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# Effect of Physiological Factors and Different Growth Media on *Alternaria solani* under *in vitro* Condition and Eco-friendly Management of Early Blight of Tomato (*Solanum lycopersicum* L.)

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**ABSTRACT**

Tomato (*Lycopersicon esculentum* L.) is one of the most important economic vegetable crops cultivated in India. Among all the fungal diseases, early blight caused by *Alternaria solani* (Ellis and Martin) is one of the world's most catastrophic diseases. The present research work was conducted with the aim to determine the effect of three *Trichoderma* species and five fungicides by using dual culture assay against *Alternaria solani*. Effect of different pH levels, temperature, light intensity and media were tested against the growth of *A. solani* under *in vitro* conditions. *Trichoderma harzianum* was very effective in managing early blight of tomato. Combination of azoxystrobin 11.4%+difenoconazole 18.2% SC was found most effective by showing 96.60% inhibition against the pathogen under *in vitro* condition, followed by propiconazole 25% EC (81.70%) and mancozeb 75% WP (74.60%), respectively. *A. solani* was maximum in pH range of 6.00-6.50 and temperature range of 25-30°C. The exposure of the fungus to alternate cycles of 12-h light and 12-h darkness resulted in the maximum mycelial growth of *A. solani* compared to continuous light and dark. Among the different media tested, potato dextrose agar (PDA) medium supported significantly the maximum growth of test pathogen followed by oat meal agar (OMA). On the basis of present study, the fungal bioagents, might be exploited for future plant disease management programs (DMP) to save environmental risk.

**Key words :** *Alternaria solani,* biocontrol, dual culture technique, early blight, management

## INTRODUCTION

Tomato (*Solanum lycopersicum)* is one of the most widely grown vegetable crops in the world (Gerszberg *et al.*, 2015). Tomatoes belong to the *Lycopersicum* genus and the Solanaceae family (Délices *et al.*, 2021). Tomatoes are the second most significant crop in the Solanaceous family, after potatoes. South and central America are the origins of this species. It was in Mexico when it was domesticated and used as a food source. China is the greatest tomato producer (31%), with India and the United States coming in second and third (Bais *et al.*, 2019). Tomato is rich in vitamins (K, C, and A), minerals (Fe, Ca and P), amino acids, sugars, dietary fibers, and antioxidants, as well as its 95.3% water content (Bhanage *et al.*, 2019).

Tomato crop is vulnerable to several bacterial, viral, nematode and fungal diseases. Among them, early blight (EB), caused by *Alternaria solani*, is an air-borne soil-inhabiting fungus that has a reputation for being one of the most damaging diseases of tomatoes, causing production losses up to 80% (Rasool *et al.*, 2021). Temperature and pH are most important physio-chemical factors for the growth of *Alternaria* species (Gawai and Mangnalikar, 2018). There are primarily three ways for managing early blight disease : cultural practices, fungicide treatment and the use of resistant types. Propineb, mancozeb, copper oxychloride, tebuconazole, propiconazole and selection of resistant genotypes have all been developed for the control of early blight, however, fungicide treatment is neither commercially feasible nor environmentally sound. Unfortunately, chemical use causes serious ecological and health issues, such as target pest resistance, increased human health risk and pollution (Sharma *et al.*, 2019). Moreover, different bio-control approaches are being used to manage early blight of tomato,

which is a very successful and ecological alternative approach (Kaur *et al.*, 2016; Dhal *et al.*, 2017; Rani *et al.*, 2017; Chanthini *et al.*, 2018; Youssef *et al.*, 2018). *Trichoderma* are well known bio-control agents (BCAs) being very effectively used for the control of large number of soil-borne plant pathogens (Singh *et al.,* 2018; Pavithra *et al.,* 2021). The present study was aimed at determining the efficacies of different doses of biocontrol agents and fungicides under *in vitro* condition against early blight of tomato along with the effect of different temperature, pH and light on growth and sporulation of *Alternaria solani*.

## MATERIALS AND METHODS

All the experiments were conducted at the Department of Plant Pathology, School of Agriculture, Lovely Professional University, Punjab. The management of early blight of tomato mainly followed biological method and chemical method. In biological method bio-agents and in chemical method various systemic and contact fungicides and poisoned food technique for chemicals and dual culture technique for bio-agents were used. The pure culture of *Alternaria solani* was purified from isolated Petri plates and sub-cultured on PDA slants and incubated at 25±2°C for 7 to 8 days and slants were preserved in refrigerator at 7°C for 25 days (Katyayani *et al.*, 2019; Roy *et al.*, 2019). Effect of variable temperature, pH, light and different growth media on the growth of *A. solani* was also evaluated.

The efficiency of three bio-agents viz., *T. viride, T. harzianum* and *T. koningii* was assessed against *A. solani* by using dual culture technique. The fungal bio-agents were grown in PDA media and poured in Petri plates (90 mm). The plates were kept in B.O.D. at 25±2°C and the radial mycelial growth of the test pathogen and bio-agents were observed. The tested pathogen growth was controlled by bio-agent and formed the inhibition zone, and also observed the untreated (control) pathogen growth for seven days. The per cent growth inhibition of fungus in each treatment when compared with control was calculated using equation:

$$I = (C - T/C) \times 100$$

Where, I = Per cent growth inhibition, C = Radial growth in control and T=Radial growth in the treatment.

The efficacy of systemic and non-systemic fungicides was used against *A. solani* and evaluated *in vitro* conditions by using poisoned food technique. The five fungicides used during the study were : Carbendazim 50% WP and Propiconazole 25% EC, Mancozeb 75% WP, Copper oxychloride 50% WP and Azoxystrobin 11.4%+Difenoconazole 18.2% SC. These fungicides were used at three different concentrations (50, 75 and 100 ppm) on potato dextrose agar medium against *A. solani*. The efficacy of chemicals was expressed as percentage of inhibition of growth over control. The formula i.e. I=C-T/C × 100 and measured the radial growth of the pathogen until the control covered the edges of the Petri plates.

Potato dextrose broth was used as a basal medium to study the effect of different temperatures, pH range and light on the growth of *A. solani*. 15-20 ml of PDA medium was poured in sterilized Petri plates. These plates were inoculated with 5 mm mycelia bit of seven days old isolates of *A. solani* were inoculated and incubated at different temperature viz., 15, 20, 25, 30 and 35°C in BOD incubators for seven days. Three replications were maintained for each treatment.

The pH of the PDA medium was adjusted to various levels, namely, 4.0, 4.5, 5.0, 5.5 and 6.0 by adding 0.1N sodium hydroxide and 0.1N hydrochloric acid and it was determined by electronic pH meter. Petri plates having media were inoculated with 5 mm discs taken from seven days old isolates of *A. solani* were inoculated and incubated at 25±2°C for seven days. Three replications were maintained for each treatment.

The effect of light on growth of *A. solani* was studied on potato dextrose agar medium by exposing the pure cultures to 4 h dark 20 h light, 12 h dark 12 h light, 8 h light 16 h dark and 24 h dark 24 h light. The inoculation of culture to Petri plates containing PDA was done as explained earlier. The plates were incubated at 25±1°C for nine days. All the above-mentioned observations on colony diameter were recorded and data were analyzed statistically using completely randomized design.

Five different solid culture media, namely, potato dextrose agar (PDA), oat meal agar

(OMA), malt extract agar (MEA), Richard's agar (RA) and Czapek's Dox agar (CDA) were tested to observe the growth pattern of test fungus. Fifty ml each of different solid medium to be tested was autoclaved and dispensed 20 ml of it in the sterilized Petri plates of 9 cm diameter. Each treatment was replicated into three. Mycelial discs were cut from three days old actively growing colony of fungal culture by using sterilized 5 mm diameter cork borer. One mycelial disc was inoculated at the centre of each test plates containing sterilized media and then incubated at 25±2°C. The radial growth was recorded in 24 h intervals till the fungus covered the whole plate.

## RESULTS AND DISCUSSION

Biological management of plant pathogens by employing potential bioagents was an important component of non-chemical plant disease management. Extensive study was undertaken through *in vitro* screening of three bioagents to ascertain their potential as suitable bio-pesticides against *A. solani*. *T. harzianum* was very effective in managing early blight of tomato where inhibition zone of pathogen was highest (79.33%) followed by *T. koningii* and *T. viride* with inhibition zone of 74.78 and 73.77%, respectively (Table 1).

The potential of *Trichoderma* species as a biological control agent (BCA) to control plant diseases in other pathosystems had already been shown in several studies (Metza and Hausladenb, 2022). Species of genus *Trichoderma* are able to improve growth of the plant and commonly used as bio fungicides typically against soil-borne phytopathogenic mycoflora. Ghazanfar *et al.* (2019) also found *Trichoderma* strains to be most effective against early blight of tomato rather than nitrogen rich compost and nutrient enriched compost. Most of the data in literature concerning to biological control refer to *Trichoderma* that was recognized successful and effective biocontrol agent of various pathogens as well as diseases for long period of time (Zin and Badaluddin, 2020). Members of the Ascomycetes genus *Trichoderma* are very effective producers of cell wall degrading enzymes gaining immense importance as bio-control agents (BCAs) of plant disease. Due to its antagonistic activity and as a plant growth promoter, *Trichoderma* appealing to sifting hazardous fungicides and soil fumigants for control of soil-borne plant pathogens (Kumar *et al.,* 2019; 2019a; Katyayani *et al.*, 2020).

Five different fungicides like carbendazim 50% WP, copperoxychloride 50% WP, azoxystrobin 18.2 w/w+difenoconazole 11.4% w/w SC, propiconazole 25% EC and mancozeb 75% EC by adopting poison food technique at three different concentrations i. e. 50, 75 and 100 ppm were used to evaluate efficacy of different fungicides at different concentration against test pathogen i. e. *A. solani*. Combination of azoxystrobin 11.4% + difenoconazole 18.2% SC was found most effective by showing 96.60% inhibition against the pathogen under *in vitro* condition, followed by propiconazole 25% EC (81.70%) and mancozeb 75% WP (74.60%), respectively (Table 2 and Fig. 1). From the above findings, it is also evident that copper oxychloride 50% WP was least effective against test pathogen.

Besides different control strategies, chemical control by fungicides was regarded as a predominant practice for the EB management (Ghazanfar *et al.*, 2016). Same finding was also mentioned by Kumari *et al.* (2022) in their data that azoxystrobin 18.2% w/w +difenoconazole 11.4% w/w SC 0.07% were most effective in inhibiting growth of *Alternaria*. Amisha *et al.* (2022) concluded from their results that

**Table 1.** *In vitro* evaluation of biocontrol agents against *Alternaria solani*

| Treatment | Colony diameter (mm) | | | Total (R1+R2+R3) | Average colony diameter of the pathogen | Inhibition (%) |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | | | |
| $T_1$–*Trichoderma viride* | 24.1 | 22.5 | 24.2 | 70.8 | 23.6 | 73.77 |
| $T_2$–*Trichoderma harzianum* | 18.4 | 19.1 | 18.3 | 55.8 | 43.6 | 79.33 |
| $T_3$–*Trichoderma koningii* | 22.5 | 22.1 | 23.5 | 68.1 | 22.7 | 74.78 |
| $T_4$–Control | 90 | 90 | 90 | 270 | 90 | 0 |
| C. D. | | | | | 1.233 | 1.385 |
| S. E(m) | | | | | 0.350 | 0.393 |
| S. E(d) | | | | | 0.490 | 0.555 |
| C. V. | | | | | 1.370 | 1.333 |

**Table 2.** *In vitro* evaluation of different fungicides against *Alternaria solani*

| Treatment | Colony diameter (mm) | | | Mean of colony diameter (mm) | Per cent inhibition | | | Mean of per cent inhibition |
|---|---|---|---|---|---|---|---|---|
| | ppm | | | | ppm | | | |
| | 50 | 75 | 50 | | 50 | 75 | 100 | |
| $T_1$–Carbendazim 50% WP | 32.30 | 32.51 | 33.21 | 32.67 | 64.10 | 63.87 | 63.10 | 63.69 |
| $T_2$–Propiconazole 25% EC | 18.51 | 16.35 | 16.25 | 17.03 | 79.43 | 81.80 | 84.00 | 81.70 |
| $T_3$–Mancozeb 75% WP | 23.58 | 24.94 | 23.45 | 23.99 | 73.80 | 72.20 | 73.90 | 74.60 |
| $T_4$–Copper oxychloride 50% WP | 55.65 | 54.91 | 54.31 | 54.95 | 38.16 | 38.90 | 39.60 | 38.15 |
| $T_5$–Azoxystrobin 11.4%+Difenoconazole 18.2% SC | 03.23 | 03.15 | 02.58 | 2.98 | 96.40 | 96.50 | 97.10 | 96.60 |
| $T_6$–Control | 90 | 90 | 90 | 90 | 0 | 0 | 0 | 0 |
| C. D. @ 0.01% | 0.644 | 0.739 | 0.614 | - | 0.188 | 0.322 | 0.35 | - |
| S. E(m) | 0.182 | 0.209 | 0.174 | - | 0.053 | 0.091 | 0.09 | - |
| S. E(d) | 0.258 | 0.296 | 0.246 | - | 0.075 | 0.129 | 0.14 | - |
| C. V. | 1.614 | 1.816 | 1.563 | - | 0.126 | 0.214 | 0.23 | - |

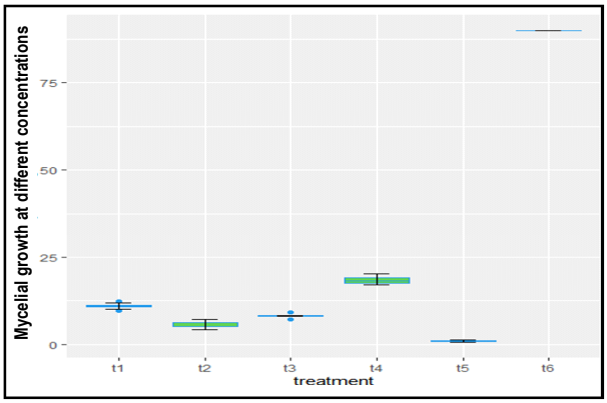


Fig. 1. Mycelial growth of *Alternaria solani* against different treatments.

maximum inhibition (100%) of test pathogen was recorded with propiconazole 25 EC at all the tested concentrations as compared to control after 15 days of incubation.

Study was undertaken to find out the optimum as well as the best temperature for the growth of *A. solani* by growing at different temperatures (15, 20, 25, 30 and 35°C) on potato dextrose agar medium. After seven days of incubation, the average radial growth in mm was recorded (Table 3). Maximum growth of the test pathogen was recorded at 25°C temperature followed by 30°C. So, it is clear from the above findings that room temperature which ranges between 25±2°C is very much suitable for the growth of *A. solani*. Parvin *et al.* (2021) evaluated the effect of different temperature range on mycelial growth of *A. solani*, on potato dextrose agar (PDA) media revealed that 25°C temperature was the most surviving temperature for *A. solani*. On the contrary; the fungus did not grow at all at the temperature of 40°C.

The growth of the fungus might be inhibited or prevented by the medium which was acidic or saline. Satisfactory medium may he prepared by addition of N/10 HCl and NaOH for lower and higher pH values, respectively. In order to find out the range of pH suitable for growth of the pathogen, five different pH levels were tried for the test fungus. Effect of different pH level on colony diameter of *A. solani* on PDA medium is presented in Table 3 indicating that maximum colony diameter (70.50 mm) after seven days of inoculation was observed at 5.5 pH level followed by 6.0 pH level (68.00 mm), and it was found at par with pH 5.0 (67.30 mm), while minimum colony diameter (45.25 mm) was observed at 4.0 pH. Same findings were also furnished by Parvin *et al.* (2021) who reported that the pH level 5 to 6.5 was appropriate for the growth *of A. solani*. Hydrogen ion concentration of the PDA culture medium had a profound effect on the amount of growth and many other life processes of the fungus (Rahmatzai *et al.*, 2016). The results of the present investigation indicated that optimal and good colony growth of the fungus was also noted at pH 5.0, 5.5 and 6.0. This showed that *A. solani* isolates preferred acidic pH rather than alkaline pH indicating its acid tolerance (Prakash *et al.*, 2022).

Test fungus was exposed to alternate cycles of dark and light and continuous light and continuous darkness for different period of time up to seven days as described in materials and methods. The maximum growth of 87.86 mm was noticed when pathogen exposed to 12 h dark and 12 h light followed by 24 h light and 24 h dark, 4 h dark and 20 h light and least radial growth of 78.25 mm was recorded in

**Table 3.** Radial growth (mm) of *Alternaria solani* at different temperature, pH range, light and culture media

| Parameters | | Mean colony diameter (mm) of test pathogen after 7 days of inoculation |
|---|---|---|
| **Temperature (°C)** | 15 | 25.45 |
| | 20 | 57.71 |
| | 25 | 88.47 |
| | 30 | 73.77 |
| | 35 | 32.01 |
| S. E(d)± | 1.88 | |
| C. D. (P=0.05) | 5.78 | |
| **pH** | 4.0 | 45.25 |
| | 4.5 | 51.50 |
| | 5.0 | 67.30 |
| | 5.5 | 70.50 |
| | 6.0 | 68.00 |
| S. E(d)± | | 0.05 |
| C. D. (P=0.05) | | 0.15 |
| **Exposed intervals (h)** | 4 h dark 20 h light | 82.23 |
| | 12 h dark 12 h light | 87.86 |
| | 8 h light 16 h dark | 78.25 |
| | 24 h light 24 h dark | 85.52 |
| S. E(d)± | | 1.36 |
| C. D. (P=0.05) | | 4.29 |
| **Culture media** | Potato dextrose agar (PDA) | 77.66 |
| | Oat meal agar (OMA) | 67.83 |
| | Malt extract agar (MEA) | 43.83 |
| | Richard's agar (RA) | 50.93 |
| | Czapeck's dox agar (CDA) | 66.33 |
| S. E(d)± | | 1.03 |
| C. D. (P=0.05) | | 3.11 |

treatment with exposure to 8 h light and 16 hrs dark (Table 3).

Among the media statistically significant and maximum growth of *A. solani* was found in potato dextrose agar (77.66 mm) followed by oat meal agar (67.83 mm) and Czapeck's Dox agar (66.33 mm), while minimum growth was found in malt extract (43.83 mm) and Richard's medium (50.93). Our present investigation is in corroboration with that of Koley and Mahapatra (2015) who reported that maximum growth of *A. solani* was observed on potato dextrose agar and oat meal agar among solid media. PDA had the simple formulation which allowed the best mycelial growth of the fungus, but it contained too much nutrients that led to ultimate loss of sporulation (Sarda Devi *et al.*, 2018).

## CONCLUSION

The early blight disease of tomato caused by *A. solani* is known to be one of the most destructive diseases. So, it is very important to develop different management strategies against Alternaria blight of tomato which includes use of several biocontrol agents as well as fungicides. From the above findings, it was proved that *Trichoderma harzianum* and *Trichoderma koningii* were very effective against early blight of tomato. Earlier it was proved by many scientists that *T. harzianum* was effective against several plant diseases. During the evaluation of agrochemicals against the test pathogen, azoxystrobin 11.4%+difenoconazole 18.2% SC showed best results followed by propiconazole and mancozeb under *in vitro* condition. But an indiscriminate use of fungicides not only polluted the environment but had an adverse residual effect on soil health and fertility. Keeping this in mind, it was concluded that eco-friendly management practices i. e. use of bio-control agents gave better results and these practices can be economical, long lasting and free from residual side effects.

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