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Effect of temperature on morphology and cultural characteristics of *Corynespora* pathogen of cotton under South Gujarat of India

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Abstract
Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops playing a key role in the economic and social scenario of the globe. India is one of the major cotton growing countries in the world. India ranks first in area and second in the total production of cotton in the world. Cotton is grown worldwide for its natural fiber and oil. As cotton seed contains 30 percent starch, 25 percent oil and 16.20 percent protein. Looking to the overall situation, it is felt necessary further to investigate its potential in terms of morphology and cultural characteristics. In this experiment, effect of different temperature on morphological and cultural characteristics of the *Corynespora cassiicola* pathogen was studied in cotton. Result showed that at 30 °C temperature, there was the maximum dry mycelium weight (60.33 mg) and abundant (++++) sporulation was noticed. The size of conidia was maximum at 30 °C (126.00 × 8.30 µm) followed by 25 °C (118.23 × 7.82 µm) was recorded. The cultural studies of *C. cassiicola* was made by growing single spore cultures on PDA medium at various temperatures *in vitro* yielded the largest colony diameter (90.00 mm) at 30 °C temperature.

Keywords: Cotton, *Gossypium*, temperature, morphology, cultural, pathogen, disease

Introduction
Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops playing a key role in the economic and social scenario of the globe. It is the oldest among all the commercial crops of the world, providing fiber for the clothing of mankind. It is also known as "The white gold" or "The king of fibers". Cotton is a premier cash crop of our country and belongs to the family Malvaceae (Anonymous, 2017a) [2]. It is one of the most ancient and important commercial crops next only to food grains and is the principal raw material for flourishing the textile industry.

Cotton is grown worldwide for its natural fiber and oil. Cotton seed contains 30 percent starch, 25 percent oil and 16.20 percent protein. It is also being used in the manufacture of medicinal supplies, tarpaulin, cordage and belting. The cotton hulls serve as roughage for livestock and the fuzz (short seed hair) is used in the manufacture of papers, plastics, carpets, rayon, explosives and cotton wools *etc.* (Prasad, 2015) [11].

India is one of the major cotton growing countries in the world. India ranks first in area and second in the total production of cotton in the world. Hence, India has a large domestic textile industry. It is chiefly grown in Maharashtra, Gujarat, Andhra Pradesh, Madhya Pradesh, Punjab, Tamil Nadu and Karnataka. India is the largest cotton growing country in the world with an area of around 134.77 lakh hectare with production of 360.65 lakh bales and productivity of 455 kg/ha, (Anonymous 2017b) [3]. In Gujarat, cotton is cultivated in an area of 26.55 lakh hectare and the production of 86.17 lakh bales with productivity 552 kg/ha (Anonymous (2017b) [3].

The *C. cassiicola* is a cosmopolitan fungal plant pathogen that infects 530 plant species from 380 genera, including cotton and soybean. It has a large geographical distribution from Japan, the tropics of Brazil to North America and can be found on leaves, stems, roots and within nematode cysts, among monocots, dicots and on one species of cycad. The fungus is ubiquitous in nature and can act as an endotroph or saprotroph means it can act as a pathogen or present on the plant material with no pathogenic effect (Seaman and Shoemaker, 1965) [13]. The pathogen produces conidiophores, which are solitary or in clusters that generate a single conidium at the broad apical pore. This single conidium is the spore of the fungus. The conidium can adopt a variety of different shapes and shades from hyaline and straight, to

brown and slightly curved and further proving genetic diversity in the fungus. Pathogenic infection of susceptible species roots will lead to root rot, while pathogenic infection of susceptible species leaves produces distinguishable necrotic target shaped spots. The border region of the target spot is a light yellow to light green halo and these lesions, if not controlled by fungicides or unfavorable environments, will lead to premature defoliation, otherwise known as leaf fall (Seaman and Shoemaker, 1965) [13].

Target spot has been a concern for farmers and researchers due to its increasing occurrence especially on cotton (Sumabat *et al*., 2018) [14] owing to the monoculture farming, adoption of conservation tillage systems, susceptibility of current cultivars, lack of crop rotation and optimal weather patterns for disease development (Koenning *et al*., 2006 [9]; Avozani *et al*., 2014) [4].

The initial symptoms of target spot in cotton are characterized by small spots on the leaves located in the lower stratum of the plant (Conner *et al*., 2013) [6]. The symptoms were observed in the lower canopy, which progressed upward to cover the entire plant. Initially, leaves exhibited circular to irregular, dark red, small and numerous lesions, which over the time became brown (5-10 mm) surrounded by a dark border. As lesions matured, alternating rings of light and dark brown bands developed. The most mature lesions presented like a target type appearance.

Lesions may present as concentric rings (Fulmer *et al*., 2012) [7] and in case of great severity, the leaves acquire a yellowish colour and easily detach from the branches resulting in defoliation (Corner *et al*., 2013) [6]. Looking into the occurrence of the *Corynespora* leaf spot disease in cotton crop, it has the potential to spread drastically over a large area. So, it is felt necessary to find out this experiment. Thus, the present study has been taken up with the specific objectives.

Material and Methods

Morphological variation

The isolate was cultured in liquid media in a 100ml flask containing 20 ml of Potato Dextrose Broth (PDB) medium in different temperatures as 15, 20, 25, 30, 35, 40 and 45 ºC for 15 days. After incubation, average measurements were taken by the micrometry method (Patel *et al*., 2020) [10].

The morphological characters like size (length and width) of conidia were recorded. The observations were recorded in three repetitions of each isolate in different temperatures. The study was carried out using an ocular and stage micrometer after mounting them on the slides containing sterile distilled water at required magnification of 40X. Data were analysed statistically using complete randomized design.

The following morphological characters were recorded under different temperatures on PDB medium after 15 days of incubation.

- Dry mycelium weight (mg)
- Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant
- Size (µm) and no. of conidia

Design: Completely Randomized Design (CRD)

Treatments: 7 and

Repetitions: 3

Location: Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat

Cultural variation

The isolate was separately cultured on PDA medium in different temperatures as 15, 20, 25, 30, 35, 40 and 45 ºC for 10 days. The 5 mm disc of *C. cassiicola* isolate was inoculated on the PDA medium containing Petri plates and incubated at different temperatures. After 10 days of incubation period, the diameter of the fungal mycelial growth, colony characters and sporulation were recorded.

Following cultural characters were recorded under the different temperatures on PDA medium.

- Colony diameter (mm)
- Sporulation category: -Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant
- Colony characters

Design: Completely Randomized Design (CRD)

Treatments: 7 and

Repetitions: 3

Location: Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat

Result & Discussions

Morphological characteristics

Morphological investigations of *C. cassiicola* using PDB medium indicated the differences in growth and sporulation as well as the size of conidia and conidiophores. (Photograph 1).

Growth and sporulation

At 30 °C temperature, the maximum dry mycelium weight (60.33 mg) was detected along with the abundant sporulation (++++) category, however at 45 °C neither growth nor sporulation was recorded (Table 1).

Conidia

Conidia were borne singly, ranging from subhyaline, olivaceous and obclavate to cylindrical, straight to slightly curved and containing 2 to 14 pseudosepta. The size of the conidia was maximum at 30 °C (126.00 X 8.30 µm) followed by 25 °C (118.23 X 7.82 µm), 35 °C (98.50 X 6.90 µm), 40 °C (96.50 X 6.60 µm), 20 °C (71.63 X 6.57 µm) and 15 °C (63.33 X 4.70 µm) was recorded. At 45 °C temperature, no conidia were produced (Table 1 and Fig.1).

Conidiophore

Conidiophores were simple, erect and intermittently branching with septate and gave rise to single and subhyaline conidia.

The findings of the morphological variations such as dry mycelium weight, and sporulation are compatible with those of Ahmed *et al.* (2013) [1], Hailmi *et al*. (2019) [8] and Salunkhe *et al*. (2019) [12]. They discovered that maximum dry mycelium weight (mg) and sporulation of *C. cassiicola* occurred between 25 and 30 °C.

The results of size of conidia and no. of septa are corroborated with the research findings obtained by Flumer *et al*. (2012) [7], Conner *et al*. (2013) [6] and Butler *et al*. (2016) [5].

Photograph 1: Growth and sporulation of *C. cassiicola* under different temperature

Table 1: Growth, sporulation and size and no. of septa of conidia of *Corynespora cassiicola* under different temperature on PDB medium after 15 days of incubation

Temperature (°C)	Dry mycelium weight (mg)	Sporulation Category	Conidia	
			Size (µm)	No. of septa
15	2.00	++	63.33 X 4.70	2-8
20	37.00	+++	71.63 X 6.57	2-10
25	52.83	++++	118.23X 7.82	4-12
30	60.33	++++	126.00 X 8.30	4-14
35	28.50	++	98.50 X 6.90	3-10
40	19.20	+	96.50 X 6.60	2-10
45	00.00	-	-	-
S.Em±	0.53			
CD at 5%	1.66			
CV%	2.78			

Sporulation category: Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant
Note: Those treatment values are zero in all the repetitions are discarded from the ANOVA

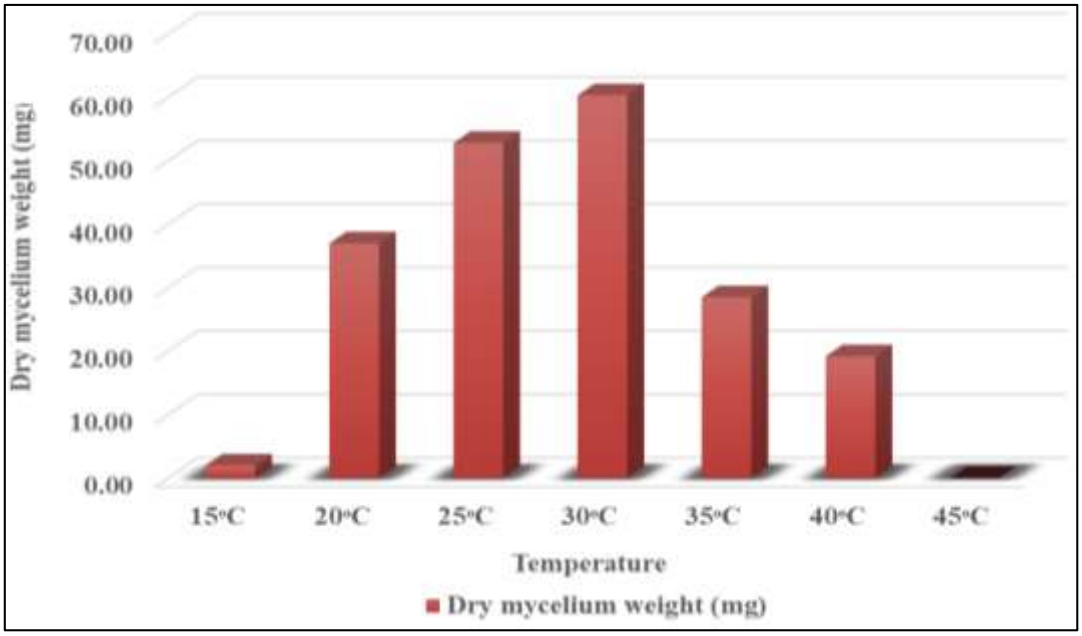

Fig 1: Colony diameter of *Corynespora cassiicola* at different temperature

Cultural characteristics

The cultural studies of *C. cassiicola* was made by cultivating the single spore culture on PDA medium at various temperatures and recorded the colony diameter (mm), cultural characteristics and sporulation (Table 2, Photograph 2 and Fig 2).

After 10 days of incubation, the colony diameter recorded the maximum of 90.00 mm at 30 °C, followed by 84.26 mm at 25 °C, 77.96 mm at 20 °C, 60.70 mm at 35 °C, 32.00 mm at 40 °C, 21.33 mm at 15 °C and 10.00 mm at 45 °C temperature.

C. cassiicola was different in colony characters at different temperatures. At 15 °C produced flat, dim gray with dark brown center, at 20 °C gray, dense and velvet with reddish brown center, at 25 °C outer side light gray, dense and velvet, raised with dark brown center, at 30 °C light gray turning to dark gray, colony dense and velvet, raised dark brown center and outer side tan brown, at 35 °C produced gray, dense and velvet, raised with dark brown center, at 40 °C produced whitish gray, colony dense and velvet with dark brown center and at 45 °C whitish gray with light brown center colony was

observed.

The findings of the cultural variations such as mycelial growth, colour and sporulation are compatible with those of Ahmed *et al.* (2013) [1], Hailmi *et al.* (2019) [8] and Salunkhe *et al.* (2019) [12]. They discovered that maximum mycelial growth and sporulation of *C. cassiicola* occurred between 25 and 30 °C.

Table 2: Colony diameter, sporulation and cultural characteristics of *Corynespora cassiicola* under different temperature on PDA medium after 10 days of incubation

Temperature (°C)	Colony diameter (mm)	Sporulation category	Cultural characteristics
			Colony characters
15	21.33	++	Flat, Dim gray with dark brown center
20	77.96	+++	Gray, colony dense and velvet with reddish brown center
25	84.26	++++	Outer side light gray, colony dense, velvet and raised with dark brown center
30	90.00	++++	Light gray turning to dark gray, colony dense and velvet, raised dark brown center with outer side tan brown
35	60.70	++	Gray, colony dense and velvet, raised with dark brown center
40	32.00	+	Whitish gray, colony dense and velvet with dark brown center
45	10.00	-	Whitish gray and light brown center
SEm±	0.94		
CD at 5%	2.89		
CV%	3.04		

Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant

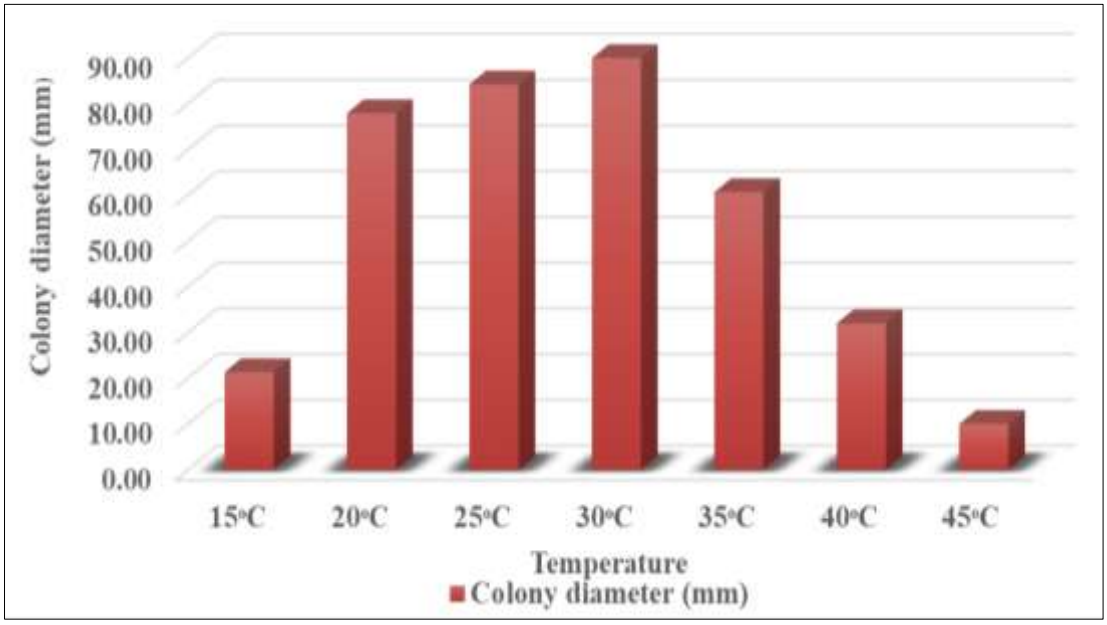

Fig 2: Colony diameter of *Corynespora cassiicola* at different temperature

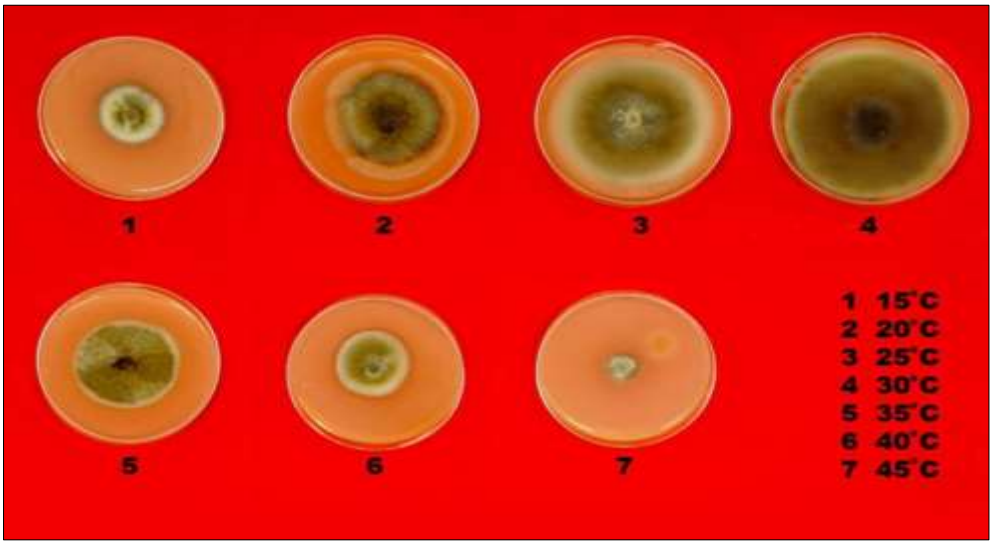

Photo 2: Growth and pigmentation of *Corynespora cassiicola* under different temperature

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