Original Research Article

Morphological and Pathogenic Variability in *Alternariasolani* Causing Early Blight Disease in Tomato (*Lycopersiconesculentum*Mill.) in Chhattisgarh, India

Abstract

Seven isolates of *Alternariasolani* were collected from different district of Chhattisgarh and studied on morphological and pathogenic variations among the isolates of *Alternariasolani*. Longest conidia with beak (39.17–74.35µm)were observed in isolate AS-6, while smallest conidia (28.12–58.35µm) were recorded in isolate AS-3. However, width of conidia was maximum (8.82–24.23µm) in isolate AS-3 and thin conidia (8.13 – 19.48µm width) in isolate AS-7. Maximum beak length (7.64–14.78µm) was observed in isolate AS-4 and minimum beak length was recorded in isolate AS-3 (4.18–7.62µm). Highest vertical septa 2–11 was recorded in isolate AS-7 followed by AS-2. Whereas, maximum horizontal septa were recorded in isolate AS-6 followed by isolate AS-3 and AS-4. Pathogenic variability test was performed to analyze the virulence of different isolates of *Alternariasolani* on Pusa Ruby variety of tomato under pot culture. Most virulent isolate was found AS-6 which exhibited maximum PDI (88.89%) and AUDPC (2302.22) followed by AS-5 and AS-7, while AS-1 was found least virulent which showed minimum PDI (40.00%) and AUDPC (995.56).

Keywords: AUDPC,Conidia,Isolates,Disease Intensity,Morphological Variability, Pathogenic Variability

Introduction

Early blight of tomato is a threat to the profitable cultivation of tomato. The disease causes reduction in quantity and quality of tomato fruits drastically. Early blight is one of the most important foliar disease of tomato which causes about 80 – 86 percent reduction in fruit yield (Pandey and Pandey, 2003). It is caused by *Alternariasolani*(Ellis and Martin) Jones and Grout. According to morphological characters and physiologic analysis of *A. sol* ed of

Comment [hs1]: Try not to repeat the words.

septate, branched, light brown hyphae, which turned darker with age (Simmons, 2000). *Alternariasolani*produceuniformed, beaked, single separate conidia on simple conidiophores. Like other members of the genus *Alternaria*, *A. solani*has transverse and longitudinal septate conidia, multinucleate cells and dark-coloured (melanized) cells (Arunakumara, 2006). Conidia contained 5–10 transverse septa and 1–5 longitudinal septa. The conidia of *Alternariasolani* were observed 35–75 µm in length and 10–20 µm in width and 2–7 transverse septa and 1–4 longitudinal septa (Singh, 1987). Its multicellular pigmented spores are produced in chains or in branching fashions. The spores are broadest near the base and taper gradually to an elongate beak under natural epiphytotic condition.

The variability is a well-known phenomenon in genus *Alternariasolani*and may be noticed as changes in conidia shape and size, growth and sporulation, pathogenicity etc. In *A. solani*, there is a variation between the populations from different areas have been reported by several workers (Ahmad, 2002; Naiket al., 2010). *Alternariasolani* found to be a highly variable pathogen (Castro et al., 2000; Pryor and Michailidesz, 2002). Moreover, it is well known that the variation in populations of plant pathogens direct affects disease control, especially when the method related to the development of resistant cultivars and fungicide usage. Analysis of morphological and pathogenic variation within pathogenpopulations is helpful in understanding host-pathogen interaction,epidemiology and developing strategies forresistance against pathogen (Leung et al.,1993). Besides, these there are no much information available on variability of *Altertnariasolani*in different locality of Chhattisgarh. Therefore, present investigation was carried out on 'morphological and pathogenic variability of *Alternariasolani* causing early blight in Tomato (*Lycopersiconesculentum*Mill.) in Chhattisgarh (India)'.

Comment [hs2]: Please, be aware of grammatical mistakes.

Comment [hs3]: Not clear what is the author meaning!!!

Materials and methods

Sample collection:

An intensive roving survey was conducted during *Rabi* 2016-17and 2017-18 in seven districts of Chhattisgarh *viz.*Kabirdham, Durg, Bemetara, Rajnandgaon, Bilaspur, Raipur and Mungelito know the diversification in *Alternariasolani* on tomato. One sample of diseased leaves showing typical early blight symptoms were collected from growing tomato plants from each district. Diseased sample collected from various places were designated as follows:

Location	Designation
Village -Arasnara, District - Durg	**AS**-1
Village -Pipariya, District - Bemetara	**AS**-2
Village -Saleghori, District - Mungeli	**AS**-3
Village –Sakari, District - Bilaspur	**AS**-4
Village -Kalegondi, District - Rajnandgaon	**AS**-5
Village -Khadhauda, District - Kabirdham	**AS**-6
Village – Horticulture Farm, IGKV, Raipur	**AS**-7

Isolation of *Alternariasolani*

Standard tissue isolation technique was followed to obtain *A. solani*culture described by Naik*et al*. (2010). The leaves were microscopically examined to confirm the presence of the fungus. After confirming for the presence of fungal spores, isolation was done by following standard tissue isolation method. The infected leaves were brought to laboratory and cut into small bits measuring about 2mm. Surface of spaceman was sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 1 minute and wash thrice with sterile distilled water. Then one piece of specimen was transferred on Potato Dextrose Agar (PDA) medium in the center of Petri dish. These petri dishes ware incubated under B.O.D. incubator at 25±2°C. Pure culture of the *A. solani*was obtained by hyphal tip culture and single spore culture technique.

Purification of*Alternariasolani*

The culture was purified by single spore technique described by Johnson and Booth (1983). A single block of 5 mm diameter was cut out from the fungal colony near the margin with the help of sterilized cork borer and transferred to 5 ml sterile distilled water in a test tube. It was mixed thoroughly to make a uniform spore suspension. One milliliter of spore suspension was spread on the surface of plain agar medium in Petri plates and incubated at 25±2°C for 24 h. The plates were observed for germinating spores under

stereoscopic microscopic and finally germinating spores were lifted by inoculation needle and transferred aseptically to potato dextrose agar slants for further growth. The pure cultures obtained were maintained by repeated sub-culturing at an interval of 30d for further studies. The stock cultures of all isolates grown on PDA slants stored at 5°C in refrigerator.

Identification of the pathogen

Spores of *Alternariasolani*were taken from the pure culture and mounted on the clear glass slide. Spores were mixed with thoroughly with lactophenol in order to obtain a uniform spread over slide and covered with cover slip. The spores and hyphae of the fungus were observed under compound microscope. *Alternariasolani* was identified following the cultural and morphobiometric characteristics criteria as per described by Ellis (1971).

Morphological variability

The morphological characteristics of respective isolate of *A. solani*including was studies on PDA medium. *Alternariasolani*was grown on PDA medium. Observations were also recorded on length of beak, conidia size (length and width) and septation in conidia (vertical and horizontal) from 10d old culture of each isolate. Seven slides from edge of active pure culture of each isolate were prepared using of light microscope, all the observed fungal feature of *Alternariasolani*were recorded.Conidia size was measured with the help of Ocular and stage micrometer using 20× power objective of light microscope. Numbers of spore were also counted per microscopic field with the help of compound microscope in which single block of 5 mm diameter disc was cut out from the 10 days old fungus colony near the margin by sterilized cork borer and was transferred to 5 ml sterile distilled water in a test tube. Later it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on a slide and average spore count of three microscopic fields under low power (10×) objective of the microscope. Sporulation was categorized as below:

No. of spore per microscopic field	Designation
0	- (nil)

1-10	+ (poor)
11-20	++ (moderate)
21-30	+++ (good)
31 and above	++++ (excellent)

Pathogenic variability

In order to confirm the identification of early blight disease and its causal agent, the pathogenicity test was carried out under pot culture. Four wk old seedlings of Pusa Ruby varietyof tomato were transplanted into the pot. Three replications were maintained for each isolate and nine seedlings of tomato were growing in each replicate. Pathogenic variability of seven isolates of *A. solani*was studied by applying detached leaf technique. The spore suspension (3×10^6spore/ml) of each isolate was separately prepared from 10 days old pure culture of the representative isolates. Healthy growing nine tomato plants were inoculated at room temperature by spraying of spore suspension of each isolate. The spore suspension was sprayed on tomato plants 20 days after transplantation of seedlings, and the inoculated tomato plants were covered by polythene bags for about 30 h to retain optimum humidity. The observed symptoms on plants for each isolate of the fungus were compared to the original symptoms of early blight. Observations on first appearance of symptoms, disease severity and typical symptoms were recorded. The pathogen was re-isolated from artificially inoculated tomato leaves and the morphological and cultural characteristics were compared with original pathogen. Based on these characteristics, the test isolates were categorized as highly virulent, moderately virulent and a mildly virulent. To study the virulence potential variability of each isolate, the disease severity was continuously measured at 7days intervals after inoculation. Disease severity was recorded using 0 – 5 disease rating scale described by Pandey *et al.*(2003) with little modifications. Disease rating scale as follows:

Comment [hs4]: Please correct it.

Comment [hs5]: Were grown!!

Comment [hs6]: seven

Rating	**Reaction Description**

0 Free from infection

1 One or two necrotic spots on a few lower leaves of plants, covering nearly 1 – 10% of the surface area of the plant

2 A few isolated spots on leaves, covering nearly 11 – 25% of the surface area of the plant

3 Many spots coalesced on the leaves, covering 26 – 50% of the surface area of the plant

4 Irregular, blighted leaves and sunken lesion with prominent concentric rings on the stem petiole, fruit, covering 51 – 75% leaf area of the plant.

5 Whole plants blighted, leaf and fruits starting to fall, covering more than 75% leaf area of plant.

Percent disease index (PDI) for each isolate were calculated as follows:

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of individual disease rating}}{\text{Total no. of plant examined} \times \text{Maximum no. of disease rating}} X100$$

Area under the disease progress curve (AUDPC) was calculated by using following formula:

$$\text{AUDPC} = \sum_{i-1}^{n}\left[0.5(x_{i+1} + x_i)[t_{i+1} + t_i]\right]$$

Whereas,

x_i = Cumulative disease severity expressed as a proportion at the i^{th} observation

t_i = Time (days after planting) at the i^{th} observation

n = Total number of observations

Results and Discussion

Morphological characterization of the *Alternariasolani*isolates

Investigations on morphological characteristics of thetest isolates of *A. solani*showed highly variability based on their conidial size, beak length and septation.

Conidia length with beak was varies from 39.17–74.35µm in isolate AS-6, 29.86–73.23µm in AS-7, 34.34–73.14µm in AS-2, 33.61–71.43µm in AS-4, 32.12–68.63µm in AS-1, 30.53–63.14µm in AS-5 and 28.12–58.35µm in AS-3. However, beak length of conidia was ranged 6.68–13.23µm in isolate AS-1, 5.76–12.37µm in AS-2, 4.18–7.62µm in AS-3, 7.64–14.78µm in AS-4, 4.25–8.87µm in AS-5, 7.23–14.16µm in AS-6 and 3.63–13.65µm in AS-7 8. Variation in width of conidia was also observed among the isolates of *A. solani*. Width of conidia was varied from 8.12–21.3687µm in isolate AS-1, 7.91–19.5687µm in AS-2, 8.82–24.2387µm in AS-3, 9.32–22.8687µm in AS-4, 7.05–20.5687µm in AS-5, 8.63–21.6387µm in AS-6 and 8.13–19.4887µm in AS-5. In case of septation, horizontal septation was found maximum (2–11) in isolate AS-7 followed by AS-2 (3–10), AS-6 (3–9) and AS-4 (3–7), while least horizontal septa (3–6) was observed in isolate AS-1 and AS-5. However, maximum vertical septa (1–3) was observed in isolate AS-3, AS-4 and AS-6 and minimum vertical septa (0–2) was found in isolate AS-1, AS-2, AS-5 and AS-7 (Table 1; Fig. 1).

The microscopic examination was done on septation in conidia of different isolates of *Alternariasolani*. Highest horizontal septa were recorded in isolate AS-7, while least horizontal septa were observed in isolate AS-1 and AS-5. However, maximum vertical septa were observed in isolate AS-3, AS-4 and AS- and minimum was found in isolate AS-1, AS-2, AS-5 and AS-7. Conidia length with beak and beak length were varying among the isolate. Highest width of conidia was recorded in isolate AS-3 and minimum width of conidia was found in isolate AS-7. These are indicated that the morphology of the *Alternariasolani* was varied from location to location. The conidial features of *A. solani*test isolates investigated in the present study agreed with the findings of Alhussaen (2012) who reported that the mycelia width between 0.8 – 1.5µm and conidia are 35 – 75µm in length and 10 – 20µmin width and 2 – 7 transverse septa and 1 – 4 longitudinal septa.Nikam*et al*. (2015) also reported that, amongst test isolate of A. *solani*, the highest mycelial width was recorded in AsJI (64.2µm). The average conidial size (L×B) and their beak length were highest in the isolate AsBd (42.18×15.18 and 13.1µm) respectively. Conidial septation of horizontal and vertical septa was also found to be varied among the test isolates of *A. solani*. Naik*et al*. (2010) found that, the conidia were solitary, straight or slightly flexuous, 19.5 – 70 µm in length and 6 – 22 µm width with 2 – 7 transverse septa and 1-4

Comment [hs7]: In this part, there is no need to mention all measurements, just the most significant ones.

Comment [hs8]: isolates

Comment [hs9]: grammatical mistake.

longitudinal septa. Similar type result is also obtained by several workers (Ellis, 1971;Kaul and Saxena, 1988; Kumar *et al*.,2012;Marak*et al*., 2014).

Pathogenicity test and pathogenic variability

Data pertaining to appearance of symptoms, PDI and AUDPC on tomato plants inoculated with different isolates have been presented in Table 2. Typical symptoms of early blight were appeared on the leaves after 3–4 days from inoculation of tomato seedlings, which was similar to those observed on naturally infected tomato plants and the morphological and cultural characteristics of the *A. solani*were compared with original pathogen. Appearance of symptoms was early noticed 3 – 4 days after inoculation in isolate AS-6 followed by isolate AS-5 and AS-7 (3 – 5 days after inoculation), AS-3 and AS-4 (4 – 5 days after inoculation), AS-2 (6 – 7 days after inoculation), while late symptoms was appeared in isolate AS-1(6 – 8 days after inoculation). Disease severity was recorded up to 42 days of inoculation at 7 days intervals and PDI was calculated as per formula described in materials and methods. At 42 d after inoculation, highest PDI was observed 88.89 percent in isolate AS-6 followed by AS-5 (84.44%). However, least PDI was observed in isolate AS-1. In case of Area Under Disease Progress Curve (AUDPC), isolate AS-6 showed maximum AUDPC (2302) followed by AS-5(2147), AS-7(1836), AS-3(1672), AS-4(1423), AS-2(1260). However, minimum AUDPC was observed in isolate AS-1 (996). Present investigation indicated that the isolate AS-6 was found most virulent followed by AS-5 and AS-7. While, isolate AS-1, AS-2 and AS-4 was found moderately virulent (Figs. 2, 3).

Pathogenic variability regarding to appearance of symptoms, PDI and AUDPC among the isolates of *Alternariasolani*were recorded on variety Pusa Ruby. Symptoms were early noticed in isolate AS-6, while a delay symptom was appeared in isolate AS-1. Highest PDI was observed in isolate AS-6, while least PDI was observed in isolate AS-1. Isolate AS-6 showed maximum AUDPC and minimum AUDPC was observed in isolate AS-1. On the basis of pathogenic variability, it is concluded that the isolate AS–6 (Kabirdham isolate) was found more virulent as compare to other isolates. The result obtained in the present study agreed with the findings of Castro et al.(2000) study on variability of *A. solani*undergreenhouse conditions based on the inoculation of 7 isolates on 14 tomato genotypes and reveal that all the isolates showed different degree of virulence on 14 tomato

Comment [hs10]: Introduce the abbreviations.

genotypes,demonstrating the existence of high level of variability in the fungus. Singh et al.(2014) reported that out of 10 isolates, only three major groups were recorded on the basis of SAS analysis likewise Group-A were highly virulent (MF-4 and PN-4), Group-C indicate virulent (BG, AF-2, EC-1 and RF-1) and Group-E were less virulent (BHU-1, IIVR, SF-1 and BX-2) with all the five varieties. Similar results were also reported by Rahmatzaiet al.(2016). They observed that the isolate AS1 of *A. solani* was mostly virulent with maximum level of disease incidence (53.5%) and disease severity (32%), whereas, the AS2 isolate noted the least early blight incidence of 27% and disease severity of 18%. AS1 isolate produced properly clear early blight symptoms on leaves, stem and even fruits and was found to be virulent causing sever disease in tomato plants.

Acknowledgements

The authors acknowledge the financial grant from Indira Gandhi KrishiVishwavidyalaya, Raipur Chhattisgarh, India

References

Ahmad, S.(2002). Conidial morphology of *Alternariasolani* and its variation in tomato.Annals of Agricultural Research.23: 514–515.

Alhussaen, K.M.(2012). Morphological and phiysiological characterization of *Alternariasolanai* isolated from Tomato in Jordan Valley. Research Journal of Biology Science. 7: 316–319.

Arunakumar, K.T.(2006). Studies on *Alternariasolani* (Ellis and Martin) Jones and Grout causing early blight of tomato. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India).

Castro, M.E.A., Zambolim, L., Chanes, G.M., Cruz, C.D. andMatsuoka, K. (2000). Pathogenic variability of *Alternariasolani*, the causal agent of tomato early blight.Summa Phytopathologica. 26: 24–28.

Ellis, M.B.(1971). *Dematiaceous hyphomycetes*. Commonwelth Mycological Institute, Kew, Surry, England, pp 608.

Johnson, A. andBooth, C. (1983). Plant Pathologist's Pocket Book, Oxford and IBH Pub. Co. Calcutta.

Kaul, A.K. andSaxena, H.K. (1988). Physiologic specialization in *Alternariasolani*causing early blight of tomato. Indian Journal of Mycology and Plant Pathology. 1: 128–132.

Kumar, A., Gour, H.N., Sharma, P. andShah, R. (2012). Investigations on variability and eco-friendly management of *Alternariaalternata*causing tomato blight. Society of Plant Research. 25:52–58.

Leung, H., Nelson, R.J. andLeach, J.E. (1993). Population structure of plant pathogenic fungi and bacteria. Advanced Plant Pathology. 10: 157–205.

Marak, T.R., Ambesh, B.S. andDas, S. (2014). Cultural, morphological and biochemical variations of *AlternariaSolani* causing diseases on solanaceous crops. The Bioscan. 9: 1295–1300.

Naik, M.K., Prasad, Y., Bhat, K.V. andRani, D.G.S. (2010). Morphological, physiological, pathogenic and molecular variability among isolates of *Alternariasolani*from tomato. Indian Phytopathology. 63: 168–173.

Nikam, P.S., Suryawanshi, A.P., A.A. andChavan.(2015). Pathogenic, morphological and molecular variability among eight isolates of *Alternariasolani*, causing early blight of tomato. Africa Journal of Biotechnology.14(10): 872–877.

Pandey, K.K. andPandey, P.K. (2003). Survey and surveillance of vegetable growing area for prevalence of major diseases in this region. Vegetable Science. 30: 128–134.

Pandey, K.K., Pandey, P.K., Kallo, G. andBanerjee, M.K. (2003). Resistance to early blight of tomato with respect to various parameters of disease epidemics. Journal of General Plant Pathology.6: 364–371.

Pryor, B.M. andMichailides, T.J. (2002). Morphological, pathogenic and molecular characterization of *Alternaria*isolates associated with*Alternaria*late blight of pistachio. Phytopathology.92: 406–416.

Rahmatzai, N., Zaitoun, A.A., Madkour, M.H., Ahmady, A., Hazim, Z. andMousa, M.A.A. (2016). Morphological, pathogenic, cultural and physiological variability of the isolates of *Alternariasolani*causing early blight of tomato.International Journal of Advanced Research. 4: 808–817.

Simmons, E.G.(2007). *Alternaria*: An Identification Manual. CBS Fungal Biodiversity Centre, Utrecht, Netherlands

Singh, A., Singh, V. andYadav, S.M.(2014). Cultural, Morphological and Pathogenic Variability of *Alternariasolani* Causing Early Blight in Tomato.Plant Pathology Journal.1: 167–172.

Table 1: Morphological variability among the isolates of *Alternariasolani* on PDA medium

Isolates	No. of horizontal septation	No. of vertical septation	Length of conidia with beak (µm)	Beak length (µm)	Width of conidia (µm)
AS-1	3 – 6	0 – 2	32.12 – 68.63	6.68 – 13.23	8.12 – 21.36
AS-2	3 – 10	0 – 2	34.34 – 73.14	5.76 – 12.37	7.91 – 19.56
AS-3	2 – 7	1 – 3	28.12 – 58.35	4.18 – 7.62	8.82 – 24.23
AS-4	3 – 7	1 – 3	33.61 – 71.43	7.64 – 14.78	9.32 – 22.86
AS-5	3 – 6	0 – 2	30.53 – 63.14	4.25 – 8.87	7.05 – 20.56
AS-6	3 – 9	1 – 3	39.17 – 74.35	7.23 – 14.16	8.63 – 21.63
AS-7	2 – 11	0 – 2	29.86 – 73.23	3.63 – 13.65	8.13 – 19.48

Table 2: Pathogenic variability among the isolates of *Alternariasolani*on variety Pusa Ruby

Isolates	Symptoms appearance (Days after inoculation)	PDI at 42 days after inoculation	AUDPC
AS-1	6 – 8	40.00	996
AS-2	6 – 7	48.49	1260
AS-3	4 – 5	64.44	1672
AS-4	4 – 5	55.56	1423
AS-5	3 – 5	84.44	2147
AS-6	3 – 4	88.89	2302
AS-7	3 – 5	75.56	1836

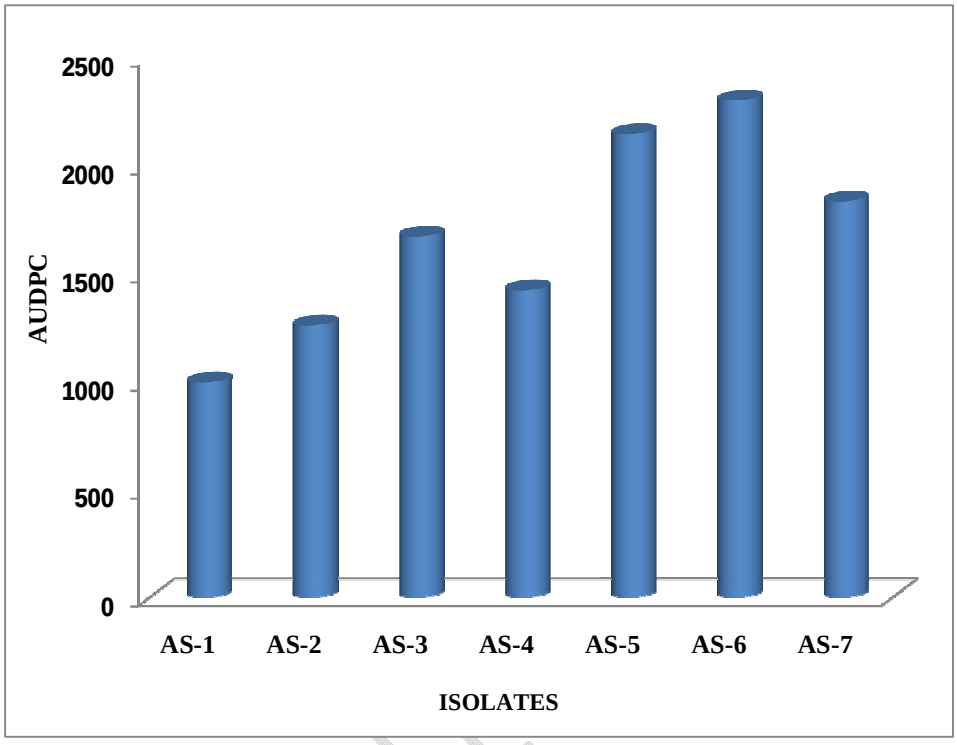
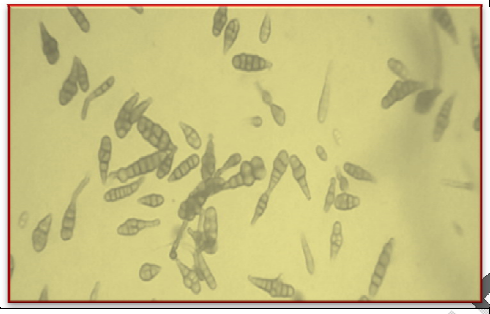


Fig. 1:Area Under Disease Progress Curve (AUDPC) on tomato variety Pusa Ruby

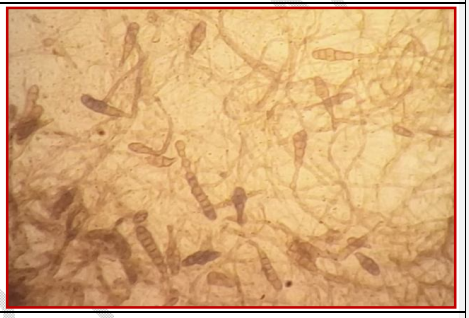
Comment [hs11]: Add error bar for each

Isolate AS-1

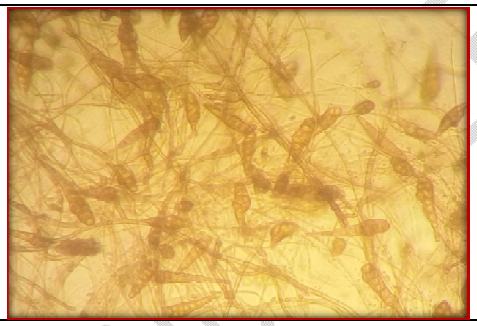
Isolate AS-2



Isolate AS-3



Isolate AS-4



Isolate AS-5

Isolate AS-6

Isolate AS-7

Plate 1: Morphological variability among the isolates of *Alternariasolani* on PDA medium

Plate 2: Pathogenic variability among the isolates of *Alternariasolani*on variety Pusa Ruby