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Effect of physical parameters on the growth of *Alternaria alternata* causing Alternaria leaf blight of carrot

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Abstract
Carrot (*Daucus carota* subsp. *sativus*) is most vital vegetable root crop with enormous medicinal, health and nutritional value. Carrot comes under *Umbelliferae* family and has a large edible conical root. Afghanistan is the main centre of origin. Among the foliar diseases of carrot, the Alternaria Leaf Blight is major one, which occurs worldwide. An *in vitro* study was conducted on effect of different temperatures, relative humidity and pH on growth of *Alternaria alternata* under laboratory of Department of Plant Pathology, S.K.N. College of Agriculture, Jobner. Results disclosed that among the physical parameters, temperature, pH and relative humidity, the mycelial growth was best at 25 ^{0}C (89.00 mm), more dry mycelial weight at pH 6.0 (810 mg) and relative humidity 100% (89.25 mm) showed better mycelial growth. Single spore technique was used for the purification of the fungus. Pathogenicity was proved by spore cum mycelial suspension spray on the carrot leaves showed that *Alternaria alternata* was pathogenic to carrot crop.

Keywords: Alternaria leaf blight, carrot, temperature, PH, relative humidity and *Alternaria alternata*

Introduction
Carrot (*Daucus carota* subsp. *sativus*) is most vital vegetable root crop with enormous medicinal, health and nutritional value. It comes under *Umbelliferae* family and has a large edible conical root. Afghanistan is the main centre of origin, from where the carrot originated for cultivation purpose, Mackevic (1929) and Banga (1976) [13, 5] from Persia the carrot was introduced to India, according to Shoemaker (1947) [17].

Carrot grown as a biennial crop. A rosette of leaves grow above at first while building the enlarged taproot below the soil. Fast-growing cultivars ripen within three months (90 days) of sowing the seed, while slow-growing cultivars require an extra month (120 days).It is cultivated almost worldwide, round the year in temperate countries and in tropical and subtropical regions during winter. It is used as a part of mixed vegetables, steamed vegetables and can also be mixed with different vegetables to prepare soup and stew (Anjum and Amjad, 2002) [1].

It is an excellent source of alpha and beta carotene, the forerunner of vitamin A (Pantastica, 1975). Gopalan *et al.* (1985) [14, 11] reported that carrot have more amount of thiamine and riboflavin. Vitamin A in carrot prevents night blindness. It is a tremendous source of Fe, vitamin-A, vitamin-B, ascorbic acid and sugar (Yawalkar, 1985) [20]. A special type of beverage called Kanji is prepared from Black carrot, which is used as appetizer (Singh, 2011) [19]. In North India, the Gajar halwa is very famous sweet dish.

According to Bose *et al.* (1986) [7] the edible portion of carrot root (per 100 g) contains carbohydrate (10.6 g), fibre (1.2 g), moisture (86 g)and carotene (1890 µg).The carrot green (per 100 g) contains carbohydrate (8.3 g), protein (5.1 g) and moisture (83.9 g).

In India the most important carrot growing states are Haryana, Punjab, Uttar Pradesh, Karnataka, Tamilnadu, Assam, Andhra Pradesh.

Carrot is attacked by a vast number of fungal pathogens along with some bacterial and few physiological disorders. Almost around ten seed borne pathogens attack the carrot crop, as it is propagated by the seed (Richardson, 1990) [15]. The most important fungal and bacterial diseases of carrot are given below:

Material and Methods

Isolation of pathogen

For isolation of the pathogen, small pieces of the leaves were cut from the diseased portion along with healthy tissues. These pieces were surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) solution for 1-2 minutes. After three consecutive washings with sterilized distilled water, the pieces were transferred to autoclaved Potato Dextrose Agar medium in Petriplates and incubated at 25±1^{0}C in B.O.D. incubator. After 7 days of incubation mycelial growth emanating from leaf bits was transferred aseptically to fresh PDA slant with the help of sterilized inoculation needle and reincubated for next days to obtain further mycelial growth and sporulation for their purification.

Purification of pathogen

Purification of the pathogen was done by single spore and hyphal tip techniques.

Effect of physical parameters on the growth and sporulation of the *Alternaria alternata*

All the glass wares were thoroughly cleaned and rinsed with distilled water and sterilized in hot air oven at 160^{0}C for 2 hours.

Effect of temperature

Effect of temperature on mycelial growth of *Alternaria alternata*, was studied under *in vitro*. Twenty ml of sterilized PDA medium was poured in each sterilized Petriplates. Inoculation was made with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer and Petriplates containing PDA medium were inoculated with 5mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer. Inoculated Petriplates without lid were immediately accommodated in glass dessicators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 25±1 ^{0}C for 7 days. Observations of mycelial growth was recorded on 7th day of incubation.

incubated at 4 different levels of temperature *viz*., 20, 25, 30 and 35 ^{0}C for 7 days. Observations of mycelial growth of fungus was recorded after 7 days of incubation.

Effect of pH on mycelial growth

Stock solutions

Solution A: 0.1 M solution of citric acid (192.1 mol.wt.) in one litre of distilled water 19.21 gm of citric acid was dissolved.

Solution B: 0.2 M solution of dibasic sodium hydrogen phosphate (293.9 mol.wt.) in one litre of distilled water 58.78 gm of dibasic sodium hydrogen phosphate was dissolved.

The effect of pH on the growth of *Alternaria alternata* was determined by adjusting the pH of Potato dextrose broth at 5.0, 6.0, 7.0, 8.0 by using citrate phosphate buffer (Singh *et al*., 2005) [18] before sterilization with the help of pH meter. Aliquate of 20ml medium were dispended in 100ml conical flask and autoclaved at 1.045kg/cm^2 for 20 minutes. Inoculations were made with 5mm disc of mycelia obtained from 7 days old culture of *Alternaria alternata*. An observation of dry mycelial weight was recorded after 14 days of incubation at 25±1 ^{0}C.

Effect of relative humidity on mycelial growth

To study the effect of relative humidity on mycelial growth of *Alternaria alternata*, five different levels of relative humidity *i.e*. 60,70,80,90 and 100 percent were maintained by using the concentrated sulphuric acid and sterilized distilled water in different proportions in glass dessicators by the method suggested by Buxton and Mellanby (1934) [8]. The composition of the acid solution was used as follows:

Relative humidity (%)	Stock solution (ml)*	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	0.00	Only distilled water

*50 percent v/v solution of concentrated sulphuric acid.

Results and Discussion

Impact of temperature on mycelial growth

To find out the role of temperature on the spore germination of (*Alternaria alternata)*, four different temperatures 20, 25, 30, 35 ^{0}C were taken. Here we found the highest mycelial growth (89.00 mm) at 25 ^{0}C followed by 30 ^{0}C (75.25 mm), then at 20^{0}C (71.75 mm) and mycelial growth was least found at 35^{0}C (69.75 mm) as given in table 1, fig 1 and plate 1.

Table 1: Impact of temperature on mycelial growth of *Alternaria alternata* after 7 days of incubation

S. No.	Temperature (^{0}C)	Mycelial growth*(mm)
1	20	71.75
		(57.89)
2	25	89.00
		(70.63)
3	30	75.25
		(60.17)
4	35	69.75
		(56.63)
	S.Em+	2.78
	CD (p=0.05)	8.56

*Average of three replications

Figures given in parenthesis are angular transformed values

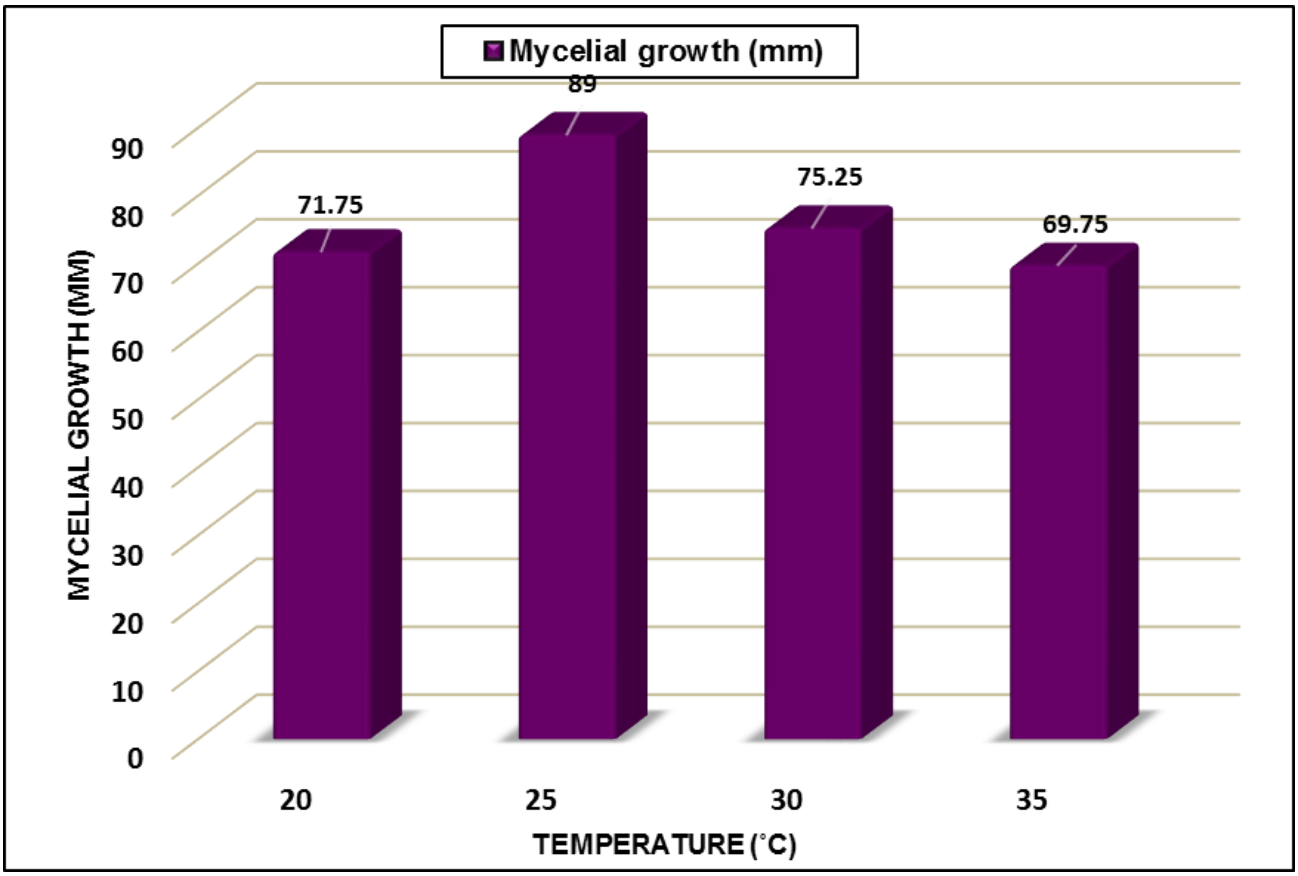
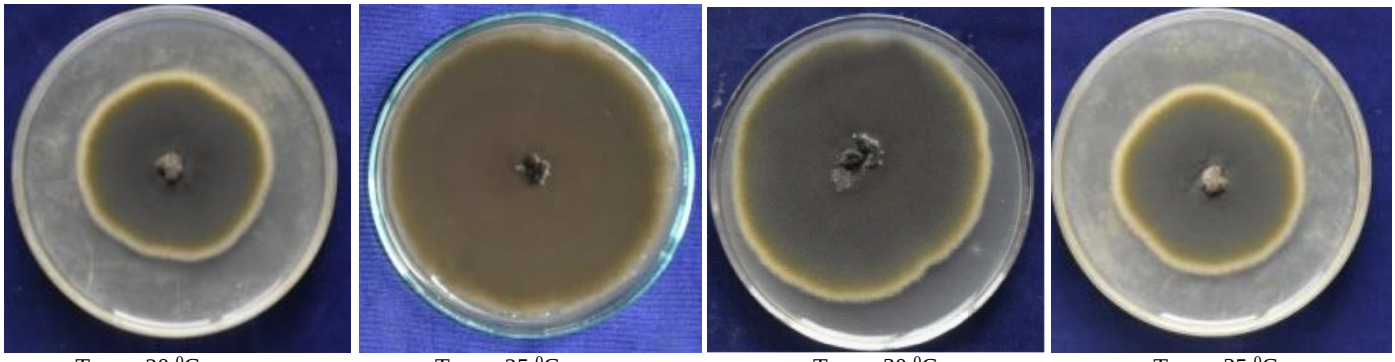


Fig 1: Impact of temperature on mycelial growth of *Alternaria alternata* after 7days of incubation



Temp- 20 ^{0}C Temp- 25 ^{0}C Temp- 30 ^{0}C Temp- 35 ^{0}C

Plate 1: Effect of temperature on mycelial growth of *Alternaria alternata* on Potato Dextrose Agar medium

Effect of pH on mycelial growth

Generally, fungus grows best at Acidic pH. Out of the four pH values 5.0, 6.0, 7.0, 8.0 selected for the experiment, the maximum dry mycelial weight was found at pH 6.0 (810 mg) after 14 days of incubation on Potato dextrose broth, followed by pH 7.0 (735 mg), pH 5.0 (720 mg) and least mycelial weight was at pH 8.0 (275 mg) as given below in table 2, fig 2, plate 2.

Table 2: Impact of pH on dry mycelial weight of *Alternaria alternata* after 14 days of incubation at 25±1^{0}C

S. No.	pH level	Dry weight of mycelial growth(mg)*
1	5.0	720
2	6.0	810
3	7.0	735
4	8.0	275
	S.Em+	2.68
	CD (p=0.05)	8.24

*Average of three replications

Figures given in parenthesis are angular transformed values

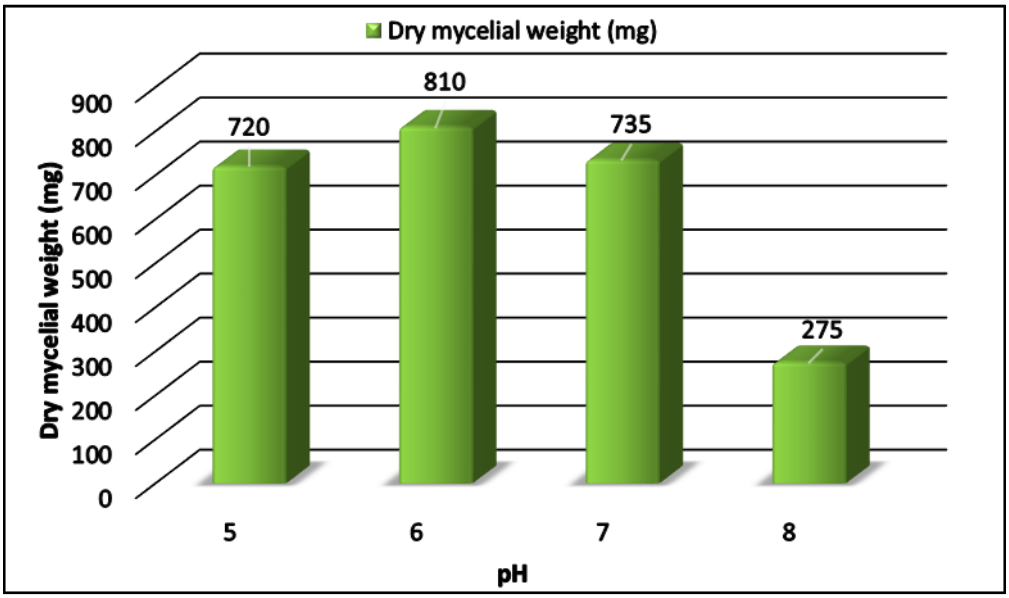
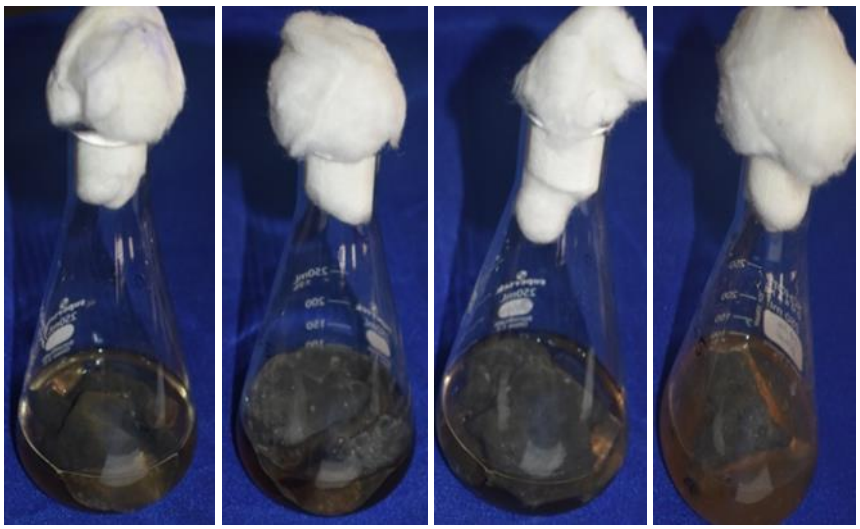


Fig 2: Impact of pH on dry mycelial weight of *Alternaria alternata* after 14 days of incubation at 25±1 ^{0}C



pH- 5.0 pH- 6.0 pH- 7.0 pH- 8.0

Plate 2: Effect of pH on dry mycelial weight of *Alternaria alternata* on Potato Dextrose Broth medium

Effect of relative humidity on mycelial growth of *Alternaria alternata* after 7-daysof incubation at 25±1 ^{0}C

To know the effect of relative humidity on the mycelial growth of fungus, five different range of relative humidity 60, 70, 80, 90,100 were adjusted in the laboratory using concentrated sulphuric acid and distilled water in different proportions. At 100% (89.25 mm) relative humidity, the highest sporulation was observed, next to it was at 90% (85.75 mm) followed by 80% (78.00 mm), 70% (63.50 mm) and least mycelial growth was noticed at 60% (45.25 mm) as given below in table 3, fig 3, plate 3.

Table 3: Impact of relative humidity on mycelial growth of *Alternaria alternata* after 7-days of incubation at 25±1^{0}C

S. No.	Relative humidity (%)	Mycelial growth* (mm)
1	60	45.25
		(42.27)
2	70	63.50
		(52.83)
3	80	78.00
		(62.03)
4	90	85.75
		(67.82)
5	100	89.25
		(70.86)
	S.Em+	2.01
	CD (p=0.05)	6.19

*Average of three replications

Figures given in parenthesis are angular transformed values

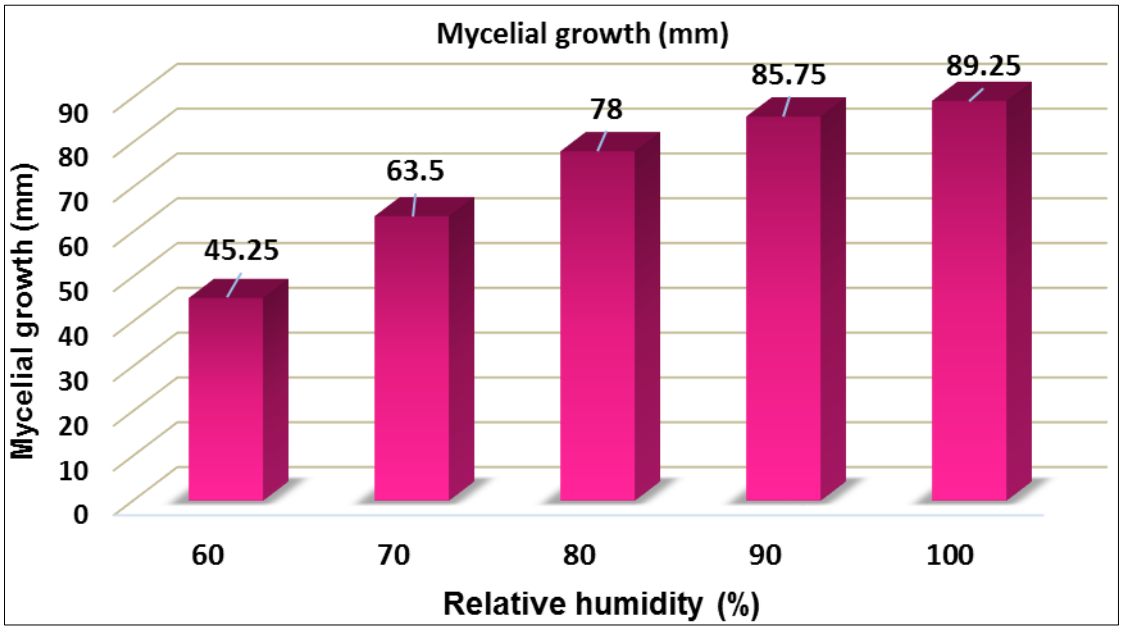
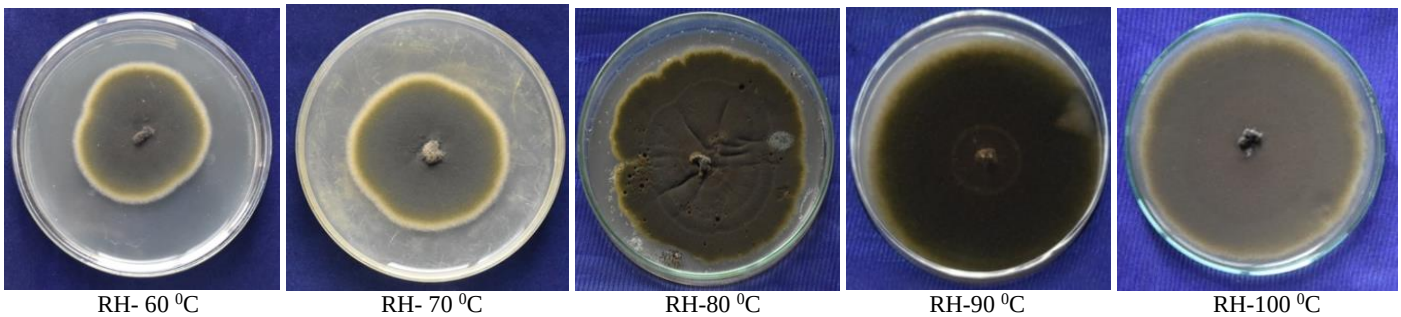


Fig 3: Impact of relative humidity on mycelial growth of *Alternaria alternata* after 7 days of incubation at 25±1^{0}C



RH- 60 ^{0}C | RH- 70 ^{0}C | RH-80 ^{0}C | RH-90 ^{0}C | RH-100 ^{0}C

Plate 3: Effect of relative humidity on mycelial growth of *Alternaria alternata* on Potato Dextrose Agar medium

Conclusion

Temperature plays a crucial role in regulating the growth and reproduction of fungi. Out of the selected four different range of temperature 20, 25, 30, 35^{0}C the *Alternaria alternata* grows best at 25^{0}C (89.00 mm). The studies of Singh (2005) [18] also showed that the 25^{0}C was best for the conidial germination of *Alternaria alternata* causing blight of adusa. The results were parallel with the results observed by Hubbali *et al*. (2010) [12] on the mycelial growth of *Alternaria alternata* causing leaf blight of noni. The results were at par with the results observed by Balai and Ahir *et al*. (2013) [6] on the mycelial growth of *Alternaria alternata* causing leaf spot of brinjal which was best at 25^{0}C. Even Choudhary *et al*. (2017) [9] reported that the *Alternaria alternata* show the maximum mycelial growth at 25^{0}C causing leaf blight of isabgol.

Generally, fungi grows best at Acidic pH. Out of the four pH values 5.0, 6.0, 7.0, 8.0 choosen for the experiment, the maximum dry mycelial weight was found at 6.0 pH (810 mg) after 14 days of incubation on Potato dextrose broth, followed by 7.0 pH (735 mg), least mycelial weight is at 8.0 pH (275 mg). The results of the Gholve *et al*. (2015) [10] revealed that the *Alternaria* grows best at pH 6.0 followed by pH 7.0. As alkalinity increases the growth of fungus subsequently decreases.

To know the impact of relative humidity on the mycelial growth of fungus five different range of relative humidity *viz.*, 60, 70, 80, 90 and 100 were taken. At 100% (89.25 mm) relative humidity the highest sporulation was observed, next to it was at 90% (85.75 mm) followed by 80% (78.00 mm), 70% (63.00 mm) and least mycelial growth was noticed at 60% (45.25 mm), the results were in accordance with the findings of Sharma and Ahir (2018) [16] where at 100% and 90% relative humidity the mycelial growth was (88.70 mm) and (85.10 mm) respectively.

By considering the results of physical parameters it was observed that the dry mycelial weight was more at pH 6.0 (810 mg) relative humidity with 100% (89.25 mm) and at temperature 25^{0}C (89.00 mm) the growth and sporulation of the mycelium was high.

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