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Study of garlic leaf essential metabolites content change after *Stemphylium vesicarium* (Wallroth) E.G. Simmons (1969) infection

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Garlic leaves were collected and analysed for their primary food content for a total of ten years from the year 2011 to 2020 from all over Maharashtra. The content of leaves shows variations as the locality, season, and environment change. This research shows the effect of pathogenic fungus, *Stemphylium vesicarium*, which causes change after its growth and later disease development. During the growth of the fungus, the food from leaves was consumed or absorbed for survival and made leaf nutrients deficient. Maharashtrian people, especially in tribal villages, consume raw salads or mixed with others as food adjuncts.

Keywords: Garlic, fungi, metabolites, nutrients, pathogen, *Stemphylium*

INTRODUCTION

Allium sativum L., garlic belongs to Family Aliaceae (onion family). In the world scenario, China is top in production while India ranks second. More than 100 countries are known to cultivate garlic (Atlasbig.com). Most people use heads or bulbs, plant parts growing below the earth. In many countries like India, green leaves are consumed uncooked or mixed with other food for flavouring and taste.Garlic cultivation practice is the oldest in the world, and Indian as well as Egyptian peoples cultivated it around 5000 years ago. History also suggests Babylonians used it 4500 years ago and the Chinese 2000 years ago. According to Rivlin (2001), garlic is used as medicine and to treat various diseases in Egypt and Greece, Rome, China and India; it is also used to increase work strength and use as performance-enhancing material.

Maharashtra ranks seventh among the Indian states of garlic production (FAO-Shrivastava *et al*., 2012) and is now in tenth position in 2022 (https://www.statista.com, 2022).

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Cultivation for commercial purposes is found in Khandesh, Vidharbha, Marathwada and Western Maharashtra region; the Konkan region is also growing this but not commercially.Garlic leaves are rich in antioxidants, showing medicinal properties like antibiotics as found in the bulb. The active constituent of garlic extract is allicin (Wanyika *et al*. 2011). Garlic leaves are a rich source of dietary fibre, vitamin C, carbohydrates, lipids and many minerals.

Many pathogens attack garlic plants and among them, *Stemphylium vesicarium* is one; this pathogen causes purple blotch disease on leaves (Dongre and Borse, 2015). All living organisms, including plants and pathogens, require nutrients for growth (Fatimaand Senthil-Kumar, 2015), and hence when pathogens start growing on the plant part from where it is trying to absorb nutrients and weaken that area.

Stemphylium vesicarium forms purple-coloured spots, which are numerous and close to each other, and look like an irregular area; hence, the disease is a purple blotch. Generally, it is growing along with another pathogen, *Alternaria porri* and both were found in mixed conditions throughout Maharashtra in this study.

MATERIALS AND METHODS

Methodology was divided into collection of the sample, disease characters, pathogenicity test, and isolation of nutrients from the sample following the method described by Dongre (2019, 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). For the 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. The samples from the selected site were collected on the spot and kept in sterilised plastic bags. The sample was sealed on the spot and placed in a chilled condition to avoid contamination and spoilage (Table 1)

Disease symptoms

Stemphylium leaf blight on Onion and Garlic was first described by Rao and Pavgi (1975) from India, especially from Varanasi, Uttar Pradesh. Infections were occurring on the aerial part of plant leaves and inflorescence stalk.

A small yellowish to orange colour streak is a sign of the initial stage of the disease; later, this enlarges and soon converts to an oval elongate spot. Oval, tan to brown lesions resembling purple blotches, Stemphylium leaf blight can be seen on leaf blades. The margin of the spot shows a pinkish or yellowish shade. Yellow striations cover the blade in both directions from the lesion before turning brown. As they spread, purple blotch lesions might develop dark-brown concentric circles. Lesions with purple blotches may grow more prominent, encircling the Leaf and killing it. Similarly, Stemphylium leaf blight develops many minute lesions that form blighted leaves. This infection starts from the Leaf's tip and proceeds toward the garlic base.

Mostly *Alternaria porri* and *Stemphylium vesicarium* appear simultaneously. Dew or rain enhances the severity of the disease. Winter Summer transition season, i.e., January to March, is a more favourable season for the disease in Maharashtra.

Pathogen morphology

Mycelium branched, septate, darkly pigmented, generally yellowish, ranges in diameter from 2.8 to 3.5 µm. Conidiophores are branched, cylindrical, straight or slightly curved, and they enlarge to a diameter of 3 to 5 µm at the tip. Muriform conidia exhibit vertical and horizontal septa. 2 to 5 transverse and 4 to 8 longitudinal septate, a brownish tint. Conidia range in size from 14.2 to 20.6 µm in diameter and 30.5 to 36.3 µm in length. The morphological data confirm the pathogen as *Stemphylium vesicarium Simmons* (1969) (Table2;Fig.1)

The colony on PDA shows dark brown colour and a yellowish margin. The colony at 32^0C grow very fast and, within seven days, occupy the entire plate. The reverse of the colony is yellow coloured.

Table 1: Sample collection site showing the region and coordinate

Collection site Region	Site Coordinate
Khandesh	21°04'56.8"N 74°50'33.5"E
Khandesh	21°00'04.3"N 74°18'16.5"E
Vidharbha	21°04'25.3"N 80°22'01.7"E
Vidharbha	21°24'19.8"N 79°56'00.9"E
Western Maharashtra	19°05'11.0"N 74°44'21.9"E
Western Maharashtra	17°40'23.5"N75°55'02.0"E
Konkan	19°04'18.0"N 73°00'31.7"E
Konkan	19°04'18.0"N 73°00'31.7"E
Marathwada	19°53'24.9"N 75°20'08.5"E
Marathwada	18°24'20.3"N 76°34'42.9"E

Pathogenicity test

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

Table 2 : Morphological characters of *Stemphylium vesicarium*from garlic leaves

Collection site	Size of conidia Length X Diameter (µm)	Numbers of septa Transverse	Longitudinal
21°04'56.8"N 74°50'33.5"E	35.6 X 19.8	4	7
21°00'04.3"N 74°18'16.5"E	34.8 X 20.1	3	5
21°04'25.3"N 80°22'01.7"E	31.5 X 17.6	2	6
21°24'19.8"N 79°56'00.9"E	34.7 X 18.2	4	8
19°05'11.0"N 74°44'21.9"E	36.3 X 20.6	5	8
17°40'23.5"N 75°55'02.0"E	35.1 X 16.5	5	7
19°04'18.0"N 73°00'31.7"E	30.5 X14.2	2	5
19°04'18.0"N 73°00'31.7"E	32.6 X 15.9	4	8
19°53'24.9"N 75°20'08.5"E	33.8 X 20.1	5	7
18°24'20.3"N 76°34'42.9"E	36.1 X 20.4	4	8

Table 3 : Nutrient content in Healthy and Diseased vegetables and % alteration of content by *Stemphylium vesicarium*on Garlic leaves

Nutrients	Content (g/100 gfresh material)	Content (g/100 g diseased vegetable)	% alteration due to disease
Water content	69.00	75.10	8.840%
Total carbohydrate	6.80	2.90	-57.352%
Reducing sugar	3.80	2.10	-44.736%
Fibre	3.10	2.40	-22.580%
Protein	1.39	1.60	15%
Amino acids	2.80	3.46	23.571%
Lipids	0.20	00	-100%
Vitamin C	0.062	0.013	-79.687%
Chlorophyll content(total)	0.0134	00	-100%
Dry matter	31.00	24.90	-19.677%

Nutrient estimations

Utilising guidelines and methods provided by several workers; various nutrients are estimated. The method described by Hedge and Hofreiter (1962) was used to estimate the amount of carbohydrates. Using an acid-alkali treatment method, the crude fibre content was analysed by weighing the sample's initial and final weights before and after ignition at 600^{0}C. Using Nelson-Somogyi's arsenomolybdate reagent technique, reduced sugar is estimated. Protein was estimated using Lowry's (1951) technique. Oil was extracted using the Soxhlet method with Petroleum ether as the solvent, following the instructions provided by Blighand Dyer(1959). Ninhydrin technique as

described by Mahesha (2012),was used to measure free amino acids. The dry matter and water content were evaluated using the method proposed by Ruck (1969). The vitamin C concentration was measured using a 2,6-dichlorophenol-indophenol dye solution and the method described by Sadasivam and Manickam (2006). Values were calculated as needed using a Systronics 2202 double-beam UV-visible Spectrophotometer.

RESULTS AND DISCUSSION

Conidiophore, conidia, and colony morphology were compared with pertinent literature while the pathogen was cultured in culture conditions, confirming the name *Stemphylium vesicarium* (Wallroth), E.G. Simmons (1969). Details are provided in Table 2.

Pathogenicity was confirmed after inoculating the pathogen from the diseased sample on the healthy potted plant (Fig.1).

Nutrient's qualitative loss or gain is explained by comparing healthy and diseased material(table 3, Fig.2) . It was evident that protein (15%) and free amino acids (23.571%) rose from the control sample, respectively. Dry matter (-19.667%), total carbohydrate (-57.352%), reducing sugar (-44.726%), fibre (-22.580%), fat (- 100%), vitamin C (-79.687), and total chlorophyll content (-100%) all experienced percentage losses, accordingly.

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

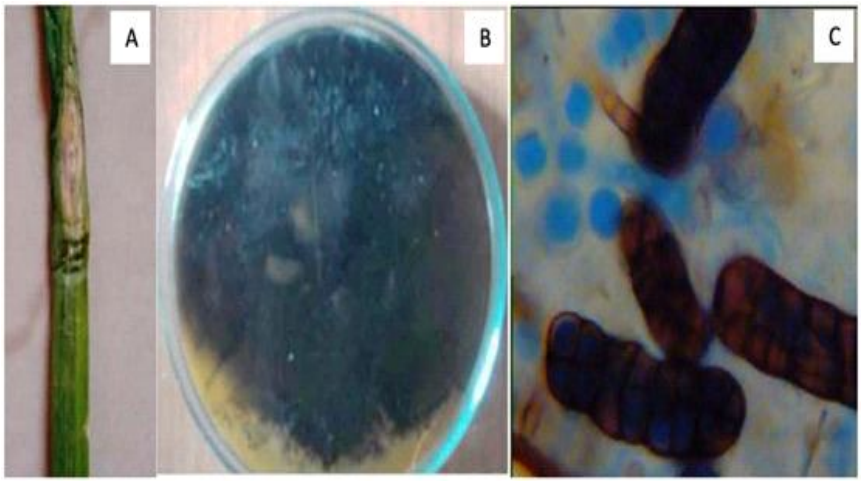

Fig. 1 : A- Leaf showing symptoms of disease; B- Growth of isolated pathogen in Petri dish; C- Conidia of *Stemphylium vesicarium*

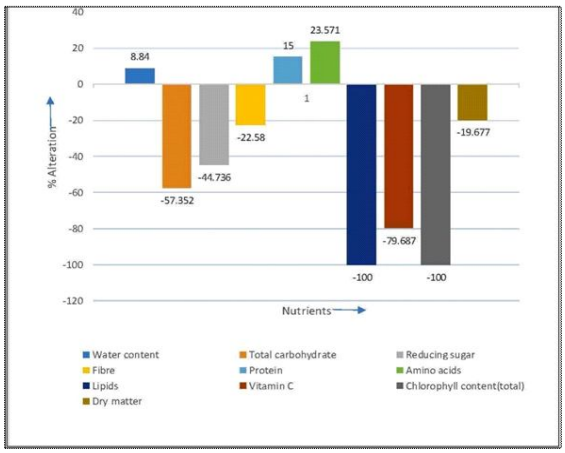

Fig. 2: Percentage loss or gain of nutrients in garlic leaves after disease development by *Stemphylium vesicarium*

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