Eco. Env. & Cons. 30 (4) : 2024; pp. (1604-1610)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i04.024

Pathogenic variability among the isolates of *Alternaria alternata* causing Alternaria Blight in Tomato (*Solanum lycopersicum* L.)

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(Received 15 May, 2024; Accepted 23 July, 2024)

ABSTRACT

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown throughout the world because of its wider adaptability, high yielding potential and suitability for variety of uses in fresh as well as processed food industries. Among all fungal diseases, early bight / leaf blight/ Alternaria blight disease of tomato is one of the widest spread and devastating disease occurring worldwide. It is caused by several species of *Alternaria* including *Alternaria solani*, *A. tomatophila* as well as *A. alternata*. The present study was undertaken to detect the major pathogen involved in Alternaria Blight in Tomato in Rajasthan. The considerable variation in characteristics symptoms of the blight disease can be seen on all plant parts *i.e.*, on stem, twigs, leaves, fruit etc in the field condition. For the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for the isolation and culturing pathogenic fungi. Because of cultural characteristics of fungus was identified as *Alternaria alternata* is prominently involved in Alternaria Blight of Tomato in Rajasthan. The pathogen was inoculated through conidial suspension (1 x 10^6 spore/ml) for testing pathogenicity.

Key words: *Symptomatology, Pathogenicity, Isolation, Alternaria Blight, Tomato and Alternaria alternata.*

Introduction

Tomato (*Solanum lycopersicum* L. [syn. *Lycopersicon esculentum* Mill.] belongs to the night shade family *Solanaceae* is a number one processing vegetable in the world. It is most remunerative vegetable crop. Tomato is also known as "Poorman's Orange" in India and the world second largest vegetable crop next to potato and it is universally treated protective food because of its major dietary source of the anti-oxidant lycopene. Tomato originated from the Andes, now called Peru, Bolivia, Chili and Ecuador where they grow wild (Mehta, 2017). It was introduced in India by the Portuguese during 1700 (Kale and Kale, 1994).

Tomato being a moderate nutritional crop is considered as an important source of vitamin A, C and minerals which are important ingredients for table

[7]Director Resident Instruction

purpose, sambar preparation, chutney, pickles, ketchup, soup, juice, puree etc. (Sekhar *et al.*, 2010).

India is a second largest producer of tomato crop after China having an area of 865.29 million hectares with a production of 21055.85 million tonnes. (Source: Agricultural Statistics at a Glance 2021). In Rajasthan, tomato is being cultivated in area of 20504 ha with a production of 232864 quintal. (Source: GOR, Directorate of Horticulture, 2020-21).

Tomato is highly sensitive to abiotic stresses especially extreme temperature, salinity, drought, excessive moisture and environmental pollution. Tomato plants suffer with large number of biotic stresses including insect pests and diseases from the time of emergence to harvest. Tomato crop is highly susceptible to plant pathogens *viz.* bacteria, fungi and viruses which deteriorate the yield and quality of tomato fruit (Pattnaik *et al.* 2012).

Tomato crop is affected by several diseases caused by fungi, bacteria, viruses, nematodes etc. (Mark and Brooke, 2006). Important fungal diseases like *Alternaria* blight (*Alternaria alternata*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), collar rot (*Sclerotium rolfsii*) and damping off (*Pythium* sp.) cause considerable losses in tomato crop. Among all fungal diseases, early bight / leaf blight/ Alternaria blight disease of tomato is one of the widest spread and devastating disease occurring worldwide in major tomato growing area (Akhtar *et al.,* 1994). It is caused by several species of Alternaria including *Alternaria solani*, *A. tomatophila* as well as *A. alternata* (Adhikari *et al.,* 2017). This disease occurring in three phases causing damping off of seedling, leaf blight and stem canker (Gilchrist and Grogan, 1976). *Alternaria alternata* is a mitosporic fungus which belongs to kingdom Fungi, phylum Ascomycota, class Dothideomycetes, order Pleosporales, family Pleosporaceae and genus *Alternaria*. It is a necrotrophic fungus with over 380 host species and can infect important vegetable crops like tomato, eggplant, potato and pepper and several others crop plant (Tozlu *et al.,* 2018).

Alternaria is a soil inhibiting air-born foliar pathogen (Ellis, 1971), which affect the crop severely during fruit maturity stage (Momel *et al*., 2006). The characteristics symptoms of the pathogen appear first on older leaves of lower part and then after, move above towards younger tissue (Agrios, 2005). Under suitable environment conditions, *Alternaria* causes defoliation, drying off of twigs and premature fruit drop and thus causing loss up to 79 per cent in fruit yield (Adhikari *et al.,* 2017).

Bessadat *et al.* (2014) reported 46-90 per cent disease severity in tomato, caused by *A. alternata* in Algeria. Datar and Mayee (1985) recorded 78.0 per cent yield loss of tomato at 72.0 per cent disease intensity due to *A. solani* and each 1 per cent increase in disease intensity, reduced tomato yield by 1.36 per cent.

The blight disease of tomato caused by *Alternaria* sp.is also being observed in major tomato producing area of Rajasthan and increasing problem for growing farmers. Keeping in view of the seriousness of the disease, the studies were planned to undertake on the following aspects which could be of help in the brief about symptomatology, identification and management of the disease.

Materials and Methods

Present investigation was carried out at laboratory and cage house of Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur-MPUAT, Rajasthan during *Kharif*, 2022 & 2023. The experimental details, methodology followed and criteria adopted during investigations are described below-

Collection of diseased samples: The symptoms of Alternaria blight of tomato on different locations

Table 1. Rating scale for assessment of early blight per cent disease index on tomato plants (Pandey *et al.,* 2003).

Rating	Reaction Description
0	Free from infection
1	< 10% surface area covering leaf, stem and fruit
2	11-25% foliage of plant covered with few isolated spots
3	Many spots coalesced on the leaves, covering 25-50% surface area of plant
4	51-75% area of the plants infected, fruits also infected at peduncle end, defoliation and blightening started. Sunken lesions with prominent concentric rings on stems, petioles and fruits
5	<75% area of plant part blighted, sever lesions on stem and fruit rotting on peduncle end

showed considerable variations. A roving survey was conducted during *Kharif* 2022 and 2023 during survey plant showing typical Alternaria blight symptoms were collected from farmer's fields of respective districts of Rajasthan *viz*., Jaipur, Bundi, Kota, Chittorgarh, Sirohi and Udaipur districts to explore the prevalence of Alternaria blight incidence and distribution of pathogen population in collected samples from farmer's fields. Samples were brought in to laboratory of Department of Plant Pathology, Rajasthan College of Agriculture, (MPUAT) Udaipur for isolation and further studies. For all the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for isolation and culturing *Alternaria alternata*.

Isolation of fungus: Standard tissue isolation technique followed for pathogen isolation. Later, careful separation by transferring the newly appearing growth from each sector on to fresh sterilized PDA Petri plates by hyphen tip culture technique was done and these were maintained on PDA slants for further studies under aseptic conditions was used to purify the culture, which were isolated from collected samples of each different districts purified and used for further studies. The culture was stored in a refrigerator at 4±1°C and sub cultured once in a month. These cultures were further designated as Jaipur (Alt-J1 & Alt-J2), Bundi (Alt-B1 & Alt-B2), Kota (Alt-K1 & Alt-K2), Chittorgarh (Alt-C1 & Alt-C2), Sirohi (Alt-S1 & Alt-S2) and Udaipur (Alt-U1 & Alt-U2).

Mass multiplication: For preparation of the inoculum, the pure cultures of different isolates were grown on PDA for 10 days on 26±1 °C in Petri plates to allow profuse sporulation. The spores were harvested by flooding with sterile distilled water and gently scrapping the colony with the help of a sterilized plastic loop and the conidial suspension was strained through muslin cloth. Final concentration of the spores was calibrated 1 x10^6 conidia ml^{-1} through hemocytometer. This is used as inoculum for pathogenicity test.

Proving the pathogenic variability: The pathogenic potential of different isolates of *Alternaria alternata* isolated from different six districts of Rajasthan was examined on tomato susceptible variety S-22 through conidial suspension spray inoculation technique on pot transplanted plants of tomato. The healthy seedlings of tomato were transplanted in sterilized sand: soil: FYM (3:1:1) @ 3 per pot keeping three replications for each isolate in completely randomized design (CRD). Three pots were separately

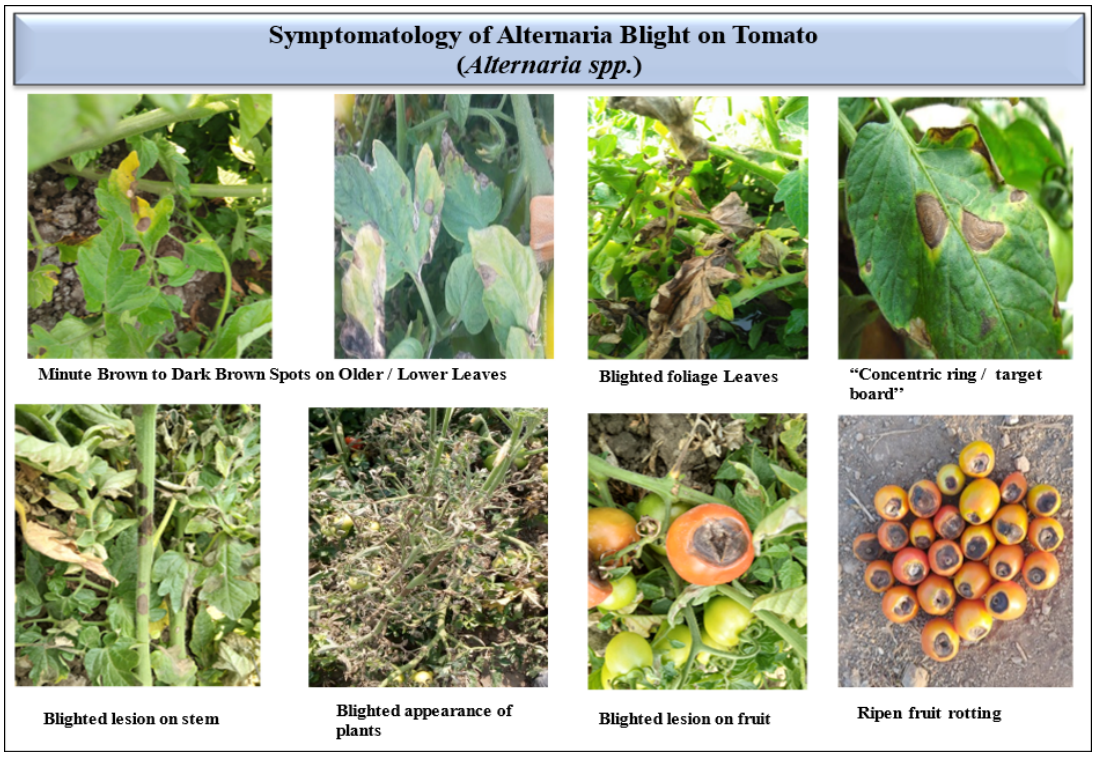

Plate 1. Symptomatology variation in Alternaria blight disease of Tomato.

inoculated 30 days after transplanting by the individual culture of twelve isolates of *A. alternata* Jaipur (Alt-J1 & Alt-J2), Bundi (Alt-B1 & Alt-B2), Kota (Alt-K1 & Alt-K2), Chittorgarh (Alt-C1 & Alt-C2), Sirohi (Alt-S1 & Alt-S2) and Udaipur (Alt-U1 & Alt-U2) by spore inoculation technique (1×10^6 spores ml^{-1}) with hand held automizer and suitable un-inoculated control pots were also maintained for tomato. Three replications of each isolate were maintained and three pots were kept as un-inoculated as control pots. Each pot was labelled, watered as and when required and left undisturbed in cage house for development of the symptoms. The pots were irrigated with distilled water on alternate days to give adequate moisture for symptom development. Detailed symptoms produced by each isolate were recorded. Observations for recorded individually using 0-5 rating scale described by Pandey *et al.* (2003). Percent disease intensity/index (PDI) using formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of All Numericals Ratings}}{\text{Total Number of Leaves Observed} \times \text{Maximu m Rating}} \times 100$$

Results and Discussion

The symptoms of Alternaria blight of tomato on different locations showed considerable variations each variable symptoms were collected through pictures, the first symptoms of the Alternaria blight disease caused by *Alternaria alternata* on tomato was appeared as small brown water-soaked lesions on the older leaf. Older leaves get infection first and later it progresses upward. The spots were oval or angular in shape ranged from 1 to 5 mm diameter. Narrow chlorotic zone was also noticed around the spot. Later stage spots were enlarged and concentric rings were formed in the centre. In advanced stage colour of the spots were changed from brown to dark brown and finally adjacent spots coalesced to form large irregular spots. Severe attacks of the disease cause drying and defoliation of foliage. When plants were old, symptoms appeared on stem and petioles as brown to dark brown elongated target board type spots. These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepals and fruits resulting in pre-mature dropping of fruits. The symptoms on fruits appeared first at stem end as black or brown sunken spots both on green and ripe fruits which enlarged within eight days involving most of the fruits, finally the fruits were rotted. Similarity with Peralta *et al.* (2005) reported that early blight disease can lead to complete defoliation in severe case in regions. Chaerani *et al.* (2006) noticed symptoms on tomato due to early blight as collar rot on stem of seedlings, lesions on stem of adult plant and rotting on fruits. Foolad *et al.* (2008) also described early blight symptoms on potato and tomato foliage as small, dark, circular lesions becoming distinctly zonate as they develop. Stem lesions was occur on diseased tomato plants as roughly circular, sunken, dark and zonate. Appearance of rot spots on green and ripe fruit of tomato were also reported by Blancard *et al.* (2012).

The pure culture of most virulent isolate, recovered from Alt-U1 (Udaipur) was submitting to ITCC for identification and confirmation. Thus, the pathogen causing Alternaria blight of tomato has been identified as *Alternaria alternata* (ITCC, Division of Plant Pathology, IARI, New Delhi confirmation with ID NO. 11973.23). Our study has identified *Altrnaria alternata* as the predominant causal agent of Alternaria blight diseases on the tomato plants cultivated in different regions of Rajasthan. In accordance with Sahi and Shyam (1993) isolated *A. solani* and *A. alternata f. sp. lycopersici* from tomato plants in Himachal Pradesh, India. In laboratory test, *A. alternata f. sp lycopersici* produced symptoms on leaflets, stems and branches following inoculation, while *A. solani* produced only on leaflets.

Among twelve isolates of *A. alternata* inoculated on tomato plants, significantly maximum PDI was observed with the isolates from Udaipur district, where isolate Alt-U1 exhibited 58.80% PDI. This was

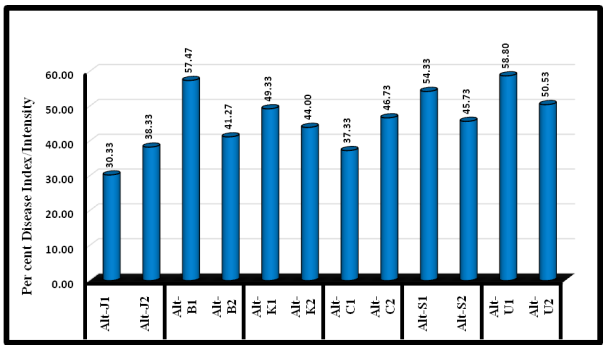

Fig. 1. Pathogenic variability among twelve isolates of *Alternaria alternata* on tomato variety S-22 underpot conditions.

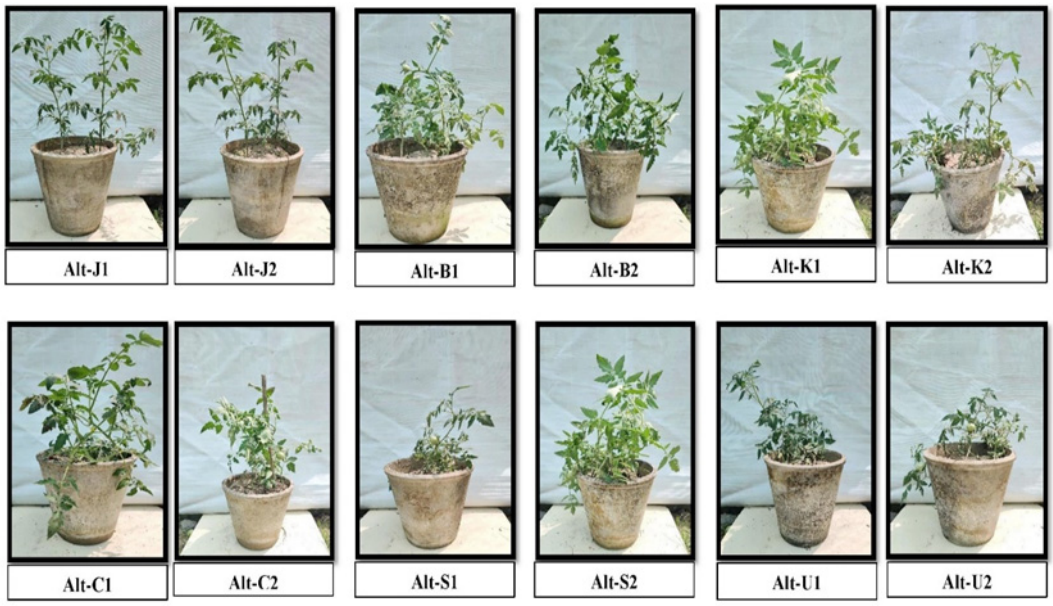

Plate 2. Pathogenic variability among twelve isolates of *Alternaria alternata* on tomato variety S-22 underpot conditions.

Table 2. Pathogenic variability among twelve isolates of *Alternaria alternata* on tomato variety S-22 under pot conditions

Sr. No.	Location	Isolates designation	PDI (Percent Disease Index) *
1.	Jaipur	Alt-J1	30.33 (33.42)
2.		Alt-J2	38.33 (38.25)
3.	Bundi	Alt-B1	57.47 (49.30)
4.		Alt-B2	41.27 (39.97)
5.	Kota	Alt-K1	49.33 (44.62)
6.		Alt-K2	44.00 (41.55)
7.	Chittorgarh	Alt-C1	37.33 (37.66)
8.		Alt-C2	46.73 (43.13)
9.	Sirohi	Alt-S1	54.33 (47.49)
10.		Alt-S2	45.73 (42.55)
11.	Udaipur	Alt-U1	58.80 (50.07)
12.		Alt-U2	50.53 (45.31)
S Em ±			0.42 (0.24)
CD at 5%			1.23 (0.71)
CV (%)			1.58 (0.99)

*Mean of three replications,
Figure in parentheses"arcsine percent angulartrans formed values.

followed by isolate from Bundi Alt-B1 was exhibited 57.47% PDI. Further, it was followed by isolates from Sirohi with PDI 54.33% in isolate Alt-S1 and 50.53% PDI in isolate Alt-U2 from Udaipur. Further next to follow isolates was Alt-K1 recovered from district Kota having PDI 49.33%, Isolate Alt-C2 from Chittorgarh district exhibited PDI 46.73%, Isolate Alt-S2 recovered from Sirohi depict 45.73% PDI and 44.00% PDI recorded in isolate Alt-K2 recovered from Kota District. In isolate Alt-B2 recorded 41.27% PDI isolated from Bundi district. While, in Alt-J2 exhibited 38.33% PDI recovered from Jaipur district and isolate Alt-C1 procured from Chittorgarh district recorded 37.33% PDI. Significantly minimum PDI was observed with isolates from Jaipur district where isolate Alt-J1 is having PDI 30.33%. The differences in PDI of different isolates procured from different districts were found to be statistically (*P*d"0.05) significant. Similarly, the pathogenic variability studies have also been carried out by Varma *et al.* (2007), Kumar *et al.* (2008) on *A. solani*. They recorded pathogenic variability among different isolates of *A. solani*. Tetarwal *et al.* (2008) observed variability among six isolates of *A. alternata* infecting Senna (*Cassia angustifolia*). The results are also in similarity with the results obtained by Meena *et al.* (2014) and Nikam *et al.* (2015). Parvin *et al.* (2021) conducted a pathogenicity test on excised leaves surface disinfected by dipping in 70% ethanol for 30 second and placed onto moistened blotting paper in a petridish. Each leaf was inoculated with conidial suspension of *Alternaria solani* having 1×10^6 conidia/ml suspension and incubated at 25°C±1°C for appearance of symptoms.

Summary and Conclusion

Characteristic symptoms of Alternaria blight of tomato on different locations showed considerable variations, the first symptoms of the Alternaria blight disease caused by *Alternaria alternata* on tomato was appeared as small brown water-soaked lesions on the older leaf. In advance stage colour of the spots were changed from brown to dark brown and finally adjacent spots coalesced to form large irregular spots. Severe attack of the disease cause drying and defoliation of foliage. Under pathogenic variability of isolates maximum PDI was observed from Udaipur district's isolate, where isolate Alt-U1 exhibited 58.80% PDI. This was followed by isolate from Bundi Alt-B1 exhibited 57.47% PDI. Significantly minimum PDI was observed with isolates from Jaipur district where isolate Alt-J1 is having PDI 30.33%. The pathogen was identified based on cultural and morphological characters and further identification got confirmed from ITCC, Division of Plant Pathology, IARI, New Delhi with ID No. 11973.23.

Acknowledgement

The authors thankful to Department of Plant Pathology, Rajasthan College of Agriculture, (MPUAT) Udaipur for financial support and Dr. Pokhar Rawal, (Major Guide), Department of Plant Pathology, Rajasthan College of Agriculture, (MPUAT) Udaipur for their moral support and providing for all facilities.

Authors' contributions: All authors contributed equally.

Conflict of Interest: The authors declare no conflict of interest.

References

Adhikari, P., Yeonyee, O. and Panthee, D.R. 2017. Current status of early blight resistance in tomato an update. *International Journal of Molecular Sciences.* 18(10): 2019.

Agrios, G.N. 2005. Plant Pathology. *5th* edition. *Elsevier Academic Press*, New York. 452.

Akhtar, K.P., Martin, M., Mirja, J.H., Shakir, A.S. and Rafique, M. 1994. Some studies on the postharvest diseases of tomato fruits and their chemical control. *Pakistan Journal of Phytopathology.* 9(6): 125.

Anonymous, 2021. Area Production Year (2020-21), Directorate of horticulture, Government of Rajasthan. https://agriculture.rajasthan.gov.in

Anonymus, 2021. Agricultural Statistics at a Glance 2021. Directorate of Economics & Statistics, DA& FW, 4th Advance Estimates, Govt. of India. Ministry of Agriculture & Farmers welfare, (Dept. of Agriculture, Corporation & Farmers Welfare). 42-43pp. visit us at:www.agricoop.nic.in&http://eands.dacnet.nic.in

Bessadat, N., Benichous, S., Kihal, M. and Henni, E.H. 2014. Aggressiveness and morphological variability of small spore *Alternaria* spp. isolated from Algeria. *Journal of Experimental Biology and Agricultural Sciences.* 2(2S): 265-278.

Blancard, D., Laterrot, H., Marchoux, G. and Candresse, T. 2012. *Tomato Diseases: Identification, Biology and Control - A Colour Handbook* (2nd edition). London Manson Publishing Ltd., United Kingdom, London. pp. 688. https://doi.org/10.1201/b15145

Chaerani, R. and Voorrips, R.E. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology.* 72(6): 335-347.

Datar, V.V. and Mayee, C.D. 1985. Chemical management of early blight of tomato. *Journal of Maharashtra Agriculture University.* 10(3): 278–280.

Dhiman, J.S., Bedi, P.S. and Bombawale, O.M. 1980. An easy method of preparing inoculum of *Alternaria solani* for mass inoculation. *Indian Phytopathology*. 33: 359-362.

Ellis, M.B. 1971. Dematiaceous hyphomycetes. *Common Wealth Mycological Institute*, Kew, England. 464-497.

Foolad, M.R., Merk, H.L. and Ashrafi, H. 2008. Genetics, genomics and breeding of late blight and early blight resistance in tomato. *Critical Reviews in Plant Sciences.* 27(2): 75–107.

Gilchrist, D.G. and Grogan, R.G. 1976. Production and nature of a host-specific toxin from *Alternaria alternata* f. sp. *lycopersici*. *Phytopathology.* 66: 165-171.

Kale, P.N. and Kale, S.P. 1994. *Bhajipala Utpadan* (Vegetable production) Continental Publication,C.O. Pune. 29-30.

Kumar V., Haidar, S., Pandey, K.K., Singh, R.P., Singh, A. and Singh, P.C. 2007. Cultural,morphological Pathogenic, and molecular variability amongst Tomato isolates of *Altemariasolan i* in India. *World Journal of Microbiology and Biotechnology.* 24(7): 1003-1009.

Mark, L.G. and Brooke, A.E. 2006. Tomato diseases and disorders. *Physiological Disorder.* pp12.

Meena, R.K., Sharma, S.S. and Singh, S.K. 2014. Studies on variability in *Alternaria alternata* (kessler) causing leaf blight of isabgol (*Plantago ovata*). *Saarc Journal of Agriculture.* 12(2): 63-70.

Mehta, R. 2017. History of Tomato (Poor Man's Apple). *Journal Humanities Social Science (IOSR-JHSS).* 22(8): 31-34.

Momol, T.M. and Pemezny, K.L. 2006. Florida plant dis-

ease management guide: Tomato. Florida Cooperative Extension Service, *Institute of Food and Agricultural Sciences*, University of Florida. http://edis.ifas.ufl.edu.

Nikam, P.S., Suryawanshi, A.P. and Chavan, A.A. 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria solani*, causing early blight of tomato. *African Journal of Biotechnology*. 14(10): 872-877.

Pandey, K.K., Pandey, P.K., Kalloo, G. and Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. *Journal of General Plant Pathology*. 69(6): 364-371.

Parvin, I., Mondal, C., Sultana, S., Sultana, N. and Aminuzzaman, F.M. 2021. Pathological survey on early leaf blight of tomato and *in-vitro* effect of culture media, temperature and pH on growth and sporulation of *Alternaria solani*. *Open Access Library Journal*. 8: e7219. https://doi.org/10.4236/oalib.1107219.

Pattnaik, M.M., Kar, M. and Sahu, R.K. 2012. 'Bio-efficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. *Asian J. Plant Sci. Res.* 2(9) : 129-142.

Peralta, I.E., Knapp, S. and Spooner, D.M. 2005. New species of wild tomatoes (*Solanum section Lycopersicon*: Solanaceae) from Northern Peru. *Systematic Botany.* 30(2): 424-434.

Sahi, H.P.S. and Shyam, K.R. 1993. Occurrence of Alternaria leaf spots on tomato in Himachal Pradesh - a new record. *Plant Disease Research.* 8(2): 140-141.

Sekhar, L., Prakash, B.G., Salimath, P.M., Channayya, P., Hiremath, S.O. and Patil, A. A. 2010. Implication of heterosis and combining ability among productive Single cross Hybrids in tomato. *Electronic Journal of Plant Breeding*. 1(4): 706-711.

Tetarwal, M.L., Rai P.K. and Shekhawat, K.S. 2008. Morphological and pathogenic variability of Alternaria infecting senna. *Journal of Mycology and Plant Pathology*. 38(2): 375-377.

Tozlu, E., Tekiner, N., Kotan, R. and Ortucu, S. 2018. Investigation on the biological control of *Alternaria alternata*. *Indian Journal of Agricultural Sciences.* 88(8): 1241.

Varma, P.K., Singh, S. and Gandhi, S.K. 2007. Variability among *Alternaria solani* isolates causing early blight of tomato. *Indian Phytopathology*. 60(2): 180-186.

Wheeler, B.E.J. 1969. *An Introduction of Plant Diseases.* John Willey and Sons Ltd. London. pp.301.