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INFLUENCE OF CARBON, NITROGEN, TEMPERATURE AND PH ON THE GROWTH AND SPORULATION OF SOME INDIAN ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING ANTHRACNOSE DISEASE OF PAPAYA (*CARRICA PAPAYA* L)

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ABSTRACT

The paper gives an account of the variations in nutritional and physiological characteristics found in different isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya, in India. The pathogen under study varied in its ability to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and sporulation of most of the isolates. Among the nitrogen sources tested, aspartic acid supported the maximum growth of isolates followed by potassium nitrate and proline. In contrast to this, isolates sporulated better in media containing potassium nitrate, ammonium nitrate or sodium nitrate as the sole nitrogen source. The response of different isolates to different temperature levels were found to be vary. However, most of the isolates preferred temperature range of 28 ^{0}C to 30 ^{0}C for the growth and sporulation when grown on Richard's agar medium. *C. gloeosporioides* isolates grew well at pH 5 while sporulation was better at pH 6.

Key-words: Carbon, *Colletotrichum*, nitrogen, papaya, pH, temperature

INTRODUCTION

Tropical and sub-tropical fruit production is significantly affected by anthracnose. Of these, greater economic importance is the huge losses due to post harvest anthracnose infection of tropical and sub-tropical fruits such as banana (*Musa* spp.), papaya (*Carica papaya*), mango (*Mangifera indica*), cashew (*Anacardium occidentale*) and avocado (*Persea americana*) (Mordue *et al.* 1971). Papaya anthracnose, caused by *Colletotrichum gloeosporioides* Penz. (Penz. and Sacc.) in Penz. (Anamorph: *Glomerella cingulata* Stonem), is one of the most important diseases of papaya (*Carica papaya* L) prevalent in most tropical and sub tropical countries. *Colletotrichum gloeosporioides* has a large host range particularly in tropical areas causing diseases most frequently in warm moist environments encountered in the humid and sub-humid tropical zones.

Currently around 40 species are accepted based on more detailed studies on morphology, cultural characters, and pathogenic abilities (Cannon *et al.* 2000). The variations among *Colletotrichum* species have been described (Sutton 1992; Sutton 1980). Morpho-taxonomic criteria such as morphological characters, *i.e.*, conidial shape and size, appressoria morphology and size, setae morphology and temperature response on potato dextrose agar (PDA) medium and host specificity are being currently used for the identification of *Colletotrichum* spp. (Gunnel and Gubler 1992; Jeffries *et al.* 1990; Smith and Black 1990; Sutton 1992). Morphology will always form the basis of separation between species or groups of species. However, morphological features vary considerably with environmental conditions, and comparing cultures with material observed directly from infected plant tissue is especially difficult.

Currently available classification system for *Colletotrichum* does not work effectively. There is great potential for augmentation of morphological, physiological and nutritional characteristics with molecular data for a better classification system. Such data will allow the development of proper, objective and even automated identification techniques (Canon *et al.* 2000). Therefore, the objective of this experiment was to study nutritional and physiological requirements of different isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya with the aim of understanding differences among the isolates for the proper identification of the pathogen.

MATERIALS AND METHODS

Nutritional study

The utilization of carbon and nitrogen nutrition was studied by replacing the sucrose and potassium nitrate in the basal medium with various nitrogen and carbon compounds on the molecular weight basis. Richards's broth was used as a basal medium for studying carbon and nitrogen. Thirty milliliters of the medium dispensed in 150 ml conical flasks were sterilized and used for inoculation with the fungus. All the flasks were inoculated with 5 mm diameter mycelial disk obtained from 7 day old single spore cultures of *C. gloeosporioides* isolates

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and incubated at 28±1^{0}C for ten days. The cultures were filtered through Whatman No. 42 filter paper and the dry mycelial weight was measured by subtracting the initial weight of the filter paper from the weight of the filter paper along with the mycelial mat.

Effect of carbon source: Carbon compounds tested in the study were glucose, sucrose, fructose, maltose, manitol and lactose. Richard's medium without adding sucrose was used as a control. Potassium nitrate was used as a source of nitrogen for all treatments. Carbon sources were added to the basal medium (Richard's medium) at 21.053g of carbon per litre of medium. Each flask containing different carbon sources was inoculated with a 5 mm mycelial disk of seven day old fungal cultures and incubated for ten days.

Effect of nitrogen source: Six different nitrogen sources used in this study were Ammonium nitrate (NH_4NO_3), Potassium nitrate (KNO_3), Sodium nitrate ($NaNO_3$), L-Asparagine, Aspartic acid, and L-Proline. Different nitrogen sources were added into Richard's medium at 1.3855g of nitrogen per liter of the medium. Sucrose was used as the source of carbon in all the treatments. Richard's broth without adding Potassium nitrate (nitrogen source) was used as the control. All flasks were inoculated with 5 mm mycelial disks of seven day old fungal culture under aseptic condition and incubated for ten days.

Physiological study

Effect of temperature: The pathogen was subjected to different temperature conditions to study the best-suited temperature level for the growth and sporulation of the fungus. Richard's agar medium was used in one experiment to study the growth and sporulation in solid medium. Twenty-five milliliters of Richard's agar medium was poured into each petriplate under aseptic condition and inoculated with 5 mm diameter identical culture discs of different monoconidial isolates grown for seven days. The experiment was replicated thrice. In the other study, thirty milliliters of Richards's broth was poured into each 150 ml conical flask and inoculated with 5 mm mycelial disc of each isolate.

Inoculated petriplates and conical flasks containing Richard's medium were incubated at 15, 20, 25, 28 and 30 ^{0}C. The colony diameter and the level of sporulation were recorded in solid medium seven days after inoculation. Dry mycelial weight and the extent of sporulation, were recorded in the liquid cultures ten days after the incubation.

Effect of pH: Effect of pH on the growth of those isolates was also tested in the laboratory using liquid cultures containing different pH levels. Richard's broth medium was used to study the effect of pH of medium on the growth and sporulation of different isolates of *C. gloeosporioides*. Thirty milliliter of liquid medium was poured into a 150ml conical flask under aseptic conditions. The Reaction of the medium was adjusted to the desired pH by adding 0.1N NaOH or 0.1N HCl (Naik *et al* 1988). The medium was buffered with Disodium hydrogen phosphate citric acid buffer according to the schedule of Vogel (Vogel 1951). Flasks were sterilized at 121 ^{0}C at 15 *psi* for 20 minutes. Each flask was inoculated with each isolate using 5 mm diameter mycelial disc in sterile conditions. Inoculated flasks were incubated at 28±1 ^{0}C for ten days and the dry mycelial weight and extent of sporulation were obtained**.**

Evaluation: The extent of sporulation in all experiments was observed visually and recorded as – for no sporulation, + for poor sporulation, ++ for moderate sporulation; +++ for heavy sporulation. All experiments were arranged as 2 factor factorial with 3 replicates in a completely randomized design (CRD). Experimental data was statistically analyzed using SAS software (SAS Institute, Cary, NC) and means were separated by Duncan's multiple range test (P=0.05).

RESULTS AND DISCUSSION

Effect of carbon source

The growth of the pathogen was different among isolates (P=0.05) and among different carbon sources (P=0.05). Glucose and sucrose were the least utilized carbon compounds by these isolates (Table 1). The pathogen grown on fructose recorded significantly higher dry mycelial weight followed by media containing maltose, manitol and lactose. IIHR-0 had significantly higher mycelial growth followed by AP-FB, CB and NM-F isolates while HD-3 had the least mycelial growth and statistically on par with the other isolates (Table 1). The interaction between isolates x carbon sources was also significant. All isolates sporulated when fructose was used as a sole carbon source where heavy sporulation was shown by HD-3, AP-FP and NM-P isolates (Table 1). Manitol and lactose supported poor sporulation for all the isolates. There was no sporulation observed when those isolates were grown on a medium without any carbon source (control).

Fungi meet their carbon requirement mainly from various organic sources, and the nature of the organism largely determines the range of substrates (Bilgrami & Verma 1978; Steinberg 1950). Hegde *et al.* (1990) found dextrose and sucrose as good carbon sources for the growth of *C. gloeosporioides* isolated from arecanut. Naik *et al.* (1988) reported

sucrose as the better carbon source followed by glucose and dextrose for the growth of betel vine anthracnose pathogen C. *gloeosporioides*. Sangeetha (2003) observed manitol followed by fructose and sucrose among the best carbon sources for the growth of C. *gloeosporioides* of mango. Apart from these, several other reports showed that sucrose was a better carbon source for various species of *Colletotrichum* (Durairaj 1956; Ramakrishnan 1941; Reddy 2000; Verma 1979). However, glucose has also been reported as the best carbon source for the growth of *C. gloeosporioides* in other studies (Chandra and Tandon 1962; Jeffries *et al.* 1990; Prasad 1965). There were vast differences in the utilization of carbon sources for the growth and sporulation of these isolates tested. However, in general, fructose was found to be the best carbon source in the current study both for the growth and sporulation of the fungus.

Carbon source also affects the sporulation of *C. gloeosporioides*. Sangeetha (2003) in her studies with *C. gloeosporioides* isolated from mango, observed heavy sporulation of the fungus when maltose was used as a sole carbon source. According to Chaturvedi (1965), glucose, fructose, maltose and starch supported good sporulation of *C. gloeosporioides*, the incitant of leaf spot of *Polyscias balijuriana.* Similarly, Saxena (2002) reported that sucrose was a good sporulating compound for *C. gloeosporioides* isolated from pomegranate. Mannitol has been utilized efficiently for both growth and sporulation of *C. gloeosporioides* isolated from grapes (Manjunatha Rao and Rawal 2002). Lactose was also reported to be a good carbon source for sporulation of *C. gloeosporioides* (Reddy 2000).

Effect of Nitrogen source

Mycelial growth of the fungus was influenced by all the nitrogen sources and was statistically on par with the control, which had no nitrogen source (Table 2). Aspartic acid supported the maximum growth followed by potassium nitrate and proline. Asparagine was the least efficiently utilized nitrogen source by *C. gloeosporioides* isolates. Utilization of nitrogen sources differ significantly among different isolates as presented in the Table 2. Growth of IIHR-1 on different nitrogen sources was significantly higher than the rest of the isolates. HD-3 isolate did not grow well in any of the nitrogen sources tested. The interaction between isolates and nitrogen source was significant (P=0.05).

All the nitrogen sources supported sporulation of all isolates, except sodium nitrate and aspartic acid on the sporulation of IIHR-1 (Table 2). Medium without any nitrogen source added (control) didn't favour the sporulation of any of the isolate except NM-P.

Nitrogen is an important element for protein synthesis but all the sources of nitrogen are not equally good for the growth of the fungi. Tandon and Chandra (1962) reported that several nitrogen compounds except nitrites, had supported varying degrees of growth of *C. gloeosporioides* and the fungus could not grow on nitrites of potassium or sodium. Peptone and tyrosine were found to be a better nitrogen source for *C. gloeosporioides* isolated from arecanut (Hegde *et al.* 1990) and a poor growth was observed in the medium containing methionine. *C. gloeosporioides* of *Amomum villosum* could utilize many nitrates and amino acids as a N source (Lai *et al.* 1993) except ammonia, which inhibited the growth of the fungus. In another study, ammonium phosphate supported maximum growth of *C. gloeosporioides* followed by organic nitrogen sources such as urea and asparagine (Durairaj 1956). Both, potassium nitrate and D L - methinine supported maximum growth of *C. gloeosporioides* of betelvine (Naik, *et al.* 1988). Similarly, asparagine, peptone, and potassium nitrate supported good growth of *C. falcatum* while, ammonium sulphate and urea provided poor growth of *C. indicum* (Ramakrishnan 1941).

Certain sources of nitrogen favour the sporulation of some fungi, which are not necessarily the same as those which are favourable for the growth

Table 1. Growth and sporulation of different isolates of *C. gloeosporioides* on different carbon sources

Isolate	Mean mycelial weight (mg) and the extent of sporulation (is given in parenthesis)							
	Fructose	Glucose	Lactose	Maltose	Manitol	Sucrose	Control	Mean[b]
IIHR-0	1.097(+)	0.966(++)	1.053 (-)	1.047(-)	1.054(-)	0.889(++)	0.204(-)	0.901[a]
IIHR-1	1.153 (+)	0.794 (-)	0.931 (++)	1.049 (-)	1.102 (+)	0.891 (-)	0.132 (-)	0.865[ab]
CB	1.054 (+)	0.833 (-)	0.793 (-)	0.940 (-)	1.075 (-)	0.798 (-)	0.115 (-)	0.801[cd]
HD-3	0.868 (+++)	0.940 (++)	0.148 (+1)	0.868 (++)	0.836 (-)	0.719 (+)	0.145 (-)	0.646[f]
AP-FP	0.879 (+++)	0.718 (+++)	0.878 (+)	0.812 (+++)	0.710 (-)	0.844 (+)	0.239 (-)	0.726[e]
AP-FB	1.145 (++)	0.930 (-)	1.000 (+)	0.834 (-)	0.892 (+)	0.968 (+)	0.127 (-)	0.842[bc]
NM-F	1.391 (++)	0.748 (++)	0.864 (-)	0.935 (++)	0.825 (+)	0.737 (++)	0.149 (-)	0.807[cd]
NM-P	1.002 (+++)	0.575 (-)	0.864 (-)	0.981(+)	1.048 (-)	0.826 (-)	0.147 (-)	0.777[de]
Mean[b]	1.073[a]	0.813[c]	0.816[c]	0.933[b]	0.943[b]	0.834[c]	0.157[d]	

[a] "-" = No sporulation; + = Poor sporulation; ++ = Moderate sporulation; +++ = Heavy sporulation.
[b] Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

Table 2 : Growth and sporulation of different isolates of *C. gloeosporioides* in different nitrogen sources

Isolate	Mean mycelial weight (mg) and the extent of sporulation (given within parenthesis)							
	KNO_3	$NaNO_3$	NH_4NO_3	Asparagine	Aspartic acid	*Proline*	Control	Mean[b]
IIHR-0	0.568 (+++)	0.599 (+)	0.55 (+++)	0.399 (+++)	0.8 (+)	0.483 (+)	0.348 (-)	0.535[g]
IIHR-1	0.913 (+)	0.851 (-)	0.698 (+++)	0.852 (+)	0.961 (-)	0.623 (+)	0.376 (-)	0.753[a]
CB	0.831 (+++)	0.618 (++)	0.987 (+)	0.673 (+)	0.811 (++)	0.887 (+)	0.406 (-)	0.744[b]
HD-3	0.599 (++)	0.459 (+++)	0.397 (+++)	0.539 (+++)	0.532 (+)	0.585 (+++)	0.197 (-)	0.472[h]
AP-FP	0.71 (+++)	0.625 (++)	0.479 (+)	0.7 (++)	0.839 (++)	0.888 (++)	0.415 (-)	0.665[d]
AP-FB	0.717 (+++)	0.751 (++)	0.864 (++)	0.521 (+)	0.861 (++)	0.785 (+)	0.326 (-)	0.689[c]
NM-F	0.603 (+)	0.555 (+++)	0.853 (++)	0.619 (+++)	0.54 (+)	0.591 (++)	0.308 (-)	0.581[f]
NM-P	0.772 (+++)	0.762 (++)	0.562 (+)	0.687 (++)	0.503 (+)	0.812 (+)	0.304 (-)	0.623[e]
Mean[b]	0.714[b]	0.652[e]	0.669[d]	0.624[f]	0.731[a]	0.706[c]	0.335[g]	

[a] "-" = No sporulation; + = Poor sporulation; ++ = Moderate sporulation; +++ = Heavy sporulation
[b] Values within a column or row followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test

(Lilly and Barnett 1951). Mishra and Mahmood (1960) found abundant sporulation of *C. capsici* on the medium containing peptone as a nitrogen source. According to Ekbote (1994), *C. gloeosporioides* of mango utilized potassium nitrate more efficiently and ammonium nitrate less efficiently for the growth and sporulation. Manjunatha Rao and Rawal (2002) reported ammonium nitrate as a better source for the growth and sodium nitrate favoured better sporulation among six different nitrogen sources tested on *C. gloeosporioides* of grapevine. Current study also suggests this variation in utilizing nitrogen sources for the growth and sporulation of this Genus.

Effect of temperature

Temperature differences among all isolates were significant (P=0.05). Mean colony diameter of isolates on solid medium, was maximum at 28 ^{0}C and 30 ^{0}C followed by 25 ^{0}C and were significantly higher than other two temperature levels (Table 3). Growth of all the isolates was inhibited at 15 ^{0}C.

Different isolates of *C. gloeosporioides* responded differently to various temperature regimes as shown in the table 3. Growth of IIHR-0, IIHR-1, CB, AP-FP, NM-F and NM-P isolates was maximum at 28 ^{0}C. HD-3 isolate had better growth on 30 ^{0}C, while AP-FB had better growth at 25 ^{0}C. There were significant interactions between isolates and temperature levels.

The growth of *C. gloeosporioides* in liquid media among different temperature levels was significant at P=0.05 (Table 4). The maximum growth of the fungus was observed at 28^{0}C and differed significantly with other temperature levels. The least mycelial growth of the fungus was observed at 15 ^{0}C on liquid medium.

The temperature range from 28 ^{0}C to 30 ^{0}C was found to be favourable for the spore production of different isolates of *C. gloeosporioides* when grown on solid medium (Table 3). There was no spore production observed when these isolates were grown at 15 ^{0}C. Extent of sporulation was high in most isolates at 28 ^{0}C in liquid culture (Table 4). Temperature of 15 ^{0}C and 20 ^{0}C found to be not suitable for the sporulation of those isolates. Among the external factors which influence the growth of fungi, temperature plays an extremely important role. Temperature affects almost every function of the fungi (Lilly and Barnett 1951). Mathur *et al.* (1950) reported that 15-20 °C favoured the conidia formation by *C. lindemuthianum* in culture. They further reported that the sporulation was less at 25 °C and ceased at 30 °C. In another study, *in vitro* tests on growth of *C. gloeosporioides* showed that maximum growth reached after 10 day incubation on potato dextrose broth, with optimum temperature in the range of 25-35 °C (Hegde *et al.* 1990).

Estrada *et al.* (1993) reported that two mango isolates of *C. gloeosporioides* differed in their appressoria production at different temperature levels. They observed 20 °C was optimum for the production of appressoria for one isolate while the other isolate required optimum temperature of 25 °C. Sangeetha (2002) recorded maximum growth of different mango isolates of *C. gloeosporioides* at a temperature range of 25-30 °C while good sporulation was seen at an optimum range of 25-28 °C.

Table 3 : Effect of temperature on the growth and sporulation of different isolates of *C. gloeosporioides* grown in solid media 7 DAI

Isolate	Mean mycelial growth (mm) and the extent of sporulation (given in parenthesis)					
	15^{0}C	20^{0}C	25^{0}C	28^{0}C	30^{0}C	Mean[b]
IIHR-0	39.66 (-)	70.33 (+)	73.66 (++)	89.00 (++)	80.33 (++)	70.6[ab]
IIHR-1	41.66 (-)	72.66 (-)	80.33 (+)	88.33 (++)	83.33 (+)	73.23[a]
CB	44.33 (-)	71.66 (-)	76.66 (-)	84.66 (+++)	75.66 (++)	70.60[ab]
HD-3	39.00 (-)	47.33 (-)	78.33 (+)	86.33 (+++)	87.00 (++)	67.60[ab]
AP-FP	37.00 (-)	45.00 (+)	78.33 (-)	86.66 (++)	85.00 (++)	66.40[b]
AP-FB	35.66 (-)	44.00 (+)	80.00 (-)	59.66 (++)	76.00 (++)	59.07[c]
NM-F	36.00 (-)	66.33 (+)	69.66 (++)	85.00 (+++)	75.00 (++)	66.40[b]
NM-P	20.33 (-)	35.33 (-)	68.66 (++)	85.00 (++)	77.00 (+)	57.27[c]
Mean[b]	36.71[d]	56.58[c]	75.71[b]	83.01[a]	79.92[ab]	

[a] "-" = No sporulation; + = Poor sporulation; ++ = Moderate sporulation; +++ = Heavy sporulation.
[b] Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

Table 4: Effect of temperature on the growth and sporulation of different isolates of *C. gloeosporioides* grown in liquid media 10 DAI

Isolate	Mean mycelial weight (mg) and the extent of sporulation (given in parenthesis)					
	15^0C	20^0C	25^0C	28^0C	30^0C	Mean[b]
IIHR-0	0.336 (-)	0.657 (+)	0.622 (+)	1.127 (+++)	1.030 (++)	0.750[bc]
IIHR-1	0.393 (-)	0.584 (+)	0.600 (+)	1.189 (++)	0.798 (++)	0.710[c]
CB	0.351 (-)	0.444 (-)	0.650 (+)	1.258 (++)	1.284 (++)	0.797[ab]
HD-3	0.372 (-)	0.514 (+)	0.603 (+)	1.240 (+++)	0.866 (++)	0.719[c]
AP-FP	0.391 (-)	0.466 (-)	0.507 (++)	1.288 (+++)	0.849 (+)	0.700[cd]
AP-FB	0.433 (-)	0.578 (-)	0.755 (+)	1.028 (++)	0.514 (+)	0.662[d]
NM-F	0.363 (-)	0.712 (+)	0.736 (++)	1.016 (+++)	0.769 (+)	0.719[c]
NM-P	0.570 (-)	0.718 (-)	0.952 (+)	1.020 (++)	0.792 (+)	0.810[a]
Mean[b]	0.400[e]	0.584[d]	0.678[c]	1.146[a]	0.863[b]	

[a] "-" = No sporulation; + = Poor sporulation; ++ = Moderate sporulation; +++ = Heavy sporulation.
[b] Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

Rajak (1983) reported optimum temperature for the growth of *C. gloeosporioides* as 25 °C however, optimum temperature of 29 °C has been reported for the maximum growth of *C. gloeosporioides* by Ekbote (1994). *In vitro* studies showed that growth of *C. gloeosporioides*, the causal organism of mango anthracnose was maximum at 28 °C (Banik *et al.* 1998).

Jayasinghe and Fernando (1998) reported that slower growth at temperatures ranging from 15 to 32.5 ^{0}C along with reaction to different fungicides were shown to be one of the reliable characteristics in distinguishing *C. acutatum* from *C. gloeosporioides* isolated from rubber.

Our findings are also in agreement as observed by other scientists that the species and isolates within the genus *Colletotrichum* respond differently in their growth and sporulation when exposed to different temperature conditions.

Effect of pH

The mycelial growth was different among isolates and different pH levels (P=0.05). The interaction between isolates and pH levels was also significant (P=0.05). *C. gloeosporioides* grew significantly better on medium with pH of 5 followed by pH 6 and pH 7 (Table 5). Growth at pH 4 and 8 were similar, but significantly less than the other pH levels.

Table 5: Effect of pH on the growth and sporulation of different isolates of *C. gloeosporioides*

Isolate	Mean mycelial weight (mg) and the extent of sporulation a given in parenthesis)					
	pH 4	pH 5	pH 6	pH 7	pH 8	Mean[b]
IIHR-0	0.554 (-)	1.001 (+)	1.065 (+)	0.792 (+)	0.305 (-)	0.740[e]
IIHR-1	0.564 (-)	1.092 (+)	0.914 (++)	0.991(++)	0.703 (+)	0.853[b]
CB	0.532 (-)	0.629 (+)	0.903(+)	0.285 (-)	0.799 (-)	0.630[f]
HD-3	0.638 (-)	0.886 (+)	1.412 (++)	1.581 (-)	0.729 (-)	1.049[a]
AP-FP	0.261 (+)	0.371 (++)	1.219 (+++)	1.001 (++)	0.278 (-)	0.626[g]
AP-FB	0.517 (-)	0.917 (++)	0.971 (+++)	0.986 (++)	0.275 (-)	0.733[f]
NM-F	0.573 (-)	0.908 (-)	1.074 (+)	1.012 (++)	0.523 (-)	0.818[c]
NM-P	0.514 (-)	1.025 (-)	1.015 (++)	0.867 (+)	0.542 (-)	0.792[d]
Mean[b]	0.520[d]	1.071[a]	0.939[b]	0.853[c]	0.519[d]	

[a] "-" = No sporulation; + = Poor sporulation; ++ = Moderate sporulation; +++ = Heavy sporulation.
[b] Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

At all pH levels tested, HD-3 grew significantly better than the other isolates. IIHR-0, CB, AP-FP and NM-F recorded maximum growth at pH 6 while pH 5 supported the maximum growth of IIHR-1 and NM-P isolates. HD-3, AP-FB and NM-F isolates grew better at pH 7 (Table 5).

Sporulation of tested isolates differed with the level of pH of the medium. All the isolates preferred pH 6 for better sporulation except NM-F isolate which had better sporulation at pH 7 (Table 5). No isolate preferred pH 8 and pH 4 for the sporulation except IIHR-1 at pH 8 and AP-FP at pH 4 with poor sporulation.

Hydrogen ion concentration is one of the most important factors influencing the growth of the fungi. The pH of the medium determines the rate and amount of growth and many other life processes (Lilly and Barnett 1951). A medium may have a pH which is favourable for the growth and unfavourable for sporulation or other processes. Lilly and Barnett (1951) reported that a medium having pH values between 5 and 6 at the time of inoculation was suitable for most fungi which are also in accordance with our study for most of the isolates. According to them, fungi generally tolerate more acid than alkali. Similar observations were also reported by some other authors with different species of *Colletotrichum* (Naik *et al.* 1988; Ramakrishnan 1941).

CONCLUSION

Isolates of *Colletotrichum gloeosporioides* under study varied in their ability to grow in different temperature and pH levels and to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and sporulation of this pathogen. Among the nitrogen sources tested, aspartic acid supported the maximum growth of the fungus followed by potassium nitrate and proline. In contrast to this, fungus sporulated better in media containing potassium nitrate, ammonium nitrate or sodium nitrate as the nitrogen source. Most of the isolates preferred temperature range of 28 to 30 ^{0}C for the growth and sporulation. *C. gloeosporioides* isolates grew well at pH of 5 while pH 6 was found preferred for the sporulation.

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