Mansoura University
Faculty of Science
Botany Department

Control of chocolate spot disease of faba bean using different bio-agents

Thesis
Submitted in partial fulfillment for
M.Sc. degree in Botany (Microbiology)

By

Eman Othman Zedan Kassem
B.Sc. Botany & Chemistry (1999)
Faculty of Science, Zagazig University

Under supervision of

Dr. Hoda Mohamed Soliman
Prof. of Mycology and Microbiology, Faculty of Science
Mansoura University

Dr. Kamar Mohamed Abd El-Hai
Prof. Plant Pathology
Plant Pathology Research Institute, ARC.

2020

List of Contents

List of Tables

List of Figures

Summary

The present investigation aimed at evaluation of some biological control agents such as *Rhizobium Leguminosarum, Trichederma* spp., *Saccharomyces Cerevisiae* on control of chocolate spot disease of *Vicia faba* L. caused by *Botrytis fabae*.

So, experiments were carried out in the laboratory, greenhouse and field of Tag El-Ezz Research Station, Dakhlia, Egypt. The most important results are summarized follows:

A- Laboratory experiments and pathogenicity test:

1- Twenty-five isolates of *B.fabae* were isolated from diseased *Vicia faba* plants collected from five sites in Dakhlia district were found to be pathogenic on *Vicia faba* cv. Giza 402. The isolate B4 which isolated from Bilkas found to be the most aggressive.

2- All of the tested twenty five *Trichoderma* isolates related to four species (*T. viride, T. harzianum*, *T. hamatum* and *T. album*) showed some sort of antagonism against the pathogen *B. fabae* B4. While the isolate *T. viride* T2 was the most potent antagonist followed by *T. harzianum* E3.

3- The most potent antagonists *T. viride* T2 and *T.harzianum* E3 caused a significant inhibition of linear growth and sporulation of the pathogen *B. fabae* B4. While *T. harzianum* E3 was more effective followed by *T. viride* T2.

4- Both non-volatile and volatile metabolites of *T. viride* T2 and *T.harzianum* E3 significantly inhibited the linear growth of *B. fabae* B4. The non-volatile metabolites of both *Trichoderma* species were more potent in inhibition of mycelial growth of *B. fabae* B4 than the volatile metabolites.

5- Culture filtrates of all tested bio-agents possessed antifungal activity and inhibited growth and sporulation of *B. fabae.* Yeast was the most effective followed by *T. harzianum* then *T. viride*.

6- Both of the tested *Trichoderma* spp. produced cellulolytic and chitinolytic activitiy more than the pathogen *B. fabae*. On the other hand, *B. fabae* produced much more proteolytic activity than *Trichoderma* spp. There was no significant difference in amylolytic activity of all the three tested fungi.

B- Greenhouse experiments:

7- In vivo studies showed that all tested individual bio-agents significantly reduced disease severity of chocolate spot disease. *S. cerevisiae* was the most active followed by *T. viride* T2 then *T. harzianum* E3.

8- The treatment of *Vicia faba* seeds and foliar spraying with the trio combination of (R + *T. viride* T2 + yeast) was the best in reduction of chocolate spot disease severity and disease incidence followed by the trio combination of (R + *T. harzianum* E3 + yeast).

9- All used bio-agents and their combinations had a marked effect on growth parameters (plant height, plant fresh weight, plant dry weight and number of branches) of faba bean plants infected with *B. fabae* B4. The highest values of the growth parameters after 70 days from sowing were obtained by using trio combination of R + *T. v* + yeast followed by the trio combination of R + *T. h* + yeast.

10- All tested bioagents and their combinations increased significantly nodulation status (nodules f.w and nodules d.w) and nitrogenase activity. The dual combination of (R + *T. v*) was the most effective followed by (R + *T. h*) then trio combination of (R + *T. v* +yeast). *Rhizobium* recorded the highest values of the tested parameters followed by *T. v* then *T. h*. While, the fungicide Kocide 101 decreased significantly nodules fresh weight and has non-significant impact on nitrogenase activity.

11- The treatment with bio-agents counteracted the negative effect of the pathogen on the pigmentation of faba bean plants with different degrees. The trio combination of (R + *T. v* + yeast) was the most effective and efficient in increasing the contents of pigments of infected bean plants followed by trio combination (R + *T. h* + yeast). Also,

the dual combination of (R + yeast) gave a significant effect on increasing the pigmentation of faba bean infected plants.

12- The treatments with all bio-agents individually or in dual or trio combinations significantly increased the levels of total phenols, phytoalexins and proline in faba bean plants infected with *B. faba* B4with different degrees. The trio combination of (R + *T. v* + yeast) gave the most significant increase in the levels followed by the dual combination of (yeast + *T. v*). The effects of the most effective combinations of bio-agents were nearby the same as the effect of the fungicide Koside 101 on increasing the levels of total phenols and phytoalexins.

13- All treatments inhibited the activity of polyphenol oxidase. The trio combination of (R + *T. v* + yeast) was the most effective in this respect followed by (R + *T. h* + yeast). On contrast, peroxidase activity increased significantly with all used treatments. The highest stimulation of peroxidase activity occurred under the application of (R + *T. v* + yeast) followed by dual combination of (*T. v* + yeast).

14- All treatments of bio-agents and their combinations increased the levels of minerals (NPK) with different degrees in faba bean plants infected with *B. fabae* B4. Interestingly, *Rhizobium* alone or in dual combinations or in trio combinations significantly increased all the minerals generally and nitrogen specially.

15- All tested bio-agents and their combinations increased significantly total soluble carbohydrates and total soluble sugars in faba bean plants in different degrees. The trio combinations of bio-agents gave the most significant increase in both traits. The combination of (R + *T. v* + yeast) was the best for total soluble sugars. For total soluble carbohydrates the trio combination of (R + *T. h* + yeast) was the best.

16- All individual bio-agents and their combinations increased the thickness of faba bean leaflet blade due to corresponding increase in palisade and spongy tissues thickness. The highest values of leaflet tissue thickness in term of palisade and spongy tissues thickness were recorded by using the trio combination of (R + *T. v* + yeast) followed by (R + *T. h* + yeast) then the dual combination of (R + yeast). The dimensions of the midrib

vascular bundle as well as xylem and phloem area were increased with all used treatments except Kocide 101 which decreased these parameters.

C- Field experiments:

17- Under natural infection with *B. fabae* conditions, it can be easily noticed that the individual, dual and trio inoculations of seed and foliar spraying of grown faba bean with *Rhizobium*, *Trichoderma* and yeast extract as well as Kocide 101 significantly reduced chocolate spot disease severity and disease incidence as compared with control. The maximum reduction of disease severity was recorded by trio combination (R + *T. v* + yeast) followed by dual combination of (yeast + *T. v*) then trio combination of (R + *T. h* + yeast). The highest reduction in disease incidence occurred under the application of *T. v* then *T. h* followed by dual combination of (R + *T. v*).

18- The three bio-agents and their combinations significantly increased faba bean yield components [No. of pods plant^{-1}, seed yield (g plant^{-1}) and weight of 100 seed (g)]. In this respect, the trio combinations of (R + *T. viride* T2+ yeast) and (R + *T. harzianum* E3+ yeast) gave the highest increase in productivity traits.

19- All tested treatments significantly increased seed protein percentage except the fungicide treatment which showed non-significant effect in this respect. The highest seed protein % occurred under the application of trio combinations in general followed by dual combination of (R + *T. v*). *Rhizobium* treatment came first from individual treatments as compared with non-treated infected faba bean plants.