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Physiological and Morphological Studies of *Colletotrichum capsici* under Different Culture Media Temperature and Light Regimes

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Abstract

Pepper (capsicum annum l.), a reasonably economic crop is severely affected by anthracnose caused by colletotrichum spp. This comparative study was aimed to investigate the physiological, morphological and pathological variability of colletotrichum isolates. Isolates were subjected to different temperature range, ph levels, light intensity and growth media. A completely randomized experimental design was used, with three replicates for mycelia growth (one plate per replicate) and replicates for spore production (8 readings per plate). Data on culture media and lighting condition were submitted to statistical analysis; means were compared by tukey test = 0.05). The study showed that colony diameter, cultural characteristics (texture, surface and reverse pigmentation) and sporulation of fungi were greatly influenced by the type of growth medium used. Survival period of fungi in relation to different temperature and ph conditions, media and natural habitats differs amongst isolates. Variation in mycelial growth and fungal sporulation was observed with media tested. The pathogens were identified based on their morphological and cultural characteristics. Cultural studies revealed that among solid media, pepper dextrose agar and potato dextrose agar were found to be good for radial growth and sporulation of colletothrichum isolates four days after incubation. Nutritional studies revealed that sucrose and potassium nitrate were better among the carbon and nitrogen sources, respectively. Physiological studies revealed that optimum ph of 6 were favorable for growth; maximum radial growth was obtained at optimum temperature of 25°c. Alternate cycles of 12hours light and 12 hours darkness favored the maximum radial growth of colletotrichum isolates followed by continuous light. In the light of the present study, farmers could be suggested the practice of fertilizer management, combination of management practices and avoidance of environmental conditions favoring the pathogen, thus result in significant production of pepper.

Key words: *anthracnose, light, colletotrichum spp, temperature, nutrient media*

Introduction

Pepper (*Capsicum* spp) is one of the most crucial cash crop in Ethiopia that dominate the spice production and marketing in Ethiopia [1].It is a crop of high value in both domestic and export markets. Since it is a commercial and industrial crop, it generates employment to urban and rural workers. In recent years, the area of production of hot pepper in south west Ethiop ia is increasing due to its high price in the market as compared to other major food crops such as maize that are known to be widely cultivated in the region[2].

The crop is also one of the important spices that serve as the source of income particularly for small holder producers in many parts of rural Ethiopia[3], with an income of 509.44 million Birr per season [4]. Ethiopia's share in the world, however, is insignificant (5%) compared to India (36%) and China (11%) with a production of 1.25, 0.39 and 0.17 million tones [5, 6]. The decline of hot pepper production (0.4 tones fruit yield/ha) is attributed to the prevalence of fungus among others [7]. Anthracnose has been spread in major pepper producing areas of southern Ethiopia, causing a significant decline in quantity and quality of pepper yield [8]. Anthracnose is cosmopolitan and attacks many crops[9]. It affects peppers at any stage of development [10], and may threaten the profitability of pepper crops in areas where it becomes established. Diseased fruit act as a source of inoculum, allowing the disease to spread from plant to plant within the field [11].

C. capsici isolates usually produce cottony colonies on PDA with a color of grayish-white to dark grey on the ventral surface whereas the reverse of the colonies were mainly black [12-14]. Paweena [15] observed the growth rate of forty five *C. capsici* isolates on PGA ranged from 6.0 to 11.1 mm/day, mean = 8.5 ± 33.4 mm. Regarding the physiological characterizations, Vinod[13] reported that maximum and minimum growth of *Colletotrichum gloeosporioides* was found at an optimum pH of 6.5 and 4.0, respectively. Mycelial growth of Colletotrichum spp was reported to have been affected by temperature [16; 17; 13]. Prema *et al*. [18] reported that the maximum growth of the*C. Musae* isolates was observed at a temperature of 30^0C followed by 25 and 20^0C. Ashutosh *et al*. [19], reported that higher (53.17 mm) radial growth of mycelia was observed at 28°C. The exposure of *C. gloeosporioides* to alternate cycles of light and darkness showed maximum growth and sporulation [16]. Ashoka[17] and Vinod [13] reported that the exposure of the *C. gloeosporioides* to alternate cycles of 12 hr light and 12 hr darkness for twelve days resulted in maximum dry mycelia weight (452.85mg) and maximum radial growth (89.35 mm). Cultural studies indicated that considerable effects were observed on mycelia weight and growth with varying nutrients [20- 22, 17].

The objective of this work was to evaluate the effect of the composition of some culture media, temperatures, and light regimes on mycelial growth and conidia production of *Colletotrichum* spp aimed at determining conditions that favor development and inoculum production for conducting future assays that would require artificial mass inoculation.

Materials and methods

Isolation of the causal pathogens

Culturing Colletotrichum spp isolates from infected pepper

One piece (~2-5 mm) of infected tissues was cut from the edge of the lesion on the collected infected pepper using sterilized sharp scalpel. The tissue was then surface sterilized by dipping the tissue in 70% ethanol for 2 min followed by washing twice with distilled water[15]. Five up to eight tissues were then wiped dry with sterilized filter paper, and placed on potato dextrose agar (PDA) containing 0.1mg of chloro-aphinicol in 1000ml of PDA.

Maintenance of the culture

The fungus was sub-cultured on PDA slants and allowed to grow at 25 ^{0}C for one week. The slants were preserved in refrigerator, at 4^0C and renewed once in one month. The preserved culture was used for further studies.

Identification of Pepper Pathogen

The morphological characters of the fungus such as colony color, surface mycilum and length and width of conidia were studied and recorded. Fungal spores were identified and counted under microscope [15].

Designation of Anthracnose (Colletotrichum spp) isolates

Totally seven isolates of anthracnose (*Colletotrichum* spp) isolates were identified and designated as Addis Ababa University Pepper anthracnose isolates (AUPEP-1, AUPEP-2 AUPEP-6, AUPEP-7, AUPEP-8, AUPEP-9 and AUPEP-10).

Pathogenicity test (in vitro)

Inoculation method

Preparation inoculum load/concentration

Conidia suspension of each *Colletotrichum*isolate was harvested from 10 day old cultures inoculated on potato dextrose agar and incubated at 25°C. The *Colletotrichum*sppculture plate was flooded with sterilized water, and the conidia were gently scraped from the culture plate. The number of viable spores/ml of suspension was counted using a haemocytometer under a microscope. The spores were first counted under low magnification (10x) then counted under high magnification (40x) of the 16 cubes (per set) were counted then total number of spores per ml was calculated and adjusted to 6×10^5 conidia/ml [23].

Cell concentration per ml = n x dilution factor x 10^4 spores/ml

Where, n=Average cell count per square of the four chamber counted.

Pepper fruit inoculation (In vitro)

Five fruits at ripe stage (matured) were randomly bought from market, and had their pedicels and calyces removed. The fruits were surface sterilized with 70% alcohol for 2 min, and washed twice with distilled water and then wiped dry with sterilized filter paper. Each pepper fruit was injected once with 1 µl of the prepared conidial suspension in the middle of the fruit. The injection was performed using a needle with 1mm diameter and 1 mm depth. The inoculated pepper fruits were placed in a Petri-dish containing filter paper, and then incubated at 25°C. Sterilized distilled water was added every three days until symptoms were observed on pepper fruits [24].

Size of lesions produced on the inoculated pepper fruits were measured every other day for 10 days using a transparent ruler. The mean lesion size for each isolate was computed for analysis. Disease severity was assessed on a 5 - point scale similar to that used by Waller *et al*. [24], *where;*

1 = O mm (no lesion)
2 = 1 - 5 mm lesion size (low virulence)
3 = 6 - 10 mm lesion size (moderate virulence)
4 = 11 - 25 mm lesion size (high virulence)
5 = More than 25 mm lesion size (very high virulence)

Morphological characterization of Colletotrichum spp
A loopful of culture of *Colletotrichum* spp obtained from fourteen days old culture was placed on the slide. A cover slip was placed over it; length and width of spores were measured under high power objective (40×) scanning microscope. The average size of the spores was calculated.

Colony growth rate and characteristics Colletotrichum spp
Colony diameter of each isolate was measured at one day after inoculation (DAI) daily, and mean colony growth rate was determined. Colony characteristics including surface mycelium and conidia color were recorded.

Physiological studies Colletotrichum spp
Several studies were carried out to test variation among the isolates under different temperature, pH, and light intensity.

The effect of temperature
Different temperature values were adjusted for the growth of *Colletotrichum* spp were 20, 25, 30, 35 and 40ºC. The pure isolated fungus was inoculated under aseptic condition and incubated for 4 days and subsequently the mycelial growth was measured and recorded.

The effect of pH
Adjustment of pH was done by adding 0.1 N NaOH or 0.1 N HCl. Reaction of the medium was adjusted to the desired pH values of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8, by using 0.1 N NaOH or 0.1N HCl. The pure isolated fungus was inoculated under aseptic conditions and incubated for 4 days at 25 ^{0}c. Their mycelia radial growth were measured and recorded.

Effect of light intensity
The effect of light on the growth of seven pathogens were studied by exposing the inoculated culture to alternate cycles of twenty four hour light, twenty four hour dark and twelve hour light and twelve hour dark in an environment chamber maintain at room tempe rature (~15-25°C). Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and florescent light intensity is adjusted to required level. The mycelial growth was record on fourth day after inoculation.

Cultural studies on Colletotrichum spp
Composition and preparation of culture media
The culture characters of *Colletotrichum* spp were studied using the following six solid media: Potato dextrose agar, Malt extract agar, Corn meal agar, Potato carrot agar, Papaya fruit agar and Pepper dextrose agar.
The composition of the above media were obtained from "Ainsworth and Bisby's Dictionary of the fungi" by Ainsworth [25]. The composition and preparation of different media are as given below:
1. Potato dextrose agar (Potato: 200 g, Dextrose: 20 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
2. Malt extract agar (Malt extract: 25 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
3. Corn meal agar (Maize: 30 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
4. Potato carrot gar (Grated potato: 20 g, Grated carrot: 20 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
5. Papaya fruit agar (Papaya fruit piece: 200 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
6. Pepper dextrose agar (Pepper: 200 g, Dextrose: 20 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).
7. Richard's agar media (Sucrose: 50 g, Potassium dihydrogen phosphate: 5 g, Potassium nitrate (KNO_3): 10 g, Magnesium sulphate ($MgSO_4.7H_2O$): 2.5 g, Ferric chloride ($FeCl_2.6H_2O$): 0.02 g, Agar agar: 20 g and Distilled water to make up: 1000 ml).

Nutritional studies
Carbon utilization
Sucrose, glucose, fructose, lactose and maltose were used. For this study the carbon in the basal medium (Richard's solution) was used by equivalent amount of the carbon source individually (as mentioned in section 3.9.1.7). Each treatment was replicated three times. After four days of incubation radial growth of the fungus was recorded and the data were statistically analyzed.

Nitrogen utilization
Potassium nitrate (KNo_3), sodium nitrate ($NaNo_3$), ammonium sulphates ($(NH_4)_2So_4$), ammonium chloride (NH_4Cl), ammonium dihydrogen phosphate ($(NH_4)_2HPo_4$ and diammonium sulphates (NH_4So_4) were used for the study. The quantity of nitrogen compound used was determined on the basis of their molecular weight so as to provide an equivalent amount of nitrogen as that of potassium

nitrate present in the basal medium, as shown in 3.9.1.7. Three replications were maintained for each treatment. Petri dishes were inoculated under aseptic conditions and incubated at 25ºC for 4 days. There after mycelial growth, (mycelia length) was measured and the data were analyzed statistically.

Result

In Vitro evaluation of pathogenicity test:

Pathogenicity test of the isolates from pepper fruits showed that the lesion size produced by *Colletotrichum* isolate AUPEP-10 and AUPEP-6 was 75.25mm and 64.2mm, respectively giving it the highest virulence for disease severity (Table-1). The sizes of lesions caused by *Colletotrichum* isolates AUPEP-1, and AUPEP-8 were 9.25mm, and 4.50 mm, respectively (Table-7). Three *Colletotrichum* isolates (AUPEP-2, AUPEP-7 and AUPEP-9) did not produce any symptoms when inoculated onto pepper fruits.

Table 1: Evaluation of differential reactions of four peppers infected by four *Colletotrichum* isolates (*in vitro*) in petri-dishes that were bought randomly from market

Isolate	Disease severity					Sizeof lesions
	Capsicum-1	Capsicum-2	Capsicum-3	Capsicum-4	Capsicum-5	
AUPEP-1	-	-	- -	-	-	9.25mm
AUPEP-6	-	- - -	-	-	-	64.2mm
AUPEP-8	-	-	-	-	-	4.5mm
AUPEP-10	- -	-	-	-	-	75.25mm
Control	-	-	-	-	-	-

Fig.1: Disease scoring scale of anthracnose of pepper using 1-5 scale (AUPEP-10 AUPEP-6 AUPEP-1 AUPEP-8 and control)

Morphological studies

The fungal colony from 14 days old culture on potato dextrose agar was white, gray and black to white. The conidia from the culture measured 9-16.5 µm length and 3-5.2 width µm (Table 8). Based on the morphology of the fungus the pathogen was identified as *Colletotrichum* spp.

Colony growth rate and characteristics

Colony characteristics were classified based on 1) colony growth rate 2) Surface mycelium and 3) colony color. Mean growth rate of 7 *Colletotrichum* isolates on PDA ranged from, 1.67±1.15 mm/day to 5.67±1.53 (Table-8). The 7 isolates could be divided into three groups according to colony growth rate, the slow growing with growth rate less than 3.33±.578 mm/day, medium growing with growth rate more than 3.33±.578 to 4.32±2.52 and the fast growing with growth rate greater than 4.32±2.52 (Table-8). One *Colletotrichum* isolate belonged to the slow growing group and five isolates of *Colletotricum* isolates belonged to the medium and one isolate of *Colletotricum* isolate belonged to fast growing group (Table-2). The *Colletotricum* isolates could be divided into four groups according to the surface mycelium: uniform, concentric rings, sector and irregular (Table-2). Surface mycelium of 4 *Colletotricum* isolates were uniform, 1 was concentric rings, one was sector and one was irregular (Table-8). Colony color was divided into 3 groups whitish, gray and black to white. Three *Colletotrichum* isolates were whitish, three were gray and the remaining one was black to white colony color, as shown in Table -2.

Table 2: Colony growth rate, Surface mycelium, Colony color and Conidial size of the 7 *Colletotrichum* isolates

Colletotrichum isolates	Growth rate in mm	Surface mycelium	Colony color	Conidial size	
				Length(µm)	Width(µm)
AUPEP -1	3.33±.578	Sector	White	9	5.2
AUPEP-2	3.67±1.53	Uniform	Gray	9.22	4.3
AUPEP-6	4.32±2.52	Uniform	Black to white	11.11	3.3
AUPEP-7	3.33±1.53	Uniform	Gray	12	4.51
AUPEP-8	1.67±1.15	Irregular	White	11.52	3
AUPEP-9	4±1	Concentric	White	15	3.02
AUPEP-10	5.67±1.53	Uniform	Gray	16.5	3.44

Growth characters on solid media

Among the six different solid media, maximum radial growth of the fungus was observed in pepper dextrose agar, potato dextrose agar and malt dextrose agar media (90mm) in isolate AUPEP-10 and AUPEP-6 respectively. Which was significantly superior over all other tested medium and the lowest were papaya fruit agar (7mm) followed by potato-carrot agar (5mm) on isolate AUPEP-9 and AUPEP-1 respectively (Fig. 2).

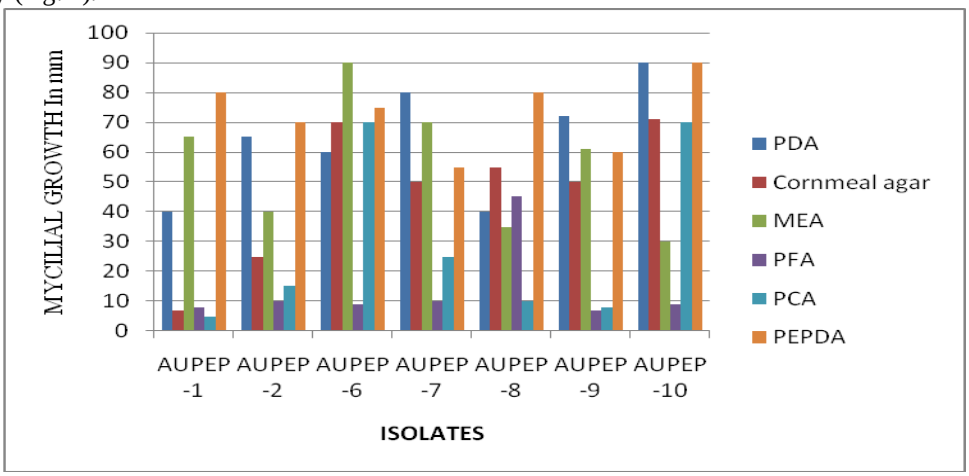

Fig 2. Effects of different solid media on the growth of *Colletorichum* isolates 4 DAI mycelial diameters in millimeter (mm).

Nutritional studies

Carbon utilization

The data from Table-3 has indicated that the effect of different carbon sources on the growth of the fungus was significant. The maximum growth of the test fungal isolate was obtained in sucrose, fructose and maltose (19mm±00 mm) on isolate AUPEP-6 followed by glucose (16±1mm), lactose (15.67±0.58mm), where as the least mycelial growth was observed on lactose (1.67±.58mm) on isolate AUPEP-9 (Table- 3).

Table 3: Effect of carbon sources on mycelial growth of *Colletotrichum* isolates after4 days of incubation(mm) (Mean and Standard deviation)

Isolates	Maltose	Lactose	Fructose	Glucose	Sucrose
AUPEP -1	10^{c}±1	11^{d}±1	11^{b}±1	14.67^{c}±.58	**13.67^{c}±.58**
AUPEP -2	15.67^{d}±.58	4.67^{b}±.58	16.67^{c}±.58	12.67^{b}±.58	**15.67^{de}±.58**
AUPEP -6	19^{e}±00	15.67^{e}±.58	19^{e}±00	16^{cd}±1	**19^{f}±00**
AUPEP -7	7^{b}±1	6.67^{c}±.58	14.67^{c}±.58	15.67^{cd}±.58	**11^{b}±1**
AUPEP -8	9.67^{c}±.58	12.67^{d}±.58	9^{b}±1	11.67^{b}±.58	**14.67^{cd}±.58**
AUPEP -9	3.67^{a}±.58	1.67^{a}±.58	2.67^{a}±.58	2.67^{a}±.58	**2.67^{a}±.58**
AUPEP -10	3.33^{a}±.58	12.67^{d}±.58	15.67^{d}±1	16.67^{d}±.58	**17^{e}±1**

**Values with the same letters are not significantly different*

Nitrogen utilization

The utilization of six different nitrogen sources by *Colletotrichum* isolates is presented in (Table 4). The data indicate that the effect of different nitrogen sources on the growth of the test fungal isolate was significant (p=0.05). The mean value which was obtained by sodium nitrate and ammonium dihydrogen phosphate (18±00mm) supported the maximum growth followed by potassium nitrate (17±00mm) and ammonium sulphate (16.67±.58mm) on isolate AUPEP -6. Potassium nitrate was found to be the least utilized nitrogen source on isolate AUPEP -9 (Table 4).

Table 4: Effect of nitrogen sources on mycelial growth of *Colletotrichum* isolates after 4 days of incubation.(Mean and Standard deviation)

Colletotrichum isolates	KNo_3	$NaNo_3$	NH_4cl	$(NH_4)_2So4$	$(NH4)_2Hpo4$	$NH4So4$
AUPEP -1	13^{bc}±1	15.67^{c}±.58	9.67^{b}±.58	6.67^{a}±2.08	7.67^{b}±.58	3.67^{a}±.58
AUPEP -2	16.67^{cd}±.58	8^{b}±00	13.67^{c}±.58	16.67^{b}±2.08	17.67^{c}±.58	15.33^{c}±1.15
AUPEP -6	17^{d}±00	18^{d}±00	13.67^{c}±.58	16.33^{b}±2.89	18^{c}±00	16.67^{c}±.58
AUPEP -7	11.33^{b}±3.21	3^{a}±1	13.67^{c}±.58	16^{b}±2.65	17^{c}±1	15.33^{c}±1.15

AUPEP -8	13.67^{bcd}±.58	3.67^{a}±.58	9.67^{b}±.58	7.33^{a}±2.31	7.67^{b}±.58	5.67^{ab}±.58
AUPEP -9	1.67^{a}±.58	9.67^{b}±.58	1.67^{a}±.58	1.67^{a}±.58	1.67^{a}±.58	6.33^{b}±.58
AUPEP -10	16.67^{cd}±.58	16.67^{c}±.58	17^{d}±1	15.67^{b}±.58	16.67^{c}±.58	16.67^{c}±.58
Overall Mean	12.86±5.24	10.57±5.81	11.28±4.73	11.48±6.07	12.33±6.25	11.38±5.58

**Values with the same letters are not significantly different*

Physiological studies

Temperature requirement

In the present study, the effect of different temperature levels on the growth of *Colletotrichum* isolates was evaluated and recorded (Figure 3). The data showed that, there was a significantly difference (p=0.05) in the growth of the fungal isolates at different temperature levels. The maximum growth of *Colletotrichum* isolates was observed at 25ºC, followed by the growth at 30ºC. However, growth of some fungal isolates was noted at 20ºC, 35ºC and 40ºC.

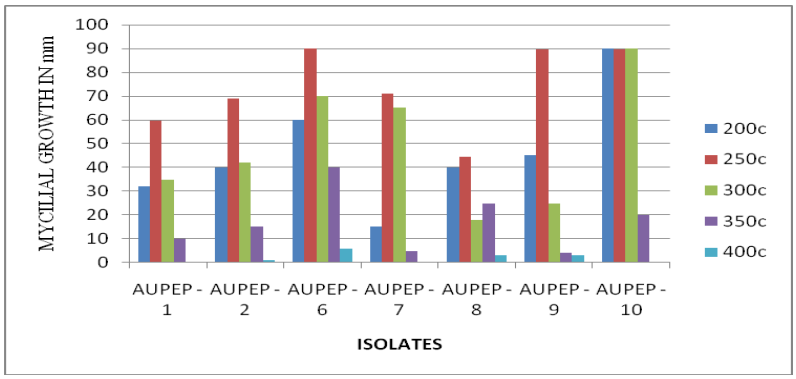

Fig .3 Effect of different temperature levels on mycelial growth of *Colletotrichum* isolates after 4 days of incubation (mm).

Hydrogen ion concentration

The effect of pH on the growth of the *Colletotrichum* isolates was studied (Table 5).The effect of different pH levels on the growth of *Colletotrichum* isolates was significant (p=0.05). The maximum growth of the fungal isolate was recorded at a pH level of 6.0 and 6.5 followed by 7.0, 5.5, 5, on isolate AUPEP -10 but the least was observed at 8.0 on isolate AUPEP -9 and AUPEP -8.

Table 5. Effects of pH on the mycelial growth of different isolates of *Colletotrichum* colony diameter (mm) at different pH after four days of Inoculation (Mean and standard Dev.)

	Mean and Standard Deviation							
Isolates	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**
AUPEP -1	3.33^{ab}±.58	4.67^{a}±1.53	6..67^{b}±.58	8^{a}±1	6^{a}±1	6^{a}±1	3.67^{ab}±.58	4.33^{a}±.58
AUPEP -2	5.67^{bc}±1.53	10^{b}±1	11.67^{d}±.58	12^{cd}±00	10^{b}±1	8.67^{b}±.58	8.33^{c}±.58	5.67^{cd}±.58
AUPEP -6	13^{e}±1	14.67^{cd}±.58	10.67^{d}±.58	11^{cd}±2	17.67^{c}±.58	12.67^{c}±.58	12.33^{d}±.58	6.67^{d}±.58
AUPEP -7	8.67^{d}±.58	12^{bc}±2	11^{d}±1	12.67^{d}±.58	10.67^{b}±.58	12^{c}±00	5^{b}±1	9^{e}±1
AUPEP -8	1.67^{a}±.58	5.67^{a}±.58	4.67^{a}±.58	6.67^{a}±.58	6.67^{a}±.58	4.67^{a}±1.53	2^{a}±00	1.67^{a}±.58
AUPEP -9	7.67^{cd}±.58	10.67^{b}±.58	8.67^{c}±.58	10^{bc}±00	12^{b}±2	8.67^{b}±.58	9.33^{c}±.58	1.67^{a}±.58
AUPEP -10	14^{e}±1	16.67^{d}±.58	17^{e}±00	18^{e}±00	18^{c}±00	17.33^{d}±.58	9.67^{c}±.58	3^{ab}±1

**Values with the same letters are not significantly different*

Light requirement

The exposure of the fungal isolates to alternate cycles of 12 hrs light and 12 hrs darkness nd continuous light for four days observed that the maximum growth mean colony diameter (90mm), followed by continuous dark on isolates AUPEP -6 and AUPEP -10. (Fig 4).

Discussion

The result of the pathogenicity test on the 7 Colletotrichum isolates that was established by artificial inoculation had shown that a significant variation among healthy pepper fruits. Diseased fruits were observed which were few and low in quality for use. In these lesions black acervuli with chain of conidia were found in concentric rings. The typical small circular spots appeared on the fruit surface; enlarged in advance stage became more or less elliptical and caused fruit rot. These spots expanded in the direction of long axis on the fruit and became black or dark brown in color. The spots were markedly delimited by a thick and sharp black outline. Similarly, Singh *et al*. [26] collected plant part showing anthracnose symptoms, particularly dieback and fruit rot were collected and

brought to laboratory for microscopic examination and isolation of pathogen. Purified cultures were tagged and tentatively based on location of sample and proved *in vitro* pathogenicity on detached fruits of pepper cultivar ''Kandhari'. Roat *et al*. [28]collected the infected fruits and isolated and identified as *Colletotrichium* spp. Fresh pepper fruit samples were surface sterilized and incubated at 25 ^{0}C for seven days and confirmed pathogenicity test of the pathogen.

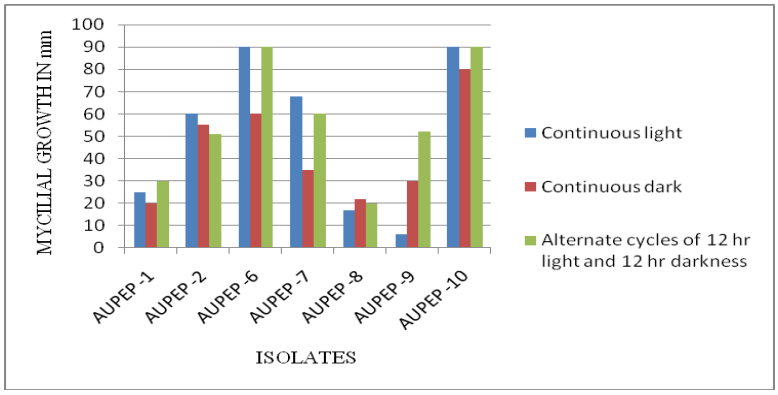

Fig:4 Effect of light intensity levels on mycilial growth of *Colletotrichum* isolates in mm.

The fungi generally utilize substrate in the form of solution, only if the reaction of the solution is conducive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for a better fungal growth. In the present study maximum growth of the *Colletotrichum* isolates was obtained at pH 6.0 followed by 6.5 and 5.5 values, where as optimum pH range was found to be 5.5 to 6.5 Similarly, Ashoka[17] observed that the maximum dry mycelial weight of the fungus was noticed at a pH level of 6.0 which was significantly superior to the rest of the pH levels tested. The least growth was recorded at a pH of 4.0. Vinod[13] has reported that maximum and minimum growth of *Colletotrichum gloeosporioides* was found at an optimum pH of 6.5 and 4.0, respectively.Temperature also affects almost every function of fungi, including growth and reproduction. In the present study, maximum growth of *Colletotrichum* spp was obtained at 25^{0}C, whereas optimum temperature ranged between 20-30^{0}C.Similarly,Ashoka[17] also reported 20-30^{0}C as optimum temperature range for *C. gloeosporioides.* In the present investigation, *Colletotrichum* isolates showed a maximum growth when exposed to alternate cycles of light and darkness which is in conformity with Vinod[13] observed that exposure of *C. gloeosporioides* to alternate cycles of 12 hours light and 12 hours darkness resulted in maximum growth. Similarly, Sudhakar[16] have showed that exposure of *C. gloeosporioides* to alternate cycles of 12hr light and 12 hr darkness yielded maximum growth and sporulation of the pathogen.

Among the six solid culture media evaluated, maximum radial mycelial growth of *Colletotrichum* isolates was observed on pepper dextrose agar (prepared from leaves, stems and fruits of pepper) and potato dextrose agar medium followed by,malt extract agar, papaya fruit agar, corn meal agar and potato carrot agar. However, Ashoka[17] observed that maximum radial mycelial growth of *C. gloeosporioides* was observed on potato dextrose agar and Richards's agar and was onpar with oat meal agar. This was followed by Czapek's agar, potato carrot agar, Tochinai's agar and V8 juice agar supportedmoderate growth of the fungus [17]. Akthar[27] has also recommended that the fresh potato extract was best source for routine isolation and growing of *C. gloeosporioides* causing mango anthracnose.

Vinod [13] reported that dextrose supported maximum growth of fungi, followed by fructose, glucose and soluble starch. The least growth of the pathogen (*Colletotrichum gloeosporioides*) was reported by lactose.

Nitrogen is an essential element and like carbon, it is also used by fungi for functional as well as structural purposes. But all the source of nitrogen is not equally good for the growth of fungi. *Colletotrichum*species have showed variation in its ability to utilize different nitrogen sources. In the present study, potassium nitrate supported the maximum growth of fungi followed by ammonium dihydrogen phosphate, diammonium sulphate, ammonium sulphate and ammonium chloride. The least growth was reported in the case of sodium nitrate. Similar observation was been made by Ekbote[29] in case of *C. gloeosporioides* who reported that potassium nitrate supported the maximum growth followed by sodium nitrate, L-asparagine but ammonium nitrate showing the least growth.

Conclusion

On the basis of morphological and cultural studies, the pathogens were identified as *Colletotrichum* spp. Cultural studies conducted on solid media, pepper dextrose agar and potato dextrose agar was the best for the growth and excellent sporulation of the *Colletotrichum* isolates .Among the carbon sources tested, sucrose was found to be the best for the growth of *Colletotrichum* isolatesof all carbon sources and lactose was utilized the least. Among the nitrogen sources tested, potassium nitrate was found to be the best for growth of *Colletotrichum* isolates. The optimum ranges of temperature and pH levels for the fungus were 25^{0}C to 30^{0}C and 5.5 to 7.0,

respectively. However, maximum growth and sporulation of fungus was recorded, at 25^{0}C temperature and pH 6. Alternate cycles of 12 h light and 12 h darkness resulted in maximum mycelia growth.

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