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IMPACT OF TEMPERATURE AND RELATIVE HUMIDITY ON CONIDIAL GERMINATION OF THE CAUSAL SPECIALIST OF CUCUMBER FINE MILDEW

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ABSTRACT

This study conducted in plant protection department. Faculty of Agriculture and Botany Department, Faculty of Art and Science in Al. Gubba. Omar Al-Moktar University during season 2019-2020. The effects of relative humidity (RH) and temperature on conidial Germination of *E.cichoracearum* were studied in controlled environments to define conditions that affect disease development of cucumbers in Al. Gabel Al-Akhder region. Angles of RH (20-90%) at consistent temperatures (20-30°C) were created in single development chambers to decide their impact on buildup advancement on develop cucumber plants, temperature from 5 to 35 are evaluated their effect of Temperatures of 30°C and above were deleterious for spore germination, germ tube elongation, and disease development Lesion growth and rate of disease progress were significantly higher at 20°C than at 25°C. Low RH levels (20-40%) reduced spore germination and lesion growth, accelerated host tissue death and reduced disease progress. Intermediate RH levels (50-70%) increased spore germination and optimized disease development, provided temperatures were maintained within favorable limits. Tall RH levels (80-90%) were favorable for spore germination but proceeded introduction to these conditions driven to a restricted injury development and malady advance. Brief every day periods (two or three day by day exposures of at slightest 2 h) of tall temperatures (35°C) smothered illness improvement by 70-92%.

KEYWORDS

Cucumber powdery mildew, Conidial germination and Environmental factors.

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INTRODUCTION

Powdery mildew is a term derived from the general observation that plant parts affected by the disease usually appear as if they have been dusted with fine white powder, This fine white powder is, in reality, a mass of abounds out mycelia, conidiophores and conidia of the fungus, which normally grows on the

surface of the host. The causal fungi themselves are known to spend the whole of their life cycle as obligate parasites. Powdery mildew is common, wide spread and very present among crop plants and ornamentals. The total losses in plant growth and crop yield it causes each year on all crops probably, surpass the losses caused by any other single type of plant disease¹. At one time *Erysiphe cichoracearum* D.C. was believed to be the only causal organism of powdery mildew on Cucumber in Libyan²⁻⁴. Later, *Sphaerotheca fuliginea* (Schlecht) was recognized on the basis of conidial characters, as a causal organism of powdery mildew on cucumber which is wide spread in Libyan country and a similar finding had been reported earlier from Sudan by^{5,6} and⁷. Powdery mildew disease occurs most readily in dry, warm areas with cool nights. Germination begins when the conidia land on the leaf during warm, dry conditions⁸. After sunset, germ-tube generation happens and the disease prepare continues as stickiness level rises⁸. The impacts of dampness on conidial germination and entrance, and fine buildup malady in general, are controversial⁹. The disease can reduce photosynthetic area of leaves, and in severe cases causes defoliation of plants, effects that are likely to reduce yield and quality of fruit¹⁰. According to proficient and logical writing this organism is exceptionally hurtful since of its coordinate impact on plants supplement take-up from leaf tissue and roundabout hurtful impact since its epiphytic mycelium reduces assimilation by covering the leaf surface¹¹. During the vegetation period, the fungus forms several micro cycles of conidial stage, so its infectious potential on the leaves increases exponentially in favorable conditions, new conidia are formed from a mature conidium in 3-4 days. Since of its destructive impacts, this organism was and still is the subject of intrigued and seriously think about from different perspectives. A less examined angle is the portion of its life cycle from the time of conidial development to hyphal infiltration into have leaf tissue This period is critical for assist advancement of the organism and in this manner contamination of the have plant Amid an interim of favorable conditions, which in nature don't final exceptionally long, conidia sprout and

frame germ tubes that taint the leaf tissue by creating auxiliary hyphae. In expansion to outside conditions, a basic figure in contamination is the vulnerable phenological arrange, i.e. the presence of young leaves¹²⁻¹⁶. Different powdery mildew fungi are reusing different optimum condition for disease development¹⁷, the environment factor also influences germination, formation release and survival of spores as well as mycelium development¹⁸. The impacts of different natural components on fine molds shift with the species considered and the conditions beneath which it is considered. This variation has led to considerable confusion as to the effect of environment on the development of powdery mildews in general the factors that will be treated are temperature, moisture. In spite of the fact that the fine molds start contamination from ascospores, conidia, and overwintering mycelia in torpid buds the conidial organize is the foremost imperative spore shape in auxiliary spread. Most of the studies on environmental effects have been made with this spore form. The aim of this study was to evaluate the importance of basic environmental factors such as temperature and relative humidity, on the germination of cucumber powdery mildew conidia and, based on the gotten comes about, to clarify their parts and put within the study of disease transmission of this pathogen.

MATERIAL AND METHODS

Source of inoculum

Inoculum was collected from diseased plants in EL-Wasiata region and identified in plant protection Lab.

Identification of the causal agents

Collected samples were tested in plant pathology Lab. For presence conidial stage, measurement of conidia thickness of mycelium and shape of germ tube¹⁹.

Effect of temperature on conidial germination

Conidia of *E.cichoracearum* from a diseased leaf Figure No.1 were dusted onto glass microscope slides using a paint brush. Two slides were suspended on a elastic bung sited over water in a fixed plastic holder (10 x 50cm profundity).

Individual containers were placed in incubating rooms at a range of temperatures from 5-35°C (Figure No.2), with five replicate containers for each temperature treatment. After 3 days incubation of conidia developed in full light and relative humidity was set at 90%, the percentage of conidial germination was determined using a microscope^{20,21}.

Effect of relative humidity on conidial germination

Conidia of *E. cichoracearum* were dusted onto microscope slides which were suspended over the solutions in plastic containers (as above). The containers were then sealed and placed in an incubator (°C) to give the required relative humidities (NH₄NO₃, 63%, NaCl, 75%; KCl, 86%; KNO₃, 94%; H₂O, 100% Percentage germination was determined with a microscope after 3 days incubation (O'Brien 1948).

The conidial germination elements and germ tube lengths were decided minutely after 4, 8, 12 and 24 h after the onset of hatching, utilizing the tests were conducted in three reproduces for each treatment. In each reproduce, germ tube lengths of 30 conidia were measured to get their normal lengths.

Growth and inoculation of host plants

Seeds of *Cucumis sativus* cv. Beit alpha and M2/E6811-42191 were grown in a mixture of Levington's compost and sand (1:1) in growth room conditions (20 ± 2°C, 16 h irradiance, 80µmol m⁻² s Uniformly developed seedlings were transplanted into plastic pots (12cm diameter) containing compost, and allowed to continue growth under similar conditions. When the primary leaf was completely expanded, plants were set within the base of a settling tower (diameter 60cm; tallness 80cm) and immunized by uncovering them to an even distribution of *S. cichoracearum* conidia from heavily at different degree of temperature and relative humidity as mention above. Infected leaves of cucumber, which had been shaken 6 h previously to remove older spores (16; 3). Essential germ tubes developed within 3-6 h, to begin with haustoria inside 12 h after immunization, and a moment germ tube created amid the taking after 6 h Inside 24 h after immunization, septate hyphae were initiated from the primary and secondary germ tubes, giving

rise to surface mycelium from which conidiophores began to develop by 5 days after inoculation , with conidi densely covering the leaf surface by 7 days Small pieces of host leaves having numerous ascospores of the powdery mildews were submerged in sterilised distilled water for varying periods and after drying were subjected to dessication These leaves were then transferred to filter paper which helped in mounting the ascospores on slides for subsequent observations.

Disease development on individual plants

Disease Assessment

Disease Incidence

Percentage of each foliar disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each treatment, then the average of disease incidence in each treatment was calculated.

Disease Severity

Percentage of each foliar disease severity was recorded as following equation

$$\text{Disease Severity \%} = \Sigma (a \times b) / N \times K \times 100$$

Where: a = Number of infected leaves in each category.

b = Numerical value of each category .

N = Total number of examined leaves.

K = The highest degree of infection category .

N = Total examined leaves

Numerical value from 0 to 4 according to Cohen *et al*, (1991) was followed, whereas: 0 = No leaf lesions; 1 = 25% or less; 2 = 26-50 %; 3 = 51-75 %; and 4 = 76-100% infected area of plant leaf. At the end of growing season the accumulated yield was calculated for each particular treatment

Disease assessment

Fourteen days after challenge inoculation, powdery mildew disease development - as affected by the different tested treatment - was evaluated by counting the number of mildew colonies on leaves surface with the naked eye.

Disease development was monitored on five individual Cucumber plants at the lab. Plant pathology. As the plants grew, each leaf was numbered and the date of emergence recorded. Leaf length measurements (from leaf base along the central vein) were made at 3-4 day intervals (ref).

Disease development was also monitored on individual leaves. These measurements were carried out over two growing seasons (2019\ 2020) and (2019/ 2018).

Data analysis

Data was subjected to statistical analysis using randomized complete block design (RCBD) and ANOVA was used to determine Relationship of powdery mil dew with environmental conditions was determined by correlation regression analysis²².

RESULTS AND DISCUSSION

Identification of the causal agents. The causal agent identified according to microscopic examination to the conidial stage which appears chains of parallel spores on short branched conidiophores, absences of ferionic bodies in conidia germ tubes no branched, according to these data the causal agent identified as *E.cichoracearum*. And confided in plant pathology institute Elgiza -Egypt.

Effect of temperature on conidium germination

Greatest germination (up to 55.2%) was recorded at 25°C, although germination was generally low after 3 days of incubation (Figure No.1). Some conidia germinated at 20°C and 30°C but no germination occurred at 5 or 35°C. The leaves with infection were submerged in sterilised distilled water and were incubated at 0, 5 and 15°C. For varying periods and were afterwards incubated at laboratory temperature (25°C; f20°C.). The results are presented in Table No.1. At all the temperatures, the best results were obtained in material subjected to low temperatures hours and alternating with exposure to laboratory temperature (25°C).

It is generally accepted that germination of conidia of *E. cichoracearum* occurs between 15o and 30°C, and is greatest at 25°C according^{23,24}.

The highest average length of germ tubes was recorded at 25°C at all of the stated time intervals (Table No.1). At temperatures lower and higher than optimal, germ tubes growth was significantly lower, while at 5°C and at 35°C the conidia did not germinate to this environmental factor. Therefore temperature is not a restricting factor in the germination of conidia, as powdery mildew occurs in temperate zones with prevailing favorable

temperature conditions for its occurrence and spreading our experiment observations indicate that the disease does not appear on cucumber.

Until the air temperature emits above 20°C. The disease spreads rapidly during the hot period. The best conditions for disease development are 35°C and more than 70% relative humidity. According to 50 powdery mildew infects plants within a temperature range of 11-28°C²⁵. Reported that powdery mildew is generally favoured by relatively dry atmospheric conditions, moderate temperature and plant growth

This temperature range falls within the summer range in the Al. Gabel Al. Akhder region, when powdery mildew starts to appear on Cucumber crops. These laboratory results may partially explain our field observations that powdery mildew symptoms appear first on leaves under dense canopies where relative humidity is usually high. Development of mildew in relation to weather factors Weather conditions play an important role in appearance and rapid buildup of especially the maximum temperature

Effect of relative humidity on conidium germination Results in Table No.3 indicated that germination high was directly proportional with relative humidity, high conidium germination 28.4 occurred at 100% relative humidity.

Relative humidity expressed very weak correlation with development of powdery mildew of Cucumber The infection process of *Erysiphe cichoracearum* on host cucumber was studied in this paper. The appressoria of conidia formed at similar rates on cucumber leaves, indicating that no resistance was expressed during the pre-penetration stage of *Erysiphe cichoracearum*²⁷ reported that the effect of relative humidity on spore germination. Relative humidity as low as 3% showed 34% germination and the highest 92% germination was observed at 100 percent relative humidity Conidia germinate best at relative humidity of 97-100%²⁸, but not below, indicating that they require moist air to germinate. Butt (1978) who demonstrated that germination decreased or was delayed in the presence of free water our results is in agreed also with²⁹ who found that conidial germination of powdery mildew of

cucumber was maximum at a temperature of 25°C, 100% R.H These laboratory results may partially explain our field observations that powdery mildew symptoms appear first on leaves under dense canopies where relative humidity is usually high development of mildew in relation to weather factors Weather conditions play an important role in appearance and rapid buildup of especially the maximum temperature²⁵. Reported that powdery mildew is generally favoured by relatively dry atmospheric conditions, moderate temperature and plant growth²⁷ reported that the effect of relative humidity on spore germination. Relative humidity as low as 3% showed 34% germination and the highest (92% germination was observed at 100 percent relative humidity³⁰. Found sunshine to be more important than R.H. for development of powdery mildew on mung bean in Madhya Pradesh Sharma⁵ studied the development of powdery mildew and found that early October sowing favours more disease development due to favourable weather conditions. Lack of information on the effect of humidity on development of different stages of powdery mildew keeps us from knowing whether the phenomena described for *E. cichrocerum* on Cucumber apply to other powdery mildews as well With *E. cichrocearum* the colonization-sporulation and dispersal were favored by dryness while infection and survival phenomena were favored by high RH or even wetness. In any case, in all cases these were inclinations but not fundamental conditions, as buildup created too beneath less favorable conditions. This circumstance was reflected within the generally little contrasts between last levels of plagues actuated in development chambers beneath different conditions of RH. The fact that these epidemics reached the highest level at low RH, suggested that under the temperature conditions of 25~ by day and 15~ by night, dryness slightly favored the epidemiological patterns Conidia germination was optimum at 18 and 20°C and with a relative humidity of 70 and 90%. The association between relative humidity and temperature varied at different temperatures, with a nonsignificant effect of RH.

Effects of environmental factor on disease development

There was a similar pattern of powdery mildew development on all Cucumber plants (Figure No.2). Symptoms did not appear on the first leaves after planting until 7-8 weeks after emergence, but appeared progressively earlier, relative to the age of the leaf, on the later formed leaves (2 weeks). Symptoms rarely developed on leaves that had not grown to their full expansion and were never recorded on rapidly growing leaves Growth and rate of disease progress increase in maturation of ascospores was obtained in experimentally when the duration was increased, time at 25 Small pieces of host leaves having numerous ascospores of the powdery mildews were submerged in sterilised distilled water for varying periods and after drying were subjected to dessication at, 25 and 30°C. For periods in incubators. These leaves were then transferred to filter paper which helped in mounting the ascospores on slides for subsequent observations the results are presented in Table No.2. The data in the Table No.2 show that alternate wetting. And drying of the ascospores material gave the best results on the ascospore formation when subjected to 30°C. But not at other temperatures³¹ have reported similar results with *Erysiphe graminis*.

Most extreme frequency of fine buildup was watched at discuss temperature 34-35°C and 18-19°C, greatest and least temperature separately.

Figure No.2 and Figure No.3). At these temperature rate of PM proceeded to increment. Our Experiment observations indicate that the disease does not appear on cucumber. So the air temperature emits above 20°C. The disease spreads rapidly during the hot period. The best conditions for disease development are 35°C and more than 70% relative humidity. These results are in line with earlier reports on the effect of accurate bleaching on environmental factors³². These results and observations are in accord with those obtained by³³ with *Erysiphe graminis* var. hordei through exposure of the material to 9°C alternating at 21*[^]C³⁴. While studying role of weather factors for development of powdery mildew on URD bean in Rabi season found maximum temperature between 21.0-26.1°C as

favourable helped significantly in disease development Incidence of powdery mildew was recorded low when temperature decreased from 35°C (Figure No.3). So 34-35°C was found to be optimum Temperature for the development of powdery mildew influenced the conidia's ability to germinate. Under these conditions their highest germination rate was 72% to exclude a change in the germination rate of conidia as a result of diurnal rhythm, inoculated plants were cultivated at a daily rhythm of 24/14°C. Conidia that development under these conditions already germinated at 15°C and reached their highest rate of germination (80%) at 20°C (Figure No.3). This germination rate was equivalent to that of conidia developing at a constant temperature of 25°C. This temperature range falls within the summer range in the Al. Gabel Al. Akter region, when powdery mildew starts to appear on Cucumber crops. Conidia germinate best at relative humidity of 97-100%²⁸, but not below, indicating that they require moist air to germinate³⁵. Who demonstrated that germination decreased or was delayed in the presence of free water our the result is an agreed also with²⁹. Who found that conidial germination of powdery mildew of cucumber was maximum at a temperature of 25°C, 100% R.H these laboratory results may partially explain our field observations that powdery mildew symptoms appear first on leaves under dense canopies where relative humidity is usually high.

It has also been reported that cucurbit powdery mildew develops better in shade than in the full sunlight^{35,36}. Development of mildew in relation to weather factors Weather conditions play an important role in appearance and rapid buildup of especially the maximum temperature³⁷ found sunshine to be more important than R.H. for development of powdery mildew on mung bean in Madhya Pradesh³⁷ while studying role of weather factors for development of powdery mildew on URD bean in Rabi season found maximum temperature between 21.0-26. 1°C as favourable helped significantly in disease development⁵ studied the development of powdery mildew and fund that early october sowing favours more disease development due to favourable weather conditions It has also been reported that cucurbit powdery mildew develops better in shade than in the full sunlight^{35,36}. Lack of information on the effect of humidity on development of different stages of powdery mildew keeps us from knowing whether the phenomena described for *E.cichrocerum* on.

Table No.1

S.No	Temperature (°C)	Average length of germ tubes (µm)
1	5	Isolate 0.0
2	15	22.9
3	20	36.7
4	25	55.2
5	30	18.9
6	35	0.0

Table No.2: Saturated-salt solutions used and relative humidity as measured in chamber studies

S.No	Relative humidity (%)	Average length germination (µm)
1	NH4NO3 (63%)	11.5
2	Nacl (75%)	13.5
3	Kcl (86%)	16.5
4	KNO3 (94%)	21.1
5	HO2 (100%)	28.4

Table No.3: The percentage of germination of Cucumber powdery mildew conidia at different temperatures after culturing under 25°C after 24 h incubation, according to the results of Duncan's test²⁶

S.No	Relative humidity (%)	Average length of germ tubes μm
1	10	12.5a
2	30	25.0b
3	50	47.6c
4	70	60.0d
5	90	75.8e



Figure No.1: *E. cichoracearum* from a diseased leaf of Cucumber

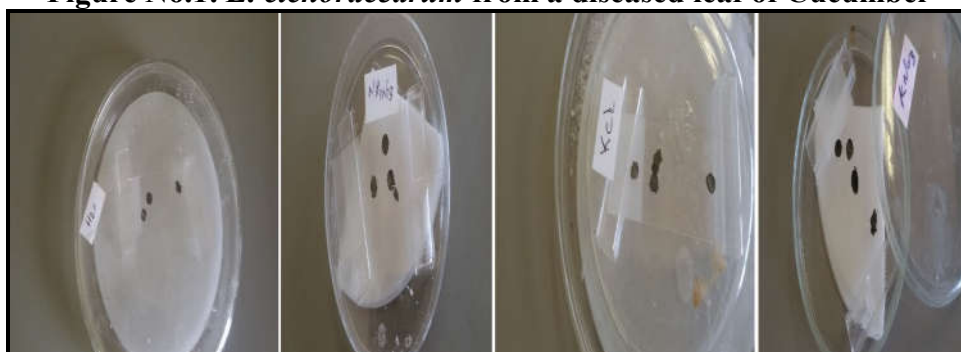


Figure No.2: Incubation of conidia in the climate chamber

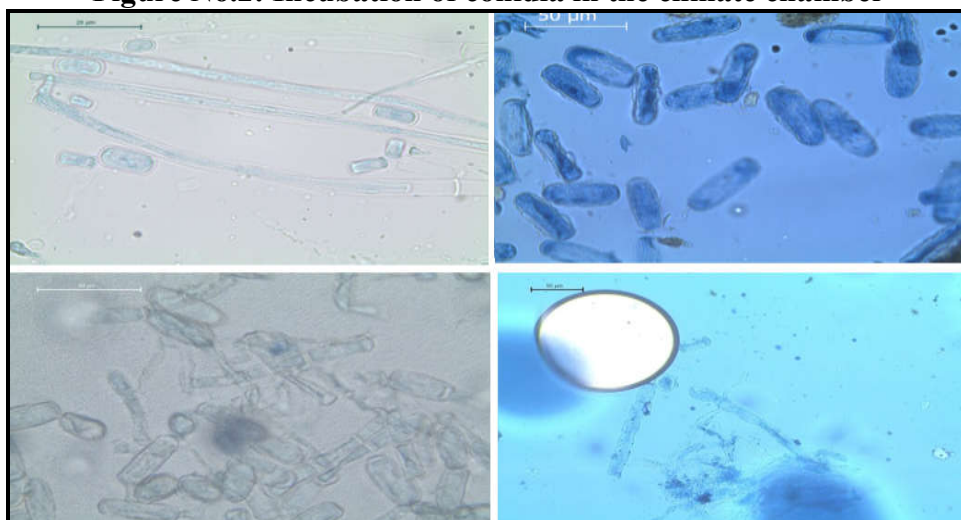


Figure No.3: Conidia, mycelium and conidiophores of *E.cichoracearum* the causal of powdery mildew on *Cucumis sativus* L

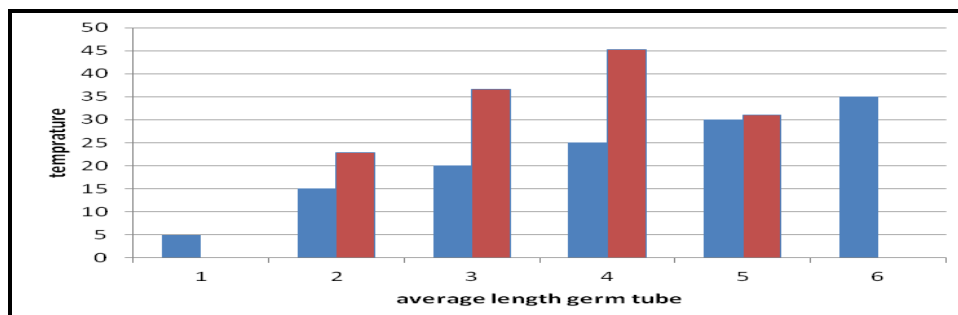


Figure No.4: Effect of temperature (°C) on the germ tubes occurrence from the of *Erysiphe cichoracearum* conidia

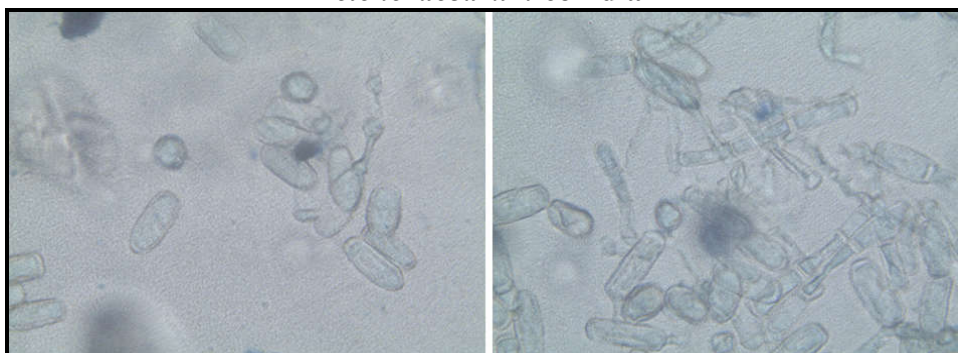


Figure No.4: Powdery mildew germ tubes and appressoria of *Erysiphe cichoracearum*

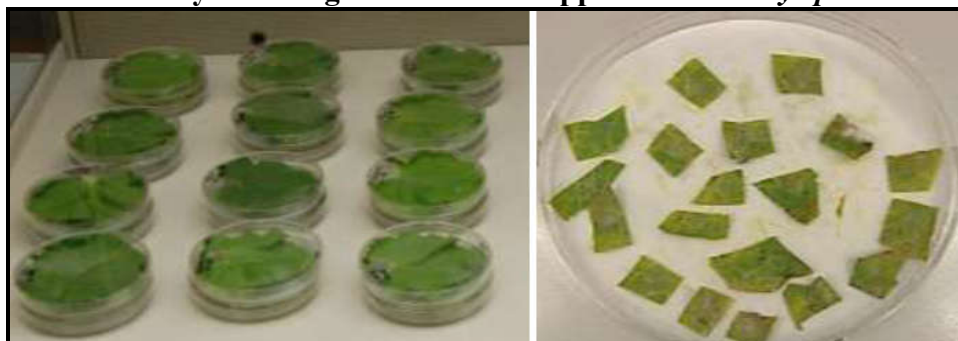


Figure No.5: Effect of alternate wetting and drying on ascospore formation

CONCLUSION

Temperature and relative humidity appeared to influence rate of growth of germ tubes after germination more markedly than percentage germination. The maximum length of germ tubes occurred between 15 to 30°C at temperatures 15, 30°C. With relative humidity 94 and 100% in the studied the shift in germination rate according to the temperature during spore development suggests that the fungus adjusts to the temperature, i.e., if the spores develop at low temperature, their germination rate at low temperature is higher than that of spores which develop at high temperatures. If, on the other hand, spores develop at high temperature, their

germination is reduced at low temperatures at very high temperatures, germination occurs, but only at a rather low rate.

Our findings coincide largely with the results of other authors, so it can be concluded that natural conditions can be simulated in a climate chamber and reliable data can be obtained on the impact of various factors on the development of the fungus.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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