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Research Article

Control of powdery mildew in vineyards by Milastin K, a commercial formulation of *Bacillus subtilis* (KTBS)

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ABSTRACT: Field experiments were conducted during the vegetative season of April–October 2008 and 2009 and fruiting season of October–April 2008–09 and 2009–10, to study the efficacy of Milastin K, a formulation containing *Bacillus subtilis (*KTBS)*,* for the control of powdery mildew on grapes. Five to six sprays of Milastin K @ 1.0 ml l^{-1} (1.0 l ha^{-1}), from 30–119 days after pruning at 7–22 days interval (depending on weather based disease risk) could effectively control powdery mildew on leaves and bunches in grapes and increase yield. The untreated control recorded PDI in the range of 4.00–82.13 during different seasons, Milastin K treatment significantly reduced PDI to 1.62–20.69. Under low to moderate disease pressure conditions, the efficacy of Milastin K in controlling powdery mildew was on par with sulphur 80 WG @ 2.0g l^{-1}. Under high disease pressure condition, Milastin K was not so effective when used alone, but was effective when used in integration with fungicide sprays. Harvestable yield of 4.55 kg per vine was recorded in Milastin K while there was nil yield in untreated control. There was no phytotoxic effect of Milastin K on vines when it was sprayed at a dose up to 2.0 ml l^{-1}.

KEY WORDS: Grapes, *Erysiphe necator*, *Uncinula necator, Vitis vinifera*, Thompson seedless, harvestable yield, sulphur, flusilazole

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INTRODUCTION

Grape (*Vitis vinifera* L.) is an important fruit crop of India with good nutritional value and is consumed fresh or processed into raisins and wine. However, the commercial cultivars are highly susceptible to powdery mildew disease which causes huge losses both in fruit quality and yield (Chadha and Shikhamany 1999). The disease is caused by *Uncinula necator* (Schwein.) Burrill (= *Erysiphe necator*) and occurs during humid and cloudy weather. In Maharashtra and adjoining regions of Andhra Pradesh and Karnataka, where 'two prunings – one yield' system of grape cultivation is followed, powdery mildew can be a serious disease during the entire year, especially during the period from flowering to fruit set. If the bunch is infected before fruit set, it becomes difficult to control the disease at later stages in the vineyard. Although berries are not infected after berry softening stage, the green stalks of bunches can be infected at any stage till harvest, which reduces shelf life of bunches during storage.

For effective disease management, fungicides are to be applied whenever weather conditions are favorable for disease development. Usually 4 to 6 sprays at an interval of 5 to 15 days are applied during both the vegetative and the fruiting seasons. However, most of the recommended fungicides cannot be safely used in the post-veraison stage; firstly because the pre-harvest intervals (PHI) of the systemic fungicides are above 30 days (www.nrcgrapes.nic.in/zipfiles/Pesticide List.pdf.); and secondly, there are apprehensions in the growers' mind that spray of the non-systemic fungicide, sulphur, may cause yellowing of berries at higher temperatures. Hence there is a need for an alternative and safer method of disease control.

Bacillus subtilis is a promising biological control agent for the control of diseases caused by fungal pathogens. There are reports of reduction in powdery mildew of grapes by a *B. subtilis* formulation 'Serenade' (Schilder *et al.,* 2002). In grapes, *B. subtilis* was also tried for control of post-harvest diseases (Benato *et al.,* 1988) and for control of *Eutypa lata* (Ferreira *et al.,* 1991). Even the application of metabolites of *B. subtilis* strains have controlled powdery mildew of cucumber (Bettiol *et al.,* 1997) and induced tolerance in wheat and barley (Wittmann and Schonbeck, 1996; Kehlenbeck and Schonbeck, 1995). *B. subtilis* formulations may vary in their efficacy (Raguchander *et al.,* 2005; Schilder *et al.,* 2002).

In the present study, the bio-efficacy of Milastin K, a formulation containing more than 10^9 cfuml^{-1} of a naturally occurring competitive strain of *Bacillus subtilis* (KTBS), was tested against powdery mildew of grapes during 2008-2010, which covered two vegetative and two fruiting seasons.

MATERIALS AND METHODS

To study the efficacy of Milastin K, a commercial formulation containing more than 10^9 cfu ml^{-1} of *Bacillus subtilis* (KTBS)**,** against powdery mildew on leaves and fruits, four field trials were conducted during 2008-2010, two during the vegetative season (April to October) and two during the fruiting seasons (October to April). A 10-year-old vineyard of Thompson Seedless grapes trained on Y trellises at the research farm of the NRC for Grapes, Pune was selected for the study.

Sulphur 80 WG (Wokovit), a contact fungicide, was included as positive control. Other fungicides, *viz*. Bordeaux mixture, flusilazole 40% (Nustar 40EC), tebuconazole 25.9% (Folicur 250EC), tridemefon 25%WP (Bayleton 25%WP), and mycobutanil 10%WP (Systhane) were also included in the trials for comparison. The doses per L were, Milastin K @ 1.0 and 2.0ml, sulphur @ 2.0g, flusilazole @ 0.125ml, tebuconazole @ 0.5ml, tridemefon @ 1.0g, mycobutanil @ 0.4g and Bordeaux mixture @ 0.5%. Another treatment, where the plants were sprayed with plain water, the same that was used for preparing suspensions of bio- or chemical pesticides, was also used for comparison. Vines, that did not receive any spray were treated as the 'negative' control.

Water volume used for sprays was about 1000 l ha^{-1}. The weather data was recorded on µMetos automatic weather station and sprays were given before the outbreak of the disease and subsequently whenever the weather was found favorable for disease development.

The experiment was laid out in randomized block design with four replications. Each replication consisted of a block of 12 vines (four rows and three vines in each row), planted at a distance of 304 x 183 cm. The ten vines on the edge of each block were treated as guard vines and observations were recorded only on the two centrally located vines. Disease incidence on leaves and bunches were recorded 3–4 days after each spray adopting 0–4 disease rating scale, where 0 = nil, 1 = trace to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = more than 75 per cent leaf area infected (Horsfall and Heuberger, 1942). Per cent Disease Index (PDI) was calculated by following the formula of McKinney (1923). The ratings on ten leaves and a bunch were recorded on five randomly selected canes from each vine.

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

For observations on berry diameter twenty berries from each replicate were selected randomly and the diameter was read using the berry diameter measuring scale. The harvestable yield was recorded by taking the weights of bunches which had market value.

To check for probable phytotoxic effects, vines were sprayed as above with Milastin K at 1.0 and 2.0 ml l^{-1}, water spray and control (no spray). The vines were critically observed for the presence of phytotoxic effects such as chlorosis, tip burning, necrosis on leaves and berries, epinasty and russeting on berries up to seven days after the spray. Observations were recorded in the form of visual ratings in 0–10 scale, where 0 = 0, 1 = 01–10, 2 = 11-20, 3 = 21–30, 4 = 31–40, 5 = 41–50, 6 = 51–60, 7 = 61–70, 8 = 71–80, 9 = 81–90 and 10 = 91–100 percent plant parts showing phytotoxic symptoms.

All data were subjected to ANOVA (Panse and Sukhatme, 1978). The PDI data was transformed using arcsine transformation. Only the results significant at $P = 0.05\%$ are discussed.

RESULTS AND DISCUSSION

i. April to October 2008

During the vegetative season 2008, the control had the highest PDI value (3.81), which was on par with the PDI of 3.38 in water spray (Fig. 1). The PDI of 2.94 in Milastin K (2.0ml l^{-1}) was less than the PDI in control, on par to that in water spray, but higher than the PDI of 1.94 in the fungicide treatment (Bordeaux mixture 0.5% + sulphur 2g l^{-1}) on the first observation. Though there was not much increase in disease incidence in control and water spray during the period, it was reduced in the Milastin K and the fungicide treatments and reached the value of 0.00 PDI, indicating complete control of disease.

ii. October 2008 to April 2009

The PDI on leaves after the first spray was least (1.50) in Milastin K at 2.0ml l^{-1} dose and maximum (3.38) in the control (Table 1). The PDI in this treatment was on par to that in the flusilazole (1.94) and sulphur (1.56) treatments and less than that in Milastin K at 1.0ml l^{-1} dose (2.13). In subsequent observations, the PDI at both the doses of Milastin K were on par to each other and also to that in sulphur (except in the third observation when it was lower than that in sulphur); and less than the PDI in the control

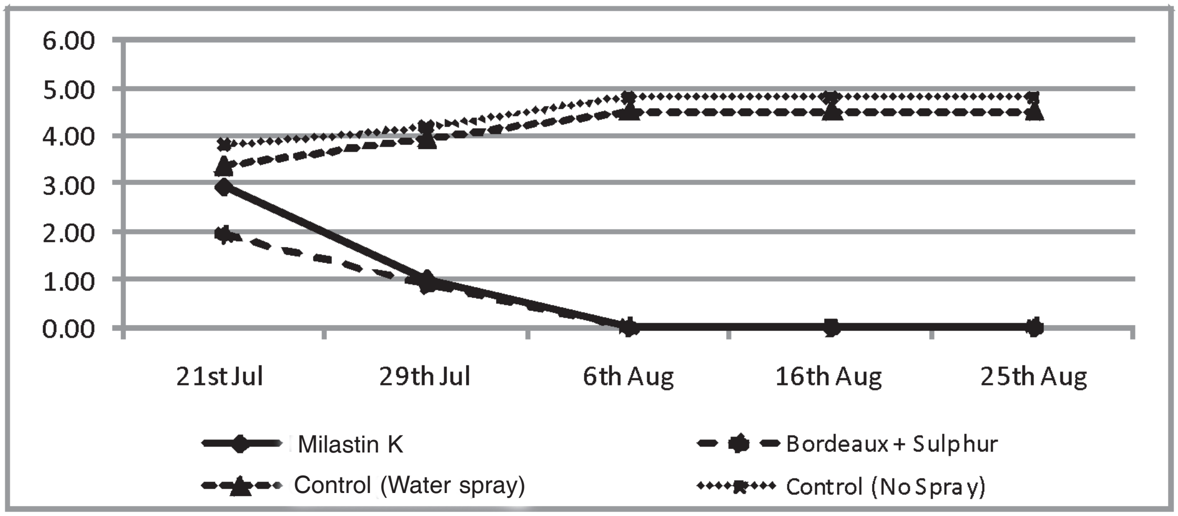

Fig. 1. Reduction of PDI of powdery mildew by Milastin K sprays

Table 1. Bio-efficacy of Milastin K against powdery mildew on grape leaves (Oct. 2008–Apr. 2009)

Treatment	Percent Dose (per 1)	Disease Index (PDI) on leaves				
		08.12.08	22.12.08	30.12.08	10.01.09	28.01.09
Milastin K	1.0 ml	2.13 (8.30)[b]	4.06 (11.59)[c]	2.88 (9.73)[bc]	2.00 (8.05)[b]	1.88 (7.74)[bc]
Milastin K	2.0 ml	1.50 (7.02)[a]	3.69 (11.05)[bc]	2.69 (9.42)[b]	1.63 (7.27)[ab]	1.56 (6.78)[b]
*Flusilazole 40EC + Milastin K	0.125 ml + 2.0 ml, respectively	1.94 (7.99)[ab]	3.50 (10.74)[bc]	2.50 (9.06)[b]	1.31 (6.48)[a]	1.44 (6.87)[bc]
Sulphur 80 WG	2.0 g 2.0 g	1.56 (7.12)[a]	4.00 (11.50)[c]	3.81 (11.23)[d]	2.19 (8.47)[b]	2.00 (8.10)[bc]
Flusilazole 40EC	0.125 ml 0.125 ml	1.94 (7.97)[ab]	2.25 (8.60)[a]	1.56 (7.15)[a]	1.50 (7.00)[ab]	0.00 (0.00)[a]
Water Spray	——	1.68 (7.43)[ab]	3.13 (10.16)[b]	3.44 (10.68)[cd]	3.44 (10.65)[c]	4.38 (12.06)[cd]
Control (No Spray)	——	3.38 (10.58)[c]	3.50 (10.77)[bc]	3.88 (11.35)[d]	4.00 (11.52)[c]	4.69 (12.48)[d]
SEM ±		0.37	0.44	0.38	0.52	0.70
CD (P = 0.05)		1.10	1.31	1.13	1.55	2.09
CV (%)		9.20	8.30	7.75	12.26	18.20

Figures in parentheses are arcsine transformed values of percentages; values followed by the same letters in a column are not significantly different at P = 0.05; *first four sprays were of flusilazole followed by two sprays of Milastin K (except on the second day of observation when they were on par).

Significantly, on the first day of observation, the PDI in water spray treatment was on par with Milastin K at both the doses and less than that in the control, but subsequently, it was more than the PDI in the Milastin K at 2 ml l^{-1} (though being on par with the PDI at the lower dose of 1 g l^{-1}), indicating that water alone could not control the disease effectively. Rain or free water is known to be inhibitory for development of powdery mildew disease and it was thought that spray of plain water might also provide some disease control, especially under low disease pressure. Hence it was included as one of the treatments.

The PDI in flusilazole treatment was less (0.00 to 2.25) than all the other treatments and the control, except on the fourth observation where it was on par with the PDI in the combined treatment of flusilazole and Milastin K and with the Milastin K 2.0ml l^{-1} dose (Table 1). The zero PDI in flusilazole treatment on the last observation is only of academic importance, as it was recorded after two sprays given at post veraison stage, when normally flusilazole is not applied, as it leads to residues above MRL at harvest (the recommended PHI of flusilazole is 50 days) (www.nrcgrapes.nic.in/zipfiles/Pesticide List.pdf).

On bunches, initially the PDI in all treatments, including Milastin K, were on par with that of control, except flusilazole, where the PDI was less than that in the control (Table 2). The water spray treatment had lower PDI than control, though being on par to most of the treatments. The disease later stabilized at a low level (9.38 PDI in control) in the vineyard. There was a reduction in disease in all treatments, including Milastin K, which were on par and less than control. However, in the last observation, the PDI in the water spray increased to the level observed in control, while that in the flusilazole treatment was reduced to 0.00. However, as discussed earlier this observation is only of academic importance. The harvestable yield was in the range of 2.13 to 2.85 kg per vine and there was no difference among different treatments (Table 2). Thus Milastin K at both 2.0 and 1.0ml/L doses provided disease control on par to that of sulphur, and also to that of flusilazole. Water spray also provided disease control at the early stages of bunch development but was not effective at the later stages.

Table 2. Bio-efficacy of Milastin K against powdery mildew on grape bunches and its effect on yield (Oct. 2008–Apr. 2009)

Treatment	Dose (per l)	Percent Disease Index (PDI) on Bunch					Harvestable Yield (kg / vine)
		08.12.08	22.12.08	30.12.08	10.01.09	28.01.09	
Milastin K	1.0 ml	5.63 (13.45)^{bc}	5.63 (13.66)^{a}	3.13 (10.05)^{a}	5.63 (13.66)^{a}	3.13 (10.05)^{b}	2.69
Milastin K	2.0 ml	6.25 (14.40)^{bc}	5.63 (13.66)^{a}	5.00 (12.70)^{a}	4.38 (11.96)^{a}	3.13 (10.05)^{b}	2.74
*Flusilazole 40EC + Milastin K	0.125 ml + 2.0 ml, respectively	6.88 (15.14)^{c}	4.38 (11.75)^{a}	3.13 (10.05)^{a}	5.63 (13.66)^{a}	5.00 (12.92)^{b}	2.85
Sulphur 80 WG	2.0 g	6.25 (14.40)^{bc}	5.63 (13.66)^{a}	5.00 (12.92)^{a}	4.38 (11.96)^{a}	2.50 (9.09)^{b}	2.68
Flusilazole 40EC	0.125 ml	4.38 (10.43)^{a}	4.38 (11.96)^{a}	3.75 (11.00)^{a}	4.38 (11.96)^{a}	0.00 (0.00)^{a}	2.88
Water spray	——	4.38 (11.96)^{ab}	6.25 (14.40)^{a}	8.75 (17.16)^{b}	10.00 (18.36)^{b}	10.63 (18.88)^{c}	2.13
Control (No Spray)	——	8.13 (16.35)^{c}	9.38 (17.72)^{b}	9.38 (17.61)^{b}	9.38 (17.72)^{b}	9.38 (17.72)^{c}	2.14 2.14
SEM ±		1.01	1.00	1.09	0.76	1.61	0.26
CD (P = 0.05)		3.01	2.96	3.23	2.27	4.78	NS
CV (%)		15.91	14.72	15.47	10.90	27.48	20.32

Figures in parentheses are arcsine transformed values of percentages; NS, not significant at P = 0.05; values followed by the same letters in a column are not significantly different at P = 0.05; *first four sprays were of flusilazole followed by two sprays of Milastin K

iii. April to October 2009

Initially, PDI in Milastin K at both the doses were on par and significantly less than the PDI in flusilazole and sulphur treatments. Highest PDI values (42.31 to 82.13) were observed in control, followed by water spray (27.94 to 64.88) in the first and the subsequent observations (Table 3). The weather conditions were favorable for disease development and there was a gradual increase in disease incidence during the period of trial, as evident by the increasing PDI values in control (42.31, 66.81 and 82.13) and in water spray (27.94, 42.19 and 64.88). In the second observation, the PDI in Milastin K at both doses was on par to sulphur and flusilazole treatments. All above treatments were better than the control and water spray. In the last observation both treatments of Milastin K recorded PDI less than that recorded in flusilazole and sulphur treatments. Interestingly the PDI in flusilazole was always on par to that observed in sulphur, indicating that during rainy period there is no added advantage of using a systemic compound. Water spray alone provided some control of the disease but it was less than the fungicide and Milastin treatments (Table 3).

Milastin K provided better disease control as compared to both the systemic and the non-systemic fungicide at high level of disease, indicating that the slightly warm and humid weather during the monsoon season was very conducive for the activity of *B. subtilis*. Milastin K at 1.0 or 2.0ml l^{-1} doses was equally effective in the control of powdery mildew disease on the leaves providing about 80-90% disease control. Earlier workers also found *B. subtilis* formulation to be as effective as the fungicide carbendazim in reducing the severity of powdery mildew and in enhancing the yield in case of urdbean (Raguchander *et al.*, 2005).

ii. October 2009 to April 2010

All treatments reduced the disease on the leaves and the fruits as compared to the control (Table 4). On the leaves, initially, when the disease level was comparatively lower (17.88 PDI in control), the PDI in Milastin K at the higher dose of 2.0ml/L was on par to that in sulphur, but later when the disease level increased (42.38 PDI in control), the PDI in Milastin K, at both doses was less than that in sulphur. On the fruits, initially the PDI in all the treatments was on par and less than control but subsequently the PDI in Milastin K, at both doses was less than that in sulphur. The PDI in the treatment, where the first four sprays were of different fungicides and the last two sprays of Milastin K, was on par with sulphur on leaves and fruits in both observations. The PDI in Milastin

Table 3. Bio-efficacy of Milastin K against powdery mildew on grape leaves (Apr. 2009–Oct. 2009)

Treatment	Dose (per l)	Percent Disease Index (PDI) of powdery mildew on leaves		
		14.07.09	31.07.09	28.08.09
Milastin K	1.0 ml	1.63 (7.32)[a]	3.06 (10.02)[b]	1.62 (7.32)[a]
Milastin K	2.0 ml	1.31 (6.57)[a]	2.69 (9.40)[b]	1.31 (6.57)[a]
*Flusilazole 40EC + Milastin K	0.125 ml + 2.0 ml, respectively	2.0 (8.19)[a]	1.31 (6.57)[a]	1.19 (6.14)[a]
Fusilazole 40EC	0.125	6.38 (14.44)[b]	2.69 (9.43)[b]	5.12 (13.06)[b]
Sulfur 80WG	2.0	7.38 (15.71)[b]	3.00 (9.96)[b]	5.75 (13.85)[b]
Water spray	——	27.94 (31.84)[c]	42.19 (40.48)[c]	64.88 (53.63)[c]
Control (No spray)	——	42.31 (40.56)[d]	66.81 (54.88)[d]	82.13 (64.98)[d]
SEM ±	0.75	0.86	0.44	
CD (P = 0.5)	2.23	2.55	1.32	
CV (%)	8.44	8.53	3.76	

Figures in parentheses are arcsine transformed values of percentages; values followed by the same letters in a column are not significantly different at P = 0.05; *first four sprays were of flusilazole followed by two sprays of Milastin K

K at both the doses were on par except on first observation on leaves (Table 4).

There was no harvestable yield in control (Table 4). All treatments increased yield and Milastin K at both 1 and 2ml l^{-1} doses were on par with sulphur. Thus under high disease pressure, too, when there was total loss in harvestable yield in control plots, application of Milastin K provided yield on par to sulphur. Maximum yield was obtained in treatment where the first four sprays were of different fungicides and the last two of Milastin K (7.48 kg/vine). Maximum berry diameter was recorded in Milastin K at 1 ml l^{-1} dose.

The study indicates that during the vegetative season, which coincides with the monsoon season, Milastin K at 1.0 or 2.0 ml l^{-1} dose could provide control of powdery mildew of grapes at a level better than that provided by sulphur or flusilazole. Thus Milastin K can substitute for sulphur during vegetative season. In the fruiting season of 2008-09, when the disease level in the vineyard was not very high, as evident by the low PDIs in control during the period of study, Milastin K at both 1.0 and 2.0ml l^{-1} doses was equally effective in control of powdery mildew on bunches and the PDIs were on par with sulphur though less than the systemic fungicide, flusilazole. But in the fruiting season of 2009-10, when the disease levels were higher, Milastin K was not as effective as sulphur in reducing PDI, though the yields were on par (Table 4). The yield of 4.55 and 5.81 kg obtained at 1.0 and 2.0 ml l^{-1} doses of Milastin K were on par, as were the PDIs (18.44 and 19.69, respectively). However, under these conditions of high disease levels, the PDI in fungicide + Milastin K treatment was on par to that obtained in sulphur treatment, while the yield was much higher (7.48) in the fungicide+Milastin K treatment than in the sulphur treatment (4.58). Thus Milastin K can be used in integration with the fungicides as the early stage sprays of different fungicides, including flusilazole, did not affect the efficacy of Milastin K sprays given at later stages. It can thus be used instead of sulphur during late stages during the fruiting season. Vines sprayed with Milastin K at 1.0 or 2.0 ml l^{-1} doses did not show any phytotoxic symptoms.

Thus, results of the trials conducted during four successive seasons indicate that effective control of powdery mildew could be obtained in vineyards by spray applications of Milastin K (a bio-formulation containing *Bacillus subtilis* (KTBS). Under low to moderate disease pressure conditions, Milastin K appeared as promising as sulphur, but under high disease pressure condition Milastin K alone was not as effective, but its efficacy was enhanced when used in integration with fungicide sprays. In an earlier study on powdery mildew of cucurbits, strains of *B. subtilis* provided disease control similar to that achieved with the fungicide azoxystrobin. The bacterial strains efficiently colonized leaf surfaces and had antagonistic interactions with *Podosphaera fusca* structures (Romero *et al.*, 2007).

Table 4. Bio-efficacy of Milastin K against powdery mildew on grape leaves and bunches (Oct. 2009–Apr. 2010)

Treatment	Dose (per l)	Percent disease index (PDI) on leaves		Percent disease index (PDI) on bunches		Harvestable yield (kg / vine)	Berry diameter (mm)
		30.12.09	22.01.10	30.12.09	22.01.10		
Milastin K	1.0ml	7.69 (15.95)^{c}	20.69 (26.52)^{b}	0.00 (0.00)^{a}	18.44 (25.30)^{b}	4.55^{b}	17.00^{a}
Milastin K	2.0ml	3.50 (10.69)^{b}	17.44 (24.60)^{b}	0.63 (3.21)^{a}	19.69 (26.25)^{b}	5.61^{b}	16.00^{b}
Different sprays *	As given below	1.19 (6.21)^{a}	0.75 (4.80)^{a}	0.00 (0.00)^{a}	2.50 (8.94)^{a}	7.48^{a}	15.15^{c}
Sulfur 80WG	2.0g	2.31 (8.64)^{b}	2.38 (8.70)^{a}	0.31 (1.60)^{a}	4.06 (10.97)^{a}	4.58^{b}	15.38^{bc}
Control		17.88 (24.99)^{d}	42.38 (40.59)^{c}	2.19 (8.04)^{b}	32.50 (34.74)^{c}	0.00^{c}	15.95^{bc}
SEM ±		0.72	1.95	1.21	1.21	0.56	0.28
CD (P = 0.05)		2.22	6.00	3.74	3.74	1.73	0.84
CV (%)		10.83	18.51	94.34	94.34	25.29	3.58

*1st spray = fusilazole 0.125ml l^{-1}, 2nd spray = tebuconazole 0.5ml l^{-1}, 3rd spray tridemefon 1.0g l^{-1}, 4th spray mycobutanil 0.4g l^{-1} and 5th and 6th spray = Milastin K 2.0ml l^{-1}

The advantage of using *B. subtilis* over other biocontrol agents like the fungus *Ampelomyces quisqualis* will be that the survival of *B. subtilis* will not be affected by fungicide sprays in the vineyard, whereas the population of *A. quisqualis* will be reduced (Pertot *et al.*, 2009). Further, as *B. subtilis* forms endospores, which are dormant resting structures with great resistance to external environmental factors, it would have longer survivability in the vineyards (Zhang and Dou, 2002).

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REFERENCES

Benato, E. A., Sigrist, J. M. M., Oliveira, J. J. do-V, Dias, M. S. C. and Correa, A. C. C. 1998. Postharvest disease control of 'Italia' grapes and the evaluation of residual levels of SO_2 and thiabendazol. *Brazilian Journal of Food Technology*, **1**: 107–112.

Bettiol, W., Garibaldi, A. and Migheli, Q. 1997. *Bacillus subtilis* for the control of powdery mildew on cucumber and zucchini squash. *Bragantia*, **56**: 281–287.

Chadha, K. L. and Shikhamany, S. D. 1999. *The Grape-improvement, production and post-harvest management.* Malhotra Publishing House, New Delhi, India, 579 pp.

Ferreira, J. H. S., Matthee, F. N. and Thomas, A. C. 1991. Biological control of *Eutypa lata* on grapevine by an antagonistic strain of *Bacillus subtilis*. *Phytopathology*, **81**: 283–287.

Horsfall, J. G. and Heuberger, J. W. 1942. Measuring magnitude of a defoliation disease in tomatoes. *Phytopathology*, **32**: 226–232.

Kehlenbeck, H. and Schonbeck, F. 1995. Effects of induced resistance on disease severity/yield relations in mildewed barley. *Journal of Phytopathology*, **143**: 561–567.

McKinney, H. H. 1923. A new system of grading plant diseases. *Journal of Agriculture Research*, **26**: 195–218.

Panse, V. G. and Sukhatme, P. V. 1978. *Statistical methods for agricultural workers*. Indian Council of Agricultural Research, New Delhi, India, 347 pp.

Pertot, I., Fiamingo, F., Tizianel, A., Fratton, S. and Elad, Y. 2009. Effect of the timing of applications of control agents on *Podosphaera aphanis* and effect of fungicides on the survival of biocontrol agents on strawberry leaves. *Acta Horticulturae*, **807**: 33–738.

Raguchander, T., Prabakar, K. and Samiyappan, R. 2005. Field evaluation of *Pseudomonas fluorescens* and *Bacillus subtilis* on the management of *Cercospora* leaf spot and powdery mildew in urdbean. *Legume Research*, **28**: 137–139.

Romero, D., Vicente, A de., Zeriouh, H., Cazorla, F. M., Fernandez-Ortuno, D., Tores, J. A. and Perez-Garcia, A. 2007. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. *Plant Pathology*, **56**: 976–986.

Schilder, A. M. C., Gillett, J. M., Sysak, R. W. and Wise, J. C. 2002. Evaluation of environmentally friendly products for control of fungal diseases of grapes, pp. 163–167. In: *Proceedings of the10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit Growing and Viticulture,* 4–7 Feb., Weinsberg, Germany.

Wittmann, J. and Schonbeck, F. 1996. Studies of tolerance induction in wheat infested with powdery mildew or aphids. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **103**: 300–309.

Zhang, J. and Dou, H. 2002. Evaluation of *Bacillus subtilis* as potential biocontrol agent for postharvest green mold control on 'valencia' orange. *Proceedings of the Florida State Horticulture Society*, **115**: 60–64.