Management of early blight of tomato caused by *Alternaria solani* by using Bio-inoculants and Chitosans under greenhouse conditions

Abstract

Tomato (*Lycopersicon esculentum*.L) is one of the most important economic vegetable crop cultivated in India. Among various diseases caused by fungal, bacterial and virus pathogens, early blight caused by *Alternaria solani* is a major concern for the farmers as it not only affects vegetative parts but also fruits. The study was aimed at developing eco-friendly alternatives for chemicals currently used for the management of *A. solani* by using biocontrol agents and chitosans with a spin-off benefit of plant growth promotion and activate innate defense mechanisms. The potential biocontrol agents identified in the previous experiments *viz. Trichoderma* (T4), *Pseudomonas* (P28) isolates and effective chitosans DA10, 134 were used either singly or in combinations for their efficacy against *A. solani in vivo* against Propiconazole @ 0.1% and water spray, as positive and negative controls treatments, respectively. Propiconazole @ 0.1% was highly effective in reducing the disease over other treatments. However, the treatments *viz.* seed coating with *Trichoderma* (T4) coupled with DA 10 chitosan (simultaneously applied) or DA 10 chitosan alone were also significantly effective and on par with the fungicide treatment indicating that alternatives to fungicides could be explored after field evaluation and large-scale demonstration of these treatments and their economic viability.

Key words: Tomato, Chitosans, biocontrol agents, early blight and *Alternaria solani*

Introduction

Tomato (*Lycopersicon esculentum* Mill) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetable in the world. Tomato is grown for its edible fruits, which can be consumed either fresh or in processed form and is a very good source of vitamins A, B, C and minerals. Tomato cultivation has become more popular since mid ninteenth century because of its varied climatic adaptability and high nutritive value. Tomato crop is usually susceptible to many diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard,1992).Among several production constraints, diseases take a considerably heavy toll and farmers resort to spraying several fungicides to manage these diseases. Among the fungal diseases, early blight also known as target spot disease caused by *Alternaria solani* (Ellis and Martin) is one of the world's most catastrophic diseases incurring losses both at pre- and post-harvest stages causing 35 to 78 per cent reduction in fruit yield in the tropical and subtropical regions (Jones *et al.*, 1993). The disease appears on leaves, stems, petioles, twigs and fruits under favourable conditions resulting in defoliation, drying-off of twigs and premature fruit drop and thus causing loss from 50 to 86 percent in fruit yield (Mathur and Shekhawat, 1986). The mechanisms of biocontrol include out-competing the phytopathogen, production of hydrolytic enzymes that inhibit the phytopathogens, parasitization of the pathogens (popularly known as mycoparasitism in case of fungal antagonists), physical displacement of the phytopathogens, secretion of the siderophores to prevent pathogens in the immediate vicinity from proliferating, synthesis of antibiotics, synthesis of variety of small molecules that can inhibit phytopathogen growth, and stimulation of the systemic resistance of the plants. Biocontrol agents including *Trichoderma* *viride*, *T. harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* have already been commercially exploited for management of important pathogens like *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and other soil borne plant pathogens. Developing climate-friendly and cost-effective management options for the management of *A. solani* will be a boon for tomato farmers and result in decreased use of expensive fungicides that add to the cost of cultivation and thereby enhanced profitability.

Chitosan is a natural non-toxic biopolymer derived as a major component of the shells of crustacean such as crab, shrimp, and crawfish. In recent years, applications of chitosan in the fields of agriculture medicine, food, chemical engineering, pharmaceuticals, nutrition, and environmental protection have received considerable attention (Li *et al*., 2007 and El Hadrami *et al.* 2010). Applications of chitosan in environmental protection and agriculture include its use as a biocontrol agent for controlling plant disease in its deacetylated form i.e. chitosan (Khan *et al*., 2006). Some chitosan molecules are also known to induce host plant resistance. Treating plant tissue with chitosan intensifies the natural defense mechanisms and consequently helps the tissue in restricting fungal colonization (Ghauoth *et al.* 1994). Hence, the present investigation has been planned to develop eco-friendly management of the disease using bioagents, biopolymers and other eco-friendly management option will help in reducing residues of fungicides as well as environmental pollution.

Materials and Methods

Two potential antagonists (*Pseudomonas* / *Trichoderma*) and two effective chitosans (Chitosan DA 10 and 134), identified after preliminary screening (Ramakrishna *et al*, 2018, Ramakrishna *et al*, 2017 and Ramakrishna *et al*, 2018. Further that evaluated either singly or in combinations for their efficacy against early blight of tomato under greenhouse conditions. Seedlings of the susceptible tomato cultivarUS440 were raised in nursery poly bags filled with field soil amended with vermicompost. Two seeds were sown per pot. The poly bags were arranged in a completely randomized design. Recommended doses of fertilizers were applied to the plants. The plants were artificially inoculated with the pathogen and early blight intensity was recorded at regular intervals. Disease severity was assessed by using 0-5 scale (Mayee and Datar, 1986) and described in below.

Per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969). Per cent disease index (PDI):

$$\frac{\textbf{Sum of the individual disease ratings}}{\textbf{Number of fruits or leaves observed} \times \textbf{Maximum disease grade}} \times 100$$

Seed treatment with biocontrol agents

The seeds were treated with 1×10^8 cfu/ml (bacteria) or 1×10^7 spores/ml (fungus) suspension of potential biocontrol agents for 2hr. The treated seeds were spread on a sheet and shade dried completely. The dried seeds were used for sowing in poly bags filled with soil and vermin-compost in 4:1 ratio.

Seed treatment with chitosans

The seeds were primed with effective concentration of two chitosans in Eppendorf tubes and placed on magnetic stirrer at 100rpm and 25°Cfor 12h as described by Paulin *et al*., 2013. After the priming treatment, the treated seeds were shade-dried and used for sowing in poly bags filled with soil and vermi-compost in 4:1 ratio.

Combined seed treatment with chitosans and biocontrol agents

The seeds were soaked in effective concentration of chitosans for 12h followed by treatment with biocontrol agent for 24h. Then the seeds were sown in poly bags after shade drying.

Soil application with biocontrol agents and seed priming with chitosans

The soil mix was treated with $1x10^8$cfu/ml (bacteria) or $1x10^7$ spores/ml (fungus) of potential biocontrol agent into which the seeds coated with each chitosans were sown.

Seed treatment with fungicide

The seeds were soaked in 0.1% fungicide (Propiconazole) solution and shade dried. The dried seeds were sown in polythene bags filled with soil mix.

Statistical analysis

The data obtained in different experiments was statistically analyzed following completely randomized block design (CRD) as per the procedures suggested by Snedecor and Cochran (1967) and Pans and Sukhatme (1978). The data pertaining to percentage were angular transformed wherever necessary.

Results and Discussion

Evaluation of combination of biocontrol agents and chitosans for management of early blight of tomato *in-vivo*

The experiments were carried out to find the effect of potential biocontrol agents in combination with effective chitosans and to assess their ability to manage early blight pathogen. A total of 14 treatments were configured based on the results from preliminary screening (Ramakrishna *et al,* 2018, Ramakrishna *et al,* 2017 and Ramakrishna *et al*, 2018.
The potential biocontrol agents *Pseudomonas* (P28), *Trichoderma* (T4), chitosan DA 10 and 134 were used as treatments either individually or in combinations. Commercial fungicide (Propiconazole) was used as a positive control. Per cent disease index was recorded in all the treatments and the data is tabulated in Table 1. The plants were artificially spray-inoculated with the test pathogen, *A. solani* to ensure disease development. All the treatments were effective in reducing the disease severity. Lowest per cent disease index (PDI) of 36.63% was recorded in T13 where seed treatment with propiconazole @ 0.1% was administered and it was significantly superior over other treatments except T7 (41.1%) and T3 (43.3%). The next best treatments were T10 > T11 > T1 > T4 > T6 with 44.4%, 44.43%, 46.63, 47.73 and 47.76% PDI, respectively and on a par. The remaining treatments were also significantly superior to control though the disease reduction was not relatively less. Highest disease index was recorded in T14 (control) with 67.76%. The PDI recorded results suggest emphatically that alternatives such as chitosans and biocontrol agents in combination with chitosans could

provide effective alternative to fungicides. El-Mohamedy *et al.* (2014) studied the effect of combination of chitosans and biocontrol agents *in vitro* on tomato and they reported that combination of *T. harzianum* and chitosan (1 g/l) as root dipping treatment combined with chitosan (0.5 g/l) as foliar spray reduced FCRR incidence and severity by 66.6 and 47.6%, respectively. However, in their study, only chitosan treatments were least effective. The present findings are in line with these findings. The present study showed that under net-house conditions, the plants became more susceptible to *A. solani* with increase in the age. Initial symptoms were observed after 2 weeks of inoculation (45 DAS) and the maximum per cent disease index was recorded in 55-day old plants. The results agreed with Moore (1942), who demonstrated that susceptibility of tomato plants to *A. solani* infection was age dependent. Rowell (1953) reported that *A. solani* invaded the leaves at all stages, but mostly confined to older leaves. The susceptibility of tomato plants to infection by *A. solani* was determined by the age of the host (Rotem, 1994). Jones *et al.* (1993) also reported that all growth stages of tomato plants were susceptible to *A. solani* infection. Increased susceptibility to infection with increasing host age has been reported in many *Alternaria* host systems, such as *A. porri* on onions (Gupta and Pathak, 1986), *A. macrospora* on cotton (Rotem *et al.*, 1990) and *A. brassicae* and *A. brassicicola* on brassica crops (Babadoost and Gabrielson, 1979).

Table 1.Management of early blight of tomato with different treatments under greenhouse conditions

Trt. No.	Details of Treatments	Per cent Disease Index (PDI)
T1	*Pseudomonas* (P28)	46.63
T2	*Trichoderma* (T4)	52.86
T3	DA 10	43.30
T4	Chitosan 134	47.73
T5	P28 + DA 10 (Simultaneous Application)	53.30
T6	P28 + Chitosan 134 (Simultaneous Application)	47.76
T7	T4 + DA 10(Simultaneous Application)	41.10
T8	T4 + Chitosan 134 (Simultaneous Application)	58.86
T9	P28 (Soil Application) + DA 10 (Seed Priming)	54.40
T10	P28 (Soil Application) + Chitosan 134 (Seed Priming)	44.40
T11	T4 (Soil Application) + DA 10 (Seed Priming)	44.43
T12	T4 (Soil Application) + Chitosan 134 (Seed Priming)	54.43
T13	Propiconazole @0.1%	36.63
T14	Control (Untreated)	67.76
SE (m)		2.54
SE (d) ±		3.59
CD		7.35
CV (%)		0.63

DA-De acetylated form of chitosan, **T4**: *Trichoderma* isolate, **P28**: *Pseudomonas* Isolate

Conclusion

The experiment demonstrated that biocontrol agents and chitosans, whether used alone or in combination, significantly reduce the severity of early blight in tomato plants. These alternatives could potentially reduce reliance on chemical fungicides, offering a sustainable approach to managing early blight.

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