ROLE OF SOME BIOAGENTS IN THE PATHOGENSIS OF *Botrytis fabae* THE CAUSAL OF FABA BEAN CHOCOLATE SPOT DISEASE

By

YASER HASSAN EL-SAID EL-GAMMAL

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THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

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(Plant Pathology)

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APPROVAL SHEET

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Approval Committee

Dr. HAMED ABDEL-RAHIM EISA .....
Head Researcher of Plant Pathology, Plant Pathology Res. Institute, ARC, Giza

Dr. EFFAT ABDEL-MAGEED ZAHER .....
Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. KHAIRY ABDEL-MAKSOUD ABADA .....
Professor of Plant Pathology, Fac. Agric., Cairo University

Date: 10 / 12 /2013

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. FAROUK MOHAMED BARAKAT
Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. KHAIRY ABDEL-MAKSOUD ABADA
Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. NAGI MOHAMED ABOU-ZEID
Head Researcher of Plant Pathology, Plant Pathology Res. Institute, ARC, Giza

Name of Candidate: Yaser Hassan El-Said El- Gammal **Degree:** Ph.D.
Title of Thesis: Role of Some Bioagents in the Pathogensis of *Botrytis fabae* the Causal of Faba Bean Chocolate Spot Disease
Supervisors: Dr. Farouk Mohamed Barakat
Dr. Khairy Abdel-Maksoud Abada
Dr. Nagi Mohamed Abou-Zeid
Department: Plant Pathology
Approval: 10 / 12 / 2013

ABSTRACT

Surveying of faba bean chocolate spot disease during 2007/2008 and 2008/2009 growing seasons in this investigation was varied from governorate to another where Nubaria (El-Beheira Governorate) was the most infected area 76.7% disease severity. The tested *Botrytis fabae* isolates varied clearly in their virulence onto faba bean plants (c.v.Giza-429) under greenhouse conditions. Thirty antagonistic bacterial isolates (B) and 30 antagonistic fungal isolates (F) were isolated from faba bean phylloplane and assayed for their antagonistic activity against *B. fabae in vitro* and *in vivo*. Results showed that the highest growth inhibition against *B. fabae* was obtained by *Trichoderma album*-$_2$ and *Bacillus megaterium*-$_8$. Light micrographs showing different types of *T.album* parasitism on *B.fabae* hyphae, which caused *B. fabae* hyperparasitism, degradion, malformation and colour changes of the growing hyphae. Volatile and non volatile compounds from *T. album*-$_2$ and *T.harziunum*-$_6$ having significant effect in reducing the radial growth and sporulation of *B. fabae*, 6 and 12 days after incubation. Culture filtrates of *T.album*-$_2$ and *T.harziunum*-$_6$ at the different concentrations significantly decreased the linear growth of *B. fabae* by 66.58 and 71.50%, respectively in comparison with control treatment.

T.album-$_2$ application gave the highest enzymatic activity of chitinase and β-1,3-glucanase in faba bean plants, which related to plant defense against pathogens infection. Meanwhile application of *B. megaterium*-$_8$ significantly increased the activity of Protease followed by *T. album*-$_2$ compared to the control.

Active antimicrobial compound bacteriocin (megacin) from the most effective antagonistic bacterial isolates (*B. megaterium*-$_8$) was separated and purificated. The extracted bacteriocin (megacin) was identification and characterization by Mass Spectrometry, Nuclear Magnetic Resonance (NMR) and Infra Red (IR), which have high potential effective as biocontrol agent against *B. fabae*.

The autoclaved or non-autoclaved semi-purified toxin(s) of *B.fabae*, culture filtrate extract at different concentrations caused different damage degrees to faba bean.

All treatments four biocides (Bio-Arc, Bio-Zeid, Plant Guardgard and Ulva), two effective antagonistic isolates (*T. album*-$_2$ and *B. megaterium*-$_8$) and chemical control (Dithane-M45 and Tridex-80) reduced the severity of *B. fabae* chocolate spot disease on faba bean using detached leaves, under greenhouse and field conditions compared with control. Also, under field conditions they gave the highest yield components compared with control.

Key words: Antimicrobial compound, bacteriocin, biocides, biocontrol agents, chocolate spot disease, enzymatic activity, Megacin, toxin, volatile and non volatile compounds.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my family, The spirit of my dear father, my mother, my wife and my daughters for their patience and help, as well as to my brothers and my closed friends for all the support they lovely offered along the period of my post graduation.

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INTRODUCTION

Faba bean (*Vicia fabae*) is considered the most important legume crop in Egypt. It is considerd the fifth food legume in the world after dry bean, dry pea, chickpea and lentil (Adak *et al.*, 1998 and Ahmed, 2005). In Egypt, it is grown mainly for human food consumption as green pods or dried seeds. Meanwhile, it is planted in some other parts of the world essentially as animal feed. Seeds of faba bean are rich in protein (28%), carbohydrates (56%) and some other compounds thus; it is a rich available source of food for both human and animals (Tewati and Virk, 1996).

The total cultivated area of faba bean in Egypt during season 2012-2013 reached about 111000 feddan with total production of 1.035.630 ardab (ardab=155 Kg) at the rate of 9.33 ardab/feddan. **(**Anonymous, 2013**)**.

Faba bean is liable to be attacked by many foliar diseases as chocolate spot (*Botrytis fabae* and *B. cinerea*), rust (*Uromyces fabae*), Ascochyta blight (*Ascochyta fabae*), leaf spots (*Cercospora zonata* and *Alternaria alternata)*, downy mildew (*Peronospora viciae*) and root-rots as well as viral diseases, which are responsible to cause considerable losses in the yield and its components.

Foliar diseases are the most common diseases specially in Delta region due to the high humidity, rain fall and favourable temperature which are prevailing during the season. Therefore, chocolate spot disease of faba bean caused by *Botrytis fabae* and *B. cinerea* is considered the most important disease in Egypt, which causes serious damage to the crop, where the yield losses could be more than 50% of the crop according to (Hussein, 1963 and Mohamed, 1982).