EvaluationofAntimicrobialactivityofNeemcakeandbio-agents onEarlyblight(*Alternariasolani*)oftomato(*Lycopersiconesculentum*L.)

Abstract

Early blight is the important disease of tomato because it causes huge economic loss to the farmer every year.*Alternariasolani*causeearlyblightoftomatoanditisconsideredweedoffieldbecauseofitswideadaptabilityunder different environment. Use of fungicides for the management of disease in crop puts a large number ofnegative health and environmental effects therefore, the urgent need for a more sustainable and ecologicalapproach to manage disease without fungicides. To avoid relying solely on chemicals and to identify a viablealternative component,this experiment was conducted to conclude the Evaluation of antimicrobial activity ofNeem cake and bio-agents on early blight (*Alternaria solani*) of tomato (*Lycopersicon esculentum* L.) by soilapplication with Neem cake @ 250kg/ha and seedling treatment with bio agents in single and combination *vizPseudomonasfluorescens*@10g/lit,*Bacillusthuringiensis*@10g/lit,*Trichodermaviride*@10g/lit,*Pseudomonasfluorescens*@5g/lit+*Bacillusthuringiensis*@5g/lit,*Pseudomonasfluorescens*@5g/lit+*Trichoderma viride*@5g/lit and *Trichoderma viride*@ 5g/lit + *Bacillusthuringiensis*@5g/lit both *in-vivo* and*in-vitro* for their effectiveness to manage early blight of tomato caused by *Alternaria solani* .All the treatmentswere found significantly reduced the severity of the disease and increased growth parameters, Among all thetreatments Neem cake@250kg/ha+ *Pseudomonas fluorescens* @5g/lit + *Trichoderma viride*@ 5g/lit followedby Neem cake@250kg/ha + *Trichoderma viride*@5g/lit + *Bacillus thuringiensis* @5g/lit were significantlysuperioroverotherthreatmentsinreducingearlyblightinfectionandalsoinincreasinggrowthparametersof thecrop. For *in-vitro* studies only bio agents were selected among the treatments *Pseudomonas fluorescens* +*Trichoderma viride*showed highest mycelia inhibition (89.3%) followed by *Trichoderma viride*+ *Bacillusthuringiensis* (86.6%). The highest cost benefit ratio was obtained with Neem cake 250kg/ha + *Pseudomonasfluorescens* @5g/lit + *Trichoderma viride*@5g/lit treatment (1:3.85). while otherthreatments also showedsignificantlyeffectiveforthecheckingofdisease intensityand yield overcontrolin thefieldcondition.

Keywords:*Alternariasolani*,tomato,soilapplication,seedlingtreatment,neemcake,bio-agents,*in-vivo*,*in-vitro*.

1. INTRODUCTION

"Tomato (*Lycopersicon esculentum* Mill) is one of the most significant vegetable crop in the world which is amemberoftheSolanaceaefamily.Tomatoisreferredtoasthe"poorman'sapple.Itisa veryversatileplantthatisused in both natural (raw material) and as an ingredient in other products. Tomato ranks first world wide amongcrops used for processing. Products made from tomato are an important part of the human diet. It is a plentifulsource of minerals, carbohydrates, vitamins, and amino acids (A, C & K). Tomato contains lycopene, a verypotent antioxidantthatpreventscancers"**(AgarwalandRao,2000)**[1]

"Thecropiscultivatedacross allcontinentsinthefieldsas wellasinprotectedconditions.Theannualproductionof fresh tomatoes accounts to approximately 180 million tonnes" **(FAO, 2019). "**India the total production oftomato is 20.70 million tonnes from 796.87 thousand hectares area" **(FAOSTAT 2019-2020)**[7]**, "**The major threetomatoproducingstatesin thecountryareMadhyaPradesh,AndhraPradeshandKarnataka.InUttarPradeshthetomato iscultivatedin23000Haandtheproductionis902000tonnes"**(Anonymous,2022)**[3]**.**

"There have been reports of more than 200 diseases that affect tomatoes worldwide" **(Sharma *et al*., 2022)[16] . "**Among the fungal diseases, early blight caused by *Alternaria solani*is one of the most important and frequentoccurring disease of the crop nation and worldwide" **(Jones *et al.*, 1991)[9] "**Symptoms of early blight are small,dark,necroticlesionsthatusuallyappearontheolderleavesandspreadupwardoftheplants.Aslesionsenlarge,they commonlyhaveconcentricringswithatarget-likeappearance,andtheyareoftensurroundedbyayellowingzone" **(Sherf and MacNab, 1986)[17]. "**It directly harms the plant and reduces both the quantity and quality of theeconomicyield.Ithasasignificantimpactoncropgrowthatallstagesduringboth*Kharif*and*Rabi*season.Thisdisease, which can cause severe defoliation in severe condition, is most damaging to tomato in areas with heavyrainfall,high humidityandfairlyhigh temperatures 24-29°C"**(Peralta*etal*.,2009)[13].**

"Accordingtomorphologicalcharactersandphylogeneticanalysis,*Alternariasolani*bearlarge,long-beakedandnon-catenatedspores"**(Simmons,2000)[18]. "**Themyceliumconsistedofsepta,branched,lightbrownhyphaewhichturned darker with age. The conidiophores are short, 50-90µmlong and dark in colour. Conidia are 120-296 ×12-20 µm in size It has beaked, muriform, dark colour and born singly Conidia contained 5-10 transversesepta and1-5 longitudinal septa" **(Seyoun*et al*., 2021)**[15]. "*A.solani*was most destructive causing heavy losses in yield oftomatosometimes as highas 78 percent of fruitloss"**(DatarandMayer, 1981)[6].**

Forthemanagementofearlyblightoftomato,thereisneedtoincorporatealternativecontrolcomponentsthatareeffectivei nfield.Theuseofbioagentsandbotanicalsarethebestalternativesformanagementof*Alternariasolani*useofindigenouss ourcesandbio-agentsforthemanagementoftheplantdiseasewhichislesscostlyanddoesn't affect public health and environment. Considering the effects of this disease, the present paper discusses theefficiencyofneemcake andbio-agents forthe managementofearlyblight disease intomato.

2. MATERIALSANDMETHODS

2.1 ExperimentDetails

The field experiment was conducted in randomized block design (RBD) with plot size 2x1m^2 and there werethreereplicationforeachtreatment.TreatmentsusedfortheexperimentwereT_1Neemcake@250kg/ha+

Pseudomonas fluorescens @10g/lit ,T_2 Neem cake @250kg/ha + *Bacillus thuringiensis* @10g/lit, T_3Neem cake @250kg/ha+ *Trichoderma viride*@10g/lit,T_4Neem cake @250kg/ha+ *Pseudomonasfluorescens*@5g/lit+*Bacillusthuringiensis*@5g/lit,T_5Neemcake@250kg/ha+*Pseudomonasfluorescens*@5g/lit +*Trichoderma viride*@5g/lit,T_6Neem cake @250kg/ha + *Trichoderma viride*@5g/lit+ *Bacillus thuringiensis* @5g/lit in which Neem cake was treated as soil application and bioagentsweretreated as seedlingdip.

2.2 Isolationandpurificationoffungalpathogen.

"Fungal pathogen was isolated from the infected tomato leaves. The infected sections were cut-off from themargins of leaf lesions together with some portions of the healthy plant and surface sterilized in 1% sodiumhypochloritefollowedbyrinsinginthreeseriesofdistilledwater.Insidelaminarflowchamber,thesectionswerecarefully and quickly transferred onto the sterilized PDA media, and incubated at 20-25^{0}C for 5-7days, andobtained the culture. The culture of *Alternaria solani*was purified and maintained by periodic sub- culturing onPDApetriplates and slants,and incubatedat25±2^{0}Ctemperature". [24]

2.3 SourceofbioagentsandTomatoSeedlingTreatment.

"CommercialbasedPowderformulationsof*Trichodermaviride*(2×10^{7}c.f.u/g),*Pseudomonasfluorescens*(2×108 c.f.u. /g) and *Bacillus thuringiensis* (2×10^{8}c.f.u. /g) obtained from Yash Green Land Pvt. Limited, Allahabad,was used as slurry seedling treatment for this experiment. All the formulations contained 0.5% w/w Carboxymethylcellulose(CMC)asanadhesivematerial.Insinglebio-agenttreatments,10gofformulationwasmixedin1liter water for seedling dip. However, in mixtures of bio-agents, combinations of the two powders were formedinratio1:1tomakethesame quantityas recommended bythe manufacturer" (**Suleiman *etal*.,2016**)[20]

2.4 IsolationofbioagentsfromTalcformulation

"Serial dilutions and spread plate techniques were adopted on the talc formulations to obtain the pure cultures ofthe bio-agents. For *T. viride*, PDA was used as medium and incubated at 25^0 C for 3 days while *Pseudomonasfluorescens*and *Bacillusthuringiens*is KBand NAwereused respectivelyat 37^{0}Cfor 48hours". [24]

Plate1Purecultureofbio-agents

2.5 Dualculturetechnique

"Dualculturemethodwasappliedtoevaluatetheinhibition(%)ofthepathogenbythebio-agents*invitro*.Amyceliaplugfromanactivelygrowing*A.solani*onPDAwastakenwithacorkborer(7mmdiameter)andkeptat1cmfromonesideoftheagar mediumcontainedin90mmdiameterpetridish. Anothermyceliadisc of*T. viride*was keptatthe other side. The bacterial isolates were streaked on the other side of the pathogen. In consortial treatments,however, both mycelial plug and bacterial streaks were used. The experimental design was complete randomizeddesign(CRD) with three replications. PDA medium was used in the experiments in order to favor the growth of*A.solani*and the potential antagonists" [24]. Petri plates without antagonist served as control. After that plates wereincubatedattemperature28±1°Cfornextfivedays.Thedatawasanalysedstatistically.Theefficacyofbiocontrolagentswasexpressedaspercentageinhibitionofmyceliagrowthovercontrolandcalculatedas**(Vincent,1927)[21].**

$$I = \frac{C - T}{C} \times 100$$

Where,

I=PercentInhibition,

C=Radialgrowthincontrol,T=Radialgrowthintreatment.

2.6 Assessmentofdiseasesymptomsandplantgrowthparametersinthefield

Plants were observed weekly for the symptoms of early blight, and plant growth promoting activity. Percentdisease intensity was recorded at 60, 75 and 90 DAT. It is calculated by using the following formula: **(Wheeler,1969)[23].**

$$\text{DiseaseIntensity} = \frac{\text{Sumofalldiseaserating} \times 100}{\text{TotalnumberofratingxMaximumdiseases grade}}$$

Also, disease severity was estimated by scoring individual plants on a 0-5 visual scale described by (**Vincent,1927**)[21].0-Freefrominfection,1-Oneortwonecroticspotsonafewlowerleavesofplants,coveringnearly1-10%2-Afewisolatedspotsonleaves,coveringnearly11-25%3-Manyspotscoalescedontheleaves,covering26-50%4-Irregular,blightedleavesandsunkenlesionwithprominentConcentricringsonthestempetiole,fruit,covering51-75%leafareaoftheplant5-Wholeplantsblighted,leafandfruitsstartingtofall, coveringmorethan75%leafarea of plant. "Yield was also recorded in respectto challenged control. The experiment was designed inRandomized Block Design (RBD). Data were analyzed statistically using analysis of variance according to SASprocedurefor a completelyrandomized design". [24]

3. RESULTSANDDISCUSSION

Table1EffectofTreatmentsonDiseaseintensity(%)ontomatoatdifferentdaysofintervals

Treatments		MeanofthreereplicatesofDiseaseintensity(%)		
		60 DAT	75DAT	90DAT
T0	Control	20.95	31.83	50.47
T1	Neemcake@250kg/ha+*P.fluorescens* @10g/lit	16.18	26.72	43.07
T2	Neemcake@250kg/ha+*B.thuringiensis* @10g/lit	17.18	27.40	43.63
T3	Neemcake@250kg/ha+*T.viride*@10g/lit	15.15	25.94	39.67
T4	Neemcake@250kg/ha+*P.fluorescens*@ 5g/lit+*B.thuringiensis*@5g/lit	14.08	24.12	33.57
T5	Neemcake@250kg/ha+*P.fluorescens*@5g/lit+ *T.viride*@5g/lit	11.76	20.42	32.21
T6	Neemcake@250kg/ha+*T.virid*@5g/l it+*B.thuringiensis*@5g/lit	13.39	22.62	33.20
C.D.(5%)		0.96	0.71	0.73
C.V.		3.45	1.56	1.04
SE(d)		0.44	0.33	0.33

DAT-DaysAfterTransplantation

3.1 EffectofTreatmentsonearlyblightintensityoftomatounderfieldconditions

TheDatapresentedintable1revealstheresponseofdifferenttreatmentsondiseaseintensityoftomatoat60,75and90DATunderfieldcondition.Thestatisticalanalysisofdatashowedthat all treatments were significantly reduced from control. Results showed that among thetreatments T_5- Neem cake + *Trichoderma viride*+ *Pseudomonas fluorescens* (32.20%)significantly reduced the disease intensity of*Alternaiasolani*as compared with T_6 – Neemcake+*Trichodermaviride*+*Bacillusthuringiensis*(33.20%),T_4-Neemcake+*Pseudomonasfluorescens*+*Bacillusthuringiensis*(33.56%),T_3-Neemcake+*Trichoderma viride*(39.66%), T_1- Neem cake + *Pseudomonas fluorescens* (43.06%), T_2-Neem cake +*Bacillus thuringiensis* (43.63%) and non - significant results were found inbetweenthe treatments (T_2, T_1) and (T_4, T_6).

Table2EffectoftreatmentsonPlantheight(cm)andyieldoftomatounderfieldconditions

Treatments		Meanofthreereplicatesof Plantheight(cm)			Yield(t/ha)
		30DAT	60DAT	90DAT	Total
T_0	Control	18.67	37.07	48.40	5.66
T_1	Neemcake@250kg/ha+ *P.fluorescens*@10g/lit	24.33	41.93	58.93	10.0
T_2	Neemcake@250kg/ha+ *B.thuringiensis*@10g/lit	23.80	40.83	57.40	9.83
T_3	Neemcake@250kg/ha+ *T.viride*@10g/lit	25.30	42.60	63.20	10.5
T_4	Neemcake@250kg/ha+ *P.fluorescens*@5g/lit +*B.thuringiensis*@5g/lit	27.00	45.33	71.97	14.6
T_5	Neemcake@250kg/ha+*P.fluorescens* @5g/lit+*T.viride*@5g/lit	28.67	52.67	74.13	16.1
T_6	Neemcake@250kg/ha+*T.viride*@5g/lit+ *B.thuringiensis*@5g/lit	27.60	46.67	72.73	15.1
C.D.(5%)		0.72	0.97	1.61	2.19
C.V.		1.62	1.25	1.42	0.18
SE(d)		0.33	0.45	0.74	1.16

3.2 EffectoftreatmentsonPlantheight(cm)oftomatounderfieldcondition

The data presented in table 2reveals the response of different treatments on plant height oftomato at 30,60,90 DAT under field condition. The statistical analysis of data showed that alltreatments were significantly increased plant height from control. Among the treatments T_5– Neem cake + *Trichoderma viride*+*Pseudomonas fluorescens* (74.13), significantly increasedplant height followed by T_6– Neem cake + *Trichoderma viride*+ *Bacillus thuringiensis* (72.73),T_4–Neemcake+ *Pseudomonasfluorescens*+*Bacillusthuringiensis*(71.96),T_3-Neemcake+*Trichodermaviride*(63.20),T_1-Neemcake+*Pseudomonasfluorescens*(58.93)andT_2-Neemcake+ *Bacillus thuringiensis* (57.40) Among the treatments (T_5,T_6),(T_6,T_4) and (T_1,T_2) werefoundnon-significantto each other

3.3 FruitYield(t/ha)oftomatoatharvest

Theanalysisofdatapresentedintable2revealedthatallthetreatmentssignificantlyincreasedinfruit Yieldt/hafrom the control.Among the treatments T_5- Neem cake+*Trichoderma viride*+*Pseudomonasfluorescens*(16.1t/ha)significantlyincreasedinfruityieldfollowedbyT_6– Neemcake+*Trichodermaviride* +*Bacillusthuringiensis*(15.1t/ha),T4–Neemcake+Pseudomonas

fluorescens + *Bacillus thuringiensis* (14.6 t/ha), T_3- Neem cake + *Trichoderma viride*(10.5), T_1- Neemcake+*Pseudomonasfluorescens*(10.0t/ha)andT_2- Neemcake+*Bacillusthuringiensis*(9.8t/ha).Amongthetreatments(T_1,T_2andT_3)(T_5,T_6)foundnon -significantto eachother.

Table3*Invitro*evaluationofbioagentsontheradialgrowth(mm)of*Alternariasolani*at72hrsafterincubation

Tr.No	Treatments	Meancolonydiameter(mm) of *Alternariasolani*	Inhibitionpercentage(%)
T_1	*Pseudomonasfluorescens*	25	66.6
T_2	*Bacillusthuringiensis*	28	62.6
T_3	*Trichodermaviride*	20	73.3
T_4	*P.fluorescens+B.thuringensis*	16	78.6
T_5	*T.viride+P.fluorescens*	8	89.3
T_6	*T.viride+B.thuringensis*	10	86.6
T_0	Control	75	00.0
	CD(5%)	1.62	
	SE(d)	0.75	

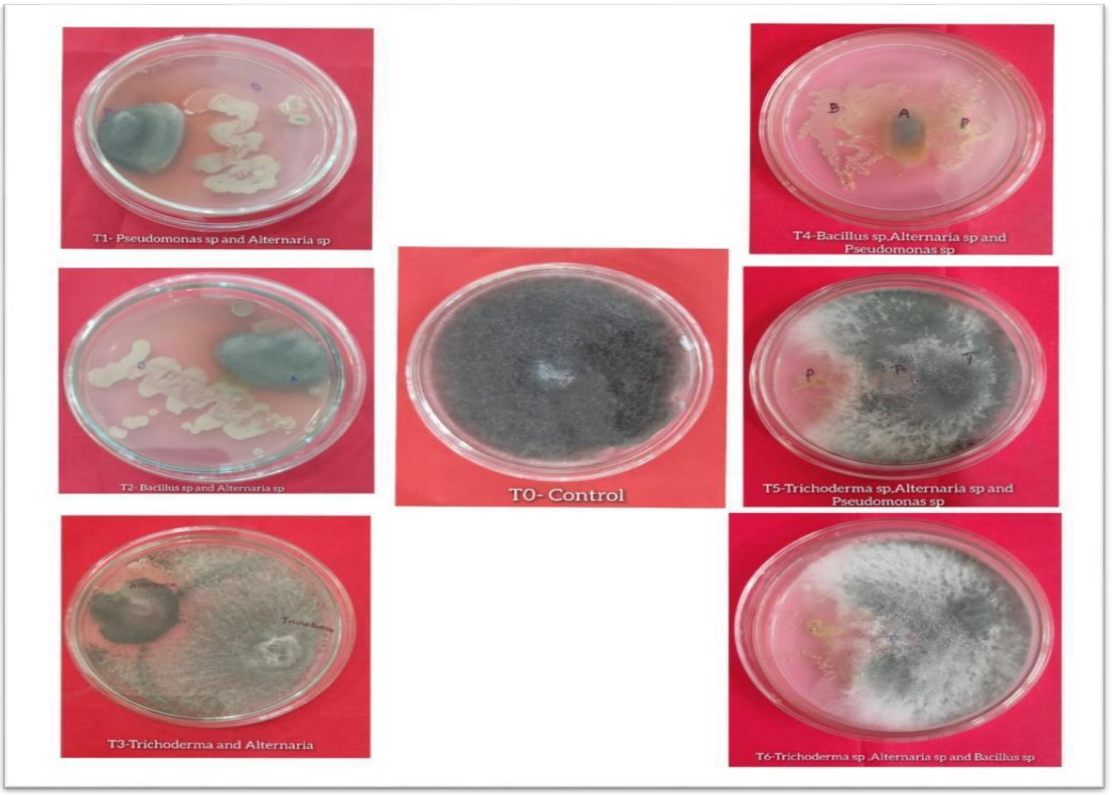

Plate2Antagonisticpotentialofbioagentson*Alternariasolani*

3.4 In-vitro evaluation of antagonistic potential of bioagents on radial growth (mm) of *Alternaria solani*

Following the dual culture technique between the *A. solani* and the biological control agents, *in vitro* analysis revealed that the six treatments, except control, inhibited the growth of *A. solani* significantly. The results obtained (Table 3) showed that T_5 - *T. viride* + *P. fluorescens* inhibited the growth of *A. solani* most by 89.3% with only 8.00mm growth in diameter of the pathogen. This is followed by T_6 - *T. viride* + *B. thuringiensis* with inhibition of 86.6 and 10.00mm diameter of pathogen growth, and then T_4 - *P. fluorescens* + *B. thuringiensis* with 78.6% inhibition and pathogen diameter of 16.00mm, T_3- *Trichoderma viride* with 73.3% inhibition and pathogen diameter of 20.00mm, T_1- *P. fluorescens* with 66.6% inhibition and pathogen diameter of 25.00mm and the least inhibition is on T_2 - *B. thuringiensis* with 62.6% inhibition and pathogen diameter of 28.00mm. Based on this in vitro study, the mixture between the three bioagents gave higher inhibition (%) of *A. solani* in comparism with single bio-agents. This is in agreement with the work of **(Suleiman *et al*., 2016)**[20].

4. CONCLUSION

Based on the results obtained from present investigations it was found that Soil application of Neemcake along with combination of bio-agents *Trichoderma viride* + *Pseudomonas fluorescens* seedling treatment was found most effective against *Alternaria solani* which cause early blight disease in tomato therefore it may be recommened for the better management of early blight of tomato. In field conditions Neemcake + *Trichoderma viride* + *Pseudomonas fluorescens* showed significant reduction of disease intensity (%) and significant increase in plant height, number of branches and yield.

In *in-vitro* condition dual culture method *Trichoderma viride* + *Pseudomonas fluorescens* showed heighest growth inhibition percentage as compared to other treatments including control. The use of plant extract and biocontrol agent in alternation with the fungicides could be suggested and recommended to be applied especially in order to manage fungicide residues

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