ENGLISH SUMMARY

SUMMARY

Pink root rot disease of onion caused by *Pyrenochaeta terrestris* (Hansen); Gorenz; Walker and Larson is considered an important disease which seriously attacks onion plant roots in both nurseries and permanent plantations, causing serious damage for roots; great losses of bulbs weight and in turns of seed production.

The results of this research could be summarized as follows:

1. Survey study indicated that the disease was widly prevalent in sandy soils of Ismailia and Fayoum governorates in the seasons 1986 and 1987 as the average percent of infection ranged from 17.66-26.28% whereas the infection was limited in the heavy clay soils of Upper Egypt governorates (Giza; Beni-Suef and Assiut) as the infection ranged from 3.47-8.94% only.

2. There was no obvious correlation between the onion pink root rot incidence and the meterological elements i.e. the average of maximum; minimum and mean of temperature degrees; relative humidity and rain fall occurred during the surveyed times.

3. Two distinguished groups of fungi were obtained from onion transplants showing typical pink root rot symptoms from different fifteen localities belonging to the five mentioned governorates. The first group included 15 isolates were identified as *Fusarium oxysporum* Schl. f. s. *cepae* (Hanz.); Snyder and Hansen, and the second group included seven isolates were identified as *Pyrenochaeta terrestris* (Hans.); Gorenz; Walker and Larson.

4.a) Frequency study indicated that *F. oxysporum* f. sp. *cepae* was isolated only (100% frequency) from all samples collected from all localities belonging to Giza and Beni-Suef governorates and one locality from each of El-Fayoum and Ismailia governorates.

b) Both *F. oxysporum* f. sp. *cepae* and *Pyrenochaeta terrestris* were isolated from one locality of Assiut governorate; three localities from each of El-Fayoum and Ismailia governorates, as the percent frequency of the former ranged from 73.1-90.5% whilest that of the later ranged from 9.4-24.1%.

c) The percentage of the colonies comprising the both pathogens-isolated simultaneously from the same root segments ranged from 1.1 to 7.1%.

5.a) Pathogenicity test of all *P. terrestris* isolates on the natural susceptible onion Cv. Giza-20 indicated that isolate No. 3 collected from Ismailia governorate was the most pathogenic isolate causing pink root rot infection to 61.94 % of the tested plants with percent severity 41.9% and 33.79 % according to infected and tested plants, respectively. Whereas the least pathogenic isolate No. 1, infected not more than 26.40% of tested plants with a severity 19.1% and 3.49% concerning to the infected and tested plants respectively.

b) All *F. oxysporum* f. sp. *cepae* isolates were pathogenic inducing typical symptoms of basal rot disease, imparting the roots with reddish colour in earlier stages and causing dry rot of the stem plates of the infected plants in the later ones.

6. The conventional onion Cvs. namely, Shandaweel-1; Giza-6 and Giza-20 showed clearly significant differences in the susceptibility against the artificial inoculation with the causal agent. Shandaweel-1 proved to be resistant; Giza-6 was moderately resistant and Giza-20 was highly susceptible.

7. The morphological; cultural and physiological characteristics of the all isolates of *P. terrestris* were studied and could be summarized as follows :

a) The isolates varied in the linear growth; dry weight of mycelium; the colour and the nature of growth; and the production of pycnidia; pycnidio and conidio-spores. All isolates produced such structures with great similar morphology, when grown on solid or liqued PDA and C'zapek's media at 27°C. However the isolates were greatly differed in their measurements of the length and/or the width of pycnidia; bristles; pycnidio and conidio-spores.

b) The complicated carbon sources greatly affected the rate of growth; dry weight of mycelium and the production of pycnidia; pycnidio and conidio-spores of all *Pyrenochaeta terrestris* isolates, as the starch or cellulose containing media were better for the growth and sporulation than pectin containing one. Also, the high stimulation effect of either starch or cellulose decreased once pectin was added with starch and cellulose singly or together.

c) Natural media i.e. onion dextrose agar (ODA); potato dextrose agar (PDA); corn meal agar (CMA) and synthetic one

medium, sucrose ammonium nitrate were the best for giving the highest linear growth and production of pycnidia; pycnidio and conidio-spores of isolate No. 3. Natural media produced more vigorous growth with huge number of pycnidia compared with those obtained on synthetic media.

d) Reduced monosaccharides (glycerol; mannitol; mannose; glucose and dextrose) and glucose plus sucrose, encouraged the fungal growth more than the non-reduced disaccharides (maltose and lactose). Whereas the reverse effect was concluded on pycnidia production. Glucose; dextrose; sucrose and glucose plus sucrose were better for pycnidiospores production. All carbon sources were suitable for the production of conidiospores with the exception for glycerol; mannitol and lactose.

e) Glycerol or mannitol containing media gave very weak colonies with irrigular margins. Mannose containing one produced yellowish lemone growth particularly arround the main inocula. Glucose or dextrose containing media induced normal white greyish colonies surrounded by yellow zones. Non-reduced disaccharide sugars containing media greatly varied in their effect, as maltose containing medium gave white yellowish colonies while sucrose containing one produced vigorous colonies with some yellow pigment arround the main inocula, meanwhile lactose containing medium gave very weak colonies which looked alike that occurred on the plane agar medium.

f) Concerning the effect of nitrogen sources, the nitrogenous salts $NaNO_3$; KNO_3 and NH_4NO_3 and amino acid arginine

increased the linear growth rather than other nitrogen sources. Only NH_4NO_3 containing medium was the best for increasing pycnidia; pycnidio and conidio-spores. As for cultural morphology, the media containing $NaNO_3$; KNO_3 or NH_4NO_3 gave very strong growth; grey yellowish in colour with regular margins and contained great number of pycnidia. Arginine containing medium produced vigorous growth with yellowish pigment far from the main inoculum. Treptophan containing one gave relatively vigorous; aerial and greyish growth, whereas the growth was very poor and looking alike the growth on plane agar on the media containing either Glycine or methionine. Ammonium tartarate gave limited growth which was flattened; completely connected with the medial surface and had an irregular margin. Urea was completely considered a non suitable nitrogen source for nitrogen nutrition.

g) The C/N ratios 30/3 (the normal); 40/3 and 50/3 were the most suitable for giving the highest linear growth and producing the maximum number of pycnidia; pycnidio and conidio-spores. The ratios (30/5); (30/10) and (30/15) also gave considerable mycelial growth but greatly varied in their effects on sporulation. However omitting either carbon or nitrogen gave hyaline colonies lacking from any of pycnidia; pycnidio and conidio-spores.

h) Optimum temperature for the maximum growth and production of different spores was 25°C. and the increasing or decreasing the temperature above or down the optimum temperature resulted a sharp reduction in the growth and formation of such structures. However, a complete inhibition of both mycelial

growth and spore production occurred at 10 and 40°C.

i) The suitable range of pH for the growth and sporulation of *Pyrenochaeta terrestris* was 6.9-7.7. However the fungal growth did not affect when the pH decreased to very acidic condition (till 3.2), whereas the growth was greatly decreased as pH values increased to very alkaloide conditions (till 10.0).

8. The role of hydrolytic enzymes, Pectin-Methyl-Estrase (PME); Poly-Galacturonase (PG) and Cellulase (CX) as well as the role of oxidative enzymes, Polyphenoloxidase and Peroxidase were studied *in vivo* and *in vitro* in relation to the virulence of *P. terrestris* isolates on both the susceptible Giza-20 onion Cv. and modified C'zapek's medium, respectively.

a) The activities of PME; PG and CX were higher in diseased than healthy onion transplants.

b) There was a clear correlation between the isolates virulence and the production of such pectinolytic and cellulolytic enzymes through the root and bulb tissues, as the most virulent isolate (No. 3) produced the highest activities. Moderate virulent isolate (No. 1;4 and 6) showed moderate activities, whilest the least virulent ones (No. 2;5 and 7) produced the least quantities of such enzymes.

c) The activities of pectinolytic enzymes produced by all isolates were rather high in diseased roots than those determined in the bulb tissues of the diseased transplants. Meanwhile all isolates approximately produced Cellulase enzyme at equal high levels in the root tissues and relatively low ones in the bulbs.

b) Polyphenoloxidase and Peroxidase activities were obviously increased in diseased rather than those determined in healthy tissues of both roots and bulbs. Also, there was a possitive correlation between the virulence of the causal organism isolates and the increasing of Polyphenoloxidase activity after inoculation, whereas no correlation was observed according to Peroxidase activity and the virulence of the tested isolates.

e) Polyphenoloxidase activity was higher in the bulb tissues of transplants infected with any of the tested isolates than those determined in the root tissues. Whereas the Peroxidase activity was highr in the root tissues of diseased transplants with isolates No. 1;2;6 and 7 than those of its bulbs, whereas the reverse was true concerning to isolates No. 3;4 and 5.

f) The seven isolates of *P.terrestris* greatly differed in their PG enzyme activity *in vitro* . Isolate No. 3 secreted the highest quantity; isolates No. 1;2;4;6 and 7 showed moderate ones whilest isolates No. 5 secreted the least quantity of PG. The secretion of PME was also greatly differed by such isolates, as isolates No. 1 and 2 showed higher PME activity than that of isolates No. 3;4;5;6 and 7 which relatively produced the same quantity of such enzyme. As for Cx activity, all isolates approximately showed the same activity level in degrading CMC containing medium. As regard to oxidative enzymes, Peroxidase and Polyphenoloxidase activities in filterates of all tested isolates were very limited.

9.a) Hydrolytic enzymes, PG; PME and CX were clearly increased in root and bulb tissues of diseased onion transplants of all tested Cvs. than those of healthy ones but in different levels concerning to their resistance and/or susceptibility.

b) Gradual increasing of such activities were correlated with the pink root rot disease development, reaching the maximum after 90 days from inoculation, meanwhile such increase was very limited in healthy tissues.

c) PME activity was higher in the root tissues of diseased and healthy transplants of all Cvs. than in its bulbs.

d) PG activity increased in the root tissues rather than in the bulbs of diseased transplants in both susceptible (Giza-20) and moderately resistant (Giza-6) Cvs. whereas the reverse was true in the resistant Cv. Shandaweel-1. On the other hand the activity of such enzyme in the healthy bulbs of susceptible and moderately resistant Cvs. was relatively higher than those recorded in its roots.

e) CX activity was higher in the root tissues than those occurred in the bulb ones of diseased transplants of all tested Cvs. Meanwhile the reverse was enough in the case of healthy tissues.

10.a) Pink root rot infection increased Polyphenoloxidase activity in the infected roots of resistant Cv. Shandaweel-1 particularly after 90 days from inoculation.

b) The bulbs of healthy and diseased transplants of all Cvs. showed higher activity of such enzyme than those of their

roots either after 30; 60 or 90 days from inoculation.

c) The activity of this enzyme decreased after 60 days in the root tissues of both diseased and healthy transplants for all tested Cvs. then a remarkable increase was assayed after 90 days, whilest a continual increase of such enzyme was generally recorded in the their bulbs.

11. a) The infection also clearly increased Peroxidase activity in the root and bulb tissues of diseased transplants than healthy ones, but the rate of increasing was higher in the root than in the bulb tissues of all tested Cvs. either after 30; 60 or 90 days from inoculation.

b) The activity of Peroxidase was generally increased in both the root and bulb tissues of diseased and healthy transplants after 60 days rather than after 30 days, then dropped after 90 days.

12.a) The activities of hydrolytic enzymes, PG; PME and Cx were decreased in the root tissues of transplants treated with any of the tested fungicides rather than in untreated ones transplanted to infested soil.

b) There was a positive correlation between the fungicidal effectiveness and the inhibition of such hydrolytic enzymes.

c) The activities of such enzymes were rather high in the roots than those of the bulbs of transplants treated with the least effective fungicides, whereas the reverse was true, particularly in the case of PG and CX, when transplants treated with the most effective fungicides.

13. a) On the other hand the infection increased oxidative enzymes, Polyphenoloxidase and Peroxidase particularly in root than bulb tissues in the transplants treated with any of the tested fungicides.

b) Oxidative enzyme activities were generally higher in the root tissues than those assayed in the bulb ones.

14. There was a positive correlation between the isolates agressiveness of *P. terrestris* and the mycotoxin production.

15. *In vitro* , Bavistin (50%); Benlate (50%) and Folicure (25%) EC prevented the fungal growth and sporulation at 3;5 and 10 ppm respectively. Topsin-M (70%); Vitavax (75%) and Vitavax/captan (75%) at 50 ppm gave the same effect. Vitavax/thiram (75%) gave complete inhibition for the fungal growth at 100 ppm, whereas both Ronilan (50%) W.P. and Ronilan (5%) dust lead to the same results at 300 ppm. The fungicides KZ (50%) 120-W.P.; Sumisclex (50%) W.P. and Sumisclex (50%) D.Fl. were the least effective and failed to inhibit the fungal growth and spores production even at 500 ppm.

16. The fungicides applied in greenhouse as dip treatments, Folicure (25%) EC.; Sumisclex (50%) W.P.; Sumisclex (50%) D.Fl. and Ronilan (50%) W.P. minimized the disease infection as well as the severity of pink rotted roots of the susceptible onion Cv. Giza-20 followed by Ronilan (5%) dust (as soil treatment) and KZ (50%) 120 W.P. (as dip treatment).

17. Under field conditions, Folicure (25%) E.C.; Sumisclex (50%) D.Fl. and Sumisclex (50%) W.P. were significantly the

most effective fungicides which reduced the infection of the pink root rot disease incidence to minimum possible level and gave the highest yield followed by Ronilan (50%) W.P.; Ronilan (5%) dust and KZ (50%) 120 W.P.

18. In NPK nutrition trial under greenhouse conditions, exceeding nitrogen to a level equal the double of recommended rate, increased the percentage of pink root infection whilest the severity of infection was decreased when either the nitrogen decreased or the phosphorus increased. However, no differences in disease severity were recorded when the level of potassium either increased or decreased above or down its recommended rate.

19. Under field conditions, increasing nitrogen over the recommended rate increased the infection of pink root rot disease and obvious reduction in onion yield. Increasing or decreasing either phosphorus or potassium- in combinations with nitrogen recommended rate- resulted opposite and satisfactory results as the disease incidence was clearly decreased and the yield either increased or at least remained constant. In unfertilized treatment ($N_0P_0K_0$), highest infection and lowest onion yield could be occurred.

20. a) The tenth of January was the best for minimizing the pink root rot disease incidence and giving the highest onion yield. The infection was increased and the yield was decreased with the earliness of planting time.

b) Under open field conditions and natural infection, Shandaweel-1 showed relatively resistance Cv. and gave the highest yield. Giza-20 Cv. showed high susceptibility and yielded the lowest crop yield meanwhile, Giza-6 showed intermediate reaction.

21. a) After 30 days from inoculation, the total individual free amino acids was decreased in the whole diseased transplants of the susceptible Cv. Giza-20 and the resistant Cv. Shandaweel-1 but to a limited level in the later, whilest the inoculation increased the total amino acids in the whole transplants of the moderately resistant Cv. Giza-6.

b) Total free amino acids was sharply decreased in the roots of the susceptible Cv. and slightly decreased in both resistant and moderately resistant Cvs.

c) Such totals remained constant in the bulb tissues of inoculated resistant transplants and increased in both susceptible and moderately resistant ones.

d) All amino acid groups were decreased in the roots of inoculated resistant transplants except for sulphoric group (especially L-Cystine) which sharply increased. This group was decreased as well as all amino acid groups in the susceptible roots. However sulphoric; amino; and aromatic groups were clearly decreased and hydroxylic; alephatic; emino and basic groups were obviously increased in the moderately resistant Cv.

e) Sulphoric; hydroxylic and emino groups were increased in the bulb tissues of inoculated resistant Cv., whereas amino;

basic and alephatic groups were decreased, meanwhile only aromatic group remained constant. Concerning to both susceptible and moderately resistant Cvs., most amino acid groups were increased.

22.a) After 60 days from inoculation, the total free amino acids of the whole transplants were increased in the resistant Cv. whereas, reverse reaction was observed in both moderately resistant and susceptible Cvs.

b) Only sulphoric group was increased in the roots of resistant and susceptible Cvs. and the rest groups were decreased. Whilest slight increase in the amino group was observed only in moderately resistant Cv. and all the rest groups were also decreased.

c) In the infected bulb tissues of the susceptible Cv., sulphoric; alephatic; amino and basic groups were decreased, meanwhile hydroxylic; aromatic and amino groups were increased. However, sulphoric; alephatic; amino; emino and basic groups were decreased rather than those detected after 30 days from inoculation. The bulbs of infected transplants of the resistant Cv. contained higher cons. of amino and emino groups and less cons. of the rest groups rather than those detected in the healthy bulbs. Hydroxylic; aromatic; amino; basic and alephatic groups were sharply decrease, meanwhile sulphoric and emino groups were remained constant in the bulbs of inoculated moderate resistant Cv.

23. a) After 90 days from inoculation, the total free amino acids in the whole susceptible transplants was sharply increased compared with those detected in the control transplants or those determined after 60 days. Slight increase in such total was observed in the resistant transplants, meanwhile the total free amino acids was decreased in the transplants of moderate resistant Cv.

b) Only sulphoric group (particularly L-Cystine) and emino group (especially L-Hydroxy proline) were decreased in the root tissues of the resistant Cv. meanwhile its infected bulb tissues contained higher concentrations of sulphoric; hydroxylic; aromatic and emino groups in particular with L-Cystine; DL-Thrionine and DL-Tyrosin; Treptophan and L-Proline respectively.

c) In the infected roots of the susceptible Cv., only the sulphoric group (particularly L-Cystine) was found with higher concentration, whilest all amino acid groups were concentrated in its bulb tissues, except for emino group.

d) With regard to the moderate resistant Cv., non-polar alephatic group; amino and sulphoric groups especially DL- α - alanine; Glutamic acid and L-Cystine respectively were concentrated in the inoculated roots whilest non-polar alephatic group (particularly Glycine) was accumulated in its bulbs.

24. a) After 30 days from transplantation, the total phenols were higher in the healthy roots of resistant Cv. Shandaweel-1 than those of moderate resistant Cv. Giza-6 and the susceptible Cv. Giza-20.

b) Inoculation increased the total phenols in both the roots and the bulbs of the moderate resistant and susceptible Cvs. rather than those of the resistant one.

c) Also, free phenols were clearly increased in the root tissues of the moderate resistant and susceptible Cvs. after inoculation whereas the reverse was true according to resistant Cv.

d) Conjugated phenols were icnreased in the root tissues of all tested Cvs. as the result of infection.

e) Free phenols were obviously decreased in the bulb tissues of the resistant and the moderate resistant Cvs. whereas such phenols were clearly increased in the bulbs of susceptible one.

25. a) After 60 or 90 days from inoculation, the diseased roots of both the resistant and moderate resistant Cvs. contained total phenolic compounds less than in healthy ones, whilest these compounds were accumulated in the diseased roots of the susceptible Cv., however such compounds were increased in the bulb tissues of all diseased Cvs. but to high; moderate and slight levels in susceptible; resistant and moderate resistant Cvs., respectively.

b) Free phenols were greatly decreased, moderately increased and greatly increased in the diseased roots of the resistant, moderate resistant and susceptible Cvs. respectively. Reverse reactions were recorded concerning free phenols in the

bulb tissues of the tested Cvs. respectively too.

c) Conjugated phenols were increased in the diseased roots of resistant Cv. and were decreased in the moderate resistant and susceptible ones, whilest the reverse was true in the bulb tissues of these Cvs.

26. a) Reducing sugars were slightly increased in the roots and bulbs of diseased transplants of all Cvs. after 30 days from inoculation.

b) Non-reducing sugars were decreased in the root and bulb tissues of inoculated transplants of the resistant Cv. Shandaweel-1. Such sugars were slightly increased in the inoculated roots of the moderate resistant Cv. Giza-6 and decreased in its bulb tissues. Whereas these compounds were increased in both root and bulb tissues of the susceptible Cv. Giza-20.

c) Total soluble sugars were increased in the diseased roots of either susceptible or moderate resistant Cvs. and was being constant in the inoculated resistant Cv. However, the reverse reaction was found in the bulb tissues of the tested Cvs.

27. After 60 or 90 days from inoculation, reducing; non-reducing and total soluble sugars were decreased in the root and bulb tissues of inoculated transplants of resistant and moderate resistant Cvs. Such sugars were generally increased in the root and bulb tissues of the susceptible Cv. particularly after 90 days from inoculation.