Kafrelsheikh University
Faculty of Agriculture
Agricultural Botany
Department

Pathological and Biochemical studies on controlling of Cercospora leaf spot disease in sugar beet plants

By

Shimaa Anwar Darwesh EL-Emary

B.Sc. (plant pathology), Fac. Agric., Tanta University, 2003, Egypt
M.SC. (plant pathology), Fac. Agric., Kafrelsheikh University, 2013, Egypt

THSIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy in Agriculture Science
IN
Plant Pathology

Agricultural Botany Department
Faculty of Agriculture
Kafrelsheikh University

(2022)

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPREVIATIONS

APX	ascorbate peroxidase
DHA	dehydorascorbate
DHAR	dehydorascorbate reductase
GSH	reduced glutathione
HR	hypersensitive response
MDA	monodehydroascorbate
MDHAR	monodehydroascorbate reductase
PM	plasma membrane
PR	pathogenesis-related
BABA	ß-Amino-n-butyric acid
dpi	Days post inoculation
GC	Giant cell
J2	Second-stage juveniles
PBS	Phosphate-buffered saline
INA	Isonicotinic acid
RCPD	Randomized complete plot design
EC50(IC50)	The effective concentration of treatment that inhibited C. beticola by 50%
Ext.	Extract
L.G.	linear growth
KOH	Potassium Hydroxide
BABA	Beta- Amino butyric acid
OXA	Oxalic acid
TCZ	Tetraconazole
TPM	Thiophanate methyl
CSA	Copper sulfate anhydrous
EMOE	Ethanol *Moringa oleifera* extract
EAVE	Ethanol *Ammi Visnaga* extract
EPNE	Ethanol *Piper nigrum* extract
ESO	*Eruca sativa* Oil
SAO	*Syzygium aromaticum* Oil
JOO	*Jasminum officinale* Oil
RCO	*Ricinus communis* Oil

EOs	**Essential oils**
g	Gram
ROS	Reactive oxygen species
BTH	Benzothiadiazole
RAPD	Randomly amplified polymorphic DNA
SA	Salicylic acid
SAR	Systemic acquired resistance
H_2O_2	Hydrogen peroxide
O_2.-	Superoxide
SOD	Superoxide dismutase
CAT	Catalase
APX	Ascorbate peroxidas
PPO	Polyphenol oxidase
w/v	Weight/ volume
%	Percentage
mM	Mille mole
TRIS	Tris buffer
UV	Ultra violet
CO_2	Carbon dioxide
POX	Peroxidase
fed	Feddan
Kg/fed.	Kilogram/ feddan
Min.	Minute
˚C	Celsius
Sec.	Second
Hr.	Hour
mg/g	Milligram/ gram
v/v	Volume/ volume
mL	Mill liter
nm	Nanometer
SD	Standard deviation
CVS	Sugar beet cultivars
CLS	Cercospora leaf spot
C. betiola	*Cercospora beticola*
Cont.	Control
AChE	Acetyl cholinesterase
ABA	abscisic acid
PHE	Phenol

6- SUMMARY

Sugar beet (*Beta vulgaris* var. *saccharifera*) is belongs to Chenopodiaceae family and recently considers the most important sugar crop for sugar production in Egypt. Sugar beet crop is conceded to be most promising and economically crop around the world. The global cultivated area from sugar beet is about 10.6 millions feddan while it arrival about 481 thousand feddan in Egypt which produced 1.248 million tons of white sugar to contributed 57.7 % from sugar production in Egypt.

Sugar beet plants attacked with many diseases throughout growth periods such as seedling damping off disease which caused during the first growth stage in plant life, leaf spot diseases are consider the most attacking sugar beet plants and effect on root weight, sugar percentage and consequently the amount of sugar yield production.

The survey was carried out in the average 2015/2016 and 2016/2017 growing seasons in different locations in Northern and mid - Delta of Egypt i.e. Kafr El- Sheikh, El-Behaira, El-Gharbia, governorates. Seven districts from Kafr El- Sheikh governorate and three districts from both El-Gharbia and El- Behaira governorates as well as one location from of tested were chosed.

The highest disease severity (77.6 and 75.2%) was noticed on sugar beet at El-Hamoul and Sidi-Salem districts, in Kafr El-Sheik governorates. While the lowest disease severity (41.7 %) was recorded on sugar beet at Damanhour district.

The causal organism of the disease were isolated from all location the surveyed Kafr El- Sheikh, El-Behaira and El-Gharbia, governorates , these isolates were identical in this morphological characteristics on sugar beet leaf extract dextrose agar medium.

Data indicate that, the number of isolates was isolated, 2 and 1 isolates when the highest disease severity (78.4and 77.8%) were noticed on sugar beet at El-Zafaran and Sakha location, kafrelsheikh District, respectively. While, the number of isolates, 2, 8 isolates was lowest the disease severity (33.2% and 38.7%) were recorded on sugar beet at El-Mandoura and Damanhour location.

Results showed that, all the tested plant extracts have reduced significantly the linear growth of *C. beticola.* Increasing the concentration of both the plant extracts reduced the linear growth significantly. ethanol *Piper nigrum* extract and ethanol *Ammi Visnaga* extract by concentrations, 1, 10 and 20 ppm were more effective Plant extracts tested. There percentage of inhibition reached, 46.7% and 44.4 %, respectively.

Results Show that, all the tested essential oils have reduced significantly the linear growth of *C. beticola.* Increasing the concentration of both the reduced the linear growth significantly. *Ricinus communis* oil by inhibited concentrations, 1 10 and 20 ppm were the most effective by inhibition 52.2 %, while, *Eruca sativa* oil occupied the 2th rank in inhibition the liner growth of *C. beticola*, the percentage of inhibition 46.7 %, followed by, *Syzygium aromaticum* oil and *Jasminum officinale* oil, respectively.

Results indicate that, all the tested chemical inducers resistance have reduced significantly the linear growth of *C. beticola.* Increasing the concentration of both the **chemical inducers resistance** reduced the linear growth significantly. potassium hydroxide (KOH) and salicylic acid (SA) by inhibited concentrations, 1, 10 and 20 ppm were the most effective by inhibition 62.2 and 52.0 %, respectively.

Results showed that the fungicide Eminent was the most effective

fungicide against *Cercospora beticola* with IC $_{50}$= 1.2 ppm, followed by **Topsin M-70** and **Crunch** with IC_{50}= 3.2 and 47.0 ppm, respectively.

Data showed that ethanol *Piper nigrum* extract was the most toxic solvent extract against *Cercospora beticola* (IC_{50}= 15.0 ppm), followed by ethanol *Ammi Visnaga* extract (IC_{50}= 21.0 ppm) and ethanol *Moringa oleifera* extract (IC_{50}= 50.0 ppm).

Data showed that potassium hydroxide (KOH) was the most toxic chemical inducer resistance against *C. beticola* (IC_{50} = 1.2 ppm), followed by, salicylic acid (SA) (IC_{50} = 5.2 ppm),

Data showed the joint toxic effects against on the tested fungi. The values of co-toxicity factor indicated that additive effects were (KOH + TCZ) , (KOH + SA+ EAVE + EPNE + RCO) and (KOH + SA**)**, the values of co- toxicity factor of these combinations were additive effect, -8.9, -9.0 and -16.7%, respectively.

Data showed that the tested fungicides significantly reduced disease severity of *cercospora beticola* in comparison with the untreated plants on sugar beet.

As regard to disease severity%, (TCZ), (TPM) and (CSA) were the most effective fungicides against *C. beticola*, 15.0, 20.0 and 30.0 % compared to untreated control 60.0%, respectively, at mean two seasons, (2018-2019 and 2019- 2020).

As for the efficiency % of test fungicides, (TCZ), (TPM) and (CSA) were the highest efficiency% on disease reduction and recorded 75.0, 66.7 and 50.0%, respectively.

The chlorophyll content of test fungicides, (TCZ), (TPM) and (CSA) were the highest content recorded on leaves, 33.0, 30.9 and 21.7, respectively to untreated control 14.0 at mean two seasons, (2018-2019 and 2019- 2020).

Data showed that the tested fungicides significantly of reduced

disease severity of *C. beticola* in the comparison with the untreated plants on sugar beet.

As regard to leaf area (cm^2), fungicides of (TCZ), (TPM) and (CSA) increase the leaf area, 254.52, 237.80 and 194.51 cm^2, respectively, at mean two seasons, (2018-2019 and 2019- 2020).

As recorded to total soluble solids (TSS%), treatments of (TCZ), (TPM) and (CSA) increase the (TSS%) were, 22.01, 21.8 and 21.1 %, respectively, compared to control was, 20.1%.

As for sucrose %, treatments with tested fungicides, (TCZ), (TPM) and (CSA) were 17.53, 17.08 and 15.50 %, , respectively, compared to untreated control was, 13.92% at mean two seasons.

As for sugar content and purity, the tested fungicides, (TCZ), (TPM) and (CSA) were, 79.4, 78.3 and 73.4 %, respectively, compared to untreated control, 69.1 %.

All treatments showed significant differences between enzyme activity of leaves after spraying, 72 hour during the mean at two seasons. Catalase activity was the best treatments were within 72 hour of spraying, (TCZ), (TPM) and (CSA), respectively, compared to untreated control.

Peroxidase activity was the best treatments within 72 hour of spraying were (TPM), (TCZ) and (CSA), respectively, compared to untreated control.

Polyphenol oxidase enzyme activity, the best treatments were within 72 hours of spraying of (TCZ), (TPM) and (CSA), respectively, compared to untreated control.

As for the efficiency % of test Plant extracts, (EPNE), (EAVE) and (EMOE) were the highest efficiency% on disease reduction and recorded 66.7, 58.3 and 25.0 %, respectively.

The chlorophyll content of test Plant extracts, (EPNE), (EAVE) and (EMOE) were the highest content recorded on leaves, 28.0, 27.5 and

21.6, respectively, compared to untreated control 14.0 at mean two seasons, (2018-2019 and 2019- 2020).

Data showed that the tested Plant extracts significantly of reduced disease severity of *C. beticola* in the comparison with the untreated plants on sugar beet.

As regard to leaf area (cm^2), fungicides of (EPNE), (EAVE) and (EMOE) increase the leaf area, 267.42, 219.35 and 191.43 cm^2, respectively, at mean two seasons, (2018-2019 and 2019- 2020).

Data showed different of combined treatments significantly effected of enzyme activity of Catalase, Peroxidase and Polyphenol oxidase enzyme compared with the untreated plants on sugar beet during mean at two seasons.

All chemical inducer showed significant differences between enzyme activity of leaves after spraying, 72 hour during the mean at two seasons.

Screening trials of the infested fields with cercospora leaf spot disease resulted in four isolates of *Cercospora beticola*. Pathogenicity of these isolates showed comparable degrees between 98–100% against sugar beet plants cv. Pleno. So, one of them coded as Cer1was selected as main isolate for the further experiments.

Due to their antagonistic effect, *Bacillus subtilis* (B1) and *Bacillus subtilis* B_2 were selected for the field experiments. For fungal antagonists, *Trichoderma koningii* T1 was ranked in class 1, by which growth of the pathogen was strongly suppressed of 88.12 %. *Epicoccum nigrum* 1 and *E. nigrum* 2 were ranked at class 2 with about two third inhibition of the pathogen, each (62.17 and 64.47 %, respectively).

In vitro results indicated that the majority of the tested bioagents i.e., *Bacillus subtilis* (B1), *B. subtilis (*B2), *Trichoderma koningii* T1, *Epicoccum nigrum* E1 and *E. nigrum (*E2) were found to have a great ability to inhibit growth of the fungal pathogen.

In the natural infested field experiment, chemical fungicide and biological antagonists were also verified using spraying treatment during 2018/2019 and 2019/2020 seasons. Disease severity (DS) were estimated as indication for the disease index parameters using percentage units. As well as, enzyme activities of polyphenol oxidase (PPO), peroxidase (POX) and catalase (CAT), chlorophyll, sucrose, TSS and root yield were also determined. Applicability of the tested control agents of sugar beet cercospora leaf spot disease in the natural infested open field.

For the bacterial antagonist, *Bacillus subtilis* (B1) showed low percentages of disease severity (27.0 and 23.0 %) compared with 60.0 and 65.0 % for control during both seasons, respectively. Accordingly, reduction of disease severity due to the other treatments showed lower magnitudes.

It illustrated that all treatments were pronounced in comparison with control of all enzymes. As well as, lower activation levels of PPO in comparison with POX were noticed. It showed also that *T. koningii* T1 followed by *B. subtilis* (B1) induced great activations of POX, PPO and CAT, respectively. Data indicated that phenols were oxidized by *T. koningii* and *B. subtilis* (B1) higher than Eminents for controlling *C. beticola*.

Under open field conditions, tested control agents were also evaluated during the two seasons via investigate their effects on some plant growth and yield parameters . T.S.S, sucrose%, Chlorophyll, root productivity data showed superiority of all treatments compared with control. It might be due to induce formation of some substances in the plants, by which

sugar beet plants become strong under pathogenic conditions. In comparison with all tested treatments, Eminent fungicide followed by *T. koningii* (T1) and *B. subtilus* (B1) considered the superiors, by them the estimated parameters reached their maximal. For TSS, differences between treatments were less significant and varied from its maximum due to Eminent to minimal by *Epicoccum nigrum* (E2) during both seasons. Sucrose concentrations were reached their maximal of 17.5 - 17.6 % due to Eminent during both seasons, respectively. It was followed by *Trichoderma koningii* (T1), by which sucrose reached 16.9 and 17.1 during 2018/2019 and 2019/2020, respectively.

For total chlorophyll, superiority of Eminent fungicide followed by *T. koningii* T1 and *B. subtilus* (B1) was also done, indicating enhancement of physiological activities. Data were well reflected to increase productivity of root yield of sugar beet during both seasons. In which, total yield was reached its maximal of 43.75 and 48.33 ton fed.$^{-1}$ due to Eminent and *T. koningii* T1, each during both seasons, respectively, indicating great superiority.