Tomato leaf spot caused by *Ulocladium* botrytis Preuss. Pathological and physiological studies.

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ABSTRACT

Ulocladium botrytis Preuss was isolated from spotted tomato leaves of "Pritchard variety" Symptoms are characterized by irregular lesions, dark-gray becoming black with time, mostly begin at the margins and extending to the main vein of leaflets. Also longitudinal dark-gray necrotic lesions are noticed on petioles and on the stem, and some axillary buds were blackened and necrosed.

Pathogenicity tests showed typical disease symptoms after 4 - 7 days incubation**.** "Cal Ice" variety was highly susceptible to infection, whereas "Super Marmande" was resistant and "Pritchard" moderately resistant.

Morphological characteristics of the pathogen were found to fit with the species description of *Ulocladium botrytis* Preuss. This identification was confirmed by the Commonwealth Mycological Institute, Kew, Surrey, England.

Profuse sporulation and good growth rate of the pathogen were obtained on Czapecks-Dox agar containing glucose or sucrose as a carbon source and sodium nitrate as a nitrogen source followed by asparagine. Optimum pH was pH 6.0 or slightly lower and temperatures from 21-25°C were most favourable. Continuous light exposure seemed to be essential for maximum sporulation. Red light was also optimum while the blue light and continuous darkness seemed to be suppressive.

INTRODUCTION

Tomato plant is known to be one of the major vegetable crops in A.B.E. It is used for local consumption and as an export crop. The crop is subjected to many destructive diseases affecting the plant at all stages of growth **(Walker, 1957).** From these, diseases affecting the foliage are particularly of great importance.

In the last 3 years a new leaf spot disease was usually observed in the farm of the Faculty of Agricultural Science at Moshtohor, Qalubia Governorate. The disease was characterized by irregular dark-gray lesions; which usually begin from the margins of leaflets and extend to the main vein. As a new disease, almost no literature was recorded about it, however **El-Zarka (1976)** reported that *Ulocladium botrytis* and *U. septosporum* together with other organisms were associated with leaf spotting organisms on sunflower plants.

This work was thus made to study the causal of this disease and to examine the susceptibility or resistance of some tomato varieties. The morphology and physiology of the pathogen were also studied. However, further pathological and epidemiological studies on the disease must be continued.

MATERIALS AND METHODS

Isolation and Identification of the 'Causal Organism:

Leaves of tomato plants, variety " Pritchard" growing in the Experimental Station of the Faculty of Agricultural Science at

Moshtohor, Qalubia Governorate, and showing the newly recognised symptoms were collected. Isolation of the causal organism was carried out by thoroughly washing the infected leaves in running tap water, dried between filter paper, than placed in a moist chamber at room temperature (about 21-28°C) in the laboratory to induce fungal growth and sporulation. Using a dissecting microscope the conidia were picked up with the tip of an a gar-moistened sterile loop and were carefully streaked on plain agar plates. After 24 hrs incubation at 26°C, single germinating spores were individually transferred to PDA slants. All cultures were intubated at 26°C in diffused normal daylight unless otherwise specified,

Morphological description of the fungus was made after growing it on PDA cultures. Genus identification was specified according to the description of **Barnett and Hunter (1972),** Species description was compared by that provided by **Simmons (1967)** and **Ellis (1971),** A pure culture was sent to the Commonwealth Mycological Institute, Kew, Surrey, England for identification.

Pathogenicity tests:

Pathogenicity tests were carried out in the greenhouse on mature potted tomato plants. Inocula were prepared from 15-day-old culture plates. Each plate was flooded with 20 ml of sterile tap water and conidia were scraped with a carnal-hair brush, than filtered through sterile cheesecloth. The suspension resulting from all plates

was mixed thoroughly and spore density was determined using a haemocytometer. Spore suspension used for pathogenicity tests was usually prepared, to contain about 6×10^4 conidia/ml. The spore suspension was sprayed using a hand atomizer, than plants were irrigated and covered with polyethylene bags to maintain high relative humidity necessary for infection. The bags were removed after 24-48 hrs incubation. Temperature range in all pathogenicity experiments was 25-30°C. A laboratory test was also employed by inoculating 6-leaf tomato seedlings kept in 250 ml beakers with the roots immersed in tap water. In other tests detached tomato leaves were picked from grown tomato plants, washed in tap-water and held in 50 ml beakers with their petioles immersed in tap water. Inoculation of seedlings and detached leaves was carried out by spraying with the spore suspension and covering the beakers with suitable bell jars, in order to maintain high relative humidity, until disease symptoms appeared after 4-7 days.

Disease readings were determined for each leaf according to disease severity rating which include the size and frequency of the lesions/leaf **(Fahim *et al.*, 1976).** Then readings were converted to disease indexes according to the equation suggested by **Towns end and Heuberger (1943**) as follows;

$$\text{Disease index\%} = \frac{\text{Sum } (n \times r)}{3N} \times 100$$

where; n is the number of leaves in which numerical rate "r" and "N" is the total number of inoculated leaves multiplied by the maximum numerical

Physiological Studies:

Physiological studies if ere carried out by using Czapeck's-dox agar **(Riker and Riker, 1936)** as a basal medium. Inocula were usually 6 mm discs taken from 15 day-old cultures. Readings for linear growth were taken as the average colony diameter (in mm) after 9-10 day incubation at 26°C in diffused daylight, Sporulation was determined by preparing a spore suspension by immersing a 6 mm disc taken ID mm apart from the inoculum site of the culture plate, then transferred to one ml of sterile water in a small glass watch, and freeing most of the conidia by a camel-hair brush. One uniform micro drop (about 0.01 ml) of the suspension was transferred to a clean glass slide then covered with 18 mm circular cover slip. The number of conidia per microscopic field (x 90) was determined in 10 fields replicated 3 times for each treatment.

Effect of light on growth and sporulation was studied using inoculated Czapeck's-dox agar plates. A set of 4 plates was exposed to continuous darkness by firmly covering with black craft paper to provide complete: darkness and another set to continuous light. Two other sets, covered with either red or blue transparent cellophane paper to provide continuous red or blue light. All the inoculated plates were incubated at 26°C in illuminated incubator.

Effect of H-ion concentration (pH) on linear growth and sporulation was tested in a buffered Czapeck's agar medium prepared according to **McLean and Cook (1952),**

Czapeck's agar was used as the basal medium for studying the effect of carbon and. nitrogen sources on growth and. sporulation. Carbon sources were added as the equivalence of 3% sucrose. Nitrogen sources ware incorporated in the basal medium equivalent to the amount of 2,0 g sodium nitrate/litre. Control medium. in both experiments was the basal medium after the omission of either the carbon or the nitrogen source.

RESULTS AND DISCUSSION

Symptoms:

Disease symptoms almost appeared on mature leaves of infected plants. On leaflets first signs appear as irregular lesions, mostly beginning from the margins and extending towards the main vein of the leaflet. Lesions were dark gray at first, becoming darker with time and finally black lesions were observed. Lesions were irregular in size and enlarge rapidly under favourable conditions (*i.e.* warm temperatures of 28-30°C and high humidity).

Pathogenicity tests:

The isolate of *Ulocladium botrytis* isolated from naturally infected tomato plants was highly pathogenic to potted tomato plants, seedlings and detached tomato leaves. Typical necrotic lesions **(Fig. 1)** were observed after 4**-7** days under laboratory conditions (*i.e.* 26 ~2°C and 90-95% relative humidity). Under these controlled favourable conditions, longitudinal dark gray necrotic lesions were also observed on leaf petioles and on the stem. On the

7th day after inoculation, some of the axillary buds were noticed to be blackened and necrosed.

Susceptibility of different tomato varieties:

The effect of artificial infestation with *U. botrytis* was studied on 3 tomato varieties (*i.e.* Pritchard, Super Marmande and Cal Ice). It is apparent that the tested varieties react differently to fungal infection. The introduced variety "Cal Ice" was the highest susceptible as the disease index was 30.0% whereas " Super Marmande " was the most resistant one as the disease index was 2.2%. Pritchard tomato variety, which is commonly planted in Egypt was intermediate in this respect 15.3%). These results clearly show that "Super Marmande" tomato variety could be recommended for growing tomato in A.R.E. and also could be used as a source for resistance in breeding programs.

The Causal Fungus:

A-Morphology

Morphological characters of the fungus were studied oil PDA cultures at different stages of fungal growth **(Figs, 2, 3 and** 4). Mycelium was sub hyaline to olive in colour and the hyphae were about 3-4 µ diameter, conidiophores were erect or ascending and short of about 8-10 µ or more, their colour was pale olivaceous when young, becoming dark olive when old. They may be simple or branched with smooth walls, conidia are born solitary, obovoid to broadly ovoid. Young conidia are one or 2-celled, sub spherical to

obovoid, smooth and the portion of the conidium which is proximal to the conidiophore is tapered and narrower than the distal portion. Mature conidia are olive-brown to dark olive or black, closely roughened to verrucae and rarely smooth. They almost have 2-3 transverse septa with one or two longitudinal or oblique septa in one or more of the transverse divisions. Dimensions of mature conidia measured 7.1-14.3µ by 13.7-25.9µ.

According to the previous characteristics of the fungus and species descriptions .given by **Simmons (1967)**; **Ellis (1971) and Barnett and Hunter (1972),** it was identified as *Ulocladium botrytis* Preuss.

Moreover, this identification was verified by the CHI, Kew, Surrey, England.

The fungus had the synonyms *Stegpliyliuia botayosum* Waller var., *Ulocladium* Sacc., and *Stemplylium botryosum* var., *botrytis* (Pr.) Lindau.

B-Physiology

As *Ulocladium botrytis* is considered a new pathogen to tomatoes in the country, it was thought of importance to study the effect of environmental conditions and nutritional requirement; on growth and sporulation of the fungus.

Effect of temperature: -

Data in **Table 1** evidently show that *U. botrytis* can grow within a temperature range from 18-31°C, maximum linear growth occurred at 28±1°C and growth decreased to both extremes.

Maximum sporulation occurred at 21-25°C. Higher or lower temperatures were mostly unfavourable for sporulation.

Table 1: Effect of temperature on growth and sporulation of *Ulocladium botrytis*.

Temperature (°C)	Av. colony diam, (mm) after 9 days	Average No. of conidia/ field (x 90)
8	-*	-
18	48	85.7
21	56	146.6
25	68	123.1
28	73	76.9
31	51	49.3
35	--	--
L.S.B. at 0.05	1.8	18.2

*= No growth occurred.

Effect of light:-

Preliminary observations indicated that cultures of *U. botrytis* produce better sporulation when incubated in indirect daylight than in the dark. This study was therefore carried out to study the effect of different light exposures on linear growth and sporulation of the fungus.

Data presented in **Table 2,** obviously show insignificant variations between growth rate at different light regimes. However, red rays gave the maximum linear growth. Significant abundant sporulation occurred in cultures incubated in continuous light or covered with red transparent paper (103.5 and 98.5

conidia/microscopic field respectively). Either continuous darkness or blue light significantly decrease sporulation rate being 29.5 and 43.1 conidia/microscopic field respectively.

Table 2: Effect of light on growth and sporulation of *Ulocladium botrytis*.

Light treatment	Av. colony diam, (mm) after 9 days	Average No. of conidia/ field (x 90)
Continuous darkness	73	29.0
Continuous light	77	103.5
Continuous red	80	98.5
Continuous blue	75	43.1
L.S.B. at 0.05	NS*	19.3

*Non-significant

Effect of H-ion concentration

Data, **Table 3,** indicate that *U. botrytis* can grow and sporulate within a wide range of pH values (*i.e.* pH 4.2-9.0**).** 1'hximnm growth was obtained at lower pH values (pH 4.2-6.0) and growth was retarded by the neutral and the alkaline range.

Effect of pH was more pronounced on fungal sporulation. Abundant sporulation was obtained at pH 6.0 and decreased gradually at pH 4.8 and 4.2 and sharply at the alkaline range (*i.e.* pH 8.2 and 9.0).

Table 3: Effect of H-ion concentration of media on growth and sporulation of *Ujocladium botrytis*.

pH values	Av. colony diam, (mm) after 9 days	Average Ho. of conidia/ field (x 90)
4.2	85	109.4
4.8	85	101.2
6.0	80	116.8
6.8	70	92.8
8.2	70	54.0
9.0	73	17.8
L.S.B. at 0.05	5.3	20.5

Utilization of different carbon sources:

Data in **Table 4** show that mannitol, maltose and sucrose produced the best mycelial growth. However, they differed greatly in their effect on sporulation. In this respect, sucrose enhanced profuse sporulation while maltose and manrito1 gave poor yield. Glucose, galactose, arabinose and starch supported moderate or poor growth. Glucose, however, enhanced good spore production followed by arabinose, while on galactose and starch poor spore yield was obtained. In the absence of carbon source, scanty growth and no sporulation was obtained.

Table 4: Effect of different carbon sources on growth and sporulation of *Ulocladium botrytis*.

Carbon sources	**Av. colony diam. (mm) after 10 days**	**Average No. of** conidia/ **field (x 90)**
Glucose	63	94.1
Galactose	62	58.6
Maltose	69	63.9
Sucrose	69	99.4
Arabinose	61	77.1
Mannitol	72	46.4
Starch	59	40.2
Without Carbon	25	0.0
L.S.B. at 0.05	2.3	15.8

Utilization of different nitrogen sources:

It is clear from results presented in **Table 5** that excellent radial growth was obtained in media containing organic nitrogen sources (*i.e.* peptone and casein) measuring 84 and 81 mm respectively. Moderate growth was obtained on asparagine, sodium nitrate and potassium nitrate (71, 69 and 66 mm, respectively). Poor growth was obtained in media containing ammonium salts, however, considerable growth was enhanced when nitrate ion was incorporated, as in ammonium nitrate. Scanty growth and poor sporulation were only obtained in nitrogen-free basal medium.

Excellent sporulation was particularly obtained, on sodium nitrate followed by asparagine, Other sources were more or less the same in their effect on sporulation capacity (32.5- 50.0

conidia/microscopic field). Ammonium chloride and ammonium phosphate gave aggregates of conidia which were particularly difficult to be counted.

From the previous studies, it is recommended for obtaining profuse sporulation, to grow the fungus on Czapeck's-dox agar containing sucrose or glucose as a carbon source sodium nitrate as a nitrogen source with pH 6.0 or slightly lower and incubated at 21-25°C under continuous or rod lights Sons of these conditions were shown to be favourable for the growth and sporulation of many fungi **(Cochrane, 1958).** Importance of light exposure to sporulation was reported by some investigators **(Abdou, 1966 and Fahim *et al.*, 1975).**

Table 5: Effect of different nitrogen sources on growth and sporulation of *Ulocladium botrytis.*

Nitrogen sources	**Av. colony diam. (mm) after 10 days**	**Average No. of** conidia/ **field (x 90)**
Ammonium chloride	28	-/1
Ammonium phosphate	24	-
Ammonium nitrate	46	44.4
Potassium nitrate	66	47.5
Sodium nitrate	69	103.2
Peptone	84	50.0
Asparagine	71	86.9
Casein	81	32.5
Without Nitrogen	-/2	3.0
L.S.B. at 0.05	3.5	23.3

-/1- Spore aggregates were formed which could not be particularly counted.
-/2- Scanty growth due to mycelial extensions from the inoculum.

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دراسات على مرض تبقع أوراق الطماطم المتسبب عن الفطر يولوكليديم بوطرايتس

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عزل الفطر يولوكليديم بوطرايتس من أوراق الطماطم عليها أعراض تبقع أوراق لم يسبق تعريفها. وتظهر الأعراض على شكل بقع غير محددة الشكل لونها رمادي داكن يميل إلى الاسود وتبدأ الاصابة من حافة الوريقات عادة وتمتد إلى العرق الوسطي.

بينت تجارب العدوى الصناعية بالفطر ظهور أعراض تبقعات على الأوراق - تشبه أعراض الاصابة الطبيعية في الحقل – خلال ٤ -٧ أيام من الحقن على درجة حرارة المعمل (٢٦±٢°م) ورطوبة نسبية ٩٠ -٩٥٪ مع ظهور بقع طولية لونها رمادي داكن إلى أسود على أعناق الأوراق والساق. كما لوحظ تعفن بعض البراعم الطرفية وتحول لونها إلى الأسود.

درست قابلية بعض أصناف الطماطم للإصابة بالفطر ووجد أن الصنف سوبر ماراماند كان أكثرها مقاومة ، في حين كان الصنف بريتشارد متوسط القابلية. وعلى ذلك فينصح بزراعة الصنف سوبر ماراماند في المناطق التي ينتشر بها المرض.

درست الصفات المورفولوجية للفطر المسبب ووجد أنها تتطابق مع الوصف المورفولوجي للفطر "يولوكليديم بوطرايتس". وأكد هذا التعريف بواسطة معهد الفطريات التابع للكومنولث بإنجلترا لعزلة نقية من الفطر المسبب.

أثبتت الدراسات الفسيولوجية للفطر أنه ينتج أكبر كمية من الجراثيم وأحسن نمو ميسليومي على بيئة تشابك المحتوية على الجلوكوز أو السكروز كمصدر للكربون ونترات الصوديوم أو الاسبراجين كمصدر للنيتروجين. وكانت أنسب درجة حموضة هي ٦ أو أدنى قليلاً. أما درجة الحرارة المناسبة فكانت ٢١ -٢٥°م. كما أن الضوء الأحمر كان أكثر تشجيعاً للتجرثم من الإضاءة الزرقاء التي كانت مثبطة للتجرثم.

ويتضح من هذه الدراسات أفضل الظروف لنمو وتجرثم الفطر المسبب وهذه ذات أهمية كبيرة في دراسة وبائية هذا المرض.