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Characterization and Chemical Control of *Neopestalotiopsis rosae* the Causal Agent of Strawberry Root and Crown Rot in Egypt

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Strawberry (*Fragaria x ananassa* Duch.) is a widely grown vegetable crop in Egypt. During a routine survey, wilt and root rot symptoms were observed on strawberry plants cv. Festival. Samples were collected and isolation was done on potato dextrose agar (PDA) medium. The frequently isolated fungi were *Pestalotiopsis* spp. along with other associated fungi. Koch's postulates were fulfilled against two strawberry cultivars Fortuna and Festival under greenhouse conditions. Pestalotiopsis fungal isolates were identified by amplification and sequencing of ITS, TEF-1α and *β*-tubulin gene regions. The BLASTn search of GenBank database revealed that all obtained sequences exhibited similarity of 99–100% to *Neopestalotiopsis rosae*. Four fungicides were evaluated against *N. rosae* through *in vitro* and *in vivo* trials. *In vitro* trials revealed that fungicides Thiram and Hymexazole inhibited the mycelial growth of *N. rosae* at concentrations 1000 and 1250 ppm, respectively. *In vivo* trials showed that Thiram reduced significantly disease severity of crown and root rot on both cultivars.

Keywords: Chemical control, Crown rot, Molecular characterization**,** *Neopestalotiopsis rosae*, Strawberry

Strawberry (*Fragaria x ananassa* Duch.) is a popular fruit eaten raw or consumed in prepared foods and drinks including puddings, jams, pies, ice cream, milk shakes and juices. Egypt is one of 15 strawberry exporting countries with 3.5 % of total exported fresh strawberries with value 90 million $ and 7% of total exported frozen strawberries with value 65.8 million $ (Workman 2018). Fungal diseases negatively affect the strawberry production in Egypt and cause severe economic losses each year. Several fungi have been reported to cause strawberry crown and root rot worldwide (Golzar *et al.*, 2007). *Pestalotiopsis* species have been recently reported to be associated with root and crown rot of strawberry. The earliest available references to Pestalotiopsis on strawberry plants are from the Netherlands in 2013 where the disease was detected in propagation material by BASF (Van Hemelrijck *et al.*, 2017), Later, it was also detected in commercial plantations of strawberry in Huelva province, Spain during the cropping year 2013-14 (Chamorro *et al.*, 2016). A closely related species, *P. longisetula*, was previously identified as the causal agent of strawberry fruit rot in Egypt (Embaby, 2007). Leaf spot on

strawberries caused by *Pestalotiopsis* species have been reported in China, Brazil, USA, Morocco, Egypt and Spain (Zhu *et al.*, 1994; Mouden *et al.*, 2014 and Rodrigues *et al.*, 2014). Furthermore, Pestalotiopsis species have also been reported to be associated with the decay of petioles and stolon (Royse and Ries, 1976), root rot in the USA (Mertely *et al.*, 2013), necrotic spots on the leaves and petioles in Brazil (Teixeira *et al.*, 2015) and root and crown rot in Spain (Chamorro *et al.*, 2016). In Belgium, this disease was also reported on crown tissues of strawberry plants (Ceustermans *et al.*, 2015). In Brazil, the disease considered as a major threat to strawberry production during the last decade and could cause complete crop failure in some regions (Rodrigues *et al.*, 2014 and *Teixeira et al.*, 2015).

Characterization of *Pestalotiopsis* species have relied mainly on the morphological features such as conidia size, septation, presence or absence as well as number of appendages, colony texture and color (Keith *et al.*, 2006 and Sutton, 1980) along with the molecular DNA-based techniques (Lee *et al.*, 2006; Wei *et al.*, 2007 and Jeewon *et al.*, 2004). In the first attempt of Jeewon *et al.* (2003), they relied on Internal transcribed spacer (ITS) sequence data to evaluate the phylogenetic status of *Pestalotiopsis* species. On the contrary, Hu *et al.* (2007) pointed out that *β*-tubulin (TUB) gene is more informative than ITS gene in resolving phylogenetic differences among different *Pestalotiopsis* spp. They proposed that a combination of both ITS and *β*-tubulin could be much more informative than single locus. This was an evident in the study of Maharachchikumbura *et al.* (2012) who demonstrated that ITS, TUB and translation elongation factor (TEF) were the preeminent molecular markers among 10 gene region screened to resolve species limits in the *Pestalotiopsis* spp. In the recent study of Maharachchikumbura *et al.* (2014), the multi-locus phylogenetic revision introduced two new genera *i.e.*, *Neopestalotiopsis and Pseudopestalotiopsis*, which are morphologically correlated but phylognetically distinct from Pestalotiopsis.

This study was carried out to characterize *Pestalotiopsis* species associated with strawberry root and crown rot. This study is supplemented with *in vitro* and *in vivo* evaluation of four fungicides to control strawberry root and crown rot disease.

Materials and Methods

Symptoms observation and fungal isolation:

Symptoms of complete or partially dried leaves on edges were observed on strawberry cv. Festival during the growing season of 2017. Cross-section of the crown area showed browning of vascular tissues. Samples were collected from symptomatic and asymptomatic (apparently healthy) plants at four locations (Meet Kenana, El-Deer, El- Hsania and El-Sohby) at Kalyobia, Governorate, Egypt. All the samples were surface sterilized by washing in 10 % bleach solution (0.5 % NaCl) for 1 min followed by washing with sterile distilled water (SDW) and dried between two layers of sterilized filter paper. Small pieces between the healthy tissues and infected one were cut into (3–5 mm2) and placed in Petri dishes 9-cm-

diameters containing potato dextrose agar medium (PDA) (Difco Laboratories, Detroit, MI) supplemented with streptomycin sulfate (0.1g/L-1) to inhibit any bacterial growth. Plates were incubated for 5-7 days at 25±2°C in the dark. Fungal hyphae from the margin of developing colonies were transferred into PDA to have pure culture. These fungal colonies were initially identified using morphological traits following Maharachchikumbura *et al.,* 2012 and Maharachchikumbura *et al.,* 2014).

Morphological characterization:

Pestalotiopsis isolates were initially identified up to the genus level based on conidia morphology as described by Maharachchikumbura *et al.,* 2012 and Maharachchikumbura *et al.,* 2014). Isolates were then transferred into PDA and incubated at 25°C in the dark for 7-10 days. Colony morphology and color on the front as well as the reverse side of plates were examined. Conidia characteristics *i.e.*, shape, cell number and size (length and width), color of median cells, length of apical and basal appendages and number of apical appendages of 30 arbitrarily selected conidia of each representative isolate were determined.

Molecular characterization:

The obtained isolates of Pestalotiopsis were subjected to PCR amplification of three gene regions. The partial *β*-tubulin gene region was amplified using T1 (O'Donnell *et al.*, 1997) and Bt2b primers (Glass *et al.*, 1995). The nuclear RNA operon containing; the 3' end of the 18S rRNA gene, the internal spacers, the 5.8S rRNA gene and a part of the 5' end of the 28S rRNA gene were amplified using ITS1 and ITS4 primers (White *et al.*, 1990). The primers EF1-728F (Carbone *et al.*, 1999) and EF2 (O'Donnell *et al.*, 1998) were used to amplify partial sequence of the translation elongation factor 1-alpha (TEF-1α) gene.

Mycelial mats were collected from 7-days old culture incubated at 25°C, dried with sterile filter paper, frozen in liquid nitrogen and grounded to a fine powder. Genomic DNA extraction was performed using Wizard Magnetic DNA Purification System kit (Promega) following the manufacturer's protocol. PCR amplifications and conditions were carried out as described by Maharachchikumbura *et al.* (2014). Sequencing of the PCR product was performed for both directions using the same primers of amplification using Big Dye Terminator cycle sequencing kit (version 3.1; Applied Biosystems), following the manufacturer's manual. PCR amplification and sequencing were done at Institute of Sciences of Food Production National Research Council, Bari, Italy. The highly similar sequences for *β*-tubulin, ITS and TEF-1α regions were retrieved from the NCBI GenBank database and aligned together with the obtained sequences using the software MEGA v.7 (Kumar *et al*., 2016). The evolutionary history and correction of sequences were done using the software MEGA v.7 (Kumar *et al.*, 2016).

Pathogenicity tests:

Pestalotiopsis isolates were tested for their pathogenicity using two strawberry cvs. Fortuna and Festival under greenhouse conditions. Two healthy strawberry seedlings from each cultivar were planted in plastic pots 25cm diameter filled with

sterile substrate mix of 1:2 sand and peat moss. Inoculation was done before planting by dipping the roots for 10 minutes in a spore suspension (106 spores/ml) prepared from 10-12 days-old cultures. Strawberry plants dipped in sterilized water were used as a negative control. All plants were kept under controlled conditions in greenhouse at 25±2°C and 50-60% relative humidity. Each pot contained two plants and serve as one replicate. Fifteen replicates were used for each treatment. Re-isolation from the roots was carried out to fulfill the steps of etiology studying as Koch's postulates.

Evaluation the inhibitory effect of some fungicides against N. rosae:
In vitro experiment:

Efficacy of four commercially available fungicides for root rot diseases was evaluated against *Neopestalotiopsis rosae* on PDA medium. The fungicides used were Vitavax 200 40% FS (Carboxin + Thiram), Mission 3.5 % FS (Fludioxonil+ Metalaxyl), Tachigaren 30% SL (Hymexazole) and Flowsan 42.7% FS (Thiram). The fungicides were prepared according to their respective commercial formulations and suspended directly into the liquid PD medium at 50–55°C. Mycelium agar plugs (6 mm) were excised from the leading edge of 7-days old culture and inverted onto 90-mm Petri-plates containing PDA medium supplemented with the tested fungicides at concentrations *i.e.*, 500, 750, 1000 and 1250 ppm. Control plates contained only PDA medium and mycelial plug of the fungi. The four concentrations of each fungicide were replicated four times. All plates were then incubated at 25±2°C in darkness for 7 days. One measurement of mycelial growth for each concentration was done with the original plug diameter (5-mm) subtracted. Fungal growth was measured and fungal-toxicity was recorded as percentage of colony inhibition (Pandey *et al.*, 1982). Percentage of growth inhibition was measured using the formula: $[(Dc - Dt)/ Dc] \times 100$, where (Dc) was the average diameter increase of the fungal colony in the control, and (Dt) was the average diameter of the fungal colony in the treatment (Weitang *et al.*, 2004).

In vivo experiment:

The effect of the four fungicides was also investigated under greenhouse conditions using apparently healthy strawberry seedlings of cvs. Fortuna and Festival. Inoculations were carried out as mentioned before in the pathogenicity test. The fungicides were applied one week after inoculation at the concentrations of 500, 750, 1000 and 1250ppm/liter. Control plants were equally infested with the tested fungus however, instead of fungicide sterile distilled water was applied to each control. Fifteen replicates (pots, each pot has two plants) were used for each treatment.

Disease assessment:

Disease index calculated and severity were determined on the foliar part using 0−5 disease scale as suggested by Fang *et al.* (2011). The plants were harvested after 60 days of inoculation and assayed for vascular discoloration of longitudinally sectioned of crowns following Fang *et al.* (2011).

Plant growth parameters:

Shoots number, length (cm) and fresh weight (g) and new emerged roots number and length (cm) were recorded 60 days after planting for both cultivars.

Data analysis:

The experiments were designed as completely randomized block design. Data were subjected to analysis of variance (ANOVA) test using Cropstat software version 7.2 (Crop Stat, 2009), after transforming all percentage with arc singe. Mean values were compared using the least significant difference (LSD) test at P< 0.05.

Results

Symptoms observation and fungal isolation:

The symptomatic strawberry plants of cv. Festival exhibited discolored crown tissues were collected from the four locations (Meet Kenana, El-Deer, El-Hsania and El-Sohby) Kaliobiya governorate, Egypt (Fig. 1). Four fungal genera were isolated purified and identified according to the examined morphological criteria as *Pestalotiopsis* spp., *Fusarium solani*, *F. oxysporum* and *Rhizoctonia solani*. *Pestalotiopsis* spp. were the most prevalent fungi with 47.62 % of the total isolates. *F. solani* was also frequently isolated at frequency was 22.22 %. Whereas, *R. solani* and *F. oxysporum* were 17.46 and 12.70 %, respectively (Table 1).

Fig.1 Symptoms observed in the filed started as drying of the edges of the leaves and finally death of the whole plant. B: Longitudinal sections of the crown area exhibited browning and necrosis of vascular tissues of cv. Festival.

Table 1. Frequency of the isolated fungi recovered from strawberry plants infected by root and crown rot.

Location / Isolates	Meet Kenana		El-Deer		El-Hsania		El- Sohby		Total	
	No.	Frequency%	No.	Frequency %	No.	Frequency %	No.	Frequency %	No.	Frequency %
Pestalotiopsis spp.	6	46.15	8	50.00	11	52.38	5	38.46	30	47.62
F. solani	5	38.47	2	12.50	4	19.04	3	23.08	14	22.22
F. oxysporum	1	7.69	2	12.50	3	14.29	2	15.38	8	12.70
R. solani	1	7.69	4	25.00	3	14.29	3	23.08	11	17.46
Total	13	100	16	100	21	100	13	100	63	100

Means followed by the same letter were not significantly different at P <0.05

Morphological characterization:

Ten-days after incubation at 25°C, Pestalotiospsis isolates developed whitish, somewhat raised, and olivaceous color on the underside of the plates (Fig. 2A and B). Conidiomata (acervuli) were mostly solitary, scattered and semi-immersed, up to 250-µm diameters. Conidia were fusiform, slightly curved and 4-septate, (avg. 20.5 × 5.7) µm (n = 50) (Fig. 2C). Apical cell was bared 2–3 un-branched, tubular, hyaline and appendages were 16–23µm long. The basal cell bared single hyaline, centric with un-branched appendage (4.5–7.5 µm long). According to the described morphological criteria, the fungus was identified as *Pestalotiopsis rosae*= *Neopestalotiopsis rosae* (Maharachchikumbura *et al.*, 2014).

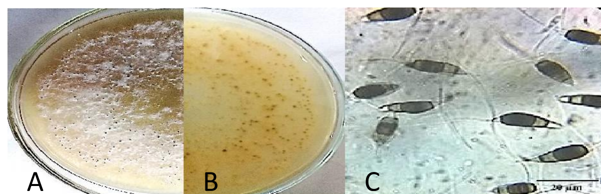

Fig 2. Colony morphology of 7-day-old culture of *N. rosae* on PDA medium; A, front side; B, reverse side; C, five-celled fusiform conidia with 2-3 apical appendages and one basal appendage.

Molecular characterization:

The PCR amplification using total genomic DNA revealed amplicons of 749, 559, 497 bp for ITS, *β*-tubulin and TEF-1α regions, respectively. The BLASTn search in NCBI GenBank database exhibited similarity of 99–100% to *Neopestalotiopsis rosae*. Sequences of three gene regions; *β*-tubulin and TEF-1α and ITS of representative isolate (PEST-1) were deposited in the GenBank under accession numbers KY688073, KY688074 and KY688075, respectively. The analysis involved 15 nucleotide sequences. There were a total of 1635 positions in the final dataset and positions containing gaps and missing data were eliminated. The tree with the highest log likelihood (-3723.63) is shown in Fig. (2). The phylogenetic analysis revealed that four isolates *N. rosae* obtained in this study sub-clustered together in sub-clade supported with bootstrap value 62% along with two isolates of *N. rosae* CBS 101057 and CBS 124745 in a well-supported clade with bootstrap value 88 %.

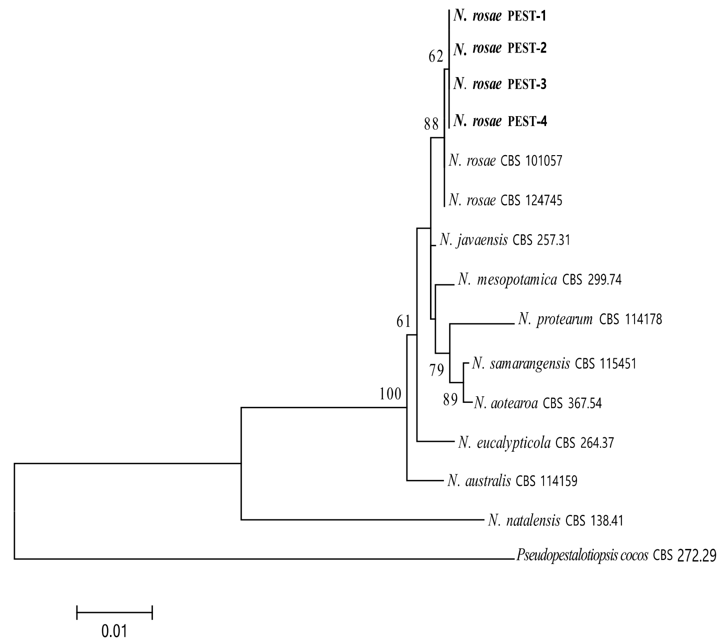

Fig 3. Maximum Likelihood tree based on the combined data set of ITS, TEF-1α and *β*-tubulin sequences of 15 *Neopestalotiopsis* strains. ML bootstrap support values (ML) are given at the nodes. ML bootstrap support values (ML) are given above the nodes. The isolates in bold face are obtained in this study. The tree was rooted to *Pseudopestalotiopsis cocos* CBS 272.29.

Pathogenicity test:

The illustrated data in Table 2 show that both cvs. Fortuna and Festival were susceptible to the infection by *N. rosae* and exhibited symptoms similar to those observed in the field as drying of the leaves edge to completely dried leaves (Figs. 4 A and D) and discoloration of the internal tissues of the crown (Fig. 4F). The *N. rosae* was successfully re-isolated from symptomatic tissues. Isolates PEST-4 and PEST-1 displayed significant high disease incidence (DI) on cvs. Fortuna and Festival with values 73.3 and 60.0%, respectively. However, low DI was observed on plants of cv. Fortuna inoculated with isolate PEST-3.

Disease severity (DS) was assessed 8 weeks after inoculation on cvs. Fortuna and Festival (Table 2). The external symptoms appeared as drying of the leaves edge to complete dry leaves and ultimately complete death of the plants. Isolate PEST-4 was the most aggressive one and caused DS on both cvs. Fortuna and Festival with values of 58.91 and 51.60 %, respectively. While, isolate PEST exhibited low DS (22.76%) cv. Fortuna. Isolate PEST-4 was also more aggressive than the rest of isolates in causing discoloration in crown tissues of both cvs. Fortuna and Festival being, 55.64 and 50.36%, respectively.

Table 2. Response of strawberry plants cultivars, Fortuna and Festival to *N. rosae* under greenhouse conditions.

Cultivar	Isolate	% Disease incidence (DI)	%, Disease severity (DS)	
			Foliar part	Vascular tissue
Fortuna	PEST-1	56.7 b	31.72d	28.53 d
	PEST-2	40.0 c	22.76 f	23.76 e
	PEST-3	16.7 d	13.43 g	12.98 g
	PEST-4	73.3 a	58.91 a	55.64 a
	Control	0.0 e	2.64 h	0.00 h
Festival	PEST-1	60.0 b	48.32 c	42.76 c
	PEST-2	36.7 c	28.97 e	27.35 d
	PEST-3	30.0 c	22.65 f	18.05 f
	PEST-4	56.7 b	51.06 b	50.36 b
	Control	0.0 e	1.58 i	0.00 h

Means followed by the same letter were not significantly different at $P < 0.05$

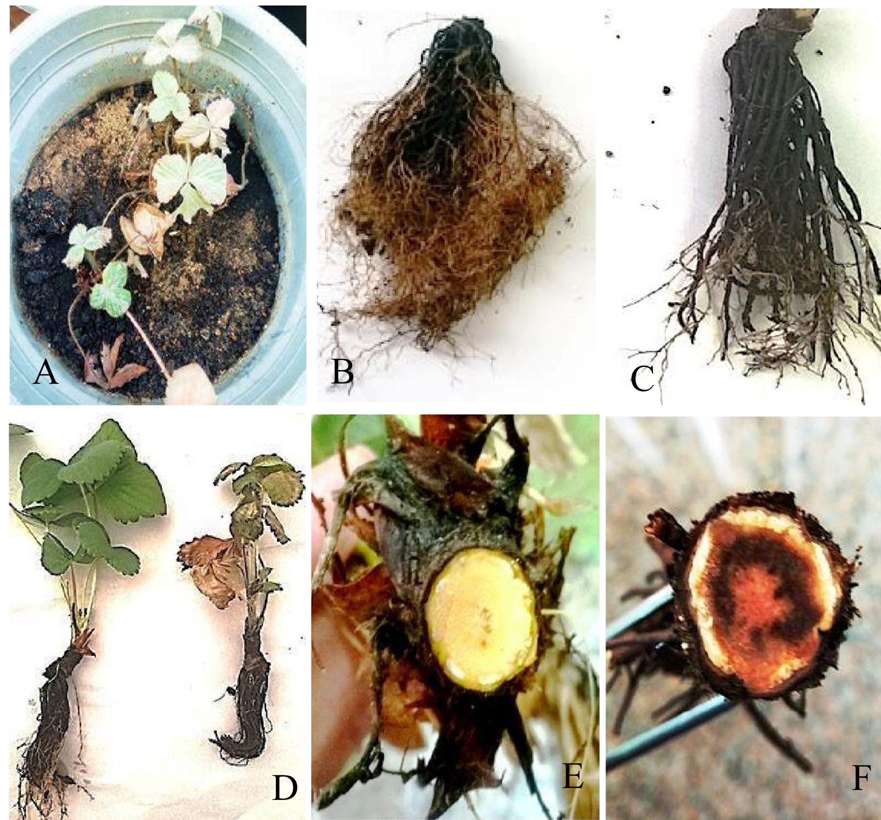

Fig 4. Pathogenicity test and symptoms developed; A, Root and crown rot symptoms of inoculated plants; B, Healthy roots of control plants; C, Root rot of inoculated plants; D, Healthy un-inoculated plant (left) and inoculated (right); E, healthy vascular tissues of cross section of the crown of control plant; F, Browned vascular tissues of cross section of crown of the inoculated plant.

Evaluation of the inhibitory effect of some fungicides against N. rosae:

In vitro experiment:

The efficacy of four fungicides was evaluated for their inhibitory effect on the mycelial growth of *N. rosae* at four concentrations (Table 3). The results show that all tested fungicides at the concentration of 1250 ppm significantly reduced the mycelial growth of *N. rosae.* However, Flowsan 42.7% FS (Thiram) revealed the best results at all concentrations compared to the other fungicides, followed by Tachigaren 30% SL (Hymexazole). However, Mission 3.5% FS (Fludioxonil + metalaxyl) and Vitavax 200 40% FS (Carboxin + Thiram) caused the lowest reduction to the mycelial growth of *N. rosae.*

Table 3. Efficacy of four fungicides on the inhibition of mycelial growth of *N. rosae* at four concentrations.

Fungicides	%, Mycelial growth inhibition at ppm				
	500	750	1000	1250	Means (A)
Vitavax 200 40 % FS	24.4	41.1	48.1	63.8	44.35
Mission 3.5 % FS	30.0	39.2	60.3	70.8	50.08
Tachigaren 30 % SL	70.8	81.1	83.3	93.3	82.13
Flowsan 42.7 % FS	82.7	93.3	94.4	94.4	91.20
Control	0.0	0.0	0.0	0.0	0.00
Means (B)	41.58	50.94	57.22	64.46	

LSD $_{at\ 0.05}$ A = 1.777 B = 1.590 A×B = 3.554

In vivo experiment:

Symptoms of drying and dying leaves were observed 8 weeks after inoculating the untreated plants. Application of the tested fungicides reduced root and crown rot of inoculated strawberry plants of both cvs. Fortuna and Festival. The fungicide Flowsan 42.7 % (Thiram) was the most effective in controlling the disease comparing to the other fungicides. The results of *in vivo* trials indicated that Thiram was considerably effective in reducing disease incidence (DI) to 23.3, 16.7% and diseases severity (DS) to 7.06, 5.0% with efficacy of 80, 82.05% on Fortuna and Festivals, respectively. The Tachigaren 30 % (Hymexazole) was also significantly able to reduce DI to 26.7% and DS to 5.83% with efficacy of 79.07 % on cv. Festival. Vitavax 200 40% (Carboxin + Thiram) was not effective in reducing the disease as DI was 40.0% and DS 13.63 with low efficacy 61.39% on cv. Fortuna and 53.3% DI, 12.36% DS with 55.63% efficacy on cv. Festival.

Strawberry growth parameters:

Data in Table 5 show that the best growth parameters of strawberry plants were obtained with Flowsan treatment for both cvs. Fortuna and Festivals. Strawberry plants of cv. Fortuna treated with Flowsan displayed a mean number of 2.33 shoots while, plants treated with any of the other fungicide gave 2.00 shoot. Similar results were obtained with cv. Festival that gave a mean number of shoots of 3.33, 2.67, 2.67 and 2.0 when the plants treated with Flowsan, Tachgarin Mission and Vitavax, respectively. All fungicidal treatments showed longer shoots than in the infected control. In cv. Fortuna treatments had a good plant shoot length without significant differences in between. On the other hand, plants were differed in plant shoot length

in cv. Festival where plants treated by Flowsan and Tachgarin displayed shoot lengths of 17.00 and 16.67 cm without significant difference in between but still significantly different than the untreated plants which showed shoot length of 18.33 cm. Regarding to shoot fresh weight, plant treated with Flowsan, Tachgarin and Mission had higher weights being 12.62 and 11.33g for cv. Fortuna and 23.76 and 23.9g cv. Festival.

Table 4. Efficacy of four fungicides against *N. rosea* on two cultivars of strawberry under greenhouse conditions.

Fungicide	Fortuna			Festivals		
	DI %	DS %	Efficacy %	DI %	DS %	Efficacy %
Vitavax 200 40 % FS	40.0 b	13.63 b	61.39	53.3 b	12.36 b	55.63
Mission 3.5 % FS	36.7 bc	10.53c	70.16	40.0 c	7.23 c	74.04
Tachigaren 30 % SL	33.3 c	8.43d	76.12	26.7 d	5.83 cd	79.07
Flowsan 42.7% FS	23.3 d	7.06 e	80.00	16.7 e	5.00 d	82.05
Control (ifection)	93.3 a	35.3 a	0.0	96.7 a	27.86 a	0.0
Control (un-infection)	0.0 e	0.01 f	-	0.0 f	0.01 e	-

Means in the same column followed by the same letter were not significantly different at $P < 0.05$

Table 5. Growth parameters of strawberry plants artificially inoculated with *N. rosea* and treated with the tested fungicides under greenhouse conditions.

Fungicide	Fortuna			Festivals		
	No. of Shoot /plant	Shoot length (cm)	Shoot fresh weight (g)	No. of shoots /plants	Shoot length(cm)	Shoot fresh weight (g)
Vitavax 200 40 % FS	2.00 c	15.43 b	11.0 c	2.00 d	14.67 c	12.67 d
Mission 3.5 % FS	2.00 c	16.66 b	10.15 d	2.67 c	16.00 bc	14.33 c
Tachigaren 30 % SL	2.00 c	15.66 b	11.33 c	2.67 c	16.67 ab	23.9 ab
Flowsan 42.7% FS	2.33 b	16.66 b	12.62 b	3.33 a	17.00 ab	23.76 b
Control (infected)	1.33 d	12.33 c	7.03 e	1.67 e	11.33 d	12.96 e
Control (un-infected)	2.67 a	19.66 a	14.43 a	3.00 b	18.33 a	24.33 a
$LSD_{0.05}$	0.299	1.388	0.630	0.293	1.962	0.516

Means in the same column followed by the same letter were not significantly different at $P < 0.05$

Number and length of new emerged roots:

Data in Table 6 show the indirect effect on the formation of new roots of plants inoculation with *N. rosae* and the both controls. The number of new roots of control plants (un-infested soil) reached 14.0 and 16.3 emerged from old roots and 17.0 and 23.6 emerged from crown in cvs. Fortuna and Festival, respectively. While, the infected plants that none treated with fungicides failed to form suitable number of new roots neither from old roots nor the crown. The new roots formed from old roots and crown were differed in number and their length according to the used fungicide. Plants treated with Flowsan or Tachgarin exhibited the higher number and their length of new roots emerged from old roots and crown than plants treated with the other fungicides (Mission and Vitavax 200).

Table 6. **Effect of some fungicides on the formation of new roots of strawberry plants artificially inoculated with *N. rosea* under greenhouse conditions.**

Fungicide	Fortuna				Festival			
	New roots from old root	Length of roots /plants	New roots from crown	Length of root	New roots from old root	Length of roots /plants	New roots from crown	Length of root
Vitavax 200 40 % FS	5.3 de	7.3 b	11.0 c	8.6 c	15.3 c	11.6 d	13.6 d	15.0 c
Mission 3.5 % FS	6.3 d	7.0 b	9.3 c	9.0 c	18.3 b	13.0 c	15.0 c	19.0 a
Tachigaren 30 % SL	8.3 c	10.0 a	11.0 c	8.6 c	18.6 b	13.7 b	16.3 b	23.6 a
Flowsan 42.7% FS	10.6 b	11.0 a	21.0 a	13.6 a	27.3 a	14.3 a	19.0 a	10.6 d
Control (infected)	4.6 e	4.8 c	8.6 c	7.3 d	7.0 d	9.8 e	11.4 e	14.4 b
Control (un-infected)	14.0 a	10.3 a	17.0 b	12.0 b	16.3 c	14.0 ab	23.6 a	9.3 e
LSD at $_{0.05}$	1.431	1.79	3.721	0.902	1.857	0.462	0.810	0.859

Means in the same colum followed by the same letter were not significantly different at P <0.05

Discussion

Root and crown rot diseases are the most important problem facing strawberry producers in Egypt. This study was conducted to investigate the most common fungal pathogens associated with these diseases. Isolation from disease strawberry plants including mortality, stunting, and wilt confirmed the association of *N. rosae*. Cross section of these symptomatic plants showed black, brown or red discoloration in the internal tissues of crown while healthy plants did not exhibit any vascular tissue or necrosis in the crown tissues. Many studies inferred that *Pestalotiopsis* species are generally not host-specific and can be associated with wide range of hosts and substrates (Lee *et al.*, 2006). The earliest study of McQuilken and Hopkins (2004) stated that *P. sydowian*a (Bresad) was a causal agent of stem-base and roots of diseased container of ericaceous crops (Calluna, Erica, Pieris and Rhododendron) in UK nurseries. A recent study by Grantina-Ievina and Kalniņa (2016) reported that *Pestalotiopsis* spp. were abundantly isolated from 5-42% of the examined

strawberry plants exhibiting crown rot disease. Van Hemelrijck *et al.* (2017) empirically concluded that *Pestalotiopsis* spp. could cause crown rot disease on strawberry plants in Belgium. Moreover, Ara *et al.* (2017) reported that *Pestalotiopsis* spp. is responsible of crown rot disease of strawberries in Bangladesh. Thus, our results also corroborate with the previous findings.

In our study, *F. oxysporum* and *R. solani* were also associated with crown and root rot, but they were isolated at low frequency 17.38 and 9.59 %, respectively. Similarly, Grantina-Ievina Kalniņa (2016) stated that *F. oxysporum* and *R. solani* were also isolated from crown rot of 5-30% and 8-22% of the examined plants, respectively. *Fusarium* species are widely devastating pathogen on strawberry and *F. oxysporum* is responsible for wilt on strawberry and it has been early reported in Australia (Winks and Williams, 1965). The only forma specialis causing strawberry wilt is *F. oxysporum* f.sp. *fragariae* (Nagarajan *et al.*, 2006). Also, Martin (2000) declared that the most common associated fungi with root rot of strawberry are binucleate Rhizoctonia belongs to AG-A, AG-G, and AG-I.

*Pestalotiopsi*s genus is a complex and its classification to species level is severely hampered by the enormous variation in morphology (Karakaya 2001). *N. rosae* isolates showed very little variation in colony and conidia morphology and looked very similar. Molecular markers tools played a significant role in the discrimination and clarifying species boundaries in Pestalotiopsis. Therefore, we coupled morphological and sequence data of ITS, TUB and TEF gene regions, which revealed a discrimination of *N. rosae* from other members of *Neopestalotiosis* species through the phylogenetic analysis. This molecular tool could be used be used in further studies to detect *N. rosae*

The *in vitro* trials indicated that Thiram and Hymexazole were able to inhibit mycelial growth of *N. rosae* at four concentrations. Conversely, the trials of Bin *et al.* (2013) showed that among 7 fungicides tested, only mancozeb, azoxystrobin and prochloraz could inhibit the mycelial growth of *P. microspora*, with inhibitory values of 66.77, 65.56% and 71.11%, respectively.

In vivo trials indicated that Thiram, the active ingredient of Flowsan was the most effective in controlling the disease comparing to the other fungicides. In a similar study, Lisboa- Padulla *et al.* (2009) stated that captan and carboxim fungicides reduced the incidence of *Pestalotiopsis* spp. in seeds of Brazil wood. However, in our study Carboxin + Thiram were less effective in reducing DI and DS of strawberry crown and root rot. Shin *et al.* (2010) reported that copper hydroxide and carbendazim are highly effective against *P. longisetula* and *P. theae*. While, Sanjay *et al.* (2008) observed that combinations between fungicides mancozeb, carbendazim and copper oxychloride exhibited 22.6 % reduction in disease incidence of grey blight disease of tea caused by *P. theae*. However, using these fungicides individually revealed only 20.7 % reduction in disease incidence. Moreover, Carrie-Missio *et al.* (2010) reported that spraying of azoxystrobin and

mancozeb has a potent role in reducing the symptoms of leaf blight on strawberry caused by *P. longisetula*. Later on, Bin *et al.* (2013) found out through *in vitro* and *in vivo* trials that mancozeb, azoxystrobin and prochloraz could be used to control *P. microspora* leaf spot disease. Furthermore, McQuilken and Hopkins (2004) found that alternate use of prochloraz and carbendazim reduced the disease incidence and foliar browning caused by *P. sydowiana*.

Conclusion

The infection by *N. rosae* has not been studied so far, but it is supposed to have arisen from the extensive marketing and movement of seedlings amongst farmers in the popular area Kaliobiya, where cultivation of strawberry is common. This pathogen might be present also in other areas in Egypt, so an extensive survey is needed to understand its spread and epidemiology.

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التـــوصيـــف والمكافحـــــة الكيميــائيـــة الفطـــر *Neopestalotiopsis rosae* المسبب لعفن الجذور والتاج لنباتات الفراولة فى مصر

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** وحـدة بحـوث أمراض وآفـات النباتــات كليــة الأغذيـــة والزراعة جامعة الملك فيصل السعودية

تُعتبر الفراولة أحـد محاصيل الخضر واسعة الانتشار فى مصر. وخلال الفحص الدورى لنباتات الفراولة لوحظ إصابة النباتات بأعراض أعفان الجذور والذبول فى صنف فيستيفال. جُمعت عينات مصابة وتم عزل المسببات المرضية على بيئة آجار دكستروز البطاطس. تم عزل فطر Pestalotiopsis وكان الأكثر تكراراً عن باقى الفطريات المصاحبة. تم تطبيق افتراضات العالم كوخ على صنفى الفراولة فورتونا وفيستيفال تحت ظرف الصوبة. تم تعريفه من خلال تفاعل تكبير ثلاث مواقع جينية TS, TEF-1α و β-tubulin وعمل تتابعات القواعد النيترجينية لها. أشار تتابع القواعد النيتروجينية للجينات عند إيداعها في البنك الدولي للجينات تشابهها بنسبة ٩٩-١٠٠% مع فطر *Neopestalotiopsis rosae*. تم تقييم أربع مبيدات فطرية ضد *Neopestalotiopsis rosae* في تجارب المعمل والصوبة. أوضحت تجارب المعمل أن المواد الفعالة الثيرم وهيمكسازول قادران على تثبيط نمو ميسليوم كاملاً عند تركيزي ١٠٠٠و ١٢٥٠ ppm على الترتيب. أما بالنسبة لتجارب الصوبة فقد حققت المادة الفعالة الثايرم خفض معنوي للشدة المرضية لكل من أعفان الجذور والتاج على كلا الصنفين.