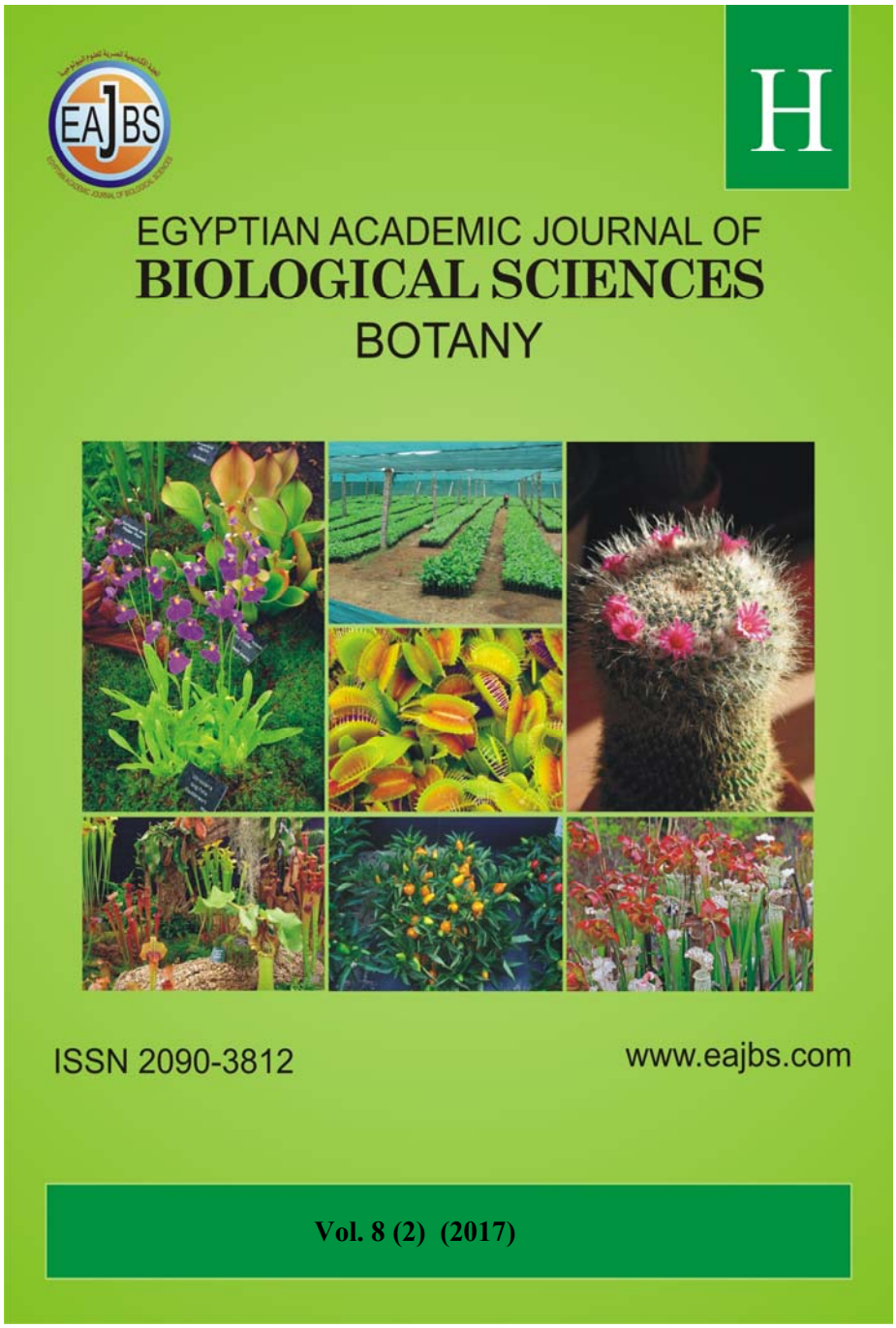

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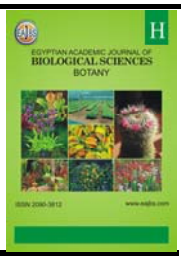

Studies on Growth and Yield Losses in Strawberry and Its Relation to Soil Borne Diseases in Major Producing Governorates in Egypt

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ABSTRACT

These experiments were carried out at Ismaelia, Beheira and Kalubia Governorates on Nuclear, foundation and classical strawberry plants. The aim of the work to study the effect of strawberry transplant order i.e., mother plants or super elite plants, on some vegetative growth and transplant productivity Moreover to collect strawberry samples from roots and runners showing disease symptoms stunting root rots, wilt and death also to isolate and identify the causal organisms and their effects on growth and yield losses. Results show that number of runners and leaves increased significantly in tube plants and nuclear plants compared with the other tested orders. Number of transplants increased significantly in nuclear and foundation plants. Cultivars and transplant order were affected by soil borne diseases whereas, Tamar and Yael cultivars showed some infected plants as stunting, root rots, wilt and death in either open field as classical plants and greenhouse as foundation plants. Higher root system diseases was recorded in Tamar compared with Yael in both greenhouse and open field plants. The percentage of naturally diseased cv. Tamar were 3.6 % and 1.6 % in open field (classic plants) and greenhouse (foundation plants) respectively. While the Yael cultivar recorded 0.8 % and zero in either classic plants and foundation ones respectively. The causal agents were *Fusarium solani*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani.* Results show also that *Fusarium* genus was the most fungal frequency occurred which recorded 41.2% followed by *Macrophomina* which gave 35.3% frequency while *Rhizoctonia* was less which recorded 11.8%. Roots of strawberry transplants were more infected organs compared to crowns as they recorded 44.7% and 33.3% respectively. Results indicate also that the highest infection percent was found in Kalubia followed by Ismailia and the lowest was Behira governorate. Finally, results indicated clear reduction in yield and fruit quality due to the different root rot diseases.

INTRODUCTION

Strawberry *(Fragaria X ananassa Duch*.) is one of the most important vegetable crops in Egypt for local consumption and exportation. Also, it is grown mainly in five governorates i.e. Kalubia, Ismailia, Sharkiya, Behira and Menofiya. The cultivated area were 8571.1 feddan for frigo plantations with an average of 11.5 ton / feddan (local market) and about 21573 feddan for fresh plantations with an average 18.5 ton / feddan, mainly for exportation (Ministry of Agriculture 2015/2016). Strawberries are an excellent source of vitamin C,. The major sugars in strawberry fruit are sucrose, glucose, and fructose, accounting for more than 99% of the total sugars in ripe fruit (Agulheiro-Santos, and Barreto, 2008). The soluble solids and total sugars content of strawberries tends to increase as the fruit matures (Nam *et al.* 2008 and Perez, *et. al.*, 2008). Strawberry cultivars differ significantly in their resistance to soil born diseases (Browne and Bhat 2011).Several microorganisms attack strawberry plants. Fungi are the major microorganisms attack strawberry and caused sever diseases in different development stages in either nursery and field production as stunting, root rot, wilt, spots and fruit rot. Both root rot and wilt (Strawberry fungal soil borne diseases) were reduced quantity and quality of strawberry productivity and caused yield losses in addition economic losses. *Alternaria spp., Aspergillus spp., Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani, Phytophthora cactorum, Fusarium spp., Penicillium spp.* and *Sclerotinia sclerotiorum* are the most fungal isolates causing strawberry fruit rots in Egypt (Tarek, 2004 and Aye and Matsumoto, 2011). In addition to Pestalotia longisetula (Embaby, 2007 a & b). Moreover, other fungi were isolated from different locations all over the world i.e. *Aspergillus, Botrytis, Colletotrichum, Geotrichum, Mucro, Penicillium, Pestaliopsis, Phytophthora and Rhizopus stolonifer* were found to infect the crop (Paivi, *et. al.,* 2008).

Colletotrichum spp. cause a wide range of strawberry diseases ranging from root-rot, crown and fruits infections resulted serious in yield losses (Garrido, *et. al.*, 2008 and Grantina-levina and Kalnina (2016)). *C. acutatum Simmonds ex Simmods* causes serious losses of strawberry fruits in Australia, Suth Africa, Uk and California. Other species recorded on strawberries in the USA include *C. dematium, C. fragaria* Brooks and *C. gloeosporioides*. Fruit can be severely affected by *Colletotrichum spp,* lesions on fruit are sunken and may be lined with a sticky mass of pink-colored spores. Black spot is first visible as a circular, slightly sunken, brown water soaked lesion, enlarging rapidly over a period of 2 or 3 days to involve most of the fruit. Mould growth may appear at the edges of the lesion, while at the centre there is copious production of spores. These are initially salmon- colored but after a period lesions become dark brown to almost black. Infected fruits may dry up completely, but usually they are further rotted by other fungi (Snowdon, 1990). The causative agent was confirmed to be *C. acutatum* by PCR amplification using the time-specific primers TBCA and TB5 (Porta-Puglia, and Mifsud, 2006, and Nam, *et. al.,* 2008). Strawberry fruit rot with black and brown spots was observed in forcing culture in Japan. These were identified as *Colletotrichum acutatum and C. gloeosporioides* (Ivanovic *et al,* 2007). In Egypt, Anthracnose of strawberry caused by *C. acutatum* was recorded for the first time by El-Marzoky, 2008, however, these report did not rely on molecular analysis for accurate and reliable identification of the causal agent of disease (*C. acutatum*). Typical anthracnose symptoms were observed in cultivated strawberry fields in Kalubia and Ismailia governorates, Egypt. This is thought to be the first reliable and accurate report, based on molecular identification, of *C. acutatum and C. gloeosporioides* causing anthracnose on strawberry in Egypt (Embaby, *et. al*. 2010). Therefore, the aim of this work was to study the effect of of strawberry

transplant order, i.e., mother plants or super elite plants, on some vegetative growth and transplant productivity.

MATERIALS AND METHODS

This experiment was carried out at Ismailia Beheira and Kalubia Governorates in 2016 and 2017 to evaluate the effect of the class of strawberry transplant; mother plants or super elite plants, on some vegetative growth and transplant productivity for Festival, Sweet Charlie, Tamar and Yael cultivars .Mother plants were produced from Tissue Culture Lab. of AL Kanater Research Station. Super elite plants were produced from mother plants grown under proof screen house. In Ismailia all transplants of both classes for both cultivars were treated with Vitafax at 0.2% against damping-off and were cultivated. Mother plants were cultivated under a proof screen house while super elite plants were cultivated in open field conditions. The size of cultivated transplants was 0.8 – 1.2 cm in diameter. The plants were spaced at 1x1 m. The soil type was sandy and was irrigated with both drip and spray systems. All cultural practices were conducted following the recommendations of the Egyptian Ministry of Agriculture for this zone. A randomized complete block design with four replicates was adopted. Each plot was 12 m^2 in area. Data were recorded on plant height; number of leaves/plant, number of runners/plant, and number of transplants/plant were recorded in two different sampling dates. The first one was at the third week of July and the second sampling date was at the third week of August. By the end of the experiment in the first week of December, total number of transplants/plant was recorded.

1- Survey of the natural soil borne fungi attached strawberry plants :

$$\text{Percentage of diseased plants} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

2- Isolation, purification and identification :

Naturally rotted fruit samples of strawberry Festival, Sweet-Charlie, Tamar and Yael showing various types of rot symptoms i.e. discoloration, a circular black spot, slightly sunken, brown water soaked lesion, salmon- colored, turn dark brown to almost black were collected from the different location. Samples were surface disinfested using 70% Ethyl alcohol for 2min, washed with sterile water (SW) and dried at room temp., then cut into small pieces. Sterilized pieces were sown on sterilized PDA medium amended with 0.1% streptomycin sulphate then incubated at 26 ± 2°C for 5 days. All fungal colonies were transferred and purified on PDA medium. Fungal purified were transferred on PDA slant medium and kept at 5°C. All isolated fungi were identified in Plant Pathology Dept. National Research Centre NRC, El-Dokki, Egypt. Based on cultural and morphological characteristics, and pathogenicity test were identified according to Freeman, & Katan, 2002. Total of infected fruits, percentage of infected fruits and percentage of yield losses of strawberry rotted fruits were calculated and recorded as follow:-

$$\% \text{ Infected fruits} = \frac{\text{Total of infected fruits}}{\text{Total of fruits}} \text{ x100}$$

$$\%\text{Loss} = \frac{\text{Infected weight (g) - Total weight of fruits (g).}}{\text{Total weight of fruits (g).}} \text{ x100}$$

RESULTS AND DISCUSSION

1- Effect of different strawberry plant orders on runner formation and transplant production of Tamar cultivar.

Data in Table (1) show that number of runners and leaves increased significantly in tube plants and nuclear plants compared with the other tested orders while plant height increased significantly in nuclear plants compared with the other tested orders. Classical plants produced the lowest number of transplants until 28th August (Data record date). Number of transplants increased significantly in nuclear and foundation plants.

Table 1: Effect of different strawberry plant orders on runner formation and transplant production of Tamar cultivar.

Plant orders	Growth characteristics			
	No. of runners	No. of leaves	Plant height (cm)	No. of transplants
Tube plants	15.00a	16.78a	17.11c	26.67b
Nuclear plants	19.63a	16.89a	18.80a	27.44a
Foundation plants	11.22c	13.89b	17.66b	28.44a
Classical plants	12.78b	15.44a	15.78d	18.89c

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

2- Fungal frequency in plants isolated from strawberry transplants:

First sample of strawberry transplants resulted that three fungal genera were identified. These were Fusarium, *Macrophomina* and *Rhizoctonia* in addition to unknowns. Data in Table (2) presented that *Fusarium* genus was the most Fungal frequency occurred which recorded 41.18 % followed by *Macrophomina* which gave 35.30 % frequency while *Rhizoctonia* was less which recorded 11.76%. On the other hand, root of the strawberry transplants was a more infected organ compared to the crown as they recorded 44.70% and 35.30% respectively.

Table (2): Fungal frequency in plants isolated from strawberry transplants (Festival), El-Nubaria nursery.

Pathogenic Fungai		The part of the plant		Total
		Crowns	Roots	
Fusarium	***solani***	00.00 b	23.53 a	41.18 a
	Spp.	00.00 b	17.65 b	
Macrophomina phaseolina		35.30 a	00.00	35.30 b
Rhizoctonia solani		00.00 b	11.76 c	11.76 c
Unknown		00.00 b	11.76 c	11.76 c
Total		35.30	64.70	100.00

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

3- Fungal Frequency in plants isolated from different strawberry transplants cultivars in different strawberry governorate Nursery.

During June, July and August, a lot of samples from infected fields in Ismailia, Kalubia and Behera were collected. Isolated fungai were identified according to their microscopic characteristics, from the different cultivars and results are presented in Table (3) as follows and the dominant fungus was macrophomina:

Table (3): Fungal frequency in plants isolated from different strawberry transplants cultivars in different strawberry Governorate Nursery.

Governorate	Cultivar	Isolated fungi
Ismailia field 1	Festival	*Macrophomin*
Ismailia field 2	Festival	*R.solani* *Macrophomina* *F.oxysporum*
Ismailia field 1	Festival	*Macrophomina*
Ismailia field 2	Festival	----------
Ismailia	Festival Sweet Charlie	---------- *R.solani*
Beheera field 1	Festival	*Macrophomina*
Beheera field 2	Festival	*Macrophomina* *Fusarium*
Kalubia field 1	Sweet Charlie	*R.solani* *Fusarium*
Kalubia field 2	Festival	*R.solani* *Macrophomina*
Kalubia	Festival Sweet Charlie Diamonte	*Macrophomina* *Macrophomina* *F.oxysporum* *Macrophomina*

4- Percentage of strawberry rotted fruits in two different localities i.e. Kalubia and Ismailia regions:-

Percentage of strawberry rotted fruits were survived in two different localities in Egypt i.e. Kalubia and Ismailia Governorates with four strawberry cultivars as Festival, Sweet Charlie, Tamar and Yael. Data in Table (4) show that, Kalubia samples gave higher of total number of infected fruits as well as infection percent compared with Ismailia samples. Yael cv. was the most infection percent 25.9% in Kalubia sample than other strawberry cultivars followed by Tamar and Festival which recorded 24.4 and 21.1% respectively. Sweet Charlie gave the lowest infection percent 18.3%. On the other hand, Festival fruit rots gave higher infection percent 26.8% than other cvs. with Ismailia samples. Also, data show that, moderate infection percent was recorded with Yael cv. 22.5% followed by Tamar cv. 20.0%. Sweet Charlie was the lowest infection percent which gave only 3.0%. Strawberry cultivars differ significantly in their resistance to soil borne diseases (Browne and Bhat 2011).

Table (4): Percentage of strawberry rotted fruits in two different localities Kalubia and Ismailia Governorate.

Location	Cultivar	Total of fruits	Rotted fruits	Healthy fruits	Infection %
Kalubia	Festival	450 b	95 c	355 c	21.1
	Sweet. Charlie	186 d	34 d	152 d	18.3
	Tamar	90 e	22 d	68 e	24.4
	Yael	471 b	122 b	349 c	25.9
Ismailia	Festival	410 c	110 b	300 c	26.8
	Sweet. Charlie	600 b	17 d	430 b	3.0
	Tamar	400 c	80 c	320 c	20.0
	Yael	800 a	180 a	620 a	22.5

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

5- Percentage of strawberry yield losses caused by some fungal rotted fruits:

Percentage of strawberry yield losses were estimated with four strawberry cultivars fruits namely Festival, Sweet Charlie, Tamar and Yael in the same regions i.e. Kalubia and Ismailia Governorates. Data were tabulated in Table (5). Data in this Table presented that, higher percentage of yield losses were recorded with Kalubia of strawberry rotted fruit samples compared with Ismailia samples. On the other hand in Kalubia region, Yael cv. was the most yield losses which recorded 59.1% than other cvs., followed by Festival 42.3% and Tamar 18.2%. Sweet Charlie cv. was the lowest yield losses 16.6%. But in Ismailia region, Festival cv. was higher yield losses 60.8% than others. Tamar was the lowest yield losses 10.7%.

Many investigators reported that, fruit rot disease caused by fungi is one of the major problems to strawberry cultivation and production reducing their quantity and quality and causing economic losses in the field, at harvest time, during marketing and exportation (Tarek, 2004). Also, the same results were recorded by Berrie Burgess, (2003) the highest incidence of black-spot on fruit (30%) and petioles (50%). Also, Timudo-Torrevilla, *et. al.,* (2005) reported that, the New Zealand strawberry industry experiences losses due to fungal diseases that can cost up to $4.4 million per annum or 20% of the crop value. The average fruit rot incidence (expressed per day) was 3.6, 2.2 and 1.6% for grey mould, leak and anthracnose, respectively, when ripe fruit was incubated at room temperature under high humidity. Also, Yakovenko*, et. al.,* (2006), reported that, first occurrence of black rot *(Colletotrichum spp.)* causing 25% fruit loss in cv. Marmolada. In addition, the incidence of anthracnose fruit rot (*Colletotrichum acutatum and Botrytis cinerea*) ranged from 2 :75% 8 : 96% and 1 : 83% (Chandler*, et. al.,* 2006). The percentage of the diseased plants differed, but in certain plots it was from 5 to 20%, because of significant economic damages. Based on the obtained results, it was shown that these all belonged to the plant pathogenic fungus *Colletotrichum acutatum,* which is the cause of anthracnose of strawberry (Trkulja*, et. al.* 2008).

Table (5) Percentage of strawberry yield losses caused by some fungal rotted fruits in Kalubia and Ismailia Governorate.

Location	Cultivar	T.W(g)	W.I(g)	W.H(g)	Yield loss%
Kalubia	**Festival**	930.4 b	393.9 b	536.5 c	42.3
	Sweet Charlie	900.0 b	150.0 c	750.0 b	16.7
	Tamar	550.0 c	100.0 d	450.0 c	18.2
	Yael	200.0 d	130.0 a	900.0 b	65.0
Ismailia	**Festival**	790.0 b	480.0 b	310.0 d	60.8
	Sweet Charlie	719.1 b	141.2 c	577.9 c	19.6
	Tamar	1000.0 b	100.7 d	899.3 b	10.07
	Yael	2800.0 a	330.4 b	2469.6 a	11.8

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

T.W= total weight of fruits (g) W.I = Weight of Infected fruits. W.H = Weight of Healthy fruits.

6- Percentage of total fungal count associated with strawberry rotted fruits:-

Rotted strawberry fruits which collected from two different localities i.e. Kalubia and Ismailia Governorates cultivated with four strawberry cultivars Festival, Sweet Charlie, Tamar and Yael yielded three hundred of fungal isolates belonging to one hundred (equal 33.33%) from Kalubia samples and two hundred (equal 66.67%) of fungal isolates from Ismailia samples. Also, percentage of total fungal count were tabulated in Table (6). Data show that, five fungal genera were found to be associated with strawberry rotted fruits. These fungi were identified as *Aspergillus niger, Botrytis cinerea, Pestalotia longisetula, Rhizopus stolonifer and Colletotrichum spp.,* the causal agent of strawberry *anthracnose.* Data also presented that, the occurrence of strawberry fruit rots in Ismailia location recorded higher percentage of total fungal count compared with Kalubia location. Also, Yael cv. was harbored higher frequency occurred of the total fungal count which gave 39% and 40% in both Kalubia and Ismailia location respectively, comparing with other cultivars.

Table (6): Total count and percentage of fungal frequency associated with strawberry rotted fruits in Kalubia and Ismailia Governorates:-

Location	Cultivar	TFC	A	B	C	P	R	Total %
Kalubia	Festival	TFC	2	10	0	4	3	19
		%	2.0	10	0.0	4.0	3.0	19.0
	Sweet Charlie	TFC	0	10	0	6	10	26
		%	0.0	10.0	0.0	6.0	10.0	26.0
	Tamar	TFC	3	5	1	2	5	16
		%	3.0	5.0	1.0	2.0	5.0	16.0
	Yael	TFC	1	8	5	5	20	39
		%	1.0	8.0	5.0	5.0	20.0	39.0
	Total	TFC	6	33	6	17	38	100
		%	6.0	33.0	6.0	17.0	38.0	100.0
Ismailia	Festival	TFC	6	20	0	5	4	35
		%	3.0	10.0	0.0	2.5	2.0	17.5
	Sweet Charlie	TFC	5	20	0	10	10	45
		%	2.5	10.0	0.0	5.0	5.0	22.5
	Tamar	TFC	5	21	4	5	5	40
		%	2.5	10.5	2.0	2.5	2.5	20.0
	Yael	TFC	10	22	8	20	20	80
		%	5.0	11.0	4.0	10.0	10.0	40.0
	Total	TFC	26	83	12	40	39	200
		%	13.0	41.5	6.0	20.	19.5	100.0

A= *Aspergillus niger.* B= *Botrytis cinerea*.
C= *Colletotrichum spp.* P= *Pestalotia longisetula.*
R= *Rhizopus stolonifer*. TFC= Total Fungal Count.

Colletotrichum spp. gave less percentage of total fungal count and occurred at 6.0% in both Kalubia and Ismailia samples respectively. Also data in the same Table presented that, Sweet Charlie cv. record moderate percentage of the total fungal count in Kalubia samples followed by Festival with 19.0% and Tamar cv.with 16%. Als, Sweet-Charlie cv. gave moderate percentage of total fungal count in Ismailia samples which record 22.5% followed by Tamar cv. with 20% and Festival record 17.5%. *Rhizopus stolonifer* was the most frequent in Kalubia samples which record 38.o% of total fungal count followed by *Botrytis cinerea* with 33.o%. Moderate percentage of total fungal count was recorded with *Pestalotia longisetula* fungus with 17.o%. Both *A. niger and Colletotrichum spp.* were less and either record 6.o%. In Ismailia samples, *B. cinerea* gave higher percentage of total fungal and record 41.5%. Pestalotia longisetula was moderate which record 20.0% followed by *R. stolonifer* with 19.5% and *A. niger* with 13.0%.

Many authors reported that, *Alternaria spp., Aspergillus spp., Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani, Phytophthora cactorum, Fusarium spp., Penicillium spp. and Sclerotinia sclerotiorum* are the most fungal isolates causing strawberry fruit rots in Egypt (Tarek, 2004). In general plant vitality decreased during the season, as well reduced yield was obtaind, and root crown rot decreased also fruit quality (Grantina-levina &Kalnina (2016)). In addition, *Pestalotia longisetula* was isolated and identified from strawberry rotted fruits (Embaby, 2007 a & b). Morever, other strawberry fungal rotted fruits were isolated from different locations in all over the world i.e. *Aspergillus, Botrytis, Colletotrichum, Geotrichum, Mucro, Penicillium,*

Pestaliopsis, Phytophthora and Rhizopus stolonifer were found infecting the crop (Fraire-Cordero, *et. al.,* 2003 and Paivi, *et. al.*, 2008). Two local species of *Colletotrichum* fungus i.e. *C. acutatum and C. gloeosporioides* that were identified and based on the cultural and morphological characteristics and by Polymerase Chain Reaction technique (PCR). The same results were obtained by Parikka, & Lemmetty, (2006) and Maymon, *et. al.,* (2009), they reported that, the *Colletotrichum* isolates were identified and characterized by classical morphological criteria and by various molecular methods. Morphological characterization identified all the tested isolates as *C. acutatum*, which was further confirmed by species-specific polymerase chain reaction (PCR) amplification using the universal ITS4 and CaInt2 primers.

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ARABIC SUMMARY

دراسات علي الفقد فى نمو و محصول الفراولة في المحافظات الرئيسيه لانتاجها في مصر و علاقته بفطريات التربة الممرضه

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إجريت عدة تجارب في محافظات الاسماعيليه والبحيره والقليوبية علي شتلات الفراولة رتبتي النوية (الأساس) والعادية وهدف هذه الدراسة تأثير الرتب المختلفه لشتلات الفراوله على جودة الشتلات والانتاجيه . جمع عينات من جذور وتيجان نباتات الفراولة التي تظهرعليها مظاهرالتقزم أو العفن أو الزبول ثم عزل وتنقيه وتعريف المسببات الممرضه ودراسة تأثيرها علي النمو والمحصول . تظهر النتائج ان وجود إختلافات معنويه بين الرتب المختلفه فى النمو و محصول الشتلات .صنفي الفراولة تمار ويائيل أظهرت بعض اعراض الاصابه في التقزم والتعفن والذبول سواء في الرتبة العادية للمزارعين في المشتل أو رتبتي الأساس في الصوبة . بالنسبة للاصناف لوحظت نسبة اصابة عالية في صنف تمار عنها في الصنف يائيل سواء في الصوبة أو المشتل المفتوح . وقد بلغت نسبة الاصابة الطبيعية في الصنف تمار في الشتلات العادية في الحقل وكذلك في الصوبة رتبة سوبر ايليت ٦,٣ ، ٦,١ % علي الترتيب بينما سجل صف يائيل نسبة اصابة بلغت ٨, % ، صفر% في كل من شتلات الرتبة العادية ورتبة السوبر ايليت علي الترتيب . وبحصر المسببات المرضية تبين إنها *F.oxysporum* ، F *solani* و*Macrophomina R.solani*. وتشير النتائج ايضا الي أن اعلي نسبة اصابه كانت بفطر *Fusarium* والتي بلغت ٤١.٢ % ويلية*Macrophomina* والتي سجلت ٣. و ٣٥ اما *Rhizoctonia* فقد كانت نسبتها ١١.٨ % وعند مقارنة نسبة الاصابة في الجذور و التيجان فقد وصلت الاصابة فيهما ٤٤.٧ و٣٣.٣ % علي الترتيب.بالنسبة لنسب الاصابة في محافظات تركيز زراعة الفراولة فكانت اعلي نسبة اصابة في القليوبية يليها الاسماعيلية واقلهم البحيرة وعموماً لوحظ نقص في النمو والمحصول وجوده الثمار مع وجود الاصابة بامراض أعفان الجذور .