International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 4, No. 4, p. 202-206, 2014

INNSPUB

RESEARCH PAPER OPEN ACCESS

Effect of different pH and temperature levels on *in vitro* growth and sporulation of *Phytophthora colocasiae*, taro leaf blight pathogen

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Article published on April 29, 2014

Key words: *Phytophthora colocasiae*, temperature, pH, *in vitro* development

Abstract

Taro leaf blight disease caused *Phytophthora colocasiae* Racib is the most destructive disease which can cause significant economic losses of taro. The research was aimed at examining the effect of temperature and pH on the *in vitro* growth and sporulation of the fungus. V8-agar medium was adjusted to various levels of pH by adding appropriate amount of sodium hydroxide or hydrochloric acid before autoclaving and incubation of the fungus took place at five different temperatures. Colony diameters of the fungus were measured every day for 7 days and the number of sporangia was assessed after 21 days. The results showed that a pH of 7 and a temperature of 27 °C were the optimum conditions for pathogen growth while those of sporulation were 6 and 18 °C respectively . It is suggested that these factors would play a role in disease development.

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Introduction

Phytophthora colocasiae is a pathogenic causal agent of leaf blight on taro (*Colocasia esculenta* L.). The attack of *P. colocasiae* on the leaves reduces significantly the number of functional leaves which leads to yield reduction. Taro leaf blight was found for the first time in 2010 in all taro plantations in Cameroon (Guarino, 2010) and is actually the main constraint in taro production in the country.

In vitro growth of *Phytophthora* is affected by several factors such as temperature and pH. Optimum temperature and pH for the growth of *Phytophthora* differed depending on the species. It is well known that temperature influences pathogen development as well the expression of host resistance. The effect of temperature on aggressiveness component has been established for many pathogen species and presents an optimum for spore germination, lesion development and sporulation. However, the response to temperature may differ among individuals. For instance, Milus and Line (1980) showed that the spore production rate of two leaf rust isolates (*P. triticina*) were identical at 2-18 °C but different at 10-30 °C. On the other hand, Hydrogen ion concentration (pH) is among the most important parameter which influences sporangium production. The optimal pHs differed among *Phytophthora* species, with the optimal pH for *P. citricola* at pH 9, the optimal pH for *P. tropicalis* at pH 5, and the optimal pH for *P. citrophthora*, *P. insolita*, *P. irrigata*, *P. megasperma*, and *P. nicotianae*, was at pH 7 (Kong et al., 2009).

According to Fullerton and Tyson (2004), *P. colocasiae* is affected by temperature and grows well between 20-25 °C. Growth is faster between 27-30 °C (Scot et al*.,* 2011). Minimum and maximum temperatures for growth are respectively 10 and 35 °C (Brooks, 2005, Scot et al*.,* 2011). *In vitro*, the optimum temperature for growth of the pathogen is approximately 25 °C (Brooks, 2005, Fullerton and Tyson, 2004).

Various studies based on laboratory tests have pointed out the influence of temperature (Phillips and Weste, 1985, Zentmeyer et al*.,* 1979), and pH (Benson, 1984) on the variability in *Phytophthora* growth and formation of its reproductive structures. Little is known about the laboratory conditions of *P. colocasiae* in Cameroon. The aim of this study was to analyse the *in vitro* effect of temperature and pH on the mycelial growth and sporangia production of *P. colocasiae.*

Material and methods

Isolation of the fungus

P. colocasiae was isolated from blighted taro leaves as described by Tsopmbeng et al. (2012). Leaf tissue fragments 1 cm^2 in size were excised from lesion margins, sterilized in 70 % ethanol for 30 sec., rinsed twice with sterile distilled water and placed onto modified V-8 agar medium (200 ml V-8 juice, 3 g $CaCO_3$ and 15 g agar and 800 ml of distilled water) amended with 250 mg l^{-1} penicillin G, 250 mg l^{-1} ampicillin and 20 mg l^{-1}Nystatin. Following incubation in Petri dishes for 2-3 days at 24 °C, mycelia growth from leaf fragment was transferred to and maintained on V8 agar medium.

Determination of the effect of pH and temperature on the growth and sporulation of the fungus.

Disks of *P. colocasiae* (diameter 4 mm) were cut from the 7-day-old culture margin with a sterilize cork borer. The cut disk was placed in V-8 agar plates (9 cm) containing 20 ml of the medium with known pH. The pH was adjusted to various levels namely; 6, 7, 8, 9 and 10 by adding appropriate amount of sodium hydroxide or hydrochloric acid and it was determined by electronic pH meter before autoclaving (Table 1). Incubation followed at 18, 21, 24, 27 and 30 °C. The colony diameters of the fungus were measured in fifteen replicates each day after inoculation for 7 days. When the culture reached the age of 21 days, the number of sporangia was assessed from prepared sporangial suspension with the aid of a haematocymeter under light microscope at magnification 40X. The experiment was a factorial in a completely randomized design.

Table 1: Quantities of acid and sodium added to the medium V8

V-8 agar medium	Volume of acid (ml)	Volume of NaOH (ml)	pH
1	27,5	-	6
2	5	-	7
3	0	0	8
4	-	75	9
5	-	150	10

Results

Effect of pH and temperature on the growth of the fungus

Radial growth of *P. colocasiae* cultured at pH 10 was lower than that of all the other pH for all temperatures tested. The highest radial growth was recorded at 27 and 30 °C, followed by 21 and 24 °C while the lowest was obtained at 18 °C (Fig. 1).

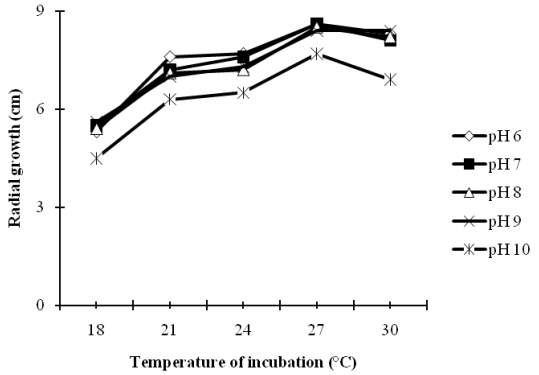

Fig. 1: Radial growth of *P. colocasiae* on V8 agar medium at different pH, seven days after incubation at various temperatures. Means of 15 replicate plates per temperature and pH included.

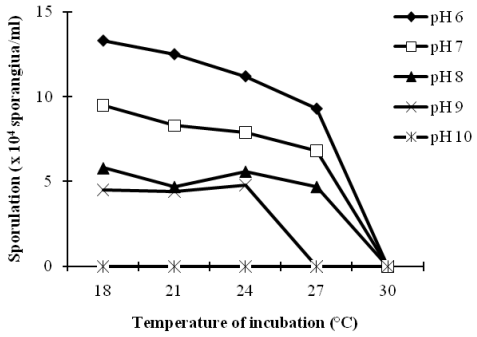

Fig 2: Sporulation of *P. colocasiae* on V8 agar medium at different pH, 7 days after incubation at various temperatures. Means of 15 replicate plates per temperature and pH included

Likewise, the highest number of spores was recorded at pH 6 and temperature 18 °C although there was no significant difference among temperatures 18, 21 and 24 °C. No sporulation was observed at pH 10 for all the temperatures and at temperature 30 °C for all the pH tested (Fig. 2).

Effect of pH and temperature on the sporulation of the fungus

Sporulation of the pathogen for temperatures of 18, 21, 24 and 27 °C evolved decreasingly with increasing pH. At 30 °C, no sporulation was observed for all pH. However, the quantities of spores produced at temperatures 18, 21 and 24°C were larger than those at 27 °C. Sporulation of the pathogen decreased with increasing temperature for all except for pH 10 where no sporulation was observed. The number of spores at pH 6 and 7 were greater than those obtained at pH 8 and 9. However, the decrease of sporulation with the increase in temperature was more constant at pH 6.

P. colocasiae exhibited different growth patterns in culture medium at different pH and temperatures (Fig. 3). At 18 °C, mycelia were whitish, cottony and areal at all pH, moderate cottony, whitish and areal at 21, 24 and 27 °C and creeping whitish with slight growth zone at 30°C (Fig. 3).

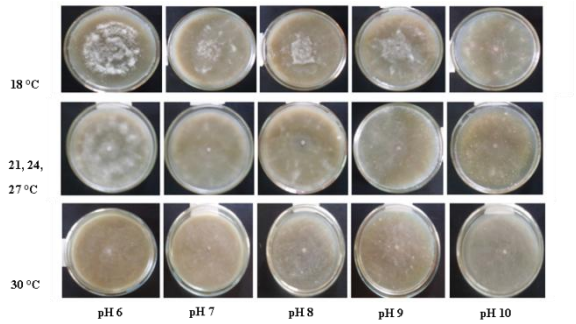

Fig 3: Growth patterns of *P. colocasiae* in culture medium at different pH and temperatures.

Discussion

P. colocasiae radial growth and sporulation was statistically different in respect to temperature and pH. The highest radial growth of the fungus was supported by temperature 27 °C for pH 6 and 7, while the highest number of spores was recorded at pH 6 and temperature 18 °C.

The results of the study revealed that hydrogen ions concentration (pH) of the medium has an effect on the growth and sporulation of *P. colocasiae*. It also indicated that optimum pH for the growth of the fungus was in the range of 6 to 7. However, good growth of the fungus was recorded at pH 6, 7, 8 and 9. This shows that *P. colocasiae* prefers acidic pH to alkaline pH indicating its acid tolerance. Bilgrami and Verma (1978) have also opined that in contrast to bacteria and actinomycetes, fungi are relatively more tolerant to acidic ions (H) than to basic ions (OH). The results of the present study are in accordance with the results obtained by Sahu et al. (2000) who reported that pH 6.5 was best for *P. colocasiae*. Kong et al. (2009) also observed that pH 5 was best for *P. tropicalis*. This pH range obtained for the growth of *P. colocasiae* was similar to that of *P. clandestina* which had a faster growth in acidic media (Simpfendorfer et al., 2001).

The growth of the pathogen increased with temperature and the maximum was obtained at 27 and 30 °C independently of the pH. Temperature seems to be the most important physical environmental factor for regulating vegetative and reproductive activity of the fungus. These results are consistent with those of Scot et al. (2011) who found that *P. colocasiae* grows well at temperatures between 20-25 °C and growth is faster between 27-30 °C. Accordingly, Sahu et al. (2000) defined a pH of 6.5 and a temperature of 28 °C as favourable for the growth of *P. colocasiae*. Based on this study, it can be suggested that growth of *P. colocasiae* is strongly influenced by temperature and pH.

As concerns the pH of the culture medium, it was noted that acidic or slightly alkaline medium had a better growth of the pathogen at all temperatures. This was confirmed in terms of growth obtained at pH of 10, where high alkaline medium greatly slowed the growth of the pathogen. However, growth in acidic environments was slightly higher than that obtained on slightly basic environments. The amount of spores decreased with increasing pH but was important in acidic than in basic media. Acidic environments are more conducive than basic media for the production of spores. This was confirmed by the important number of spores produced at pH 8 and 9 and the complete absence of spores at pH 10.

Spores production was significant at temperatures of 18, 21 and 24 °C to 27 °C low and none was observed at 30 °C. This variation could be due to the gradual increase in temperature that led to the reduction of the relative humidity, which is essential for proper sporulation. Sporulation at 18 °C was obtained at the average relative humidity of 80 % during incubation time. Fullerton and Tyson (2004) and Brooks (2005) found that sporangia production in *P. Colocasiae* is optimal between 20-22 °C with relative humidity close to 100 %. It was also noted that the quantities of spores obtained at room temperature of 24 ± 2 °C were lower than those reported by Tsopmbeng *et al.* (2012) on the same pathogen. This difference could be attributed to environmental conditions since this test was conducted in dry season. This preliminary studies carried out in the present investigation with *P. colocasiae* indicated a maximum growth and sporulation, when the inoculated plates were exposed to temperature 27 °C and pH 6 and temperature 18 °C with pH 7 respectively.

Conclusion

The present research focused results on pH of culture medium and temperature of incubation for *P. colocasiae*. Based on this result, *P. colocasiae* grew well at pH 6 and 27 °C, while pH of 7 and temperature of 18 °C favoured sporulation.

Acknowledgement

The authors are greatly indebted to God Almighty and to Dr. Yaouba Aoudou and Lekeufack Martin for their critical review of the manuscript.

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