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Prevalence, Pathogenicity and Physiological Studies of *Cercospora tageticola* Ellis and Everhart Causing Leaf Spot in Marigold

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ABSTRACT

A study was undertaken during first week of October to second week of October for two consecutive years i.e. 2017 and 2018 at Dr. YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh (173 230), India. The objective of the investigation was to find out the prevalence of of *Cercospora tageticola* through morphological analysis, pathogenicity and physiological studies for developing future control strategies. Highest disease incidence and severity (62.4% and 70.4%) was recorded in Nauni while, least incidence was observed in Chhogtali (8.9% and 8.6%) of Sirmaur district. The disease exhibited characteristic symptoms, starting as circular to angular, dark reddish brown or purplish spots that turned almost brown black, the centre of which is often greyish in colour. On advancement, symptoms slowly develop from lower to upper leaves giving blighted appearance. Flowers displayed small but noticeable light to dark brown lesions as they grow, initially circular and later becoming unequal to irregular blotches. Morphological analysis of the test pathogen revealed hyaline, septate and branched mycelia (8.54–11.34 µm) in older cultures. The pathogen produced geniculate conidiophores (3.5–6×50–300 µm) with hyaline conidia (2.7–5×70–200 µm). The identified pathogen was *Cercospora tageticola*. Koch's postulates confirmed its pathogenicity on "Pusa Narangi Gainda" variety, with symptoms appearing 8 days after inoculation, indicating a 192-hour incubation period. Cultural and physiological factors such as Carrot decoction agar as the optimal medium, 25°C as the temperature and pH 5.5 for maximum radial growth and dry mycelial weight significantly influenced pathogen growth *in vitro,* with black to greyish mycelial growth displaying good sporulation.

KEYWORDS: *Cercospora tageticola*, fungus, identification, physiological studies, *Tagetes* spp.

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1. INTRODUCTION

The kingdom of fungi is regarded as a crucial element of biodiversity. Worldwide production of floriculture is challenging due to the lethal biotic stress known as Cercospora leaf spot (CLS), which is caused by the fungus *Cercospora* spp. Cercospora is one of the largest genera in the Hyphomycetes family, mostly infects the aerial parts of its host plants quite prevalent and extremely destructive across the nation (Haque et al., 2013; Barro et al., 2023; Azevedo et al., 2017). The majority of the primary flora including cereals, pulses, vegetables, plantation crops, medicinal and aromatic plants and woods are affected by leaf spots caused by Cercospora species (Crous and Braun, 2003; Groenewald et al., 2013; Bakhshi et al., 2015; Kang et al., 2019; Gupta, 2022). Marigold flowers gained popularity amongst gardeners and dealers on its easy cultivation and wide adaptability (Priyanka et al., 2018). Himachal Pradesh is well known for the floriculture venture since 1990 and now the area under marigold cultivation has expanded enormously. Marigold (*Tagetes* spp.) is an Asteraceous plant and is native to South and Central America, specifically from Mexico. The genus *Tagetes* comprises of 55 species and the most commonly cultivated species are *Tagetes erecta* L. (African marigold) and *Tagetes patula* (French marigold) (Kumar et al., 2019). The area under flower crops in India is about 0.37 tha which accounts for the production of 1.30 mt of loose flowers and 10.45 mt of cut flower (Anonymous, 2022). Marigold plants have antinematicidal activity (Olabiyi and Oyedunmade, 2007) and found most effective against the nematode species *Pratylenchus penetrans*. The flowers are used to make food pigments as they are rich in carotenoid pigment. The powder of flower petals are used in poultry feed which ensure a good colouration of egg yolks and broiler skin. Marigold is commercially cultivated in Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Haryana, West Bengal, Maharashtra, Chhattisgarh, Tamil Nadu, Sikkim and Himachal Pradesh. In Himachal Pradesh, area under marigold is 51.91 ha with production of 780 t (Anonymous, 2022). Recently, its cultivation has been taken up on a large scale in Sirmaur district with an area of 17.82 ha (Anonymous, 2021) where summer season is long and dry and irrigation facilities are available. Seeing its success in Sirmaur district, farmers are taking up its cultivation in other districts of the state and every year area under marigold is increasing. This disease causes a 70% yield loss in cultivars that are sensitive to it because it drastically reduces the amount of photosynthetic tissues in plant foliar regions (Thakur et al., 2013; Islam et al., 2015; Stewart, 2016; Mohammed et al., 2018; Tedford et al., 2019). Most scientists now have an inexhaustible desire to explore methods to mitigate the severity of the disease due to its economic significance (Zongo et al., 2019; Denwar et al., 2021; Nana et al., 2022). Cercospora leaf spot in marigold for the first time in Himachal Pradesh during the rainy season, when the intensity is quite high and an estimated loss to the crop of up to 62% was documented due to the favourable climatic conditions. Due to the future demand for less pesticide residues, *Cercospora* spp. prevalence may rise in conventionally maintained major floriculture crops. Due to the paucity of research on Cercospora leaf spot of marigold (*Cercospora tageticola* Ellis & Everhart), the current investigation was conducted to observe the prevalence of the disease, isolation and identifying the *Cercospora tageticola* pathogen through morphological analysis, pathogenicity and physiological studies for developing future control strategies.

2. MATERIALS AND METHODS

Periodic surveys were conducted during first week of October to second week of October for two consecutive years i.e. 2017 and 2018 in cultivated marigold habitats in Solan and Sirmaur districts of Himachal Pradesh, India at the time of the onset of the disease to record the disease incidence/severity. The per cent disease incidence was recorded by counting infected plants and flowers over normal healthy plants and flowers while the per cent disease severity was recorded by leaf spot scoring system (0–9 scale) used for symptom appearance in plant with slight modification (Chiteka et al., 1988). Per cent disease index (PDI) was worked out using the formula given by Mckinney (1923) and Wheeler (1969):

Disease incidence (%)=(No. of diseased plants/or flowers)/(Total no. of plants/or flowers observed)×100……(1)

For isolation of plant pathogen, infected leaves and fruits showing characteristic symptoms were collected and isolation were made by following standard isolation method under aseptic conditions. Morphological characters of the fungus were studied by observing cotton blue stained slides under compound light microscope {Stereo zoom binocular microscope (SZ2, Olympus make, Japan)} with Magna pro software by using 10x objective and 40x eyepiece lenses. On the basis of morphological characters such as mycelium, shape and size of conidiophores, conidia and cultural characters as reported by Ellis and Everhart in 1902 and described in "Illustrated Genera of Imperfect Fungi" (Barnett, 1955) and "Monograph of the Fungus Genus *Cercospora*" (Chupp, 1954), the identification of *Cercospora tageticola* Ellis & Everhart was done.

Pathogenicity test was conducted by creating artificially inoculated conditions in pot culture on the raised marigold plants. Preparation of spore suspension were performed by inoculating 10 g of mycelium scraped from a 14 day-old-colony which was washed in sterilized water and

homogenized in a blender with 100 ml of sterile 0.2% water agar for 40 s and were sprayed on both sides of leaves. After inoculation, the plants were covered with plastic bags for 48 h and kept in a greenhouse at temperature ranging from 28–33°C. The variation in cultural characters of *Cercospora tageticola* Ellis & Everhart was studied on the following synthetic (Czapeck's (Dox) agar) and non-synthetic solid media (Carrot decoction agar, Potato dextrose agar, Marigold leaf decoction agar, V8 juice agar). Physiological studies were conducted at different temperature (10, 15, 20, 25, 27 and 30°C) and Hydrogen-Ion Concentration (pH) (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0), respectively.

3. RESULTS AND DISCUSSION

3.1. Morphological characters and identification of the pathogen

In order to monitor the prevalence and incidence of the disease, extensive surveys of the African marigold-producing regions in Himachal Pradesh's Solan and Sirmaur districts were carried out throughout during the years 2017 and 2018in growing seasons. Marigold leaves with the classic signs of Cercospora leaf spot were gathered and brought to the laboratory for the pathogen isolation. In the Solan district, the disease incidence and severity appeared in severe form in Nauni (62.4% and 70.4%) while moderate to minimal proportion in Kayaratu (17.8% and 20.5%). In the Sirmour district, disease was recorded in moderate form in Rajgarh (46.7% and 52.6%), Shirgulli (32.9% and 33.5%), whereas Habban valley, Thanadhar, Bhadoli, and Chhogtali recorded significantly less disease (Figure 1).

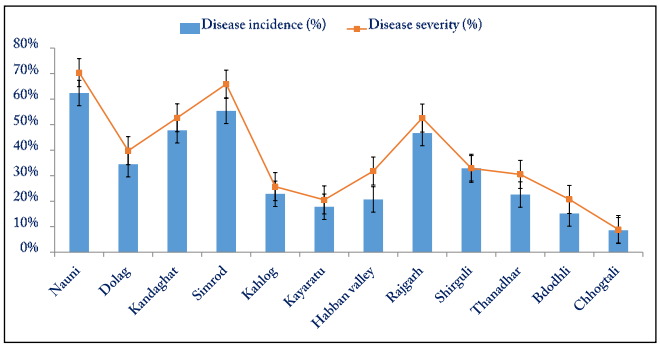

Figure 1: Graphical representation of Solan and Sirmaur districts surveyed for Cercospora leaf spot of marigold in Himachal Pradesh

Surveys revealed that the disease affected all above ground of the plants. The leaves and flowers were affected more frequently than the other parts. Initial symptoms on leaves included a raised, circular to sub-circular lesion with a diameter of 5–9.5 mm on upper surface. Later on, light brown raised lesion appeared on upper surface become brown with greyish growth and their margins formed by a definite dark purple zone. On advancement, dark to blackish brown zones developed outside the whitish centre as a result, leaves start drying and get blighted and symptoms gradually develops in upward direction. On flowers, initially circular to olivaceous, water-soaked spots which were found covered with greyish brownish growth on lower surface; gradually disease goes to above ground part and cause blightening appearance. The results of symptoms were in accordance with earlier workers (Chupp, 1953, Vasudeva, 1963, Ayala-Escobar et al., 2009, Kang et al., 2019). During surveys, marigold plants showing characteristic symptoms were brought to laboratory and the associated pathogen was isolated by placing surface-sterilized bits of infected leaves on Carrot decoction agar medium plates. The fungus grew profusely on Carrot decoction agar medium with white fluffy mycelium at 25±1°C temperature. The mycelium grew at a very fast rate attaining 90 mm colony diameter within 21 days (504 days). These results were agreement with Thirumalachar and Chupp (1948), Chupp (1953) and Vasudeva (1963) who reported that conidia are hyaline, a circular, straight to curved, with truncate base and acute tips. They are multi-septate measuring 3–5×80–250 µm. The identification of the pure culture of isolated pathogen was carried out on the basis of morphological characteristics represented in Table 1. Mycelial colonies of isolated fungus were brown to olivaceous brown in colour with fluffy or cottony dense aerial growth. Fungus was fast growing and took 21 days to completely cover the Petri plates (90 mm) with mycelium (Plate 1).

Table 1: Morphological characters of the pathogen (*Cercospora tageticola* Ellis & Everhart)

Morphological character(s)		Description
Mycelium colour	Young	Hyaline, septate and branched
	Old	Brown to olivaceous brown
	Diameter (µm)	8.54–11.34
Septation		Oblique septa
Conidiophore (µm)		Emerge either through stomata or ruptured epidermis. Conidiophores borne singly or in fascicles of 2–12, pale to medium brown, not branched, straight or curved and show distinct geniculate (knee like) bends and measured 3.5–6×50–300 µm.
Conidia size (µm)		2.7–5×70–200
Conidial shape		Hyaline, acircular, straight to curved, multi-septate (transverse septa), hyaline or brownish in colour.
Number of oblique septa		0–2

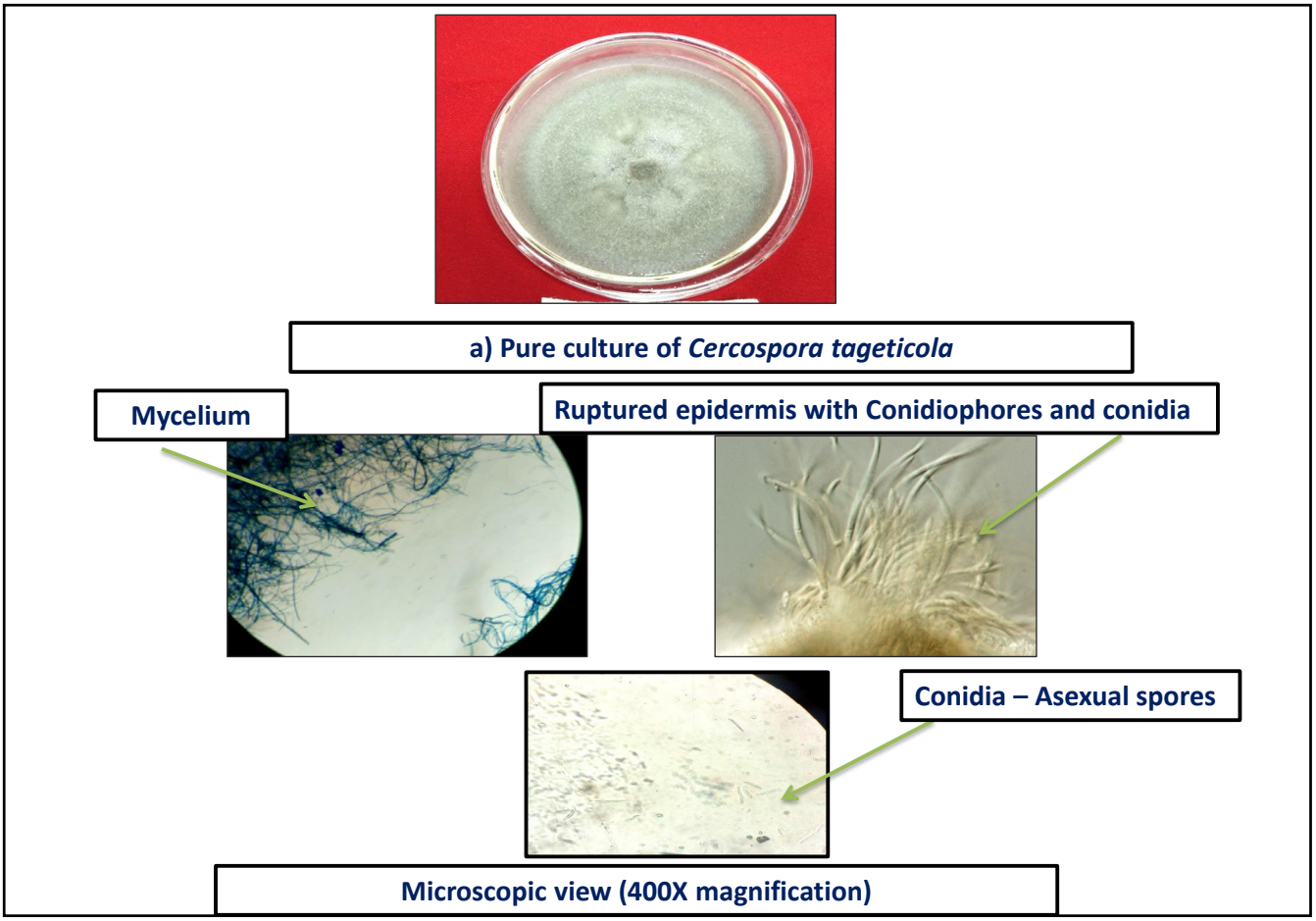

Plate 1: Cultural and microscopic view of *Cercospora tageticola*

3.1.1. Pathogenicity test

The pathogenicity of *Cercospora tageticola* inone month old seedlings of African marigold cv. "Pusa Narangi Gainda" was obtained within 8 days of inoculation. The observations on incubation period (h) and symptom development were recorded and presented in Table 2. These results were in consonance with Stahel (1937) and Sridharan and Rangaswamy (1968) who proved that aqueous suspension of viable spores of *Cercospora musae* and *Cercospora abelmoschi* when inoculated onto healthy detached leaves of banana and okra produced infection (Plate 2).

3.2. Physiological studies and cultural characteristics

3.2.1. Effect of solid culture media on mycelial growth, characteristics and sporulations of Cercospora tageticola

Effect of solid culture media on mycelial growth, characteristics and sporulations of *Cercospora tageticola*

Table 2: Pathogenicity test of *Cercospora tageticola* under pot culture

Pathogenicity test		
Incubation period (h)	Pot culture	192 hours (8 days)
Symptom development	Initial stage	Appeared as circular to sub-circular irregular lesion having 5–9.5 mm diameter raised on upper surface
	Later stages	Light brown raised lesion appeared on upper surface which become brown with greyish growth and marginated by a definite dark purple zone.
	Advanced stages	Dark to blackish brown zones developed outside the whitish centre as a result leaves started drying and get blighted. The symptoms usually on older leaves and gradually develops upward direction. Flower also showed similar circular to olivaceous, water soaked spots which are covered with greyish brownish growth of the pathogen.

Plate 2: Pathogenicity symptoms produced by Cercospora leaf spot symptoms (Spore suspension method)

were studied by preparing six different synthetic and non-synthetic media and data on radial growth of the test fungus was recorded and presented in Table 3. The maximum radial growth (50.67 mm) of fungus was recorded on Carrot decoction agar followed by Potato dextrose agar (45.48 mm). However, minimum radial growth was observed on Czapek's-Dox agar media (4.81 mm). The results pertaining to the effect of different solid cultural media at different time intervals indicated that the growth steadily increased starting from 7th day and attained maximum growth (73.33 mm) at 21st day in case of Carrot decoction agar whereas no growth on 7th day in case of Czapek's-Dox agar was seen and was found minimum even after 21st day of observation (9.60 mm). Out of six solid media tested, good sporulation i.e above 10 conidia per microscopic field was observed on Carrot decoction agar media where the mycelium growth produced was of raised type and black to light brown in colour while in rest of the media, poor to no. sporulation was observed presented in Table 4. These results were in agreement with Kilpatrick and Johnson (1956) and Khandar et al. (1985) (Plate 3).

3.3. Effect of different temperatures on mycelial growth, characteristics and sporulations of Cercospora tageticola

In order to study the effect of different temperature on mycelial growth, characteristics and sporulations of *Cercospora tageticola*, the test fungus was grown on the best solid media i.e. carrot decoction agar obtained from the solid cultural media studies and incubated at different

Table 3: Effect of different solid cultural media on the development of *Cercospora tageticola* under *in vitro*

Type of media	Radial mycelial growth (mm) after days of incubation			
	7th day	14th day	21st day	Mean
Carrot decoction agar	27.67	51.00	73.33	50.67
V-8 juice agar	14.50	28.67	32.50	25.22
Czapek's-Dox agar	0.00	4.83	9.60	4.81
Potato dextrose agar	23.67	48.44	64.33	45.48
Yeast extract agar	7.42	31.50	27.33	22.08
Marigold extract agar	21.10	42.43	59.22	40.92
Mean	15.73	34.48	44.39	
		C.D.	SE(d)	SE(m)
Media		1.02	0.50	0.35
Incubation period		0.72	0.35	0.25
Media×Incubation period		1.77	0.87	0.61

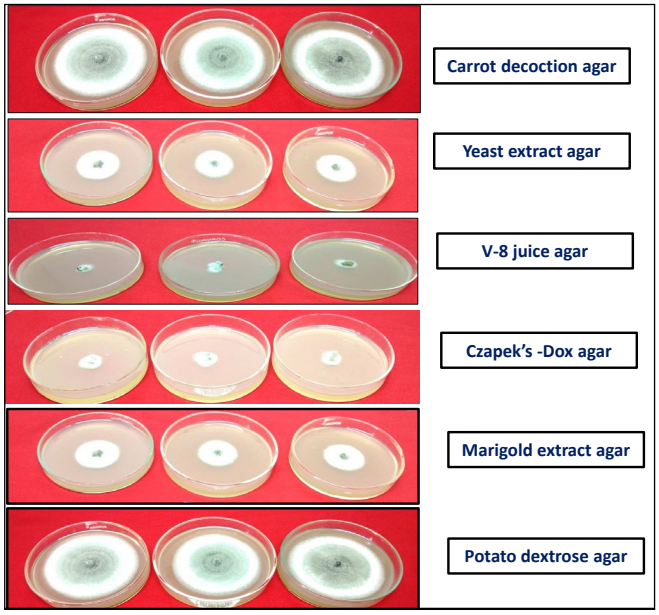

Plate 3: Effect of different solid media on growth of test pathogen

Table 4: Effect of solid media on growth characteristic and sporulation of *Cercospora tageticola*

Type of media	Growth characteristics	Sporulation*
Carrot decoction agar	Good mycelial growth, black to light brown colour, raised growth.	Good
V-8 juice agar	Average mycelial growth, dark grey centre with brown substrate color, raised growth with irregular margin	Poor
Czapek's-Dox agar	Slow mycelial growth, greyish brown colour, slightly raised growth with regular margin.	No sporulation
Potato dextrose agar	Good mycelial growth, grey to light brown colour, somewhat raised growth.	Average
Yeast extract agar	Slow growth, greyish color, yellow substrate color with grey centre	No sporulation
Marigold extract agar	Good mycelial growth, dull white, raised growth with irregular margin	Poor

*Sporulation microscopic field^{-1}: Good: >10 conidia; Average: 5–10 conidia; Poor: <5 conidia; 0: No sporulation (10x)

temperatures viz. 10°C, 15°C, 20°C, 25°C, 27°C and 30°C. Observations regarding radial mycelial growth were recorded and presented in Table 5. The results showed that the disease was able to develop at all temperature regimes. With increase in temperature regimes there was a corresponding increase in radial mycelial growth and maximum mean radial mycelial growth was recorded at 25°C (50.67 mm) which was significantly superior to other treatments thereafter a gradual decline was observed in growth pattern as the temperature reached to 30°C. Similar findings were also reported by Dinesha (1984) and Khandar et al. (1985) who reported that maximum growth and spore germination of *Cercsopora sorghi* occurred at 25°C.

Table 5: Effect of different temperatures on the development of *Cercospora tageticola* under *in vitro*

Temperature (°C)	Radial mycelial growth (mm) after days of incubation			
	7th day	14th day	21th day	Mean
10	3.15	6.50	12.14	7.26
15	6.47	13.58	19.47	13.17
20	17.10	22.84	31.55	23.83
25	27.67	51.00	73.33	50.67
27	21.67	45.13	65.67	44.15
30	11.90	19.58	25.58	19.01
Mean	14.69	26.44	37.96	
		C.D.	SE(d)	SE(m)
Temperature		0.81	0.40	0.28
Incubation period		0.58	0.28	0.20
Temperature×Incubation period		1.41	0.69	0.49

*Sporulation microscopic field^{-1}: Good: >10 conidia; Average: 5–10 conidia; Poor: <5 conidia; 0-No sporulation (10x)

3.4. *Effect of pH level on the growth of Cercospora tageticola*

The pH level of the liquid media (Carrot decoction) on the growth of *Cercospora tageticola* was adjusted to 4.0, 4.5, 5.0, 5.5, 6.5 and 7.0 presented in Table 6. The maximum mean (765.15 mg) dry mycelial weight of the test *fungus Cercospora*

Table 6: Effect of different pH levels on the development of *Cercospora tageticola* under *in vitro*

pH	Mean dry mycelial weight (mg)		
4.0	176.23		
4.5	205.39		
5.0	511.03		
5.5	765.15		
6.5	430.48		
7.0	305.25		
	CD (p=0.05)	SE(d)	SEm±
	1.19	0.54	0.38

tageticola was obtained at pH 5.5 which was significantly higher while the least dry mycelial weight of the fungus was recorded at pH 4.0 (176.23 mg). These results were in agreement with Dayal and Ram (1968) and Khandar et al. (1985) recorded the highest percentage of spore germination of *Cercospora jasmincola* at pH 5.5 whereas Khandar et al. (1985) reported that sporulation was very good in pH 5.9 (Plate 4).

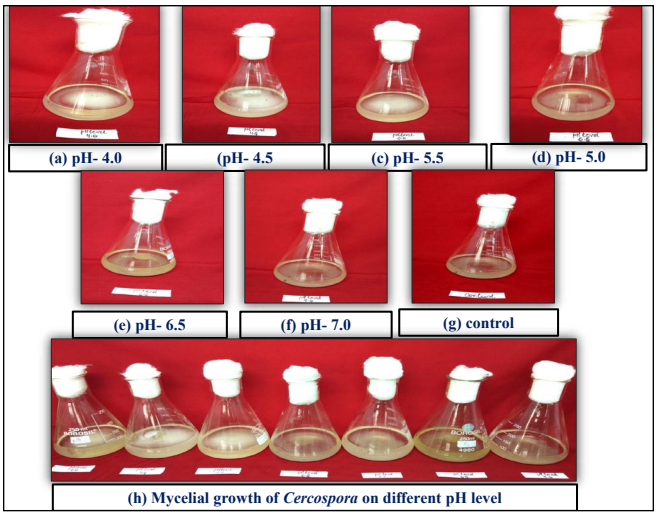

Plate 4: Effect of different liquid media on growth of test pathogen

4. CONCLUSION

In recent years, Cercospora leaf spot disease became a significant issue for marigold cultivation, causing substantial losses ranging from 8.9% to 70.4% in terms of disease severity and flower incidence (8.6% to 62.48%) in Solan and Sirmaur districts, particularly during the monsoon season. The pathogenicity of Cercospora tageticola was observed in one-month-old seedlings of African marigold just 8 days after inoculation. The pathogen thrived best on Carrot decoction agar media and potato dextrose agar media at a temperature of 25°C and pH 5.5.5.

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