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

**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 (*Paspalum scrobiculatum*) 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-1and fodder yield of 70338.39 kg ha-1 and greater B:C ratio.

**Keywords**: morphological characterisation, cultural characterisation, banded blight, fungicides,

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 caused by *Rhizoctonia solani* (Kuhn.) (Basidial stage: *Thanatephorus cucumeris*) is one of the emerging disease 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* isolate causing banded leaf and sheath blight disease in kodo millet, Management of banded blight was done under field conditions.

1. **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 and 3 replications were maintained for each media. And parameters like, Colony diameter, growth rate, mycelial margins, sclerotial initiation, sclerotia number, weight of sclerotia, colour and texture of sclerotia were taken. 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 agents such 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 SiO2was 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.

List1: Experimental model

Design : Randomized block design

Treatments : Seven (along with control)

Replications : Three

Plot size : 3.37 sq.m

Spacing : 22.5 x 10 cm2

Variety : Co-2 (susceptible)

Season : *kharif*, 2024

Dates of sowing : 21-07-2024

Location : Agricultural Research Station, Vizianagaram.

Disease severity was recorded by following SOP for BLSB (SOP, 2022) and Per cent disease index (PDI) was calculated for individual treatments based on the following formula given by Wheeler (1969).

**PDI** (%) = Sum of individual disease ratings

No. of observations assessed × maximum disease rating.

Per cent increase or decrease in disease severity over control was calculated as per the formula described by Vincent (1927).

I = x 100

Where,

I = Per cent decrease of the disease

C = PDI in control

T = PDI in treatment

**List 2: Disease scoring scale for sheath blight (ICAR-SOP, 2022)**

|  |  |
| --- | --- |
| **Score** | **Description** |
| 1 | <1% of plant area covered by lesion |
| 2 | 1-5% of plant area covered by lesion |
| 3 | 6-10% of plant area covered by lesion |
| 4 | 11-20% of plant area covered by lesion |
| 5 | 21-30% of plant area covered by lesion |
| 6 | 31-40% of plant area covered by lesion |
| 7 | 41-50% of plant area covered by lesion |
| 8 | 51-75% of plant area covered by lesion |
| 9 | >75% plant area covered by lesion |

**Area Under Disease Progress Curve (AUDPC)**

The efficacy of different treatments was assessed based on AUDPC values using the formula given by Wilcoxson *et al*. (1975).

Where,

*Si* = Disease incidence at ith day of evaluation

*k* = Number of successive evaluations of the disease

d = Interval between i and i-1evaluation of disease

**Yield Parameters**

Observations on yield attributing parameters like gain yield and fodder yield   
(kg ha-1) were recorded after harvesting and benefit cost ratio (BC ratio) was calculated for each treatment by using the formula given below and compared with untreated check.

Net returns

Benefit cost ratio = ---------------------------------- ×100

Cost of cultivation

1. **Results And Discussion**

The cultural traits and mycelial growth of *R. solani* were examined on 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. 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) 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 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. 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 Margins** | **Mycelium** |
| **3 DAI** | **4 DAI** | **5 DAI** |
| **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*.**

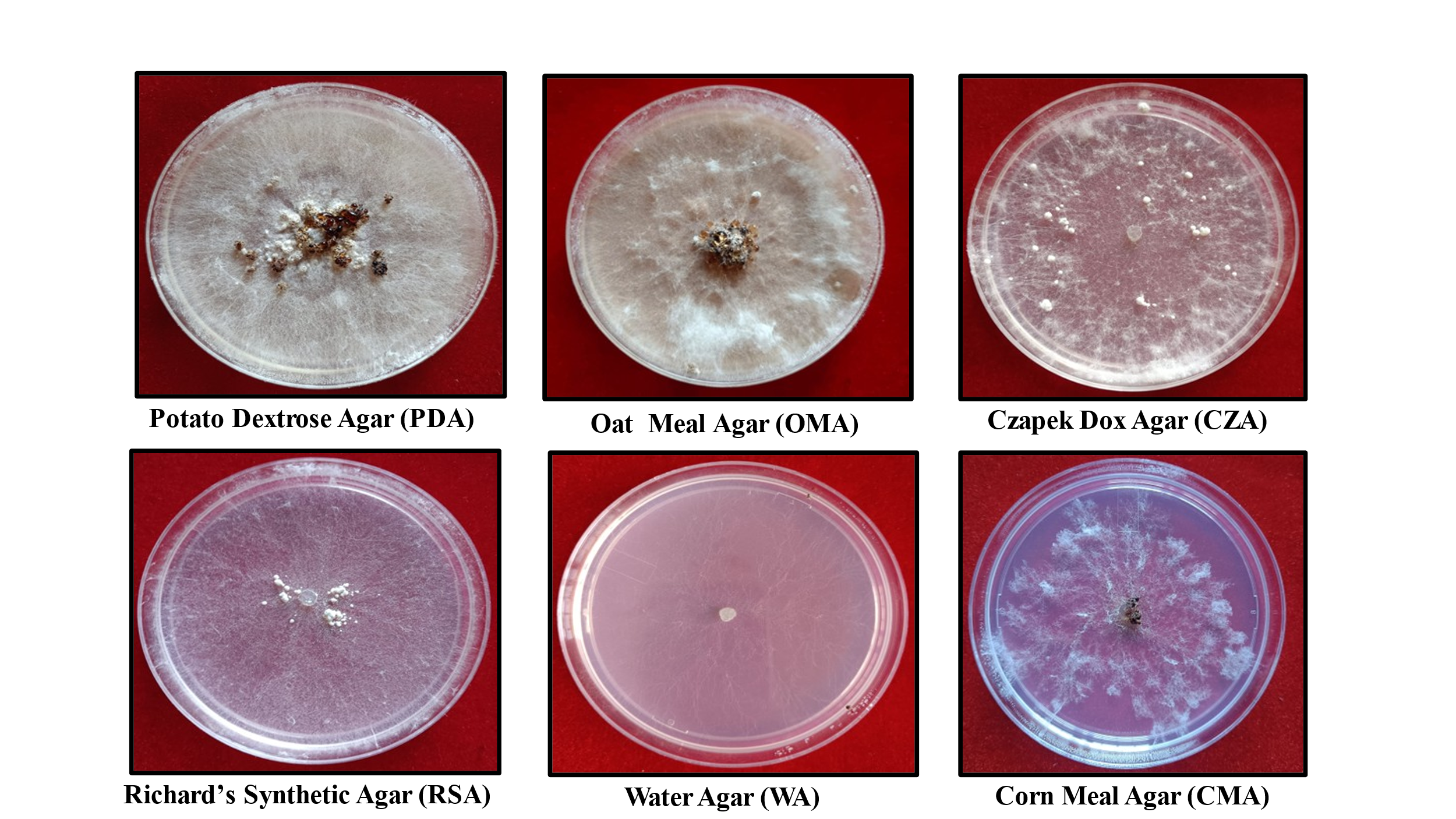
\*Mean of three replications

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

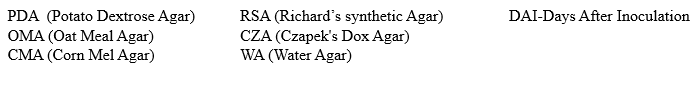
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Media** | **Sclerotia initiation** | **\*\*Sclerotia number** | **25 sclerotia**  **Weight (mg)** | **Sclerotia colour\*** | **Sclerotia texture** |
| **PDA** | 4 DAI | 112 | 52 | Light Brown (6/3 7.5 YR) | Rough |
| **OMA** | 3 DAI | 96 | 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



**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*.**

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 T6 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 and singh (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 T1- 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 ratio nor in net returns. Furthermore, among the treatments SiO2 nanoparticles was imposed as T7 which has recorded the grain yield of 1625.00 kg ha-1 and fodder yield of 3453.42 kg ha-1 with 1.43 B:C ratio which is more or less effective with combination of biocontrol agents examined (T4) (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 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) recorded the lowest AUDPC of 261.12 followed by T6 (372.79) and T4 (522.23) whereas the highest AUDPC was recorded in T8 (983.33) (Table 5; Fig 2).

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **PDI (%)** | | |
| **37 DAS** | **52 DAS** | **% inhibition over control @ 52 DAS** |
| **T1: ST + SA of *Trichoderma asperellum* @ 10g kg-1** | 29.63 (32.96) \*\*b | 48.15 (43.93) c | 35.64 |
| **T2: ST + SA of *Pseudomonas fluorescens* @ 10g kg-1** | 36.30 (37.02) c | 56.30 (48.64) d | 24.75 |
| **T3: ST + SA of *Bacillus subtilis* @ 10g kg-1** | 37.04 (37.42) c | 63.70 (53.01) de | 14.85 |
| **T4: ST with *P. fluorescens* + SA of *T. asperellum* + FS of *B. subtilis*** | 27.41 (31.49) b | 42.22 (40.50) bc | 43.56 |
| **T5: ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%** | 14.08 (22.02) a | 20.74 (27.04) a | 72.28 |
| **T6: ST+FS with Propiconazole @ 0.1%** | 18.52 (25.45) a | 31.85 (34.34) ab | 57.42 |
| **T7: FS of Silicon dioxide @ 0.15%** | 33.33 (35.26) bc | 51.85 (46.06) cd | 30.69 |
| **T8: 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

\*\*Values within the parenthesis are arcsine transformed values

\*Mean of three replications

**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** |
| **T1: ST + SA of *Trichoderma asperellum* @ 10g kg-1** | 1632.41d | 3287.81c | 1.28 |
| **T2: ST + SA of *Pseudomonas fluorescens* @ 10g kg-1** | 1474.07d | 3055.95c | 1.11 |
| **T3: ST + SA of *Bacillus subtilis* @ 10g kg-1** | 1401.85d | 2732.29cd | 1.01 |
| **T4:ST with *P. fluorescens* + SA of *T. asperellum* + FS of *B. subtilis*** | 1903.70c | 4161.80b | 1.44 |
| **T5: ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%** | 3094.44a | 7038.39a | 3.12 |
| **T6: ST+FS with Propiconazole @ 0.1%** | 2835.19b | 6301.23a | 3.07 |
| **T7: FS of Silicon dioxide @ 0.15%** | 1625.00d | 3453.42b | 1.43 |
| **T8: Control** | 1037.96e | 2252.11d | 1.22 |
| **SEm±** | 81.90 | 266.66 |  |
| **CD (P ≤ 0.05)** | 248.37 | 808.69 |  |
| **CV (%)** | 7.56 | 11.45 |  |

\*Mean of three replications

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

|  |  |
| --- | --- |
| **Treatment** | **AUDPC** |
| **T1: ST + SA of *Trichoderma asperellum* @ 10g kg-1** | 583.33 |
| **T2: ST + SA of *pseudomonas fluorescens* @ 10g kg-1** | 694.45 |
| **T3: ST + SA of *Bacillus subtilis* @10g kg-1** | 755.55 |
| **T4:ST with *P. fluorescens* + SA of *T. asperellum* + FS of *B. subtilis*** | 522.23 |
| **T5: ST + FS with Trifloxystrobin + Tebuconazole @ 0.05%** | 261.12 |
| **T6: ST+FS with Propiconazole @ 0.1%** | 372.79 |
| **T7: FS of SiO2 @0.15%** | 638.88 |
| **T8: Control** | 983.33 |

**Table 5. Effect of bioagents and fungicides application on AUDPC during *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%) 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 T6 (372.79) and T4 (522.23).

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