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Effect of Environmental Factors on the Growth of Pathogenic Fungi Causing Fruit Rot in Tomato (*Lycopersicon esculentum*)

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A B S T R A C T

Effect of different temperature ranges, pH levels and light intensity were tested against the growth of *Rhizopus stolonifer* (Ehrenberg ex. Fr.) Lind, *Aspergillus niger* Van Teighem, *Alternaria alternata* (Fr.) Keissler, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, and *Phytophthora nicotianae* Breda de Haan under *in vitro* conditions which were isolated from the tomato samples brought from various locations. The temperature of 25 °C was best for the growth of *Rhizopus stolonifer*, *Alternaria alternata*, *Fusarium oxysporum* f. sp. *lycopersici* and *Phytophthora nicotianae* while as the temperature of 35 °C was best for the growth of *Aspergillus niger.* Maximum growth of *Rhizopus stolonifer*, *Aspergillus niger*, *Alternaria alternata* was obtained when exposed to alternating light and alternating dark. *Fusarium oxysporum* f. sp. *lycopersici* recorded maximum growth when exposed to dark. Maximum growth of *Phytophthora nicotianae* was observed at twelve hours light. The pH 5.5 was best for the growth of *Rhizopus stolonifer*, *Aspergillus niger* and the pH6.5 was best for the growth of *Alternaria alternata* and *Phytophthora nicotianae* while as the pH 7.0 was best for the growth of *Fusarium oxysporum* f. sp. *lycopersici.*

***Key words*:** Environmental factors, Growth, Pathogenic fungi, Tomato

Solanaceous crops are economically important in both tropical and temperate regions. Tomato (*Lycopersicon esculentum* L.) is considered as one of the most economic vegetable crops either for local consumption or exportation purposes. World losses in tomato yield can be referred to soil-borne pathogens. Temperature, pH and light plays an important role on the growth and reproduction of fungi. All the fungi have minimum temperature, below which they cannot grow and above which they are inactivated or killed. Each fungus has its temperature range for the growth. Light has the profound effect on growth and sporulation of fungi. Similarly, pH of the medium has a profound effect upon the rate and extent of growth and many other life processes of fungi. An understanding of the role of environmental conditions have on the infection and survival of these pathogens is necessary to develop cultural disease management practices. Therefore, the objectives of this study were collection, isolation, purification and identification of pathogenic fungi causing fruit rot of tomato and to determine optimum cultural conditions for mycelial growth by these fungi viz., temperature, pH and light. The understanding of the effect of temperature on fungal growth is an essential part of fungal physiology. Two applied fields where this is also of interest are building science and food science. Mould growth indoors and inside of constructions is a major problem both in tropical and temperate climates as both mould spores and fragments are suspected to contribute to indoor related health problems, e.g., sick building syndrome, allergy etc. [1].

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MATERIALS AND METHODS

The diseased tomato fruits were collected from the local markets andalso from Shalimar Campus. The diseased fruits were kept in sterilized polythene bags and brought to the laboratory for the purpose of isolation of the pathogen.

Isolation of fungal pathogens

Isolation of fungi was made in Laminar Air Flow Chamber (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) by following the standard tissue bit method [2]. The infected portion of the tomato fruits were cut into small bits measuring about 2 mm and surface sterilized with 0.1% mercuric chloride for one minute. The bits were then rinsed thrice in sterilized distilled water and then aseptically transferred to the plates containing the Potato Dextrose Agar (PDA) media, incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at 28±1 °C and observed periodically for fungal growth.

Purification of fungal pathogens

The culture obtained was purified in Laminar Air Flow Chamber (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) by single spore and hyphal tip isolation methods. Hyphal tip isolation was done on water agar plates. Ten ml of clear, two per cent water agar was poured into sterile Petri plates and allowed to solidify. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates. Single spore was then marked under the microscope (Leica Hiplan 1359500, China) with ink on the glass surface of the plate and it was allowed to germinate. Such plates were incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at 27±1 °C and periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants under aseptic conditions and incubated at temperature of 27±1 °C for 5 days. Later, mycelial bits of the fungus were placed in the center of Petri plates containing PDA medium and incubated at 27±1 °C for 5 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus.

Identification of the pathogens

The culture thus obtained was observed under the microscope (Leica Hiplan 1359500, China) for various cultural and morphological characters and the effect of different environmental factors was observed on the isolated test fungi.

Environmental factors affecting growth of isolated fungal pathogens causing tomato fruit rot

Effect of incubation temperature on growth of fungal pathogens

Potato Dextrose Agar was used as a basal medium to study the effect of temperature on the growth of pathogenic fungi. Petri plates containing 20 ml of PDA medium were inoculated with 5 mm diameter discs from 5 days old culture of each test fungus. The inoculated plates were then incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at different temperatures 5, 15, 25, 35, 45 °C and control (laboratory temperature). Three replications were maintained for each treatment. Observations on colony diameter were recorded and the data was analysed statistically.

Effect of duration of light on growth of fungal pathogens

Potato Dextrose Agar was used as a basal medium to study the effect of light on the growth of pathogenic fungi. Petri plates containing 20 ml of PDA medium were inoculated with 5 mm diameter discs from 5 days old culture of each test fungus. The Petri plates were incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at 26±1 °C under eight hours light, eight hours dark, alternating light and alternating dark, twenty-four hours dark and twelve hours light (control). Three replications were maintained for each treatment. Observations on the colony diameter was recorded and the data was analysed statistically.

Effect of pH on growth of fungal pathogens

Potato Dextrose Agar was used as a basal medium to study the effect of pH on the growth of pathogenic fungi. The pH of the medium was adjusted to various concentrations *viz*., 5.5, 6.5, 7.5, 8.5 and 9.5 and control (pH = 7) by adding 0.1 N sodium hydroxide and 0.1N hydrochloric acid and it was determined by electronic pH meter (Hanna HI 98127, Mauritius). Petri-plates containing 20 ml of PDA medium were inoculated with 5 mm diameter discs from 5 days old culture of each test fungus. The inoculated plates were incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at 26±1 °C. Three replications were maintained for each treatment. Observations on colony diameter were recorded and the data was analysed statistically.

RESULTS AND DISCUSSION

On the basis of cultural and morphological characteristics the pathogens were identified as *Rhizopus stolonifer* (Ehrenberg ex. Fr.) Lind, *Aspergillus niger* Van Teighem, *Alternaria alternata* (Fr.) Keissler, *Fusarium oxysporum* f. sp. *Lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, and *Phytophthora nicotianae* Breda de Haan (Fig 1-2). The identity of the pathogens was confirmed from the Division of Plant Pathology, SKUAST-K, Shalimar. The pathogens viz., *Rhizopus stolonifer*, *Aspergillus niger*, *Alternaria alternata*, *Fusarium oxysporum* f. sp. *lycopersici* were isolated from the tomato samples brought from local markets, where as the pathogen *Phytophthora nicotianae* was isolated from the tomato samples brought from Shalimar campus. The cultural and morphological observations also agreed with the description for *Alternaria alternata* [3], *Phytophthora nicotianae* [4], *Fusarium oxysporum* f. sp. *Lycopersici* [5-6], *Rhizopus stolonifer* [7], *Aspergillus niger* [8].

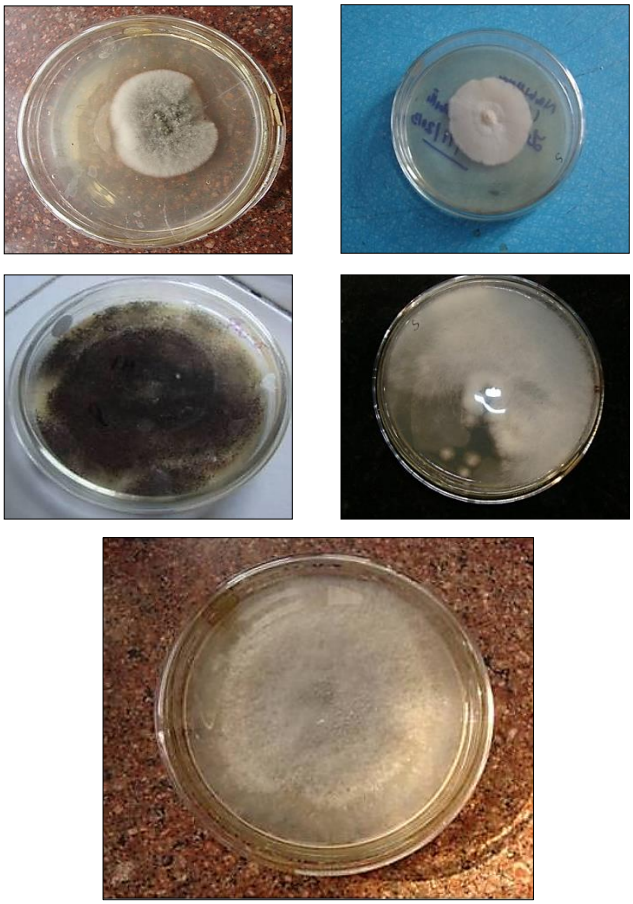

Fig 1 Pure cultures of *Alternaria alternata, Phytophthora nicotianae, Rhizopus stolonifer, Aspergillus niger* and *F. oxysporum* f. sp. *lycopersici*

Effect of temperature

Temperature plays an important role on the growth and reproduction of fungi. All the fungi have minimum temperature, below which they cannot grow and above which they are inactivated or killed. Each fungus has its temperature range for the growth. In the present study the fungi were grown at

different temperature levels and significant differences on the growth have been observed at all the temperature levels studied. Data in (Table 1, Fig 3) indicate that 25 ºC resulted in maximum growth of all tested fungi except *A. niger* which grew best at 35 ºC. The maximum growth (85.30 mm) of *R. stolonifer* was obtained at 25 ºC while no growth was observed at 5, 35 and 45 ºC [7]. The maximum growth (77.36 mm) of *A. niger* was recorded at 35 ºC followed by 25 ºC, being favorable temperature for its growth. However, no growth occurred at lower temperature of 5 ºC and at higher temperature of 45 ºC which is unfavorable temperature to the growth of *A. niger*. Minimum growth (20.40 mm) of *A. niger* was recorded at 15 ºC [9]. The best growth (79.01 mm) of *A. alternata* was observed at 25 ºC whereas lowest growth (6.99 mm) was observed at 45 ºC. At 5 ºC growth (8.01 mm) of *A. alternata* was observed [10]. Maximum mycelial growth (61.90 mm) of *Fusarium oxysporum* f. sp. *lycopersici* was obtained at 25 ºC and minimum (10.50 mm) at 35 ºC. However, no growth was observed at 5 and 45 ºC [11]. *P. nicotianae* grew best at 25 ºC where 55.42 mm growth was observed. Poor growth of *P. nicotianae* occurred at 15 ºC (12.34 mm) and 35 ºC (17.10 mm). However, no growth was observed at 5 ºC and 45 ºC. It is showed that this is slow growing fungus which resulted in minimum growth rate and it might be due to its nature [12]. It is thus revealed that very low and high temperatures are not suitable for the growth of the test fungi.

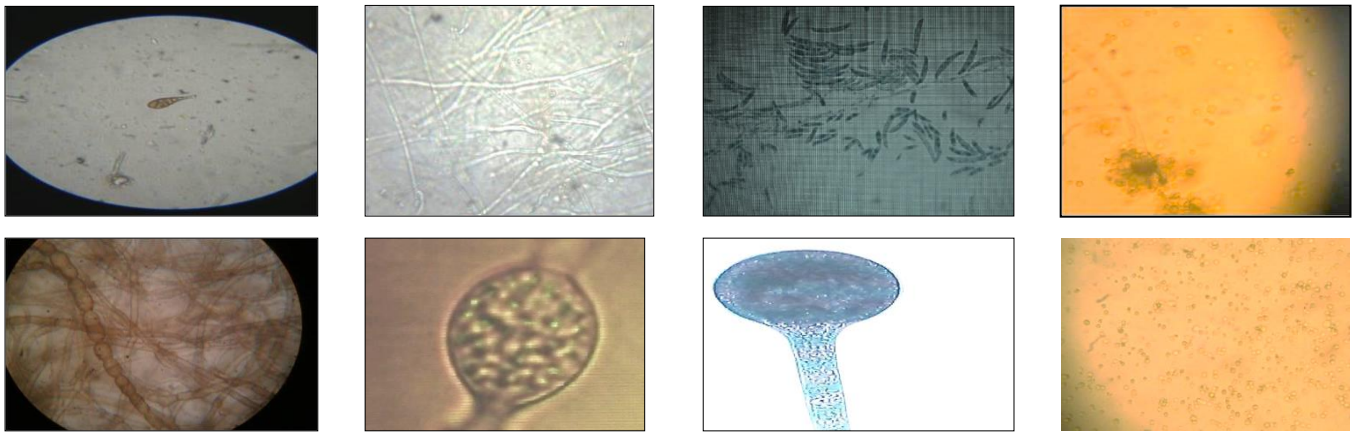

Fig 2 Microscopic observations of conidia of *Alternaria alternata,* Brownish septate conidiophores with simple olive-brown septate of *Alternaria alternata,* Hyphae of *Phytophthora nicotianae,* Sporangia of *Phytophthora nicotianae* and Conidia of *Fusarium oxysporum* f. sp. Lycopersici, Sporangia and sporangiophores of *Rhizopus stolonifer,* Conidia of *Aspergillus niger,* Conidiophore of *Aspergillus niger*

Table 1 Effect of different temperature on growth of pathogenic fungi

Temperature (ºC)	*Rhizopus stolonifer*	*Aspergillus niger*	*Alternaria alternata*	*Fusarium oxysporum* f. sp. *lycopersici*	*Phytophthora nicotianae*
5	NG	NG	8.01	NG	NG
15	42.50	20.40	44.00	28.11	12.34
25	85.30	52.90	79.01	61.90	55.42
35	NG	77.36	62.90	10.50	17.10
45	NG	NG	6.99	NG	NG
Control (Laboratory temperature 25±2)	79.40	49.40	75.50	58.70	36.42
$CD_{0.05}$	1.63	0.96	0.85	1.58	1.13

Data (mm) are mean of three replications. NG = No growth

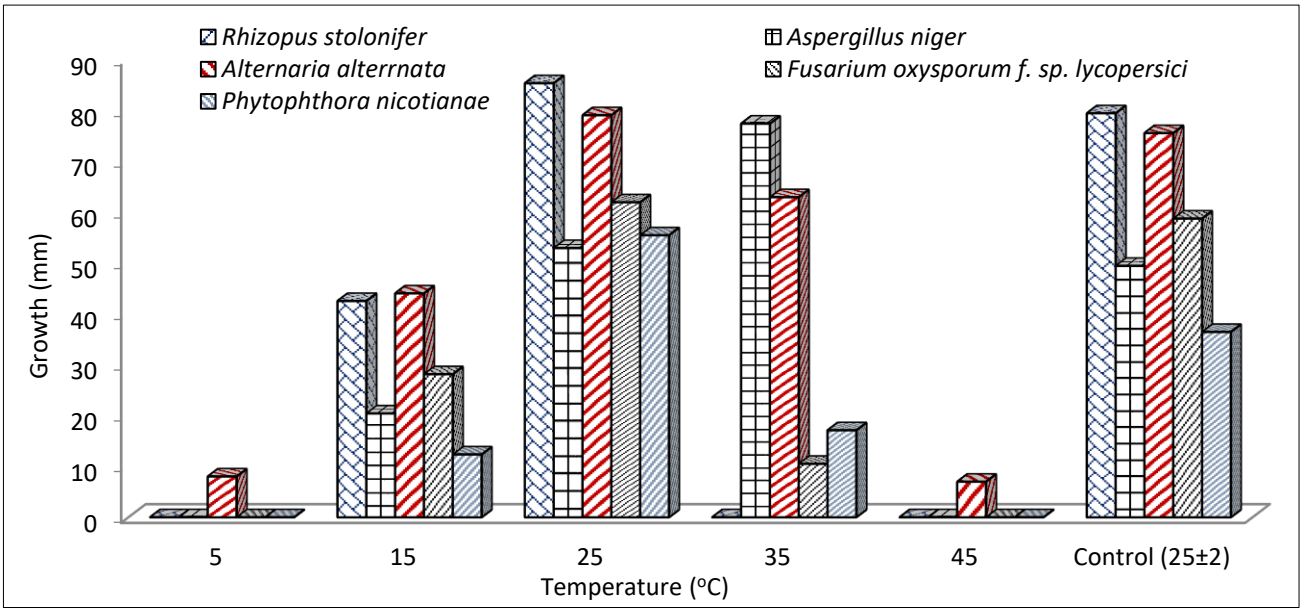

Fig 3 Effect of different temperature on growth of pathogenic fungi

Effect of light

Light has profound effect on growth and sporulation of fungi. Data in (Table 2, Fig 4) show that light regime had a significant effect on culture growth of test pathogens. Maximum growth (86.31 mm) of *Rhizopus stolonifer* exhibited under alternating light and alternating dark, whereas lowest growth (20.40 mm) of the same pathogen was observed under exposure of twenty-four hours dark. The maximum growth of (82.80 mm) of *Aspergillus niger* was recorded at alternate cycles of light and dark. Minimum growth (17.30 mm) was recorded at eight hours light [13]. Highest growth (84.80 mm) of *Alternaria alternata* was observed under exposure to alternating light and dark, whereas lowest growth (14.40 mm) of *Alternaria alternata* occurred at eight hours dark condition. This is in agreement with Hubballi *et al*. [10] who reported that fungus exposure to alternate cycles of light and dark resulted in maximum growth compared to continuous light and dark exposure. Maximum growth (78.23 mm) of *Fusarium oxysporum* f. sp. *lycopersici* occurred under dark and minimum growth (53.00 mm) occurred at alternate cycles of light and dark. This is in agreement with Benaouli *et al*. [14] who reported that maximum growth of fungi occurred under dark. Continuous light promoted maximum growth of *Phytophthora nicotianae* followed by alternating light and alternating dark. Lowest growth of *Phytophthora nicotianae* occurred at twenty-four hours dark [15].

Table 2 Effect of duration of light on growth of pathogenic fungi

Exposure	*Rhizopus stolonifer*	*Aspergillus niger*	*Alternaria alternata*	*Fusarium oxysporum* f. sp. *lycopersici*	*Phytophthora nicotianae*
Eight hours light	35.40	17.30	28.70	58.00	39.80
Eight hours dark	24.30	24.20	14.40	73.00	28.10
Alternating light and alternating dark (24 h)	86.31	82.80	84.80	53.00	34.32
Twenty-four hours dark	20.40	31.40	19.20	78.23	24.30
Twelve hours light (control)	51.40	21.20	31.20	66.00	45.20
$CD_{0.05}$	1.582	0.851	2.357	1.562	0.756

Data (mm) are mean of three replications

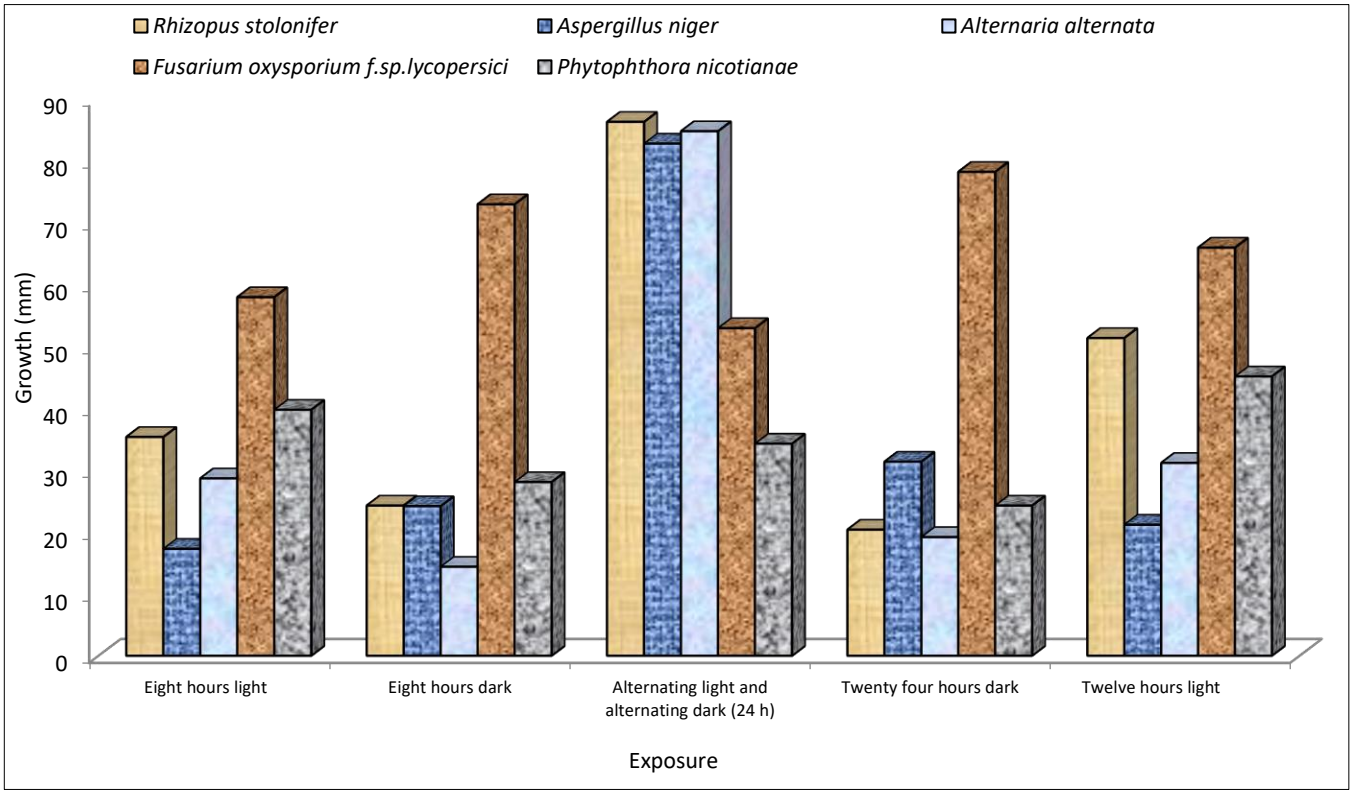

Fig 4 Effect of duration of light on growth of pathogenic fungi

Table 3 Effect of pH on growth of pathogenic fungi

pH	*Rhizopus stolonifer*	*Aspergillus niger*	*Alternaria alternata*	*Fusarium oxysporum* f. sp. *lycopersici*	*Phytophthora nicotianae*
5.5	78.64	85.50	42.10	53.40	43.20
6.5	23.20	72.00	85.40	42.31	55.30
7.5	NG	59.40	60.40	60.20	35.10
8.5	NG	36.00	48.50	30.01	23.20
9.5	NG	10.11	27.00	28.32	7.10
7.0 (Control)	NG	61.90	68.10	62.40	41.30
$CD_{0.05}$	t-test performed p value < 0.05 hence the two differ significantly	1.717	2.051	1.595	0.963

Data (mm) are mean of three replications. NG = No growth

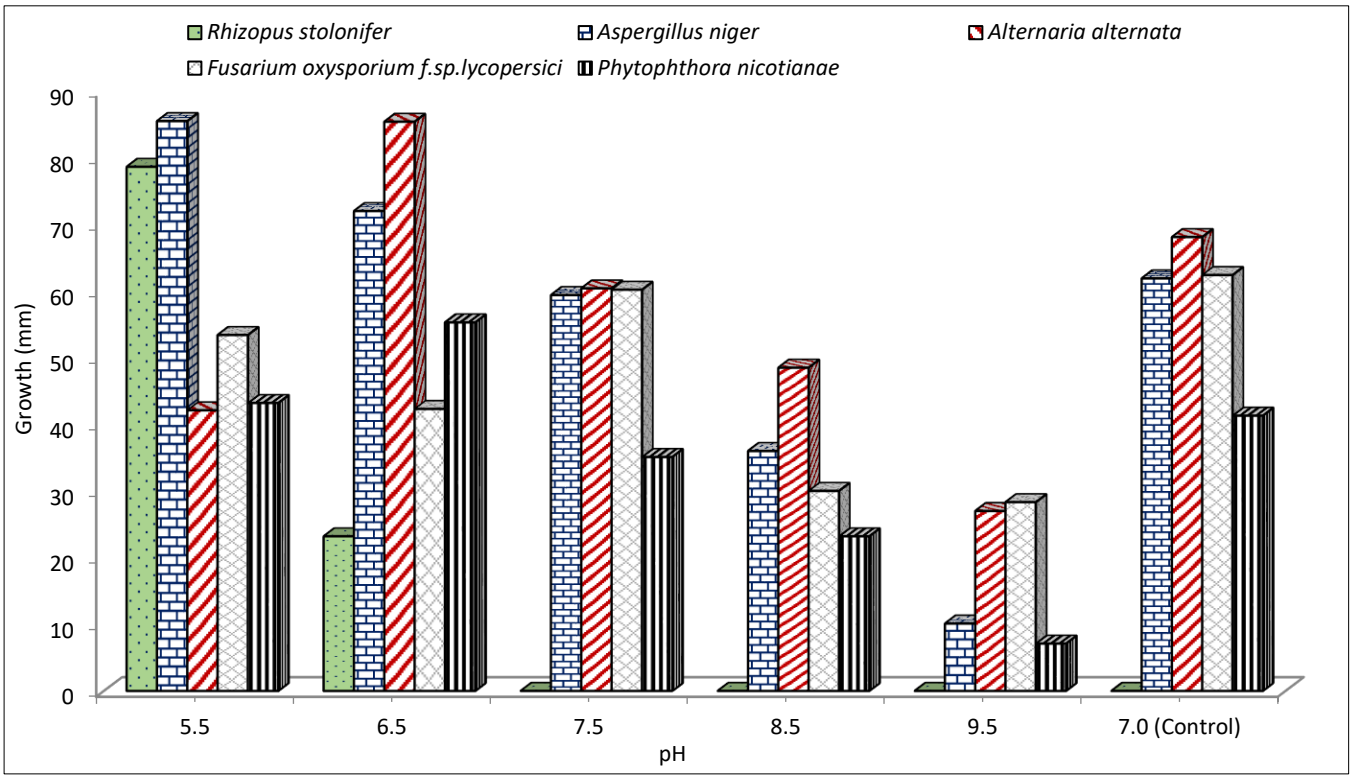

Fig 5 Effect of pH on growth of pathogenic fungi

Effect of pH

pH of the medium has a profound effect upon the rate and extent of growth and many other life processes of fungi. The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to the fungal growth and metabolism. This shows importance of hydrogen ion concentration for better growth of fungi. The results of the present study (Table 3, Fig 5) indicate that highest growth of *R. stolonifer* (78.64 mm) occurred at pH 5.5 whereas lowest growth (23.20 mm) was observed at pH 6.5. However, as the pH increased towards a neutral range, fungal growth rate declined until mycelial growth was not supported at pH 7.0 and beyond [16]. Maximum growth of *A. niger* (85.50 mm) was observed at pH 5.5 followed by pH 6.5 where growth of 72 mm was recorded. Lowest growth (10.11mm) of fungi was observed at a pH 9.5. This is supported by the findings of Al-Gabr *et al*. [17] who reported that the pH levels 5.5-6.5 showed best growth of fungi as compared to the other pH levels which proved that the highest pH levels could not be favorable to fungus so these were not grown profusely. *A. alternata* showed maximum growth at pH 6.5 (85.40 mm) followed by pH 7.0 which recorded 68.10 mm growth [10]. Maximum growth (62.40 mm) of *F. oxysporum* f. sp. *lycopersici* was recorded at pH 7.00. Lowest growth of test fungus was observed at pH 9.5 [18]. Slightly acid conditions favoured the growth of *P. nicotianae*. The optimum growth was at pH 6.5 (55.30 mm). Lowest growth (7.10mm) occurred at the pH 9.5 [12]. The inhibitory action of certain pH level for a specific test pathogen can be attributed to the unconducive reaction of the media. It is thus revealed that the pathogenic fungi prefer acidic and neutral conditions to alkaline condition indicating their acid tolerance. Bilgrami and Verma [19] have also opined that in contrast to bacteria and actinomycetes, fungi are relatively more tolerant to acidic ions than to basic ions.

CONCLUSION

The temperature of 25 °C and 35 °C was best for the growth. In terms of light and pH maximum growth was obtained when exposed to alternating light and alternating dark, dark and twelve hours light, whereas pH 5.5, 6.5 and 7.0 was best for the growth of species tested.

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