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Control of onion basal rot disease using endophytes

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THESIS

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Summary

Onion (*Allium cepa* L.) is worldwide and cultivated crop; it also has an economic, medicinal and nutrient importance. Different diseases attack onion during the growth stage such as fungal disease which causes huge crop losses due to climatic conditions which are suitable for plant infection. Onion basal rot disease caused by *Fusarium oxysporum* species is one of the economically important diseases of onion in Egypt. So, the aim of this work was isolation of endophytic bacteria and fungi from three endemic *Allium* species and characterization of their antagonistic activity against *Fusarium oxysporum* the causal pathogen of basal rot disease in onion.

Results of the current study can be summarized as follows:

1. Thirty infected onion samples showing typical symptoms of basal rot were collected from onion fields in Assiut, Sohage (Sohag, AL-Maragha, Tahta, Gerga, Al-Mensha) Qena (Qous and Qena) and Monofya (Menouf) governorates.
2. Pathogenicity tests of *Fusarium sp* isolates on Giza 6 onion cultivar carried out under greenhouse conditions at Shandaweel Agriculture Research Station, Sohag in 2017/2018 onion growing season. Obtained results revealed that significant differences were observed in disease levels amongst the 30 *Fusarium spp.* isolates, there were 18 pathogenic isolates resulting in 13.33–100 % percentage of infection with the control (0%), and isolate No. (17) showed the highest virulent bility among the isolates.
3. The identification of fnngi showed that all of them are different species belonging to the genus *Fusarium*, and the isolate with the highest pathogenicity was known as *Fusarium oxysporim*.

4. Nineteen isolates of endophytic bacteria isolated from healthy *allium* species: onion (*Allium cepa*), garlic (*Allium sativum* L.) and leek (*Allium porrum*) plants.
5. The antagonistic capability against *Fusarium oxysporum* investigated in *vitro*. Bacterial isolates exhibited inhibition percentages that were more than 50%. Isolate SH2 caused the highest percentage of inhibition (83.33%) followed by isolate SH1 (66.66%) then isolate SH3 (57.77%) and isolate SH4 (52.96%).
6. Based on 16S rRNA, data revealed that isolate SH1 belonged to *Bacillus velezensis*, isolate No. SH2 showed 100% of similarity with *S.maltophili*, isolate No.SH3 showed 100% of similarity with *Bacillus velezensis*, isolate SH4 was *Bacillus velezensis*.
7. Twenty-nine endophytic fungi isolated from healthy *allium* species: onion (*Allium cepa*), garlic (*Allium sativum* L.) and leek (*Allium porrum*) plants, fungal species belonging to 9 genera and 17 species.
8. Three fungal isolates exhibited inhibition percentages on pathogen mycelium growth that were *T. roseum* caused the highest percentage of inhibition (58.11%) followed by *C. globosum* (44%) then *S. chartarum* (41.33%) while other tested isolates having no effect.
9. Four of bacterial strains namely *B.velezensis* SH1, *S.maltophilia* SH2, *B.velezensis* SH3, *B.velezensis* SH4 and three fungal namely *Chaetomium globosum* , *Stachybotrys chartarum* , *Trichothecium roseum* were selected for greenhouse studies. The application of the tested powder formulations of endophytic fungi and bacteria significantly reduced the incidence of basal rot on onion under greenhouse conditions.
10. The evaluation of the tested powder endophytic fungi and bacteria of onion under greenhouse led to the selection of five of the most

- promising formulations *B.velezensis* SH1, *S.maltophilia* SH2, *B.velezensis* SH3, *B.velezensis* and *Trichothecium roseum* for field studies. All formulations significantly decreased the disease percentage of basal rot disease on onion compared with the control.
11. All the tested endophytic bacterial isolates gave a positive response to siderophore production. Among the bacterial isolates tested in this study, *B. velezensis* SH4 produced the maximum percentage of (94.34%) over the control, followed by *B. velezensis* SH1 which gave 93.50%. and *B. velezensis* SH3 93.48% where it provided an increase of in the siderophore production compared with control.
 12. All bacterial isolates tested for the ability to produce IAA proved positive results. The highest production rate was found by *S.maltophili* SH2, giving an increase of 93.2% over control, followed by *B. velezensis* SH1 (92.6%).
 13. Among the four bacterial isolates tested for the production of salicylic acid, *B. velezensis* SH4 produced the maximum SA (94.43%), followed by *B. velezensis* SH1 (93.50%), *B. velezensis* SH3 (93.48%) compared to the control.
 14. Among the four bacterial isolates tested for the production of chitinase, The highest yield of chitinase was achieved about 80.1 IU/ml by *B velezensis* SH4 While others isolate produced only low levels of chitinase.
 15. All selected isolates showed pectinase producing ability in the range of 126.91 to 152.5 IU/ml . The highest yield of pectinase was achieved by *B. velezensis* SH3 (152.5 IU/ml) and *B. velezensis* SH4(151.8 IU/ml) the lowest values were obtained by *B. velezensis* SH1.

16. Salicylic acid contents were higher in treated plants than the infected and healthy control plants. The highest value of salicylic acid accumulation was obtained by application of *S.maltophili* SH2 and *B. velezensis* SH4 formulations while the lowest values were obtained by application of *T. roseum* formulation.
17. Results showed that the total phenolic content of onion plants treated with the endophytic bioagents formulations was significantly higher than that of infected and healthy control. The highest phenolic content in plants treated with *B. velezensis* SH4. While the lowest total phenolic was *T. roseum* formulation.
18. Onion plants treated with *B. velezensis* SH1 exhibited the highest increase in peroxidase activity (5.80 unit/mg protein) followed by *B. velezensis* SH3 (5.53 unit/mg protein) and *B. velezensis* SH4 (5.71 unit/mg protein). No significant differences in enzyme activity were found between *S.maltophili* SH2 and *T. roseum* formulations.
19. Data showed that an increase in the activity of polyphenol oxidase in onion plants treated with all formulations. However, Application of *B. velezensis* SH3 and *B. velezensis* SH4 showed the highest increase in polyphenoloxidase activity in onion plants, while *T. roseum* showed the lowest activity of the enzyme. No significant variations in polyphenoloxidase activity were found between 30 and 45 days from the application.
20. Application of either bioagents formulations increased the activity of phenylalanine ammonia-lyase compared to both infected and controls except *T. roseum*. Application of *B. velezensis* SH1 showed the highest increase in phenylalanine ammonia-lyase followed by *B. velezensis* SH3 and *B. velezensis* SH4. No significant variations

phenylalanine ammonia-lyase activity was found between 30 and 45 days from the application.

21. Results of catalase activity revealed that no significant decrease was in catalase activity of the onion plants treated with the endophytic bioagents formulations than that of Healthy control. Results obtained also showed that *T. roseum* caused the highest catalase activity after 30 days of the application.