

CONTENTS

Page

1-INTRODUCTION .	1
2- REVIEW OF LITERATURE .	2
3- MATERIALS AND METHODS .	46
4- RESULTS AND DISCUSSION .	55
1 : The causal organism.	55
2 : Pathogenicity test .	56
3 : Estimation of the recent acquired fungicidal resistance level of mycelium of the natural population of <i>R.solani</i> to different fungicides .	59
3.1: Resistance to Rizolex .	59
3.2: Resistance to Rizolex.T .	62
3.3: Resistance to Vitavax.T.	65
3.4: Resistance to Monceren .	68
3.5: Resistance to Thiram .	71
4: Cross resistance among the different tested fungicides	75
5: Effect of different temperatures on acquired resistance to fungicides .	77
5.1: Effect of temperatures on the resistance and sensitive isolates to Rizolex .	77
5.2 : Effect of temperatures on the resistance and sensitive isolates to Rizolex.T .	77
5.3 : Effect of temperatures on the resistance and sensitive isolates to Vitavax.T . .	78
5.4 : Effect of temperatures on the resistance and sensitive isolates to Monceren .	78
5.5 : Effect of temperatures on the resistance and sensitive isolates to Thiram .	78
6: Effect of exposure of sensitive isolate (R3) of <i>R.solani</i> to U.V rays	84
7: Effect of an acquired resistance on the physiological characters :	87
7.1: Correlation between resistance to different fungicides and virulence of <i>R.solani</i> isolates .	88
7.2: Effect on oxidative enzymes .	89
SUMMERY	94
LITERATURE CITED	100
ARABIC SUMMARY	

Summery

Rhizoctonia solani fungus is the causal organism to damping-off and root rot in cotton. it is also attacking a lot of cultivars. Pesticides are the most important way to control this disease, but there is some isolates of this fungus appeared resistance to fungicides this made a big problem. The resistance is dynamic matter didn't static, different from time to time and from place to another, so this work aimed to illiminate the problem of a acquired resistance in the natural populations of *R. solani* . all isolates were isolation , purification and identification of fungal isolates in different governorates in Egypt . El-dakahlia . El Gharbia. Kafr El Shikh . El-Behera . Domietta . El Sharkia and El-Monofia , all isolates were able to attack cotton seeds.

The present work aims to :

- 1- Study thepathogenicity of all isolates of the fungal .
- 2- Study the current situation of the acquiered resistance problem in *R.solani* in Egypt .
- 3- Study relation ship between the different isolates of *R.solani* isolated from different locations .
- 4- Studying possible cross resistance fungicides in the different isolates .
- 5- Effect of different temperatures on acquiered resistance to fungicides .
- 6- Study the effect of exposure U.V rays on sensitive isolate .
- 7- study Correlation between % virulence of *R.solani* isolates and resistance index to different fungicides .
- 8- Study the activity of oxidative enzymes in *R.solani* isolates .

1) Studied the pathogenicity of *R.solani* isolates .

There were a significant differences among the virulence of the different isolate of *R.solani* R₅ , R₆ and R₇ were the most virulent isolates since not survival plants , R₂ , R₃ , R₈ isolates showed moderate virulence R₁ , R₄ ,R₉and R₁₀ showed the lowest degrees of virulence .

2) Fungicidal resistance of *R. solani* isolates :

The ten *R.solani* isolates representing 7 governorates were tested for their resistance to 5 fungicides, representing the different fungicide groups to which belong most of the fungicides used to control damping off and root rot disease.

a) Resistance to organophosphorus compounds:

a-1) Resistance to Rizolex :-

The isolates classified to three groups depending on their acquired resistance to Rizolex .The first group were 8 isolates very sensitive which IC₅₀ ranging from 0.4 to 1 p.p.m and resistance factor (RI) were ranged to 1 to 2.5 (R₁, R₂ , R₃ , R₄, R₅, R₈ , R₉ and R₁₀ isolates) . The second group contain one isolate (R₇) moderate resistance with IC₅₀ 3.6 p.p.m and resistance factor 9. the last group was only one isolate R₆ with IC₅₀ 100 p.p.m and RI (250) this isolate is very resistant.

a.2) Resistance to Rizolex.T :-

The isolates classified into 4 groups, the first one containing sensitive isolates with resistance factors ranging form 1 to 2.5 i.e (R₂ , R₃ , R₄ and R₇) isolates . The second group included two moderately resistant isolates R₁ and R₅ with (R I

6.25 and 11.5 respectively . the third class includes 3 high resistance isolate .R₈, R₉ and R₁₀ showed (RI)27.5, 27.5 and 29 respectively. The last group contains very high resistant isolate R₆with IC₅₀ 51 p.p.m and (RI) 157.5.

b) Resistance to carboxinalide compounds (Vitavax.T) :

The isolate (R₆) had a very high resistance index 17.14 and the isolate R₂ was high resistance with (RI) 13.6 the moderate resistance (R₁ , R₄, R₅, R₇ and R₉) with (RI) (8.6 , 8.6 , 3.7 , 7.4 and 4.11) respectively .

The isolates R₃, R₈ and R₁₀ were sensitive with (RI) (1.1.5 and 1.43) respectively .

c) Resistance to phenylurea compound (Monceren) :

The isolates (R₁, R₅ and R₈) respectively were very resistance . The isolates R₃, R₄, R₆ ,R₉ and R₁₀ were sensitive with (RI) (1,9,4,2.8 and 4) respectively .

d) Resistance to Thiram :

The IC₅₀ ranged from 0.5 , 0.7 p.p.m for isolates R₁₀ , R₃. This was reflected on the resistance factor ranged from 0.8 to 1 for sensitive isolates. All the other isolates showed high IC₅₀ , the most isolate resistance were two can be ranged IC₅₀ 198 R₂ and R₇ with 270 p.p.m .We suggest that the resistance to this fungi , is anultigenic trait .

3) Cross resistance among the different fungicides :

There were positive correlation between cross resistance of fungicides Rizolex and Rixolex.T (0.974) , Rizolex and

Vitavax.T (0.675) , Rizolex and Thiram (0.069) , Vitavax.T and Rixolex.T (0.592) and thiram with Vitavax.T(0.555) .

On the other hand there were negative correlated coefficient of cross resistance between Monceren and Rixolex (-0.198) , Monceren and Rixolex.T (-0.226), Monceren and Vitavax.T(-0.384) , thiram and Rizolex.T (-0.046) and thiram with Monceren (0.066) .

4) Effect of different temperatures on acquired resistance to fungicides:

The resistance isolate (R_6) to fungicide Rizolex didn't be any more resistance at 20° and 33°C comparison with at 25°C (R_1) was 250 at 25°C and become 1.5 at 20° and 33°C. So at optimum temperature the resistance increased, the sensitive isolate IC_{50} was 0.4 p.p.m at 25°C become 0.04 p.p.m at 20°C but at 33°C IC_{50} was 0.6 p.p.m. About Rizolex.T the same thing to resistant isolate like Rizolex . The resistance decreased at 20°, 33°C than 25°C. The sensitive isolate become more resistance it 33°C than 25°C. Vitavax.T at 20°, 33°C the resistance isolate become sensitive while at 25°C it was resistance. The sensitive isolates become more resistance at 20° and 33°C the IC_{50} was 1.4 at 25°C it become 11 and 25.5 at 20° and 33°C respectively. Fungicide Monceren the resistance increased with increasing the temperature since IC_{50} were 40, 74 and 1260 p.p.m at 20°, 25°, 33°C respectively. The sensitive isolate became more resistance at 20° and 33°C than at 25°C since IC_{50} were 1.93, 0.1 , and 8.34 p.p.m at 20°, 25° , 33°C respectively .

The fungicide Thiram :

The isolate resistance and sensitive become more resistance at 20°, 33°C more than it were at 25°C .

5) Effect of exposure sensitive isolate (R₃) to U.V radiation on the acquisition of fungicidal resistance :

The IC₅₀ increased from 0.4 p.p.m in case of Rizolex the mother isolate to 0.8, 0.8, and 0.9 p.p.m at (5, 15, and 15 minutes of U.V rays) respectively, this effect can be mutagen regarding the second fungicide (Rizolex.T), the IC₅₀ were increasing, since it were 0.4 p.p.m the mother isolate to 1.6, 1.8 and 2 p.p.m) at (5,10.and 15 min .) , concerning the IC₅₀ for Vitavax increased from 1.4 to 11 at 15 min. The same thing happened in Monceren. The IC₅₀ was 0.1 in the mother isolate and became 1 at 15 min. In case of thiram noticed that the mother isolate become resistance in all concentrations at all periods exposure to U.V rays . That means that the effect can be mutagene .

6) Correlation between % virulence of *R.solani* isoates and resistance index to different fungicals:

The correlation were very different from fungicide to another we found that there were a high significant variation between RI of (Rizolex, Rizolex.T and thiram) and virulence , there was significant variation between RI of Vitavax and virulence . There were non significant variation between RI of Monceren and virulence of the fungi.

7-Effect on oxidative enzymes:

All the resistant isolates were had very high activity of enzyme polyphenoloxidase than the sensitive isolate since the activity were 0.060, 0.070 and 0.057 to (R₆ R₈ and R₇) respectively comparing with R₃ 0.056 .The same things were in fungicide

Monoceren , but in thiram the level was not very high , the activity in R₇ was 0.057 and in R₃ 0.026.

Enzyme activity of peroxidase in isolates (R₆ , R₋ and R₈) was also clearly resistant isolate (R₆ , R₋ and R₈) were very high activity than the sensitive isolates .The activity were (0.339,0.130 and 0.182) respectively in (R₆ , R₈ and R₇) Comparing with R₃ (0.098) . So this enzyme was very high level on resistant isolate than sensitive isolate. On the other hand Catalase enzyme and Ascorbic acid were low level in resistant isolate than in sensitive isolate to all fungicides. That means that there were positive correlation between the activity of enzymes Polyphenoloxidase and Peroxidase with resistant isolates. On the other hand there is negative correlation between the activity of enzymes Catalase and Ascorbic acid with resistant isolates.