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EFFECTS OF TEMPERATURE ON THE GERMINATION, SPORULATION, AND *IN - VIVO* INFECTION OF *Sphaerotheca fuliginea* (POWDERY MILDEW) ON WATER MELON (*Citrullus lanathus.* L)

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ABSTRACT
This research was conducted to investigate the effect of temperature and on the germination, sporulation and in vivo infection of Sphaerotheca fuliginea on healthy water melon. The experiment was laid out in completely Randomized Design (CRD) in the laboratory with 5 replications in each case. Mycelium growth was observed to be highest with 20˚C and the control. This was also with 40˚C. At 50˚ however there was no mycelium growth. Significant difference (P<0.05) was observed among the temperature levels on mycelium length with highest length observed at 20˚C, but least was at 40˚C. Temperature influence on mycelium width had not shown any significant difference (P>0.05). Spores are formed at all temperature levels, with highest spores' number obtained at 20˚C, and least was observed at 50˚C. The highest effect of temperature on disease incidence was observed at 20˚C (85%) and also at 20ºC, while lowest percentage was at 40˚C and 50˚C. On the disease severity the highest temperature effect was obtained at 20˚C, 30˚C, and at optimum temperature 25˚C (4, severe infection), while at 40˚C was (1, i.e. Mild infection) and at 50˚C was observed to be no infection, significant differences (P<0.05) was not shown among the temperature levels on disease severity. Therefore, understanding the optimum ranges of temperature for the development of powdery mildew fungus, May minimized the high rate of infection to occur as well as damages caused on Water Melon.
Key words. Sporulation, In vivo, Spaerotheca fuliginea, Mycelium, Temperature.

INTRODUCTION

The different species of Cucurbitaceae have served humans for over 10,000 years as important foods and as many useful products. In Nigeria, they are used for different purposes in different parts of the country. They occupy a special place in the life and culture of many ethnic groups (Okoli, 1984). They are very interesting and an outstanding family of dicotyledons, distributed widely over the tropical parts of the world (Cobbley *et al.*, 1976). About three genera of Cucurbitaceae bear the common name, melons. These are *Cucumis*, *Citrullus* and *Cucumeropsis*. According to Agrios (2005) powdery mildews are probably the most common, conspicuous, widespread, and easily recognizable plant diseases. They are found to affect all kinds of plants except gymnosperms. Powdery mildews appear as spots or patches of a white to greyish, powdery, mildew growth on young plant tissues or as entire leaves and other organs being completely covered by the white powdery mildew. Tiny, pinhead-sized, spherical, at first white, later yellow-brown, and finally black cleistothecia may be present singly or in groups on the white to grayish mildew in the older areas of infection. Powdery mildew is most common on the upper side of leaves, but it also affects the underside of leaves, young shoots and stems, buds, flowers, and young fruit. 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 C*ucurbits*: among them, are temperatures, 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). Various types of synthetic chemicals are used as fungicides to control the development of powdery mildew on crops which in most cases become a source of pollution to our immediate environment, and this can lead to so many problems to the human health. However the knowledge of the environmental factors may help to understand the best period for cultivating crops which may not be favorable for some certain pathogens development. The aim of this research was to evaluate the effect of temperature on the germination, sporulation and in vivo infection of *Sphaerotheca fuliginea* on water melon.

METHODOLOGY

Recognisance survey and Sourcing of Inoculums

This research was carried out with the purpose of determining the optimum temperature for the development of powdery mildew on water melon. Survey of water melon cultivated fields was done in Bagauda area of Bebeji Local Government in Kano state, Nigeria for two months i.e. from 5th September to 5th November. 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 white later yellow brown and finally black *cliestothecia*; this exist either singly or in group on the whitish to greenish affected area (Robert and Kucharek, 2005). Survey was done to recognize and identify the disease, as well as to obtain the inoculum.

Collection of Infected and Healthy Leaves

A total of ten samples were collected each of infected and healthy leaves in November, 2014 during a survey of farm fields at random. The samples were labeled appropriately in white plastic containers, then brought to laboratory and kept in a well ventilated place (Stein *et al*., 1985)

Treatments and Experimental Design

Determining the Effect of Temperature on Conidia Germination.

Conidia of *S. fuliginea* from a diseased leaf were dusted onto glass microscope slides using a paint brush (10µm). Two slides were suspended on rubber bung seated over water in a sealed plastic container (10 x 50 cm depth). Individual containers were placed in incubator rooms at different temperatures of 20, 30, 40 and 50°C, and a control (25°C). These were arranged in a Completely Randomized Design with five replicate containers for each temperature. After 3 days incubation, the percentage of conidial germination, mycelium length and width were determined using a microscope (O'Brien, 1994)
by cutting the most infected leaf, dusting the available spores from the lesions using a, paint brush ,and placed on the glass slides. Two slides were suspended on rubber bung seated over water in a sealed plastic container (10 x 50 cm depth). Individual containers were placed in incubating rooms at different temperatures of 20, 30, 40 and 50°C with five replicate containers for each temperature treatment. After 3 days incubation, the numbers of spores were enumerated using Heamocytometer under a microscope (O'Brien, 1994).

Determining the Effect of Temperature on *in vivo* Infection

The young leaves of *Citrullus lanatus* were removed and cutted to a one millimeter square each, leaf cuttings were then placed in 60 ml specimens' containers containing 30ml of water agar, which was subsequently covered with paraffin liquid to prevent evaporation. Each cutting were sprayed with a spore suspension (5 X 10' spores per milliliter, 0.25 ml per plant) obtained by using heamocytometer on both surfaces with conidia from 25 day old *in vivo* cultures of *Sphaerotheca fuliginea* collected from the same cultivar. Inoculated cuttings were then incubated at 20, 30, 40 and 50°C, for 3days (O'Brien, 1994)

Determination of Disease Parameters

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 (%) = $\frac{Number\ of\ diseased\ leaf\ cuttings}{Number\ of\ the\ whole\ leaf\ cutting} \chi \frac{100}{1}$

As recommended by Chaube and Pundhir (2005).

Disease Severity

Disease severity was determined as reported by Chakravarti (1977) with little modification by
Bem (2010) using a numerical scale of 0 – 4 as follows.
0%= No infection
1–20% = Mild Infection
21–40% = Moderate Infection
41–60% = High Infection
61% and above = Severe Infection
This was done carefully by examining the infected area of the leave cuttings at each temperature and treatments. Data collected were subjected to analysis of variance and significance means were separated using LSD at 5% probability level.

RESULTS

Figure1 shows that highest mycelia growth was observed at 20°C this was same at optimum temperature (control) and the least germ tubes occurrence was observed at 40°C, but at 50°C there was no germination.
Similarly table 1, showed the highest mycelia length at 20^0C, which was higher than at optimum temperature (control, i.e. 25°C) and least mycelia length was at 40°C, while at 50°C there was no mycelia growth. showed significant differences ($P<0.05$) among the temperatures level on mycelia length. But on the width of the mycelia,the result showed almost the same width at all temperature levels, and based on the there was no significant differences ($P<0.05$)
Fig 2 shows the number of spores at all temperature levels used in this research with highest spores number was obtained at 20°C, but least at 50°C.

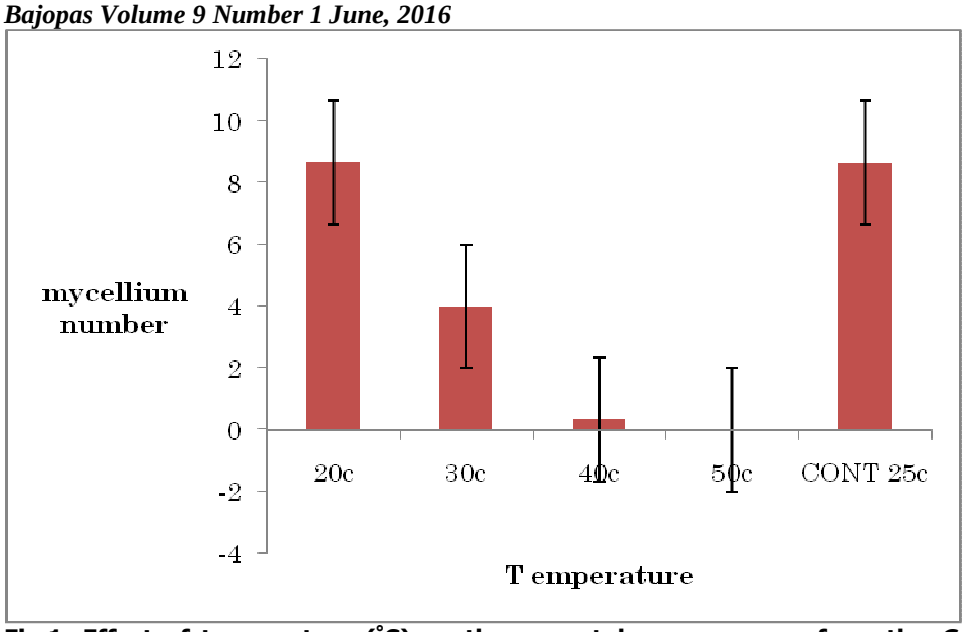

Fig.1, Effect of temperature (˚C) on the germ tubes occurrence from the *Sphaerothca fuliginea* conidia.

Table 1:Effect of Temperature (˚C) on the length (µm) and width (µm) of germ tubes (*Mycelium)* of *S. fuliginea*

Temperature (˚C) Treatments	Length (µm)	Width (µm)
20	105.8	0.23
30	88.16	0.30
40	3.96	0.30
50	0	0
25(Control)	98	0.07
Mean	47.60	0.24
S.E.	26.43	0.06
LSD (5%)	46.28	NS

N.S. = Not Significant.

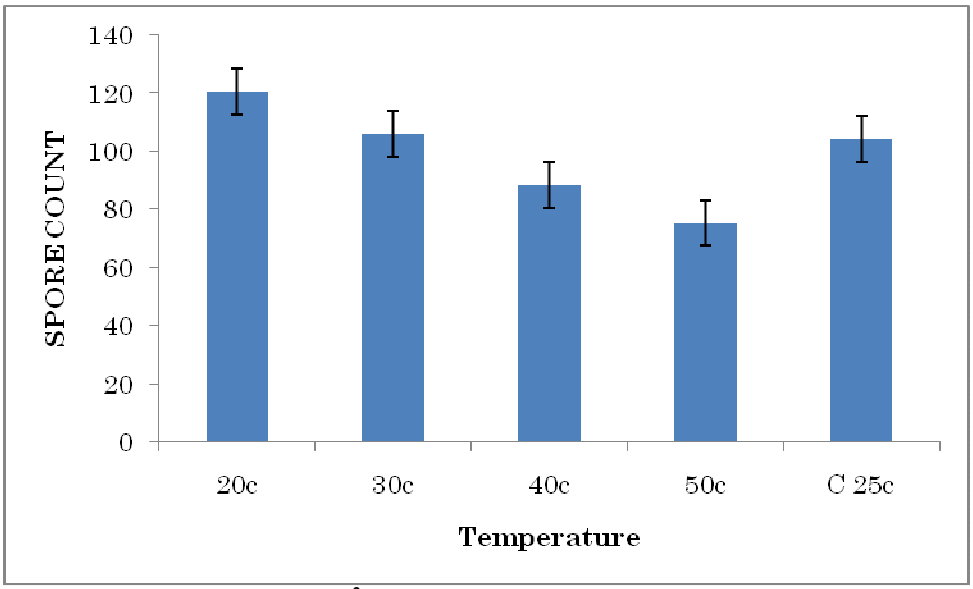

Fig.2 Effect of temperature (˚C) on the sporulation of *Sphaerotheca fuliginea*.

Table 2 showed the highest disease incidence (%) at temperatures of 20˚C, (85%) and also 25˚C, (80%) (I.e. control temperature), but showed lower percentage at 40˚C and 50˚C (2.5% and 0.0% respectively)

Table 3 showed results on the disease severity. From the table, it is clear that the highest severity rating was obtained at 20˚and 30, (4, 4 i.e. SI, SI, respectively) and at optimum temperature 25˚C (4, SI as severely infection), while at 40˚C was (1, MI) and at 50˚C was observed to be (0, No infection).

Single factor ANOVA was used, to statistical analyzed the data which showed high significant differences ($P<0.05$) among temperature levels on disease severity

Table (2): Effect of temperature (˚C) on disease incidence (%) of *S.fuliginea* infection

Temperature (C) Treatments	Disease Incidence (%)
20	85
30	75
40	2.5
50	0
25(Control)	80

Table (3): Effect of temperature on disease severity of *S.fuliginea* infection

Temperature (ºC)	Disease Incidence	S.R
20	4	SI
30	4	SI
40	1	MI
50	0	NI
25(Control)	4	SI
Mean	46	
S.E	18.17	
LSD (5%)	12.00	

Key. SI = Severely Infection, MI = Mild Infection, NI = No Infection

DISCUSSION

Result in figure1. showed that the temperature effect on mycelium growth was highest at 20˚C. This finding was in agreement with report made by Hussain and Akran. (1995) that germination of *S. fuliginea* conidia was optimum at 20˚C and this indicated that 20˚C temperature level is condusive for the germination of *S. fuliginea*.

Table 1, showed the significant differences ($P<0.05$) among the temperature levels, with highest mycelium growth at 20˚C, which also indicated that 20˚C temperature is favorable for the development of *S. fuliginea* and this was supported with the work by Husain and Akram. (1995) where the highest mycelium length was observed at 20˚C. but mycelium width was not shown significant differences ($P>0.05$) among the temperature levels influence, which explained that mycelium width is not an important feature for the disease spreading . Doubrava, (2007) stated that mycelium length grows rapidly during the warm summer months with an optimum temperature of about (10˚C-32˚C).

From the study spores formation were observed at all temperature levels, with the highest spores number obtained at 20˚C and least was observed at 50˚C. This indicates that lower temperature level favor the spore formation than higher temperatures. George, (2005) stated that temperature affects the number of spores formed in a unit plant area, more especially the lower temperature (20º–35º).

Table 2 Shown the effect of temperature on the disease incidence which ranged from 2.5% -85% with the highest percentage at 20˚C and 30°C, but at 40˚C was 2.5%. This explained that higher temperature level does not favor the occurrence of the infection.

Table 3 Shown the significant different occurred among the temperature levels for the disease severity, in which the highest severity rating was obtained at 20˚C and 30˚C but at 50˚C, there was no infection. This also emphasized on the influence of lower temperature level on the infection to occur and possibly spread. But Charles *et al.*, (2013) stated that powdery mildew colonies form on the leaf both temperature and moisture is less important to the pathogen ability to further spread.

CONCLUSION

Results obtained from this research showed that temperature plays important role on the development of powdery mildew. It was also shown that temperature is an important factor which affects all the developmental stages of powdery mildew such as conidial germination, and based on this research it was observed that lower temperature level of 20º – 30º favour severity of *in vivo* infection by powdery mildew (*Sphaerotheca fuliginea*) on water melon (*Citrullus lanatus*) in Kano, Nigeria.

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