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Some Physiological and Control Studies on *Septoria pisi*, the Causal Pathogen of Pea Leaf and Pod Spots

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Leaf and pod spot disease of pea (*Pisum sativum* L.) caused by *Septoria pisi* Westend is considered as a common factor that reduces yield production. Three isolates of *Septoria pisi* were isolated from naturally infected leaves and pods of pea showing spots collected from Minia and Matai Provinces, Minia Governorate. Pathogenicity test indicated that these isolates were differed in their virulence; isolate S_1 was the most virulent one. Master B, Qena 1 and Qena 3 pea cultivars were susceptible to infection, whereas Jaguar and Palmoral cvs were more resistant. Pycnidia formation and sporulation of *S. pisi,* which are frequently used as propagules for infection and also are necessary to identify the species, can be scarce and are often reduced when the fungus is cultivated *in vitro*. A series of experiments were conducted to assess the effect of temperature, culture media, carbon and nitrogen sources on *S. pisi* growth and pycnidial production. Best results were obtained when fungal colonies were grown at $22\pm2^{o}C$ on oat meal, malt and pea dextrose of natural media and Czapek's medium from the synthetic media, in 12 hours-photoperiod at pH 6.5. The lowest growth and sporulation was recorded on basil and garlic extracts media. The best growth and spore formation were obtained when fructose, glucose, maltose and sucrose represented as the sole carbon sources, followed by raffinose, dextrin and starch. Pectin, sorbitol, manitol and sodium citrate proved to be poor sources of carbon for the growth of the tested fungus. Ammonium nitrate and ammonium sulphate mostly favored fungal growth. Minimum growth was observed when each of urea or asparagine was used as a sole nitrogen source. Peptone and the amino acid threonine were the best nitrogen sources for sporulation and forming pycnidia of *S. pisi.* Whereas, no spores or pycnidia were formed when each of sodium nitrite, cystine or cysteine was used as a sole nitrogen sources.

Keywords: Carbon and nitrogen sources, media, pea leaf and pod spots, *Septoria pisi*.

Pea (*Pisum sativum* L.) is one of the most important legume crops in several parts of the world. It cultivated either in protected cultures or in fields. Pea is considered a valuable food source for many people throughout the world. It belongs to Family *Fabaceae* (*Leguminosae)*. Like many legumes, it has the ability to fix nitrogen from the air through a symbiotic relationship with *Rhizopium* spp. (bacteria housed in root nodules) making it very rich in protein. Pea seeds are high in carbohydrates, protein, fibers, vitamins and many important minerals, *viz.* iron,

magnesium, phosphorus and zinc. There are a number of diseases which infect pea plants in Egypt and cause fairly extensive damage. In addition, there are a number of minor diseases which, causing considerable damage to pea crop. Septoria leaf spot of pea is considered one of this latter category of these diseases (Cruikshank, 1947). *Septoria* is a widespread genus of fungi, estimated to contain about 1072 species (Kirk *et al*., 2008), which attacking various plant species belonging to different families causing numerous leaf spot, blotch and leaf-blight diseases and is responsible for yield losses (Zitter, 1987).

Leaf spot or blight of pea caused by *Septoria pisi* Westend is one of the common factors that reduce yield production (Anonymous, 2014). This pathogen infects the older plant leaves, and causes destructive necrotic symptoms, that lead to yield loss. During the last few years, septoria leaf spot on pea has been occurring almost every year, essentially due to the survival of pycnidia and spores of the fungus, which can persist from one season to the next on debris of diseased plants incorporated in the soil, cultivation of susceptible varieties and favorable environmental conditions. The fungus can also overwinter on leguminous weeds (Cooke and jones, 1970 and Lee and Jones, 1974). Septoria blight (*S. pisi*) can cause problems in some localities in the world, for example in parts of New South Wales (Hawthorne *et al*., 2012). Fungal sporulation is a key component for several purposes because fungal spores are frequently used as propagules to infect plants and they are necessary to identify the species. *In vitro* experiments, spores are usually used to study the nature and control of plant diseases caused by biotic agents. In case of leaf and pod spots caused by *Septoria* spp, conidial suspensions are commonly used as inoculum, but sporulation *in vitro* can be scarce (Saidi *et al.*, 2012). It is well known that *in vitro* sporulation of *Septoria* spp. requires special conditions and that conidial production tend to decrease after periodic subculturing of the pathogen. Considerable attention has been directed towards improving conidial production of *Septoria* species by using favorable and unfavorable conditions for vegetative growth (Richards, 1951; Eyal, 1999; Kema and Annone, 1991; Guo and Verreet, 2008 and Saidi *et al*., 2012). Three major factors that influence sporulation *in vitro* are commonly wielded by nutrition, light spectrum, and temperature (Rodrigues *et al*., 2010). Natural culture media are suitable for sporulation, storage, and to maintain viability of the colony after subculturing (Dhingra & Sinclair, 1995). For example, V8 juice medium, PDA (potato-dextrose-agar) and media with parts or extracts of plants are used in protocols to encourage sporulation of *Septoria* spp and other fungi (Miller, 1955 and Shahin and Shepard, 1979).

This study aimed to isolate the causal of leaf and pod spot of pea to investigate the favourable temperature, media, sources of carbon and nitrogen for the growth and sporulation, also the ability of the fungus to infect pea plants (pathogenicity test) and to study the response of different pea cultivars and lines to the infection by the isolated fungus.

Materials and Methods

1- Sampling, isolation, purification and identification of the pathogenic fungus:

Naturally diseased pea (Master B cv.) plants and pods were collected from different fields and popular markets at Minia and Matai counties, Minia governorate, Egypt, during September – December, 2015 and 2016 growing seasons. The collected samples were used for isolating the pathogenic associated fungi with leaf, stem- and pod-spot symptoms. The diseased plant organs were surface sterilized by dipping in sodium hypochlorite solution (2%) for two minutes. The surface sterilized plant organs were washed twice thoroughly with sterilized water and dried between two folds of sterilized filter papers. Small pieces of the sterilized spotted leaves, stems and pods were cut with sterilized scalpel and aseptically plated on potato dextrose agar (PDA) medium. To prevent bacterial contamination, 200 mg mixture of Streptomycin sulfate with Penicillin ($1x10^6$ unit of Penicillin in 3g Streptomycin sulfate) were added to one liter medium when medium temperature was approximately at 40-45°C (Saidi *et al*., 2012).

The plates were incubated at 20 and 25±2°C and checked daily until 7 days. The developed mycelium fungal colonies were purified by hyphal-tip technique, and sub-cultured on PDA medium. Inoculated test tubes containing slants of PDA medium were incubated and kept at 5±2°C as stock cultures of the isolated fungus for further studies. Identification of the purified fungus was accomplished according to the keys given by Cunfer and Ueng (1999), Crous (2009) and Verkley *et al.* (2013). Also, identification was confirmed at the Center of Fungi, Faculty of Science, Assiut University.

2- Pathogenicity test:

Pot experiment was carried out in the experimental open field of the Plant Pathology Department, Faculty of Agriculture, Minia University, Minia, Egypt. Surface disinfected pea (Master B cv.) seeds, obtained from Egyptian Ministry of Agriculture and Land Reclamation, Central Managing for Seed Examining and Credited, were sown at 30th October 2015, in sterilized clay pots (30 cm in diameter x 40 cm in height) containing autoclaved Nile clay soil. Pots were sterilized, 18 days before sowing, by dipping in formalin solution (5%) for about 5 minutes then aerated for 15 days before being used. Surface seed sterilization was performed through dipping in sodium hypochlorite solution (2%) for 2 minutes followed by washing in several changes of sterilized tap water.

Eight seeds were sown in each pot and immediately after emergence, seedlings were thinned to five seedlings/pot. Pea plants, 5 and 7 weeks old, were sprayed with a fungal propagules suspension ($1x10^5$ propagules/ml). The spores and the mycelia were collected from 2-weeks-old- oat liquid culture by filtration and shaken well. About 20 ml of the fungal suspension were foliar sprayed to each individual plant in the early morning using a manual atomizer (an adequate amount to cover the plant leaves). One drop of Tween-20 was added to the spore suspension to enhance the adhesion of spores on the leaves. To insure high humidity around inoculated organs, plants were covered with polyethylene covers for 24 hours. Check control plants were sprayed with distilled water, instead of spore suspensions. Three pots, with five

plants / pot, were served as a replicate and three replicates/treatment were applied. Seeds were mixed with pea symbiotic bacteria (*Rhizopium* sp., commercial package obtained from Institute of Soil and Water Res. Ins., ARC, Giza, immediately before planting. Plants were fertilized as recommended by Min. of Agric., and plants were irrigated when necessary.

Disease assessments:

Twenty days after inoculation, the disease was scored by determine the disease incidence (DI,%). Disease severity (DS,%) also was determined. The disease index described by Cohen *et al.* (1991) was used to classify the disease severity in this study. However, the leaves and pods (15 pods/ treatment were chosen in random) infection was rated on a scale of 0 to 4 categories as follows:
0 = healthy (no visible symptoms), 1= less than 25% of the leaflet or pod with scattered flecks, 2= from 26 to 50% of the leaflet or pod with flecks, 3= from 51 to 75% of the leaflet or pod with flecks and 4= more than 75% to 100% of the leaflet or pod with flecks.

The readings were converted to disease severity index using the formula developed by Liu *et al*. (1995) as follows: Disease severity (DS, %)= Sum (n x r)/4N X100, Where, n = frequency number /category, r = 0 – 4 categories, and N = the total number of examined leaflets or pods. Reisolation from the artificially diseased leaves and pods was carried out and the resultant fungus was compared with the original culture.

2- Response of different pea cultivars and lines to the isolated fungus:

Three cultivars and four lines of pea, namely Master B, Jaguar, Palmoral, Qena line1, Qena line3,Yellow line ,Yemeni line, were used to study their reaction to the infection by the isolated fungus, which caused leaf and pod spots of pea. Seeds of the previous mentioned cultivars and lines were kindly taken from Prof. Dr. Yasser Mostafa, Branch of Vegetable Crops, Dept. Hort., Fac. Agric., Minia Univ. Experimental pots were applied at 2015-2016 and 2016-2017 growing seasons in the open experimental field of Plant Pathology Dept., Fac. of Agric., Minia Univ. Inocula and plants inoculation were applied as mentioned before in pathogenicity test. As well as, pots, soil and seed preparation were carried out as described in pathogenicity test. The percentages of disease incidence (DI, %) and disease severity (DS,%) on leaves and pods were calculated 60 days after planting as described before.

3- Physiological studies:

Due to the reducing number of *Septoria pisi* mature pycnidia, that discharged conidiospores, some laboratory studies were carried out to courage the isolated fungus for spores formation, seven degrees of temperature, nine different liquid media, twelve carbon sources and eleven nitrogen sources were tested. In the following experiments, mycelial dry weight (d. wt. in mg) or linear growth (LG in mm) was measured and number of formed pycnidia was counted as a criterion for evaluating the effect of treatment. Except when otherwise mentioned, all experiments were performed at pH 6 - 6.5 (Saidi *et al*., 2012). The pH value of the media was adjusted before sterilization with 0.1N NaOH or 0.1N HCl. Media were

autoclaved (for 15 minutes at a pressure of ½ Kg/cm^2, and then inoculated with 5 mm discs cut out with a sterile cork-borer from the growing edges of 7-days-old cultures of a high virulent isolate (S_1) of the isolated fungus to be tested. After inoculation, flasks and/or plates were incubated at 22±2°C with a photoperiod of 12 h light and 12 h darkness (Saidi *et al*., 2012). Three Erlenmeyer flasks (replicates) were used for dry weight determination; also, three plates were used to measure the linear growth and/or to count mature pycnidia.

1- Effect of temperature on the growth and pycnidial formation of the tested fungus:

The effect of different degrees of temperature on the linear growth and produce of pycnidia of the isolated pathogenic fungus was studied by keeping inoculated Petri plates containing oat medium at 5, 10, 15, 21, 27, 30 and 35°C. The linear growth was measured, 7 days after inoculation. Also, the number of pycnidia of *S. pisi* was counted, 25 days after inoculation on agar-solid media using a microscope (10x10 magnified lens). The number of pycnidia was counted at 1.5 and 4 cm away from the center of inoculated plate.

2-Effect of different media on growth of the tested fungus:

The effect of different liquid media *i.e.,* potato dextrose, pea dextrose, malt dextrose, oat meal dextrose, garlic dextrose, onion dextrose, basil dextrose, Czapek's and Waxman's, on the amount of growth (in mg/100 ml medium) of the pathogenic fungus was studied by incubated the inoculated conical flasks (100 ml each containing 50 ml medium) at 22±2°C. In each treatment, the mycelial mats were harvested, 10 days after inoculation, dried at 70°C for 24 hours, and then weighed. Pycnidia were counted at 1.5 and 4cm from the center of plate, 25 days after incubation at 22±2°C, using the same media supplemented with agar as described before.

3-Effect of different carbon sources on growth and pycnidial formation of the tested fungus:

This study was carried out using Czapek's liquid medium lacking sucrose as a basic medium. Different carbon sources (listed in Table 1), except dextrose, pectin and starch; whose molecular formula are uncertain, were added separately at a concentration that provided the same carbon content as in regular Czape's medium *i.e.*, 12.63 g C/l, w/v) Whereas dextrose, pectin and starch were added at a concentration that was similar to that of sucrose in the regular medium (3%). Total growth was evaluated using liquid medium contained in 100 ml conical flasks (50 ml/flask) after 10 days incubation at 22±2°C. Control free of carbon source was run additional in parallel. Pycnidia were counted in Petri dishes contained the same media supplemented with agar to be solid as described before.

Table 1. Molecular formula, and amounts of the used carbon sources

Carbon source	Molecular formula	Amount in g/l
Fructose	$C_6 H_{12} O_6$	31.58*
Glucose	$C_6 H_{12} O_6$	31.58
Maltose	$C_{12} H_{22} O_{11}$	30.00
Raffenose	$C_{18} H_{32} O_{16}$	30.00
Sucrose	$C_{12} H_{22} O_{11}$	30.00
Ascorbic acid	$C_6 H_8 O_6$	30.88
Sodium citrate	$C_6 H_5 O_7Na_3$	45.26
Manitol	$C_6 H_{14} O_6$	31.96
Sorbitol	$C_6 H_{14} O_6$	31.96
Dextrin	-----------	30.00
Pectin	-----------	30.00
Starch	-----------	30.00

**Amount of each source provides either the same carbon content (12.63 g), or, if the molecular formula is uncertain, the same source ratio (3%) in the regular Czapek's medium.*

4-Effect of different nitrogen sources on growth and pycnidia formation of the tested fungus:

This study was carried out using Czapek's liquid medium lacking potassium nitrate as a basic medium. Eleven inorganic or organic nitrogen sources (listed in Table 2) were added separately to the basic medium. Inorganic nitrogen sources were provided in form of nitrite (sodium nitrite), nitrate (potassium and sodium nitrate), ammonium (ammonium sulphate), or both latter N forms (ammonium nitrate). Organic nitrogen sources were provided in the form of sulphoric amino acids cysteine and cystine or non sulphoric (Asparagine and Methionine) or non-amino acid (Urea and peptone).

The nitrogen source of uncertain molecular formula (peptone) was added at the same level of KNO_3 (2 g/l) as in regular Czape's medium; other nitrogen sources were added at a concentration that provided 0.329 g N/l (the same nitrogen content in the regular Czape's medium). Total growth (in mg) was evaluated using liquid media contained in 100-ml conical flasks (50 ml/flask) after 10 days incubation at 22±2°C. Also, the formed pycnidia were determined using agar solid media, 25 days after inoculation as mentioned in carbon source evaluation.

Table 2. Molecular formula, and amounts of the used nitrogen sources

Nitrogen source	Molecular formula	Amount in g/l
Ammonium nitrate	NH_4NO_3	0.79*
Ammonium sulphate	NH_4SO_4	4.51
Potassium nitrate	KNO_3	2.00
Sodium nitrate	$NaNO_3$	1.68
Sodium nitrite	$NaNO_2$	1.36
Peptone	------------	2.000
Urea	$CO (NH_2)_2$	0.59
Asparagine	$C_4 H_8 O_3 N_2$	1.31
Threonine	$C_4H_9NO_3$	2.35
Cystine	$C_6H_{12}N_2O_4S_2$	2.37
Cysteine	$C_3H_7NO_2S$	2.39

**Amount of each nitrogen source provided either the same nitrogen content (0.329 g/l), or, if the molecular formula is uncertain, the same source ratio of sodium nitrate (0, 2%) in the regular Czapek's medium.*

Statistical analysis:

The experimental designs of all experiments were completely randomized blocks with 3 replicates, analysis of variance (ANOVA) of the data was performed using the Statistical Analysis System (Anonymous 2004). Means were compared according to the least significant differences (L.S.D $_{0.05}$) following Doncan's test.

Results and Discussion

1-Isolation, purification and identification of the pathogen:

Naturally infected leaves and pods of pea (Master B cv.) were collected from different fields and popular markets at Minia and Matai counties, Minia Governorate, Egypt. Potato dextrose agar (PDA) medium was used to isolate the pathogenic fungus from the different parts; leaves, stems, internal and external spotted pods, of pea plants. Purification of the isolated fungus was carried out by using the hyphal tip technique. Identification depending on the morphological and microscopic characters was also performed on PDA medium. Identification was accomplished according to the keys given by Cunfer and Ueng (1999), Crous (2009) and Verkley *et al.* (2013) and was kindly further verified by the Mycological Center, Faculty of Science, Assiut University under Code No. AUMC10995. The isolated fungus formed a hyaline, thin, septate mycelium, abundant with several branches. Pycnidia produce needle-like pycnidiospores. Under wet conditions, numerous spores (conida) are produced in the pycnidia and are exuded when the fruiting structures are mature. Three isolates, designated as S_1 – S_3, were identified as *Septoria pisi* Westend. Isolation showed that the pathogen was isolated from naturally infected leaves and pods collected from Minia and Matai counties. Isolates S_1 and S_2 were obtained from Minia county but isolate S_3 was isolated from pea samples collected from Matai county. S_1 and S_3 isolates were isolated from naturally infected pods whereas S_2 was isolated from naturally infected leaves of pea.

Pathogenicity test:

The results in Table 3 show that all isolates of *S. pisi* could infect leaves and pods of pea (Master B cv.) causing spots with different degrees of severity depending on the isolate. The highest percentages of leaf and pod infection were caused by isolate S_1, which caused the highest values of disease severity, followed by isolates S_2 and S_3 for leaves. In addition, isolate S_3 showed height disease severity than S_2 on pods infection. Data in Table 3 indicate that their was no significant difference between isolates S_2 and S_3. Yellow spots on infected leaves and stems were observed on the lower, senescing parts of the plant and the pods. Spots became necrotic and covered with numerous light brown spots. At first, they appear yellow, later becoming straw-colored. Lesions vary in their sizes, roughly circular and had no district margins. Several such spots might join to cover the entire leaf forming blotches. As the blotches dry out, many pinpoint-sized black to dark brown pycnidia may be seen scattered widely on infected plant parts. Infection of seeds suggests that the fungus may be seed-borne and transmission by infected seeds can occur.

Table 3. Percentages of infected pea (Master B cv.) leaves and pods after spraying with spore suspensions of different isolates of *Septoria pisi* isolated from naturally diseased plants or pods

Isolates	Disease incidence (DI,%) and disease severity (DS,%), 60-65 days after sowing on			
	Leaves		Pods	
	DI%	DS,%	DI%	DS,%
S_1	71.1a*)	39.4a	89.56a	48.9a
S_2	55.6b	31.7ab	71.1b	30.5b
S_3	62.2ab	28.9b	60.0b	27.2b
Control	0.0c	0.0c	0.0c	0.0c

*) *Figures represent an average of 15 plants and 15 pods replicated 3 times. Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05*

The quantity and quality of the herb material are reduced by pathogenic fungi infection. In the vegetation periods the fungus occurs with varying intensity, which is related to the requirements of its life (Zalewska, 2012). *Septoria pisi* is the pathogen in the case of field pea. Septoria leaf and pod spot (Septoria blotch) of pea is considered as a common disease in some areas of the world *i.e.*, Midwest of USA, is rarely significant because it occurs primarily on the old foliage, pods and stems (Hagedorn, 1991). He reported that the fungus *Septoria pisi* and, more rarely, *Septoria flagellitera* cause the disease.

2-Response of different pea cultivars and lines to the isolated pathogen:

Seven cultivars and lines of pea; namely Master B, Jaguar, Palmoral, Qena line 1, Qena line 3, Yellow line, and Yemeni line, were used to study their susceptibility against infection with isolate S_1; the most virulent isolate of leaf and pod spots of pea. Data presented in Table 4 show that all tested cultivars and lines of pea infected by the pathogen show spots with significant differences of infection values on leaves and pods. Data show that Master B, Qena 1 and Qena 3 showed the highest degrees of susceptibility than Jaguar and Palmoral, which were more resistant showing the least degrees of leaves and pods infection. In the second season, 2016-2017, similar results as those recorded in the first season were obtained, but the symptoms and degrees of infection were more severe than those appeared in the first season of study. This may be due to the accumulation and increasing inoculum sources in the same locations from a season to another.

Nearly all field pea cvs. are moderately susceptible to Septoria, however, the disease appears to be most severe on the short, semi-leafless varieties (Cruikshank, 1947). The later-maturing pea cvs. are more tolerant to *S. pisi* than those maturing early, and perfection-type canning peas are more tolerant than market-garden types (Hagedorn, 1991), immunized cvs. to *S. pisi* has not been reported. Testing susceptibility of pea cultivars to infection by *Erysiphe pisi*, the causal pathogen of powdery mildew of pea, isolates revealed that Yasmen, Lincoln and Jaguar cvs. are demonstrated high levels of resistance. On the contrary, Master B, Kareem and Little Marvel were very susceptible to *Erysiphe pisi* (Abd-Alla, 2012).

The period (latent period) from inoculation of *Septoria nodorum* on Kolibri and Maris Butler cvs. of wheat to first production of spores was similar over a range of constant temperatures (12-18°C). This period was shorter for Kolibri cv. at 6 and 24°C. Pycnidia production was greatest during the period of stem extension and was twice as great on Kolibri cv. as on Maris Butler cv. Some of tomato genotypes were screened under epiphytotic condition and Pusa Ruby cv. was found to be the most susceptible to *Septoria lycopersici*. (Madalgeri *et al.,* 1988).

3- Effect of some environmental factors on growth and pycnidial formation of S. pisi:

In these studies, the effect of various temperature degrees, various media, carbon and nitrogen sources on growth of isolate $S_{1,}$ the most pathogenic isolate of *S. pisi* was tested in the laboratory.

3-a. Effect of different temperature degrees:

Effect of different degrees of temperature on growth and producing of pycnidia of isolate S_1 of *S. pisi* was estimated, 7 and 25 days culturing using oat medium to measure linear growth (in mm) and number of pycnidia, respectively.

Table 4. Relative susceptibility of pea cvs. and lines to infection by isolate S_1 of *Septoria pisi,* the pea leaf and pod spots pathogen

Pea Cultivars and lines	Incidence (DI, %) and severity (DS, %) of the disease during growing seasons 2015-2016 and 2016- 2017.							
	2015-2016 cultivated season				2016- 2017 cultivated season			
	Leaf infection		Pod infection		Leaf infection		Pod infection	
	DI,%	DS, %	DI,%	DS, %	DI,%	DS, %	DI,%	DS, %
Master B.	73.3a	38.3a	80.0a	46.7a	80.0a	40.0a	86.7a	48.3a
Jaguar	60.0a	25.0bc	53.3ab	21.7cd	60.0ab	28.3ab	60.0bc	26.7de
Palmoral	53.3a	23.3c	46.7b	18.3d	46.7b	21.7b	53.3c	20.0e
Qena line 1	73.3a	35.0ab	73.3ab	38.3ab	73.3ab	38.3a	80.0ab	46.7ab
Qena line 3	73.3a	36.7a	80.0a	36.7b	73.3ab	40.0a	73.3abc	41.7abc
Yellow line	66.7a	36.7a	66.7ab	35.0b	80.0a	36.7a	80.0ab	38.3bc
Yemeni line	60.0a	31.7abc	60.0ab	30.0bc	66.7ab	33.3ab	66.7abc	31.7cd

[*]Each reading is average of 5 plants or 5 pods, replicated three times. Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05

The obtained results in Table 5 indicate that the fungus can grow at a wide range of degrees of temperature between 10 and 30ºC. The optimum temperature was 21ºC for both growth and pycnidia formation. Also, *Septoria pisi* showed the best growth at 15 and 27ºC, being 80 and 75 mm for S_1 and 44 and 62 mm for S_3, respectively. The fungus failed to grow at 5ºC but poor growth and producing pycnidia were over 30ºC in case of isolate S_1. The fungus formed immature pinky–colored pycnidia at 1.5 cm distance from the center of the plate, but mature black pycnidia were found at 4 cm. Most formed pycnidia for isolate S_1 at 30ºC were pinky or bright-brown colored immature and failed to form pycnidiospores.

Table 5. Effect of different degrees of temperature on linear growth (mm) and pycnidia formation of *S pisi*, isolate S_1

Temperature (ºC)	Mycelial growth (in mm) and number of pycnidia		
	Mycelial linear growth (in mm)	No of pycnidia	
		3cm	8cm
5	0.0e	0.0f	0.0f
10	45d	20e	54e
15	80b	240b	130b
21	90a	670a	472a
27	75c	199c	111c
30	42d	198c	0.0d
35	0.0e	0.0d	0.0f

*) *Each reading is an average of 3 replicates. Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05.*

These results are to somewhat in agreement with those reported by several researchers. Sohi and Sokhi (1972) found that *S. lycopersici* showed good growth between 15-28ºC and the optimum was 25ºC for growth and pycnidial formation. Whereas, the optimum temperature for growth of this fungus was 22-25ºC (Marcinkowska, 1977). Wolcan (1988) reported that the temperature ranged between 21ºC and 27ºC for growth and development of the *S. lycopersici*. The temperature range for *S. lycopersici* sporulation varied from 15-27ºC, with 25ºC being the optimal (Anonymous, 1987). The growth of *S. carvi* Syd., the causal of blight of caraway, grow at the temperatures from 0-30ºC, the optimum being from 20-25ºC, and sporulation is possible at the temperatures from 10-30ºC, with the optimum at 25ºC (Zalewska, 2012). Also, *S. humuli*, the causal pathogen of hop leaf spot, can grow well at 5-25ºC, and the optimum temperature for sporulation was 20-25ºC, optimum pH for growth was 6 with maximum sporulation at 5.5 (Punithalingam, 1985). Hagedorn (1991) reported that Septoria blotch of pea development favors prolonged high humidity (at least 24 hours) and moderate temperatures at 21-27ºC.

3-b. Effect of different media on dry weight and pycnidia formation of S. pisi, isolate S_1:

The obtained data in Table 6 show that oat meal, malt and pea dextrose media were almost the best natural media for both growth and pycnidia formation. The V-8 juice and PD broth media were the next favorable natural media. The poorest growth was obtained on basil garlic extracts media. Much growth was produced on the synthetic media; Czapek's and Waksman's. A poor formation of pycnidia was

recorded on PDA and Waksman's solid media, but on Czapek's medium the fungus gave substantially higher number of pycnidia when compared with other media. No pycnidia were formed on garlic and onion media, whereas the least number of pycnidia was formed on basil medium.

Table 6. Effect of different media on the amount of growth (in mg) and formation of pycnida of S. pisi, isolate S_1

Medium	Mycelial dry wt. (mg)	No. of pycnidia on the agar media solid at,	
		3 cm	8 cm
Basil dextrose	153*) g	30.0 g	3.0 h
Garlic dextrose	118 h	0.0 h	0.0 h
Malt dextrose	570 a	507 b	397 b
Oat dextrose	562 a	667 a	487 a
Onion dextrose	251 f	0.0 h	0.0 h
Pea dextrose	530 b	480 c	370 c
PD broth	450 d	257 f	59 g
V-8 juice	490 c	309 e	165 e
Waxman's	353 e	257 f	75 f
Czapek's	521 b	370 d	202 d

*) *Each reading is the average of 3 replicates. Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05*

Several researches studied the favorable media, temperature and period of incubation needed fer growth and sporulation of different fungi attacking pea plants. Zalewska (2012) reported that the most useful medium for the isolation of *Septoria carvi* from caraway plants is malt medium and malt medium supplemented with decoction of caraway's leaves. Pycnidia and conidia of the fungus were formed the fastest on the malt medium with decoction of the leaves or schizocarps of caraway and on PDA medium. The solid and liquid media were investigated for their efficiency in quantitative sporulation of *S. tritici* (Saidi *et al*., 2012). The inoculated media were incubated at 18 to 20°C with a photoperiod of 12 h light and 12 h darkness and investigated after 4 to 6 days. Among solid media, YMDA (yeast extract + malt extract + dextrose + agar) and liquid media YMB (yeast extract + malt extract + glucose in 1 lit distilled water) were the best with conidia production rates of 1.7×10^9 spore/ ml and 2.3×10^9 spore/ ml,

In the available literature no information on the growth conditions of *S. pisi* was found, although they have been tested for other species of *Septoria* spp. (Richards, 1951 and Marcinkowska, 1977). Determination of the conditions for growth and development of *S. pisi*, especially the optimum and minimum temperatures for the mycelium and spores formation was considered as purposeful and necessary for understanding the epidemiology of the fungus. Moreover, the study of the growth and development of *S. pisi* on different culture media let us determine the most suitable medium for the isolation of the pathogen from the plant tissue and substrates to culture for diagnostic purposes.

Septoria lycopersici showed profuse growth with excellent sporulation on potato dextrose agar medium with excellent sporulation but poor mycelial growth on

oatmeal agar, coon's agar and Richard's agar, whereas, cornmeal agar gave excellent sporulation with a comparatively poor mycelial growth. On the other hand, both mycelial growth and sporulation were scanty on Czapeck's solution, Sohi and Sokhi (1972). The linear growth of the fungus was best on tomato leaf extract agar, potato dextrose agar and carrot agar (Marcinkowska, 1977). Rawal and Sohi (1983) noticed abundant mycelium on Czapek's Dox agar and potato dextrose agar. Whereas, Wolcan (1988) found that PDA was the best and V-8juice was the poor medium for *S. lycopersici* growth and sporulation.

3-c. Effect of different carbon sources on fungal growth and pycnidia formation of Septoria pisi:

The effect of different carbon sources was evaluated by substituting the sucrose by different carbon compounds to prove 12.63 grams C/l equivalent to 30 g/l of sucrose, except dextrose, pectin and starch, because they are of uncertain molecular formula, were added at 3%; similar to that of sucrose in the regular medium (Czapek's medium). Control without additional carbon source was used at 22±2°C and pH 6 for 10 days incubation. Also, the formed pycnidia were counted. 25 days after inoculation. Data obtained and presented in Table 7 point out to the following:

1- The tested isolate of *Septoria pisi* grew on all tested media, but the growth varied from excellent to fairly good on all tested media.

2- The best growth was obtained when simple sugarsi.e., fructose, glucose, maltose and sucrose, represented as the sole carbon sources, followed by raffinose (from oligosaccharides); dextrin and starch came next to them. Whereas, pectin, sorbitol, and manitol proved to be a poor sources of carbon for growth of tested fungus.

3- Sodium citrate when used as a sole carbon source gave a less growth.

4- In the same line, *Septoria pisi* produced the highest amount of pycnidia on medium provided with sucrose or glucose, followed by that contained fructose or maltose. Whereas, the least amounts of pycnidia were formed on media containing dextrin, starch, manitol, sorbitol .The least number of pycnidia was formed on sodium citrate amended medium.

Carbon is an essential element for the growth and sporulation of fungi. Hence, its requirement and utilization were studied. The fungus may utilize certain complex form of carbon compound into simple form as reported by Bias *et al*. (1970). Hence increase or decrease in sugar content in plant tissues may affect the disease development. Dextrose, glucose, sucrose, maltose supported the excellent growth of *Septoria* sp. whereas mycelial growth was comparatively poor in lactose, glycerine and mannitol whereas no growth was observed in tartaric acid (Sohi and Sokhi, 1972). Rawal and Sohi (1983) reported maximum growth and sporulation of *Septoria vignicola* were observed when sorbose was used as carbon source, whereas, Grzybowska (1977) found that D-galactose, D-glucose, sucrose and maltose were the best source of carbon for the growth of *Septoria digitalis*.

Table 7. Effect of different carbon sources on growth and Pycnidial formation of *S. pisi* (isolate S_1)

Carbon source	Effect of carbon source on growth and number of pycnidia		
	Mycelial dry wt. (mg)	No. of pycnidia formed on solid medium at 1.5 and 4 cm from the plate center	
		At1.5 cm	At 4 cm
Fructose	519ab[(1)]	352b[(2)]	142bc
Glucose	532ab	359ab	139c
Maltose	545a	351b	145b
Sucrose	521cd	370a	202a
Raffinose	492abc	291c	95d
Manitol	232e	101f	60e
Sorbitol	252e	110f	32g
Sod. Citrate	125f	65g	20h
Dextrin	451abc	293c	59e
Pectin	293e	131e	43f
Starch	395d	162d	93d
Control[(**)]	73f	76g	32g

1) Average of 3 replicates, growth was recorded 10 days after incubation.[2)] No. of pycnidia was recorded on solid agar media, 25 days after incubation.
Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05

3-d. Effect of different nitrogen sources on growth and pycnidia formation of S.pisi, the causal pathogen of pea leaf and pod spot.

Czapeck's medium free from potassium nitrate was used as a basic medium. Eleven organic or inorganic nitrogen sources were added (Table 8). Total growth (in mg of dry weight) and numbers of pycnidia of isolate No_1 of *S. pisi*, were determined, 10 and 25 days of incubation at 22±2°C, respectively.

Data obtained and presented in Table 8 point out to the following:

1- The growth of the tested fungal isolate varied in the response to different nitrogen sources used. The best growth occurred when medium contained sodium or potassium nitrate, followed by peptone or threonine.

2- Ammonium nitrogen sources (ammonium nitrate and ammonium sulphate) mostly favored fungal growth. Minimum growth was observed when urea or asparagine was used as a sole nitrogen source.

3- Among the tested nitrogen sources, amino acids Cysteine and Cystine completely inhibited the growth of the tested fungus, when used as a sole nitrogen sources.

4- The poorest growth was obtained when the fungus was allowed to grow on medium contained sodium nitrite.

5- Peptone and the amino acid threonine were the best nitrogen sources for sporulation and forming pycnidia of *S. pisi*, followed by the Ammonium nitrogen source. Nitrate nitrogen sources mostly favored fungal sporulation, for example, a best sporulation and pycnidial formation for the tested isolate of S. pisi were found on media contained potassium and sodium nitrate.

6- No spores or pycnidia were formed when sodium nitrite or amino acids cystine or cysteine was used as a sole nitrogen source, but on medium supplemented with asparagine or urea as a sole nitrogen source, a few number of pycnidia, less than that formed in control, were formed.

Nitrogen is also one of the important elements, required for the growth of the fungi. Among the nitrogen sources, urea has been reported to support good mycelial growth and sporulation for *Septoria humuli* (Punithalingam, 1985). Bhandari and Singh (1976) studied the nitrogen requirements for *S. lycopersici* and reported that asparagines and glutamine supported good growth. In another study, asparagine and urea were significantly superior to others and showed good growth of the fungus. The media containing ammonium nitrate proved unfavourable for pycnidia formation (Sohi and Sokhi, 1972). Rawal and Sohi (1983) observed the excellent growth of the fungus when aspartic acid and potassium nitrate were used as organic and inorganic nitrogen sources.

Table 8. Effect of different nitrogen sources on growth and pycnidia formation of *S. pisi* (isolate S_1), the causal of leaf and pod spots of pea

Nitrogen source	Effect of nitrogen source on,		
	Mycelial dry wt. (mg)	No. of pycnidia, at	
		1.5 cm from the center	4 cm from the center
Ammoium. Nitrate	395[(1)]c	192[(2)]e	131c
Ammoium sulphate	392c	270c	186b
Potassium nitrate	521a	370a	202a
Sodium nitrate	518a	361b	205a
Sodium nitrite	25g	0.0j	0.0g
Peptone	420b	257d	53e
Urea	70e	58h	23f
Asparagine	53f	33i	21f
Threonine	419b	135f	113d
Cystine	00h	0.0j	0.0g
Cysteine	00h	0.0j	0.0g
Control	185d	84g	53e

1) Average of 3 replicates, growth was recorded 10 days after inocubation.[2)] *No. of pycnidia was recorded on agar solid media, 25 days after incubation.*
Treatments with the same letter are not significantly different from each other according to Fisher's protected least significance test at p=0.05

Ammonia is known to leave the cells by passive diffusion as undissociated ammonia molecules. Rapid fall in pH due to assimilation of ammoniacal nitrogen has been observed in several fungi (Cochrane, 1958). The maximum mycelial growth was recorded in Potassium nitrate amended medium followed by sodium nitrate, ammonium nitrate and thiourea. Asparagine was found to be the best source of nitrogen for the growth of *S. lycopersici* showing maximum dry mycelial weight, followed by calcium nitrate and potassium nitrate, urea and ammonium sulphate. The least growth was noticed in case of ammonium nitrate (Aria, 2010).

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بعض الدراسات الفسيولوجية لمقاومة الفطر *Septoria pisi* مسبب تبقعات الأوراق والقرون في البسلة

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يُعتبر مرض تبقع الأوراق والقرون السبتورى فى البسلة المتسبب عن الفطر سيبتوريا بيسي من الأمراض الشائعة التى تسبب نقص فى المحصول. تم الحصول على ثلاثة عزلات من الفطر سيبتوريا بيسى من أوراق وقرون بسله مصابة طبيعيا وتظهر عليها أعراض البقع وقد اختلفت هذه العزلات عن بعضها من حيث شدة الإصابة حيث كانت العزلة S1 أشدها قدرة مرضية. اظهرت الأصناف المختبرة من البسلة درجات متفاوتة من حيث قابليتها للإصابة حيث كان الصنف ماستر ب، والخطوط قنا ١ وقنا ٣ أكثرها قابلية للاصابه ، بينما كان الصنفان جاكوار وبالمورال أكثرها تحملا للمرض. إن الأوعية والجراثيم البكنيدية التى تسبب إصابة النباتات وذات ضرورة لتشخيص الفطر يقل تكوينها عادة عند تنمية الفطر خارج العائل. وقد أجريت فى هذا البحث عدة تجارب لمعرفة درجة الحرارة والبيئة ومصادر الكربون والنتروجين المناسبة لتكوين البكنيديات والجراثيم. وقد وجد أن أفضل نمو وتجرثم حدث عند نمو الفطر عند درجة حرارة ٢٢± ٢o م لفترة ١٢ ساعة ظلام و١٢ ساعة ضوء ودرجة حموضة ٦,٥، على بيئات مستخلص بذور الشوفان، الشعير والبسلة مع الدكستروز من البيئات الطبيعية وبيئة زابكس من البيئات التركيبية. وقد سجل أقل نمو وتجرثم للفطر عند تنميته على بيئتى مستخلص الثوم أومستخلص الريحان. كان أفضل نمو وتجرثم للفطر على بيئة زابكس التى تحتوى على السكريات البسيطة مثل الفركتوز، الجلوكوز والمالتوز والسكروز كمصدر وحيد للكربون متبوعة بالرافينوز والدكسترين والنشا. بينما قل نمو وتجرثم الفطرعلى البيئة التى تحتوى على البكتين، السوربيتول ، المانيتول أو سترات الصوديوم كمصدر للكربون. كما أن نترات الأمونيوم وكبريتات الأمونيوم كانتا أفضل مصادر النتروجين المختبرة للنمو بينما ظهراقل نمو عندما استخدمت اليوريا والأسبراجين كمصدر وحيد للنتروجين. كان الببتون والحمض الأمينى ثريونين أفضل مصادر النتروجين للتجرثم ، ولم تتكون بكنيديات أو جراثيم عند استخدام نيتريت الصوديوم أو الأحماض الأمينية الكبريتية "سيستين أو سيستاين" .