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Pathological, morphological and chemical differences among sclerotia of *Sclerotinia sclerotiorum* affecting some vegetable plants

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ABSTRACT

The pathogenecity test, fungal linear growth measurement, morphological characters and lipid composition of the sclerotia, from different isolates of *Sclerotinia sclerotiorum*, expressed variable pattern of behaviour. Pepper and Eggplant (Solanaceae); isolates were similar, Squash and Cucumber (Cucurbitaceae) were, also, similar and Pea (Fabaceae) had its own characterized behaviour. Generaly, the larger the sclerotia the more pathogenic the isolate. Pepper and Eggplant isolates produced the largest sclerotia followed by Squash and Cucumber isolates and, then, Pea isolate, Five fatty acids were detected in the sclerotia of tested isolates namely; palmitic, palmitoleic, stearic, oleic and linoleic acids, but at different rates. Higher virulency was associated with lower Tu/Ts ratio, e.g.; higher palmitic and oleic and lower linoleic acids.

INTRODUCTION

Sclerotinia sclerotiorum (Lib-) De Bary is an important soilborne pathogen in temperate and subtropical areas of the world that causes diseases on a wide variety of crops and vegetables (Walker, 1952 and Chupp and Sherf, 1960). The fungus produces sclerotia, which have a major role in the biology of the organism. Sclerotia are the principle survival structure under adverse conditions. Inocula sources for most diseases caused by *S. sclerotiorum* originate from sclerotia that germinate myceliogenically or carpogenically (Nelson *et al.* 1988). The disease occurs on root, stem and fruits of infected host plant (Echandi and Walker, 1957). Sclerotinia stem rot is characterized by the development of white, fluffy mycelial growth of the pathogen. Lesion may girdle the stem and kill plant. Large black sclerotia develop in the mycelial felt (Morgan and Ledieu, 1979).

Several investigators reported the occurrence of Sclerotinia disease on different vegetable plants grown in Egypt (El-Zarka, 1967; Assawah *et al.*, 1969 and El-Tobshy and Zayed, 1978).

Many reports have emphasized the importance of moisture in the development of *S. sclerotiorum* (Moore, 1955; Adams, 1975 and Adams and Tate, 1976). Tyrrell (1967) recorded that quantitative differences in fatty acid composition occurred among different isolates of the same fungus. Sumner and Colotelo

(1970) compared the fatty acid composition of sclerotia of various fungi including *S. borealis* and *S. sclerotiorum* produced under specific culture conditions with those of sclerotia isolated from natural environments. Khalil and Ragab (1988) found that sclerotia of *S. sclerotiorum* contained higher percentage of oleic fatty acid than their "parent" mycelium. Sclerotia contained higher proportion of unsaturated fatty acids Tu than the mycelium. Fatty acids in one month old sclerotia were more unsaturated than in those of two years old. Sclerotia accumulated high amount of Tu, in particular oleic and linoleic to be used for new fungal growth. Sclerotial survival and differences between young and old sclerotia, in their germination rate, linear growth, mycelial dry weight, might be affected by the fatty acid composition of the fungal lipid.

This work was carried out to study the morphological differences among sclerotia of *Sclerotinia sclerotiorum* affecting some vegetable plants. The relationship between fatty acid composition of sclerotia produced on tested hosts, and their pathogenecity was studied.

MATERIALS AND METHODS

Isolation of the pathogen

Stem samples of five vegetable plants showing symptoms of Sclerotinia rot were collected from

different localities in Ismaillia Governorate during 1992 season. Tested hosts were: *Pisum sativum* L. (Pea), *Cucurbita pepo* L. (Squash), *Cucumis sativus* L. (Cucumber), *Capsicum frutescens* L. (Pepper) and *Solanum melongena* L. (Eggplant). Infected portions of stem were washed using tap water before being cut into small pieces and surface sterilized with 2% sodium hypochlorite solution for 10 min. Pieces were then rinsed several times in sterile water and dried between two sterile filter papers. Pieces were plated on water agar medium and incubated at 25°C for 1 month. The growing fungus was identified on the bases of its morphological and cultural characters according to identification keys of; Domsch *et al.* (1980) and Hanlin (1990).

Pathogenecity test

Pathogenecity of fungal isolates was checked under greenhouse conditions. Inocula were prepared by growing the fungus on water agar medium under sterile conditons in Petri dishes for 1 month. Sterile potted soil, pots 25 cm in diameter, were infested with the tested fungal isolates by mixing 50 sclerotia which were randomly chosen from each isolate/kg soil, 4 days before planting. Seeds of tested hosts were, also, sterilized in 2% sodium hypochlorite for 5 min. Seeds were then washed in several changes of sterilized water. The treatment for each host comprised of 5 pots. Each pot was sown with 10 seeds. In addition, 5 pots of uninoculated soil served as a check treatment.

Diseased plants were counted two months after sowing.

Morphological and chemical studies

One month old sclerotia, from hyphal tips, of tested fungal isolates grown on water agar media were used for morphological and chemical studies.

Measurement of the fungus linear growth

A sclerotium 1 month old was collected from each of various tested hosts, 5 replicates, and transferred to PDA medium in Petri dishes 9 cm in diameter. All Petri dishes were incubated at 25°C until the fungal growth of one of the isolates completely covered the whole dish. This occurred after 7 days with Pepper isolate.

Morphological characters of sclerotia

Shape, colour and growth nature of the sclerotia were recorded. A representative sample of 50 sclerotia was collected in duplicate from each tested host to determine the specific weight of sclerotia and their size (by difference in water size placed in a 10 ml. measurement cylinder resulted when adding the sclerotia).

Extraction of crude oil

Representative samples, 2g each, of sclerotia

obtained from various investigated vegetable plants were soaked in a mixture of chloroform: methanol (2:1 , v/v) for 24 hrs. The miscella of various samples were collected and filterated. The solvent was evaporated under vacuum at 40–50°C. (Sahl, 1967).

Preparation of free fatty acid methyl esters

The methyl esters of free fatty acids were prepared using benzene: methanol: conc. sulphuric acid (10:86:4). Methylation was carried out for 1 hr at 80–90°C (Stahl, 1967).

Identification of free fatty acid methyl esters by Gas liquid chromatographic technique (GLC)

Hewlett Packard gas liquid chromatograph (Model 5890) equipped with a flame ionization detector and 6 ft coiled stainless steel column packed with 10% DEGS (Di–ethylene glycol succsinate) was used. One ul of fatty acid methyl esters was injected into the column using a Hamilton microsyringe. The gas chromatographic conditions used for isothermal analysis were as follows. Temperatures were; detector 300°C, injector 250°C and oven 170°C. Flow rates were ; hydrogen 30 ml/min., nitrogen 30 ml/min. and air 330 ml/min. Peak areas were measured using Hewlett Packard 3392 A integrator.

RESULTS AND DISCUSSION

Pathogenecity test

Symptoms on artificially inoculated plants, started at the basal portion of the stem as brown and water-soaked spot. Development of white fluffy mycelial growth of the pathogen occurred on the lesions. A histogrammatic representation of the disease incidence of tested hosts is shown in Fig. 1. Data indicate that tested hosts could be classified into two categories on the basis of the percentage of infection: a-Highly sensitive; Pepper (88%) and Eggplant (84%); b- Moderately sensitive; Cucumber (80%), Pea (78%) and Squash (74%).

Measurement of the fungus linear growth

The fungul linear growth was measured for the five studied isolates. Results are shown in Fig. 2. Data indicated that rate of linear growth coincided with those of the disease incidence, i.e.; the higher the growth rate the more virulent the isolate.

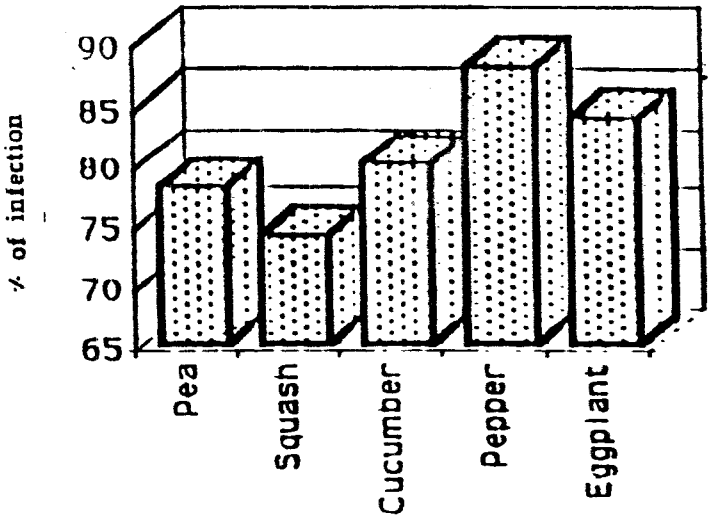


Fig. 1 : Disease incidence (infection %) on five vegetable plants infected with Sclerotinia sclerotiorum .

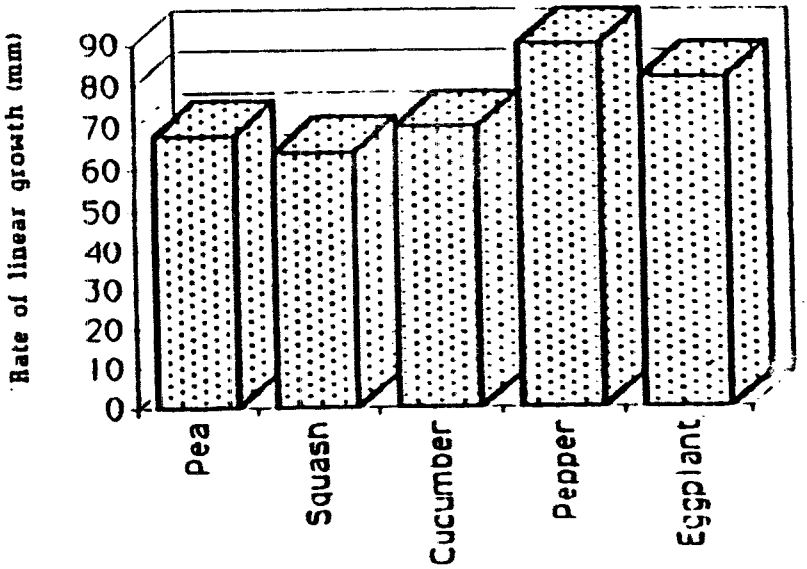


Fig. 2 : Linear growth (mm) of different Sclerotinia sclerotiorum isolates from five vegetable plants .

Morphological characters of sclerotia

Sclerotial bodies gradually formed superficially on vegetative mycelium grown on the water agar edium in Petri dishes. Pattern of sclerotial evelopment varied according to the tested isolates. Generaly sclerotial bodies gradually formed, first as grey-coloured compact masses which darken gradually until they became black and hard. Pea, isolate developed their sclerotia at the priphery of the plate and continued inward to the centre. In contrast, Pepper and Eggplant isolates developed their sclerotia at the centre of the plate and continued out wards. Squash and Cucumber isolates, however did not follow a definite pattern and were scattered allover the medium.

Table (1) represents morphological characters of the sclerotia of different fungal isolates. Weight and size of sclerotia varied according to tested isolate. The behaviour pattern of both weight and size of sclerotia was, almost, similar to that forementioned for the infection percentage and fungal linear growth. Solanaceae (Pepper and Eggplant) were on top. Fabaceae (Pea) recorded the least values. Cucurbitaceae (Squash and Cucumber) were intermediate compared to others.

Sclerotia of Pepper and Eggplant were elongated in shape and developed in groups. Those of other tested isolates developed solitary being flattened globe in case of Pea and elongated for

Squash and Cucumber. Sclerotia of various isolates were more or less black in colour.

Previous investigators reported that sclertia varied in their morphological characters according to certain factors, such as the species of pathogen involved, used media, temperature and their chemical composition (Walker, 1952; Bedi, 1956 and 1962; Ragab 1980 and Khalil and Ragab 1988).

Table 1. Morphological characters of sclerotia of *Sclerotinia sclerotiorum* affecting five vegetable plants (Average of 50 sclerotia)

Host	Weight, g.	Size, cc.	Shape	Colour
Pea	0.5	0.7	Flattened globe	Faint black
Squash	1.2	1.5	Elongated	Black
Cucumber	1.2	1.5	Elongated	Black
Pepper	2.0	3.5	Elongated (in groups)	Black grey
Eggplant	1.3	1.8	Elongated (in groups)	Black

Chemical characters of sclerotia

Fatty acid composition of sclerotia of the tested isolates are represented in Table 2 and illustrated in Figs 3 and 4. Five fatty acids were detected in the sclerotia of tested isolates but at

various levels. The percentage of fatty acids recorded in various sclerotia followed the pathological and morphological pattern of behaviour previously mentioned; i.e, Eggplant and pepper were similar, Squash and Cucumber were, also, similar and Pea showed a distinct pattern.

Sclerotia of Pepper and Eggplant which recorded a high disease incidence recorded the least ratio of TU/TS being 1.410 and 1.373; respectively, compared with 3.288 (Squash), 2.791 (Cucumber) and 4.035 (Pea).

The major fatty acids were palmitic, oleic and linoleic. However, stearic was detected at relatively low level (3 to 10%). On the other hand, palmitoleic was only detected (6.073%) in Sclerotia of Eggplant and was completely absent in the others.

From quantitative standpoint, sclerotia of various isolates contained different proportions of fatty acids. Sclerotia of Pea, Squash and Cucumber showed high proportion of oleic and linoleic. Pepper and Eggplant sclerotia, however, had high proportion of palmitic and oleic.

It might be concluded that, the high percentage of infection, recorded for Pepper and Eggplant isolates was accompanied by lower ratio of TU/TS, higher proportion of palmitic and lower proportion of linoleic. However, sclerotia of all isolates were characterized by having high level of

oleic acid specially in case of Pepper (48.068%) and Eggplant (47.257%) which recorded the highest disease incidence. These findings indicate that oleic (an unsaturated fatty acid) being the major constituent in lipids of sclerotia, played an important role in disease incidene followed by palmitic (saturated fatty acid); i.e., both oleic and palmitic are major fatty acids specific to *Sclerotinia sclerotiorum*.

Shaw (1965) supported the view that the presence of certain fatty acid is probably genus or even species spcific. Tyrrell (1967) showed that different isolates of the same fungus quantitatively differed in fatty acid composition. Farag *et al.* (1981) concluded that variation in fatty acid pattern could be used as a criterion for fungal taxonomy. Khalil and Ragab (1988) indicated that sclerotia contained a high proportion of unsaturated fatty acids than the mycelium and young sclerotia developed considerably more unsaturated fatty acids than the older sclerotia. They found that the proportion of oleic acid in sclerotia was high irrespective of their age. This emphasizes the role of oleic acid in disease incidence reported in the present study.

Table 2. Free fatty acid composition, %, in sclerotia of *Sclerotinia sclerotiorum* affecting five vegetable plants.

Fatty acids	%				
	Pea	Squash	Cucumber	Pepper	Eggplant
*16:0 Palmitic	16.107	15.899	19.561	31.740	30.451
16:1 Palmitoleic	0.0	0.0	0.0	0.0	6.073
18:0 Stearic	3.264	5.564	4.313	9.751	9.024
18:1 Oleic	33.509	32.009	22.495	48.068	47.257
18:2 Linoleic	44.655	38.558	44.144	10.441	0.874
TU/TS**	4.035	3.288	2.791	1.410	1.373

* Number of carbon atoms : number of bonds per molecule.

** TU/TS refers to the ratio between the total unsaturated fatty acids to the total saturated fatty acids.

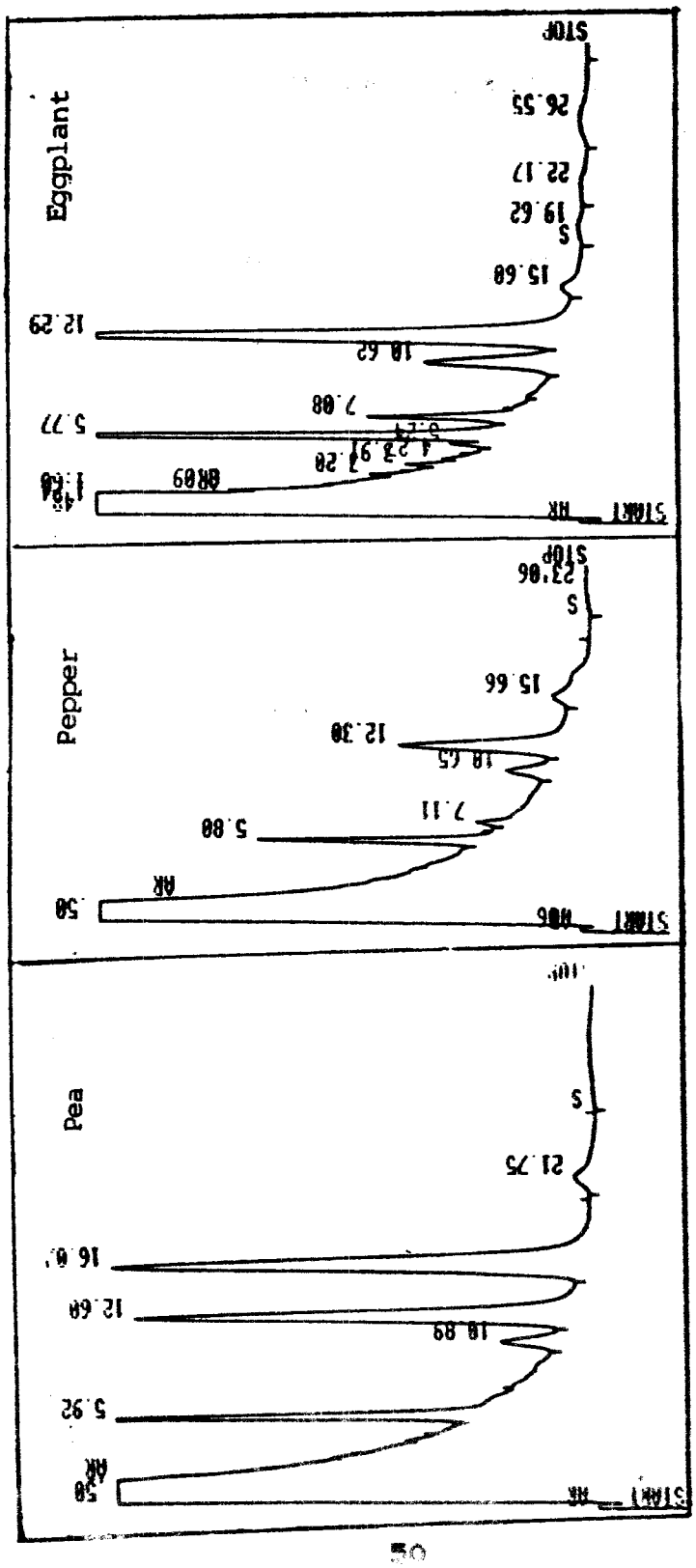
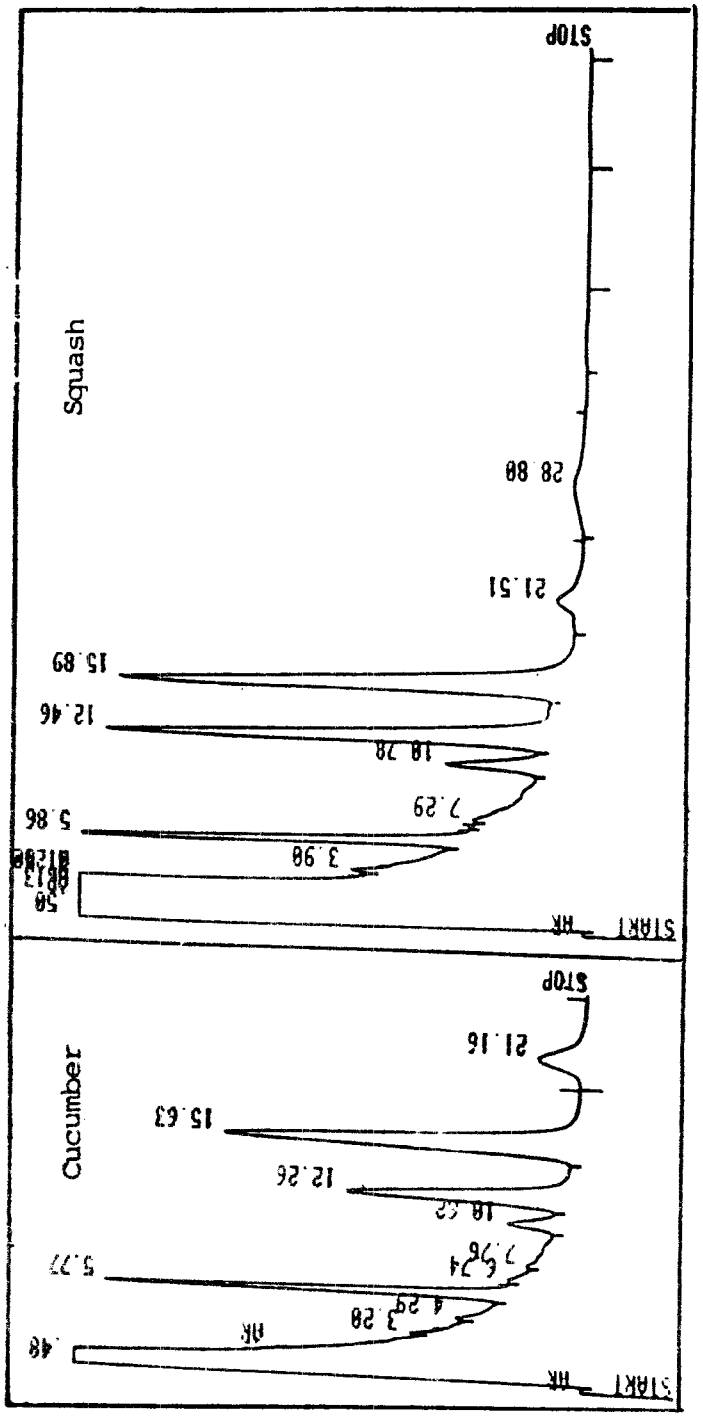


Fig. 3 : GLC chromatograms of free fatty acid methyl esters in sclerotia of Sclerotinia sclerotiorum affecting Pea , Pepper and Eggplant.



Retention time (min.)

Fig. 4 : GLC chromatograms of free fatty acid methyl esters in sclerotia of Sclerotinia sclerotiorum affecting Cucumber and Squash

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الإختلافات المرضية والظاهرية والكيميائية بين الأجسام الحجرية لفطر سكليروتينيا سكليروتيورم الذى يصيب بعض نباتات الخضر

الملخص العربى

أظهرت الدراسة المرضية ، وقياس قطر نمو الفطر ، والصفات الظاهرية ، وتركيب الأحماض الدهنية للأجسام الحجرية لعزلات مختلفة لفطر سكليروتينيا سكليروتيورم نمط مختلفا في سلوكها ، حيث تشابهت العزلات المأخوذة من الفلفل ، والباذنجان (الفصيلة الباذنجانية) ، وتماثلت ، كذلك ، الكوسة والخيار (الفصيلة القرعية) ، وكان للبسلة (الفصيلة الفراشية) سلوكها المتميز .عموما ، صاحب زيادة حجم الأجسام الحجرية زيادة الإصابة المرضية للعزلات . سجلت عزلات الفلفل ، والباذنجان أعلى نسبة للإصابة ، وكانت الكوسة ، والخيار متوسطة ، والبسلة معتدلة في هذا الصدد . أمكن الكشف عن خمسة أحماض دهنية بالأجسام الحجرية المختبرة ، وهى أحماض البالمتيك ، والبالمتوليك ، والستياريك ، والأوليبك ، واللينوليك . وكانت تختلف في نسبتها تبعا لإختلاف العزلات . صاحب العزلات ذات القدرة المرضية العالية إنخفاض في نسبة الأحماض الدهنية الغير مشبعة إلى تلك المشبعة ، وكذلك محتوى عالى من أحماض البالمتيك والأوليبك ، وإنخفاض محتوى اللينوليك .