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Full Length Research Paper

Growth and Conidiation of *Colletotrichum lindemuthianum* under Conditions of Different Water Activity.

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Laboratory experiment was conducted to determine the effect of water availability on growth and conidiation of *C. lindemuthianum* using unmodified Potato Dextrose Agar (PDA) media, water activity,(a_w) = 0.995 and PDA modified with non-ionic solute glycerol, a_w =0.97, 0.964 and 0.91. The results from the study shows that the rate of growth and conidiation varied significantly at different levels of water availability (a_w) being highest when water was freely available (a_w = 0.995) and decreased with water stress (a_w = 0.97-0.91). The rates of growth of the fungus at 0.995 and 0.91 a_w were 4.34 and 3.38 mmday^{-1} respectively.

Keywords: *Colletotrichum lindemuthianum*, water activity, growth rate, and conidiation.

INTRODUCTION

Colletotrichum lindemuthianum, the pathogen causing anthrancnose disease in cowpea belongs to the family melanconiaceae and order melanconiales. It is an important disease of cowpea that is prevalent in the tropics when grown as monocrops (Ayotunde, 2015). The disease produces cankers on petioles and stem thus causing defoliation and rotting of fruits. Yield loss caused by this disease is about 75% when not controlled (Enyiokwu, 2014).

This pathogen like any other fungi requires Oxygen, Nitrogen, suitable PH, temperature and water for growth. Severity of infection is known to be affected by water availability (water activity) (Gideon, K.O. And Anita, 2013). Water availability significantly affects growth, sporulation and infectivity of phytopathogenic fungal species.

Therefore, the relative humidity of the environment or irrigation regimes in field crops have important roles to play in modulating aw (Borisade and Magan, 2014). It has been reported that the fungus requires 10 hours wet period and 22.2-22.8^{0}C temperature range for establishment and survival (Roberto et al., 2013). Based on this finding, experiment was conducted to determine the growth and conidiation of the fungus under conditions of different water activities.

MATERIALS AND METHODS

Preparation of laboratory apparatus

Glasswares used in these studies were washed in Tapol detergent containing 0.05% hypochlorite, rinsed with tap water and dried inside an oven (Gallenkamp 300) at 50°C. Thereafter, Erlenmeyer flasks, beakers, glass pipettes and Petri- dishes were wrapped in aluminum foil and autoclaved at 121°C for 35 minutes. Inoculating loop, cork borers and scalpels were sterilized by dipping in 70% ethanol and flaming to red hot before and after use. Laminar flow cabinet and all other working surfaces were sterilized by swabbing with 70% ethanol. Distilled water was autoclaved inside Erhlenmeyer flask plugged with non- absorbent cotton wool and autoclaved at 121°C for 45minutes.

Preparation of media

Potato Dextrose Agar (PDA) was prepared by dissolving 39g dehydrated PDA (E. Merck, Darmstadt Germany) in one litre of distilled water in an Erlenmeyer flask. The flask was stoppered using cotton wool plug, wrapped with aluminum foil and autoclaved at 121°C for

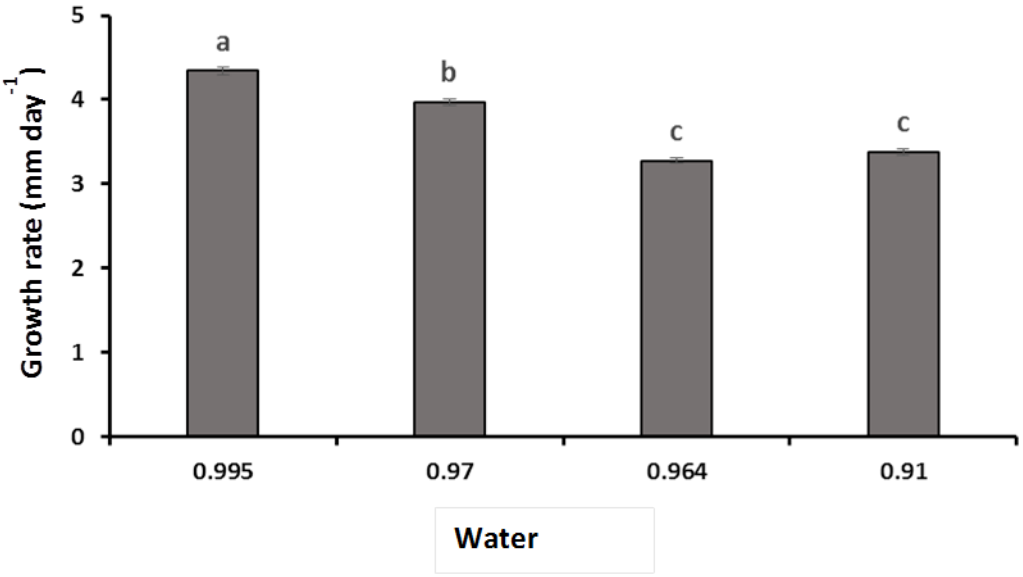


Figure 1: Effect of water activity on growth rate of *C. lindemuthianum* under ambient temperature

35 minutes Thereafter, the agar was allowed to cool and amended with 300µg/L streptomycin sulphate and 20ml of cooled media were poured into 9cm sterile petri dishes (Sterlin Product, UK) inside a laminar flow cabinet and left for 20 minutes to solidify.

Effect of water activity on growth of *C. lindemuthianum*

Conidia from 10-day old culture were harvested by flooding the surface of the agar plate with distilled water containing 0.02% Tween 80. The conidia suspension was poured into universal bottles and centrifuged at 1500rpm for 30 minutes. The supernatant was discarded and the conidia suspension was made to 1ml. Serial dilutions were made and conidia were counted with Improved Neubauer Haemocytometer under x400 objective of Microscope. Thereafter, the conidia suspension was standardized to 10^4 conidia ml^{-1}. Standard Potato Dextrose Agar (PDA, a_w=0.995) and modified PDA media containing calculated amounts of non-ionic solute glycerol at three water activity (a_w) levels; 0.97, 0.964 and 0.91were prepared and poured into 9cm Petri-dishes. Three replicate plates at each a_w were inoculated at the center with one micro-litre of the standardized conidia suspension. The plates were sealed with parafilm and incubated at ambient temperature for 10-12 days or until ¾ of the surface of the agar in the 9 cm Petri dish was covered with the growing colony. Measurement of radial extension of the colony along two pre-marked orthogonal axis was done daily for the entire incubation period. Growth rate was calculated by plotting the graph of radial extension against the period of growth. The slope of the log phase of growth (growth rate) was estimated using the regression equation of the linear model (Borisade and Magan, 2014).

Effect of water activity on conidiation of *C. lindemuthianum*

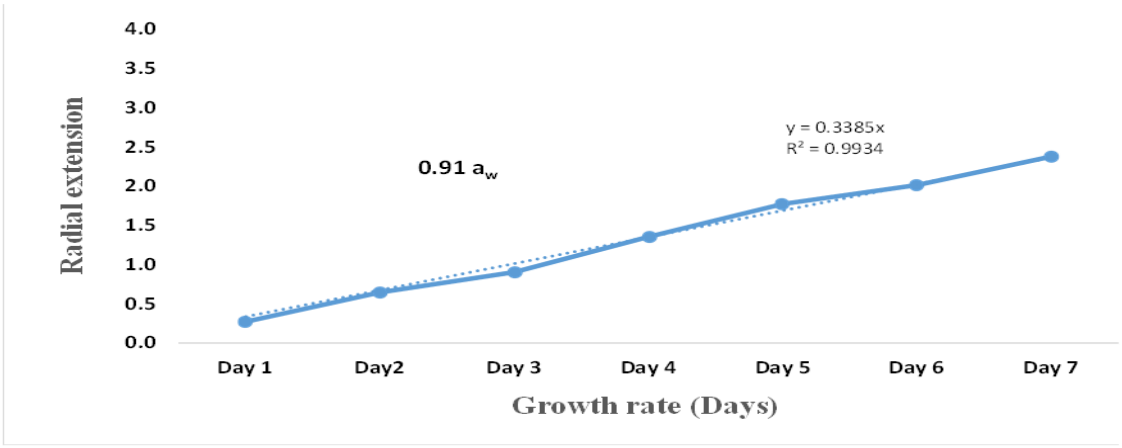
One centimeter agar disks from the culture used to estimate growth were taken randomly from three portions on the PDA plate into 10 ml disposable universal bottles. 1 ml sterile distilled water containing 0.02% Tween 80 was added into each bottle and vortexed for 1-2 minutes to dislodge the spores. The conidia suspension was thereafter made to 10 ml and spore count was done using x40 objective of light microscope and Haemocytometer. Sporulation density was calculated as the number of conidia cm^{-2} of fungal colony.

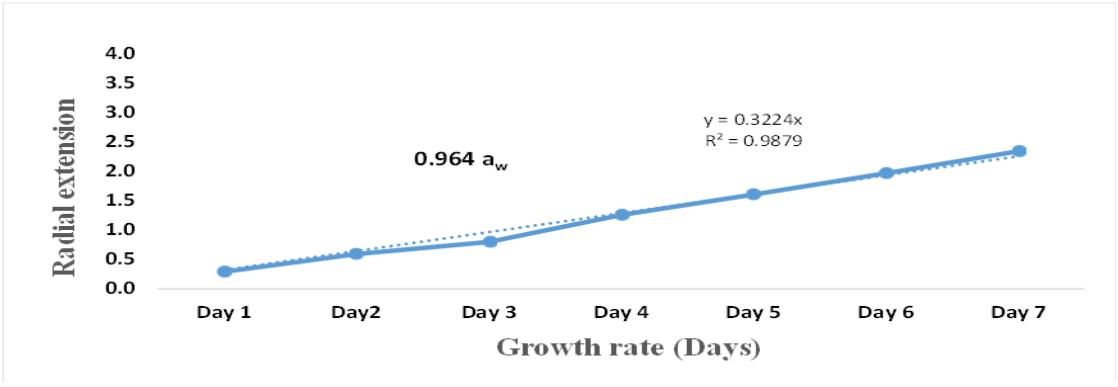
RESULTS

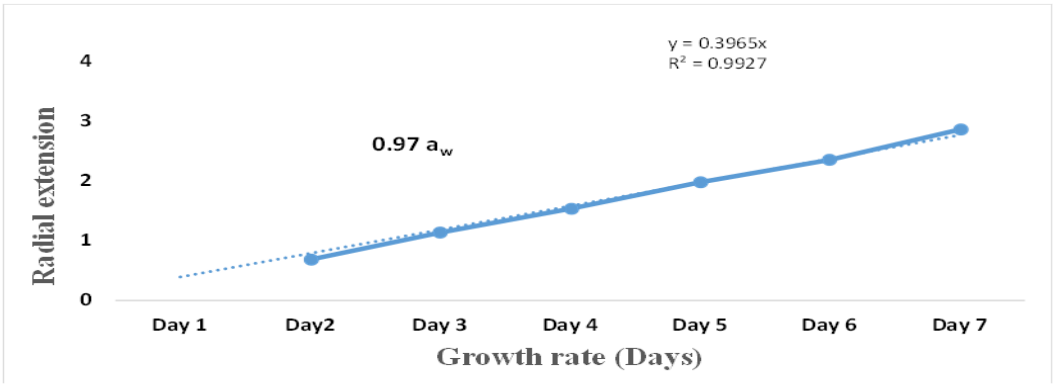
Figure 1 shows the rates of growth of *C. lindemuthianum* on glycerol modified-PDA at different a_w levels (0.995, 0.97, 0.964 and 0.91). The highest rate of growth was observed at 0.995 a_w and this reduced significantly as the a_w reduced from 0.995-0.964. However, further decrease in a_w from 0.964-0.91 had no significant effect on growth rate.

Figure 2 shows the linear model for the estimation of growth rate of *C- lindemuthianum* at different water activities. Growth rate was faster at a_w of 0.995 and decreased with water stress.

Figure 3 shows the effect of different a_w on conidia







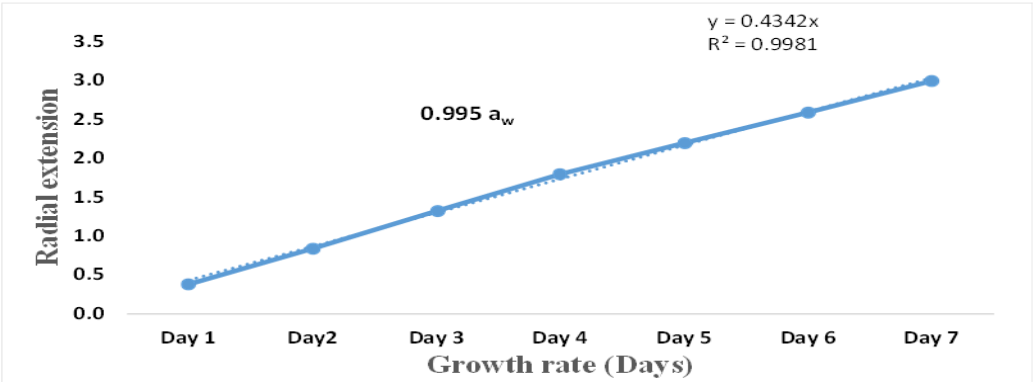


Figure 2: Linear model for estimation of growth rates of *C. lindemuthianum* at different a_w

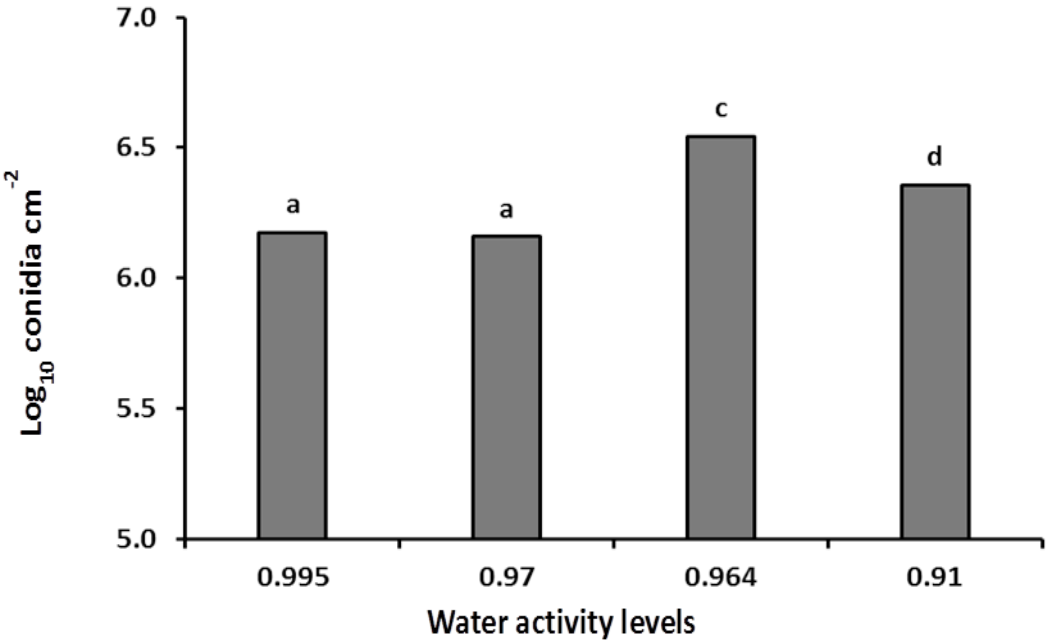


Figure 3: Effect of water activity on *C. lindemuthianum* conidia density.

density. At high water availability conidia count was low but at low available water (a_w= 0.91) more conidia were produced.

DISCUSSION

The result from this study shows that growth of *C. lindemuthianum* increases as the water availability increases and decreases with water stress this is because water plays important roles in the physiological processes of micro-organisms and to some extent, it affects their shelf life (Amani *et al.,* 2016). However, as the water activity decreases from 0.995-0.91, growth rate was reduced. Water availability significantly affected the conidiation of *C. lindemuthianum*. More conidia were formed at low a_w (0.964a_w) whereas conidiation reduced as the amount of available water increased. It has been reported that when available water for fungi growth is reduced, spores are produced at the expense of mycelia formation (Mousa *et al.,* 2011). However, when the a_w was further reduced to 0.91, there was no reduction in conidiation. Probably, the fungi have reached the critical water limit. It has been reported that many fungi do not sporulate below water activity of 0.55 which is the critical limit. (Samapundo *et al.,* 2010). Based on the effect of water relations on growth and conidiation, it may be suggested that water stress could increase incidence of secondary spread of fungal conidia while freely available water may contribute to severity of infection under field conditions. The effect of aw on growth and conidiation of *C. lindemuthianum* has not been previously reported. However, Borisade and Magan (2014) reported that a_w significantly affected the growth rate and conidiation of anamorphs of Hypocreales, *Metarhizium anisopliae, Beauveria bassiana, Isaria farinosa and I. fumosorosea. Therefore, m*anagement of irrigation regimes may be a useful cultural method in reducing incidence of anthracnose disease.

However, further studies should be carried to investigate the effect of interactions of water activity and temperature modulation as it affects the growth of *C. lindemuthianum* in the laboratory and on the field.

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