International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1791-1794

Received: 10-01-2018
Accepted: 12-02-2018

DB Sheth
Department of Plant Pathology, B.A. College of Agriculture, Anand, Gujarat, India

PR Patel
Department of Plant Pathology, B.A. College of Agriculture, Anand, Gujarat, India

RK Patil
Department of Plant Pathology, B.A. College of Agriculture, Anand, Gujarat, India

Correspondence
DB Sheth
Department of Plant Pathology, B.A. College of Agriculture, Anand, Gujarat, India

Influence of media, temperatures and pH on the growth and sporulation of *Aspergillus niger*

DB Sheth, PR Patel and RK Patil

Abstract
Highest radial growth and dry weight of *A. niger* was obtained on Malt extract, Corn meal, and Oat meal agar (90.00 mm) and Czapek's broth (361.62 mg) with excellent sporulation. The 30^0C temperature found best for the mycelial growth (90.00 mm) and sporulation of A. *niger* on PDA. The pH 6 found most suitable for the mycelial growth (86.05 mm & 262.05 mg) and sporulation in solid and liquid potato dextrose broth media, respectively.

Keywords: *Aspergillus niger*, media, temperature, pH, growth, sporulation

Introduction
Citrus is an important fruit crop in the world and is cultivated in more than 100 countries covering all six continents, and it is often regarded as golden fruit or queen of all fruits (Nito, 1996) [9]. It is also known as Kagzi-lime, Acid lime, Sour lime, Mexican lime and in Hindi as "Neebu" and in Gujarati as "Leembu". Acid lime plant is a perennial profusely branched thorny shrub or small tree which bears more or less round oval, smooth fruits having thin rind attached lightly. Mature fruits of lime are light yellow in colour (Singh, 1995) [10]. Lime is a potential source of vitamin – C and it also extensively used for medicine and culinary purposes (Singh, 1995) [10]. The most popular citrus products prepared in India are squash, juice cordial, marmalade, pickles as well as essential oils from citrus peels is used for flavouring and perfumery trade, also in manufacturing of soap and resins etc. (Randhawa and Srivastava, 1986) [11]. Kaur and Verma (2002) [12] observed a soft rot of citrus caused by *Aspergillus niger* up to 20 per cent in the orchard as well as in Punjab markets. A perusal of the available literature scanned revealed that a very meager research work has been carried out on *in vitro* studies of *Aspergillus niger*.

Materials and Methods
Media
To find out the most suitable medium for the mycelial growth and sporulation of test pathogen, total eight media (solid and liquid) were studied.

Solid media
Composition of media

i) Potato Dextrose Agar (PDA)

Peeled Potatoes	:	200.0 g
Dextrose	:	20.0 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml

ii) Malt Extract Agar (MEA)

Malt extract	:	20.0 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml

iii) Corn Meal Agar (CMA)

Corn Meal	:	20 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

iv) Oat Meal Agar (OMA)

Oat Meal	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

v) Nutrient Agar (AMA)

Meat or Beef extract	:	3.00 g
Peptone	:	5.00 g
Agar agar	:	20.00 g
Distilled water	:	1000 ml

vi) Richard's Agar Medium (RAM)

Potassium nitrate	:	10.0 g
KH2PO4	:	5.0 g
MgSO4. 7H2O	:	2.5 g
Ferric chloride	:	0.02 g
Sucrose	:	50.0 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml

vii) Czapek's Agar Medium (CAM)

Sodium nitrate	:	2.0 g
MgSO4. 7H2O	:	0.5 g
KCl	:	0.5 g
KH2PO4	:	1.0 g
Ferrous Sulphate	:	0.01 g
Agar agar	:	20.0 gm
Distilled water	:	1000 ml

viii) Citrus Leaves Extract Dextrose Agar Medium (CLEDAM)

Green citrus leaf	:	200.0 g
Dextrose	:	20.0 g
Agar agar	:	20.0 g
Distilled water	:	1000 ml

To avoid bacterial contamination, the media were supplemented with 50 ppm streptomycin sulphate. Twenty milliliter sterilized medium was poured into sterilized Petri plates (90 mm diameter) separately. The Petri plates were inoculated with 5 mm diameter mycelial discs of 7 days old mycelial culture with the help of sterilized cork borer and placed at the centre and incubated at $30^{o} \pm 1^{0}$C for 7 days. Four replicates were maintained for each medium. Observations on mycelial growth (mm) and sporulation of the pathogen on solid media were recorded after 7 days of incubation by making average of two observations by measuring colony diameter at right angle to one another. The sporulation was recorded by following the method used by Patel (1990) (Table 1).

Liquid media

Eight liquid media were studied for the growth and sporulation of the test pathogen. The compositions of liquid media were same as of solid media except agar-agar.

Twenty milliliter liquid media was poured into 100 ml conical flasks separately. The flasks were plugged with sterilized cotton and autoclaved at 121^{0}C for 20 min. The flasks were inoculated with five millimeter disc of 7 days old culture of test pathogen with the help of sterilized cork borer and incubated at 30 ± 1^{0}C for 14 days. The mycelial mat was harvested and filtered through weighed Whatman's filter paper No. 42. Filtered mycelial mat along with filter paper was dried in oven at 50^{0}C for 24 hrs, cooled and then weighed. Four replicates were maintained for each medium. Observations on dry mycelial weight (mg) and sporulation were recorded after 14 days of incubation. Dry weight of the mycelium (mg) was calculated by following formula:

$$\text{Dry mycelial weight} = B - A$$

Where

A = Weight of filter paper (mg)

B = Weight of filter paper (mg) + Weight of dried mycelial mat (mg)

Temperatures

To study the optimum temperature requirement for the mycelial growth and sporulation of test pathogen, temperatures ranging from 0 to 40^{0}C were studied on PDA medium. Twenty milliliter sterilized PDA media was poured in sterilized Petri plates. The Petri plates were inoculated as mentioned earlier in solid media and the inoculated plates were separately incubated at 0, 5, 10, 15, 20, 25, 30, 35 and 40^{0}C. Four replications were maintained for each treatment. Observations on mycelial growth (mm) and sporulation of the pathogen were recorded after 7 days of incubation.

pH

Different pH levels i.e. 3, 4, 5, 6, 7 and 8 were evaluated to study the influence of pH on the mycelial growth and sporulation of test pathogen on both solid (PDA) and liquid (PDA broth) media. The required pH levels were adjusted by adding 0.1 N HCl /NaOH with the help of electronic pH meter. The poured Petri plates were inoculated with 7 days old culture and incubated at 30 ± 1^{0}C for 7days. Four replications were maintained for each pH level. Observations on the mycelial growth (mm / mg) and sporulation of the pathogen were recorded after 7 (solid media) and 14 days (liquid media) of incubation.

Results and Discussion

Media

Solid media

To find out the best medium, eight different solid media were selected to study their effect on the mycelial growth and sporulation of *A. niger*. The results obtained are presented in Table 2.

Significantly highest mycelial growth was recorded in Malt extract, Corn meal and Oat meal agar (90.00 mm) over all other media and it was at par with Czapek's agar medium (89.75 mm). Potato dextrose agar and Richard's agar (87.75 & 83.00 mm) stood next in rank to support the mycelial growth of *A. niger*. Significantly lowest mycelial growth was recorded in Nutrient agar medium (30.25 mm).

Excellent sporulation was noted in Malt extract, Corn meal, Oat meal, Potato dextrose and Czapek's agar media. Whereas, good sporulation was observed in Richards agar medium while, citrus leaf extract dextrose agar and Nutrient agar media showed fair and poor sporulation, respectively.

Results similar to the present investigations giving best mycelial growth of *Aspergillus quadrilineatus* on Czapek's agar and Malt extract agar media (80 & 75 mm) was observed by Polacheck *et al.* (1992) [13]. Shashikala and Krishnamurthy

(2005) [14] observed excellent mycelial growth and dense sporulation of *A. flavus* on Czapek's dox agar medium. Further Cabrera *et al*., (2005) [15] noted highest mycelial growth of *A. niger* on Czapek's yeast extract medium after seven days of inoculation.

Liquid media

Eight different liquid media were selected to study their effect on the mycelial growth and sporulation of *A. niger*. The results achieved are presented in Table 3.

The results presented in Table 3 revealed that, significantly highest dry mycelial weight was recorded in Czapek's broth (361.62 mg) over all other media. The next in order of merit were Oat meal (335.60 mg) and Corn meal (334.68 mg), followed by Malt extract (330.46 mg), Richards broth (289.43 mg) and Potato dextrose broth (286.13 mg). Significantly lowest dry mycelial weight was recorded in Nutrient broth medium (121.70 mg).

Excellent sporulation of *A. niger* was observed in Corn meal, Oat meal, Malt extract, Czapek's and Potato dextrose broth media. Richard's, Citrus leaf extract dextrose and Nutrient broth showed good, fair and poor sporulation, respectively.

Results of present investigation corroborate with the results obtained by Patel (1990) recording the best mycelial growth and excellent sporulation of *A. niger* in Czapek's broth medium. Saradava (1984) reported highest dry mycelial weight of *A. niger* in Richard's medium followed by Czapek's medium. Czapek's dox medium supported maximum mycelial growth of *Aspergillus niger* (410 mg) after two weeks of incubation (Narasimha *et al.*, 2006).

Temperatures

Influence of temperatures on the mycelial growth and sporulation of *A. niger* were studied on PDA. The inoculated Petri plates with *A. niger* were incubated at 0, 5, 10, 15, 20, 25, 30, 35 and 40^{0}C. Observations on mycelial growth (mm) and sporulation were recorded after seven days (Table 4).

It is clear from the results that pathogen grew well between 25 to 35^{0}C. Significantly highest mycelial growth (90 mm) with excellent sporulation was observed at 30^{0}C, which was followed by 35 (83.97 mm) and 25^{0}C (65.05 mm). The lower temperatures (0, 5 & 10^{0}C) failed to produce mycelial growth of *A. niger*. While very meager mycelial growth was obtained at 15^{0}C (15.97 mm). It is concluded that higher temperatures (30 & 35^{0}C) found more conducive for the mycelial growth and sporulation than lower one on PDA. Further it is noted that mycelial growth was found declined after 30^{0}C.

The present findings are quite confirmative with the results obtained by Nath (2006) [18] reporting maximum mycelial growth of *A. niger* at 30^{0}C. Further, he noted that pathogen could grew between 15 to 35^{0}C. Nagerabi and Ahmed (2003) [19] recorded maximum growth and sporulation of *A. niger* at 30 – 35^{0}C. Akhtar *et al*., (1999) [20] found that *A. niger* could grew well on PDA at temperatures ranging from 20 – 35^{0}C and it was optimum at 25 – 35^{0}C.

pH

Six pH levels were studied to determine the effect of different hydrogen ion concentrations on mycelial growth and sporulation of *A. niger* in both solid and liquid potato dextrose medium.

Effect of pH on the mycelial growth and sporulation of *A. niger* on PDA

It is clear from the results presented in Table 5 that the significantly highest mycelial growth with excellent sporulation was obtained in pH 6 (86.05 mm) followed by pH 5 (82.70 mm) and 7 (77.92 mm). The acidic pH was found more favourable for the mycelial growth of the pathogen as compared to alkaline one. However, the pathogen had produced lowest mycelial growth at pH 3 (64.52 mm). Good and fair sporulation were observed at pH 8, 5 and 3, 4 respectively.

Results similar to the present investigation had been reported by Brancato and Golding (1953) indicating *A. niger* could grew well in wide range of pH from 4.4 to 7.5. While, Iwamoto *et al*., (1958) reported better growth of *A. niger* at pH ranging from 2 to 8. The pH range between 4 to 6 was observed most conducive for mycelial growth of *A. niger* (Chohan, 1969) [23].

Effect of pH on the mycelial weight and sporulation of *A. niger* in potato dextrose broth

The data presented in Table 6 revealed that significantly highest mycelial dry weight was recorded in pH 6 (262.05 mg) followed by pH 7 (257.42 mg). The next best treatment in order of merit were pH 5 (250.17 mg) and 4 (230.25 mg). The acidic pH (3) produced lowest mycelial weight (206.27 mg).

The excellent sporulation was recorded at pH 6 and 7. Whereas, good sporulation was observed at pH 5 and 8. While, fair sporulation was recorded in pH 3 and 4. From the results obtained, it is concluded that acidic pH is more favourable for mycelial growth than the alkaline one.

The results of present findings are confirmative with the results obtained by Patel (1990) reporting best mycelial growth and excellent sporulation of *A. niger* at pH 6. Pass and Griffin (1974) [24] observed conidial germination of *Aspergillus flavus* in glucose plus peptone medium at pH 3.0 – 7.5. Further, Paster and Chet (1979) noted best mycelial growth of *Aspergillus ochraceus* at pH ranging from 7.0 – 9.0.

Table 1: Statement showing criteria used to record the sporulation of *Aspergillus niger*.

S. No	Particulars	Sign indication	Average no. of spores / microscopic field
1.	No sporulation	-----	-----
2.	Poor sporulation	+	> 10
3.	Fair sporulation	++	11 to 25
4.	Good sporulation	+++	26 to 50
5.	Excellent sporulation	++++	< 50

Table 2: Influence of solid media on the mycelial growth and sporulation of *Aspergillus niger*

S. No.	Media	Mycelial growth (mm)	Sporulation
1	Potato Dextrose Agar	87.75	++++
2	Malt Extract Agar	90.00	++++
3	Corn Meal Agar	90.00	++++
4	Oat Meal Agar	90.00	++++
5	Nutrient Agar	30.25	+
6	Richard's Agar	83.00	+++
7	Czapek's Agar	89.75	++++
8	Citrus Leaf Extract Dextrose Agar	40.75	++
S.Em. ±		0.571	
C.D. at 5 %		1.665	
C.V. %		1.56	

Table 3: Influence of liquid media on the mycelial growth and sporulation of *Aspergillus niger*

S. No	Media	Dry mycelial weight (mg)	Sporulation
1	Potato Dextrose Broth	286.13	++++
2	Malt Extract Broth	330.46	++++
3	Corn Meal Broth	334.68	++++
4	Oat Meal Broth	335.60	++++
5	Nutrient Broth	121.70	+
6	Richard's Broth	289.43	+++
7	Czapek's Broth	361.62	++++
8	Citrus Leaf Extract Dextrose Broth	186.01	++
	S.Em. ±	2.918	
	C.D. at 5 %	8.516	
	C.V. %	2.08	

Table 4: Effect of temperatures on the mycelial growth and sporulation of *Aspergillus niger*

S. No	Temperature	Mycelial growth (mm)	Sporulation
1	0^0C	0.00	---
2	5^0C	0.00	---
3	10^0C	0.00	---
4	15^0C	15.97	+
5	20^0C	43.82	++
6	25^0C	65.05	++++
7	30^0C	90.00	++++
8	35^0C	83.97	++++
9	40^0C	57.97	++++
S.Em. ±		0.155	
C.D. at 5 %		0.450	
C.V. %		0.78	

Table 5: Effect of pH on the mycelial growth and sporulation of *Aspergillus niger* on PDA

S. No.	pH Level	Mycelial growth (mm)	Sporulation
1	3	64.52	++
2	4	76.62	++
3	5	82.70	+++
4	6	86.05	++++
5	7	77.92	++++
6	8	74.45	+++
S. Em. ±		0.431	
C.D. at 5 %		1.282	
C.V. %		1.12	

Table 6: Effect of pH on the dry mycelial weight and sporulation of *Aspergillus niger* in potato dextrose broth

S. No.	pH	Dry mycelial wt (mg)	Sporulation
1	3	206.27	++
2	4	230.25	++
3	5	250.17	+++
4	6	262.05	++++
5	7	257.42	++++
6	8	213.55	+++
S. Em. ±		1.053	
C.D. at 5 %		3.129	
C.V. %		0.89	

References

1. Nito N. Second international crop science congress, Nov 17-25, New Delhi, 1996, 40.
2. Singh SP. *Sour lime*, In: Commercial Fruits, Kalyani Publishers, New Delhi, 1995, 107-113.
3. Randhawa GS, KC Srivastava. In: Citriculture in India, Hindustan Publishing Corporation Press, New Delhi, 1986, 432-434.
4. Kaur P, KS Verma. Prevalence of post harvest rots of kinnow in Punjab, Pl. Dis. Res. 2002; 17(2):329-331.
5. Polacheck I, Nagler A, Okon E, Drakos P, Plaskowitz P, Kwon Chung KJ. *Aspergillus quadrilineatus*, a new causative agent of fungal sinusitis, J of Clinical Microbiol. 1992; 30(12):3290-3293.
6. Shashikala J, Krishnamurthy YL. Growth pattern and colony characteristics of *Aspergillus flavus* isolates from soybean seeds, J Mycol. Pl. Pathol. 2005; 35(2):228-232.
7. Cabrera HP, Taniwaki MH, Hashimoto JM, Menezes HCD. Growth of *Aspergillus ochraceus, A. carbonarius* and *A. niger* on culture media at different water activities and temperatures, Brazilian J of Microbiology. 2005; 36:24-28.
8. Saradava MR. Investigations on seed-rot and collar rot of groundnut caused by *Aspergillus niger*. M. Sc. (Agri.) Thesis submitted to Gujarat Agricultural University, Sardar Krushinagar, Dantiwada campus, 1984.
9. Narsimha G, Sridevi A. Viswanath B, Subhosh Chandra M, Reddy RB. Nutrient effect on production of cellulolytic enzymes by *Aspergillus niger*, African J of Biotech. 2006; 5(5):472-476.
10. Nath K. Post harvest diseases of aonla fruits and their management under north Gujarat conditions. M. Sc. Thesis submitted to Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar (Gujarat), 2006.
11. Nagerabi SA, El F, Ahmed AHM. Storability of onion bulbs contaminated by Aspergillus niger mould, Phytoparasitica. 2003; 31(5):515-523.
12. Akhtar KP, Shakir AS, Sahi SJ. Physiological studies of the fungi causing post harvest losses of tomato fruits, Pak. J of Phytopathol. 1999; 11(1):25-29.
13. Brancato FP, Golding NS. The diameter of the mold colony as reliable measure of growth, Mycologia. 1953; 45(6):848-864.
14. Iwamoto H, Kunhara K, Shingu M. Physiology of Aspergillus niger, Chem. Abstr. 1958; 52(11):9594-9595.
15. Chohan JS. A sclerotial strain of *Aspergillus niger* Van Tieghem., the causal agent of collar rot disease of groundnut, J Res. Ludhiana. 1969; 6(2):349-352.
16. Pass T, Griffin GJ. Interaction of pH and temperature with exogenous carbon and nitrogen nutrition in conidial germination by *Aspergillus flavus*, Phytopathology. 1974; 64(8):1151-1152.
17. Paster N, Chet I. Effect of environmental factor on growth and Sclerotium formation in *Aspergillus ochraceus*, Canadian J Bot. 1979; 58(16):1844-1850.