Journal of Sustainable Agricultural Science
http://jsas.journals.ekb.eg/

Potential Impacts of Eminent Fungicide, Certain Bacterial and Fungal Antagonists for Controlling Cercospora Leaf Spot Disease in Sugar Beet.

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Applicability of some isolates and Eminent for control sugar beet cercospora leaf spot disease which cused by C.*ercospora beticola* was inestigated. The fungicide, Eminent was the superior *in vitro* treatment in suppressing growth of the pathogenic fungus. *Bacillus subtilis* (B1), *B. subtilis* (B2) and *Trichoderma koningii* (T1) were the most antagonistic against the pathogen. Mycelia of *Cercospora beticola* were *in vitro* partially prevented due to application of Eminent, by which its IC_{50} reached 1.20 ppm. Whereas 88.12 % of the pathogenic fungus were inhibited due to *Trichoderma koningii* (T1). Additionally, Relative power of antibiosis (RPA) reached their maximal of 2.11 by *B. subtilis* (B1). Under field conditions, disease severity was reduced to 20.0 and 21.0 % by Eminent during both seasons, respectively, followed by *B. subtilis* (B1) and *T. koningii* (T1). Efficiencies of the tested *Epicoccum nigrum* isolates for controlling sugar beet Cercospora leaf spot disease were not as expected. The enzyme activities of Peroxidase (POX), Catalase (CAT), and Polyphenol oxidase (PPO) was increased in sugar beet to control *C. beticola*, and induce systemic acquired resistance. Accordingly, chlorophyll content, total soluble solids (T.S.S), sucrose % and root productivity per ton fed.$^{-1}$ were also enhanced due to the use the superior control of Eminent, *Trichoderma koningii* (T1) and *Bacillus subtilis* (B1), respectively.

Keywords: *sugar beet*, *Cercospora beticola*, Biocontrol agents، Eminent, Enzymes.

1. Introduction

Cercospora leaf spot disease(CLS) caused by *Cercospora beticola* Sacc. (Mukhopadhyay and Rao, 1978) is one the most damaging disease in sugar beet fields worldwide and affect more than a third of the growing regions with sugar beet (Weiland and Koch 2004; Kaiser *et al*., 2010). Sugar beet is subject to attach certain diseases that affect dramatically sugar productivity (windels *et al*, 2004). Cercospora leaf spot disease caused by *Cercospora beticola* has been considered among the most common fungal leaf pathogens in sugar beet fields worldwide. The causal pathogen is responsible for significant reduction in the root yield as well as for decrease sucrose content and juice purity in the affected roots (Elfahar, 1997; El-Kazzaz, 2002; Elfahar. 2003; Morsy, 2022).

Sugar beet cercospora leaf spot disease causes gradual destruction against the photosynthesis apparatus, decreasing root yield and sugar content. It causes significant economic losses up to 43% in total sugar beet productivity (Skaracis *et al*., 2010). Application of new agricultural techniques, rotation with non-host crops, using a resistant sugar beet cultivar and frequent use of fungicides can help in controlling the disease (Tedford *et al*., 2019; Morsy *et al*. 2022). *Cercospora beticola* can survive up to 22 months in the soil, depending on soil depth (Khan *et al*., 2008).

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Received: 27/06/2022; Accepted: 01/08/2022
DOI: 10.21608/JSAS.2022.147464.1362

Biological control is the most effective alternative and environmentally safe strategy instead of chemical compounds (Derbalah *et al*., 2013). *Bacillus mycoides* can reduce the symptoms of Cercospora leaf spot (Bargabus *et al.*, 2002 and Derbalah, *et al.*, 2013). Also, the well-known *Trichoderma* spp were found to be the most antagonistic fungal agent successfully used in controlling different plant diseases. Competition, antagonism, hyper parasitism, Induction of and defence responses are the most well-known mechanisms due to *Trichoderma* spp. (Harman *et al*., 2004; Galletti *et al.*, 2008). Role played by *Trichoderma*, *Epicoccum* and *Bacillus* antagonists in reducing symptoms of different plant diseases were formerly applied by Hjeljord *et al*. (1998), Tronsmo and Hjeljord (1998), Shalaby *et al*. (2013), Shalaby *et al*. (2014) and Shalaby *et al*. (2015). Upregulation of catalase, peroxidase and polyphenol oxidase is very important in the defensive system of host plants under pathogenic conditions (Hatcher, 1995).

The application of chemical fungicides causes harmful effects to the environmental system and human health, so alternative sources of resistance must be provided. Therefore, the presented work aimed to evaluate the potential impacts of Eminent fungicide and certain biocontrol agents on *Cercospora beticola* to reduce symptoms of Cercospora leaf spot disease and enhancing productivity of sugar beet plants.

2. Materials and Methodes

The presented work was conducted during 2017/2018 to 2019/2020 growing seasons at Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University and at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt.

Tested Materials

Fungicide

The tested fungicide used in this study were Tetraconazole with trade name Eminent (EME) EC 12.5% and common name tétraconazole (m) F-ISO); tetraconazole (BSI, E-ISO). IUPAC name (RS)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl) propyl1,1,2,2-tetrafluoroethyl ether.

The sensitivity of *C. beticola* to Eminent was *in vitro* tested by calculating the effective concentration of Eminent that have ability to inhibit 50% of its growth in relative to control treatment which represented by IC_{50} (Weiland and Halloin, 2001; Karaoglanidis and Bardas, 2006). To determine its sensitivity to Eminent, mycelial growth of *C. beticola* was categorised based on IC_{50} of Eminent according to Karaoglanidis and Ioannidis (2010); Secor *et al*. (2010) as follows: S, sensitive (< 1 ppm); RS, reduced sensitivity (1–10 ppm); MI, moderately insensitive (10–50 ppm); I, insensitive (50–100 ppm); and R, resistant (> 100 ppm).

Biocontrol agents

Four bacterial strains of *Bacillus subtilis* (B1), *B. subtilis* (B2), *B. subtilis* (B3), *B. subtilis* (B4), and three fungal species of *Trichoderma koningii* (T1), *Epicaccum nigrum* (E1) and *E. nigrum* (E2) as biocontrol agents were kindly obtained from Plant Pathology Research Institute, Agricultural Research Centre (ARC), Giza, Egypt.

Isolation, Identfication, Pathogencity of the pathogen

Pathogenic fungus of Cercospora leaf spot disease was isolated from collected leaves of sugar beet (Pleno cultivar) with typical symptoms from Sakha Agricultural Research Station. To isolate the pathogenic fungus of Cercospora leaf spot disease, up to 20 infected leaves were collected and disinfected with a 10% Na ClO solution (v/v) during 2017/2018, 2018/2019 and 2020/2021seasons. Leaf samples were placed in plastic bags with damp paper towels to maintain the humidity near 100% under fluorescent lamps with an 8-h photoperiod at 24 °C for 7 d to promote ovulation. Spore-bearing lesions of the same size and ovulatory stage were selected. Conidia of the pathogenic fungus were transferred to glass Petri dishes (9 cm) containing sugar beet leaf extract (100 ml), dextrose (20 g) and Agar (15 g) medium (SBLEDA), Streptomycin was added to the to prevent the contamination. Petri dishes were incubated at 27 ± 2 °C for 7-10 days and inspected daily for signs of fungal growth. The fungi were examined and purified using the hyphal tip technique (Dhingra and Sinclair, 1995). The isolates were screened to avoid loss of sensitivity caused by sub-culturing and/or long-term storage (Morsy *et al.*, 2022). According to Alexopoulos and Mims (1979). The tested isolates were found to be belonged to *Cercospora beticola* according to cultural, phytopathological and microscopic properties, one of them was selected for the further studies. Pathogenicity test was carried out in 30 cm diameter pots under greenhouse condition. Pots were filled with sandy-loam (1:2 w/w).Pure fungal isolate from diseased plants was tested for its pathogenicity using Pleno sugar beet cultivar, against the sensitive cultivar to C. beticola. The isolates were growing in liquid CZ-apeks medium and incubated at 27±2°C for 15 days to obtain the required inoculate. Ninety days-old plants were sprayed with 50 x 10^3 conidia (spore/ml) of each isolate using an atomizer according to Crane and Calpouzos (1984) in four replicates, comprising four plants for each. Before inoculation, plants were sprayed with water two make a thin film of water on the leaf surface. Two grams sucrose and 0.1 ml tween 80 per litre were added to spore suspension to enhance infection. Inoculated

plants were kept in most polyethylene champers for 7 days.

***In Vitro* antagonistic trials**

Bacterial antagonists

Petri dishes (9 cm in diameter) of PDA-medium (15 ml/dish) were inoculated in their center with agar discs (5 mm) bearing mycelium of 15-days-old cultures of *Cercospora beticola*. Each plate was inoculated periphery by standard amounts of the tested 4 bacterial strains using sterile toothpicks. Plates inoculated with the pathogen without antagonists were used as control. Experiments were represented by five replicates. Plates were incubated at 27±2°C until full growth of the control treatment. The diameter of the inhibition zone surrounding each antagonistic agents was recorded, and the Relative Power of antibiosis (RPA) for each strain was estimated by the ratio described by Ibrahim *et al.* (1987) as follows: RPA = Z / C

Where:

Z = Diameter of inhibition zone.

C = Diameter of spotted antagonistic isolate.

Fungal antagonists

Antagonistic efficacies of the three well known fungal species against *C. beticola* were tested using the dual culture method. Agar discs (5 mm in diameter) bearing mycelium of 7-day-old cultures of one of the isolated fungal antagonists and *C. beticola* were placed on the opposite sides on15 ml PDA-medium in Petri-dishes, plates containing *C. beticola* alone were used as control. Plates of control were incubated at 27±2 °C until full growth. Degree of antagonism was scored based on the well-known scale of 1–5 classes (Bell *et al*.,1982)

Percentage inhibition (I %) was calculated according to the formula adopted by Topps and Wain (1957) as follows:

$$I\ \% = [(D1-D2) / D1] \times 100$$

Where I% is the percentage inhibition, D1 is the growth of the pathogen in the absence of antagonist (control), while D2 is the growth of the pathogen in the presence of antagonist (treatment).

Field trials

The field experiments were performed in a randomized complete block design with four replicates, each replicate contained 6 rows with a length of 900 cm and width 60 cm. Each row contained 45 mounds with an interval of 20 cm. All recommended agricultural practices were implemented in a timely manner. Plants naturally infected with leaf spot disease were sprayed with each treatment. Spraying was started when symptoms of the disease were detected (90 days after planting). Untreated plots were acted as control. All treatments were applied three times with an interval of 10 days between each one. Bacterial antagonists were sprayed by using 0.8×10^8 CFUml^{-1} each. As well as *Trichoderma koningii* (T1), *Epicaccum nigrum* (E1) and *E. nigrum* (E2) were sprayed by using 0.2×10^7, 0.5×10^7 and 0.8×10^7 spore ml^{-1}, respectively.

The disease severity (DS %) was recorded according to Verreet *et al.* (1996). Efficacies of the tested treatments were expressed as percentages and calculated based on the following equation:

Efficacy% = ((DS% in control – DS% in treatment) / DS% in control) × 100

Fresh leaf and root weights as well as total soluble solids (T.S.S %) in fresh roots of sugar beet were estimated using a manual refractometer according to McGinnis (1982). Sucrose was estimated according to Delta Sugar Company laboratories (Kafr El-Sheikh Governorate, Egypt) according to A.O.A.C. (1990). Yield (t fed^{-1}) was determined for a random subsample of 10 roots representing approximately 27–72% of the total harvest, depending on root size. The average root weights were evaluated to calculate the average number of plants per unit area (Mahmoud *et al.*, 2012). The chlorophyll content was determined using method of Moranr (1982).

Enzyme activities

Activity of Peroxidase (POX), Catalase (CAT), and Polyphenol oxidase were assessed by homogenizing 0.5 g of fresh leaves at 0–4 °C in 3 ml of 50 mM TRIS buffer (pH 7.8) containing 1 mm EDTA-Na2 and 7.5% (w/v) polyvinylpyrrolidone (PVP) made. Homogenizers were centrifuged at 17.709 g (20 min. 4 °C). Total soluble enzyme activity was measured using a spectrophotometer, at 480 nm for peroxidase activity, and at 440 nm for catalase activity (Hafez, 2014). CAT activity was determined according to Aebi, (1984). POX was determined according to Hammerschmidt *et al.* (1982). PPO activity was measured according to the method described by Malik and Singh (1980).

Chlorophyll contents

The concentrations of chlorophyll pigments (chl.a, b, and total chl.) were calculated as follows: 1 cm^2 from leaf after 120 days from planting in both seasons. The pigments were extracted with 5 mL N-N dimethyl formamide and then kept in the dark bottle for 24 hours in the refrigerator. The absorbency of the chosen samples was measured using a spectrophotometer at 664 (nm) and 647 (nm) wavelengths. According to **(Moran, 1982),** the concentration of photosynthetic pigments was determined as follows:

1- Chl. (a) = $12.64\ A_{664} - 2.99\ A_{647}$. µg mL^{-1}

2- Chl. (b) = $23.26\ A_{647} - 5.6A_{664.}$ µg mL^{-1}

3- Total Chl. = $7.04\ A_{664} + 20.27\ A_{647}$ µg mL^{-1}

So, chlorophyll contents in relative to the leaf area per µg (cm^2)$^{-1}$ were recalculated. Then using the following formula: -

Chl. (µg / cm^2) = reading (µg / ml) x

$$\frac{\text{Extraction solution volume (ml)}}{\text{Sample area (cm}^2\text{)}}$$

Statistical analysis

Analysis of variance (ANOVA) and the Least Significant Difference (LSD) test was used. Differences were considered significant at $P = 0.05$ and highly significant at $P = 0.01$ (Gomez and Gomez, 1984). The data in Table 2 and Figure 2, 3,4,5,6,7 and 8 were subjected to ANOVA, followed by Duncan's multiple range test at $P = 0.01$. Data were processed in Computer Statistical Package (CoStat) v6.45.

3-Results and dissection

***In Vitro* antagonistic trials**

Pathogen:

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 *Cer1*was selected as main isolate for the further experiments.

Biological antagonists:

Efficiency of the selected bacterial and fungal antagonists against *C. beticola* was determined using standardized tests. Relative power of antibiosis (RPA) for the tested four bacteria and inhibition of the linear growth for the three fungi were represented in **Table (1)** and **Fig. (1**).

Table 1. **Effect of certain biogent (bacterial and fungal isolates) on liner growth of *C.beticola* on PDA medium *in vitro*.**

Antagonistic isolates	Antagonistic test	
Bacteria:	**RPA**	
Bacillus subtilis B1	2.11 a	
Bacillus subtilis B2	2.01 a	
Bacillus subtilis (B3).	1.68 b	
Bacillus subtilis B4	1.52 b	
LSD 0.05	0.09	
	Growth inhibition	
Fungi:	**I %**	**Scale 1-5**
Trichoderma koningii T_1	88.12 a	1
Epicoccum nigrum E1	62.17 b	2
Epicoccum nigrum E2	64.47 b	2
LSD 0.05	7.54	

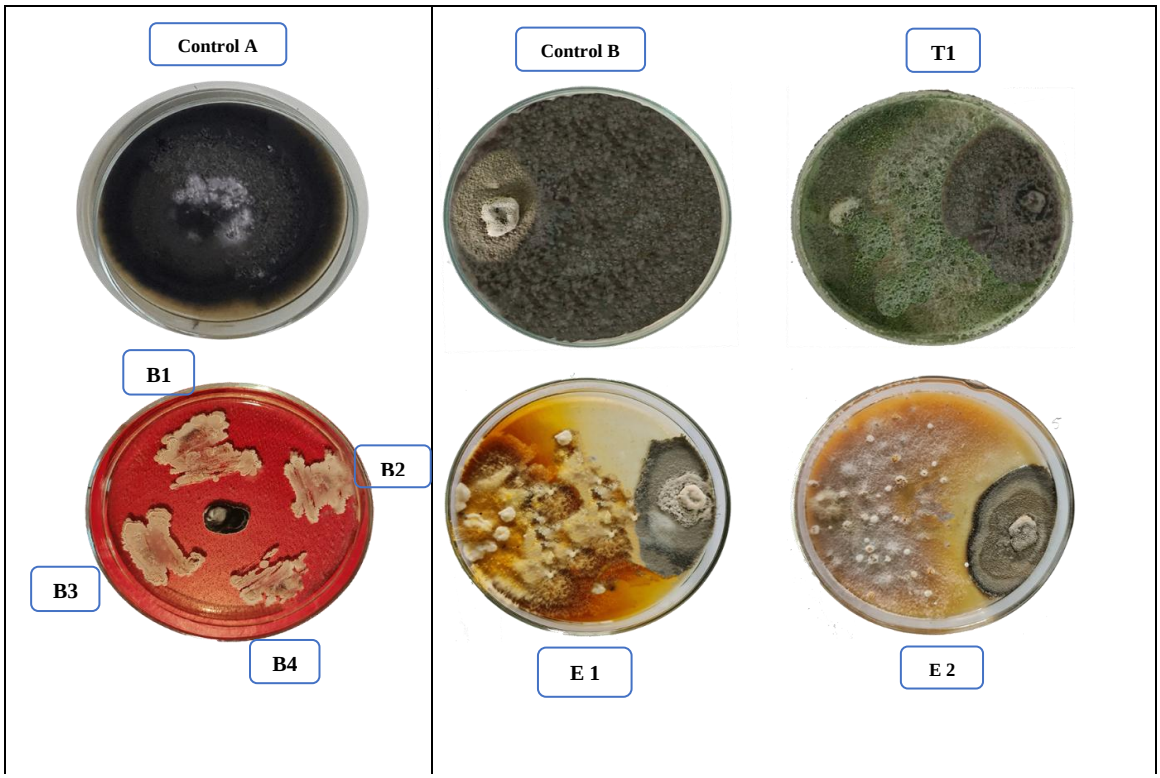
The same letters are not significantly different according to DMRT at 0.05 level. RPA= Relative power of antibiosis, I %= Inhibition percentage, Scale 1-5 according to Bell *et al*. (1982).

For bacteria, it showed that *Bacillus subtilis* B_1 and *Bacillus subtilis* B_2 proved to have the highest antagonistic effect against *C. beticola* (*Ser1*), by which highest RPA values (2.11 and 2.01, respectively) were recorded.

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 (Derbalah *et al.,* 2013; Esh *et al.,* 2011; El-Kazzaz *et al.,* 2002).

Different isolates of *Bacillus* species were known as producers of lipopeptides (Zuber *et al*., 1993). Antagonistic mechanism of *B. subtilis* against *C. beticola* suggested production of effective antibiotics can cause damage to the fungal cells. These compounds are amphilic, membrane active surfactants and peptide antibiotics with specific antimicrobial potential. Peptide antibiotics represent the predominant class and exhibit highly rigid, hydrophobic and/or cyclic structures with unusual constituents like D-amino acids and are generally resistant to hydrolysis by peptidase and proteases (Kowall *et at*., 1998; Stein, 2005; El-Khateeb and Ketta 2019).



(Control A for Bacteria and B for fungi , only pathogen).

B1	*Bacillus subtilis* (B1)	B4	*Bacillus subtilis* (B4)
B2	*Bacillus subtilis* (B2)	T1	*Trichoderma koningii* (T1)
B3	*Bacillus subtilis* (B3)	E1	*Epicoccum nigrum* (E1)
		E2	*Epicoccum nigrum* (E2)

Fig. 1. Effect of the most effective doses of the tested biological antagonists on the mycelium linear growth of *C. beticola* compared to control on PDA medium. Bacterial (*Bacillus subtilis* (B1), (B2), B_3 and B_4) and fungal antagonists (*Trichoderma koningii* T1, *Epicoccum nigrum* E1 and *E. nigrum* E2).

These results agreed with McLean and Stewart (2000) who observed strong antagonistic effects of *Trichoderma* spp against most of the pathogenic fungi. They stated that *Trichoderma* depends on competition for nutrients or space, mycoparasitism or antibiosis and/or antibiotic excretion. Mycoparasitism is the main mechanism recorded by different *Trichoderma* species against pathogenic fungi (Margni *el al.*, 2002). Also, *Trichoderma* can secrete antibiotic and toxins such as trichothecin and a sesquiterpine, trichodermin, which have a direct effect on other organisms (E1-Kazzaz *et al.*, 2002). *Trichoderma* spp. may secrete different lytic enzymes such as glucanase, chitinase, and pectinase which can dissolve cell wall of the pathogen (McLean *et al.* 2001; El-Khateeb, 2004).

Chemical fungicide:

Due to use sequential doses of Eminent, radial growth of *C. beticola* was *in vitro* measured. Based on the obtained data, inhibition percentages were calculated and recorded in **Table (2).** It illustrated that the tested fungicide has antifungal value started up to use 1.0 ppm, by which mycelium growth was inhibited for about 48.90 % and reached its maximal of 76.70 % using 20.00 ppm of Eminent. It is worthy to note here that the inhibitory effect of the fungicide was increased gradually, and it did not reach its maximal yet, indicating reach it by using Eminent more than 20.00 ppm. To determine concentration of Eminent that inhibit 50 % of mycelium growth known as IC_{50}, data and their lineal fitting were plotted in **Fig. (2).** It showed the lower IC_{50}, the more efficient antagonistic agent and vice versa. Based on slop of the resulted curves, IC_{50} of Eminent was subjected to be 1.20 ppm.

Table 2. Effect of Eminent on the linear growth of *C. beticola*.

Fungicide	Concentration (ppm)	Net diameter of linear growth (cm)	Inhibition (%)
Control	0.00	8.50	0.00
Eminent	1.00	4.60	48.90
	5.00	3.20	64.40
	10.00	2.90	67.80
	15.00	2.50	72.20
	20.00	2.10	76.700
IC50	**1.20**		

The antagonistic effect of Eminent may be due to striking changes and disorder in the cell wall of hyphae and conidiophores (Abd El-Ghany and Tayel, 2009). These changes in the wall were not noticed with the untreated hyphae. Moreover, vacuoles were completely disappeared because of the fungicides effect (Amer and El-Shenawy, 2003).

In vivo experiment

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 was determined **(Table 3)**.

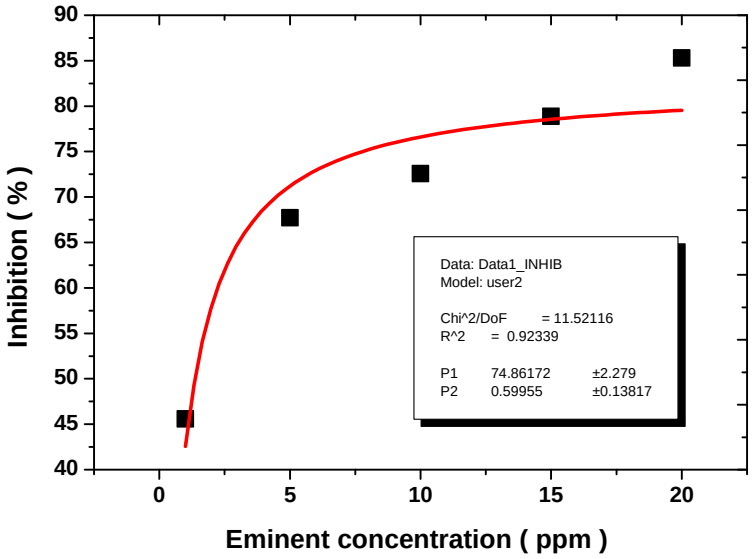


Fig. 2. Modelled data describing the inhibitory effects of the tested chemical fungicide Eminent against *C. beticola* in relation to their concentrations. Symbol refers to the experimental data and line refers to lineal fitted data.

Disease severity

Table .3. Disease severity and efficacies of different tested treatments against *C.beticola* during 2018/2019 and 2019/2020 seasons.

Treatments	Season (2018/19)		Season (2019/20)	
	Disease severity %	Efficiency %	Disease severity %	Efficiency %
Eminent (fungicide)	20.0 g	66.7	21.0 g	67.7
Bacillus subtilis (B1)	27.0 e	55.0	23.0 f	64.6
Bacillus subtilis (B2)	32.0 d	46.7	28.0 e	57.0
Bacillus subtilis B3	36.0 c	40.0	34.0 c	47.7
Bacillus subtilis B4	35.0 c	41.7	30.0 d	53.8
Trichoderma koningii T_1	22.0 f	63.3	21.0 g	67.7
Epicoccum nigrum E1	32.0 d	46.7	38.0 b	41.5
Epicoccum nigrum E2	42.0 b	30.0	38.0 b	41.5
Control	60.0 a	0.0	65.0 a	0.0
LSD (0.01)	1.831	-	1.541	-
LSD (0.05)	1.354	-	1.140	-
CV	2.740	-	2.369	-
P_{value}	0.00**	-	0.00**	-

Means followed by same letter do not significantly. Means were compared by the least significant difference (LSD) test, differences being considered significant at $P = 0.01$ and highly significant at $P = 0.05$ respectively.

It showed that Eminent was the superior control agent with fewer percentages of disease severity (20.0 and 21.0 %) compared with 60.0 and 65.0 % for control during both seasons, respectively. *T. koningii* T1 was the most effective biological antagonist reduced severity of sugar beet Cercospora leaf spot disease to 22.0 and 21.0 % with great efficiencies reached 63.3 and 67.7 % during 2018/2019 and 2019/2020 seasons, respectively. 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.

The locally systemic fungicide Eminent (tetraconazole) belonged to sterol demethylation inhibiting group (DMI) and triazole class. The DMI fungicides inhibit one specific enzyme, C14-demethylase, which plays an important role in sterol production, such as ergosterol. Ergosterol are required for membrane structure and function as well as cell walls. Thus, these fungicides cause abnormal growth and eventually death for fungi (Morsy *et al.*, 2022, Gouda and El-Naggar, 2014). This result might be due to the successful antagonism to plant pathogens by saprophytic microorganisms which was operated by nutrient competition, hyper parasitism, antibiosis and/or induced host resistance. One of the methods used to control pathogens is mycoparasitism, whereby a species or strain of fungus directly attacks and feeds on other fungi. Another mechanism is the production of antibiotics or enzymes that can inhibit the growth of other organisms. Bioagents could also encourage changes in the plant which increase resistance (El-Sayed *et al*., 2017; Derbalah *et al*., 2013; Bolton *et al*., 2012; El-Kazzaz *et al*., 2002).

Enzyme activities

As defensive indicators to the phytopathogens, enzyme activities of PPO, POX and CAT of sugar beet plants were also determined under natural infested field conditions. Activities of the tested enzymes were blotted in **Fig. (3).** 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*. It was expressed very well via induction of the systemic acquired resistant (SAR) is a suggested phenomenon by Hatcher (1995) to define the systemic induction of resistance against a broad spectrum of phytopathogens. Scalbert (1991), found that the highly oxidized phenols are the more inhibitory effect to the pathogen. Although strong suppression of disease by Eminents was appeared, lowest activations of PPO, POX and CAT were achieved, indicating no SAR coincided with the antifungal effects. Similar results were obtained by Hafez *et al.* (2014). Previous studies reported that POX and PPO may participate in the defense system by inducing plant resistance against pathogenic agents (da Silva *et al.*, 2017;

Omara *et al.*, 2019; Omara and Abdelaal, 2018; Esmail *et al.*, 2019; El-Dengawy *et al.*, 2016).

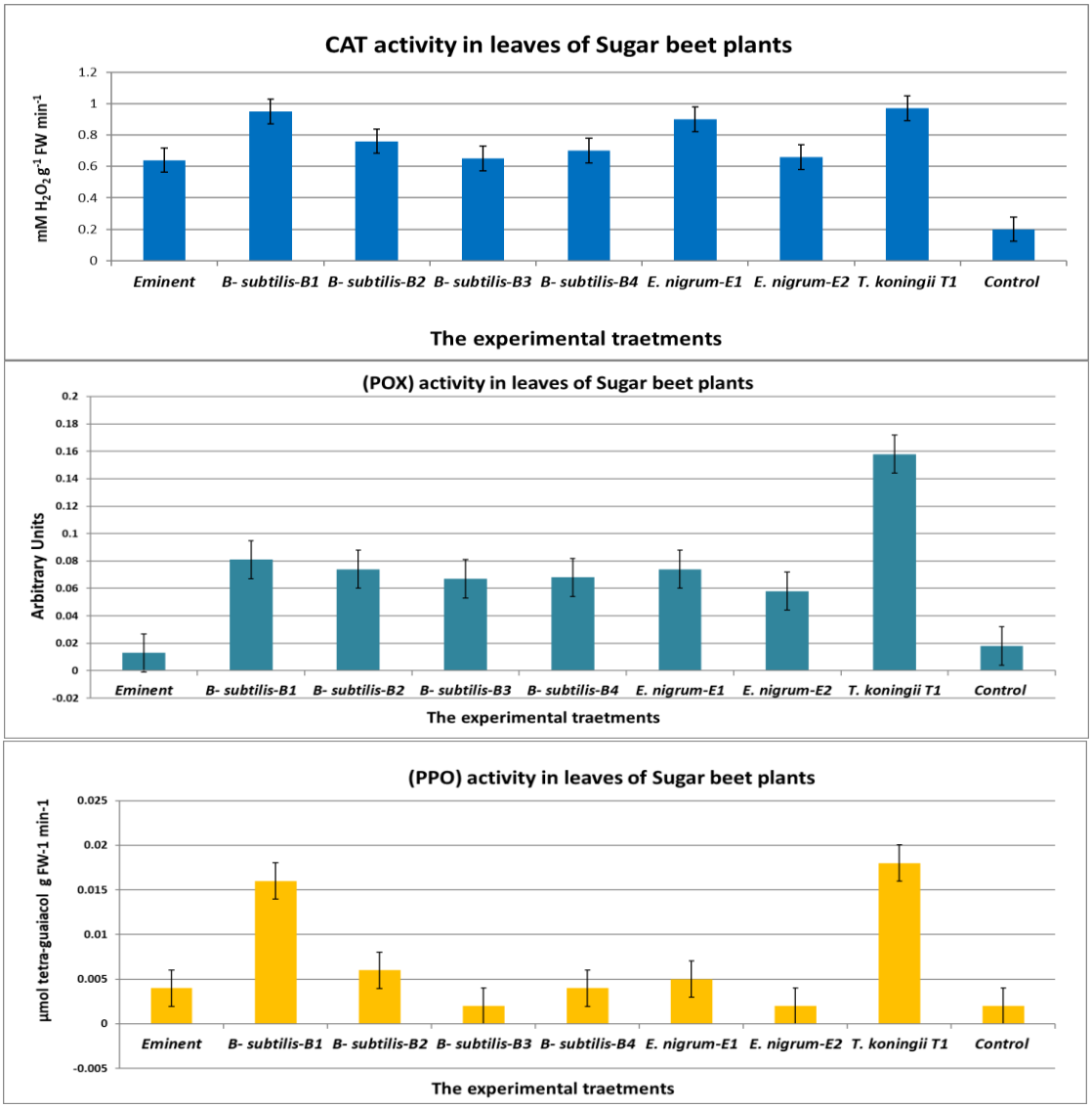


Fig. 3. Activities of enzymes catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) in Sugar beet leaves infested with *C. beticola* at average two seasons, 2018/2019 and 2019/2020.

Chlorophyll contents and yield parameters:

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 **(Table 4)**. 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.

Table 4. Effects of Eminent and certain biological control antagonists on TSS%, sucrose content, total chlorophyll and yield of sugar beet during both seasons of 2018/2019 and 2019/2020.

Treatments	T.S.S %		Sucrose %		Total Chlorophyll µg $(cm^2)^{-1}$		Yield Ton fed^{-1}	
	Season 2018/19	Season 2019/20	Season 2018/19	Season 2019/20	Season 2018/19	Season 2019/20	Season 2018/19	Season 2019/20
Bacillus subtilis 1	21.1b	21.5b	16.0c	16.2c	29.4b	30.0c	40.83 b	43.75 b
Bacillus subtilis 2	20.5b	21.1c	15.8c	16.2c	27.0c	28.0d	37.92 c	43.75 b
Bacillus subtilis B4 e	21.0b	21.4b	15.2de	15.4e	26.8c	27.5d	32.08 e	35.00 d
Bacillus subtilis 4.	20.3cd	20.5e	14.8f	15.2e	21.9e	23.0f	32.08 e	32.08 e
Trichoderma koningii T_1	21.9a	22.1a	16.9b	17.1b	30.0b	32.0b	43.75 a	48.33 a
*Epicoccum nigrum*1	20.8bc	20.7d	15.4d	15.8d	25.5d	25.0e	35.00 d	37.92 c
*Epicoccum nigrum*2	20.3cd	20.5e	15.0ef	15.4e	22.2e	23.0f	32.08 e	32.08 e
Eminent	22.0a	22.02a	17.5a	17.6a	33.0a	35.0a	43.75 a	48.33 a
Control	19.8d	19.6f	14.24g	13.6f	16.0f	14.0g	29.17 f	28.33 f
LSD (0.01)	0.791	0.150	0.407	0.287	0.990	1.468	3.088	1.585
LSD (0.05)	0.589	0.111	0.301	0.213	0.732	1.086	2.284	1.172
CV	0.162	0.363	1.325	0.924	1.957	2.831	1.810	0.865
P_{value}	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**

Means followed by same letter do not significantly. Means were compared by the least significant difference (LSD) test, differences being considered significant at $P = 0.01$ and highly significant at $P = 0.05$ 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. Obtained results agreed with the findings of Morsy *et al.,* (2022); Gouda and El-Naggar, 2014), who found that when applied Eminent and bioagents, consistently provided effective against Cercospora leaf spot disease. It was in agreed with Amaresh and Bhatt (1998), they reported that bioagents may increase the nutrients and essential components required to improve photosynthesis in the host plants to decrease the negative effects of the pathogen.Manal, and Fathia, (2017)

CONCLUSION

It can be concluded that chemical fungicides are still the superior for controlling plant diseases under field conditions. Utilization of biological control as an alternative to fungicides has achieved good results in controlling Cercospora leaf spot disease under field conditions. *Trichoderma koningii* (T1) and *B. subtilis* (B_1) proved to be effective promising agents not only as biocontrol agents, but also for enhancing growth and productivity of sugar beet plants.

Acknowledgements

The authors thank the Environmental Remediation of Agricultural Pollutants Laboratory (ERAP) for technical, Department of Chemistry and Toxicity of Pesticides, Faculty of Agriculture, Kafrelsheikh University.

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التأثيرات المحتملة للمبيد الفطري إيمنينت وبعض المضادات البكتيرية والفطرية لمكافحة مرض تبقع الأوراق السركوسبوري في بنجر السكر

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تم اختبار تأثير بعض العزلات الميكروبية والمبيد الفطرى إيمنينت على أكثر العزلات الممرضة من الفطر *Cercospora beticola* المسبب لمرض تبقع الأوراق السركوسبوري فى نباتات بنجر السكر. وقد كان المبيد الفطري إيمنينت هو المعاملة الأكثر فعالية تحت ظروف المعمل لإيقاف نمو المسبب المرضي. كما أن العوامل المضادة Bacillus *subtilis* (B2) و*Bacillus subtilis* (B1) و*Trichoderma koningii* (T1) الأكثر تضادا للفطر المسبب للمرض . حيث تم تثبيط نمو ميسيليوم المسبب المرضى بسبب المبيد الفطرى إيمنينت بمعدل IC_{50} يعادل 1.20 .ppm في حين ٨٨.١٢ من الميسيليوم تم تثبيطها بواسطة *Trichoderma koningii* T1 . في حين وصلت نسبة RPA قيمتها القصوى ٢.١١ بواسطة *B. subtilis* B1 . وتحت ظروف الحقل المعدى طبيعيًا، فقد انخفضت الشدة المرضية إلى ٢٠، ٢١% باستخدام إيمنينت خلال موسمي النمو على الترتيب، متبوعا بـ B_1 *Bacillus subtilis* وT1 *Trichoderma koningii*. فى حين كانت فعالية فطر *Epicoccum nigrum* لمكافحة مرض تبقع الأوراق السركوسبورى على غير المتوقع. كما ثبت وجود علاقة وثيقة بين التأثير البيولوجى وزيادة النشاط الإنزيمى لكل من الكاتاليز والبوليفينول أكسيديز والبيروكسيديز والذى يؤدى إلى زيادة قابلية نباتات بنجر السكر لمكافحة المسبب المرضى .*C. beticola*، مما يدل على تحفيز المقاومة الجهازية المكتسبة. كما ارتفعت نسبة الكلوروفيل، والمواد الصلبة الذائبة والسكروز وكذا إنتاجية جذور بنجر السكر نتيجة استخدام المعاملات المتفوقة مثل إيمنينت و*T. koningii* (T1) و*B.subtilis* (B1)، على التوالي.

الكلمات المفتاحية: بنجر السكر، التبقع السركسبوري، المقاومة الحيوية، الإيمينت، الإنزيمات.