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Effects of Temperature and Relative Humidity on the Germination and *in vivo* Infection Dynamics of *Sphaerotheca fuliginea* fungus on Watermelon (*Citrullus lanatus*) in Sokoto, Nigeria

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Abstract

Powdery mildew (PM) caused by the fungus Sphaerothe cafuliginea is a major foliar disease affecting greenhouse and field-grown watermelons worldwide. Infection at an early stage of watermelon plant by S. fuliginea reduces seedling and vigor, causing premature desiccation of leaves. This research investigates the effects of temperature and relative humidity on the conidial germination and in vivo infection of Sphaerotheca fuliginea on healthy watermelon plants. The experiment was laid out on Completely Randomized Design (CRD) in the laboratory with (5) replications in each case. Conidia of Sphaerotheca fuliginea were subjected to different temperatures and relative humidity of 30, 35, 40, 45 °C and 52%, 63%, 86%, and 94%, respectively, and observed for conidial germination, mycelium length, width, and in vivo infection. The results showed that lower temperature ranges (30 °C-35 °C) and higher relative humidity (>70%) often enhance the conidial germination of Sphaerotheca fuliginea and in vivo infection. Moreover, powdery mildew be triggered by a low temperature of 30 °C and a high relative humidity of ~94% in watermelon (Citrullus lanatus) in Shagari Local Government Area of Sokoto State. Based on the results obtained, the best time for watermelon cultivation around Shagari Local Government Area should be from February to May, when the temperature and relative humidity levels are not favourable for the development of powdery mildew due to Sphaerothe cafuliginea.

Keywords: Completely Randomized Design, Sphaerotheca fuliginea, Temperature, Relative Humidity

INTRODUCTION

A prominent foliar disease that affects watermelon and other cucurbit crops farmed in greenhouses and fields worldwide is powdery mildew (PM), which is caused by *Sphaerotheca fuliginea* (McGrath, 2017; Kousik *et al.*, 2019). According to Kousik *et al.* (2008) and McGrath (2010), P. xanthii infection of watermelon plants at an early stage decreases seedling vigor, induces premature leaf desiccation, and predisposes the plants to various secondary infections. It is also recognized that fruit infections lower commercial quality and yield (Kousik *et al.*, 2011). According to Zhang *et al.* (2022), a common sign of PM is the development of whitish, talcum-like colonies on the front of the leaves, which can lead to decreased photosynthesis, plant chlorosis, and even plant mortality. As a sink for plant photosynthesis, powdery mildew infections reduce plant development and cause untimely (Mossler and Nesheim, 2005). However, foliar diseases, which affect the leaves, cause melons to be exposed to sunburn, lowering the quality of the fruit. Furthermore, healthy foliage is necessary for normal ripening and fruit taste. Fruits affected by powdery mildew are typically unmarketable and prone to rotting, leading to losses during shipping and ultimately resulting in a significant profit loss to the farmer. In various watermelon-growing regions of Nigeria, Powdery mildew has been found to be more common on watermelons and other cucurbits. (Keinath and Rennberger, 2017). The illness is severe when there is high humidity and low temperature, and if crops are grown continuously under these conditions, the disease will occur more frequently and cause enormous losses each year (Milod *et al.*, 2021).

Fungicides continue to be the most crucial instrument for preventing and managing watermelon powdery mildew (Xu *et al.*, 2015). On the other hand, fungicide use results in pesticide residues being deposited in fruit and contaminating the environment (Rubio *et al.*, 2015). The persistent toxicity of fungicides makes their long-term use unfavourable for the ecosystem. Alternative management techniques are therefore required (Mandal *et al.*, 2018). Therefore, understanding the favorable ranges of temperature and relative humidity for developing Powdery mildew on Watermelon may reduce the chances of disease occurrence. Based on the forgoing, this study aimed at elucidating the Effects of Some Selected Abiotic Conditions on the Germination and *in vivo* infection dynamics of *Sphaerotheca fuliginea* fungus on Watermelon (*Citrullus lanatus*) in Sokoto, Nigeria. The specific objectives include: Determining the effect of temperature and relative humidity on the conidial germination of *Sphaerotheca fuliginea* in watermelon, determining the effect of temperature and relative humidity on the germ tube length and width, determining the effect of temperature and relative humidity on *in vivo* infection of *Sphaerotheca fuliginea* and to determining disease incidence and severity.

MATERIALS AND METHODS

Survey and Sourcing of Inocula

A survey of watermelon-growing fields in four distinct locations was conducted in Shagari, Sokoto State, Nigeria, for two months, from June 5 to August 5, 2023, according to the recommended timing by Robert and Kucharek (2005), who reported that this kind of survey should be carried out between the third week following planting and the onset of the disease. The disease was easily identified by the small, pinched-sized, spherical cleistothecia, which are either found alone or in groups on the whitish-to-greenish affected area. They initially appear white, then yellow, brown, and finally black.

Collection of Infected and Healthy Leaves

Samples of both healthy and infected leaves were carefully collected separately to avoid cross-contamination at random by hand from four different locations during a survey of farm fields, labelled appropriately in white plastic containers, and transported to a laboratory and kept in a well-ventilated environment for further analysis (Marleen, 2022).

Identification of Watermelon Powdery mildew Pathogen

The Powdery mildew pathogen *S. fuliginea* was identified by adding a single white spot of the Powdery mildew pathogen Conidia to one millilitre of 0.3% KOH solution. After the mixture was poured onto a microscope slide, the form, size, and structure of the fungal spores and hyphae were examined under a (microscope at ×40) to identify the morphological characteristics of the pathogen; moreover, to improve visibility under a light microscope, lactophenol cotton blue was used.

Determination of the effect of Temperature and Relative humidity on Conidia Germination of *S. fuliginea* in watermelon

A spore suspension containing approximately $4\times10_3$ conidia/mL was made by dusting *S. fuliginea* conidia from diseased leaves into sterile distilled water with a paintbrush (10µm). A few drops of the spore suspension were then placed on glass slides and maintained in a Petri dish with wet filter paper. The prepared Petri dishes were plastered shut and then incubated at 30, 35, 40, and 45 degrees Celsius, with the control temperature set at 25°C. Results were taken after 48 hours of incubation (Each temperature was maintained with five duplicates). According to Sangeetha *et al.* (2022), the procedure graticule was used under a microscope to measure the length and width of mycelium. Sulfuric acid and distilled water were used to determine relative humidity at (52, 63, 86, and 94 percent, with 75% as the control) were established and tracked using a schycrometer, as documented by Sangeetha *et al.* (2022). A paintbrush (10µm) was used to dust the conidia of *S. fuliginea* off damaged leaves into sterile distilled water. A few drops of the prepared spore suspension (4×10^3 conidia/mL) were deposited on glass slides and stored in a Petri dish lined with moist filter paper. The Petri dishes were then placed in desiccators with the appropriate relative humidity level and incubated at 25 ± 1°C. Paraffin was used to seal each desiccator to stop evaporation; observations were made after 48 hours of incubation. Each treatment was kept in five replicates. A graticule was used to measure the mycelium's breadth and length under a microscope.

Determination of the effect of Temperature and Relative humidity on *in vivo* Infection of *S. fuliginea* in watermelon

A conidial suspension (4×10^3 conidia/ml, 0.25 *mL* per leaf cutting) obtained from 25-day-old in vivo cultures of *Sphaerotheca fuliginea* collected from the same cultivar was sprayed onto the young, healthy leaves of *Citrullus lanatus*, which were then cut to a one-millimeter square each.

The prepared Petri dishes were sealed with plaster and then incubated at various temperatures, i.e., 30, 35, 40, and 45 ℃, with 25℃ serving as the control temperature. Results were recorded after 48 hours of incubation. (Sangeetha *et al.*, 2022). Fifteen replicates were maintained for each treatment. The young *Citrullus lanatus* leaves were cut to a millimeter square. The leaf cuttings were then placed in a petri dish and sprayed on both surfaces with a conidial suspension (4×10^3 conidia/ml, 0.25 *mL* per leaf cutting) that was taken from the same cultivar's 25-day-old in vivo cultures. For 48 hours, inoculated cuttings were incubated at 25 ˚C under four distinct relative humidity (RH) levels—52, 63, 86, and 94%—with 75% serving as the control. These levels were established using sulfuric acid and distilled water and tracked using a schycrometer, as described by (Sangeetha *et al.*, 2022). Each treatment was in five replicates.

Determination of Disease Incidence and Severity

Diseases Incidence

Disease incidence was obtained by carefully counting the number of the affected leaves cutting on *in vivo* infection, which was physically recognized by leaves discoloration to yellow-brown or black for both temperature and relative humidity effects. Using the following equation, Incidence (%) = $\frac{Number\ of\ diseased\ leaves\ cuttings}{number\ of\ the\ whole\ leaves\ cutting}\ x\ \frac{100}{1}$ (Robert and Kucharek 2005) (Suleiman *et al.*, 2016).

Disease Severity

This was done carefully by examining the infected area of each leave cutting, which showed discoloration to yellow-brown or black over the total surface area of each leaves cutting at each temperature and relative humidity treatments under a microscope using an Overlay grid, following the adoption of the following equation and numerical scale. (Bem, 2010, Suleiman *et al.*, 2016).

Disease Severity (%) = $\frac{Number\ of Affected\ Squares}{Total\ Number of\ Squares} \times 100$

A numerical scale of 0 – 4 is as follows.

0=0%= No infection

1=1–20% = Mild Infection

2= 21–40% = Moderate Infection

3= 41–60% = High Infection

4= 61% and above = Severe Infection

Statistical Analysis

A total of five biological replicates were performed for each experiment, the numerical data sets were recorded on SPSS for descriptive and ANOVA statistical analysis conducted.

RESULTS

Microscopic examination

The conidial shape and structure of S. *fuluginea* were observed to be oval in shape, and Hyphae is septate with segmentations (Figure 1),

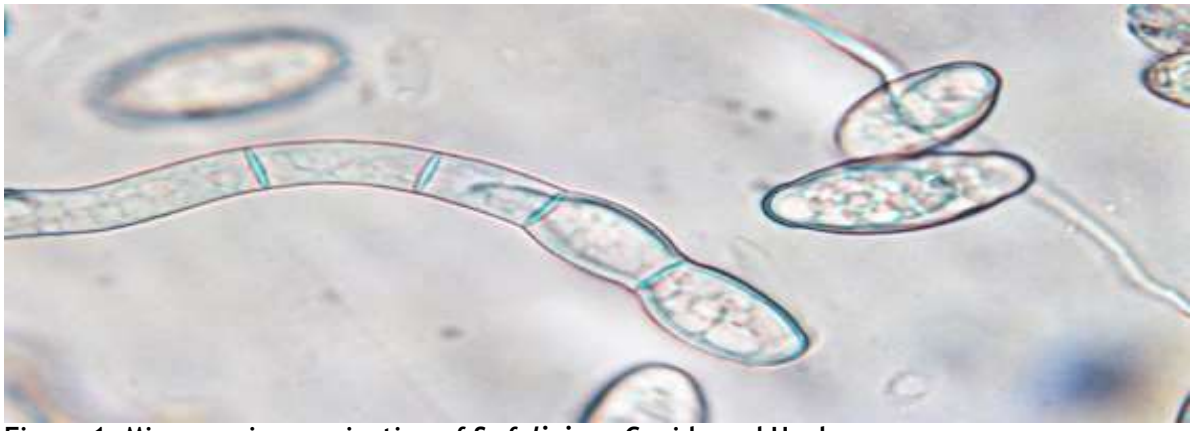

Figure 1. Microscopic examination of S. *fuliginea* Conida and Hyphae

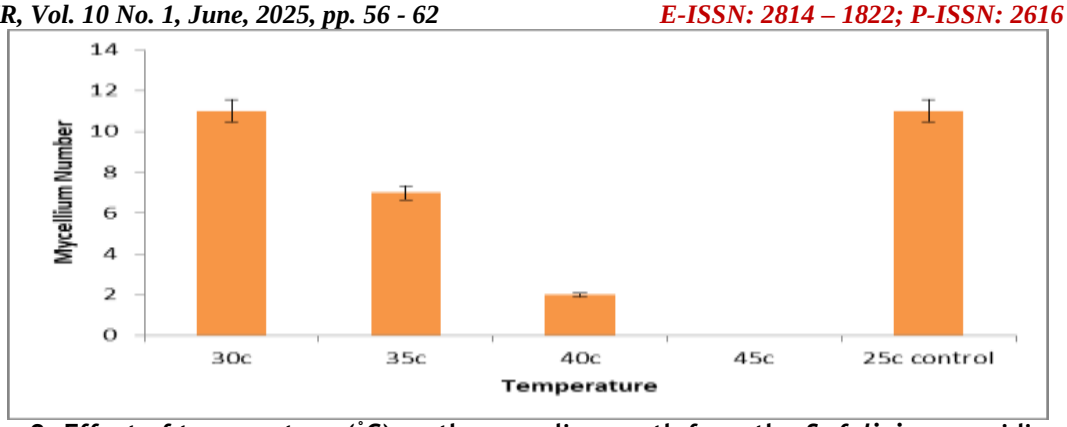

Figure 2: Effect of temperature (˚C) on the mycelia growth from the *S. fuliginea* conidia

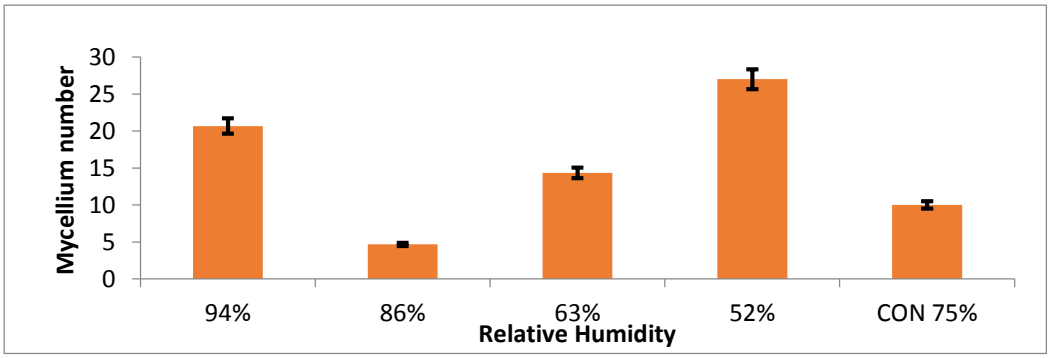

Figure 3: Effect of relative humidity on the mycelia growth from the *S. fuliginea* conidia.

Effect of Temperature and Relative Humidity on Germ Tube Length and Width

The result of temperature effects on mycelium length showed the highest mycelia length recoded at 30 ˚C, and the least mycelia length was at 40 ˚C. Significant differences (P<0.05) were observed among the temperature level on mycelia length, while no significant differences in width among the temperature levels (See Table 1). The result of relative humidity influence Revealed the highest length of (32.9)at 94%, followed by (23.2) at86%and least was recorded as (18.8) at 52%, and this indicated significant differences among Relative humidity values on mycelia length, no significant differences (P>0.05) among the relative humidity values effect on mycelia width (Table 2)

Table 1: Effect of Temperature (˚C) on the Length (µm) and Width (µm) of Germ Tubes (*Mycelium*) of *S. fuliginea*

Temperature (˚C)	Length (µm)	Width (µm)
30	29.8±1.30^{b}	0.29±0.23^{a}
35	27.4±2.07^{c}	0.13 ± 0.28^{b}
40	7.8±2.59^{d}	0.13 ± 0.27^{b}
45	0.0±0.00^{e}	0.0± 0.0^{c}
25(Control)	31.8± 1.79^{a}	0.21± 0.16^{a}

*Values are stated as mean of five replications, P value ≤ 0.05, and the letters a-e indicate LSD values. Values sharing similar letters in the same column indicate that there is no significant difference between them.

Table 2: Effect of Relative Humidity on the Length and Width of Germ Tubes (*Mycelium) S. fuliginea*

Relative humidity (%)	Length (µm)	Width (µm)
94	32.9 ±1.59^{a}	0.38 ±0.27^{a}
86	27.2 ±3.04^{c}	0.31 ±0.34^{b}
63	23.2 ±2.41^{d}	0.27 ±0.25^{b}
52	18.8 ±2.45^{e}	0.13 ±0.19^{b}
75Control	31.7 ±1.54^{b}	0.42 ±0.31^{a}

*Values are stated as mean of five replications, P value ≤ 0.05, and the letters a-e indicate LSD values. Values sharing similar letters in the same column indicate that there is no significant difference between them.

Effect of Temperature and Relative Humidity on *in vivo* Infection

The result of disease incidence showed the highest disease incidence (%) at 30°C (85%) and also at 25°C (80%) (Control temperature), but the least percentage was observed at 40 °C and no infection was recoded at 45 °C (2.5% and 0.0% respectively) as shown on (Table 3). The result of temperature influence on disease incidence revealed the highest severity rating at 30 °C, 35 °C, (4, 4 SI, SI, respectively) and at optimum temperature 25 °C (4, SI as severe infection), while at 40 °C was (1, MI) and at 45 °C was observed to be (0, No infection) (See Table 4).

Results obtained on the influence of relative humidity influence on disease incidence revealed the highest disease incidence percentage (97) at 94%, followed by (85) at 86%, but least was observed (40) at 52% R/H value as reflected as shown on (Table 5). The result of relative humidity effects on disease severity showed the highest disease severity rating at, 94%, (2, Highly infection), but at 86% and control value 75% (2,2 moderate infection, respectively), the least severity was observed at 63% and 52% relative humidity value respectively (1, 1 mild infection) as shown on (Table 6).

Table 3: Effect of Temperature (°C) on Disease Incidence (%) of *S. fuliginea* Infection

Temperature (°C)	Disease Incidence (%)
30	85
35	70
40	25
45	0
25(Control)	80

Table 4: Effect of Temperature on Disease Severity of *S. fuliginea* Infection

Temperature (°C)	Disease Incidence	S.R
30	++++	SI
35	++++	SI
40	+	MI
45	0	NI
25(Control)	++++	SI

Table 5: Effect of Relative Humidity (%) on the Disease Incidence (%) of *S.fuliginea* Infection

Relative humidity (%)	Disease Incidence (%)
94	97
86	85
63	52
52	41
75(Control)	78

Table 6: Effect of Relative Humidity on Disease Severity of *S.fuliginea* Infection

R/Humidity (%)	Disease severity	S. R
94%	+++	HI
86%	++	MDI
63%	+	MI
52%	+	MI
C75%	++	MDI

HI=Highly Infection, MD=Moderate Infection, MI=Mild Infection, + = Level of infection

DISCUSSION

In this investigation, the temperature effect on conidial germination data showed that 30 °C was the ideal temperature for mycelium growth. Some fungal diseases are more common in areas, seasons, or periods of the year with lower temperatures (Velázquez *et al.*, 2018). However, as the largest germination conidia

were observed around 25 °C, similar research by Sangeetha *et al*. (2022) discovered that the optimal temperature for fungal conidial germination is < 30 °C. According to Milod *et al*. (2021), PM disease is dangerous when there is high humidity and low temperature. Persistent cropping in these conditions causes the disease to occur more frequently, which causes significant losses each year. Sangeetha et al. (2022) reported that the highest mycelium growth was observed at 85% relative humidity value; this may be because powdery mildew is independent of moisture value, especially during the early stages of conidial germination (Pap *et al*., 2013). The result obtained from the influence of relative humidity on the conidial germination revealed the highest mycelium number at 52%, as the lowest humidity value used.

The present findings, however, did not support the conclusions of Plazola *et al*. (2003), who found that temperatures of 30 °C and higher were detrimental to conidial germination. High RH levels (80–90%) were favorable for conidial germination, but low RH levels (20–40%) inhibited spore germination. Similar findings were published by Kim *et al*. (2009) and Ramesh (2011), who found that 25 ºC and 85% relative humidity were the ideal temperatures for conidial germination.

The results obtained were consistent with those of Zayan (2016), who found that the ideal temperature for powdery mildew fungus conidial germination was 30 °C and >90% relative humidity. The results on mycelium length revealed significant differences (P<0.05) among the temperature levels, with the longest mycelium length at 30 °C. This is consistent with Doubrava's (2007) findings that the mycelium length of *S. fuliginea* grows rapidly during the warm summer months, with an optimum temperature of roughly 10 °C to 32 °C. Significant differences (P<0.05) were found between the relative humidity values and the mycelium length as a result of the relative humidity effect. With the maximum percentage at 30 °C (75%), and the lowest at 40 °C (25%), the results of temperature impacts on disease incidence ranged from 25% to 75%. This explained why an infection is less likely to occur at high temperatures. According to McGrath (2001), the relative humidity influence on disease incidence produced the highest percentage incidence (74) at 94% relative humidity value and the lowest at 52%, the lowest value taken into consideration. Though infection may happen at relative humidity as low as 50%, it is said that high humidity encourages the growth of disease. The highest severity rating was attained at 30 °C and 35 °C, according to the results of the temperature influence on disease severity (SI = Severely Infection).The influence of relative humidity on illness severity revealed that no infection was seen at 63% and 52%, but the severity level was high at 94% and moderate at 86%. This highlighted the potential impact of lower temperatures on the transmission of illness. However, according to Charles *et al*. (2013), when powdery mildew colonies develop on leaves, climate and moisture have less of an impact on the pathogen's capacity to spread.

CONCLUSION

The findings of this research revealed that lower temperature ranges of 30 °C-35 °C promote conidial germination while higher relative humidity (>70%) enhances rate of conidial germination and mycelium length of *Sphaerotheca fuliginea*. However powdery mildew severity in watermelon was shown to be triggered by lower temperature and high relative humidity of ~94% in watermelon (*Citrullus lanatus*). in Sokoto, Nigeria. Based on the results obtained, it is recommended that the best time for watermelon cultivation around Shagari Local Government Area should be from February to May, when the temperature and relative humidity levels are not favourable for the development of powdery mildew due to *Sphaerothe cafuliginea*.

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