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RESEARCH

ASSESSMENT OF DIFFERENT GROWTH MEDIA ON *CORYNESPORA CASSIICOLA* UNDER *IN VITRO* TEST

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Abstract: Cotton (*Gossypium hirsutum* L.) is one of the world's most important fiber crops with a significant economic and social impact. It is the world's oldest commercial crop, which provides fiber for mankind's garments. It is also known as "The king of fibers" or "The white gold". Cotton is primarily a raw material for a thriving textile industry and is also one of the most ancient and essential commercial crops, next to food grains. Among which, the Corynespora leaf spot of cotton caused by *Corynespora cassiicola* is observed in moderate form causing considerable damage in recent times but the disease has the potential to be severe and it could become significant concern for the cotton growers. Here in this experiment, seven different growth media were tested for their suitability for the mycelial growth, colony colour, cultural characteristics and sporulation of the *C. cassiicola* under *in vitro* test. Among the seven different growth media tested, Potato dextrose agar and V-8 Juice media proved the best growth media for the mycelial growth of the pathogen. The best sporulation of *C. cassiicola* was observed in Potato dextrose agar media, while poor sporulation was observed in Nutrient agar media.

Keywords: Cotton, *Gossypium spp*, Treatment, Disease, Media, Sporulation

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops playing a key role in economic and social scenario of the globe. 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. Cotton is one of the most ancient and important commercial crop next only to food grains and is the principal raw material for a flourishing textile industry. It provides employment and sustenance to a population of nearly 42 million people, who are involved directly or indirectly in cotton production, processing, textiles and related activities (Manickam and Sankaranarayanan, 2013).

Currently, Gossypium includes 50 species, four of which are cultivated, forty four is wild diploids and two are wild tetraploids. Out of the four cultivated species, *Gossypium hirsutum* L. and *Gossypium barbadense* L. commonly called as new world cottons are tetraploids (2n = 4x = 52). Whereas, *G. herbaceum* L. and *G. arboreum* L. are diploids (2n = 2x = 26) and are commonly called as old world cottons. India with its unique distinction is the only country in the world which cultivates all four cultivable species of cotton (Khadi *et al*., 2010).

Cotton is an important fiber yielding crop of global importance, which is grown in tropical and subtropical regions of more than 80 countries of the world. The major cotton producing countries are USA, China, India, Pakistan, Uzbekistan, Egypt, Argentina, Australia, Greece, Brazil and Turkey. At global level, during the year 2022-23 China was estimated to produce 59.80 lakh tonnes of cotton followed by India 52.00, USA 31.96 and Brazil 29.46 lakh tonnes. Around 71 per cent of the world cotton production comes from these countries from 64 per cent of the world cotton area (Anonymous, 2022). For many developing and underdeveloped countries cotton export is the main source of foreign exchange earnings (Patil, 2007).

Cotton is grown worldwide for its natural fiber and oil. Cotton seed contain 30 per cent starch, 25 per

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cent oil and 16.20 per cent 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 wool (Prasad, 2015).

Cotton is grown in varied or diverse agroclimatic conditions varying from 8-32ºN latitude and 70-80ºE longitude in three areas *viz*., Northern, Central and Southern zones of India. Approximately 65 per cent of India's cotton is produced under rain fed conditions and 35 per cent on irrigated lands. Cotton is a warm climate crop sown in spring or early summer (April/May) and harvested in early winter (October/November). The optimum temperature is required for the growth of cotton ranged between 20 to 30ºC and primarily grown on black to alluvial soil with the pH range 5.5-8.5 (Chidambaram, 2007).

India is the largest cotton growing country in the world with an area of 130.49 lakh ha with an annual production of 337.23 lakh bales. In Gujarat, cotton is cultivated in an area of 25.49 lakh ha and the production of 87.12 lakh bales (Anonymous, 2023).

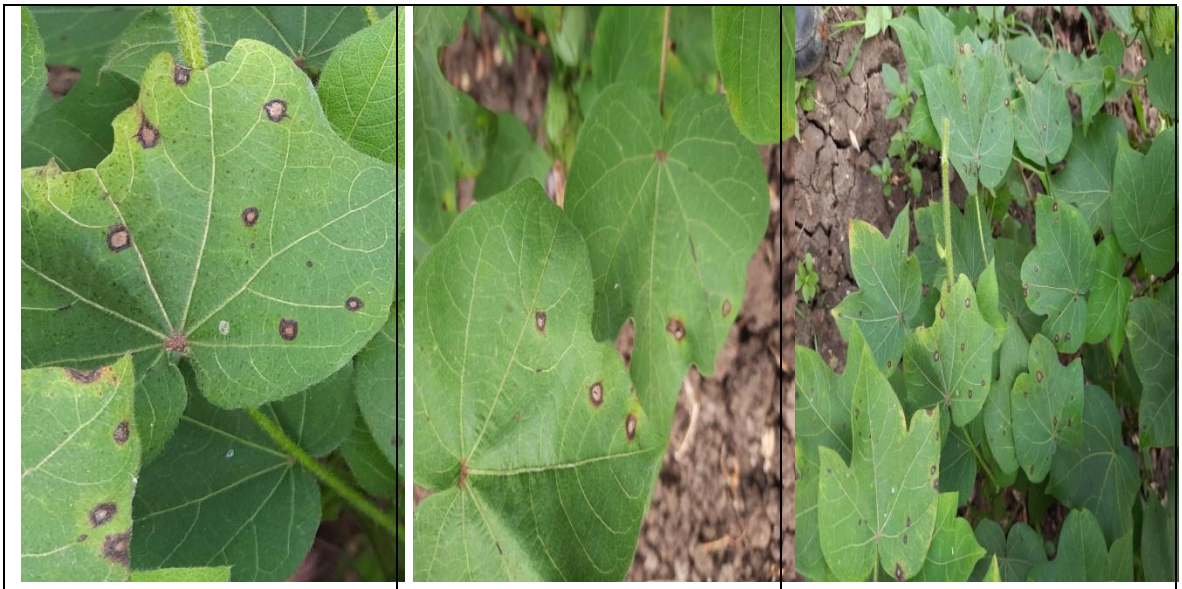
In India, the productivity of cotton is very low due to many constraints including diseases. Diseases are inherent compounds of the agro ecosystem that must be dealt with continuously and on a knowledge basis. Cotton is affected by various diseases caused by fungi, bacteria and viruses. The most common cotton diseases reported in India are Wilt (*Fusarium oxysporum f. sp. vasinfectum* (G. F. Atk.) W. C. Snyder & H. N. Hansen), Root rot (*Rhizoctonia bataticola* (Taubenh.), Verticillium wilt (*Verticillium dahliae* Kleb.), Anthracnose (*Colletotrichum gossypii* Southworth. or *C. capsici* (Syd.) Butler & Bisby), Grey mildew (*Ramularia areola* G. F. Atk.), Blackarm (*Xanthomonas campestris pv. malvacearum* (Pammel) Dowson), Corynespora leaf spot (*Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, Leaf blight (*Alternaria macrospora* Zimm) and Leaf curl (Cotton leaf curl virus), Boll rot and Physiological disorders as Para wilt, Leaf reddening and sometimes Leaf elongation due to improper use of weedicides *etc*. Among them Corynespora leaf spot of cotton is one of the important and serious disease of cotton. Looking to the history target leaf spot caused by *C. cassiicola* was reported in Alabama for the first time in 1959 on upland cotton (*Gossypium hirsutum* L.), the most widely planted species of cotton in the world (Jones, 1961, Onesirosan *et al*., 1975). In 1995, target spot was reported on upland cotton in Brazil but under the name of Corynespora leaf spot (Mehta *et al*., 2005). The first occurrence and re-emergence of the disease on upland cotton appeared from China (Wei *et al*., 2014), Brazil (Galbieri *et al*., 2014) and several states in the United States including Georgia (Flumer *et al*., 2012), Alabama (Campbell *et al*., 2012, Conner *et al*., 2013), China (Wei *et al*., 2014), Brazil (Galbieri *et al*., 2014, Dias *et al*., 2016), Louisiana (Price *et al*., 2015), Tennessee (Butler *et al*., 2016), Central India (Salunkhe *et al*., 2019) and South India (Bandi *et al*., 2022).In 2017, an unusual leaf spot on cotton (*Gossypium hirsutum* L.) was recorded from the Nagpur district, Maharashtra, India. Disease symptoms were observed in the lower canopy, which progressed upward to cover the entire plant. Initially the leaves exhibited circular to irregular, dark red, small, numerous lesions, which over time became brown (5 to 10 mm) and surrounded by a dark border. As lesions matured, alternating rings of light and dark brown bands developed. The most mature lesions presented a target-type appearance (Salunkhe *et al*., 2019).

Target spot has been a concern for farmers and researchers due to its increasing occurrence especially on cotton (Sumabat *et al*., 2018) 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, Avozani *et al*., 2014).

Corynespora leaf blight of cotton (*Gossypium hirsutum* L.) also referred to as "target spot" is caused by *Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, was reported in the cotton plant for the first time in 1959 in the state of Alabama, USA (Jones 1961, Onesirosan *et al*.,1975). After this occurrence, it has frequently appeared in cotton crops in the United States (Fulmer *et al*., 2012, Corner *et al*., 2013), China (Wei *et al*., 2014) and Brazil (Galbieri *et al*., 2014).

Diseases incited by this pathogen cause yield losses on several crops, *e.g*., cucumber (Blazquez, 1967), cowpea (Rodriguez and Melendez, 1984) tomato (Pernezny *et al*., 2002) and rubber (Fernando *et al*., 2010). Corynespora leaf spot has dominated Alternaria leaf spot in recent years and emerged as major leaf spot in cotton. Estimated yield losses due to target spot in selected cultivars of cotton (Deltapine 1050 and Phytogen 499) exceeded 336 kg/ha seed cotton (Conner *et al*., 2013). Lint yield loss due to target spot on apparently susceptible cotton cultivar had been estimated to be as high as 448 kg lint/ha (Hagan *et al*., 2015).

The initial symptoms of target leaf spot in cotton are characterized by small spots on the leaves located in the lower stratum of the plant (Conner *et al*., 2013). The primary symptoms of target spot in cotton observed as the lesions on the leaves were found to irregular, brick-red, tiny and numerous. Lesions grew, enlarged and often took on a target like look with the concentric ring formation within the spots. As the lesions merge, the leaves developed severe necrosis, which is followed by premature senescence, mortality and defoliation (Patel *et al*., 2022) (Photograph a, b and c).



Photograph a | Photograph b | Photograph C

Lesions may present as concentric rings (Fulmer *et al*., 2012) and in case of great severity, the leaves acquire a yellowish colour and easily detach from the branches, resulting in defoliation of the plant (Corner *et al*., 2013). This disease is newly introduced into South Gujarat region and has a wide host range including vegetables, ornamentals, weeds, yams, medicinals and other cash crops so, there is a high risk of cross infection possibilities (Peiris *et al*., 2015).Hence, it is felt necessary to conduct systemic investigation in the respective topic.

MATERIALS AND METHODS

With a view to find out the superior media for the mycelial growth, colony colour, cultural characteristics and sporulation of fungus, seven different media were studied. All the glass wares and plastic wares were soaked overnight in potassium dichromate sulphuric acid solution. After 24h, the glass wares were washed thoroughly in running water and rinsed with distilled water. The plastic wares were air-dried and the glass wares were sterilized in hot air oven at 160-180ºC for about 90min.

Isolations and purification were conducted under the aseptic condition of laminar air flow cabinet. Before working under the hood, the working surface was uniformly sterilized using 70 per cent alcohol. The blades, forceps and inoculation loop *etc*. were sterilized by incineration on the flame of the spirit lamp.

Each culture media was prepared in 1 liter of sterilized water and autoclaved at 121ºC at 15lb psi (1.036kg/cm^2) for 20min.These was cooled to 45ºC and then poured in 90mm Petri dishes for solidification. 20ml of melted media was poured into each sterilized Petri plates. Then 5mm disc of the test fungus was cut with the help of sterilized cork borer from the margin of seven days old culture grown on Potato dextrose agar (PDA) Petri plate. One disc was placed in the centre of each Petri plates. Three repetitions of each media were maintained for each of the pathogen and were incubated at 27±2ºC temperature.

Following growth media (Table: 1) was used to find out the most suitable media for the mycelial growth, colony colour, cultural characteristics and sporulation of *C. cassiicola*. All the observation was recorded after seven days of sub-culture.

Table 1. List of different media as follows

Tr. No.	Media	Composition
T_1	Potato dextrose agar	Potato :200g Dextrose :20.0g Agar :20.0g Distilled water:1000ml
T_2	Richard's agar	Potassium nitrate (KNO3) :10.0g Magnesium sulphate (MgSO4.7H2O) :2.50g Sucrose :50.0g Agar :15.0g Distilled water :1000ml
T_3	Malt extract agar	Malt extract :20.0g

		Agar :20.0g Distilled 5water :1000ml
T_4	Nutrient agar	Beef extract :3.0g Peptone :5.0g Sodium chloride :8.0g Agar :15.0g Distilled water :1000ml
T_5	V-8 Juice agar	V-8 Juice :200ml Calcium carbonate :3.0g Agar :20g Distilled water :800.0ml
T_6	Sabouraud agar	Pepton :10g Dextrose :40.0g Agar :20.0g Distilled water :1000ml
T_7	Oat-meal agar	Oat :150g Agar :20g Distilled water :1000g

Design: Completely Randomized Design
Treatments: 7
Repetitions: 3
Location: Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat

RESULTS AND DISCUSSION

Seven different growth media were tested for their suitability for the mycelial growth, colony colour, cultural characteristics and sporulation of the *C. cassiicola* under *in vitro* test and the results are presented (Table: 2).

Among the various growth media tested, the growth of *C. cassiicola* was significantly maximum in the Potato dextrose agar (PDA) (83.73mm) as compared to the rest of the growth media, which was followed by V-8 Juice agar (V8A) (75.43mm). The next best growth media in order of merit was Sabouraud agar (SA) (64.8mm) and Richard's agar (RA) (55.8mm) media. The remaining of the growth media *viz*., Oat-meal agar (OA) (47.66mm), Malt Extract agar (MEA) (40.43mm) and Nutrient agar (NA) (25.53mm) also supported the growth of the pathogen.

Among the seven different growth media tested, PDA and V8A proved the best growth media for the mycelial growth of the pathogen followed by Sabouraud agar.

The colony colour and cultural characteristics of *C. cassiicola* of cotton was different on different growth media. On PDA media fungus produces greyish, moderate, smooth circular, fibrous and arial mycelium, on RA media greenish white, cottony and arial mycelium, on MEA media whitish gray, velvety with spreading margin, on NA media white, oily, transparent and no arial growth, on V8A whiteish gray floccose or woolly, dense and tuft, on SA media white to grey, profuse mycelium and on OA media milky white to light gray moderate, circular, fluffy and raised mycelium was observed (Table: 2, Fig.: 1, Photograph: 1).

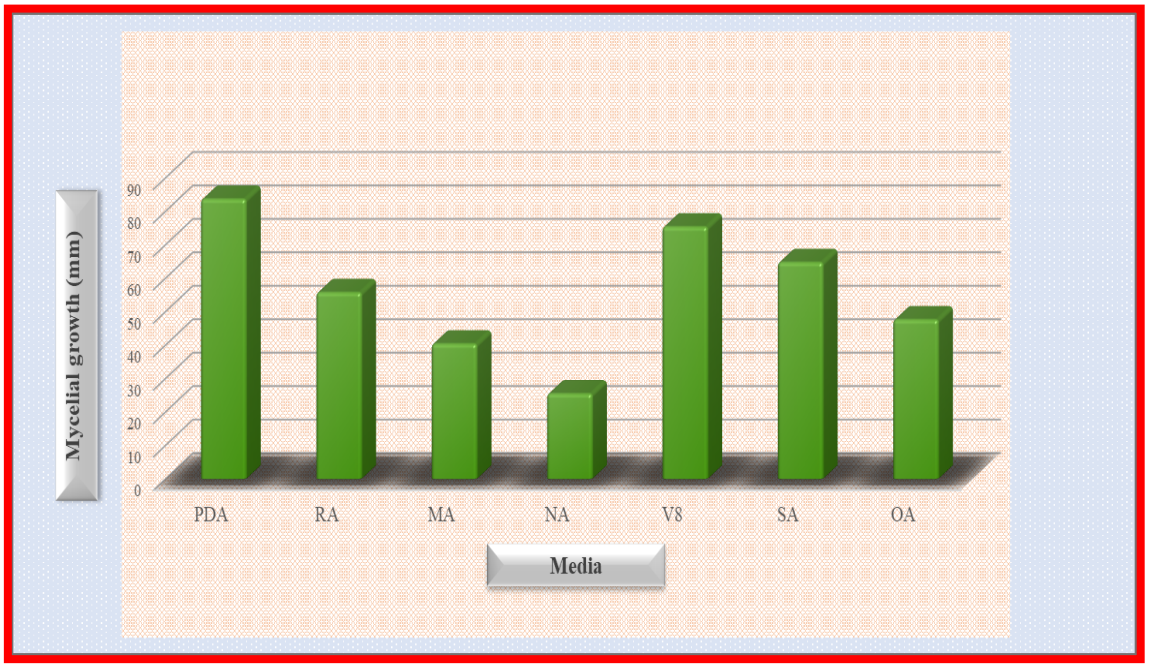
The sporulation of *C. cassiicola* was abundant on PDA followed byV8A and SA. Moderate sporulation was recorded in RA, MEA and OA while, scanty in NA (Table: 2).

The findings of cultural variation such as mycelial growth, colony colour, cultural characteristic and sporulation are compatible with the research findings of Meléndez and Pinero (1971) who reported that PDA and V8A are the best media for the growth of *C. cassiicola* of papaya. The best media for *C. cassiicola* of papaya growth was PDA and V8A media. Patel (2005) reported that the best media for growth and sporulation of *C. cassiicola* of soyabean was PDA. Fernando *et al*. (2012) reported that the highest conidia ($10^4/cm^2$) production of *C. cassiicola* causing leaf fall disease of rubber was obtained from PDA. Ahmed *et al*. (2013) reported that the PDA media was found to be the most suitable for optimum growth and sporulation of *C. cassiicola* of okra. Puia *et al*. (2021) also reported that the mycelial colour was dark grey to light brown and the maximum mycelial growth rate was found in V-8 Juice agar with 8.40 to 8.82mm. The production of conidia in PDA medium was higher in the isolates of cotton with 88.50 to 90.60 conidia mL^{-1}.

Table 2. Effect of different growth media on the mycelial growth, colony colour, cultural characteristics and sporulation of *Corynespora cassiicola*.

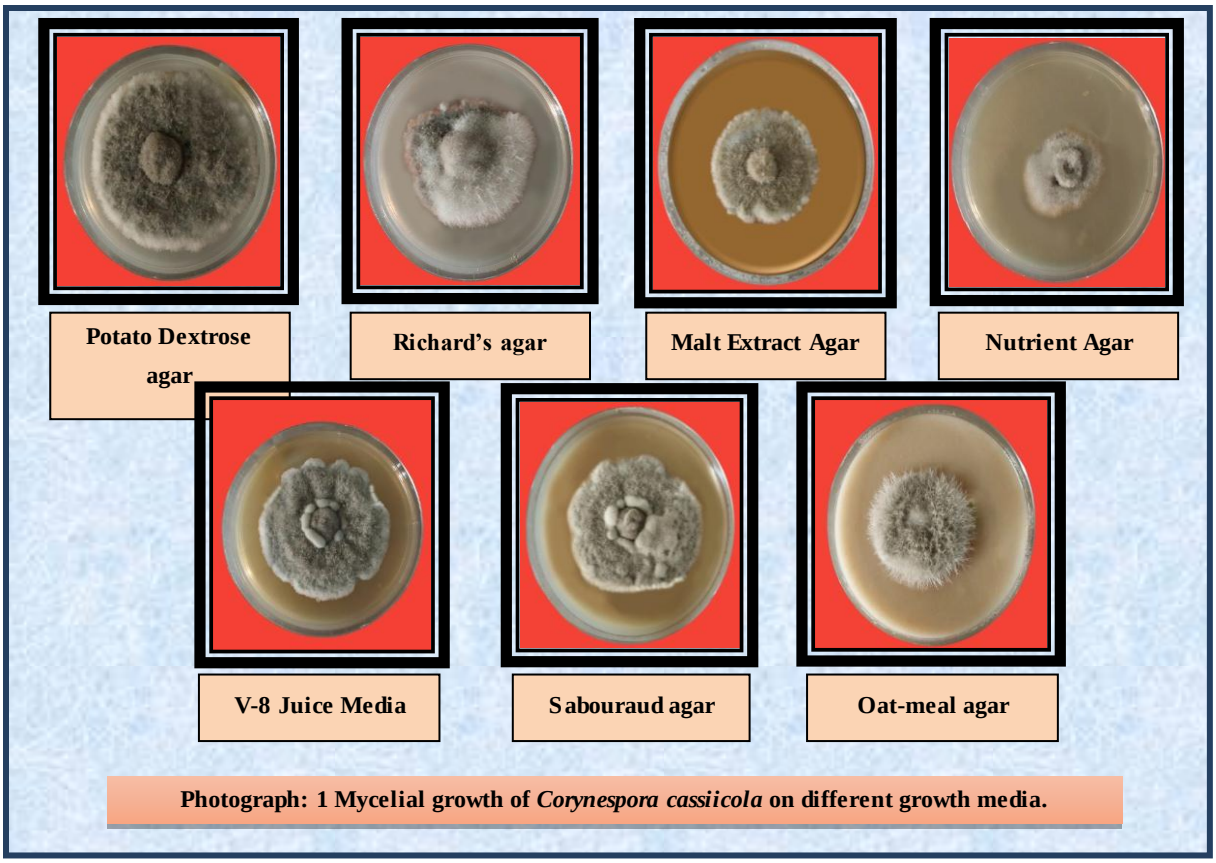
Culture media	Mycelial growth (mm)	Colony colour	Cultural characteristics	Sporulation category*
			Colony character	
Potato dextrose agar	83.73	Grayish	Moderate, smooth and circular, fibrous and arial mycelium	++++
Richard's agar	55.80	Greenish White	Cottony, arial mycelium	++
Malt extract agar	40.43	Whiteish Gray	Velvety with spreading margin	++
Nutrient agar	25.53	White	Oily, transparent, no aerial growth,	+
V-8 Juice media	75.43	Whiteish Gray	Floccose or woolly, dense, tuft	+++
Sabouraud agar	64.80	White to Gray	Profuse mycelium	+++
Oat-meal agar	47.67	Milky White to Light Gray	Moderate, circular, fluffy and raised mycelium	++
SEm±	1.23			
CD at 5%	3.76			
CV%	3.79			

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



PDA: Potato dextrose agar **RA: Richard's agar** **MA: Malt extract agar**
NA: Nutrient agar

Fig.: 1 Mycelial growth of *Corynespora cassiicola* on different growth media.



Photograph: 1 Mycelial growth of *Corynespora cassiicola* on different growth media.

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