

CONTENTS

	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
1. The causal organisms	3
2. Pathogenicity tests	5
3. Cultivar reaction	6
4. Biological control	8
5. The antagonism	13
6. Carrying materials for bioagents	15
7. Production of a biofungicide	17
8. Chemical control	18
9. Integrated control	19
III. MATERIALS AND METHODS	22
1. Isolation of the pathogenic fungi	22
2. Pathogenicity test	22
2.1. Preparation of pathogens inocula and soil infestation	23
2.2. Disease assessment	23
2.3. Identification of pathogens	24
3. Isolation of antagonistic microorganisms	24
4. Screening and identification of the antagonistic microorganisms ...	25
4.1. Screening of bacterial antagonists <i>in vitro</i>	25
4.2. Screening of fungal antagonists	26
4.3. Identification of antagonists	26
5. Disease control studies	27
5.1. Cultivar reaction	27
5.2. <i>In vitro</i> biological control studies	27
5.3. <i>In vitro</i> chemical control studies	27
5.4. <i>In vivo</i> biological control studies	29
5.4.1. Bacterial antagonists	29
5.4.1.1. Preparation of the bacterial inocula and soil inoculation	29
5.4.1.2. Design of the experimental treatments	29
5.4.2. Fungal antagonists	30
5.4.2.1. Preparation of fungal inoculum and soil inoculation	30
5.4.2.2. Design of the experimental treatments	30

5.5. <i>In vivo</i> chemical control studies	30
5.5.1. Preparation of fungicides and seedlings treatment	30
5.5.2. Design of the experimental treatments	31
5.6. Effect of carrying materials on the biocontrol agents survival	31
5.7. Integrated control experiment.....	32
5.8. Application of biocontrol agents for controlling root-rot of tomato in non sterilized infested soil	33
IV. EXPERIMENTAL RESULTS	34
1. Isolation of the causal pathogens	34
2. Pathogenicity test	36
3. Disease control studies	38
3.1. Isolation of antagonistic microorganisms.....	38
3.2. <i>In vitro</i> experiments	38
3.2.1. Biological control studies	38
A. Effect of the different antagonists on the growth rate of the tested pathogens	38
B. Mode of action of the antagonistic isolates toward the root-rot pathogens.....	47
3.2.2. Chemical control studies	50
3.3. <i>In vivo</i> experiments	53
3.3.1. Cultivar reaction.....	53
3.3.2. Biological and chemical control experiments.....	64
3.3.3. Effect of carrying materials on the biocontrol agents survival.....	83
3.3.4. Integrated control experiment	86
V. DISCUSSION.....	89
VI. SUMMARY.....	98
VII. REFERENCES	102
VIII. ARABIC SUMMARY	

SUMMARY

Tomato (*Lycopersicon esculentum* Mill) plants are vulnerable to several soil-born pathogens causing damping-off, wilt and root-rot diseases. These pathogens cause damage to plants and hence subsequent reduction in fruit yield. Seed and soil treatment with fungicides are commonly used for controlling such diseases. Because of the hazardous effect of chemicals to the human and the environment, new approaches were necessary for controlling these pathogens which attack tomato plants. Biological control of such disease by certain biocontrol agents was the most essential approaches in this respect. The obtained results of the present study are summarized as follows:

1. Isolation trials from naturally infected rotted roots and crowns of tomato plants collected from different Governorates in the Delta of Egypt i.e., Kafr El-Sheikh, Gharbiya, Behira and Minufiya, nurseries and glass houses, yielded fungal isolates belong to 3 genera i.e., *Fusarium*, *Rhizactonia*, and *Pythium*.
2. Occurrence and frequency of fungi associated with diseased samples differed according to the locality from which the samples were collected. The highest number of fungi was isolated from samples collected from Kafr El-Sheikh Governorate (33 isolates). The most frequent fungal isolates were *Fusarium* spp. which obtained from all localities. While *R. solani* was only isolated from Minufiya samples and *P. ultimum* from Kafr El-Sheikh.
3. The pathogenicity test and identification of the isolated pathogens revealed that *F. oxysporum*, *F. solani*, *Rhizoctonia solani* and *Pythium ultimum* are the major soil-borne pathogens of tomato. These

Summary

pathogens are implicated in damping-off, root-rot and wilt of tomato plants.

4. The preliminary screening of several soil samples collected from the rhizosphere of healthy tomato plants resulted in isolation of 8 bacterial isolates and 17 fungal isolates exhibiting marked antifungal activity. These antagonistic bacteria were belong to *Bacillus subtilis*, *Bacillus marinus* and *Bacillus firmus*, whereas fungal isolates were belong to *Trichoderma harzianum*.
5. These antifungal isolates obtained in the preliminary screening were subjected to a standardized test to select those having the highest effect against the tested pathogenic fungi. They were identified as, *Trichoderma harzianum* (T₅), *Trichoderma harzianum* (T₁₆), *Bacillus subtilis* (B₅), *Bacillus firmus* (B₇), *Bacillus marinus* (B₈).
6. All the tested antagonistic fungi significantly reduced the linear growth of the four pathogens, *in vitro*. *T. harzianum* was the most effective antagonist which sharply inhibited the growth of all studied pathogens.
7. About mode of action of the most tested antagonists caused overgrowth. All most (T) isolates gave overgrowth especially with *Fusarium solani* followed by *Fusarium oxysporum* compared with *R. solani* and *Pythium ultimum* couldn't gave any overgrowth.
8. The tested antagonistic bacterial isolates significantly inhibited the growth of all pathogens *in vitro* as compared with control. Of eight bacterial isolate. *Bacillus subtilis*, *Bacillus firmus* and *Bacillus marinus* were the most effective ones.
9. Topsin M 70% was the most effective fungicide against all tested pathogens. It completely inhibited the growth of all tested pathogens

Summary

at concentration of 20 ppm. However; Vitavax and Bafry showed moderately toxicity while Prevecure was in effective except in case of *Pythium*.

10. The four tested tomato cultivars, i.e., Super Strain B, Castle Rock, Floridide and 448 (Al-Qudse) were susceptible to any of the studied pathogens. However; 448 (Al-Qudse) cultivar was the least susceptible, where it showed the least damping-off of plants and significantly higher number of survival plants as compared with the other three cultivars. Castle Rock cultivar insignificantly affected by the infection with the four pathogens 15 days after transplanting but it was highly susceptible to infection with *Fusarium lycopersici* and *R. solani* 30 days after transplanting.
11. Under controlled greenhouse conditions; application of any of the antagonistic fungi to the previously artificially infested soil with and of the four pathogens; *F. solani*, *F. oxysporum* ; *Pythium ultimum* and *R. solani*; decreased root rotted plants. *T. harzianum* was the best antagonist reduced dead plants caused by such tested pathogens. Plant height was significantly increased in response to application of fungal antagonists. On the other hand; the tested antagonistic bacterial isolates significantly increased plant survival grown in artificially infested soil with each pathogen.
12. In comparison studies between the best tested antagonists i.e., *T. harzianum* (T₅ and T₁₆) *B. subtilis* (B₅), *B. firmus* (B₇) and *Bacillus marinus* (B₈), and the two effective fungicides (Topsin (M) 70% and Vitavax thiram 75%), it could be noticed that the biocontrol agents were more satisfactory efficient than the fungicides. Using the biological control agents improved the growth of tomato plants and

Summary

significantly decreased damping off and root rot diseases. The best results were achieved when bioagents T₅ and B₅ were applied.

13. All tested carrying materials showed significant difference in colony forming units and survival time of both *T. harzianum* and *B. subtilis*. Barely was the best carrier to *T. harzianum* as compared with the other five materials while wheat bran was the best carrier for *B. subtilis*. In general sugar-cane baggase was the worst carrying material for both tested antagonists.
14. The integrated control studies proved that planting the least susceptible cultivar (Al-Qudse) and applying either *T. harzianum* or *B. subtilis* to the infested potted soil gave very good results, both for disease control and plant growth parameter.
15. Generally, application of B₅ isolate was the best; both for disease reduction and plant height. While application of T₅ was also satisfactory for disease reduction. However; combined application of B₅ and T₅ resulted less satisfactory disease control than the individual bioagent application.