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Effect of pH on Mycelial Growth and Sporulation of Postharvest Pathogen *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. and *Pestalotiopsis mangiferae* (Henn.) Steyaert

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Abstract

Among the plethora of postharvest pathogens causing enormous losses C*olletotrichum gloeosporioides* is an important polyphagous pathogen, *Pestalotiopsis mangiferae* is monophagus to mango. A study was conducted with these two pathogens obtained from banana and mango to find out the optimum pH level for growth and sporulation as well as the possible utilization of this knowledge in developing management strategy of quiescent infections by them. Both fungi grew at Potato Dextrose Broth and PDA within the pH regime 4.0–9.0 tested at 28±1 °C, although growth rate differs significantly at different hours of incubation. The optimum pH for growth and sporulation differs significantly. The optimum pH for both growth and sporulation was 5.0 in PDA. In case of potato dextrose broth pH 6.0 was optimum for *C. gloeosporioides* and *P. mangiferae* has wide adaptability within ph range 4.0–6.0. Both pathogens sporulate in all the pH regime 4.0–9.0, however *C. gloeosporioides* best sporulate at pH 5.0 and *P. mangiferae* at pH.8.0 The role of pH modulated host resistance has been discussed.

Keywords: *Colletotrichum gloeosporioides*, *Pestalotiopsis mangiferae*, pH, fungal growth, mycelia, sporulation

1. Introduction

Fruits and vegetables are susceptible to various postharvest diseases during transit; market and storage caused by various biotic and abiotic factors, thus ultimately reduce the quality and quantity. Banana and mango are two most preferred tropical fruits all over the world, many European countries import them. Among many pathogens, *Colletotrichum gloeosporioides* infects both banana and mango whereas *Pestalotiopsis mangiferae* is restricted to only on mango affecting fruits (preharvest and postharvest stage) and leaf. These two fungal pathogens, *Colletotrichum gloeosporioides* and *Pestalotiopsis mangiferae* remain quiescent in the young stage at field (Swinburne, 1983) and on maturity of the fruit infection progresses and causes exhaustive damage and loss. Differential varietal behaviour of mango in terms of susceptibility to *C. gloeosporioides* both at pre- and postharvest stage under Indian condition have been documented (Srivastva,1965; Tandon and Singh, 1969; Dasgupta and Mandal, 1989). Fruits undergo several changes including pH between growth and development of young stage and subsequently maturity in the late phase just before harvest (Dasgupta and Mandal, 1989). In mosambi infected with *C. gloeosporioides,* acidity of the fruit was enhanced. Similarly, in vegetables pH is also known to be linked with host resistance factor as in Tomato–*Alternaria* system (Swinburne, 1983). The quiescence to necrotrophy has been explained in the light of genetic control of pH regulation. Fungal toxic products are capable to terminate biotrophic phase towards preparation to undergo subsequent necrotrophic growth. Although the fungal pH modulation is host induced yet the specific virulence factors of pathogen is dictated/ modulated in the environment (Alkan et al., 2013, Prusky et al., 2013).

Various parameters influence greatly to fungal morphology, physiology and pathogenicity. Among them temperature, pH, humidity, light etc. are factors, which influence their growth and sporulation of field fungi.The work have been extensively studied and compiled by Cochrane (1958); Griffin (1981) and still vigorously continued in different parts of the world. *In vitro* study of these factors on fungi may be helpful for various strategy developments on management particularly toxicogenic fungi (Pardo et al., 2006). 'Negative Hydrogen ion concentration' (pH) of the medium either directly by its action on the cell surfaces or indirectly by its effect on the availability of nutrients affects growth dynamics. Most fungi have optimum pH around 5.0 within a range of 3.0 to 8.0.

Published reports from different authors indicate that there are variations in optimum pH for dry mycelial weight and sporulation of fungi (Esteban et al., 2006; Khan et al., 2011) in most cases. In case of *Colletotrichum gloeosporioides,* the optima for growth and sporulation were pH 5.5 and pH 6.5, respectively (Deshmukh et al., 2012). But Pandy et al. (2012) recorded both growth and sporulation at pH 6.0. Present isolate of *C. gloeosporioides* may behave differently due to geographical isolation and relevant studies in respect of *Pestalotiopsis mangiferae* is lacking Therefore the research has been conducted. Therefore, give your justification to conduct the research.

2. Materials and Methods

The invitro experiments were carried out in Plant Protection department of Palli Siksha Bhavana, Sriniketan, West Bengal, India during 2017.

2.1. Isolation and identification of fungi

Naturally infected ripe banana and mango fruits were collected from local Bolpur market (23.6686° N, 87.6827° E) and Palli Siksha Bhavana orchard (23.6693° N, 87.6593° E). Isolation of fungi was performed by standard surface sterilization method followed by incubation on PDA at 25±1 °C for 24–48 hrs. The fungal colonies that appeared on the PDA surface surrounding fruit bits after incubation period were aseptically transferred on fresh sterilized petri plates containing PDA. Isolate from a single colony were maintained for further studies including identification with the help of standard literature.

2.2. Preparation of potato dextrose broth and pda media of varying ph levels

Potato dextrose broth (PDB) was prepared by standard method and in order to avoid bacterial contamination 0.05 g of chloramphenicol l^{-1} were added to the medium after sterilization. The prepared unsterilized medium was distributed within 100 ml conical flasks at the rate of 50 ml $flask^{-1}$. The required pH was adjusted either by adding Hydrochloric acid (0.1N HCl) or Sodium hydroxide (0.1 N 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. Two percent agar was added for solidification of PD broth to PDA medium. The final pH was measured using electrical pH meter before sterilization within autoclave at 121 °C. Prepared media was immediately used for growth studies of both fungi.

2.3. Inoculation and mycelial development of c. gloeosporioides and p. mangiferae in the broth media

A disc (5 mm diameter) of the culture of test pathogens was transferred into each flask aseptically. The inoculated flasks were incubated within BOD for 10 days at 28±1 °C. The culture media alongwith mycelia growth were separately decanted from each flask and filtered using pre-weighted Whatman filter paper No. 1 to recover the mycelia after washing several times with distilled water. The mycelia were oven dried at 60 °C until constant dry weight was attained. The dried mycelia were weighed (g) using electronic balance. The solidified PDA medium within petri dishes was inoculated at the centre by a disc (5 mm diameter) of the culture of test pathogens. The average of the triplicates for these two experiments was recorded.

2.4. Inoculation and sporulation of C. gloeosporioides and P. mangiferae in the agar media

Five mm discs of the culture of *C. gloeosporioides* and *P. mangiferae* were obtained from the growing edges of PDA cultured colonies using sterilized cork borer. The agar plugs were transferred to the centre of PDA plates (one plug per plate) at different pH levels and incubated at temperature of approximately 28±1 °C for six (6) days. Each treatment was in triplicate. The spores in each treated Petri dish was washed in 50 ml (25 ml+25 ml) sterilized distilled water by using of sterile slide and rubbed on the surface of mycelia growth on media to dislodge the spores and poured into beaker. 0.2 ml drop of this solution was placed in the haemocytometer and mounted for counting on compound light microscope. Each treatment was in triplicate. The number of spore ml^{-1} was computed.

2.5. Statistical analysis

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

3. Results and Discussion

Although the teleomorph state of *C. gloeosporioides* and *P. mangiferae* taxonomically belong to the same class sordariomycetes (Division:Ascomycota) but they differ at family level Glomerellaceae and Sporocadaceae, respectively, yet ascopores production is more common in the former one. In the anamorph state too both produce conidia of different shape (pigmented conidia in *Pestalotiosis* and hyaline in *Colletotrichum*) within acervuli for enormous sporulation alongwith deep blue glutinous substances for the first one and light salmon/orange for latter. The pathogenic behaviour particularly symptoms development and ecological *niche* of them is entirely different in spite of both genera are polyphagous in nature (Lee et al., 2003). *P. mangiferae* is a monophagous pathogen. Despite the genetic control mechanism, the nutritional uptake and balance perhaps play significant roles in the growth behaviour and dynamics as well as kind and quantity of spore production (Bailey and Jegar, 1992).

3.1. Effect of different ph levels on radial growth and mycellial dry weight

It is revealed from results presented in Table 1 and 2 and Figure 1 and 2 both *C. gloeosporioides* and *P. mangiferae* radially grew in the tested pH ranges of 4–9, but growth rate differs significantly at different hours of incubation. Radial growth became faster in *C. gloeosporioides* as compared to

Table 1: Radial growth of *Colletotrichum gloeosporioides* in different pH and incubation time*

pH	Radial growth (mm) after different hours of incubation					
	24	48	72	96	120	144
4.0	1.17	16.00	40.00	60.83	86.00	95.00
5.0	1.33	27.00	53.33	76.00	89.67	95.00
6.0	1.17	19.00	43.33	66.00	85.33	95.00
7.0	1.17	16.00	39.67	61.67	81.33	91.33
8.0	1.50	14.33	37.00	58.33	69.67	79.33
9.0	0.17	11.33	31.33	50.50	66.00	87.33
SEm±	0.25	0.61	0.89	0.55	1.62	0.71
CD (*p*=0.05)	0.78	1.88	2.75	1.70	5.00	2.18

*Average of three replicates

Table 2: Radial Growth of *Pestalotiopsis mangiferae* in different pH and incubation time*

pH	Radial growth (mm) after different hours of incubation					
	24	48	72	96	120	144
4.0	8.50	23.67	39.67	52.33	65.67	78.17
5.0	8.17	24.67	44.67	59.17	73.00	84.67
6.0	8.67	22.50	41.17	54.83	68.33	84.00
7.0	9.00	22.50	40.33	49.00	57.67	72.00
8.0	7.50	20.33	39.50	50.00	62.67	79.33
9.0	7.17	16.67	35.83	46.00	54.67	67.83
SEm±	0.24	0.89	0.33	0.75	2.21	3.26
CD (*p*=0.05)	0.73	2.75	1.01	2.30	6.80	10.03

*Average of three replicates

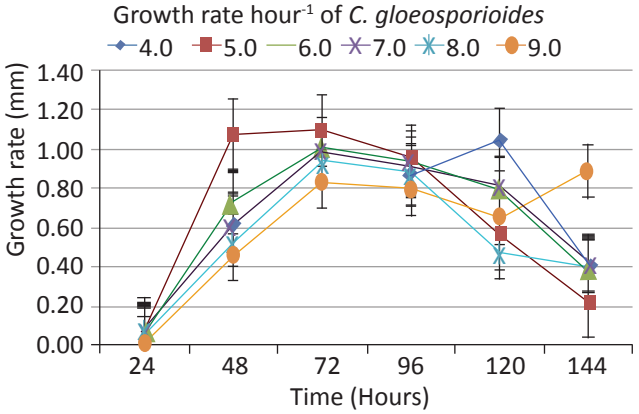

Figure 1: Growth rate of *C. gloeosporioides* in different pH level (hr^{-1})

P.mangiferae although up to 72 hrs of incubation growth rate of *P.mangiferae* was higher. The highest growth was obtained at pH 5.0 for both *C. gloeosporioides* and *P. mangiferae*.

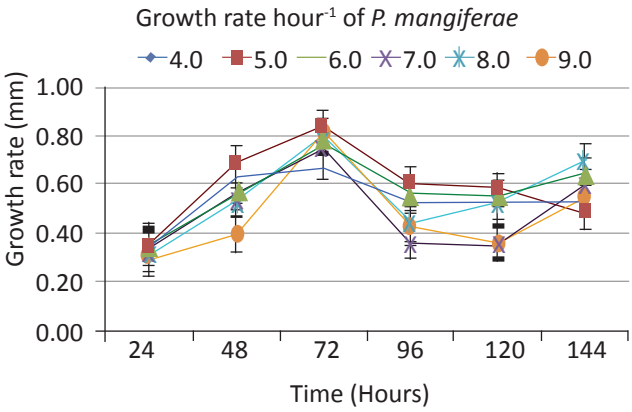

Figure 2: Growth rate of *P. mangiferae* in different pH level (hr^{-1})

Griffin (1981) although opined that pH probably have little effect on growth of fungi rather than components of the medium, but this study indicates otherwise as fresh medium prepared was utilized for the study thus avoiding the variation in components and composition. The genetic makeup of these two fungi seems responsible for uptake behaviour of nutrients which ultimately has been reflected in variation of growth rate. At higher pH of 9.0 the uptake of nutrients seems to be partially impaired which may draw attention of future researchers. Earlier, many researchers have pointed out that most fungi can generally tolerate acidic pH than alkaline condition (Lilly and Barnett, 1951). Excellent growth of various isolates of *C. gloeosporioides* obtained from chrysanthemum, turmeric and chilli were recorded at pH 5.5 to 6.0. (Naik et al., 1988; Thakare and Patil, 1995; Patel, 2000; Prashanti and Kulkarni, 2003; Patel, 2004). Under *in vitro* condition the growth dynamics of certain species of foliicolous *Pestalotiopsis* has been found to differ after 5 days of incubation at 25 °C with different pH levels. These fungi grew at pH range of 4.5–7.0 with an optimum growth at pH 7.0. The conspicuous decline in growth occurred at an increase pH from 7.5–9.0. In contrast to our finding, in Libya three species of *Pestalotiopsis* did not grow at pH 4.0. These isolates required 5 days to reach maximum growth where as our isolate reached in 3days and after words start decline (El-Gali, 2017).

Data presented in Table 3 indicate that both fungi grew in all pH ranges (4.0–9.0) tested, however, maximum dry weight of mycelia in *C. gloeosporioides* was obtained at pH 6.0 followed by pH 7.0. (Deshmukh et al., 2012; Kumara and Rawal, 2008) The higher CD value for *Pestalotiopsis mangiferae* indicates there was no significant variation in dry weight of mycelia when cultured at pH 8.0 and 9.0 tested. The highest radial growth for *P. mangiferae* was at pH 5.0 followed by pH 6.0 . Either the much precision becomes apparent or the organism has the capacity to overcome the impact of pH under liquid culture technique.

3.2. *Effect of different ph levels on sporulation of C. gloeosporioides and P. mangiferae*

The study of sporulation of many fungi under *in vitro* condition

Table 3: Mycelial dry weight of two quiescent pathogens of mango after 240 hrs of incubation

pH	*Colletotrichum gloeosporioides*	*Pestalotiopsis mangiferae*
4.0	126.00	527.00
5.0	132.67	530.33
6.0	238.33	553.33
7.0	206.67	498.33
8.0	136.33	424.33
9.0	91.667	344.33
SEm±	9.2155	42.566
CD (*p*=0.05)	28.396	131.16

was a matter of interest among many researchers of the past. In *C. gloeosporioides* maximum sporulation was obtained in pH 5.0 followed by pH 9.0 and pH 7.0. On the other hand the highest sporulation for *P. mangiferae* was recorded in pH 8.0 followed by pH 5.0 and pH 4 (Table 4). Kumara and Rawal (2008) reported that pH 5.0 was suitable for mycelia growth while pH 6.0 preferred for the sporulation of *C. gloeosporioides*. Exellent sporulation of *C. gloeosporioides* was recorded by Deshmukh et al. (2012) at pH 5.0 to 6.0. Similar observation recorded by Thakare and Patil (1995); Prashanti and Kulkarni (2003).

Table 4: Sporulation of *Colletotrichum gloeosporioides* and *Pestalotiopsis mangiferae* under *in vitro* condition

pH	Spore concentrations ml^{-1} ($\times 10^6$)	
	Colletotrichum gloeosporioides	*Pestalotiopsis mangiferae*
4.0	17.99^{b} Low	2.44^{bc}
5.0	33.30^{a} High	2.78^{b}
6.0	19.44^{b} Low	1.89^{cd}
7.0	27.98^{a} High	1.63^{d}
8.0	18.35^{b} Low	4.05^{a}
9.0	31.39^{a} High	0.88^{e}
SEm±	1.89	0.25
CD (*p*=0.05)	5.60	0.73

If we compare the growth and sporulation it is clear that (Figure 1) *C. gloeosporioides* in pH 5.0 had maximum mycelia growth and sporulation but pH4.0 and 6.0 had more mycelia growth as compared to sporulation. Since growth and sporulation are independent phenomenon, the impact of growth on sporulation seems insignificant although in *P. mangiferae* pH 6.0 had less mycelial growth with more sporulation. Since the pH has expected relations with growth and sporulation of fungi, a study was undertaken to define the nature of relationship by simple correlation studies and the parameter of same is correlation coefficient (r). The relationship between pH and growth of *C. gloeosporioides* is extremely negative (r=-0.91) and the highly negative for *P. mangiferae* (r=-0.76) at 120 hours of incubation. Interestingly, the relationship between pH and sporulation was slightly positive for *C. gloeosporioides* (r=0.24) but slightly negative for *P. mangiferae* (r=-0.21). This may be possible because of the inherent variation in the method of spore formation between these two fungi.

In *Diplocarpon mali*, Zhao et al. (2010) recorded optimum pH 5.0–7.0 for mycelia growth and pH 5.0–8.0 for conidial production.

4. Conclusion

The pH requirement of growth and sporulation differs between the two fungi *C. gloeosporioides* and *P. mangiferae* and sporulation may be the effect of higher pH shock in *P. mangiferae.* The optimum pH can be used for any further culture. The synthesis and continued presence of pre-infectional chemical compounds within young green fruits of many crops have been found to be modulated with changing pH along with the fruit age and progression of maturity. This study partially lends supports to the mechanism of quiescence and subsequent infection at maturity.

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