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COMPARATIVE STUDY OF SPINACH LEAF NUTRIENT CONTENT FROM LEAF SPOT DISEASE *CAUSED BY CERCOSPORA BETICOLA* SACC.(1876) BEFORE AND AFTER DISEASE.

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Abstract:

Spinach is a leafy vegetable grown for its nutrient content. Throughout the world, it is consumed raw and cooked. *Spinaciaoleracea* L. is reported to be native to central Asia (Persia). Leaves and tender shoots are consumed fresh, processed, or mixed with other vegetables. Spinach (*Spinaciaoleracea* L.) is from the family*Chenopodiaceae*. *Cercospora beticola* Sacc. ***(***1876***),***causes leaf spot disease on the leaves of spinach. When inoculated with isolated Cercospora, healthy leaves will develop the same leaf spot disease. Once the disease grew then, nutrients are isolated and compared with control spinach leafcontent.*Cercosporabeticola*, a pathogen, alters, and reduces the concentration of total carbohydrates 8.571 %, reducing sugar 22 %, dietary fibre 17.391 %, lipid 100 %, vitamin C 100 % and chlorophyll content 99.791 % of Spinach leaves. The protein level increases by 0.690 % and amino acids by 8.334 % in Spinach leaves.

Keywords: Spinach, Cercospora, Nutrients and Disease

Introduction:

Throughout World, it is consumed raw and cooked. *Spinaciaoleracea* L. is reportedly native to central Asia (Persia) (Ribera*et al.*, 2021). Leaves and tender shoots are consumed fresh, processed, or mixed with other vegetables.Grown worldwide for its nutrient contents, likewise in India also.Maharashtra stands first in the production of spinach among Indian states. Cercospora leaf spot is a prevalent pathogen causing leaf spot disease in spinach worldwide. When grown properly, 80 to 100 quintals per acre in the Indian environment (www.agrifarming.in). Spinach is a leafy vegetable grown for its nutrient content.

Spinach (*Spinaciaoleracea* L.) is from the family *Chenopodiaceae*. *Cercospora beticola* Sacc. ***(***1876***),***causes leaf spot disease on the leaves of spinach. When inoculated with isolated *Cercospora*, healthy leaves will develop the same leaf spot disease. Once the disease grew then, nutrients are isolated and compared with control spinach leafcontent.

Material and methods:

For this study, the material and method were divided into Collection of the sample, Disease characters, Pathogenicity test, Isolation of nutrients from the sample.(Dongre 2021; Dongre and Borse 2021).

Collection of samples:

The collection of samples was done based on geographical zone. Maharashtra is divided into five Geographical zones(Singh *et al.*, 2004); forthe study, at least two collection sites are considered from each zone (except in a few cases). Collecting samples for the nutrient assay was done from every zone of Maharashtra. At least two samples were selected from each zone, the location of the collected spot was recorded, and all samples were kept in

sterilised plastic bags.As the laboratory was very long from the collected spot, the sample was sealed on the spot and placed in a chilled condition to avoid contamination and spoilage. (Table-I)

Table 1: Sample Collection site Cercosporabeticola on Spinach leaves

Place of collection	Geographical data
Jalgaon	21°00'20.9"N 75°33'23.1"E
Shirpur, dhule	21°20'42.4"N 74°52'45.3"E
Ratnagiri	16°59'16.3"N 73°18'42.7"E
Palghar	19°41'53.9"N 72°46'15.8"E
Aurangabad	19°54'17.6"N 75°21'42.1"E
Nandel	19°11'06.8"N 77°17'52.9"E
Wadgaonsheri, pune	18°32'38.3"N 73°55'23.4"E
Satara	17°41'00.9"N 73°59'16.8"E
Amravati	20°51'11.7"N 77°43'50.2"E
Goregaon	21°20'43.2"N 80°12'23.7"E

Disease symptoms:

The symptom of this disease starts with small brown spots on both sides of the leaf. The disease spots wereoval or circular, with a reddish margin and greyish core.The spot diameters were up to 4-6 mm. later, the spots enlarged and formed necrotic spots.

Pathogen Characters:

Pathogen identification was by observation of culture characters and morphology. Dense fascicles of conidia are emerging from stomata. Conidiophore dark coloured and septet (1-3).The length of the conidiophore was 20 – 65 µm, and the diameter of the conidiophore was 3.5 - 4.5 µm conidiophores septate and showed distinct knee bending. Conidia are borne singly, conidia hyaline and needle-like. Multiseptated, slightly broader at one end and tapered at another end.The length of conidia ranges from 40 - 125 µm in length and 2.5 - 4 µm in diameter (Kim & Shin, 1998). (Table-2;Figure-1)

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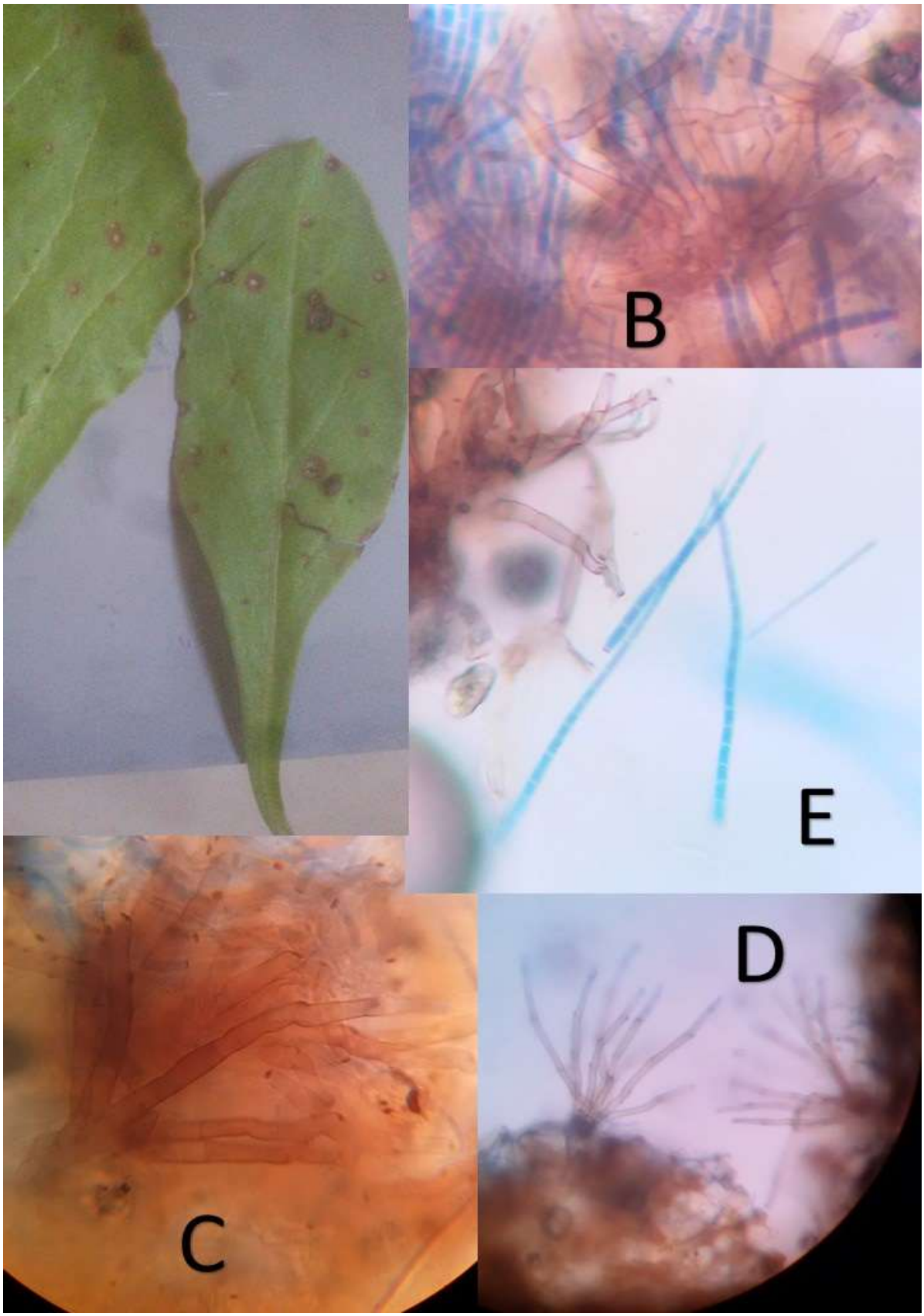

Figure 1Cercosporabeticola on Spinach leaf (A), section showing Hyphae, Conidiophore (B) and Conidiophore high power (C) and Low power resolution (D).

The colony on PDA shows greyish or ash colour, smooth margin, and white in the centre region. The colony diameter was found to be 90 mm. 28^{O}C ± wasa suitable temperature for growth.

Pathogenicity test:

Isolated pathogen directly inoculated on healthy plotted spinach leaf and later observed for change at the site. The same disease spot developed after 5 days of inoculation confirms the pathogen's ability.

Table 2:Culture and morphological characters of Cercosporabeticola on Spinach leaves

Geographical site of collection	Conidiophore size µm	Conidia size µm
21°00'20.9"N 75°33'23.1"E	37 X 4.1	65 X 3.1
21°20'42.4"N 74°52'45.3"E	65 X 4.5	125 X 4.0
16°59'16.3"N 73°18'42.7"E	52 X 4.1	89 X 3.8
19°41'53.9"N 72°46'15.8"E	40 X 4.1	60 X 3.1
19°54'17.6"N 75°21'42.1"E	20 X 3.5	40 X 2.5
19°11'06.8"N 77°17'52.9"E	28 X 3.6	56 X 2.7
18°32'38.3"N 73°55'23.4"E	60 X 4.3	116 X 3.9
17°41'00.9"N 73°59'16.8"E	48 X 4.2	69 X 3.1
20°51'11.7"N 77°43'50.2"E	52 X 4.2	92 X 3.6
21°20'43.2"N 80°12'23.7"E	58 X 4.3	102 X 3.8

Nutrient estimations:

Various nutrients are estimated by using protocols and processes given by various workers. Carbohydrate was estimated according tothe method given by Hedge, J E and Hofreiter, B T (1962). Crude fibre is estimated by acid alkali treatment method in which e initial andfinal weight after ignition at 600 ^{O}C give the crude fibre content in the sample. Reducingsugar estimated using Nelson-Somogyi'sarsinomolybdate reagent method.Lowry's (1951) method helped to estimate protein. Oil was extracted with Soxhletapparatusand Petroleum ether as a solvent, a protocol given by Bligh,E.G., andDyer,W.J. (1959). The Ninhydrin methodwas applied to estimate free aminoacids (Mahesha,2012). Dry matter content and watercontent were analysed by using the procedure given by Ruck (1969).Vitamin C content was estimated using2,6- dichlorophenol-indophenol dyesolution, the procedure given by Sadasivam andManickam, (2006). Whenever required, Systronics 2202 double-beam UV-visible Spectrophotometer was used to calculate values.

Observation:

Table 3: Nutrient analysis from healthy and diseased Spinach leaves and percentage comparison of nutrient content.

Sr. no.	Nutrients	Content in 100 Gram Healthy material	Content in 100 Gram diseased vegetable	% alteration due to disease
1.	Water content	91.0 Grams	85.00 Grams	-6.593
2.	Total carbohydrate	3.5 Grams	3.2 Grams	-8.571
3.	Reducing sugar	0.5 Grams	0.39 Grams	-22
4.	Fibre	2.4 Grams	1.90Grams	-17.391
5.	Protein	2.9 Grams	2.92 Grams	0.690
6.	Amino acids	2.4 Grams	2.6 Grams	8.334
7.	Lipids	0.3 Grams	00 Grams	-100
8.	Vitamin C	29 mg	00 mg	-100
9.	Chlorophyll content (total)	96 mg	0.20 mg	-99.791
10.	Dry matter	9 Grams	15 Grams	66.666

Result :

When the pathogen was grown in culture media, conidiophore, conidia and colony morphology compared with relevant literature confirmed the name *CercosporabeticolaSacc. (1876).*

After inoculation of the pathogen isolated from the diseased sample on the healthy plotted plant, confirm pathogenicity.

Comparison between healthy and diseased nutrientsexplains qualitative loss or gain of that specific nutrient. Here it was clear that dry matter (66.667 %), free amino acids (8.334%) and protein (0.690%) get elevated from its control sample, respectively. While percentage loss was seen in total carbohydrate (-6.593%), reducing sugar (-22%), fibre(-17.391%), lipid(-100%), vitamin C (-100) and total chlorophyll content (-99.791%) respectively.

Conclusion:

After fully developing the disease, most nutrients decline, but it was noticed that protein content and free amino acid content elevated from normal one. The water content declines, resulting in a quantitative increase of dry matter.

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