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Integrated Disease Management of Early Blight in Organically grown Tomato under Hill Condition of Sikkim, India

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Abstract— Tomato (*Lycopersicon esculentum* Mill.) is the most widely cultivated vegetable crop in the world and early blight caused by *Alternaria* spp. is one of the major production constraints. Survey was conducted during kharif seasons of 2018-19 &2019-20 in four districts of Sikkim. Maximum early blight disease severity was recorded in south district of Sikkim. Pathogenicity test of isolates collected from different location were established. Morphological, cultural, and molecular identification of the pathogen reveal pathogen as *Alternaria* spp. Five different bio-control agents i.e., *Trichoderma harzianum*, *T. Viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus cereus* were evaluated against the test pathogens. Among the bio-control agents tested, *Trichoderma harzianum* (74.89%) and *Trichoderma viride* (69.41%) showed maximum percent growth inhibition. Open field experiment was conducted with eleven treatments formulated by integrating the different organic inputs, biocontrol agents and copper-based fungicide as chemical check. Organic treatment T_9 {Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg *Trichoderma* + 25 Kg of FYM) + cow dung slurry 10% + Nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%} consistently showed the best result among the bioresources tested.

***Keywords*— Tomato, Organic, Bio agent and Integrated disease management**

INTRODUCTION

Tomato (*Lycopersicon esculent*um Mill, n = 12) belongs to the family Solanaceae and is one of the most remunerable and widely grown vegetables in the world. Among the fungal diseases, leaf blight disease incited by Alternaria spp. is one of the world's most catastrophic disease incurring losses both at pre- and post-harvest stages in tomato growing tracks of India (Gondal *et al.*, 2012). Early blight symptoms appear as small, circular, light brown necrotic areas with or without yellow halo, mostly on the lower leaves. Some spots are enlarged with characteristic concentric rings giving a target board effect. Some irregular spots also observed to have been restricted by veins. Seedling, stem, blossom blight and fruit drop symptoms also produced by this pathogen (Agrios, 2005). Presently, many systemic as well as non-systemic fungicides have been recommended to manage this disease (Patil *et al.*, 2002) and farmers mainly rely upon systemic fungicides to control most of the diseases in tomato cultivation due to unavailability of any concrete organic management technology to tackle the prevailing biotic stress. On the other hand, the current global scenario firmly emphasizes the need to adopt eco-friendly

agricultural practices for sustainable agriculture. Chemical management has made an adverse impact not only on soil health but also on the beneficial soil microbial communities and the plants cultivated in these soils. Therefore, keeping these views in mind, the present investigation on *in-vitro* efficacy of bio-control agents against eight isolates of A*lternaria* spp. was studied. Thereafter, field evaluation of different combination of bioresources against early blight of tomato was conducted.

MATERIALS AND METHODS

The field experiments were conducted in the farmers field of Sikkim for two successive kharif seasons of 2019 & 2020. The lab experiment was conducted in the Department of Plant Pathology, Bidhan Chandra Krish Vishwavidyalaya, Mohanpur, West Bengal during the year 2019,2020 and 2021. To isolate the pathogen, infected tissues from leaves, stems and fruits of tomato plants showing typical symptoms of early blight were collected and isolation was done by standard isolation protocol.

A. *In vitro* evaluation of biocontrol agents against *Alternaria* isolates

Bio control agents were evaluated for their efficacy against *Alternaria* isolates using dual culture technique. Twenty ml of PDA was poured into 90 mm diameter petri dishes and allowed to solidify. Five mm discs of isolates were taken from seven days old culture and placed at one end of petri dish. Respective antagonistic organism (5mm disc) was also inoculated at the opposite side of same petri plate. In case of bacterial antagonist *Alternaria* isolates was placed at centre of the petri plates and bacterial culture was streaked at the centre of petri plates. Each treatment was replicated thrice and incubated at 25±2°C. The activity of antagonistic organisms was recorded by measuring the colony diameter of each isolate in each treatment and compared with control. Mean % inhibition was calculated by the following formula:

$Mean\ \%\ Inhibition = \frac{C-T}{C} \times 100$(Where, **C**= Colony diameter in control, **T**= Colony diameter in treatment) **Details of bio-control agents studied are as follows**:1. *Trichoderma harzianum*2. *Trichoderma viride* 3. *Pseudomonas fluorescens*,4. *Bacillus Subtilis* 5. *Bacillus cereus* 6. Control(1 & 2 Collected from CNG Agrocare Pvt. Ltd., Kolkata and 3, 4 & 5 collected fromNational Bureau of Agriculturally Important Microorganisms (NBAIM), UP).

B. In Vivo management of Early blight

An experiment was conducted in farmers field at Namthang, South Sikkim during 2019 and 2020 with treatments formulated by integrating the different organic inputs, biocontrol agents in single and in combination. The safe fungicides like copper-based fungicide was taken as check and the untreated plot as control. The experiment was laid out in a randomized block design with different treatments replicated thrice. The susceptible hybrid variety Romeo which is widely grown by farmers of Sikkim was grown under different treatment combinations**.**

Twenty-five-day old seedlings were transferred in 5m X 4 m plot with 60 X 60 spacing. At 10 DAP, treatments were applied. Foliar sprays with bioagent and organic amendments commenced from 20 DAT. Spray applications with *P. fluorescens* talc formulation ($1x10^9$cfu/g) @ 2 g/l and copper oxychloride @ 3 g/l were given as per the schedule explained in treatments.

Data on per cent disease severity was assessed on five tagged plants selected randomly in each plot at 10 days interval from the first appearance of disease. Disease severity was measuredby using 0-5 scale given by (Mayee and Datar, 1986) as described in **Table 1**. The

cumulative fruit yield for all pickings per plot was recorded and yield in t ha^{-1} was calculated. Per cent disease reduction, per cent yield increase and per cent yield loss were calculated by using following formulas.

$$\%\ disease\ reduction = \frac{Disease\ severity\ in\ check - Disease\ severity\ in\ treatment}{Disease\ severity\ in\ check} X\ 100$$

$$\%\ yield\ increase = \frac{Yield\ in\ treatment - yield\ in\ check}{yield\ in\ check} X\ 100$$

$$\%\ Yield\ loss = \frac{Attainable\ yield - Actual\ yield}{Attainable\ yield} X\ 100$$

Table 1.: Description of disease scale (0-5)

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot-on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, somespots on petiole
3	21-40 per cent leaf area infected and covered by spot, spotsalso seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spotsalso seen on petiole, branches, stem
5	>71 per cent leaf area infected and covered by spot, spotsalso seen on petiole, branch, stem, fruit

Percent Disease Index (PDI) was worked out by using formula given by Wheeler (1969).

$$rating\ PDI = \frac{Sum\ of\ individual}{Number\ of\ leaves\ scored\ x\ Maximum\ rating\ in\ the\ scale} X\ 100$$

Table.2: List of different treatments for field trial in Sikkim

Sl.No.	Treatment	References
T_1	Five spray of *Allium sativum* @5% at 10 days interval	(Attri*et al.*, 2020)
T_2	Susceptible Variety +Cow urine @ 10 % at 10 days interval	(Ghosh and Biswas, 2018)
T_3	Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval	(Jagadeesh and Jadadeesh, 2009)
T_4	Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg *Trichoderma* +25 Kg of FYM) and foliar spray at 10 days interval	(Hosagoudar and Chattannavar ,2013)
T_5	Susceptible Variety + Botanical pesticide Neem @ 0.2% at 10 days interval	(Hassanein *et al.*, 2010)
T_6	Susceptible Variety + Panchagavya 10% at 10 days interval	(Patilkulkarni, 2013)
T_7	Susceptible Variety + Vermi wash @ 10% at 10 days interval	(Latha and Zacharai, 2021)
T_8	Susceptible Variety + Soil treatment with *Trichoderma harzianum* @ 1:25(1kg *Trichoderma* +25 Kg of FYM) + spray of neem oil 5% + spray of cow urine 10%	(Devi, 2013)
T_9	Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg *Trichoderma* + 25 Kg of FYM) + cow dung slurry 10% + nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%	(Patilkulkarni, 2013)
T_{10}	Foliar spray of Seaweed (Red and Brown Algae, Sagarika) @ 1ml/l water thrice @ 20 days interval	(Chanthini *et al.*, 2012)
T_{11}	copper oxychloride 0.3% at 10 days interval Chemical check	(Hosagoudar and Chattannavar ,2013)
T_{12}	Untreated control	-

C. Statistical analysis

Data obtained of various experiments were analysed statistically by using OPSTAT software and their means were separated by the test of CRD and RBD at the 0.05% of the probability level.

RESULTS AND DISCUSSION

Pathogen associated with the early blight disease of tomato was isolated in pure culture and identified as *Alternaria* spp.on the basis of morphological i.e., conidiophores, size, shape, septation and beak length of conidia and cultural characteristics i.e., colour and type of colony of the pathogen (Simmons, 2007). Molecular characterization of different isolates was done by PCR amplification and nucleotide sequence analysis of two unlinked loci – rDNA containing ITS1 and ITS4.The isolates were designated as ASS1 (South Sikkim, 27^0 10’ 53.4” N, 88^0 28’ 33.2” E), AWS2 (West Sikkim, 27^0 18’ 55.2” N, 88^0 16’, 02.6” E), AES3 (East Sikkim, 27^0 17’ 46.9”N, 88^0 36’ 30.6” E), ANS4 (North Sikkim, 27^0 24’ 28.4” N, 88^0 34’ 22.2” E), AJF5 (Jaguli farm, 22^0 59’ 21” N, 88^0 27’ 22” E), AGF6 (Gayespur farm, 22^0 58’ 22.4” N, 88^0 29’ 42.5” E), AIF7 (InCheck Farm, 22^0 19’ 55.3” N, 88^0 30’ 35.4” E) and AMF8 (Madanpur, farmers field, 23^0 00’ 26” N, 88^0 29’ 28” E).

A. Efficacy of bio - control agents against eight isolates of *Alternaria* spp.

Experimental data pertaining to *in-vitro* efficacy of bio-control agents against eight isolates of *Alternaria* spp. are presented in **Table 3**

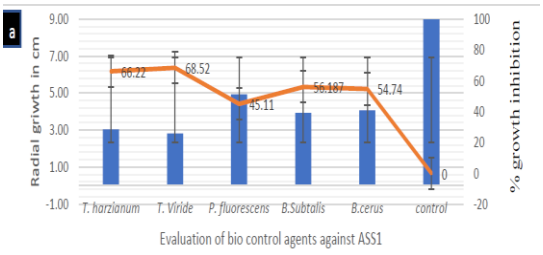
Table 3: *In vitro* evaluation of bio agents against eight isolates of *Alternaria* spp.

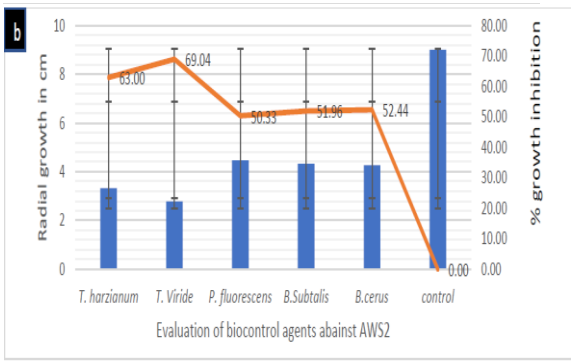
	ASS1 (South Sikkim)		AWS2 (West Sikkim)		AES3 (East Sikkim)		ANS4 (North Sikkim)	
Treatment	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**
Trichoderma harzianum	3.04	66.22 (54.44)	3.33	63.00 (52.53)	3.43	61.85 (51.86)	2.51	72.11 (58.14)
T. Viride	2.83	68.52 (55.85)	2.78	69.04 (56.18)	3.05	66.15 (54.39)	3.08	65.81 (54.19)
P. fluorescens	4.94	45.11 (42.17)	4.47	50.33 (45.17)	4.35	51.70 (45.96)	4.32	52.00 (46.13)
B.Subtills	3.94	56.19 (48.54)	4.32	51.96 (46.11)	4.46	50.48 (45.26)	4.11	54.29 (47.45)
B.cereus	4.07	54.74 (47.70)	4.28	52.44 (46.38)	4.87	45.89 (42.62)	4.13	54.07 (47.32)
Control	9.00	0 .00	9.00	0.00	9.00	0.00	9.00	0.00
C.D.	0.405	2.623	0.338	2.232	0.518	3.361	0.776	5.033
SE(m)±	0.13	0.842	0.108	0.716	0.166	1.079	0.249	1.615
	AJF5 (Jaguli farm)		**AGF6 (Gayespur farm)**		**AIF7 (InCheck Farm)**		**AMF8 (Madanpur, farmers field)**	
Treatment	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**	**Mycelial growth**	**% Growth Inhibition**
Trichoderma harzianum	2.50	72.22 (58.24)	2.86	68.22 (55.68)	2.55	71.67 (57.83)	2.26	74.89 (59.92)
T. Viride	2.75	69.41 (56.43)	2.85	68.37 (55.76)	2.79	69.00 (56.15)	2.40	73.29 (58.87)
P. fluorescens	3.32	63.07 (52.57)	3.35	62.81 (52.40)	3.04	66.26 (54.47)	2.82	68.70 (55.96)
B.Subtalis	3.95	56.11 (48.49)	3.81	57.70 (49.41)	3.44	61.78 (51.79)	3.35	62.78 (52.42)
B.cerus	4.18	53.48 (46.98)	4.14	54.04 (47.29)	3.99	55.59 (48.19)	3.83	57.44 (49.26)
Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00
C.D.	0.506	3.532	0.289	1.955	0.244	1.663	0.525	3.54
SE(m)±	0.162	1.134	0.093	0.628	0.078	0.534	0.168	1.136

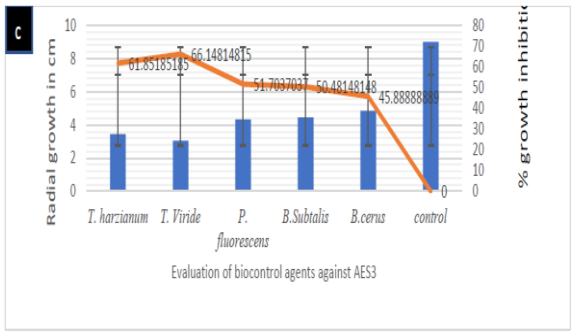
Among the bio-control agents tested against eight isolates, all the bioagent did suppress the mycelial growth, however, *Trichoderma harzianum* (74.89%)and *Trichoderma viride* (69.41%)showed maximum percent growth inhibition, which was followed by *Pseudomonasfluorescens* (66.26%).

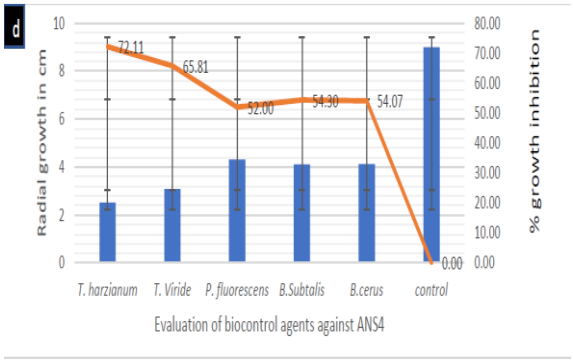
The findings obtained from the present dual culture assay clearly reveals that, *Trichoderma viride* and *Trichoderma harzianum* are superior in inhibiting the growth of all the isolate tested. *Pseudomonas fluorescens, Bacillus subtilis* and *B. cereus* also moderately inhibit the mycelial growth of the pathogen tested but unlike *Trichoderma viride* and *Trichoderma harzianum.* Pandey (2010) studied the antagonistic properties of *Trichoderma viride* and *Trichoderma harzianum* against *Alternaria alternate* and found that, *Trichoderma harzianum* reduced the growth of *Alternaria alternate* by 67.07 % and was found to be more effective in controlling the growth of test pathogen. *Trichoderma viride* reducing the mycelial growth by 66.67% was also found to be suitable bio-control agent. Similar results wherein efficacy of *Trichoderma* spp. against *Alternaria*

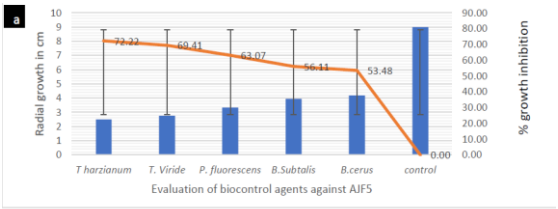
species was previously reported by Martinez and Solano (1995), Deshmukh and Raut (1992), Rao (2006) and Dalpati *et al.*, (2010).

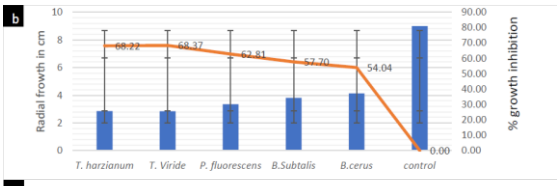


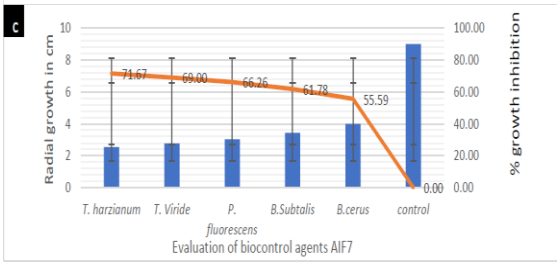












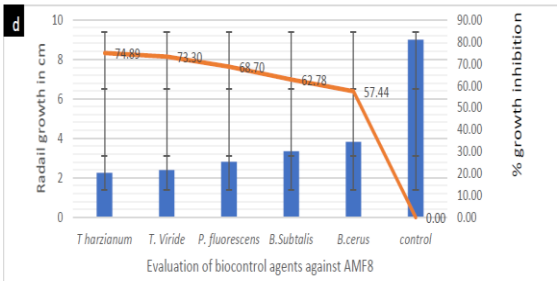


Fig.1. In vitro evaluation of bioagents against Alternaria spp isolate ASS1, AWS2, AES3, ANS4, AJF5, AGF6, AIF7, AMF8.

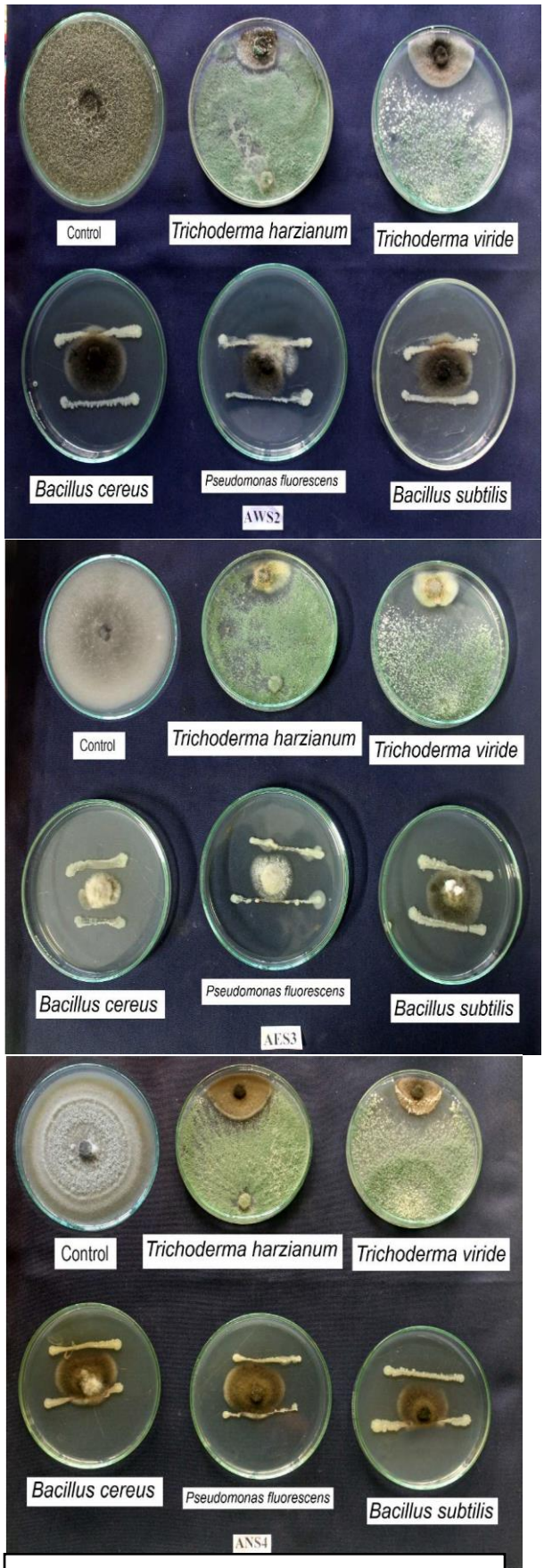


Plate.1. *Invitro* evaluation of biocontrol agents against Alternaria isolates. a= ASS1; b= AWS2; c= AES3; d= ANS4

Plate.2. *Invitro* evaluation of biocontrol agents against Alternaria isolates. a= AJF5; b= AGF6; c= AIF7; d= AMF8

B.Integrated disease management of Alternaria leaf blight of tomato

A fixed plot survey was carried out during kharif season of 2018-19 &2019-20 in four districts of Sikkim. The least disease severity (16.67 %) and AUDPC of 614.28 was recorded in Sakyong village under West District of Sikkim and the maximum severity (34.67%) and maximum AUDPC (1447.70) were recorded in Namthang village located under South District of Sikkim. An experiment was conducted in Farmer's field in farmer's field at Namthang, South Sikkim during 2019 and 2020.The treatments were formulated by integrating the different organic inputs, bio control agents in sole and in combination, copper oxychloride @ 0.3% was taken as chemical check and untreated plot as control, as described in materials and methods. First spray was imposed immediately after appearance of the disease which was followed by sprays at an interval of 10 days. The data on disease appearance, disease severity, per cent disease reduction, yield, per cent yield increase over control and per cent yield loss were recorded. Cost benefit ratio was also calculated to know the efficacy of each treatment.

The results presented in the **Table 4** indicates that, during 2019 in Sikkim, all the bioresources tested under field condition significantly reduced the disease severity and increased the yield as compare to the T_{12} (untreated control). The data also signified that the least disease severity (16.00%), maximum disease reduction (58.20%), highest yield (15.72t/ha), maximum per cent yield increase (44.83%) and least yield loss (21.40%) and highest cost benefit ratio 3.16:1 were recorded in T_9 {Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg *Trichoderma* +25 Kg of FYM) + Cow dung slurry 10% + Nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%} except chemical check (T_{11}).

T_1 (Susceptible Variety + Five spray of *Allium sativum* @5%) also showed significant effect in respect to disease severity (17.33%), per cent disease reduction (54.73%), yield (15.14t/ha), per cent yield increase (39.54%), per cent yield loss (24.30%) and cost benefit ratio (1: 3.05) followed by T7 (Susceptible Variety + Vermi wash @ 10% at 10 days interval),in which disease severity (18.67%), per cent disease reduction (51.48%), yield (14.63t/ha), per cent yield increase (34.86%), per cent yield loss (26.85%) and cost benefit ratio (1:2.95) which recorded, which is also a promising result as compared to other treatments.

Two treatments were composed only with biocontrol agents, T_3 (Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval) and T_4 {Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg Trichoderma +25 Kg of FYM) and foliar spray at 10 days interval}. T_4 {Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg Trichoderma +25 Kg of FYM) and foliar spray at 10 days interval} performed better in respect to disease severity (19.33%), per cent disease reduction (49.81%), yield (14.33t/ha), per cent yield increase (32.04%), per cent yield loss (28.35%) and cost benefit ratio (1:2.89) than T_3.

The maximum disease severity (26.67%), minimum percent disease reduction (30.54%), lower yield (12.32t/ha), minimum percent yield increase (13.59%), maximum percent yield loss (38.40%) and least cost benefit ratio (1:2.49) was recorded in T_2 (Susceptible Variety +Cow urine @ 10 % at 10 days interval) as compared to all other treatments except untreated control (T_{12}).

Similarly, during 2020 in Sikkim, the data presented in**Table 5**reveals that, all the organic

management strategies implemented so far, significantly reduce the disease severity and increased the yield as compare to the T_{12} (untreated control). In exception to chemical check (T_{11}), the least disease severity (15.33%), maximum disease reduction (56.52%), highest yield (16.93t/ha), maximum percent yield increase (51.65%) and least yield loss (15.35%) and highest cost benefit ratio 1:3.41 was recorded in T_9 {Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg *Trichoderma* + 25 Kg of FYM) + Cow dung slurry 10% + Nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%}, which was projected superior to all other treatments.

T_1 (Susceptible Variety + Five spray of *Allium sativum* @5%) with disease severity (18.67%),disease reduction (47.05%), yield (14.95t/ha), percent yield increase (33.92%), percent yield loss (25.20%) and cost benefit ratio (1:3.01) and T_7 (Susceptible Variety + Vermi wash @ 10% at 10 days interval) with disease severity (19.33%), disease reduction (45.31%), yield (15.00t/ha), percent yield increase (34.54%), percent yield loss (24.85%) and cost benefit ratio (1:3.02) were at par with each other in terms of both the disease and yield related parameters respectively. Wherein, significant difference between the two treatments was not observed as per their respective CD values.

Finally, the maximum disease severity (24.67%), minimum percent disease reduction (30.06%), low yield (12.24t/ha), minimum percent yield increase (9.69%), maximum percent yield loss (38.75%) and least cost benefit ratio (1:2.47) was recorded in T_3 (Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval) as compared to all other treatments except untreated control (T_{12}).

From the two successive years trial it is noticed that, organic treatment T_9 {Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg *Trichoderma* + 25 Kg of FYM) + cow dung slurry 10% + Nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%} consistently showed the best result among the bioresources tested so far.

Though, the sole application of *Trichoderma harzianum* (T_4), Nimbicidine (T_5), Panchagavya (T_6) and Cow urine (T_2) were able to manage the early blight disease to certain extent as compared to untreated control (T_{12}), but the combined effect of the above resources (T_9) showed a better result than individual application.

Combined effect of Panchagavya and neem in respect to growth, yield and pest & disease management parameter have been studied with significant effect (Sireesha, 2013).

Patilkulkarni (2013) concluded that among field evaluation of organics, among nine treatments, T2 (S.T. with *T. harzianum* @ 10 g/kg + spray of Panchagavya 5%+ NSKE 5%) with least disease index score was the most effective in controlling purple blotch of onion and there by recorded highest yield. Panchagavya when combined with cow urine or plant extracts, its antifungal activity enhanced remarkably.

In respect to sole application of bio-control agent i.e., treatment T_3 and T_4, both the treatments were able to restrict the disease to certain extent in comparison with untreated crop, T_4 {Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg Trichoderma +25 Kg of FYM) and foliar spray at 10 days interval} showed better in performance than T_3 (Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval)in both the successive years . This result is in contradiction with the findings of Jagadeesh and Jadadeesh (2009), where they found that *Pseudomonas* spp. was able to control early blight disease of tomato by 60.2% which is at par with chemical control. Instead, the result was

in line with the findings of Hosagoudar and Chattannavar (2013), where they found that, seed treatment plus foliar spray of *Trichoderma* spp. was able to suppress the *Alternaria* leaf spot of cotton drastically.

It is noted that, the efficiency of biocontrol agent may be determined by various factors viz., the soil temperature and soil reaction (Harman *et al.*,1980, 1981 ; Knudsen and Bin, 1990), the kind of soil and its microbiota (Hadar *et al.*, 1984), the nutritional status of the inoculants (Harman *et al.*,1981), the inoculum density on the seed (Papavizas*et al.*, 1982), the inoculum potential of the pathogen in soil (Wu, 1982), the rate of application of the antagonist to soil (Elad *et al.*, 1980; Kommedahl*et al.*, 1981).

Treatment T_1 (Susceptible Variety +Five spray of *Allium sativum* @5%) showed the second-best result among the various bio-resources tested in terms of disease severity suppression. The result of present study is in consonance with the findings of various earlier workers who reported that garlic extract was highly effective against diseases reduction in addition to increase the fruit yield. (Naswa and Abo-Elyousr,2012; Mahapatra and Das 2013; Debbarma *et al.*,2017, Attri*et al.*,2020). The reduction of disease in present investigation may be due to the presence of compound allicin and ajoene in garlic. Shekhawat and Prasad (1971) reported that garlic extract was effective against *Alternaria* and concluded that allicin in garlic was responsible for bursting in young hyphae of fungus. Singh *et al.*, (1990) reported an ajoene compound derived from garlic inhibited the spore germination of *A. solani*.

Table 4: Field evaluation of different organic management strategies in tomato in the hills of Sikkim during 2019 cropping season

Treatments	Cropping period	Time of Disease onset (DAT)	Time of Max. severity (DAT)	PDI	%Disease reduction	Yield t/ha	% Yield increase	%Yield loss	B:C ratio
Susceptible Variety +Five spray of *Allium sativum* @5%	July-Nov	40	80	17.33 (24.58)	54.73 (47.71)	15.14	39.54	24.30	3.05:1
Susceptible Variety +Cow urine @ 10 % at 10 days interval	July-Nov	30	100	26.67 (31.07)	30.54 (33.44)	12.32	13.59	38.40	2.49:1
Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval	July-Nov	35	90	25.33 (30.20)	34.17 (35.73)	12.87	18.57	35.65	2.60:1
Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg Trichoderma +25 Kg of FYM) and foliar spray at 10 days interval	July-Nov	40	100	19.33 (26.06)	49.81 (44.88)	14.33	32.04	28.35	2.89:1
Susceptible Variety + Botanical pesticide Neem @ 0.2% at 10 days interval	July-Nov	35	90	21.33 (27.49)	44.59 (41.88)	13.62	25.57	31.90	2.74:1
Susceptible Variety + Panchagavya 10% at 10 days interval	July-Nov	35	90	22.67 (28.42)	41.05 (39.81)	13.57	24.98	32.15	2.73:1
Susceptible Variety + Vermi wash @ 10% at 10 days interval	July-Nov	40	100	18.67 (25.58)	51.48 (45.83)	14.63	34.86	26.85	2.95
Susceptible Variety + Soil treatment with *Trichoderma harzianum* @ 1:25(1kg Trichoderma +25 Kg of FYM) + spray of neem oil 5% + spray of cow urine 10%	July-Nov	35	90	23.33 (28.87)	39.38 (38.84)	13.07	20.41	34.65	2.6:1
Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg Trichoderma +25 Kg of FYM) + cow dung slurry 10% + nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%	July-Nov	45	80	16.00 (23.54)	58.20 (49.74)	15.72	44.83	21.40	3.16:1
Foliar spray of Seaweed (Red and Brown Algae, Sagarika)@ 1ml/l water thrice @ 20 days interval	July-Nov	35	90	20.67 (27.02)	46.27 (42.83)	13.56	25.04	32.20	2.73:1
copper oxychloride 0.3% at 10 days interval Chemical check	July-Nov	45	80	12.67 (20.82)	67.12 (55.01)	16.32	50.40	18.40	3.23:1
Untreated control	July-Nov	30	90	38.67 (38.42)	0.00 (0.00)	10.85	0.00	45.75	2.19:1
CD	-	-	-	1.678	3.004	0.59	5.48	-	-
SE(m)	-	-	-	0.568	1.018	0.20	1.86	-	-

Table 5: Field evaluation of different organic management strategies in tomato in the hills of Sikkim during 2020 cropping season

Treatments	Cropping period	Time of Disease onset (DAT)	Time of Max. severity (DAT)	PDI	%Disease reduction	Yield t/ha	% Yield increase	%Yield loss	B:C ratio
Susceptible Variety +Five spray of Allium sativum @5%	July-Nov	40	80	18.67 (25.58)	47.05 (43.28)	14.95	33.92	25.20	3.01:1
Susceptible Variety +Cow urine @ 10 % at 10 days interval	July-Nov	30	100	24.00 (29.32)	32.03 (34.44)	13.35	19.51	33.25	2.69:1
Susceptible Variety + Seedling root dip and eight foliar sprays with *Pseudomonas fluorescens* 0.3% at 10 days interval	July-Nov	35	90	24.67 (29.76)	30.06 (33.16)	12.24	9.69	38.75	2.47:1
Susceptible Variety + Soil treatment (ST) with *Trichoderma harzianum* culture mixed with well rotten FYM @ 1:25 (1kg Trichoderma +25 Kg of FYM) and foliar spray at 10 days interval	July-Nov	40	100	20 (26.55)	43.35 (41.16)	14.91	33.528	25.45	3.00:1
Susceptible Variety + Botanical pesticide Neem @ 0.2% at 10 days interval	July-Nov	35	90	20.67 (27.02)	41.50 (40.08)	13.01	16.525	34.95	2.62:1
Susceptible Variety + Panchagavya 10% at 10 days interval	July-Nov	35	90	23.33 (28.86)	33.87 (35.55)	12.5	12.022	37.45	2.51:1
Susceptible Variety + Vermi wash @ 10% at 10 days interval	July-Nov	40	100	19.33 26.068	45.31 42.29	15.0	34.541	24.85	3.02:1
Susceptible Variety + Soil treatment with *Trichoderma harzianum* @ 1:25(1kg Trichoderma +25 Kg of FYM) + spray of neem oil 5% + spray of cow urine 10%	July-Nov	35	90	21.33 (27.49)	39.65 (39.01)	12.95	15.982	35.25	2.61:1
Susceptible Variety + Soil treatment with *Trichoderma harzianum* @1:25(1kg Trichoderma +25 Kg of FYM) + cow dung slurry 10% + nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%	July-Nov	45	80	15.33 (23.03)	56.52 (48.75)	16.93	51.645	15.35	3.41:1
Foliar spray of Seaweed (Red and Brown Algae, Sagarika)@ 1ml/l water thrice @ 20 days interval	July-Nov	35	90	22.67 (28.42)	35.84 (36.74)	12.78	14.556	36.10	2.58:1
copper oxychloride 0.3% at 10 days interval Chemical check	July-Nov	45	80	11.33 (19.65)	67.97 (55.52)	17.03	52.515	14.80	3.43:1
Untreated control	July-Nov	30	90	35.33 (36.45)	0 (0.00)	11.17	0	44.15	
CD	-	-	-	1.318	3.144	0.49	4.41	-	-
SE(m)	-	-	-	0.446	1.065	0.16	1.49	-	-

Plate.3. Graph a= PDI and % disease reduction in 2019; b= yield and % yield increase in 2019; C= PDI and % disease reduction in 2020; d= yield and % yield increase in 2020 in Sikkim

Fig.2. Filed evaluation of different organic treatments in Sikkim

CONCLUSION

Five different bio-control agents i.e., *Trichoderma harzianum*, *T. Viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus cereus* were evaluated against the test pathogens. Among these biocontrol agents tested against different isolates, *Trichoderma viride* showed maximum percent growth inhibition which was followed by *Trichoderma harzianum*. Studies on integrated disease management with the application of organic inputs conducted during the year 2019 and 2020, in South Sikkim revealed that out of 12 treatments including chemical check and untreated control, treatment T9 {Susceptible Variety + Soil treatment with Trichoderma harzianum @1:25(1kg Trichoderma +25 Kg of FYM) + cow dung slurry 10% + nimbicidine 0.3% + Panchagavya 10% + Cow Urine 10%} was the most effective in terms of least disease severity, maximum per cent disease reduction, maximum yield and maximum per cent yield increase followed by treatment T1 (Susceptible Variety + Five spray of Allium sativum @5%) and T7 (Susceptible Variety + Vermi wash @ 10% at 10 days interval) except chemical check T11

REFERENCES

Agrios, G.N., 2005. Plant pathology. 5th Edition, Academic Press, New York, USA, 2005, 1−922.

Attri, K., Gupta, P., Kansal, S., Gupta, M. & Goswami, M. (2020). Early blight disease management of tomato through use of native bioresources in mid hills of Himachal Pradesh. *International Journal of Bio-resource and Stress Management.*, 11(2): 153-158.

Chanthini, K., Kumar, C.S. and Kingsley, S.J. (2012).Antifungal activity of seaweed extracts against phytopathogen *Alternaria solani*.*Journal of Academia and Industrial Research*.,1(2):86-90

Dalpati, N. N. S., Parate, R. L. and Ingle, S. T. (2010). Efficacy of some bioagents and botanicals against *Alternaria macrospora* causing leaf spot of cotton. *Journal of Plant Disease Sciences*., 5 (1): 95-97

Debbarma, S., Zacharia, S., Simon, S. (2017). Effect of plant extracts on early blight of potato (*Solanum tuberosum* L.). *Journal of Pharmacognosy and Phytochemistry*. 6(4): 1415−1417.

Deshmukh, P. P. and Raut, G. J. (1992). Antagonism by *Trichoderma* spp. on five plant pathogenic fungi. New Agriculture., 3: 127-130

Devi, P.R. (2013). Biology, Epidemiology and Management of *Alternaria* spp. causing early blight of tomato (*Lycopersicon esculentum* Mill.). *Ph.D. Thesis*. Dr. YSR Horticultural University, Andhra Pradesh.

Elad, Y., Chet, I. and Katan, J. (1980). *Trichoderma harzianum*: A biological agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology., 70: 119-121.

Ghosh, T and Biswas, M.K. (2018). Evaluation of antibacterial and antifungal activity of cow urine against some seed borne microflora.*International Journal of Current Microbiology and Applied Science*., 7(5):1714-1727.

Gondal, A.S., Ijaz, M., Riaz, K and Khan, A.R. (2012). Effect of different doses of fungicide (Mancozeb) against *Alternaria* leaf blight of tomato in Tunnel. *Plant Pathology and Microbiology*. 3(3): 1-3.

Hadar, Y., Harman, G. E. and Taylor, A. G. (1984). Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. *Phytopathology*., 74: 106-110.

Harman, G. E., Chet, I. and Baker, R. (1980). *Trichoderma hamatum* effects on seed and seedling diseases induced in radish and pea by *Pythium* spp. or

Rhizoctonia solani. Phytopathology., 71: 1167-1172.

Harman, G. E., Chet, I. and Baker, R. (1981). Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. Phytopathology., 71: 569-572.

Hassanein, N. M., AbouZeid, M.A., Youssef, K.A. and Mahmoud, D.A. (2010). Control of tomato early blight and wilt using aqueous extract of neem leaves. *PhytopathologiaMediterranea.*, 49: 143–150.

Hosagoudar, G. N., and Chattannavar, S. N. (2013). Management of *Alternaria* leaf spot disease on Bt cotton through bio agents and botanicals. *Bioinfolet.*10(3a) :774-778.

Jagadeesh, K.S. and Jagadeesh, D.R. (2009). Biological control of early blight of tomato caused by *Alternaria solani* as influenced by delivery methods of *Pseudomonas gladioli* B 25. *Acta Horticulture*., 808: 327-332.

Knudsen, G. R. and Bin, L. (1990). Effects of temperature, soil moisture and wheat bran on growth of *Trichoderma harzianum* form alginate pellets. Phytopathology., 80: 724-727.

Kommedahl, T., Windles, C. E., Sarbini, A. and Wiley, H. B. (1981). Variability in performance of biological and fungicidal seed treatment in Corn, Peas and Soybean. Protection Ecology., 3: 55- 61.

Latha, R.D. and Zacharia, S. (2021). Eco-friendly management of early blight of tomato (*Lycopersicon esculentum* Mill.). *Pharma Innovation*., 10(10):819-822.

Martinez, B. and Solano, T. (1995). Antagonism of *Trichoderma* spp. to *Alternaria solani* (Ellis and Martin) Jones Grout. *Revista de proteccion Vegetal*., 3: 221-225

Nashwa, S.M.A., Abo-Elyousr, K.A.M. (2012). Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science*., 48(2): 74−79.

Papavizas, G. C., Lewis, J. A. and Abd-El-Moity, T. H. (1982). Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to Benomyl and enhanced biocontrol capabilities. *Phytopathology*., 72: 126-130.

Patil, M.J., Ukeyand, S.P., Raut, B.T., 2002. Evaluation of fungicides and botanicals for the management of early blight (*Alternaria solani*) of tomato. PKV Research Journal 25(1), 49−51.

Patilkulkarni, V.G. (2013). Organic management of purple blotch of onion caused by *Alternaria porri*. *M.Sc. Thesis*. University of Agricultural Science, Dharwad, Bangalore.

Rao, M. S. L. (2006). Management of *Alternaria* leaf spot in sunflower. Annual Report 2005- 2006. Direc. Oilseeds Res. 54p.

Shekhawat, P.S. and Prasad, R. (1971). Antifungal properties of plant extracts in inhibition of spore germination. *Indian Phytopathology*., 24(4): 801−802.

Simmons, E.G. 2007. *Alternaria*: an identical manual.CBS Biodiversity Series **6**: 1−775

Singh, U.P., Pandey, N., Wagner, K.G. and Singh, K.P. (1990). Antifungal activity of agoene. A constituent of garlic (*Allium sativum*). *Canadian Journal of Botany.*,68(6): 1354−1356.

Sireesha, O. 2013. Effect of plant products, Panchagavya and bio-control agents on rice blast disease of paddy and yield parameters. *International Journal of Research in Biological Sciences*, ***3*(1)**: 48-50.

Wu, W. S. (1982). Seed treatment by applying *Trichoderma* spp. to increase the emergence of soybean. *Seed Science and Technology*., 10: 557-563