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Temperature Effects on Cultural and Morphological Aspects of *Ascochyta fabae* Sand, Agent of Ascochyta Blight on Faba Bean (*Vicia faba* L. Subsp. Major)

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Abstract: *Ascochyta fabae* is a major parasite of faba bean causes ascochyta blight disease. Two controlled condition experiments were conducted to study the temperature effect on mycelial growth and sporulation of *A. fabae*, 5 days after incubation. The maximum average of mycelial growth was 75 mm on 5 days and sporulation was 24×10^3 conidia ml^{-1} and mycelial growth was increasing by 15 mm day^{-1}, were recorded at 22°C. The least values of these parameters were obtained in 26 and 30°C. Such parameters were linearly increased with temperature to maximum and declined progressively in both reaction evaluation tests. The two evaluation tests indicated that the optimum temperature for mycelial growth and sporulation development of *A. fabae* was 22°C.

Key words: Faba Bean · *Ascochyta fabae* · Ascochyta Blight · Mycelial Growth · Sporulation

INTRODUCTION

Faba bean (*Vicia faba* L.), is one of the oldest domesticated food legumes have been cultivated for at least 5, 000 years [1]. Its exact geographical origin is unknown, although Central Asia and the Mediterranean region have been proposed as possible centers [2]. It is an important food legume in the Middle East and North Africa region is a good source of protein and also a strategic crop due to its income contribution to the farmers and its significance in the intensification of wheat-based system. It is important for soil fertility because of its nitrogen fixation properties. It also tolerates acid soil types and water logging better than other grain legumes [3]. World production is 3, 5 Million of Tonnes [3]. China is currently the world's leading producer, accounting for approximately 60% of the total [4]. In Algeria, Faba bean is grown on 2.5 million of hectares with an annual production of about 610845 tonnes [4]. The average yield of this crop under small-holder farmers is 2.4 t ha^{-1}, while world average grain yield of Faba bean is around 1.8 t ha^{-1} [3].

Though the agronomic and economic importance of faba bean is well known, its cultivation and productivity are affected by different biotic and abiotic stresses. The major biotic constraints of faba bean productivity and production in Mediterranean type environments are parasitic weeds (*Orobacnche* and *Phelipanche* spp) and foliar diseases like chocolate spot, rust and Ascochyta blight [5]. Ascochyta blight (*Ascochyta fabae*, sexual stage: *Didymella fabae* Jellis and Punithalingam), is the most prevalent disease where faba bean is grown in winter, either in Mediterranean-type or mild oceanic climates and spreads most rapidly in the early spring, before the crop starts flowering. The losses reported can reach 34 and 35% on green pod and dry seed yields, respectively [6].

Climate influences the pathogen and host environments separately and in interaction throughout the period of crop growth from infection to host death [7]. It is well known that temperature governs the rate of reproduction of fungi and the physiological conditions of the host and has a marked effect on the incidence of diseases [7]. Temperature also affects the growth and

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aggressiveness of pathogens and expression of disease symptoms in the plants [8]. Moreover, it has been demonstrated that inoculum density has been closely related with temperature and disease development [9].

The objectives of this study were to study the effect of five levels of temperature on mycelial growth and sporulation of fifteen isolates of *Ascochyta fabae* originated from western north of Algeria, collected in the periode 2015-2016 seasons.

MATERIALS AND METHODS

Collection of Faba Bean Leaves Samples and Isolates Conservation: Fifteen samples of faba bean leaves were collected from Western North of Algeria, in the periode 2015-2016 springs, showing ascochyta blight symptoms, were isolated, purified, monospored and conserved in PDA medium (Potato Dextrose Agar), (Table 1). *Ascochyta fabae* was isolated from faba bean leaves naturally infected by this disease. Leaves were surface disinfected with 1% sodium hypochlorite for 2 min and rinsed in two changes of sterile water, placed on PDA, incubated at 20°C for 7 days [10] and purified by repeated sub-culturing. Likewise, antagonistic fungal isolates residing on faba bean leaves were isolated on PDA media. The collected healthy looking leaf samples were washed in two changes of sterile water for 10 minutes each and macerated using mortar and pestle. The suspension was diluted at 10^{-2}, poured on PDA and incubated at 25°C for 7 days. All visible fungal colonies were isolated, purified, coded and stored at 4°C. The fungal isolates which were later found effective were identified [11].

Evaluation of Mycelial Growth and Sporulation of *A. fabae*: An agar disc, 6 mm in diameter, was taken from the actively growing margin of 10 day-old culture using a sterile cork borer and placed in the center of a 9 cm Petri dish containing 20 ml of PDA. All isolates were arranged together in completely randomized design with four replications and incubated at five temperature (20, 22, 24, 26 and 30°C) levels, for study its mycelial growth and sporulation [12]. The colony diameters (mm) were measured in two directions at right angles to each other at every 24 h interval until the mycelium fully covered the Petri dish. Colony morphology, texture and shape were characterized at full plate colony growth (6-10 days after incubation (DAI)). Sporulation and sclerotial production were estimated from 12 day-old culture for each temperature level per plate. For conidial size, the length and the width of 20 conidia per sample were measured [13]. Each plate was flooded with 10 ml of sterile distilled water and its entire surface was gently rubbed with a glass rod several times to release all the conidia. The spore suspension obtained was filtered through two layers of sterile gauze and was poured into a small beaker, the plate rinsed thoroughly and the final volume was adjusted to 20 ml by adding sterile distilled water [14]. Sporulation was determined under the microscope by counting 4 samples (0.1 ml each) per replicate [15]. Number and size of spores were counted and measured using the Malassez haemacytometer slide and micrometer under an optical microscope field of vision (10x eye piece and 40x objective) [16].

Table 1: Fifteen isolates of *Ascochyta fabae* with its origin and year of sampling

Isolate name	Province	Year of sampling
Af1	Mascara	2015
Af2	mascara	2015
Af3	Mostaganem	2015
Af4	Mostaganem	2015
Af5	Relizane	2015
Af6	Relizane	2015
Af7	Ain Temouchent	2015
Af8	Ain Temouchent	2015
Af9	Ain Temouchent	2015
Af10	Ain Temouchent	2015
Af11	Sidi Bel Abbes	2015
Af12	Sidi Bel Abbes	2015
Af13	Sidi Bel Abbes	2015
Af14	Tlemcen	2015
Af15	Tlemcen	2015

Measuring of Mycelial Growth and Sporulation: To estimate the mycelia growth, the technique used is that indicated by Kuçuk and Kivanç [17]. This method initially consists in measuring the mycelial growth linear day laborer of the colonies until the seventh day, according to the formula:

$$L = (D - d) / 2$$

L: Mycelial growth (mm),
D: Colony diameter (mm),
d: Explant diameter (=5mm).

The averages of mycelial growth are calculated by the formula:

$$C = \frac{\sum i^{\frac{(D-d)}{2}}}{n(j)} (mm / j)$$

C : Average of mycelial growth (mm/day);
D: Mycelial growths on the day *i*;
D: Explant diameter;
n : Days number.

Statistical Analysis: Data from two runs of experiments were pooled after confirming homogeneity of variances for growth and sporulation evaluation. Analysis of Variance (ANOVA) was performed to determine effects of incubation temperature on colony radial growth rate and sporulation. Regression analysis of diameters of colony radial growth against time after inoculation were performed and the slopes were used as measures of growth rates (mm day^{-1}) for each temperature treatment [18].

RESULTS

The averages of mycelial growth (C) of the isolates at different incubation temperatures is presented in Table 2. The results showed a significant ($P \leq 0.05$) reduction in the mycelial growth at 26 and 30°C compared to the temperatures at 20, 22 and 24°C. These averages of mycelial growth increased from 25 mm at 30°C to 75 mm at 22°C on 5 days. Averages of mycelial growth were affected by temperature. The isolate grew faster (15 mm day^{-1} at 22°C and 10 mm day^{-1} at 24°C) and relatively slower (9 mm day^{-1} at 26°C and 5 mm day^{-1} at 30°C), than at other temperatures tested (Table 2). The averages of mycelial growth followed a linear increasing trend at each incubation temperature over time (Figure 1).

Mycelial growth patterns included both light and dense extending mycelium. Highly dense mycelium with pycnids concentric rings associated with black colour was observed at both 20 and 22°C at later incubation period. Such characteristics were intermediate at 24°C, but a very thin mycelium with a very slow extending rate with few pycnids was obtained at 26 and 30°C (Figure 1).

The colony colour was similar at 20, 22 and 24°C. However, the pycnid production was very important at 20 and 22°C, due to rate of sporulation and distribution of conidia. The whitish colony (Composed of white mycelium), was observed nearly in the whole cycle of the incubation period at 26 and 30°C, with few pycnids production. Mycelial growth resumed when inoculums plugs were placed on PDA medium and incubated at the optimum (22°C) temperature was identified.

The isolate highly (2, 5×10^3 conidia ml^{-1}) sporulated at 22°C followed by 20 and 24°C temperature levels, with sporulation at 2, 3 and 1, 8×10^3 conidia ml^{-1}, respectively. No sporulation was recorded at 26 and 30°C (Table 3).

Table 2: Effect of five levels of temperature on mycelial growth of *A. fabae*.

	Averages[1] of mycelial growth (mm day^{-1})					
Temperature °C	24h	48h	72h	96h	120h	Mycelial growth increasing (mm day^{-1})
20	7[b]	22[b]	42[b]	60[b]	72[b]	13
22	8[a]	30[a]	50[a]	65[a]	75[a]	15
24	6[b]	20[b]	45[b]	58[b]	62[b]	10
26	6[c]	15[c]	20[c]	35[c]	50[c]	9
30	4[c]	10[c]	15[c]	25[c]	28[c]	5
Mean	6.2	19.4	34.4	48, 6	57, 4	
LSD[2]	2.85*	4.5*	4.65*	4.62*	3.32*	
CV %	23	13.2	8.5	6.67	4.55	

[1] Mean of four replicate; [2] Temperature levels are significant at $P_{0.05}$; * significant at $P_{0.05}$; CV: Coefficient of variation.

Table 3: Effect of five levels of temperature on sporulation of *Ascochyta fabae*.

Temperature C°	Sporulation[1] ($\times 10^3$ conidia ml^{-1})
20	1, 8
22	2, 5
24	2, 3
26	0
30	0
LSD[2]	0.26*
CV%	2, 32

[1] Mean of four replicate; [2] temperature levels are significant at $P_{0.05}$, *significant at $P_{0.05}$; CV: Coefficient of variation

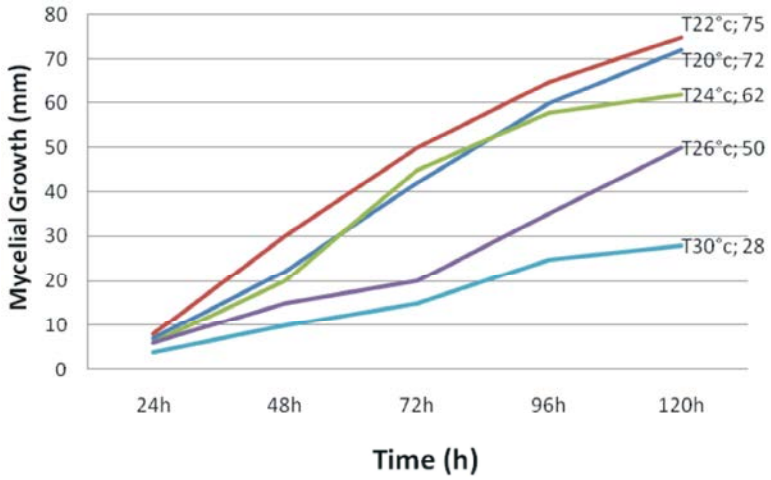

Fig. 1: *In vitro* effect of five levels of temperature (°C), on mycelial growth of *Ascochyta fabae* from 1 to 5 days after incubation

DISCUSSION

Many reports were published in the world on the importance of temperature on the cultural proprieties of phytopathogenic fungi [19-26]. This study was indicated differences in the effect of temperature levels on mycelial growth and sporulation of *Ascochyta fabae*. Most mycelial growth, sporulation and pycnidial formation were early observed at 22°C, but progressively these perfect morphological and cultural characters declined under or upper 22°C, with absence of sporulation at 26 and 30°C.

Fernandez *et al.* [27] found that temperature highly affected the mycelial growth of *B. cinerea* isolates and discriminate isolates based on their temperature optima. Pefoura *et al.* [23] showed that radial growth of *Trachysphaera fructigena* decreased to minimum at higher temperatures, which can be considered as lethal for radial growth of the pathogen. The investigators found that sporulation increased to optimum temperature and then declined till nil at higher temperature levels. Similarly, Sehajpal and Singh [28] noted that temperature of 20±1°C was the best for mycelial growth of *Botrytis gladiolorum* and the least was observed at 30±1°C. No conidial and sclerotial production was recorded at lower and extreme temperatures [12]. The rate of mycelial growth of *Sphaeropsis pyriputrescens* increased as temperature increased up to 20°C and then decreased rapidly as temperature increased. Slight changes in colony morphology were observed at lower and higher temperatures than the optimum temperature [29]. Fernando *et al.* [30] also reported that *Corynespora cassiicola* sporulated freely on PDA at 10 to 35 °C with a peak at 30 °C. However, no sporulation or growth of the colonies of the isolates was observed at temperatures below 5 and above 35°C.

Ondrej and Hunady [31] reported that the pycnids abundance of *Ascochyta fabae* has an important effect on virulence of the pathogen in the faba bean field. Ahmed *et al.* [32] showed that temperature of 22±2 °C was the optimum for ascochyta blight of faba bean development in Lebanon and Morocco.

CONCLUSION

These results indicated that temperature strongly influenced growth and sporulation of *Ascochyta fabae* which can effects on the development of ascochyta blight in faba bean varieties. Temperature at 22°C was the optimum temperature for the growth and sporulation development of *A. fabae*; whereas, low mycelia growth and nil sporulation were recorded at 26 and 30°C.

These methods are also useful for studying various aspects of epidemiology of chocolate spot disease and behavior of *A. fabae* under natural conditions. Such studies can improve our understanding of conditions required for epidemic onset, disease progress rate over time and eventual decline of epidemics; and could allow us to predict the initiation and potential severity of chocolate spot epidemics in the growing season. Absence of sporulation and highly reduced disease levels at 26°C. This information can help us to use type of faba bean varieties, winter or spring sowing. However, it is difficult to exactly predict the effect of increasing temperature on the host/pathogen and their interactions under controlled

conditions. Therefore, investigations on the effects of temperature on infection and disease development in faba bean due to *A. fabae* has to be further tested in the field conditions to valid these results.

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