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Research Article

Biocontrol potential of three antagonists against downy mildew of pearl millet caused by *Sclerospora graminicola* (Sacc.) Schoret

K. K. SAINI[1*], A. C. MATHUR[1], R. S. SHARMA[1], VIRENDRA KUMAR[1], R. K. BAGRI[1] and VISHNU GAUTUM[2]

[1]*Division of Plant Pathology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur – 302018, Rajasthan, India*

[2]*Residue Analysis Laboratory, Division of Entomology, Durgapura, SKN Agriculture University, Jobner, Jaipur – 303329, Rajasthan, India*

**Corresponding author E-mail: kamalsainipathology@gmail.com*

ABSTRACT: The efficacy of three bio-control agents *viz*., *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated against downy mildew of pearl millet in glasshouse as well as in field conditions at research farm, Rajasthan Agricultural Research Institute, Durgapura (Jaipur) during cropping seasons of *kharif* 2016 and 2017. The seed treatment with *Trichoderma viride* (@ 6 g/kg seed) followed by one spray of *Pseudomonas fluorescens* (@ 1 x 10^8 cfu/ml) 21 days after sowing significantly reduced the downy mildew incidence and increased the grain and fodder yield. Seed treatment with *Trichoderma harzianum* followed by one spray of *Pseudomonas fluorescens* (@ 1 x 10^8 cfu/ml) 21 days after sowing were the next in reducing the downy mildew incidence at significant level.

KEY WORDS: Biocontrol agents, downy mildew, pearl millet, *Pseudomonas, Trichoderma*

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INTRODUCTION

Pearl millet is a staple food for millions of poor people living in the semi-arid tropical regions of Africa and Asia (Rachie and Mujumdar, 1980). Pearl millet is grown for both fodder and grain in Asian and African countries, whereas it is solely cultivated as a forage crop in South America, U.S.A., Canada, South Africa, Italy, Japan, Australia, etc. (Johnson *et al.,* 1976). Besides food for human consumption, the grain is also used for poultry feed and some industrial uses. A large number of diseases *viz.,* downy mildew, ergot, smut, blast and rust (Raj *et al.,* 2014) are reported to attack pearl millet crop. Among these, downy mildew is one of the most important diseases of pearl millet crop and cause considerable economic yield losses. It is the most widespread and destructive disease of pearl millet in India and western Africa. The disease, first reported in India (Butler, 1907), is present in more than 20 countries (Safeeulla, 1976), and is a major factor limiting the full exploitation of the high yielding hybrids in India. The disease normally appears in the form of chlorosis at the base of infected leaf followed by production of sporulation on the lower side of leaves known as the "half-leaf" symptom. Abundant white asexual sporulation on the lower leaf surface of infected chlorotic leaf produce white downy growth under high relative humidity (>95%) and moderate temperature (20-22^{0}C). Subsequently the leaves turn reddish brown due to oospore production and dry ultimately. Downy mildew causes 57% reduction in the yield besides heavy losses in the fodder yield (Gupta and Singh, 1996). Subsequent to this epidemic, grain yield losses continue to occur quite frequently due to downy mildew epidemics in India (Singh *et al.,* 1987). It has been demonstrated that the losses in yield can be directly proportional to disease severity (Williams and Singh, 1981). Although, chemical fungicidal interventions are reported effective against this disease, growing concern for environmental pollution and residue problems in the pearl millet necessitate the search for alternative means of control. In this direction, the present investigation was carried out to study the efficacy of antagonistic organisms *viz*., *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* against *Sclerospora graminicola* caused downy mildew of pearl millet.

MATERIALS AND METHODS

Biocontrol potential of antagonists on downy mildew of pearl millet in glasshouse

Three known antagonists' *viz*., *Trichoderma viride, Trichoderma harzianum and Pseudomonas fluorescens* procured from Biocontrol laboratory, RARI, Durgapura, Jaipur

and tested as seed dresser as well as spray of *Pseudomonas fluorescens* in glass house for their antagonistic effect against *Sclerospora graminicola*. These fungal bio-control agents were cultured for seven days on Potato Dextrose Agar (PDA) in order to get fresh and active growth of each fungus. In case of bacterial fresh and active growth King`s B medium was used. For this purpose, soil + compost (2:1) was sterilized in autoclave at 30 psi for 30 minutes and filled in earthen pots of 30 cm diameter. Pearl millet cultivar (7042 S) seeds were sterilized with bleaching agent (NaOCl, 0.5% w/v) for 30 minutes and rinsed thoroughly with sterilized water. To prepare seed treatment inoculants, a suspension of conidia in sterile water was prepared for seed treatment and sterilized seeds of highly susceptible cultivar 7042 S were treated separately with the conidial suspension at the rate of 20 µl g^{-1} to deposit 2 × 10^7 CFU g^{-1} of seed. Control seed were treated with an equal amount of sterilized water. Treated seed were allowed drying them for 30 minutes before sowing in pots. Fifty seeds were sown in each pot at a depth of 0.5 cm from soil surface. The pots were watered regularly as and when needed and 2 sprays of *Pseudomonas fluorescens* (@ 1 x 10^8 cfu/ml) were done at 7 and 14 days after sowing. The experiment was conducted in Completely Randomized Design (CRD) with three replications for each treatment. Downy mildew incidence was recorded 14 days post sowing and the Per cent disease incidence was calculated using the below given formula.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Evaluation of biocontrol agents against downy mildew of pearl millet under field conditions

The effective biocontrol agents were assessed as seed treatment for management of pearl millet downy mildew. The experiment was conducted for two seasons during *kharif* 2016 and kharif 2017 at Research Farm, Rajasthan Agricultural Research Institute, Durgapura (Jaipur). The moderately resistant cultivar (Nokha Local) seeds were treated with each biocontrol agent separately. The treatment without dressing with biocontrol agent served as check and seeds were sown in row-to-row 50 cm and plant to plant 15 cm apart in plot size of 3 × 4 m. The treatments were replicated in a Randomized Block Design (RBD) and FYM (Farmyard Manure) was applied @ 10 to 12 tones/ha at two to three week before sowing. The fertilizers, 90:30 (N:P_2O_5) kg/ha were applied, in which nitrogen was applied in two equal doses as basal and top dressing at 30 days after sowing. Experiment was kept free from weeds and insect pests. Irrigation was applied as and when necessary. The other details of the experiment are as follows:

- Replications - 4
- Treatments - 6

T_1: Seed treatment with *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) on 21 days old plants

T_2: Seed treatment with *T. harzianum* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) on 21 days old plants

T_3: Seed treatment with *T. viride* (@ 6 g/kg seed)

T_4: Seed treatment with *T. harzianum* (@ 6 g/kg seed)

T_5: Seed treatment with *P. fluorescens* (@ 8 g/kg seed)

T_6: Control

Observations recorded

i. Downy mildew incidence (%)

The total number of plants was recorded at the time of thinning i.e. fifteen days after sowing, while the number of downy mildew infected plants was recorded at 30 and 60 days after sowing. Percent incidence of downy mildew was calculated with the help of the following formula:

$$\text{Downy mildew incidence (\%)} = \frac{\text{Downy mildew infected plants}}{\text{Total number of plants}} \times 100$$

ii. Seedling emergence

$$\text{Percent seedling emergence} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

iii. Grain Yield q/ha

iv. Fodder Yield q/ha

RESULTS AND DISCUSSION

Biocontrol potential of antagonists on downy mildew of pearl millet in glasshouse

The data on seedling emergence (%) and downy mildew incidence were recorded (Table 1). Maximum seedling emergence (83.50%) was observed in seed treatment with *T. viride* (@ 6g/kg seed) + 2 sprays of *P. fluorescence* (@ 1 x 10^8 cfu/ml) at 7 days old plant and 14 days old plant. Other treatments showed seedling emergence in the range of 71.5-79.5%, while untreated control showed the least seedling emergence (40.50%). All treatments were reduced the downy mildew incidence significantly over control but recorded minimum (11.23%) DM incidence in seed treatment with *T. viride* (@ 6 g/kg seed) + 2 sprays of *P. fluorescence* (@ 1 x 10^8 cfu/ml) and the untreated control showed maximum of 40.43% downy mildew incidence. Other

treatments showed downy mildew incidence in the range of 13.56-19.45%.

Evaluation of biocontrol agents against downy mildew of pearl millet under field conditions

The pooled data on seedling emergence (%), downy mildew incidence (%), grain yield (q/ha) and fodder yield (q/ha) recorded during *kharif* seasons of 2016 and 2017 are presented in Table 2.

The perusal of the data indicated that all the bio-agent treatments were significantly superior to untreated control in reducing the downy mildew incidence and increasing the yield of grains and fodder during *kharif* 2016 and *kharif* 2017 crop seasons.

Seed treatment with *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) recorded the maximum seedling emergence of 52.1% followed by seed treatment with *T. harzianum* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) (50.69%), seed treatment with *T. viride* (@ 6 g/kg seed) (49.68%), seed treatment with *T. harzianum* (@ 6 g/kg seed) (48.73%) and *P. fluorescens* (@ 8 g/kg seed) (47.66%) in comparison to the seedling emergence in untreated control (40.63%).

Amongst different treatments, seed treatment with *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) was significantly effective in reducing the incidence of pearl millet downy mildew to the minimum of (6.0%) in comparison to 31.15% in control at 30 days after sowing. Seed treatment with *T. harzianum* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) ranked next (7.6%) followed by *T. viride* (@ 6 g/kg seed) (9.15%), *T. harzianum* (@ 6 g/kg seed) (14.10%) and *P. fluorescens* (@ 8 g/kg seed) (11.95%).

At 60 days after sowing, seed treatment with *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) was significantly superior in reducing the pearl millet downy mildew incidence to the minimum of 13.71 in comparison to 39.35% in the control. Seed treatment with *T. harzianum* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) and seed treatment with *T. viride* (@ 6 g/kg seed) ranked next (14.71% and 16.42% respectively) in managing pearl millet downy mildew and treatment T_1-seed treat with *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) was at par with treatment T_2- Seed treatment with *T. harzianum* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml).

The results revealed that grain yield in all the treatments of biocontrol agents was significantly superior in comparison to control of 4.44 q/ha. The maximum grain yield of 8.94 q/ha was recorded with seed treatment of *T. viride* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) was at par with seed treatment of *T. harzianum* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) (8.81 q/ha).

The fodder yield also in all treatments of biocontrol agents was significantly superior in comparison to control (37.75 q/ha). The maximum fodder yield of 51.80 q/ha was recorded on seed treatment with *T. viride* (@ 6 g/kg seed)

Table 1. Antagonistic effect of various biocontrol agents against *S. graminicola* in glasshouse

Tr. No.	Microorganism	Seedling emergence (%)	Downy mildew Incidence (%)
T_1	**Seed treatment with *T. viride* (@ 6 g/kg seed) + spray of *P. fluorescence* (@ 1 x 10^8 cfu/ml)**	83.50^a (66.01)*	11.23^a (19.56)
T_2	**Seed treatment with *T. harzianum* (@ 6 g/kg seed) + spray of *P. fluorescence* (@ 1 x 10^8 cfu/ml)**	79.5^b (63.07)	13.56^b (21.59)
T_3	**Seed treatment with *T. viride* (@ 6 g/kg seed)**	76.50^c (60.99)	15.89^c (23.47)
T_4	**Seed treatment with *T. harzianum* (@ 6 g/kg seed)**	71.50^d (57.72)	17.34^d (24.59)
T_5	**Seed treatment with *P. fluorescens*(@ 6 g/kg seed)**	73.50 (59.00)	19.45 (26.15)
T_6	**Control**	40.50 (39.50)	40.43 (39.46)
	SEm±	(0.59)	(0.27)
	CD at 5%	(1.77)	(0.81)
	CV%	(2.05)	(2.11)

Average of four replications
*Figures in parentheses are angular transformed value

Table 2. Evaluation of various biocontrol agents against downy mildew of pearl millet during kharif 2016 and 2017 under field conditions

Treatments	Seedling emergence (%)	Downy mildew incidence (%) at 30 DAS (Pooled)	Downy mildew incidence (%) at 60 DAS (Pooled)	Pooled grain yield (q/ha)	Pooled fodder yield (q/ha)
T_1- Seed treatment with *T. viride* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml)	52.10 (46.69)*	6.0a (14.14)	13.71a (21.71)	8.94	51.80
T_2- Seed treatment with *T. harzianum* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml)	50.69 (45.38)	7.6b (15.98)	14.71b (22.53)	8.81	49.95
T_3- Seed treatment with *T. viride* (@ 6 g/kg seed)	49.68 (44.49)	9.15c (17.57)	16.42c (23.88)	7.85	47.25
T_4- Seed treatment with *T. harzianum* (@ 6 g/kg seed)	48.73 (44.25)	11.95d (20.20)	16.45d (24.29)	6.72	43.42
T_5- Seed treatment with *P. fluorescens* (@ 8 g/kg seed)	47.66 (43.64)	14.1 (22.04)	19.43 (26.14)	6.48	42.12
T_6- Control	40.63 (39.58)	31.15 (33.91)	39.35 (38.83)	4.44	37.75
SEm±	0.65	0.44	0.47	0.21	0.66
CD @ 5%	1.99	1.36	1.44	0.65	2.02
CV%	2.96	4.34	3.61	5.93	2.93

All data are means of 4 replications
*Figures in parentheses are angular transformed value

+ spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) which was at par with seed treatment of *T. harzianum* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) (49.95 q/ha). These findings are closely agreement with the findings of other scientists viz., Umsesha *et al.* (1998), Nandakumar *et al*. (2001), Niranjan *et al*. (2004), Raj *et al*. (2005), Latake and Kolase (2007), Mane *et al*. (2007), Mani and Hepziba (2009), Mani *et al.* (2009), Raj *et al.* (2011), Nandini *et al*. (2013), Sangwan and Kushal Raj (2016) and Sasode *et al.* (2018) who evaluated bioagents viz., *P. fluorescence, T. viride* and *T. harzianum* as seed dresser as well as spray for the control of downy mildew incidence and recorded *T. viride* and *P. fluorescence* as best in reducing the downy mildew incidence and increased the grain and fodder yield.

CONCLUSION

The present studies indicated that the treatments *T. viride* (@ 6 g/kg seed) + 1 spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) at 21 days after sowing/ *T. harzianum* (@ 6 g/kg seed) + spray of *P. fluorescens* (@ 1 x 10^8 cfu/ml) at 21 days after sowing are the most effective treatments in reducing the pearl millet downy mildew incidence and increasing the grain and fodder yield.

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