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**Pathological and biochemical studies on the
controlling of onion white rot disease caused
by *Sclerotium cepivorum***

A THESIS

Submitted in Partial Fulfillment for the Requirements for the Degree of

Doctor of philosophy

IN

AGRICULTURAL SCIENCES

(PLANT PATHOLOGY)

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2020

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6. SUMMARY

The pathogenic fungus of onion white rot disease identified as *Sclerotium cepivorum* Berk was isolated from diseased onion plants grown at different locations within Kafr El-Sheikh and El-Gharbia Governorates. Different isolates of the pathogen were resulted using samples collected from the selected previous locations. Pathogenic abilities of fourteen pathogenic isolates to infect onion plants (Giza 6 cv.) were tested. They were varied in their pathogenicity under sterilized pots conditions. Isolate Sc₁₁ was found to be the most pathogenic with fulfilled degree of 100 % followed by the isolate Sc₁ incited severity reached 91.25%. On the other hand, isolate Sc₁₄ was less aggressive by which its pathogenicity was reduced to 73.75%. However, isolates Sc₂, Sc₃, Sc₄, Sc₅, Sc₆, Sc₇, Sc₈, Sc₉, Sc₁₀, Sc₁₂ and Sc₁₃ were varied in their pathogenicity between 80.00 % and 87.50%. Due to its massive pathogenicity, Sc₁₁ was selected as the main pathogenic isolate of *S. cepivorum* to achieve the purposes of the presented study.

***In vitro* studies were carried out and the following results were achieved:**

1- As a result of the microbial screening of rhizosphere of healthy plants grown nearby the diseased ones, 40 bacterial and 15 fungal isolates were found to have antagonistic impacts against *S. cepivorum*. Antagonistic isolates were varied in their antagonist against the elected pathogenic isolate (Sc₁₁) of the causal agent using the dual culture technique on PDA medium.

2- For bacterial antagonists, RPA (Relative power of antibiosis) values showed that B₁₀ isolate was the superior with highest antagonistic effect against the pathogen,

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by which the highest 1.6 RPA-value was recorded. It followed by B₁₈ isolate with 1.4 RPA-value and each of B₂, B₁₀ and B₁₄ reached 1.2 RPA-value. On the other hand, lower RPA-values by the other isolates were obtained. According to its superior antagonistic effect, cultural, morphological and biochemical properties of B₁₀ isolate was tested and it was found to be identified as *Bacillus* sp. To confirm its identification, genomic 16S rRNA sequencing test of B₁₀ was carried out. Accordingly, tree and similarity index of the related bacterial species were plotted and it could be clearly seen that the *Bacillus* isolate B₁₀ was exactly linked to *B. nakamurai* strain NRRL B-41091 with the highest similarity percentage.

3- For fungal antagonists, massive percentages of inhibition (I %) against the pathogen were obtained by the fungal isolates of T₂, T₁ and T₈, respectively. By them, mycelium growth of the pathogen was massive inhibited to 92.22, 90.00 and 88.89 %, respectively. Whereas, inhibition reduced to 78.89 % due to isolate T₇. The remainder 11 antagonists were varied between both poles. Based on its superior antagonism, T₂ isolate was chosen for identification and complete the further experiments. Based on its microscopic and cultural examinations, T₂ was found to be related to *Trichoderma* sp. This identification was confirmed by using genomic DNA technique. So, Phylogenetic tree was constructed to graphically represent the genetic relationship of the strains. It could be clearly seen that the *Trichoderma* isolate T₂ was closely related to *Trichoderma asperellum* strain BT with the highest similarity percentage.

4- Results illustrated also that the antifungal activity of the chemical inducers of citric acid, salicylic acid, ascorbic acid, ZnSO₄, CuSO₄ and H₂O₂ on the diameter

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growth of *S. cepivorum* (Sc_{11}) were varied. Results indicated that 0.4 g L^{-1} was the most effective dose of all inducers, but 0.8 g L^{-1} for H_2O_2 . Inhibition percentages of all inducers substances were not exceed to 83.00 % and not reached their maximum. As well as, potential impacts of the chemical fungicides of Topsin M, and Maxim Fs were reached to their fulfilled inhibition percentage (100%) by using 1.00 g L^{-1} and by using 3.00 g L^{-1} of Moncut 25, Uniform and Rhizolex-T in comparison with control.

5- Percentages data of pathogen inhibition obtained by the tested chemical inducers were modeled and found to be very well described by Monod's equation, which explains the relationship between concentrations of the tested agents and their inhibition percentages on the metabolic activity of the pathogen. Experimental data were fitted completely with the kinetic parameters of Monod's equation with regression values (R^2) ranged from 95.44 to 98.05 %. Kinetic model was also used to devise IC_{50} concentrations of each control agents. Lower value of IC_{50} indicates a large antagonistic efficacy. Data showed that citric acid was one of the most effective antagonists achieved 0.06 % as IC_{50} , followed by ZnSO_4 with 0.09 % and 0.11 % for both CuSO_4 and salicylic acid each. Antifungal activities of the tested fungicides confirmed fulfilled inhibition (100.00 %) against growth of *S. cepivorum*(Sc_{11}) using the recommended doses. By them, mycelium growth was totally prevented compared to control, indicating absolute antifungal activities.

6- For germination of sclerotia, *in vitro* results confirmed that soaking of sclerotia in the tested chemical fungicides led to prevent their germination totally due to use Topsin M, Moncut 25, Uniform, Rhizolex-T and Maxim Fs, by which no sclerotia

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were germinated for each. On the other hand, CuSO_4 was ranked at the second position after the chemical fungicides due to its efficiency, by which only 3.67 % of sclerotia were germinated. Concerning the biological agents, their efficiencies against germination of sclerotia were varied between 5.00 to 7.33 % and ranked at the third and fourth order. Whereas, the other chemical inducers were less effect, by which 8.33 to 9.33 % of sclerotia were germinated in comparison with 10.00 % for control.

7- Due to their expected role in controlling white rot disease and/or promoting plant growth, GC-Chromatography analysis of Cyanobacteria mix strains, *Azospirillum brasiliense*, *Trichoderma asperillum* and *Bacillus nakamuri* was carried out. Data indicated existence huge amounts of active ingredients occupied large area and extended retention time under the curve of Gas chromatography mass-spectrum (GC-MS). Based on the data obtained from screening of the active ingredients compounds of each agent, the compounds extracted from the tested samples were varied. Octadecene, Phorbol-dihexanoate, Dimethoxyglycerol docosylether, Tridecene, Tetradecanol and $\text{C}_{29}\text{H}_{38}\text{FeMoO}_4\text{P}_2$ were the most common in the cultures of *Bacillus nakamuri*. Tetracosahexaene-hexamethyl, N,N-Dimethyl-aminocarbonyl, Hexaethyltricarboxamide, Methylpropanoate, Dimethyl-2, 4-dioxo-6-methyl-triazolo-purine and Bis-pyrimidine were found to be the most common of *Trichoderma asperillum* cultures. For *Azospirillum brasiliense*, carotene, Flavone, Phytofluene, Rhodopin and growth regulator such as Gibberelline-Methyl ester were produced. Whereas, Cyanobacteria was found to form too much quantities of Diphenyl-2-pyrrole representing chlorophyll rings, $\text{C}_{15}\text{H}_{24}\text{Cl}_3\text{GeMoO}_2\text{P}$ and Methoxy-4-hydroxy-5-bromobenzylidene (cyano) acetyl-4-butanediamin from the chromatography analysis.

Based on the laboratory findings, it is worthy to test effect of such treatments for controlling onion white rot disease in both pots and natural infested field trials, the results were as follows:

I. Pots trials:

1- Results confirmed that bury of sclerotia in depth of 30 Cm from soil surface was the superior treatment as physical strategy, by which no disease incidence and consequently no disease severity were recorded. Rhizolex-T and Topsin-M were the superior control agents reduced disease incidence to 6.67 % each with pronounced effect for Rhizolex-T gave less severity of 2.23 % compared with 2.98 % for Topsin-M. Disease incidence data for the other agents were varied, but reduced to 13.33 % for Maxim Fs, 20.00 % for *Azosperillum brasiliense* and *Trichoderma asperellum* each.

2- For growth and yield parameters, results indicated absence or tiny effect of the physical treatment of sclerotial bury and chemical fungicides. Data showed remarkable elongation of onion roots and shoots due to use *Saccharomyces cerevisiae*, compost tea and mixture composed of cyanobacteria and compost tea, respectively, indicating rich nutrient supply. It was reflected positively to enhance dried matter of both shoots and bulbs. Application of Yeast and cyanobacteria caused high accumulation of dried matter in both shoots and bulbs. Hydrogen peroxide and salicylic acid were found to have great abilities to form dried tissues in the shoots reached 12.80 and 13.07 %, respectively, but not in the bulbs, indicating majority role of ROS induced by both inducers. Tosin-M was found to have a great effect to form maximum total chlorophyll reached 42.90 %, followed by 39.60, 39.30 and 38.77 % due to CuSO₄, mix of cyanobacteria and compost tea

and separate application using compost tea, respectively. As well as, the highest weight of onion bulbs was reached due to use *Saccharomyces cerevisiae* and compost tea reached 0.60 and 0.59 kg pot⁻¹, respectively, indicating high metabolic activity.

II. Natural infested field trials

a) During 2015 season:

1- Results obtained in pots were also confirmed under natural infested field conditions. Data proved that bury of sclerotia in depth of 30 Cm from soil surface was the superior treatment, by which no disease incidence or disease severity were noticed. As well as, Rhizolex-T and Topsin-M were the superior control agents reduced disease incidence to 12.50 and 15.00 %, respectively. Consequently, low disease severity values due to Rhizolex-T and Topsin-M reached 5.21 and 7.71 % were correspondingly obtained, respectively. Disease incidence data for the reminder agents were varied, but reduced to 19.83 % for Maxim Fs with 9.17 % disease severity. Here, role played by *Azosperillum* and *Trichoderma* in reducing disease parameters were not enough clear in the experimental fields. As well as, less significant effect of the reminder agents for controlling onion white rot disease was observed.

2- Results indicated also lower effect of both the chemical fungicides and bury of sclerotia on growth and yield parameters. Data showed remarkable elongation of onion roots and shoots reached 11.33 and 67.93 Cm due to use *Saccharomyces cerevisiae* and 12.00 and 68.37 Cm by compost tea, respectively, indicating rich

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nutrient supply. Dry matter of shoots was increased clearly and reached its maximum of 14.67 g plant⁻¹ by using *Trichoderma asperillum* compared with 8.73 g plant⁻¹ for control, indicating plant growth promoting fungi (PGPF). By dipping onion transplants in mixture composed of cyanobacteria and compost tea, large amount of bulb dried matter reached 11.67 g plant⁻¹ was accumulated, indicating high metabolic activity. For total chlorophyll, chemical fungicides and inducers were found to have high potential varied between 71.50 to 75.40%, which represented about twice value recorded in the pots trials, indicating availability of the required elements. As well as, the highest weight of onion bulbs was reached due to use *Saccharomyces cerevisiae* and compost tea reached 5.50 and 5.00 kg plot⁻¹, respectively, indicating high metabolic activity.

b) During 2016 and 2017 seasons:

1- Data proved also that bury of sclerotia in depth of 30 Cm from soil surface was the superior treatment, by which no disease incidence or disease severity were noticed during both 2016 and 2017 seasons. As well as, Topsin-M and Rhizolex-T were the pronounced control agents reduced disease incidence in reduction average of 82.38 and 80.24 % during both seasons respectively. Consequently, low mean of reduction values of disease severity due to Rhizolex-T and Topsin-M reached 89.96 and 89.26 % were correspondingly obtained, respectively. Disease incidence and disease severity data for the other agents were varied, but not reached to their expected, indicating low efficiencies.

2- The results showed significant improvement of plant growth and bulb yield parameters as a result of tested treatments in comparison with control. Chlorophyll contents (A, B and total), root and shoot lengths and dry matter of both tube leaves

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and bulbs were clear increased due to treatments compared to control, referring induction of defensive mechanism in the treated plants required to overcome the harmful effects under disease conditions. The inhibitory role played by the chemical inducers and fungicides against *S. cepivorum* not reflected to enhance onion growth or productivity. Data showed remarkable elongation of onion roots due to bury sclerotia 30 Cm under soil surface, indicating healthy not infected roots. As well as, onion plants reached to their longest mean of shoots of 67.09 Cm during both seasons compared with 49.65 Cm for control. Due to use *Saccharomyces cerevisiae* and *Azospirillum brasilense*, dry matter of the bulbs was remarkable increased to 12.63 g plant⁻¹ each compared with 7.84 g plant⁻¹ for control, indicating plant growth promotion and high anabolic activity. Consequently, onion bulb productivity reached its maximum of 5.92 Kg plot⁻¹ due to use yeast application in comparison with 3.65 Kg plot⁻¹ for control.

Data illustrated superiority of chemical fungicides, especially Rhizolex-T followed by Topsin-M, to form high concentrations of chlorophyll contents, indicating chlorophyll protected. By Rhizolex-T, great mean values of chlorophyll A, B and total reached to 21.04, 16.24 and 37.28 mg g⁻¹ fresh weight were obtained, respectively.

3- Data of the enzymatic activities of Both peroxidase (PO) and polyphenol oxidase (PPO) showed comparable values during both seasons and they seem to be constant during the measurement time courses. Therefore, average data of both enzyme activities were recalculated. All tested control agents recorded high activation rates of both enzymes compared to non-treated plants, referring to increase induction of the systemic acquired resistance (SAR). As well as, lower

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activity levels of PPO in comparison with PO were also noticed. Both PPO and PO enzyme activities showed superiority of fungicides in comparison with the chemical inducers, biological agents and bury of sclerotia. It showed also that Topsin-M and Moncut were the superior treatments, by which great activities of PPO enzyme of 0.226 OD/Sec/g fresh weights were induced each, followed by Maxim Fs, Rhizolex-T and uniform. For PO, Rhizolex-T and Topsin-M induced high activities reached 0.965 and 0.926 OD/Sec/g fresh weight, respectively. For sclerotial depth, low enzyme activities were obtained, indicating physical resistance. For chemical inducers and biological agents, both PO and PPO enzymes showed lower activities than the chemical fungicides.

4- To confirm induction of defensive mechanism, phenolic compounds were also assessed. Data illustrated that chemical fungicides are still the superior for enhancing accumulation of phenolic compounds in onion tissues in comparison with the other treatments and control. Conjugated, free and total phenols in the onion leaves were determined during 2016 and 2017 seasons and averages data were recalculated. It indicated that conjugated phenols were reached to their highest values of 7.43 and 6.29 mg g⁻¹ fresh weights by Rhizolex-T during 2016 and 2017 seasons, respectively, with superior mean reached 6.86 compared to 2.01 mg g⁻¹ fresh weights for control, indicating high oxidative activities. As well as, free phenols were large accumulated in the cells due to Topsin-M followed by *Azospirillum braselense*. The highest free phenols were resulted during 2016 season of 6.79 and 6.75 mg g⁻¹ fresh weights due to use *Azospirillum braselense* and Topsin-M, respectively. During 2017 season, both values were reduced, but their averages reached to 6.03 and 5.40 mg g⁻¹ fresh weights during both seasons due to Topsin-M and *Azospirillum braselense*, respectively. For total phenols,

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larger amounts were accumulated in onion tissues due to Rhizolex-T and Topsin-M of 11.98 and 11.70 mg g⁻¹ fresh weights, respectively than the other treatments, indicating induction of non-enzymatic defensive mechanism.

5- Results of the nutritional value of onion bulbs indicated no significance differences between all tested control agents in moisture contents. Citric acid and the mixture composed of cyanobacteria and compost tea were found to be more effective in forming higher ash contents of 0.875 and 8.65 % respectively, compared to 0.625 % for control, indicating availability of minerals. The major fiber components of onion bulbs of 0.785 and 0.755 % were achieved by using compost tea and *Azospirillum braselense* respectively, indicating hardness tissues required for induce resistance under pathogenic conditions. Differences due to all treatments in carbohydrate contents were not observed, but relatively high contents reached 8.690 and 8.585 % were obtained by Topsin-M and ZnSO₄ respectively, showing less effective metabolic activity. Role played by H₂O₂ was noticed via obtaining high contents of fats reached 0.165 % followed by 0.145 % for each of cyanobacteria, sclerotial depth and mix composed of cyanobacteria and compost tea. Data obtained for crude protein indicated no remarkable differences between treatments, but relatively high amount of 1.80 % due to H₂O₂ was formed, indicating antioxidant activity of protein. Similar behavior, but lower values of 1.775 and 1.765 % protein were resulted by CuSO₄ and ascorbic acid, respectively.

From the obtained results, it is worth to note that the massive expected antifungal efficacy of the chemical fungicides was and will still the best suggested strategies for controlling different plant diseases, of them onion white rot. Role

Summary

played by Rhizolex-T and Topsin-M for controlling onion white rot disease was clear appeared in this study. Due to their harmful effects on human health and the environment, utilization of efficient and environmentally safe control agent's alternative to chemicals which been produced from vital sources (bacteria - fungi – cyanobacteria- waste bioprocess products) became necessary.

In conclusion, bury of sclerotia of *S. cepivorum* at 30 Cm depth from soil surface, as physical control strategy, could be recommended in the presented study as superior treatment for reducing onion white rot disease. It represents a promising environmentally safe effective strategy for controlling onion white rot disease under Egyptian conditions. But it has not a full capacity to induce complete acquired resistance. So, further studies are required in this aspect. Despite being their varied efficient, the tested control agents proved not only to enhance enzymatic and non-enzymatic defense mechanism in onion plants, but also worked to improve growth and productivity of bulbs yield under pathogenic stress conditions.