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Assessment of a novel strain of *Bacillus* against powdery mildew of grapes

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Powdery mildew is a major constraint in grape production, requiring sustainable alternatives to chemical fungicides. The present study evaluated the biochemical traits, fungicide compatibility, and field bio-efficacy of *Bacillus subtilis* DR39 for the management of grape powdery mildew. Biochemical characterization revealed that *B. subtilis* DR39 lacked indole production, phosphate solubilization, and siderophore activity, but displayed strong extracellular hydrolytic enzyme production, including cellulase, protease, and lipase, indicating its antagonistic mode of action. The strain also demonstrated surface-associated biofilm formation, supporting its colonization and persistence on plant surfaces. *In vitro* compatibility assays showed that *B. subtilis* DR39 was compatible with most commonly used fungicides, including strobilurins, triazoles, SDHIs, sulfur, and several ready-mix formulations, while incompatibility was observed with copper-based and certain multi-site fungicides. Field evaluations conducted across three locations (Warkheda, Nashik; ICAR-NRCG,Pune and Theni, Tamil Nadu) demonstrated that *B. subtilis* DR39 applied at 2.0 and 3.0 g (mL)/L significantly reduced powdery mildew severity on leaves and bunches, achieving 72–76% disease control with statistically comparable yields. The 2.0 g (mL)/L dose was found to be as effective as the higher dose, signifying optimal performance at a lower input level. The study confirmed that *B. subtilis* DR39 is an effective and economical biocontrol agent that can be successfully integrated into Good Agricultural Practices (GAP) integrated disease management programs for sustainable control of grape powdery mildew.

Keywords : *Bacillus subtilis*, biocontrol agent, fungicide, grape, powdery mildew

INTRODUCTION

Grape (*Vitis vinifera* L.) is a key commercial fruitcropcultivatedworldwide with a global productionof 28.87 million metric tonnes in 2024-2025. As reported by the National Horticulture Board, India exported around 343,982 tons of grapes valued at Rs 3.461 billion during 2023-24 (Anonymous, 2024).

Maharashtra accounted for 96% of the exports, contributing 324,441 tons worth Rs 3.395 billion (Anonymous, 2024). Although grape is an essential commodity for the economy of the country, it faces numerous challenges due to both biotic and abiotic factors. Disease incidence causes substantial losses, often requiring multiple pesticide applications which accounts for 30% to 35% of the total production cost (Anonymous, 2023). Powdery mildew (causal agent: *Erysiphe necator*) hadresulted in considerable reduction in economy of grape industry (McDaniel *et al.* 2023) although several other diseases like downy mildew, anthracnose, bacterial leaf spot are prevelant as well (Bhosale *et al*. 2024).

Application of fungicides is one of the most effective and widely recommended methods for disease control.Due to economic, environmental and resistance-related concerns associated with chemical fungicides, biocontrol agents have emerged as sustainable substitutes in integrated disease management (IDM) (Harman *et al.* 2004). In viticulture, the biocontrol agents used largely include*Trichoderma* sp., *Bacillus subtilis*, *Bacillus*

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licheniformis, *Pseudomonas fluorescens* and entomopathogens such as *Verticillium lecanii*, *Beauveria bassiana* and *Paecilomyceslilacinus*. They inhibited the pests through the combined processes of antagonism, competition, and parasitism (Suryawanshi*et al.,* 2018). They are also responsible for targeted pest control while reducing harm to non-target organisms, thereby safeguarding the ecosystem. However their effectiveness as standalone treatments might be a constraint under of high pest pressure (Chandler *et al.,* 2011).Therefore, combining biopesticides with chemical pesticides had become an increasingly popular approach to improve overall pest management effectiveness, and is in congruence with biointensive disease management strategies.

Bacillus subtilis is a proven biological control agent for the management of diseases caused by fungal pathogens in grapes (Sawant *et al.* 2016). In earlier studiesit was also found that *Bacillus* DR-39 can also influence degradation kinetics and reduce the half-lives of different classes of pesticides, like profenofos which is an organophosphate insecticide, carbendazim which is a benzimidazole fungicide and myclobutanil, tetraconazole and flusilazole which belong the triazole group of fungicides (Salunkhe *et al*. 2015) *in vitro*and *in vivo*. The mechanism of antagonistic activity of *Bacillus* species is comprised of production of toxic secondary metabolites and lytic enzymes.Keeping in view the growing importance of biointensive disease management strategies, the present study was undertaken to evaluate a novel strain, *Bacillus subtilis* DR39 against powdery mildew of grapes. In addition to evaluating field-level bio-efficacy, the study also focused on the compatibility of *B. subtilis* with chemical fungicides registered in grape (Anonymous, 2025) as well as biochemical characterization of the strain.

MATERIALS AND METHODS

Bacterial culture

The novel strains, *B. subtilis* DR39 (Accession No. NAIMCC- SD-0009) was collected from the repository of ICAR-National Research Centre for Grapes, Pune. The cultures were maintained on nutrient agar slants (NA, M002, HiMedia) at 4°C and were retrieved and grown on NA plates as and when required.

Biochemical characterisation of B. subtilis DR39

Indole production test

Four mL of sterile tryptone broth (1%) was inoculated with a loopful of test organism and incubated at 37°C for 48h. An uninoculated tube was taken as a control. After incubation, 1 mL of Kovac's reagent was added and observed for the formation of cherry red coloured ring which denoted the positive result (Vashist *et al.*2013).

Phosphate solubilization

Sterile Pikovskaya's agar plates supplemented with tricalcium phosphate were streaked with the bacterial isolates and incubated at 37°C for 10 days. Clear zone of phosphate solubilization around the growth was analysed (Tariq *et al.* 2018).

Lipase production test

Bacteria was streaked on sterile Tributyrin agar (TBA) plates and incubated at 37°C for 24h. Lipolytic activity was observed by visualizing the zone around the bacterial colony (Shaini and Jayasree, 2016).

Casein hydrolysis test or protease test

Bacteria were streaked on sterile skim milk agar plates and were incubated at 37°C for 24h. Zone of hydrolysis was observed for proteolytic activity (Vashist *et al.*2013).

Cellulase test

Bacteria were streaked on sterile carboxymethyl cellulose agar plates and were incubated at 37°C for 24 h. Zone of hydrolysis was observed for cellulase enzyme activity (Vashist *et al.* 2013).

Siderophore production test

Chrome azurol sulphonate agar medium was used to identify siderophore production by

selected bacterial strains. After 5 days of incubation at 28°C, the change in medium color from green to yellow indicates positive results (Shaini and Jayasree, 2016).

Chitinolytic activity

The selective media for isolation of chitinolytic bacteria was prepared following, containing g/L: colloidal chitin 1%(W/V): Na_2HPO_4, 6.0; K_2HPO_4, 1; NH_4Cl, 0.5; KH_2PO_4 3; $MgSO_4.7H_2O$ 0.12; NaCl, 0.5; yeast extract 0.05; agar 15 and at (pH 7) and autoclaved at 121°C for 15 min. The sterilized medium was poured into sterilized plates and cooled to room temperature and inoculated with 100 µL of the 10^{-4} to 10^{-7} dilutions of soil suspension by pour plating techniques and incubated at 37°C for 4 days. The chitin-degrading bacteria were selected based on clean diameter after 72 h of incubation.

Quantification of biofilm using crystal violet assay

Bacteria was grown overnight in nutrient broth (NB). The growth was diluted to get absorbance of 0.02 at 600nm. The bacterial suspension was transferred to a 96-well polystyrene plate, and160 µL of broth was added to each well. The plate was incubated at 28 ± 1°C for 48 h. After incubation the broth was removed from the well gently and washed twice with sterile distilled water followed by resting the plate for 30 min. The growth was then stained by 0.1% crystal violet solution and the plate was incubated at 28 ± 1°C for 20 min. The stain was drained off and 70% ethanol was added to each well. The intensity of crystal violet was measured at 590 nm on ELISA plate reader.

Compatibility studies of Bacillus subtilisDR39 withfungicide under in vitro conditions

The study was conducted under laboratory conditions at ICAR-National Research Centre for Grapes, Pune with *B. subtilis* DR39 and fungicides mentioned in Annexure 5 published by ICAR-NRCG, Pune (Anonymous, 2025). These fungicides are registered against particular diseases of grapes by The Central Insecticides Board and Registration Committee (CIB&RC), Government of India.All treatments were replicated thrice and laid out in completely randomized design (CRD).The compatibility of bacteria with pesticides was determined by following well diffusion method. Recommended concentrations for each fungicide were prepared. NA plates were prepared and swabbed with bacterial culture. Wells were prepared on NA plates embedded with bacteria using cork borer. Further 100 µL of fungicide concentrations were poured into the wells. Well with water was considered as a control. All the plates were incubated at 27±2°C for 72 hours and subsequently examined for the radial growth of the bacterial culture.

Field evaluation of B. subtilis DR39 against powdery mildew

Inoculum preparation and field treatments

B. subtilis was inoculated in 200 mL of NB with loopful of 24-h-old culture grown on NA medium. Broth was incubated at 28±1°C on rotatory shaker with 150 rpm for 72h. After incubation, the broth was centrifuged at 3000 rpm for 10 min. Discarding the supernatant, the pellets were washed thrice with sterile distilled water (SDW) and finally re-suspended in 5 mL SDW. Suspension was diluted with SDW containing 10 µL of Tween 80 to adjust the count at 1×10^8 CFU/mL.

The bacterial suspension was standardized to 1×10^8 CFU/mL prior to field application. Treatments consisted of application of B. subtilis DR39 at 1, 2 and 3 mL/L, corresponding to approximately 1×10^8, 2×10^8 and 3×10^8 CFU/L of spray solution, respectively, for the management of grape powdery mildew. The field trial was carried out invineyard spaced at 300 cm×180 cm and trained on extended Y trellis in a field at three different locations (Table. 1) during two consecutive seasons (2016-17 and 2017-18). Sulphur 80% and commercial formulation of *B. subtilis* (Mildown) were used as standard check fungicides at their recommended doses. Untreated control should be sprayed with water. Volume of water used was 1000L/Ha. To avoid spray drift to neighboring plots, sprayings were carried out with knapsack sprayers. Field

experiment was conducted using randomized block design with five replications, each consisting of two treatment vines surrounded by guard vines.

Assessment of powdery mildew in the field

Powdery mildew incidence on leaves was noted visually adopting the 0-4 scale (Horsfall and Heuberger 1986) and percent disease index (PDI) was calculated by following the formulae of McKinney (1923). All data obtained were subsequently analyzed statistically after PDI data of both seasons were pooled for convenience and significant interpretation.

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

Observation was recorded in terms of disease rating from 0-4, where 0=no symptom, 1=trace to 25%, 2=26-50%, 3=51-75% and 4= more than 75% symptom. Disease ratings were recorded from ten leaves on randomly selected canes, with ten canes assessed per vine, resulting in a total of 100 observations per replicate. The experiment included four replications. Only actively developing powdery mildew lesions were considered for disease assessment.The marketable yield from all the treatments was recorded at harvest and expressed in Kg /vine and further deduced to yield in t/ha basis.The percent disease control (PDC) was tabulated using the formula of (Vincent, 1947)

I= C-T/C ×100

Where,

I=percent disease control; C=PDI in untreated control; T= PDI in fungicide treatment

Statistical Analysis

The PDI data was transformed by using arcsine transformation for leaves and analyzed statistically following Randomized Block Design (RBD) using WASP 2.0 (Central coastal Agricultural Research Institute). All data obtained were subsequently analyzed statistically.

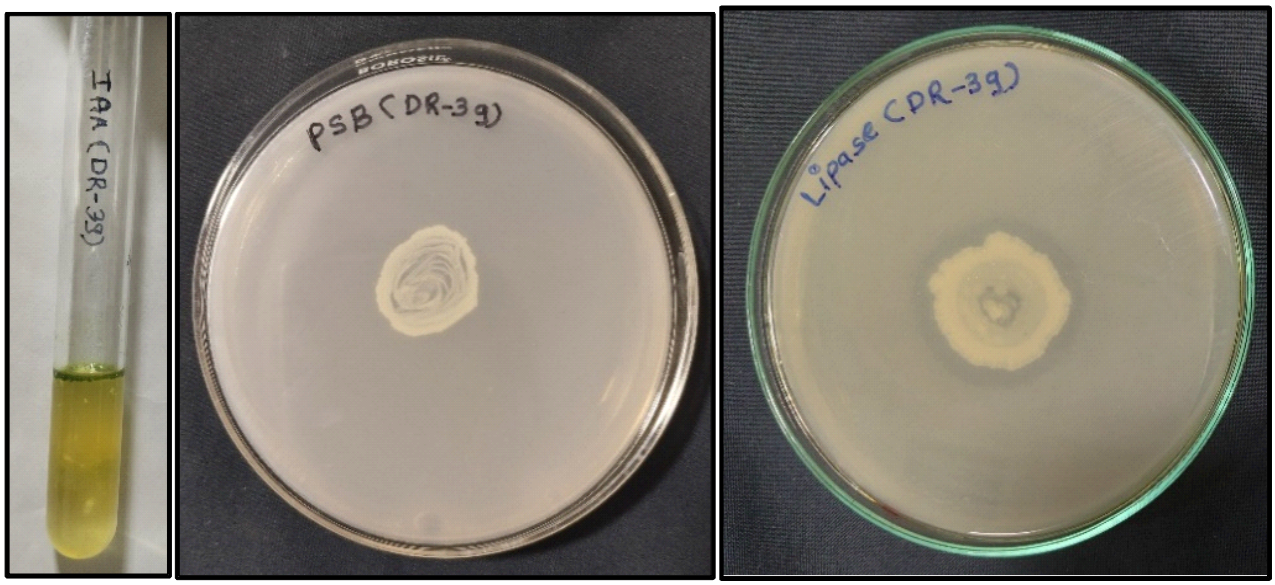
RESULTS AND DISCUSSION

Biochemical test of Bacillus subtilis DR39

The biochemical characterization of *Bacillus subtilis* DR39 revealed a distinct metabolic and enzymatic profile indicative of its functional role in microbial antagonism. The isolate tested negative for indole production and phosphate solubilization, suggesting the absence of tryptophan degradation and inorganic phosphate mobilization. Siderophore production was not detected, which disclosed that iron chelation was not associated with this strain. *B. subtilis* DR39 manifested strong extracellular enzymatic activity, with positive reactions for cellulase, protease, and lipase production. These hydrolytic enzymes are widely recognized as critical components of antagonistic mechanisms involved in bacteria (Bhosale *et al.* 2026). Cellulases and proteases contribute to the degradation of fungal cell wall polysaccharides and structural proteins, while lipases disrupt lipid components of pathogen membranes. Similar enzymatic profiles have been reported in several biocontrol strains of *Bacillus*, highlighting enzyme-mediated antagonism as a major factor in disease inhibition (Rahman *et al.* 2017). The absence of indole production, phosphate solubilization, and siderophore activity suggested that *B. subtilis* DR39 did not have any plant growth–promoting mechanisms. Instead, its primary mode of action appeared to be direct antagonism against phytopathogens. This observation was parallel with previous studies reporting that *Bacillus* strains depended predominantly on extracellular enzyme secretion rather than nutrient mobilization or phytohormone production to achieve biocontrol efficacy (Zhang *et al.* 2010; Rahman *et al.* 2017).It was observed that *B. subtilis* DR39 produced clear zone around the colony indicating positive chitinolytic activity. The finding of this study was analogous with the previous reports that the clear zone surrounding the colony shows chitinase activity to break down chitin compounds in the medium (Gonfa *et al.* 2023). In the study *B. subtilis* DR39 produced surface-associated biofilms which is a crucial trait for effective colonization and persistence on plant surfaces, as it enhances bacterial survival and stability

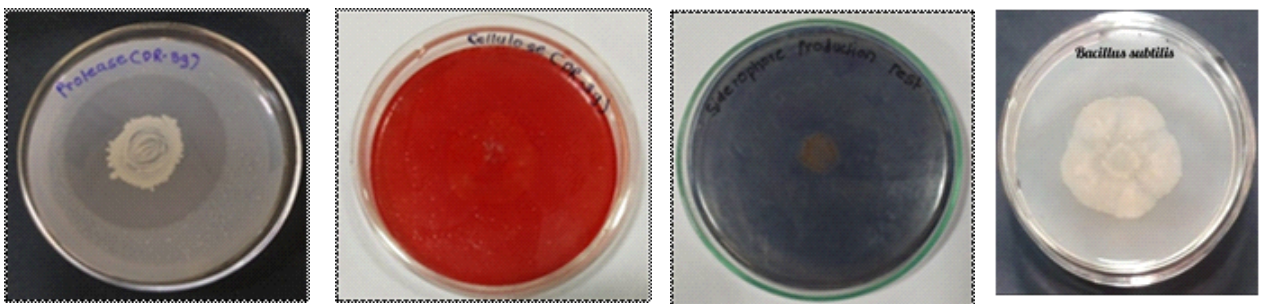
Table 1: Details of locations for trials of *B. subtilis* DR39 against powdery mildew of grapes

State	District	Variety	Village Position Name	Latitude (N)	Longitude (E)	Mean Sea Level (meters)
Maharashtra	Nashik	Manik Chaman Seedless	Warkheda	20.234	73.893	700
Maharashtra	Pune	Manik Chaman Seedless	ICAR-NRCG	18.492	73.986	559
Tamil Nadu	Theni	Black panner-Black muscut	Theni	10.011	77.478	300



IAA test Phosphate solublizing test Lipase test

Fig. 1: Biochemical characterization of *Bacillus subtilis* DR39



Protease test Cellulase test Siderophore production test Chitinolytic activity

Table 2 : Compatibility of *B. subtilis* DR39 with different registered powdery mildew fungicides

Chemicals	Results	Sr no.	Chemicals	Results
Amisulbrom 17.7% SC w/w (20% SC w/v)	Compatible	31	Metrafenone 50% SC	Compatible
Azoxystrobin 23 SC	Compatible	32	Myclobutanil 10 WP	Compatible
Captan 50 % WP	Compatible	33	Penconazole 10 EC	Compatible
Cyazofamid 34.5% SC	Compatible	34	Polyoxin D zinc salt 5% SC	Compatible
Dimethomorph 50 WP	Compatible	35	Sulfur 55.16 SC	Compatible
Fosetyl Al 80 WP	Compatible	36	Sulfur 80% WDG	Compatible
Kresoxim methyl 44.3 SC	Compatible	37	Sulphur 58.5% w/w + Azoxystrobin 4.2% w/w SC	Compatible
Mancozeb 75 WP	Compatible	38	Tetraconazole 3.8 EW	Compatible
Mandipropamid 23.4% SC	Compatible	39	Azoxystrobin 25 % + Boscalid 35 % WG	Compatible
Metiram 70% WG	Compatible	40	Boscalid 25.2% + Pyraclostrobin 12.8% w/w WG	Compatible
Ametoctradin 27 + Dimethomorph 20.27 SC	Compatible	41	Fluopyram 200 + Tebuconazole 200 SC	Compatible
Azoxystrobin 8.3% + Mancozeb 66.7% WG	Compatible	42	Fluxapyroxad 25% + Pyraclostrobin 25% SC	Compatible
Azoxystrobin 11 % + Tebuconazole 18.3% w/w	Compatible	43	Fluxapyroxad 75 g/L + Difenoconazole 50g/L SC	Compatible
Benalaxyl-M 4% + Mancozeb 65% WP	Compatible	44	Tebuconazole 50% + Trifloxystrobin 25% WG	Compatible
Cymoxanil + Mancozeb 8 + 64 WP	Compatible	45	Carbendazim 50 WP	Compatible
Dimethomorph 12% + Pyraclostrobin 6.7% WG	Compatible	46	Thiophanate methyl 70 WP	Compatible
Famoxadone 16.6 % + Cymoxanil 22.1 % SC	Compatible	47	Carbendazim 12% + Mancozeb 63% WP	Compatible
Fenamidone + Mancozeb 10 + 50 WG	Compatible	48	Kasugamycin 5% + Copper Oxychloride 45% WP	Non-Compatible
Fluopicolide 4.44% + Fosetyl-Al 66.67% WG	Compatible	49	Pyraclostrobin 5% + Metiram 55% 60 WG	Non-Compatible
Kresoxim methyl 18% + Mancozeb 54% WP (72 % WP)	Compatible	50	Oxathiapiprolin 4.61% w/w + Amisulbrom 23.08% SE	Non-Compatible
Metiram 44% + Dimethomorph 9% WG	Compatible	51	Oxathiapiprolin 0.6% w/w + Mancozeb 60% w/w WG	Non-Compatible
Oxathiapiprolin 3% + Mandipropamid 25% w/v (280 SC)	Compatible	52	Metalaxyl + Mancozeb 8 + 64 WP	Non-Compatible
Picarbutrazox 9.53% SC	Compatible	53	Metalaxyl-M + Mancozeb 4 + 64 WP	Non-Compatible
Valifenalate 6 % + Mancozeb 60% WG	Compatible	54	Iprovalicarb + Propineb 5.5 + 61.25 WP	Non-Compatible
Bupirimate 25% w/v (26.7% w/w) SC	Compatible	55	Copper Sulphate 47.15% + Mancozeb 30% WDG	Non-Compatible
Cyflufenamid 5% EW	Compatible	56	Copper Sulphate Pentahydrate 23.99% SC	Non-Compatible
Difenoconazole 25 EC	Compatible	57	Propineb 70 WP	Non-Compatible
Flusilazole 40 EC	Compatible	58	COC 50 WP	Non-Compatible
Hexaconazole 5 EC	Compatible	59	Copper hydroxide 53.8 DF	Non-Compatible
Meptyldinocap 35.7% EC	Compatible	60	Copper hydroxide 61.41% WG	Non-Compatible

Table 3 : Bio-efficacy of *B. subtilis* DR39against powdery mildew of grapes on leaves at Warkheda, Nashik

Tr. No.	Treatment	Dose (g or mL/L)	PDI of powdery mildew of leaves			Percent disease control	PDI of powdery mildew of Bunches			Percent disease control	Pooled yield
			2016-17	2017-18	Pooled data		2016-17	2017-18	Pooled data		
T1	*B. subtilis* DR39	1.0	9.13 (17.56)c	9.63 (18.06)c	9.69 (18.13)c	68.12 (55.64)c	9.69 (18.11)c	10.94 (19.28)c	10.63 (19.00)c	64.81 (53.66)c	9.96c
T2	*B. subtilis* DR39	2.0	7.44 (15.82)b	7.94 (16.36)b	7.69 (16.09)b	74.72 (59.83)b	8.13 (16.52)b	8.75 (17.18)b	8.44 (16.86)b	72.05 (58.13)b	12.57b
T3	*B. subtilis* DR39	3.0	6.94 (15.27)ab	7.44 (15.82)ab	7.19 (15.55)ab	76.35 (60.92)ab	6.88 (15.16)ab	8.13 (16.53)ab	7.50 (15.86)ab	75.20 (60.20)ab	13.40ab
T4	Sulphur 80WDG	2.0	6.19 (14.39)a	6.69 (14.97)a	6.44 (14.68)a	78.89 (62.67)a	6.25 (14.44)a	6.56 (14.80)a	6.41 (14.62)a	78.76 (62.63)a	13.94a
T5	Commercial formulation of *B. subtilis* (Mildown)	2.5	9.44 (17.88)c	9.94 (18.37)c	9.38 (17.81)c	69.25 (56.34)c	10.00 (18.42)c	11.25 (19.56)c	10.31 (18.71)c	66.02 (54.37)c	10.38c
T6	Untreated control	-	30.00 (33.20)d	31.00 (33.83)d	30.50 (33.51)d	0.0 (0.0)d	28.75 (32.42)d	31.88 (34.37)d	30.31 (33.40)d	0.0 (0.0)d	5.16d
	CD (*P = 0.05*)		1.22	1.19	1.21	2.18	1.47	2.02	1.67	3.26	0.97

Table 4 : Bio-efficacy of *B. subtilis* DR39against powdery mildew of grapes on leaves at ICAR-NRCG

Tr. No.	Treatment	Dose (g or mL/L)	PDI of powdery mildew of leaves			Percent disease control	PDI of powdery mildew of Bunches			Percent disease control	Pooled yield
			2016-17	2017-18	Pooled data		2016-17	2017-18	Pooled data		
T1	*B. subtilis* DR39	1.0	12.75 (20.91)c	10.13 (18.55)c	11.72 (20.01)c	69.55 (56.53)c	13.44 (21.48)c	11.56 (19.86)c	12.81 (20.97)c	66.23 (54.48)c	11.26c
T2	*B. subtilis* DR39	2.0	10.75 (19.13)b	8.88 (17.32)b	9.81 (18.25)b	74.60 (59.74)b	10.94 (19.29)b	10.00 (18.42)b	10.47 (18.87)b	72.74 (58.54)b	13.47b
T3	*B. subtilis* DR39	3.0	10.25 (18.67)ab	8.44 (16.88)ab	9.34 (17.79)ab	75.76 (60.53)ab	10.31 (18.69)ab	9.38 (17.82)ab	9.84 (18.27)b	73.90 (59.29)b	14.46ab
T4	Sulphur 80WDG	2.0	9.06 (17.51)a	7.75 (16.15)a	8.41 (16.84)a	78.24 (62.21)a	9.38 (17.82)a	8.13 (16.55)a	8.75 (17.21)a	77.72 (61.84)a	15.17a
T5	Commercial formulation of *B. subtilis* (Mildown)	2.5	13.06 (21.16)c	10.69 (19.07)c	11.59 (19.89)c	70.02 (56.82)c	13.75 (21.76)c	11.88 (20.15)c	12.50 (20.70)c	67.57 (55.29)c	11.77c
T6	Untreated control	-	40.13 (34.75)d	37.00 (37.46)d	38.56 (38.39)d	0.00 (0.0)d	42.19 (39.41)d	35.00 (36.27)d	38.59 (34.41)d	0.00 (0.0)d	5.08d
	CD (*P = 0.05*)		1.51	1.17	1.29	2.18	1.42	1.31	1.00	1.36	1.08

under environmental stress conditions. Biofilms also facilitated sustained production of antagonistic metabolites and enzymes, thereby contributing to consistent biocontrol performance in field conditions (Fessia*et al,* 2022).

Compatibility of Bacillus subtilis against different registered powdery mildew fungicides

The compatibility of *Bacillus subtilis* DR39 with commonly used fungicides in grape disease management was evaluated under *in vitro* conditions (Table 2). The results indicated that the biocontrol agent was compatible with the majority of the tested fungicides, encompassing systemic, contact, and combination formulations. Complete compatibility was observed with fungicides such as azoxystrobin, kresoxim methyl, mandipropamid, dimethomorph, fosetyl-Al, fluopyram, fluxapyroxad, carbendazim, thiophanate methyl, sulfur formulations, and several combination products, as evidenced by the absence of inhibition zones. These findings indicate that these fungicides do not significantly interfere with bacterial growth or viability, supporting their potential co-application with *B. subtilis* DR39 in disease management programs

Table 5 : Bio-efficacy of*B. subtilis* DR39bio-formulation against powdery mildew of grapes on leaves at Theni, Tamilnadu

Tr. No.	Treatment	Dose (g or mL/L)	PDI of powdery mildew of leaves			Percent disease control	PDI of powdery mildew of Bunches			Percent disease control	Pooled yield
			2016-17	2017-18	Pooled data		2016-17	2017-18	Pooled data		
T1	*B. subtilis* DR39	1.0	8.69 (17.13)c	9.75 (18.19)c	9.59 (18.03)c	65.67 (54.16)c	9.06 (17.49)c	10.00 (18.42)c	9.84 (18.23)c	64.76 (53.62)c	10.29c
T2	*B. subtilis* DR39	2.0	6.94 (15.27)b	8.13 (16.56)b	7.53 (15.93)b	73.03 (58.73)b	7.19 (15.51)b	8.13 (16.50)b	7.66 (16.02)b	72.65 (58.50)b	11.70b
T3	*B. subtilis* DR39	3.0	6.38 (14.62)ab	7.56 (15.96)ab	6.97 (15.30)ab	75.06 (60.07)ab	6.88 (15.12)ab	7.81 (16.20)ab	7.34 (15.68)ab	73.46 (59.09)ab	12.56ab
T4	Sulphur 80WDG	2.0	5.75 (13.87)a	7.00 (15.34)a	6.38 (14.63)a	77.24 (61.51)a	5.63 (13.61)a	6.56 (14.80)a	6.09 (14.23)a	78.03 (62.17)a	13.28a
T5	Commercial formulation of *B. subtilis* (Mildown)	2.5	9.00 (17.43)c	10.19 (18.60)c	9.22 (17.67)c	67.12 (55.02)c	9.38 (17.78)c	10.31 (18.66)c	9.53 (17.97)c	65.70 (54.17)c	10.58c
T6	Untreated control	-	27.00 (31.30)d	29.00 (32.58)d	28.00 (31.94)d	0.00 (0.00)d	27.19 (31.41)d	28.44 (32.21)d	27.81 (31.82)d	0.00 (0.0)d	5.49d
	CD (*P = 0.05*)		1.18	1.11	1.14	2.16	1.84	1.65	1.70	3.54	0.80

(Rajesh *et al.,* 2023). In contrast, partial incompatibility was recorded with certain copper-based and multi-site fungicides. Copper oxychloride, copper hydroxide, copper sulphate based formulations and some fungicide combinations containing mancozeb, propineb, or metiram exhibited varying degrees of growth inhibition. The incompatibility with copper-based fungicides were similar with earlier reports exhibiting the non-selective antimicrobial nature of copper compounds, which can negatively affect beneficial microorganisms (Lamichhane *et al.* 2018).

The high compatibility of *B. subtilis* DR39 with aforementioned fungicides suggested that these fungicides can be safely used alongside the biocontrol agent without compromising its activity. Similar observations had been reported previously, where strobilurin- and sulfur-based fungicides exerted minimal adverse effects on *Bacillus* spp., likely due to their specific modes of action targeting fungal respiration or surface activity rather than bacterial metabolism (Harsha *et al.*, 2023). The inhibitory effects of fungicides containing mancozeb, propineb or metiram might be due to their broad-spectrum activity against bacteria (Rajkumar *et al.*, 2018).Future studies may explore the use of *Bacillus subtilis* DR39 in microbial consortia with other compatible biocontrol agents such as *Trichoderma* spp. and *Pseudomonas fluorescens*. Such consortium-based approaches may enhance disease suppression through complementary mechanisms including antibiosis, competition, induced resistance, and improved rhizosphere/ phyllosphere colonization.

Bio-efficacy of Bacillus subtilis DR39 against powdery mildew of grapes at different locations

At Warkheda, Nashik, the pooled PDI of powdery mildew on leaves under *B. subtilis* DR39 @ 2.0 g/L was 7.69%, while the 3.0 g/L dose (T3) recorded 7.19%. Similarly, pooled PDI on bunches was 8.44% and 7.50% under T2 and T3, respectively. The corresponding percent disease control ranged from 74.72–76.35% on leaves and 72.05–75.2% on bunches, with no significant difference between the two doses. The pooled yield under these treatments (12.57 t/ha and 13.4t/ ha, respectively) was also statistically comparable, indicating similar field performance (Table 3).

A similar trend was observed at ICAR-NRCG, where pooled leaf PDI values of 9.81% (T2) and 9.34% (T3) and bunch PDI values of 10.47% (T2) and 9.84% (T3) were recorded. The percent disease control under both doses ranged from 74.6–75.76% on leaves and 72.74–73.9% on bunches, further confirming their comparable efficacy. The pooled yields of 13.47 t/ha (T2) and

14.46 t/ha (T3) did not differ significantly, suggesting that increasing the dose beyond 2.0 g/L did not result in a proportionate yield advantage (Table4).

At Theni, Tamil Nadu, *B. subtilis* DR39 @ 2.0 g/L recorded pooled PDI values of 7.53% on leaves and 7.66% on bunches, while the 3.0 g/L dose recorded 6.97% and 7.34%, respectively. Percent disease control under these treatments was 73.03–75.06% on leaves and 72.65–73.46% on bunches, with statistically similar pooled yields of 11.7 t/ha and 12.56 t/ha, reinforcing the consistency of results under diverse agro-climatic conditions (Table5).

In contrast, *B. subtilis* DR39 @ 1.0 g (mL)/L consistently recorded higher PDI values, lower disease control, and reduced yields across all locations, indicating sub-optimal efficacy at the lower dose. The commercial *B. subtilis* formulation (Mildown) was also inferior to DR39 at comparable doses, highlighting the strain-specific superiority of DR39. Although the check fungicide, Sulphur 80 WDG, recorded the lowest disease intensity and highest yield across locations, the performance of *B. subtilis* DR39 @ 2.0 mL/L and 3.0 mL/L closely approached the chemical standard, emphasizing its potential as an effective biological alternative.

Overall, the results unequivocally illustrated that *Bacillus subtilis* DR39 @ 2.0 g (mL)/L is as effective as 3.0 g (mL)/L in managing powdery mildew of grapes. Considering reduction in disease, yield response, and input optimization, the 2.0 g mL/L dose emerged as the most efficient and economical biological option for powdery mildew management across locations. The observed reduction in disease severity was accompanied by a corresponding increase in yield, indicating that effective disease reduction directly translated into economic benefits (Sawant *et al*. 2019). The results align with previous studies demonstrating that *Bacillus* based bio-formulations can effectively manage foliar diseases when applied preventively or under favourable environmental conditions (Seenivasagan *et al.* 2021).

CONCLUSION

The findings indicated that *B. subtilis* DR39 possessed strong antagonistic potential driven by hydrolytic enzyme activity. It exhibited broad compatibility with commonly used fungicides, and provided effective field-level control of grape powdery mildew. Its integration into GAP, particularly in combination with compatible fungicides and appropriate spray scheduling, could contribute to reduced chemical inputs and sustainable grape disease management under diverse production systems.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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