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Original Research Article

Biological Approach in Management of Grape Powdery Mildew Caused by *Erysiphe necator*

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A B S T R A C T

Grapevine powdery mildew is caused by the fungus *Erysiphe necator* (formerly *Uncinula necator*) which is an obligate parasite and is one of the most important diseases causing considerable losses in Grape. In present study, five bio-agents were evaluated under natural inoculum conditions, at recommended concentrations for their efficacy against *E. necator* during 2016-17 and 2017-18. The results revealed that all the biocontrol agents tested were found effective and significantly reduced the disease severity with maximum disease control in all the two season. Among all the bio control agents *Trichoderma harzianum* recorded the least 23.78 and 24.27 per cent disease severity on leaves and bunches respectively and recorded maximum per cent disease control 62.14 and 55.83 per cent on leaves and bunches respectively followed by plants treated by *Ampelomyces quisqualis* with 52.67 percent disease control. The maximum disease severity was recorded on plants sprayed with *Pseudomonas fluorescens* 36.64 and 31.59 per cent disease severity on leaves and bunches respectively and recorded least per cent disease control in all two seasons.

Keywords

Powdery mildew, *Erysiphe necator*, Bio-agents, *Trichoderma harzianum*, *Pseudomonas fluorescens*

Introduction

Grape (*Vitis vinifera* L.) a refreshing fruit, is rich in sugars and vitamins, especially vitamin C. It contains high level of glucose, proteins, vitamins, amino acids, lecithin and minerals as well as flavonoids which are good antioxidants, eliminate free radicals, and prevent aging (Choudhary *et al.*, 2014). Grape is grown in almost all parts of the country and excels other fruit crops in productivity, hardiness and adaptability. Grape is known for its cultural dualism between subsistence-oriented growers and export oriented large corporate growers in India. It has become the most remunerative commercial farming enterprise and as such, India exports a large quantity of fresh grapes.

Grape is prone to a number of fungal, bacterial and viral diseases which significantly affect its quality and production (Nimbalkar *et al.*, 2005). However, fungal diseases inflict huge losses to the crop. Among these, the powdery mildew is second important fungal disease after downy mildew. It is caused by the obligate fungus *Erysiphe necator* Schw (formerly *Uncinula necator* (Schw.) Burr.) and is favoured by warm, humid and cloudy weather (Pearson and Goheen, 1988). The disease is quite severe in India. Though reported from most parts of

India, the disease is more serious in Maharashtra. Foliar infections reduce the photosynthetic activity of grapevines (Chadha and Shikhamany, 1999), while fruit infections impair wine characteristics or reduce the yield and market value of table grapes (Sawant and Sawant, 2008). Additionally, *E. necator* causes direct effects on yield if the infection occurs prior to fruit maturation; the skin of the berries can become necrotic, preventing the epidermis from expanding, and the actively growing flesh causes the fruit to crack (Kassemeyer and Berkelmann, 2009) Hence, sustainable and effective management options for powdery mildew are essentially required for cultivars of both table and wine grapes.

The currently available control methods of powdery mildew include the use of chemical fungicides, but the repeated and heavy dose application of chemical fungicides results in development of pathogen resistance, environmental pollution and also have adverse effect on beneficial organism. Powdery mildew management in India is largely dependent on sulphur, triazole and strobilurin fungicides (Sawant *et al.,* 2017). The best alternative strategy for chemical control includes the use of biocontrol agents to reduce the disease severity caused by powdery mildew. The present study was planned to test the potential of five bio-agents namely, *Trichoderma viride*, *T. harzianum*, *Ampelomyces quisqualis*, *Bacillus subtilis* and *Pseudomonas fluorescens* to control or cure powdery mildew disease of grape plants in an open field.

Materials and Methods

Experiments were conducted at grape garden of AICRP on Fruits, MPKV Rahuri in two different seasons to study the bio efficacy of bio-agents, plant extracts and fungicides against grapevine powdery mildew. The evaluation was carried out on susceptible grape cultivar "Thompson Seedless". Vines of uniform size and age were selected for this purpose. A single vine represented one replication and the neighbouring vines were considered as separating one treatment from the other. A knapsack sprayer was used for application of bio-agents, plant extracts and fungicides in the vineyard ensuring complete coverage of the foliage. The applications were started when the first signs of natural infection by powdery mildew were visible on the leaves and were repeated at 10 day intervals. Details of the experiment are given below

A field experiment for testing the efficacy of Bio agents against *E. necator* was conducted during 2016-17 and 2017-18 at grape garden of AICRP on Fruits, MPKV, Rahuri. The experiment was laid out in a Randomized Block Design with six treatments and four replications.

All the cultures of given bio agents were obtained from the culture collection bank of Department of Plant Pathology and Agricultural Microbiology, PGI, M.P.K.V. Rahuri. The fungal cultures *Trichoderma viride*, *T. harzianum* and *Ampelomyces quisqualis* were grown on potato dextrose broth in 200 ml conical flask for for 10 days under natural daylight conditions at 28 ± 4 °C. The fungal growth was blended, filtered through single layer of muslin cloth and the suspension was appropriately diluted by adding required quantity of sterile distilled water (SDW), to provide a spore count of 2 $\times 10^8$ spore per ml as counted on a haemocytometer.

The bacterial cultures *Bacillus subtilis* and *Pseudomonas fluorescens* were grown on nutrient broth medium and incubated at 28 ^{0}C for 24 h in an orbital shaker. Bacterial cultures were centrifuged at 5000 rpm for 10

min and the pellet was re-suspended in sterile distilled water to reach approximately 10^6 CFU/ml for further treatment.

The disease severity was recorded by visual observations using 0-4 scale (Horsfall and Heuberger, 1942). As detailed below.

Further these scales were converted to per cent disease intensity using formula given by Wheeler (1969):

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves examined} \times \text{Maximum grade}} \times 100$$

Methods of recording observation

Forty leaves (four cane) and ten bunches per vine were selected randomly for recording disease severity of powdery mildew.

The disease severity was recorded immediately before each spray application and seven days after the last spray by applying 0–4 rating scale, (Horsfall and Heuberger, 1942). The ratings were converted to per cent disease intensity (PDI) using the formula given by Wheeler (1969)

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves/bunches observed} \times \text{Maximum grade}} \times 100$$

The per cent disease control over control was calculated by using following formula.

$$\text{PDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Results and Discussion

Bio-efficacy of bio-agents against grape powdery mildew, during 2016-17

It is evident from the data (Table 1) that during the year 2016-17, all of the test bio-agents significantly reduced the disease severity on both leaves and bunches, as compared to control. However, the magnitude of reduction varied from bio-agents to bio-agent.

After third spray, the range of disease severity on the leaves in different bio-agent treatments varied from 22.37 to 33.87 in comparison to 59.42 per cent recorded in control. Minimum disease severity of (22.37 %) was observed in plants sprayed with *Trichoderma harzianum* which was significantly at par with *Ampelomyces quisqualis* (23.20 %) and *Trichoderma viride* (24.82 %). The next best treatment was *Bacillus subtilis* exhibiting disease severity of 26.14 per cent. The maximum disease severity was recorded on plants sprayed with *Pseudomonas fluorescens* (33.87 %) which was significantly least effective in comparison to all the test bio-agents but was significantly superior to control (59.42 %). In case of per cent disease control, results revealed that (Table 1), all the bio-agents tested were found effective and significantly reduced the disease severity. The disease control in percentage ranged from 62.35 to 42.99 per cent. The maximum per cent disease control was found in *T. harzianum* which was 62.35 per cent followed by *A. quisqualis* having 60.95 per cent and *T. viride* having 58.22 per cent disease control over unsprayed control treatment. The minimum per cent disease control was recorded in the treatment of *P. fluorescens* over unsprayed control treatment.

After third spray, the range of disease severity on bunches in different bio-agent treatments varied from 23.83 to 31.17 in comparison to 52.70 per cent recorded in control. Minimum disease severity of (23.83 %) was observed in plants sprayed with *T. harzianum* which was significantly at par with *A. quisqualis* (24.94 %) and *T. viride* (26.03 %). The maximum disease severity was recorded on plants sprayed with *P. fluorescens* (31.17 %) which was significantly least effective in comparison to all the test bio-agents but was significantly superior to control (52.70 %). The disease control in percentage was ranged from 54.78 to 40.85 per cent. The maximum per cent disease control was to be found in *T. harzianum* which was 54.78 per cent followed by *A. quisqualis* having 52.67 per cent and *T. viride* having 50.60 per cent disease control over unsprayed control treatment. The minimum per cent disease control was recorded in the treatment of *P. fluorescens* over unsprayed control treatment.

Bio-efficacy of bio-agents against grape powdery mildew during 2017-18

During the year 2017-18 the experiment was repeated. The data on powdery mildew severity on grapes plants during 2017-18 (Table 2) followed the same pattern as recorded during previous crop season. All the test bio-agents significantly reduced the disease on both the leaves and bunches as compared to control.

After third spray, the range of disease severity on leaves in different bio-agent treatments varied from 25.19 to 39.40 in comparison to 66.23 per cent recorded in control. Least disease severity of (25.19 %) was observed in plants sprayed with *T. harzianum* which was at par with *A. quisqualis* (27.10 %) and *T. viride* (29.12 %). The next best treatment was *B.subtilis* exhibiting disease severity of 29.30 per cent. The maximum disease severity was recorded on plants sprayed with *P. fluorescens* (39.40 %) which was significantly least effective in comparison to all the test bio-agents but was significantly superior to control (66.23 %). In case of per cent disease control, results revealed that (Table 2), all the bio-agents tested were found effective and significantly reduce the disease severity.

The disease control in percentage was ranged from 61.96 to40.51 per cent. The maximum per cent disease control was to be found in *T. harzianum* which was 61.96 per cent followed by *A. quisqualis* having 59.08 per cent and *T. viride* having 56.03 per cent disease control over unsprayed control treatment. The minimum per cent disease control was recorded in the treatment of *P. fluorescens* over unsprayed control treatment.

After third spray, the range of disease severity on bunches in different bio-agent treatments varied from 24.72 to 32.01 in comparison to 57.21 per cent recorded in control. Minimum disease severity of (24.72 %) was observed in plants sprayed with *T. harzianum* which was significantly at par with *A. quisqualis* (26.16 %) and *T. viride* (27.16 %). The next best treatment was *B. subtilis* exhibiting disease severity of 28.88 per cent. The maximum disease severity was recorded on plants sprayed with *P. fluorescens* (32.01 %) which was significantly least effective in comparison to all the test bio-agents but was significantly superior to control (57.21 %). In case of per cent disease control, results revealed that (Table 2), all the bio-agents tested were found effective and significantly reduce the disease severity with maximum disease control. The disease control in percentage was ranged from 56.79 to 44.04 per cent.

Table.1 Bio-efficacy of bio-agents against grape powdery mildew on leaves and bunches during 2016-17

Tr. No.	Treatments	Conc. (%)	PDI on leaves*(%)				PDC (%)	PDI on Bunches*(%)				PDC (%)
			Before spray	After 1st spray	After 2nd spray	After 3rd spray		Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T_1	*Trichoderma viride*	0.5	6.25 (14.42)	13.81 (21.70)	19.40 (26.04)	24.82 (29.81)	58.22	3.35 (10.52)	12.26 (20.41)	19.22 (25.92)	26.03 (30.58)	50.60
T_2	*Trichoderma harzianum*	0.5	7.19 (15.43)	12.20 (20.40)	17.52 (24.69)	22.37 (28.13)	62.35	2.88 (9.76)	11.90 (20.10)	18.67 (25.45)	23.83 (29.10)	54.78
T_3	*Bacillus subtilis*	0.5	6.62 (14.81)	13.42 (21.38)	20.22 (26.60)	26.14 (30.67)	56.00	4.16 (11.74)	14.48 (22.33)	21.92 (27.82)	28.76 (32.30)	45.42
T_4	*Pseudomonas fluorescens*	0.5	6.09 (14.23)	16.22 (23.68)	26.10 (30.68)	33.87 (35.54)	42.99	3.70 (11.08)	14.96 (22.71)	23.17 (28.70)	31.17 (33.88)	40.85
T_5	*Ampelomyces quisqualis*	0.5	5.80 (13.90)	12.38 (20.52)	19.20 (25.94)	23.20 (28.73)	60.95	3.06 (10.07)	13.15 (21.21)	19.17 (25.89)	24.94 (29.84)	52.67
T_6	Control (untreated)	-	6.70 (14.96)	22.42 (28.21)	36.62 (37.20)	59.42 (50.47)	-	3.97 (11.39)	24.12 (29.35)	35.23 (36.36)	52.70 (46.56)	-
SE ±			**0.67**	**1.18**	**1.43**	**1.58**		**0.81**	**1.37**	**1.57**	**1.94**	
CD at 5 %			**NS**	**3.56**	**4.32**	**4.75**		**NS**	**4.12**	**4.73**	**5.85**	
CV (%)			**9.18**	**10.41**	**10.04**	**9.30**		**15.21**	**12.07**	**11.07**	**11.51**	

* Mean of four replication * Figures in parenthesis are arc-sin transformed value * PDC- Per cent disease control

Table.2 Bio-efficacy of bio-agents against grape powdery mildew on leaves and bunches during 2017-18

Tr. No.	Treatments	Conc. (%)	PDI on leaves*(%)				PDC (%)	PDI on Bunches*(%)				PDC (%)
			Before spray	After 1st spray	After 2nd spray	After 3rd spray		Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T_1	*Trichoderma viride*	0.5	9.47 (17.83)	17.20 (24.42)	23.30 (28.79)	29.12 (32.61)	56.03	3.17 (10.23)	13.49 (21.49)	21.20 (27.30)	27.16 (31.32)	52.52
T_2	*Trichoderma harzianum*	0.5	10.80 (19.17)	16.12 (23.64)	21.19 (27.37)	25.19 (30.08)	61.96	4.21 (11.75)	13.92 (21.82)	19.73 (26.31)	24.72 (29.76)	56.79
T_3	*Bacillus subtilis*	0.5	9.21 (17.59)	18.68 (25.55)	23.07 (28.62)	29.30 (32.69)	55.76	3.92 (11.40)	14.28 (22.08)	21.46 (27.50)	28.88 (32.43)	49.51
T_4	*Pseudomonas fluorescens*	0.5	9.77 (18.16)	21.25 (27.42)	29.22 (32.69)	39.40 (38.86)	40.51	3.64 (10.89)	13.61 (21.58)	25.16 (30.04)	32.01 (34.42)	44.04
T_5	*Ampelomyces quisqualis*	0.5	10.78 (19.15)	16.22 (23.73)	22.46 (28.25)	27.10 (31.32)	59.08	4.22 (11.78)	12.26 (20.42)	20.84 (27.11)	26.16 (30.72)	54.27
T_6	Control (untreated)	-	11.34 (19.58)	25.22 (30.09)	42.73 (40.80)	66.23 (54.60)	-	3.83 (11.19)	23.43 (28.93)	38.16 (38.12)	57.21 (49.18)	-
SE ±			**0.97**	**1.37**	**1.47**	**1.61**		**0.77**	**1.25**	**1.52**	**1.88**	
CD at 5 %			**NS**	**4.14**	**4.43**	**4.84**		**NS**	**3.77**	**4.59**	**5.68**	
CV (%)			**10.48**	**10.67**	**9.45**	**8.76**		**13.72**	**11.00**	**10.36**	**10.88**	

* Mean of four replication * Figures in parenthesis are arc-sin transformed value * PDC- Per cent disease control

Table.3 Bio-efficacy of bio-agents against grape powdery mildew pooled mean severity on leaves and bunches (2016-17 and 2017-18)

Tr. No.	Treatments	Conc. (%)	Mean PDI on leaves *(%)				PDC (%)	Mean PDI on Bunches *(%)				PDC (%)
			Before spray	After 1st spray	After 2nd spray	After 3rd spray		Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T_1	*Trichoderma viride*	0.5	7.86 (16.24)	15.50 (23.14)	21.35 (27.47)	26.97 (31.23)	57.06	3.26 (10.38)	12.87 (20.96)	20.21 (26.62)	26.60 (30.95)	51.59
T_2	*Trichoderma harzianum*	0.5	8.99 (17.41)	14.16 (22.09)	19.36 (26.08)	23.78 (29.16)	62.14	3.55 (10.81)	12.91 (20.98)	19.20 (25.89)	24.27 (29.44)	55.83
T_3	*Bacillus subtilis*	0.5	7.91 (16.29)	16.05 (23.58)	21.64 (27.68)	27.72 (31.73)	55.87	4.04 (11.57)	14.38 (22.21)	21.69 (27.66)	28.82 (32.37)	47.55
T_4	*Pseudomonas fluorescens*	0.5	7.93 (16.33)	18.74 (25.63)	27.66 (31.71)	36.64 (37.23)	41.67	3.67 (11.00)	14.28 (22.15)	24.16 (29.38)	31.59 (34.15)	42.51
T_5	*Ampelomyces quisqualis*	0.5	8.29 (16.72)	14.30 (22.21)	20.83 (27.14)	25.15 (30.09)	59.96	3.64 (10.96)	12.70 (20.83)	20.00 (26.51)	25.55 (30.29)	53.50
T_6	Control (untreated)	-	9.02 (17.46)	23.82 (29.19)	39.68 (39.03)	62.82 (52.48)	-	3.90 (11.29)	23.77 (29.14)	36.70 (37.24)	54.95 (47.87)	-
SE ±			**0.80**	**1.26**	**1.45**	**1.55**		**0.74**	**1.25**	**1.54**	**1.90**	
CD at 5 %			**NS**	**3.80**	**4.36**	**4.69**		**NS**	**3.78**	**4.63**	**5.71**	
CV (%)			**9.62**	**10.40**	**9.70**	**8.81**		**13.48**	**11.05**	**10.64**	**11.09**	

* Mean of four replication * Figures in parenthesis are arc-sin transformed value * PDC- Per cent disease control

The maximum per cent disease control was to be found in *T. harzianum* which was 56.79 per cent followed by *A. quisqualis* having 54.27 per cent and *T. viride* having 52.52 per cent disease control over unsprayed control treatment. The minimum per cent disease control was recorded in the treatment of *P. fluorescens* over unsprayed control treatment.

Bio-efficacy of bio-agents against grape powdery mildew pooled mean severity (2016-17 and 2017-18)

Two years pooled mean data on per cent disease severity (Table 3) revealed that all of the bio-agents were significantly effective in reducing powdery mildew severity on both the leaves and bunches over control. Before spraying it was observed that the disease severity ranged from 7.86 to 9.02 per cent which were non significant. After first spray the minimum severity was recorded in the plants sprayed with *T. harzianum* (14.16 %) and the maximum severity was observed in control. Similarly after the second spray also the least severity was recorded in *T. harzianum* with 19.36 per cent. After third spray, the average powdery mildew disease severity on the leaves in all the treatments was ranged from 23.78 to 62.82 per cent. Among all the test bio control agents *T. harzianum* recorded the least 23.78 disease severity. The second and third best biocontrol agents found were *A. quisqualis* and *T. viride* with 25.15 and 26.97 per cent disease severity respectively. Among the bio-agents maximum 36.64 per cent disease severity was recorded in the treatment of *P. fluorescens* (Table 3). In case of per cent disease control, results revealed that all the biocontrol agents tested were found effective and significantly reduce the disease severity with maximum disease control. The disease control in percentage was ranged from 62.14 to 41.67 per cent. The maximum per cent disease control was to be found in *T. harzianum* which was 62.14 per cent followed by *A. quisqualis* having 59.96 per cent and *T. viride* having 57.06 per cent disease control over unsprayed control treatment. The minimum per cent disease control was recorded in the treatment of *P. fluorescens* over unsprayed treatment.

On bunches before spraying, it was observed that the disease severity ranged from 3.26 to 4.04 per cent which were non significant. After first spray the minimum severity was recorded in the plants sprayed with *A. quisqulis* (12.70 %) and the maximum severity was observed in control (23.77 %). After the second spray the least severity was recorded in *T. harzianum* with 19.20 per cent.

After third spray, on bunches the average powdery mildew disease severity in all the treatments were ranged from 24.27 to 54.95 per cent. The disease severity before first spray was to be found non -significant. Among all the biocontrol agents *T. harzianum* recorded the least 24.27 per cent disease severity. The second and third best biocontrol agents found were *A. quisqualis* and *T. viride* with 25.55 and 26.60 per cent disease severity respectively. Among the bio-agents maximum 31.59 per cent disease severity was recorded in the treatment of *P. fluorescens* (Table 3). In case of per cent disease control, results revealed that all the biocontrol agents tested were found effective and significantly reduce the disease severity with maximum disease control. The disease control in percentage was ranged from 55.83 to 42.51 per cent. The maximum per cent disease control was to be found in *T. harzianum* which was 55.83 per cent followed by *A. quisqualis* having 53.50 per cent and *T. viride* having 51.59 per cent disease control over unsprayed control treatment. The minimum per cent disease

control was recorded in the treatment of *P. fluorescens* over unsprayed treatment.

However, the efficacy of bio-agents in controlling grape powdery mildew disease was reported earlier by several workers. In the present investigation *T. harzianum* and *A. quisqualis* were found best in reducing the disease. Puzanov (1988) reported the effectiveness of antibiotic prepared from *A. quisqualis* against *U. necator* was 70-80 per cent. Kikkort *et al.,* (2000) reported that chitinase from *T. harzianum* inhibit spore germination of *Uncinula necator* causing powdery mildew of grapes. Sudha and Lakshmanan (2007) observed that *Trichoderma harzianum* recorded maximum disease reduction of 66.00 per cent in chilli powdery mildew. Surwase *et al.,* (2009) found that *Trichoderma harzianum* was highly effective and economical against *Erysiphe polygoni,* causing pea powdery mildew.

Legler *et al.,* (2016) observed that strain of *Ampelomyces* spp., RS1-a and AQ10 significantly delayed and reduced early-season development of grapevine powdery mildew in the next year. Sawant *et al.,* (2017) showed that *T. afroharzianum* strains NAIMCC-F-01938 and NAIMCC-F-01965 were superior in reducing grapevine powdery mildew by 43.67–50.36 per cent and 35.71–53.40 per cent, respectively. Similar result also obtained by Singh *et al.,* (2017) reported that the cell free culture filtrate of *T. harzianum* gave higher effectiveness against the grapevine powdery mildew followed by *A. quisqualis.*

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