Efficacyof*Bacillussubtilisagainst*aerialblightdiseaseofstevia crop (*Steviarebaudiana* caused by *Rhizoctonia solani*kuhn)

ABSTRACT

Stevia (*Stevia rebaudiana*Bertoni) has become a valuable crop due to its natural sweetness andwide-ranging applications in both the food and pharmaceutical industries. Despite its potential,steviacultivationishinderedby diseaseslikeaerialblight,causedby thefungal pathogen*Rhizoctoniasolani*Kuhn,whichleadstosignificantyieldlosses.Thisstudyexploresthepotential 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- 4l/ha) of the bioagent (*Bacillus subtilis*) and their effects on plant health, disease resistance, andoverall yield. The results indicate that the application of *Bacillus subtilis* not only reduced theseverity of aerial blight but also enhanced key growth parameters, including plant height andsucker production. During evaluation, all the seven treatments were found to be significantlysuperior over control in managing the disease. Among all the treatments the growth parameterswere maximum in T_5 - *Bacillus subtilis* @ 3 l/ha, such as such as plant height (56.83 cm) numberof suckers (55), fresh leaves yield (4.51) and dryleaves yield (4.00) and minimize the diseaseintensiy @ 12.23 % followed by T_4- *Bacillus subtilis* @ 2.5 l/ha,T_6 -*Bacillus subtilis* @ 3.5l/ha ,T_7- *Bacillus subtilis* @ 4 l/ha ,T_3- *Bacillus subtilis* @ 2 l/ha ,T_2- *Bacillus subtilis* @ 1.5l/ha ,T_1- *Bacillus subtilis* @ 1 l/ha and T0 (untreated). Furthermore, an economic analysisconfirmedthecost-effectivenessof thisbiological treatment,highlightingitspotential asasustainable alternative tochemicalfungicidesinsteviafarming.

Keywords: Aerial blight, *Bacillus subtilis,* biocontrol agent, growth parameters, *Rhizoctoniasolani.*

1. Introduction:

Stevia *(Stevia rebaudiana Bertoni)* commonly known as sweet leaf or honey leaf, is a perennialherb widely cultivatedfor its sweet-tastingcompounds,which are usedas natural sweeteners.The main producer of stevia are India, Japan, China, Taiwan, Thialand, Korea, Brazil, Malaysiaand paraguay (Singh and Verma, 2015). In india farmers have started growing stevia in somepartsofRajasthan,Punjab,Uttar-Pradesh,West-Bengal,MadhyaPradesh,Karnataka,Chhattisgarh, Maharashtra and Tamil-nadu (Maiti *et al.*,2007). Differentspecies of steviacontain several potential sweetening compounds ,*Stevia rebaudiana*beingthe sweetest of all(Goyal *et al.*, 2010). Stevia contains several potential sweetening compounds,six sweet –tastingcompoundshavebeenfoundintheleavesof*Steviarebaudiana*Bertonii.e;stevioside,rebaudiosides A, rebaudioside D, rebaudioside E, dulcosides A and dulcosides B which haveinsulin balancing properties .These sweetners impart 250 times sweetness than table sugar and300 times more than sucrose (Kassahun *et al.*, 2012). Despite its economic potential, steviacultivation is challenged by aerial blight disease caused by the fungal pathogen *Rhizoctoniasolani*Kuhn. *Rhizoctonia solani*is very destructive plant pathogen and responsible forcausingseedling damping off, root rot, collar rot, stem canker, sheath blight, bended leaf, bud and fruitrots, black scurf and aerial blight of differentagricultural crops. (Chauhan *et al.*, 2019). Thisdisease can lead to significant crop losses. Traditional methods of managing plant diseases ofteninvolvechemicalfungicides,whichhaveenvironmentalandhealth-relateddrawbacks.Therefore, there is growing interest in biological control agents like Bacillus subtilis, known foritsantagonisticpropertiesagainstvariousplantpathogens.AmongPGPR(plantgrowthpromoting rhizobacteria), *Bacillus* spp. is one of the most effective genera foe enhencing thegrowth and yield of crops under biotic and abioticconditions due to their spore forming propertyandproductionofseveralmetabolitessuchasindole3-aceticacid(IAA),siderophore,solubilizedpotassium,phosphateandexceptthis*Bacillus*sp.hastheabilitytoproducebiocontrol metabolitessuch as salicylic acid, chitinase and β, 1,3- glucanase (Prakash *et al.*,2022).

2. Materialsandmethod:

- **Efficacyoftreatmentsongrowthparameter**

The study was conducted at the Central Research Field of Sam Higginbottom University ofAgricultureTechnologyandSciences,Prayagraj,duringthe*kharif*seasonof2023.Theexperimentfoll owedaRandomizedBlockDesign (RBD)with threereplicationsandeighttreatments namely Treatment 1 - *Bacillus subtilis* @1 l/ha, Treatment 2 - *Bacillus subtilis* @1.5l/ha,Treatment3-*Bacillus subtilis@ 2*l/ha,Treatment4-*Bacillus subtilis@* 2.5l/ha,Treatment 5 - *Bacillus subtilis @* 3 l/ha, Treatment 6 - *Bacillus subtilis @3.5* l/ha, Treatment 7 - *Bacillussubtilis@4*l/ha*and*Control(withouttreatments).Steviaplantsweretransplantedwitha spacing of 30 cm between plants and 45 cm between rows. The treatments were applied at 30,60, and 90 days after transplanting (DAT). Plantheight, number of suckers,disease intensity,andyieldwererecorded.

Disease intensity was assessed using a rating scale, and the Percent Disease Intensity (PDI) wasworkedoutbyapplyingtheformula ofwheeler(1969)

$$PDI = \frac{Sumofallneumericalratings}{Highestratingsofthescale \times Totalnumbersofleafobserved}$$

Table1:Ratings/gradesbasedonpercentleafareainfected

Rating scale	Description	Severityindex
0	Nolesions/spots	0
1	1 %leafareacovered withlesions/spots	0.1-1%
3	1.1to10%leafareacoveredwithlesions/spots,nospots onstem	1-10%
5	10.1to 25%ofleafareacovered,nodefol iation;little damage	10-25%
7	25.1 to 50 % leaf area covered; some leaves drop;deathofa few plants,damageconspicuous	25-50%
9	More than 50 % area covered, lesions/spots verycommononallplants,defoliationcommon;deatho fplantscommon;damagemorethan50%.	50-100%

[AccordingtoAmrate*et al.*2020)

- **Isolation andidentification ofpathogen *Rhizoctoniasolani***

Potato Dextrose Agar (PDA) was prepared, and 80 mg of streptomycin, an antibiotic, was addedto each 500 ml of the medium to prevent bacterial contamination. Diseased leaf portions were cutinto small pieces under aseptic conditions using scissors, which were sterilized by flaming over aspirit lamp. The leaf segments were then surface sterilized in 0.2% mercuric chloride and rinsedwith 70% ethanol before being placed on petri dishes containing solidified PDA medium. Theplates were incubated at room temperature until fungal growth became visible. The resultingfungal colonies were subsequently transferred to fresh medium to obtain pure cultures. Thehyphae of *Rhizoctonia solani*were observed to be long and tubular, with internal septa. Thebranches of the hyphae formed at perpendicular angles, tapering at the point of branching, wherethe septum was slightly narrowed or curved. The young hyphae of *Rhizoctonia solani*exhibitedbranchesformingat45°angles.

3. ResultandDiscussion:

The details aboutthe efficacy of treatments on growth parameteranddiseaseintensity arementionedinthefollowingtables:

Table2: Efficacyoftreatmentsongrowthparameter:

Treatments	**Plantheight**			**Numberofsuckers**		
	30 DAT	**60 DAT**	**90 DAT**	**30 DAT**	**60 DAT**	**90 DAT**
T0Control	17.16	25	36.66	24	28.5	36.5
T1 (*Bacillussubtilis*@1 l/ha)	18.44	27	39	26	30.5	39
T2(*Bacillussubtilis*@1.5l/ha)	20	29.53	43.5	29	32	41.66
T3(*Bacillussubtilis*@2 l/ha)	21	31.94	44.83	30	36.83	41.66
T4(*Bacillussubtilis*@2.5l/ha)	27.5	36.55	59	36	39.5	51.33
T5 (*Bacillussubtilis*@3 l/ ha)	28.83	39.16	61.33	38.33	43.5	55
T6(*Bacillussubtilis*@3.5 l/ha)	25.83	34.91	56.83	34.33	37.33	49

T7(*Bacillussubtilis*@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 showed different 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 observedwith 39.61 cm followed by 36.55cm, 34.91cm, 33cm, 31.94cm, 29.53cm, 27cm and 25. At 90DAT,thehighestheightwasobservedwith61.33cmfollowedby59cm,56.83cm,51cm,44.83cm, 43.5cm, 39cm and least observed in 36.66cm (Fig:1).The number of suckers at 30DAT,was bestwith38.33perplantfollowedby36,34.33,32.66, 30, 29, 26and24perplant.At 60DAT,thehighestnumberwas43.5perplantfollowedby39.5,37.33,36.83,35,32,30.5and 28.5perplant.At90DAT,thehighestnumber was55per plantfollowedby51.33,49,46, 41.66, 41.66,39 and 36.5(Fig: 2)

Table3:Efficacyoftreatmentsongrowthparameter:

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

Table 3showed that the maximum disease reduction rate at 60, 75 and 90 DAT was observed inT_5[*Bacillus subtilis* @ 3 l/ha] with the value of (8.25 % ,10.98% and 12.23%) followed by $T_4$with(10.06%,13.56%and14.19%),$T_6$with(11%,14.97%and18.81%),$T_7$with(13.93%,17.04% and23.52%),$T_3$with(11.40%,15.82%and21.5%),$T_2$with(14.72%,18.61%and24.89%),T_1 with (16.51%,20.66% and 26.32) and T_0 with (18.54%,22.58% and 28.28%) (Fig: 3).The highestyield was shown in the treatment 5 with the value of 4t/ha followed by 3.64 t/ha, 3.19 t/ha, 2.38t/ha,2.76t/ha,2.03t/ha,1.6t/haand1.15t/ha.

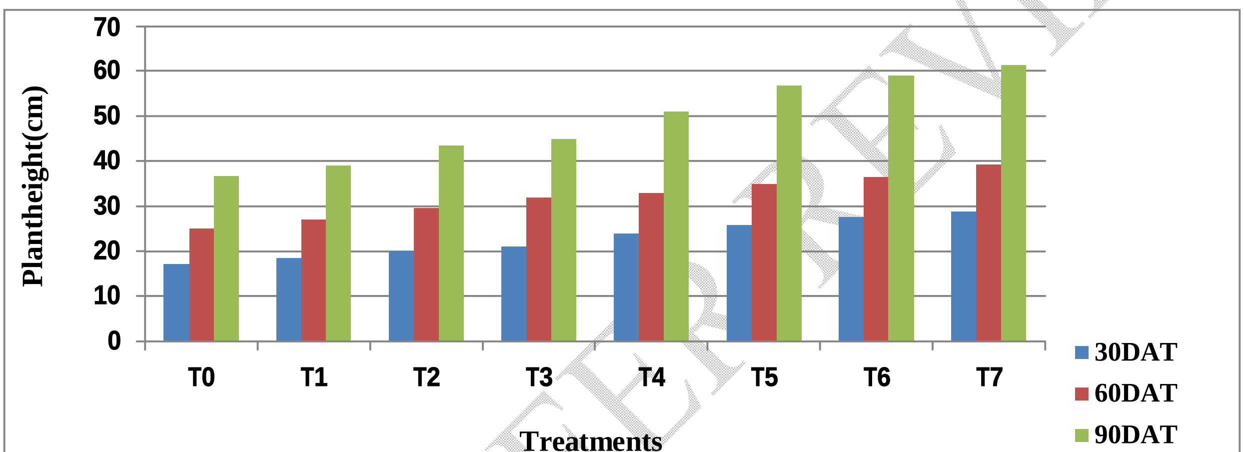

Fig1Graphicalrepresentationofeffectoftreatmentsonplantheight

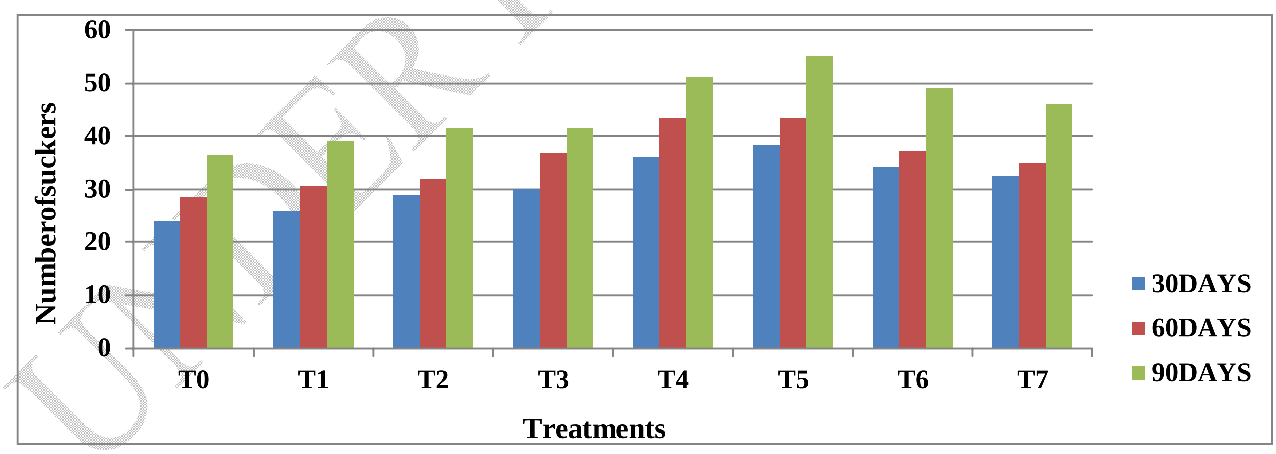

Fig 2:GraphicalrepresentationofEffectoftreatmentsonnumberofsuckers

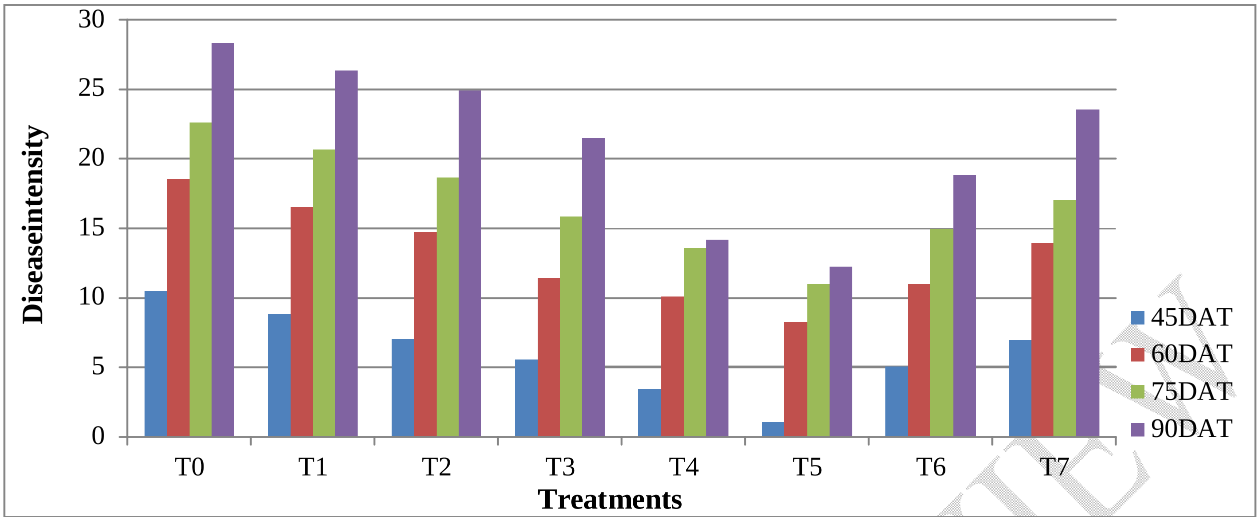

Fig3:Graphicalrepresentationofplantdiseaseintensity

Discussion:

The treatment significantly improved plant height, increased the number of suckers, boostedyield, and reduced plant disease intensity. These benefits are primarily due to *Bacillus subtilis*, aplantgrowth-promotingrhizobacterium(PGPR)thatproducesvariousgrowth-enhancing metabolites,includingindole-3-aceticacid(IAA),siderophores,andsolubilizedpotassium,phosphate, and zinc. The likely explanation for the observed results is attributable to the plantgrowth-promoting bacteria (Bacillus spp.), known for its capacity to synthesize a variety of plantgrowth-promoting metabolites, such as indole-3-acetic acid (IAA), siderophores, and solubilizedformsofpotassium,phosphate,andzinc.Additionally,Bacillusspp.producesbiocontrolmetabolites,includingchitinaseandβ-1,3-glucanase.Salicylicacid,anaturallyoccurringphenolic compound, plays a critical role in preventing the fungal pathogen *Rhizoctonia solani*byinducing systemic resistance, as documented by Prakash *et al*. (2022). Furthermore, Abbas *et al*.(2019)establishedthat theplantgrowth-promotingrhizobacteria(*Bacillussubtilis*)producevarious antibiotics, such as iturin A and surfactin, which are instrumental in suppressing thefungal pathogen*Rhizoctoniasolani*.However,excessivelyhigh dosescanleadtonegativeeffects, such as hormonal imbalances with auxins, cytokinins, and gibberellins, which can inhibitgrowth and reduce plant height. Moreover, high doses of *Bacillus subtilis* might cause nutrientimbalancesbyoverproducingsiderophores,whichbindironandlimitnutrientavailability,potentially reducing plant growth. According to Blake *et al*. (2021), increasing the dose beyond acertainpointmaynotfurtherenhancediseasecontrol,asthebioagentmaysaturatetheenvironmentandadditionalcellsoffernoextrabenefit.Thissaturationcandiminishthebioagent'seffectivenessandincreasepathogensusceptibility, asobservedbyQiao*etal*.(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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