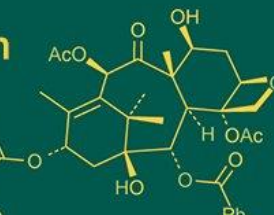


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## Status of early blight of tomato around East Nimar, cultural and morphological variability of the pathogen in Khandwa district

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### Abstract

Tomato (*Lycopersicon esculentum*) belongs to the Solanaceae family is one of the most remunerable and widely grown vegetables in the world. The early blight of tomato incited by *Alternaria solani* (Ellis and Martin) is one of the serious diseases in Tomato growing areas of the country. A roving survey was carried out during 2024-25 for the disease severity of early blight of tomato in major growing areas of Khandwa district. Maximum disease intensity of early blight of tomato observed in the block Punasa (61.11%) followed by Pandhana, Khalwa, and new Harsud (55.10%, 41.23%, and 35.24% respectively) whereas minimum disease intensity was observed in block Khandwa (32.55%). The isolates were divided in two color categories viz., Creamy White and white. Category-I, is Creamy White color of colony and the growth pattern were varied from Cottony cultural growth to Fluffy Cottony growth. The maximum average conidial length X Width of *Alternaria solani* isolates was in SAS-V (54.15× 13.68 µm), followed by SAS-II (47.83× 09.57 µm), and SAS-IV (41.24× 13.71 µm) while minimum average size of conidia length X Width was observed in SAS-I (37.50× 08.75 µm). The maximum number of horizontal septation found in SAS-V ranges 3-7 and the minimum horizontal septation was (2-4) septa observed.

**Keywords:** Early blight, cultural, morphological, pathogen, *Alternaria solani*

### Introduction

It is the second most consumed vegetable after potato. It is grown for its edible fruits, which can be consumed either fresh or in processed form and is a very good source of vitamins (A, B and C) and minerals. In India, the production and productivity of the crop is very low which mostly attributes to diseases and pest of crop. Among the major diseases of tomato early blight caused by *Alternaria solani* is one of the important foliage and fruit disease causing yield losses up to 79 percent over the World". The disease produces a wide range of symptoms and manifestations on foliage named as early blight and the symptoms on stems, seedlings and fruits are termed as stem lesions, collar rot and fruit rot, respectively. Initial symptoms of disease appeared as small, dark necrotic lesions on the older leaves which subsequently spread upward as the plants become older. In later stages, the lesions enlarge and they generally resulted in concentric rings giving a target board like appearance which are often surrounded by a yellowing zone. *Alternaria* can also live as an endophyte inside plants without causing disease (DeMers, 2022) [4]. The early blight/brown spot disease complex produces symptoms are included foliar lesions that first present as brown spots with characteristic concentric rings depending on the species, which eventually lead to severe defoliation and considerable yield loss (Schmey *et al.*, 2024) [10]. In a global study of soil borne fungi, *Alternaria* was the most abundant plant pathogen and it is also reported in a field warming experiment showed that the abundance of the genus *Alternaria* increases with warming and its importance grows under climate-change scenarios (Delgado Baquerizo *et al.*, 2020) [3]. Disease affects crop production as they cause premature defoliation and result in heavy losses in production by reducing quality and quantity of fruit. Crowded plantation, high rainfall and extended period of leaf wetness are responsible factors to induce disease development (Gondal *et al.*, 2012) [5]. The early blight of tomato is very difficult to manage in its advance stages and causes reduction in yield. Yield losses due to the disease were reported up to 79% from various countries like Canada, India, USA, and Nigeria (Sherf and

MacNab 1986; Gwary and Nahunnaro 1998)<sup>[13, 6]</sup>.

### Materials and Methods

A roving survey was carried out during 2024-25 to now the disease severity of early blight of tomato in major growing areas of Khandwa district to assess the disease intensity of early blight in farmers' fields. PDI recorded at farmer's fields of each Block from the district of Khandwa.

Fields were selected randomly in a village on the survey route. In each field, plants were selected at random and the severity of the disease was recorded.

The percent disease incidence was assessed by counting the number of plants showing *Alternaria solani*. The incidence and severity of early blight of tomato was recorded by using 0-4 scale (Sheo Raj 1988)<sup>[12]</sup>.

### Intensity of disease was calculated by the formula

$$\text{Percent disease intensity (\%)} = \frac{\text{Sum of all disease rating scale}}{\text{Total no. of rating} \times \text{Maximum disease rating}} \times 100$$

### Single spore isolation

The clear filtered of two percent water agar was poured into sterile petri plates and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from 15 days old culture. One milliliter of such suspension was spread uniformly on agar plate. These plates were incubated at 24±1 °C for 12 hrs. Then such plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the plates. The growing hyphal tip portion was transferred to PDA slants with the help of corkborer under aseptic conditions and incubated at 24±1 °C. These culture tubes were used for further studies.

### Proving the pathogenicity

Surface sterilized with 0.1 percent Sodium hypochlorite and sown in earthen pots containing sterilized soil. They were allowed to grow for a month. Prior to inoculation, the plants were exposed to 95 percent humidity for 24 hours. Thereafter, they were inoculated with spore suspension of ( $5.4 \times 10^6$  spores/ml) the fungus, by using atomizer. After inoculation, the plants were exposed in the same conditions for 24 hours. Suitable control plants were maintained by spraying of sterile distilled water. After the symptom expression, the organism was re-isolated from these artificially infected leaves and the culture obtained was compared with the original culture for confirmation.

### Maintenance of the culture

The fungus was sub cultured on Potato Dextrose Agar (PDA) slants and allowed to grow at 27±1 °C for 15 days. These slants were then preserved in the refrigerator at 54 °C and sub-cultured once in two months. This pure culture was used for further studies.

### Potato dextrose agar (PDA)

In most of the experimental studies, the potato dextrose agar (PDA) was used. The composition of PDA is as follows. Potato peeled 200 g Dextrose 20 g Agar-agar 20 g Distilled

water 1000 ml (volume to make up) 21 Two hundred gram of peeled potatoes were cut into small bits and boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose 20 g and agar 20 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized as described earlier and preserved for further use.

### Results and Discussion

The symptomatology of the disease was studied on five randomly selected plants of susceptible cultivars the selected plants were marked for continuous monitoring of disease development. Plants were kept unsprayed throughout the growing seasons to study the symptoms of early blight leaf spot under natural epiphytotic conditions. First observation was taken as soon as the disease appeared. Periodic observations were recorded besides size, shape, coalescing and color of the lesion on leaves. Characteristic dark, target-like lesions with concentric rings on leaves and stems, and brown, sunken spots with raised borders on fruits. Brown spots with concentric rings bull's eye pattern with yellow margin Brown concentric rings of fruit, which was in accordance to the present worker (Yuldosheva *et al.*, 2025)<sup>[14]</sup> Early blight affects the leaves, stems, and tubers. The symptoms on leaves usually appear 5-20 days before flowering as brown or dark brown spots, often surrounded by concentric rings.

### Pathogenicity

Pathogenicity test of (*A. solani*) pathogen were conducted on tomato in field. The major symptoms were noted as typical disease, bulls eye patterns, stem cankers, and fruit rot and after drying of plant was observed. The *A. solani* was successfully isolated from the diseased samples all the collected isolates of the pathogen were found pathogenic in shifting grade with spot blight symptoms after 6-7 days of inoculation later small blighting, light brown to blackish in color occurred on the whole leaf and canker in stem. The spots later converted in to typical circular spot or irregular spots and lesions. Spore was visible on microscopic observation of *A. solani*. The leaves were inoculated actively growing mycelial block of test fungus and were incubated for 6-7 days. In control plates 5 mm agar blocks were inoculated on the leaves. The fungus was tested to find out their pathogenic ability.

### Survey of disease intensity (*A. solani*)

Survey on farmer's field of tomato crop was conducted in five blocks of Khandwa district in 5 locations to trace disease intensity of Early blight of Tomato during 2024-25. The data depicted in Table-1 and Fig-2 revealed that *Alternaria solani* early blight of tomato be of widespread disease and intensity varying between 32 percent to 61 percent at different crop stages in almost all the fields. Among the blocks, maximum disease intensity of early blight of tomato observed in the block Punasa (61.11%) followed by Pandhana, Khalwa, and new Harsud (55.10%, 41.23%, and 35.24% respectively) whereas minimum disease intensity was observed in block Khandwa (32.55%). The variation of disease intensity is given in table-1

**Table 1:** Early blight of intensity in Tomato fields in different blocks of Khandwa district.

| Blocks     | Villages  | Disease severity |
|------------|-----------|------------------|
| Khandwa    | Gondwadi  | 32.55            |
| Khalwa     | Khedi     | 41.23            |
| New Harsud | Navalpura | 35.24            |
| Pandhana   | Borgaon   | 55.10            |
| Punasa     | Sulgaon   | 61.11            |
| Mean D.I.  |           | 45.04            |
| Range      |           | 32.55-61.11      |

Out of 5 locations of Khandwa, highest percentage of disease severity outbreak *Alternaria solani* was observed in Sulgaon in block Punasa (61.11%), followed by Borgaon in block Pandhana (55.10%), Khedi in block Khalwa (41.23%), and Navalpura in block New Harsud (35.24%). Although in minimum disease intensity was observed in Gondwadi block Khandwa (32.55%) recorded intensity respectively. The mean intensity was calculated 45.04 percent and range is 32.55-61.11%. similar with the findings of (Bhokse *et al.*, 2025)<sup>[2]</sup> he described that the disease was appeared in all the major potato crop growing areas under Western Maharashtra surveyed during the 2022-23 and 2023-24, and appeared in moderate form. The maximum intensity (42.98%) was described in Peth village, Ambegaon tehsil, while the minimum intensity (18.43%) was observed in Pait village, Rajgurunagar tehsil, Pune. The survey also denoted that disease intensity varied, with fields practicing good agricultural practices and continuous fungicide use showing lower disease intensity. Similarly the findings of Wongamthing (2023)<sup>[15]</sup> was in accordance to the present findings he conducted survey in Thrissur and Palakkad district of Kerala state during December 2021 record the disease severity on foliage varied from 23 to 78% at various locations.

**Table 2:** Variability of Cultural of *Alternaria solani* isolates from blocks of Khandwa district.

| S.N.       | Region (Blocks) | Isolate Code | Growth of Mycelium (in mm) * | Cultural pattern | Color of colony | Pigmentation  |
|------------|-----------------|--------------|------------------------------|------------------|-----------------|---------------|
| 1.         | Khandwa         | SAS-I        | 36.85                        | Cottony          | Creamy White    | Reddish brown |
| 2.         | Khalwa          | SAS-II       | 42.11                        | Fluffy cottony   | Creamy white    | Pale yellow   |
| 3.         | New Harsud      | SAS-III      | 53.12                        | Cottony          | White           | brown yellow  |
| 4.         | Pandhana        | SAS-IV       | 45.22                        | Cottony          | Creamy white    | Reddish brown |
| 5.         | Punasa          | SAS-V        | 48.11                        | Fluffy cottony   | White           | Light yellow  |
| SEm(±)     |                 |              | 1.20                         |                  |                 |               |
| C.D. at 5% |                 |              | 3.11                         |                  |                 |               |

\* Mean of three replications

### Pigmentation of colony

There are four categories in pigmentation of *Alternaria solani*, it ranges from Reddish Brown to Light yellow Table-2. Different isolates are having variations on the basis of Pigmentation. Isolates SAS-I and SAS-IV releases the pigmentation of Category-I Reddish brown. Category-II Pale yellow belongs to the isolate SAS-II, Category-III isolate SAS-III releases brown yellow and Category-IV Light yellow belongs to isolate SAS-V.

In the colony characteristics of 5 isolates were expressed as cottony and fluffy cottony. Cultural variability was studied among 5 isolates of the isolated pathogen (*A. solani*) from samples collected in different blocks of Khandwa District of Madhya Pradesh, growing on PDA media by. The isolates of *A. solani* were classified into two categories on the basis of Cultural pattern. Cottony pattern consists three isolates (SAS-I, SAS-III, SAS-IV, Fluffy cottony pattern consists

### The Morphological and Cultural characters of the pathogen.

*Alternaria solani* is obliging for early blight of tomato, which are highly uncertain pathogen. Cultural characteristics of *Alternaria solani* were studied on the Potato Dextrose Agar media which were discussed in previous.

Different cultural and morphological characters studied like color of colony are as creamy white and white, cultural pattern are cottony to fluffy cottony and the pigmentation of colony are reddish brown, pale yellow, and brown yellow to light yellow where observed. In the morphological characters, conidia length width and septation were observed while studying cultural and morphological variability. Growth of mycelium is measured in mm and various observations and its result were concocted in Table-2 respectively.

### Cultural pattern of *Alternaria solani*

The basis of cultural pattern, two patterns were found and each pattern belongs to different isolates Table-2. SAS-I, SAS-III and SAS-IV belongs to pattern-one, which has Cottony type cultural pattern. SAS-II, SAS-V belongs to pattern-II, which has Fluffy Cottony type cultural pattern. The maximum radial growth 53.12 mm in isolate SAS-III procure from new Harsud followed by Punasa SAS-V 48.11mm, Pandhana SAS-IV 45.22 mm and the minimum radial growth 36.85 mm was recorded in isolate-SAS-I.

### Color of colony

The five isolates those discussed in Table-2 which is known by Color of colony and divided in two color categories viz., Creamy White and white colony color. Category-I, is Creamy White color of colony in the isolates SAS-I, SAS-II, and SAS-IV and Category-II, is white consist of isolate SAS-III and SAS-V.

only two isolates SAS-II and SAS-V. sequently, isolates are classified on the basis of Colour of colony. Creamy White colour colony observed in isolates SAS-I, SAS-II and SAS-IV white colour colony observed in two isolates SAS-III, SAS-V. Next, was classified on the basis of Pigmentation. Reddish brown colony were found in two isolates SAS-I, SAS-IV. Light yellow colony was found in one isolate SAS-V. brown yellow colony was one isolate SAS-III one isolate in colony was pale yellow and the least size of colony varied from 36.85-53.12 mm, where highest mycelial growth is observed in SAS-III (53.12 mm) and least mycelial growth is measured in SAS-I (36.85 mm). The findings of Sharma *et al.*, 2022<sup>[11]</sup> In a case of study of Seven days old culture of isolate inoculated (5 mm diameter disc) on PDA and incubated at 25±1 °C. The radial growth of fungal mycelium, colony characters of each isolate, such as colony growth and colony colour observations are in accordance to

the present findings. Similar findings were described by Kumar *et al.* (2022) <sup>[7]</sup> that the isolates of the *A. solani* from Kanpur showed Light green colony colour, hyphae and septate conidia. The Faizabad isolate showed light green to green colony colour and conidia muriform. The Varanasi isolates found dark green colony colour, ovoid to obclavate conidia. These findings are in accordance with present investigations. These differences are showing due to the variations in the climatic conditions.

The current findings are in accordance to the findings of Rahmatzai *et.al.* (2016) <sup>[9]</sup> who described cultural characters of isolates of *A. solani* were collected in 2015 from different tomato growing fields of Hada al sham region of Jeddah, Saudi Arabia. Observed cultural and morphological characteristics of the test isolates of *A. solani*. The isolate was produced cottony growth which is dark, ranging from grey to black with tints of brown or olive. Colonies are spreading hairy and grey brown to black; the whole texture of the fungus was similar to cotton.

### Morphological Variability *Alternaria solani*

After the survey of different blocks of East Nimar region, Khandwa district of Madhya Pradesh, collected several samples and isolated organism from 5 Samples of Khandwa district. Separated pathogen from these isolates and made it

grow in the laboratory to study morphological characters. The length, width of the conidia and their septation were observed.

### Conidia (µm) of Size (Length and Width)

The conidia of the pathogen were observed and found elliptical with ends and most of these conidia were typically club-shaped to pear in shape. The average size of conidia ranges from 40.29× 9.81 µm to 54.15× 13.68 µm. The data revealed in Table-3 that the maximum size (Length and Width) of conidia are different in each isolate, with the highest length SAS-V (41.08-67.22 µm) with width 09.11-18.25 µm followed by SAS-III length (28.17-52.42 µm) with width 06.10-13.52 µm and SAS-I (29.8-45.21 µm) length with width 07-10.5 µm while minimum size of conidia was observed in SAS-III ((28.17-52.42 µm). It is revealed that horizontal septation of isolates varies in each of the isolates and it ranges between 2 to 7. The maximum number of horizontal septation found in SAS-V ranges 3-7 followed by SAS-I and SAS-IV (2-4). The minimum horizontal septation was (2-4) septa. Highest number of vertical septation was found in SAS-IV and SAS-V (1-3 septation) and the minimum vertical septation was recorded in isolate SAS-I and SAS-II (0-2 septation).

**Table 3:** Morphology of conidia among Isolates of *Alternaria solani* around Khandwa district.

| S. No. | Isolates Code | Conidial Length Range (µm) | Conidial Width Range (µm) | Conidial Length Avg. (µm) | Conidial Width Avg. (µm) | No. of Septa horizontal | No. of Septa vertical |
|--------|---------------|----------------------------|---------------------------|---------------------------|--------------------------|-------------------------|-----------------------|
| 1.     | SAS-I         | 29.8-45.21                 | 07.00-10.5                | 37.50                     | 08.75                    | 2-4                     | 0-2                   |
| 2.     | SAS-II        | 32.12-63.55                | 05.00-14.15               | 47.83                     | 09.57                    | 3-5                     | 0-2                   |
| 3.     | SAS-III       | 28.17-52.42                | 06.10-13.52               | 40.29                     | 09.81                    | 2-6                     | 1-2                   |
| 4.     | SAS-IV        | 35.07-47.41                | 07.11-20.32               | 41.24                     | 13.71                    | 2-4                     | 1-3                   |
| 5.     | SAS-V         | 41.08-67.22                | 09.11-18.25               | 54.15                     | 13.68                    | 3-7                     | 1-3                   |

\* Means three replications

Among the isolates of *A. solani* pathogen collected from different locations of Khandwa district. The conidia of the pathogen were observed and found elliptical with ends and most of these conidia were typically club-shaped to pear in shape. The average size of conidia ranges from 37.50× 8.75 µm to 54.15× 13.68 µm. The data revealed that the maximum size (Length and Width) of conidia is different in each isolate, with the highest length SAS-V (41.08-67.22 µm) with width 09.11-18.25 µm while minimum size of conidia was observed in SAS-III ((28.17-52.42 µm). The maximum number of horizontal septation found in SAS-V ranges 3-7 and the minimum horizontal septation was (2-4) septa. The present findings are in accordance to (Bharath *et al.*, 2024) <sup>[1]</sup> who described under the microscope, morphological features like dark brown to blackish mycelium with prominent septations and muriform conidia with transverse septations were observed. The similar findings are also recorded by Khalaf M. Alhussan (2012) <sup>[8]</sup> in their experiment they identified *A. solani* on the bases of morphological characteristics. The conidia were observed muriform or ellipsoidal, solitary straight and slightly oblong with tapering to beak and pale or olivaceous brown. The length of conidia 35-75 µm and 10-20 µm width with 2-7 transverse and 1-4 longitudinal septa in potato dextrose agar media supporting good fungal growth. Similarly, the findings of Rahmatzai *et.al.* (2016) <sup>[9]</sup> are in accordance to the present findings, he described that Tomato leaves showing typical early blight symptoms were collected in

2015 from different tomato growing fields of Hada al sham region of Jeddah, Saudi Arabia. Observed cultural and morphological characteristics of the test isolates of *A. solani* showed high variability on their mycelial width, conidial size, beak length and septations. The conidiophores of the fungus were formed singly or in groups, strait or flexuous brown to olivaceous and solitary, muriform and ellipsoidal tapering to a beak and pale or olivaceous brown. The average conidial size (L × W) was 25-44 × 7-15 µm and comparatively lowest 20-30 × 5-13 µm. The conidial vertical septa and horizontal septations were maximum 2-11 and 1-4 in the while it was (2-8 and 1-3). The maximum length of beak was (6-10 µm) and the minimum was 4-8 µm.

### Conclusions

All blocks of East Nimar region Khandwa district of Madhya Pradesh were surveyed in 2024-25, in which disease intensity (severity) percent were ranges between 32.55-61.11 percent. The highest Disease intensity was seen in Punasa block, while the lowest Disease intensity was seen in Khandwa block of Khandwa district. Radial growth of isolate culture was highest in SAS-III followed by SAS-V and SAS-IV, whereas lowest cultural radial growth noticed in SAS-I followed by SAS-II.

The growth patterns of Isolates of *Alternaria solani* around Khandwa district varied between cottony cultural pattern to concentric cottony cultural pattern and the colony color of

isolates were varied between Creamy White to white color. The pigmentation was varied among the reddish-brown color, pale color, brown yellow and light-yellow color pigment. The average size of conidia ranges from  $37.50 \times 8.75 \mu\text{m}$  to  $54.15 \times 13.68 \mu\text{m}$ . The maximum size (Length and Width) of conidia is different in each isolate, with the highest length SAS-V ( $41.08\text{--}67.22 \mu\text{m}$ ) with width  $09.11\text{--}18.25 \mu\text{m}$  while minimum size of conidia was observed in SAS-III ( $28.17\text{--}52.42 \mu\text{m}$ ). The maximum number of horizontal septation found in SAS-V ranges 3-7 and the minimum horizontal septation was (2-4) septa.

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