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LIST OF ABBREVIATIONS

Fungi

<i>Pythium debaryanum</i>	Py
<i>Rhizoctonia solani</i>	RS
<i>Fusarium solani</i>	FS
<i>Fusarium semitectum</i>	FST

Cultivars

Victory Freezer	VF
Lincoln	L
Master-B	M-B
Sinnary	S

Symptoms

Pre-emergence	PRD
Post-emergence	PTD
Seedling survival	SS

CHAPTER 6

SUMMARY

Pea (*Pisum sativum* L.) is one of the important vegetable crops in Egypt for local consumption and exportation. It has cultivated for green pods and dry seeds, which contain high value of protein. However, pea seedlings are subjected to the attack by numerous pathogens causing damping-off and root rot diseases which reduce the quantity and quality of the crop.

The present investigation was planned to isolate and study the pathogenicity of soil-borne fungi associated with Pea (*P. sativum* L.) roots of many cultivars. The investigation includes also the study of the effect of some biological agents, plant oils and extracts, plant residues in addition to fungicides and some IPM elements on controlling damping-off and root rot pathogens. The following results were obtained.

Pathogenicity experiment:

1. Causal agents were isolated from pea roots collected from different location in Egypt, purified and identified as: *Pythium debaryanum*, *Rhizoctonia solani* isolate I, *Rhizoctonia solani* isolate II, *Fusarium solani* isolate I, *Fusarium solani* isolate II, and *Fusarium semitectum*.
2. The pathogenicity tests proved that all the tested fungi were pathogenic and able to produce pre-emergence damping-off (PRD), post-emergence damping off (PTD) and root rot symptoms in different degrees on pea cultivars, namely; Victory Freezer (VF), Lincoln (L),

Masher-B (M-B) and Sinnary (S).

3. The highest PRD values were obtained on VF cultivar inoculated with PY and FSI (39.23 compared to control), L cultivar inoculated with RSII and FSI (43.08 and 41.15), M-B cultivar inoculated with RSII (41.15). The least infection values less than 26.57 were obtained on Sinnary cultivar, whereas L cultivar PRD values were the highest. FST was the least in producing PRD among the tested fungi.
4. The highest PTD incidence ratios were obtained by PY on L, M-B and S cultivars (56.79%) and on VF cv. (50.77%) compared with control. In addition, relatively high PTD values were realized by RSII on VF cv. (33.21%) and L cv. (37.22%).
5. Inoculation experiments using PY led to significant reduction in SS values (9.097). On the other hand, the highest SS values obtained from M-B and S cultivars inoculated with FSII (48.93 and 50.77) and S cv. inoculated with FST (56.79).
6. All the tested isolates were able to induce root rot symptoms on the all tested cultivars. However, PY was the most pathogenic (more than 85%), followed by the group of RSII, RSI and FSI (70-84%) then FSII and FST (55-69%).

Biological control:

I. *In vitro*

7. All the tested species of *Trichoderma* and the bacteria *B. subtilis* were able to inhibit the growth of all the tested damping-off and root rot pathogens on petri dishes but at different degrees. *Trichoderma viride* induced more sound antagonistic effect on FSI (77.78% less than control) while the other tested bioagents *B. subtilis*, *T. harzianum* and *T. koningii* were more antagonistic against all the tested species of *Fusarium* (67.41% - 77.04% less than control). PY isolate was the

least affected bioagent.

II. *In vivo*

8. Significant reduction in PRD and PTD values was recorded due to treatment with the tested bioagents. However, reduction % differed according to the applied bioagent, the tested pathogen and the cultivated cultivar as follows:
 - a. Seed treatment with *T. viride* significantly decreased percentage of the PRD values produced by PY isolate by 46.11% less than control, whereas seed treatment with *T. harzianum* realized 43.85% reduction less than control.
 - b. The highest reduction % in PRD values (32.2%) was obtained in L seeds treated with *B. subtilis* or *T. koningii* and inoculated with RSII, compared with that (57%) realized with S cultivar.
 - c. PRD produced by FS was reduced by 20% less than control with *T. koningii* in L cultivar, whereas the ratio was 65% less than control with *B. subtilis*.
 - d. The percentage of PRD values produced by FST significantly decreased when seeds were treated with any of the tested bioagents (64.9% to 67.7% less than control).
9. Seed treatment with any of the biocontrol agent significantly decreased PTD values, depending upon the bioagent, pathogen and cultivar as shown below:
 - a. In spite of the significant decrease in PTD values developed by PY (61.6-78.2% less than control), however, differences in PTD values between treatments and cultivars were insignificant.
 - b. Seed treatment of L cultivar with *T. harzianum* significantly reduced PTD (21% less than control) caused by RS, whereas PTD values in S cultivar treated with *B. subtilis* was 82% less than

control.

- c. Seed treatment with *T. viride* and *T. harzianum* decreased the percentage value of PTD incidence caused by FS (43% and 53% less than control).
 - d. PTD values produced by FST were significantly decreased by 42% less than control when seeds were treated with the bioagent *T. viride* and to 35.71% with *T. koningii*.
10. All the tested biocontrol agents considerably increased the seedling survival rates escaping infection with damping-off and root rot diseases. Increasing percentages of SS values in case of PY, RSII and FSI were 261%, 91.18% and 138.33%, respectively, more than control. The lowest increasing percentages of SS were obtained in case of FST (20% more than control).
 11. Seed treatment with the tested bioagents significantly decreased root rot incidence (20-40% less than control).

Control by mean of plant oils

I. Effect of crude oils *in vitro*

The antagonistic effect of some crude plant oils, i.e. cinnamon, clove, spearmint, lemon, onion, garlic and thyme oils were tested against damping-off and root rot pathogens *in vitro*.

12. Crude spearmint oil completely inhibited hyphal growth of both PY and RS. On the other hand, radial growth of both FS & FST was reduced by 88.9% and 67.8% less than control. Cinnamon oil completely inhibited the growth of RS and significantly reduced the growth of the other tested pathogens by 65 to 68.5% less than control. Crude clove oil characterized by its inhibitory effect against PY, RSII, FST and FSI, where radial growth was reduced by 72.29%, 66.7%,

65.9% and 52.6%, respectively, less than control. Onion, garlic and thyme oils were ineffective.

II. Antagonistic doses of the tested oils *in vitro*:

13. Spearmint oil completely inhibited growth of PY at conc. 100% to 50%, whereas RS growth was inhibited completely at 100% and to 75% only. The growth of each of PY and RS was completely ceased due to the effect of lemon oil at conc. from 100% and to 50%. The other oils showed less effect and the reduction rates decreased with the decrease of oil concentration.

III. *In vivo* studies

14. Seed treatment with all the applied plant oils, generally, led to significant reduction in PRD incidence caused by the tested pathogens. Seed treatment with spearmint oil at conc. 100% gave the best results. However, at conc. 50% reduction rates in PRD incited by PY, RS, FS and FST were 57.57%, 46.77%, 30.99% and 29.24%, respectively, less than control. The other oils were ineffective except at 100%. Moreover, response of S cv. to the treatments was higher than that of L cv.
15. All the tested oils exhibited a significant reduction in PTD values, however, reduction percentage varied according to the applied oil and its concentration and the tested pea cultivar. Spearmint oil proved to be the most effective in reducing PTD incidence caused by PY, RS, FS and FST (41%, 75.17%, 36.18% and 47.5% less than control) than the other tested oils at conc. 100%. However, at conc. 50% the percentage values were 27%, 18.22%, 20.96% and 32.04%, respectively, less than control. Lemon oil was the least effective oil in

decreasing PTD incidence compared with the other tested oils. Sinnary cv. responded to these treatment better than L cv.

16. Treatment with any of the tested oils pronouncedly increase the total SS values compared with the infected control. The highest SS values were obtained when Spearmint oil was applied at 100% conc., where increasing rates in PY, RS, FS and FST treatment attained 89.45%, 143.21%, 80.73% and 63.99%, respectively, more than control. The lowest increasing rates in SS values were obtained with lemon oil.
17. All the tested plant oils significantly decreased root rot diseases incited by the four tested pathogenic agents. The Spearmint oil at conc. 100% successfully reduced root rot incidence caused by PY, RS, FS, and FST by 80%, 76.5%, 68.8%, and 58.3%, respectively, in Lincoln cv. and 85%, 76.9%, 73.3% and 60%, respectively in S cv. less than control. Reduction rates at 50% conc. treatment were less than those of the conc. 100%.

Control by mean of plant residues

I. Amendment with some summer crops grinds

In order to achieve this aim fine grinds of well dried corn or soybean plants were applied to soil to check their effect on infection of pea seedlings with damping-off and root rot diseases.

a. Effect on PRD

18. Amendment of soil with corn grinds significantly reduced PRD incidence produced by the tested damping-off and root rot agents, i.e. PY, RS, FS, and FST. Reduction rates in PRD were, respectively, 50.56%, 46.77%, 45.75%, and 22.5% in L cultivar and 35.79%, 48.55%, 48.16%, and 22.5% in S cv less than control.

19. Amendment of soil with soybean grinds significantly reduced PRD incited by PY, RS, FS and FST. Reduction percentages were 40.53%, 17.10%, 28.15%, and 31.3%, respectively, in L cultivar and 22.76%, 35.79%, 35.96% and 41.24%, respectively, in S cultivar less than control.

b. Effect on PTD

20. Amendment of soil with corn grinds significantly reduced PTD incited by PY, RS, FS and FST. Reduction percentages were 48.63%, 48.5%, 32.25%, and 32.25% in L cultivar and 67.12%, 45.4%, 38.51%, and 6.52% in S cultivar, respectively, less than control.

21. Amendment of soil with soybean grinds significantly reduced PTD incidence, but still efficiently less than corn grinds. Reduction rates of PTD value incited by PY, RS, FS, and FST were 30.06%, 41.90%, 32.25%, and 32.25% in L cv. and 35.76%, 27.70%, 20.47%, and 15.33%, respectively in S cv. less than control

c. Effect on SS values:

22. Seedling survival (SS) values were greatly affected by the amendment of corn and soybean grinds to the soil infected by the tested pathogenic agents. Moreover, amendment with corn grinds was significantly more effective in increasing SS values than soybean grinds, except for FST, which was affected more by soybean grinds. Increase of SS values was more sound in PY isolate where these values increased in L cv. by corn or soybean by 132.4%, and 92.50%, and in S cv. by 87.87%, and 49.12%, respectively, more than control.

d. Effect on root rot infection:

23. Amendment of corn residues to the soil successfully reduced root rot incidence caused by PY, RS, FS, and FST by 80%, 70.6%, 68.8%, and 50%, respectively, in L cv. and by 85.%, 69.2%, 73.3%, and 50% in S cv., respectively, less than control.
24. Soybean residues successfully reduced root rot incidence caused by the same pathogenic agents by 75%, 64.7%, 68.8%, and 58.3% in L cv. and 80%, 69.2%, 66.7%, and 60% in S cv., respectively, less than control.

II. Amendment with some winter crops residues

Fine grinds of dried cabbage and turnip plant were applied to soil to check their effect on reduction damping-off and root rot incidence.

a. Effect on PRD

25. Amendment of soil with cabbage grinds significantly reduced PRD incidence produced by PY, RS, FS, and FST. Reduction percentages were 60.60%, 46.8%, 63.20%, and 50.2% in L cv. and 65.8%, 54.05%, 70.6%, and 53.2% in S cv., respectively, less than control.
26. Amendment of soil with turnip grinds significantly reduced PRD incidence produced by PY, RS, FS, and FST in which reduction percentages were 23%, 26.5%, 45.7%, and 22.5% in L cv. and 20.4%, 54%, 50.6%, and 29.3% in S cv., respectively, less than control.

b. Effect on PTD

27. Amendment of soil with cabbage grinds significantly reduced PTD incited by PY, RS, FS, and FST. Reduction % were 62.80%, 70.3%, 65.3%, and 56.50% in L cv. and 75.5%, 72.4%, 70.6%, and 70.6% in S cv., respectively, less than control.

28. Amendment of soil with turnip grinds significantly reduced PTD incited by PY, RS, FS, and FST. Reduction % were 34.40%, 41.9%, 32.3%, and 32.50% in L cv. and 58.8%, 35.9%, 23% and 50.6% in S cv., respectively, less than control.

c. Effect on SS values

29. Seedling survival values were greatly affected by the amendment of cabbage grinds to soil infected by the tested pathogenic agents namely; PY, RS, FS, and FST. Increasing percentages were 143.93%, 144.73%, 143.54%, and 106.9% in L cv. and 102.29%, 96.84%, 118%, and 85.08% in S cv., respectively more than control.

30. Seedling survival values (SS) were also affected by the amendment of turnip grinds to soil infected by PY, RS, FS, and FST. Increasing percentages were 80.55%, 84.9%, 86.56%, and 55.29% in L cv. and 77.5%, 66.35%, 61.41%, and 55.67% in S cv., respectively, more than control.

d. Effect on root rot incidence

31. Amendment of soil with cabbage residues realized significant reduction in root rot incidence caused by PY, RS, FS, and FST. Reduction percentages were 75%, 70.6%, 75%, and 58.3% in L cv. and 80%, 76.9%, 80%, and 70% in S cv., respectively, less than control.

32. Soil amendment with turnip residues also significantly reduced root rot caused by PY, RS, FS, and FST by 70%, 58.8%, 50%, and 50% in L cv. and by 75%, 53.8%, 53.3%, and 50% in S cv., respectively, less than control.

Chemical control

I. *In vitro*

The effect of three fungicides namely; Rizolex-T, Topsin-M and Vitavax/captan, on the linear growth of the applied damping-off and root rot agents was carried out. Different concentrations of the tested fungicides were applied (0.0, 2.5, 5, 10, 25, 50, 100, 200, 500, 1000 and 2000 ppm). The following results were recorded:

33. Topsin-M at all the tested concentrations and Rizolex-T and Vitavax/captan up to 100 ppm were ineffective against PY growth *in vitro*. Rizolex-T and Vitavax/captan at 500 and 1000 ppm completely inhibited PY growth, whereas at 200 ppm they reduced the growth by 81.7% and 12%, respectively, less than control.
34. Topsin-M and Vitavax/captan were completely ineffective against RS isolate at all the tested concentrations. However, Rizolex-T showed a reasonable reduction in the growth of the fungus. Reduction increased as the concentration increased, attaining complete inhibition of the RS growth at 200 ppm.
35. FS was greatly affected by all the tested fungicides at relatively low concentrations attaining 2.5 ppm. Complete inhibition of the fungus was achieved by Topsin-M, Rizolex-T and Vitavax/captan at the concentrations 50, 500 and 2000 ppm, respectively.
36. Topsin-M was the most effective against FST isolate growth and development at relatively low concentrations (10 ppm), at which reduction of growth was 56.6% less than control attaining complete inhibition at 200 ppm. Both Rizolex-T and Vitavax/captan have reduced the growth of FST to 45% and 29.4% less than control at conc. 25 and 50 ppm, respectively. Moreover, full inhibition was attained at conc. 500 and 1000 ppm, respectively.

II. *In vivo*

This experiment was carried out to investigate the efficiency of seed dressing with the three tested fungicides at the rate of 2 or 3 g/kg seeds on the control of the tested damping-off and root rot agents on Lincoln and Sinnary cv. of pea.

a. Effect on PRD

37. Seed treatment with Topsin-M at 2 g/kg seeds was not significantly effective in reducing PRD produced by PY and RS. However, at the same dose, Topsin-M and Rizolex-T were able to reduce PRD incidence caused by FS (68.39% less than control in both L and S cvs) and FST (40.14% and 54.05% less than control for L and S cvs, respectively).

Application of the three tested fungicides at the rate 3 g/kg seeds significantly reduced PRD values produced by PY, RS, FS, and FST agents. Moreover, seed treatment with Rizolex-T exhibited the highest reduction percentages in PRD values produced by the same pathogenic agents by 61.8%, 57.57%, 72.61% and 70.45%, respectively, less than control.

Seed treatment with Vitavax/captan at 3 g/kg seeds significantly decreased PRD values but at lower rates compared with the other tested fungicides. Reduction rates were 70.6%, 26.54%, 44.5% and 50.24% in L cv. and 61.8%, 11.39%, 55.5%, and 54.05% in S cv., respectively, less than control.

b. Effect on PTD

All fungicidal seed treatments at the applied concentrations were significantly effective in reducing PTD incidence. However, the most

promising results were obtained when seeds were treated with Rizolex-T at the rate of 3 g/kg seeds, where reduction percentages of PTD produced by PY, RS, and FS were 77.89%, 77.85%, and 74.17% in L cv. and 73.16%, 72.60% and 74.17% in S cv., respectively less than control.

Topsin-M was the most effective fungicide against PTD incidence caused by FST in both tested L and S cultivars. Reduction rates were 65.33%, and 70.45%, respectively, less than control.

c. Effect on SS

All fungicidal seed treatments significantly increased SS values but at different degrees. Rizolex-T at the rate 3 g/kg seeds was the most effective in raising SS produced from infection by PY, RS. Increasing rates were 112.39%, and 166.03% in L cv and 188.65%, and 91.05% in S cv, respectively more than control. On the other hand, Topsin-M was more effective against FS and FST, raising SS to 163.86% and 135.16%, respectively more than control. The other fungicides were less effective.

d. Effect on root rot incidence

40. The fungicide Rizolex-T at 3 g/kg seed treatment successfully reduced root rot incidence caused by PY, RS, FS, and FST to 15% in L cv and by 10% in S cv. Seed treatment with Topsin-M fungicide at the rate 3 g/kg seed successfully reduced root rot incidence caused by PY, RS, FS, and FST by 40%, 35%, 15% and 15% in L cv. and to 35%, 30% 10%, and 10% in S cv., respectively less than control

Control by the application of some IPM elements

41. Applying some IPM elements, i.e. soil treatment with dried cabbage leaves, seed treatment with *T. viride* as a bioagent, and soaking seeds

in spearmint oil before sowing successfully exhibited a highly significant reduction in PRD values (74.17% less than untreated control inoculated with *R. solani*). It also exhibited a pronounced significant reduction in PTD values (70.33% less than the untreated inoculated control). Moreover, SS values were greatly increased due to the application of the tested IPM elements (242.39% more the untreated inoculated control).

Application of the tested IPM elements fully prevented the incidence of root rot disease caused by RS isolate. It significantly reduced root rot incidence (70.59% less than the untreated inoculated control).