International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **17**(04): 687-694, December 2024
DOI: 10.30954/0974-1712.04.2024.6

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

RESEARCH PAPER

Biocontrol of Sheath Blight (*Rhizoctonia solani* Kühn) in Rice (*Oryza sativa* L.) by Native *Trichoderma* Isolates

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Paper No. 1186 **Received:** 26-08-2024 **Revised:** 23-11-2024 **Accepted:** 01-12-2024

ABSTRACT

Rice sheath blight caused by *Rhizoctonia solani* Kühn is one of the major diseases occurring in all rice growing areas of Nepal. Management of this pathogen is difficult due to its broad host range and high survival ability under various environmental conditions. This present study aims to evaluate native *Trichoderma* isolates for eco-friendly management of the disease. Thirty *Trichoderma* isolates from the soils of rice fields of different parts of Nepal terai, and three *Rhizoctonia solani* isolates from sheath blight-infected rice plants grown in the terai region were isolated. The *R. solani* isolates were tested for pathogenicity in rice cv. Sona Mansuli and selected a virulent one for further use. The *Trichoderma* isolates were evaluated for antagonism against *R. solani* by dual culture technique. Of the 30 isolates, RN-1, RN-5, RN-4, RN-10, and RN-12 significantly inhibited the mycelial growth of *R. solani*, thus selected for an *in-vivo* experiment in a screenhouse. The experiment included four rice varieties recommended for Nepal terai (Sabitri, Bahuguni 2, Sworna Sub-1, and Sona Mansuli), seed and seedling treatments by the selected five *Trichoderma* isolates following challenge inoculation with *R. solani*, and assessment of disease reduction and plant growth. All five selected *Trichoderma* isolates (based on dual culture results) significantly reduced the relative lesion height of sheath blight in treated rice plants as compare to untreated control. Highest reduction of the disease was obtained with the isolate RN-1 in all four rice varieties. The *Trichoderma* isolates also significantly enhanced the growth of rice plants. Plants treated with the isolates RN-10 and RN-1 had significantly highest plant height, maximum flag leaf length, number of tillers, and root length. The present results clearly showed that native Trichodermas have potential in controlling sheath blight disease and enhance growth of rice plants.

HIGHLIGHTS

- Native *Trichodermas* are found effective against *Rhizoctonia solani* that causes sheath blight disease in rice.
- *Trichodermas* exerted plant growth promoting effects on rice (cv Sona Mansuli, Sabitri, Sworna Sub-1 and Bahuguni-2).
- Native *Trichodermas* can be utilized as an important component in the integrated crop management of rice.

Keywords: Biological control, dual culture, *Oryza sativa, Rhizoctonia solani, Trichoderma*

Rice (*Oryza sativa* L.) is the most important staple food for more than half of the world (Prasad *et al.* 2017). In Nepal, rice is considered as staple crop and an important source of income for farmer. About 73% of rice is produced in the Terai, 24% in the hills and 4% in high hills (Joshi *et al.* 2020).

Sheath blight of rice caused by *Rhizoctonia solani* Kühn, is considered as a major economically

How to cite this article: Yadav, R.N., Manandhar, H.K., Dahal, K.C., Gopal Bahadur, K.C. and Yadav, L. (2024). Biocontrol of Sheath Blight (*Rhizoctonia solani* Kühn) in Rice (*Oryza sativa* L.) by Native *Trichoderma* Isolates. *Int. J. Ag. Env. Biotech.*, **17**(04): 687-694.

Source of Support: None; **Conflict of Interest:** None

important fungal disease worldwide (Singh *et al.* 2019) including Nepal (Manandhar 2017). Crop damage by the disease can result in yield reduction up to 45%, depending on the plant growth stage and the environmental conditions (Kumar *et al.* 2016). *R. solani*, a polyphagous competitive saprophyte, has wide host range (Singh *et al.* 2004), which made it as an enigmatic pathogen to control. More than 30,000 rice germplasms have been screened against the pathogen around the world and no desirable level of resistance to sheath blight has been found except in few wild relatives (Molla *et al.* 2020).

Non judicious applications of the chemical pesticides in crop plants have caused pollution to environment, and hazards to human and animal health (Sharma *et al.* 2012). These unacceptable effects have brought attention of the plant pathologists in developing the effective bio-control measures to manage plant diseases (Waard *et al.* 1993; Yadav *et al.* 2018).

The genus *Trichoderma* (Ascomycota) is identified for its antagonistic activity against several plant pathogens, including *R. solani*. Studies show that *Trichoderma* are effective in minimizing the incidence of sheath blight in rice (Rashid *et al.* 2020; de Franca *et al.* 2015; Chaudhary *et al.* 2020). This present study aims to evaluate native *Trichoderma* isolates from rice fields of Nepal terai for the control of rice sheath blight pathogen both under laboratory (*in-vitro*) and screenhouse conditions and also to investigate the growth promotion potential of *Trichoderma* spp. in rice plants.

MATERIALS AND METHODS

The present study involved (i) isolation of *Trichoderma* as biocontrol agent, (ii) isolation of *Rhizoctonia solani* as sheath blight pathogen, (iii) pathogenicity testing of *R. solani*, (iv) dual cultures of *Trichoderma* and *R. solani* isolates, and (v) evaluation of *Trichoderma* isolates against *R. solani* in rice and for enhancing growth in rice plants. All experiments were conducted at the laboratory and screenhouse of Nepal Plant Disease and Agro Associates (NPDA), Balaju-Chakrapath, Kathmandu.

Isolation and identification of *Trichoderma*

Thirty-two soil samples were collected in polythene bags from rice fields (up to 15 cm depth) of different terai region from east to far west for isolation of *Trichoderma* (Annexure-1). Each sample weighing about 500 g was composed of 4-5 sub-samples from each location. The soil samples were air-dried to remove excess moisture, mixed well, placed in the respective polythene bags, and stored at room temperature until use.

For the isolation of *Trichoderma*, working samples (about 25 g each) were prepared from the stored soil samples. One gram soil was drawn from each working sample and added to 9-ml of sterile water in a test tube and left for about 15 minutes. The soil suspension was serially diluted up to 10^{-4}. Finally, from the dilution number 10^{-4}, 100 µl of suspension was plated onto Petri plates (9 cm diameter) containing *Trichoderma* selective medium (TSM) in a laminar flow. These plated Petri dishes were then incubated at 28±2°C for 4-6 days. Fungal colonies, including green circular colonies appeared on TSM. Fungal colonies resembling *Trichoderma* were examined under stereo and compound microscopes and the confirmed Trichodermas (Bissett 1991) were transferred to Petri plates containing potato dextrose agar (PDA) for purification. Single spore culture of each isolate was made and maintained in PDA slants at 4°C for further use.

Isolation of *R. solani* and pathogenicity test

Four samples of rice sheaths showing typical sheath blight symptoms were collected from naturally infected rice plants, grown in different rice growing areas of the terai regions of Nepal. The infected plant parts were cut into small pieces, surface sterilized with 1% sodium hypochlorite for one minute, washed with sterile water, blot-dried, and plated onto water agar (WA) Petri plates. The plates were incubated at 25°C for 2-3 days, and examined for the hyphal growth under compound microscope. A typical hyphae with braching at 90 degree was subcultured in PDA using single hyphal tip method and the cultures were maintained in PDA slants at 4°C for further use.

Pathogenicity test of the *R. solani* was done on rice cv. Sona Mansuli by inserting a 5-mm mycelial disc of the pathogen between the joint of basal leaf sheath and stem and covering it by moist cotton. Re-isolation of *R. solani* was done from artificial inoculated Sona Mansuli rice plants showing distinctive lesion of sheath blight. *Trichoderma*

isolates and *R. solani* isolates were grown on 'PDA' at 28°C for 5 days and stored at 4°C for further use.

Dual cultures between *Trichoderma* and *Rhizoctonia solani* isolates

The antagonistic activities of *Trichoderma* isolates were tested against *R. solani* by dual culture technique (Morton and Stroube, 1955). Five-mm mycelial discs of *R. solani* and *Trichoderma* spp. were cut from actively growing periphery of 7-day-old cultures on PDA, and separately positioned opposite to each other 1.5 cm away from the edge of 90 mm Petri plates containing PDA. Plates inoculated with *R. solani* alone and *Trichoderma* alone served as control. Each pair was replicated four times and incubated at 28 ± 2°C. The mycelial growth of *R. solani* and *Trichoderma* was recorded for every 48 h after incubation and the percentage of inhibition over control for each treatment was calculated according to formula given by Vincent (1947).

$$\text{Percent inhibition (PI) \%} = \frac{C-T}{C} \times 100$$

Where, *C* = Pathogen radial growth in cm in control

T = Radial growth in cm in treated plates

In-vivo evaluation of *Trichoderma* against sheath blight in screenhouse

1. Preparation of *Trichoderma*

Five *Trichoderma* isolates (RN-1, RN-4, RN-5, RN-10 and RN-12) were selected based on the performance shown in dual cultures against *R. solani*. They were grown on PDA for seven days and harvested the spores in sterilized distilled water and filtered through muslin cloth. Spore concentration was adjusted to 10^6 using hemocytometer.

2. Rice seed treatment and seedling raise

Rice seeds of four varieties (Sona Mansuli, Sabitri, Sworna Sub-1 and Bahuguni-2) were procured from National Rice Research Program, Hardinath Dhanusha, Nepal. Rice seeds were soaked in clean water for 12 h. and the pre-soaked seeds were then dipped in the *Trichoderma* suspension containing for 12 h. Untreated control seeds were soaked in water for 24 h. The seeds treated with *Trichoderma* and untreated control were sown in plastic pots containing sterilized field soil, fine sand and vermicompost (at the rate of 2:1:1). The pots were placed in a screenhouse and watered as needed for growing rice seedlings.

3. Seedling treatment and transplanting

The seedlings when reached 24-day-old were pulled out gently from the pots and dipped the root portion into *Trichoderma* suspension (prepared as described earlier) for 8 h. Untreated control seedlings were dipped in water. The treated seedlings were transplanted in pots (17 cm dia. × 15 cm height) containing soil, sand and vermicompost (2:1:1) under wet conditions and watered regularly to keep soil saturated. The pots were arranged in a completely randomized design (CRD) and replicated six times for each treatment and kept in a screenhouse (25 to 36°C temperature and 85 to 90% relative humidity). Two seedlings per pot was transplanted and 1 g of urea was applied after 30 days of transplantation.

4. Challenge inoculation with the pathogen

Rice plants were inoculated at maximum tillering stage (60 days after transplanting) by placing 5-mm mycelium disc of 5-day-old pure culture of *R. solani* at the sheath by removing sheath gently and covering it by moist absorbent cotton so that moisture would be maintained for 24 h for proper growth of fungus.

5. Assessment of disease and growth parameters of rice plant

Lesion height (cm) of sheath blight was recorded 20 days after inoculation. Relative lesion height was calculated using the following formula (Sharma *et al.* 1990):

$$\text{RLH} = \frac{\text{Lesion height}}{\text{Plant height}} \times 100$$

Besides the disease, plant height, total number of tillers, flag leaf length and panicle length were recorded at 90 days after transplanting. Root length was taken after harvesting.

Statistical analysis

Web Agri Stat Package 2.0 (WASP) was used for the statistical analysis of all the data obtained. Post hoc test with Duncan's multiple range test (DMRT) at 1% and 5% (CD = 0.01 and 0.05) significance level was applied for one-way analysis of variance (ANOVA).

RESULTS

Isolation and identification of *Trichoderma*

Green circular colonies resembling *Trichoderma* appeared on TSM from 4 to 6 days after incubation. Altogether 30 isolates were isolated from 32 soil samples. All isolates grew well and covered 9-cm Petri plates within four days and sporulated within a week.

Isolation, identification and pathogenicity of *Rhizoctonia solani*

Of the four sheath blight-infected samples, three showed mycelial growth in a radiating manner on water agar 24 h after incubation. The mycelia had branching at 90 degree angle, which is characteristic of *R. solani*, when observed under compound microscope.

All three *R. solani* isolates produced disease on rice cv. Sona Mansuli, showing typical symptoms of sheath blight. Of the three isolates, isolate RS-3 comparatively produced more disease (data not shown), thus selected for further studies (dual culture and *in-vivo* efficacy).

Dual cultures between *Trichoderma* and *Rhizoctonia solani* isolates

All 30 *Trichoderma* isolates significantly inhibited the growth of *R. solani* (Table 1). Isolate RN-1 gave highest inhibition (68.81%) followed by RN-12 (63.82%), and RN-10 (60.084%) and RN-5 (56.30%). Isolates RN-12, RN-10 and RN-1 grew over *R. solani* by 53.3 mm, 52.60 mm, and 52.8 mm, respectively. The initial growth rate of *Trichoderma* and *R. solani* differed in dual cultures. For example, *Trichoderma* (RN-1) grew slower than *R. solani* till 48 h after inoculation, they formed interaction zone on 72 h and the former completely overgrew the latter one on 96 h (Fig. 1).

Table 1: Effect of *Trichoderma* isolates against *Rhizoctonia solani* (RS-3) in dual cultures, 96 hours after incubation

Trichoderma Isolates	*R. solani*		*Trichoderma* grown over *R. solani* (mm)
	Radial growth (mm)	Inhibition (%)	
RN-1	25±0.57	68.81	52.08±2.16
RN-2	56.25±0.40	29.83	35±5
RN-3	51.83±0.42	35.34	31.6±5
RN-4	38.17±0.45	52.69	46.67±3.3
RN-5	35.67±0.39	56.30	43.33±4.8
RN-6	57.83±0.46	27.86	37.5±8.29
RN-7	64.83±0.29	19.13	31.66±1.66
RN-8	61.75±0.09	22.97	38.33±2.88
RN-9	66.17±0.12	17.46	30±0
RN-10	32±0.12	60.08	52.60**±1.44**

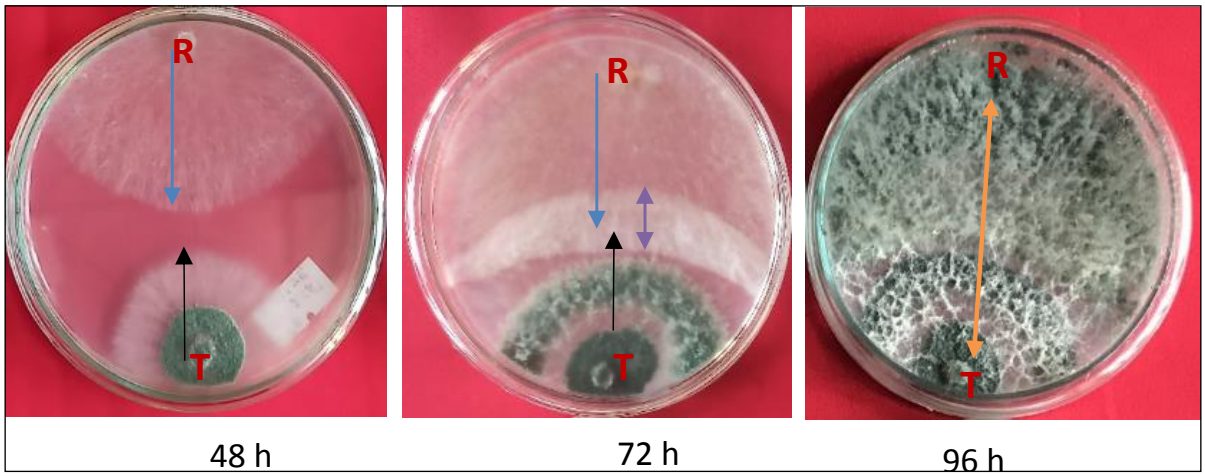

Fig. 1: *Trichoderma* (RN-1) inhibiting the growth of *Rhizoctonia solani* (RS-3) in dual culture at 48, 72 and 96 h after incubation. Black arrow shows *Trichoderma* growth, blue arrow shows *R. solani* growth, yellow arrow shows interaction zone, green arrow shows *Trichoderma* growth over *R. solani*.

RN-11	60.67±0.24	24.32	44.16±2.76
RN-12	29±0.08	63.82	53.3±1.17
RN-13	54±0.07	32.64	30.83±1.44
RN-14	61.25±0.04	23.59	26.58±3.57
RN-15	62.75±0.08	21.72	34.66±3.80
RN-16	57.25±0.08	28.58	29.16±2.76
RN-17	58.25±0.08	27.34	27.5±4.33
RN-18	57.75±0.04	27.96	22.5±4.33
RN-19	65.3±0.16	18.54	25.58±1.01
RN-20	58.25±0.08	27.34	25.58±1.01
RN-21	61.5±0.11	23.28	31.08±1.7
RN-22	63.5±0.11	20.79	23.5±1.0
RN-23	65.75±0.19	17.98	33±0.91
RN-24	55.25±0.29	31.08	26.41±5.5
RN-25	54.5±0.16	32.01	32.08±0.72
RN-26	61.25±0.04	23.59	33.83±1.28
RN-27	64±0.17	20.16	31.25±0.4
RN-28	65.5±0.15	18.29	28.66±2.3
RN-29	62.25±0.67	22.35	32.25±0.4
RN-30	56.75±0.19	29.21	35.41±2.9
Control	80.17±0.48		

Data depicts the mean of four replications. Numerical value indicates mean ±standard deviation. Significant difference at 1% and 5 % level of significance. CD (0.01) = 0.560 CD (0.05) = 0.426.

In-vivo evaluation of *Trichoderma* against sheath blight in screenhouse

All five selected *Trichoderma* isolates (based on dual culture results) significantly reduced the relative lesion height of sheath blight in treated rice plants as compare to untreated control (Table 2). The highest reduction of the disease (66.94% mean of 4 varieties) was obtained with the isolate RN-1 in all four rice varieties.

The *Trichoderma* isolates also significantly enhanced the growth of rice plants (Table 2). Plants treated with the isolates RN-10 (66.25 cm, mean of 4 varieties) and RN-1 (65.74 cm) had significantly highest plant height. Similarly, isolate RN-10 recorded maximum flag leaf length (22.65 cm), number of tillers (21.16), and root length (26.02 cm) (Fig. 2).

DISCUSSION

In the present study, dual culture results showed the rapid growth of *Trichoderma* isolates against *R. solani*. Efficacy of *Trichoderma* was tested against *R. solani* by using dual culture technique, during this technique average diameter growth of *R. solani* was calculated for further analysis. The initial growth of *R. solani* was found faster, but later after 4th day of incubation colony of *R.*

Fig. 2: Rice plant root of different varieties treated by *Trichoderma* isolates RN-1 (T1), RN-12 (T2), RN-10 (T3), RN-4 (T4), and RN-5 (T5); root of untreated control of Sabitri (V1), Bahuguni-2 (V2), Swarna Sub-1 (V3), and Sona Mansuli (V4)

Table 2: Effect of *Trichoderma* isolates on relative lesion height of sheath blight and growth of rice in screen-house

Treatments	Relative lesion height (cm)	Lesion size reduction (%)	Plant height (cm)	Flag leaf length (cm)	Root length (cm)	Total number of tillers	Panicle length (cm)
T1V1	2.13±0.2	76.84	71.33±4.6	28.38±4.2	28.1±1.4	19.33±1	23.1±0.6
T1V2	3.96±0.2	56.04	66.33±2.6	20.25±0.8	27.5±2.3	18.00±1	21.93±1.1
T1V3	3.70±0.1	60.76	61.66±0.8	20.88±2.0	22.66±5.1	21.00±1	22.96±0.8
T1V4	2.43±1.3	74.14	63.66±0.5	22.41±0.6	23.5±1.6	14.66±0.5	22.76±1.7
T2V1	2.54±0.2	72.39	71±3.3	26.3±4.2	23.5±2.2	19.00±1.6	22.56±0.6
T2V2	3.14±0.2	65.14	68.6±2.8	17.8±0.3	27±2.5	16.66±2.3	22.6±0.4
T2V3	4.04±0.06	57.15	61.33±2.0	20.46±0.9	21.1±1.4	23.66±2	23.63±0.4
T2V4	4.21±0.2	55.21	62.5±0.5	23.41±0.7	27±2.0	20.66±2.2	23.6±0.1
T3V1	4.19±0.9	54.45	72±0.5	28.66±2.7	28±1.7	25.66±1.3	23.7±0.9
T3V2	2.43±0.2	73.02	71.1±0.7	17.73±1.1	27.6±1.8	18.00±0.4	22.6±2.1
T3V3	4.94±0.3	47.61	61±1	22.71±0.6	24.83±1.9	21.66±0.8	22.63±0.3
T3V4	3.97±0.2	57.76	62.5±0.5	21.5±1.7	23.5±1.0	19.33±1.5	23.73±0.4
T4V1	3.10±0.2	66.30	70±0.5	20±0.6	19.33±3	18.00±2.9	22.2±0.2
T4V2	3.90±0.06	56.71	63.6±4.6	17.33±0.7	26.16±2.4	15.66±2.7	22.3±0.08
T4V3	4.13±0.09	56.20	59.66±2.8	20±2.6	20.66±3.4	22.66±1.5	23.23±0.1
T4V4	4.27±0.2	54.57	61.66±2.3	22.18±2.7	24.5±1.6	20.66±1.1	23.9±0.08
T5V1	2.65±0.2	71.19	68.33±1	26.66±0.8	21±2.8	22.33±2	22.1±0.08
T5V2	4.49±0.1	50.16	66±3.4	20.5±0.4	25.8±0.7	16.66±0.8	22.33±0.2
T5V3	3.87±0.09	58.96	63.6±0.5	21.66±0.7	26.5±1.8	19.33±0.4	22.4±0.2
T5V4	3.45±0.2	63.29	64.66±5	23.25±0.6	25.16±4.4	19.33±2.3	23.76±0.2
V1	9.20±1.0	—	56.66±1	19.33±1.6	13.83±1.4	19.00±1.5	19.8±0.4
V2	9.01±0.1	—	57.33±1.4	19.91±0.3	14.33±1.8	12.66±0.8	20.83±1.1
V3	9.43±0.4	—	55.66±1.5	15.66±0.9	15.33±0.5	15.33±3.6	20±0.3
V4	9.40±0.7	—	59±1	19.08±1.0	15±2.2	12.33±1.8	21.5±0.1

T1 – Isolate RN-1, T2 - RN-12, T3 - RN-10, T4 - RN-4, T5 - RN-5, V1 – var. Sabitri, V2 - Bahuguni-2, V3 - Swarna Sub-1, and V4 - Sona Mansuli.

solani was completely covered by the growth of *Trichoderma* isolates (Fig. 1). Due to overgrowth of *Trichoderma* isolates, growth of *R. solani* was found to be highly suppressed. No inhibition zone was observed between *Trichoderma* isolates and *R. solani*, indicated that the antagonistic effect was due to competition for space and nutrient (Fig.1). Yadav *et al.* (2018) reported that *Trichoderma* isolates showed maximum percent growth inhibition against *R. solani*. The *Trichoderma* isolates were not only able to inhibit the growth of pathogen in laboratory experiment (Table 1) but also capable of suppressing the disease in screenhouse experiment (Table 2) confirming that versatile defensive mechanisms' with 'demonstrating potential disease management by the bioagents. Chaudhary *et al.* (2020) reported that *Trichoderma* spp. have the potential to biocontrol of sheath blight disease caused by *R. solani*.

In the present study, *Trichoderma*-treated rice plants showed higher plant growth in terms of plant height, tiller numbers, flag leaf length, panicle length, and root length compared to untreated control plants. These results suggested that *Trichoderma* treatments had the plant growth promoting effects in rice plants. These findings are similar to those reported by Chaudhary *et al.* (2020) 'in which *Trichoderma* spp. were found to enhance plant growth parameters.

Among the *Trichoderma* isolates RN-1 showed higher inhibition of *R. solani* over control, while, *Trichoderma* isolates RN-10 showed highest boost in plant height, flag leaf length, root length, total number of tillers and panicle length. This might be due to native and well adaptation of *Trichoderma* isolates to the environment. The result is supported

by similar result reported by Swain *et al.* (2021) where the seed of rice treated with *Trichoderma* strains not only promoted germination, seedling vigor, and growth of the plant, but also increase plant defense. Moreover, *Trichoderma* spp. can effectively manage plant pathogen, therefore it is an option for farmers to use in sustainable cropping and increase in yields and to produce quality produce (Kubheka *et al.* 2022). Recently, Rokaya *et al.* (2023) reported efficacy of *Trichoderma* for the management of late blight disease in potato. *Trichoderma* spp. can be used as an ecofriendly and sustainable tool to enhance yield of different crop plants, including rice (Hossain *et al.* 2017).

CONCLUSION

Trichoderma isolates showed the efficacy to biocontrol of sheath blight disease caused by *R. solani* and boost rice plant growth parameters. Among the *Trichoderma* isolates RN-1, RN-10, RN-12, RN-5 and RN-4 were the most effective isolates having potential to control sheath blight and promote rice plant growth compared to other isolates tested. Therefore, these isolates can be used for production and development of *Trichoderma* based biopesticide-cum biofertilizers to manage *R. solani* and growth regulators for rice plants.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Institute of Agriculture and Animal Science, Tribhuvan University, Kathmandu, Nepal. Also grateful to the University Grant Commission, Sanothimi, Bhaktpur Nepal for providing research grant for this research. Authors are also grateful to Nepal Plant Disease and Agro Associates (NPDA) Balaju, for providing necessities.

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Annexure 1: Details of soil sample collections

Sample	Location and district	Latitude	Longitude	Altitude	No of Isolations	Remark
1	Gauradaha, Jhapa	26.5589738	87.716765	100	1	
2	Birtamod, Jhapa	26.629305	87.98245	99	0	
3	Tarahara, Sunsari	26.42092	87.16347	136	2	
4	Ithari, Sunsari	26.6646	87.2718	116	0	
5	Inaruwa, Sunsari	26.6094	87.1572	90.55	0	
6	Jatuwa, Morang	26.484086	87.266211	78	1	
7	Biratnagar, Morang	26.47762	87.613028	79	1	
8	Sunbarsi, Morang	26.4556	87.5484	86	1	
9	Dainiya, Morang	26.2719	87.3252	85	1	
10	Belbari, Morang	26.6676	87.4307	118	0	
11	Urlabari, Morang	26.6649	87.6137	121	1	
12	Mirchaiya, Siraha	26.084260	86.02586	112	1	
13	Golbazar, Siraha	26.795701	86.29709	138	1	
14	Hardinath, Dhanusha	26.04746	85.05749	75	3	
15	Janakpur, Dhanusha	26.7271	85.9407	74	1	
16	Jatahi, Dhanusha	26.6238	85.9279	68	0	
17	Jaleshwar, Mahottari	26.6471	85.8008	56	1	
18	Bardibas, Mahottari	26.8757	85.8690	105	0	
19	Malangawa, Sarlahi	26.85511	85.5542	79	1	
20	Haripurwa, Sarlahi	26.5450	85.4106	78	1	
21	Phenhara, Sarlahi	26.4817	85.48913	75	1	
22	Parwanipur, Bara	27.0716	84.9180	70	1	
23	Paklihawa, Rupandehi	27.6523	84.3508	116	2	
24	Bhairahawa, Rupandehi	27.32	83.25	105	1	
25	Badhiyatal, Bardiya	28.2128	81.446772	88	1	
26	Madhuwan, Bardiya	28.15057	81.16369	112.6	1	
27	Raptisonari, Banke	28.02192	81.758998	80.3	0	
28	Khajura, Banke	28.0645	81.3558	182	2	
29	Khajura, Banke	28.0645	81.3558	181	1	
30	Chatakpur, Banke	28.06616	81.73955	182	1	
31	Dangpur, Bardiya	28.2009	81.1933	111.5	1	
32	Tikapur, Kailali	28.3119	81.0723	243	1	