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EFFECT OF MEDIA, TEMPERATURE AND RELATIVE HUMIDITY ON THE MYCELIAL GROWTH OF *Claviceps fusiformis* CAUSING ERGOT DISEASE IN PEARL MILLET

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ABSTRACT

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important warm-season cereal crop widely cultivated in the arid and semi-arid regions of Asia and Africa. It is valued for its ability to withstand drought and poor soil conditions and is recognized as nutritious cereal rich in carbohydrates, protein, healthy fats and essential minerals. However, its productivity is severely affected by ergot disease caused by *Claviceps fusiformis*, which can lead to substantial grain yield losses under favourable environmental conditions. The effects of different culture media, temperature and relative humidity on mycelial growth of *Claviceps fusiformis* inciting agent of ergot of pearl millet were studied *in vitro*. Among the six solid media tested, Potato Dextrose Agar (PDA) supported the maximum mycelial growth (90.00 mm), followed by Oat Meal Agar (87.20 mm). The fungus exhibited comparatively lower growth on Czapek's Dox Agar, Richard's Agar, Potato Carrot Agar and Corn Meal Agar. Temperature significantly influenced fungal growth, with the highest mycelial growth (90.00 mm) recorded at 30°C, followed by 25°C (87.92 mm). Growth declined at 35°C and 20°C and was minimum at 40°C (35.68 mm), indicating that 25–35°C is the optimum temperature range for fungal growth. Relative humidity also had a marked effect, with maximum mycelial growth observed at 90% RH (90.00 mm), which was statistically at par with 95% and 100% RH. The lowest growth (58.30 mm) was recorded at 70% RH.

***Keywords*:** Media, Temperature, Relative humidity, *Claviceps fusiformis*, Ergot disease, Pearl millet.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), commonly known as bajra, is also referred to as spiked millet and bulrush millet in different parts of the world. It belongs to the family *Poaceae* and subfamily *Panicoideae* and has a diploid chromosome number of 2n = 14. Pearl millet is the third most important cereal crop after rice and wheat and serves as a major source of food, fodder and livelihood for millions of people living in the arid and semi-arid regions. It is mainly cultivated in the tropical regions of Asia and Africa, covering approximately 27 million hectares. The crop is a warm-season, cross-pollinated cereal with excellent adaptation to drought, high temperatures and low-fertility soils, making it suitable for cultivation under rainfed conditions where other cereal crops often fail. Pearl millet originated in West Africa. (Vavilov, 1950). Pearl millet is well adapted to acidic, saline and aluminium-toxic soils (Yadav and Rai, 2013). It is a hardy crop that grows successfully under arid and semi-arid conditions, making it suitable for regions with limited water availability. Pearl millet is considered a "nutri-cereal" because of its high nutritional value. The grains contain about 60–78% carbohydrates, 11–19% protein and 3.0–4.6% fat. It is also a good source of essential minerals such as iron and phosphorus (Reddy *et al*., 2016). Pearl millet contains about 74% polyunsaturated fatty acids (PUFAs) and is a good source of beneficial omega-3 fatty acids. In India, the major pearl millet-growing states are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana. Although the crop is well

adapted to harsh climatic conditions, its productivity is affected by several factors, among which diseases are one of the major constraints. Ergot of pearl millet was first reported in India by Thomas et al. (1945). The first major outbreak of the disease occurred in 1956 at Satara, Maharashtra (Bhide and Hegde, 1957). According to Natarajan et al. (1974), the average disease incidence was about 62.4%, resulting in nearly 58% grain yield loss. Later, Khairwal *et al*. (2007) reported that grain yield losses in pearl millet hybrids may reach 58–70% under severe disease conditions. For laboratory studies, successful artificial cultivation of the pathogen requires a suitable nutrient medium that supports its growth and development. Besides nutritional requirements, environmental factors such as temperature and relative humidity play a vital role in influencing fungal growth, metabolism and physiological activities. Therefore, the present study was conducted to assess the effects of different culture media, temperature, and relative humidity on the mycelial growth of *Claviceps fusiformis*.

Materials and Methods

Effect of media

The growth characteristics of *Claviceps fusiformis* were studied on seven different solid media (Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Potato Carrot Agar (PCA), Richard's agar, Czapeck's Dox Agar (CzDA), Yeast Malt Agar (YMA) and Oat Meal Agar (OMA) under *in vitro* conditions. All the media were sterilized at 121.6°C (15 psi) for 20 minutes. About 20 ml of each medium was poured into sterilized Petri dishes and allowed to solidify. The plates were inoculated with a 5 mm culture disc of the *Claviceps fusiformis* and incubated at 28 ± 1°C. Each treatment was replicated three times. Observations on radial mycelial growth were recorded at 8th day of inoculation.

Effect of temperature

Effect of temperature on mycelial growth of the pathogen was studied *in vitro* conditions. Twenty ml of sterilized media was poured in each of sterilized Petri plates. Inoculation was made with 5 mm diam. disc of 8 days old culture of *Claviceps fusiformis* cut with the help of sterilized cork borer and incubated at different levels of temperature *viz*., 20, 25, 30, 35 and 40°C for optimum growth. Each experiment was arranged in completely randomized design (CRD) with four replications. The observations on radial mycelial growth were recorded by averaging two diameters of colony at right angles to one another and subtracting 5 mm of the mycelial bit. The data on mycelium growth was analyzed statistically. Observations on radial mycelial growth of the test pathogen was recorded at 8th day of inoculation.

Effect of relative humidity

Studies were conducted with a view to determine the optimum range of relative humidity for mycelial growth of *Claviceps fusiformis*. Twenty ml sterilized Potato Dextrose Agar was poured into each Petri plate under aseptic conditions. The Petri plates were inoculated with 5 mm mycelial discs of inoculum cut with the help of sterilized cork borer from 8 days old culture of *Claviceps fusiformis* raised on PDA. Three replications were kept for each treatment. The inoculated Petri plates were kept at different relative humidity levels *i.e*. 70, 75, 80, 85, 90, 95 and 100 per cent were maintained in by mixing distilled water and concentrated sulphuric acid (specific gravity 1.84) in definite proportion by adopting the method suggested by Buxton and Mellanby (1934). To maintain different humidity conditions, equal volume of concentrated sulphuric acid and double distilled water were mixed to get stock solution. It was found that 100 ml of acid + 100 ml of water gave 183.5 ml. The double distilled water + concentrated sulphuric acid in different proportion. Different concentrations of the acid and double distilled water were poured at the bottom of desiccators and a porcelain plate was used to keep the inoculated Petri plates above the solution. The lids of the desiccators were tightly closed and made air tight by applying the grease. The desiccators were incubated at 28±1ºC in the BOD incubator. Observations of radial mycelial growth of the test pathogen was recorded at 8th day of inoculation.

Results and Discussion

Effect of different media

The result presented in table 1 and plate 1 revealed that among all solid media tested, maximum mycelial growth was obtained in potato dextrose agar medium (90 mm), which was statically at par with oat meal agar (87.20mm). The rest of the media *viz*., Czapeck's Dox Agar (68.00 mm), Richard's agar base (59.40 mm), Potato Carrot Agar (58.00 mm) and Corn Meal Agar (45.30 mm), were also supported excellent growth of the fungus *Claviceps fusiformis*. The present result supported by the finding of Hajano *et al*. (2013) reported that *Pyricularia oryzae* exhibited maximum mycelial growth and sporulation on Potato Dextrose Agar (PDA), followed by Potato Carrot Agar (PCA), whereas oat meal agar was the least supportive medium. Similarly, Lal *et al*. (2014), who evaluated different solid and liquid media for the growth of *Curvularia lunata*. They reported that Potato Dextrose

Agar (PDA) supported the maximum mycelial growth and sporulation followed by host extract agar, whereas comparatively lower growth was recorded on Conn's agar and Czapek-Dox agar.

Effect of temperature

Temperature plays an important role in infection and disease development. Data presented in table 2 and plate 2 revealed that, maximum mean mycelial growth of test fungus was recorded at temperature of 30 ^{0}C (90.00 mm) which was significantly superior over all other temperature. This was followed by 25°C, which recorded 87.92 mm mycelial growth. A gradual reduction in mycelial growth was observed at 35°C (78.50 mm) and 20°C (56.10 mm). The minimum mycelial growth of 35.68 mm was recorded at 40°C. In the present study, it was observed that temperature range of 25 °C to 35 °C can be recommended to obtain excellent fungal growth. The present result agrees with Lal *et al*. (2014) studied the effect of different temperatures (8–37°C) on the growth of *Curvularia lunata*. They reported that the fungus grew over a wide temperature range, with 28°C being the optimum for maximum mycelial growth, followed by 30°C, while growth declined at temperatures below and above the optimum. Similarly, Bhatt and Kumar (2018) reported different temperature levels significantly influenced the mycelial growth of *Curvularia lunata under in vitro* conditions.

Effect of relative humidity

Relative humidity is one of the most important environmental factors influencing the growth and development of plant pathogens after temperature. The findings of the present study showed that mycelial growth of *Claviceps fusiformis* cultures causing ergot of pearl millet were significantly influenced by different RH levels. Significantly highest mean mycelial colony growth was observed at 90 per cent RH (90.00mm) of *Claviceps fusiformis* (Table 3 and plate 3), followed by statistically at par with 95 per cent RH (89.40 mm) and 100 per cent RH (89.30 mm). The mycelial growth decreased significantly at 85 per cent RH (82.10 mm), followed by 80 per cent RH (78.20 mm) and 75 per cent RH (67.50 mm). The minimum mycelial growth of 58.30 mm was recorded at 70 per cent relative humidity.The present findings are in agreement with the results reported by Kumar and Singh (2021)**,** who reported maximum mycelial growth of *Cercospora canescens* at 90 per cent relative humidity (89.00 mm)**,** followed by 100 per cent RH (86.30 mm)**,** whereas the least growth **(**45.30 mm) was observed at 50 per cent RH. Likewise, Shivran *et al*. (2020) observed that 90 per cent RH was the most favourable for the mycelial growth of *Rhizoctonia solani*, recording maximum radial growth (90.00 mm). Similarly, Sharma *et al*. (2019) reported that 90 to 100 per cent RH was highly favourable for the growth of *Alternaria cyamopsidis*, with maximum mycelial growth at 90 per cent RH (88.90 mm) followed by 100 per cent RH (85.67 mm)**.**

Table 1: Effect of different solid media on mycelial growth of *Claviceps fusiformis*

S.NO.	Media	Mycelial growth (mm) *
1	Potato dextrose agar	90.00
2	Corn meal agar	45.30
3	Potato carrot agar	58.00
4	Richard's agar	59.40
5	Czapek dox agar	68.00
6	Malt extract agar	37.30
7	Oat meal agar	87.20
	SEm±	0.74
	CD(p=0.05)	2.28
	CV (%)	2.03

*Average of three replications

Table 2: Effect of temperature on mycelial growth of *Claviceps fusiformis*

S.NO.	Temperature (°C)	Mycelial growth (mm) *
1	20	56.10
2	25	87.92
3	30	90.00
4	35	78.50
5	40	35.68
	SEm±	0.70
	CD(p=0.05)	2.14
	CV (%)	2.02

*Average of four replications

Table 3: Effect of relative humidity on mycelial growth of *Claviceps fusiformis*

S.No.	Relative humidity (%)	Mycelial growth (mm) *
1	70	58.30
2	75	67.50
3	80	78.20
4	85	82.10
5	90	90.00
6	95	89.40
7	100	89.30
	SEm±	0.94
	CD(p=0.05)	2.87
	CV (%)	2.05

*Average of three replications

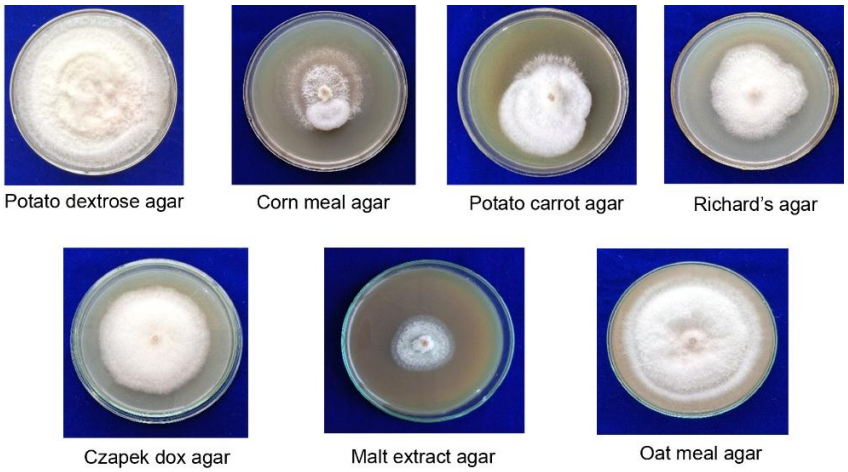

Plate 1: Effect of different solid media on mycelial growth of *Claviceps fusiformis*

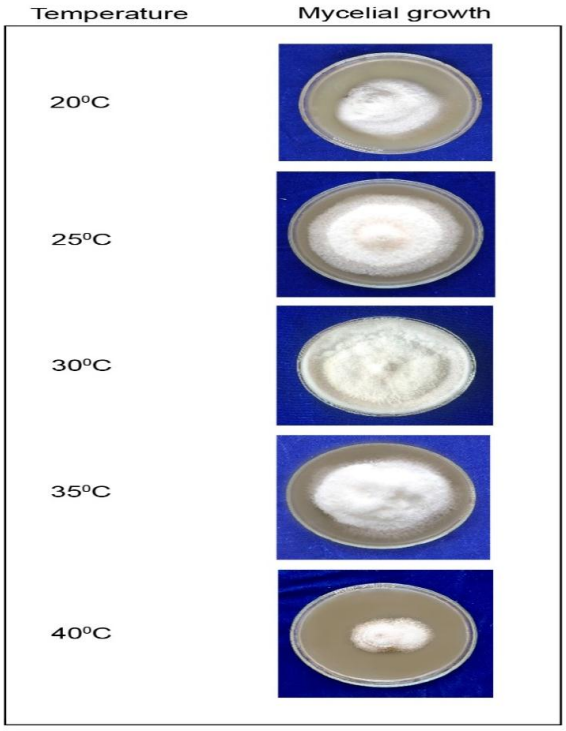

Plate 2: Effect of temperature on mycelial growth of *Claviceps fusiformis*

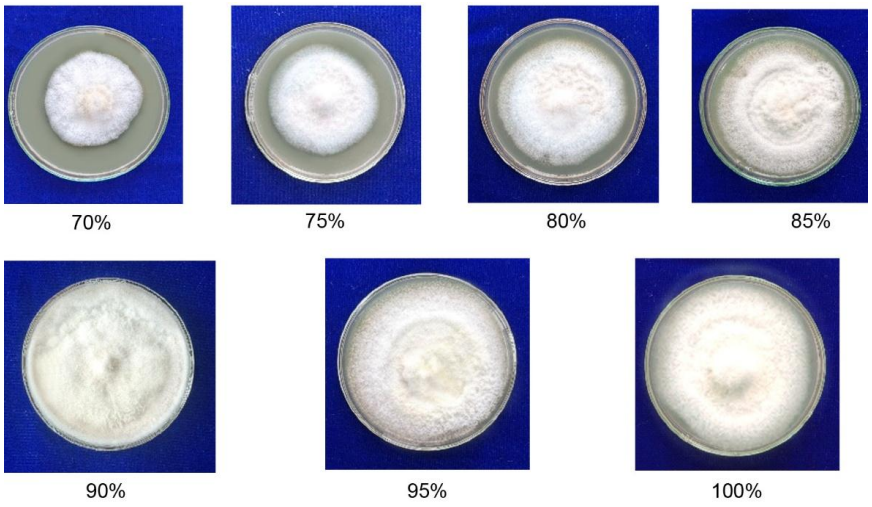

Plate 3: Effect of relative humidity on mycelial growth of *Claviceps fusiformis*

Conclusion

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal crop cultivated in the arid and semi-arid regions of India. However, its productivity is greatly affected by ergot disease caused by *Claviceps fusiformis*. The present investigation revealed that potato dextrose agar (PDA) was the most suitable medium for the growth of *C. fusiformis*, followed closely by oat meal agar. The pathogen showed maximum mycelial growth at 30°C, while good growth was also observed at 25°C and 35°C. Among the different relative humidity levels tested, 90% RH was found to be the most favourable for fungal growth, followed by 95% and 100% RH. These findings indicate that PDA medium, a temperature of 30°C and 90% relative humidity provide the optimum conditions for the *in vitro* growth of *C. fusiformis*. The information generated from this study will be useful for maintaining the pathogen under laboratory conditions and will support future research on the epidemiology and management of ergot disease in pearl millet.

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