Himachal Journal of Agricultural Research 34(2):50-54,2008

Biorational management of rice blast

A.S. Kapoor and Vinay Sharma
Department of Plant Pathology
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

Abstract

***In vitro* evaluation of five bioagents against *Pyricularia grisea, Pseudomonas fluorescens* showed maximum inhibition of mycelial growth of *P. grisea* (73.76%) followed by *Trichoderma viride* (64.48%) and *T. viride*- E (63.71%). Similarly *in vivo* and field conditions *P. fluorescens*, as seed treatment followed by spray gave maximum control of leaf blast disease. Among biopesticides, neem based biopesticide; Wanis (@ 0.5%) was found most effective under *in vitro*, pot and in field conditions. Among botanicals *Saussurea lappa* was most effective against rice blast.**

Key words: Rice blast, rice, biorational management, botanicals, bioagents.

Introduction

Rice crop suffers from a number of diseases but the blast disease caused by the *Pyricularia grisea* (Cooke) Sacc. is the most destructive among all diseases. Production losses of US $ 55 million each year in South and Southeast Asia is estimated due to rice blast (Robert, 1991). In India, damage due to rice blast to the rice crop has been as high as 75 per cent (Padmanabhan, 1959). In Himachal Pradesh, rice blast appears in epidemic proportion in many areas year after year and is the main yield limiting factor especially in the mid - hill region, where rainfall is very high during rice growing season. This disease can be managed by using systemic and non systemic fungicides but due to poor cost-benefit ratio, washing away of fungicides especially, during excessive rains and environmental pollution, extensive sprays of agro chemicals are not advisable. Host plant resistance has tended to be unreliable because of its ephemerality. Therefore, emphasis is being laid on low level of resistance (slow blasting resistance) in combination with other management practices such as non chemical fungicides, biocontrol agents (Soytong, 1990; Hossain *et al.* 2002) and botanical pesticides.

Material and Methods

P. grisea was isolated from naturally infected leaves of rice cultivar Himalaya 2216, a susceptible cv, and maintained on potato dextrose agar medium (Dhingra and Sinclair, 1985). Mycelial culture of the pathogen was purified by mono conidial culture. Stock cultures were stored at 10°C temperature and maintained by regular sub culturing at 15 days interval.

Cultures of *Trichoderma harzianum, T. viride, T. viride*-E and *Gliocladium virens* were maintained by sub culturing on PDA at 23 ±1°C, whereas *P. fluorescens* was maintained on nutrient agar medium.

***In vitro* evaluations**

Bioagents: Cultures of *T. viride, T. harzianum, T. viride*-E and *G. virens* were evaluated against *P. grisea* on PDA using dual culture technique (Huang and Hoes, 1976). Whereas seeded medium of *P. fluorescens* was evaluated against mycelial growth of *P. grisea*. The cultures were incubated at 23±1 °C. Each treatment was replicated thrice. The linear growth of fungal bioagents and the pathogen from the centre of the disc towards the centre of the plate was recorded after the control plates were completely covered by the pathogen. Growth inhibition of pathogen by bioagent over control was calculated using the formula given by Vincent (1947).

Botanicals and biopesticides

Fifty gram fresh leaves each of *Ranunculus muricatus* L., *Lantana camara* L., *Eupatorium adenophorum* Spreng. , *Colocasia esculenta* (L.) Schott , and *Saussurea lappa* Clarke, were crushed in a pestle and mortar with 50 ml sterilized distilled water (1:1 W/V). The extract was obtained by squeezing the macerated leaves. The plant extracts were centrifuged at 3000 rpm for 30 minutes. The pellet was discarded and the supernatant was taken.

Double strength of potato dextrose agar medium sterilized at 1.05 kg cm^{-2} pressure for 20 minutes was prepared. Requisite solution of 5

neem based biopesticides (a.i azadirachtin): Neem Azal (M/S EID Parry's India Ltd. Chennai 600001), Tricure, Spictaf, Wanis (M/S Southern Petrochemical Industries Corporation Ltd. Chennai 600116), and Achook (M/S Godrej Agro Ltd. Sachin Surat Gujrat) (double strength) and aqueous plant extracts (1:1 w/v) were prepared separately. The double strength medium was mixed thoroughly with biopesticides and aqueous plant extracts solutions separately and poured in Petri plates. These were then allowed to cool and solidify. Five mm dia mycelial agar disc of 7 days old fungal culture was placed on the centre of each Petri plate under aseptic conditions. The Petri plates containing PDA medium without any biopesticide/plant extract served as control. Each concentration of the biopesticide was replicated thrice and the Petri plates were incubated at 23±1°C for 7 days. The growth of the mycelium was measured and per cent inhibition was calculated (Vincent, 1947).

Field evaluations

Efficacy of five biocontrol agents was also evaluated under field conditions as seed treatment, as spray and seed treatment plus spray. Seeds of rice blast susceptible cv Himalaya 2216 of rice were treated with five biocontrol agents as talc based formulation @ 4g/ kg seed and sowing were done in 6 m^2 size plots with row to row distance of 20 cm and plant to plant 15 cm. Similarly biocontrol agents were sprayed thrice i.e. at tillering, booting and panicle initiation stage as spore suspension (2-3x10^8 spores per ml) and *P. fluorescens* formulation (Bioshield) @ 0.2 per cent. Field evaluation of biocontrol agents was also done as seed treatment followed by three sprays i.e. at tillering, booting and panicle emergence stage. The experiments were conducted in a Randomized Block Design (RBD) and replicated thrice.

Efficacy of the five biopesticide formulations i.e. Achook, Neem- Azal, Spictaf, Tricure and Wanis @ 0.5 per cent concentration and aqueous extracts of five plants viz; *R. muricatus, L. camara, E. adenophorum, C. esculenta* and *S. lappa* as three foliar sprays at tillering, booting and panicle emergence stages, were evaluated under field conditions. The experiment was conducted in a RBD with three replications. Twenty five days old seedlings of Himalaya 2216 were transplanted in plot size 6 m^2 with spacing 20cm x15cm between rows and plants respectively. Data on leaf blast severity were recorded on ten randomly selected plants from each plot (IRRI, 1996). Data on neck blast incidence was recorded as percentage of tillers having neck blast in 1m^2 area using quadrant at two spots per plot. Recommended doses of fertilizer N: P: K (90: 40: 40) were applied. N was applied as three split doses, whereas P and K applied at the time of planting.

Results and Discussion

***In vitro* evaluations**

In vitro evaluation of biocontrol agents revealed maximum inhibition of mycelial growth of *P. grisea* with *P. fluorescens* (73.36%) followed by *T viride* (64.48%) and, *T. viride* E (63.71%) (Table 1) though statistically both isolates of *Trichoderma* were at par in restricting the growth of the pathogen. *T. harzianum* inhibited the growth of fungus by 52.11 per cent, whereas, least inhibition was observed with *G. virens*.

As many species of fungi and other microbes have been reported to be antagonists of *P. grisea*, but very few of them have been studied extensively. All the five biocontrol agents used in the present study showed antagonistic activity under *in vitro* conditions.

Table 1. Evaluation of biocontrol agents, biopesticides and botanicals against *Pyricularia grisea* under *in vitro* conditions

Biocontrol agents	Mycelial growth inhibition (%)	Bio-pesticides	Mycelial growth inhibition (%)		Botanicals	Mycelial growth inhibition (%)
			0.1%	0.5%		
Trichoderma viride	64.48 (53.39)	Neem -Azal	35.23 (36.39)	61.49 (51.62)	*Colocasia esculenta*	38.13 (38.12)
T.viride -E	63.71 (52.94)	Tricure	26.52 (30.98)	34.21 (35.78)	*Eupatorium adenophorum*	42.93 (40.92)
T. harzianum	52.11 (46.19)	Spictaf	53.28 (46.87)	78.29 (62.21)	*Lantana camara*	18.18 (25.23)
Gliocladium virens	50.09 (45.03)	Achook	31.31 (34.01)	53.16 (46.79)	*Ranunculus muricatus*	38.64 (38.42)
Pseudomonas fluorescens	73.36 (58.91)	Wanis	89.02 (70.62)	99.24 (87.07)	*Saussurea lappa*	57.70 (49.41)
CD(P= 0.05)	(1.08)		(1.16)	(3.76)		(0.78)

Figures in parentheses are arc sine transformed values

Similar results were reported by Hossain *et al.* (2002). Gnanamanickam *et al.* (1994) reported out of 1085 strains of *P. fluorescens* 207 strains induced growth inhibition and 8 strains inhibited mycelial growth of *P. grisea* up to a maximum of 35 mm diameter.

In the present investigations, the results of evaluation of five neem based biopesticides against *P. grisea* under *in vitro* conditions showed that all the biopesticide formulations inhibited mycelial growth of the pathogen at 0.1 and 0.5 per cent concentrations as compared to control (Table 1). However, Wanis (0.5 %) was found to be the most effective and resulted 99.24 per cent inhibition of mycelial growth of *P. grisea* followed by Spictaf (78.29%), Neemazal (61.49%), Achook (53.16%) and Tricure (34.21%). Similar reports on the effectiveness of these biopesticides have been reported against *P. grisea* in rice (Anonymous, 2000). The difference in the toxicity of the different neem based formulations may be due to the solubility of the active compound (s) in the solvent used by the manufacturing firms or to the presence of inhibitors to the fungitoxic principle (Tewari and Nayak, 1991). This could also be due to extractions made from different parts of the plant.

Five aqueous extracts of plants evaluated under *in vitro* conditions; *S. lappa* gave 57.70 per cent inhibition of mycelial growth of *P. grisea* followed by *E. adenophorum* (42.93%) (Table 1). However, *L. camara* was found least effective (18.18%). Sharma and Kapoor (1999) observed the antifungal property of *S. lappa* and *L. camara* against *Sclerotinia sclerotiorum*. Tewari (1995) found inhibitory activity of *Ocimum sanctum* against rice blast under *in vitro* conditions.

Field evaluations

All the bioagents, biopesticides and botanicals were also evaluated under field conditions. Seed treated with Bioshield (*P. fluorescens*) gave maximum (45.53%) disease control followed by *T. viride* (38%), *T. viride* E. (34.36%) and *T. harzianum* (32.89%), least control was observed with *G. virens* (Table 2).

Table 2.Effect of biocontrol agents against leaf blast as seed treatment, foliar spray and seed treatment + foliar spray against leaf blast under field conditions*

Treatments^	Seed treatment		Foliar spray		Seed treatment + foliar spray	
	Leaf blast severity (%)	Disease control (%)	Leaf blast severity (%)	Disease control (%)	Leaf blast severity (%)	Disease control (%)
Trichoderma viride	14.57 (3.94)	38.00	12.07 (3.61)	50.79	7.30 (2.88)	69.10
T. harzianum	15.77 (4.09)	32.89	14.27 (3.91)	41.82	10.63 (3.41)	55.01
T. viride (E)	15.40 (4.05)	34.46	13.20 (3.77)	46.18	8.73 (3.12)	63.05
Gliocladium virens	16.30 (4.16)	30.63	15.70 (4.09)	35.99	12.03 (3.61)	49.09
*Pseudomonas fluorescens*** (0.2%)	12.80 (3.71)	45.53	10.10 (3.33)	58.82	6.73 (2.39)	71.51
Control	23.50(4.95)	-	24.53 (5.05)	-	23.63 (4.96)	-
CD (P= 0.05)	0.13	-	0.88	-	0.90	

Figures in parentheses are square root transformed values, *Average of four replications

** Commercial formulations- Bioshield; ^Dose=2-3 x 10^8 spores/ml (fungal bioagents) and for seed treatment, seeds thoroughly covered with spores of biocontrol agents

Biocontrol agents as foliar spray though exhibited reduction in leaf blast but *P. fluorescens* gave maximum per cent disease control (58.82%) followed by *T. viride* (50.79%), *T. viride*-E (46.18%) and *T. harzianum* (41.82%) (Table 2). The effect of seed treatment followed by foliar spray of biological control agents revealed maximum (71.51%) disease control by *P. fluorescens* followed by *T. viride* (69.10%), *T. viride* E (63.05%), *T. harzianum* (55.01%) and *G. virens* (49.09%). Ngueko *et al.* (2002) found pre-inoculation of *T. harzianum* prior to inoculation of TH16 strain of *M. grisea* led to significant decrease in blast severity. Krishnamurthy and

Gnanamanickam (1998) found rice seed treatment with *P. fluorescens* strain pf 7-14 followed by twice foliar sprays of same bioagent reduced rice blast by 68.5 per cent in nursery and 59.6 per cent in field experiments. Dang *et al.* (2002) reported that eight isolates of bacteria suppressed the rice blast by 50- 60 per cent under field conditions.

Clung (1988) found that seed treatment with *Trichoderma* spp. reduced the incidence of seedling blast, whereas, Gnanamanickam *et al.* (1989) found reduction of leaf and neck blast infection by spraying with fluorescent *Pseudomonas* bacterial cell suspension. Vidyasekaran *et al.* (1997) reported the effectiveness of talc based formulation of *P. fluorescens* when applied as seed treatment. Bacteria spread to roots, stem and leave of the plants and thus gave effective control of the disease and also increased the grain yield.

Five neem based biopesticide formulations were also evaluated at 0.5 per cent concentration under field conditions against rice leaf and neck blast disease. Three sprays of each biopesticide were given at tillering, booting and panicle initiation stages of crop. All the treatments were found significantly superior over untreated control (Table 3). Wanis resulted 67.15 per cent disease control followed by Spictaf (60.92%), Neem-Azal (59.09%), Achook (50.97 %, and Tricure (41.26 %). However, none of the formulations were found superior to Bavistin (0.1%).

Table 3. Evaluation of biopesticides at 0.5% concentration against leaf and neck blast under field conditions on cv. Himalaya 2216*

Biopesticides	Leaf blast severity (%)	% disease control	Neck blast severity (%)	% disease control
Achook	13.43 (3.79)	50.79	5.00 (2.44)	74.58
Neem Azal	11.17 (3.48)	59.09	6.33 (2.71)	67.81
Spictaf	10.67 (3.41)	60.92	5.33 (2.52)	72.90
Tricure	16.03 (4.12)	41.26	5.66 (2.58)	71.22
Wanis	8.97 (3.15)	67.15	1.99 (1.73)	89.88
Bavistin(@ 0.1%)	5.77 (2.59)	78.87	1.33 (1.24)	93.23
Control	27.30 (5.32)	-	19.67 (5.06)	-
CD (P= 0.05)	0.34		0.23	

Figures in parentheses are square root transformed values *Average of three replications

Similarly all the biopesticides were also found significantly effective against neck blast disease however; Wanis gave 89.89 per cent disease control followed by Achook (74.58%), Spictaf (72.22%), and Tricure (71.22 %). Jayarajan (1997) highlighted the importance of neem based biopesticides as important component of integrated disease management, as these are less phytotoxic and there is no risk of the pathogen developing resistance. Thangavelu *et al.* (1995) controlled rice blast disease by using neem leaf extract. Similarly, Hooda and Srivastava (1988) managed rice blast by using oil extracts of seeds of neem plant (*Azadirachta indica*). Hossain and Kulkarni (2001) found Neem based formulations Nimbicidine and Neemgold as most effective in reducing the rice blast incidence.

Evaluation of five aqueous plant extracts revealed that all the treatments were found significantly superior in reducing leaf blast severity over untreated control. *S. lappa* controlled the disease by 53.84 per cent (Table 4), followed by *E. adenophorum* (51.23 %), *C. esculenta* (44.71%) and *R. muricatus* (43.28%).

Table 4. Evaluation of botanicals against leaf and neck blast under field conditions on cv. Himalaya 2216*

Treatments^	Leaf blast severity (%)	% inhibition	Neck blast severity (%)	% inhibition
Colocasia esculenta	14.13 (3.89)	44.71	7.00 (2.82)	68.80
Eupatorium adenophorum	12.47 (3.67)	51.23	6.33 (2.71)	71.79
Lantana camara	17.50 (4.29)	31.55	7.66 (2.94)	65.86
Ranunculus muricatus	14.50 (3.94)	43.28.	6.33 (2.71)	71.79
Saussurea lappa	11.80 (3.57)	53.84	6.00 (2.65)	73.26
Bavistin	4.33 (2.29)	83.05	2.10 (1.02)	90.64
Control	25.57 (5.15)	-	22.44 (4.84)	-
CD (P =0.05)	0.35	-	0.27	-

Figures in parentheses are square root transformed values; *Average of three replications ^Aqueous plant extracts (1: 1, w/v)

However, *L. camara* was least effective. None of the aqueous plant extracts were found superior to Bavistin (0.1%). Similarly *S. lappa* gave maximum neck blast control (73.26%) over untreated check followed by *E. adenophorum* (71.79%) and *R. muricatus* (79.26%).

Homma *et al.* (1980) found anti fungal properties in lecithin content of soybean seeds which effectively controlled blast fungus.

References

Anonymous 2000. Fourteenth Annual Progress Report. Department of Plant Pathology, CSK HPKV, Palampur.

Clung HS 1988. Recent advances of study on rice fungal diseases. World situation of rice diseases and recent advances in their studies. *5th International Phytopathological Congress,* Kyoto, pp. 6.

Dang H, Po Wei L, Xian Juan C and Peng HX 2002. Biological control of rice blast with *Bacillus* species. Chinese J. Biol. Control **18**: 25-27.

Dhingra OD and Sinclair JD 1985. *Basic Plant Pathology Methods.* CRC Press.

Gnanamanickam SS, Reys SS and Mew TW 1989. Biological control of rice blast with antagonist bacteria. International Rice Research Newsletter **14**: 34-35.

Gnanamanickam SS, Valasubramanian R, Chaterjee A, Chaterjee AK and Mew TW 1994. Antibiotic production mediates the biological control of rice blast by *Pseudomonas fluorescens. Rice blast disease.* Los Banos, Laguna IRRI, pp 601-02.

Homma Y, Arimoto Y and Mijato HT 1980. Effect of soybean lecithin on each growth state of rice blast fungus. *Pyricularia oryzae* Cav. in its life cycle. J. Pesticide Sci. **5**: 611-13.

Hooda KS and Srivastava MP 1988. Impact of neem coated urea and potash on the incidence of rice blast. Plant Dis. Res. **13**: 28-34.

Hossain MM, Kulkarni S and Kulkarni S 2002. *In vitro*: evaluation of biological agents against blast disease of rice. Plant Pathology Newsletter **20**: 7-8.

Hossain MM and Kulkarni S 2001. Field evaluation of fungicides, neem based formulations and biological agents against blast of rice. J. Mah. Agric. Univ. **26**: 148-50.

Huang HC and Hoes JA 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans.* Canadian J. Bot. **54**: 406-10.

IRRI 1996. *Standard Evaluation System for Rice.* International Rice Research Institute Manila, Philippines.

Jayarajan R 1997. Low cost components of IPM. The Hindu Survey of Indian Agri. pp 165-67.

Krishnamurthy K and Gnanamanickam SS 1998. Biological control of rice blast by *Pseudomonas fluorescens* strain Pf 7-14: evaluation of a marker gene and formulations. Biol. Control: Theory and Application Pest Management **13**: 158-65.

Ngueko RB, ShenY and Xu T 2002. Induced resistance in rice plants by *Trichoderma harzianum* P1 and its efficiency to *Magnaporthe grisea.* Chinese Rice Res. Newsletter **10**: 16-18.

Padmanabhan SY1959.The present position and control of rice diseases in India. Proc. Indian Acad. Sci. **49**: 349-62.

Robert WH 1991. Research priorities for Rice Biotechnology. In: *Rice Biotechnology,* G.S. Khush and G.H. Toenniessen (eds.), Alden Press, Ltd., London, 35-37 pp.

Sharma CL and Kapoor AS 1999. *In vitro* evaluation of herbicide and plant extracts against *Sclerotinia sclerotiorum* causing white rot in pea. Pl. Dis. Res. **14**: 79-81.

Soytong K 1990. Biological control of rice blast pathogen by coating seeds with *Chaetomium cochlioides* and *C. cuniculorum.* Khon Kaen Agri. J. **18**: 89-96.

Thangavalu R, Padma N, Satyanaryana K and Reddy APK 1995. Effect of different neem based formulations on rice blast disease. Indian J. Pl. Prot. **23**: 93-94.

Tewari SN and Nayak M 1991. Activity of four plant leaf extracts against three fungal pathogens of rice. Tropical Agri. (Trinidad) **68**: 373-75.

Tiwari SN 1995. *Ocimum sanctum* L., a botanical fungicide for rice blast control. Tropical Sci. **35**: 263-73.

Vincent JH 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature **15**: 850.

Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K and Rajappan K1997. Development of a powder formulation of *Pseudomonas fluorescens* for control of rice blast. Pl. Pathol. **46**: 291-97.