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Pestalotia Root Rot : A New Disease on Strawberry Plants in Egypt

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A SURVEY of soil borne fungi attacking strawberry plants in Ismailia Governorate, Egypt resulted 593 fungal isolates; among of them 71 isolates belong to *Pestalotia longisetula* (*Pestalotiopsis longisetula*) out of these isolates (15.49%) were isolated from soil and 60 isolates (84.51%) from diseased strawberry plants, (Tamar and Yael cvs.). *Pestalotia longisetula* was higher frequency with Yael cv. than Tamar cv., which recorded 35.52 and 30.99% respectively. Also, higher frequency occurred with *Pestalotia longisetula* in nursery stage (65%) compared with the field production (35%.) On the other hand, less *Pestalotia longisetula* frequency occurred with diseased runners (8.33%) in comparison with diseased strawberry roots (61.67%) in both Tamar and Yael cvs.

Examination of *Pestalotia longisetula* by light microscope (LM) and (SEM) examination studies showed presence of fungus acervular conidimata, conidial septate, single conidia has appendage with 2-3 branches and five cells. Transmission electron microscope examination of conidia showed thick cell wall and cell membrane. The cytoplasm contains nucleus, mitochondria, vacuoles and cytoplasmic granules.

No pores were found between apical and subapical cell and spezenkorper region was observed in the apical cell of spore in longitudinal section. Longitudinal section of fungal hypha showed moderate thick wall, thin membrane, mitochondria and other organelles.

Four enzymes, *i.e.*, pectinase, cellulase, chitinase and dehydrogenase were estimated in Pestalotia culture medium.

Also, *Pestalotia longisetula* was found to produce two mycotoxin, *i.e.* ochratoxin A and mycophenolic acid.

Keywords: *Fragaria x ananassa*, Fungal diseases, Root rot, *Pestalotia longisetula* , *Pestalotiopsis.*

Strawberry plants (*Fragaria x ananassa* Duch.) are suffering from many diseases caused by fungi, bacteria, viruses, mycoplasma...etc. Fungi are the major microorganisms attacking strawberry plants and causing severe diseases at different development stages. Runner, roots and crown rots of strawberry consider one of the most important diseases in Egypt.

Etiological studies of the caused agents of runner, roots and crown rot of authors in Egypt recorded that,i.e., *Fusarium oxysporum*, *F. solani* and *F. equisti* were isolated from diseased strawberry roots (Michail *et al.*, 1980). *Fusarium oxysporum* caused 50% wilt to mature strawberry plants (Abou-Taleb *et al.*, 1988). *Cephalosporium* sp.; *F. oxysporum* and *Verticillium* sp. caused wilt, while others cause root and crown rots (Fahim *et al.*, 1994 and 1998). *Fusarium solani* and *Rhizoctonia solani* gave higher percentage of root rot disease (Aref, 2005).

Moreover, other root and crown rot fungi were isolated from different locations all over the world *i.e. Rhizoctonia solani, F. moniliform and Verticillium dahlia* were isolated from diseased strawberry plants with wilt symptoms (Teliz, *et al.*,1986). *Pestalotiopsis, Fusarium, Rhizoctonia, Robillarda* and *Acremonium* spp. were isolated from diseased strawberry roots in severely affected areas in China. *Rhizoctonia* occurred in 50.4 % of samples and *Pestalotiopsis* in 21.9% (Zhu, *et al.*, 1994). The seedlings infected by *Pestalotia longisetula* came from a commercial fruit area in Jarinu, Brazil. The pathogenicity of *P. longisetula* on strawberry plants and fruit was confirmed (Camili *et al.*, 2002), Black root rot complex of strawberry crop caused by several facultative saprophytic pathogens by *Rhizoctonia* spp , *Pythium* spp. *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Fusarium solani*, *Pestalotia* sp. and others (Manici *et al.*, 2005 and Embaby, 2007 a).

This work is focused to isolate pathogen(s) associate with strawberry root, runner and crown rots, study some morphological of *Pestalotia longisetula* using Lm, SEM, TEM and study the ability of *Pestalotia longisetula* to secret some hydrolysis enzymes and toxins.

Material and Methods

Isolation, purification and identification

Diseased strawberry plants (Tamar and Yael cvs.) showed wilt, root rot, runner rot and death symptoms were collected from different locations of Ismailia Governorate, Egypt. Wilt and root rot symptoms of diseased plants are shown in Fig. 1 and 2.

Diseased runner, root and crowns were washed with tap water, cut into small pieces (1-2 cm), and sterilized with 1% sodium hypochlorite solution for 2 min., then, washed several times with sterilized water and dried by using sterilized filter papers. Surface disinfected pieces of both root and crowns were transferred separately to sterilized PDA medium with traces of streptomycin, and then

incubated at 23 ± 2 °C for 5-7 days. All fungal colonies were transferred and purified on PDA solid medium by using hyphal tip and/or single spore techniques. Purified fungi were kept on PDA slants at 5°C for further studies. All isolated fungi were identified at Plant Pathology Dept., National Research Center (NRC), El-Dokki, Egypt, based on cultural characteristics by using specific media (Ronald, 1995). Identification was carried out by light microscope, electron microscope (SEM) and the literature according to Gilman (1957); Guba, (1961); Barnett & Hunter (1972) and Nelson *et al.*, (1983). Percentage of fungal soil borne associated with each of root and crowns of diseased strawberry plants were recorded.

Symptoms of wilt and root rot of strawberry plants

Fig. 1. Healthy (left) and wilted (right) plants.

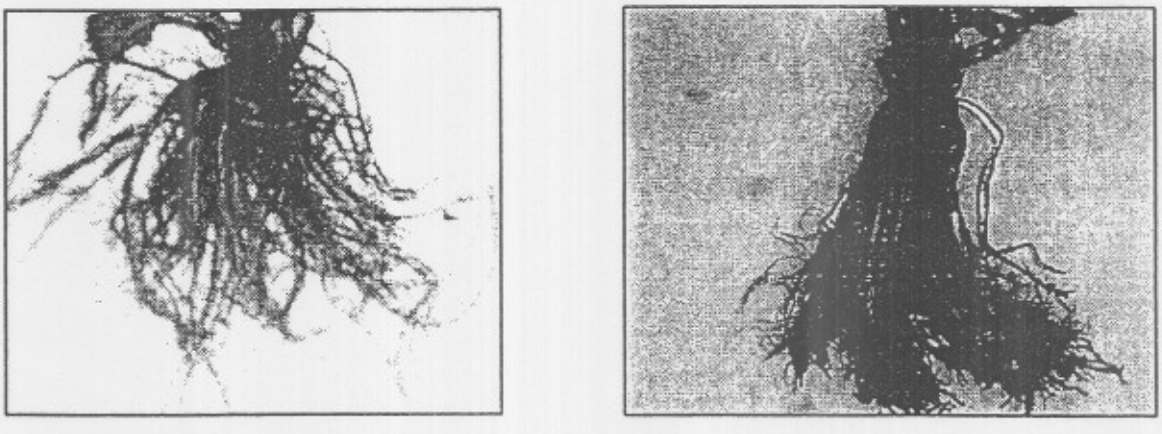


Fig. 2. Healthy (left) and rotted roots (right) .

Pathogenicity tests

Sterilized potato dextrose broth in conical flasks was inoculated with a disk 7 days old culture of *Pestalotia longisetula*, incubated at 23 ± 2 °C for two weeks. The liquid cultures were filtered to separate the mycelium mat from the suspension then conidial suspension was diluted to be 10^4 spore/ ml., Strawberry transplants (Yael cv.) were inoculated by dipping their roots in 1 x 10^4 suspension of *Pestalotia longisetula* (Chang *et al.*, 1998) and transplanted in pots (25 cm) contained sterilized clay-sand soil. Some of transplants were

planted in sterilized soil (inoculated and non-inoculated pots) as control. Three replicates (2 transplants / pot) were used for each treatment . Disease was recorded after 90 days from transplanting.

Preparation of Pestalotia longisetula for microscopic examination

Morphological and cytological studies : Identification and morphological studies were carried out by using Light microscope (LM) The fungus was cultivated on malt extract agar medium (MEA) at 23 ± 2 °C. The fungus was prepared for fixation and dehydration procedures using the programmable Scanning Electron Microscopy (SEM) tissue processor model (Lynx ™el) at the Regional Center for Mycology and Biotechnology(RCMB) according to (Zain, 1998).

Thin sections were examined with a JEOL 1010 Transmission Electron Microscope at 80 kv at the RCMB according to Reynolds, (1963).

Detection of enzymatic activities

Total pectinase and total cellulase : Pectinase and cellulase activities were measured by the cup plate clearing zone (PCZ) technique, the assay plates were prepared by pouring 20 ml aliquots of assay medium; pectin clearing zone medium for pectinase and cellulase clearing zone medium for cellulase activity.

Three cups were made in each plate by sterile cork porer (0.9 cm) and 0.1 ml of the tested cultural filtrate was introduced into each cup.

Plates were incubated over night at 30 c° and at the end of inoculation period; plates were flooded with lugols iodine solution prepared, according to method of (Cowan and Steel, 1979).

Pectin clear zone medium (PCZ): The medium contained: pectin (1.0), Gum Arabic (1.0) agar (20.0) in citrate phosphate buffer per liter at pH 6.0.

Cellulase clear zone medium (CCZ): This medium contained: cellulose (10.0) agar (20.0), in phosphate buffer (one liter) at pH 6.6.

Chitinase activities: Chitinase activity was determined according to Lingappa and Lockwood (1961). Chitin added to water agar at concentration of about 0.25% and pour as a thin layer on Petri dishes, after solidification, a well of (0.9 cm) was made using cork porer. By adding 0.1 ml of cultural filtrate and incubating at 30°C. Chitin hydrolysis is shown by measuring a clear zone around the added sample.

Dehydrogenase activity: Dehydrogenase activity was determined using triphenyl tetrazolium chloride according to the methods described by Thalmann (1968).

Mycotoxin production

Mycotoxin produced by *Pestalotia longisetula* was determined according to A.O.A.C. (2000). Ten flasks, each containing 50ml yeast extract liquid medium were inoculated using 7days old cultures of *Pestalotia longisetula* and incubated at 23°C ± 2 for 15 days. Culture was extracted using chloroform : methanol 95% (2:1v/v) in a separating funnel, shaken well and left for at least 6 hours until complete separation. The lower layer was separated, the solvent was evaporated and the metabolites were dissolved in methanol (Zhu, *et al.*, 2003). Different mycotoxins (as aflatoxin, fumonisin, ochratoxin A and mycophenolic acid) were tested using HPLC Analysis {Column: Hypersil C18 250 x 4.6 mm, Buffer: methanol + acetonitrile (0.75): water (0.25), Detector: fluorescence Ex 364 – Em 430} at RCMB.

Results

Occurrence and total count of soil mycoflora

Soil mycoflora were counted with three different dilutions, *i.e.*, 10^3, 10^4 and 10^5. The fungal genera isolated from soil sample were tabulated in Table 1. Data show that the survey of soil mycoflora yielded 163 of total fungal count belong to 6 genera. These are: *Aspergillus*, *Alternaria*, *Fusarium*, *Pestalotia*, *Rhizoctonia* and *Trichoderma*. Aspergilli was the most frequent genus in the three dilutions of soil sample than others, it accounted 50 and 31 (81 of total count) with 30.67% and 19.02% of density population followed by *Alternaria* which recorded 21 of total count with 12.88% density. *Pestalotia longisetula* was moderate count and gave 11 of the total genera with 6.75% of density. *Fusarium oxysporum* was the lowest fungal count (5) with 3.07% percentage of density followed by either *Rhizoctonia* or *Trichoderma* sp., each gave 6 of total count and 3.68% density of population.

TABLE 1. Total count of soil mycoflora and percentage of soil fungi frequency (density percentage of soil fungi occurred in Ismailia Governorate).

Organisms	Dilution			Population density	
	10^3	10^4	10^5	Total count	Percentage %
Aspergillus niger	35	11	04	50	30.67
Aspergillus sp.	25	06	00	31	19.02
Alternaria sp.	15	04	02	21	12.88
Fusarium oxysporum	02	02	01	05	3.07
Fusarium solani	04	03	02	09	5.52
Fusarium sp.	05	03	02	10	6.14
Pestalotia sp.	05	04	02	11	6.75
Rhizoctonia solani	03	02	01	06	3.68
Trichoderma sp.	03	02	01	06	3.68
Unknown	12	02	00	14	8.59
Total count of fungi	109	39	15	163	100.00

Isolation and identification of the causal agent from diseased strawberry plants

Table 2 listed the frequency occurred of isolated fungi. Isolation yielded 430 fungal isolates.

TABLE 2. Fungal occurrence of diseased strawberry, Tamar and Yael cvs. Ismailia Governorate, Egypt.

Fungal isolate (s)		Tamar cv.				Yael cv.				Total	
		T.c*	%	T.c*	%	T.c*	%	T.c*	%	T.c*	%
Pestalotia sp.	nursery	12	30.8	2	66.67	23	56.1	2	7.69	38	8.84
	field production	-	-	-	-	10	6.6	-	-		
Alternaria sp.	nursery	5	12.8	-	-	9	22	14	53.8	34	7.91
	field production	10	10.2	-	-	10	6.6	-	-		
Fusarium oxysporum	nursery	12	30.8	-	-	2	4.8	-	-	158	36.75
	field production	50	51.0	20	66.67	60	39.5	20	48.8		
Fusarium solani	nursery	7	17.9	-	-	1	2.4	-	-	25	5.81
	field production	10	10.2	-	-	10	6.6	-	-		
Rhizoctonia sp.	nursery	2	5.13	-	-	3	7.3	-	-	4	0.93
	field production	-	-	-	-	-	-	-	-		
Trichoderma sp.	nursery	1	2.56	1	33.33	2	4.9	-	-	111	25.81
	field production	20	20.4	10	33.33	50	32.9	20	48.8		
Unknown	nursery	-	-	-	-	1	2.4	10	38.5	430	100
	field production	98	100	30	100.0	152	100	41	100		
Total	nursery	39	100	3	100.0	41	100	26	100	100	100
	field production	22.79	100	6.98	100.0	35.35	100	9.54	100		
%	nursery	9.06	100	0.69	100.0	9.54	100	6.05	100	60	13.95
	field production	8	8.2	-	-	12	7.9	1	2.4		

The isolated and identified fungi were belong to five genera as i.e. *Pestalotia* sp., *Alternaria* sp. *Fusarium oxysporum, F.solani, Rhizoctonia* sp. and *Trichoderma* sp., in addition some isolates, which were not completely identified, recorded as unknown. Also, data in the same Table 2 show that, the percentage of associated fungi in nursery with Tamar cv. are 9.06% in root and 0.69% in runner, while Yael cv. resulted 9.54% and 6.05% fungal isolates associated with root and runner, respectively. Most strawberry fungi begin on plants growing in nursery (first stage) and continue to develop in the second stage (field production). The percentages of associated fungi in the field production with Tamar cv. were 22.79% and 6.98% with diseased roots and runner, respectively. Yael cv. yielded 35.35% and 9.45% fungal isolates associated with infected roots and runner, respectively in the same period. Also, the frequency of isolated fungi in the same Table presented that, *Fusarium solani* was the most frequent fungus with 36.75% followed by *Pestalotia* sp. with

13.95%, *Alternaria* sp. with 8.84%, *Fusarium oxysporum* with 7.91% fungal occurrence, while *Rhizoctonia* sp. was the least (5.81%).

Among 593 fungal isolates, obtained 71 isolates of *Pestalotia* were found from strawberry cultivation in Ismailia Governorate. Table 3 resulted 11 isolates of *Pestalotia longisetula* with 15.49 % while diseased strawberry (runners, roots and crowns organs) yielded 60 isolates of *Pestalotia longisetula* which recorded 84.51%. Frequency of Pestalotia fungus isolated from diseased strawberry plants. Also Table 3 presented that Yael cv. was the most susceptible and frequency occurred for *Pestalotia longisetula* than Tamar cv. with 53.52 and 30.99 respectively.

TABLE 3. Occurrence of *Pestalotia longisetula* in soil and diseased strawberry Tamar and Yael cvs. Ismailia Governorate, Egypt.

Diseased strawberry plants				Soil assays Soil mycoflora		T.C
Tamar cv.		Yael cv.				
T.C*	%	T.C	%	T.C	%	
22	30.99	38	53.52	11	15.49	71

* T.C: total count

Data in Table 4 presented that, *Pestalotia* fungus were isolated from all different diseased organs, *i.e.,* runner, root and crowns. Also data presented that *Pestalotia* was isolated from two different diseased cultivars Tamar and Yael cvs. as well as in two different periods i.e. nursery stage and field production. Higher frequencies of *Pestalotia longisetula* were recorded in strawberry of nursery stage compared with the field production which recorded 65 and 35 % respectively.

The same Table 4 shows that, diseased runners were less frequency occurred with *Pestalotia longisetula* (8.33%) compared with diseased roots which recorded 61.67% in both Tamar and Yael cvs, in two different stages, *i.e.,* nursery stage and field production.

TABLE 4. Frequency of *Pestalotia longisetula* isolated from diseased strawberry plants cv. Tamar and Yael in both nursery stage and field production, Ismailia Governorate, Egypt.

Stage(s)	Cultivars								T.C	%
	Tamar cv.				Yael cv.					
	Root		Runner		Root		Runner			
	T.C*	%	T.C	%	T.C	%	T.C	%		
Nursery stage	12	60	2	100	23	65.71	2	66.67	39	65
Field production	8	40	0	0	12	34.29	1	33.33	21	35
%	33.3	-	3.33	-	58.34	-	5	-	100	-

*T.C: total count.

Pathogenicity test

All tested fungi, except *Trichoderma* sp., were pathogenic to strawberry transplants (Yael cv.), *Fusarium oxysporum* caused wilt symptom, while, other tested fungi, *i.e.*, *Pestalotia* sp., *Rhizoctonia solani* and *Fusarium* solani caused root and crown rots of strawberry transplants (Table 5).

TABLE 5. Symptoms occurred by the tested fungi.

Organism	Symptom
Alternaria sp.	Root rot
Fusarium oxysporum	Wilt
Fusarium solani	Root rot
Pestalotia sp.	Root rot
Rhizoctonia solani	Root rot
Trichoderma sp.	N.S.

N.S. = no symptoms

Morphological studies

Culture characteristics show that, *Pestalotia longisetula* produced numerous acervuli Fig. 3a.

Also light microscope (LM) studies showed the presence of fungus *Pestalotia longisetula* produced only acervular conidimata, conidial septa, the conidia had a single appendage with 2-3 branches and five cells, Fig. 3b.

Morphologically, Fig. 3c showed fusiform conidia, straight and curved, with 3-5 celled or loculate and crowned with one three branched setula arising from the base of the apical cells. It showed also a characteristic acervulus bearing phragmospores .

Identification of Pestalotia sp. (Pestalotiopsis)

Pestalotia sp. fungus has a white aril mycelium which is more branched and produced numerous acervuli especially in the oldest culture Fig. 3a, 3b & c shows clearly septate conidia 3-5 cells the conidia had single appendages with 2-3 branched with typical morphology, characterizing the anamorph genus *Pestalotia* sp. *(Pestalotiopsis)*. Based on symptoms cultural characteristics, morphological studied by using light microscope (LM) as well as electron microscope (EM), pathogenicity test and available literature, the pathogen was identified as *Pestalotia longisetula (Pestalotiopsis longisetula)*

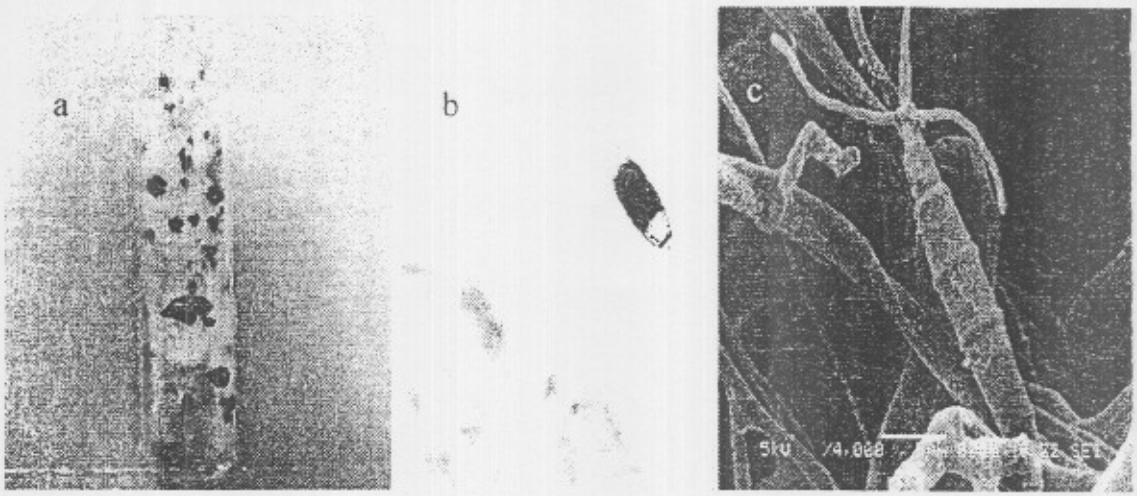


Fig. 3. Culture of *Pestalotia longisetula* with black *acervuli* (a) conidia under light (microscope (400 X) (b) and SEM of conidial and mycelial micrograph (1600X) (c)

Ultrastructural Electron Microscopic examination by (TEM)

Stained ultrathin sections (50 nm) were examined with TEM at 80 kV. Figure (4) shows conidial longitudinal section. It showed thick cell wall and thin cell membrane. No any pores between apical and subapical cell and spezenkorper region was observed in the apical cell of spore in longitudinal section. The cytoplasm contains: nucleus with nucleolus, mitochondria, vacuoles, cytoplasmic granules. Also, Fig. 4 (a and b) showed mycelial longitudinal section with moderate thick wall, thin membrane, mitochondria and other cytoplasmic materials.

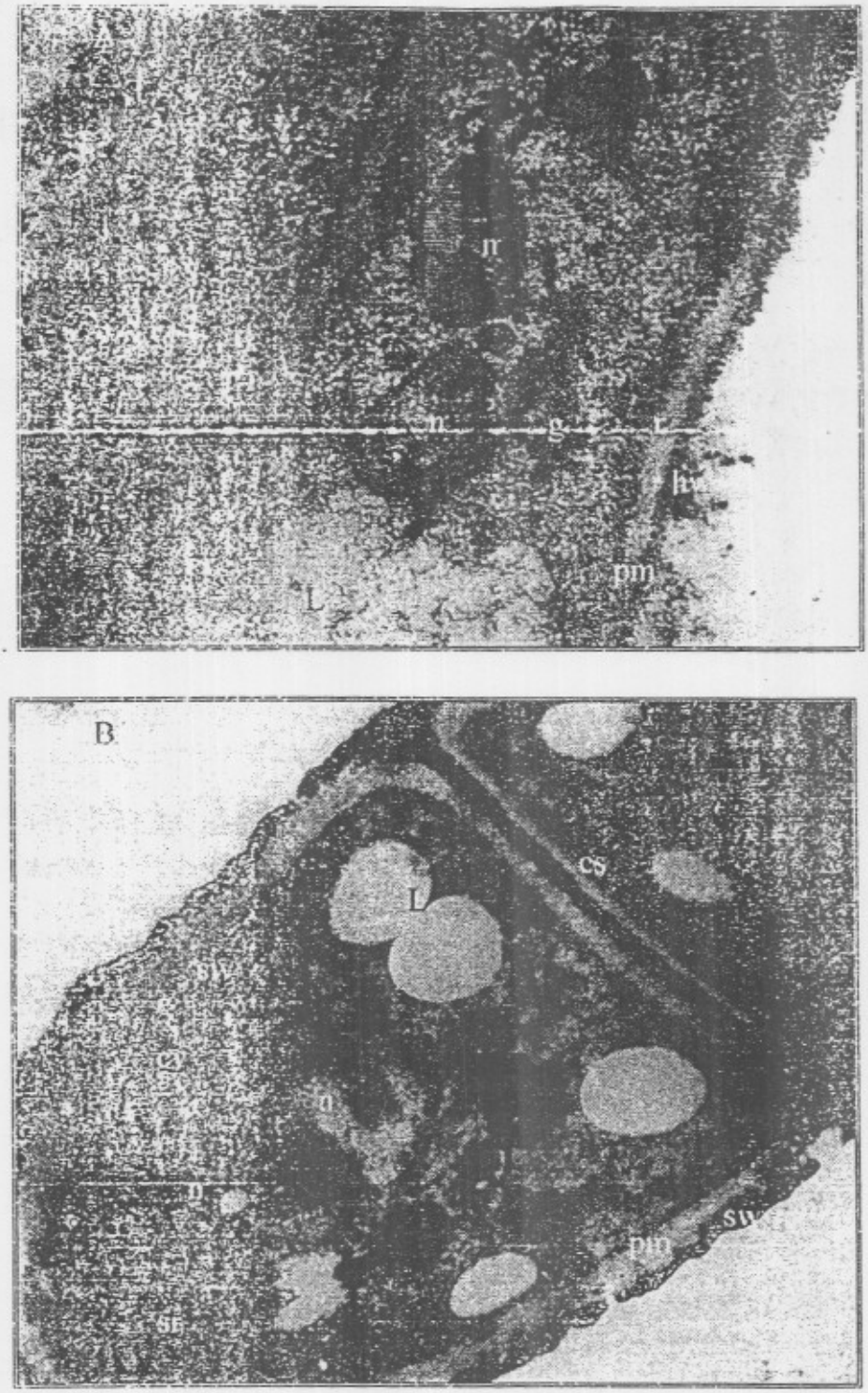
Enzymatic activity of Pestalotia longisetula

Enzyme assays revealed that four enzymes, *i.e.,* pectinase, cellulase (C_X), chitinase and dehydrogenase were estimated in culture medium of *Pestalotia longisetula* and recorded in Table 6. Data presented that, *Pestalotia longisetula* produced pectinase, cellulase and chitinase. Determination of clear zone gave 26, 25 and 10 expressed as the main diameter (mm), respectively. On the other hand, dehydrogenase was 38 µg %.

TABLE 6. Enzyme activities and mycotoxin production with *Pestalotia longisetula.*

Enzyme activities		Mycotoxin production (ppm)	
Pectinase *	26	Aflatoxin	N.d
Cellulase *	25	Fumonisin	N.d
Chitinase *	10	Ochratoxin A	4.2
Dehydrogenase	38	Mycophenolic acid	3.2

Note: * Data are expressed as the mean diameter of cleared zone (mm).
Data of dehydrogenase is expressed as µg %.
N.d = Not detected



Mitochondria (m), ribosome (r); golgy apparatus (g); hyphal wall (hw); plasma membrane (pm); closed cross septum (cs); lipid droplet (L); spore wall (sw); chitesomal vesicle (cv); nucleus (n); spezenkorper region (sr).

Fig. 4. TEM micrograph of *Pestalotia longisetula* (A) conidial longitudinal section (L.S.) (40000X) and (B) mycelial L.S. (35000X).

Mycotoxin production

Detection of mycotoxin production with *Pestalotia longisetula* extracts of liquid culture media was tabulated in Table 6. Data show that, *Pestalotia longisetula* produced both ochratoxin A and mycophenolic acid which determined 4.2 and 3.2 ppm of toxin, respectively.

Discussion

Strawberry (*Fragaria x ananassa* Duch.) is one of the most important vegetable crops in Egypt for local consumption and exportation. Fungi are the major microorganisms attack strawberry roots causing sever diseases in different development stages in either nursery or field production, causing yield and economic losses. *Pestalotia longisetula* was isolated for the first time from diseased strawberry as well as rhizoplane and soil samples in Egypt. Moreover, strawberry infection in Egypt with this fungus *(Pestalotia longisetula)* has been investigated for the first time in Egypt and pathogenicity test was confirmed by Embaby (2007a & b). Similar results were obtained by Zhu, *et al.* (1994) and Camilli, *et al.* (2002).

Fungi are the major microorganisms attack strawberry roots causing sever diseases in different development stages. A survey of soil borne fungi (soil mycoflora) in strawberry cultivation, at Ismailia Governorate resulted 163 of fungal isolates colony. Sex fungal genera were identified as *Alternaria, Aspergillus, Fusarium, Pestalotia, Rhizoctonia* and *Trichoderma.* Aspergilli were the most frequent followed by *Alternaria* sp. While, genus *Pestalotia* was moderate in a count and density. *Fusarium oxysporum* was the lowest fungal count and population density followed by *Rhizoctonia* and *Trichoderma.* Ito & Nakagri (1997) and Mirkova & Margia (2003).

A survey of soil borne fungi infecting strawberry plants, Ismailia Governorate, Egypt. Yielded 593 fungal isolates, among of them 71 isolates were identified as *Pestalotia longisetula* Out of these 11 isolates (15.49%) from soil assaying (rhizoplane) and others 60 isolates (84.51%) from diseased strawberry in both Tamar and Yael cvs. *Pestalotia longisetula* recorded higher frequency with Yael cv. than Tamar cv. which record 35.52 and 30.99 % respectively. In addition, runners were less frequency occurred with *Pestalotia longisetula* 8.33% comparing (in comparison) with diseased strawberry roots (61.67%) in both Tamar and Yael cv. In two different stages, *i.e.,* nursery and field production. Many investigators reported that *Pestalotiopsis* was isolated from strawberry root diseases in severely affected area in China with 21.9% (Zhu, *et al.*, 1994). Pestalotiopsis was isolated from mangrove rhizoplane mycoflora (Ito and Nakagri, 1997)., *Pestalotia longisetula* was isolated from infected roots and seeds of bird of paradise plants in Egypt (Hilal and Helmy 1998). Also, *Pestalotia longisetula* was found with other soil fungi from infecting aromatic and medicinal plants as *Melissa officinal* (Mirkova and Margia, 2003). *Pestalotia longisetula* was isolated from strawberry root rot and rhizoplane of Tamar and Yael cvs. for the first time in Egypt (Embaby, 2007a).

Pestalotia species were identified based on their morphological characters (Guba, 1961 and Nag, 1993). It includes approximately 220 published names (www.indexfungorum.org). Many of these were established based on slight morphological differences and host affiliation (Jeewon *et al.*, 2004).

Light microscope (LM) studies showed that *Pestalotia longisetula* fungus produced only acervular conidimata, conidial septa, the conidia has a single with appendage 2-3 branches and five cells (Howard, 1973, Howard & Albregts, 1973 and Camilli *et al.*, 2002) Electron microscope (EM) showed also a characteristic of acervulus bearing phragmospores. Examination of sections showed thick cell wall and cell membrane. The cytoplasm contains: nucleus, mitochondria, vacules and cytoplasmic granules. No any pores were found between apical and subapical cell Spezenkorper region was observed in the apical cell of spore in longitudinal section. Longitudinal section of mycelium showed moderate thick wall, thin membrane, mitochondria and other organelles.

Longitudinal section results are similar to results obtained by Purohit & Chawla (1989), Sun *et al.* (1990), Kathirvan & Muthumary (1998) and Murugan & Muthumary (2003).

Activity of enzymes, *i.e.*, pectinase, cellulase (C_X), chitinase and dehydrogenase were determined in *Pestalotia longisetula* culture medium. Results showed that hydrolyic enzymes produced by *Pestalotia longisetula* are of the main mechanism for fungal infection, these enzymes have been investigated as they may play a role in modifying the degree of plant infection under natural conditions. Many investigators found that *Pestalotia malicola* produced enzyme (s) with cutinase and non-specific esterase activities (Sugui, *et al.*, 1998). Dehydrogenase activity increased suddenly in inoculated guava fruits, the increase being greater with *Pestalotiopsis versicolor* than with *Rhizoctonia solani* (Madhukar and Reddy, 1990). *Pestalotiopsis disseminata* produced large amounts of cellulase and moderate quantities of polymethylgalacturonase *in vitro* as well as *in vivo*. Production of polygalacturonase was insignificant (Ghani and Tandon, 1991). From 15 tested fungi for their pectolytic enzyme activity and growth response to tannin, *Pestalotiopsis* sp. and *Myrothecium cinctum* showed the least enzyme activities and the most sensitive to tannin, respectively (Purkatt & Purkayastha, 1996 and De *et al.*, 1999).

It is clear that, hydrolysis enzyme(s) produced by the pathogenic fungus, *Pestalotia* sp, is one of the main mechanisms for fungal infection.

Pestalotia longisetula was found to produce both ochratoxin A and mycophenolic acid. Pulici *et al.* (1997) found that two strains of *Pestalotiopsis* spp. (JCM 9685 and JCM 9686) produced several new compounds when grown in liquid culture. Detailed investigation of JCM 9685 revealed that, 5 of the metabolites were sesquiterpenes. Of these, 3 were of the caryophyllene types, Pestalotiopsin A, B and C, one possessed the humulane skeleton, and another

was a drimane derivative. The remaining compounds were 2 C- methylated acctogenins :(45*, 5R*)-(6Z, 8E) - 4, 5-dihydroxy-6-hydroxy-6-hydroxymethyl-2, 6, 8-decatriene. An aldehyde closely related to these 2 triols was obtained from JCM 9686. The isolation and characterizations of Pestalotiopsin C and of the 3 new C-methylaited acetogenins. The culture filtrate of *Pestalotia funerea* was primarily fractioned by ethanol precipitation. High molecular weight substances, such as proteins, were nonpathogenic, whereas, the nonproteinic components were pathogenic to the cut root seedling of *Pinus massoniana* and *P. elliotti*. Nine fractions separated by gel H_{60} column chromatography. Among them, three fractions with Rf values of 0.83, 0.79 and 0.80, respectively, damaged leaves of *Pinus massoniana* and *P. elliotti* (Zhu *et al.*, 2003).

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عفن الجذور البستالوشى : مرض جديد علي نباتات الفراولة في مصر

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تم الحصول على ٥٩٣ عزله فطريه من نباتات الفراوله المصابة وكذلك فطريات التربة بمحافظة الأسماعليه ، مصر، منها ٧١ عزله لفطر ، *Pestalotia longisetula* وكانت نسبه عزله من التربة ١٥,٤٩٪ و نسبة عزلة من النباتات المريضة ٨٤,٥١٪ لكل من صنفي الفراوله تمار و ويانيل.

سجل الصنف يانيل أعلى نسبة ظهور لفطر . *Pestalotia longisetula* ٥٣,٥٢ ٪ مقارنه بالصنف تمار ٣٠,٩٩٪ ، وأيضا كان نسبة تواجد ال *Pestalotia longisetula* في المشتل أعلى منها فى الحقل حيث سجلت النسبة ٦٥٪ ، ٣٥٪ على التوالي. على الجانب الآخر كانت أقل نسبه تواجد على المدادات المصابة حيث سجلت ٨,٣٣٪ مقارنه بنسبه التواجد على جذور الفراو له المصابة بالأعفان وهى ٩١,٦٧٪ .

أظهر الفحص بالميكروسكوب الضوني والألكترونى الماسح أن فطر *Pestalotia longisetula* ينتج أسرفيولا وكونديات فرديه مقسمه الى خمس خلايا والطرفيه بها زائده ذات ٢-٣ أفرع. وأظهر الفحص بالميكروسكوب الألكترونى النافذ وجود جدار خلوى سميك و السيتوبلازم يحتوى النواه ، الميتاكوندريا ، الفجوات والحبيبات السيتوبلازمية. لا يوجد ثقوب بين الخلية الطرفية و التي تليها (التحتية)، كما توجد في قمم الخلية Spezenkorper region .

تم التعرف على أربع أنزيمات يفرزها الفطر فى البيئه السائله وهى البيكتناز ، السيليوليز، شيتناز و الديهيدروجينيز.

أيضا وجد أن فطر الـ *Pestalotia longisetula* يفرز كل من الأوكراتوكسين وحمض الميكوفينوليك .