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Growth and Sporulation Physiology of Postharvest Pathogen *Thielaviopsis paradoxa* (De Seynes.) Höhn

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A B S T R A C T

Thielaviopsis paradoxa is an ascomyceteous fast growing fungus has broad host range under tropical condition. The growth dynamics and sporulation of this pathogen have been investigated in relation to various media, physical factors like temperature and pH with significant variation among the treatments. All 12 media supported growth although with a change in their dynamics. Maximum radial growth were recorded on PDA, PSA, MEA and OMA media and the optimum temperature for growth was 30^{o}C although the cardinal temperatures were 10^{o}C and 40^{o}C. No growth was found on PDA at temperature 40^{o}C and very scanty growth at 10^{o}C, after 96hrs of incubation. Optimum pH for growth was 5.0 and 6.0. Least growth recorded both on CDA medium and at pH of 9.0. Highest sporulation recorded on OMA medium and temperature at 30^{o}C and pH 6.0 on PDA whereas lowest sporulation found at pH 8.0 on PDA and on CDA medium.

Introduction

Thielaviopsis paradoxa is an important postharvest fungal pathogen, which was encountered to cause enormous economical loss to different fruits both in transit and storage. Generally, it is a soil inhabiting fungus, yet it has the capacity to affect various parts of the plant as a wound parasite (Py *et al.,* 1987) .In pineapple, three distinct phases leaf spot, bud rot and fruit rot have been recognized and Cho et al, (1977) found that it severely affects both buds and fruits as bud rot and fruit rot diseases, respectively as compared to leaf spot infections. The fruit rot phase of pineapple was variously named and recognized as black rot, soft rot, stem end rot or water blister and this became serious at the postharvest phase with worldwide distribution (Ploetz, 2003) limiting long distance transport or long term storage. It is a polyphagous fungus (Frossard, 1968) and the major hosts include pineapple, corn, sorghum, sugarcane, cocoa, date palm, banana and sweet potato. In the postharvest state it was also obtained from guava, pear, chilli, potato bean tuber (Mandal, 1981) and musk melon (Rao and subramaniam, 1975). *Thielaviopsis paradoxa*

causing blight in mango in Brazil (Piza,1966; Ribiero,1980**).** Research conducted on artificial inoculation by different isolates obtained by many other authors suggest isolates are cross inoculable and produce various kinds of effects like seedling decay following planting causing poor stand in sugar cane (Bautista,2014). Black scorch of date palm is long known (Klotaz, 1932). Because of its faster growth rate and as a wound pathogen it has considerable potential for damage. Although the growth and extent of rotting are greater in mature banana fruit, it can invade stem tissue and green fruit as well. When rot occur in harvested ripe or near ripe fruit, the invaded flesh become soft and water soaked (Kader, 2002). In India including West Bengal, *T. paradoxa* affecting mango has not been recorded earlier under natural condition although cross inoculability of both sugarcane and arecanut isolates to mango has been reported.(Sundararaman,1928; Sundararaman *et al.*, 1928).

The anamorph state *T. paradoxa* is much common with abundant matured (brown coloured) and immature (hyaline) conidial production however, the teleomorph state *Ceratocystis paradoxa* (Dade)Moreau with ascospore is available in natural state under Indian condition. The physiology of growth and sporulation of *T. paradoxa* have not been investigated in details.

Materials and Methods

Isolation and Identification of pathogen

Naturally infected fruits were collected from the orchard of University farm, Palli-Siksha Bhavana, Visva Bharati (23.6693^{o} N,87.6593^{o} E). Isolation of fungi was performed by standard surface sterilization method followed by incubation on PDA at 25±1°C for 24 hours. The fungal colonies that appeared on the PDA surface surrounding infected fruit bits after incubation period were aseptically transferred on fresh sterilized petri plates containing PDA. Isolates from a single colony were maintained for further studies including identification with the help of standard literature (Ellis, 1971).

Effect of selective nutrient media on *T. paradoxa*

The cultural characters of *T. paradoxa* were examined on different agar media namely PDA (Potato Dextrose Agar), PCA(Potato carrot Agar), PSA(Potato Sucrose Agar), OMA(Oat Meal Agar),MEA(Malt Extract Agar),YDA(Yeast Extract Dextrose Agar), GPA(Glucose Peptone Agar), MPDA (Malt Extract Peptone Dextrose Agar), RA (Richard's Agar), SA(Sabouraud's Agar),CDA (Czapek dox Agar),WA(Water Agar) which were prepared as standard methods and composition. The composition of different media mentioned above is available in Dhingra and Sinclair, 2012. After that 5 mm disc from *T. paradoxa* culture was cut by using a sterilized cork borer and a single disc was placed at the centre of cool sterilized petri plate containing media. Each set of experiment was replicated thrice and the plates were incubated at 28±1°C for 5 (five) days. The cultural characters such as colony diameter, colour, type of margin, aerial growth were recorded.

Effect of pH levels on physiology of *T.paradoxa*

Potato dextrose Agar (PDA) was prepared by standard method and in order to avoid bacterial contamination 0.05g of chloramphenicol per litre were added to the medium. The prepared unsterilized medium was distributed within 100ml conical flasks at the rate of 50ml per flask. The required pH was adjusted either by adding Hydrochloric acid (0.1HCl) or Sodium hydroxide (0.1N

NaOH) solution. In order to stabilize the pH, 20 % phosphate buffer was used. Six pH treatment levels 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were maintained and replicated thrice. The final pH was measured using electrical pH meter before sterilization within autoclave at 121^0C. Prepared media was immediately used for growth studies of the fungi. Optimum pH was determined by measuring fungal mycelial growth (mm).

Effect of various temperature levels on *T.paradoxa*

Sterilized media poured into the petriplates @ 20ml per plate was inoculated with 7days old culture of *T.paradoxa* and kept at temperature levels viz., 10, 15, 20, 25, 30, 35 and 40°C for fungal colony development and three replications were maintained for each treatment. Most suitable temperature for *T.paradoxa* was determined by measuring mycelial growth (mm).

Statistical analysis

Data obtained were analysed using one way Analysis of Variance (ANOVA) using the SPAR 3.0 for data analysis.

Results and Discussion

Determination of mycelial growth of *T.paradoxa* under different media

Fungal growth alongwith its certain morphological characters are highly influenced on the composition of the various types of media used by the researchers. The preferential uptake of nutrients is strongly determined not only by the qualitative aspects but also the kind and quantitative nutritional values. Isolate from a host either may or may not be media specific. In case of *T. paradoxa,* sugarcane isolate can infect mango, banana, date palm and other *Saccharum* sp.(Sundararaman,1928) but media specificity was not determined. The growth of *T.paradoxa* was found to be influenced by all 12 media tested and data are presented in Table 1 and 2. The data of the experiment presented in Table 1 revealed that mycelia growth substantially differ between scanty and fluffy. While media like PCA, SA, RA and WA support only scanty growth, on the other hand PDA, PSA, MEA and OMA media results in luxuriant and profuse growth. The moderate growth was obtained on YDA, MPDA and GPA media. The amount of available energy source (sugar) perhaps played significant contribution on the mycelial growth (Griffin, 1994). Therefore, for initial isolation and to avoid contamination the most common media PDA can be used. Since *T.paradoxa* is a very fast growing fungus the colony diameter was attained maximum within 72 hours of incubation incase of PDA, PSA, YDA and MPDA although extremely poor radial growth on CDA medium. Similar observation was recorded on CDA medium by Bachiller *et al.,* (1998) in Philippines.

As the colony colour is not a stable character among fungi obviously here too this parameter appeared after 120 hours of incubation was found to be changed among the media used. While light colonies almost white can be recorded on CDA and RA media, on the contrary light blackish (WA) to extremely deep black colonies observed on PDA, PSA, MEA and MPDA. This criterion of deep black coloured colony can be used for selective isolation of this fungus as similar observation of pink colony is common on *Fusarium moliniformae* on PDA. (Booth, 1971). Inconsistency in colony colour (dull white to light brown) was noted in YDA medium and the reason is not clear (Plate-1). The fungus produce maximum spore in OMA followed by YDA, PSA, PDA and MEA whereas least sporulation was observed on CDA.

Table.1 Effect of media on morphological character of *T.paradoxa*

Media	Mycelial Growth	Colony dia(mm) after 72hours	Colony colour	Sporulation
PDA	Profuse	95.00	Black pigmentation	Very High
PCA	Scanty filamentous	91.00	light blackish growth; pigmentation absent	Poor
PSA	Profuse growth	95.00	off white to black pigmentation	Good
OMA	Fluffy mycelial growth	83.33	Brown	heavy
MEA	Smooth growth; advancing margin regular	90.00	Deep black	good
YDA	mycelia growth advancing margin regular	95.00	Dull white to light brown	heavy
GPA	Fluffy mycelial growth	85.00	Off white to greyish	medium
MPDA	Smooth fast growth advancing margin regular	95.00	Deep black in periphery with dull white centre	medium
RA	Low fluffy mycelial growth	21.33	White mycelial growth	Very poor
SA	Filamentous mycelia advancing margin irregular	77.83	light blackish	very poor
CDA	smooth slow growth	15.67	White	very poor
WA	Scanty, filamentous irregular advancing margin	60.00	Dull white growth,	poor

Table.2 Effect of media on radial growth and sporulation of *T.paradoxa*

Media	24	48	72	Sporulation per ml at 120hrs (x 10^6)
PDA	26.17	95.00	95.00	6.27
PCA	12.00	51.00	91.00	0.96
PSA	23.67	84.33	95.00	7.00
OMA	20.17	70.33	83.33	8.54
MEA	19.00	66.67	90.00	5.20
YDA	18.00	81.00	95.00	7.20
GPA	11.00	51.67	85.00	2.80
MPDA	17.00	65.33	95.00	2.60
RA	0.00	8.00	21.33	0.11
SA	0.50	38.50	77.83	0.66
CDA	0.00	4.67	15.67	0.07
WA	1.17	28.50	60.00	0.21
SEm	0.68	0.94	1.40	0.61
CD < 0.05	1.98	2.74	4.07	1.75

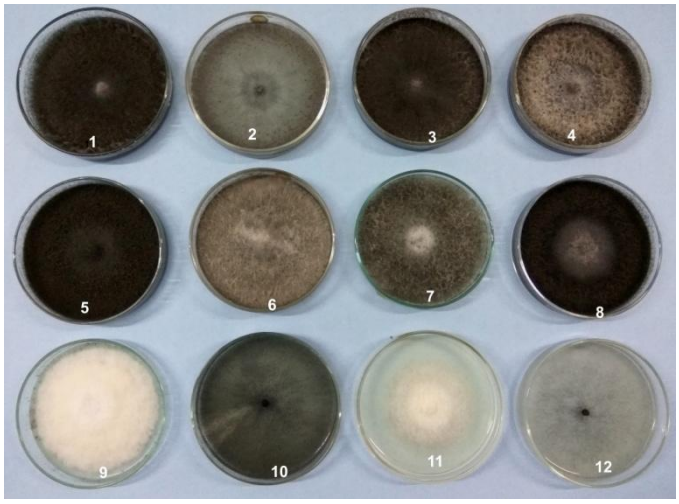
Table.3 Radial growth and sporulation of *T.paradoxa* at different pH levels

pH	24	48	72	Sporulation per ml at 120hrs (x 10^6)
4.0	33.17	89.25	91.33	4.80
5.0	32.83	90.83	94.50	4.89
6.0	31.08	89.25	94.17	5.24
7.0	29.83	82.75	90.33	4.87
8.0	20.00	65.50	84.33	3.82
9.0	16.00	46.92	58.83	4.40
SEm	0.60	1.11	0.96	0.22
CD < 0.05	1.84	3.41	2.94	0.66

Table.4 Effect of temperature on radial growth and sporulation of *T. paradoxa*

Temp (^{o}C)	24	48	72	96	120	Sporulation per ml at 120hrs (x 10^6)
10	0.00	0.00	0.00	0.50	1.17	0.50
15	2.00	18.50	43.18	69.33	84.17	2.52
20	15.83	56.17	95.00	95.00	95.00	4.27
25	28.00	83.00	95.00	95.00	95.00	4.40
30	27.50	84.67	95.00	95.00	95.00	5.38
35	7.33	50.67	85.00	95.00	95.00	3.69
40	0.00	0.00	0.00	0.00	0.00	0.00
SEm	0.30	0.74	0.07	0.17	0.18	0.20
CD < 0.05	0.92	2.25	0.21	0.51	0.54	0.59

Plate.1 Growth of *T. paradoxa* on different media (1. PDA, 2.PCA, 3.PSA, 4.OMA, 5.MEA, 6.YDA, 7.GPA, 8.MPDA, 9.RA, 10.SA, 11.CDA, 12.WA)



The density of sporulation was not very poor on WA medium despite of the poor nutritional status. Mubarak.et.al.,(1994), have recorded that (*T. paradoxa* isolate from date palms) sporulation was best on PDA or malt extract agar, among six media tested, at optimum incubation temperature of 30°C.

Effect of pH levels on radial growth of *T.paradoxa*

T.paradoxa growth was observed on various pH levels on different time intervals as presented in Table 3. After 72 hours of incubation all pH levels tested were found to support both statistically significant growth and sporulation. Although the best growth was obtained on pH 5.0 and 6.0, However at pH 9.0 the percent reduction of growth was 37.74 as compared to pH 6.0. Colony colour and the nature of growth were found to be unaffected due to pH variations used. The trend of growth at different pH levels was not similar which were found to be declined from 7.0 onwards indicating the fungus is slightly acid loving in nature. Bachiller *et al.,* (1998) also obtained decline in growth and sporulation at pH 8.0 and above. Sporulation was found to be seriously unaffected by different pH levels tested, although statistically significant.

Effect of various temperature levels on *T.paradoxa*

Fungal growth is affected due to variation in temperature of incubation and found to be significant at 95% confidence level. The mean colony diameter of *T.paradoxa* on PDA medium was 95.0 mm at 72 hours of incubation temperature ranges between 20^{o}C and at 30^{o}C, although there were initial variation in growth at 24 and 48 hours. The pathogen did not grow at 40^{o}C & very scanty growth of 1.17mm at 10^{o}C (Table 4). *T.paradoxa* grew well in a temperature range of 21-22^{o}C and completely inactive below 10^{o}C (Agrios, 2005). Mubarak *et al.,* (1994) obtained optimum temperature for germination of conidia of *T. paradoxa* at 25^{o}C - 30^{o}C and under this temperature regime, the growth was so fast that entire petri plate was covered at 48 hrs. The fungus did not sporulate to the extreme temperature of 40^{o}C and insignificant at 10^{o}C. Maximum sporulation observed at temperature of 30^{o}C followed by 25^{o}C and 20^{o}C. It has a higher growth rate in culture than most other tropical fruit rotting fungi at optimum temperature and this rate is reduced at 12-14^{o}C (Kader, 2002).

Both in India and abroad, effect of temperature, pH and chemical composition of nutrients on growth and sporulation of different fungi were investigated by many authors. (Dhingra *et al.,* 1979; Prasad and Sinha, 1987; Awadhuya, 1991; Maheshwari *et al.,* 1999; Alam *et al.,* 2001; Rani and Murthy, 2004; Kim *et al.,* 2005; Saha *et al.,* 2008; Gadgile and Chavan, 2010). Therefore, present findings are in agreement with the findings of the earlier workers. The fungus remain found to be extremely viable on PDA medium when sub culturing undertaken after a year of storage at 4^{o}C.

It is concluded, since growth was obtained best in natural media like PDA, PSA, MEA and OMA could be utilized for both multiplication and any other future studies. The optimum pH 5.0 and 6.0 was found to support best growth and media can be adjusted accordingly. The fungus can be easily cultured at 20 to 30^{o}C. Both philoconidia and aleuroconidia were produced however abundant aleuroconidia was observed in old cultures. These two types of conidia at anamorph phase were recorded in all 12 media used, pH 6.0 and 30^{o}C temperature. *In vitro* condition none of the media used supported the ascospore production.

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