

Eco-friendly management of sheath blight in rice under organic conditions

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ABSTRACT

Sheath blight is the most important diseases in rice. In the study, various eco-friendly treatments were attempted to manage sheath blight in rice both *in-vitro* and *in-vivo* conditions. Among the various *Trichoderma* isolates tested, the isolate T2 collected from Pangthang (27.73° N, 88.63° E) was very effective in inhibiting the growth of sheath blight pathogen with 78.88 per cent inhibition over control and among the plant extracts fern leaves (5%)+garlic bulb (5%)+neem leaves (5%) extract combination @ 15% showed maximum percent inhibition (74.44%) over control. All the fungicides tested under field conditions performed well and better than biocontrol agents and botanicals. Among treatments, copper oxychloride @ 0.25%, was effective in the variety PD 10 with no incidence of disease. Similar result was also observed in the variety TN 1 in which copper oxychloride @ 0.25% registered lowest PDI of 7.41 per cent. Among the biocontrol agents *Pseudomonas fluorescens* recorded less PDI i.e 27.77 per cent and 26.65 per cent, respectively in PD 10 and TN 1.

Keywords: Disease, Eco-friendly, Rice, Sikkim, *Rhizoctonia solani*

INTRODUCTION

Rice is one of the most important crops of Sikkim. In Sikkim, the cultivated area under this crop is around 12140 ha producing 20970 tons (Anonymous 2011). Various diseases like blast, sheath rot, sheath blight, BLB, Rice tungro virus (RTV) and bacterial leaf streak have been reported in rice grown in Sikkim. Sheath blight, caused by the fungus *Rhizoctonia solani* J.G. Kühn AG1-1A (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk.), is one of the major diseases of rice and considered as a globally significant one, second-most prevalent to the blast disease. The yield losses due to sheath blight disease varied from 5-54.3%. No rice varieties have been found to be completely resistant to this particular disease. Symptoms usually develop in the later tillering or early internode elongation stage of growth. Characteristic symptoms of the disease are water-soaked, spherical to oval-shaped or irregularly elongated discoloured, greyish to light brownish lesions with brown margin on the leaf sheath and blades. Under favourable conditions, infection spreads rapidly to the upper plant parts (**Fig. 1**). Heavy infection results in reductions in grain yield, quality and increased lodging of plants. Disease severity depends on the amount of inoculum, crop growth stage, environment, and variety susceptibility. The disease spread by either sclerotia or runner hyphae from the infected plants. These sclerotia are the resting structures and may remain dormant for over the years in the soil and infected plant stubbles and can re-infect healthy rice plants in the subsequent crop season. By virtue of their buoyancy, sclerotia can float long distances in the field with irrigation water and aid in the spreading of the disease.

This disease became a worldwide major threat after introduction of high yielding varieties and associated high dose of nitrogen fertilizers. Higher crop densities and resultant humid canopies have long been considered as important factors which favour increasing sheath blight

incidence. The pathogen is generally soil-waterborne, infectious to a broad range of plants from ~32 taxonomic families. In addition to the broad host range and variability of the pathogen, the main obstacle in managing sheath blight disease is the lack of an identified germplasm with an adequate level of resistance for using in the resistance breeding programme. Due to the lack of high level of rice varietal resistance to sheath blight, it has become necessary to find alternative means to manage the disease.



Fig. 1: Symptoms of sheath blight on rice

There are so many chemical fungicides available to manage this disease in conventional agriculture, however, the use of chemicals in organic agriculture is either banned or restricted. Moreover, many of the systemic fungicides have inherent drawbacks, like not ecofriendly, not affordable to the farmers and development of resistant among the pathogens. As Sikkim being an organic state, it is very much necessary to identify some eco-friendly options or to study the effect of

organically permitted fungicide for the management of various diseases including sheath blight in rice to infuse confidence in the minds of resource poor farmers about the organic agriculture. Keeping in view, the present investigation was undertaken to assess the efficacy of different organic treatments against *R. solani* under *in-vitro* and field conditions.

MATERIALS AND METHODS

A field experiment was laid out in ICAR Research farm, Sikkim Centre, Tadong, Gangtok (1350 a.m.s.l) to test the efficacy of different plant extracts and bio-control agents, organically permitted fungicides against sheath blight (*R. solani*) in rice. Pathogen was isolated from the infected leaf sheath using potato dextrose agar medium. Crude extracts of various plants were used to test the efficacy against the pathogen using Poison food technique. 7 day old culture of *R. solani* was used in this study. The plant extracts used were local fern leaves, garlic, onion, neem leaves, titapati (*Artemisia* spp.) leaves, banmara (*Chromolaena odorata*) leaves, geranium leaves, pudina leaves, chiloney leaves etc. Here, plant extract alone or mixture of plant extracts were used (Table 3). Per cent inhibition was studied using the formula.

Per cent inhibition =

$$\frac{\text{Radial growth in control(C)} - \text{Radial growth in treatment (I)}}{\text{Radial growth in control(C)}} \times 100$$

Similarly, various *Trichoderma* isolates collected from different places in Sikkim was tested against 7 day old culture of *R. solani* using dual culture technique. Soil samples were collected from different places of Sikkim and soil samples were brought to the lab and were kept at 4°C in refrigerator. *Trichoderma* was isolated by serial dilution technique. Five-fold serial dilutions of each soil samples were prepared in sterilized distilled water and 1 ml diluted sample was poured on the surface of *Trichoderma* Specific Medium (TSM). Plates were incubated at $28 \pm 2^\circ\text{C}$ for 96 h. Morphologically, different colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA) medium. The purified isolates were preserved at 4°C and used for further study. The spore suspension prepared using sterile water having the concentration of 10^6 was used for field study. The varieties used were PD10 and TN 1 with the spacing of 20×15 cm. the plot size was 3×1 m² and the design adopted was Randomized Block Design (RBD) with three replications. Sowing was done in the third week of June for both years. All the treatments were maintained organically by adding required FYM, vermicompost and hand weeding.

For the disease management study, the pathogen was isolated and cultured on PDA plates. Artificial inoculation (Park *et al.*, 2008) was done in late tillering stage using agar bits of 3 day old culture beneath leaf sheath and covered with moist cotton and aluminum foil (Fig. 2). Aluminum foil was removed 3 day after inoculation. The inoculated plants were observed for disease development. The treatments used were 1.

hexaconazole 0.1%, 2. copper oxychloride 0.25%, 3. sulfex 0.25% , 4. copper hydroxide 0.25%, 5. garlic bulb 10%, 6. neem oil 0.3%, 7. garlic+neem+fern 15%, 8. banmara (*Chromolaena odorata*) 10%, 9. *Pseudomonas fluorescens* 0.25% (Commercial formulation) 10. *Trichoderma* spp-1 (T2), 11. *Trichoderma* spp-2 (T3), 12. *Trichoderma* spp-3 (T5), 13. *Trichoderma* spp-4 (T9), 14. control, the standard fungicide hexaconazole @ 0.1% and untreated control were used for comparison. Total 5 spraying was done. First spray before inoculation and 2nd one three days after inoculation and remaining at 7 day interval. Data recording was done on per cent disease index after the final spray. To make the standard plant extracts solution (100%) required quantity of fresh leaf/bulb extract of each plant was thoroughly washed with tap water followed by distilled water and was ground @1 g of tissue / ml of water (1:1 w/v) and filtered through doubled layered cheese cloth. Required quantity of sticker was added to the plant extracts before spraying.



Fig. 2: Inoculation of *R. solani* on rice plant

In each plot 10 plants were selected randomly, labelled and the data on occurrence of sheath blight was recorded one

week after the last application of treatments by using the disease rating scale of 0-5 (Park *et al.*, 2008) (**Table 1**) and the Percent Disease Index (PDI) was calculated using standard formula. The data was analysed statistically using analysis of variance (ANOVA).

The per cent disease index (PDI) was calculated using the formula given below :

$$\text{PDI} = \frac{\text{Total sum of numerical ratings}}{\text{Total number of tillers observed} \times \text{Maximum disease category}} \times 100$$

Table 1: Scale/description of the symptom for sheath blight

Scale	Description of symptom
0	no lesion
1	appearance of water soaked lesions
2	appearance of necrotic lesions
3	less than 50% necrosis on the leaf cross-section
4	more than 50% necrosis on the leaf cross-section
5	necrosis across the entire leaf section resulting in leaf death

RESULTS AND DISCUSSION

Various plant extracts and different combinations were tested against the pathogen *in-vitro*. Most of the plant extracts were not inhibiting the pathogen growth and they are at par with control. However, among the extracts tested, fern leaves (5%)+garlic bulb (5%)+neem leaves (5%) extract combination @ 15% showed maximum per cent inhibition (74.44%) over control (0%) followed by fern leaves (3.3%)+garlic bulb (3.3%)+neem leaves (3.4%) @10% (37.40%) (**Table 2**). Use of plant extracts for the management of various diseases in crop plants were documented by various authors. Use of neem and its products are well known and its fungitoxic activity is due to the compounds like azadirachtin, azadiradione, etc. (Sehajpal *et al.*, 2009). Similarly, *Allium sativum* also has role in disease management due to the presence of sulphur compounds and allicin (Singh and Singh 2005). Effect of *Allium sativum* in inhibiting *R. solani* pathogen was reported in rice (Sehajpal *et al.*, 2009). *Allium sativum* has shown strong fungitoxicity against the sheath blight pathogen even at low concentration i.e. 100 ppm. Fern extracts were found effective in controlling the growth of *Cercospora* spp. of groundnut under *in-vitro* conditions (Sahayaraj *et al.*, 2009). Plant extracts such as polyalthia (*Polyalthia longifolia* Sonn.) and rhizomes of ginger (*Zingiber officinale*) were found most active against the *R. solani* (Choudhury *et al.*, 2017). The presence of

phytochemical compounds such as steroid, tannin, flavonoid, alkaloid, and saponin were reported for antimicrobial activity in plant extracts. Antibacterial activity and antifungal activity for the various fern leaf extracts were also reported.

Table 2. Efficacy of plant extracts against *R. solani in-vitro*

Sl. No	Plant extracts	Per cent Inhibition Over Control (%)
1.	Chiloney+Ginger+Artemisia+ Chromolaena+Neem	0(0.47) *
2.	Garlic+Neem+Fern+Artemisia	0(0.47)
3.	Artemisia(3.4%)+Neem(3.4%)+Chromolaena(3.3%)	0(0.47)
4.	Geranium+Fern+Neem+Artemisia+Chromolaena-10% (2% each)	0(0.47)
5.	Garlic+Onion+Ginger+Chromolaena-10%(2.5% each)	0(0.47)
6.	Pudina(<i>Mentha</i> spp) 10%	0(0.47)
7.	Geranium 10%	0(0.47)
8.	Garlic+Onion+Ginger+Artemisia-10%(2.5% each)	0(0.47)
9.	Ginger+Neem+Fern+Artemisia-10%(2.5% each)	0(0.47)
10.	Garlic(3.3%)+Fern(3.3%)+Neem(3.4%)	74.44(59.65)
11.	Garlic+Fern+Neem-15%(5% each)	37.40(38.38)
12.	Control	0(0.47)
CD (0.05)		3.50

*The figures in parenthesis are arcsine transformed values

Different *Trichoderma* isolates obtained from various places of the Sikkim was studied for their antagonistic effect against *R. solani* using dual culture technique. A total of 39 isolates were isolated from the soil for *in-vitro* study on *R. solani*. Among the various isolates tested the isolate T2 collected from Pangthang was very effective in inhibiting the growth of sheath blight pathogen with 78.88 per cent inhibition over control followed by T5 (75.18%), T3 (73.70%), T9 (73.33%), collected from Today busty, Nandugaon, ICAR, Tadong respectively (**Table 3**). Biocontrol approach is considered to be a practical and economical alternative for managing diseases in rice (Suryadi *et al.*, 2013). Srinivas *et al.* (2013) reported that *Trichoderma viride* (72.65%) followed by *Penicillium notatum* (64.07%)> *T. atroviride* (62.51%) stopped the growth of *R. solani* under *in-vitro* conditions.

Sheath blight disease appeared 3 day after inoculation. All the treatments used in this study showed significant difference with respect to disease severity. Among the treatments studied, copper oxychloride @ 0.25%, was effective (7.40%) in the variety followed by copper hydroxide (2.22%) on PD 10 with no incidence of disease on the plots treated with copper oxychloride. Similar result was also observed in the variety TN 1 in which copper oxychloride @ 0.25% registered lowest PDI of 7.41 per cent (**Table 4**). Copper hydroxide treated plots recorded PDI of 8.14%. Copper compounds such as, copper oxychloride, copper hydroxide is permitted in organic agriculture with some restrictions and these fungicides should be used as last resort when all other methods fail.

Table 3. Effect of different *Trichoderma* isolates on *R. solani* *in-vitro*

Sl. No.	Isolate name	Per cent inhibition over control (%)	Sl. No.	Isolate name	Per cent inhibition over control (%)
1.	T1	67.77	21.	T21	49.62
2.	T2	78.88	22.	T22	59.62
3.	T3	73.70	23.	T23	59.62
4.	T4	56.66	24.	T24	45.18
5.	T5	75.18	25.	T25	70.73
6.	T6	65.59	26.	T26	61.47
7.	T7	68.88	27.	T27	57.77
8.	T8	69.25	28.	T28	71.10
9.	T9	73.33	29.	T29	51.47
10.	T10	53.32	30.	T30	61.47
11.	T11	57.03	31.	T31	65.55
12.	T12	69.99	32.	T32	56.29
13.	T13	54.44	33.	T33	42.22
14.	T14	54.81	34.	T34	46.29
15.	T15	59.25	35.	T35	45.55
16.	T16	55.92	36.	T36	68.88
17.	T17	69.25	37.	T37	48.88
18.	T18	53.33	38.	T38	42.96
19.	T19	55.55	39.	T39	42.59
20.	T20	48.14		CD at 5%	7.06

Table 4: Effect of different organic treatments on per cent disease index of sheath blight

Sl. No.	Treatments	Percent Disease	Index (PDI)
		TN 1	PD 10
1.	Hexaconazole 0.1%	2.96(9.76)	0.00(0.44) *
2.	Blitox (Copper oxychloride) 0.25%	7.40(15.53)	0.00(0.44)
3.	Sulfex 0.25%	37.76(37.56)	18.88(22.62)
4.	Copper hydroxide 0.25%	8.14(16.32)	2.22(7.15)
5.	Garlic 10%	42.95(40.92)	33.75(35.42)
6.	Neem oil 0.3%	48.14(44.09)	23.69(28.80)
7.	Garlic+Neem+Fern	48.14(43.95)	42.95(40.83)
8.	Banmara(<i>Chromolaena odorata</i>)10%	46.66(43.03)	38.51(38.28)
9.	<i>Pseudomonas fluorescens</i>	26.65(30.78)	18.14(23.73)
10.	<i>Trichoderma</i> spp-1	34.06(35.27)	39.99(38.85)
11.	<i>Trichoderma</i> spp-2	48.14(43.83)	48.88(42.87)
12.	<i>Trichoderma</i> spp-3	51.84(46.19)	48.14(48.14)
13.	<i>Trichoderma</i> spp-4	47.39(43.18)	22.59(25.85)
14.	Control	48.14(43.93)	61.47(51.69)
	CD (0.05)	14.36	20.51

*The figures in parenthesis are arcsine transformed values

Control of various diseases under organic conditions using copper fungicides was studied against diseases like rhizome rot of ginger, blast in paddy and also tomato late blight (Gopi *et al.*, 2020). Among the biocontrol agents *P. fluorescens* registered less PDI i.e. 27.77 per cent and 26.65 per cent, respectively in PD 10 and TN 1, followed by *Trichoderma*

spp-1 (T2) collected from Pangthang (27.73 °N, 88.63 °E). *Trichoderma* sp-1 (T2) was found effective under *in-vitro* conditions also. Bio-pesticides are potential alternatives to chemical pesticides. Microbial bio-pesticides are living natural enemy organisms and/or their products including plant and microbial products and/or their by-products and they could reduce pathogen populations. In the present decade, bio-pesticides are widely acceptable and demanded for sustainable agriculture and for production of safe foods.

Several microorganisms belonging to genera *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma* were used as bio-control agents to control sheath blight (ShB) in rice. Various *Trichoderma* spp. and PGPR were found effective under field conditions against sheath blight in rice. Application of *P. fluorescens* in combination with *Trichoderma* and FYM and zinc sulphate + lime recorded less incidence of sheath blight (Prasad and Kumar, 2016). Lenka and Pun (2016) found that *Trichoderma viride* was effective and recorded maximum sheath blight reduction. Lowest sheath blight intensity (49.33%) was recorded in T7 i.e. soil application of value added *P. fluorescens* + *T. asperellum* + *B. subtilis* (Patro *et al.*, 2018). Bashar *et al.* (2010) showed bacterial isolates exhibited comparatively higher growth inhibition of *R. solani* and reduced sheath blight disease development upto 35% over to control. PGPR strains screened in the greenhouse, *Bacillus subtilis* strain MBI600 provided the most significant and consistent suppression of ShB (Zhou *et al.*, 2021). None of the botanicals were found effective against sheath blight under field conditions. In the present study, the botanicals and bio-control agents performed well in case of *in-vitro* did not perform well under field conditions. Similar results of variation *in-vitro* and field conditions were already reported by Gopi *et al.* (2020). This may be due to lower inhibitory activity under field conditions, lack of persistence on the rice plant, unfavourable climatic conditions and also poor adoptability of bio-control, when we artificially apply on the field.

CONCLUSION

In our study, copper oxychloride was most effective followed by copper hydroxide in managing sheath blight in rice. Among the bio-control agents *P. fluorescens* was effective. Therefore, these two chemicals and biocontrol agents i.e., *P. fluorescens* and *Trichoderma* spp. can be used in an integrated way with other organically approved treatments for the effective management of sheath blight in rice. Employing integrated disease management consisting of the use of resistant or moderately resistant varieties, good cultural practices, and permitted fungicides, is an economic and effective means to reduce yield losses. As copper fungicides are being protective in nature it requires repeated application for better protection. Some varieties of rice may show phytotoxicity, therefore, testing is required before applying copper based fungicides. The results obtained will be very useful for the farmers cultivating rice under organic conditions and will lead to healthier food and environment.

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