

A Study on the Effect of Certain Factors on Growth of *Colletotrichum fragariae*, the Causal Agent of Anthracnose in Strawberries

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ABSTRACT

The results showed that the leaf spot diseases on strawberry plants in Syrian Coast and El-Beheira government in Egypt caused mostly by *Colletotrichum fragariae*. The percentage of infection in strawberry plants in greenhouses in Tartous and Lattakia was about 10.7 – 17.5%, whereas the severity was 1.5 – 2.2%. The optimum temperature degree for germination of conidiospores and mycelial growth was 25°C. Germination of conidiospores increased when 1% glucose was added at pH 6-8 to the growth media. Four types of genus *Colletotrichum* were defined which infect strawberry plants: i.e. *C. fragariae* isolated from leaves, *C. gloeosporioides* isolated from stem, flowers and fruits, *C. dematium* and *C. acutaum* isolated from fruits.

Keywords: Strawberry plants-anthracnose-spore-germination–*Colletotrichum fragariae*.

INTRODUCTION

The cultivation of strawberry in the Syrian Coast is considered a modern greenhouse crop which began as individual locations, then spread and concentrated in two other major regions on the Syrian Coast in South Tartous (Meayar Shaker; Sahl Akar; etc.), and some regions of Lattakia (Jabla; Ein Elarous), then spread all over Syria.(Gamal Eldin, *et.al.*, 1985). Strawberry cultivation in Egypt is located between El-Qaliubiya, El-Ismailia, El-Sharkia and some other regions such as Giza and El-Beheira Cultivated area of strawberry in Egypt its estimated area about 6 thousand acre, yielde which is valued about thirty million pounds per year (Khafagy, 2000). Strawberry plants are exposed to infection with many fungal diseases that lead to significant quantitative and qualitative reduction of yield. The most important fungal disease that infects strawberry cultivated

in the greenhouses is anthracnose (Al Gany, 2008; Peresipken, 1989; Mass, 1984).

Anthraco disease of strawberry is caused by four species of the genus *Colletotrihum* (Brooks, 1931; Beraha and Wright, 1973) i.e. *C. acutatum*, *C. dematium*, *C. gloriosporoides* and *C. demalium*, causing fruit and root rots. *C. acutatum* and *C. gloeosporioides*, cause crown rots, which lead to the death of strawberry seeding in nurseries (Barrau and Romero, 2003; Mass, 1984).

Colletotrichum fragariae Br. (Howard, 1972; Horn and Carver, 1963; Brook, 1931) attacks and infects the shoot system of stem crown, fruits, flowers and leaves of strawberry (Munoz *et.al.*, 2000). The infected strawberry plants were the main source of primary infection. The disease causes fruit circular, 32- mm in diameter spots, which appeared, low and brown spots later on enlarged and became bronze in color and eventually became black (Bost and Strow, 2010). In high humid conditions the center of the spot is covered a mass of orange spores and fruits became shrunken and rotted. Immature fruits may be infected and then became hard, small with brown wounds. Infected leaves showed gray spots which later on became black in color, with time. The symptoms on the flowers under humid conditions were similar to those of leaves and stolons. Infection lead to wilt and death of the infected organs. Infected crown may show Brown lesions (Horn and Carver, 1963; Barrau and Romero, 2003). Warm humid weather encourages the spread of the disease (Mass, 1984; Ullio and Macarthur, 2004).

Due to the economic nutritional and medical importance of strawberry and the great damage caused by this disease in the greenhouse, this research was carried out to:

1. Study on the causal agent of strawberry leaf spot disease and El-Beheira government in greenhouse plantation.
2. Determine the incidence and severity of the disease on strawberry leaves and to investigate the effect of temperature, pH and cultivar on mycelial growth and germination of conidiospores.

MATERIALS and METHODS:

The infected strawberry plants were collected during the growth seasons of 2007/2008 and 2008/2009 from the fields of Tartous region and from greenhouse in the area of Safaita and Draykish. Samples were collected from 50×8m greenhouses. Samples from greenhouses of Lattakia – Jabla regions were collected during the growth seasons of 2010/2011. In Egypt, infected samples were collected from Tahrir region in El-Beheira

governorate. Samples were kept in bags and samples were numbered, also, locations and dates were defined.

The cultivated strawberry cultivar "Osogrande", produced in California in 1987, which was susceptible to root rot red disease (red Stele), verticillium wilt disease and viruses (Lous, 2004). (Potato – Dextrose – Agar- PDA medium) was applied throughout the experiment for isolation and observation of pathogens (Rappilly, 1968; Kerelinco, 1978, Agrios, 2005). Samples that collected from greenhouses in Safaita, Draykish, Jabla and Beheira were depended on morphological symptoms of the disease. Isolation, purification and identification studies were carried out in laboratories. Faculty of agriculture, University of Tishreen and in the laboratories of plant pathology, Faculty of Agriculture, Saba Basha, Alexandria University.

Fungal isolates were isolated, purified and transplanted on PDA slants and identification was carried out on the basis of colony shape and color and spores color, shape and dimension (Ellis, 1971; Barnett and Barry, 1972; Fuller 1978; Nelson *et.al.*, 1983; Agrios, 2005, Hassan and Ataf, 2004).

Percentages of infection were estimated according to equation: (Shkalecova, 2004), " $R = n \times 100 / N$ " where:

R = Percentage of disease spread.

N = Total number of healthy and diseased plants and,

n = The number of infected plants.

Severity of the infection was estimated on the basis of the following infection grades = 0 Zero- No infection.

- 1- Infection covered from 2 – 10% of the total surface of leaves.
- 2- Infection covered from 11 – 25% of the total surface of leaves.
- 3- Infection covered from 26 – 50% of the total surface of leaves.
- 4- Infection covered from 51 – 75% of the total surface of leaves.
- 5- Infection covered from 76 – 100% of the total surface of leaves.

Severity of the infection was then estimated according to the following equation:

$R = \sum (a \times b) \times 100 / N \times K$ where,

R = Severity of infection (%),

$\sum (a \times b)$ = sum of multiplying the number of the infected leaves (a) by grades of infection (b).

N = Total number of leaves.

K = The number of infection degrees (K = 6).

Pathogenicity was estimated by the method recommended by Smith and Black (1990), using 7 days old colonies as inoculum. Discus of the pathogen were placed in the center of the inoculation dish and incubated

for 24 hours before exposing to different temperatures at the rate of 40 °C - 5 °C intervals at the rate of four dishes for each replicate. Time required to start the germination of spores was then estimated according to Al-Gany, (2008). The growth of fungi were estimated by Baracanto and Golding (1953). The effect of the pH on the germination of the conidiospores of the fungus (*Colletotrichum fragariae*) was studied according to Baracanto and Golding (1953) and El-Gany (2008). Statistical analysis was carried out by SPSS testing of ANOVA, then the differences behance means was conducted using significant differences (L.S.D, 0.05).

RESULTS and DISCUSSION

Disease symptoms and morphological characteristics of the disease agent:

The infected leaves, stolons, flower, crown and fruits of strawberry plants were characterized via symptoms on mature fruits include the formation of semicircular, brown color to black spots, whereas in immature fruits, spots were harsh by small incisions, brown in color and elongated. These detected symptoms coincide with those of strawberry anthracnose disease as described by Bost and Strow (2010). Leaf spots incubated for four days showed white creamy and the acervuli, contained short crowded conidiophores bearing the conidiospores. Fungal growth on the upper surface of PDA media was white creamy in color, whereas at the bottom surface of the Petri dish showed green growth. As for the morphological feature of the isolated fungus it could be concluded that it is *C. fragariae*. Moreover, four species belonging to the genus *Colletotrihum* were isolated as shown in table (1).

Table (1): The isolated species of the genus *Colletotrichum* in relation to the infected plant organs.

User plant	<i>C.fragariae</i>	<i>C.gloeosporioides</i>	<i>C.dematium</i>	<i>C.acutum</i>
Root	-	-	-	-
Crown	-	-	-	-
Stem	-	+	-	-
Flowers	-	+	-	-
Leaves	+	-	-	-
Fruits	-	+	+	+

(+) positive isolation , (-) negative isolation).

From results shown in table (1) it was found that the fungus *C. fragariae* has been isolated from leaves, while the two fungi *C. acutatum* and *C. dematium* have been isolated from fruits; whereas; the fungus *C. gloeosporioides* has been isolated from stem, flowers and fruits of strawberry plants. These results cops with those reported by Dyko and Mordue, (1979), Mass, (1984), Gunnell and Gubler, (1992), Munoz *et.al.* (2000).

Table (2): The percentage and severity of infection of leaf spot disease in strawberry in Syrian coast during the growth season 2007/2008.

Site	Percentage of infection	Severity of infection
Jabab / Draykish	10.5	1.5
Bat Alian/Tartous	14.2	2.1
Meayar Shaker/Tartous	16.3	2.4
Safsafa / Safaita	17.3	1.9
Jabla / Jabla	13.5	1.8
Jabla/Ein Elarous	9.7	1.2
L.S.D (0.05)	1.33	0.06

Results of table (2) revealed that the percentage of infection by leaf spot disease on strawberry grown in greenhouses of Syrian coast during the growth season 2007-2008, ranged between 9.7 to 17.3 and its severity ranged between 1.2 to 2.4%. The highest percentage of infection (17.3%) was obtained from Safsafa/Safaita region, while the lowest percentage (9.7%) was found in Jabla/Ein Elarous and Draykish/Jabab regions (10.5%). Differences in disease incidence may be explained on the basis that the fact that strawberry cultivation was recent in Jabla/Ein Elarous and Draykish/Jabab region.

Table (3): The percentage and the severity of infection by leaf spot disease in strawberry in Syrian coast during the growth season 2008/2009.

Site	Percentage of infection	Severity of infection
Jabab / Draykish	11.8	2.1
Bat Alian/Tartous	12.3	1.9
Meayar Shaker/Tartous	14.8	2.1
Safsafa / Safaita	18.8	2.2
Jabla / Jabla	14.5	1.6
Jabla/Ein Elarous	11.7	1.8
L.S.D (0.05)	1.31	0.05

During 2008-2009 growth season in the Syrian coast, the percentage of infection by leaf spot disease in greenhouses ranged between 11.7 to 18.8% and its severity ranged between 1.6 to 2.2%. The highest percentage of infection (18.8%) was detected in Safsafa/Safaita region, whereas the lowest percentage (11.7%) was recorded in Jabla/Ein Elarous and in the Draykish/Jabab (11.8%). Differences in disease incidence may be attributed to the fact that strawberry cultivation was recent in Jabla/Ein Elarous and Draykish/Jabab region.

Table (4): The effect of temperature on spores germination and growth of mycelium of *Colletotrichum fragariae*, the causal agent of leaf spot disease on strawberry. (in Saba Basha).

Degrees of (°C)	Growth of mycelium	Germination of spores
10	0	0
15	35	14
20	75	55
25	91	65
30	70	41
35	15	16
40	0	0
L.S.D.(0.05)	2.5	3.12

Results of table (4) showed that the optimum temperature for mycelial growth of *colletotrichum fragariae* was 20-25°C, where the percentage of growth was 75-91%, respectively. No mycelial growth was detected at 10°C; however, poor growth (35%) was obtained at 15°C. Rates of growth significantly decreased as temperatures raised or lowered than 25°C. Optimum temperature of germination of *Colletotrichum* spores was the same as their of mycelial growth (20-25°C), whereas, the percentages of germination were 55-65%, each in turn. No spore germination was observed at 10 or 40°C, however, poor germination (14%) was obtained at 15°C.

Table (5): The effect of cultivation methods at (25°C) on conidiospores of *Colletotrichum fragariae* (in Saba Basha).

Cultivation method	The percentage of germination of conidiospores			
	Periods after cultivation (hours)			
	12 hours	24 hours	36 hours	48 hours
Distilled water	35	41	77	91
Water tap (Napa Baqin)	21	32	56	77
Distilled water +% Glucose	39	47	85	93
Distilled water +1% strawberry leaves extract	43	52	89	95
Drenched strawberry leaves (unwashed) in distilled water	41	48	55	67
L.S.D (0.05)	2.09	3.12	2.99	1.43

According to the results presented in table 6, conidiospores of *Colletotrichum* significantly grew on all the applied cultures. Maximum germination % was attained on cultures amended with the applied nutrients, i.e. distilled water + leaf extract or distilled water + 1% glucose, where maximum germination % was obtained after 48 hrs after inoculation (95 and 93%, respectively). The germination % on the rest of the tested cultures ranged from 67 to 91%, however, lower germination % were attained on culture of Drenched strawberry leaves and water tap. Decrease of germination on water tap culture may be due to the effect of water tap salts, which resist water absorption by conidia.

Table (6): The effect of pH of the growth medium on germination of conidiospores of *Colletotrichum fragariae*.(in Saba Basha).

pH	The percentage of germination of conidiospores			
	Periods after cultivation (hours)			
	12 hours	24 hours	36 hours	48 hours
2	0	0	0	0
4	0	0	0	0
6	12	23	34	78
8	28	39	53	87
10	0	0	0	0
12	0	0	0	0
L.S.D. (0.05)	1.60	2.24	1.92	2.31

Results of table 6 showed that pH of the growth medium, significantly, affected germination of conidiospores of *Colletotrichum fragariae* maximum germination percentage (78-87%) were attained at pH 6 pH 8 respectively. No germination occurred at pH 2, 4, 10 and 12.

CONCLUSIONS and RECOMMENDATIONS:

- 1- The leaf spot disease (anthracnose) on the strawberry leaves in Syrian coast is caused by fungus *Colletotrichum fragariae*.
- 2- The four species of the genus *Colletotrichum* that infect strawberry plants were: *C. fragariae*, *C. gloeosporioides*, *C. dematium* and *C. acutatum*.
- 3- The percentages of infection by anthracnose ranged from 10.7 to 18.8% and the severity from 1.5 to 2.3%. The optimum temperature for the germination of conidiospores and growth of mycelium was 25°C. Amendment of culture media with some nutrients increased the percentage of germination.
- 4- The optimum pH value for germination of conidiospores and mycelial growth was in the range pH 6 – pH 8.
- 5- Further study has to be carried out to investigate the disease incidence in other locations in the country, selection of more resistant cultivars and studying the dynamics of disease development in the greenhouse.

REFERENCES

- Al Gany, Z. (2008).** Study of the strawberry leaf spot disease – strawberries in El Gabal El Agdar region – Libyan –plant protec. 26: 160 -162 p. In arabic
- Agrios, G. (2005).** Plant Pathology. Univ. publication, Kar Unis 948p.
- Gamal El-din , I. F. , T.Abd El Hag, A, Siry, M.M, El Zayat, and W Ashour, . (1985) .** Diseases of horticultural crops. Vegetables- fruit- Accessories– medicinal plants and aromatic. Cairo press, Victor Cyrils, 336p.
- Hassan, M., and E. Alaf, . (2004).** The principal of fungi: (practical part) university press Tashrin, 145 p.
- Khafagy, Y. (2000).** Strawberries (red gold in the new century) Agricultural Research Center, Egypt, publication and distribution. First printing, 409 P.
- Barnett, H.L., and Y. B. H. Barry.(1972).** Illustrated genera of imperfect fungi, Burgess publishing company California, USA, 241 p.
- Barrau, C., and F. Romero, . (2003).** Strawberry fungal diseases, 129-132P.
- Beraha, L., and W.R. Wright. (1973).** A new anthracnose of strawberry caused by *Colletotrichum dematium* .Plant Disease Reporter , 57:445-448.
- Bost, S., and,R.A, Strow. (2010).** Strawberry diseases in tennessee, P6 21-5-2010 .
- Baracanto, F.P., and N.S .Golding.(1953).** The diameter of the mold colony as a reliable measure of growth. Mycologia ,45:848.
- Brooks,A.N.(1931).** Anthracnose of strawberry caused by *Colletotrichum fragariae*, n . sp., Phytopathology ,21:739-744.
- Ellis, M.B. (1971).** Dematiaceous Hyphomycetes commonwealth mycological institute kew, surrey ,England ,608p.
- Fuller,M.S. (1978).** Fungi in the laporatory Athens ,USA,University of Georgia ,213 p.
- Gunnell, P.S., and W.D Gubler.(1992).** Taxonomy and morphology of *Colletotrichum species* pathogenic to strawberry. Mycologia 84:157-165.
- Shkalecova, V.A. (2004) .** Thesheta restane atpelezne .Masqua. Kolas, 255p.
- Horn , N.L., and R.G. Carver, . (1963).** A new crown rot of strawberry plant caused by *Colltotrichum fragariae* . Phytopathology , 53:768-770
- Howard , C.M. (1972).** A strawberry fruit rot caused by *Colletotrichum fragariae* . Phytopathology ,62:600-602.

- Dyko, B. J., and J. E. M Mordue. (1979).** *Colletotrichum acutatum*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, No. 630.
- Kerelinco ,T.C. (1978).** Classification of fungi in soil. Kiev, Domca, 264 P .
- Lous, D.F. (2004).** Managing diseases in strawberry ,plug production url adrees ; htp.
- Mass, J.L. (1984).** Compendium of strawberry Diseases . The American Phytopathological Society, St.Paul, MN, P 57-60 and 85-87.
- Nelson, P.E., T.A.Toussoun, and,W.F. O .Marasos. 1983).** *Fusarium* species and illustrated Manual for identification. University Park Pennsylvania U.S.A., 193p
- Munoz, J., G.Anres, and M.B Suarez. (2000).** A physiological and biochemical approach to the systematics of *Colletotrichum* species pathogenic to strawberry. *Mycologia*,92:488-98 .
- Peresipken, F.(1989).** *Phytopathology*. Kolos kiev, 480 P.
- Rappilly , F. L. (1968).** Technique de mycologien pathologie vegetable ,Annules des epiphyties 19 numero hors serie, Institut national de la recherché agronomique ,Paris ,France,102 P.
- Smith, B.J., and L.L Black. (1990).** Resistance of strawberry plant to *Colletotrichum fragariae* affected by environmental conditions. *Plant Disease*, 1990, 71:834-837.
- Ullio and Macarthur. (2004).** Strawberry disease control guide , Agfact H3.3.1 third edition,8 p.

الملخص العربي

دراسة تأثير بعض العوامل في نمو فطر *Colletotrichum fragariae*

المسبب لمرض تبقع أوراق (أنثراكنوز) الفراولة.

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أظهرت النتائج أن مرض تبقع أوراق الفراولة في الساحل السوري وفي منطقة البحيرة - جمهورية مصر العربية يتسبب عن الفطر *Colletotrichum fragariae* ، وبلغت نسبة الإصابة به على الفراولة في الزراعة المحمية في محافظتي طرطوس واللاذقية 10.7-17.5% وشدتها 1.5-2.2%. تعتبر درجة الحرارة 25م هي الدرجة المثالية لإنبات الأبواغ الكونيدية ونمو مشيجة الفطر المسبب للمرض. تنبت الأبواغ الكونيدية بنسبة عالية عند إضافة جلوكوز (1%) وفي مدى درجة حموضة وقلوية pH (6-8). كما تم تعريف أربع أنواع من جنس *Colletotrichum* تصيب نباتات الفراولة وهي:

1- عزل من الأوراق الفطر *C. fragariae*

2- عزل من الساق والأزهار والثمار الفطر *C. gloeosporioides*

3- عزل من الثمار الفطر *C. dematium*.

4 - عزل من الثمار الفطر *C. acutatum*.

كلمات مفتاحيه : الفراولة - أنثراكنوز - إنبات الجراثيم - *C. fragariae*