

INCIDENCE, DISTRIBUTION AND MAPPING OF SOIL AND SEED BORNE FUNGI ASSOCIATED WITH WHEAT IN EGYPT

(¹) **IMBABY IBRAHIM A.**, (²) **SHERIF M. AYOUB**, (¹)
FATEN K. EL NASHAR and (²) **AMR A. HAFEZ**

(¹) *Plant pathology Research Institute, ARC, Giza, Egypt*

(²) *Syngenta Agro Egypt*

Abstract

The importance of Wheat production in world Economy is proven by its share of 15% from 1500 million hectare arable land in world. This rate is equivalent to 225 million hectare wheat area based on FAO figures 2009. In Egypt Wheat is the most important cereal crop (planted area cultivated approximately 3.25 Fadden). Which used as staple food. Wheat is susceptible to several diseases which cause significant losses on the grain yield and quality of seeds. Survey was conducted in the main wheat growing areas: Gharbia, Qalubia, Menofia, and Kafr El Sheikh (Delta) and Beni-Sweif, Menia (Upper Egypt). Which indicated the presence of Soil borne diseases associated with wheat plant Roots. The Fusarium species group was the most common fungal diseases associated with wheat roots in all six governorates (62.29%) followed by Helminthosporium (29.30%), Epicoccum (5.94%) and Alternaria group (5.50%) respectively. This findings based on Nine Hundred soil and root samples collected from soils and plants in the first Mapping record for Soil-borne diseases associated with plant roots in Egypt. On the other hand Alternaria SP. Was the major occurring pathogen upon evaluation of seed borne diseases by infection ranging from (80.21%) Beni-Sweif, and (51.49%) Dakhalia, followed by Stemphylium SP. As the second major pathogen with infection ranging from (38.62%) Dakhalia to (7.37%) Beni-Sweif. Epicoccum SP. Was the third major pathogen with infection ranging from (9.91%) Beni-Sweif to (2.27%) Menia, Fusarium SP. was isolated from five Governorates, with infection ranging from (3.27%) Sharkia to (0.57%) Gharbia. It is obvious that there were differences between the pathogens associated with the Roots and those associated with Seeds. Soil. However it was not found in the seeds, possibly due to the lack of rain. Soil-borne Fusarium SP. is known to cause Root/ foot crown Rot by infecting the crop the roots, crown and or lower stem. The Seed Care solution revealed that Celest Top was superior on all treatments in the terms of Germination, Plant vigor, Tillering, Number of heads and yield. These results followed by Celest Extra, Dividend Extreme and finally by Premise.

Key Words: Wheat, Mapping, Fusarium SP. Alternaria SP. Celest Top

INTRODUCTION

Fungi are the most important common cause of plant disease (Persley 1993) since they are the most wide spread and destructive parasites of plants (Ingold and Hudson 1993).

Seed borne fungi are one of the most important biotic constraints in seed production worldwide.

Infection rate of seeds depending on some environmental conditions such as high relative humidity, suitable temperature and also high level of moisture content in seed is variable.

Yield losses caused by seed borne pathogens to wheat are reported between 15 to 90% of untreated seeds grown in fields (Wiese 1984)

A complex of seed borne fungi including genera of *Bipolaris*, *Fusarium*, *Alternaria*, *Drechlera*, *Stemphylium*, *curvularia*, *cladosporium*, *Rhizopus*, *Aspergillus* and *penicillium* has been convincingly reported as the most frequent seed-borne fungi of Wheat through the world (Nirenberg *et al.* 1994, Glazek 1997, Hasmi and Ghaffar 2006 and Rehman *et al.* 2011).

Fusarium species play an important role as plant pathogens causing a wide range of diseases such as vascular wilt and stem rot in a diversity of hosts (Schollenberger *et al.* 2005).

Fusarium Solani is a soil saprophyte and soil borne plant pathogen which is responsible for variety of seedling diseases including seed rots, pre and post emergence damping off (Allen *et al.* 2004)

Alternaria Solani is a necrotrophic fungus responsible for causing early blight in tomato and potato whenever these crops are grown (Neergard 1945). On the other hand seed health testing to detect seed borne pathogens is an important step in the management of crop diseases.

Little information is known about the status of this group of pathogens in wheat growing regions in Egypt.

Controlling such diseases are mainly dependent on seed care treatments (Saad *et al.* 2014) as reported that Ridomil Gold MZ and Tridex showed maximum inhibition of *Alternaria Solani* mycelial growth at a concentration of 400 pp as compared to other tested fungicides.

Therefore the aim of the study is to survey the soil borne diseases that associated to wheat plant roots in the main growing areas and drawing map for fusarium distribution all over the particular governorates, and identify seed borne mycoflora of wheat.

This studies represent the most important knowledge information for the local growers of wheat to understand the seed fungal pathogen associated with wheat.

Materials and Methods

Survey of the Soil borne diseases associated to wheat plant Roots

The Survey were carried out from December to January 2014 where the wheat plants at tailoring in the main wheat growing governorates (Menia and Beni sweif Upper Egypt) and Sharkia, Dakhliya, Gharbia and Behaira (Lower Egypt). Sixteen Plant samples were collected from each grower. Three growers from each district and three districts from each governorate. The samples were transported in ice box to the lab for isolation and identification. Latitude, longitude and evaluation of the sampling filed were recorded.

Root of the plant samples were washed gently with tap water to remove the soil particles, sterilized with alcohol then washed in distilled water. Each root was cut three parts (basal stem part, nodal root part and seminal root part) and planted on petri dishes containing PDA media under sterilized condition.

The petri dishes were incubated at 20° C in cooling incubator for 10-15 days. The cultures grown were purified on glass tubes with PAD media and incubated at 20° in cooling incubator.

The obtained cultures were classified based on the color of the fungal mycelium.

Six groups i.e., *Fusarium oxysporum*, *F.semitectum*, *F.avenaceum*, *Epicocceum*, *Alternaria* and *Helminthosporium* were obtained and the percentage of each group was estimated for each governorate as well as all over the six governorates.

Survey of the Seed borne Fungus associated to wheat Seeds

The Survey was carried out for end of May to the end of July 2014 while the Wheat Plants were at ripening.

A total of 200 wheat seeds were collected from three wheat threshed spikes samples for each grower, three growers for each district and three districts for each governorate.

The samples were tested by the standard agar plate method.

The seeds were subjected to surface sterilized with 5% Sodium Hypochlorite (Naocl) for 4 min, washed two times in distilled sterilized water and dried with filter paper. Five seeds per plate were incubated at 23° C for 7 Days and in the 8th day, the seeds in petri dishes were examined and the primary identification of fungi grown on wheat seeds was performed on the basis of their typical colony characteristics.

The primary colonies of growing fungi were transferred on to PDA slants for identification the percentage of seed infection in each sample and the percentage of infection in each region were determined by the following formula:

$$\text{Mean rate of seed infection} = \frac{\text{number of seed on which a fungi species identified}}{\text{number of seed tested}} \times 100$$

$$\text{Mean of Region infection} = \frac{\text{frequency of samples on which a fungus identified}}{\text{number of sample collects}} \times 100$$

Seed care treatments

Two trials were conducted to evaluate the biological performance of certain seed care treatments, one in clay soil and the second in sandy soil.

Treatments

Dividend Extreme (Metalaxyl M + Difenconazole)

Celest Top (Fludioxonil + Difenconazole + Thiamethoxam)

Celest Extra (Fludioxonil + Difenconazole)

Premis (Triticonazole)

Table 1. Seed care treatments on wheat in term of controlling soil borne diseases (Fusarium& Pythium) clay soil

No	Treatment List	Formulation	ml prod./ 100 kg seeds
1	Dividend Extreme	23 g/l Metalaxyl-M, 92 g/l difenoconazole	95 ml./100 kg seeds
2	Celest Top	25 g/l fludioxonil, 25 g/l difenoconazole, 262.5g thiamethoxam	200 ml./100 kg seeds
3	Celest Extra	25 g/l fludioxonil, 25 g/l difenoconazole	200 ml./100 kg seeds
4	Premis 2.5% FS	Triticonazole	200 ml./100 kg seeds
5	Check untreated	_____	_____

RESULTS AND DISCUSSION

Wheat is represents the most important cereal crop in Egypt which is used as staple food. Furthermore the yield losses caused by seed borne pathogens are reported as

high percent. Meanwhile the little information known about *Fusarium* species causing a wide range of diseases to wheat in Egypt draw the attention to survey the soil borne diseases that associated to wheat plant roots in the main growing area and drawing map for *Fusarium* distribution and identify seed borne mycoflora of wheat.

Survey of the Soil borne diseases associated to wheat plant Roots

The result of survey was found that the most predominance pathogens associated with wheat roots was illustrated in table 2, figure 1, and map 1. It is obvious that *Fusarium* species group was the most Fungal diseases associated with wheat roots in all six governorates (62.29%) followed by *Helminthosporium* (29.30%), *Epicoccum* (5.94%) and *alternaria* group (5.50%) respectively. As a conclusion there were six fungal groups were isolated from wheat root plants in the six surveyed governorates namely: *Fusarium oxysporium*, *Fusarium semitectum*, *Fusarium avericeum*, *Epicocum* SP., *Helminthosporium* SP and *alternaria* SP.

Survey of seed borne fungus associated to wheat seeds

The results of the survey of the seed borne fungus associated to wheat seeds are presented in table 3, figure 2 and map 2. It is obvious that seven seed borne pathogens were detected from fifty from wheat samples were collected from six major wheat growing governorates. Seven seed-borne pathogens were detected from fifty-four wheat seed samples that were collected from six major wheat-growing governorates in Egypt during 2013/2014.

The *Alternaria* SP were the major occurring pathogen, with infection ranging from 51.49% (in Dakhliya) to 80.21% (in Beni-Swief). *Sremphylium* SP was the second major pathogen, except for Beni-Sweif- with infection ranging from 7.37% (Beni-swief) to 38.62% (Dakhliya). *Epicoccum* SP. was the third major pathogen with infection ranging from 2.27% in Menia to 9.91% in Beni-swief, while *Fusarium* SP was isolated from five governorates only (Beni-swief, Sharkia, Dakhliya, Gharbia, and Beheira) with infection ranging from 0.57%, in Gharbia, to 3.27% in Sharkia. In addition *Aspergillus niger* was isolated from almost districts of five governorates (Menia, Beni-swief, Dakhliya, Gharbia, and Beheira) with infection ranging from 0.45%, in BeniSwief to 5.87% in Dakhliya. *Penicillum* SP. was isolated from Menia governorate only with infection 0.90%, while *Helminthosporium* SP. was isolated from some districts in five governorates (Menia, Beni-swief, Sharkia, Dakhliya, and

Beheira) with infection ranging from 0.16%, in Beheria, to 2.40% in Sharkia governorate.

The Variation in number of pathogens and their incidence was found from one governorate to the other, as the highest infection rate % of the tested seeds was found in Menia (78.74%), followed by Gharbia (69.65%), Sharkia (64.23%), Beni-swief (63.35%), Sharkia (63.95%), then Beheira (61.56%).

Generally it was found that the most predominance pathogens associated with seeds was as follow:

- *Alternaria* SP. (51.4 to 80.2% of Isolates), *Stemphylium* SP. (7.3 to 38.62% of Isolates), while *Epicoccum* SP. (2.2 to 9.0% of Isolates)

On the other hand the data showed that there was a difference between the pathogens associated with roots at the first trial and the pathogens associated with seeds in the second trial.

The results emphasize the presence of *Fusarium* SP in the Soil, however it did not found in the seeds, this is due to the lack of rains which carries the spores of *Fusarium* pathogen from the soil to wheat head.

Table 2. percentage of the six fungal groups associated with wheat roots all over the six governorates.

No	Fungal group	% governorate						% all over the governorates
		1	2	3	4	5	6	
1	<i>Fusarium oxysporium</i>	9.5	25	41.1	29.6	28.9	24.6	26.45
2	<i>F. semitectum</i>	0.0	12.5	5.4	24.1	15.8	20.0	12.97
3	<i>F. avenicium</i>	23.8	15.0	24.1	16.7	14.5	43.1	22.87
4	<i>Epicoccum</i> SP.	0.0	18.75	4.5	1.9	10.5	0.0	5.94
5	<i>Helminthosporium sativum</i>	52.4	18.8	22.3	27.8	30.0	24.6	29.3
6	<i>Alternaria</i> SP	14.3	10.0	2.7	0.0	0.0	3.1	5.5

1- Menia, 2- Beni-swief, 3- Sharkia, 4- Dakhliya, 5- Gharbia, 6- Beheira

Map 1. distribution of the most three predominance fungal group in six governorates:

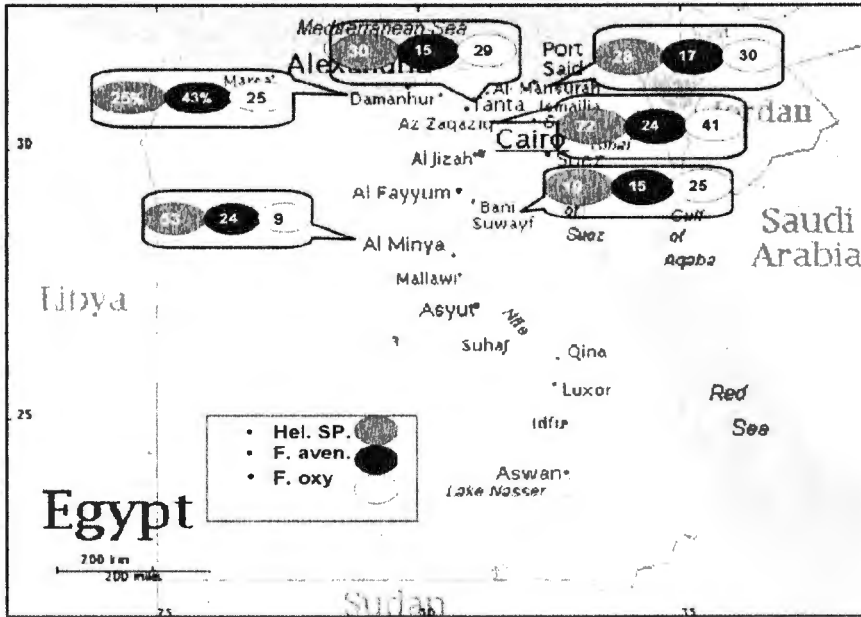


Figure 1. percentage % of the six fungal groups associated with wheat roots all over the six governorates

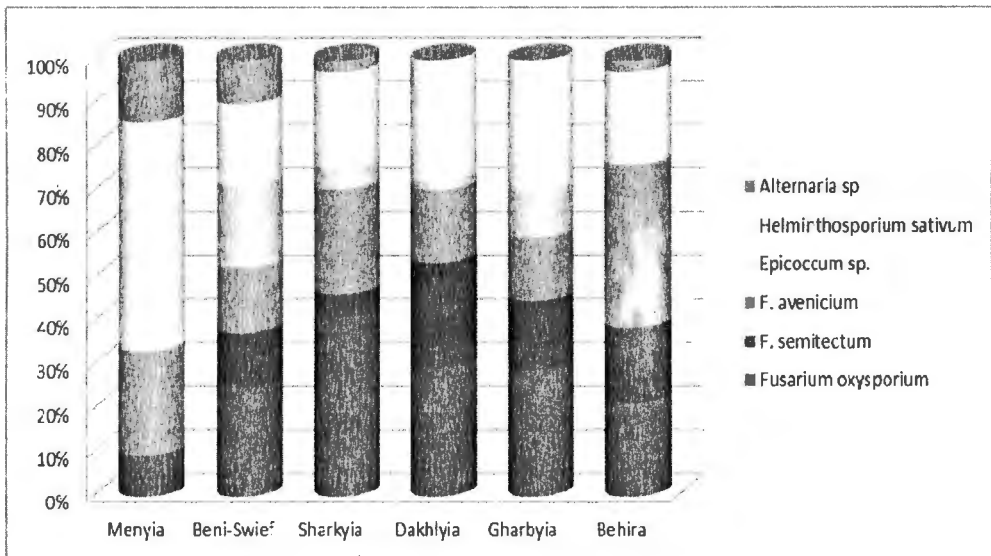


Table 3. incidence and frequency % of fungi associated with wheat seeds in six governorates.

Identified species	Mean rate of seed infection % / governorate						Total mean rate of infection %
	Menia	Beni-Swief	Sharkia	Dakhliia	Gharbia	Beheira	
Alternaria SP.	76.64	80.21	57.56	51.49	68.26	79.78	68.99
Stemphylium SP.	19.94	7.37	32.22	38.62	27.37	14.36	23.31
Epicoccum SP.	2.27	9.91	4.66	3.11	2.82	3.45	4.37
Asperagillus niger	0.06	0.45	—	5.87	0.97	0.47	1.30
Fusarium SP.	—	1.19	3.27	0.61	0.57	1.78	1.24
Helminthosporium SP.	0.19	0.87	2.40	0.30	—	0.16	0.65
Penicillium SP.	0.90	—	—	—	—	—	0.10
Total	14.29	14.29	14.30	14.29	14.28	14.30	14.29

Map 2. The Incidence and frequency% of fungi associated with wheat seeds in Six governorate.

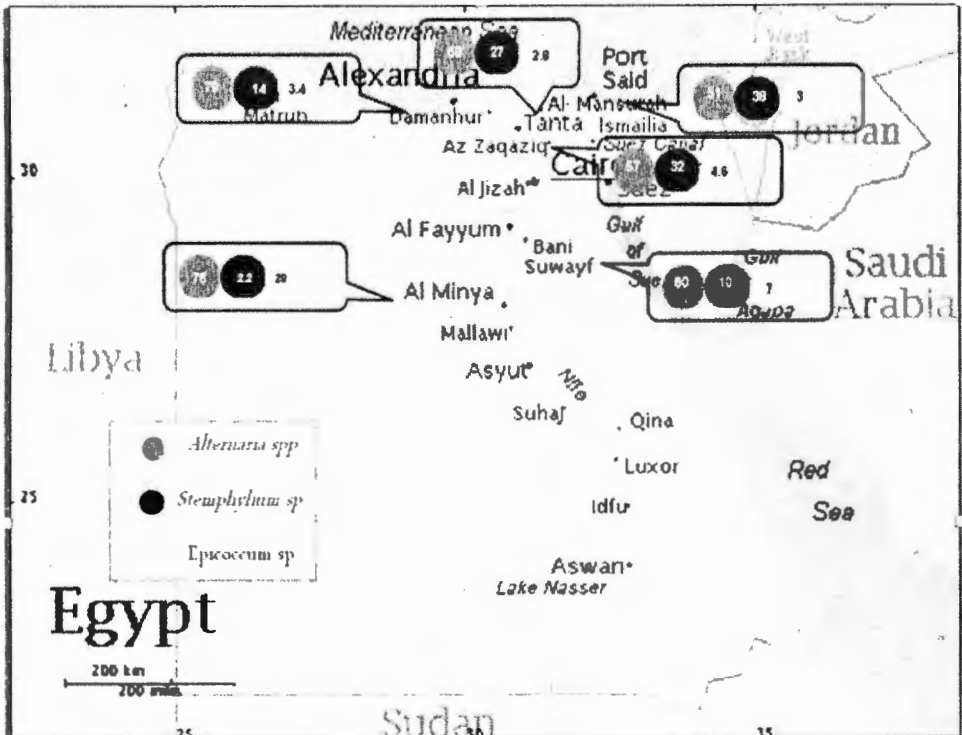
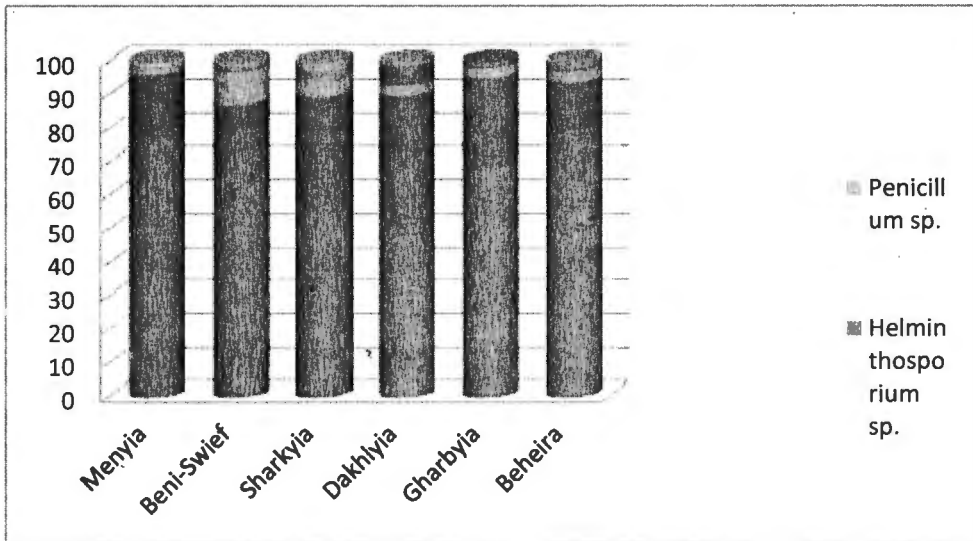


Figure 2. frequency% of fungi associated with wheat seeds in six governorates



Many strategies to control the fungal pathogen have been investigated in the field (Khan *et al.* 2007) currently, the most effective method for preventing soil-borne diseases is to apply chemical fungicides which could be harmful to other living organisms and reduce useful soil microorganisms (Khalifa *et al.* 1995 and Lewis *et al.* 1996)

Hawamdeh and Ahmed (2001) reported that fungicides had significantly reduced the colony growth of early blight pathogen comparing with control. Tridex and Ridomil Gold MZ were the most efficient fungicides, since they covered complete inhibition to the fungus lines growth of early blight pathogen.

Table (4) illustrated the effect of seed care on treatments (the crop density), plant height, tiller density, number of heads and grain yield.

Celest top was superior on the other seed care treatments regarding all the investigated criteria, followed by Celest extra while the premise 2.5% F.S was the least effective ones. On the other hand all treatments affect positively on the characteristic subjected to the study than the check untreated.

Celest Top (200 ml/ 100 kg seeds) was superior in all treatments in term of germination, plant vigor, No. of tillers, No. of heads and tiled. Furthermore the Celest Top was the best product for controlling soil borne diseases (fusarium and pythium) compared to all treatments.

Dividend extra (95 ml/ 100 kg seeds) and premise (200 ml/ 100 kg seeds) are nearly have the same performance in term of germination plant vigor, No. of tillers, No. of heads and the tiled.

On the other hand it could be concluded that Celest Top is the promising candidate to protect the wheat and produce more yield.

Table 4. Effect of CELEST TOP and other treatments on wheat seed characteristics

Treatments	Crop Denisty	Plant Height	Tiler Denisty	# of Heads	Grain Yield
	pl/m ²	cm/60 plt	# of tiller / 60 plt	# of Heads / 4 m ²	Arddab / Fed
Dividend Extreme	103	64	4.6	205	18.62
Celest Top	106	78	5.6	235	20.58
Celest Extra	104	66	5	210	18.87
Premis 2.5% FS	93	55	4	190	17.80
Check	74	40	3	170	15.50

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مدى تأثير وتوزيع خريطه إصابه التربه بالفطريات التي تصيب جذور وبذور نباتات القمح في مصر

إمبابي إبراهيم^١ - شريف أيوب^٢ - فاتن النشار^١ - عمرو علي^٢

١- معهد بحوث أمراض النباتات- مركز البحوث الزراعيه
٢- سينجنتا أجرو

تتأكد الأهميه الأقتصاديه لإنتاج محصول القمح حسب تقديرات منظمه الأغذيه والزراعه عام ٢٠٠٩ من أن النسبه المئويه للمساحه المنزرعه من القمح هي ١٥% من ١٥٠٠ مليون هكتار من المساحه الزراعيه العالميه وهذه النسبه تعادل ٢٢٥ مليون هكتار قمح في مصر يعتبر القمح من أهم محاصيل الحبوب فالمساحه المنزرعه من القمح (حوالي ٣.٢٥ مليون فدان) حيث يعتبر القمح المحصول الرئيسي للتغذيه، هذا ويصاب القمح بالعديد من الأمراض الفطريه التي تسبب فقد جوهر في المحصول وجودته، وقد بينت دراسه المسح الميداني في مناطق زراعه القمح في محافظات الغربيه- القليوبيه والمنوفيه- كفر الشيخ (الدلتا) وبني سويف واهنيا (مصر العليا)

هذا وقد بينت الدراسه التي أجريت في هذه المحافظات أن الأمراض الفطريه التي تصيب جذور نباتات القمح والموجوده في التربه هي:

مجموعه فطريات الفيوزاريوم وهي المجموعه المنتشره في المحافظات السنه التي أجرى بها عمليه المسح ونسبه هذه الفطريات (٦٢.٢٩%) وتليها مجموعه أمراض هلمينسبوريم (٢٩.٣٠%) ثم مجموعه أبيكوكم (٥.٩٤%) ثم مجموعه الألترناريا (٥.٥٠%) على التوالي. وهذه النتائج أمكن الحصول عليها من دراسه وفحص ٩٠٠ عينه من الأراضي والجذور التي جمعت من أماكن الدراسه، هذا وقد وضعت خريطه توضح الإصابه بأمراض القمح المرتبطه بالتربه من خلال الجذور لأول مره في مصر.

على الجانب الأخر بينت الدراسه لأمراض بذور القمح أن مجموعه الألترناريا هو المرض الفطري الأكثر إنتشارا فقد تصل الإصابه (٨٠.٢١%) في محافظه بني سويف بينما كانت الإصابه (٥١.٤٩%) في محافظه الدقهليه تليها مجموعه *Stemphylium* وهو ثاني مرض يؤثر على بذور القمح بنسبه ٣٨.٦٢% في محافظه الدقهليه إلى ٧.٣٧% في محافظه بني سويف.

ويلي ذلك *Epicoccum* في المرتبه الثالثه بنسبه (٩.٩١%) في محافظه بني سويف بينما كانت نسبه الإصابه (٢.٢٧%) في محافظه المنيا.

وقد عزلت مجموعه *Fusarium* من الخمس محافظات بنسبه إصابه ٣.٢٧% في الشقيه إلى ٠.٥٧% في محافظه الغربيه.

هذا ويلاحظ أن الإصابه بأمراض التربه التي تصيب الجذور تختلف عن نسبه الأمراض التي تصيب البذور والنتائج تؤكد أن وجود أمراض الفيوزاريوم في التربه على الجذور أعلى من أمراض الفيوزاريوم في البذور وهذا يحتمل إلى قله الأمطار.

وأنه من المعلوم أن أصابه التربه بأمراض الفيوزاريوم تسبب أمراض كثيره للقمح مثل أعفان الجذور- الأعفان التاجيه- وأصابه سيقان نباتات القمح.

ومن خلال دراسه حل مشاكل إصابه القمح بالأمراض الفطريه وذلك بالعنايه بالبذور أثبت مركب سليست توب أنه يتفوق على كل العاملات التي أختبرت من حيث التأثير على نسبه الإنبات- والنشاط - والخلفه- وأعداد رؤوس السنابل- والمحصول وتلى ذلك مركبات السليست إكسترا- وديفيداند إكستريم وأخيرا بريمس.