Assessment of aerial blightdisease and different growth parameter of stevia crop (*Stevia rebaudiana*)by usingbioagents (*Bacillus subtilis*)

ABSTRACT

Stevia (*Stevia rebaudiana*Bertoni) has become a valuable crop due to its natural sweetness and wide-ranging applications in both the food and pharmaceutical industries. Despite its potential, stevia cultivation is hindered by diseases like aerial blight, caused by the fungal pathogen *Rhizoctonia solani* Kuhn, which leads to significant yield losses. This study explores the potential of Bacillus subtilis, a biological control agent, in mitigating the effects of this disease. Conducted during the *kharif* season of 2023-24, the experiment assessed various dosages (1- 4 l/ha) of the bioagent (*Bacillus subtilis*) and their effects on plant health, disease resistance, and overall yield. The results indicate that the application of *Bacillus subtilis* not only reduced the severity of aerial blight but also enhanced key growth parameters, including plant height and sucker production. During evaluation, all the seven treatments were found to be significantly superior over control in managing the disease. Among all the treatments the growth parameters were maximum in T_5 (*Bacillus subtilis* @ 3 l/ha) such as plant height (56.83 cm) number of suckers(55), fresh leaves yield (4.51) and dry leaves yield (4.00) and minimize the disease intensiy @ 12.23 %followed by T_4 (*Bacillus subtilis* @ 2.5 l/ha), T_6 (*Bacillussubtilis* @ 3.5 l/ha),T_7 (*Bacillus subtilis* @ 4 l/ha),T_3 (*Bacillus subtilis* @ 2 l/ha),T_2(*Bacillus subtilis* @ 1.5 l/ha),T_1(*Bacillus subtilis* @ 1 l/ha) and T0 (untreated).Furthermore, an economic analysis confirmed the cost-effectiveness of this biological treatment, highlighting its potential as a sustainable alternative to chemical fungicides in stevia farming.

Keywords: Aerial blight, *Bacillus subtilis*,bio-control agent, growth parameters, *Rhizoctonia solani*.

1.Introduction:

Stevia *(Stevia rebaudianaBertoni)* commonly known as sweet leaf or honey leaf, is a perennial herb widely cultivated for its sweet-tasting compounds, which are used as natural sweeteners.

The main producer of stevia are India, Japan, China, Taiwan, Thialand, Korea, Brazil, Malaysia and paraguay (Singh and Verma, 2015). In india farmers have started growing stevia in some parts of Rajasthan, Punjab, Uttar-Pradesh, West-Bengal, Madhya Pradesh, Karnataka, Chhattisgarh, Maharashtra and Tamil-nadu (Maiti *et al.*, 2007). Different species of stevia contain several potential sweetening compounds , *Stevia rebaudiana* being the sweetest of all (Goyal *et al.*, 2010). Stevia contains several potential sweetening compounds,six sweet –tasting compounds have been found in the leaves of *Stevia rebaudiana* Bertoni *i.e*; stevioside, rebaudiosides A, rebaudioside D, rebaudioside E, dulcosides A and dulcosides B which have insulin balancing properties .These sweetners impart 250 times sweetness than table sugar and 300 times more than sucrose (Kassahun *et al.*, 2012).Despite its economic potential, stevia cultivation is challenged by aerial blight disease caused by the fungal pathogen *Rhizoctonia solani* Kuhn.*Rhizoctonia solani* is very destructive plant pathogen and responsible for causing seedling damping off, root rot, collar rot, stem canker, sheath blight, bended leaf, bud and fruit rots, black scurf and aerial blight of different agricultural crops.(Chauhan *et al*., 2019).This disease can lead to significant crop losses. Traditional methods of managing plant diseases often involve chemical fungicides, which have environmental and health-related drawbacks. Therefore, there is growing interest in biological control agents like Bacillus subtilis, known for its antagonistic properties against various plant pathogens.Among PGPR (plant growth promoting rhizobacteria), *Bacillus* spp. is one of the most effective genera foe enhencing the growth and yield of crops under biotic and abiotic conditions due to their spore forming property and production of several metabolites such as indole 3- acetic acid (IAA), siderophore, solubilized potassium, phosphate and except this *Bacillus* sp. has the ability to produce biocontrol metabolites such as salicylic acid, chitinase and β, 1,3- glucanase (Prakash *et al*., 2022).

2. Materials and method:

- **Efficacy of treatments on growth parameter**

The study was conducted at the Central Research Field of Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, during the *kharif* season of 2023. The experiment followed a Randomized Block Design (RBD) with three replications and eight treatments namelyTreatment 1 - *Bacillus subtilis* @1 l/ha, Treatment 2 -*Bacillus subtilis* @1.5l/ha, Treatment 3 -*Bacillus subtilis @ 2* l/ha, Treatment 4 -*Bacillus subtilis* @ 2.5l/ha, Treatment 5 -*Bacillus subtilis* @ 3 l/ha, Treatment 6 -*Bacillus subtilis @3.5* l/ha, Treatment 7 -*Bacillus subtilis @4* l/ha *and* Control (without treatments).Stevia plants were transplanted with a spacing of 30 cm between plants and 45 cm between rows. The treatments were applied at 30, 60, and 90 days after transplanting (DAT). Plant height, number of suckers, disease intensity, and yield were recorded.

Disease intensity was assessed using a rating scale, and the Percent Disease Intensity (PDI) was worked out by applying the formula of wheeler (1969)

$$PDI = \frac{Sum\ of\ all\ neumerical\ ratings}{Highest\ ratings\ of\ the\ scale\ \times\ Total\ numbers\ of\ leaf\ observed}$$

Table 1:Ratings/grades based on percent leaf area infected

Rating scale	Description	Severity index
0	No lesions/spots	0
1	1 % leaf area covered with lesions/spots	0.1-1%
3	1.1 to 10 % leaf area covered with lesions/spots, no spots on stem	1-10%
5	10.1 to 25% of leaf area covered, no defoliation; little damage	10-25%
7	25.1 to 50 % leaf area covered; some leaves drop; death of a few plants, damage conspicuous	25-50%
9	More than 50 % area covered, lesions/spots very common on all plants, defoliation common; death of plants common; damage more than 50 %.	50-100%

[According to Amrate*et al.*, 2020)

The randomized block design which were used during field trial:

Sub-irrigation Channel			
Replication 3rd	**Replication 2nd**	**Replication 1st**	**Main Irrigation Channel**
T_0	T_6	T_7	
T_2	T_0	T_6	
T_4	T_5	T_1	
T_7	T_4	T_2	
T_5	T_1	T_3	
T_3	T_2	T_4	
T_1	T_3	T_5	
T_6	T_7	T_0	

- **Isolation and identification of pathogen*Rhizoctonia solani***

Potato Dextrose Agar (PDA) was prepared, and 80 mg of streptomycin, an antibiotic, was added to each 500 ml of the medium to prevent bacterial contamination. Diseased leaf portions were cut into small pieces under aseptic conditions using scissors, which were sterilized by flaming over a spirit lamp. The leaf segments were then surface sterilized in 0.2% mercuric chloride and rinsed with 70% ethanol before being placed on petri dishes containing solidified PDA medium. The plates were incubated at room temperature until fungal growth became visible. The resulting fungal colonies were subsequently transferred to fresh medium to obtain pure cultures. The hyphae of *Rhizoctoniasolani* were observed to be long and tubular, with internal septa. The branches of the hyphae formed at perpendicular angles, tapering at the point of branching, where the septum was slightly narrowed or curved. The young hyphae of *Rhizoctoniasolani* exhibited branches forming at 45° angles.

3. Result and Discussion:

The details about the efficacy of treatments on growth parameter and disease intensity are mentioned in the following tables:

Table 2: Efficacy of treatments on growth parameter:

Treatments	Plant height			Number of suckers		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90DAT
T0Control	17.16	25	36.66	24	28.5	36.5
T1(*Bacillus subtilis* @ 1 l/ ha)	18.44	27	39	26	30.5	39
T2 (*Bacillus subtilis* @1.5 l/ha)	20	29.53	43.5	29	32	41.66
T3 (*Bacillus subtilis* @ 2 l/ha)	21	31.94	44.83	30	36.83	41.66
T4(*Bacillus subtilis* @ 2.5 l/ha)	27.5	36.55	59	36	39.5	51.33
T5 (*Bacillus subtilis* @ 3 l/ ha)	28.83	39.16	61.33	38.33	43.5	55
T6(*Bacillus subtilis* @ 3.5 l/ ha)	25.83	34.91	56.83	34.33	37.33	49
T7 (*Bacillus subtilis* @ 4 l/ha)	23.83	33	51	32.66	35	46
CD (0.05%)	**1.03**	**1.53**	**1.85**	**1.05**	**1.26**	**1.58**

Table 2 showeddifferent effect of treatments on plant height and number of suckers. At 30 DAT, the highest height was observed with 28.83 cm followed by 27.5cm, 25.83cm, 23.83cm, 21cm, 20cm, 18.44cm and least observed in 17.16 cm. At 60 DAT, highest plant height was observed with 39.61 cm followed by 36.55cm, 34.91cm, 33cm, 31.94cm, 29.53cm, 27cm and 25. At 90 DAT, the highest height was observed with 61.33cm followed by 59cm, 56.83cm, 51cm, 44.83cm, 43.5cm, 39cm and least observed in 36.66cm (Fig:1).The number of suckers at 30 DAT, was best with 38.33 per plant followed by 36, 34.33, 32.66, 30, 29, 26 and 24 per plant.At 60 DAT, the highest number was 43.5 per plant followed by 39.5, 37.33, 36.83, 35, 32, 30.5 and 28.5per plant. At 90 DAT, the highest number was 55 per plant followed by 51.33,49,46,41.66,41.66,39 and 36.5 (Fig: 2)

Table 3: Efficacy of treatments on growth parameter:

Treatments	**Plant disease intensity**			**Yield**
	60 DAT	**75 DAT**	**90 DAT**	
T0Control	18.54	22.58	28.28	1.15
T1(*Bacillus subtilis* @ 1 l/ ha)	16.51	20.66	26.32	1.6
T2(*Bacillus subtilis* @1.5 l/ha)	14.72	18.61	24.89	2.03
T3 (*Bacillus subtilis* @ 2 l/ha)	11.40	15.82	21.5	2.76
T4(*Bacillus subtilis* @ 2.5 l/ha)	10.06	13.56	14.19	3.64
T5 (*Bacillus subtilis* @ 3 l/ ha)	8.25	10.98	12.23	4.0
T6(*Bacillus subtilis* @ 3.5 l/ ha)	11.00	14.97	18.81	3.19
T7 (*Bacillus subtilis* @ 4 l/ha)	13.93	17.04	23.52	2.38
CD (0.05%)	0.87	1.11	1.21	0.32

Table 3 showed that the maximum disease reduction rate at 60,75 and 90 DAT was observed in T_5[*Bacillus subtilis* @ 3 l/ha] with the value of (8.25 % ,10.98% and 12.23%)followed byT_4 with(10.06%,13.56% and 14.19%),T_6 with (11%,14.97% and 18.81%),T_7 with (13.93%,17.04% and 23.52%) ,T_3 with(11.40%,15.82% and 21.5%),T_2 with (14.72%,18.61% and 24.89%),T_1 with (16.51%,20.66% and 26.32) and T_0 with (18.54%,22.58% and 28.28%) (Fig: 3).The highest yield was shown in the treatment 5 with the value of 4t/ha followed by 3.64 t/ha,3.19t/ha,2.38t/ha,2.76t/ha,2.03t/ha,1.6t/ha and 1.15t/ha.

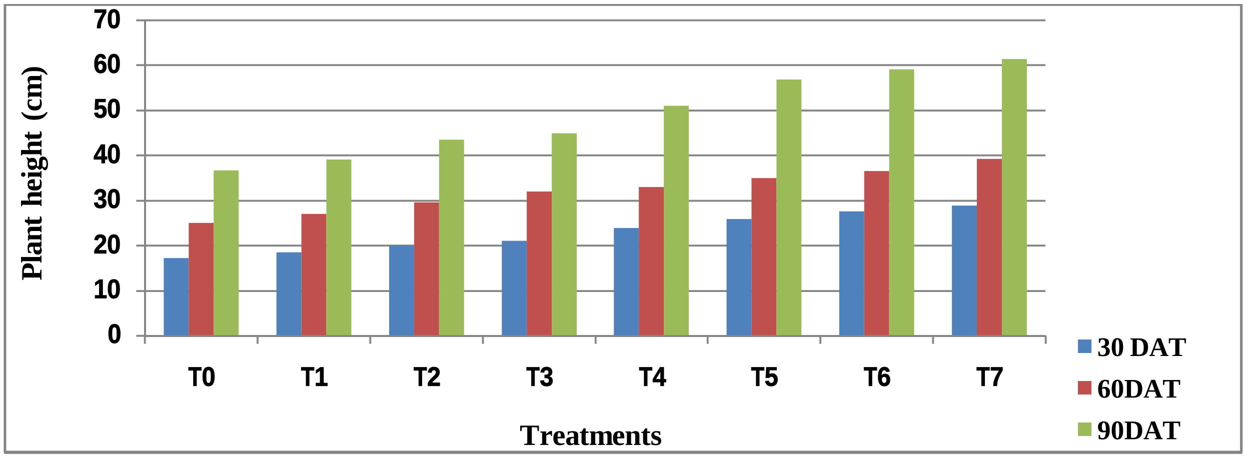

Fig 1Graphical representation of effect of treatments on plant height

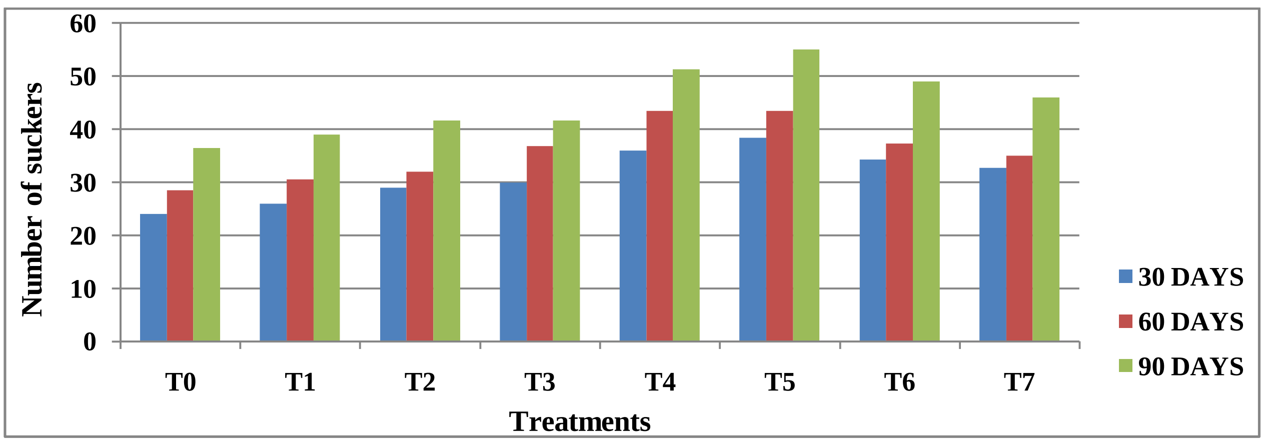

Fig 2: Graphical representation of Effect of treatments on number of suckers

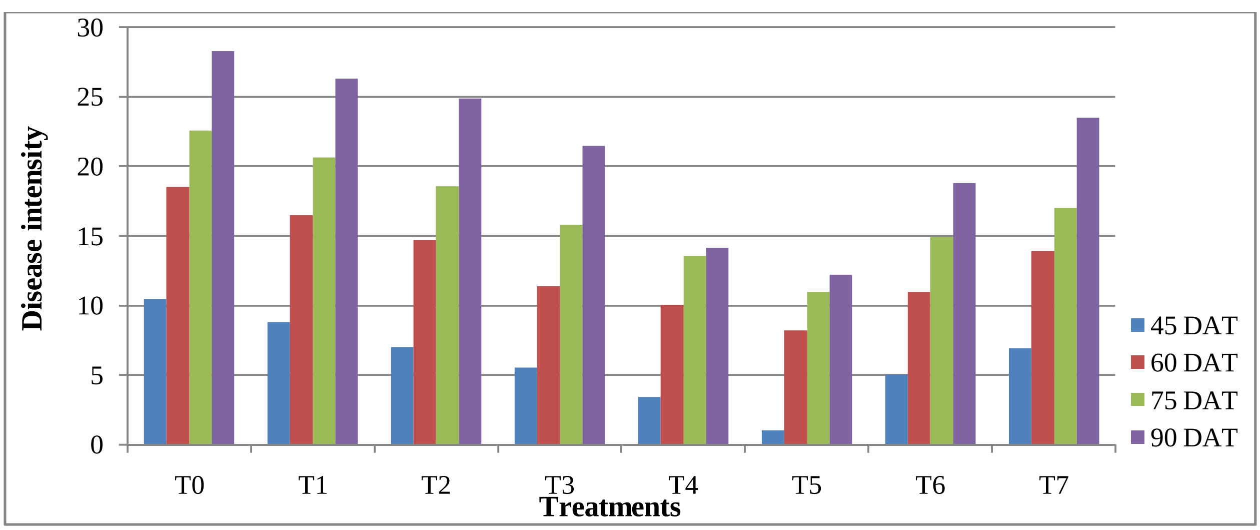

Fig 3: Graphical representation of plant disease intensity.

Discussion:

The treatment significantly improved plant height, increased the number of suckers, boosted yield, and reduced plant disease intensity. These benefits are primarily due to *Bacillus subtilis*, a plant growth-promoting rhizobacterium (PGPR) that produces various growth-enhancing metabolites, including indole-3-acetic acid (IAA), siderophores, and solubilized potassium, phosphate, and zinc. The likely explanation for the observed results is attributable to the plant growth-promoting bacteria (Bacillus spp.), known for its capacity to synthesize a variety of plant growth-promoting metabolites, such as indole-3-acetic acid (IAA), siderophores, and solubilized forms of potassium, phosphate, and zinc. Additionally, Bacillus spp. produces biocontrol metabolites, including chitinase and β-1,3-glucanase. Salicylic acid, a naturally occurring phenolic compound, plays a critical role in preventing the fungal pathogen *Rhizoctoniasolani*by inducing systemic resistance, as documented by Prakash *et al*. (2022). Furthermore, Abbas *et al*. (2019) established that the plant growth-promoting rhizobacteria (*Bacillus subtilis*) produce various antibiotics, such as iturin A and surfactin, which are instrumental in suppressing the fungal pathogen *Rhizoctonia solani*.However, excessively high doses can lead to negative effects, such as hormonal imbalances with auxins, cytokinins, and gibberellins, which can inhibit growth and reduce plant height. Moreover, high doses of *Bacillus subtilis* might cause nutrient imbalances by overproducing siderophores, which bind iron and limit nutrient availability, potentially reducing plant growth. According to Blake *et al*. (2021), increasing the dose beyond a certain point may not further enhance disease control, as the bioagent may saturate the environment and additional cells offer no extra benefit. This saturation can diminish the bioagent's effectiveness and increase pathogen susceptibility, as observed by Qiao *et al*. (2017).

Conclusion:

This study underscores the potent efficacy of *Bacillus subtilis* as a biological control agent in mitigating aerial blight disease in stevia, caused by the fungal pathogen *Rhizoctonia solani*. The strategic application of *Bacillus subtilis* markedly diminished disease severity while simultaneously enhancing critical agronomic parameters, including plant stature, sucker proliferation, and both fresh and dry biomass yield. Among the tested regimens, the application of *Bacillus subtilis* at 3 l/ha emerged as the most efficacious, culminating in a superior plant height of 61.33 cm, an optimal sucker count of 55 per plant, and a minimum disease intensity of 12.23%. Furthermore, this treatment yielded the highest dry leaf biomass at 4.00 tons/ha. Economic analysis further corroborated the cost-efficiency of *Bacillus subtilis*, positioning it as a sustainable alternative to conventional chemical fungicides. These findings advocate for the integration of *Bacillus subtilis*into stevia cultivation practices, promising enhanced crop productivity alongside environmental stewardship.

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