Egypt. J. Phytopathol., Vol. **44**, No. 1, pp. 175-189 (2016)

Effect of Infection by *Sclerotinia sclerotiorum* on Constituents of Cucumber Plants of Phenols and Amino Acids

M.A. Khalil, Z.M.M. Mustafa and Abeer A. El-Ghanim
Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

Over the last few years, white mould disease was one of the most important diseases causing severe losses on cucurbit plants in many Egyptian governorates, particularly under commercial plastic houses. Investigation was carried out to isolate and identify the causative agent (s).Five fungal isolates were obtained from different governorates and were pathogenic to Delta-star RZ hybrid of cucumber. Moreover, the isolates obtained from the naturally infected cucumber plants grown in Giza Governorate (Gizerat El-Dahab) were the highly virulent. On the other hand, plants of eight cucumber hybrids were evaluated for their susceptibility to infection by the white mould disease. Results indicated that Delta-star RZ (hyb.) gave the highest percentage of disease susceptibility, while Marmar hybrid showed the less percentage. Data elucidated that the amounts of free, total phenols and conjugated compounds were somewhat higher in the non-infected healthy highly resistant hyb, Marmar than those observed in the more susceptible hyb. Delta-star RZ particularly at 40 days after infection. On the other hand, total free amino acids were increased in response to infection with any highly virulent or low virulent isolate, Delta-star RZ showed the highest figure particularly after 40 days than the less susceptible hyb, Marmar.

Keywords: Amino acids, cucumber; hybrids; phenols and *Sclerotinia sclerotiorum.*

Cucumber (*Cucumis sativus* L.) is considered one of the most economically important cucurbitaceous crop in Egypt. It occupies wide area of the total area cultivated with the different cucurbits in open fields and also under commercial plastic houses. It's subjected to invasion by numerous diseases, in particular under commercial plastic houses, such as wilt, root rot and stem base rots diseases which cause severe damage and reduce the quantity and quality of fruit production. *Sclerotinia sclerotiorum* (Lib.) De Bary causes white mould; stem rot and fruit rot diseases in 408 plant species of 275 different families (Boland and Hall, 1994).The pathogen overwinters as sclerotia in soil or within the infected tissues, or as mycelia in dead or alive plant tissues (Agrios, 1997).

Eight cucumber hybrids were tested in this experiment using soil infested by the highly virulent isolate (Giza) under greenhouse conditions. This experiment showed differences in host reaction to white mould infection.

Materials and Methods

1. Source of the fungal isolates:

Five different isolates of *Sclerotinia sclerotiorum* were obtained from naturally infected cucumber (*Cucumis sativus*) plants. These plants were grown during season 2013/2014 under plastic houses located at Giza (Gizerat El-Dahab), (Etledem), Ismailia (El-Kassasen), Beheira (El-Nubaria) and Kalubia (Sheben El-Kanater) governorates. Pure cultures of the pathogen were obtained following the procedure adopted by Ricker and Ricker (1936). The fungus was identified according to Barnett and Hunter (1972) and Purdy (1979). Visually diseased root plants were thoroughly washed with running tap water. Then small pieces of the diseased roots were surface sterilized by dipping in 0.1% mercuric chloride solution for 2 minutes and washed smoothly with sterilized water, then plated under aseptic conditions on PDA medium in petri dishes, then dishes were incubated at 27 ^{o}C for three days, after which the growing fungi were microscopically checked (Barnett and Hunter, 1972). The pathogens were identified according to their macroscopically and morphological characters. The identification was confirmed by the fungal Mycology and Plant Diseases Survey Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. On the other hand, two types of pathogen inocula of *S. sclerotiorum* (De Figueiredo *et al*., 2010) were used, the first type of inocula consisted of sclerotia and the second one was the fungal mycelium suspended in sterilized distilled water.

2. Pathogenicity test:

Sandy-barley medium inoculated by mycelium of *S. sclerotiorum* isolates was separately mixed with the disinfested sand-clay soil (1:1, w/w) in pots (30-cm-diam) at the rate of 4% by weight. Twenty five cm pots were partially filled with the inoculated soil of each isolate and watered daily for two weeks before planting. Six seeds of cucumber Delta-star RZ hybrid per pot were planted (at the depth of 2 cm.). Plants were irrigated and fertilized when it was necessary. Four pots were used as replicates for each treatment. The same numbers of pots containing sterilized non-inoculated soil were served as control. Plants were kept under greenhouse conditions where air temperature ranged between 20 to 25^{o}C. As shown in Table (1), 45 days after planting, the mean averages of disease incidence percentage and disease severity were calculated according to Horsfal and Barrat (1945).

Table1. An improved grading system for measuring plant disease

Rating scores	Interval (%) (Disease incidence)	(%) (Disease severity)
0	0	1.17
1	0-3	2.34
2	3-6	4.68
3	6-12	9.37
4	12-25	18.75
5	25-50	37.50
6	50-75	62.50
7	75-88	81.25
8	88-94	90.63
9	94-97	95.31
10	97-100	97.66
11	100	98.82

3. Reaction of different cucumber hybrids to white mould disease under greenhouse conditions:

Eight cucumber hybrids; Delta-star RZ, Sweet kranch, Amera 2, Super-star, Novo, Hana, Primo and Marmar were kindly obtained from Vegetable Research Dept., Horticulture Res. Instit., ARC. Plants were tested for their reactions against the highly virulent isolate of *S. sclerotiorum* as mentioned before under pathogenicity test. On the other hand, free, total phenols and conjugated compounds and free amino acids (mg/g dry weight) were determined in extracts of infected and healthy cucumber plants, of the more resistant Delta-star RZ hyb and the less susceptible Marmar hyb at 30 and 40 days after inoculation. Recently, Bozarth and Diener (1963) recommended that preparation of the stem-base extracts as follows: Samples of 5.0 grams of fresh stem-base tissues were obtained from healthy cucumber hybrids Delta-star RZ (more resistant, hyb.) and Marmar (less susceptible, hyb) inoculated with the highly virulent and low virulent isolates of *S. sclerotiorum* at two plant ages (30 and 40 days old) as mg/1g dry weight were cut into small portions and immediately stored in 50ml. of 95% ethanol in brown bottles and kept in the dark at room temperature for one month until the tissues were colourless then the ethanol extracts were subjected to air current at room temperature till approximately dryness, then the extracts were quantitatively transferred into 5ml of 50% isopropanol and stored in vials at 1 Co. The obtained ethanolic extracts were used for the following determinations.

3.1. Phenol contents:

Free, total phenols and conjugated were colourimetrically determined using the "Folin and Ciocaltei" reagent as described by Snell and Snell (1953). The reagent was prepared by adding 100g sodium tungstate and 25g sodium molybdate to 700ml distilled water in conical flask then 50ml phosphoric acid (85%) and 100ml of concentrated hydrochloric acid (HCL) were added. Flask containing this mixture was attached to a reflex condenser. The mixture was left to boil gently in water-bath for 10hrs.' then it was left to cool. After that 150g lithium sulphate and 50 ml distilled water were added. Few drops of bromine were also added and the mixture was heated again to remove access bromine. Finally, the mixture was completed to 1000 ml with distilled water. Free, total phenols and conjugated were determined in extracts of infected and healthy cucumber plants. Determination was calculated as catechol in terms of mg phenols per 1g dry weight.

To determinate the free phenols, 1 ml of sample extract was put in a sterilized test tube with 1ml distilled water, 5 ml Folin-Ciocaltei reagent and 15 ml Na_2CO_3 20% (w/v). The mixture was completed to 50 ml with distilled water and the colour density was recorded at 520 nm using a spectrophotometer (Snell and Snell, 1953).

Total phenols were determined by treating 1 ml of a sample extract with 0.25 ml Folin-Ciocaltei and 25 ml Na_2CO_3. The mixture was completed to 100 ml with distilled water and the values of colour density were recorded at 520 nm using the same apparatus (Snell and Snell, 1953).

Conjugated phenols were determined by subtracting the amount of free phenols from that of the total. Results were expressed as milligrams equivalent of catechol per 1g dry weight of stem-base.

3.2. Free amino acids contents:

Free amino acid contents were determined qualitatively and quantitatively in extracts of infected and healthy cucumber plants at 30 and 40 days after inoculation. Paper chromatography was used. Extracted samples as well as solutions of 24 pure amino acids were separately spotted on chromatograms (Whatman No.1) using micropipette, then chromatography was flown in the solvent system consisted of n-butanol: glacial acetic acid: water (4:1:2) for 24 hrs. After that, chromatograms were air dried and then flown once more in the same solvent. Then chromatograms were immediately dipped in a solution of 0.2% ninhydrin in acetone (w/v) (Smith, 1969). Purple colour development of the separated amino acid spots was obtained by heating the air dried- chromatograms for 30 minutes at 70^{o}C.RF (Rang value) was determined for each spot. According to rang value of each pure amino acid, It was possible to identify the amino acids in samples of cucumber extracts. Amino acids in cucumber stem-base were determined quantitatively; also concentrations of different amino acids in stem-base extracts were calculated according to standard curves prepared for each pure amino acid. Series of different concentrations were prepared for each pure amino acid, spotted and developed on chromatograms as described before. Coloured zones of each sample of each pure amino acid on the chromatograms were marked, cut off from the paper and extracted for 25-30 min. in 50% methanol. The colour density was immediately recorded using colourimeter at 570 nm for all amino acids except proline which was determined at 420 nm (Ambe and Toppel, 1961).

Statistical analysis:

The obtained data were subjected to statistical analysis using MSTATC computer program (Michigan Statistical Program Version C). Least significant difference (L. S. D.) at confidences was determined according to Gomez and Gomez (1984).

Results

1. Pathogenicity test:

Data presented in Table (2) and Fig. (1) indicate that all the tested isolates proved to be pathogenic to the tested Delta- star RZ cucumber hybrid. In this regard, isolate Gizerat El-Dahab (Gizag gov.) was significantly the highly virulent isolate causing the highest percentage of disease incidence and disease severity (83.3, 31.2%, respectively), followed by isolates from Etledem (Minia gov.) (50.0, 30.9%) and El-Kassasen (Ismailia gov.) and (41.7, 14.1%), respectively. while, the two isolates El-Nubaria (Beheira gov.) and Sheben El-Kanater (Kalubia gov.) recorded the lowest percentage of the disease incidence and disease severity, being 33.3, 4.7% and 16.7, 1.6%, respectively.

2. Reaction of different cucumber hybrids to white mould:

Eight cucumber hybrids were tested in this experiment using soil infested by the highly virulent isolate Gizerat El-Dahab. Data in Table (3) and Fig. (2) show that Delta-star RZ hyb showed the highest percentage of the disease incidence and disease severity (66.7, 43.7%), respectively, followed by hybrids, Sweet-kranch

Table 2. Pathogenicity test of *S. sclerotiorum* isolates on Delta-star RZ cucumber hybrid under greenhouse conditions

Source of fungal isolate	Disease incidence%	Disease severity%
Gizerat El-Dahab (Giza gov.)	83.3	31.2
Etledem (Minia gov.)	50.0	30.9
El-Kassasen (Ismailia gov.)	41.7	14.1
El-Nubaria (Beheira gov.)	33.3	4.7
Sheben El-Kanater (Kalubia gov.)	16.7	1.6
Control	0.0	0.0
L.S.D. at 0.05%	12.7	7.8

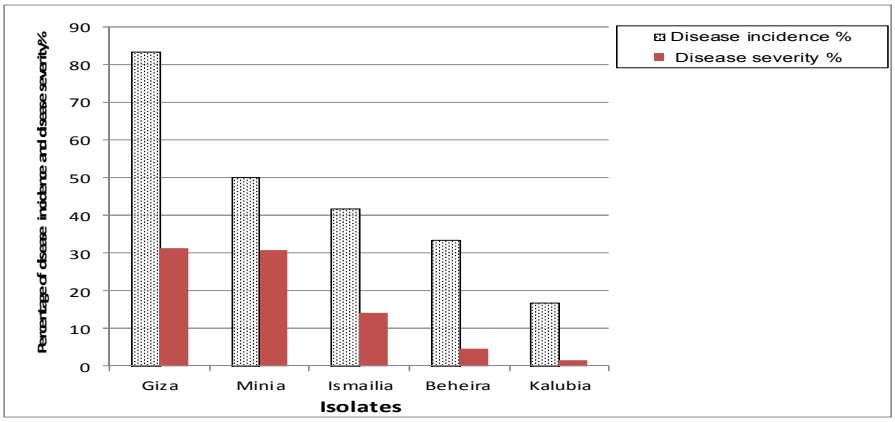

Fig. 1. Pathogenicity test of five fungal isolates of *S. sclerotiorum* on Delta-star RZ cucumber hybrid.

and Amera 2 (50.0, 37.5%) and (41.7, 21.9%), respectively, while Super-star; Hana and Novo hybrids were less susceptible. On the other hands, Primo and Marmar hybs were resistant, where disease severity was 3.1 and 2.3%, respectively. Therefore, Delta-star RZ, the more susceptible and Marmar the highly resistant were chosen for further studies.

Table 3. Susceptibility of different cucumber hybrids to infection by the highly virulent isolate of *S. sclerotiorum* under greenhouse conditions

Hybrids	Disease incidence (%)	Disease severity (%)
Delta- star RZ	66.7	43.7
Sweet-kranch	50.0	37.5
Amera 2	41.7	21.9
Super- star	33.3	17.2
Novo	25.0	9.4
Hana	25.0	5.5
Primo	16.7	3.1
Marmar	8.3	2.3
L.S.D. at 0.05%	10.8	6.9

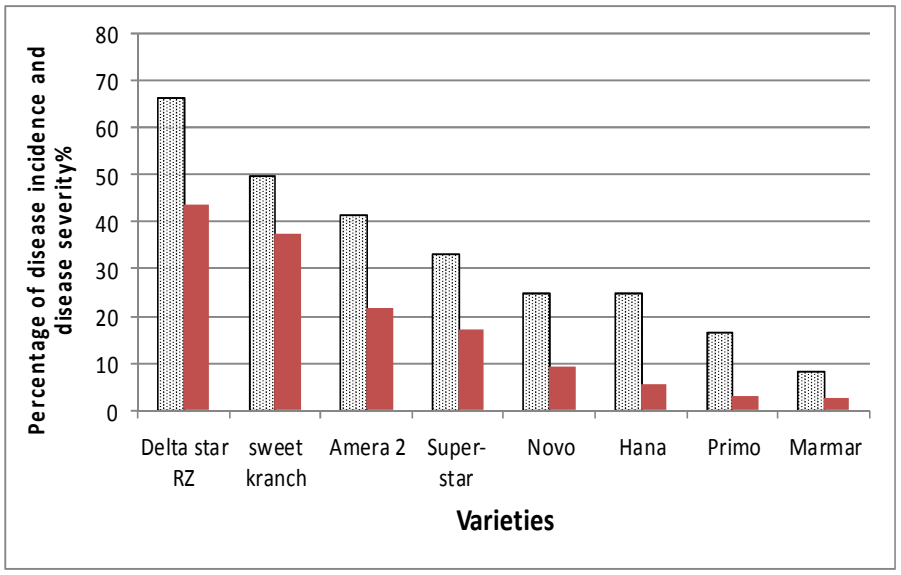

Fig. 2. Susceptibility of different cucumber hybrids to infection by the highly virulent isolate of *S. sclerotiorum*.

3. Effect of the causal organism on some constituents of cucumber plants:
3.1. Phenols content:

Data in Table (4) and Figs. (3 and 4) indicate that the amounts of free phenolic compounds were somewhat higher in non-infected healthy cucumber plants of the more susceptible Delta-star RZ at 30 days after planting. Meantime, the infection with each of the two isolates (highly virulent and low virulent), generally stimulated production of the phenolic compounds in both tested Marmar hyb and Delta-star RZ hyb particularly in the highly resistant one (Marmar) at 40 days of planting. The highly virulent isolate outclassed low virulent one in this respect. Obtained results signified that the amounts of phenolic compounds (free, conjugated and total phenol) were rather higher in non-infected healthy cucumber plants of Marmar hyb than those observed in the Delta-star RZ at 40 days after sowing in infested soil.

Table 4. Free, conjugated and total phenol contents of plant stem base of two cucumber hybrids infested with highly virulent and low virulent isolates of *S. sclerotiorum* as mg /1g dry weight

Amount of free (F), conjugated (C) and total phenols (T) in mg												
Soil infested by	Delta-star RZ (highly susceptible)						Marmar (highly resistant)					
	30days after sowing			40days after sowing			30days after sowing			40days after sowing		
	F	C	T	F	C	T	F	C	T	F	C	T
Highly virulent isolate	0.10	0.85	0.95	1.25	0.80	2.05	1.50	0.80	2.30	1.80	1.20	3.00
Low virulent isolate	0.80	0.45	1.25	1.00	0.80	1.80	0.85	0.40	1.25	1.10	1.02	2.12
non-infested	0.85	0.40	1.25	0.85	0.40	1.25	0.70	0.35	1.05	0.85	0.85	1.7
L.S.D. at 0.05%	A (Soil infestation) = n..s. B (30days after sowing) = 0.105 AB=0.149 C (40days after sowing) = 0.111 AC=0.158 BC=0.158 ABC=0.224						A (Soil infestation) = 0.158 B (30days after sowing) = 0.158 AB= n..s. C (40days after sowing) = 0.185 AC=0.262 BC= n..s. ABC= n..s.					

F, free phenols; C, conjugated phenols; T, total phenols

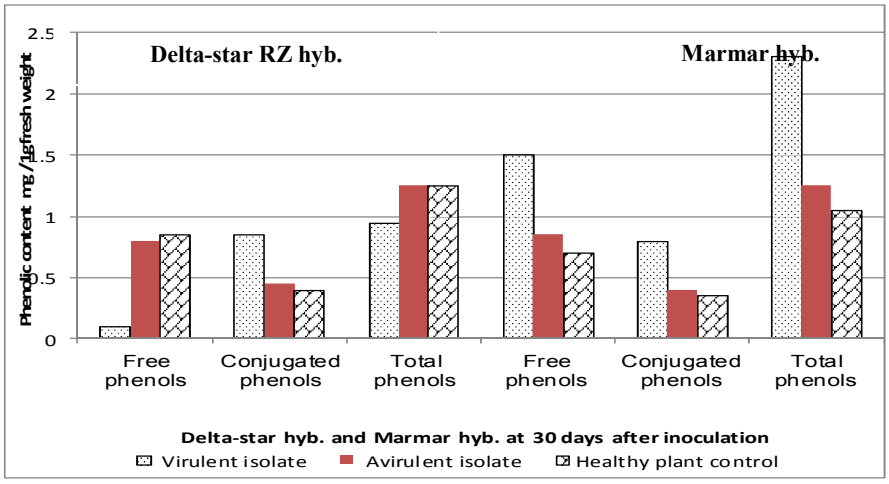

Fig. 3. Free, conjugated and total phenol contents in plant stem base of Delta-star RZ (highly susceptible hyb.) and Marmar (highly resistant hyb.) cucumber plants infested by highly virulent and low virulent isolates of *S. sclerotiorum* at 30 days after sowing as mg /1g dry weight.

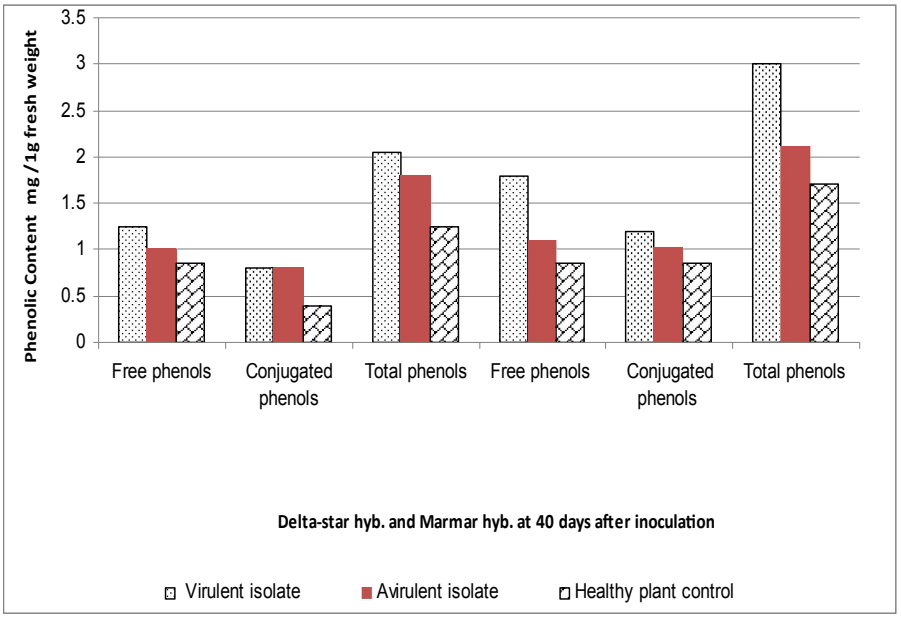

Fig. 4. Free, conjugated and total phenol contents in plant stem base region of Delta-star RZ (highly susceptible hyb.) and Marmar (highly resistant hyb.) cucumber plant infested by highly virulent and low virulent isolates of *S. sclerotiorum* at 40 days after sowing as mg /g dry weight.

3. 2. Free amino acids content:

Data in Tables (5 and 6) and illustrated in Figs. (5 and 6) indicate that the total free amino acids were increased in response to infection with any highly virulent or low virulent isolates. This increase was more pronounced after sowing of hybrid Delta star RZ in soil infested by the highly virulent isolate particularly after 40 days than the highly susceptible, hyb. (Marmar).Results also indicated that the highly susceptible, hyb. Delta star RZ contained higher levels of total free amino acids than the less susceptible, hyb. (Marmar) after sowing in soil infested with each of the two isolates of *S. sclerotiorum*.

DL-Alanine, Tryptophan, DL-Aspartic and DL-Metionine were increased as a result of infection by the highly virulent and low virulent isolate of *S. sclerotiorum* in both hybrids, Delta star RZ and Marmar after 30 and 40 days of sowing, particularly in the more susceptible, hyb Delta star RZ after 40 days of sowing than the less susceptible, hyb.(Marmar).

Table 5.Total and free amino acids (mg/g dry weight) determined at 30 and 40 days after sowing Delta star RZ (highly susceptible, hyb) in soil infested with the highly virulent and low virulent isolate of *S. sclerotiorum*

Amino acid	Delta-star RZ (highly susceptible)					
	30days after sowing			40days after sowing		
	Highly virulent	Low virulent	Control	Highly virulent	Low virulent	Control
Cystine	0.0	0.0	0.0	0.0	0.0	0.0
L-Cystein	0.0	0.0	0.0	0.0	0.0	0.0
DLOrnithineHCL	0.0	0.0	10.0	0.0	0.0	15.0
L-Lycine HCL	0.0	0.0	20.0	0.0	0.0	35.0
L-Histidine HCL	0.0	0.0	60.0	75.0	0.0	65.0
L-Arginine HCL	0.0	15.0	0.0	130.0	15.0	0.0
DL-Aspartic	120.0	120.0	0.0	200.0	60.0	0.0
DL-Serine	630.0	110.0	0.0	700.0	170.0	0.0
Glycine	80.0	0.0	0.0	100.0	55.0	0.0
L-Hydroxyproline	0.0	0.0	0.0	70.0	0.0	0.0
L-Glutamice	1.0	0.0	63.0	20.0	0.0	63.0
DL-Thrionine	0.0	0.0	0.0	0.0	0.0	0.0
Dihydrox alanine(3,4)	0.0	0.0	0.0	0.0	0.0	0.0
DL-Alanine	330.0	70.0	0.0	405.0	170.0	0.0
L-Proline	0.0	0.0	0.0	0.0	0.0	0.0
DL-Amino butyric	55.0	0.0	90.0	155.0	0.0	90.0
L-Tyrosine	40.0	0.0	0.0	8.0	0.0	0.0
Tryptophan	165.0	95.0	40.0	165.0	30.0	130.0
DL-Metionine	40.0	0.0	30.0	75.0	60.0	45.0
DL-Valine	0.0	0.0	10.0	0.0	0.0	10.0
L-Leucine	0.0	0.0	0.0	0.0	0.0	0.0
DL-Isoleucine	0.0	0.0	0.0	0.0	0.0	0.0
DL-Noxleucine	15.0	0.0	30.0	25.0	0.0	30.0
DLPhenylalanine	10.0	0.0	15.0	25.0	0.0	15.0
Total	1486.0	410.0	368.0	2153.0	560.0	498.0

Table 6.Total and free amino acids (mg/g dry weight) determined at 30 and 40 days after sowing Marmar (highly resistant, hyb) in soil infested with the highly virulent and low virulent isolate of *S. sclerotiorum*

Amino acid	Marmar (highly resistant)					
	30days after sowing			40days after sowing		
	Highly virulent	Low virulent	Control	Highly virulent	Low virulent	Control
Cystine	0.0	0.0	0.0	0.0	0.0	0.0
L-Cystein	0.0	0.0	85.0	0.0	0.0	80.0
DLOrnithineHCL	0.0	0.0	20.0	0.0	0.0	0.0
L-Lycine HCL	0.0	0.0	55.0	0.0	0.0	50.0
L-Histidine HCL	60.0	90.0	0.0	15.0	15.0	87.5
L-Arginine HCL	135.0	10.0	0.0	160.0	22.5	0.0
DL-Aspartic	170.0	130.0	0.0	205.0	95.0	0.0
DL-Serine	111.0	465.0	0.0	905.0	670.0	0.0
Glycine	85.0	150.0	0.0	155.0	50.0	0.0
L-Hydroxyproline	70.0	0.0	0.0	0.0	0.0	0.0
L-Glutamice	20.0	0.0	0.0	12.5	2.2	0.0
DL-Thrionine	0.0	0.0	65.0	0.0	0.0	0.0
Dihydrox alanine (3,4)	0.0	0.0	0.0	0.0	0.0	0.0
DL-Alanine	290.0	120.0	0.0	285.0	265.0	0.0
L-Proline	0.0	0.0	0.0	0.0	0.0	0.0
DL-Amino butyric	0.0	105.0	0.0	43.0	0.0	0.0
L-Tyrosine	0.0	15.0	0.0	72.5	0.0	150.0
Tryptophan	145.0	10.0	0.0	175.0	40.0	0.0
DL-Metionine	105.0	0.0	0.0	50.0	35.0	5.5
DL-Valine	0.0	0.0	0.0	0.0	0.0	0.0
L-Leucine	0.0	0.0	0.0	0.0	0.0	0.0
DL-Isoleucine	0.0	0.0	15.0	0.0	0.0	0.0
DL-Noxleucine	17.5	2.5	10.0	2.2	7.5	10.0
DLPhenylalanine	4.0	10.0	35.0	17.5	10.0	25.0
Total	1212.5	1107.5	285.0	2097.7	1211.7	408.0

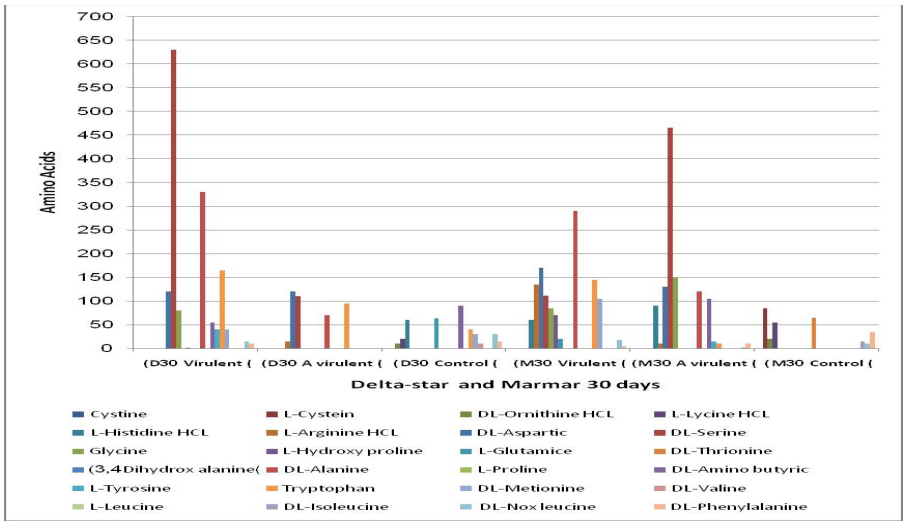

Fig. 5. Total free amino acids content (mg/g dry weight) of stem base tissues of cucumber Delta-star RZ (highly susceptible hyp.) and Marmar cucumber (highly resistant hyp.) grown in artificially infested soil with highly virulent and low virulent isolates of *S. sclerotiorum* at 30 days after sowing.

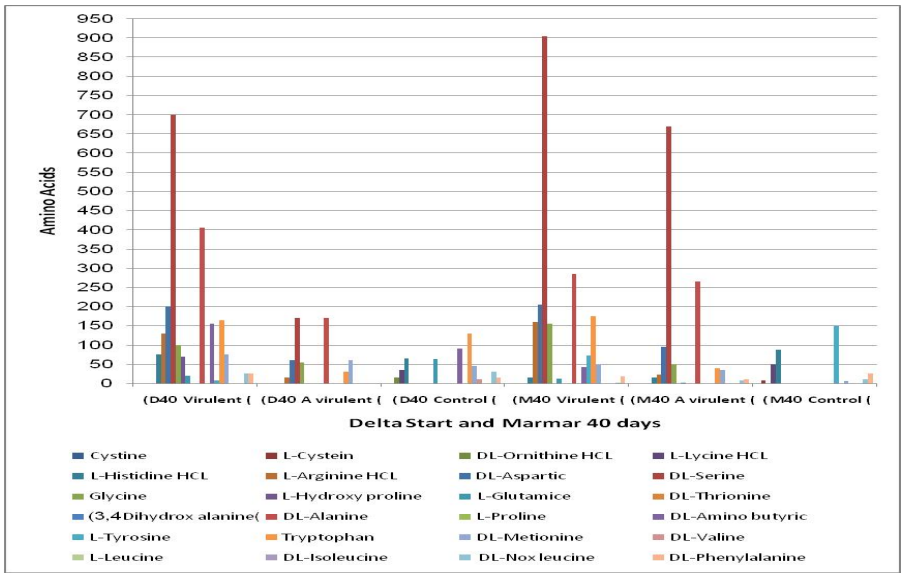

Fig. 6. Total free amino acids content (mg/g dry weight) of stem base tissues of Delta-star RZ (highly susceptible hyp.) and Marmar cucumber (highly resistant hyp.) grown in artificially infested soil with highly virulent and low virulent isolates of *S. sclerotiorum* at 40 days after sowing.

Discussion

In the present study, five different isolates of *Sclerotinia sclerotiorum* were obtained from cucumber (*Cucumis sativus*) plants naturally infected with white mould disease. These plants were grown during season 2013/2014 at Giza (Gizerat El-Dahab), Minia (Etledem), Ismailia (El-Kassasen), Beheira (El-Nubaria) and Kalubia (Sheben El-Kanater) governorates. Jnr *et al*. (2000) mentioned that Sclerotinia rot caused by *S. sclerotiorum* is one of the most important diseases and widespread which causes severe losses to cucurbit plants. Yanar and Onaran (2011) isolated one hundred nineteen isolates of *S. sclerotiorum* from infected greenhouse grown cucumber in the growing seasons of 2007 to 2009.

The pathogenicity test of the five isolates of *S. sclerotiorum* indicated that (Gizerat El-Dahab) isolate obtained from Giza proved to be the most virulent one followed by both isolates; Etledem (Minia) and El-Kassasen (Ismailia), while the two isolates El-Nubaria (Beheira) and Sheben El-Kanater (Kalubia) recorded the lowest percentages of disease severity. Bedlan (1986 and 1987), Monnet and Thibault (2000) and Rego (1994) found that *S. sclerotiorum* can attack all cultivars of cucurbit plants causing severe damage.

The highly susceptible hybrid was Delta-star RZ hyb which showed the highest percentage of disease severity followed by hybrids, Sweet kranch and Amera 2, while Super-star and Novo hybrids were less susceptible. On the other hand, Primo and Marmar hybs were resistant to the disease. In addition, host parasite interaction test which was carried out in this work pointed to some constituents as phenols and amino acids.

Likewise, phenol contents play an important role in plant resistance. Previous results are in agreement with the findings of Sinch and Chohan, (1979) who found that susceptibility of mature and ripe fruits of cucurbits to *Macrophomina phaseolina* was correlated with the concentration of total phenols, which was decreased with increasing maturity. In addition, results revealed also that the infection with each of the two isolates, generally stimulated production of the phenolic compounds in both tested Marmar hyb and Delta-star RZ hyb particularly in the less susceptible one (Delta-star) at 40 days after inoculation. The highly virulent isolate outclassed the low virulent one in this respect. It could be concluded that the high levels of phenols became toxic and inhibited the growth of the invading fungi indicating that these compounds may play an important role in plant resistance.

Furthermore, total and individual free amino acids were studied. The results obtained showed that the more susceptible, hyb. Delta star RZ contained higher levels of total free amino acids than the less susceptible one hyb Marmar at 30 and 40 days after inoculation with the highly virulent isolate. Results indicated that susceptibility was accompanied with high content of amino acids. Also results obtained showed clearly that total free amino acid was increased in response to infection with each of the tested isolates. Similar results were obtained by El-Deep *et al*. (1987) who found that methionine and valine were increased as a result of infection in more susceptible, hybrids of cucumber stem base than in the less susceptible ones. Results also showed that Dl- Serine, Tryptophan, DL-Aspartic and

Glycine were increased in the highly susceptible hyb. Delta-star with the highly virulent isolate at 30 and 40 days after sowing in the infested soil.

Similar results were obtained by Dulermo *et al*. (2009) who found that some amino acids were changed when an analysis of sunflower cotyledons amino acid content during infection with the necrotrophic fungus *Botrytis cinerea*. The authors concluded that a rapid disappearance of plant amino acids was observed due to fungal assimilation. Glutamate depletion was correlated to an enhanced sunflower glutamate dehydrogenase (GDH) transcription level in the area invaded by pathogen. Glutamine, Glutamate, Arginine and Asparagine were the main amino acids detected in healthy sunflower cotyledons. During infection, amino acid concentration was drastically decreased to be negligible. In this context, our results suggest that plant amino acids could be consumed by *S. sclerotiorum* during infection. Moreover, asparagine could be less favorably metabolized than other amino acids.

R e f e r e n c e s

Agrios, G.N. 1997. *Plant Pathology,* Academic Pres, California, 635pp.

Ambe,K.S. and Toppel,A.I. 1961.Improved separation of amino acids with new solvent system for two dimensional chromatography, *J. Chromatography*, **5**:546-549.

Barnett, H.L. and Hunter, B.B. 1972. Illustrated Genera of Imperfect Fungi 3th ed. *Burgess Publishing Company*.Minnesota, 241 pp.

Bedlan, G. 1986. The most important fungal diseases of cucumber. Die wichtigstenpilzkrankheiten der GurkenPflanzenschutz, (**9)**: 8-11.

Bedlan, G. 1987. The most important of pickling cucumbers. Die wichligstekrankheiten der Einlegeurken, **3** (3-4): 6-8.

Boland, G.J. and Hall, R. 1994.Index of plant hosts of *Sclerotinia sclerotiorum*.*Cand. J. Pl. Pathol.*, **16**: 93-108.

Bozarth, R.F. and Diener, T.O. 1963. Changes in concentration of free amino acids and 12-amids in tobacco plants by potato virus X and potato virus Y. *Virol.*, **21**: 188-193.

De Figueiredo, G.S.; De Figueiredo, C.; Cavalcanti, F.C.N.; Dos Santos, A.C.; Da Costa, A.F. and De Oliveira, N.T. 2010. Biological and chemical control of *Sclerotinia sclerotiorum* using *Trichoderma* spp. and *Ulocladium atrum* and pathogenicity to bean plants. *Brazilian Archives Biol. Technol*., **53**(1): Curitiba Jan/Feb. 2010.

Dulermo, T.; Bligny, R.; Gout, E. and Cotton, P. 2009. Amino acid changes during sunflower infection by the necrotrophic fungus *Botrytis cinerea. Plant Signal Behavior*, **4**(9): 859-861.

EL-Deep, A.A.; Ahmed, K.G.M.; Ghobrial, E. and Elian, M.I. 1987. Biochemical changes associated with resistance to spot blotch disease of barley. 2-Amino acids. *Agric. Res. Rev.*, **65(2)**: 191-201.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical Procedures for Agriculture Research*. A Wiley-Interscience Pub. NY., USA. 678pp.

Horsfall, J.G. and Barratt. R.W. 1945. An improved grading system for measuring plant disease. *Phytopathology* (Abstr.), **55**: 655.

Jnr, M.L.; Lopes, C.A.; and Silna, W.L.C. 2000. Sclerotinia rot losses in processing tomatoes grown under centre pivot irrigation in central. Brazil. *Plant Pathol.*, **49** (1): 51-56.

Monnet, Y. and Thibault, J. 2000. Diseases and pests of courgette. *PHM Revue, Horticole.,* **415**: 33-34.

Purdy, L.H. 1979. *Sclerotinia sclerotiorum:* History, disease and symptomatology, Host range, geographic distribution and impact. *Phytopathology*, **69**: 875-880.

Rego, A.M. 1994. Diseases caused by fungi on cucurbitaceae. *Inform. Agropecuario (Belo Horizonte)*, **17** (182): 45-54.

Ricker, A.J. and Ricker, R.S. 1936. *Introduction to Research on Plant Disease. A Guide to the Principles and Practice for Studying Various Plant Disease Problems*. Publ. John S. Swiff Co. Inc., St., Louis Chicago, New York, **117** pp.

Sinch, R.S and Chohan, J.S. 1979. Total phenolic in the fruits of cucurbits and their relation to development of charcoal rot. *Indian Phytopathol.*, **31**(4): 529-530.

Smith, L. 1969. *Chromatographic and Electrophoretic Techniques.* I. 250 William Heinemanon. Medical Books.Lid. 15616. Queen Street May Faire, London, W.L. and Inter Science. Publishers.Inc. New York, USA.

Snell, F.D. and Snell, C.L. 1953. *Colorimetric Methods of Analysis Including some Tubidimetric and Nephalometric Methods*. Vol. **111**, D.VanNostrand Company Inc., Pirnceton, New Jersey, Toronte-New York, London. 606 pp.

Yanar, Y. and Onaran, A. 2011. Mycelial compatibility groups and pathogenicity of *Sclerotinia sclerotiorum* (Lib) De Bary causal agent of white mold disease of greenhouse grown cucumber in Antalya, Turkey. *African J. Biotechnol.*, **10**(19): 3739-3746.

(Received 24/02/2016;
in revised form 31/03/2016)

تأثير الاصابة بالفطر *Sclerotinia sclerotiorum* على محتوى نباتات الخيار من الأحماض الأمينية والفينولات

محمد علي خليل ، زكى مصطفى محمد مصطفي ، عبير عبد الرحمن الغانم
معهد بحوث أمراض النباتات- مركز البحوث الزراعية – الجيزة.

أجريت هذة الدراسة فى نهاية موسم 2013 وخلال موسم 2014 تحت ظروف الصوبة الزراعية بهدف إختبار القدرة المرضية لبعض عزلات الفطر سكليروتينيا سكليروتيورم المسبب لمرض العفن الأبيض وتقييم بعض هجن الخيار ضد الاصابة بهذا المرض مع دراسة تأثير الاصابة على محتوى نباتات الخيار من الأحماض الأمينية والفينولات.تم في هذه الدراسة الحصول علي خمس عزلات من فطرسكليروتينيا سكليروتيورم من نباتات الخيار المصابة بمرض العفن الأبيض والمنزرعة تحت ظروف الصوب البلاستيكية جمعت من خمس محافظات هي: الجيزة (جزيرة الدهب) ، المنيا (أتليدم) ، الإسماعيلية (القصاصين) ، البحيرة (النوبارية) القليوبية (شبين القناطر).

تم إجراء إختبار القدرة المرضية للعزلات علي نباتات الخيار (هجين دلتاستار) وتبين أن عزلة جزيرة الدهب هي أقوى العزلات حيث أعطت أعلى شدة إصابة بالمرض يليها عزلة أتليدم ثم عزلة القصاصين بينما عزلتى النوبارية وشبين القناطر كانتا أقل قدرة على إحداث الإصابة بالمرض.أظهرت ثمانية هجن من الخيار ردود أفعال مختلفة تجاة العدوى بأقوى العزلات قدرة على إحداث الإصابة بالعفن الأبيض وكان الهجين دلتا ستار أكثرها حساسية للإصابة بالمرض يتبعة الهجين سويت كرانش ثم الهجين أميرة 2 بينما شوهدت ردود أفعال متوسطة ضد الإصابة بالعفن الأبيض مع الأصناف سوبر ستار ونوفو. وكانت هجن الخيار الأخرى : هنا ، بريمو، مرمر أقل قابلية للإصابة على الترتيب.

أظهرت النتائج أنة عند العدوى بأى من العزلتين القوية والضعيفة كلا على حدة للهجين دلتا ستار والهجين مرمر، حدثت زيادة فى كمية الفينولات الحرة والمرتبطة والكلية فى كلا الهجينين إلا أن الزيادة كانت أوضح في الهجين مرمر الأقل قابلية للإصابة مقارنة بالهجين دلتا ستار الأكثر قابلية للإصابة خاصة مع العزلة الأقوى في القدرة المرضية بينما كانت كمية الفينولات الحرة والمرتبطة والكلية عالية نوعا ما في نباتات الخيار السليمة للهجين مرمر مقارنة بالهجين دلتا ستار خاصة بعد 30 يوم من العدوى مقارنة بما لوحظ بعد 40 يوم من العدوى.

أظهرت النتائج أيضا أن الهجين دلتا ستار يحتوى على كميات عالية من الأحماض الأمينية الكلية مقارنة بالهجين مرمر وذلك عند عدوى كلا الهجينين بالعزلة الأقوى والعزلة الأقل في القدرة على الإصابة خاصة مع العزلة الأقوى في القدرة المرضية بعد 40 يوم من العدوى. ولقد سببت الاصابة بكل من العزلتين القوية والاقل قدرة مرضية زيادة فى كمية الأحماض الأمينية: سيرين ، الانين ، تربتوفان ، اسبارتيك ، جليسين ، امينوبيوتريك وذلك فى كلا الهجين دلتا ستار والهجين، مرمر بعد 30 يوم أو 40 يوم من العدوى وكانت الزيادة فى الأحماض الأمينية أكثر وضوحا فى الهجين دلتا ستار بعد 40 يوما من العدوى بالعزلة الاقوى فى القدرة المرضية.