STUDIES ON THE EFFECTS OF RELATIVE HUMIDITY ON THE GERMINATION, SPORULATION, AND *IN - VIVO* INFECTION OF *SPHAEROTHECA FULIGINE* (POWDERY MILDEW) ON WATER MELON (*CITRULLUS LANATHUS.L*)

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ABSTRACT
This research was conducted to investigate the effect of Relative humidity (%) on the germination, sporulation and in vivo infection of Sphaerotheca fuliginea on healthy watermelon plants. The experiment was laid out on Completely Randomized Design (CRD) around February 2018 in the laboratory with 5 replication in each case. The relative humidity effect on mycelium shows the highest mycelium number at 52 %(27), but least was observed at 86 % (4). There were no significant differences (P>0.05) observed among the relative humidity values on both mycelium length as well as width. Relative humidity influence on spore formation showed the highest spores number obtained at 94% (120) and least at 52% (80). The highest disease incidence among the relative humidity values was observed at 94 % (96%), but least was at 52% (40%). Significant differences (P<0.05) obtained among the Relative humidity influence on disease severity, which shows the highest severity rate at 94%, 86% (2, 2 i.e. Moderate infection respectively) and control value 75% (2, i.e. Moderate infection), but least was observed at 63% and 52% (1, 1 i.e. Mild infection). Therefore, understanding the optimum ranges of relative humidity for the development of powdery mildew fungus, May minimized the high rate of infection to occur as well as damages caused on cucurbits.

Keywords: Sporulation, Germination, *Sphaerotheca, fuliginea,* Relative Humidity, Mycelium.

INTRODUCTION

The powdery mildews are a group of pathogens that can cause disease over a wide range of environmental conditions. However, several environmental factors may directly affect the development of this disease in cucurbits: among them, temperature, relative humidity and light (Jarvis *et al*., 2002). Temperature and humidity are very important because it is the water vapor pressure deficit (VPD) that has the greatest effect on host-parasite interactions. For example, temperatures between 75-85 °F and elevated levels of relative humidity (80-95%) in the absence of rainfall promote the development of this disease (Jarvis *et al*. 2002). Powdery mildew diseases are caused by many different species of fungi in the order *Erysiphales*. It is one of the easier diseases to spot, as its symptoms are quite distinctive. Infected plants display white powdery spots on the leaves and stems. The lower leaves are the most affected, but the mildew can appear on any above-ground part of the plant. As the disease progresses, the spots get larger and denser as large numbers of asexual spores are formed, and the mildew may spread up and down the length of the plant. (Huang, 2000)

METHODOLOGY

This research was carried out with the purpose of determining the optimum temperature and relative humidity for the development of powdery mildew on watermelon. Survey of watermelon cultivated fields was done in Shagari Local Government in Sokoto state, Nigeria for two months i.e. from5thDecemberr 2017 to 5th February 2018. The survey was done from the 3rd weeks after planting to the time the disease started developing which was easily recognized by their physical appearance as tiny, pinched sized, spherical at first and white later yellow-brown and finally black *cleistothecia*, which exist either singly or in group on the whitish to greenish affected area (Robert and Kucharek, 2005). The survey was done to recognize and identify the disease, as well as to obtain the inoculums.

Collection of Infected and Healthy Leaves

A total of ten samples were collected each of infected and healthy leaves in February, 2018 during a survey of a farm field at random. The samples were labeled appropriately in white plastic containers, then brought to laboratory and kept in a well-ventilated place.

Determining the Effect of Reative Humidity on Conidia Germination

Saturated solutions of different chemicals were prepared to give a range of relative humidity in sealed containers. Fifty ml of these solutions were poured into individual plastic containers (10 x 50 cm depth). Conidium of *S. fuliginea* was then dusted onto microscope slides which were suspended over the solutions in plastic containers (as above). The containers were later then sealed and placed in an incubator at 25°C to give the required relative humidity of (Mg(NO3)2, 52%; NH4NO3, 63%;KCl, 86%; KNO3, 94%; Percentage germination, mycelium length, and width were determined and measured using microscope after 3 days incubation. NaCl, 75% was used as control.

Determining the Effect of Relative Humidity on Sporulation

Saturated solutions of different chemicals were prepared to give a range of relative humidity in sealed containers. Fifty ml of these solutions were poured into individual plastic containers (10 x 50 cm depth). Plant cuttings with matured sporulating lesions after which the available spores are dusted on the glass slides were suspended over the solutions in plastic containers. The containers were later then sealed and placed in an incubator at 25°C to give the required relative humidity of (Mg(NO_3)2, 52%; NH4NO_3, 63%;KCl, 86%; KNO3, 94%;). The numbers of spores were enumerated with a microscope after 3 days incubation using heamocytometer. NaCl, 75% was used as control.

Determining the Effect of Relative Humidity on in *vivo* Infection

The young leaves of *Citrullus lanatus* were removed and cut to one-millimeter square, leaves cuttings were placed in 60ml specimens' containers containing 30ml of water agar, which was subsequently be covered with paraffin liquid to prevent evaporation. Each leaf was inoculated on both surfaces with conidia from 25 days old *in vivo* cultures of *Sphaerotheca fuliginea* collected from the same cultivar. Inoculated cuttings will then incubated at 25c for 3days, under different ranges of relative humidity which were prepared as (.Mg $(NO_3)2$, 52%; $NH4NO_3$, 63%;KCl, 86%; KNO3, 94% , NaCl saturated solution (75%) was used as control.

2.6 Determination of Disease Parameters

Were done as disease incidence as well as disease severity.

Diseases Incidence

Disease incidence was obtained by carefully counting the number of the affected leaves cutting on in vivo infection for both temperature and relative humidity effects

Using this equation Incidence (%) = $\frac{Number\ of\ diseased\ leaves\ cuttings}{Number\ of\ the\ whole\ leaves\ cutting}\ x\ \frac{100}{1}$

As recommended by Chaube and pundhir (2005).

Disease Severity

Disease severity was determined following Chakravarti (1977) method with little modification by Bem (2010) using a numerical scale of 0 – 4 as follows.

0=0%= No infection

1=1−20% = Mild Infection

2= 21−40% = Moderately Infection

3= 41−60% = High Infection

4= 61% and above = Severely Infection This was done carefully by examining the infected area of the leave cuttings at each temperature and relative humidity treatments.

RESULT

Figure (1) revealed the mycelia growth at all relative humidity values, with the highest at 52% followed by 94%, then at 63%. But least was observed at 86% relative humidity value.

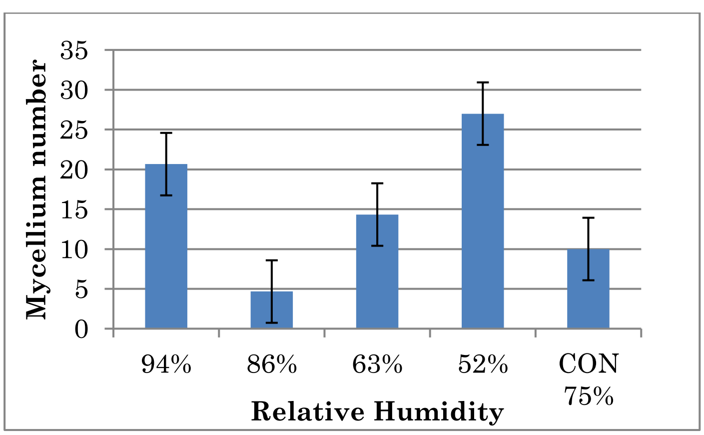

Fig (1) Effect of relative humidity on the germ tubes occurrence from the *Sphaerotheca fuliginea* conidia.

Figure (2) showed the number of spores obtained at all relative humidity values, with the highest spores number at 94% (120) but least was observed at 52% (80)

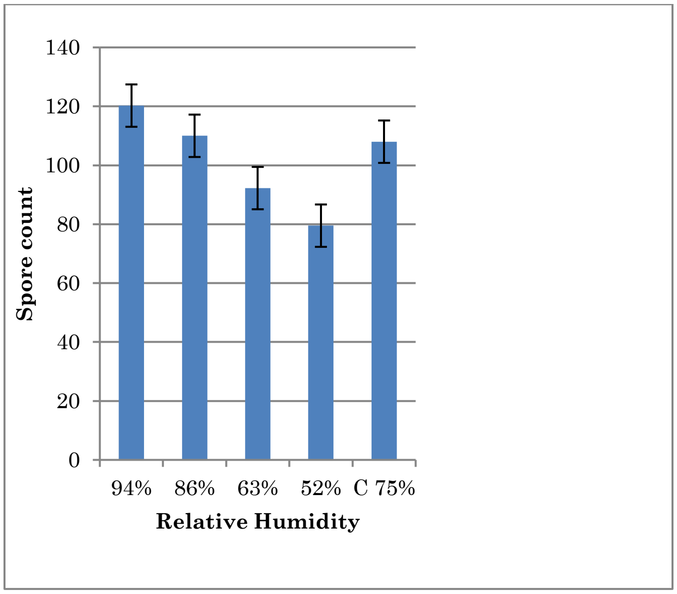

Fig (2) Effect of relative humidity on the sporulation of *Sphaerotheca fuliginea.*

Table (1) showed the effect of Relative humidity on mycelia length and width, there were no significant differences (P>0.05) among the relative humidity values effect on both mycelia length and width.

Relative humidity (%)	Length (µm)	Width (µm)
94	94.43	0.38
86	75.56	0.30
63	60.56	0.23
52	54.16	0.30
75Control	98.26	0.30
Mean	76.6	0.29
S.E.	8.80	0.02
LSD	NS	NS

Table 1: Effect of R/Humidity on the Length and Width of Germ Tubes (*Mycelium) S. fuliginea*

Table (2) showed the highest Disease incidence percentage at 94%, i.e. (96%) followed by 86% i.e. (83%), but least was observed at 52% R/H value as (40%)

Table 2: Effect of relative humidity (%) on the disease incidence (%) of *S.fuliginea* infection

Relative humidity (%)	Disease Incidence (%)
94	96
86	83
63	50
52	40
75(Control)	77

Table (3) showed the highest disease severity rating at, both 94%, 86% (2, 2 i.e. moderate infection respectively) and control value 75% (2 moderate infections), least was observed at 63% and 52% (1, 1 i.e. mild infection) .Significant differences ($P< 0.05$) among the relative humidity values were observed

Table 3 Effect of relative humidity on disease severity of *S.fuliginea* infection

R/Humidity (%)	Disease severity	S.R
94%	2	MDI
86%	2	MDI
63%	1	MI
52%	1	MI
C75%	2	MDI
Mean	23.33	
S.E	5.05	
LSD(5%)	11.67	

KEY. MDI = Moderate Infection, MI = Mild Infection, S.R = Severity Rate

DISCUSSION

Relative humidity on the mycelium germination, where the highest mycelium number was obtained at 52% which is the lowest relative humidity value and the least mycelium growth was at 94% and this explained that the relative humidity is not a limiting factor in the germination more especially at the early stage of germination i.e. with the optimum temperature level germination can occur at any given relative humidity value. As it was also stated by (Pap *et al*., 2013), powdery mildew is independent in terms of moisture value, especially during the early stages of conidial germination.

No significant difference ($P>0.05$) among the temperature levels on the Mycelium length and width. This may be because relative humidity has a limited influence on the spread of infection which normally occurs by the increased in Mycelium length and based on the theoretical evidence powdery mildew development generally occurred in the presence of moisture or dew even from the name implies as powdery mildew, this was the reason why there is no difference in the mycelium length and width.

The humidity influence of the spore, which occurred at all relative humidity values with the highest spore's number produced at 94% and least, was observed at 52%. This indicated that high level of relative humidity with optimum temperature level favors the formation of large number of spores and this was in accordance with the work reported by (Pap *et al*; 2013) that the highest spores number was obtained at the highest relative humidity value.

Relative humidity effect on disease incidence which ranged as 40% -96% with the highest percentage at 94%, but least was observed at 52%. This explained that high humidity favors infection more to occur than the lower value, not like in the case of temperature effect on infection. (McGrath, 2001) stated that high humidity favors the development of the disease, but infection can occurs at relative humidity as low as 50%.

Significant difference occurred among the relative humidity value, with high severity at 94% and 86%, but no infection was observed at 52%. This also explained the influence of high moisture content on the increase in disease severity under an optimum temperature condition finally powdery mildew as recognized to decrease plant canopy, reduce yield through decreased fruit size and number of fruits per plant and also reduce fruit quality, flavor and storage life (Donald,2008). The reduced canopy may result in sunscald of the remaining fruits making them unmarketable. The fungus causes the powdery mildew are of different races, therefore resistance screening to emerging these races and pathotypes and development of differential lines that may be used to detect these differential form of fungus of great advantage.

CONCLUSION

Based on the results obtained from this research, it is pertinent that environmental factors such as temperature and relative humidity play some important roles in the development of powdery mildew. It was also shown that temperature is an important factor that affects all the developmental stages of powdery mildew such as conidial germination, spore formation as well as the occurrence of the infection. Also relative humidity plays important role, especially for the infection occurrence, spore formation, and germ tubes occurrence. Finally, understanding the optimum ranges of these environmental factors for the occurrence of powdery mildew fungus may quietly help in reducing the risk associated with powdery mildew infection, particularly powdery mildew of cucurbits, which is mostly caused by *Sphearotheca fuliginea.*

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