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# Morpho-physiological Studies, Management and Screening of Tomato Germplasm against *Alternaria solani*, the Causal Agent of Tomato Early Blight

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## Abstract

Early blight (*Alternaria solani*) is a potential disease of tomato that reduces its production globally both in conventional and tunnel cultivations. Due to variability in pathogenic isolates, prolonged active disease cycle phase and broad host range early blight is very difficult to manage. *A. solani* isolate collected from tunnel grown under vegetable area, Bahauddin Zakariya University, Multan was subjected to different temperature range, pH levels, light intensity and growth media (*in vitro)*. The results indicated that *A. solani* grew the maximum at 25°C (7.50 cm) on PDA medium at 6.5 pH (8.34 cm) under continuous light condition (9.00 cm). On PDA medium the pigmentation varied from creamy yellow, brown black to olivacious brown while on HLEA medium it was light brown. Varietal screening of six tomato varieties was carried out against early blight disease. No variety was found resistant. Concerning severity, out of six tomato varieties Roma showed maximum susceptibility (70.50%) while in Nagina it was least (29.38%). Efficacy of three fungicides (Topsin M, Bavistin and Ridomil Gold MZ) at 1 g/L, 2 g/L and 3 g/L concentration and two bio-agents (*Trichoderma harzianum* and *T. viridae*) was evaluated against *A. solani in vitro* and under tunnel cultivation. In *in vitro* assay Ridomil Gold MZ inhibited *A. solani* (47.06%) at 2 g/L, while at 3 g/L concentration Topsin M was more effective (64.71%) as compared to control. Comparing both bioagents inhibition percentage (67.78%) was recorded in *T. harzianum* whereas *T. viridae* showed less inhibition (59.63%). Under tunnel cultivation, early blight of tomato was significantly reduced by foliar applications of Topsin M and *T. harzianium* at 3 g/L and $10^8$ conidia/mL concentration, respectively comparing with untreated plants. In the light of present study farmers could be suggested the practice of resistance source, combination of management practices and avoidance of environmental conditions favoring the pathogen, thus result in significant production of tomato. 

**Keywords:** Bavistin; Conidiophores; Light regimes; Roma; Topsin M; *Trichoderma* Species

## Introduction

*Solanaceae*, commonly known as nightshade family include tomato, potato, chilli, pepper and eggplant. Tomato, (*Lycopersicon esculentum* Miller) often referred to as "fruity vegetable" is second major vegetable crop produced in Pakistan (Mirza, 2007). Tomato is considered as highly nutritious because of its high contents of vitamin A and C as well as lycopene – natural antioxidant, which is not found in the other solanaceous crops. It has niacin 0.712 mg, calcium 31 mg and water 94.28 g per 100 g weight (Anonymous, 2008). In Pakistan, tomato is cultivated over an area of 58.196 thousand hectares with a production of 574.052 thousand tons annually (AGRISTAT, 2013). Of the various constraints, responsible for low yield, tomato, like other vegetables, is also vulnerable to abiotic and biotic stresses (Abdel-Sayed, 2006). However, fungal diseases, particularly early blight caused by *Alternaria solani* is most common and destructive one causing great reduction in the quantity and quality of fruit yield wherever tomato is grown (Tewari and Vishunavat, 2012). Early blight is usually characterized by the appearance of brown to dark brown necrotic spots having concentric rings on foliage, stem and fruits. Concentric rings inside the spots produced target board effect (Singh, 1987). *Alternaria* (Nees. ex. Fr.) is a dematiaceous fungus commonly isolated from plants, soil, food, and indoor air environment. The production of melanin-like pigment on target host is one of its distinguishing characters (Bell and Wheeler, 1986). *Alternaria* sp. can easily be recognized by the morphology of their large catenate conidia formed in acropetal chains or solitary and multicelled having long apical beak. In spite of

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this, *A. Solani* bears smooth, dark olive or olive-brown**,** muriform solitary conidia, with a long filiform beak ended with a small pore (Ellis, 1976). Such uniqueness of *Alternaria* spp. has made challenging and exciting ground for exploring its morphological and cultural variations.

Tomato early blight is favored by warm temperature and extended periods of leaf wetness from dew, rain fall and crowded plantation. The plants are more susceptible to infection by the disease during fruiting period (Cerkauskas, 2005; Momel and Pemezny, 2006). An understanding of the role of environmental conditions and its consequence on infection and survival of the pathogen is needed to develop disease management practices. Earlier investigations reported the influence of environmental factors such as temperature, illumination, relative humidity (RH) and composition of the culture media on morphology of conidia produced in vitro of various fungi (Vieira, 2004).

In recent years, tunnel farming has gained popularity in Pakistan as it provides effectual way of protecting crop from low temperature both in spring and fall. Tomato, like other vegetables, cultivated in tunnels gives early crop as compared to field grown tomato. Crops grown in tunnels are as susceptible to pest and diseases as those grown under field conditions. Apart from other diseases, early blight of tomato in tunnels as well as in field is the main restraining factor for tomato production. The losses from this disease may increase significantly if no protective measures are adopted well ahead of time. However, the use of fungicides, one of the most effective and conventional method, may help in reducing disease spread, if applied to greenhouse and tunnels in addition to field. Various fungicides including captafol, mancozeb, benomyl, carbendazim, copper oxychloride and Mancozeb have been used to control tomato early blight (Mate *et al.,* 2005). Recently an ecofriendly biocontrol agent, *Bacillus subtilis* has received much attention by both conventional and organic farmers to suppress plant diseases (Zitter *et al.,* 2005; Romero *et al.,* 2007). Biological control of *A. solani* with *Trichoderma sp.* has been proved more effective and environment friendly (Mukerji and Garg, 1988; Gardener and Fravel, 2002). *Trichoderma* species besides inhibiting growth of fungi also promote growth and development of plant (Samuels, 2006). The present study has therefore been undertaken with the objective to screen tomato varieties, determine the influence of defined environmental factors on the morphology of *A. solani* conidia and to evaluate the efficacy of fungicides and biological agents against pathogenic isolate of early blight of tomato under laboratory and tunnel cultivation.

## Materials and Methods

An experiment was carried out during 2011-2012 to observe the severity of early blight disease in natural conditions on six local tomato varieties viz., Sahal, Reograndi, Salar, Roma, Nagina and Packit grown under walk-in tunnels of vegetable research area, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan.

### Isolation and Identification of Culture

The infected tomato leaves showing distinctive symptoms of leaf blight were collected from experimental area. An isolate of *A. solani* was obtained by tissue segment method (Rangaswami, 1958) on potato dextrose agar (PDA) medium. The infected leaves were cut into 1–2 cm pieces, surface sterilized with sodium hypochlorite solution (1%) for 2 min, rinsed thrice with sterile distilled water, blot dried and placed on PDA medium amended with streptomycin (250 mg/L). The petri plates were incubated at 27±2°C for 5 days. Fungal mycelia growing out from segments were subsequently transferred to fresh PDA plates. Pure culture was obtained by re-culturing of isolated fungi through single spore technique (Choi *et al.,* 1999) and maintained as stock culture on Agar slants at 4°C for further studies. Pathogen was identified following the cultural and morphobiometric characteristics criteria (Ellis, 1971; Barnet and Hunter, 1972). Cultural characteristics were observed directly by pigmentation on medium and mycelial growth pattern on PDA plates while sporulation was recorded by slides from 10-day-old culture under the microscope. Ocular and stage micrometer were used to measure the size of conidia.

### Pathogenicity Test

Pathogenicity was carried out by atomizing the conidial suspension ($5 \times 10^6$ conidia/mL) at the rate of 8-10 mL/plant, prepared from 10-day-old culture on to the 2-month old seedlings of moderately susceptible tomato variety Reograndi, grown under disease conditions. Conidia were harvested by dislodging the surface of fungal colony with glass rod, transferred to sterile distilled water and filtering through sterile cheese cloth. The resultant suspension was then adjusted $5 \times 10^6$ conidia/mL with the help of (Neubauer improved) haemocytometer. Control plants were sprayed with sterile distilled water. All test plants were covered with polyethylene bags for 48 h to retain humidity. The test plants were then uncovered and kept in green house. The experiment was run in quadruplicate with eight seedlings per replication. Observations were recorded after seven days for symptom development and re isolation were made from test plants, thus fulfilling the Koch's postulates.

### Effect of Culture Media

The cultural characteristics of the pathogen were studied on two solid media. Petri plates (9 cm) containing 20 mL of each media. Potato dextrose agar (Peeled potato = 200 g, Dextrose and Agar Agar = 20 g each) and host leaf extract agar (Healthy tomato leaves = 200 g and Agar Agar = 20 g) were seeded with 0.4 cm diameter disc from 10 days old culture of *A. solani* isolate. Three replications with three plates per replication were maintained for each media and

the inoculated plates were kept in completely randomized design at 27±2°C for 7 days. Colony diameter was recorded after 24 h.

**Effect of Temperature, Light Regime and pH on Mycelial Growth of *A. solani* (*In Vitro*)**

Petriplates comprising 20 mL of Potato dextrose Agar medium were seeded with mycelial discs of 0.4 cm in diameter from margins of 7 days old actively growing colony of *A. solani*. The inoculated plates were incubated at different temperature ranges of 20, 25, 30, 35°C under cool illumination. Similarly for light effect PDA plates were exposed to continuous light, darkness and 12 h light (Philips daylight fluorescent lamp, 20W, TLT, 75RS) altered with 12 h dark and maintained at 25°C in controlled environment. While in case of determining best pH *A. solani* isolate was inoculated on PDA medium with pH adjusted to 5.5, 6.5 and 7.5 with the help of digital pH meter by adding 0 .1N sodium hydroxide and 0.1N hydrochloric acid and buffered with standard phosphate buffer. All the inoculated plates were kept in incubator at 25±2°C for 7 days. The experiment was carried out in a completely randomized design with three replications. The colony diameter was measured daily for 7 days.

***In vitro* Evaluation of Fungicides and Biological Control agents on Growth of *A. solani***

Poisoned Food Technique (Dhingra and Sinclair, 1985) was used for *in vitro* evaluation at different concentrations (1, 2 and 3 g/L by weight of formulation) of the fungicides namely Bavistin (carbendazim), Ridomil Gold MZ (metalaxyl + mancozeb) and Topsin M (thiophenate methyl) against *A. solani*. After autoclaving the PDA media was amended with certain volumes of fungicides at 45°C before pouring to sterilized petri plates. The petri plates were aseptically inoculated in center with 0.4 cm mycelia plugs taken from growing margins of 10-day-old *A. solani* culture. Each treatment was replicated thrice and plates without any fungicide were used as check. All plates were incubated at 27±2°C for seven days. Two isolates of biological control agent *T. harzianum* and *T. viridae* isolated on *Trichoderma* special medium (Elad and Chet, 1963) from rhizosphere of cucumber and chillies were used against *A. solani* on PDA by dual culture technique (Morton and Strouble, 1955). Discs of mycelia from fungal culture plate were placed on the edge of each PDA plate containing *A. solani* at opposite direction in triplicate with control without any isolate of *Trichoderma* and these plates were incubated at 25±2°C until the *A. solani* covered whole plate in control. Both fungicide and biological control agent's trial were subjected to Completely Randomized Design in triplicate. Efficacy of fungicides and *Trichoderma* isolates was determined by measuring linear growth (cm) on daily basis and data was expressed as percent inhibition over control using formula suggested by Sundar *et al.* (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where,
X= Growth of control plate.
Y= Growth of fungicide treated plate.

**Screening of Tomato Varieties**

Six tomato varieties (Sahal, Reograndi, Salar, Roma, Nagina and Packit) were sown in nursery on 15th October 2011 and were transplanted to plastic tunnel with a dimension of 6 ft height, 12 ft width and 20 ft length covered with UV polyethylene film of 10 mm thickness on 15th November 2011. The plant to plant and bed to bed distance was of 30 cm and 70 cm respectively on both sides of bed which was covered with black plastic mulch. All recommended agronomic practices were carried out. The experiment was conducted in randomized complete block design with four replications, while each replication contains six plants. Symptoms produced by the pathogen were observed on regular basis and experiment was repeated twice.

**Disease Severity on Leaves and Fruits of Tomato**

Infected leaves and fruits of tomato were observed for detection of early blight disease severity. Ten leaves and fruits were selected randomly from each replication and tagged. The data of disease severity was assessed in randomized complete block design with four replications by using modified 0-5 disease rating scale of Mayee and Datar (1986) for leaves where 0= No symptoms on leaves, 1= 0-5% infection on leaves, 2= 6-20% infection on leaves, 3= 21-40% infection on leaves, 4= 41-70% infection on leaves, 5= >71% infection on leaves. Whereas 0-8 rating scale suggested by Dillard (1989) for fruits where0= No apparent disease, 1= 1-12.5%, 2= 12.5-25%, 3= 25-37.5%, 4= 37.5-50%, 5= 50-62.5%, 6= 62.5-75%, 7= 75-87.5%, 8= 87.5-100% were used and Percent Disease Index (PDI) was expressed by using formula suggested by Wheeler, (1969).

$$\text{Percent Disease Index (PDI) \%} = \frac{\text{Sum of individual rating}}{\text{No. of leaves x Maximum examined disease scale}} \times 100$$

***In vivo* Evaluation of Fungicides and Bio Control Agents**

Laboratory tested fungicides and *Trichoderma* isolates were applied in tunnel on susceptible tomato variety Roma that showed maximum percentage of disease severity on leaves and fruit preceding year. The same nursery and transplanting procedure as described above was adopted. After four weeks of transplanting, tomato plants were sprayed with (100 mL/plant) of *A. solani* suspension at the concentration of ($5 \times 10^6$ conidia/mL). After 10 days of inoculation, these plants were sprayed (150 mL/plant) with the fungicides Topsin M, Bavistin and Ridomil Gold MZ at

the concentration of 2 and 3 g/L of water and bio control agents *T. harzianum* and *T. viridae* at the concentration of $10^7$ and $10^8$ conidia/mL adjusted with the aid of haemocytometer. While the control treatments were sprayed with *A. solani* suspension only. The same was repeated after the development of fruit. The experiment was run in a randomized complete block design with three replications. Data of disease severity was taken after 15 days of fungicides and bio control agent's application to assess the effect of time on the antagonist activity (El-Katatny and Emam, 2012).

**Statistical Analysis**

Data were analyzed by following ANOVA to differentiate statistically significant means. Fisher's Least Significant Difference (LSD) test was used to compare and separate means at 5% level of significance (Steel *et al.*, 1997) by using statistical software Statistix 8.1.

## Results

Microscopic study revealed that the conidiophores of *A. solani* isolate were straight or flexuous and brown to olivaceous brown. The conidia were solitary straight or slightly flexuous, oblong or ellipsoidal tapering to a beak, pale or olivaceous brown, smooth, 150-300 µm in length, 13-20 µm thick in the broadest part with 8-10 transverse and none or few (1 to 4) longitudinal septa. The beaks were flexuous, pale and sometimes branched.

Two media namely Potato Dextrose Agar (PDA) and Host Leaf Extract (HLE) agar were used for the isolation and assessment of the best media for the growth of *A. solani*. Fungus grew on both media but growth was better on PDA (8.64 cm) compared to HLE agar with (6.52 cm). The color of colony was observed dark brown on PDA and light brown on host leaf extract agar.

The daily data of mycelial growth at different temperature was statistically significant. The growth of *A. solani* started slowly and then progresses day by day. But the significant growth (7.56 cm) was observed at 25°C followed by 35°C, 20°C and 30°C with 7.28, 5.36 and 5.00 cm growth respectively. Mycelial growth of the fungus was statistically significant at different light conditions. The preliminary studies carried out in the present investigation with *A. solani* indicated significant growth and sporulation, when the inoculated plates were exposed to continuous light followed by continuous dark and conditions (12 h light alternated with 12 h dark) with 9.00, 7.71 and 5.54 cm growth respectively. The optimum pH for the growth of *A solani* was in the range of 5.5 (HCl), 6.5 (PDA) and 7.5 (NaOH). However, less growth of the fungus was recorded at pH 5.5 and maximum on pH 6.5 with 5.89 cm and 8.34 cm growth respectively and pH 7.5 showed intermediate results with 6.79 cm growth and data of growth on three different pH concentrations was statistically significant. This shows that *A. solani* prefers acidic pH to alkaline pH indicating its acid tolerance (Table 1).

The percent inhibition of the growth of *A. solani* was significantly different at all fungicide concentrations compared to control. Bavistin was less effective at 1g/ liter with 17.65% inhibition while Ridomil Gold MZ was more effective at same concentration with 29.49 and Topsin M was intermediate with 29.41% inhibition. At concentration 2 g/L, Ridomil Gold MZ was most effective with 47.06% inhibition followed by Topsin M and Bavistin with 41.18 and 23.53% inhibition, respectively. Maximum growth of *A. solani* was inhibited at concentration i.e., 3 g/L by Topsin M with 64.71% inhibition followed by Ridomil Gold MZ and Bavistin with 52.94 and 35.29% inhibition respectively compared to control with 0% inhibition (Table 4). The growth of *A. solani* was checked in the presence of two antagonistic isolates of *Trichoderma* sp. The plates containing biological control agent reduced the tested fungus growth considerably. Inhibition percentage was maximum (67.78%) with *T. harzianum* followed by 59.63% inhibition in *T. viridae* compared to control with zero percent inhibition (Table 2).

At initial stage the data of disease severity on tomato leaves depicted that Nagina was resistant with only 3.75% severity followed by Sahal (5.38%), Packit (6.25%), Reograndi (18.75%), Salar (24%) and Roma (30.25%) with the passage of time, disease spread continuously. At 20$^{th}$ day, salar ranked first in susceptibility with 57% severity followed by roma, reograndi, sahal, packit and nagina with 56.88, 55.75, 29.13, 29 and 25.38%, respectively. At 35$^{th}$ day, it was observed that no line/variety were resistant to early blight disease. But maximum disease was on roma with 88% severity while less disease 66.13% was on nagina (Table 3).

Similarly disease severity on tomato fruit was also statistically significant as recorded by modified 0-8 disease rating scale suggested by Dillard (1989). During 5$^{th}$ day, reograndi, salar were resistant with 0% severity on fruits followed by low disease percentage 0.25, 0.50, 0.75 and 1.50% in roma, packit, nagina and sahal respectively. At 20$^{th}$ day, the situation of severity was changed from previous data. Fruit of roma was damaged more with 19.50% followed by sahal, salar, packit, reograndi and nagina with 18.38, 16.75, 11.75, 9.50 and 7% severity respectively. At 35$^{th}$ day roma which showed highest severity on leaves also susceptible to pathogen attack on fruit with 70.50% severity, while fruit of nagina was less affected (27.38%) by the pathogen (Table 3).

Fungicides concentrations which proved best in laboratory trial were applied in tunnel on susceptible tomato variety Roma that showed highest percentage of leaf and fruit affected. Three fungicides with two best concentrations (2 and 3 g/L) showed statistically significant results of disease inhibition on leaves and fruits compared to control. Bavistin with 65% severity at concentration 2 g/L was weaker compared to other two fungicides with 55.50% and

**Table 1:** Effect of temperature, light regime and pH on *in vitro* growth of *A. solani*

| Days | Temperature Ranges (°C) | | | | Light Conditions | | | pH Variation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | Darkness | Continuous light | 12 h light 12 h dark | 5.5 (HCl) | 6.5 (PDA) | 7.5 (NaOH) |
| 1 | 0.24 x | 0.64 v | 0.72 u | 0.60 w | 0.80 t | 1.03 s | 0.63 u | 0.70 r | 1.20 p | 0.90 q |
| 2 | 1.20 s | 1.20 s | 1.20 s | 0.96 t | 1.83 p | 2.40 o | 1.13 r | 1.67 o | 3.10 l | 2.71 m |
| 3 | 2.16 r | 3.20 n | 3.32 m | 1.20 s | 2.46 n | 3.60 l | 1.66 q | 2.34 n | 4.17 i | 3.57 k |
| 4 | 3.00 p | 3.60 l | 3.96 j | 2.64 q | 3.71 k | 5.71 f | 2.80 m | 3.10 l | 5.30 f | 4.16 i |
| 5 | 3.16 o | 4.68 f | 4.36 h | 4.20 i | 5.38 h | 6.46 e | 4.06 j | 3.94 j | 6.38 d | 5.06 g |
| 6 | 3.84 k | 5.60 c | 4.60 g | 5.36 d | 6.49 d | 8.11 b | 4.46 i | 4.76 h | 7.40 b | 5.98 e |
| 7 | 5.36 d | 7.56 a | 5.00 e | 7.28 b | 7.71 c | 9.00 a | 5.54 g | 5.89 e | 8.34 a | 6.79 c |

The values are means of three replications. Lower case letters immediately after values represent comparison within column. The fungal growth was measured in cm. $LSD_{0.05}$ for temperature ranges= 0.0154, $LSD_{0.05}$ for Light conditions = 0.0146, $LSD_{0.05}$ for pH variation = 0.1076

**Table 2:** Efficacy of different concentrations of three fungicides against *A. solani* (*in vitro*)

| Doses | Inhibition Percentage | | | | |
|---|---|---|---|---|---|
| | Topsin M* | Ridomil Gold MZ* | Bavistin * | *T. harzianum*** | *T. viridae*** |
| 1 g/L | 29.41 g | 29.49 f | 17.65 i | - | - |
| 2 g/L | 41.18 d | 47.06 c | 23.53 h | - | - |
| 3 g/L | 64.71 a | 52.94 b | 35.29 e | - | - |
| - | | | | $67.78^{ns}$ | $59.63^{ns}$ |

*Method used Poison food technique

**Method used Dual culture technique

$^{ns}$Non Significant

The values are means of three replications. Comparison was made continuously across all treatments. $LSD_{0.05}$ value for comparison = 0.0376

**Table 3:** Severity of early blight disease on tomato leaves and fruits in tunnel

| Varieties | Disease severity on Leaves (%) Days | | | | | | | Disease severity on Fruits (%) Days | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |
| Reograndi | 18.75 c | 27.00 c | 40.50 b | 55.75 a | 70.50 a | 77.75 a | 82.38 b | 0.00 b | 1.75 cd | 3.75 d | 9.50 e | 17.13 e | 35.63 c | 53.38 d |
| Salar | 24.00 b | 31.00 b | 38.63 c | 57.00 a | 66.88 c | 74.13 b | 84.13 b | 0.00 b | 2.63 b | 6.00 c | 16.75 c | 27.25 c | 43.63 b | 67.50 b |
| Roma | 30.25 a | 39.75 a | 48.38 a | 56.88 a | 68.38 b | 77.38 a | 88.00 a | 0.25 b | 4.25 a | 9.00 b | 19.50 a | 39.88 a | 60.25 a | 70.50 a |
| Nagina | 3.75 e | 11.63 f | 15.88 f | 25.38 c | 37.13 f | 52.00 e | 66.13 e | 0.75 ab | 1.50 d | 3.38 d | 7.00 f | 12.50 f | 20.00 e | 27.38 f |
| Packit | 6.25 d | 13.75 d | 19.88 d | 29.00 b | 40.88 e | 55.13 d | 69.00 d | 0.50 b | 2.25 bc | 5.63 c | 11.75 d | 17.63 d | 24.13 d | 34.00 e |
| Sahal | 5.38 d | 12.75 e | 17.88 e | 29.13 b | 44.38 d | 66.00 c | 76.88 c | 1.50 a | 4.00 a | 9.75 a | 18.38 b | 30.88 b | 43.50 b | 57.50 c |
| $LSD_{0.05}$ | 1.206 | 0.878 | 1.032 | 1.360 | 1.357 | 0.889 | 2.707 | 0.800 | 0.653 | 0.666 | 1.073 | 0.361 | 0.885 | 0.657 |

The values are means of four replications. Lower case letters immediately after values represent comparison within column

49.50% severity on leaves in Ridomil Gold MZ and Topsin M respectively. Topsin M showed exceptional control at 3 g/L concentration with only 24% disease severity followed by 30.50% and 34% severity in Ridomil Gold MZ and Bavistin, respectively compared to control 85.50% severity. Similar to control of disease on leaves, all fungicides decreased the severity at both concentrations. The best control was exhibited at 3 g/L concentration. Minimum severity (27%) was observed on plants sprayed with Topsin M followed by Ridomil Gold MZ and Bavistin with 39 and 45% severity, respectively compared to control 91% (Table 4).

Keeping in view the inhibition of *A. solani* with two *Trichoderma* isolates in laboratory, both *T. harzianum* and *T. viridae* were applied on leaves and fruits of tomato in tunnel with two different concentrations $10^7$ and $10^8$ conidia/mL. Growth of the fungus on leaves and fruit after spraying both isolates was statistically significant. *T. harzianum* decreased pathogen growth at both the concentrations compared to *T. viridae*. Tomato plants showed 65, 76% and 41, 49.50% severity on leaves sprayed with $10^7$ and $10^8$ conidia/mL concentrations of *T. harzianum* and *T. viridae*, respectively compared to control with 86.50% severity. Similarly disease severity on fruit was 59.50, 80% and 53.50, 66% at $10^7$ and $10^8$ conidia/mL concentrations of *T. harzianum* and *T. viridae* respectively compared to control with 93.50% severity (Table 5).

## Discussion

*A. solani* was initially identified on the basis of symptoms. Presently, symptomatology is not a reliable method for detection of fungal disease but it is an initial step in disease diagnosis (Batool *et al.*, 2011), while modern detection techniques e.g., PCR were found more reliable for disease identification. Pathogenicity was carried out by applying conidial suspension on seedlings for confirmation of pathogen association with host. Similar approach has been followed by different scientists. Vloutoglou and Kalogerakis (2000) and Castro *et al.* (1999) performed the pathogenicity test with conidial suspensions. Conidial suspensions of *A. solani* were more effective for preparation of inoculum load and pathogenicity test against tomato and potato (Rotem,

**Table 4:** Effect of different concentrations of fungicides on early blight severity (%) on leaves and fruits of Roma in tunnel

| Doses | Fungicides | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bavistin | Ridomil Gold | Topsin M | Mean | Bavistin | Ridomil Gold | Topsin M | Mean |
| | Leaf | | | | Fruit | | | |
| Control | 85.50 a | 85.50 a | 85.50 a | 85.50 A | 91.00 a | 91.00 a | 91.00 a | 91.00 A |
| 2g / Liter | 65.00 b | 55.50 c | 49.50 d | 56.67 B | 71.00 b | 61.00 c | 54.00 d | 62.00 B |
| 3g / Liter | 34.00 e | 30.50 f | 24.00 g | 29.50 C | 45.00 e | 39.00 f | 27.00 g | 37.00 C |
| Mean | 61.50 A | 57.17 B | 53.00 C | | 69 A | 63. 67 B | 57.33 C | |

$LSD_{0.05}$ value for interaction effect of leaves = 2.87, for fungicides = 1.09 and for concentrations = 1.65
$LSD_{0.05}$ value for interaction effect of fruits = 3.89, for fungicides = 1.92 and for concentrations = 2.24

**Table 5:** Effect of different concentrations of biological control agent's on early blight severity (%) on leaves and fruits of Roma in tunnel

| Doses | Biocontrol Agents | | | | | |
|---|---|---|---|---|---|---|
| | *T. harzianum* | *T. viridae* | Mean | *T. harzianum* | *T. viridae* | Mean |
| | Leaf | | | Fruit | | |
| Control | 86.50 a | 86.50 a | 86.50 A | 93.50 a | 93.50 a | 93.50 A |
| $10^{7**}$ | 65.00 c | 76.00 b | 70.50 B | 59.50 d | 80.00 b | 69.75 B |
| $10^{8**}$ | 41.00 e | 49.50 d | 45.25 C | 53.50 e | 66.00 c | 59.75 C |
| Mean | 64.17 B | 70.67 A | | 68.83 B | 79.83 A | |

$LSD_{0.05}$ value for interaction effect of leaves = 3.706, for biocontrol agents = 4.362 and for concentrations = 2.621
$LSD_{0.05}$ value for interaction effect of fruits = 4.432, for biocontrol agents = 3.679 and for concentrations = 3.134

1994). Light spectrum, temperature, pH and nutrition are major factors that influence the sporulation of *A. solani*. The maximum growth was observed at $25^0$C, continuous light condition and 6.5 pH. While minimum growth at $30^0$C, 12 h light and 12 h dark condition and 5.5 pH. Sodlauskiene *et al.* (2003) and Gemawat and Ghosh (1979) tested the isolate of *A. solani* on particular temperature and pH respectively.

Out of two media used both resulted in good sporulation of the pathogen but PDA performed better compared to host leaf extract agar. Similarly, Vieira (2004) used PDA and plants extracts media to induce sporulation of *Alternaria* spp. Three fungicides at three different concentrations and two species of biocontrol agent *Trichoderma* were tested on PDA under laboratory condition to check the growth of *A. solani.* Ridomil Gold MZ was efficient at concentration 1 g and 2 g/L and resulted in decreased sporulation of the pathogen. But at higher concentration i.e., 3 g of fungicide/L Topsin M depicted excellent inhibition of the pathogen compared to others. But the results showed that dose 3 g/L of water of all the fungicides inhibited growth of the pathogen significantly. Different fungicides namely Zineb, iprodione, copper oxychloride, dithianon, mancozeb, carbendazim, captafol and thiophanate-methyl were tested against *A. solani* and Mancozeb and reduced effective (77%) growth inhibited followed by captafol while Dithane M-45 was most effective to control the fungus (Elad *et al.,* 1995). Effectiveness of mancozeb (0.2%) against early blight fungus of tomato was confirmed by Choulwar *et al.* (1989), Singh *et al.* (2001) while, Babu *et al.* (2001) also found Mancozeb (0.2%) very effective against *A. solani* followed by Captafol (0.2%). *Trichoderma* species due to its antagonistic activity are considered as potential biological control agents against numerous plant pathogenic fungi (Mohamed and Haggag, 2006). The most studied fungal biocontrol agent (*Trichoderma* spp.) has been marketed as biopesticides, biofertilizers and soil amendments commercially for many years (Harman *et al.,* 2004; Lorito *et al.,* 2004). *T. harzianum* and *T. viridae* were used. *T. harzianum* showed maximum inhibition percentage compared to *T. viridae.* Mukerji and Garg (1988) described that *Trichoderma* are more reliable in biological control because it is easy to isolate and culture, while its enzyme system along with production of different antibiotics increase its efficiency to control different pathogens. Raziq and Ishtiaq (2010) confirmed that different fungicides and *Trichoderma* species effectively reduced the growth of *A. solani* under laboratory condition. Many workers confirmed that *Trichoderma* sp. control the pathogen growth due to the production of extracellular enzymes, antifungal metabolites and antibiotics (El-Katatny *et al.,* 2006; Montealegre *et al.,* 2010). El-Katatny *et al.* (2006) confirmed the antagonistic activity of two isolates of *T. harzianum* (T3 and T24) against phytopathogens. Both *T. harzianum* strains were generally effective at low concentration of $10^6$-$10^8$ conidia per ml. These concentrations are even lower than the recommended concentrations of other biocontrol agents (Wang *et al.,* 2008) thus considered suitable for commercial use. Therefore, the use of *Trichoderma* isolates offers a potential, safe and efficient mean alternative to fungicides in treatment of different fungal diseases of tomato fruits. However, such measures should be adopted in future to provide these isolates for the control of pathogens in field condition instead of fungicides to minimize the human health hazards and environmental pollution.

Among selected varieties/lines Reograndi and Salar showed resistance during early stage of infection but high disease severity was observed at later stage. Maximum infection of fungus was observed on the leaves and fruit of Roma and Packit respectively while Nagina showed

resistance against leaves and fruit infection. The yield (quality and quantity) of *Solanaceous* crop can be enhanced by cultivation of resistance varieties against, nematode, bacteria, viruses and fungus which are more effective and environment friendly (Abbas and Hameed, 2012). Serological and molecular techniques are also reliable for the screening of resistance source.

## Conclusion

Resistant or tolerant local tomato varieties, biological and chemical control strategies against *A. solani* may play a vital role in reducing yield losses and thus may increase the income of farmer. It is therefore, suggested that farmers along with resistant lines/varieties should employ recommended fungicides and bio-control agents to make tomato less vulnerable to early blight pathogen both under conventional and tunnel farming.

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