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Bacillus velezensis: Potential bioagent for the management of *Sclerotinia sclerotiorum*

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Abstract
Sclerotinia sclerotiorum, a broad host pathogen, causes humungous losses in several crops. The limited availability of resistant sources against the pathogen makes its management difficult. Biocontrol agent offers environment friendly approach for the suppression of the pathogen. Microbes like *Trichoderma*, *Bacillus* and *Pseudomonas* spp, have been successfully utilized for their antagonistic effect against the pathogen in several studies. This study emphasized on the use of some microbial formulations against the pathogen and their methods of application. Six bioformulations were evaluated using soil drenching and seedling dip method of applications. Amongst all the tested formulations, the liquid formulation consisting of *Bacillus velezensis*, *Bacillus subtilis* subsp. *subtilis* & *Bacillus subtilis* subsp. *spizizenii* (Kush Vardan-1) was found to be most affective and showed minimum disease incidence of *Sclerotinia sclerotinia* on cauliflower. On comparing the methods of application seedling dip method was observed to be more effective in minimizing disease incidence for all the bioformulations.

Keywords: *Sclerotinia sclerotiorum*, microbial, management, pot experiment, disease incidence

Introduction
Sclerotinia sclerotiorum (Lib.) de Bary is among the world's most virulent and widespread plant pathogen. The fungus is widely distributed throughout temperate, tropic, and arid region (Lehner *et al*., 2017) [11]. Diseases, named by numerous terms such as cottony rot, white mold, drop, cottony soft rot, bloom blight, stem rot, crown rot, and Sclerotinia rot (Jahan *et al*., 2022) [10]. White mold is widely used term for the disease caused by the pathogen. The mycelium of the pathogen, *S. sclerotiorum* has the ability to infect all plant parts. Critically, for any particular host this fungus does not have specificity and infects over a few hundred monocotyledonous and dicotyledonous plant species, including some economically important crops, flowers, and weeds. *S. sclerotiorum* possess a threat to crops such as sunflower, soybean, oilseed rape, edible dry bean, chickpea, peanut, dry pea, lentils and various vegetables (Bolton *et al.*, 2006) [3]. Yield losses in oilseed rape due to Sclerotinia stem rot have been reported to be as high as 80% in China (Wang *et al*., 2009) and 75% in Nepal (Chaudhary, 1993) [4, 17]. In the United Kingdom, oilseed rape invasion by *S. sclerotiorum* results in a 50% yield loss (Pope *et al*., 1989) [13], while in Germany, it caused 20–30% yield loss (Dunker and Tiedemann, 2004) [6].

The vast host range of the pathogen, its omnipresent nature and severe losses caused by the pathogen in several host crops makes it a pathogen of serious concern. Several chemical methods have proved to be effective for the management of the pathogen (Bhatt and Sharma, 2024) [2]. Some of the recent studies have also shown the efficacy of biochar for the management of the pathogen (Bhatt and Sharma, 2022) [1]. Biological control agents (BCAs) provide an effective alternative means for controlling *S. sclerotiorum* (Mao *et al*., 1997) [12]. The BCAs *Coniothyrium minitans* (Yang *et al*., 2010) [19], *Ulocladiumatrum* (Huang and Erickson, 2007) [8], *Trichoderma* spp. (Inbar *et al*., 1996) [9], and *Pseudomonas* spp. (Savchuk and Fernando, 2004) [14] have reportedly been effective in controlling Sclerotinia diseases. As antagonists, *Bacillus* spp. have also been reported to be effective for the biocontrol of multiple soil-borne plant diseases (Sharma *et al*., 2009) [15], including *S. sclerotiorum*. *B. subtilis* strain SB24 significantly reduced the mycelial growth of *S. sclerotiorum* and suppressed sclerotia formation on the surface of canola leaves (Zhang and Xue, 2010) [20]. The management of pathogen can be effectively done by bioagents but the method and time of application of the bioagent also plays an important role to suppress the pathogen activity. A very studies emphasize on the methods of application, thus the study was conducted to find out the most appropriate method of application of bioagent. The foliar application of *Bacillus amyloliquefaciens* strains has shown to be a promising strategy for the management of sclerotinia stem rot in soybean plants (Wu *et al*., 2014) [18].

Material and Methods
The culture of *Sclerotinia sclerotiorum*, was maintained at 20±2° C (Fig1). Six bacterial antagonist formulations were procured from NABIM, MAU (Table 1). The bioagents were screened using a pot experiment using completely randomized design (CRD). Thirty nine pots of size 16×16 cm size each were filled with soil. In thirty nine pots, the mycelium of *Sclerotinia sclerotiorum*, was mixed thoroughly at the depth of 7 cm in pots. After 24 hours of innoculation, four Cauliflower seedlings were transplanted in each pot. For all the bioagents, two methods of application of the bioagents were used: Soil drenching method and seedling dip method. In soil drenching method, 10 ml of bioformuation (2%) was drenched in the pots. The control pot was drenched with water without any bioagent. While, in seedling dip method, roots of the seedling were dipped in the bioformulations (2%) for 30 minutes before transplanting (Fig2). The control was maintained by dipping the seedlings in water. The disease incidence (%) was observed after 30 and 60 days of inoculation and was calculated using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

Fig 1: Culture plate of *Sclerotinia sclerotiorum*

Fig 2: Cauliflower seedlings transplanted in pots innoculated with *Sclerotinia sclerotiorum*

Table 1: List of Bioformulations used against *Sclerotinia sclerotiorum*

Sr. no.	Trade Name	Bioformulation
1	Kush Eco Pesticide	Liquid bioformulation of *Pseudomonas fluorescens* PF-08
2	Kush Bio-Care 24	Liquid bioformulation of *Bacillus subtilis* RP-24
3	Kush Crop Care 24	Liquid bioformulation of *Bacillus amyloliquefaciens*B-16
4	Kush Vardan-1	Liquid bioformulation of *Bacillus velezensis*, *Bacillus subtilis* subsp. *subtilis*&*Bacillus subtilis* subsp. *spizizenii*
5	Bio-Pulse	Talc-based bioformulation of *Trichoderma asperellum*UBSTH-501and *Bacillus amyloliquefaciens*B-16
6	Green Fungicide	Talc-based bioformulation of *Trichoderma asperellum* UBSTH-501

Table 2: Treatment details and method of application

Treatment	Bio-agent	Method of application
T_1	Kush Eco Pesticide	Soil Drenching method
T_2	Kush Bio-Care 24	
T_3	Kush Crop Care 24	
T_4	Kush Vardan-1	
T_5	Bio-Pulse	
T_6	Green Fungicide	
T_7	Kush Eco Pesticide	Seedling dip method
T_8	Kush Bio-Care 24	
T_9	Kush Crop Care 24	
T_{10}	Kush Vardan-1	
T_{11}	Bio-Pulse	
T_{12}	Green Fungicide	
T_{13}	Control	

Results and Discussion

The results revealed that liquid bioformulation of (Kush Vardan-1) *Bacillus velezensis*, *Bacillus subtilis* subsp. *subtilis* & *Bacillus subtilis* subsp. *spizizenii* was most effective against the pathogen and showed minimum disease incidence in soil drenching method (50%) and no disease was observed in seedling dip method of application (Table 3). The Talc-based bioformulation of (Bio-Pulse) *Trichoderma asperellum* UBSTH-501 and *Bacillus amyloliquefaciens* B-16 (50%) also was effective against the pathogen and was at par with liquid bioformulation of (Kush Crop Care 24) *Pseudomonas fluorescens* PF-08 after 60 days of transplanting. Liquid bioformulation of (Kush Bio-Care 24) *Bacillus subtilis* RP-24 was found to be the least effective compared to all the other bioformulations.

On comparing the two method of application of bioagents, seedling dip method was found to be more effective then soil drenching method for all the bioformulations. This may be due to direct interaction of the microbial antagonists with roots of the seedlings. Seedling dip in the T_{10} formulation (Kush Vardan-1) *Bacillus velezensis*, *Bacillus subtilis* subsp. *Subtilis* & *Bacillus subtilis* subsp. *spizizenii* lead to no disease in plants after 30 and 60 days post innoculation. Treatment (T_{11}) with Biopulse formulation was also observed to prevent the plant from any disease after 30 days on innoculation but after 60 days 50 percent of disease incidence was observed which was least compared to other treatments.

Table 3: Efficacy of bioformulations and methods of application against *S. sclerotiorum*

Treatment	Drenching Method		Treatment	Seedling Dip Method	
	Disease incidence (%) (After 30 days)	Disease incidence (%) (After 60 days)		Disease incidence (%) (After 30 days)	Disease incidence (%) (After 60 days)
T_1	33.33	83.33	T_7	16.66	50.00
T_2	50.00	100.00	T_8	33.33	83.33
T_3	41.66	91.66	T_9	25.00	66.66
T_4	8.33	50.00	T_{10}	0.00	0.00
T_5	25.00	83.00	T_{11}	0.00	50.00
T_6	33.33	83.33	T_{12}	25.00	75.00
Control	75.00	100.00	Control	75.00	100.00
SE±(m)	0.27	0.27	SE±(m)	0.19	0.30
C.D. (5%)	0.84	0.84	C.D. (5%)	0.60	0.94

a) **b)**

Fig 3: Cauliflower seedlings a) Healthy seedling b) Seedlings infected by *Sclerotinia sclerotiorum*

The result were in accordance to Teixeira *et al*. (2021) [16] who showed that *Bacillus velezensis* had antagonistic activity against *Sclerotinia sclerotiorum*, *Macrophomina phaselonia*, *Botrytis cinerea*, and *Rhizoctonia solani*, and it reduces their mycelial growth by about 60%. *B. velezensis* 20507, can damage the membranes of *S. sclerotiorum* hyphae (Cheng et 2024) [5]. This results obtained might be due to induction of resistance in plants against the pathogen by *B. velezensis*. Wu *et al* (2016) also reported that *Bacillus amyloliquefaciens* strain NJZJSB3 cell suspension reduced the disease incidence by 83.3% in *Brassica napus* due to the production of non volatile antifungal compounds such as toluene, phenol, and benzothiazole. The efficacy of Biopulse can be supported by Lopes *et al*. (2012) who observed that *Trichoderma asperellum* 11/11 and *Trichoderma harzianum* ALL-42 showed the highest inhibition (>50%) of *Sclerotinia sclerotiorum* growth. Sharma *et al*. (2016), observed maximum efficacy of *T. viride* followed by *T. harzianum*, and minimum by *Bacillus subtilis* against *Sclerotinia sclerotiorum.* Chakma *et al*. (2023) in his study also observed that *P. fluorescens* was effective against the pathogen.

The study showed that seedling dip method was more effective compared to soil drenching. The results were in alignment with El-Mohamedy (2009) [7] who observed successful control of Fusarium root rot on citrus rootstocks seedlings i.e. sour orange (SO), volkamer lime (VL), rangpur lime (RP) and cleopatra mandarin (CL) by dipping the root system of such seedlings in water suspensions of each biological treatment i.e. *Trichoderma harzianum* and *Bacillus subtilis*.

Conclusion

The study concludes that all the bioformulations were effective in minimizing the disease incidence compared to control. The mechanism involved in minimizing the disease could either be by suppressing the pathogen or by inducing the resistance in plants**.** Liquid bioformulation of Kush Vardan-1, was found to be most effective followed by Bio-Pulse. On comparing the methods of application of bioformulations, seedling dip method was found to be more effective then soil drenching method for all the bioformulations, probable reason may be direct interaction of the microbes with roots of the seedlings.

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Competing Interests

All the authors hereby declare that there is no conflict of interest.

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Data availability statement

The data related to the study is already mentioned in the manuscript.

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