FIELD EVALUATION OF *Trichoderma viride* FOR THE MANAGEMENT OF RICE LEAF BLAST DISEASE IN PYUTHAN DISTRICT, NEPAL

N.R. Bhusal[1], B. Acharya[2], A.R. Devkota[1] and J. Shrestha[2]
[1]Agriculture and Forestry University, Chitwan Nepal
[2]Nepal Agricultural Research Council

ABSTRACT

A field experiment was conducted at Prime Minister Agriculture Modernization Project (Zone office), Bijuwar, Pyuthan from 18th April, 2017 to 12nd September, 2017 to evaluate the efficacy of *Trichoderma viride* in the management of leaf blast diseases of rainy season rice under field conditions. The experiment was laid out in two factors factorial Randomized Complete Block Design with five replications. The treatment consisted of two varieties of rice of which one is improved and resistant to blast disease (Sabitri) and another is local (Radha 7) and two rates of *Trichoderma viride* (5 ml/lit. and 0 ml/lit.). The leaf blast disease was scored three times using the disease rating scale 0-9 given by IRRI, 2002 from 75 DAT to 85 DAT at 5 days interval. The disease incidence, severity and AUDPC value were calculated and analyzed by using RSTAT. The variety factor and *Trichoderma viride* factor varied significantly for leaf blast incidence, severity and AUDPC, however, the interaction of variety and *Trichoderma viride* factor varied significantly only in AUDPC value. Disease incidence, severity and AUDPC were found least in Sabitri in comparison to Radha 7. *T. viride* had significant role in reducing the disease incidence, severity and AUDPC value. The interaction of Sabitri and *Trichoderma viride* treatment was found best with the mean AUDPC value being the least 3.228. So, the application of Trichoderma viride was found efficient for leaf blast management and it is more effective when applied along with an improved and resistant variety.

Keywords: Rice blast, Biocontrol, *Trichoderma viride*, Sabitri, local variety, AUDPC

INTRODUCTION

Rice (*Oryza sativa* L.) is important staple food and cash crop of the world . In Nepal, rice ranks first on the basis of area and production with 31.4% and 21% contribution to GDP and AGDP respectively (MoF, 2017). Rice blast, caused by the ascomycete fungus, *Magnaporthe grisea* Barr (Anamorph *Pyricularia grisea* Sacc., synonym *P. oryzae* Cav.) is one of the major biotic constraints of rice known to occur in most rice producing areas of the world (Singh *et al.,* 2015) causing severe yield losses and significantly impair rice production worldwide (Vasudevan *et al.,* 2014).

The disease can strike all aerial parts of the plant. Most infections occur on the leaves, causing diamond-shaped lesions with a gray or white center to appear (Scardaci *et al.,* 1997). The disease results in yield loss as high as 70-80% (Ou, 1985) when predisposition factors (high mean temperature values, degree of relative humidity higher than 85-89%, presence of dew, drought stress and excessive nitrogen fertilization) favor epidemic development (Piotti *et al.,* 2005). In Nepal, rice blast is most serious disease in both terai and hilly region, causing an average yield loss of 125 kg/ha in the hills and 112 kg/ha in the terai (IRRI, 1996).

Although the blast diseases can be successfully controlled through the fungicidal spray and seed treatment with systemic fungicide (Prabhu & Filippi, 1993), it disturbs the rice ecosystem, pollute the environment and induce resistant mutants of the pathogen. One of the approaches, which are progressively being developed in compliance with sustainable environment issues during recent years, is biological control (Emmert & Handelsman, 1999). *Trichoderma* spp., the

well-known antagonistic fungus, is widely used in agriculture as bio fungicides (Mukherjee *et al.,* 2008). *Trichoderma viride* showed antagonistic activity under in vitro, in vivo and field condition by inhibition of mycelial growth of *P. grisea* and minimum leaf blast severity (Sharma, 2006). The *Trichoderma* treated seed showed low disease intensity as compared to untreated seed reducing the disease intensity by 10–25% (Aravindan *et al.,* 2016).

Considering all these factors, this research was carried out with the following objectives:

- To evaluate the efficacy of *Trichoderma viride* for the management of rice leaf blast diseases under field conditions.
- To compare the effect of improved and resistant variety over the local variety for the management of rice leaf blast.

MATERIAL AND METHODS

Selection of plant materials

Two types of plant materials including resistant variety Sabitri and locally cultivated variety Radha 7 were tested in both *Trichoderma* treated and not treated control condition. The seed of resistant variety Sabitri was collected from Regional Agricultural Research Station, Khajura, Banke. The locally cultivated variety Radha 7 was collected locally within the trial area.

Source of *Trichoderma viride*

Trichoderma viride with commercial concentration of 109 cfu/ml was purchased from Agricare Nepal Pvt. Ltd. Bharatpur, Nepal and its commercial concentration was confirmed from the laboratory test in Regional Agricultural Research Station, Khajura, Banke.

Field layout and treatment details

The experiment was laid out in two factors factorial randomized complete block design in rainy season rice during April to September, 2017 with five replications and four treatment combinations. The treatments consisted of combinations of factor A (Two rice varieties namely, Sabitri and Radha 7) and factor B (*Trichoderma viride*, 5 ml/lit. and control). Individual plot size comprised of 6m x 1m, the row to row and plant to plant spacing was 20 cm. The space between two replications and boarder was one and half meters.

Table 1: Treatment combinations

Factor A	Factor B	Treatment combinations	
V_1	R_1	$V_1 R_1$ = Radha 7 (Local) + 0 ml/lit. of *T. viride*	T_1
	R_2	$V_1 R_2$ = Radha 7 (Local) + 5 ml/lit. of *T. viride*	T_2
V_2	R_1	$V_2 R_1$ = Sabitri (Improved and blast resistant) + 0 ml/lit. of *T. viride*	T_3
	R_2	$V_2 R_2$ = Sabitri (Improved and blast resistant) + 5 ml/lit. of *T. viride*	T_4

Agronomical management and *T. viride* application

During 1st week of June, 2-3 seedlings/hill were transplanted with 20 × 20 cm^2 spacing. In case of treated plot, rice seedlings were dipped in *Trichoderma viride* (10^9 cfu/ml) solution of 5 ml/ litre of water for 30 min. and shade dried for 10-15 min. before transplanting. FYM and N: P: K were applied @6 ton/ha, and 100:30:30, kg/ha respectively. Total amount of phosphorus and potash and half amount of nitrogen were mixed in soil at the time of final land preparation. Remaining half

doses of nitrogen was applied in two split doses; one at the tillering stage and the other at the head emergence stage. Weeding was done two times before the top dressing of urea.

Sampling and disease scoring

10 plants were randomly selected from each plot excluding the plants of border line and tagged by a piece of ribbon for identification while taking data. Data on disease measurement was taken. The disease was scored three times at 5 days interval starting from the first appearance of leaf blast symptoms i.e. 75 days after transplanting (DAT) during tillering stage. It was taken on the basis of symptom appearance of the leaf blast which is graded in a 0-9 scale (IRRI, 2002).

Disease incidence and severity

The disease incidence and severity were recorded treatment wise by using the following formula:

$$Incidence\ (\%) = \frac{Number\ of\ infected\ plant}{Total\ number\ of\ plants\ rated} \times 100\%$$

$$Severity\ (\%) = \frac{Sum\ of\ all\ numerical\ ratings}{Number\ of\ plants\ rated\ Highest\ rating\ scale} \times 100\%$$

Estimation of area under disease progress curve (AUDPC)

The effect of disease severity on rice variety was integrated into area under disease progress curve (AUDPC) (Campbell & Madden, 1990). Area under disease progressive curve (AUDPC) determines the intensity and progress of disease development and calculated by using the following formula (Das *et al.,* 1992).

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\frac{Y_{i+1} + Y_i}{2}\right)(T_{i+1} - T_i)$$

Where, Yi = disease scored on first date, Ti= date on which the disease was scored, n= number of dates on which disease was scored.

Data of disease scoring and then disease incidence and severity were tabulated in excel data sheet. The data were processed to fit into R-studio and analyzed using R 3.0.3 (R Core Team, 2013). The data entry was done to develop ANOVA table and different treatments were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Leaf blast incidence

Leaf blast incidence was found maximum at 80 DAT. The maximum incidence of leaf blast disease was found significant ($P<0.01$ and 0.001) among the both factors, variety and *Trichoderma viride*. Disease incidence was found least in Sabitri and *Trichoderma viride* treatment.

Table 1: Incidence of leaf blast disease in rice variety factor under field condition at Pyuthan on 80 DAT, 2017

S.N.	Factor A	Maximum disease incidence
1	Radha 7	32[a]
2	Sabitri	11[b]
	Grand mean	21.5
	CV (%)	46.843
	MS Error	101.43
	LSD (0.05)	9.66
	P value	0.000046***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1 % level of significance

Disease incidence was observed at 75, 80 and 85 DAT after the first appearance of disease symptoms. At 80 DAT, highest disease incidence was observed and it was significantly lower in sabitri in comparison to Radha 7. The disease incidence was almost three times more in Radha 7 than Sabitri. It was 11% and 32% for Sabitri and Radha 7 respectively. Experiment by Chaudary *et al.***,** (2001) and Khanal *et al.***,** (2016) also presented the lowest incidence of blast disease in Sabitri.

Application of *T. viride* was found significant in reducing the incidence of leaf blast disease. Disease incidence was almost two times less in *T. viride* treatment. It was 16% and 27% for treatment and non-treatment respectively. Singh *et al.,*(2012) also showed 23.30 to 30.55% disease incidence in Trichoderma treatment and 40.50 to 48.09% in non- treatment.

Table 2: Incidence of leaf blast disease in *Trichoderma viride* factor under field condition at Pyuthan on 80 DAT, 2017

S.N.	Factor B	Maximum disease incidence
1	0 ml/lit of *T. viride*	27[a]
2	5ml/lit of *T. viride*	16[b]
	Grand mean	21.5
	CV (%)	68.131
	MS Error	215.71
	LSD (0.05)	14.088
	P value	0.007**

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 1 % level of significance

Leaf blast severity was also found maximum at 80 DAT. The maximum severity of leaf blast disease was found significant (P<0.001) among both factors, variety and *Trichoderma viride*. Disease Severity was found least in Sabitri and *Trichoderma viride* treatment.

Table 3: Severity of leaf blast disease in rice variety factor under field condition at Pyuthan on 80 DAT, 2017

S.N.	Factor A	Maximum disease severity
1	Radha 7	3.864[a]
2	Sabitri	1.472[b]
	Grand mean	2.668
	CV (%)	43.221
	MS Error	1.3297
	LSD (0.05)	1.1061
	P value	0.0000029***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1 % level of significance

Disease severity was also observed maximum at 80 DAT and it was significantly lowest in sabitri as compared to Radha 7. It was 1.472% and 3.864% for Sabitri and Radha 7 respectively. This is in line with the findings of Khanal *et al.*, (2016).

Table 4: Severity of leaf blast disease in *Trichoderma viride* factor under field condition at Pyuthan on 80 DAT, 2017.

S.N.	Factor B	Maximum disease severity
1	0 ml/lit of *T. viride*	3.547[a]
2	5 ml/lit of *T. viride*	1.879[b]
	Grand mean	2.668
	CV (%)	59.071
	MS Error	2.4838
	LSD (0.05)	1.5117
	P value	0.00016***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1 % level of significance

T. viride treatment significantly reduced the percentage of leaf blast severity. In this study, *T. viride* was applied @ 5ml/lit of water and leaf blast severity in *T. viride* treatment and non-treatment was found 1.879% and 3.547% respectively i.e. leaf blast was 52. 97 % more severe in non-treatment than that of treated one. This is in line with the result reported by the Kumar et al. (2017). Sharma, (2006) also recorded minimum leaf blast severity with *T. viride.*

Area under disease progress curve (AUDPC). The AUDPC value of leaf blast for both factor, variety and *Trichoderma viride*, and their interaction varied significantly ($P<0.001$) along with their mean AUDPC.

Table 5: AUDPC value of leaf blast on rice variety factor under field condition at Pyuthan

S.N.	Factor A	AUDPC I	AUDPC II	Mean AUDPC
1	Radha 7	10.431[a]	20.323[a]	15.377[a]
2	Sabitri	2.683[b]	7.485[b]	5.083[b]
	Grand mean	6.557	13.904	10.23
	CV (%)	78.049	49.45	57.07
	MS Error	26.19	47.273	34.085
	LSD (0.05)	4.9087	6.5949	5.5999
	P value	1.70E-06***	3.50E-08***	1.00E-08***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1% level of significance

The I, II and mean AUDPC value was least in Sabitri. The mean AUDPC value was 5.083 and 15.377 for Sabitri and Radha 7 respectively and differed significantly among Sabitri and Radha 7. Sabitri showed lowest level of AUDPC value which is supported by Chaudhary et al. (2001) suggesting that Sabitri variety to be resistant to blast pathogen. This result also coincides with the findings of Chaudhary *et al.,* (2005).

Table 6: AUDPC value of leaf blast on *Trichoderma viride* factor under field condition at Pyuthan

S.N.	Factor B	AUDPC I	AUDPC II	Mean AUDPC
1	0 ml/lit of *T. viride*	9.855[a]	18.861[a]	14.356[a]
2	5 ml/lit of *T. viride*	3.259[b]	8.947[b]	6.104[b]
	Grand mean	6.557	13.904	10.23
	CV (%)	86.396	60.616	67.449
	MS Error	32.092	71.033	47.611
	LSD (0.05)	5.4337	8.0841	6.6184
	P value	8.70E-06***	6.00E-07***	1.20E-07***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1% level of significance

The I, II and mean AUDPC value was found significant among the *T. viride* treatment and non-treatment. *T. viride* was found efficient in reducing the AUDPC value for leaf blast disease. The AUDPC value was 6.104 and 14.356 in treated and untreated cases respectively. The decreased AUDPC for sheath blight infected rice plants was also observed in the research findings of Franca et al. (2015) when sprayed with Trichoderma.

Table 7: AUDPC value of leaf blast on interaction of variety and *Trichoderma viride* factor under field condition at Pyuthan

S.N.	Treatment combination	AUDPC I	AUDPC II	Mean AUDPC
1	Radha 7 + 0 ml/lit of *T. viride*	15.976[a]	27.576[a]	21.774[a]
2	Radha 7 + 5 ml/lit of *T. viride*	4.886[b]	13.070[b]	8.980[b]
3	Sabitri + 0 ml/lit of *T. viride*	3.743[bc]	10.146[b]	6.938[b]
4	Sabitri + 5 ml/lit of *T. viride*	1.632[c]	4.824[c]	3.228[c]
	Grand mean	6.557	13.904	10.23
	CV (%)	29.103	20.547	19.157
	MS Error	3.6416	8.1613	3.8406
	LSD (0.05)	2.5586	3.8302	2.6275
	P value	0.0003***	0.00085***	5.60E-05***

DAT: Days after transplanting, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 0.1% level of significance

In the case of variety and *Trichoderma viride* interaction, AUDPC I value ranged from 1.632 to 15.976 with the least in treated Sabitri (1.632), followed by untreated Sabitri (3.743) and treated Radha 7 (4.886), and the highest in untreated Radha 7 (15.976). AUDPC II value ranged from 4.824 to 27.576 with the least in treated Sabitri (4.824), followed by untreated Sabitri (10.146) which was at par with treated Radha 7 (13.07), and the highest in untreated Radha 7 (27.576). Similarly, mean AUDPC value ranged from 3.228 to 21.774 with the least in treated Sabitri (3.228), followed by untreated Sabitri (6.938) which was at par with treated Radha 7 (8.980), and the highest in untreated Radha 7 (21.774).

CONCLUSION

From the study, it can be concluded that *Trichoderma viride* application was found efficient for leaf blast management and it is more effective when applied along with sabitri variety. The performance of Sabitri was found effective over the Radha 7 for leaf blast disease management in Pyuthan district of Nepal.

ACKNOWLEDGEMENT

We would like to express our heartfelt thanks to Agriculture and Forestry University, Rampur, Chitwan and Regional Agricultural Research Station, Khajura, Banke for providing financial and technical support to conduct this research.

REFERENCES CITED

Aravindan, S., Yadav, M. K., & Sharma, P. (2016). Biological control of rice blast disease with *Trichoderma* spp. under upland rice System. *ORYZA- An International Journal on Rice, 53*(2), 167-173.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & sons.

Chaudary, B., Shrestha, S. M., & Sharma, R. C. (2001). Resistance in rice breeding line to blast fungusin Nepal. *Nepal Agriculture Research Journal, 6*, 49-59.

Emmert, E. A., & Handelsman, J. (1999). Biocontrol of plant disease: a (Gram-) positive perspective. *FEMS Microbiology letters, 171*(1), 1-9.

Franca, S. D., Cardoso, A. F., Lustosa, D. C., Ramos, E., Filippi, M. D., & Silva, G. D. (2015). Biocontrol of sheath blight by *Trichoderma asperellum* in tropical lowland rice. *Agron. Sustain. Dev., 35*, 317-324.

IRRI. (1996). *Rice Research in Asia: Progress and Priorities.* (R. E. Evenson, R. W. Herdt, & M. Hossain, Eds.) C.A.B. International, International Rice Research Institute.

IRRI. (2002). *Standard evaluation system for rice.* Las Banos, Manila, Phillipines: International Rice Research Institute.

Khanal, S., Subedi, B., Bhandari, A., Giri , D. R., Shrestha , B., Neupane, P., Shrestha, S. M., & Gaire, S. P. (2016). Screening of Different Rice Genotypes against (*Pyricularia grisea*) Sacc. in Natural Epidemic Condition at Seedling Stage in Chitwan, Nepal. *Advances in Crop Science and Technology, 4*, 231.

Kumar, S., Lal, A. A., Kumar, N., Jaiswal, S., Kumar, H., Kumar, A., & Kumar, M. (2017). Effect of bio control agents and botanicals against Blast of Paddy caused by *Pyricularia oryzae*. *International Journal of Chemical Studies, 5*(1), 314-318.

MoF. (2017). *Economic Survey.* Singh Durbar, Kathmandu: Government of Nepal, Ministry of Finance.

Mukherjee, P. K., Nautiyal C. S., & Mukhopadhyay A. N. (2008). Molecular Mechanisms of Biocontrol by *Trichoderma* spp. Molecular Mechanisms of Plant and Microbe Coexistence. *Spr. B. Heidelberg, 15*, 243-262.

Ou, S. H. (1985). *Rice Diseases* (2nd ed.). Kew, UK: Commonwealth Mycological Institution.

Piotti, E., Rigano, M. M., Rodino, D., Rodolfi, M., Picco, A. M., Castiglione, S., & Sala, F. (2005). Genetic Structure of *Pyriculariya grisea* (Cooke) Sacc. Isolates from Italian Paddy Fields. *Journal of Phytopathologia, 135*, 80-60.

Prabhu, A. S., & Filippi, M. C. (1993). Seed treatment with pyroqilon for the control of leaf blast in Brazilian upland rice. *International Journal of Pest Management, 39*, 347-353.

Scardaci, S. C., Webster, R. K., Greer, C. A., Hill, J. E., Williams, J. F., & Mutters R. G. (1997). *Rice Blast: A New Disease in California.* University of California, Department of Agronomy and Range Science. Davis: Agronomy Fact Sheet.

Sharma, V. (2006). *Integrated Management of Rice Blast.* Ph.D. Thesis, CSK Himachal Pradesh Krishi VishvavidyalayaI, Palampur, India, pp 62.

Singh, M. K., Singh, P. Singh, R. P., & Mohapatra, C. (2015). Association Analysis for Yield and Quality Attributes in Indica Rice and Screening of Hybrids against Blast Disease (*Magnaporthe grisea* Barr.). *Journal of Plant Sciences. 8*(2):45-56.

Singh, P. K., Singh, A. K., Singh, H. B., & Dhakad, B. K. (2012). Biological Control of Rice Blast Disease with *Trichoderma harzianum* in Direct Seeded Rice under Medium Low Land Rainfed Conditions. *Environment & Ecology, 30*, 834-837.

Vasudevan, K., Vera Cruz, C. M., Gruissem, W., & Bhullar, N. K. (2014). Large scale germplasm screening for identification of novel rice blast resistance sources. *Frontiers in plant science, 5*, 505.