Temperature response and effect of different culture media on growth of *Macrophomina phaseolina* causing charcoal rot disease of sesame

ABSTRACT

Temperature plays major role in growth of fungus. Climate change response of the current biotic stresses, particularly fungi-related diseases in oilseeds is a major global issue. *Macrophomina phaseolina* is the causal agent of charcoal rot (stem and root rot/MSR) disease of sesame, resulting in large yield losses globally. Evaluation of physiological characteristics are very important to know the behaviour of the pathogen, which is helpful for its' management.

Aim: The purpose of the current investigation was to evaluate the impact of temperature, pH and different culture media on the growth of *Macrophomina phaseolina* the disease causing pathogen of sesame

Methodology: *In-vitro* experiments conducted to investigate the physiological characteristics of the devastating pathogen of sesame *M. phaseolina*. A field survey was conducted and diseased samples collected from the predominant areas. The fungus was isolated by using PDA medium and incubated at different range of temperatures to show the effect. The mycelial growth was also tested in different pH levels. The cultural characteristics were studied using three culture media i.e. potato dextrose agr, oat meal agar and host leaf extract agar.

Results: The results showed that there is no growth of pathogen or it is very negligible in 10 – 25^0C temperature in 24 and 48 hours. 25 - 35^0C temperature is the optimum temperature for the pathogen to grow and above 35^0C temperature the growth ceased. Similarly, in case of pH, 6.5 is the suitable pH for the *M. phaseolina* to grow the highest. Among the culture media the pathogen attains its highest growth rate within five days of isolation in potato dextrose agar and the oat meal agar showed better result for the morphological characters.

Conclusion: Physiological studies showed that, sesame stem and root rot pathogen *Macrophomina phaseolina* prefers high temperature i.e. 30-35ºC and pH 6.5 grow significantly as well as for disease development. Also, the *in-vitro* study on different culture media showed the growth rate is high in PDA medium but the morphological characters showed better result in OMA medium.

Keywords: Sesame, Charcoal rot, Temperature, pH, Culture media, *M. phaseolina* and pathogen.

Introduction

Macrophomina phaseolina, a plant pathogen which can infect more than 500 plant species belongs to *Basidiomycetes* family, causes charcoal rot disease. It is a soil and seed-borne imperfect fungus [1] [2]. Microsclerotia are the principal and primary source of inoculums, and the major form in which *M. phaseolina* persists in soil. Microsclerotia are vegetative propagules highly resistant to unfavourable climatic conditions which are formed on host tissues and are then dispersed to the soil after the host plant has died [3]. These microsclerotia can survive from three months to three years under stress conditions [4]. Climate is considered as a major factor in the distribution of *M. phaseolina* as they are thermophilic in nature and show strong correlations with environmental factors and soil. High temperatures, high atmospheric humidity, and available moisture are some of the key climatic factors that play an important role in activation and proliferation of *M. phaseolina*. Maximum disease was detected between 28-35°C environmental temperature and 23-35°C soil temperature [5]. Furthermore, temperatures of 15–40°C in the air and 15–25°C in the soil are said to have been critical in the development of this disease [6]. Although various other factors contribute to the occurrence of disease, such as distinct pathogen races, irregularities in disease susceptibility and resistance, and physico chemical properties of soil that modify the interaction between pathogen and host, sudden changes in the environment is considered as a major factor in increasing the incidence of Charcoal rot disease. The regular fluctuations in the present climatic conditions motivate researchers to investigate the role that environmental elements and soil in the emergence of disease. Past investigations associated

the combined effects of increased temperatures and moisture stress during reproductive growth to the severity of charcoal rot disease [7].

Sesame (*Sesamum indicum* L.) is one of the most important ancient edible oilseed crops grown around one third of World's total sesame production in India. Out of which Eastern India contributes the major part of production [8]. In spite of wide distribution of sesame in different Agroclimatic Zones of India the productivity is very low due to diseases caused by various biotic factors such as fungus, bacteria and virus. One viral, seven bacterial, one phytoplasmal and seventy-two fungal diseases for sesame have been reported from India. The crop is vulnerable to number of diseases, such as Charcoal rot of sesame (*Macrophomina phaseolina*), *Alternaria* leaf spot (*Alternaria sesami*), Bacterial blight (*Xanthomonas campestris pv. sesami*), *Corynespora* blight (*Corynespora cassiicola*), Powdery mildew (*Erysiphe cichoracearum),* Cercospora leaf spot *(Cercospora sesami*) and Sesame phyllody *(Phytoplasma like organism)* [9]. Of these, sesame's charcoal rot (Stem & root rot) caused by *Macrophomina phaseolina* (*M. phaseolina*) (Tassi) Goid solely causes 25-38% yield loss in farmers' field and experimental plots depending upon climatic conditions and severity of the disease.

A number of writers have looked into how temperature affects growth of *M. phaseolina*, however, as of yet, no satisfactory response regarding the ideal epidemiological factors of this polyphagous pathogen has been provided [10]. The temperature range of 25 to 35ºC was the most conducive for the isolates' development [11]. Das et al. [12] looked into the impact of temperature on the growth of *Macrophomina* isolates and discovered that 30ºC was the ideal temperature in India for mycelial growth and microsclerotia development. There is variation in the morphological and cultural characteristics of *M. phaseolina* isolates. Numerous efforts have been undertaken to investigate *M. phaseolina* variation. Significant morphological and physiological variations were observed in fungus [13]. The pathogen can

adapt and survive in an array of environments. However, knowledge of the population variability of pathogen is crucial for any successful disease management programme. Additionally, knowledge of the pathogen's cultural and morphological variability aids in the selection of virulent strains for host resistance identification.

The present study aims, to find out the physiological and cultural variability of the pathogen M. phaseolina using *in-vitro* methods.

Materials and methods

2.1 Study area

In-depth survey was conducted in major sesame cultivation areas of various agroclimatic zones of Odisha, India in the summer season of 2020–21 based on the information provided by different Regional Research Technology Transfer Stations and Krishi Vigyan Kendras of Odisha University of Agriculture and Technology, Odisha. Numerous diseases, such as Macrophomina stem and root rot, were noted in terms of frequency. Diseased samples and the soil surrounding the plant's rhizosphere were also gathered at the same time. In each sampling, the disease incidence was noted from five randomly selected 1 m^2 area plots. The percent disease incidence was calculated as follows.

$$PDI = \frac{Total\ number\ of\ infected\ plants}{Total\ number\ of\ plants\ present\ in\ the\ plot} \times 100$$

2.2 Climatic condition

The climate of the studied area is categorized as sub-humid and sub-tropical. The climate is hot and normally receives an annual precipitation of 1352 mm and having mean maximum summer temperature 37.8°C and mean minimum winter temperature of 11.9°C [14].

2.3 Study of symptoms and collection of diseased samples

To investigate symptomatology, regular visits to the sesame fields of a group of selected farmers, closer to the university were carefully conducted. The purpose of these visits was to gather data on disease incidence, pathogen impact, and symptoms caused by the pathogen at every stage of the plant life cycle, from seed rotting to mature plant withering. Root length, stem girth, height, and root density were accurately measured and compared in both diseased and healthy plants through plant uprooting. Samples of stems, roots and soil from rhizosphere were collected from both infected and healthy sesame plants in various locations. The tools used to collect samples were disinfected every time with 70% ethanol. Following collection, the stem samples were sealed in sterile polypropylene bags. Soil samples (~200 g) from 15−30 cm below the surface were also collected. The stem samples were stored in a refrigerator for further analysis.

2.4 Isolation and Purification procedure of the fungus

Using the tissue segment method the fungal organisms linked to stem rot were isolated from the stem on Potato Dextrose Agar (PDA) medium [15]. Fresh infected stems and roots of sesame (cv. Prachi and local) plants showing typical, well developed dark brown to black discoloration were used to isolate the pathogen from the infected parts. The infected stems were cut into small pieces, in such a way that each piece comprised of infected as well as healthy tissues, each measuring 5-7 mm. And these stems were kept in sterile Petri plates. After thoroughly rinsing with autoclaved distilled water the pieces were surface sterilising for a minute with 1 per cent sodium hypochlorite (NaOCl) solution. The pieces were transferred to Petri plates containing sterile distilled water and washed thoroughly in two changes of sterile water to remove the traces of chemicals; if any. Washed the hands with rectified spirit and wiped the table top of inoculation chamber with a cotton swab of rectified spirit. After lighting the burner hold the flask containing sterile lukewarm PDA in the right hand and remove plug near the fame. Lift the lid of pre-sterilized Petri plate gently with left hand and

pour about 20 ml of medium. Close the mouth of the flask with plug near the flame. After solidification of the medium, four sterilized pieces were placed aseptically at different distance in a single PDA plate. The inoculated plates were incubated in an inverted position (upside down) at 28 ± 2°C for three to seven days and examined for the growth of the pathogen. After 3-4 days the hyphal tips of the growing fungi were aseptically cut and the isolates were pure-cultured using the hyphal tip method [16], and the resulting purified cultures were stored on PDA slants for use in subsequent research. Lactophenol blue staining used for microscopic observations of the fungal isolates for identification based on mycelia colour, fruiting body and conidia. The isolates were stored on PDA in plates sealed with Parafilm for further investigation.

2.5 Effect of different range of temperature on radial growth of test fungus

In order to determine the optimum temperature required for radial growth the fungus, a 5 mm mycelia disc was transferred to potato dextrose agar medium in petriplates and incubated at temperature of 10ºC, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45^0C in triplicates following completely randomized design. After 24 hours, 48 hours and 7 days of incubation, the diameter of the fungal colonies was measured and the data obtained were statistically analysed.

2.6 Effect of different range of pH on mycelial growth of test fungus

In order to study best pH for mycelial growth of test fungus, it was tested in a range of pH from 3 to 10. Fifty ml of potato dextrose broth was poured in 100ml capacity conical flasks. The pH of potato dextrose broth was adjusted to various ranges from 6 to 8 by using pH meter adding standard 0.5 M NaOH or 0.5 M HCl, as the case may be. All the flasks were inoculated with 5mm agar discs and incubated at 28 ± 1ºC. Three replications were maintained for each pH value. After seven days of inoculation, it was filtered through whatsman no 1 filter paper and mycelia mat were collected. These mycelia mat were dried in

hot air oven at 60^0C for 2 hours. Dry weight of the mycelial mat was recorded. The data obtained were statistically analysed using completely randomized design.

2.7 Effect of different culture media on growth of test fungus

The pathogen was isolated from all the infected sesame stem samples collected from eight locations of Odisha viz., Dhenkanal, Angul, Ganjam, Khurda, Puri, Malkanagiri, Koraput and Kalahandi. The *M. phaseolina* pathogen screened against three different culture media i.e. Potato Dextrose Agar, Oat Meal Agar and Host Leaf Extract Agar.

Potato Dextrose Agar (PDA)

Potato	: 200 g
Dextrose	: 20 g
Agar-agar	: 20 g
Distilled water	: 1000 ml
pH	: 6.5

Oat Meal Agar (OMA)

Oat meal	: 100 g
Agar agar	: 20 g
Trace salt solution	: 1 ml
Distilled water	: 1000 ml

Host Leaf Extract Agar (HLEA)

Host leaf bits	: 200 g
Agar agar	: 20 g
Dextrose (C6H12O6)	: 20 g
Distilled water	: 1000 ml

Each of the twenty millilitre media was added to a sterilised Petri plate. The process of inoculation involved moving a 5 mm mycelia mat disc that was removed from the periphery

of 7 days old culture and comprised of various isolates. Every treatment was carried out three times. The plates were incubated at 28±10C for 10 days. Observation on fungal radial growth (mm) was recorded when the maximum growth was attained in any one of the media tested. Other cultural characters viz., type of margin, topography of colony, color of colony and sclerotial production were also recorded.

2.8 Statistical analysis

The ANOVA to study the effects of treatments among the experiment, and their interactions, was performed by using the SAS programme PROC GLM of SAS 9.3 version (SAS Institute Inc., Cary, NC). The standard methods of analysis of variance for Randomized Block Design were used in the experiments [17]. Differences were considered significant at $P<0.05$ and a tendency towards significance at $0.05 < P < 0.1$. The appropriate standard error of mean (S.Em.±) was computed in each case. For the treatments effects, which were found to be significant, the critical difference (CD) at 5 percent level of probability was worked out to compare two treatment means.

Results

3.1. Field survey

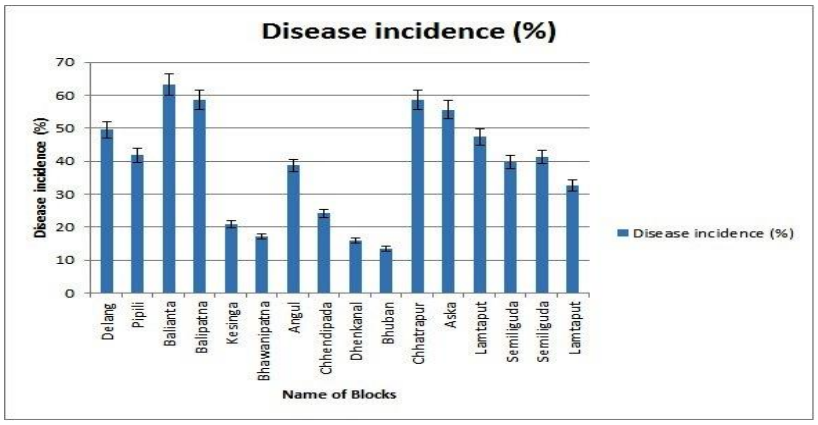
The outcomes of the field survey, which are presented in Table 1 and Fig. 1, showed that the incidence of charcoal rot varied between 13.48 - 63.27 percent in five districts of Odisha. Khurda district had the highest mean disease incidence (61.01%), followed by Malkangiri (57.09%), Puri (45.65%), Ganjam (45.65%), Koraput (36.96%), Angul (31.47%), Kalahandi (19.02%) and lowest was in Dhenkanal district (14.73%). The disease incidence also varied among the blocks and followed the sequence of decreasing order as Balianta (63.27%) > Balipatna (58.75%) > Delang (49.51%) > Pipili (41.79%) > Chhatrapur (47.37%) > Kukudakhandi (41.79%) > Semiliguda (41.28%) > Lamtaput (33.63%) > Angul (38.73%) > Chhendipada (24.21%) > Kesinga (20.83%) > Bhawanipatna (17.21%) > Dhenkanal

(15.97%) > Bhuban (13.48%). The all over mean sesame stem and root rot disease incidence of these five surveyed districts was 38.69 per cent.

Table 1: Survey and occurrence of stem and root rot incidence in sesame in different locations in Odisha

District	Block	Village	Situation	Cultivar	Soil type	Disease incidence (%)
Khurda	Balianta	Kakarudrapur	Rainfed	Local	Clay loam	63.27
Khurda	Balipatna	Jhinti	Rainfed	Local	Clay loam	58.75
						Mean 61.01
Malkanagiri	Mathili	Biralaxmanpur	Irrigated	Local	Sandy Loam	58.53
Malkanagiri	Malkangiri	MV9, MPV1	Irrigated	Local	Sandy Loam	55.64
						Mean 57.09
Puri	Delang	Gada Motari	Rainfed	Local	Sandy Loam	49.51
Puri	Pipili	Laxminarayanpur	Irrigated	Local	Sandy Loam	41.79
						Mean 45.65
Ganjam	Chhatrapur	Chamakhandi	Rainfed	Local	Sandy Loam	47.37
Ganjam	Kukudakhandi	Ankushpur	Rainfed	Local	Sandy Loam	41.79
						Mean 45.65
Koraput	Semiliguda	Semiliguda	Rainfed	Local	Red Laterite	41.28
Koraput	Lamtaput	Balel	Rainfed	Local	Red Laterite	32.63
						Mean 36.96
Angul	Angul	Angul	Rainfed	Local	Sandy Loam	38.73
Angul	Chhendipada	Khamar	Rainfed	Local	Sandy Loam	24.21
						Mean 31.47
Kalahandi	Kesinga	Gaigaon	Rainfed	Local	Sandy Loam	20.83
Kalahandi	Bhawanipatna	Malgaon	Rainfed	Local	Sandy Loam	17.21
						Mean 19.02
Dhenkanal	Dhenkanal	Mahisapat	Rainfed	Prachi	Sandy Loam	13.48
Dhenkanal	Bhuban	Dighi	Rainfed	Local	Sandy Loam	15.97
						Mean 14.73

Fig. 1: Percentage disease incidence of stem and root rot of sesame in different locations of Odisha



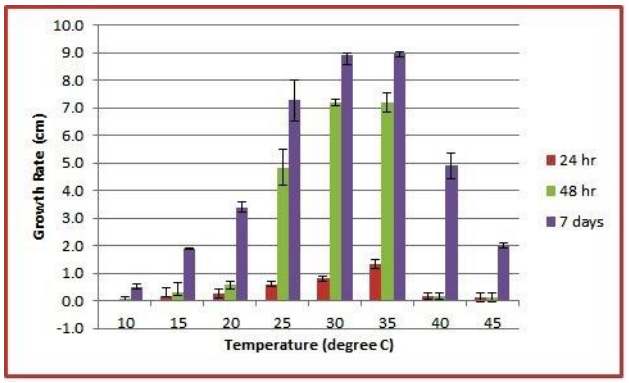
3.2. Effect of temperature

All microorganisms require a specific cardinal temperature range, with a possible minimum, optimum and maximum temperature to grow. The data (Table 2) (Fig. 2)clearly shows that the fungus grows at all temperature range of 25 and 40 ˚C. On the seventh day of incubation, the fungus showed its maximum mycelial growth (90 mm) at 35 ˚C, which was followed by 30 ˚C (90.0 mm). At 25 °C, mycelial growth gradually decreased to 72.7 mm, followed by 40 °C (49.0 mm), 20 °C (34.0 mm), 45 °C (20.0 mm), 15 °C (18.8 mm) and at 10 °C, mycelial growth was at its lowest, 5.0 mm. The optimum temperature required for maximum mycelial growth is 35 °C, after which the growth rate of fungus was ceased. Similarly, at lower temperature range the growth rate was very slow.

Table 2: Effect of temperature on mycelial growth of *M. Phaseolina*

Temp	24 hr	48 hr	7 days
10	0.0	0.8	5.0
15	1.7	3.0	18.8
20	2.7	5.7	34.0
25	6.0	48.3	72.7
30	8.0	70.0	90.0
35	13.3	72.0	90.0
40	1.7	1.7	49.0
45	1.3	1.3	20.0
Sem	0.1	0.2	0.2
CD5%	0.3	0.6	0.8
CV%	35.0	11.8	7.9

Fig. 2: Growth rate of mycelium at different temperature ranges



3.3. Effect of pH

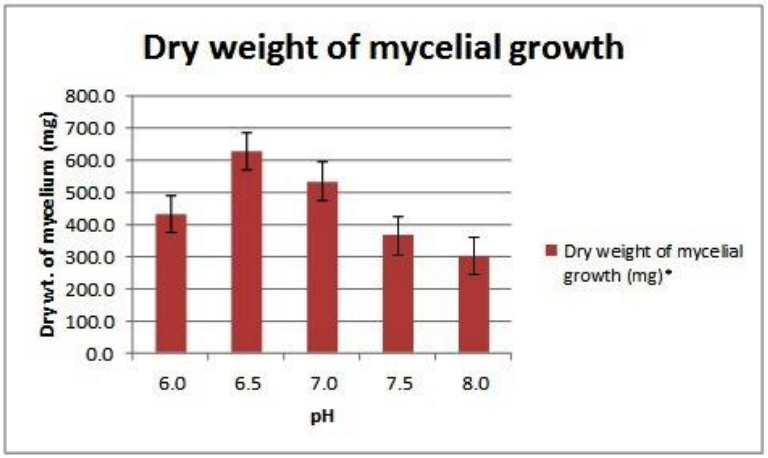
The results of the experiment showed that all the five pH levels include the growth of *M. phaseolina.* Perusal of data (Table 3) showed that maximum dry mycelial weight (624 mg) of *M. phaseolina* was observed at 6.5 pH level. A significantly decrease in dry mycelial weight was observed at pH 7.0 (533mg), at 6.0 (432 mg) and at 7.5 (364 mg). Minimum dry mycelial weight (300 mg) was observed at 8.0 of pH level.

Table 3: Effect of pH on mycelial growth of *M. Phaseolina*

Sl. No.	pH level	Dry weight of mycelial growth (mg)*
1	6.0	432.3
2	6.5	624.0
3	7.0	533.3
4	7.5	364.0
5	8.0	300.0
	Sem	4.4
	CD5%	13.6
	CV%	1.7

* Value showed the average of three replications

Fig. 3: Growth rate of mycelium at different pH level



3.3. Effect of different culture media on growth of *M. phaseolina*

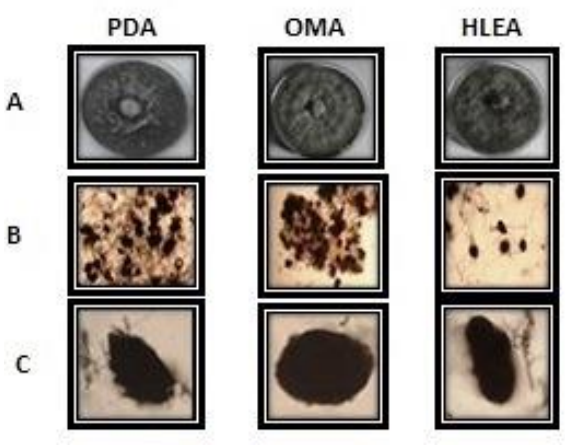
The *M. phaseolina* pathogen screened against three different culture media i.e. Potato Dextrose Agar, Oat Meal Agar and Host Leaf Extract Agar. The results shown in Table 4 and Figure 4 showed that the colony characteristics of the various isolates on the three distinct solid media varied significantly. The radial growth of all three media was same i.e. 90.00 mm and the type of margin is luxuriant uniform which is also same for all three media. Colony topography varies from dense uniform in PDA media Fluffy in OMA where as dense and flat in HLEA media. Similarly colony colour is also varies from black in PDA media and greyish black in other two media. The morphological characters like length, width and shape of sclerotia also showed variability in all three medium. The shape of sclerotia is oval in PDA medium and circular in OMA and irregular in HLEA medium. The length of sclerotia varies from 128.57 µm in OMA, 96.51 µm in HLEA and 92.32 µm in PDA medium. The width also varied from 87.53 µm in OMA, 62.13 µm in HLEA and 52.37 µm in PDA medium.

Table 4: Effect of culture media on mycelial growth of *M. Phaseolina*

Sl. No.	Characters	Potato dextrose agar	Oat meal agar	Host leaf extract agar
1.	Radial growth (mm) (Mean of three repl.)	90.00	90.00	90.00
2.	Type of margin	Luxuriant uniform	Luxuriant uniform	Luxuriant uniform
3.	Colony topography	Dense uniform	Fluffy	Dense & flat
4.	Colony colour	Black	Greyish black	Greyish black
5.	Length of sclerotia (µm)	92.32	128.57	96.51

6.	Width of sclerotia (µm)	52.37	87.53	62.13
7.	Shape of sclerotia	Oval	Circular	Irregular

Fig. 4: Cultural and morphological variation in different culture media



A. Cultural characteristics, B. Number of sclerotia and C. Shape of sclerotia

4. Discussion

Owing to its quick maturation following seeding, sesame is grown as an attractive crop in Odisha. A new disease known as charcoal rot, also known as Macrophomina stem rot, is destroying the sesame crop in Odisha. The current study found that a combination of monoculture, a lack of crop diversity, and poor management may be the reason for the higher disease prevalence in the Balianta block of the Khurda district [18]. Pathogen population dynamics, including overwintering and survival rates, population growth rates, and the number of generations in polycyclic species, can be strongly impacted by temperature, pH and different culture media. Warm, temperate, and tropical parts of the world are home to a vast array of cultivated and wild species that are harmed by the fungal pathogen *M. phaseolina*. Its pathogenicity is temperature-dependent, peaking between 28 and 35 degrees Celsius [19]. The development of microsclerotia, a hard and compact mass of hyphea, resistant to unfavourable conditions was also high on the ideal range of temperature. For certain fungi to survive, the production of sclerotia is essential [20]. Bigger microsclerotia

have a higher probability of proliferating rapidly, forming germtubes, and eventually infecting the disease-susceptible host [13]. The results of this investigation showed that *M. phaseolina* is capable of growing at temperatures between 25 and 35 degrees Celsius.

For *M. phaseolina* to grow, the ideal temperature range was 30 to 35°C (8.0 to 8.6 cm in 72 hours). While colonies of all isolates expanded to a diameter of 9 cm (the diameter of a Petri dish) within 10 days of plating in the temperature range of 20 to 38°C, growth was slower at 25 and 27°C (3–5 cm in 72 hours). Growth was negligible (up to 2 cm after 72 hours) or non-existent at 40°C. Manici et al., (1995) observed that certain isolates from the south of Italy (which has a Mediterranean climate) grew better at 35°C when they studied the in vitro growth of *M. phaseolina* isolates from sunflower grown in different climatic regions of the country. Additionally, Dhingra et al., (1973) discovered that most isolates collected in various parts of Niger grew best at 35°C, with a smaller percentage growing at 25-30°C.

In order to assess the impact of pH on the mycelium growth of fungus, it was subjected to varying pH levels directly. pH observations are in line with the findings of Kaur et al. (2013) and Sukanya et al. (2016), who discovered that the highest mycelium growth was observed at pH levels 6.5 to 7.0. The current investigation supports this result.

M. phaseolina exhibits more morphological and cultural variability than other root rot pathogens in different culture media [13]. Observations of the current investigation are consistent with those of previous researchers [23] [24] [25]. A range of cultural characteristics, including colony colour, margins, and topography, were observed among the *M. phaseolina* isolates. The sclerotial morphology of the *M. phaseolina* isolates is consistent with the descriptions made by Shekhar et al. (2006) and Prasad et al. (2011), who noted variations in the size, shape, and sclerotial production of the isolates.

5. Conclusion

The present investigation on physiological studies showed that, sesame stem and root rot pathogen *Macrophomina phaseolina* prefers high temperature i.e. 30^{o}C and pH 6.5o grow significantly as well as for disease development. Also, the *in-vitro* study on different culture media showed the growth rate is high in PDA medium but the morphological characters showed better result in OMA medeium.

Data Availability Statement: Not applicable

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