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Assessment of Bio-Control Potential of *Ampelomyces quisqualis* against Powdery Mildew of Grapes

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ABSTRACT

Powdery mildew (*Erysiphe necator*) is one of the most important biotic constraints in grape (*Vitis-vinifera* L.) limiting the commercial production of the crop in most of grape growing regions in India. Disease management with fungicides is a common practice but fungicide resistance and environmental pollution issues have brought to the fore the extensive use of biocontrol agents. *Ampelomycesquisqualis* is a naturally occurring preventive biological fungicide that hyper-parasitizes powdery mildew pathogen. Evaluating the efficacy of *Ampelomycesquisqualis* 2% WP formulation against powdery mildew of Tas-A-Ganesh variety was done. Five sprays of *Ampelomycesquisqualis* 2.0% WP @ 6 g/L, 8 g/L and 10 g/L were given at 10 days interval for disease management. Among all treatments, foliar spray application of *Ampelomyces quisqualis* 2.0% WP @ 10 g/L manifested maximum efficacy in controlling the disease on leaves and bunches with mean PDC of 24.63 and 22.48 respectively. Untreated control manifested mean PDI of 40.20 and 39.78 on leaves and bunches. A significant increase of in marketable yield was also observed. The results of this study suggest that the *Ampelomyces quisqualis* 2.0% WP 10 g/L have potential to be used for the management of grape powdery mildew.

Keywords *Ampelomyces quisqualis*, Bio-fungicide, Powdery mildew, *Erysiphe necator,* Grapes.

INTRODUCTION

Grapevine (*Vitis vinifera)* is a globally important horticultural crop cultivated all over the world. It is third most widely cultivated fruit after citrus and banana in the world (Singh *et al.* 2017). The grape itself is used for a myriad of products, ranging from fresh fruit, preserves, juice, wine and raisins. It is a rich source of nutrients like minerals, vitamins, antioxidants like resveratrol. The major medicinal properties of grape and its constituents include antioxidant, anticarcinogenic, immunomodulatory, antidiabetes, anti-atherogenic, neuroprotective, anti-obesity, anti-aging and anti-infection attributes (Yadav *et al*. 2009).

This crop occupies fifth position amongst fruit crops in India with a production of 1.21 million

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tonnes. The area under grape cultivation in India is 137 thousand hectares with production of 2,951 thousand MT (Anonymous 2019). However, grapevine is subjected to the infection of several diseases which attack susceptible grapevine varieties and cause severe loss in yield as well as to the economy (Gadoury *et al.* 2001, Calonnec *et al.* 2004, Mannini and Digiaro 2017). Grapevine powdery mildew, caused by Uncinulanecator (Schwein) Burrill. recently renamed as *Erysiphe necator* Schwein (Braun and Takamatsu 2000) is a wide spread destructive disease of grapevine. Under favorable environmental conditions, the pathogen grows rapidly and causes severe infection to the grapevine which leads to low activity of chloroplast and low efficiency of carbon dioxide fixation (Dhillon *et al.* 1992) along with scarred, disturbed and split berries. Infection caused by this fungus develops at high humidity conditions, but not by free water (Sawant *et al.* 2017). Rainy periods having cumulative rainfall ranging between 2.0 and 58.5 mm favors the release of ascospores. Hence, rain acts as a primary source of inocula as it is necessary for ascospore release (Jailloux *et al.* 1999).

This disease is observed on all green parts of vine. Mildew colonies were usually found on either both lower surface of exposed leaves or both sides of well shaded leaves. Levels of photosynthesis and transpiration of infected leaves get reduced. Berries are susceptible to infection until sugar content reaches about 8%, although, established infection continue to produce spores until the berries contain 15% sugar (Delp 1954). Grape berries are most susceptible to powdery mildew during the period from flowering to fruit set and failure to control the disease during this period can result in serious crop loss.

Current powdery mildew control methods include the use of fungicides and of resistant cultivars (Narayana *et al.* 2005, Sandipan *et al.* 2014, Bisht *et al.* 2015). The extended use of fungicide is a matter of concern as repeated application and heavy dose of chemical fungicides result in development of pathogen resistance, environmental contamination and also has non-target effects on human, plants and other beneficial organisms (Thomas 1986, Manandhar *et al.* 1988). Residue problems emerging from by the use of fungicides is a serious concern for exports (Anonymous 2019).

Biological control agents (BCAs) have received a significant attention because of their versatile modes of action to protect plants and their potential to be included in integrated disease management programs (Shoda 2000, Paulitz and Bélanger 2001, Sawant *et al.* 2011). Powdery mildew fungi are prime targets for biocontrol agents because of their superficial growth (Belanger *et al.* 1997). Among different biocontrol agents, *Ampelomyces quisqualis* is a promising and potential option against powdery mildew fungi (Kiss 2003, Kiss *et al.* 2004). Genus Ampelomyces belongs to the class Coelomycetes that are wide spread, thermophilic and adopted to various climatic conditions (Sucharzewska *et al.* 2011). *Ampelomyces* killed the parasitized powdery mildew cells by invasion and destruction of host cytoplasm and suppressed their sporulation as well (Hashioka and Nakai 1980, Sundheim and Krekling 1982).

The present work is aimed to evaluate the efficacy of a formulation of *Ampelomyces quisqualis* against powdery mildew of grapevines under field conditions.

MATERIALS AND METHODS

The experiment was conducted for two successive seasons 2017- 2018 and 2018-2019 inthe vineyards of ICAR-NRCG campus, Pune (latitude 18.31N, longitude 73.55 E) in Randomized Block Design with four replications. Tas-A-Ganesh variety grown on Bower system of training was used for the study. Different concentrations viz., 6, 8 and 10 kg/ha of commercial formulation of bio-control agent *Ampelomyces quisqualis* (Bio Dewcon 2% WP) manufactured by T-Stanes and Company Ltd, Coimbatore, Tamil Nadu, India were used for the study. The formulation contained spores @ 2 x 106 CFU/ g sulfur 80 % WP @ 3.0 g/L was kept as check fungicide along with an untreated control.

The chosen vines were twelve years old, spaced at 10.0 × 6.0 ft and irrigated, using drip irrigation system. All vines received the same agricultural practices applied in the vineyard till harvest. The applications of *Ampelomyces quisqualis* 2.0% WP at different doses and sulfur 80% WP were started when the weather conditions were favorable for development of powdery mildew. Based on the favorable weather conditions four sprays were given at ten days interval for powdery mildew management. Water volume used for spray was calculated based on requirement of 1000 L/ha at full canopy. Knapsack sprayer fitted with hollow cone nozzle was used for spray.

For powdery mildew assessment, the evaluation on leaves and bunches were carried out 10 days post the last application. The ratings on ten leaves were recorded on randomly selected canes. Ten such canes per vine were observed, thus 100 disease observations were recorded per replicate. Four replications for each treatment were considered. Only actively growing powdery mildew lesions were considered for recording ratings. Disease severity was rated on a 0 to 4 scale (where 0= No disease present, 1= 15-25% leaf area and berries infected, 2=26%-50% leaf area and berries infected, 3= 51%-75% leaf area and berries infected, 4= more than 75% leaf area and berries infected) (Horsfall and Heuberger 1942).

Percent disease index (PDI) was determined according to the formula (McKinney 1923).

$$\text{PDI} = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of leaves observed} \times \text{Maximum rating scale}}$$

At harvesting, when Total Soluble Solids (TSS) percent of berries reached about 16–170 B in control, 6 clusters/vine were weighed and an average cluster weight was multiplied by the number of clusters/vine to calculate average of yield/vine (Ahmed 2018). The yield was extrapolated to yield/ha by following the formula.

$$\frac{\text{Yield}}{\text{ha}} \ \text{No of vines per ha} \times \text{Avg yield per vine}$$

Where, [No of vines /Ha = 1808]

Statistical analysis

The data were analyzed in RBD design with analysis of variance (ANOVA) using SAS (ver 9.3; SAS Institute Inc, Cary, North Carolina, USA). The percentage data were arcsine-transformed before analysis.The yield data were analyzed without transformation. Means were compared using Least Significant Difference (LSD) Test.

RESULTS

Bio-efficacy of *Ampelomyces quisqualis* 2.0% WP in control of powdery of grapes

All the tested concentrations of *Ampelomyces quisqualis* and sulfur (80% WP) alone significantly reduced the powdery mildew severity in both the seasons as compared to untreated control (Figs.

Fig.1. Pure culture of *Ampelomyces quisqualis.*

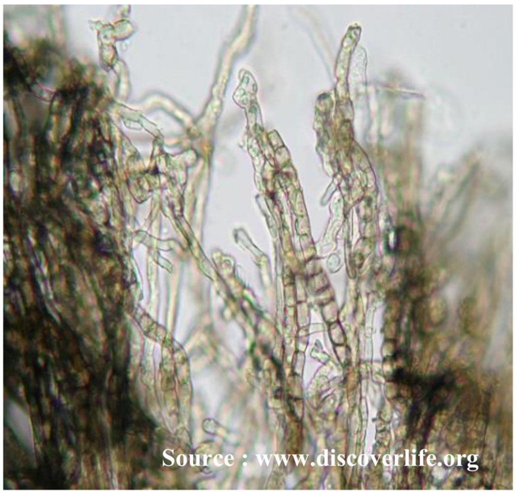

Fig. 2. Hyphae of *Ampelomyces quisqualis.*

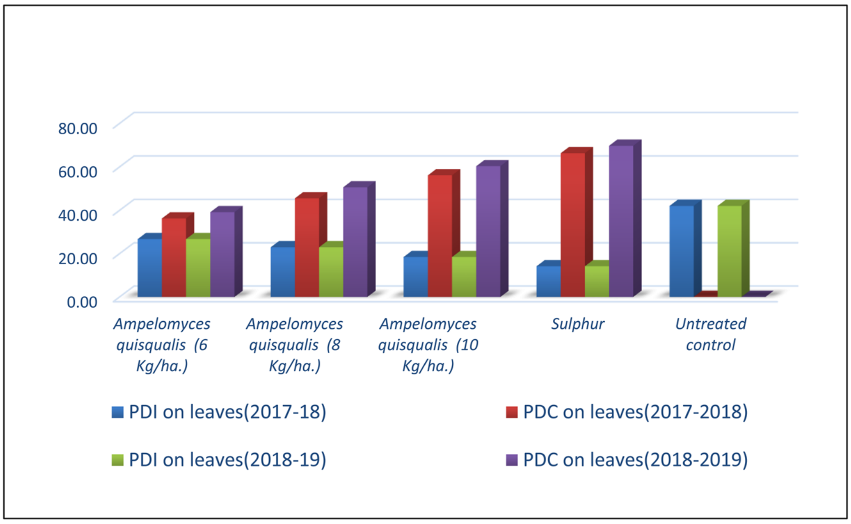

Fig. 3. Bio-efficacy of *Ampelomyces quisqualis* against powdery mildew on grapevine leaves.

1-4). In case of leaves, significant differences were also found among thebio-agents treatments (Table 1). *Ampelomyces quisqualis* 2.0% WP @ 10 kg/ha showed the highest efficacy (PDI- 25.42 and 23.76), followed by *Ampelomycesquisqualis* 2.0 % WP @ 8 g/L (PDI- 28.60 and 26.77) in controlling disease intensity during 2017-2018 and 2018-2019, respectively. On the other hand, check fungicide micronic sulfur showed the respective highest efficacy (PDI - 22.08 and 20.61) in controlling the disease. Among the different doses however, increase in dose from 6 kg/ha to 8 kg/ha and subsequently to 10 kg/ha showed significant reduction in PDI on leaves. Similar trend was observed in case of percent disease control on leaves. *Ampelomyces quisqualis* 2.0% WP @ 10 g/L showed higher mean percent disease control (49.76) among the all tested doses of biocontrol agents.

In case of bunches, *Ampelomyces quisqualis* 2.0% WP@ 6-10 kg/ha treatments recorded significantly lower PDI of powdery mildew during both the seasons i.e. 21.48-30.17 than the untreated control

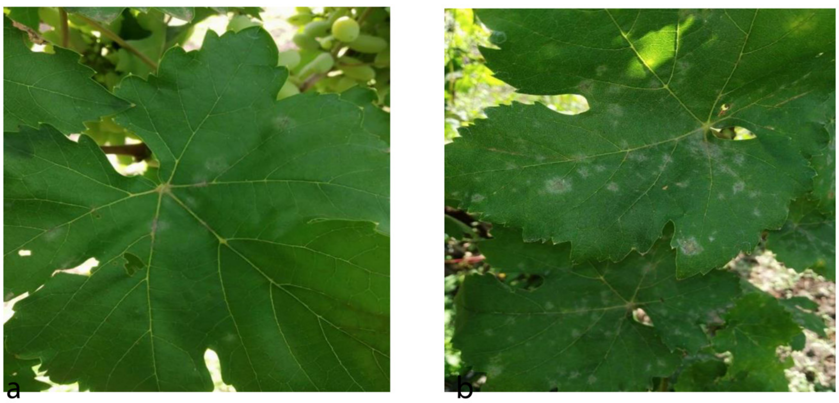

Fig. 4. Powdery mildew infection on leaves a) in treatment *Ampelomyces quisqualis* @10 kg/ha and b) in untreated control.

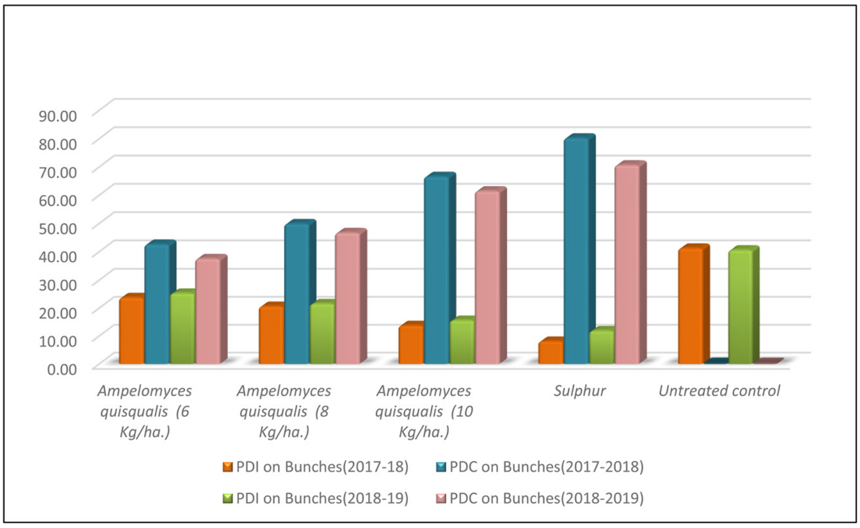

Fig. 5. Bio-efficacy of *Ampelomyces quisqualis* against powdery mildew on grapevine bunches.

with PDI 39.96-39.59 respectively (Figs. 5, 6). Sulfur 80% WP @ 3.0 kg/ha showed significantly lower PDI (15.85 and 20.12) than that of *Ampelomyces quisqualis* 2.0% WP @ 6-10 kg/ha respectively. Among the different doses however, increase in dose from 6 kg/ha to 10 kg/ha showed significant reduction in PDI on bunches. Among tested concentrations, *Ampelomyces quisqualis* 2.0% WP @ 10 kg/ha showed the highest efficacy (PDI- 21.48 and 23.26), followed by *Ampelomyces quisqualis* 2.0 % WP @ 8 kg/ha (PDI- 26.98 and 27.53) in controlling disease intensity during both the seasons respectively. However, on a comparative analogy, sulfur showed the highest efficacy (PDI – 15.85 and 20.12) in controlling the disease in both the seasons. The mean percent disease control in case of 6 kg and 8 kg per ha treatment of *Ampelomyces*

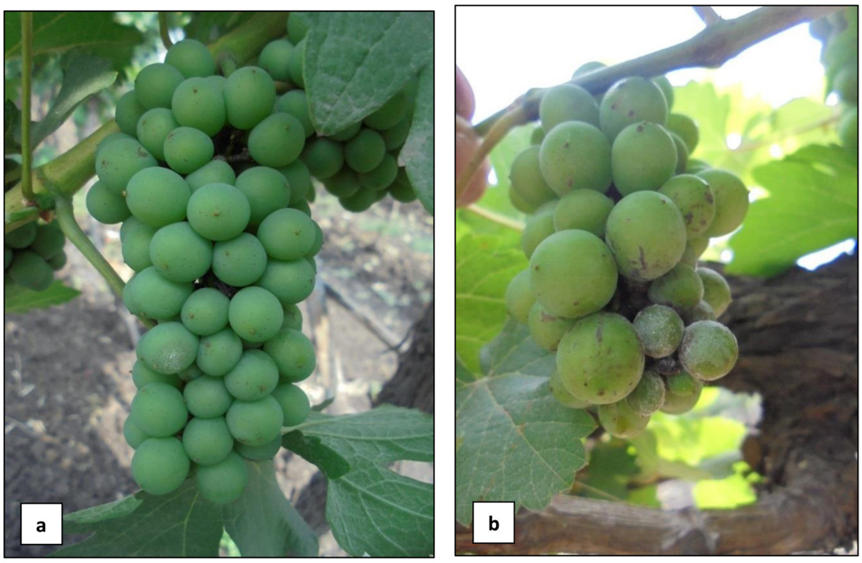

Fig. 6. Powdery mildew infection on bunches a) in treatment *Ampelomyces quisqualis* @10 kg/ha and b) in untreated control.

Table 1. Bio-efficacy of *Ampelomyces quisqualis* 2.0% WP (Bio-Dewcon) in control of powdery mildew on leaves and bunches of-grapes after fruit pruning. Values in the parentheses are angular transformed values; Means in the same column with different letters are significantly different at p≤0.05 level according to the LSD multiple range test.

Treatments	Dose (kg/ha)	Intensity of powdery mildew on leaves PDI 2017-2018	2018-2019	Mean	PDC 2017-2018	2018-2019	Mean
Ampelomyces quisqualis 2.0% WP	6.0	26.75 (31.14)d	25.00 (29.98)d	25.88 (30.57)d	36.26 (37.00)d	39.17 (38.68)d	37.72 (37.87)d
Ampelomyces quisqualis 2.0% WP	8.0	22.94 (28.60)c	20.31 (26.77)c	21.63 (27.71)c	45.54 (42.44)c	50.62 (45.36)c	48.08 (43.90)c
Ampelomycesquisqualis 2.0% WP	10.0	18.44 (25.42)b	16.38 (23.76) b	17.41 (24.63)b	56.13 (48.52)b	60.34 (51.04)b	58.23 (49.76)b
Sulfur 80% WP	3.0	14.19 (22.08)a	12.56 (20.61) a	13.38 (21.39)a	66.30 (54.54)a	69.70 (56.73)a	68.00 (55.59)a
Untreated control	-	42.06 (40.43)e	41.25 (39.95)e	41.66 (40.20)e	0.00 (0.00)e	0.00 (0.00)e	0.00 (0.00)e
LSD		1.56	2.98	1.72	2.93	5.67	3.34

Table 1. Continued.

Treatments	Dose (kg/ha)	Intensity of powdery mildew on bunches 2017-2018	2018-2019	Mean	2017-2018	2018-2019	Mean
Ampelomyces quisqualis 2.0% WP	6.0	23.75 (29.06)d	25.31 (30.17)d	24.53 (29.66)c	42.65 (40.69)c	37.55 (37.68)b	40.10 (39.26)c
Ampelomyces quisqualis 2.0% WP	8.0	20.63 (26.98)c	21.56 (27.53)c	21.09 (27.33)c	49.91 (44.95)c	46.74 (45.05)b	48.32 (44.04)
Ampelomycesquisqualis 2.0% WP	10.0	13.75 (21.48)b	15.63 (23.26)b	14.69 (22.48)b	66.64 (55.02)b	61.49 (51.66)a	64.06 (53.22)b
Sulfur 80% WP	3.0	8.13 (15.85)a	11.88 (20.12)a	10.00 (18.37)a	80.24 (64.60)a	70.68 (57.26)a	75.46 (60.39)a
Untreated control	-	41.25 (39.96)e	40.63 (39.59)e	40.94 (39.78)d	0.00 (0.00)d	0.00 (0.00)c	0.00 (0.00)d
LSD		4.78	3.09	2.75	8.30	6.23	5.19

quisqualis were at par with each other and 10 kg per ha dose showed significantly higher mean PDC over two lower doses and untreated control.

Effect of *Ampelomyces quisqualis* 2.0% WP on marketable yield of grapes

Pooled data of both the years showed that marketable yield (kg/vine) of grapes was increased in each treatment over untreated control. The yield in case of *Ampelomycesquisqualis* 2.0% WP @ 6-10 kg/ha were significantly higher (7.44-8.79 kg/vine) over untreated control (6.01kg/vine). However, the marketable yield incase of *Ampelomyces quisqualis* 2.0% WP @ 10 kg/ha was at par with *Ampelomyces quisqualis* 2.0% WP @ 8 kg/ha and was significantly more over *Ampelomyces quisqualis* 2.0% WP @ 6 kg/ha. Fungicide sulfur 80 WP @ 3.0 kg/ha showed highest yield of 10.05 kg/vine which was significantly higher over control and all doses of *Ampelomyces quisqualis* 2.0% WP (Table 2). *Ampelomycesquisqualis* 2.0% WP@ 10 kg/ha manifested 46.3% increase in yield over untreated control.

DISCUSSION

Ampelomyces quisqualis is the most studied mycoparasite of powdery mildew disease on various crops and observed as one of the important biological control agents (BCAs) of powdery mildew disease. It has a potential to work against the Erysiphaceae and restrict the growth and spread of powdery mildew fungi.

Table 2. Marketable yield in vines treated with Bio-Dewcon (*Ampelomyces quisqualis* 2.0% WP) against powdery mildew of grapes. Means in the same column with different letters are significantly different at p≤0.05 level according to the LSD multiple range test.

Tr. No.	Treatments	Dose (kg/ha)	Harvestable yield (kg/vine)			Percent increase in yield over untreated control
			2017-18	2018-19	Pooled	
T_1	*Ampelomyces quisqualis* 2.0% WP	6.0	6.15 c	8.72 b	7.44 c	23.07
T_2	*Ampelomyces quisqualis* 2.0% WP	8.0	7.26 b	9.11 b	8.19 b	36.2
T_3	*Ampelomyces quisqualis* 2.0% WP	10.0	7.82 b	9.77 b	8.79 b	46.3
T_4	Sulfur 80% WP	3.0	8.93 a	11.18 a	10.05 a	67.2
T_5	Untreated control	---	5.04 d	6.97 c	6.01 d	0.00
	LSD		1.51	1.36	0.65	

There are about 18 epithets recorded worldwide till date on variety of plant hosts (www.indexfungorum.com). The mechanism of biocontrol by the fungus has been established as hyperparasitism as this fungus possess the ability to colonize the mycelium of powdery mildew and produce reproductive structure. It does not stop radial growth of the pathogen, but it stopped sporulation of the pathogen (Philipp *et al.* 1984, Shishkoff and McGrath 2002, Kiss *et al*. 2004).

Results from the present investigation showed that *Ampelomyces quisqualis* 2% WP @ 6-10 kg/ha was significantly effective in controlling the powdery mildew of grapevines than the untreated control. *Ampelomyces quisqualis* 2% WP @ 10 kg/ha was most effective in controlling the disease. Marketable yield (MT/ha) of grapevines treated with *Ampelomyces quisqualis* 2.0% WP@ 6-10 kg/ha was significantly higher over untreated control. *Ampelomycesquisqualis* Ces. is a potential biological control agent for powdery mildew diseases on vegetable crops, apple, grapes mango and Buxus trees (Kiss *et al.* 2004,Vaidya and Thakur 2005, Naseripour *et al*. 2014). Singh *et al*. (2017) reported that cell free culture filtrate and biomass of *Ampelomyces quisqualis* were effective in prevention and control of powdery mildew of grapes. Shishkoff and McGrath (2002) showed that application of *Ampelomycesquisqualis* did not significantly reduce powdery mildew of pea (*Psidium xanthii*) colony sizes, but it reduced the amount of inoculum produced by each colony.

Falk *et al.* (1995) reported that partial control of grape powdery mildew is possible by the use of the mycoparasite *Ampelomyces quisqualis*. Bio-compound produced by the *Ampelomyces*–from A*geratum conyzoides* reduced the severity of powdery mildew disease in greenhouse experiments (Wanasiri *et al.* 2020).It was reported by Romero *et al.* (2007) that the efficacy of *Ampelomyces quisqualis* would be increased by adding a mineral oil. In the absence of mineral oil, however, severity values obtained for plants treated with mycoparasites were not statistically different from untreated control. Sundheim (1982) compared the effect of *Ampelomyces quisqualis* and fungicides in cucumber and concluded that the yield was higher when a reduced rate of fungicides was used.Shinde *et al*. (2019) reported that foliar application of *Ampelomyces quisqualis* effectively control the powdery mildew on grapes. Sztejnberg *et al*. (1989), Srivastava (2003) observed that formulation of *Ampelomyces quisqualis* is capable of parasitizing powdery mildew of mango *(Oidium mangiferae)* and lowered the severity of powdery mildew on mango trees with an increase in fruit yield. It is to be noted that, in case of high disease pressure (PDI > 25), chemical fungicides like sulfur gave a better control (Angeli *et al*. 2013). Application of *Ampelomyces quisqualis* in alternation with chitosan gave better control of powdery mildew of grapes than with chitosan alone (Thosar *et al.* 2020). So, it is advisable to use *Ampelomyces quisqualis* as a preventive application under high humidity conditions.

The overall study of these experiments proved that *Ampelomyces quisqualis* may successfully prevent, control and significantly reduce the severity of powdery mildew of grapes and may be included in the integrated disease management strategies to control the disease.

CONCLUSION

The study concluded that, *Ampelomyces quisqualis* 2.0% WP (Bio Dewcon) @ 10 kg/ha was most effective in controlling the powdery mildew disease of grapevines. It is important to use the bio agent as it suppressed the sporulation rate of its fungal hosts and prevented secondary spread of the disease. Further, it can be used with sulfur in tandem to give a wholistic management of the disease. Effect of tested bioagent on non-target hosts is required to be tested for applying to the larger areas. Integration of compatible bio agents with fungicides may enhance the effectiveness of disease control and provide better management of diseases. The combination of BCAs with fungicides would provide similar disease suppression as achieved with higher fungicide use and helps in reducing the residue level on berries hence compatibility of *Ampelomyces quisqualis* with the recommended fungicides is required to be analyzed.

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