PATHOGENS ASSIOCIATED WITH ROOT-ROT DISEASE OF WHEAT IN EGYPTIAN DRY LAND

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Abstract

In this study, a survey of root rot disease of wheat was carried out along the North West Coast (NWC) of Egypt under rainfed conditions. The survey revealed that high percentage of disease incidence was recorded at Abo-Lahow followed by Abo-Atiah and Abo-Lowh. The lowest percentage of disease incidence was recorded at El-Hafian.

The main pathogens causing wheat root-rot in NWC were *Helminthosporium sativum, Fusarium graminearum, F.moniliforme and Rhizoctonia solani. H.sativum* was the most frequently isolated fungus, while the least frequent one was *F.moniliforme.* Pathogenicity tests proved that the most virulent fungus was *F.graminearun* followed by *H.sativum* and *R.solani,* while the least virulent one was F.moniliforme. Synergistic effect was noticed when the most virulent fungi were mixed and percentage of disease incidence was increased.

Regarding the effect of soil textures on root-rot disease incidence, data obtained proved that the percentages of pre-post emergence damping-off and disease rate were decreased by increasing the percentage of clay in soil to 75%, while , percentage of survival plants and wheat yield components were increased.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major and most important cereal crop in Egypt and many other countries in the world, because it is the main source of food for human and as feed for livestock.

Common root-rot is a major disease in dry land wheat. It is widely distributed throughout the cereal-growing areas of the world. Root-rot of wheat is considered a serious problem in some regions and causes high reduction in yield. Several soil-borne pathogens of wheat cause common root-rot, with necrosis of basal stems, crowns, subcrown internodes and roots. Several pathogenic fungi, *i.e. Fusarium graminearum, F.culmorum* and *Helminthosporium sativum* are involved in this disease. Common root-rot fungi fungi are aggressive pathogens in plants under stress such as drought, warm temperature and nutritional stress. Moisture or at least high relative humidity is required for root infection, but thereafter disease development is highly dependent on

warm temperatures and moistu e stress, hence, the name "dry land root-rot" (Wiese, 1977).

Soil texture has great influence on development of root-rot disease (Zimmermann 1983) stated that sandy loam soil produced a higher degree of wheat root-rot However, incidence caused by *Gaeumannomyces graminis* var *tritici* than in loam soil. However, Mazen et al. (1991) found that occurrence of *Fusarium* spp. in Egyptain soils is influenced by soil type and locality.

In the present study, a survey of diseased plants in four locations at the Egyptian North Western Coast (NWC) was carried out to evaluate the percentage of disease incidence and to identify the causal organisms of root-rot disease of wheat. The effect of soil textures on the behaviour of the pathogenic fungi was also studied.

MATERIALS AND METHODS

I- Survey of root-rot disease of wheat:

Survey of root-rot disease of wheat was carried out during the growing season 1994-1995 in four locations, i.e. Abo-Atiah, Abo-Lahow, Abo-Lowh and El-Hafian located in the dry region of Egyptian North West Coast (NWC) which depend on rainfall.

The survey was done during second half of Febraury, at booting stage, using the method described by Horricks (1980). Percentage of diseased plants was calculated on the basis of percentage of disease incidence = (no of diseased plants/total plants) x 100. The plant samples were collected in paper bags, and transferred to the laboratory to isolate the causal pathogens.

II- Isolation and identification of the causal organisms:

Roots of plants showing disease symptoms were collected from different locations. Collected roots were washed carefully with tap water. Small portions of diseased pre washed roots were surface sterilized in 1% sodium hypochlorite for one minute followed by several rinses in sterile water. They were then carefully blotted between sterile filter paper before being transferred onto peptone-dextrose agar + rose bengal and streptomycin (Johnson *et al.,* 1960) plates and incubated at 25±1^{o}C. Pure cultures were obtained by hyphal tip (Warcup, 1955) or by single spore technique (Booth, 1971).

Identification of the purified causal organsims was carried out according to the

morphological and physiological characteristics described by Barnett and Hunter (1986). Indentification was confirmed through the Mycology Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

III-Pathogenicity test:

Two tests of pathogenicity were carried out at laboratory and outdoor pots. A susceptible variety of wheat, cultivar Sakha 69, was used in pathogenicity.

a. Laboratory test:

Three ml of sterilized Hogland agar medium (Hogland and Arnon, 1950) were poured to each glass tubes (170 ml in capacity). Three surface sterilized wheat grains were placed in each tube and incubated in a growth chamber at 20°C, until the appearance of the first leaf. Discs (4mm) of each tested fungus were placed in each tube near the wheat seedling. Three replicates were used for each treatment. Uninoculated tubes were used as control. Tubes were reincubated in growth chamber (14 hours photoperiod, 20°C) for three weeks. The tested fungus considered to be pathogenic if the whole stem base was lesioned.

b. Preparation of inocula:

Fungal inocula used for soil infestation were prepared by growing each fungal isolate on autoclaved sand/barley grains medium (1:3, w/w). The inoculated medium was incubated at 25±1°C for 21 days.

c. Outdoor pot experiment and inoculation technique:

Sterilized porous pots (20 cm in diam.) were filled with 3 Kg sandy soil transferred from the NWC fields. Soil was infested with fungal inoculum either in single or in combination, at the rate of 5% of soil weight (Soliman *et al.*, 1993). Sterilized, uninoculated sand/barley medium was added to the soil at the same rate and used as a control treatment. After one week, fifteen surface sterilized wheat grains were sown in each pot. Three replicates were used in each treatment. Plants were watered in treated plots and fertilized to maintain excellent development and growth for plants in treated plots.

d. Disease assessment:

Disease assessment was carried out at seedling and mature stages.

1-At seedling stage:

After 15 and 45 days from sowing, the percentages of pre-post-emergence damping - off and healthy survivals were calculated using the formula devised by Abd El-Moity (1985).

2-At mature stage:

To determine the severity of root-rot disease at mature stage, the perecentage of disease rating (DR) was calculated by using Mckinneys formula (Mckinney, 1923) slighlty modified by Tinline and Hunter, (1982).

e. Yield assessement:

At mature stage, two parameters were determined, *i.e.* tillers number per plant (t/p) and weight of 1000 grains (1000 KW).

IV- Effect of soil textures on pathogenic fungi:

Five different soil textures (Table 1) were used. According to pathogenicity test, only the three most aggressive pathogenic fungi were used. Preparation of inocula, inoculation, sowing, disease assessment and yield assessment were made as mentioned before.

Data obtained were analyzed using computers of special unit of CLDSA of Agricultural Research Center (ARC) according to Gomez and Gomez (1984).

Tabe 1. Prcentage of sand and clay in each type of the prpared soils.

Treatments	1	2	3	4	5
sand %	100	75	50	25	0
clay %	0	25	50	75	100

RESU_TS AND DISCUSSION

1. Disease survey at different locations along the North West Coast (NWC) of Egypt:

The percentage of disease incidence of wheat root-rot was determined by extensive survey in four locations, *i.e.* Abo-Atiah, Abo-Lahow, Abo-Lowh and El-Hafian along the NWC under rainfed conditions. The survey (Table 2) revealed that high level of dis-

ease incidence was recorded at Abo-Lahow (82%) followed by Abo-Atiah (78%) and Abo-Lowh (58%), while the lowest level of disease incidence was recorded at El-Hafian (42%).

II. Isolation and identification of the causal organisms:

The causal organism (s) of common root-rot were isolated and identified. The main pathogens causing wheat root-rot in NWC (Table 2) were *Helminthosporium sativum, Fusarium graminearum, F.moniliforme* and *Rhizoctonia solani. H.sativum* was the most frequently isolated fungus, while the lowest frequent one was F.moniliforme. These results are in agreement with those obtained by Hill et al. (1983), El-Meleigi and Al-Rukibah (1996), Bakr (1997) and Ibrahim (1997) who found one or more of these fungi associated with wheat root-rot disease.

Table 2. Prcentage of wheat root-rot disease incidence and frequency of associated pathogens in different locations along the North West Coast (NWC)

Location	disease incidence %	% of frequency of isolated pathogens				
		Fusarium gramin earum I_1	F. gramin earum I_2	F. monilifor me	*Helminthos -porium sativum*	*Rhizoc to- nia solani*
Abo-Atiah	78.0*	25.00**	0.00	0.00	75.00	0.00
Abo-Lahow	82.0	33.33	0.00	8.33	58.34	0.00
Abo-Lohw	58.0	0.00	16.67	16.67	41.66	25.00
EL-Hafian	42.0	0.00	8.33	0.00	33.33	58.34

* Percentage of disease incidence = (no. of diseased plants/total plants X 100)
** Each value represents the mean of 12 replicates.

III- Pathogenity test:

Different purified isolated fungi were tested to ensure their pathogenicity. Pathogenicity tests were carried out under laboratory or outdoor pot conditions.

a. Laboratory test:

This test was carried out in the laboratory using agar Hogland medium infested with the tested fungus. Photograph (1) illustrates that all tested fungi could infect wheat seedling causing brown lesions on the stem basis at different degrees. The most

virulant fungi were *F.graminearum* and *H.sativum*, while *R.solani* and *F.moniliforme* showed the least effect comparing with the control treatment.

b. Outdoor pot test:

In this test the pathogenic capabilities and potentialities of the isolated fungi caused root-rot disease to susceptible wheat cultivar, Sakha 69, were determined in both seedling and adult stage.

1. In seedling stage:

Data in Table (3) indicate that *F.graminearum, H. sativum* and *R.solani* caused severe pre-and post emergence damping-off (ranged from 29.24-48.59% for pre-emergence and 10.33-22.50% for post emergence damping-off), whereas *F.moniliforme* caused the lowest percentage of pre-and post-emergence damping-off (15.7%, 0.0%). According to these data, only *F.graminearum, H.sativum* and *R.solani* were considered the main pathogens and were used for further studies.

Tabe 3. Effect of fungal species isolated from naturally diseased wheat plants on disease incidence in seedling stage under outdoor conditions,

Fungal species	Disease incidence		Survival Plants (%)
	Pre-emergence damping - off	post emergence damping - off	
Fusarium graminearum I_1	48.59	17.94	35.63
F. graminearum I_2	54.73	15.70	40.04
F. moniliforme	15.70	0.00	74.29
Helminthosporium sativum	43.00	22.50	38.48
Rhizoctonia solani	29.24	10.33	57.83
F.gr.I_1 + *F.gr.* I_2	49.69	17.94	34.23
F.gr.I_1 + *F. moniliforme*	35.69	15.70	49.96
F.gr.I_1 + *H. sativum*	54.30	24.29	24.31
F.gr.I_1 + *R.solani*	44.26	19.72	38.66
F.gr.I_2 + *F.moniliforme*	34.23	15.70	51.36
F.gr.I_2 + *H. sativum*	45.63	22.50	35.78
F.gr.I_2 + *R.solani*	38.72	5.17	49.87
F. moniliforme + *H. sativum*	27.95	18.03	55.68
F. moniliforme + *R.solani*	24.38	10.33	62.17
H. sativum + *R.solani*	43.00	19.72	40.00
Combination of all fungi	52.75	26.08	24.37
Control	0.00	0.00	100.00
L. S . D .	4.58	8.31	6.60

2. In adult stage:

Data in Table (4) show that the highest percentage of disease rate (63.43 and 65.91%) was exhibited in case of *F.graminearum* mixed with *H.sativum,* and in case of mixing all tested fungi with each other.

Results presented in Tab e (4) also show that the number of tillers per plant and 1000 kw were significantly influenced by the disease rate. Data proved a negative correlation between disease rate and yield components.

Tabe 4. Effect of fungal species isolated from naturally diseased wheat plants plants on percentage of disease rating and wheat yield components in adult stage under outdoor conditions, impots.

Fungal species	Percentage of disease rating (%)	Yield components	
		Number of Tillers/plant	1000-Kernel weight (g)
Fusarium graminearum I_1	62.85	1.00	14.56
F. graminearum I_2	60.01	1.27	16.87
F. moniliforme	32.68	2.59	34.97
Helminthosporium sativum	53.52	1.00	14.48
Rhizoctonia solani	60.00	1.401.00	22.22
F.gr.I_1 + *F.gr.* I_2	54.00	1.31	13.94
F.gr.I_1 + *F. moniliforme*	63.43	1.00	31.07
F.gr.I_1 + *H. sativum*	46.91	1.23	11.54
F.gr.I_1 + *R.solani*	44.04	1.50	12.72
F.gr.I_2 + *F.moniliforme*	60.00	1.17	25.90
F.gr.I_2 + *H. sativum*	45.95	1.66	15.30
F.gr.I_2 + *R.solani*	29.44	2.39	27.77
F. moniliforme + *H. sativum*	26.20	2.56	29.37
F. moniliforme + *R.solani*	51.25	1.17	32.48
H. sativum + *R.solani*	65.91	1.00	20.11
Combination of all fungi	0.00	3.30	7.85
Control	1.41	0.25	36.51
L. S . D .			1.62

Pathogenicity tests prove that tested fungal isolates varied in their pathogenic potentialities. *F.graminearum* was the most virulent one followed by *H.sativium* and *R. solani,* while *F. moniliforme* was the virulent. On the other hand, synergistic effect was noticed when pathogenic fungi (*F.graminearum, H.sativum and R.solani*) were mixed and percentage of disease incidence was increased. This result can be explained in the light of fact that each pathogenic fungus plays different role. *R.solani* produces pectolytic enzymes, *H.sativum* works on the cortex producting host specific toxin, named Helminthosporal, which cause root-rot, stem base rot and leaf spot disease in cereal. Also, *F.graminearum* produces lethal toxic substances such as fusaric acid (Mayo *et al.,* 1961; Ellen and Daly, 1980 and Barna *et al.,* 1983). Generally, combination between these pathogens exhibited a synergistic effect and increased virulence of each other (Khan, 1966 and Hilal, 1985).

IV- Effect of soil texure on pathogenicity of the pathogenic fungi:

1. In Seedling stage:

Data in Table (5) show that the highest percentage of pre and post-emergence damping-off (ranging from 32.6 to 51.0% with an average mean of 44.49% for pre-emergence and 11.0 - 21.0% with an average mean of 18.6% for post emergence damping-off) was found in soil texture of 100% sand content. The lowest percentage of pre and post-emergence damping off was obtained in soil containing 25% sand. Data in Table (5) also show that the greatest percentage of healthy survival plants was found in soil with 75% clay contents (average 55.7%), whereas the least (average 39.0%) was obtained in soil texture of 100% sand content.

2. In adult stage:

Data in Table (6) show that the highest DR % was recorded in soil texture of 100% sand content (average 57.08%), whereas the lowest one (average 38.88%) was noticed in soil containing 75% clay. Greatest number of (tillers/plant) and 1000 KW (average 2.0 t/p and 30.0g, respectively) were found in soil of 75% clay content, while the least one (average 1.0t/p and 17.0g, respectively) was found in soil containing 100% sand.

Results presented in Tables (5 and 6) also prove that *F.graminearum* was more effective than other pathogenic fungi regarding percentage of pre-and post-emergence damping-off and disease rate, whereas *R.solani* was the least effective one.

Tabe 5. Effect of soil texture on disease incidence in seedling stage of Sakha 69 wheat plants.

Fungal species	Soil texture																	
	100% Sand			75% Sand			50% Sand			25% Sand			100% Clay			Mean		
	Pre.	Post.	Surv.	Pre.	Post.	Surv.	Pre.	Post.	Surv.	Pre.	Post.	Surv.	Pre.	Post.	Surv.	Pre.	Post.	Surv.
Fusarium graminearum	46.5	21.0	35.9	45.7	20.0	37.0	41.5	17.9	41.5	34.0	7.0	51.0	39.7	15.0	46.0	41.5	16.0	42.0
Helminthosporium sativum	41.9	16.0	43.5	41.5	15.7	44.0	35.6	15.7	50.0	26.0	5.0	62.8	32.0	10.0	54.8	35.5	12.7	50.9
Rhizoctonia solani	32.6	11.0	54.0	32.8	10.0	54.0	26.0	5.0	62.0	17.9	0.0	72.0	27.6	5.0	60.0	27.0	6.0	60.7
F.gr.1 + H. sativum	49.4	21.0	32.5	48.5	22.5	32.8	43.5	20.0	39.0	37.0	15.7	48.5	42.0	20.0	40.8	44.0	19.9	38.8
F.gr.1 + R. solani	46.5	21.0	35.9	45.7	20.0	37.0	41.5	17.9	42.9	34.0	7.0	53.0	41.0	12.9	44.7	41.8	15.9	42.8
H. sativum + R. solani	43.5	18.7	40.0	43.0	18.0	41.0	37.0	15.7	48.5	41.0	5.0	57.0	36.8	10.0	50.0	38.0	13.6	47.6
Combination of fungi	51.0	21.0	30.9	48.5	22.5	32.8	44.0	20.0	38.6	40.0	15.7	45.7	42.0	20.0	40.8	45.0	19.9	37.8
Mean	44.4	18.6	39.0	43.7	18.5	39.9	38.6	16.0	46.0	31.5	8.0	55.7	37.0	13.5	48.0	--	--	--
Controls	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0

L.S.D. at 0.05 for	Pre.	Post.	Survival
Soil texture.	1.62	3.83	0.50
Pathgen	1.92	4.54	0.66
Interaction	NS	NS	1.47

Data were transformed to arcsine values

Tabe 6. Effect of soil texture on percentage of disease rating and wheat yield components in adult stage .

Fungal species	Soil texture																	
	100% Sand			75% Sand			50% Sand			25% Sand			100% clay			Mean		
	DR%	t/p.	1000 kw(g)	DR%	t/p.	1000k w(g)	DR%	t/p.	1000k w(g)	DR%	t/p.	1000k w(g)	DR %	t/p.	1000 kw(g)	DR%	t/p	1000 kw(g)
Fusarium graminearum	58.38	1.0	14.6	57.34	1.0	19.0	53.2	1.5	23.0	39.22	2.0	30.0	46.4	1.5	25.0	50.92	1.0	22.6
Helminthosporium sativum	53.73	1.0	18.5	54.73	1.0	23.0	4	1.5	25.0	34.71	2.0	31.6	3	1.0	28.7	46.45	1.5	25.5
Rhizoctonia solani	44.52	1.0	22.0	45.47	1.0	26.0	47.3	1.8	27.0	28.88	2.9	33.0	41.6	1.7	31.5	39.16	1.8	28.0
F.gr.1 + H. sativum	63.46	1.0	11.5	62.85	1.0	12.7	9	1.0	20.9	45.95	1.7	225.	5	1.0	25.7	56.45	1.0	19.0
F.gr.1 + R. solani	58.91	1.0	12.7	57.34	1.0	18.0	41.1	1.6	26.5	39.71	2.0	0	35.7	1.6	27.5	49.96	1.6	32.0
H. sativum + R. solani	55.25	1.0	17.8	55.75	1.0	24.7	6	1.0	23.0	36.27	2.6	30.0	6	1.5	25.0	48.13	1.5	23.7
Combination of fungi	65.30	1.0	7.9	63.43	1.0	14.5	56.7	1.0	19.0	47.39	1.6	27.7	53.2	1.0	18.8	57.64	1.0	16.6
Mean	57.08	1.0	17.0	56.70	1.0	21.8	8	1.7	25.0	38.88	2.0	22.5	3	1.7	27.7	-	-	-
Controls	0.00	2.0	30.0	0.00	2.0	35.8	48.8	2.9	37.0	0.00	3.0	30.0	45.0	3.0	38.8	0.0	2.9	36.6

L.S.D. at 0.05 for	DR%	t/p	1000kW
Soil texture.	0.854	0.11	0.5
Pathgen	1.011	0.14	0.7
Interaction	3.120	0.30	1.5

Data were transformed to aresine transformation

Regarding the effect of soil texture on root-rot disease incidence, data obtained proved that the percentage of pre, post-emergence damping off and disease rate were decreased by increasing the percentage of clay in soil, up to 75%, where percentage of survival plants and wheat yield components were increased. This finding might be due to the fact that increased organic matter and clay in soil led to increase the number of saprophytes which antagonise different pathogens and reduce thier effect (Papavizas, 1963). On the other hand, fungal multiplication was rapid in sandy soil than in clay one because of better aeration of sandy soil favoured the growth of fungi and this was correlated with the increase of infection. These results are in agreement with those reported by Zimmermann (1983), Mazen et al. (1991) and Bakr (1997), who found that highest infection to roots of wheat plants caused by *Drechslera sarokiniana, Fusarium graminearum* (*F.roseum*) and *F.moniliforme* occurred in plants grown in sandy soil with low organic matter, while the lowest infection existed in clay soil.

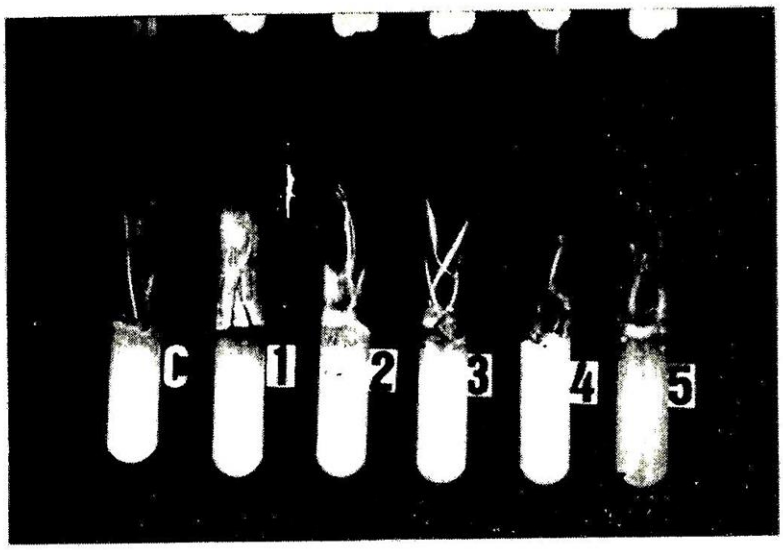

Photo (1): Pathogenicity of different soil-borne pathogens on wheat seedlings

C = Control.
1 = *Fusarium graminearum* .
2 = *Fusarium graminearum* 2.
3 = *Helminthosporium sativum.*
4 = *Rhizoctonia solani.*
5 = *Fusarium moniliforme.*

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المسببات المرضية المصاحبة لمرض عفن الجذور في القمح في الأراضي المصرية الجافة

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أجري هذا البحث بغرض دراسة الكائنات المسببة لمرض عفن الجذور في القمح والتي تهاجم كلا من البادرات والنباتات البالغة مسببة فقدا في إنتاجية محصول القمح المنزرع في الأراضي الجافة وتحت ظروف المطر علي امتداد الساحل الشمالي الغربي لمصر.

أجري حصر للمرض في أربع مناطق علي امتداد الساحل الشمالي الغربي وقد ثبت وجود المرض في جميع المناطق المختبرة وهي أبو لهو – أبو عطية – أبو لوح والحفيان ولقد سجلت أعلي نسبة حدوث بالمرض في منطقة أبو لهو يليها مناطق أبو عطية وأبو لوح بينما سجلت النسبة الأقل في منطقة الحفيان.

أظهرت النتائج أن أهم المسببات المرضية لمرض عفن الجذور في القمح في الإراضي الجافة هي الفطريات هلمنثوسبوريم ساتيفم وفيوزاريوم جراميئيرم ورايزوكتونيا سولاني و فيوزاريوم مونيليفورم. ولقد كان الفطر هلمنثوسبوريم ساتيفم اكثر الفطريات المعزولة تواجدا في كل المناطق المختبرة يلية رايزوكتونيا سولاني ثم الفطر فيوزاريوم جراميئيرم بينما كان الفطر فيوزاريوم مونيليفورم أقل الفطريات تواجدا.

ثبت من اختبار القدرة المرضية للفطريات العزوله أن اكثر الفطريات قدرة علي إحداث مرض عفن الجذور هو الفطر فيوزاريوم جراميئيرم يليه الفطر هلمينتوسبوريوم ساتيفم ورايزوكتونيا سولان بينما كان الفطر فيوزاريوم مونيليفورم أقل الفطريات تطفلا. كما أثبتت النتائج زيادة نسبه حدوث المرض وشدة الاصابة زيادة معنوية عند خلط اكثر الفطريات قدره علي أحداث المرض.

بالنسبة لتأثير نوع التربة علي شدة الإصابة بمرض عفن الجذور في القمح لوحظ نقص في شده الإصابة بالمرض بزيادة كمية الطمي المضاف للتربة الرملية حتي ٧٥٪ مع وجود زيادة في النسبة المئوية للنباتات السليمة وكذلك زيادة في مكونات المحصول.