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Strategic Management of Powdery Mildew of Grapes Using Fungicides and Bio-Control Agents

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ABSTRACT

The study was carried out at ICAR-NRCG, Pune, Maharashtra, India during November–February of 2019–2020 and 2020–2021 to evaluate the bio-efficacy of fungicides and biocontrol agents and level of tolerance against powdery mildew of grapes. Total 18 treatments categorised into three groups were taken into account. Three groups viz. fungicides, biocontrol agents (*Bacillus licheniformis*, *Trichoderma afroharzianum*, *Ampelomyces quisqualis*) along with metrafenone and commercial formulations of biocontrol agents were analysed for disease control. A total of five applications of each treatment were made during the growing season. Results showed that, in case of Group I (registered fungicides), Hexaconazole 5 EC @ 1 ml l^{-1}/ Difenoconazole 25 EC @ 0.5 ml l^{-1}/Myclobutanil 10 WP @ 0.4 g l^{-1}/Two sprays of sulphur @ 2 g l^{-1} was most effective in controlling the disease. In case of pure culture, *Trichoderma afroharzanium* had proved to be most effective in the control of powdery mildew when sprayed in alteration with Metrafenone. In Group III, alternate applications of commercial formulations of *Bacillus subtilis* (2 ml l^{-1}), *Trichoderma viridae* (2 g l^{-1}), *Ampelomyces quisqualis* (5 ml l^{-1}) and sulphur (2 g l^{-1}) was most effective in controlling the disease. It was concluded that the application of bio-control agents in alternation with chemical fungicides controlled the disease effectively.

KEYWORDS: *Ampelomyces*, *Bacillus*, BCA's, fungicides, grapevine, powdery mildew, *Trichoderma*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Grape (*Vitis vinifera* L.) is an important commercial fruit crop of India and is grown in varied climatic conditions worldwide (Otto et al., 2022, Ponti et al., 2018). In India, it is mostly consumed as fresh fruit and only a limited quantity is utilized for the production of juice, wine, raisins etc. Grape covers an area of 155.3 t ha occupying 2.24% of the total area in 2020–21 (Anonymous, 2022). India is also a major exporter of fresh grapes to the world, exported 263,075.67 mt of grapes to the world worth of $305.66 million during the year 2021–22. (Anonymous, 2022). The fruit of the grape is one of the most palatable foods, having many established nutritional and medicinal attributes for consumers Grapes have anticarcinogenic, antidiabetic, neuroprotective and cardioprotective properties (Nassiri-Asl and Hosseinzadeh, 2016, Sabra et al., 2021).

Production of grapevines is threatened by both biotic and abiotic stresses (Heidari et al., 2021). Powdery mildew (c.o.-*Erysiphe necator*) a serious disease of grapevine, causes huge losses by deteriorating the yield and quality of bunches. Chemical control measures are effective and widely adopted management strategy (Saha et al., 2012) for controlling the disease but long term use of fungicides are detrimental to the environment and often leave harmful residues in the crop. Fungicide resistance further adds to the losses and to cope up with these problems, alternative options have been looked upon by researchers such as the use of biocontrol agents (BCA) for disease control either alone or in an integrated approach with other chemicals. Biological control is the eco-friendly management of diseases which is useful in almost all agro-ecological situations (Kamal et al., 2015, Sharma et al., 2017). It has received a considerable attention as an alternative strategy (Collinge et al., 2022) and sustainable approach. Several microbial biocontrol agents have been recognized like *Trichoderma, Ampelomyces, Pseudomonas, Bacillus* and *Agrobacterium* (Fira et al., 2018, Mukhopadhyay and Kumar, 2020, Panpatte et al., 2016, Saha et al., 2021). *Trichoderma* is reported to be effective against several fungal phytopathogens including grapes and induced systemic resistance in grapevines against powdery mildew disease (Ahmed, 2018, Sawant et al., 2017, Sawant et al., 2020). *Trichoderma afroharzianum* Rifai showed a higher tolerance to fungicides commonly used in powdery mildew management (Sawant et al., 2017). *Bacillus* species are ideal candidates as bio-control agents because they have different genetic characteristics (Fira et al., 2018, Andric et al., 2020). *Bacillus species* act as bacterial antagonists to pathogens due to their ability to reproduce actively and their resistance to unfavourable environmental conditions by forming endospores. *Bacillus* strains are also known for their use in pesticide residue biodegradation (Salunkhe et al., 2015). *Ampelomyces quisqualis* is a naturally-occurring biocontrol agent which protects crops from powdery mildew (Saha et al., 2021).

The duration of active disease control can be extended by using chemical and biological control agents together in integrated management system. Even reduced amounts of the fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist (Wedajo, 2015). Chemical protectants are effective under climatic conditions in which biological antagonists are less effective, while an active biological control agent can prophylactically colonize wounds or senescing plant tissue (Hjeljord and Tronsmo, 1998, Ajay et al., 2018). The various antagonistic organisms are combined along with synthetic chemicals to reduce the development of resistance in the pathogen and decrease the application of fungicide as well (Ons et al., 2020). Bio-control agents varies in their sensitivity to different fungicides and so, the compatibility of bio-control agents to fungicides needs confirmation before its use in integrated management system. Attempts were made in the present study to assess the comparative efficacy of chemical fungicides, bio-control agents along with registered fungicides and commercial formulations of biocontrol agents against powdery mildew of grapes for enhancing consistency and level of tolerance against powdery mildew of grapes.

2. MATERIALS AND METHODS

2.1. Experimental layout

A field experiment was carried out on Thompson Seedless variety in the season November–February of 2019–2020 and 2020–2021 at the vineyard of ICAR-National Research Centre for Grapes, Pune, Maharashtra, India (latitude 18.31° N, longitude 73.55° E, 559 m above MSL) in a randomized block design. A total of 18 treatments categorised into three groups were taken into account. Each treatment consisted of 12 vines. Fungicides were applied with knapsack sprayers. Water volume used for spray was calculated based on requirement of 1000 l ha^{-1} at full canopy. The first spray was given as soon as the disease appeared in field and the successive sprays were given at 5 days interval. The disease progress in the experimental plot was monitored periodically and the disease severity observations were recorded prior to each spray. Consecutively, a total of five applications of each treatment were made during the growing season. Disease intensity was recorded in the field by following the 0–5 disease rating scales, where 0=No disease incidence, 1=1–10% leaf area covered with powdery mildew growth, 2=11–25% leaf area covered with powdery mildew growth, 3=26–50% leaf area covered with powdery mildew growth, 4=51–75% leaf area covered with powdery mildew growth, 5=>75% leaf area covered with powdery mildew growth. Randomly 4 vines of each treatment were selected for recording the observations both on leaves and bunches. The percent disease index (PDI) was calculated by using the formula of McKinney (1923).

Percent disease index (PDI)=(Sum of numerical ratings/(Total no. of observations)×(100/Maximum rating) ….(1)

The percent disease control was calculated by using the following formula

Percent Disease control (PDC)=(C-T)/C)×100 ...(2)

Where,

C=PDI in untreated control plot

T=PDI in treated control plot

2.2. Treatments

The three groups viz. fungicides, biocontrol agents along with metrafenone and commercial formulations of biocontrol agents were analysed for disease control. Group I consisted of fungicide treatments viz. Sulphur @ 2 g l^{-1}, 2.5 g l^{-1} and 3.0 g l^{-1}, Hexaconazole 5 EC (1 ml l^{-1})/Difenoconazole 25 EC (0.5 ml l^{-1})/Myclobutanil 10 WP (0.4 g l^{-1})/Two sprays of Sulphur (2 g l^{-1}), Fluopyram 200+Tebuconazole 200 SC (0.563 ml l^{-1})/ Fluxapyroxad+Difenoconazole 50 g l^{-1} SC (0.8 ml l^{-1})/ Two sprays of Sulphur (2 g l^{-1}) and Metrafenone 50% SC (0.25 ml l^{-1}). Group II included solo and subsequent applications of pure culture of biocontrol agents and fungicide viz. *Bacillus licheniformis* (2 ml l^{-1}), *Trichoderma afroharzianum* (2 ml l^{-1}), *Ampelomyces quisqualis* (5 ml l^{-1}), *Bacillus licheniformis* (2 ml l^{-1})/Metrafenone, *Trichoderma afroharzianum* (2 ml l^{-1})/Metrafenone and *Ampelomyces quisqualis* (5 ml l^{-1})/Metrafenone 50% SC (0.25 ml l^{-1}). In group III, solo application of commercial bio-formulations of biocontrol agents viz. *Bacillus subtilis* (2 ml l^{-1}), *Trichoderma viridae* (2 g l^{-1}), *Ampelomyces quisqualis* (5 ml l^{-1}) and subsequent applications of *Bacillus subtilis* (2 ml l^{-1}) /*Trichoderma viridae* (2 g l^{-1}) /*Ampelomyces quisqualis* (5 ml l^{-1}) and application of *Bacillus subtilis* (2 ml l^{-1})/ *Trichoderma viridae* (2 g l^{-1})/*Ampelomyces quisqualis* (5 ml l^{-1})/Sulphur 80% WDG (2 g l^{-1}) were applied at weekly interval and evaluated against powdery mildew of grapes. All the treatments were compared with untreated control.

2.3. Statistical analysis

The experiments were laid out as completely randomized design and randomised block design. All data were subjected to ANOVA using SAS (ver. 9.3). The percentage data were arcsine transformed before analysis.

3. RESULTS AND DISCUSSION

3.1. Field efficacy of chemical fungicides against powdery mildew of grapes

The perusal of the data (Table 1) revealed that all the treatments were effective in controlling the disease as compared to untreated control.

The pooled data of both the years revealed that Hexaconazole 5 EC @ 1 ml l^{-1}/Difenoconazole 25 EC @ 0.5 ml l^{-1}/ Myclobutanil 10 WP @ 0.4 g l^{-1}/ Two sprays of Sulphur @ 2 g l^{-1} exhibited lowest PDI i.e. 9.93 which was at par with Fluopyram 200+Tebuconazole 200 SC @ 0.563 ml l^{-1}/ Fluxapyroxad+Difenoconazole 50 g l^{-1} SC @ 0.8 ml l^{-1}/ Two sprays of Sulphur @ 2 g l^{-1} and Metrafenone 50% SC @ 0.25 ml l^{-1} with a PDI 10.23 and 10.64 respectively. Untreated control exhibited maximum PDI i.e. 17.79. In case of bunches, Hexaconazole 5 EC (1 ml l^{-1}) / Difenoconazole 25 EC (0.5 ml l^{-1}) / Myclobutanil 10 WP (0.4 g l^{-1})/two sprays of Sulphur (2 g l^{-1}) was most effective in controlling the powdery mildew with a PDI of 12.25 which was at par with Fluopyram 200+Tebuconazole 200 SC (0.563 ml l^{-1})/ Fluxapyroxad+Difenoconazole 50 g l^{-1} SC (0.8 ml l^{-1})/ Two sprays of Sulphur (2 g l^{-1}) i.e. 12.38 and Metrafenone 50% SC (0.25 ml l^{-1}).

In case of registered chemical fungicides, the results obtained from the field trials showed that Hexaconazole 5 EC @ 1 ml l^{-1}/Difenoconazole 25 EC @ 0.5 ml l^{-1}/ Myclobutanil 10 WP @ 0.4 g l^{-1}/Two sprays of sulphur @ 2 g l^{-1} was most effective in control of the disease. This was followed by the treatment where Fluopyram 200+Tebuconazole 200 SC (0.563 ml l^{-1}), Fluxapyroxad+Difenoconazole 50 g l^{-1} SC (0.8 ml l^{-1}) and Sulphur (2 g l^{-1}) were used in alteration with one another. Sulphur in different concentrations and Metrafenone 50% SC (0.25 ml l^{-1}) were also potent enough to control the disease incidence but its activity was not as good as that of above treatments, where in the combinations of chemicals were adopted. The results obtained in the present investigations were in cognizance with previous findings which reported that the triazole fungicides like Hexaconazole, Difenoconazole and Myclobutanil were excellent fungicides in controlling powdery mildew of grapes. Findings of Lavezzaro and Morando (2014) highlighted the role of Fluopyram and Tebuconazole in regulating the disease severity. The role of Fluxapyroxad was also mentioned in the work of Valente et al. (2018). Metrafenone was reported to be effective in the control of powdery mildew by Scannavini et al. (2006) and Dietz and Winter (2019). Results of Sulphur were in cognizance with previous investigations reported by Bhardwaj (1997). The rotational application

Table 1: Field efficacy of chemical fungicides in control of powdery mildew on grape

Sl. No.	Treatment details	leaves		bunches	
		Pooled PDI	Pooled percent disease control over control	Pooled PDI	Pooled percent disease control over control
1.	Sulphur 80 WDG @ 2 g l^{-1}	13.88 (21.83)[c]	21.81 (27.31)[b]	15.88 (23.47)[d]	18.67 (25.27)[c]
2.	Sulphur 80 WDG @ 2.5 g l^{-1}	13.50 (21.48)[bc]	23.94 (28.52)[b]	14.13 (22.06)[cd]	27.58 (31.56)[b]
3.	Sulphur 80 WDG @ 3.0 g l^{-1}	11.50 (19.81)[ab]	35.26 (36.41)[a]	14.00 (21.97)[bc]	28.47 (32.13)[ab]
4.	Hexaconazole 5 EC (1 ml l^{-1}) / Difenoconazole 25 EC (0.5 ml l^{-1}) / Myclobutanil 10 WP (0.4 g l^{-1}) / Two sprays of Sulphur 80 WDG (2 g l^{-1})	9.93 (18.36)[a]	43.88 (41.46)[a]	12.25 (20.48)[a]	37.37 (37.68)[a]
5.	Fluopyram 200+Tebuconazole 200 SC (0.563 ml l^{-1}) / Fluxapyroxad+Difenoconazole 50 g l^{-1} SC (0.8 ml l^{-1})/Two sprays of Sulphur 80 WDG (2 g l^{-1})	10.23 (18.62)[a]	41.83 (40.14)[a]	12.38 (20.55)[ab]	36.89 (37.31)[ab]
6.	Metrafenone 50% SC (0.25 ml l^{-1})	10.64 (19.00)[a]	39.99 (39.17)[a]	12.88 (21.01)[abc]	34.22 (35.77)[ab]
7.	Untreated Control	17.79 (24.94)[d]	0.00 (0.00)[c]	19.58 (26.26)[e]	0.00 (0.00)[d]
	CD (p=0.05)	1.71	6.86	1.46	5.75

*figure in parenthesis are angular transformed values, Figure in a column indicated with same letter are not significantly different. '/' signifies 'followed by'.

of fungicides with different mode of action at a particular stage, depending on the number of days after pruning was found to be most effective in the control of the disease both in the leaves and the bunches.

3.2. Field efficacy of pure biocontrol agents along with fungicide in control of powdery mildew on grapes

Field efficacy of pure culture of biocontrol agents along with metrafenone was assessed. All the treatments were significantly effective in controlling powdery mildew of grapes as compared to untreated control (Table 2). Application of biocontrol agents in alternation with metrafenone 50% SC performed better than solo treatments of biocontrol agents.The pooled data showed that, *Trichoderma afroharzianum*+Metrafenone 50% SC (0.25 ml l^{-1}) recorded least PDI i.e. 8.75 followed by *Bacillus licheniformis*+Metrafenone 50% SC (0.25 ml l^{-1}) with PDI of 10.60. *Bacillus licheniformis*+Metrafenone 50% SC (0.25 ml l^{-1}) and *Ampelomyces quisqualis*+Metrafenone 50% SC (0.25 ml l^{-1}) were statistically at par with each other with PDI of 10.60 and 11.7 respectively. All the treatments significantly controlled the powdery mildew disease as compared to untreated control, which exhibited the highest PDI i.e. 18.44.

In case of bunches, pooled data showed that *Trichoderma afroharzianum*+Metrafenone 50% SC (0.25 ml l^{-1}) and *Bacillus licheniformis*+Metrafenone 50% SC (0.25 ml l^{-1}) were significantly superior over other treatments and at par with each other. *Trichoderma afroharzianum*+Metrafenone 50% SC (0.25 ml l^{-1}) exhibited lowest PDI of 12.63 which was at par with *Bacillus licheniformis*+Metrafenone 50% SC (0.25 ml l^{-1}) with a PDI of 13.50. Untreated control exhibited highest PDI i.e. 20.87.

In case of pure culture, *Trichoderma afroharzanium* had proved to be effective in the control of powdery mildew both when sprayed alone or in alteration with Metrafenone. However, when used with chemical Metrafenone, its activity gets amplified and the control became significantly more prominent. The next effective organism whose effect was noteworthy in the trials was *Bacillus licheniformis,* whose efficiency in the control got accentuated with the use of the chemical Metrafenone. The organism *Ampelomyces quisqualis* though having a pronounced effect on control of the disease performed less effectively which might be because of the sensitivity of the organism with the changing climatic conditions.

Results of the present findings correlated with the findings

Table 2: Field efficacy of biocontrol agents along with Metrafenone 50% SC (0.25 ml l^{-1}) in control of powdery mildew on grape

Sl. No.	Treatment details	Percentage Disease index			
		On leaves		On bunches	
		Pooled	Pooled PDC	Pooled	Pooled PDC
1.	*Bacillus licheniformis* @ (2 ml l^{-1})	15.25 (22.94)^{d}	17.47 (24.01)^{d}	17.08 (24.41)^{c}	18.04 (25.09)
2.	*Trichoderma afroharzianum* @ (2 ml l^{-1})	12.14 (20.38)^{bc}	34.07 (35.70)^{bc}	17.36 (24.61)^{c}	16.65 (23.63)
3.	*Ampelomyces quisqualis* @ (5 ml l^{-1})	13.70 (21.63)^{cd}	26.33 (30.34)^{cd}	17.65 (24.79)^{c}	15.60 (22.62)
4.	*Bacillus licheniformis* @ (2 ml l^{-1})/Metrafenone 50% SC (0.25 ml l^{-1})	10.60 (18.98)^{b}	42.43 (40.63)^{ab}	13.50 (21.55)^{a}	35.17 (36.36)
5.	*Trichoderma afroharzianum* @ (2 ml l^{-1})/Metrafenone 50% SC (0.25 ml l^{-1})	8.75 (17.19)^{a}	52.49 (46.43)^{a}	12.63 (20.79)^{a}	39.51 (38.91)
6.	*Ampelomyces quisqualis* @ (5 ml l^{-1})/Metrafenone 50% SC (0.25 ml l^{-1})	11.7 (19.96)^{b}	36.72 (37.25)^{bc}	15.20 (22.91)^{b}	27.06 (31.05)
7.	Untreated Control	18.44 (25.42)^{e}	0.00 (0.00)^{e}	20.87 (27.17)^{d}	0.00 (0.00)
	CD (p=0.05)	1.64	7.07	1.34	5.93

*figure in parenthesis are angular transformed values, Figure in a column indicated with same letter are not significantly different. '/' signifies 'followed by'.

of previous workers. Ons et al. (2020) stated that fungal antagonists improved disease control when combined with traditional fungicides against pre-harvest pathogens. Elad et al. (1998) reported that *Trichoderma harzianum* inhibited the growth of *Uncinula necator* by 97%. Similarly, Sawant et al. (2017) and Ahmed (2018) recorded the same nature of *Trichoderma harzianum* in the control of powdery mildew. Combination of *T. virens* and thiophanate-methyl was found to be compatible and more effective than either treatment alone against *Fusarium solani* and *Fusarium oxysporum* in field trials of dry bean production (Abd-El-Khair et al., 2019). The combined application of *Trichoderma* spp. with a low dose of fluazinam was found to be more effective to control avocado white rot (caused by *Rosellinia necatrix*) than either treatment alone (Ruano-Rosa et al., 2018). Combined application of *Trichoderma afrohurzianum* with Sulfur 80% WDG were more effective in controlling powdery mildew of grapes than either treatment alone (Sawant et al., 2017).

Bacillus subtilis was recorded to control powdery mildew in the grapevine (Sawant et al., 2011) which resembled the results of the present study. Similar findings were made by Kim et al. (2012) and Sihem et al. (2015). The incomplete disease control of *Bacillus megaterium* against *F. oxysporum* on tomato could be improved when combined with a low dose of the fungicide carbendazim in plant-packs (Omar et al., 2006). In greenhouse trials, the foliar application of *B. subtilis* with azoxystrobin provided the highest yield and the best disease control against powdery mildew (caused by *Podosphaera xanthii*) on zucchini, compared to both treatments alone (Galindo et al., 2013). *Ampelomyces quisqualis* is an effective biocontrol agents against powdery mildew fungi causing disease in different crops including grapes. Dik and Wubben (2002) observed that *A. quisqualis*, *T. harzianum* effectively control the powdery mildew of rose, cucumber, tomato, pepper, etc. in greenhouse when applied individually or in combination by one or more compounds. Similar results were also observed by Lee et al. (2004) and Saha et al. (2021). *Ampelomyces quisqualis* in combination with chemical fungicide ford the management of powdery mildew of grapes was investigated for the first time under Indian conditions.

3.3. Field efficacy of commercial formulations of biocontrol agents in control of powdery mildew on grapes

In case of commercial formulations, all the formulations were significantly superior in controlling the disease as compared to untreated control (Table 3). Alternate applications of three formulations at weekly interval was found most effective than repeated application of solo formulation. In case of leaves, pooled data showed that *Bacillus subtilis* (2 ml l^{-1})/*Trichoderma viridae* (2 g l^{-1}) / *Ampelomyces quisqualis* (5 ml l^{-1})/Sulphur (2 g l^{-1}) was most

Table 3: Field efficacy of commercial bio-formulations in control of powdery mildew on grapes

Sl. No.	Treatment details	Percentage disease index			
		On leaves		On bunches	
		Pooled PDI	Pooled PDC	Pooled PDI	Pooled PDC
1.	*Bacillus subtilis* (2 ml l-1)	15.38 (23.08)d	18.79 (25.64)d	22.38 (28.21)b	15.12 (22.49)bc
2.	*Trichoderma viridae* (2 g l-1)	13.63 (21.66)c	27.91 (31.84)c	21.63 (27.69)b	17.49 (23.90)bc
3.	*Ampelomyces quisqualis* (5 ml l-1)	14.06 (22.00)c	25.89 (30.48)c	22.63 (28.36)b	14.29 (21.14)c
4.	*Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1)/*Ampelomyces quisqualis* (5 ml l-1)	12.35 (20.56)b	34.88 (36.17)b	19.50 (26.18)ab	26.04 (30.60)ab
5.	*Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1)/*Ampelomyces quisqualis* (5 ml l-1)+Sulphur 80 WDG (2 g l-1)	10.08 (18.51)a	46.62 (43.05)a	17.72 (24.89)a	32.41 (34.60)a
6.	Untreated Control	18.98 (25.81)e	0.00 (0.00)e	26.34 (30.87)c	0.00 (0.00)d
	CD (p=0.05)				

*Figure in parenthesis are angular transformed values, Figure in a column indicated with the same letter are not significantly different

effective in controlling the disease with PDI of 10.08 followed by *Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1) / *Ampelomyces quisqualis* (5 ml l-1) with PDI of 12.35. Untreated control exhibited highest PDI i.e. 18.98.

In case of bunches, similar trend was observed. *Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1)/*Ampelomyces quisqualis* (5 ml l-1)/sulphur (2 g l-1) was found superior among all treatments (PDI 17.72) followed by *Bacillus subtilis* (2 ml l-1)+*Trichoderma viridae* (2 g l-1)+*Ampelomyces quisqualis* (5 ml l-1) with PDI of 19.50.

In case of the market samples, effective control by all the three biocontrol agents when used alone was prominent and congruent to each other. However, when the three biocontrol agents were used together in alteration, there was a prominent improvement in their activity which proved their effective synergistic nature. Their activity got amplified when they were combined with sulphur. These findings had resemblance with earlier investigations. Bio-efficacy of commercial formulations of *Ampelomyces quisqualis* and *Bacillus subtilis* in controlling the powdery mildew disease was recorded by Romero et al. (2007) and Sawant et al. (2011).

In all experiments, the highest PDC were obtained with the group of chemicals but among all the treatments, *Trichoderma afroharzianum* @ (2 ml l-1)/Metrafenone 50% SC (0.25 ml l-1) treatment showed the best disease control (Figure 1).

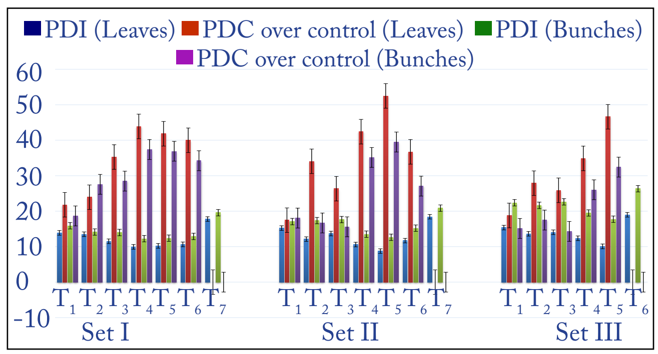

Figure 1: Comparison of the percent disease control chemical fungicides, pure culture of biocontrol agents with fungicide and commercial biocontrol agents; Set I- T_1: Sulphur 80 WDG @ 2 g l-1; T_2: Sulphur 80 WDG @ 2.5 g l-1, T_3: Sulphur 80 WDG @ 3 g l-1; T_4: Hexaconazole 5 EC (1 ml l-1)/Difenoconazole 25 EC (0.5 ml l-1)/ Myclobutanil 10 WP (0.4 g l-1)/Two sprays of Sulphur 80 WDG (2 g l-1); T_5: Fluopyram 200+Tebuconazole 200 SC (0.563 ml l-1)/Fluxapyroxad+Difenoconazole 50 g l-1 SC (0.8 ml l-1)/Two sprays of Sulphur 80 WDG (2 g l-1), T_6: Metrafenone 50% SC (0.25 ml l-1); T_7: Untreated control; Set II- T_1: *Bacillus licheniformis* @ (2 ml l-1); T_2: *Trichoderma afroharzianum* @ (2 ml l-1), T_3: *Ampelomyces quisqualis* @ (5 ml l-1); T_4: *Bacillus licheniformis* @ (2 ml l-1) / Metrafenone 50% SC (0.25 ml l-1); T_5: *Trichoderma afroharzianum* @ (2 ml l-1)/Metrafenone 50% SC (0.25 ml l-1); T_6: *Ampelomyces quisqualis* @ (5 ml l-1)/Metrafenone 50% SC (0.25 ml l-1); T_7: Untreated control, Set III- T_1: *Bacillus subtilis* (2 ml l-1); T_2: *Trichoderma viridae* (2 g l-1); T_3: *Ampelomyces quisqualis* (5 ml l-1); T_4: *Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1)/*Ampelomyces quisqualis* (5 ml l-1), T_5: *Bacillus subtilis* (2 ml l-1)/*Trichoderma viridae* (2 g l-1)/*Ampelomyces quisqualis* (5 ml l-1)+Sulphur 80WDG (2 g l-1); T_6: Untreated control

4. CONCLUSION

The group constituting of fungicides gave the best control of the disease. *Trichoderma afroharzianum*/ Metrafenone 50% SC (0.25 ml l^{-1}) showed maximum control of disease in all the tested groups. Alternation of commercial formulations with sulphur provided best disease control.

5. FURTHER RESEARCH

In light of the growing threat posed by resistant infections and the detrimental consequences of synthetic fungicide exploitation on the environment and health, alternatives must be investigated.Combining fungicides with resistance antagonists or inducers via tank-mixing or alternated applications is one of the most immediate method that farmers can implement on a commercial scale. This practice could improve disease control and reduce the risk of resistance development. Future research should focus on screening potential bio-control agent (BCA) strains, optimising BCAs and their timing of application to tolerate certain fungicides. In many cases, combining fungicides allows for lower fungicide rates and/ or applications to manage plant diseases in both sensitive and resistant populations. These advantages will directly reduce food losses and thus production costs, encouraging farmers to shift from fungicide-based agriculture to an integrated approach. Free advisory services educating farmers on the benefits of IDM methods are valuable in breaking traditional farmers' old habits, especially when uncertainty about the efficacy of alternatives to fungicides prevails.

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