Survey of cercospora leaf spot of spinach caused by *Cercospora beticola* in south Gujarat

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ABSTRACT

Cercospora leaf spot disease is one of the most widespread diseases of spinach which is caused by *Cercospora beticola* Sacc and has posed a serious threat to the successful cultivation of this crop both abroad and in India. A roving survey of major growing areas of spinach was conducted in two Takulas of Bharuch district viz Bharuch and Ankleshwar during first fortnight of September-October (reproductive stage of the crop) in the year 2020-21. It was found that higher disease index of 41.84 per cent was observed in Ankleshwar Taluka as compared to 25.11 per cent in Bharuch Taluka of Bharuch district.

Keywords: Survey; spinach; Cercospora leaf spot; *Cercospora beticola*

INTRODUCTION

Spinach (*Spinacia oleracea* L), an important leafy vegetable, belonging to family Amaranthaceae, has originated from southwest Asia. In addition to its economic importance, spinach is one of the fastest growing vegetable crops in the US and other regions in terms of per capita consumption and is considered one of the healthiest vegetables in the human diet due to its high concentration of nutrients and health promoting compounds. It is closely related to beetroot (*Beta vulgaris*) and the name *Beta vulgaris* var *bengalensis* is derived because its leaves were used first in Bengal (Morelock and Correll 2008). Spinach is native to Indo-Chinese region; the major producers being China, United States, Japan, Turkey, Indonesia, India and France. Spinach is one of the most popular leafy vegetables grown in India. It is widely grown in West Bengal, Uttar Pradesh, Bihar, Gujarat and Rajasthan. Cercospora leaf spot disease caused by *Cercospora beticola* Sacc has become very serious disease nowadays. Cercospora leaf spot, caused by the fungus *C beticola*, occurs wherever table beets, swiss chard, sugar beet and spinach are grown and is one of the most important diseases affecting the Chenopodium group. It can result in significant losses, particularly in late summer when conditions are favorable (high temperatures, high humidity, long leaf wetness periods at night) (https://ag.umass.edu/vegetable/fact-sheets/cercospora-leaf-spot-of-swiss-chard-beets-spinach). In Gujarat, during favourable environmental conditions, especially at reproductive stage of the crop, it is attacked from its early stage to maturity stage by this disease resulting in severe leaf spotting, yellowing and premature withering of the plant which is a major constraint in its successful cultivation.

MATERIAL and METHODS

Isolation of the pathogen: Fresh naturally infected diseased leaves showing typical characteristic symptoms of cercospora leaf spot were collected from the farmers' fields during the course of survey and were repeatedly used for isolation of the pathogen. Isolation of the fungus was carried out by standard tissue isolation method (Johnston and Booth 1983). Small segments of diseased leaf tissue (5 mm^2) along with some healthy portion were cut with a razor blade and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds and washed serially three times in sterilized distilled

water to remove the traces of mercuric chloride, blotted dry, placed aseptically on to the Petri plates containing V8 juice agar medium and incubated at 27 ± 2°C for 30 days.

Purification and identification of pathogen: The culture was purified by single spore isolation method (Johnston and Booth 1983). The pure culture thus obtained was maintained by repeated sub-culturing and stored in refrigerator for further study. The stock culture in V8 juice agar slants was stored at 4°C in a refrigerator. The pathogen was identified as *C beticola* based on its morphological characters as described by Bashir (2017).

Pathogenicity test of the pathogen: The plants of cultivar spinach were raised in pots containing sterilized soil. The moisture was maintained by timely irrigation of the pots and intermittent spraying with sterilized water. Thirty-day-old plants were sprayed first with distilled water and covered with polythene bags for 24 h. The mycelial bits of the fungus were smeared on leaf surface using cotton plug. Similarly control plants were sprayed with sterile distilled water for comparison. The inoculated plants were covered with polythene bags and were kept for 120 h to ensure successful penetration of the pathogen into the tissue. The inoculated plants were kept under greenhouse conditions at 25°C and 95 per cent relative humidity. Observations were made regularly for the appearance and development of symptoms. The symptoms appeared in 10 days and re-isolation was made from such spots. The isolate obtained was compared with original inoculants to satisfy Koch's postulates.

Survey for the cercospora leaf spot disease of spinach: A roving survey of major growing areas of spinach in Bharuch district of Gujarat was carried out during first fortnight of September-October (reproductive stage of the crop) in the year 2020-21 to know symptoms appearance and per cent disease index of cercospora leaf spot disease. Random selection of spinach fields, irrespective of cultivars, from five villages per Taluka and five fields per village was done for this purpose.

Ten spinach plants were randomly selected from each field and scored for the per cent disease index by following 0-5 point scale as suggested by (Mayee and Datar 1986) (Table 1).

Table 1. Disease assessment key for Cercospora leaf spot of spinach

Grade	Description
0	No symptoms on leaves
1	Up to 1% of total area of leaves covered by lesions
2	1-10% of total area of leaves covered by lesions
3	11-25% of total area of leaves covered by lesions
4	26-50% of total area of leaves covered by lesions
5	More than 50% of total area of leaves covered by lesions

Per cent disease index (PDI) was calculated by using the following formula (Wheeler 1969):

$$PDI\ (\%) = \frac{\text{Sum of individual disease rating}}{\text{Total number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

Infected leaf samples were collected for isolation of pathogen. The location of the farm was recorded by GPS coordinates and altitude of the farm. In Ankleshwar Taluka, villages selected were Diva, Sakkarpor, Divi, Pungam and Borbhata Bet and in Bharuch were Tavara, Mangleshwar, Shuklatirth, Nikora and Jhanor.

RESULTS and DISCUSSION

Isolation of the pathogen: After 7 days of incubation at 27 ± 2°C, whitish mycelial growth of the fungus started emerging from the diseased leaf tissues inoculated on V8 – juice agar medium (Fig 1).

Purification and identification of pathogen: Pure culture shown in Fig 2 was maintained by sub-culturing. The conidia formed were hyaline, many celled, needle shaped and slightly broader at one end and pointed at the other end. Based on these characters and comparison with authentic descriptions as given by Bashir (2017), the fungus was identified as *C beticola*.

Pathogenicity test: In pathogenicity test it was found that the initiation of symptoms started as small light brown spots that were pale toward the center and had a reddish edge, which later turned to dark brown colour within 10 days after inoculation on injured leaves. However, in case of un-injured leaves, the disease symptoms appeared 14 days after inoculation. The symptoms produced were identical to those produced in the field under natural conditions of disease

development. The test fungus was re-isolated from such symptoms and compared with original culture.

Survey for the cercospora leaf spot disease of spinach: The data regarding per cent disease index of cercospora leaf spot disease are presented in Table 2. The results indicate that cercospora leaf spot disease of spinach was prevalent in both the Talukas. Higher mean disease index of 41.84 per cent was observed in Ankleshwaras compared to 25.11 per cent in Bharuch Taluka. In Ankleshwar, the average disease index was highest at Diva village (56.61%) followed by Borbhata Bet, Divi and Sakkarpor (46.82, 41.84 and 37.69% respectively) and least at Pungam (26.27%), with a mean disease index of 41.84 per cent. Similarly, in Bharuch Taluka, the average disease index was highest at Mangleshwar (41.65%) followed by Shuklatirth, Nikora and Tavara (28.63, 23.00 and 17.48% respectively), while least at Jhanor (14.81%) with a mean disease index of 25.11 per cent

Mukhtar et al (2019) reported a new record of *C beticola* on spinach in Pakistan. Kumar et al (2021) recorded the disease intensity of cercospora leaf spot of mungbean from 29.38 to 51.20 per cent in all surveyed areas of Rajasthan. The highest disease intensity was recorded from Nagaur (50.33%) and least from Tonk (32.07%). Liu et al (2020) recovered *C beticola* on spinach leaves in addition to other fungi in Texas, California, Arizona and South Carolina from 2016 to 2018. *C beticola* caused significant leaf damage.

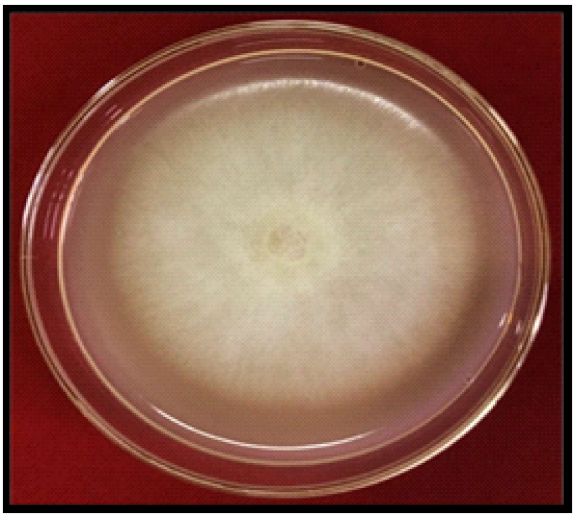

Fig 1. Pure culture of *Cercospora beticola*

Fig 2. Symptoms of cercospora leaf spot

Table 2. Disease index of cercospora leaf spot disease of spinach at different villages of Bharuch district during 2020-21

Taluka	Village	Latitude	Longitude	PDI
Bharuch	Tavara	21.7400000	73.0612607	17.48
	Mangleshwar	21.7649312	73.1252729	41.65
	Shuklatirth	21.7555513	73.1279444	28.63
	Nikora	21.7846869	73.1314926	23.00
	Jhanor	21.8388625	73.1193902	14.81
			Mean	25.11
Ankleshwar	Diva	21.6461993	72.9806982	56.61
	Sakkarpor	21.6463380	72.9281034	37.69
	Divi	21.6539036	72.9878050	41.84
	Pungam	21.6196071	72.9415235	26.27
	Borbhata Bet	21.6774791	73.0091752	46.82
			Mean	41.84
			Overall mean	33.47

PDI = Per cent disease index

REFERENCES

Bashir T 2017. Studies on cercospora leaf spot of spinach in Kashmir. MSc (Agric) Thesis, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India.

https://ag.umass.edu/vegetable/fact-sheets/cercospora-leaf-spot-of-swiss-chard-beets-spinach (Retrieved: 02.03.2023)

Johnston A and Booth C 1983. Plant pathologist's pocket book. 2nd Edn, Commonwealth Mycological Institute, Kew, Surrey England, 439p.

Kumar N, Singh M, Prajapati S, Lakhran L, Maurya S and Kumar S 2021. Pathogenic variability of cercospora leaf spot disease of mungbean caused by *Cercospora canescens* in surveyed areas of Rajasthan. Biological Forum **13(4):** 76-79.

Liu B, Stein L, Cochran K, do Toit LJ, Feng C, Dhillon B and Correll JC 2020. Characterization of leaf spot pathogens from several spinach production areas in the United States. Plant Disease **104(7):** 1194-2004.

Mayee CD and Datar VV 1986. Phytopathometry. Technical Bulletin 1, Marathwada Agricultural University, Parbhani, Maharashtra, India, 146p.

Morelock TE and Correll JC 2008. Spinach. In: Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae and Cucurbitaceae (J Prohens and F Nuez, Eds), Springer, New York, pp 189-218.

Mukhtar I, Khokhar I, Yan Y and Xie B 2019. First report of cercospora leaf spot caused by *Cercospora beticola* on spinach in Pakistan. Plant Disease **103(7):** doi: 0.1094/PDIS-12-18-2274-PDN.

Wheeler BEJ 1969. An introduction to plant diseases. John Wiley and Sons Limited, London, 374p.