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Isolation and physicochemical test studies of *Ascochyta pisi*

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Abstract

Anthracnose caused by pea *Ascocgyta pisi* is one of the most serious diseases affecting crops of peas (*Pisum sativum* L.) worldwide. In Algeria, more precisely in the north-west, quantitative data on the incidence of the disease is not available; it proved essential as a first step to isolate the pathogen and study *in vitro* the physicochemical factors affecting its growth. 10 strains were selected from 32 isolates from infected plants. The results indicate that the degree of the temperature of incubation affects the mycelia growth, at 4°C, mycelia growth was slowed and 37 °C inhibits the mycelia growth. We recorder that the optimum growth is between 22 and 25°C. Relative humidity affects the mycelia growth of the strains, the rate ≤ 50%, it is low at 74%, it is average, and other rate ≥ 80%, and it is optimal.. The usual media used (PDA, Malt, Mathur) give a good mycelia growth of the strains, but some have very low growth in the middle of pea; Overall, the usual empirical media used do not affect the mycelia growth of the strains. All strains assimilate the different carbon sources, however, is optimal mycelia growth on the (lactose, maltose and starch), the average (glucose, fructose), and weak on the cellulose. Mycelia growth is optimal on low nitrogen sources $NaNO_3$, KNO_3 and asparagine, on Valine, Leucine and L-arginine media, and slowed on the environment $(NH_4)_2SO_4$ the nature of the nitrogen source influences on the growth of the strains.

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Introduction

In Algeria, the grains of pea (*Pisum sativum* Lib.) are eaten fresh or dried and planted varieties are those with smooth or wrinkled grain (Latcha and Merveille). the scochyta blight is the most widespread disease of the pea (Kraft and Pfleger, 2001; Setti *et al.* 2008). *Ascochyta pisi* it causes characteristic symptoms that appear on all aerial parts of the plant; with lesions surrounded by a yellow chlorotic ring that turns into dark-brown spots missing separate margins on the leaves, and necrotic spots with a reddish center on pods making them unfit for consumption (Jones 1927, Champion *et al.* 1984). In Algeria the anthracnose caused by *Ascochyta* spp, a disease feared by farmers, but recent studies have shown the spread of anthracnose (Setti *et al.* 2008; Setti *et al.* 2010; Setti *et al.* 2011). During the spring of 2012, continuous rains, accompanied by lower temperatures, a period that has characterized the region of Oran (western Algeria), has fostered the emergence of anthracnose pulses, including small peas (Setti *et al.* 2012). Tivoli *et al.* (1996), reported that the free water on the leaves promotes sporulation. Differences in nutritional behavior are recorded in phytopathoènes fungi, the effects of pH, temperature and ionic concentration etc. are also considerable growth and sporulation of plant pathogens and consequently on symptom expression and pathogenesis.

The present work consists of two parts, the first is the morphological characterization based on a set of microscopic and macroscopic criteria and the second part is devoted to the study of different temperature growth factors, the water activity; different culture media; carbon source and amino acid on the mycelia growth of the isolates. This in vitro study is essential to the knowledge of *Ascochyta pisi*.

Material and methods

Fungal material

The samples are obtained from the leaves, stems and pods of two varieties with characteristic symptoms. Periods are those during the spring (February-March), the flowering time of freshness and moisture at this stage of growth, the plants are very susceptible to infection by *Ascochyta* (Allard *et al.* 1993) and also in June from the pods reached. Of each area visited, three samples were collected from different parts of the plant for laboratory analysis. The infected parts were placed in the wet paper envelopes and then dried in ambient air, then /they are stored at 10°C until used for the isolation of the causative agent.

Pathogen Isolation

Pathogens *Ascochyta pisi* are easily isolated from the plants peas which have the characteristic symptoms of anthracnose. The fragments were cut into 1 cm segments, their disinfected with sodium hypochlorite at 2% for 3 min surfaces, they are rinsed five times with distilled water, then dried on filter paper water (Rappilly, 1968). Fragments were placed in a petri dish containing a PDA medium (« 250 g of mashed potato boiled in 1L of water » 200 ml of filtrate are used, Dextrose 20 g, Agar agar 15 g; 800 ml of distilled water; pH= 5,19). Averages of three Petri dishes are incubated at 25 °C under continuous darkness for 7 days (Darby *et al.* 1986).

Purification strains d'Ascochyta pisi

In this study and unless otherwise indicated, the media used are PDA and the cultures are incubated at 28 °C under darkness. Petri dishes were inoculated centrally with small cuttings taken from 5 mm margin growth cultures *Ascochyta pisi* aged 7 days.

Preparation of inoculum

The method used, which consists in preparing the inoculum by adding 5 ml of sterile distilled water over 7 days old cultures. The pycnidiospores are released by scratching the surface with a bent Pasteur pipette, and then the spore suspension is filtered on sterile filter paper. For the dilution method, the concentration pycnidiospores is adjusted using a haemocytometer.

Monospore culture

0.5 ml of a dilution of 10^2 spores/ml was spread on Petri dishes containing agar medium at 2%. After 2-3 days of incubation, aseptically and using a binocular

microscope, the germinating spores are identified, removed one by one, and then placed on solid medium PDA. The microscopic and macroscopic identification is carried out after 7 days of incubation (Bouznad, 1978).

Identification of Ascochyta spp

On PDA, *A. pisi* is easily recognizable by her colony characters, and by her conidial morphology. The colonies are light in color, with light brown pycnidia scattered uniformly within the colony. The pycnidiospores, visiblent under the light microscope, are hyaline, septate, formed of two cells, small size approximately 11µm long, straight or slightly curved with rounded ends (Punithalingam and Holliday, 1972a). (Punithalingam and Holliday, 1972b; Maufras, 1996; Chen *et al.* 2004.). Some of these isolates are used in our experiments.

Pathogen Storage

For one short period of conservation, one stored the stocks on PDA inclined under the temperature of 4°C. According to Rapilly (1968), they to be it also in sterile water at the same temperature.

Strains used

Ten strains purified *Ascochyta pisi* were retained which represent a random sample representative of the areas and visited varieties (Setti *et al.* 2012).

Physicochemical study on the mycelia growth of Ascochyta pisi

In this study, and unless otherwise indicated, are solid medium used, the incubation temperature is 28°C in darkness, and all experiments were conducted in three replicates. The cultures in petri dishes were inoculated in the center by 5 mm smaller cuttings, taken from the margin of crop *Ascochyta pisi* aged 5 days. After 7 days incubation, growth of the colony diameter was measured in two perpendicular axes.

Effects of physical factors

Incubation temperature

The optimum temperature for mycelia growth of *Ascochyta* son culture media varies according to the origin of the isolates (Walter, 1973). In our experiments, the temperatures used are: (4, 15, 25 and 37 °C).

Medium pH

To obtain different desired (pH 4, pH 5, pH7, pH 8), the medium is adjusted with a buffer solution sterilized. The 0.05M sodium acetate buffer is used for the low pH (4 and 5), and 0.1M phosphate buffer pH 7 and 8.

Relative Humidity

Walter (1973) suggests that relative humidity parameters should be studied as a means of predicting the likelihood of infection from airborne spores.

To obtain the corresponding relative humidity (14%, 50%, 74%, 80% and 95%), we adjusted sterile saline solutions of various mixtures of NaCl and distilled water. For the humidity 100% sterile distilled water to replace the saline solution. After inoculation of petri dishes, the petri dishes are returned, and then 10 ml of the same saline solution is inserted into the lid. The boxes are sealed to prevent evaporation of the solution.

Exposure to the light

The type and duration of exposure of cultures to light plays an important role in the development of *Ascochyta* (Walter, 1973). In this experiment, the cultures were incubated in an incubator adjusted allowing the lighting (continuous light or dark and photoperiod of 12h ligh/12h dark).

Effects of chemical factors

Media empirical used

The media used: are: PDA, Pea (Flour of Pea 20g, Agar agar 15g ; distilled water 1L; pH= 5,81), Malt (Malt extract 20g, Agar agar 15g ; distilled water1L; pH= 5,5), Mathur Mathur-agar (Glucose 2,8g, $MgSO_4$ 1,2g, KH_2PO_4 2,7g, Peptone 1g, Yeast extract 0.5g, Agar-agar 15g, water1L; pH = 5,65).

Carbon source

The carbon source of the medium Czapek (Glucose 20 g, KH_2PO_3 1g, Na_2NO_3 2g, KCl 0.5g, $FeSO_4$, 0.1g, Agar agar 15g, distilled water 1L, pH= 5.5) was substituted by: monosaccharide (glucose, Fructose), disaccharides (lactose, Maltose, and Sucrose), polysaccharide (starch, cellulose).

Nitrogen source

According Jacquet (1979), most fungal organisms use nitrogen sources in the form of nitrate nitrogen and ammonia nitrogen, what led us to find the best nitrogen source for the growth of our strains. The, Nitrogen source, of the medium Czapek, was substituted by organic Nitrogen source (Peptone, Valine, Leucine, L-Arginine, Asparagine) or by minerals Nitrogen source ($NaNO_3$, KNO_3, $(NH_4)_2SO_4$).

Evaluation of mycelial growth

After seven days of incubation, and in all experiments, mycelia growth was measured by the average of two perpendicular diameters most dissimilar. Three replica plates were used.

Statistical Analysis

The values of the diameter growth on different culture media were compared statistically by analysis General Multivariate Linear Model of SPSS (Statistical Package for the Social Sciences) at 5%.

Results

Aspect microscopique

The mycelium is partitioned, Pycnidia are globular inside which will foment the asexual spores, they are provided with an ostiole through which pycnidiospores (conidia) exit outwards The pycnidiospores are hyaline, straight or slightly curved with two rounded ends, they are bicellular, monocellular and rarely consist of two cells (Champion, 1984) (Fig. 1).

Table 1. Strains Ascochyta pisi used (Terbeche, 2011).

Strain	Area	Infected organe	variety
Ap1	*Es Senia*	Stems	*latcha*
Ap2	*Hassi Bounif*	stems	*latcha*
Ap3	*Misserghin*	Stems	*merveille*
Ap4	*Es Senia*	leaves	*latcha*
Ap5	*Hassi Bounif*	leaves	*latcha*
Ap6	*Misserghin*	leaves	*merveille*
Ap7	*Es Senia*	pods	*latcha*
Ap8	*Hassi Bounif*	pods	*latcha*
Ap9	*Es Senia*	pods	*merveille*
Ap10	*Hassi Bounif*	pods	*merveille*

Table 2. Mean values the growth of mycelia (cm) and significance degree of differences between strains on different incubation temperature.

	temperature				
strain	4°C	15°C°	20°C	25°C	37°C
Ap1	1,37 cd	3,06 de	5,2 f	4,97 ab	0.0
Ap2	1,4 d	3,1 e	4,7 cd	5,01 b	0.0
Ap3	1,3 bc	3,2 f	4,87 e	5,87 d	0.0
Ap4	1,1 a	2,97 c	4,7 cd	5,4 c	0.0
Ap5	1,4 d	3,0 cd	4,87 e	5,0 ab	0.0
Ap6	1,27 b	3,06 de	4,47 a	5,06 b	0.0
Ap7	1,4 d	2,8 ab	4,5 ab	4,9 a	0.0
Ap8	1,4 d	2,77 a	4,6 bc	5,0 ab	0.0
Ap9	1,56 e	2,87b	4,8 e	5,0 ab	0.0
Ap10	1,37 cd	3,06 de	4,7 cd	4,97 ab	0.0

Means in each column followed by the same letters are not significantly different at $P < 0.05$.

Aspect morphologique

On a culture medium based PDA, *Ascochyta pisi* present mycelia colonies that appear white or creamy first time, then take various pigmentation (brown, black) following the development of pycnidia and pycnidiospores (Champion, 1984) (Fig. 2).

Effects of physical factors

Incubation temperature

Growth arrest is recorded by the strains at 4 ° C and is completely inhibited at 37 ° C (fig 3). The favorable temperature mycelia development is 25° C, the intermediate values are observed at 15 and 25 ° C. Statistical analysis shows that under the same incubation temperature, there are significant differences; in addition, between the temperature ≤ 25° C, there are no significant differences. At the interaction stem-incubation temperatures, there are significant differences. Therefore, the incubation temperatures used affect the mycelia growth (Table 2).

Table 3. Mean values the growth of mycelia (cm) and significance degree of differences between strains over different pH.

Strain	pH 4	pH 5	pH 7	pH 8
Ap1	4,01 a	4,71 bc	4,88 b	4,99 ab
Ap2	4,25 ab	4,56 a	4,98 c	5,04 bc
Ap3	4,42 b	4,63 ab	5,13 e	5,2 d
Ap4	4,3 b	4,57 a	5,0 cd	5,1 c
Ap5	4,29 b	4,82 d	4,95 bc	4,97 ab
Ap6	4,27 ab	4,67 bc	5,0 cd	4,98 ab
Ap7	4,22 ab	4,67 bc	5,08 de	4,93 a
Ap8	4,22 ab	4,72 bc	4,93 bc	5,03 abc
Ap9	4,37 b	4,75 cd	4,98 c	5,02 abc
Ap10	4,26 ab	4,83 d	4,78 a	5,02 abc

Means in each column followed by the same letters are not significantly different at P < 0.05.

Table 4. Mean values the growth of mycelia (cm) and significance degree of differences between strains according to the percentage of the relative humidity.

	humidity					
Strain	100%	95%	80%	74%	50%	14%
Ap1	4,42 ab	3,91 ab	3,99 ab	3,54 c	2,73 bc	2,61 de
Ap2	3,48 a	3,7 a	3,6 a	2,25 a	1,9 a	1,72 a
Ap3	3,96 ab	3,62 a	3,68 a	3,28 bc	2,62 abc	2,46 cde
Ap4	3,8 ab	4,49 bc	4,38 bc	3,15 abc	2,85 bc	2,43 bcde
Ap5	4,8 c	4,49 bc	4,38 bc	3,15 abc	2,85 bc	2,43 bcde
Ap6	4,13 ab	4,47 bc	4,27 bc	2,47 ab	2,05 ab	1,83 ab
Ap7	4,58 bc	4,95 c	4,56 bc	2,58 ab	2,17 ab	1,92 abc
Ap8	4,73 bc	5,01 c	4,73 c	2,67 abc	2,22 ab	2,02 abcd
Ap9	3,79 ab	4,57 bc	4,55 bc	3,55 c	3,08 c	2,64 d
Ap10	4,8 c	4,49 bc	4,38 bc	3,15 abc	2,85 bc	2,43 bcde

Means in each column followed by the same letters are not significantly different at P < 0.05.

Medium pH

Strains show varying at each pH tested (fig 4) responses. PH 7 and 8 are the most favorable for mycelia growth, however, acid pH are less favorable. The statistical analysis indicates that in different strains the pH showed significant differences between them, moreover, between pH> 4, there are no significant differences. However, at the interaction of strain-pH environment, there are significant differences. Therefore, the nature of the pH of the medium affects the mycelial growth.

Humidity relative (RH)

The relative humidity ≤ 50% rate, mycelia growth is

low at 74%, it is average, and other rate ≥ 80%, it is optimal (Tab 4). Statistical analysis shows that between the humidity ≤ 74%, there are significant differences. At the strain- humidity interaction, there are significant differences. Also, the relative humidity affects the mycelia growth of the strains.

Table 5. Mean values the growth of mycelia (cm) and significance degree of differences between strains over different duration from light.

Strain	light	obscurity	photoperiod
	continue	continue	light/dark 12h
Ap1	5,46 ab	4,55 ab	5,42 abcd
Ap2	5,5 ab	4,77 b	5,59 cd
Ap3	5,54 ab	4,38 ab	5,1 a
Ap4	5,61 ab	4,47 ab	5,56 bcd
Ap5	5,27 a	4,31 a	5,2 abc
Ap6	5,67 b	4,35 a	5,35 abcd
Ap7	5,42 ab	4,29 a	5,12 a
Ap8	5,27 a	4,37 a	5,13 ab
Ap9	5,43 ab	4,5 ab	5,15 ab
Ap10	5,63 ab	4,67 ab	5,65 d

Means in each column followed by the same letters are not significantly different at $P < 0.05$.

Table 6. Mean values the growth of mycelia (cm) and significance degree of differences between strains over different complex media.

	Medium			
Strain	PDA	Malt	Mathur	pea
Ap1	6,0 a	5,47 a	5,37 a	5,2 bcd
Ap2	6,0 a	5,73 a	5,27 a	4,9 ab
Ap3	6,1 a	5,87 a	5,37 a	5,33 bcd
Ap4	6,2 a	5,9 a	5,33 a	5,43 cd
Ap5	6,17 a	6,0 a	5,4 a	5,1 bc
Ap6	5,5 a	6,0 a	5,27 a	4,63 a
Ap7	5,47 a	6,1 a	5,23 a	5,3 bcd
Ap8	5,9 a	6,17 a	5,43 a	5,4 cd
Ap9	5,87 a	6,2 a	5,43 a	5,47 cd
Ap10	5,73 a	5,5 a	5,2 a	5,6 d

Means in each column followed by the same letters are not significantly different at $P < 0.05$.

Exposure to the light

Mycelia growth is lower in the dark, under the optimal continuous dim light under a 12/12h photoperiodicity (Table 5). Statistical analysis shows that the strains show significant in the presence or absence of light differences, on the other hand, between different photoperiodicity, there are significant differences. At the interaction strain-photoperiodicity, there are significant differences. Thus, exposure of cultures to the dark or light influences the mycelia growth of the strains.

Effects of chemical factors

It appears from the table (Table 6) that the different media used, all strains give a good mycelia growth, and however, optimal growth is obtained on PDA and malt media. The Ap6 strain gives a very low growth in the media of pea. Statistical analysis shows that strains do not show significant differences in media (PDA, Malt, Mathur), but between these environments, there are significant differences. Therefore, the empirical media used (PDA, Malt, Mathur) does not affect the mycelia growth of the strains.

Carbon source

All strains assimilate various carbon sources (Table 7), however, is optimal mycelia growth on the

(lactose, maltose and starch), the average (glucose, fructose), and weak on the cellulose. Statistical analysis indicates that between strains, growth on media (glucose, fructose, maltose and sucrose) there's no significant differences, more at the source of carbon-strain interaction, all carbon sources, there are no significant differences. Finally, the nature of the carbon source used does not affect the growth of strains.

Table 7. Mean values and significance degree (cm) of differences between strains for sugar assimilation.

strain	Glucose	Fructose	Lactose	Maltose	Sucrose	Strach	Cellulose
Ap1	5,43 a	5,8 a	5,33 a	6,27 a	5,97 a	6,0 ab	4,3 ab
Ap2	5,63 a	5,77 a	6,13 ab	6,4 a	6,0 a	5,97 a	4,33 ab
Ap3	5,6 a	5,73 a	6,07 ab	6,1 a	5,7 a	6,07 abc	4,1 ab
Ap4	5,67 a	5,73 a	6,0 ab	6,2 a	5,77 a	6,07 abc	4,13 ab
Ap5	5,67 a	5,8 a	5,73 ab	5,9 a	5,67 a	6,47 c	3,77 a
Ap6	5,7 a	5,8 a	6,23 b	6,37 a	5,93 a	6,27 abc	4,37 ab
Ap7	5,7 a	5,8 a	6,2 ab	6,17 a	5,77 a	6,2 abc	3,97 ab
Ap8	5,63 a	5,83 a	6,17 ab	6,43 a	5,9 a	5,9 a	4,27 ab
Ap9	5,37 a	5,93 a	6,17 ab	6,4 a	5,87 a	6,43 bc	4,53 ab
Ap10	5,73 a	5,97 a	5,97 ab	6,33 a	6,0 a	5,93 a	4,57 b

Means in each column followed by the same letters are not significantly different at $P < 0.05$.

Table 8. Mean values the growth of mycelia and significance degree of differences between strains for the assimilation of nitrogen source.

Strain	$NaNO_3$	KNO_3	$(NH_4)_2SO_4$	Valine	Leucine	L-arginine	Asparagine
Ap1	6,53 a	6,4 b	0,9 b	2,93 ab	2,4 b	2,3 bc	5,1 a
Ap2	6,1 a	6,17 a	0,8 a	2,97 ab	2,67 c	2,3 bc	6,0 d
Ap3	6,63 a	6,5 b	0,8 a	2,87 ab	1,9 a	2,6 d	5,13 a
Ap4	6,73 a	6,87 d	0,8 a	2,83 ab	1,9 a	1,73 a	6,0 d
Ap5	6,47 a	6,9 de	0,9 b	2,7 a	2,63 c	2,63 d	6,07 de
Ap6	6,6 a	6,93 de	0,9 b	3,07 ab	2,63 c	2,53 d	6,13 ef
Ap7	6,9 a	6,9 de	0,8 a	2,67 a	2,3 b	2,5 c	6,17 f
Ap8	6,83 a	7,0 e	1,2 e	3,53 c	2,93 d	2,23 b	5,7 b
Ap9	6,23 a	6,5 b	1,0 c	2,87 ab	1,97 a	2,7 d	5,73 bc
Ap10	6,07 a	6,73 c	1,03 d	3,23 bc	2,47 bc	2,3 bc	5,8 c

Means in each column followed by the same letters are not significantly different at $P < 0.05$.

Nitrogen source

Mycelia growth is optimal on low nitrogen sources $NaNO_3$, KNO_3 and asparagine, on Valine, Leucine and L-arginine media, and slowed on the environment $(NH_4)_2SO_4$ (Table 8). Statistical analysis indicates that except for the middle $NaNO_3$, between strains, growth on the same medium gives significant differences over the level of interaction strain-carbon sources, there are significant differences. Therefore, the nature of the nitrogen source influences on the growth of the strains.

Discussion

The degree of the temperature of incubation affects the mycelia growth of the strains. According to Henry *et al.* 1995, the temperature affects the metabolism of chemical and biological reactions mushrooms. The temperature below 15°C and the slow growth of the 37

completely inhibits, these observations are consistent with those Allard *et al.* 1993 and Tivoli and Lemarchand, 1992. According Singh and Reddy, 1990, the temperature below 6°C which prevents the infection process. The optimum growth is obtained between 22 and 25 ° C, which are consistent with the results of epidemiological studies by Bedi and Aujla, 1970. Nene *et al.* 1987 argues that the formation of pycnidia and spore germination in this pathogen are at about 20 ° C.

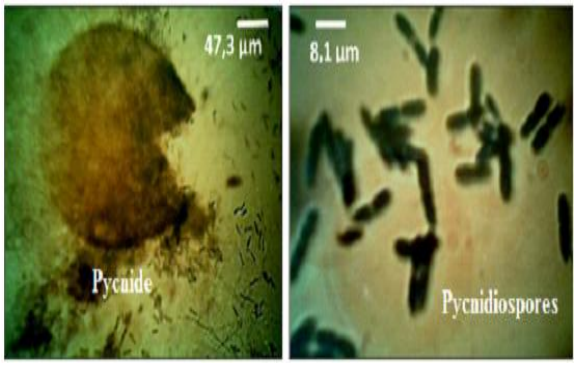

Fig. 1. Pycnidia and pycnidiospores *Ascochyta pisi (Gharbi, 2013).*

The media pH between pH 4 and 8 do not affect the mycelia growth of the strains. This is consistent with the work of Regnault, 1990 state that the optimal pH for mycelia growth of mold is between pH 2 and pH 9. Absida, 1985 noted that the amylolytic highest yield by *Aspergillus flavus* was performed at pH 7 and pH 8.

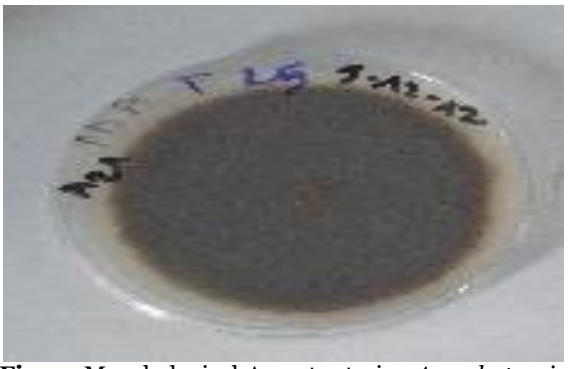

Fig. 2. Morphological Aspects strains *Ascochyta pisi* on pea media.

The relative humidity affects the mycelia growth of the strains. The relative humidity ≤ 50% rate, mycelia growth is low at 74%, it is average, and other rate ≥ 80%, and it is optimal. Also, the relative humidity affects mycelia growth strains. Dixon (1988) reported that the severity of *Ascochyta* spp. is higher in wet areas than in dry regions, and that *Ascochyta* diseases could be controlled by the production of pathogen-free seeds in arid areas. Lawyer, 1985 also reported that seed production of dry peas that most of the inoculums became external form of dust or small particles areas.

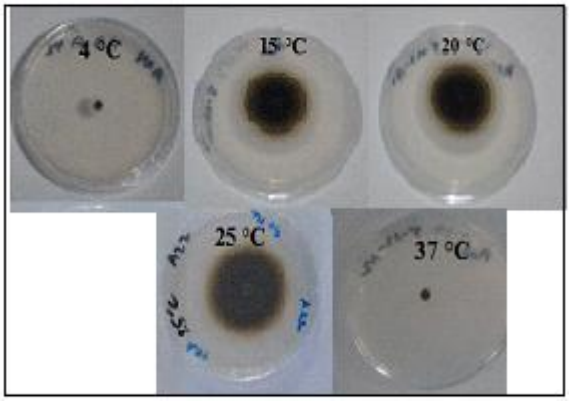

Fig. 3. Influence of temperature on the mycelia growth.

The growth of *Ascochyta pisi* was observed at darkness, light and 12 h photoperiod. Mycelia growth is lower in the dark, under the optimal continuous dim light under a 12/12h photoperiodicity. Thus, exposure of cultures to the dark or light influences the mycelial growth of the strains. Indeed, the brightness of the trigger 12 h favored infection (Singh and Reddy, 1990). It was reported that darkness favors maximum uredospore germination of legume rust pathogen *Uromyces viciae-fabae* (Emeran *et al.* 2005).

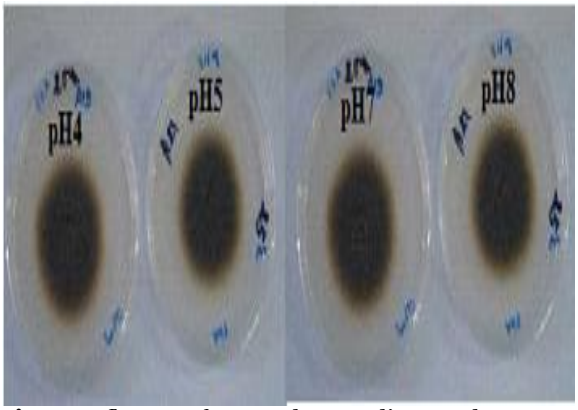

Fig. 4. Influence of pH on the mycelia growth.

The usual media used (PDA, Malt, Mathur) give a good mycelia growth of the strains. PDA is one of the most commonly used culture media because of its

simple formulation and its ability to support mycelia growth of a wide range of fungi. (Sharma and Pandey, 2010). Osman *et al.* 1992 studied the effect of different culture media on fungi and found that PDA was the best growth medium. Some strains give a very weak growth in the middle of pea. Overall, the usual empirical media used do not affect the mycelia growth of the strains.

All strains assimilate the different carbon sources, however, is optimal mycelia growth on the (lactose, maltose and starch), the average (glucose, fructose), and weak on the cellulose. Moreau, 1968 showed that most of the carbon sources may be used for fungi; however, the nature of the carbon source used affects the growth of the strains. Moreover, Jacquet, 1979 showed that some carbon sources have an influence on the growth and reproduction of fungi.

The nature of the source of nitrogen affects the growth of the strains. Mycelia growth is optimal e on nitrogen $NaNO_3$, KNO_3 and Asparagine and slowed on the environment $(NH_4)_2SO_4$ sources. Therefore, the nature of the nitrogen source influences on the growth of the strains. Generally nitrates are considered an excellent source of nitrogen for many imperfect fungi (Benaouali *et al.* 2014). Dandge, 2012 noted that nitrates of Ca, Na and Mg are less used by fungi nitrates and showed that the NaNO3, KNO3 and asparagine media, there is an excellent growth mycelia.

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