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Environmental Factors Affecting Growth of Pathogenic Fungi Causing Fruit Rot in Tomato (Lycopersicon Esculentum)

Nowsheen Hassan[1],
[1]Division of Environmental Sciences,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shalimar-190 025 Srinagar Jammu and Kashmir, India.

Shafiq-Ur-Rehman[2] ,
[2]Professor and Head,
Division of Environmental Sciences,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shalimar-190 025 Srinagar Jammu and Kashmir, India.

Haleema Bano[3]
[3]Assistant Professor
Division of Environmental Sciences,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shalimar-190 025 Srinagar Jammu and Kashmir,
India.

Abstract **- 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 pH 6.5 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, Fungi, Pathogenic, Plant extracts, Tomato.***

INTRODUCTION

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 cultura conditions for mycelial growth bythese fungi *viz*., temperature, pH and light.

MATERIALS AND METHODS

Collection of disease samples

The diseased tomato fruits were collected from the local markets and also 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 (Mamatha and Rai, 2004). 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-

International Journal of Engineering Research & Technology (IJERT)
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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 petriplates 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 petriplates 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 the 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 hourslight (control). Three replications were maintained for each treatment. Observations onthe colony diameter were r ecorded 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, SKUASTKashmir, 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 Abeer et al. (2014), Phytophthora nicotianae Mounde et al. (2012), Fusarium oxysporum f. sp. Lycopersici Nirmaladevi and Srinivas (2012) and Chopada et al. (2015), Rhizopus stolonifer Kwon et al. (2001), Aspergillus niger Diba et al. (2007).

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

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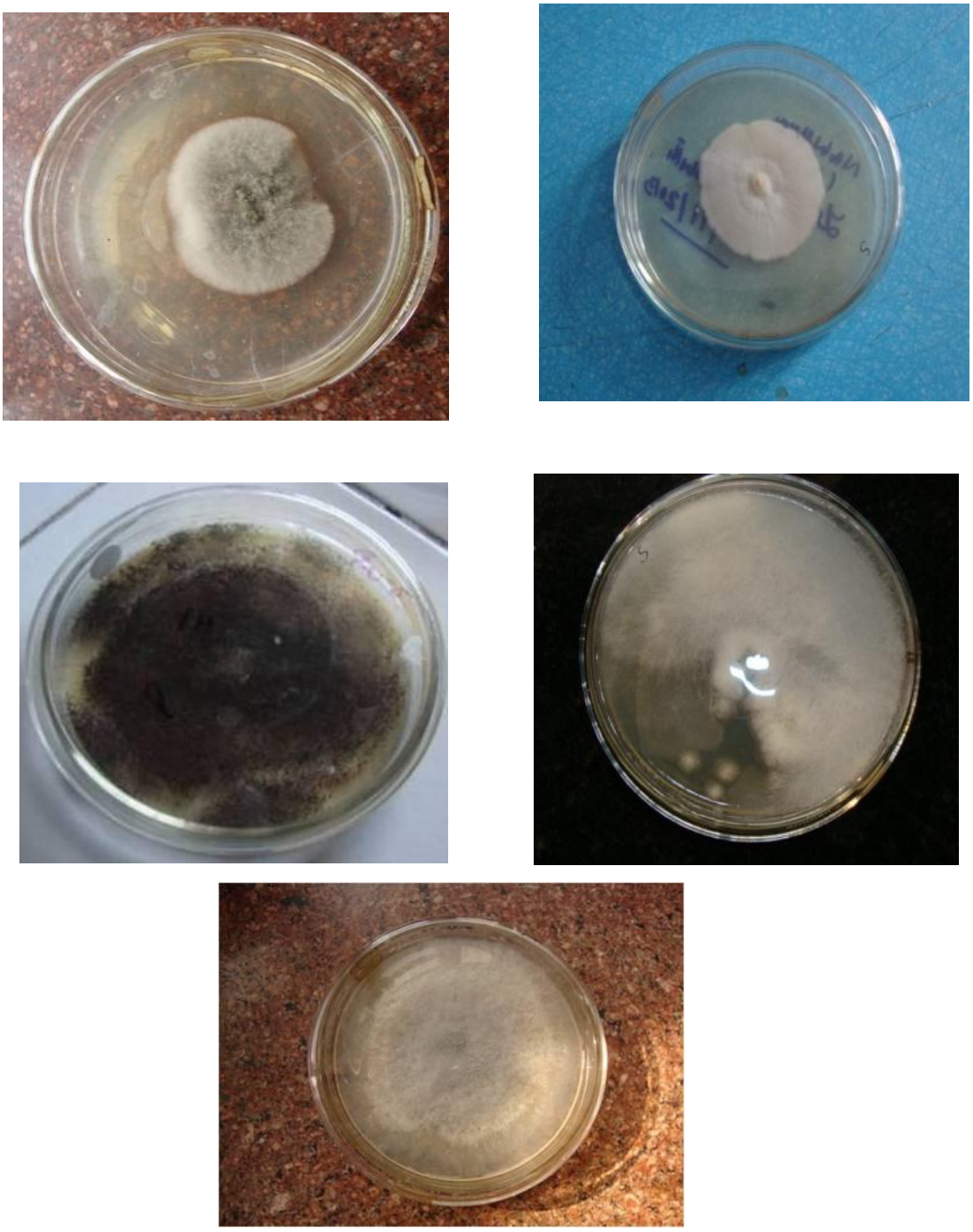
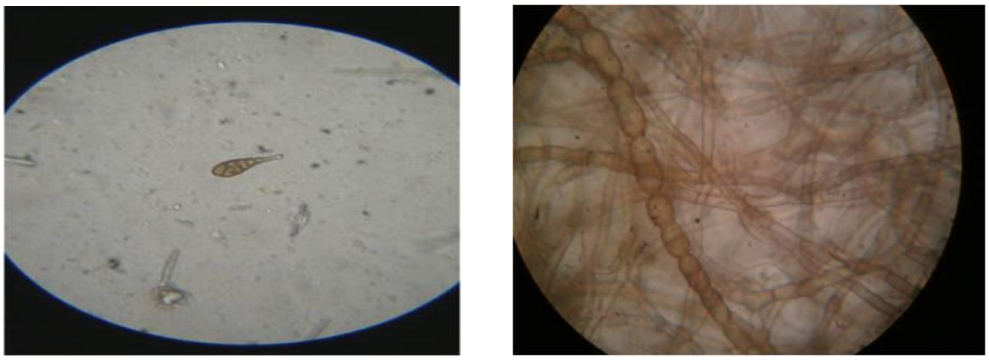


Fig. 1: Pure cultures of Alternaria alternata, Phytophthora nicotianae, Rhizopus stolonifer, Aspergillus niger and F. oxysporum f. sp. lycopersici



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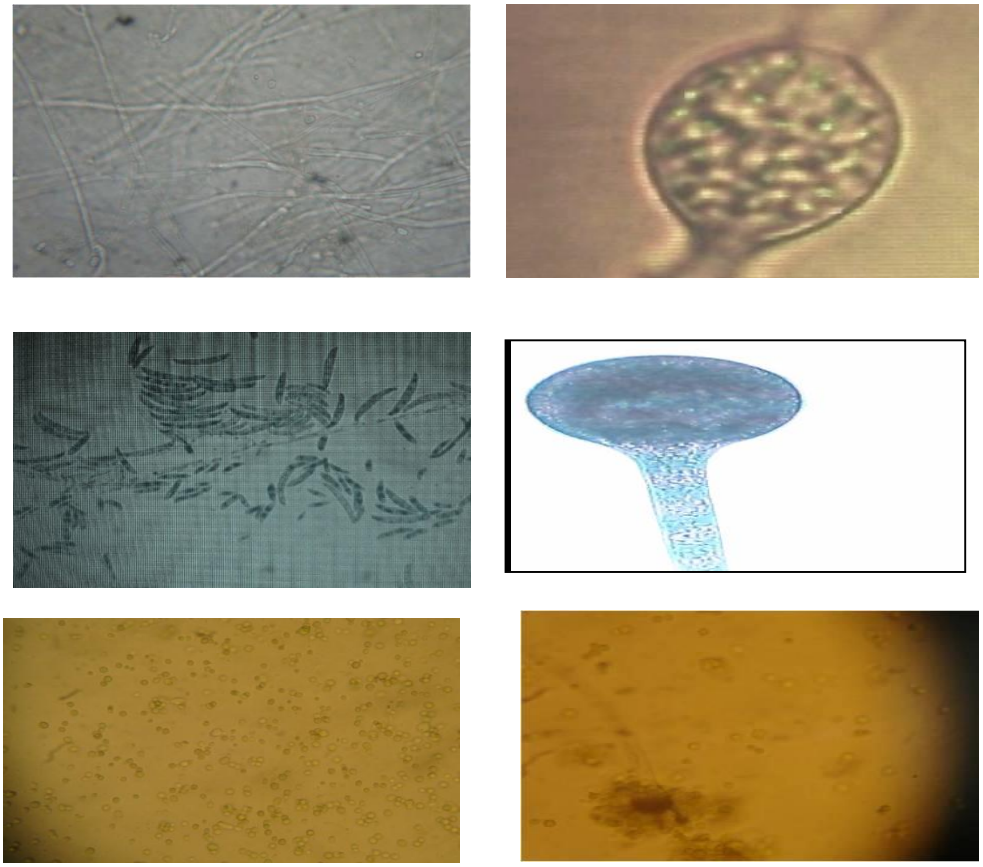

Fig. 2: Microscopic observations of Conidia of Alternaria alternata, Brownish septate conidiophores with simple olive-brown septate of Alternaria alternata, Hyphae of Phytophthora nicotianae, Sporangia of Phytophthora nicotianae and Conidia of Fusarium oxysporum f. sp. Lycopersici, Sporangia and sporangiophores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

differences on the growth have been observed at all the temperature levels studied. Data in Table-1 and 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. These findings are supported by the findings of Kwon et al. (2001). 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. These results are in concurrence with studies of Ababutain (2013). 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. These results are attributed by Hubballi et al. (2010). Maximum mycelial growth (61.90 mm) of F. 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. These findings are supported by the findings of Fayzalla et al. (2008). 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. The findings are supported by Shah and Bhat (2009). It is thus revealed that very low and high temperatures are not suitable for the growth of the test fungi.

Effect of light

Light has profound effect on growth and sporulation of fungi. Data in Table-2 and Fig. 4 show that light regime had a significant effect on culture growth of test pathogens. Maximum growth (86.31 mm) of R. 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 A.niger was recorded at alternate cycles of light and dark. Minimum growth (17.30 mm) was recorded at eight hours light. These findings are in consonance to the observations made by Shehu and Bello (2011). Highest growth (84.80 mm) of A. alternata was observed under exposure to alternating light and dark, whereas lowest growth (14.40 mm) of A. alternata occurred at eight hours dark condition. This is in agreement with Hubballi et al. (2010) 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 F. 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. (2014) who

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ISSN: 2278-0181
Vol. 6 Issue 02, February-2017

reported that maximum growth of fungi occurred under dark. Continuous light promoted maximum growth of P. nicotianae followed by alternating light and alternating dark. Lowest growth of P. nicotianae occurred at twenty four hours dark. These results are in consonance with Faisal et al. (2005).

Table 1: Effect of different temperature on growth of pathogenic fungi

Temperature (°C)	*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

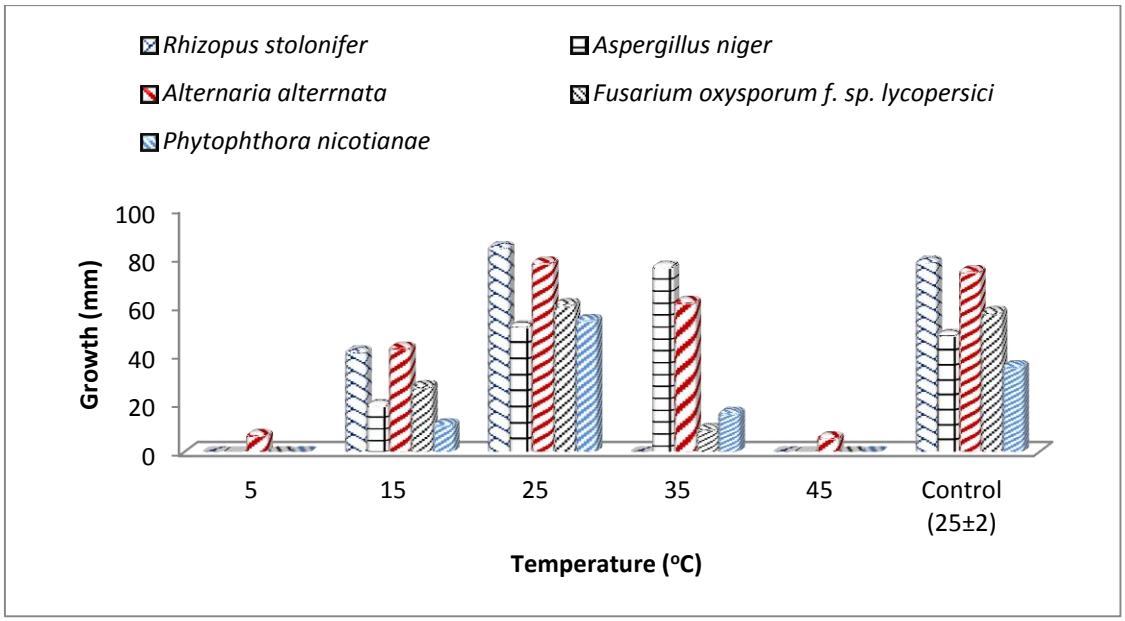

Fig. 3: Effect of different temperature on growth of pathogenic fungi

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.

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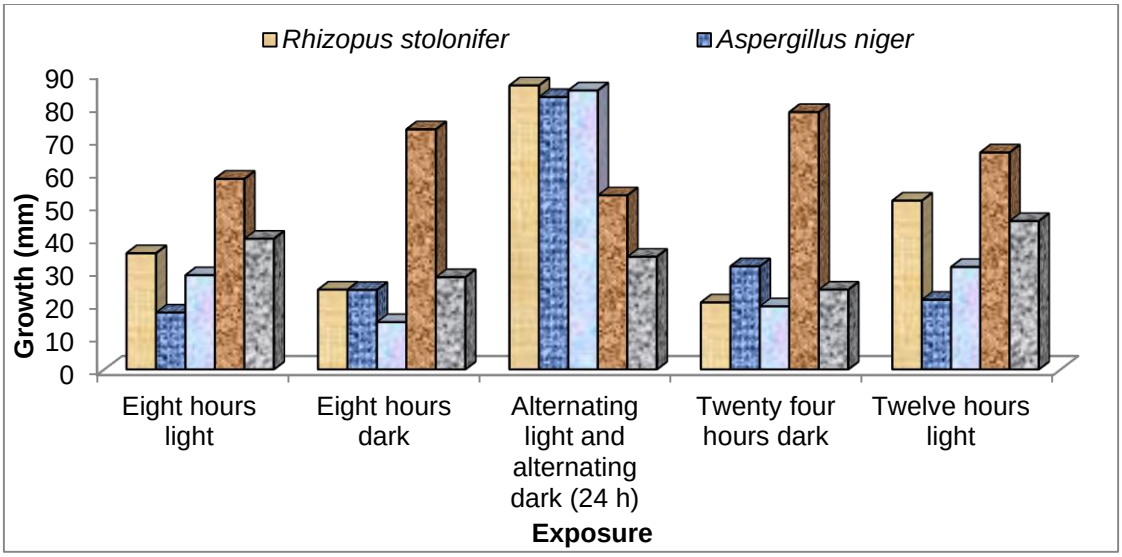

Fig. 4: Effect of duration of light on growth of pathogenic fungi

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 and Fig.5 indicate that highest growth of *R. stolonifer* (78.64 mm) occurred at pH 5.5 where as 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. These findings are in agreement with the findings of Odeniyi *et al*. (2009).

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 (10.11 mm) of fungi was observed at a pH 9.5. This is supported by the findings of Al-Gabr *et al*. (2012) 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. This is in agreement with findings of Hubballi *et al*. (2010). 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. These findings are in agreement with the findings of Mousa (2004) and Fayzalla *et al*. (2008). Slightly acid conditions favoured the growth of *P. nicotianae*. The optimum growth was at pH 6.5 (55.30 mm). Lowest growth (7.10 mm) occurred at the pH 9.5.

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

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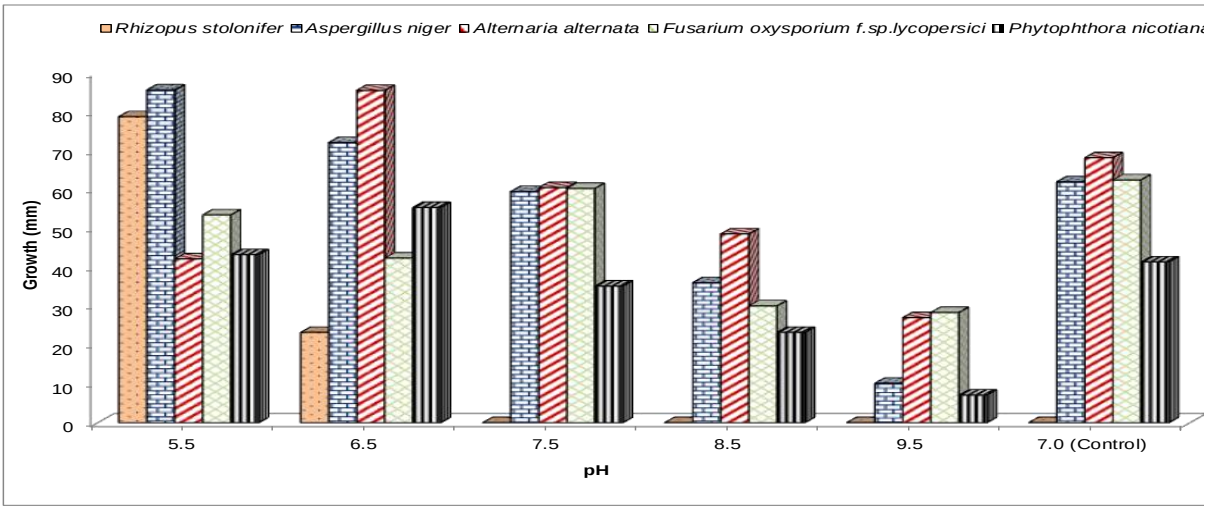

Fig. 5: Effect of pH on growth of pathogenic fungi

These findings are in accordance with those of Shah and Bhat (2009). 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 (1978) have also opined that in contrast to bacteria and actinomycetes, fungi are relatively more tolerant to acidic ions than to basic ions.

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