Pathological Studies on Barley Leaf Stripe Disease Caused by *Drechslera graminea*

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THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In
Plant Pathology

Plant Pathology Department
Faculty of Agricultural, Moshtohor
Benha University

2021

CONTENTS

SUMMARY

Results obtained from this investigation could be summarized as follows:

1- Samples of barley diseased leaves from which showing leaf stripe typical symptoms were collected from different governorates, *i.e.* El-Sharqia (Kafr El-Hmam); El-Qalyubia (Moshtouhr); El-Gharbia (El-Gemmiza); El-Behaira (Etay El-Barod); El-Giza (Giza agriculture research station) and Kafr El-Sheikh (Sakha) during growing season 2012/2013. Six isolates of *Drechslera graminea* were individually isolated, purified and microscopically identified based on its morphological characteristic and pathological symptoms. The six isolates were coded as E, G, M, F, S and K.

2- Pathogenicity of six isolates were tested using two sensitive barley cultivars *i.e.* Giza123 and Giza 124 using artificial inoculation (sandwich technique) under greenhouse condition at Barley Disease Research Department, Plant Pathology Institute, Agricultural Research Center (ARC), Giza, Egypt. Data indicated that, the tested isolates were pathogenic to both cultivars. The differences between efficiencies of tested isolates for disease incidence (DI%) and infection value (IV%) were found to be significant differences in both DI% and IV% at 15, 30 and 45 day after sowing (DAS). Forty five days after sowing, isolate E caused the highest mean of disease incidence (DI%) and infection value (IV%) on Giza 123 with 84.25% and 71.99%, respectively, followed by isolate G, M and F while, isolate S and K was found to be less disease incidence (40.00 and 33.33%) and infection value (20.00 and 16.66%)**.** Same trend was observed in virulence spectrum of the tested six isolates on cv. Giza 124**.** In this respect the disease incidence and infection value of the pathogenic isolate *i.e.* E, G, M and F were early recorded 15 DAS in both cultivars.

3- The cultural variability of *Drechslera graminea* isolates carried out under laboratory condition to examine the effect of different solid media, temperature degree and relative humidity concentrations (RH%) on growth

of *D. graminea* isolates. Results obtained from this investigation could be summarized as follows:

- Effect of different solid media: Data indicated that, there were great variations in radial growth of the tested *D. graminea* isolates on tested media. In this respect, all of tested *D. graminea* isolates *i.e.* E and G gave the maximum radial growth (90.00 mm) on PDA medium at 7 days post inoculation followed by M (89.33 mm), F (86.00 mm), S (80.67 mm) and K (77.00 mm) isolates. On the other hand, isolate E gave also the maximum radial growth (90.00 mm) on Czabek dox agar medium followed by G isolate (89.67 mm). In general, the results indicate that the most suitable media for mycelial growth of the six tested *D. graminea* isolates was PDA followed by Czabek dox agar, Malt extract agar, Nutrient agar, Tomato juice, V8 juice and Water agar respectively. Therefore, PDA media was used in the following experiments.
- Effect of different degrees of temperature: Results indicated that, temperature degrees affected greatly the growth of *D. graminea* isolates. All the tested six *D. graminea* isolates able to grow at wide range of temperature degrees (10 - 30°C) *in vitro* conditions. In this respect, the isolates E, G and M recorded the maximum mycelial growth (90.00 mm) at 22°C while F, S and K isolates recorded 85.20, 79.00 and 75.52 mm, respectively. On the other hand, the best mycelial growth of the six tested isolates was recorded at 22°C followed by 20°C and 25°C respectively, meanwhile the least mycelial growth of the six tested *D. graminea* isolates was recorded on the 10°C and 30°C at 7 days post inoculation.
- Effect of different concentration of humidity: Results revealed that different concentrations of relative humidity (RH%) affected greatly the growth of *D. graminea* isolates. It was found that six isolates varied in their response when maintained on different concentrations of RH%. The highest radial growth of six isolates was recorded on a range of RH% degrees from 70% to 90% where the average growth rate ranged from 43.50 to 54.95 mm at 7 days post inoculation. On the other hand,

the lowest radial growth with average 35.33mm was recorded at 50% relative humidity concentration.

4- Response of 18 Egyptian barley cultivars to the six isolated pathogenic isolates of *D. graminea* was recorded after 15, 30 and 45 day after sowing using artificial inoculation under greenhouse condition. Disease incidence % recorded at 15 days after sowing in 8 cvs which inoculated with isolate E, but only it was recorded on 6 cvs using the two isolates G and M and 3 cvs by isolate F. It was also noticed that, increasing the days after sowing increased the disease incidence (%) in all tested cvs using all isolates. Results showed that, the six tested isolates exhibited a large variation in their pathogenic reactions ranging from 3.33 % to 86.66 % as percent of disease incidence at 45 DAS. The most virulent isolate E caused disease on 13 cvs among the 18 cvs tested while other isolate *i.e.* G, M, F, S and K were virulent on 12, 12, 11, 10 and 8 cultivars, respectively. According to disease incidence of the tested cultivars against different isolates of *D. graminea*, barley cultivars varied in the levels of resistance. The cultivars *i.e.* Giza 127, 128, 129, 130 and 2000 were resistance to all isolates while cvs Giza 123 and 124 were susceptible to all isolates tested. Disease incidence on the other tested cultivars ranged from resistant, intermediate and susceptible to one or more isolates. Also, data showed the infection value of 18 barley cultivars inoculated with 6 isolates of *D. graminea* at 15, 30 and 45 days post sowing, respectively. The pathogenic variability between isolates ranged from 0.33 to 68.55 as a mean of infection value. Virulence spectrum of the six tested isolates different from most virulence to least virulent. The most virulent isolate E (mean infection value 68.55) could infect 13 cvs among 18 cvs tested while other isolate *i.e.* G, M, F, S and K were recorded in 12,12,11,10 and 8 cvs, respectively. Differences among the reaction of the cultivars to the isolates of the fungus were observed. The cultivars *i.e.* Giza127 Giza128, Giza129, Giza130 and Giza 2000 were resistant to all 6 isolates (mean infection value 0.00) exhibited 33.33% from the tested cultivars.

5- Response of 18 Egyptian barley cultivars to the six isolated pathogenic isolates of *D. graminea* was recorded after 15, 30 and 45 day after sowing using natural infection under greenhouse condition and open field conditions. Data showed that the effect of natural infection of *D. graminea* on disease incidence (%) of 18 barley cultivars grown under both greenhouse and open field conditions. The wide variation in the reaction of the tested barley cultivars to *D. graminea* ranging from complete resistance to susceptible**.** The incidence of barley leaf stripe disease varied significantly with barley genotype, natural infection with *D. graminea* an their interactions. Cultivars Giza127, Giza128, Giza129, Giza130 and Giza 2000 were resistant to leaf stripe disease under both greenhouse and field conditions. Results also showed that Giza123 cv. represented a highly susceptible cultivar where the disease incidence percent was 36.00%, 31.00% and 28.83% at greenhouse, El-Gemmiza and Sakha stations, respectively. In the present investigation, the pathogenic variations of *D. graminea* using natural infection under greenhouse and field conditions were studied using the same set of 18 cvs. The incidence of stripe disease varied significantly with barley genotype, natural infection with *D. graminea* and their interactions. The same resistant cultivars obtained from artificial inoculation were also resistant at greenhouse and field conditions under natural infection.

6- Effect of *D. graminea* infection on yield components of some tested barley cvs using natural infection under field condition was carried out to study the effect of infection with *D. graminea* on plant height; grains spike number, spike weight and 1000 kernel weight of 18 barley cultivars in two stations (El-Gemmiza and Sakha). Data indicated that infection with *D. graminea* reduced plant height and grain number/spike compared with their respective control (healthy plants) and their reduction differed greatly among cultivars. The disease decreased the plant height of Giza123, Giza124, Giza121, Giza118, Giza117 and Giza125 by 45.50%, 34.75%, 29.14%, 21.67%, 20.86% 9.45% and 43.23%, 31.40%, 28.70%, 20.80%, 15.80%, 7.80% in El-Gemmiza and Sakha stations, respectively. As for grains number/spike**,** the disease decreased the grains spike number of Giza123, Giza124, Giza121, Giza118, Giza117 and

Giza125 by 52.51, 41.12, 28.50, 22.23, 17.20, 15.60 and 47.64, 37.50, 26.70, 21.80, 12.60, 11.80 in El-Gemmiza and Sakha stations, respectively. No significant reduction of the plant height and grains spike number was observed in the resistant cultivars. Results revealed that infection with *D. graminea* had a negative effect on the grains spike weight and TKW of the susceptible cultivars. Significant differences in grains spike weight and TKW were observed between infected and healthy plants, and this effect was similar in both stations. Infection had significant effects on the grains spike weight and TKW of susceptible cultivars of Giza123, Giza124, Giza121, Giza118, Giza117 and Giza125, As for grains spike weight decreased in these susceptible cvs by 69.70%, 53.12%, 45.50%, 43.00%, 38.90%, 32.60% and 66.20%, 58.90%, 44.70%, 42.50%, 37.72%,30.80% in El-Gemmiza and Sakha stations, respectively, while 1000 kernel weight decreased by 69.60%, 59.40%, 44.90%, 37.00%, 36.16%, 30.90%.and 62.50%, 59.10%, 42.60%, 36.50%, 34.00%, 28.00%, respectively.

7- Two methods *i.e.* osmotic and plate agar were used using the grain of the two barley cvs *i.e.* Giza 123 and Giza 124 which used in disease control. Osmotic method was more detectable to the presense of *D. graminea* than the agar plate method in two cultivars. The data showed significant differences between osmotic and agar plate in case of grain surface sterilization. The percent of infected grain was higher in Giza123 than in Giza124 in all tested methods.

8- Five preliminary concentrations from all tested fungicides were tested on linear growth of *D. graminea in vitro* to choose the range of effective concentrations of each one. It is worthy to notice gradual increase of inhibition (%) by increasing concentration of tested fungicides. Results proved that the two fungicides *i.e* Opus 12.5% and Switch 62.5% gave complete inhibition (100%) of *D. graminea* growth at 1mg/L concentration, while it was recorded by Bellis 38% at 2mg/L. On the other hand, the highest reduction in linear growth was obtained at 12mg/L concentration of Collis 30% (88.9%) and Uniform 39% (84.8%)**.** IC_{50} was calculated

according linear relation inhibitory using EPA probit analysis program version 1.5, where lower IC_{50} value had higher fungicidal activity. Opus 12.5%, Switch 62.5% and Bellis 38% were the most effective fungicides according to IC_{50} values *i.e.* 0.127, 0.230 and 0.371 mg/L, respectively. Data also showed the minimum inhibition concentration (MIC >90%) were observed when the culture was amended with 0.8mg/L in Opus 12.5% and Switch 62.5% and more than 1mg/L and 12mg/L in the other three fungicides.

9- Effect of different concentrations of three plant oils on growth of *D. graminea* was affected with different degrees by adding each of the three plant oils tested. Data indicated that different concentrations of plant oils significantly inhibited linear growth of *D. graminea*. The highest inhibition was observed when the culture was amended with 15% of Coriander (84.44%), Marjoram (77.78%) and Caraway (67.80%) oils. The effect of the tested plant oils could be arranged according to the IC_{50} values in the following descending order, Coriander, Marjoram and Caraway. MIC values were obtained when the media was amended with more than 15% of the three plant oils**.**

10- The antagonistic activity of two bio- agents *i.e. Trichoderma harzianum* and *Bacillus subtilis* were assayed against *D. graminea* by dual culture technique. The results revealed that, the both bio-agents tested were affected inhibiting the mycelia growth of *D. graminea*. *T. harzianum* was most effective in mycelial inhibiting than *B. subtilis*. Data also showed that degree of inhibiting was maximum with *T. harzianum* (70.52%) after 4 days, but it was 55.55% with *B. subtilis* after 7days. In 4^{th} day of culture, the growth of *T. harzianum* contacted with the pathogen. At 7^{th} day *T. harzianum* was over grown the pathogen and severely inhibited the growth of the pathogen.

11- All tested concentrations of acetic acid were tested on the growth of *D. graminea*, gave full inhibition (100%) of linear growth.

12-Concerning the effect of tested fungicides on the barley leaf stripe disease incidence under greenhouse condition, data indicated that highly significant differences ($P \leq 0.5$) in disease incidence percent were observed between all fungicides and control (without fungicides) when applied at three concentrations in the Giza123 cultivar. Tested fungicides showed significant differences between their concentrations meanwhile, the highest efficacy (100%) were obtained when the seeds were applied with Opus 12.5% (at 2ml and 4ml/kg), Switch 62.5% (at 2g/kg) and Bellis 38% (at 1g/kg)**.**

13-As for the effect of plant oils on the barley leaf stripe disease incidence under greenhouse condition, results revealed that all the tested plant oils had significantly reduced in the disease incidence (%) compared to control. The most effective treatments were Coriander followed by Marjoram and Caraway oils. Tested plant oils showed significant differences between their concentrations in disease incidence percent. The most effective concentration in all tested plant oils was 15ml/kg which reduced the disease incidence (%) from 39.5% (in control) treatment to 14.88% (in Coriander treatment), 18.93% (in marjoram treatment) and 23.44 (in Caraway treatment).

14-The effect of the two bio-agents at three concentrations (at the rate of 1L:100L water, 1L:75L water and 1L:50L water prepared from original concentration "$30X10^6$ CFU/mL") on the response of barley plants against *D. graminea* infection under greenhouse conditions was conducted during 2016/2017. *Trichoderma harzianum* and *Bacillus subtilis* had a positive effect reducing incidence of leaf stripe. Data indicated that higher concentration (1L:50L water) of the two bio-agents lead to the highest efficacy in controlling disease incidence. *T. harzianum* was in general the best in reducing incidence compared with *B. subtilis*.

15-Also, effect of different combinations of doses and concentrations of acetic acid as seed treatment were tested on two barley cultivars for estimating the percent of leaf stripe incidence under greenhouse condition.. Results proved that using dilutions of acetic acid has significant control of the pathogen

without causing phytotoxicity. Data also indicated that disease incidence was reduced by 68.91% at the dose of 40ml/kg at 100% concentration of acetic acid.

16-Effect of treating Giza123 barley cultivar under greenhouse condition with five fungicides, three plant oils, two bio-agents and acetic acid as seed dressing application on activity of peroxidase (PO), polyphenole oxidase (PPO) and chitinase enzymes were estimated. Data indicated that all tested treatments positively increased the activities of Po, PPO, and chitinase enzymes in leaves of barley plants comparing with control treatment. The highest effective treatment on PO, PPO and chitinase enzymes with fungicides was recorded in Opus 12.5% followed by Switch 62.5%, Bellis 38%, Collis 30%, Uniform 39%, respectively. In case of plant oils treatment, the enzymes activity were moderately effective comparing with control treatment, but the highest activities between plant oils were recorded with Coriander, Marjoram and Caraway, respectively**.** On the other hand, the least activities of peroxidase, polyphenole oxidase and chitinase were recorded with biological control treatments *Trichderma harzianum* and *Bacillus subtilis*, respectively comparing with control. Also, results indicated that all application with combination of acetic acid between dose and concentration positively increased the activities of PO, PPO and chitinase enzymes in leaves of barley treatments comparing with control. In this respect, the highest effective on PO, PPO and chitinase enzymes with acetic acid was recorded in combination between 40 ml/kg and 100% in the tested cultivar. Meanwhile the least activities of PO, PPO and chitinase were recorded with combination between 10ml/kg and 30%.

17-Effect of treating Giza123 barley cultivar with five fungicides , three plant oils, two bio-agents and acetic acid as seed dressing application on activity of total, free and conjugated phenols was tested. Data indicated that all tested treatments positively increased the activities of total, free and conjugated phenols in leaves of barley plants comparing with control treatment. The highest effective treatment on total, free and conjugated

phenols with fungicides was recorded in Opus 12.5% followed by Switch 62.5%, Bellis 38%, Collis 30% and Uniform 39%, respectively. As for the effect of plant oils, the highest activities between plant oils were recorded with Coriander, Marjoram, Caraway oils, respectively. However, the least activities of total, free and conjugated phenols were recorded with *T. harzianum* and *B. subtilis* treatment, respectively. Also, results revealed that all application with combination of acetic acid between dose and concentration positively increased the activities of total, free and conjugated phenols in leaves of barley plants comparing with control. In this respect, the highest effective on total, free and conjugated phenols with acetic acid was recorded in combination between 40ml/kg and 100%. On other hand, the least activities of total, free and conjugated phenols were recorded with combination between 10ml/kg and 30%.

18- Concerning control of barley leaf stripe disease and yield parameters under open field conditions, experiments were carried out on barley plants (cv. Giza 123) naturally infected with barley leaf stripe disease under open field conditions at two locations *i.e.* the Experimental Station, Agriculture Research Centre, Giza, and Kafr-Elhmam Station, Agriculture Research Centre, El-Sharquia governorate during the successive winter growing season 2016/2017 to evaluate the efficacy of different tested treatments against barley leaf stripe disease caused by *D. graminea*.

19- Five different fungicides were tested against barley leaf stripe disease caused by *D. graminea* for their efficacy on disease incidence % and yield parameters at two locations *i.e.* Kafr-Elhmam and El-Giza Station during the growing season 2016/2017. Data revealed that, all treatments significantly reduced the disease incidence % in comparing with control. In this respect, Opus 12.5% treatment recorded the highest disease incidence reduction (0.0%) at both locations followed by Switch 62.5%, Bellis 38%, Collis 30% and Uniform 39%, respectively. Regarding the effect of tested fungicides on barley yield parameters, results prove that, leaf stripe disease had an extreme effect on the yield parameter in untreated barley plants. On

the other hand, all fungicide treatments significantly increased the assessed yield parameters in comparing with control treatment. Opus 12.5% treatment recorded the most effective fungicide in increasing all yield parameters at the two stations, followed by Switch 62.5%, Bellis 38%, Collis 30% and Uniform 39%.

20- Also, data revealed that, three commercial plant oils were screened against the barley leaf stripe disease caused by *D. graminea* under open field conditions during the growing season 2016/2017. The results reveal that, all tested treatments significantly reduced leaf stripe disease incidence % compared with control treatment. The lowest disease incidence in both stations *i.e.* Kafr-Elhamam and El-Giza was recorded on plant treated with Coriander oil (13.25%, 10.33%, respectively) followed by marjoram oil (19.00%, 15.60%, respectively) and Caraway oil (22.33%, 19.12%, respectively).Considering the effect of the tested plant oils on yield parameters, decreasing in disease incidence correlated negatively with assessed yield parameters. In this respect, Coriander oil treatment at the two locations (Kafr-Elhamam and El-Giza) recorded the highest yield parameters *i.e.* plant height (79.73, 81.04 cm), grains number/spike (32.0, 34.0), grains weight/spike (0.97, 1.05 g) and 1000 kernel weight (29.00, 31.00g) compared with untreated control (49.26, 50.00 cm), (18.45, 19.33) (0.54, 0.61 g) and (11.86, 12.07 g), respectively.

21- Also, data showed that, the barley plants treated with the two tested bio-agents (*Trichoderma harzianum* and *Bacillus subtilis*) resulted suppression in disease incidence as well as increment in yield component. They successfully manage the disease in the field. Using *T. harzianum* and *B. subtilis* recorded disease incidence 16.25%, 13.33% and 19.86%, 16.35% at Kafr-Elhmmam and El-Giza, respectively. As for yield components, results indicated that the two tested bio-agents increased yield components compared with control plants. The highest effect in both stations was recorded with *T. harzianum* application on plant height (70.87, 72.20 cm), grains number/ spike (27.27, 29.00), grains weight /spike (0.70, .85 g) and

weight of 1000 grains (25.00, 25.77 g) at Kafr-Elhmam and El-Giza, respectively.

22-In field trial, seed treatment with acetic acid reduced leaf stripe incidence. Data proved that the application with different combinations between doses and concentrations of acetic acid gave significant effect in reducing disease incidence. The highest efficacy (65.40%, 66.17%) in reducing disease incidence was recorded when barley plant treated with dose 40ml/kg of concentration 100% of acetic acid at Kafr-Elhmam and El-Giza, respectively. Besides, the four yield components tested were improved in two stations comparing with control plants. Results in such tables indicate that the heights effect on four yield components in both stations was observed when 40 ml/kg of 100% concentration of acetic acid applied on barley plant, as a mean at two stations caused an increase in plant height (80.00 cm), number of grains /spike (32.17), weight of grains/spike (0.96 g) and weight of 1000 grains (29.30 g). The effect of other tested combinations could be arranged in ascending order as 10ml, 20ml and 30ml /kg of 100% concentration. Seed treatment with different combination of dose and concentration of acetic acid reduced significantly disease incidence and increase yield components under field conditions in this study. The highest efficacy was recorded when barley treated with dose 40ml/kg of concentration 100% of acetic acid. Meanwhile, the percent of disease incidence was decreased from 32.5% in control to 11.12% at Kfr-Elhmam and from 12.07g to 30.33g at El-Giza.