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EFFECT OF ABIOTIC FACTORS ON THE GROWTH OF MULBERRY (*MORUS* SPP.) ROOT ROT CAUSED BY *FUSARIUM OXYSPORUM* SCHLECHT AND *FUSARIUM SOLANI* (MART.) SACC. - AN *IN-VITRO* STUDY

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ABSTRACT

Mulberry (*Morus* spp.) is a perennial crop and the sole food for silkworm (*Bombyx mori*). Among many diseases, fungal root rot caused by *Fusarium oxysporum* Schlecht. and *Fusarium solani* (Mart.) Sacc. is major one. Considering the importance of the pathogens and their growth pattern, *in-vitro* study has been conducted on abiotic environmental factors viz. temperature, pH, relative humidity and culture media by following the standard methods. The data were analyzed with one way ANOVA and Tukey HSD-Post Hoc Test ($p \leq 0.05$). From the present study it was found that, the test pathogens grew profusely on PDA medium at 25-30°C with a pH range of 5.5-5.6 under relative humidity of 85-100%.

Keywords: Mulberry, Root rot, *Fusarium oxysporum, Fusarium solani,* abiotic factors

INTRODUCTION:

Mulberry (*Morus* spp.) plant is the sole food for silkworm (*Bombyx mori*). It is a perennial crop with deep root, fast growing tree species and is widely adaptable to different environmental/agro climatic conditions. Mulberry leaf is the major economic component since the quality of the leaf produced per unit area has a direct bearing on cocoon harvest. The genus *Morus* belongs to the family Moraceae, having 68 species growing as tree in wild cultivated forms in different countries of the world **[1]**. Mulberry being an economically important tree, is cultivated in China, India, Thailand, Brazil, Uzbekistan and other Countries across the globe, for its leaves to feed monophagous mulberry silkworm. The sustainability of silk industry is directly correlated with the production and continuous supply of high-quality mulberry leaves **[2]**. Mulberry is usually cultivated for years together as a mono-crop in the same field and its production is restricted to 70 days/crop, thus completing 5 crops/year **[3]**.

Due to the continuous cultivation, repeated harvesting, absence of adequate quality of organic matter and the microbial equilibrium disruption, the crop becomes vulnerable to various diseases. Mulberry is affected by large number of diseases caused by fungi, bacteria, mycoplasma, virus and nematodes. Among the fungal diseases, dry root rot caused by *Fusarium oxysporum* Schlecht and *Fusarium solani* (Mart.) Sacc. is the major one. Literatures reveals that the disease was reported from almost all types of soils under various agro-climatic conditions throughout the year resulting in 30% of plant mortality, 14% of leaf yield loss due to wilting, defoliation, drying and plant death **[4]**. As mulberry is being exploited by sericulture, pharmaceutical, cosmetic, food and beverage industries along with its utilization in environmental safety approach; it is appropriate to call it as a most suitable plant for sustainable development **[5]**.

Any organisms require optimum environmental conditions for their growth and development. Similarly, the mulberry root rot pathogens also require optimum environmental conditions for their growth and to invade the host plant to cause disease. Considering the importance of the disease-causing agents and their growth pattern, *in-vitro* study has been conducted in various abiotic factors.

MATERIALS AND METHODS:

Two mulberry dry root rot fungal pathogens namely *Fusarium oxysporum* and *Fusarium solani* **(Figure 1, 2 and 3)** were selected to

study the effect of environmental factors on their growth in the laboratory conditions. Effect of abiotic factors *viz.,* temperature, pH, relative humidity and the culture media were tested as follows **[4, 6]**.

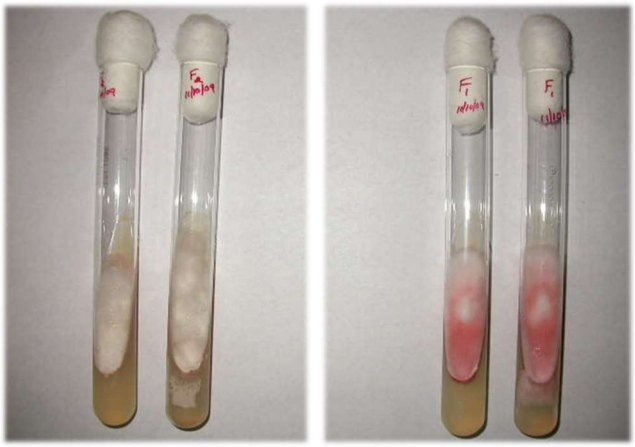

Figure 1: ***Fusarium oxysporum*** **(left) &** ***Fusarium solani*** **(right) in Agar Slants**

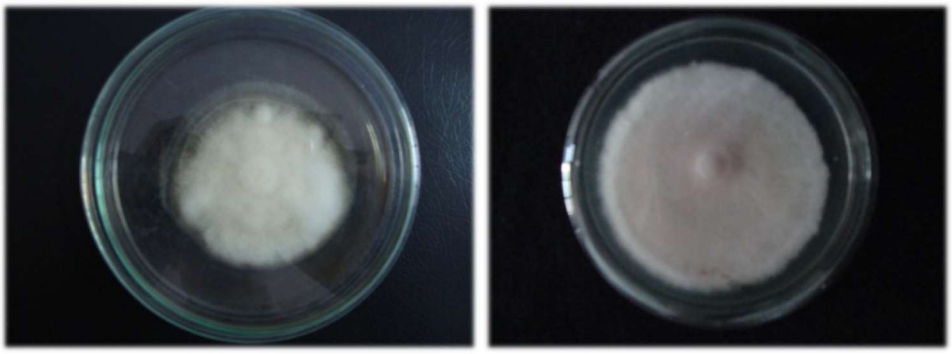

Figure 2: Pure cultures of ***Fusarium oxysporum*** **(left) &** ***Fusarium solani*** **(right) in Petri plates**

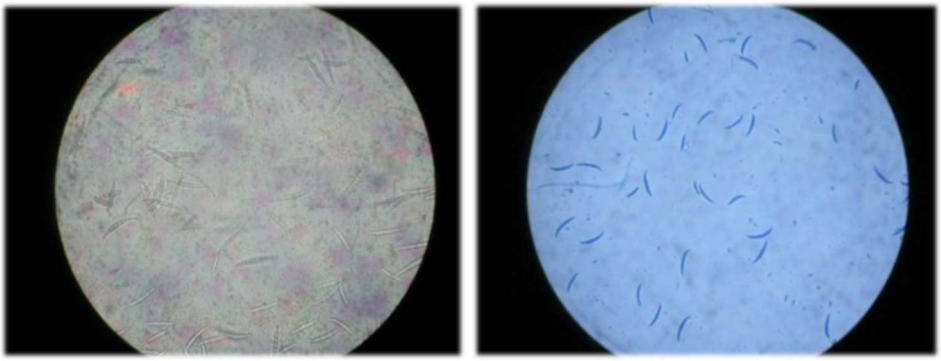

Figure 3: Microscopic View (40x) of ***Fusarium oxysporum*** **(left) &** ***Fusarium solani*** **(right)**

Effect of Temperature:

The effect of temperature on the growth of the pathogens was studied by inoculating 5 mm mycelial discs of actively growing pure cultures of both the test pathogens on PDA medium. The Petri dishes were incubated at different temperatures *viz.,* 15, 20, 25, 30, 35 and 40°C in incubator. After seven days of incubation period, the radial growth of the fungal colony was measured **[7]**.

Effect of Relative Humidity (RH):

The effect of RH on the growth of the test pathogens was studied using different concentrations of H_2SO_4 in desiccators. Different ranges of relative humidity *viz.,* 25, 50, 75 and 100% were maintained by mixing the concentrated H_2SO_4 and distilled water in the proportions of 54:46; 42:58; 30:70 and 0:100 ml respectively **[8]**. 5 mm mycelial discs of actively growing test pathogens were inoculated in Petri dishes seeded with PDA medium. The plates were incubated at 27±2°C for seven days at different humidity ranges in different desiccators. A control was also maintained in the RH of room temperature. After the incubation period, the radial growth of the pathogens was recorded.

Effect of pH:

The effect of pH on the growth of the test pathogens was studied using five different pH levels *viz.,* 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 5.6 (control). The pH of the medium was adjusted by using HCl and NaOH. All the different pH adjusted media were autoclaved and poured into sterilized petri plates. 5 mm mycelial discs of both the actively growing test pathogens were inoculated to the petri dishes containing medium. All the plates were incubated at 27±2°C for seven days. After the incubation period, the radial growth of the pathogens was recorded **[9]**.

Effect of Culture Media: The effectiveness of the culture media on the growth of the test pathogens was studied by using three different culture media namely Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) and Martin Rose Bengal Agar (MRBA). All the media were autoclaved separately and poured in Petri dishes. Upon the solidification of the medium, 5 mm mycelial discs were inoculated. The plates were incubated at 27±2°C for seven days. For each treatment, four replications were maintained. After the incubation period the radial growth of the pathogens was recorded **[10]**.

RESULTS:

Four important abiotic factors *viz.,* temperature, pH, relative humidity and culture media which influence the growth *F. oxysporum* (FO) and *F. solani* (FS) in standard *in-vitro* conditions were tested. The data obtained from the study has been

subjected to one way ANOVA, Tukey HSD-Post Hoc Test and the mean difference is significant at $p \leq 0.05$ of the results and are interpreted with small English alphabets in prefix in the respective figures which denotes the significant difference between groups.

Effect of Temperature:

After seven days of incubation period, the radial growth was measured and the results obtained are presented in **Table 1 and Figure 4.1a and Figure 4.1b**. It was observed that there was significant difference in the growth of the test pathogens between different ranges of temperatures except at 25°C and room temperature. At 25°C, both the test pathogens recorded highest growth with 65.6 mm in *F. oxysporum* and 64.3 mm in *F. solani* followed by 65.0 mm and 64.3 mm at 27 ± 2^0C respectively. It was found that average growth at 30°C with 56.2 mm and 54.5 mm mycelial growth in respective pathogens. And there was significantly lesser growth of both the test pathogens in lower and higher temperatures.

Effect of pH:

The radial growth of the respective test organisms was measured after seven days of incubation period at room temperature and recorded in **Table 2 and Figure 4.2a and Figure 4.2b**. The results showed the significant difference between different pH levels in both the test pathogens. But, pH levels 5.5 and 5.6 recorded no significant difference in the growth of both of the pathogens. pH 5.6 (control) was found to be optimum for the growth of both of the microbes with 66.0 mm and 64.6 mm radial growth respectively. The highest growth of *F. oxysporum* and *F. solani* was recorded at pH 5.6 with 66.0 mm and 64.6 mm followed by 5.5 with 64.6 mm and 63.6 mm for *F. oxysporum* and *F. solani* respectively. In case of both *F. oxysporum* and *F. solani,* there was no significant difference between pH 5.5 and 5.6. With increased pH levels, the growth of the both of the test pathogens gradually decreased.

Effect of Relative Humidity (RH):

After seven days of incubation, the radial growth was measured and recorded. The results obtained are presented in the **Table 3 and Figure 4.3a and Figure 4.3b**. It was observed that, there was significant difference in the growth rates of both the test pathogens in different RH levels. It was found that at 85% (control) was the optimum RH, in which both the test fungi recorded highest growth *i.e.*, *F. oxysporum* recorded 65.3 mm and *F. solani* 66.3 mm growth. At 100% RH, the growth was found to 61.5 mm and 63.5 mm respectively for respective pathogens. Whereas, there was no significant difference occurred between the growth of *F. solani* at

85% and 100% RH. With the decrease in RH percentage, there was gradual decrease in the growth of both the test fungi.

Effect of Culture Media:

After incubation period of seven days, the radial growth of the test fungi was measured and the results recorded of individual test pathogens are presented in **Table 4 and Figure 4.4a and 4.4b**. In this case also, there was significant difference between the growths of the pathogens observed. PDA was the effective medium for the growth of *F. oxysporum* (66.5 mm). However, there was no significant difference between CDA and PDA in case of *F. solani,* with 65.5 mm and 65.0 mm respectively. Whereas, MRBA recorded least growth in which *F. oxysporum* recorded 27.0 mm and *F. solani* 32.0 mm radial growth.

Table 1: Effect of temperature on the growth of *F. oxysporum* and *F. solani*

Sl. No.	Temperature in ^{0}C	*F. oxysporum* RGM (mm)	*F. solani* RGM (mm)
1	Control (27±2)	65.0^a	64.3^a
2	15	38.2^d	36.7^d
3	20	45.7^c	49.2^c
4	25	65.6^a	64.3^a
5	30	56.2^b	54.5^b
6	35	37.3^d	32.3^e
7	40	16.6^e	16.6^f

The mean values are replications of four RGM = Radial Growth of Mycelium
The mean values followed by different alphabets differ significantly when subjected to Tukey HSD @ p ≤ 0.05
FO: SD=16.59 & SE=3.13 & Sum of Squares=7409.4; df=6; Mean Square=1234.9; F=1127.5
FS: SD=16.64 & SE=3.14 & Sum of Squares=7450.2; df=6; Mean Square=1241.7; F=750.3

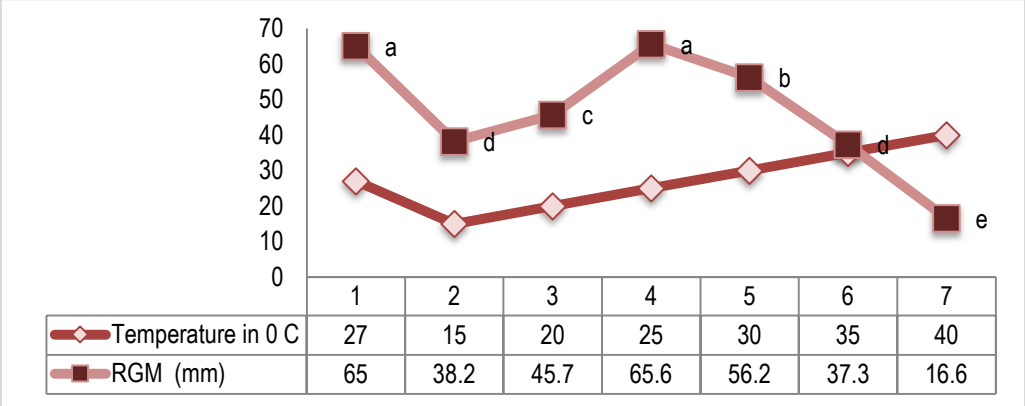

Figure 4.1a: Effect of temperature on the growth of *F. oxysporum*

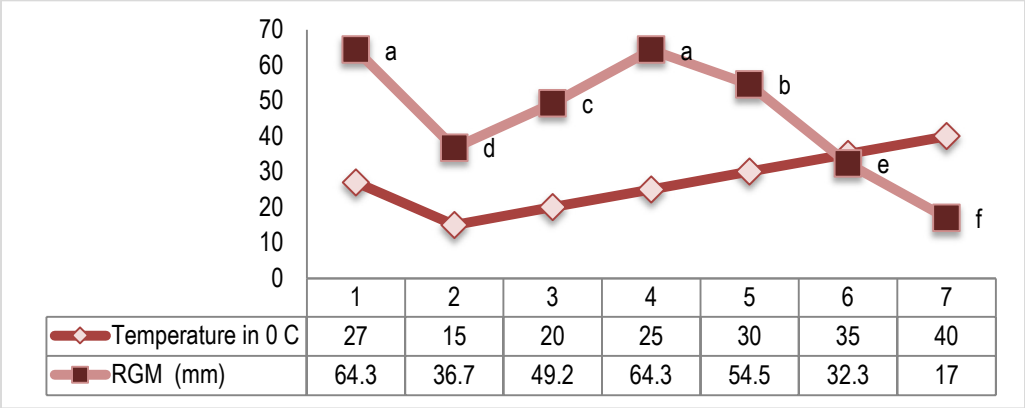

Figure 4.1b: Effect of temperature on the growth of *F. solani*

Table 2: Effect of pH on the growth of *F. oxysporum* and *F. solani*

SI. No.	pH	*F. oxysporum* RGM (mm)	*F. solani* RGM (mm)
1	Control (5.6)	66.0[a]	64.6[a]
2	5.0	47.0[b]	49.0[b]
3	5.5	64.6[a]	63.6[a]
4	6.0	48.6[b]	45.3[c]
5	6.5	31.6[c]	27.6[d]
6	7.0	19.0[d]	17.6[e]
7	7.5	10.3[e]	09.3[f]

The mean values are replications of four RGM = Radial Growth of Mycelium
The mean values followed by different alphabets differ significantly when subjected to Tukey HSD @ $p \leq 0.05$
FO: SD=20.36 and SE=3.84 & Sum of Squares=11157.2; df=6; Mean Square=1859.5; F=892.5
FS: SD=20.63and SE=3.89 & Sum of Squares=11472.8; df=6; Mean Square=1912.1; F=2006.2

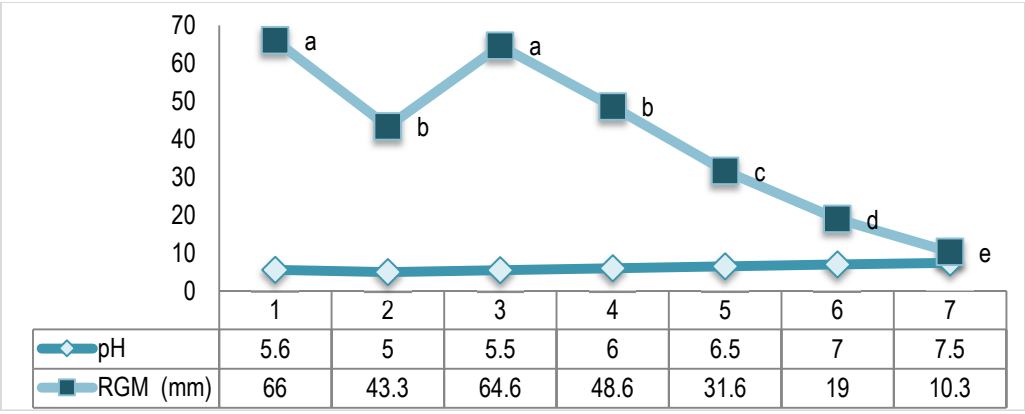

Figure 4.2a: Effect of pH on the growth of *F. oxysporum*

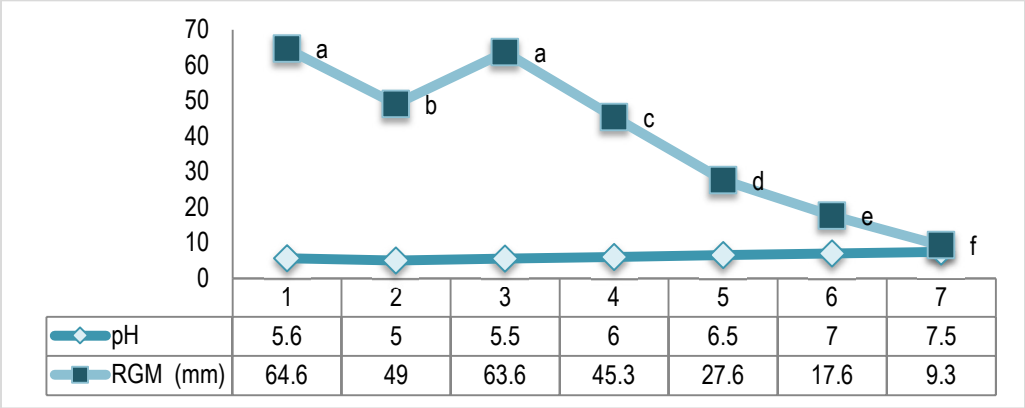

Figure 4.2b: Effect of pH on the growth of *F. solani*

Table 3: Effect of relative humidity on the growth of *F. oxysporum* and *F. solani*

SI. No.	Relative Humidity	*F. oxysporum* RGM (mm)	*F. solani* RGM (mm)
1	Control (85)	65.2[a]	66.2[a]
2	25	10.2[e]	12.0[d]
3	50	47.2[d]	46.5[c]
4	75	58.7[c]	56.7[b]
5	100	61.5[b]	63.5[a]

The mean values are replications of four RGM = Radial Growth of Mycelium
The mean values followed by different alphabets differ significantly when subjected to Tukey HSD @ $p \leq 0.05$
FO: SD=20.64 and SE=4.61 & Sum of Squares=8076.8; df=4; Mean Square=2019.2; F=1514.4
FS: SD=20.26 and SE=4.53 & Sum of Squares=7772.5; df=4; Mean Square=1943.1; F=925.2

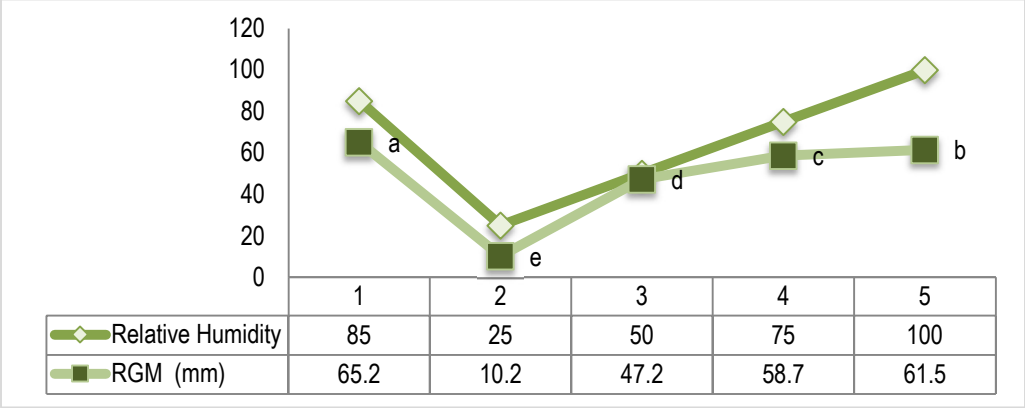

Figure 4.3 a: Effect of relative humidity on the growth of *F. oxysporum*

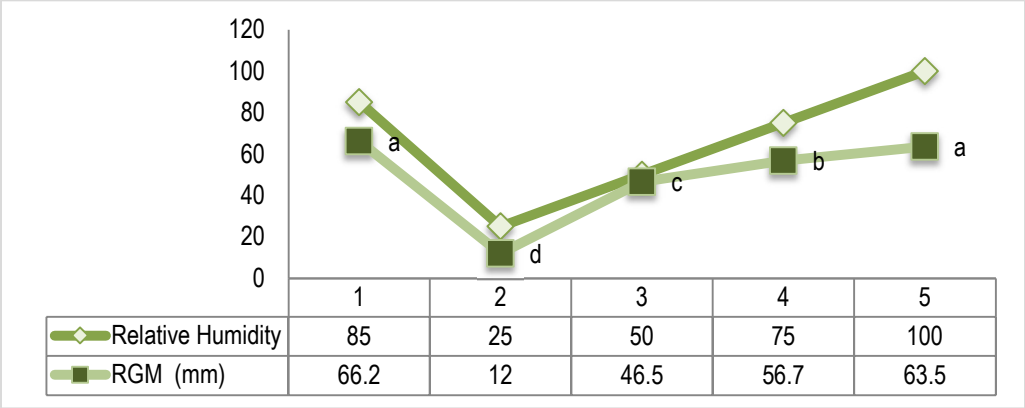

Figure 4.3b: Effect of relative humidity on the growth of *F. solani*

Table 4: Effect of culture media on the growth of *F. oxysporum* and *F. solani*

SI. No.	Culture Media	*F. oxysporum* RGM (mm)	*F. solani* RGM (mm)
1	PDA	66.5[a]	65.0[a]
2	CDA	62.2[b]	65.5[a]
3	MRBA	27.0[c]	32.0[b]

The mean values are replications of four RGM = Radial Growth of Mycelium
The mean values followed by different alphabets differ significantly when subjected to Tukey HSD @ $p \leq 0.05$
FO: SD=18.52 and SE=5.34 & Sum of Squares=3761.1; df=2; Mean Square=1880.5; F=1230.9
FS: SD=16.44 and SE=4.74 & Sum of Squares=2948.6; df=2; Mean Square=1474.3; F=530.7

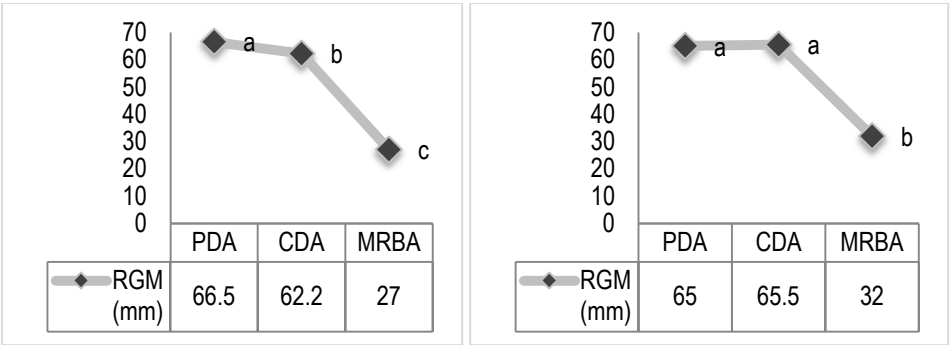

Figure 4.4a & 4.4b: Growth of *F. oxysporum* and *F. solani* on different agar media respectively

DISCUSSION:

In consideration to the factors of temperature and relative humidity, the outcome of the present study is in accordance with the work done by Gangadhara *et al*., 2010 **[9]**; Khilare and Ahmed **[6]** who have reported variability studies on *F. oxysporum* f. sp. *vanillae* and *F. oxysporum* f. sp. *ciceris,* at different temperatures and found that the maximum growth was observed at 25°C and 30°C. The results are on par with Hibar *et al.,* 2006 **[7]** who worked on the influence of temperature

and relative humidity on the culture of fungi isolated from rotting of sweet potato (*Ipomoea batatas* (L.) Lam.), in which *F. oxysporum* recorded maximum growth at 27°C. Laboratory studies of Chi and Hansen **[11]** reported that the growth of *F. solani* isolates was maximum at higher temperature of 28°C. However, growth of the fungus was drastically reduced at lower temperatures and started to decline above 30°C and become zero at 40°C, as these temperatures did not favor the growth of the fungus. At 25-26°C temperature, carnation wilt induced by *F. oxysporum* f. sp. *dianthi* was maximum **[12]**. They have also stated that, at lower temperature (18°C), the disease incidence also reduces. The results are also on par with the studies carried out by Landa et al., 2001 **[13]** on *Fusarium* wilt in chickpea (*Cicer arietinum*) caused by *F. oxysporum* f. sp. *ciceris,* in which disease development was greater at 25°C compared with 20 and 30°C. Rossi *et al.,* 2001 **[14]** studied the influence of relative humidity on the infection of wheat spike by some fungi causing *Fusarium* head blight under different regimes of relative humidity (100% to 65%). The results obtained in our present study are on par with the findings of Rossi *et al.,* 2001 **[14]**. Reduction of relative humidity during incubation increased the frequency of glumes infected by *F. culmorum*.

pH is another important abiotic factor for the growth of the pathogens, the present results obtained for the pH factor are on par with the works of Gangadhara, *et al.,* 2010 **[9]** who observed the optimum pH for the growth of *F. oxysporum* f. sp. *vanillae* isolates at pH 5. The studies conducted by Jamaria **[15]** on *F. oxysporum* f. sp. *nivium* indicated that, as the pH decreases or increases from the optimum, the rate of growth gradually decreases. Ramteke and Kamble, **[16]** reported that, the rhizome rot of ginger pathogen, *F. solani* (Mart.) Sacc. grew at temperatures ranging from 10 to 35°C, with optimum growth at 25°C and no growth was observed at 5°C and 40°C. The most suitable pH level for the growth was 4.5.

With respect to the aspect of culture media studied, the results of the present study are in accordance with the findings of Gupta *et al.,* 2010 **[10]** who has tested five solid media (Potato Dextrose Agar, Czapek Dox Agar, Corn Meal Agar, Cooke Rose Bengal Agar and Oatmeal Agar) for the cultural studies of *F. oxysporum* f. sp. *psidii* and *F. solani*. The results revealed that maximum mycelial growth was obtained in PDA *i.e*., 78.00 mm for *F. oxysporum* f. sp. *psidii*; 73.83 mm for *F. solani.* It was clearly indicated that

F. oxysporum f. sp. *psidii* is a fast-growing pathogen of guava wilt than *F. solani* and Potato Dextrose Agar was the best for the growth of *Fusarium* spp. isolates among the solid media. The results of the present study are in agreement with Chittem and Kulkarni, **[17]** who have observed the growth characteristics of *F. oxysporum.* f. sp. *gerberae* and *F. oxysporum.* f. sp. *dianthi* on eight solid media *viz.,* Czapek's Dox Agar, Host Leaf Extract Agar, Malt Extract Agar, Oat Meal Agar, Potato Dextrose Agar, Richards's synthetic agar, Rose Bengal Agar and Sabouraud dextrose Agar. It was found that, *F. oxysporum.* f. sp. *gerberae* showed maximum growth in OMA, whereas the PDA also recorded significant growth of 83.6 mm. The *F. oxysporum.* f. sp. *dianthi* recorded highest growth in PDA i. e., 90 mm, followed by RA and CDA. The current findings are also on par with the work of Rahman *et al.,* 2012 **[18]** who have studied the effect of culture media on the growth and spore production of *F. oxysporum* f. sp. *cubense* isolated from infected banana plants in Sarawak, Malaysia. Seven types of carbon sources, namely: Potato Dextrose Agar (PDA), Rose Bengal Agar (RBA), Corn Meal Agar (CMA), Water Agar with Glucose (WAG), Water Agar with Starch (WAS), Inoculation Media (IM) and Sporulation Media (SM) were evaluated. In general, *F. oxysporum* f. sp. *cubense* grew best in CMA, and also significant growth was recorded in PDA medium. The current results are also in agreement with Kulkarni **[19]** who found that, maximum dry mycelial weight of *Fusarium* sp. fungus was obtained in Potato Dextrose Medium which was followed by Oatmeal Medium.

Mulberry is also being used as a medicinal plant in improving and enhancing the life of human beings by utilizing the biologically active pharmacokinetic compounds found in leaf, stem and root parts. Further industrial exploitation of mulberry through preparation of various products in pharmaceutical, food, cosmetic and health care industries has gained the attention of industrialists **[5]**. Processed products of mulberry leaf in the form of powder with various biochemical ingredients including antioxidants are being used to prevent heat stroke **[20]**. Decoction or beverage made up of mulberry leaf powder is used as a remedy for sore throat **[21, 22]**. Mulberry leaf powder is also being used for the treatment of hypertension, hyperlipidaemia, arteriosclerosis, liver and kidney damage, cancers and as a neuroprotective agent **[23, 24]**.

Hence, it is important to know the potential threat in all aspects including the

current investigation of effect of abiotic factors on the growth of mulberry root rot caused by *Fusarium oxysporum* Schlecht and *Fusarium solani* (Mart.) Sacc.

CONCLUSIONS:

In the present study, it was found that, test pathogens grew profusely on PDA medium at 25-30°C with a pH range of 5.5-5.6 under relative humidity of 85-100%. From this investigation, the optimum environmental conditions required for the occurrence of mulberry root rot could be inferred. In future, the scope of exploring the root cause of pathogenesis with exploitation of molecular and advanced microscopy studies can be incorporated for the better management strategies of mulberry root rot disease.

Conflict of interest: None

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