



Symptomatology, Isolation and Pathogenicity of Alternaria Leaf Spot in Spinach Caused by *Alternaria alternata* (Fr.) Keissler

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ABSTRACT: Spinach (*Spinacia oleracea* L.) is one of the most important leafy vegetable belongs to the family Chenopodiaceae. This crop generally affected by many leaf spots but among all the leaf spots, the Alternaria leaf spot caused by *Alternaria alternata* (Fr.) Keissler is major threat and destructive disease. The present investigation has been carried out on the aspect of symptomatology, isolation and pathogenicity test of Alternaria leaf spot caused by *Alternaria alternata* (Fr.) Keissler.) The diseased plant produced typical symptoms of Alternaria leaf spot on spinach appeared as small and circular spots with concentric rings at initial stage which later develop in large size irregular lesions. These circular spots were dark black colour along the margins which encircled the necrotic region, with the spread of disease these necrotic spots turn to appear as blighted lesions. Initially the pathogen produced culture was grayish white in culture, which later turn into dark and becomes greenish black or olive brown with light border on PDA. The pathogenicity of fungus was tested on susceptible variety "All Green" by spore suspension spray under cage house. Initially inoculated plant produced symptoms like; small and circular spots with concentric ring which was later turned into dark black colored large size irregular lesions with necrotic regions.

Keywords: Spinach, *Alternaria alternata*, Pathogenicity, Leaf spot, Isolation.

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is one of the most important leafy vegetable belongs to the family Chenopodiaceae. It is native to South-West and central Asia and widely cultivated in all over the world including India (Roughani and Miri 2019). Spinach is produced commercially with the top nine countries in the order of production are China, USA, Japan, Turkey, Indonesia, France, South Korea, Pakistan, Belgium and India. In present China has first rank in the world and produced 29.8 million tonnes of spinach (Anonymous, 2023). It is commercially cultivated in many states of India like; Uttar Pradesh, West Bengal, Maharashtra, Rajasthan, Punjab, Haryana and Gujarat (Meena *et al.*, 2016). Total spinach production in India during 2022-23 was 0.16 million tonnes (Anonymous, 2022). Alternaria leaf spot is one of the widest spread and destructive disease cause massive losses in respect of quality and quantity of spinach. Plants infected with *Alternaria alternata* (Fr.) Keissler shows Symptoms appeared like; small and circular spots with concentric rings at first which later became irregular lesions. These circular spots were dark black colored along the margins which encircled the necrotic region. With the spread of disease, these necrotic spots turned to appear

as blight (Marraiki *et al.*, 2012). The pathogen *Alternaria alternata* is a foliar fungus associated with the plant leaves and usually spread through contact air and water. Therefore, keeping in view the economic importance of spinach present investigations has been carried out for the isolation, identification and pathogenicity of *Alternaria alternata* pathogen.

MATERIALS AND METHODS

A. Collection of Disease Samples

Disease infected samples of spinach leaf spot was collected from the farmers field and horticulture farm of RCA, Udaipur for isolation of the pathogen.

B. Culture Media

Potato Dextrose Agar (PDA) was used as basal culture medium for isolation, purification and maintenance of pure culture of *Alternaria alternata* (Fr.) Keissler and further laboratory studies.

C. Isolation of Pathogen

Fresh diseased samples of spinach leaves showing typical Alternaria leaf spot symptoms were collected from the farmer's field and field growing at Horticulture farm of RCA, Udaipur. The samples were

washed in gentle flow of water to remove dust adhering to leaves. The infected leaves tissues were cut into small bits of size 3-4 mm and these bits were surface sterilized with 0.1 per cent mercuric chloride (HgCl_2) solution for one minute under aseptic condition and rinsed in three changes of sterilized distilled water. Excess of water was absorbed by passing them through two folds of sterile blotter papers and then plated 4 bits at equal distance on petri plate containing sterilized solidified potato dextrose agar (PDA) medium. Petri plates were incubated in an incubator at $27\pm 2^\circ\text{C}$ temperature. These petri plates were observed daily for growth of fungus. After 2-3 days the fungal hyphal growth of mycelium appeared from infected leaf tissue. The culture was purified by single hyphal tip method on potato dextrose agar medium. Pure culture of isolated fungus (pathogen) was examined under microscope for identification and confirmed based on cultural and morphological characters under low (10X) and 40X power magnification.

D. Identification

For identification of pathogen on the basis of morphological and cultural characteristics like; growth pattern of fungus, colour of colony, formation of conidia in obtained culture of (*Alternaria alternata*) was studied and compared with the standard description given by Simmons (2007).

E. Mass Multiplication of Pathogen (*Alternaria alternata*)

The culture of the pathogen (*Alternaria alternata*) was multiplied on PDA media. Actively growing mycelium discs (5mm) of *Alternaria alternata* was placed on PDA poured Petri plate under aseptic condition these were incubated at temperature $26\pm 2^\circ\text{C}$ for 10 days. The spore suspension was prepared by harvesting of freshly sporulating culture with scraping of mycelial growth and then mixed into sterile water and then filtered by muslin cloth for further use. The spore suspension having 1×10^6 spores/ ml^{-1} was used for pathogenicity and field experiments.

F. Pathogenicity Test

Pathogenicity of isolated fungus (*Alternaria alternata*) was proved by using Koch's postulates. For the pathogenicity test two pots were filled with a sterilized potting mixture of soil: sand: FYM (2:1:1) and 4-5 seeds of spinach cultivar "All green" were seeded. The inoculum was prepared from 7-days old culture plates at a concentration of 1×10^6 conidia/ mL and applied by spraying on one pot with the help of hand atomizer and the inoculated plants were covered polythene bags during night hours to maintain a humid environment and establishment the pathogen. Another pot without inoculum spray was maintained as control pot for comparison with inoculated pot. Both pots were irrigated with distilled water to provide appropriate moisture for symptom development, and kept in the cage house for further observations. Symptoms of *Alternaria* leaf spot were observed and re-isolation of culture (*Alternaria alternata*) was made from diseased plant leaves and compared from original cultures for to prove Koch's postulates.

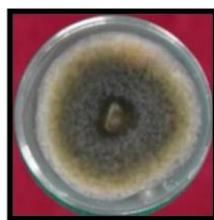
RESULTS AND DISCUSSION

A. Isolation and Identification of Pathogen

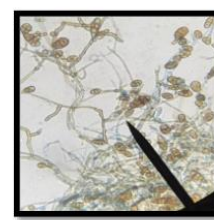
The fungus was isolated from leaf tissue of spinach plant and characters of the fungus produced on PDA and identified as *Alternaria alternata* (Fr.) Keissler. Growth of fungus was observed 3-4 days after incubation at $25\pm 2^\circ\text{C}$. The maximum colony growth was obtained in 12 days after incubation. Thereafter the growth of culture ceased. The culture was raised with grayish white culture, which later turn into dark and becomes greenish black or olive brown with light border. The fungus produced conidia with one to two longitudinal and two to eight transverse septa and were found solitary, smooth-walled and mostly ovoid in shape. septa and were found solitary, smooth-walled and mostly ovoid in shape. Hyphae were brown in color, septate and branched, on the basis of above a morphological and cultural character, the isolate was identified as *Alternaria alternata* (Fr.) Keissler which was similar mentioned by Simmons, 2007. The isolate was purified and mass multiplied for further studies. Pure culture and microscopic structure are presented in Plate 1.



A. Typical symptoms of *Alternaria alternata* leaf spot



B. Pure culture of *Alternaria alternata*



C. Microscopic structure of *Alternaria alternata*

Plate 1: Pure culture and microscopic structures of *Alternaria alternata* (Fr.) Keissler.

B. Pathogenicity Test

The pathogenicity of test pathogen (*A. alternata*) was proved by inoculating the plant with spore suspension. Results of pathogenicity test was appeared after 7-day

of spore suspension the susceptible variety of spinach "All Green" gets infected and produce symptoms. Initially inoculated plant produced symptoms like; small and circular spots with concentric ring which was

later turned into dark black colored large size irregular lesions with necrotic regions. Whereas uninoculated plants remained healthy. The fungus was re-isolated and re-identified through microscope observation based on cultural and morphological characterizes of the pathogen under 40X magnification power which was similar to inoculated culture. Further, the test pathogen submitted to ITCC New Delhi for re-confirmation.

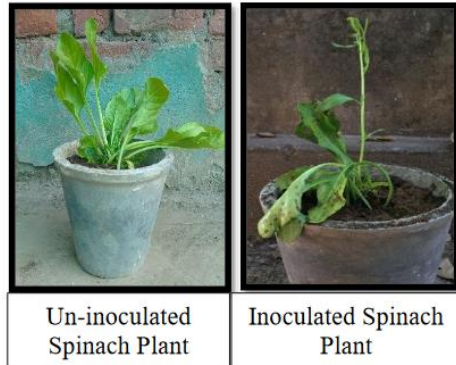


Plate 2: Pathogenicity of *Alternaria alternate* (Fr.) Keissler on “All Green” Variety of Spinach.

SUMMARY AND CONCLUSION

In present study the fungus was isolated from leaf of spinach plant and characters of the fungus produced on potato dextrose agar and identified as *Alternaria alternate* (Fr.) Keissler. The culture was raised with grayish white colure, which later turn into dark and becomes greenish black or olive brown with light border. The fungus produced conidia with one to two longitudinal and two to eight transverse septa. On the basis of above morphological and cultural character, the

isolate was identified as *Alternaria alternate* (Fr.) Keissler. Pathogenicity of isolated culture was proved by following the Koch’s postulates on susceptible spinach cultivar “All Green” in the cage house. The leaf spot symptoms including small and circular spots with concentric ring which was later turned into dark black colored large size irregular lesions with necrotic regions. Re-isolation of pathogen was attempted from diseased leaf which produced culture of *Alternaria alternata* (Fr.) Keissler, which were identical with the original one that was inoculated.

REFERENCES

- Anonymous (2022). International production: Spinach, FAO. www.agriexchange.apeda.gov.in.
- Anonymous (2023). International production: Spinach, FAO. www.agriexchange.apeda.gov.in.
- Marraiki, N., Siddiqui, I., Rizwana, H. and Javaid, A. (2012). First report of *Alternaria alternata* leaf spots on spinach in Saudi Arabia. *The Journal of Animal and Plant Sciences*, 22(1), 247-248.
- Meena, V. K., Subramanian, S., Dipti, N. V. and Simta, K. (2016). Introduction of palak (*Spinacia oleracea* L.) in Ernakulam district of Kerala through front line demonstration and on farm testing. *Agricultural Science Digest*, 36(2), 130- 133.
- Roughani, A. and Miri, S. M. (2019). Spinach: An important green leafy vegetable and medicinal herb. *The 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Pharmaceutical Ingredients*. pp-1-6.
- Simmons, E. G. (2007). *Alternaria*. An identification manual. In: Biodiversity No 6. CBS fungal diversity center, Utrecht, The Netherlands.