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# Perpetuation studies of pathogen *Alternaria solani* (Ellis and martin) in tomato crop through seed and plant debris

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**Abstract**
Perpetuation studies were conducted through both seed and plant debris. The seeds collected at harvest from diseased tomato plants (c.v Marglobe) were stored in khadi cloth bags in laboratory and assessed for the presence of the pathogen through blotter methods at monthly intervals. Tomato leaves and twigs bearing typical symptoms of early blight disease at harvest and kept separately in nylon mesh bags. The highest conidial viability 89.67 % was seen in the month of November I$^{st}$ 2014. The viability showed a gradual decrease as the storage period advanced such that after six months of storage in April 2015. The conidial viability was gradually reduced as the storage period advanced such that only 18.00 per cent was recorded in April 2015. 24.66 per cent seeds showed *Alternaria solani* growth in November 2014. The percentage of seeds exhibiting *Alternaria. solani* growth showed a gradual decrease as the storage period advanced, such that the percentage of these seeds was 4.94 per cent after six months of storage in April 2015. Leaf bits exhibiting *Alternaria solani* growth (31.13%) was recorded in November 2014 immediately after harvest followed by those in December 2014 and January 2015 (22.4 and 14.40% respectively). Conidial count was taken from November 2014, i.e. one month after storage of leaf bits under laboratory condition, revealed that presence of 68.21 ($1\times 10^3$) conidia cm$^2$ leaf area. There was a gradual decrease in conidial count such that 29.10 per cent spore count was recorded in April 2015. The number of viable conidia was maximum (67.34%), when leaf bits were placed under ambient laboratory conditions. On soil surface, the conidial viability was 58.30 per cent whereas at 5 cm soil depth (42.10%) conidia remained viable.

**Keywords:** *Alternaria solani*, perpetuation, conidial viability

## Introduction
Tomato (*Solanum lycopersicum* Mill.) is one of the most remunerable and widely grown vegetables in the world. It belongs to family *solanaceae*, commonly known as nightshade family include tomato, potato, chilli, pepper and eggplant (Mirza, 2007) [6]. The total area under crop in India is about 1204 thousand hectares with annual production of about 19402 metric tonnes which accounts for 11.5% of the total vegetable production (FAO, 2015) [8]. Globally tomato is cultivated in 140 countries of the world with an annual production of 16.82 metric million tonnes (Anonymous, 2012) [3]. In India, major tomato growing states are Maharashtra, Bihar, Uttar Pradesh, Karnataka and west Bengal. The production of tomato in Jammu and Kashmir during the year 2014 was 0.008 metric million tonnes which accounts for 28.50 per cent of the total vegetable production of the State (FAO, 2014) [7]. Inspite of quite favorable edaphic and environmental conditions for tomato cultivation in the Kashmir valley, the yield have not been encouraging. The wide gap between the yield potential of cultivars and the yields realized is chiefly attributed to a number of biotic and abiotic stresses (Balanchard, 1992; Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al*., 2008) [4, 10, 2, 1].
The perpetuation the pathogen in a crop ecosystem forms an important link for recurrence of any endemic or epidemic disease. Groves and Sholka (1994) [11] reported that the *Alternaria solani* pathogen survives on infected seeds for several months and serves as the source of inoculum for disease development. The fungal pathogen may be externally or internally seed borne, extra or intra embryal, or associated with the seeds as contaminants (Singh and Mathur, 2004) [12]. Mehrotra and Agarwal (2003) [13] confirmed that *Alternaria solani* is the seed borne fungal pathogen.

## Methods and Materials

### Perpetuation of the pathogen

### Perpetuation through seed

The seeds collected at harvest from diseased tomato plants (c.v Marglobe) were stored in khadi cloth bags in laboratory and assessed for the presence of the pathogen through blotter methods at monthly intervals.

### Conidial viability

Twenty tomato seeds were taken randomly at one month interval from harvest and crushed in pestle and mortar in 80 ml of sterilized distilled water. The crushed material was strained through double layer of cheesecloth. Twenty mililitre of filtrate was centrifuged at 6000 rpm for 15 minutes. After centrifugation, supernatant was discarded and 2 ml sterilized water was added to pellet. Two drops of 50 µl from processed sample were placed on a glass slide and incubated in a moist chamber at 24± 2 ºC. After 24 hours of incubation, one drop of cotton blue in lactophenol was added to each drop (Filajdic and Sutton, 1995). After 24 hour incubation, the slide was viewed under binocular microscope to record spore germination index of conidial viability. The spore viability was recorded using formula:

$$\text{Conidial viability} = \frac{\text{Number of conidia germinated}}{\text{Total number of conidia viewed}} \times 100$$

### Seed infection

**Blotter method:** Twenty seeds were taken randomly at monthly intervals from harvest and surface sterilized by immersing in 0.1 per cent mercuric chloride for 30 seconds. The seeds thoroughly rinsed thrice with sterilized distilled water. The seeds were then placed in sterilized petri dishes lined inside with sterilized blotter papers moistened with sterilized distilled water. Twenty seeds were placed aseptically in each petri dish maintaining three replications for each plate. The plates were incubated at 24 ± 2 ºC The blotter paper in petri dish was kept moist by carefully pouring few drops of sterilized distilled water in the plates as and when required. The seeds were examined from 7 days of incubation for the appearance of fungal colonies. The observation on per cent seeds showing *A. solani* growth was recorded using the formula:

$$\text{Per cent seed infection} = \frac{\text{Number of seeds infected}}{\text{Total Number of seeds examined}} \times 100$$

### Perpetuation through plant debris

Tomato leaves and twigs bearing typical symptoms of early blight disease at harvest and kept separately in nylon mesh bags. The presence of fungus in the diseased tissue was ensured beforehand. The material thus collected were divided into three sets of 12 bags each. One set, was placed on the soil surface in vacated tomato field under natural conditions. The other set was buried 5 cm deep in such soil and third set was stored under ambient laboratory conditions. The samples from the bags containing leaf bits were randomly drawn separately from all the placement conditions at monthly intervals starting from November 2014 to April 2015 next year, thoroughly washed with tap water, surface sterilized with 0.1% mercuric chloride for 30 seconds, and rinsed thrice with distilled sterilized water to remove the traces of mercuric chloride. Leaf bits were randomly taken with the help of a 3mm diameter cork borer, placed in a moist chamber on petri plates containing blotter paper and incubated at 24± 2 ºC for seven days at nearly 100 per cent relative humidity. The pieces were then removed, air dried and examined under stereomicroscope for the presence of conidia and conidiophore. These leaf discs were then crushed in 10 ml of sterilized distilled water and were centrifuged at 3000 rpm for 15 minutes. After discarding the supernatant, sterile distilled water was added to the pellet to make 5 ml spore suspension and the number of conidia was counted with the help of heamocytometer. To check the conidial viability in overwintering the leaf debris, spore germination method was used. Two drops of 50 µl from each processed sample were placed on glass slide and incubated in a moist chamber at 24± 2 ºC. The number of spores observed and the number that germinated were recorded after 24 hour incubation for calculating the per cent viability of conidia.

## Results and Discussion

### Perpetuation

The possibilities of perpetuation of the pathogen *Alternaria solani*, during cropless months after harvest, on tomato seeds and diseased plant debris were explored during the year 2014.

### Perpetuation in/on tomato seeds

### Conidial viability

Random seed sample collected from heavily infected crop in October 2014 and drawn at monthly intervals from November 2014 to April 2015 for the examination of conidial viability indicated 89.67 per cent conidial viability immediately after harvest in November 2014 (Table 1). The viability showed a gradual decrease as the storage period advanced such that after six months of storage in April 2015. The conidial viability was gradually reduced as the storage period advanced such that only 18.00 per cent was recorded in April 2015.

**Table 1:** Survival of *Alternaria solani* on tomato (*Solanum lycopersicum*) seeds observed at monthly intervals after harvest in 2014

| Month/Date of observation | Conidial viability* (%) | Per cent infected seeds** (Blotter method ) |
|---|---|---|
| November 1st 2014 | 89.67 (71.25) | 24.66 (4.96) |
| December 1st 2014 | 76.65 (61.08) | 23.31 (4.93) |
| January 1st 2015 | 51.67 (45.95) | 21.41 (4.73) |
| February 1st 2015 | 46.33 (42.87) | 18.07 (4.36) |
| March 1st 2015 | 21.00 (27.28) | 10.00 (3.16) |
| April 1st 2015 | 18.00 (25.10) | 6.00 (2.44) |
| Mean | 50.55 (45.58) | 17.23 (4.09) |
| C.D($P \leq 0.05$) | 0.032 | 0.010 |

*****Figures in parenthesis are arc sine transformed values.
******Figures in parenthesis are square root transformed values.

### Seed infection

The seeds showing natural infection with *Alternaria solani* were examined by using blotter method.

**Blotter method:** The seeds, collected from *Alternaria solani* infected tomato crop in October 2014, were incubated on wet blotter paper at 24±2 ºC at monthly intervals starting from November for six months and observed for *A. solani* growth.

The data recorded for the seeds collected in 2014 revealed that 24.66 per cent seeds showed *A. solani* growth in November 2014 (Table 1). The percentage of seeds exhibiting *A. solani* growth showed a gradual decrease as the storage

period advanced, such that the percentage of these seeds was 4.94 per cent after six months of storage in April 2015.

**Perpetuation through leaves**

The infected leaf bits kept under different placement conditions after harvest were examined for leaf bit showing *A. solani* growth at monthly intervals starting from November 2014 to April 2015 and the data obtained is presented in (Table 2). The data revealed that irrespective of leaf bit placements, leaf bits exhibiting *A. solani* growth (31.13%) was recorded in November 2014 immediately after harvest followed by those in December 2014 and January 2015 (22.4 and 14.40% respectively).There was a gradual decrease in the per cent leaf bits showing *A. solani* growth as storage period advanced such that a minimum of 4.78 per cent was recorded in April 2015. On an average, leaf bits placed indoor under laboratory conditions harboured the maximum leaf bit showing - growth (22.79%) followed by the placement in soil surface (15.58%) ; placement at 5 cm deep in soil (7.14%) leaf bits leaf bit showing - growth. There existed a significant interaction between the placement conditions and the storage period. The leaf bits placed under ambient laboratory conditions at harvest exhibited 37.60 and 31.10 per cent. Leaf bits showing - growth in November and December 2014, respectively. The leaf bits showed decline in *Alternaria solani* growth as the storage period advanced such that only 7.34 per cent was recorded in April 2015.The leaf bits placed on soil surface showed 35.60 and 24.20 per cent growth in November and December 2014, respectively.

**Table 2:** Survival of *Alternaria solani* on infected tomato (*Solanum lycopersicum*) leaf bits kept under different placement conditions after harvest in 2014

| Year/month | Leaf bits showing *A. solani* growth (%) | | | Mean |
|---|---|---|---|---|
| | Under laboratory conditions | On soil surface | 5 cm deep in soil | |
| 2014 | | | | |
| November | 37.60 (37.80) | 35.60 (36.61) | 20.20 (26.72) | 31.13 |
| December | 31.10 (33.88) | 24.20 (29.43) | 12.00 (20.28) | 22.40 |
| 2015 | | | | |
| January | 28.00 (31.98) | 11.20 (19.57) | 4.00 (11.58) | 14.40 |
| February | 20.60 (26.98) | 9.50 (17.91) | 3.34 (11.58) | 11.14 |
| March | 12.10 (20.35) | 7.34 (15.70) | 2.00 (8.19) | 7.14 |
| April | 7.34 (15.70) | 5.67 (13.76) | 1.34 (6.65) | 4.78 |
| Mean | 22.79 (28.49) | 15.58 (23.23) | 7.14 (15.49) | 15.16 |
| C.D(P≤0.05) | 0.028 | 0.061 | 0.083 | |

* Figures in parenthesis are arc sine transformed values

When these leaf bits buried 5 cm depth showed 20.20 per cent immediately after harvest in November 2014. There was a gradual decrease in leaf bits showing - growth was observed such that only 1.34 per cent was recorded in April 2015.

**Conidial Count**

Conidial count was taken from November 2014, i.e. one month after storage of leaf bits under laboratory condition, revealed that presence of 68.21 ($1\times 10^3$) conidia $cm^2$ leaf area (Table 3). There was a gradual decrease in conidial count such that 29.10 per cent spore count was recorded in April 2015. In case of leaf bits kept under field i.e. 0 and 5 cm depth, there was an initial increase in conidial count $cm^2$ leaf area and thereafter there was gradual decrease. Periodic observation on leaf bits kept on soil surface revealed the presence of 60.21($1\times10^3$) conidia $cm^2$ leaf area in November 2014 followed by 59.34 ($1\times10^3$) conidia $cm^2$ leaf area in December 2014. There was a gradual decrease in conidial count and minimum number of 16.40 ($1\times10^3$) conidia $cm^2$ was observed in April 2015. Similar pattern was observed on leaf bits kept at 5cm soil depth. Leaf bits showed 58.90 and 53.15($1\times 10^3$) conidia $cm^2$ in November and December 2014, when kept at 5 cm depth. Thereafter, there was gradual decrease in conidial count such that 8.15 ($1\times10^3$) conidia $cm^{2.}$ Leaf area was observed in April 2015.

**Conidial Viability**

The data (Table 4) revealed that the conidia remained viable in considerable proportions from crop harvest till next growing season under all placement conditions except 5 cm soil depth. On an overall basis, the number of viable conidia was maximum (67.34%), when leaf bits were placed under ambient laboratory conditions. On soil surface, the conidial viability was 58.30 per cent, whereas at 5 cm soil depth (42.10%) conidia remained viable. Irrespective of the condition of leaf bit placement, the maximum number of conidial viability was 86.04 per cent in November 2014. The viability gradually decreased as the placement period advanced such that only 21.99 per cent viable conidia were observed in the month of April 2015. There existed a significant interaction between conidial viability and the storage period. The leaf bits placed under ambient laboratory conditions exhibited 88.99 per cent conidial viability in November 2014 and 86.55 per cent in December 2014. The viability of conidia gradually declined as the period advanced reaches a minimum of 35.33 per cent in April 2014. Similarly the leaf bits kept on soil surface showed 83.44 per cent viable conidia in November and 80.33 per cent in December 2014. When leaf bits buried in soil showed 85.69 per cent viability in November 2014. Thereafter a gradual decrease in conidial viability was observed reaching a minimum of 42.10 per cent in April 2015.

**Table 3:** Conidial production of *Alternaria solani* on infected tomato (*Solanum lycopersicum*) leaves kept under different conditions after harvest in 2014

| Year/month | Conidia/cm$^2$ leaf area (1×10$^3$) | | |
|---|---|---|---|
| | Under laboratory conditions | On soil surface | 5 cm deep |
| 2014 | | | |
| November | 68.21 (55.65) | 60.21 (50.87) | 58.89 (50.09) |
| December | 66.52 (54.62) | 59.34 (50.36) | 53.15 (46.78) |
| 2015 | | | |
| January | 41.33 (39.98) | 34.20 (35.78) | 29.30 (32.76) |
| February | 34.20 (35.77) | 30.46 (33.48) | 24.10 (29.39) |
| March | 30.46 (33.48) | 20.79 (27.11) | 10.10 (18.52) |
| April | 29.10 (32.63) | 16.40 (23.88) | 8.15 (16.57) |
| Mean | 44.97 | 36.90 | 30.61 |
| C.D(P≤0.05) | 0.006 | 0.009 | 0.008 |

* Figures in parenthesis are arc sine transformed values

**Table 4:** Periodical observations of conidial viability of *Alternaria solani* on infected tomato leaves kept under different condition after harvest in 2014

| Observation year/moth | Conidial viability (%) | | | Mean |
|---|---|---|---|---|
| | Laboratory conditions | On soil surface | 5cm soil depth | |
| **2014** | 88.99 (9.48) | 83.44 (9.18) | 85.69 (9.31) | 86.04 |
| November | | | | |
| December | 86.55 (9.35) | 80.33 (9.01) | 75.66 (8.75) | 80.84 |
| **2015** | 75.66 (8.75) | 68.98 (8.36) | 54.31 (7.43) | 66.10 |
| January | | | | |
| February | 68.87 (8.35) | 51.33 (7.23) | 36.98 (6.16) | 52.40 |
| March | 48.65 (7.04) | 34.99 (5.99) | ** | 27.88 |
| April | 35.33 (6.02) | 30.66 (5.62) | ** | 21.99 |
| Mean | 67.34 (8.26) | 58.30 (7.70) | 42.10 (6.56) | 55.87 |

transformed values

** Material exhausted

Figures in parenthesis are square root

Since the presence and the amount of pathogen inoculum in any crop ecosystem is the first link in the infection chain or disease cycle, the perpetuation in/on plant debris and tomato seeds was studied in detail. Grover and Sholka also reported that *Alternaria solani* pathogen survives on infected seeds for survival months and serves as the source of inoculum for disease development. Robert and Boothroyd (1972) [14] also reported that *Alternaria solani* survived as long as 18 months in diseased leaves. Dorozhkin and Ivanyuk (1979) [5] also reported that the inoculum persisted in field for several months and the fungus overwintered as conidia, chlamydospores and mycelium on plant debris and in the soil.

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