table 1; Plate 1; Fig 1). Nuri and Biswas. (2021) assessed six culture media for the growth of *R. solani* and found that Potato Dextrose

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DS210]:The experimer is not clear and needs to b rewritten better

التعليق [DS211]: How was the severity of the disease assessed? What scale was used?

Agar medium recorded maximum mycelial growth, whereas Water Agar resulted in the least mycelial growth. Nandi et al. (2023) isolated R. solani from rice mat nursery and studied the growth of the pathogen on different media and reported that, the maximum growth of R. solani (60.78 mm) on PDA medium. On the contrary, the minimum growth was reported on Czapek's dox Agar (19.66 mm). The maximum cottony fluffy appearance of the colony was observed in OMA followed by PDA, CDA, CMA and RSA, whereas the least sparse or transparent growth was observed in the water agar medium. R. hizoctonia solani mycelial margins was regular in PDA, OMA and RSA and it was irregular in CMA and WA media. However, mycelium growth was 'aerial' in PDA, OMA and RSA whereas it was 'surface' in CMA, WA and CDA media. Sclerotia formation was initiated from 3 DAI in OMA and CMA whereas it was, 4 DAI in PDA and CDA and it was from 5 DAI in RSA and WA media. The sclerotial colour on different media tested was differentiated based on Munsell's soil colour chart under different categories viz., Light brown (PDA, RSA, CDA), Reddish Brown (OMA) and dark brown (CMA and WA). Sclerotial texture was determined visually as rough (PDA, CMA, CDA) and smooth (OMA, RSA, WA) textures (Table 2). Kumar et al. (2014) observed that sclerotia developed in PDA and OMA were smooth in texture whereas, those formed in CDA and RSA had a rough texture. According to Goswami et al. (2019), sclerotia did not form in two isolates out of 112 R. solani isolates when tested on PDA. Meena et al. (2001) in previous studies indicated that Potato dextrose agar medium promoted the highest level of sclerotial production, while water agar facilitated the least.

Media	*Colony diameter (cm)			Mycelial	Margalinas		
Media	3 DAI	I 4 DAI 5 DAI		Margins	Mycelium		
PDA	4.47	6.60	9.00	Regular	Profusely growing cottony aerial mycelium		
OMA	5.63	8.23	9.00	Regular	Profusely growing cottony aerial mycelium		
CMA	3.00	5.27	6.23	Irregular Sparsely growing Surface mycelium			
RSA	4.10	6.37	9.00	Regular Sparsely growing Aerial mycelium			
WA	3.73	6.30	9.00	Irregular Sparsely growing Surface mycelium			
CDA	4.67	7.00	9.00	Regular	Profusely growing cottony aerial mycelium		
CD (P≤0.05)	0.49	0.34	0.19				
SEm±	0.15	0.11	0.06				
CV (%)	6.27	2.80	1.20				

Table 1. Effect of Different Solid Media on the Morphological characterisation of

Rhizoctonia solani.

Table 2. Effect of Different Solid Media on the Cultural Characterisation of *Rhizoctonia solani*.

Media	Sclerotia initiation	**Sclerotia number	25 sclerotia Weight (mg)	Sclerotia colour*	Sclerotia texture
PDA	4 DAI	112	52	Light Brown (6/3 7.5 YR)	Rough
OMA	3 DAI	96	37	Reddish Brown (4/3 5 YR)	Smooth
CMA	3 DAI	41	23	Dark Brown (3/4 7.5 YR)	Rough
RSA	5 DAI	15	8	Light Brown (6/3 7.5 YR)	Smooth
WA	5 DAI	4	3	Dark Brown (3/4 7.5 YR)	Smooth
CDA	4 DAI	49	26	Light Brown (6/3 7.5 YR)	Rough

^{*}As per Munsell's soil colour chart

^{*}Mean of three replications

^{**}Mean of three replications

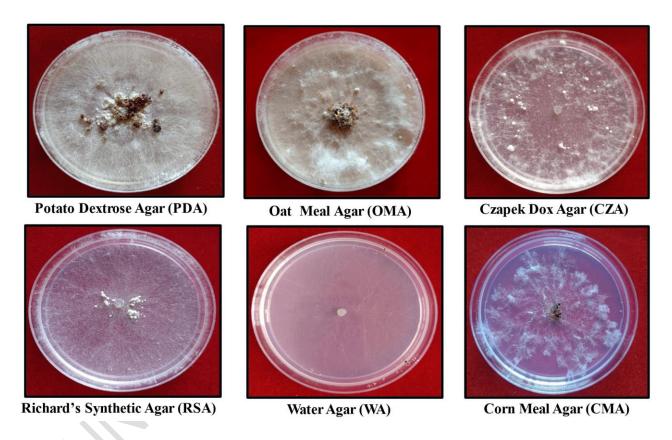


Plate 1. Effect of different solid media on the morpho-cultural characters of R. solani

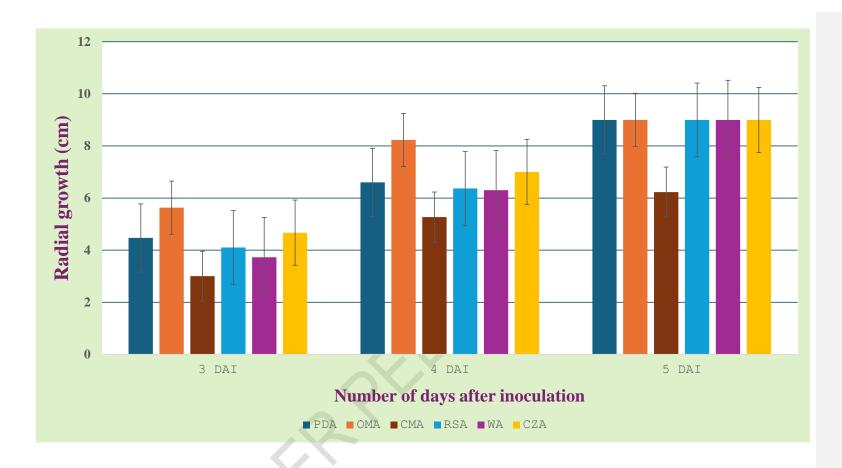


Fig 1. Effect of different media on radial growth of R. solani.

PDA (Potato Dextrose Agar) OMA (Oat Meal Agar) CMA (Corn Mel Agar)

RSA (Richard's synthetic Agar) CZA (Czapek's Dox Agar) WA (Water Agar) DAI-Days After Inoculation

التعليق [DS212]: What is the LSD value?

Are the differences significant?

Banded leaf and sheath blight disease intensity was scored on 37 DAS and 52 DAS (i.e., 7 days from 30 and 45 days after foliar spray). Among the eight treatments tested, at 37 DAS, the treatment T₅ (Seed treatment and 2 foliar sprays with trifloxystrobin 25% + tebuconazole 50 % WG @ 0.05% at 15 days interval at 30 and 45 days after sowing) was recorded minimum PDI (14.08%) which is statistically on par with T₆ (Seed treatment and 2 foliar sprays with propiconazole 25 EC @ 0.1% at 15 days interval at 30 and 45 days after sowing) (18.52%). At 52 DAS, the treatment T₅ was found to be effective recording least PDI (20.74%) and highest % inhibition over control (72.28%) which is statistically on par with T_6 which was recorded 31.85% of PDI and 57.42% inhibition over control. Kaur et al., (2022) stated that propiconazole inhibited the mycelial growth by 100% at 100 ppm and 500 ppm. In the similar pattern Neelam (2017) found that propiconazole at 20 ppm inhibited the mycelial growth of R. solani by 93.33 % over check (Table 3). Among different treatments tested, a significant variation was recorded in grain yield and fodder yield. Out of eight treatments T₅ (Seed treatment and 2 foliar sprays with trifloxystrobin 25 % + tebuconazole 50 % WG @ 0.05 % at 15 days interval i.e., 30 and 45 days after sowing) recorded the highest grain yield of 3094.44 kg ha⁻¹ which is statistically significant with other treatments. T₅ is followed by T₆ (Seed treatment and 2 foliar sprays with propiconazole 25 EC @ 0.1% at 15 days interval i.e., 30 and 45 days after sowing) which was recorded 2835.19 kg ha⁻¹ grain yield which was followed by T₄-Seed treatment with P. fluorescens @10 g kg⁻¹seed + soil application of T. asperellum + 2 foliar sprays of B. subtilis @ 10 g lit⁻¹ at 15 days interval (1903.70 kg ha⁻¹) and T_1 . Seed treatment with T. asperellum @ 10g kg⁻¹ + soil application of T. asperellum (1632.41

kg ha⁻¹). However, it was 1037.96 kg ha⁻¹ in control. Moreover, the maximum fodder yield (7038.39 kg ha⁻¹) was recorded in T₅ which was statistically on par with T₆ (6301.23 kg ha⁻¹) followed by T₄ (4161.80 kg ha⁻¹) and T₆ (3453.42 kg ha⁻¹). The minimum fodder yield was found in T₃ (2732.29 kg ha⁻¹) whereas, it was 2252.11 kg ha⁻¹ in control. Over the eight treatments, the effective and economically viable treatments is T₅, which has attained the B:C ratio of 3.12 followed by T₆ (3.07). Among the treatments with biocontrol agents tested, T₄ which is in combination with *T. asperellum, P. fluorescens, B. subtilis* has attained maximum grain yield (1903.70 kg ha⁻¹) and fodder yield (4161.80 kg ha⁻¹) with 1.44 B:C ratio. The treatments with biocontrol agents like T₂ (*P. fluorescens*) and T₃ (*B. subtilis*) were recorded. The high grain yield of 1474.07 kg ha⁻¹ and 1401.85 kg ha⁻¹ and fodder yield 3055.95 kg ha⁻¹ and 2732.29 kg ha⁻¹ respectively. However, they could not surpass T₈ (control) either in B:C

التعليق (DS213]: These studies cannot be compared to those that showed the effect of pesticides on mycelium grow in the laboratory, while the current study shows the effect of pesticide treatments on disease severity in the field.

ratio nor in net returns. Furthermore, among the treatments SiO_2 nanoparticles was imposed as T_7 which has recorded the grain yield of $1625.00 \text{ kg ha}^{-1}$ and fodder yield of $3453.42 \text{ kg ha}^{-1}$ with 1.43 B:C ratio which is more or less effective with combination of biocontrol agents examined (T_4) (Table 4). AUDPC was estimated for all the treatments tested during *kharif*, 2024 in the management of banded leaf and sheath blight in kodo millet. The treatment T_5 (Seed treatment and 2 foliar sprays with trifloxystrobin 25 % + tebuconazole 50 % WG @ 0.05 % at 15 days interval at 30 and 45 days after sowing) recorded the lowest AUDPC of 261.12 followed by T_6 (372.79) and T_4 (522.23) whereas the highest AUDPC was recorded in T_8 (983.33) (Table 5; Fig 2).

Table 3. Effect of Ffungicides and bioagents application on Percent Disease Index during kharif, 2024

	PDI (%)			
Treatment	37 DAS	52 DAS	% inhibition over control @ 52 DAS	
T ₁ : ST + SA of Trichoderma asperellum @ 10g kg ⁻¹	29.63 (32.96) ** ^b	48.15 (43.93)°	35.64	
T ₂ : ST + SA of Pseudomonas fluorescens @ 10g kg ⁻¹	36.30 (37.02)°	56.30 (48.64) ^d	24.75	
T ₃ : ST + SA of Bacillus subtilis @ 10g kg ⁻¹	37.04 (37.42)°	63.70 (53.01) ^{de}	14.85	
T ₄ : ST with P. fluorescens + SA of T. asperellum + FS of B. subtilis	27.41 (31.49) ^b	42.22 (40.50) bc	43.56	
T ₅ : ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%	14.08 (22.02) ^a	20.74 (27.04) ^a	72.28	
T ₆ : ST+FS with Propiconazole @ 0.1%	18.52 (25.45) ^a	31.85 (34.34) ab	57.42	
T ₇ : FS of Silicon dioxide @ 0.15%	33.33 (35.26) bc	51.85 (46.06) ^{cd}	30.69	
T ₈ : Control	56.30 (48.64) ^d	74.81 (60.19) ^e	-	
SEm±	1.63	2.31		
CD (P ≤ 0.05)	4.96	7.01		
CV (%)	8.38	9.05		

ST: Seed treatment, SA: Soil application, FS: Foliar spray

^{*}Mean of three replications

^{**}Values within the parenthesis are arcsine transformed values

Table 4. Impact of various treatments on grain yield and fodder yield of Kodo millet during kharif, 2024

Treatments	Grain Yield (Kg ha ⁻¹)	Fodder yield (Kg ha ⁻¹)	B:C ratio
T ₁ : ST + SA of Trichoderma asperellum @ 10g kg ⁻¹	1632.41 ^d	3287.81 ^c	1.28
T ₂ : ST + SA of Pseudomonas fluorescens @ 10g kg ⁻¹	1474.07 ^d	3055.95°	1.11
T ₃ : ST + SA of Bacillus subtilis @ 10g kg ⁻¹	1401.85 ^d	2732.29 ^{cd}	1.01
T ₄ : ST with P. fluorescens + SA of T. asperellum + FS of B. subtilis	1903.70°	4161.80 ^b	1.44
T ₅ : ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%	3094.44 ^a	7038.39 ^a	3.12
T ₆ : ST+FS with Propiconazole @ 0.1%	2835.19 ^b	6301.23 ^a	3.07
T ₇ : FS of Silicon dioxide @ 0.15%	1625.00 ^d	3453.42 ^b	1.43
T ₈ : Control	1037.96 ^e	2252.11 ^d	1.22
SEm±	81.90	266.66	
$CD (P \le 0.05)$	248.37	808.69	
CV (%)	7.56	11.45	

^{*}Mean of three replications

ST: Seed treatment, SA: Soil application, FS: Foliar spray

التعليق [DS214]: Not mentioned in the working methods

Table 5. Effect of bioagents and fungicides application on AUDPC during

Treatment	AUDPC
T ₁ : ST + SA of Trichoderma asperellum @ 10g kg ⁻¹	583.33
T ₂ : ST + SA of pseudomonas fluorescens @ 10g kg ⁻¹	694.45
T ₃ : ST + SA of Bacillus subtilis @ 10g kg ⁻¹	755.55
T ₄ : ST with P. fluorescens + SA of T. asperellum + FS of B. subtilis	522.23
T ₅ : ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%	261.12
T ₆ : ST+FS with Propiconazole @ 0.1%	372.79
T ₇ : FS of SiO ₂ @ 0.15%	638.88
T ₈ : Control	983.33

kharif, 2024

ST: Seed treatment, SA: Soil application, FS: Foliar spray

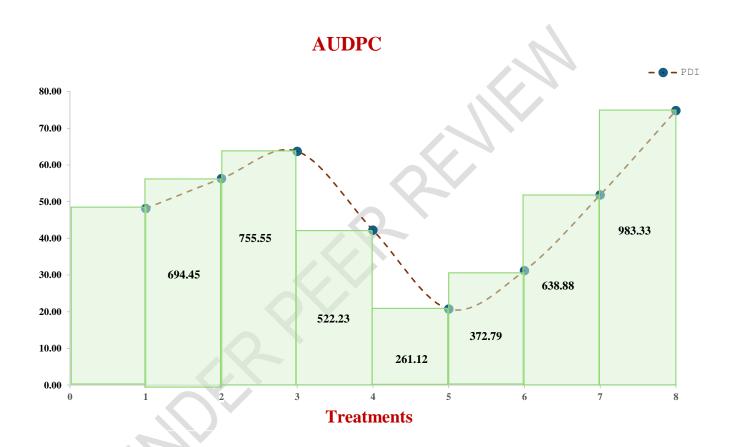


Fig 2. AUDPC for disease management of banded leaf and sheath blight

Conclusion

Morphological and cultural characters of the BLSB pathogen was determined by using, six different culture media: Czapek's Dox Agar, Oat Meal Agar, Richard's Agar, Potato Dextrose Agar, Corn Meal Agar and Water Agar. Observations at 5 days after inoculation (DAI) revealed that radial mycelial growth had reached maximum (9.00 cm) in all media except for Corn Meal Agar (6.23 cm). Among the media tested, PDA supported the highest sclerotia production (112), followed by Czapek's Dox Agar (92). An experiment was conducted on the management of kodo millet banded leaf and sheath blight disease under field conditions during kharif, 2024. Total of eight treatments were used that includes bioagents, fungicides and nanoparticles. Among them the treatment T₅ (Seed treatment and 2 foliar sprays with trifloxystrobin 25% + tebuconazole 50 % WG @ 0.05% at 15 days interval i.e., 30 and 45 DAS) was found to be effective recording highest % inhibition over control (72.28%) followed by T₆ (Seed treatment and 2 foliar sprays with propiconazole 25 EC @ 0.1% at 15 days interval i.e., 30 and 45 DAS) which has recorded 57.42% inhibition over control and T₄ (Seed treatment with P. fluorescens @ 10 g kg⁻¹ seed + soil application of T. asperellum + 2 foliar sprays of B. subtilis @ 10g lit⁻¹ at 15 days interval). with 43.56% inhibition over control. The same sequence was followed with PDI. Among the eight treatments, T₅ (Trifloxystrobin + Tebuconazole) recorded the highest grain yield (3094.44 kg/ha) and fodder yield (7038.39 kg/ha) with the highest B:C ratio of 3.12, followed by T₆ (Propiconazole) with 2835.19 kg/ha grain yield, 6301.23 kg/ha fodder yield, and 3.07 B:C ratio. Among biocontrol-based treatments, T₄ (Trichoderma + Pseudomonas + Bacillus) was the most effective, yielding 1903.70 kg/ha grain and 4161.80 kg/ha fodder with 1.44 B:C ratio. The treatment T₇ (SiO₂ nanoparticles) showed comparable results with individual biocontrol agents with 1625.00 kg/ha grain yield and 1.43 B:C ratio. The treatment T₅ recorded the lowest AUDPC of 261.12 followed by T_6 (372.79) and T_4 (522.23).

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