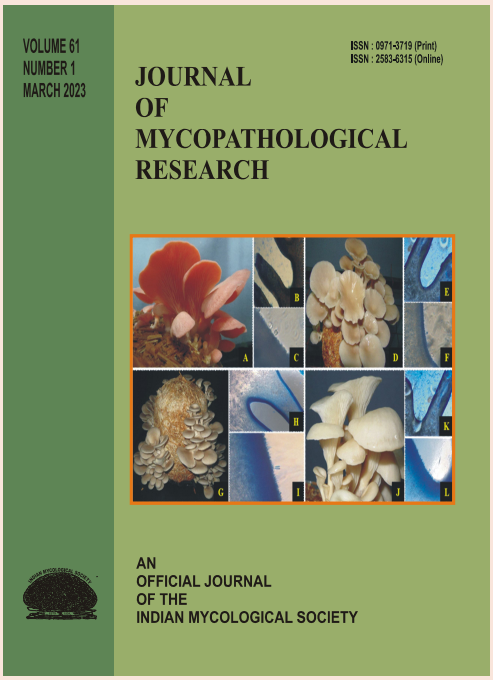
Biological management of Early blight disease of tomato caused by *Alternaria solani*

T. ANAND, [1*] G. SENTHILRAJA[1] AND P. SENTHILKUMAR[2]



J. Mycopathol. Res. 61(1) : *91-96, 2023;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. **61(1)** : 91-96, 2023; ISSN (0971-3719 (Print), 2583-6315 (Online) doi. 10.57023/JMycR.61.1.2023.091

Biological management of Early blight disease of tomato caused by *Alternaria solani*

T. ANAND, [1*] G. SENTHILRAJA[1] AND P. SENTHILKUMAR[2]

[1]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu
[2]Agricultural College and Research Institute, Vazhavachanur, Thiruvannamalai-606 753 Tamil Nadu

Received : 14.12.2022 Accepted :02.02.2023 Published : 27.03.2023

Early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is an important fungal disease of tomato. The pathogen affects all parts of plants and causes severe damage at all stages of plant growth. The use of chemical fungicides is the principal method used to control this disease. However, as an alternative to chemical fungicides, biological management is one of the most promising methods for managing plant diseases. In the present study, two field experiments were conducted in 2016-17 and 2017-18 at Regional Research Station farm, Tamil Nadu Agricultural University (TNAU), Paiyur, Tamil Nadu, India to evaluate the effectiveness of biocontrol agents and bioproduct (chitosan) against early blight disease. The findings showed that plots treated with *Pseudomonas* -TNAU-Pf1 (0.5%) had a minimum early blight incidence of 5.56 PDI, followed by chitosan at 0.3% (5.93 PDI) which were statistically on par with each other. The treatments also recorded a higher tomato fruit yield of 32.90 and 32.84 t/ha, respectively compared to the other treatments.

Key words: Chitosan, Early blight, management, *Pseudomonas*, tomato, *Trichoderma*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown in India. The crop is affected by various diseases caused by fungi, bacteria and viruses. Among the fungal diseases, early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is the most threatening (Abdel-Sayed, 2006; Abada *et al*. 2008; Anand *et al*. 2010), causing a great reduction in the quantity and quality of fruit yield. The pathogen causes diseases on the leaves (leaf blight), stem (collar rot) and fruits (fruit rot) and can cause severe damage at all stages of plant development (Foolad *et al*. 2000). It is increasingly becoming a limiting factor for successful tomato production, causing yield losses of 15-100% (Babu *et al*. 2000; Pandey *et al.* 2003).

Regarding tomato early blight control, many workers have done a lot of work based on chemical control. Although chemical control with fungicides is the most common control method, it is still too expensive for most smallholder farmers. In the long term, chemical spraying will also have adverse effects on the environment. Use of bioproducts (chitosan) and antagonistic microbes can be an efficient way to control plant diseases (Algam *et al*. 2010). The interaction between biocontrol agents/ bioproducts and plant pathogens has been widely studied and the use of biocontrol agents to protect some commercially significant crops appears promising. Fungal and bacterial antagonists have been effective in controlling a vast number of plant diseases (Ramamoorthy *et al.* 2001; Latha *et al.* 2008; Anand, 2021). Due to its excellent antagonistic action against many plant pathogens, *Pseudomonas* is one of the most effective biocontrol agents currently on the market and is predominantly utilized for seed, soil, and

* Correspondence: anandpath10@yahoo.com

foliar treatment (Latha *et al.* 2009; Anand *et al.* 2010; Manikandan *et al.* 2013). Chitosan is a natural and non-toxic biopolymer obtained from the deacetylation of chitin, an important component of the shell of crustaceans such as crab, shrimp, and crawfish. Applications of chitosan in agriculture have received much attention in recent years (El Hadrami *et al*. 2010). Use of chitosan as a biocontrol agent for plant disease management is one of the uses for the substance in environmental protection and agriculture (Khan *et al*. 2006).

The main objectives of this study were to evaluate the effect of different *Pseudomonas* isolates and chitosan at different concentrations on the mycelial growth of *Alternaria solani in vitro* and to investigate the efficacy of the talc-based formulation of *Pseudomonas* isolates and chitosan against the development of early blight and plant growth promotion in tomato under field conditions.

MATERIALS AND METHODS

Source of early blight pathogen and biocontrol agents

Using potato dextrose agar (PDA) medium, the virulent early blight pathogen was isolated from tomato leaves exhibiting typical early blight symptoms and identified as *Alternaria solani* based on the various cultural and morphological characters. Different *Pseudomonas* strains isolated from tomato rhizosphere soils using King's B (KB) medium. Bacterial isolates were characterized based on standard biochemical tests (Hildebrand *et al*. 1992). *Pseudomonas* (TNAU-Pf1) and *Trichoderma asperellum* (Ta1) isolate were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India and was maintained on KB and PDA media, respectively.

In vitro screening of fungal antagonists against A. solani

Antagonism of *Trichoderma* spp. against *A. solani* was tested by using dual-culture method. Mycelial discs (8-mm dia.) of the test pathogen and the antagonist were placed on the opposite side of Petri dishes containing PDA, 1 cm away from the edge. Petri dishes were incubated at 28 ± 2°C for 7 days and the mycelial growth *of A. solani* was measured.

In vitro screening of fluorescent pseudomonads against the mycelial growth of A. solani

For *in vitro* screening of *Pseudomonas* isolates against *A. solani,* the isolates were streaked on one side of a Petri dish (1 cm from the edge of the dish) with PDA medium and a mycelial disc (8-mm in diameter) of 7-day-old culture of *A. solani* was placed on the opposite side of the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature (28±2°C) for 7 days and the mycelial growth of the pathogen was measured. The per cent inhibition (PI) of mycelial growth was calculated by using the following formula

I= C-T/T x 100

Where,
I = Percent reduction in growth of test pathogen;
C = Radial growth (cm) in control
T = Radial growth (cm) in treatment

Preparation of chitosan

Chitosan extracted from crab shells, degree of N-deacetylation (75%, Sigma Aldrich USA) was dissolved in an aqueous solution of acetic acid at 1% to obtain a concentration of 10 mg/ml (stock solution). The solution was washed with 1N NaOH, which had been made alkaline to pH 5.6, and autoclaved at 121°C for 15 min (Algam *et al*. 2010).

Antifungal effect of chitosan

Using the agar dilution method (Hanlon *et al.* 2007), a sterilized stock solution of chitosan was incorporated into sterilized cool melted PDA at 50°C (Muñoz *et al.* 2009) to obtain concentrations of 0.05, 0.1, 0.3, 0.5 and 1.0% of chitosan-amended media; then poured into sterilized Petri dishes (9 cm diameter). PDA medium without chitosan served as a control. The plates were then inoculated with an 8-mm diameter fungal-disc from 7-day-old colony and then incubated at 28 ± 2°C for 7 days. The diameter of the mycelial growth and the percentage inhibition at various concentrations were calculated.

Preparation of talc-based formulation of Pseudomonas isolates

A loopful of *Pseudomonas* isolate was inoculated into sterilized KB broth and incubated on a rotary

shaker at 150 rpm for 48 h at room temperature (28±2°C). After 48 h of incubation, the broth containing 9 x 10^8 cfu/ml was used for the preparation of talc-based formulation. A bacterial suspension of 400 ml, 1 kg of talc-powder (sterilized at 105°C for 12 h), 15 g of calcium carbonate (to adjust the pH to neutral) and 10 g of carboxymethylcellulose (CMC) (glue) were mixed under sterile conditions, following the method described by Nandakumar *et al.* (2001). After drying in the shade overnight, it was wrapped and sealed in a polypropylene bag. At the time of application, the bacterial population in the talc formulation was 2.5 to 3 x10^8 cfu/g.

Efficacy of fluorescent pseudomonads and chitosan against early blight disease

Fungicides and bacterial bioagents effective under *in vitro* conditions have been selected for field experiments only. A field experiment to test the efficacy of fluorescent pseudomonads and chitosan against tomato early blight was conducted at Regional Research Station farm, TNAU, Paiyur, Tamil Nadu, India during September 2016 to January 2017 and November 2017 to March 2018. The experiment was conducted in a Randomized Block Design (RBD) with three replications. The tomato hybrid, Shivam which is popular and highly susceptible to early blight was used in all experiments. The treatments of the experiment were T1- *Pseudomonas* (TNAU-Pf1) (0.5%), T2- *Pseudomonas* isolate 1 (0.5%), T3- Chitosan (0.1%), T4-Chitosan (0.2%), T5-Chitosan (0.3%), T6-Chitomax (0.1%) and T7-Untreated control. The talc-based formulation of *Pseudomonas* and chitosan were sprayed at 30 and 50 days after planting (DAP). Data on the disease severity (PDI) was recorded 10 days after second spray. Disease incidence was recorded by randomly selecting 5 plants from each plot. Four leaves were selected from each plant and the area covered by the disease was measured on a scale of 0-9 and expressed as Percent Disease Index. The fruit yield per plot yield was also recorded from which the yield per hectare was calculated.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the IRRISTAT version 92-1 programme developed by the Biometrics Unit, International Rice Research Institute, The Philippines. Disease incidence data were arc-sine transformed before analysis.

RESULTS AND DISCUSSION

Effect of bioagents and chitosan on the mycelial growth of A. solani

Among the biocontrol agents, *Trichoderma harzianum* registered a maximum inhibition (79.55%) of *A. solani* followed by TNAU-Pf1 and *T. asperellum* (73.86%) which were statistically at par with each other (Table 1). Mycelial growth of *A. solani* was not significantly affected at all chitosan concentrations tested. However, chitosan at concentrations of 0.3, 0.5 and 1.0 per cent recorded no sporulation in the culture medium, while chitosan at 0.05 and 0.1 per cent recorded abundant and sparse sporulation of *A. solani,*

Table1: Effect of different bioagents on the mycelial growth of *Alternaria solani*

Biocontrol agent	Diameter of the mycelial growth (cm)	Per cent inhibition over control
Trichoderma asperellum	2.30[b]	73.86
Trichoderma harzianum	1.80[a]	79.55
Pseudomonas (TNAU-Pf1)	2.30[b]	73.86
Pseudomonas isolate 1	4.73[c]	46.25
Pseudomonas isolate 2	7.33[d]	16.70
Untreated control	8.80[e]	-

Values are means of four replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

Table 2: Effect of chitosan on the mycelial growth and sporulation of *Alternaria solani*

Chitosan at different concentrations	Diameter of the mycelial growth (cm)	Type of sporulation
0.05%	9.00[ab]	Abundant
0.1%	8.88[ab]	Sparse
0.3%	8.55[ab]	No sporulation
0.5%	8.40[a]	No sporulation
1.0%	8.32[a]	No sporulation
Untreated control	9.00[b]	Abundant

Values are means of four replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

Table. 3: Efficacy of different bioagents/chitosan against early blight disease and yield of tomato ((Pooled mean of two seasons)

Treatments	PDI	% reduction over control	Fruit yield (t/ha)	% increase over control	CBR
TNAU-Pf1(0.5%)	5.56	80.63	32.90	32.55	1:4.83
Pseudomonas isolate 1 (0.5%)	7.94	72.33	31.54	29.64	1:4.22
Chitosan (0.1%)	9.44	67.96	31.05	28.53	1:3.98
Chitosan (0.2%)	7.53	67.11	31.96	30.57	1:4.19
Chitosan (0.3%)	5.93	79.34	32.84	32.47	1:4.40
Chitomax (0.1%)	10.37	63.87	30.10	26.28	1:3.74
Untreated control	28.70	-	22.19	-	-
CD (p=0.05)	1.35	-	0.51	-	-
S.Ed	0.62	-	0.24	-	-

Values are mean of three replications

respectively (Table 2). The inhibitory effect of *Trichoderma* and *Pseudomonas* strains against *A. solani* may be due to competition, antibiosis and lysis. Several strains of *Pseudomonas* and *Trichoderma* spp. have been reported to produce a wide variety of antibiotics, namely 2,4, diacetylphloroglucinol, oligomycin, phenazine, pyoluteorin, pyrolnitrin, pyocyanin and gliotoxin are responsible for their antifungal activity. The TNAU-Pf1 strain was reported to produce siderophore, HCN and antibiotics such as DAPG and pyoluteorin (Ramamoorthy *et al*. 2001). The antifungal activity of chitosan against plant pathogens has also been reported by several workers. Chitosan has been shown to have strong antifungal activity against *A. alternata* under in vitro conditions (Algam and Elwagia, 2015).

Efficacy of fluorescent pseudomonads and chitosan against early blight disease under field conditions

The results of the field trial showed that *Pseudomona*s and chitosan significantly reduced the severity of early blight at all concentrations tested (Table 3). Minimum *Alternaria* incidence was recorded in plots treated with TNAU-Pf1 (0.5%) (5.56 PDI) followed by chitosan (0.3%) (5.93 PDI), while untreated control plot had a maximum incidence of 28.70 PDI. Fruit yield results showed that plots treated with TNAU-Pf1 had the highest fruit yield of 32.90 t/ha with a BC ratio of 1:4.22 followed by chitosan (0.3%) which gave a tomato yield of 32.84 t/ha with a BC ratio of 1:4.0 and the treatments were at par with each other. The untreated control plots recorded only 22.19 t/ha of fruit yield (Table 3). Similar reports of the use of *Pseudomonas* and chitosan for better disease control have been reported by several workers. Management of plant diseases using *Pseudomonas* isolate TNAU –Pf1 has been demonstrated by several reserachers in many crops (Saravanan *et al.* 2004; Anand *et al.* 2010; Manikandan *et al.* 2013). Algam and Elwagia (2015) reported that chitosan successfully controls early blight of tomatoes and promotes tomato growth. Application of chitosan to tomato plants increases growth parameters and reduces disease severity (EL-Tantawy, 2009).

In conclusion, *Pseudomonas*-TNAU-Pf1 (0.5%) or 0.3% chitosan sprayed at 30 and 50 days after planting had minimum incidence of early blight with the highest fruit yield. The biocontrol agent or chitosan could be recommended for early blight management in tomato especially in organic farming, as an alternative to the chemical fungicides.

REFERENCES

Abada, K.A., Mostafa S.H., Hillal, R.M. 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egypt. J. Appl. Sci.* **23**: 47-58.

Abdel-Sayed, M.H.F. 2006. *Pathological, physiological and molecular variations among isolates of Alternaria solani the causal of tomato Early blight disease*. Ph.D. Thesis, Fac. Agric., Cairo University, 181pp.

Algam, S.A.E., Xie, G. Li, B. Yu, S. Su, T., Lersen, J. 2010. Effect of *Paenibacillus* strains and chitosan on growth promotion

and control of Ralstonia wilt in tomato. *J. Plant Pathol.* **92**: 593-600.

Algam, S.A.E., Elwagia, M.E.A. 2015. Evaluation of chitosan efficacy on tomato growth and control of early blight disease. *Jordan J. Agric. Sci.* **11**: 27-36.

Anand, T., Chandrasekaran, A. Kuttalam, S., Samiyappan, R. 2010. Evaluation of azoxystrobin (Amistar 25 SC) against early leaf blight and leaf spot diseases of tomato. *J. Agric. Technol.* **6**: 463-485.

Anand, T. 2021. Effect of fungal and bacterial biofomulation mixtures on major soil borne diseases of cotton. *Int. J. Ecol. Environ. Sci.* **3**: 56-59.

Babu, S., Seetharaman, K., Nandakumar, R., Johnson, I. 2000. Effect of selected plant extracts/oils against tomato leaf blight. *Int. J. Trop. Agric.* **18**: 153"157.

El Hadrami, A.L., Adam, R., Elhadrami I., Daayf, F. 2010. Review: Chitosan in plant protection. *Marin Drugs* **8**: 968-987.

Foolad, M.R., Ntahimpera, N., Christ, B.J., Lin, G.Y. 2000. Comparison between field, greenhouse, and detached-leaflet evaluations of tomato germplasm for early blight resistance. *Plant Dis*. 84: 967-972.

Hildebrand, D.C., Schroth M.N., Sands, D.C. 1992. *Pseudomonas*. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, Schaad, N.W. (Ed.), 2nd ed., International Book Distributing Co., Lucknow, India, pp. 60–80.

Khan, M.R., Fischer, S., Egan, D., Doohan, F.M. 2006. Biological control of Fusarium seedling blight disease of wheat and barley. *Phytopathology* **96**: 386–394.

Latha, P., Anand, T., Ragupathi, N., Prakasam V., Samiyappan, R. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biol. Control* **50**: 85–93.

Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T., Samiyappan, R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. *Biol. Control* **54**: 83–89.

Muñoz, Z., Moret, A. 2010. Sensitivity of *Botrytis cinerea* to chitosan and acibenzolar-S- methyl. *Pest Manage. Sci.* **66**: 974-979.

Nandakumar, R., Babu, S., Viswanathan, R., Sheela, J., Raguchander, T., Samiyappan, R. 2001. A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biocontrol* **46**: 493–510.

Pandey, K.K., Pandey, P. K., Kalloo G., Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. *J. Gen. Plant Path*. **69**: 364-371.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam V., Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. *Crop Prot.* **20**: 1-11.

Saravanan, T., Muthusamy M., Marimuthu, T. 2004. Effect of *Pseudomonas fluorescens* on Fusarium wilt pathogen in banana rhizosphere. *J. Biol. Sci.* **4**: 192–198