Perpetuation of *Alternaria solani* of Potato under Temperate Kashmir Valley Conditions

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**Abstract** The present study on perpetuation of *Alternaria solani*, causing early blight of potato in Kashmir valley was conducted during 2009 and 2010. The perpetuation of the fungus *A. solani* was studied on diseased leaves by placing the leaves on ground surface and at 20 cm depth, as well as on diseased potatoes kept in ambient store. The pathogen perpetuated as mycelium and conidia throughout winter on diseased leaves left on the ground surface and on diseased potatoes kept in ambient store. The number of spores cm⁻² leaf area and the viability of spores decreased with increase in depth of placement in soil. Maximum spores production on overwintered leaves was observed during first fortnight of June during the year 2009 and 2010, with maximum number of 294 and 323 spores, respectively. However, by the first fortnight of July, the number gradually declined to 160 and 220 spores, respectively. The highest spore viability of 44.3 and 49.3 per cent in leaves on ground surface was recorded in the first fortnight of June, 2009 and 2010, respectively. In potatoes kept in ambient store the average number of spores increased upto first fortnight of June, both in 2009 and 2010, with a maximum number of 430 and 508 spores, respectively. The number then gradually declined to 216 and 263 spores, respectively, till last observation recorded in the second fortnight of July.

**Keywords** Perpetuation; *Alternaria solani*; Potato; Ambient store; Ground surface

**Introduction**

Potato is one of the most important crops in the world and is planted in 18.2 million ha and a total yield reached 314.1 million ton (FAO, 2010). Potato is considered ‘The King’ in food staples and hardly any domestic kitchen is available which does not use it in one or the other form as it possesses all the attributes to be a potential food crop. Potato contains significant levels of phenolic compounds and vitamin C as potent antioxidants (Brown, 2005), which inactivate reactive oxygen species, reduce oxidative damage, lead to improved immune functions and reduce risk of cardiovascular diseases, cancer, cataract, diabetes and aging (Kour et al., 2004). Potato is highly remunerative and nutritive crop in Jammu and Kashmir particularly in high altitude cold and cold arid areas of Jammu and Kashmir (J&K) where it serves as a staple food. Early blight, caused by *Alternaria solani* (Ellis and Martin), Jones and Grout, is a serious disease of potatoes that occurs in most potato growing regions world-wide (Christ 1990); Pelletier and Fry (1990); Shlienberg et al. (1990) Van der Walls et al. (2001) The disease often occurs initially on older, less productive foliage, followed by a gradual upward progression within the canopy, resulting in premature leaf senescence (Franc and Christ 2001, Rotem 1994). If the inoculum load is high during favourable environmental conditions, early blight may become severe enough to cause significant reductions in yield (Kapsa and Osowski 2003, Patel et al., 2004, Shlienberg et al., 1996, Teng and Bissonnette 1985, Van der Waals et al., 2001).

Heavy infection early in the growing season can cause yield losses of 20 - 50% (Denner and Theron, 1999). Yield loss estimates resulting from foliar damage incited by early blight on potato vary by location, cropping season, cultivar, and the stage of potato maturity. In general, yield reductions of 20-30% have been reported in USA (Christ and Maczuga, 1989, Shlienberg et al., 1990). Early blight may also cause dry rot of tubers, reducing both the quantity and quality of marketable tubers (Nnudu et al., 1982). Environmental factors such as temperature,
wetness duration and relative humidity (moisture) affect the development of early blight on potatoes (Adams and Stevenson, 1990, Vloutoglou and Kalogerakis, 2000). Temperature increases *A. solani* infection and sporulation (Vloutoglou and Kalogerakis, 2000). Water in the form of high relative humidity, rainfall or dew accumulation can increase conidia germination and pathogen infection (Rotem, 2004). Alternating low and high humidity conditions have also been shown to favour disease development (Van der Walls et al., 2001). Early blight is also enhanced through continuous potato production (Olanya et al., 2009). Symptoms are initially observed on older, senescing leaves (Rands, 1917, Pscheidt, 1985; Shuman, 1995). Characteristic symptoms are dark brown or black lesions with concentric rings on leaves, which produce a ‘target spot’ effect. Enlarging lesions are often surrounded by a narrow chlorotic halo. Lesions are similar on all hosts (Pscheidt, 1985).

*Alternaria solani* over winters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants of the same family (Pscheidt, 1985; Pelletier, 1988; Shuman, 1995). The primary inoculums, conidia, are produced in the spring, and are then splash or wind dispersed to the lower leaves of the plant. Spore germination is facilitated by free moisture, but can be induced by relative humidity close to saturation. Germ-tubes form appressoria, and penetrate the epidermis directly or through wounds or stomata. The minimum temperature for infection can be as low as 10°C, the maximum >35°C, and the optimum between 20°C and 30°C. Incubation periods vary greatly, depending on age and susceptibility of plants (Rotem, 1994). Sporulation occurs between 5°C and 30°C, with the optimum around 20°C (Pscheidt, 1985). *A. solani* is one of the few pathogens that is able to overcome a lack of prolonged humidity, by using several short wet periods (usually at night) interrupted by dry intervals during the day, otherwise known as interrupted wetting periods (Bashi and Rotem, 1975, Rotem et al., 1978). This adaptation allows *A. solani* to thrive equally well in areas with continuous humidity and in areas with alternating wet-dry conditions. Epidemics do not generally occur until late in the season, when the plants are most susceptible.

Under temperate Kashmir conditions, early blight of potato caused by *Alternaria solani* is posing a great threat for its cultivation. The systemic study on potato early blight has not been conducted so far under Kashmir conditions. Therefore keeping in view the devastating nature of disease a detailed investigation was undertaken to study the perpetuation of the causal organism.

1 Result and Analysis

The perpetuation of the fungus *Alternaria solani* was studied on diseased leaves by placing the leaves on ground surface and at 20 cm depth, as well as on diseased potatoes kept in ambient store during the year 2009 and 2010.

1.1 Survival on over-wintered diseased leaves

Diseased leaves in mesh wire bags were placed on ground surface as well as buried at 20 cm depth. Observations regarding the spore production and their viability were recorded at fortnightly intervals and are presented in Table 1, Table 2.

Perusal of data revealed that the spore production in over-wintered diseased leaves continued for comparatively longer period upto first fortnight of June in both the years (2009 and 2010) of experimentation, when kept at ground surface. On diseased leaves kept at 20 cm deep in soil, the spores were altogether absent throughout the observation period, because the leaves were decomposed. The average number of spores cm⁻² diseased leaf area increased up to first fortnight of June in the year 2009 and 2010, with maximum number of 294 and 323 spores, respectively. However, by the first fortnight of July, the number gradually declined to 160 and 220 spores, respectively.

The viability of spores exhibited a sharp decline with the increase in depth of placement. Highest spores viability was recorded in over-wintered diseased leaves at ground surface. The highest spore viability of 44.3 and 49.3 per cent in leaves on ground surface was recorded in the first fortnight of June, 2009 and 2010, respectively. However, in leaves buried at 20 cm depth the spores were altogether absent because the leaves were decomposed.
Table 1: Production and viability of *Alternaria solani* conidia on infected potato leaves kept on ground surface and at 20 cm depth in the soil during the year 2009.

| Month  | Fortnight | Number of conidia (cm² leaf area)* | Viability (%) |
|--------|-----------|-----------------------------------|---------------|
|        |           | Ground surface| 20 cm | Ground surface| 20 cm |
| March  | I         | 20         | -     | 27.0         | NA   |
|        | II        | 63         | -     | 29.4         | NA   |
| April  | I         | 86         | -     | 30.8         | NA   |
|        | II        | 123        | -     | 32.3         | NA   |
| May    | I         | 173        | -     | 37.0         | NA   |
|        | II        | 203        | -     | 40.6         | NA   |
| June   | I         | 294        | -     | 44.3         | NA   |
|        | II        | 263        | -     | 38.4         | NA   |
| July   | I         | 160        | -     | 31.7         | NA   |
|        | II        | -          | -     | -            | NA   |

Note: *Mean of three replications each comprising of 30 leaf discs of 1 cm² surface area
-= Material perished
NA= Spores not available

Table 2: Production and viability of *Alternaria solani* conidia on infected potato leaves kept on ground surface and at 20 cm depth in the soil during the year 2010.

| Month  | Fortnight | Number of conidia (cm² leaf area)* | Viability per cent |
|--------|-----------|-----------------------------------|--------------------|
|        |           | Ground surface| 20 cm | Ground surface| 20 cm |
| March  | I         | 42         | -     | 28.3         | NA   |
|        | II        | 83         | -     | 31.6         | NA   |
| April  | I         | 110        | -     | 32.0         | NA   |
|        | II        | 138        | -     | 34.4         | NA   |
| May    | I         | 206        | -     | 42.0         | NA   |
|        | II        | 240        | -     | 45.6         | NA   |
| June   | I         | 323        | -     | 49.3         | NA   |
|        | II        | 308        | -     | 40.5         | NA   |
| July   | I         | 220        | -     | 32.4         | NA   |
|        | II        | -          | -     | -            | NA   |

Note: *Mean of three replications each comprising of 30 leaf discs of 1 cm² surface area
-= Material perished
NA= Spores not available

1.2 Survival on diseased potatoes kept at ambient store

Perpetuation of pathogen *Alternaria solani* in diseased potatoes was studied by keeping them in ambient store and the production and viability of spores at fortnightly intervals commencing from the first fortnight of March was recorded. The data recorded is presented in Table 3, Table 4.

The perusal of data revealed that the diseased potatoes continuously produced spores during entire period of study in both the years (2009 and 2010). The average number of spores increased up to first fortnight of June, both in 2009 and 2010, with a maximum number of 430 and 508 spores, respectively. The number then gradually declined to 216 and 263 spores, respectively, till last observation recorded in the second fortnight of July.

Initial viability of spores from diseased potatoes in first week of March was 24.5 and 29.0 per cent during 2009 and 2010, respectively. The viability of spores gradually increased with time till first fortnight of June during the year 2009 and 2010, respectively, which then showed a gradual decrease to 23.5 and 27.6 per cent till last observation.
Table 3 Production and viability of *Alternaria solani* conidia on infected potato tubers kept in ambient store during the year 2009

| Month | Fortnight | Number of conidia (cm$^2$ slice area)* | Viability per cent |
|-------|-----------|-----------------------------------------|--------------------|
| March | I         | 30                                      | 24.5               |
|       | II        | 69                                      | 30.2               |
| April | I         | 130                                     | 33.6               |
|       | II        | 200                                     | 38.2               |
| May   | I         | 305                                     | 42.3               |
|       | II        | 363                                     | 49.7               |
| June  | I         | 430                                     | 52.5               |
|       | II        | 385                                     | 46.3               |
| July  | I         | 308                                     | 35.0               |
|       | II        | 216                                     | 23.5               |

Note: * Mean of three replications each comprising of 30 slice discs of 1 cm$^2$ surface area

Table 4 Production and viability of *Alternaria solani* conidia on infected potato tubers kept in ambient store during the year 2010

| Month | Fortnight | Number of conidia (cm$^2$ slice area)* | Viability per cent |
|-------|-----------|-----------------------------------------|--------------------|
| March | I         | 52                                      | 29.0               |
|       | II        | 91                                      | 32.5               |
| April | I         | 141                                     | 37.3               |
|       | II        | 225                                     | 42.5               |
| May   | I         | 313                                     | 45.6               |
|       | II        | 456                                     | 50.5               |
| June  | I         | 508                                     | 55.7               |
|       | II        | 436                                     | 51.3               |
| July  | I         | 370                                     | 40.2               |
|       | II        | 263                                     | 27.6               |

Note: Mean of three replications each comprising of 30 slice discs of 1 cm$^2$ surface area

2 Discussion

Perpetuation of pathogen from one season to the next in the absence of a living host is pre-requisite for successful establishment of any plant disease. Present studies conducted on the perpetuation of the pathogen revealed that *A. solani* overwintered on diseased leaves left on the ground surface and on diseased potatoes in the form of conidia and mycelium throughout the winter. These findings are supported by Rotem (1968), who reported that *A. solani* overwinters and survives as conidia and mycelia on buried host debris and potato tubers, particularly in fields with poor cultural practices such as continuous cropping of tomatoes or potatoes. Rands (1917) reported that conidia of *A. solani* are capable of surviving freezing weather on the soil surface.

Further, diseased leaves left on the ground surface were observed to be the most important source of primary infection of early blight of potato. These findings are supported by Manzer and Merriam (1974), who also reported that the first infections of the new crop are produced from overwintering inoculums. However, in our study the leaves buried in soil at 20 cm depth, the spores were altogether absent throughout the observation period during both the years of experimentation. The leaves buried at 20 cm depth, decomposed earlier than those at ground surface. This could be attributed to greater aerobic respiration, which favoured quick decomposition of leaves. The proportion of spores and their viability decreased with the increase in depth of placement in soil. Rotem (1968, 1990) reported that oversummering of *A. solani* in potato and tomato debris and overwintering of *A. macrospora* in cotton were much more successful (lasting up to 8 months) in debris deposited on the soil surface than in debris buried in soil. These findings were also supported by Pandotra (1965) who reported that in Punjab, *Alternaria* survived 8 months in debris left on the soil surface but only 2 months in debris buried in the soil.
The differences between survival on the soil surface and survival under the soil surface derive from differences in environmental and biotic conditions in the two habitats. In particular, the soil surface is drier than the soil below and less microbial activity occurs at the soil surface. These and other effects were studied in the over summering of A. solani in different plot covering the site of a diseased winter tomato field in the rainless Negev Desert (Rotem, 1968).

Perpetuation of pathogen (A. solani) in diseased potatoes kept in ambient store revealed that the diseased potatoes continuously produced spores during entire period of study in both the years (2009 and 2010). The average number of spores increased up to first fortnight of June both in 2009 and 2010, with a maximum number of 430 and 508 spores, respectively. The number then declined to 216 and 263 spores respectively, till last observation recorded in the second fortnight of July. Similar observations were reported by Vijaya Kumar and Rao (1979) who reported that the longest periods of survival of A. triticina in debris and in wheat seeds were 4 and 10 months, respectively.

*Alternaria brassicae* is known to cause seed infection and the infected seeds have already been shown to act as main source of recurrence of the disease in the field (Shrestha et al., 2000). Besides, A. brassicae was found to survive for 8 months in the seeds of rapeseed stored in local containers in the farmhouse at Nawalpur (Shrestha and Chaudhary 1999). At room temperatures (11-25°C) the fungus survived in the seeds for more than 6 months (Shrestha et al., 2003). Robert and Boothroyd (1972) showed that A. solani, the pathogen causing early blight of tomato and potato survived as long as 18 months in dry diseased leaves. A. solani inoculum persisted in field for several months (Basu, 1971). *Alternaria solani* over wintered as conidia chlamydospores and mycelium on plant debris and in the soil (Dorozhkin and Ivanyuk 1979). Mahabaleswarappa (1981) recorded that A. carthami Chowdhury survived as conidia for about four months and as mycelium for five months, when diseased leaves were kept between folds of blotting papers. According to Kvasnyuk (1986) conidia of A. solani remained viable on over wintering potato litters every year conidia were killed by wet warm weather in autumn and spring or by frequent thaws in winter followed by sharp drop in temperature. Patterson (1991) reported that chlamydospores persisted in soil for 12 months and played a role as primary inoculum for A. solani chlamydospores placed at depth of 4.8 and 12 cm in soil initiated infection and collar rot on tomato. Islam et al (1976), reported that, under natural conditions the important source of inoculum was diseased plant debris. They also reported that, conidia did not retain viability in winter but the mycelium survived to produce conidia. Bhaskaran and Kandaswamy (1980) reported that the fungus A. helianthi remained viable in plant debris for 22 weeks, which was left on soil surface continuously dry. A. helianthi was reisolated from 12 month old sunflower crop debris that over wintered in the field which showed its ability to survive during off season Lipps and Herr (1981); Shane et al., (1981); Herr and Lipps (1982); Allen et al., (1983); Jeffrey et al., (1984); Nagaraju et al., (1995) and Appaji (1995). A. tenuissima can survive under various environmental conditions and must have developed sophisticated mechanism to adapt itself to different environmental niches (Wan et al., 2008). Therefore it has a wide range of hosts (Feng et al., 2007). The humid climate of some areas in Saudi Arabia is seemed to be more favorable for infection by A. tenuissima of several crop plants. Taken et al (1994), studied the survival of E. turcicum from infested maize residue in Uganda and reported that local epidemics of northern leaf blight caused by E. turcicum usually originated from conidia on infested maize residues. The percentage leaf area blighted and area under disease progress curve were significantly higher in residue infested plots than in residue free plots.

The production of viable spores in over wintered diseased leaves and potatoes increased from the month of April to June. This suggests that in spring under favourable weather conditions, lesions on over wintered leaves and potatoes kept in ambient store resume spore formation and the inoculum may build up to levels sufficient to initiate primary infection. The environmental conditions in the early summer thus determine the extent of epidemic development. The information generated about the possible means of perpetuation of the fungus during winter and production of conidia as a source of primary inoculum during the early spring highlights the importance of removal of plant debris as one of the strategies for disease management.
3 Materials and Methods

3.1 Survival of over-wintered diseased leaves and tubers

The survival of pathogen in fallen diseased leaves and tubers of susceptible potato cultivar “Kufri Jyoti” was studied during the year 2009 and 2010 at Shalimar campus of SKUAST-K.

3.2 Survival in over-wintered diseased leaves

Diseased leaves of potato were collected on 15th of August in the year 2009 and 2010. The leaves were kept in mesh wire bags, each bag containing equal number of leaves and divided into two sets. One set of such leaves were kept on soil surface (Figure 1a) and the other at 20 cm depth (Figure 1b) in the field. The diseased leaves, in the mesh wire bags were removed at fortnightly intervals beginning from first week of March onwards. Thirty leaf bits of 1 cm² area were randomly taken from one randomly selected bag and examined for the presence of conidia. These leaf discs were then crushed separately in 40 ml of sterilized distilled water and strained through a double layer cheese cloth. Twenty milliliter of the filtrate was centrifuged at 6000 rpm for 15 minutes. After centrifugation, 18 ml of the suspension was drawn off with a pipette. The pipette was re-suspended in 2 ml sterilized water and the numbers of conidia were counted with the help of haemocytometer. To estimate the percent viability of conidia in over-wintered diseased leaves, spore germination method was used. Two drops of 50 µl from each processed sample were placed on a glass slide and incubated in a moist chamber at 23±2°C. The number of spores observed and the number of spores that germinated were recorded after 24 hour incubation for calculating the percent viability of conidia.

3.3 Survival on tubers

The tubers collected in autumn at the time of harvest from the diseased crop were kept in ambient store and were periodically assessed for the presence/viability of fungal propagules. Ten randomly selected tubers were taken, ground/crushed separately in 80 ml of sterilized distilled water and strained through a double layered cheese cloth. The rest of the procedure was same as that of leaf sample. The percent viability of spores was estimated as per the method describe in above mentioned leaf samples.

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