

## Effect of different Solid Media and Physiological factors on the Growth Pattern of *Fusarium verticillioides* Incitant of Ear Rot of Maize

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**ABSTRACT:** *Fusarium* ear rot caused by *Fusarium verticillioides* is the newly emerging disease of maize causes 10 to 30 per cent reduction in the yield. The laboratory experiments ventured to know the effect of different solid media, temperature, relative humidity and seed moisture levels on the growth and sporulation of *F. verticillioides*. Among 10 media tested, the maximum colony diameter (89.67 mm) with very good sporulation was recorded on potato dextrose agar which was significantly superior over other media. The temperature of 25°C coupled with 90 per cent relative humidity were apt for the growth and sporulation of the fungus. The maximum mycelial growth of 42.67 per cent coverage of seed surface area was recorded at 41.37 per cent of seed moisture level which is significantly superior over other seed moisture levels. Therefore, the different nutrient base as well as the abiotic factors play a major role in disease development.

**Keywords:** *Fusarium* ear rot, relative humidity, seed moisture levels, solid culture media, temperature.

### INTRODUCTION

Maize (*Zea mays* L.) is one of the four basic staple food crop of the world population and ranks first with respect to the production in the world (Anderson *et al.*, 2004). The crop is popularly known as "corn" and belongs to the tribe Maydaceae of the family Poaceae. The grain is rich in starch (72 %), followed by protein (10 %) and fat (4 %) (Hulse *et al.*, 1980), which supplies an energy density of 365 kcal/100 g and grown throughout the world. It also consists of vitamin A, vitamin E and minerals such as potassium, phosphorus, magnesium, zinc, calcium and iron (Nuss and Tanumihardjo, 2010). It has emerged as a crop of global importance and prime driver of the global agricultural economy owing to its myriad end uses as a human food (17 %), feed (61 %) and serves as an important component of varied food and industrial products (22 %) including cornmeal, grits, starch, flour, tortillas, alcohol, sweetener, oil, ethanol, glue *etc* (Mehta and Dias, 1999). Maize is the most versatile crop with wider adaptability in varied agro-ecological regions with highest genetic yield potential among the food grain crops. It is well known as "Queen of Cereals" as it is grown throughout the year due to its photo-thermo-insensitive character and high genetic yield potential (Das *et al.*, 2018).

The majority of the maize diseases are incited by fungi by affecting root, stalk, ear head and kernel. Among these diseases, ear rot caused by *Fusarium verticillioides* is the newly emerging disease due to an

erratic and heavy rainfall coincides with high relative humidity in the maize growing countries and which causes huge losses in the yield. In addition to reduction in the yield, the fungi will also adversely affects the physical, physiological, phytosanitary as well as nutritive qualities of the seed and the mycotoxins produced by the fungi which is threatening to the human and livestock health. The disease typically occurs on random kernels, group of kernels or physically injured kernels and appears tan or brown in color, in case where fungal growth is visible on the ear, infected kernels appear whitish to pink or salmon coloured and white streaks radiating from the point of silk attachment to the cap of the kernel known as the "starburst" symptom (Munkvold and Desjardins, 1997). Generally pathogen is favoured by hot, dry conditions at flowering coupled with high humidity. The fungus utilizes both organic compounds and inorganic materials as the source of their nutrient supply which is required for its growth and reproduction. Every fungus has its own specificity for its nutritional requirement. The present investigation mainly concentrates to find out the best media for the pathogen growth as well as depicts the effect of temperature, relative humidity, and seed moisture levels on the growth and sporulation of *F. verticillioides*, that will helpful for further understanding the pathogen ecological survival which in turn collaborated with the management aspects under the field conditions.

## MATERIAL AND METHODS

### A. Isolation of *F. verticillioides* from ear rot infected maize cob

The standard tissue isolation procedure was followed to recover the *F. verticillioides* from the ear rot infected cobs at Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur. Infected kernels were appeared as tan or brown in colour, later with the growth of mycelia, white to pink discoloration was noticed and in severe case white streaks radiating from the point of silk attachment to the cap of the kernel known as the “starburst” symptom was also recorded. Such infected kernels were cut into small pieces and surface sterilized in mercuric chloride (0.1 %) or sodium hypochlorite solution (1 %) for 30 seconds, then thoroughly rinsed in three changes of sterile distilled water immediately and kept on clean tissue paper for two minutes before being put on PDA medium containing 100 ppm streptomycin sulphate in Petri plates. The plates were incubated for three days at  $25 \pm 2^\circ\text{C}$ .

### Details of expression of sporulation of *F. verticillioides*.

Sr. No.	Score	Grade	Description {conidia / microscopic field (10x)}
1.	++++	Very good sporulation	> 40
2.	+++	Good sporulation	26-40
3.	++	Fair sporulation	11-25
4.	+	Poor sporulation	1-10
5.	-	No sporulation	0

### C. Physiological characterization

(i) **Effect of temperature.** The temperature study was carried by pouring 20 ml of PDA in each Petri plate under aseptic condition. Five mm of mycelial disc was cut with the help of sterilized cork borer from the margins of seven days old culture of *F. verticillioides* grown on PDA. A disc of culture was placed in inverted position at the centre of each Petri dish and incubated at 15, 20, 25, 30, 35 and  $40^\circ\text{C}$  temperatures with three replications. Colony diameter and sporulations were recorded at 7<sup>th</sup> day.

(ii) **Effect of relative humidity.** Five mm discs of seven days old culture of *F. verticillioides* was placed on the centre of Petri dish containing 20 ml of PDA under aseptic condition and Petri dish were exposed to 60, 70, 80 and 90 per cent relative humidity levels maintained in the desiccators. Different levels of relative humidity were created by using different concentration of  $\text{H}_2\text{SO}_4$  with three replications. The desiccators were kept under room temperature and observations on the colony diameter and sporulation of the fungus was recorded seven days after inoculation.

(iii) **Effect of seed moisture levels.** Healthy grains were taken for the study and initial weight of the seed was recorded. The seeds were surface sterilized with sodium hypochlorite (1 %) or mercuric chloride (0.1 %) and soaked in spore suspension for 12 hrs. Immediate seed weight was recorded and placed in to the Petri plates containing three layers of moist blotter paper and incubated at  $25^\circ\text{C}$ . The soaked seeds were further allowed for air drying at two, four, six and eight hours

### B. Effect of different solid culture media on growth and sporulation of *F. verticillioides*

The effect of diverse culture media on cultural characteristics of *F. verticillioides* and also to find out the best media for the pathogen growth and sporulation. The 10 different solid culture media (Conn’s agar, corn meal agar, Czapek’s dox agar, Richard’s agar, oat meal agar, potato dextrose agar, Sabouraud’s dextrose agar, water agar, malt extract agar and V-8 juice agar) were evaluated for the local Raichur isolate. Twenty ml of each medium was poured into 90 mm Petri plates. Five mm disc of the fungus was cut with the help of sterilized cork borer from the margins of an actively growing culture of seven days old pure culture of *F. verticillioides* grown on PDA. One disc of the culture was placed in the inverted position at the centre of each Petri dish and incubated at  $25 \pm 2^\circ\text{C}$ . Three replications were maintained for each culture media and the data pertaining to radial growth and sporulation (Tabassum *et al.*, 2020) of the pathogen was recorded.

until it reaches the original seed weight, like this different moisture levels were maintained and each time the seeds were kept in the Petri plates containing three layers of moist blotter paper. The observations were recorded at three days interval and seeds were graded by using 1-5 scale given by Machado *et al.* (2013). Observations regarding germination percentage, root, shoot and total length of the seedling were recorded. Maintenance of different moisture levels in the maize seeds at different time intervals.

Moisture level (%)	Time taken for drying (hrs)
41.37	0.00
32.14	2.00
24.14	4.00
18.89	6.00
Dried(Average moisture level 12)	8.00

Grading scale for *F. verticillioides* infection, ranging from 1-5, based on the percentage of area of maize seeds covered by fungal structure Machado *et al.* (2013).

Disease Scale	Per cent of infected kernel
1	Seeds with no visible fungal mycelium
2	Mycelium covering 1-25 % of the seed surface
3	Mycelium covering 25 -50 % of the seed surface
4	Mycelium covering 50-75 % of the seed surface
5	Mycelium covering over 75 % of the seed surface

## RESULTS AND DISCUSSION

### A. Effect of different solid culture media on growth and sporulation of *F. verticillioides*

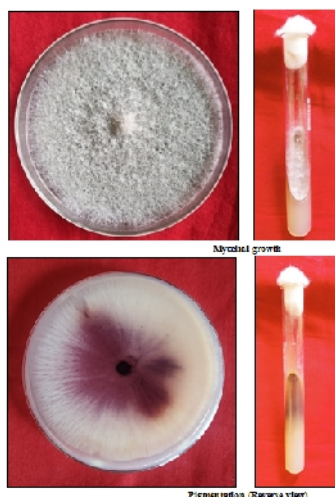
Ten different synthetic and non-synthetic solid culture media were used for studying the growth and sporulation of *F. verticillioides*. Observations with

respect to colony diameter, colony colour, pigmentation, type of margin and sporulation were recorded at seven days after inoculation. The data on the colony growth and sporulation was statistically analysed and given (Table 1, Plate 1 and 2).

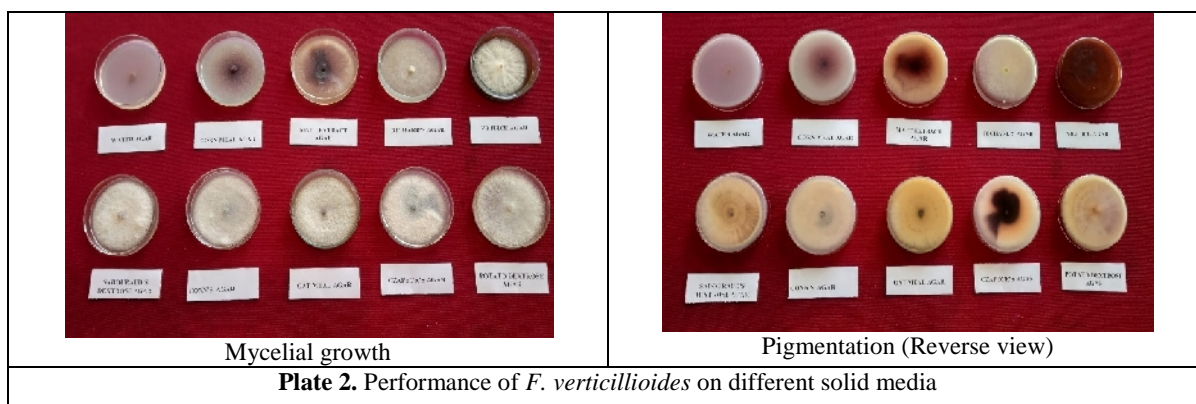
**Table 1: Effect of different solid media on mycelial growth and sporulation along with cultural characteristics of *F. verticillioides* (Fv 19).**

Sr. No.	Media	Mycelial growth (mm)*	Sporulation	Colony characters			
				Colony color	Type of mycelium	Pigmentation	Zonations
1.	Conn's agar	80.67	++	White	Medium fluffy	White	Absent
2.	Corn meal agar	67.67	+	Pinkish white	Scanty	Light purple	Absent
3.	Czapek's dox agar	81.00	+++	Purplish white	Fluffy	Purplish white	Absent
4.	Richard's agar	85.00	+++	Yellowish white	Appressed	Light yellowish white	Present
5.	Sabouraud's dextrose agar	81.67	++	White	Fluffy	Brownish yellow	Absent
6.	V- 8 juice agar	71.00	++	White	Medium fluffy	Brownish white	Absent
7.	Water agar	53.67	+	White	Scanty	Pink	Absent
8.	Oat meal agar	82.33	++	Yellowish white	Fluffy	Yellowish white	Absent
9.	Malt extract agar	72.67	+	Purplish white	Scanty	Purple	Absent
10.	Potato dextrose agar	89.67	++++	White	Fluffy	Light yellowish white	Absent
	<b>S. Em±</b>	0.69					
	<b>CD at 1%</b>	2.78					

\*Mean of three replications, ++++: Very good sporulation (>40 spores/10x), +++: Good sporulation (26-40 spores/10x), ++: Fair sporulation (11-25 spores/10x) and +: Poor sporulation (1-10 spores/10x)



**Plate 1. Pure culture of *F. verticillioides* on PDA.**



**Plate 2. Performance of *F. verticillioides* on different solid media**

The results showed that the maximum colony diameter of *F. verticillioides* (89.67 mm) and very good sporulation was observed on potato dextrose agar which was significantly superior over all the media followed by Richard's agar (85.00 mm) and oat meal agar (82.33 mm) with good and fair sporulation, respectively.

Whereas least colony diameter was obtained on water agar (53.66 mm) with poor sporulation. These findings were in confirmation with the results of Chaudhary *et al.* (2018), where they obtained maximum colony diameter of *F. udum* on PDA (82.0 mm) followed by Richard's agar (79.33 mm) and Czapek's agar (56.83

mm), respectively. Hegde *et al.* (2018) studied growth characters of *F. oxysporum* f. sp. *dianthi* on different media, they indicated that maximum growth of the pathogen was recorded on potato dextrose agar (90 mm).

Kumar *et al.* (2019) noticed that maximum mycelial growth and very good sporulation of *F. verticillioides* on potato dextrose agar. The findings were more or less in agreement with that of Poorvasandhya *et al.* (2020) who reported that *F. oxysporum* f. sp. *udum* grew the best on PDA (87.7 mm) with very good sporulation followed by Czapek's dox agar (78.0 mm) with good sporulation.

*F. verticillioides* thrives well on PDA as it consists of potato infusion which acts as nutritionally rich base encourages fungal growth and sporulation and dextrose serves as energy source, provides carbon, growth stimulant and helps in chromogenesis. On contrary least mycelial growth and poor sporulation was noticed on water agar because it consists of only water and agar but lacks the nutritional richness and complex carbohydrates. There is significant difference found between the different culture media regarding the colony diameter.

*F. verticillioides* showed variation with respect to colony characteristics on different cultural media (Table 1). On all the cultural media the feathery mycelial growth pattern with serrated margin was recorded. Mycelial growth was scanty on water agar, malt extract agar and corn meal agar, whereas appressed growth was recorded on Richard's agar, medium fluffy growth was noticed on Conn's agar and V-8 juice agar while fluffy growth was seen on rest of the media (Czapek's dox agar, potato dextrose agar, Sabouraud's dextrose agar and oat meal agar).

Difference was found with respect to colony colour on potato dextrose agar, Sabouraud's dextrose agar, V-8 juice agar, Conn's agar and water agar with white mycelial growth, whereas yellowish white mycelial growth was seen on Richard's agar and oat meal agar. Pink- purplish white colony colour was found on corn meal agar, Czapek's dox agar and malt extract agar.

*F. verticillioides* also varied with respect to pigmentation on different media from white to purple. It produced white pigmentation on Conn's agar, light yellowish white pigmentation was exhibited by the fungus on potato dextrose agar and Richard's agar.

Whereas yellowish white pigmentation was seen on oat meal agar, brownish yellow pigmentation was recorded on Sabouraud's dextrose agar, brownish white was seen on V-8 juice agar, pink pigmentation was noticed on water agar, light purple pigmentation was recorded on corn meal agar and purple pigmentation was noticed on malt extract agar. The isolate showed zonations only on Richard's agar and zonations were absent on the rest of the media.

Nithiya *et al.* (2012) described that colony character of *Fusarium* species varied from white to purple and type of mycelium aerial to compact on PDA. Pitt (2014) found that *F. verticillioides* grew rapidly and produced white to pale salmon coloured, with low and ropy mycelium and powdery texture with pale salmon, greyish violet, brownish to deep violet pigmentation. Colonies of *F. verticillioides* were fast growing, pale to bright coloured with cottony aerial mycelium. The colour of the mycelium varied from whitish to purple (Dharanendra *et al.*, 2019). The culture surface of *Fusarium* spp. was flat and white at the beginning, later typical purple, gray and light brown was developed as they matured with thick aerial mycelial growth covered the colony surface (Kalman *et al.*, 2020).

Variation in cultural characteristics of *F. verticillioides* on different media suggests that each media provides different nutrients which may or may not be so suitable for luxurious growth and expression of the pathogen to thrive well

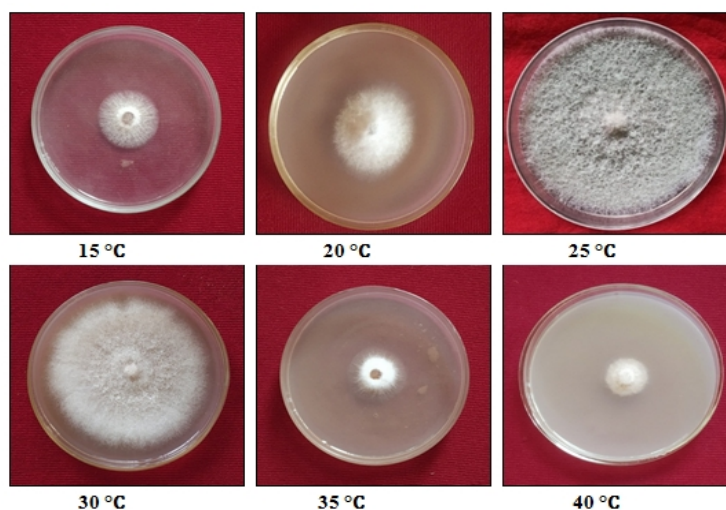
#### B. Physiological characterisation of *F. verticillioides*

(i) **Effect of different temperatures on growth and sporulation of *F. verticillioides*.** The effect of temperature from 15 to 40°C on the growth and sporulation of *F. verticillioides* was studied and result is presented (Table 2 and Plate 3). Maximum mycelial growth (86.67 mm) was observed at 25°C which was significantly superior over other temperatures followed by 30°C and 20°C which accounts for 77.33 mm and 45.66 mm, respectively. Reduced growth was found at temperatures 15°C and 35°C whereas, minimum mycelial growth was 21.33 mm recorded at 40°C. With regard to sporulation, the very good sporulation was observed at 25°C, good sporulation was noticed at 30°C while fair sporulation was seen at 20°C and 35°C. However both the extreme temperatures, 15°C and 40°C were recorded poor sporulation.

**Table 2: Effect of temperature regimes (°C) on mycelial growth and sporulation of *F. verticillioides*.**

Sr. No.	Temperature (°C)	Mycelial growth (mm)*	Sporulation
1.	15	31.67	+
2.	20	45.67	++
3.	25	86.67	++++
4.	30	77.33	+++
5.	35	24.00	++
6.	40	21.33	+
S. Em ±		0.61	
CD at 1%		2.62	

\*Mean of three replications, ++++ : Very good sporulation (>40 spores/10x), +++ : Good sporulation (26-40 spores/10x), ++ : Fair sporulation (11-25 spores/10x) and + : Poor sporulation (1-10 spores/10x)



**Plate 3.** Effect of temperature regimes on mycelial growth of *F. verticillioides*.

The results were similar with those obtained by Khilare and Ahmed (2012) where they studied the effect of temperature (10 to 40°C) on the growth of *F. oxysporum* f.sp. *ciceri*. They concluded that temperatures from 20 to 30°C were most favourable for the fungal growth. Sharma *et al.* (2014) evaluated the effect of temperature (10 to 40°C) on *F. moniliforme*. They observed that maximum growth was recorded at 28°C and minimum growth was recorded at 40°C.

Kumar *et al.* (2019) reported that the most suitable temperature for mycelial growth and sporulation of *F. verticillioides* was ranged from 24 to 30°C. Poorvasandhya *et al.* (2020) observed temperature from 20 to 30°C was found to be most congenial for growth and sporulation of *F. oxysporum* f. sp. *udum*.

Temperature is an important factor generally which affects the fungal growth, metabolic pathways and reproduction process. Several metabolic pathways involve enzymes which in turn depend on congenial temperature for their proper function. The high temperature 40°C affects the both growth and sporulation of the fungus as it suffers from desiccation of microconidia which are the important source of dissemination. As *F. verticillioides* is a mesophile which requires warm temperature for better growth. Low temperature is also not favourable for *F. verticillioides* as it stops the metabolic events which in turn slows down the growth.

(ii) **Effect of different relative humidity levels on growth and sporulation of *F. verticillioides*.** To determine the suitable relative humidity required for mycelial growth and sporulation (Table 3 and Plate 4),

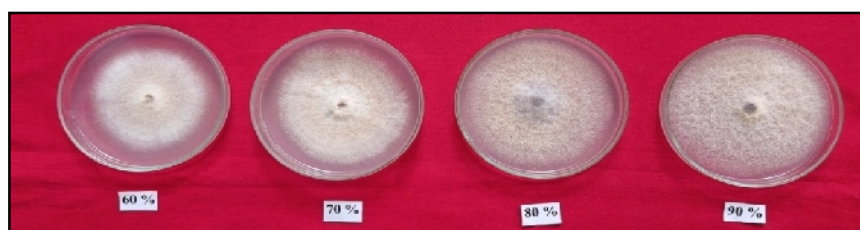
*F. verticillioides* was grown on PDA at different RH ranged from 60 to 90 per cent for seven days. The maximum mycelial growth of 87.50 mm obtained at 90 per cent RH with very good sporulation which is significantly superior over other RH followed by 80 and 70 per cent RH which accounts for 84.50 mm (good sporulation) and 73.75mm (fair sporulation), respectively. The minimum mycelial growth of 68.50 mm with poor sporulation was recorded at 60 per cent RH. These results are in confirmation with the findings of Tonapi *et al.* (2007) who observed that maximum grain mold severity and sporulation of *F. moniliforme* at 25-28 °C and RH levels from (95-98 %). The relative humidity > 75 per cent coupled with 25°C temperature benefited the outbreak of *F. oxysporum* f. sp. *vanilla* in Mexico by Quintana *et al.* (2017). Pempee *et al.* (2020) observed among the different relative humidities (60, 70, 80, 90 and 100 per cent) maximum mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri* supported by 100 per cent RH followed by 90 per cent RH.

The reason behind the variation in growth and sporulation of pathogen was attributed to the fact that, the highest relative humidity contributes to maximum fungal growth and sporulation because of availability of surface moisture, pathogen absorbs the water and digest the sugars and starch which promotes the fungal growth and it is also required for spore germination to cause the infection on the host. On contrary, 60 per cent RH inhibits the growth and sporulation of the pathogen as it suffers from desiccation because the moisture inside the Petri dish gets readily evaporated to the atmosphere.

**Table 3: Effect of relative humidity on mycelial growth and sporulation of *F. verticillioides*.**

Sr. No.	Relative humidity (%)	Mycelial growth (mm)*	Sporulation
1.	60	68.50	+
2.	70	73.75	++
3.	80	84.50	+++
4.	90	87.50	++++
S. Em ±		0.61	
CD at 1%		2.62	

\*Mean of four replications, +++++ : Very good sporulation (>40 spores/10x), +++ : Good sporulation (26-40 spores/10x), ++ : Fair sporulation (11-25 spores/10x) and + : Poor sporulation (1-10 spores/10x)



**Plate 4.** Growth response of *F. verticillioides* at different levels of relative humidity.

(iii) **Effect of different seed moisture levels on growth of *F. verticillioides*.** The growth of *F. verticillioides* at different seed moisture levels was studied and results obtained are explained in detail (Table 4 and Plate 5). The maximum mycelial growth of 42.67 per cent coverage of seed surface area was recorded at 41.37 per cent of seed moisture which is significantly superior over other seed moisture levels followed by 34.58 per cent coverage of seed surface area at 32.14 per cent seed moisture level and 28.75 24.14 per cent coverage of seed surface area seed moisture level, respectively. Whereas minimum mycelial growth (10.53 %) was noticed in the dried seeds with seed moisture level of 12 per cent.

Lauren *et al.* (2004) obtained that higher moisture contents (24-27 %) and modest temperatures (20-30°C) were favourable for growth of toxigenic *Fusarium* species and to produce mycotoxins. Naiz *et al.* (2011)

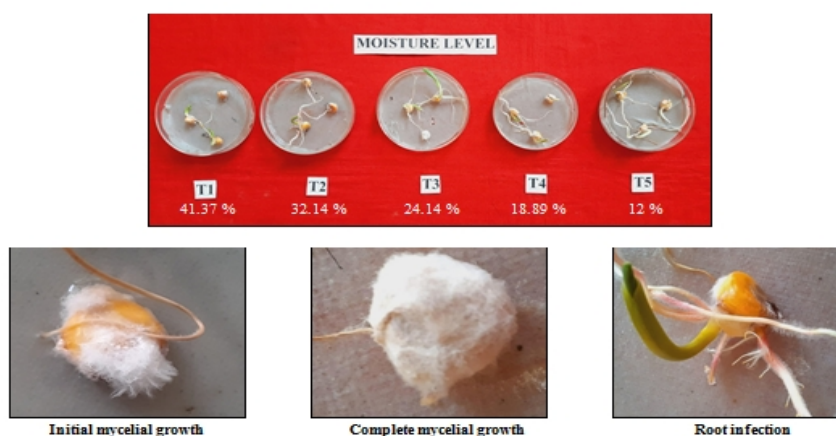
reported that heavy fungal infection by *Fusarium* spp. (98-100 %) on maize seed after 30 days of storage was noticed when seed moisture is 20 per cent and 8-12 per cent of seed moisture reduces the chances of mold growth.

*F. verticillioides* further affected the performance of maize seedling as the maximum root, shoot and total length of 7.76 cm, 5.95 cm and 13.71 cm, respectively was recorded in dried seeds as the mycelial growth was minimum (10.53 %). On contrary minimum root, shoot and total length of 2.43 cm, 2.29 cm and 4.73 cm, respectively was observed at high seed moisture level of 41.37 per cent because of maximum mycelial growth of 42.67 per cent. The results explained that as the moisture content increased, the mycelial growth also increased which in turn reduced the root, shoot and total length.

**Table 4.** Effect of seed moisture levels on mycelial growth of *F. verticillioides*.

Sr. No.	Seed moisture level (%)	Per cent of seed surface covered by mycelial growth	Mean root length (cm)	Mean shoot length (cm)	Mean total length (cm)
1.	41.37	42.67* (40.77)**	2.43	2.29	4.73
2.	32.14	34.58 (36.00)	3.22	3.57	6.79
3.	24.14	28.75 (32.41)	6.01	3.85	8.60
4.	18.89	15.00 (22.78)	6.91	4.89	11.78
5.	Dried (Approximate moisture level 12 )	10.53 (18.92)	7.76	5.95	13.71
<b>S. Em ±</b>		0.89	0.83	0.28	0.79
<b>CD at 1%</b>		2.71	3.45	1.17	3.30

\* Mean of three replications; \*\* Values in the parentheses are arc sine transformed



**Plate 5.** Mycelial growth of *F. verticillioides* at different seed moisture levels.

Lonnie *et al.* (2006) observed that when seeds inoculated with *F. verticillioides* MRC 826 produced symptoms such as necrotic leaf lesions, abnormal leaf development, stunting, and reduced root development

compared to the respective uninoculated control group. Soybean seeds were pre-soaked in culture filtrates of *F. culmorum* for 2, 4, 6, 8 and 24 hrs. The results showed that the pathogen inhibited seed germination and

seedling development where the root and seedling lengths were  $4.6 \pm 0.1$  cm and  $31.3 \pm 0.5$  cm, respectively when compared to control which had  $7.9 \pm 0.3$  cm root length and  $38.7 \pm 0.5$  cm seedling length (Haikal, 2008).

Maize kernels were inoculated with the *F. verticillioides* to understand the influence of fungal growth on seed germination, the length of root and stem. Beccaccioli *et al.* (2021) noticed necrotic areas caused by infection with *F. verticillioides* on seedling shoot and root which further reduced the root and shoot length.

The increased seed moisture content was due to the continuous rainfall towards the end of the harvest period, this moisture content inside the seed affects the cell integrity and the outer seed surface becomes soft which makes easy gate way for the infection of *F. verticillioides*. Hence as the moisture level increases, the mycelial growth also increases and they are positively correlated with each other. But when seed moisture content decreases, the seed becomes hard so that can't be breached by fungus as it experiences the structural barrier which does not even allow the spread of the fungus from one kernel to another, therefore least mycelial growth was observed. *F. verticillioides* at higher seed moisture level significantly reduced the root, shoot and total length of the seedling and affected the performance of the seedling, this shows the systemic nature of the pathogen which moves from seed to germinating seedling and causes seedling blight.

## CONCLUSION

*Fusarium* ear rot caused by *Fusarium verticillioides* is the newly emerging disease of maize which results in 10 to 30 per cent reduction in the yield as well as adversely affects the physical, physiological, phytosanitary as well as nutritive qualities of the seed and the mycotoxins produced by the fungi is threatening to the human and livestock health. The present study provides information regarding the nutritional requirement and abiotic factors affecting the pathogen performance. Among different media tested, potato dextrose agar was the best media for growth and sporulation of *F. verticillioides*. The optimum temperature of 25°C and 90 per cent relative humidity were recorded best for maximum growth and very good sporulation of *F. verticillioides*. Seed moisture also had positive effect on the *F. verticillioides* infection in the maize seeds.

## FUTURE SCOPE

The cultural and physiological characterization will be helpful for easy identification of *F. verticillioides* and to understand the epidemiological requirement of the pathogen which in turn aids to take up proper precautionary and management measures that will ensure harmless, valuable and high quality grain production.

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**Conflict of Interest.** None.

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