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Epidemiology and Survival of *Macrophomina phaseolina* and *Colletotrichum acutatum*, the Causal Organisms of Strawberry Crown and Root Rot Diseases in Egypt

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ABSTRACT

Soil-borne fungi of strawberry are distributed and infected the plants under different soils moisture,. High incidence of crown rots of strawberries caused by *Macrophomina phaseolina* and *Colletotrichum acutatum* has been observed and isolated in major strawberry production districts in Ismailia Governorate. Pathogenicity test and susceptibility of different strawberry cultivars were done. Two fungiunder study were proved.Both fungi were found as virulent pathogens and caused pathogenic effect to different tested strawberry cultivars *in vivo*.Festival was the most susceptible cv. followed by N-70 and Gavuta cvs. However, Camarosa cv. was the lowest susceptible to *C. acutatum* while the other cultivars (Sweet Charlie and Fortuna) were similar and showed moderate susceptibility to foliar inoculation with the pathogens ,respectively.*Colletotrichum acutatum* was less virulent than *M. phaseolina* either in disease incidence (DI%) or disease severity (DS%).Studying the host range of the two pathogens revealed that cotton, sesame, soybean, water melon and sunflower showed positive reactions and respond to the artificial inoculation with *M. phaseolina* (the causal of charcoal rot disease) with percentages of disease incidence ranged from 50 - 80% , respectively. On the other hand,all the testedelevin hosts showed negative response without any disease symptoms when it artificially inoculated with *C. acutatum.* Survival of both *M. phaseolina* and *C. acutatum* inocula buried in depths of 0.0, 5, 10 and 15 cm in field soil were recovered and keep its viabilitytill 120 days sthenstarted to decrease as it reached 60and80% after180days for the two pathogens under study, respectively.

Keywords: Soil-borne fungi - Strawberry crown and root rots - *Colletotrichum acutatum -Macrophomina phaseolina*- Disease incidence (DI%) - Disease severity (DS%) -*In vivo* - Strawberry cultivars .

INTRODUCTION

In Egypt, some susceptible strawberry cultivars e.g., Susana and Festival are subjected to be attacked by numerous of soil borne pathogens. This attack is due to spread of infestation; either in old Nile valley or in new reclaimed sandy soil. As result of these infestation; considerable reduction in quantity and quality of yield were noticed (Ayat - Ali 2013). Considerable amounts of high toxic fungicides are used annually to protect strawberry seedlings against these diseases. The toxic substances of fungicides lead to serious problems for environment and human health. Crown and root rots are important diseases of commercial strawberry farms. Several fungi have been reported as causal agents for strawberry crown and root rots. Strawberry plants reported to be infected by several soil-borne pathogens causing crown and root rots (Fang *et al*., 2012). These pathogens cause crown and root rot diseases of strawberry either individually or in combinations. These fungal species associated with the strawberry cultivars are become ubiquitous and can be found as soil-inhabiting microorganis the (Abdel-Sattar *et al*; 2008. Ceja-Torres *et al*., 2014).

The control of both diseases in strawberry is challenging because the causal pathogens, *M. phaseolina*, and *C.acutatum* can survive for long periods in soil as resistant structures (respectively, sclerotia and chlamydospores). They are disseminated by various means, including wind, soil and infected plant material. Chemicals currently available for pre-plant soil disinfestation are not effective for the eradication of *M. phaseolina*(Zveibil- Aida *et al.,* 2012and Chamorro *et al*, 2015), and their efficacy in treatment of root rot pathogens is yet unknown. In addition, the prohibition of most chemical fungicides for edible exported strawberry and adverse effects of their indiscriminate use on the environment and human health, have promoted research in the field of biological control of plant diseases as an effective and sustainable alternative.

The overall objective of the present investigation is to study strawberry crown rot diseases in Ismaillia Governorate. Prove pathogenicity test and susceptibility of different strawberry cultivars to infect with the isolated pathogenic fungi. Studying host range, viability and survival of *M. phaseolina*, and *C.acutatum* were also included.

MATERIALS AND METHODS

Strawberry plants showed typical symptoms of crown and root rot diseases were collected from different districts belongs to Ismailia governorate. The common planted cultivars in the different selected farms were; Camarosa, Festival, Sweet Charlie, Gafuta and N70.

1-Isolation and identification: Isolation from infected and apparently healthy strawberry plants including crown and root parts.

All fungal isolates were cultured on PDA for purification and then identification. Frequency percentages of fungal colonies were recorded for each fungus, and then purified using single spore and hyphae tip techniques suggested by Dhingra and Sinclair (1985).*Macrophomina* sp. and *Colletotrichum* sp. were isolated from host tissues and then identified following (Watanabe, 2010).

2-Susceptibility of strawberry cultivars to infect with *C. acutatum* and *M. phaseolina*

Both *C. acutatum* and *M. phaseolina* were chosen for further study because of their significance as a new pathogenic fungi started to spread in the last decade and threaten strawberry cultivation in Ismailia governorate as well as other soil- borne fungi.

Inoculum preparation of *C. acutatum* and *M. phaseolina*:

To produce inoculumof *C.acutatum* conidia from 2-weeks-old cultureson potato dextrose agar (PDA) at 25 °C under fluorescent lights 12 hrs. each day were washed from the dishes , filtered through cheesecloth, and suspended in 200 ml sterile water with two drops Tween 20 (0.01%) as a surfactant. The spore concentration was adjusted to $5x10^6$ conidia/ml-l with the aid of a hemocytometer. Inoculations

were performed in the early evening of April to maximize conditions for optimal infection. Concerning inoculum preparation of *M. phaseolina* small disks (0.5 cm in diam.) were taken from the edges of 12-days-old colonies of *M. phaseolina* grown on PDA medium. Each disk was placed in each 250-ml flask contain 100 ml of potato dextrose broth amended with Chloramphenicol at 250 mg/liter according to (Mallikarjuna, and JayapalGowdu, 2015). Inoculated checks were incubated in the dark at 25^{o}C for 10 days. Thereafter, 0.01% Tween-20 was added to the suspension containing mycelia and sclerotia, and the suspension was blended for 30 S. and filtered through Nylon cloth. The nylon cloth containing sclerotia was dried at 25^{o}C in an incubator for 3 days and filtered through a 2-mm-diameter sieve into a glass beaker. The viability of sclerotia was tested on the agar medium for *M. phaseolina*. Sclerotia were collected and used for experiments. *Macrophomina* sclerotia were suspended in sterile distilled water; at a concentration of 5×10^{5}sclerotia per ml. Amount of 100ml of the microsclerotia suspension was used for plant infestations.

3- Host range of *C. acutatum* and *M. phaseolina*:

The host range of strawberry crown and root rot pathogens, *C. acutatum* and *M. phaseolina* were studied in pots. A volume of inocula (100 ml) of *M. phaseolina* and *C. acutatum* cultures were applied as spray and soil drench to roots grown in sterilized peat moss soil at 1:20 ratio (w/w) a week before planting the host seedling (each ml contains conidial suspensions (10^{6}/ml of *C. acutatum* and 5×10^{5} /ml sclerotia of *M. phaseolina*).

A total of eleven plant species belong to 7 botanical families commonly grown in Ismailia governorate *i.e.* Yellow maize, White maize, Cotton, Sesame , Soybean , Faba bean , Beans , Peanut , Water melon , Sun Flower and Strawberry were tested. The surface sterilized seeds or healthy seedlings of the tested host species were planted at five seedlings per pot. Three replicates were used for each treatment. The pots were maintained in the greenhouse with careful, uniform and regular irrigation. The disease incidence was recorded at maturity stage of the plants and expressed as percent of crown and root rot incidence.

4-Survival of *C. acutatum*and *M. phaseolina* in artificially strawberry plants buried at different depths in soil.

Survival of *C. acutatum*and *M. phaseolina* was studied as a part of epidemiological work during 2015-2016 at Suez Canal Agric. Research Farm in Ismailia governorate. Information about the perpetuation of the pathogens during the off-season and evaluate their implication in the survival and transmission of crown and root rot pathogens of strawberry were studied.Under field conditions,the experiment was performed at the off-season of strawberry cultivation to evaluate their implication in the survival and transmission of this crown and root rot pathogens of strawberry. Also, to determine whether the inoculum could survive and thus serve as a potential infection source.

To evaluate *C. acutatum* and *M. Phaseolina* survival in naturally infected crowns an experment was conducted at Expermintal Farm of Suez Canal University, Faculty of Agriculture. The inocula were buried in untreated soil. Individual crowns (10 crowns) were placed in bags of Gauze Fabric Muslin cheese cloth, mixed with chopped soil and buried for a longer period in strawberry field soil at the depth of 0.0 as control, 5, 10 and 15 cm. The trial started in late 2015 (November) and it was lasted after 180 days (from the end of season to the start of next strawberry cultivation season in 2016).The first group of samples was dug up after 20 days (late November 2015), the second sample after 40 days (December 2015) and so on every 20 days. Recovering of sclerotia samples from soil at different depths was started after 20 days, and continued every 20 days intervals (20, 40, 60, 80, 100, 120, 140, 160 and 180 days) till the beginning of next growing season on which environmental conditions support strawberry infection. The crown pieces were retrieved and washed under running tap water, surface sterilized in 3% sodium hypochlorite for 2 min, rinsed in sterile distilled water for 1 min, dried in a laminar flow hood. After that, these cut pieces were transferred into the potato dextrose agar (PDA) medium on Petri plates. Then, Petri plates were incubated at 25±1ºC for 7 days. After 7 days, survival of pathogen was observed by the growth of viable fungal colonies on the medium. Means of 5 crowns per depth were calculated. The percent viability of *C. acutatum* or *M. phaseolina* was calculated by using following formula given by (Freeman *et al.,* 2002)

$$\% \text{ of viability} = \frac{\text{Number of pieces showing fungal colonies}}{\text{Total number of pieces plated}} \times 100$$

Experimental Results

Susceptibility of cultivar reactions of strawberry to *C. acutatum*and *M. phaseolina*

Data present in Table (1) illustrate the results obtained from the experiments carried out to test pathogenicity and susceptibility of six common strawberry cultivars cultivated in Ismailia Governorate to infect with *C. acutatum* and *M. phaseolina* by different inoculation and infestation methods ofstrawberry plants and soil under greenhouse conditions.Data show that *C. acutatum* caused variable percentages of disease incidence (DI%) and disease severity (DS%). Inoculation method of foliar spray was the most effective one of infection in case of*C. Acutatum*.

Festival was the most susceptible cv. and showed higher DI and DS% (85 and 75%) followed by N-70 cv. (78 and 52%)and Gavuta cv. (78 and 45%).However, Camarosa cv. was the lowest susceptible to *C. acutatum* (66 and 45%), while the other cultivars (Sweet Charlie and Fortuna) were similar and showed moderate susceptibility to foliar inoculation with the pathogen as they recorded (65 and 43%) and (66 and 56%); respectively.

Root dipping method occupied the 2nd order effective in artificial inoculation after foliar spray. Also, Festival, N-70 and Gavuta cvs. were the most susceptible to *C. acutatum* in both DI and DS values .The other cultivars (Sweet Charlie, Camarosa and Fortuna) showed less and moderate susceptibility to root dipping inoculation.

The third method of soil infestation with the pathogen was less effective as inoculation method tested in this study with *C. acutatum* but also showed significant infection in DI and DS assessment among cultivars. Also, Festival cv. was the most susceptible (74 and 50%) followed by N-70 (50 and 34.5%). The other tested

cultivars (Sweet Charlie, Fortuna and Gavuta) were less susceptible and their DI and DS values recorded (38 and 16.5%), (43 and 15%) and (50 and 11%), respectively. Camarosa was the lowest susceptible cv. in this inoculation method (30 and 10.8%).

Data in the same Table (2) show that *M. phaseolina* caused also variable percentages of disease incidence (DI%) and disease severity (DS%). Inoculation method of root dipping and soil infestation were the most effective artificial inoculation method with this pathogen while foliar spray caused also significant infection damage but less than the other mentioned methods. All tested cultivars showed severe DI% ranged from 34 to 100% and DS% ranged from 35 to 85% in root dipping method. In the soil infestation method, Festival, N-70, Sweet Charlie and Fortuna cvs. recorded values of DI% and DS% (100, 79%), (88, 70%), (78, 55%) and (70, 62.5%), respectively.

Table 1. Susceptibility of strawberry cultivars using conidial suspensions (10^6/ml) of *C. acutatum* and sclerotia suspensions (100 ml of 10^5/ml) of *M. phaseolina* by different inoculation methods in greenhouse.

Cultivar	Pathogens															
	C. acutatum								*M. phaseolina*							
	Pathogenicity method															
	control		Foliar spray		Root dipping		Soil infestation		control		Foliar spray		Root dipping		Soil infestation	
	DI.	DS.	DI.	DS.	DI.	DS.	DI.	DS.	DI.	DS.	DI.	DS.	DI.	DS.	DI.	DS.
Festival	0	0	85a*	75a	80a	65a	74a	50a	0	0	68a	45a	100a	85a	100a	79a
N 70	0	0	78b	52d	66b	46b	50b	34.5b	0	0	66a	30d	95b	85a	88b	70b
Sweet Charlie	0	0	65c	43e	48d	35e	38d	16.5c	0	0	45bc	35c	85d	75b	78c	55d
Fortuna	0	0	66c	56c	54c	42c	43c	15d	0	0	44c	23e	90c	72c	70d	62.5c
Gavuta	0	0	78b	45e	64b	39d	50b	11e	0	0	34d	28.5de	75e	63d	40f	35e
Camarosa	0	0	66c	45e	48d	38d	30e	10.8e	0	0	47b	33.5c	100a	55e	50e	25f

*** Each value represents the mean of 3 replicates. Values within same column followed by the same letter are not significantly different according to Duncan's multiple range test (P= 0.05).**

2- Host range of *C. acutatum* and *M. phaseolina*.

Data present in Table (2) show the pathogenic effect of the causal organisms of crown and root rot pathogens, *C. acutatum*and *M. phaseolina* on total of eleven plant species belong to 7 botanical families commonly grown in Ismailia governorate. Results of this experiment indicated that all the host showed negative response without any disease symptoms when it artificially inoculated with *C. acutatum.* On the other hand, white maize, yellow maize, faba bean, bean and peanut showed negative response to artificial inoculation with *M. phaseolina* while cotton, sesame, soybean, water melon and sunflower showed positive reaction and respond to the artificial inoculation with *M. phaseolina* with percentages of disease incidence ranged from 50 – 80% , respectively. Sesame, sunflower and strawberry had the highest percentage of disease incidence (80%) followed by cotton (70%), watermelon (50%) and soybean (30%).

Table 2. Host range of pathogenic *C. acutatum*and *M. phaseolina* fungi isolated from infected strawberry plants.

Host Common name	Host Latin name	Host Family	Tested pathogens	
			C. acutatum	*M. phaseolina*
			Disease incidence (%)	
Yellow maize	*Zea mays* L	Gramineae	0	0
White maize	*Zea mays* L	Gramineae	0	0
Cotton	*Gossypium hirsutum*L	Malvaceae	0	70
Sesame	*Sesam umindicum*L	Pedaliaceae	0	80
Soybean	*Glycine max (*L.*) Merr*	Fabaceae	0	30
Faba bean	*Vicia faba*L	Fabaceae	0	0
Water melon	*Citrullus lanatus*(Thunb.)	Cucurbitaceae	0	50
Sun Flower	*Helianthus annuus*L.	Asteraceae	0	80
Beans	*Phaseolus vulgaris* L	Fabaceae	0	0
Peanut	*Arachis 425oloniza* L.	Fabaceae	0	0
Strawberry	*Fragaria ananassa* Duchesne	Rosaceae	70	80

3- Viability and Survival of *C. acutatum* and *M. phaseolina* in artificially inoculated strawberry plants buried at different depths in soil.

In this study, inocula of *C. acutatum* and *M. phaseolina* were evaluated under field conditions. The experiment was performed during the off-season of strawberry cultivation and to evaluate their implication in the survival and transmission of this crown and root rot pathogen of strawberry. Also, to determine whether the 425olonizat could survive and thus serve as a potential infection source. Data presented in Table (3) showed the recovery percentages of viable 425olonizat buried at different depths in field soil at the depth of 5, 10 and 15 cm. It was lasted for 180 days (from the end of season to the start of next strawberry cultivation season in 2016). Samples were examined every 20 days interval (20, 40, 60, 80, 100, 120, 140, 160 and 180 days) to determine the recovery (%) of viable 425olonizat.

Results showed that 100 % of both *C. acutatum* and *M. phaseolina* inocula were recovered after periods of 20, 40 and 60 days except *M. phaseolina* buried in 15 cm depthwas 80% after 60 days. Both *C. acutatum* and *M. Phaseolina* samples buried in depth of 5 cm in field soil keep its viability till 120 days then started to decrease to 60

and 80% after 180 days, respectively. After 80 days at depths of 0, 10 and 15 cm both pathogens had 80% of viable inocula.

After 100 days at depths of 0, 10 and 15 cm *C. acutatum* showed 80, 70 and 70 % of viable inocula while *M. phaseolina* were 70, 80 and 80%, respectively. After 120 days at depths of 5, 0, 10 and 15 cm *C. acutatum* showed 80, 60 and 50 % of viable inocula while *M. phaseolina* were 70, 70 and 60%, respectively. After 140 days at depths of 0, 5, 10 and15 cm *C. acutatum* showed 70, 80, 50 and 50 % of viable inocula while *M. phaseolina* were 60, 80, 70 and 60%, respectively. After 160 days at depths of 0, 5, 10 and15 cm *C. acutatum* showed 50, 70, 50 and 40 % of viable inocula while *M. phaseolina* were 60, 80, 60 and 60%, respectively. After 180 days at depths of 0, 5, 10 and15 cm *C. acutatum* showed 50, 60, 40 and 40 % of viable inocula while *M. phaseolina* were 60, 80, 60 and 50%, respectively.

Table 3. Survival of *C. acutatum* and *M. phaseolina* in artificially strawberry plants buried at different depths in soil.

Inoculum burial period (Day)	*C. acutatum*				*M. phaseolina*			
	Inoculum burial depths (cm)							
	0	5	10	15	0	5	10	15
	(%) Recovery of viable inocula							
20-Days	100a*	100a	100a	100a	100a	100a	100a	100a
40-Days	100a	100a	100a	100a	100a	100a	100a	100a
60-Days	100a	100a	100a	100a	100a	100a	100a	80b
80-Days	80b	100a	80b	80b	80b	100a	80b	80b
100-Days	80b	100a	70c	70c	70c	100a	80b	80b
120-Days	80b	100a	60d	50d	70c	100a	70c	60c
140-Days	70c	80b	50e	50d	60d	80b	70c	60c
160-Days	50d	70c	50e	40e	60d	80b	60d	60c
180-Days	50d	60d	40f	40e	60d	80b	60d	50d

*** Each value represents the mean of 5 replicates. Values within same column followed by the same letter are not significantly different according to Duncan's test (*P= 0.05*).**

DISCUSSION

Susceptibility of strawberry to *C. acutatum* and *M. phaseolina* was tested under greenhouse experiments using different methods. Results indicated that Festival was the most susceptible cv. followed by N-70 cv. and Gavuta. Camarosa cv. was the lowest susceptible to *C. acutatum* while the other cultivars (Sweet Charlie and Fortuna) were similar and showed moderate susceptibility to foliar inoculation with the pathogen.On the other hand; *M. phaseolina* caused also variable percentages of disease incidence and disease severity. Inoculation method of root dipping and soil infestation showed that all tested cultivars recorded severe DI% up to 100% and DS% ranged from 55 to 85%. Inoculation method of foliar spray was the less effective method compared with the other tested methods.

Our findings are also in conformity with the work of Ragab, *et al*., (2017) who reported 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. Avilés, *et al*., (2009) in Spain reported that the response of the cultivars :Camarosa, Candonga and Ventana (the most common strawberry cultivars planted in Spain) toward *M. phaseolina* was characterized by severe infection by artificial different inoculation methods under greenhouse conditions. MacKenzie, *et al*., (2006) showed that resistance of strawberry cultivars to crown rot caused by *C. gloeosporioides* from Florida was nonspecific, with significant differences among cultivars and isolates, but without significant cultivar × isolate interactions. Our studies suggest that the differential responses of cultivars observed in relation to the incidence and severity of disease reflect significant effects of relative cultivar resistance or susceptibility to these individual pathogens.

Survival of *C. acutatum* and *M. phaseolina* in artificially strawberry plants buried at different depths in field experiments soil showed that 100 % of both *C. acutatum* and *M. phaseolina* inocula were recovered after periods of 20, 40 and 60 days except *M. phaseolina* buried in 15 cm depth after 60 days was 80%. Both *C. acutatum* and *M. phaseolina* samples buried in depth of 5 cm in field soil keep its viability till 120 days then started to decrease to 60 and 80% after 180 days, respectively.The ability of the strawberry anthracnose pathogen to survive for extended periods as reported by Freeman *et al*., (2002). Therefore, potential infections in developing daughter plants may start from splash dispersal of 426olonizat (Madden *et al*., 1996 and Ntahimpera, *et al*., 1999). They found no significant differences in survival between the two *Colletotrichum* spp., except for *C. gloeosporioides* in MB-fumigated soil at the lower moisture content. This may improve fitness in terms of *C. gloeosporioides* survival in soil relative to *C. acutatum* was demonstrated in Florida, USA.

Therefore, the contribution of these inocula to disease outbreaks should be considered because the period between cultivations of the fruiting crop is approximately 4 months; termination in May to June and new plantings in September to October. The results of other researchers in Israel (Zveibil- Aida *et al.*, 2012) are in agreement with the obtained results of our study. They studied the viability of *M. phaseolina* sclerotia which declined in control plots by approximately 55 and 67% at 10- and 30-cm depths, respectively, relative to survival of sclerotia maintained in the laboratory at 25°C. Similarly, viability of inocula in stolons declined in control plots by 71 and 75% at 10- and

30-cm. depths, respectively, comparedto stolon'sstored in soil in the laboratory.

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وبائية وحيوية الفطرين *Colletotrichum acutatum, Macrophomina phaseolina* المسببان لإمراض عفن التاج واعفان الجذور فى الفراولة بمصر

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نسبه حدوت أعفان التاج فى الفراولة المتسببة عن الفطر *M. phaseolina* والفطر *C.acutatum* تم ملاحظتها فى مزارع إنتاج الفراولة الرئيسية بمراكز محافظة الإسماعيلية . العدوى الصناعية ومدى قابلية أصناف الفراولة المختلفة للاصابة بالفطرين المذكورين تحت الدراسة ثم أتباتها . وجد أن كلا الفطريين كانا ذا قدرة عالية على أحداث المرض بأصناف الفراولة المختبرة فى الحقل . صنف الفستيفال كان أكثر الأصناف قابلية للإصابة بفطر . *M phaseolina* يليه صنف N70وصنف سويت شارلي كما كان الصنف كماروزا متوسط القابليه للإصابة بينما صنف جافيوتا أقل قابلية للإصابة . فى حين كان الصنف كماروزا أقل الأصناف قابلية للإصابة بالفطر*C.acutatum* كذلك أظهرت الأصناف الأخرى سويت شارلى و فورتونا قلة قابليتها للإصابة . أوضحت النتائج أن الفطر *M. phaseolina* كان الأقوى مقارنة بفطر *C. acutatum* سواء فى نسبة أو شدة الاصابة . أوضحت نتائج اختبار المدى العوائلى مقدرة الفطر *M . phaseolina* مسبب مرض العفن الفحمي على إصابة القطن والسمسم وفول الصويا والبطيخ وعباد الشمس بنسب اصابة تتراوح من ٥٠-٨٠% على الجانب الاخر لم يستطع الفطر *C.acutatum* أظهار أعراض المرض على العوائل المختبرة تحت الدراسة . أوضحت نتائج البقاء الحي لكل من الفطرين *C. acutatum* و *M . phaseolina* داخل أنسجة الفراولة المصابة والتى ثم دفنها على أعماق ٠,٥ ,١٠ ,١٥سم تحت سطح التربة بالحقل أنهما ظلا محتفظين بحيويتهما حتى ١٢٠ يوم ثم بدأت حيوية الجراثيم تتناقص الى ٦٠, ٨٠ % بعد ١٨٠ يوم على التوالى لكلا الفطرين تحت الدراسة .