ROLE OF EMERICELLA NIDULANS AND EPICOCCUM NIGRUM IN CONTROLLING SUGER BEET LEAF SPOT DISEASE

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Submitted August 16, 2021; Accepted October 13, 2021; Published November 21, 2021

SYMMARY

Two fungal isolates from commercial sugar beet phyllospher in Kafr-Elshaikh governorate, Egypt were tested for its antagonistic activity against Cercospora beticola the causal pathogen of sugar beet cercosporia leaf spot (CLS) disease in vitro.

Three methods were used to evaluate its antagonistic activity (Conventionalstreak, culture filtrates and volatile gases). The ability of the tested isolates to produce PR-proteins and growth-promoter indole acetic acid were studied. The tested isolates Emericella nidulans (Aspergillus nidulans) and Epicoccum nigrum found to have the potential to produce chitinase and β-1, 3 glucanase while Emericella nidulansthe only found to have the potential to produce indole acetic acid (IAA). The tested fungal isolates showed a remarkable antagonistic activity in both green-house and open field against C. beticola compared with the healthy control and the infected control.

Also it was found that the control efficiency of the two isolates was almost equivalent to the tested fungicide. The present data show that phyllospheric fungi can play as a good candidate to be used as a bio-control agent against sugar beet leaf spot disease.

Key words: Phyllospheric fungi, Antagonism, Chitinase, β 1, 3 glucanase, Indole acetic acid (IAA) and Salicylic acid (SA).

Introduction

Epiphytic microbes have been documented for numerous phyllosphereand rhizosphere inhabiting organisms and/or stimulating the induction of systemic resistance mechanisms within the plant (Bargabus *et al*. 2002). Filamentous fungi (Punja and Utkhede, 2003), and Oomycetes (Picard *et al*., 2000) have been employed as biological control agents. Bio-control agents can be effective through the production of hydrolytic enzymes and antibiotics, niche colonization and competition for host nutrients, induction of plant host defense mechanisms, and interference with pathogenicity factors (Punja and Utkhede, 2003). Integration of Bacillus-based biological control agents (BCAs) with breeding for disease resistance has proven to be useful in management of several disease problems, particularly where high yielding; highly resistant cultivars are not available. Jacobsen *et al*., (2002). Larson, (2004) published examples include Cercospora leaf spot(CLS) of sugar beet and other plant diseases they revealed that, disease control involving a Bacillus-based bio-control was better on the more resistant cultivars. Larenaa *et al*., (2005) E. nigrum application, alone or in combination with fungicides can be considered in a disease control strategy for reducing fungicide treatments and residues. A further reduction of brown rot may be possible by a better formulation of the biological product. Since fungicides may affect human health and the environment and since pathogens can develop resistance to fungicides, the attention to environmentally friendly alternatives to the use of chemical pesticides as biological control of cercospora has begun. Epiphytic microbes has been documented for numerous phyllosphere and rhizosphere inhabiting organisms and/or stimulating the induction of systemic resistance mechanisms within the plant (Bargabus *et al*., 2002).

Materials and Methods

1- Isolates source:

Phyllospheric fungiisolates Emericella nidulans (Aspergillus nidulans) and Epicoccum nigrum were obtained from the isolates collection of Plant pathology laboratory, Sugar Crops Research Institute, ARC. The isolates were obtained from a former survey of phyllosphieric fungi of sugar beet in Egypt.

2- Source of Cercospora beticola isolate:

A virulent isolate of *C.* beticola (isolated from Kafr El-Sheikh) was obtained from the Cercospora collection in the Department of Sugar Crops Pest and Diseases, Sugar Crops Research Institute, ARC, Giza to be use in this study.

3-Antagonistic effect of the different tested fungi on linier growth of Cercospora beticola in vitro:

The possible antagonistic potential of fungal isolates against C. beticola was studied using different dual culture techniques.

3.1. Conventional streak method:

The possible antagonistic potential of the different phyllosphric microorganism isolate against C. beticola was studied as described by (Douglas *et al*., 2003).Petri-dishes (9 cm in diameter) containing 10 ml of the PDA medium were inoculated with a disk of C. beticola (5.0-mm in diameter) obtained from the margin of an actively growing 14 days old culture of C. beticola placed on one edge of a 9 cm in diameter Petri-dish containing PDA medium, while the tested fungal isolate placed on the opposite side of the plate. The plates incubated at 28±2°C for 30 days under weekly observations. At the end of incubation period when the growth of the control reach 9 cm diameter, the average of longest and shortest diameter of Cercospora colony was measured in the treated plates.

The antagonistic potential of the bio-agents expressed according to the following formula:

Antagonistic potential (%) = [(C – T)\ C] X 100

Where: C = diameter of radial growth of the pathogenic fungus on plates containing it only, T = diameter of radial growth of the pathogenic fungus on plates paired with each antagonistic fungus (dual cultures).

3.2. Effect of volatile gases produced by tested fungal bio-agents on the growth of Cercospora beticola(in vitro):

A discs (0.5 cm in diameter) of Cercospora isolate and the fungal isolates obtained from the margin of an actively growing 14 days old culture of each culture were inoculated in the center of a separate petri-dishes containing PDA medium. Under aseptic conditions both half plates of cercospora and tested fungal isolates were placed face to face without any physical contact between the inoculum, then sealed using parafilm to isolate the inside atmosphere and to prevent loss of volatiles formed (Fig. 2). Plates were incubated at 28±2°Cfor 30 days and the liner growth of C. beticola was measured and compared to controls developed at the same condition with absence of the bio-antagonist (mocked inoculation with an 5.0-mm disk of PDA) (Montealegre, *et al*., 2003). Each experiment considering a single fungal isolate was run in triplicate.

3.3. Effect of culture filtrates of the selected fungal isolates on the growth of C. beticola (in vitro):

The tested fungal isolates Emericella nidulans and Epicoccum nigrumwere grown in 250 ml containing sterilized 100 ml potato dextrose broth (PDB) then incubated at 28±2°Cfor 7 days. The growth mass was picked up and then the supernatant centrifuged and filter sterilized as mentioned before. The sterile fungal filtrates mixed (1:1) with warm PDA medium then inoculated with C. beticolaas mentioned above. The inoculated plates were incubated at 28±2°Cfor 30 days. The liner growth of C. beticola was measured compared to the control treatment to calculate the percentage in growth reduction.

4. Physiological studies on the tested fungi isolates:

4.1. Indole acetic acid production (IAA):

To determine the ability of the tested fungal isolates Emericella nidulansand Epicoccum nigrumto produce IAA, a colorimetric technique (Bric *et al*., 1991) was performed using the Van Urk Salkowski reagent (1 ml of 0.5 M $FeCl_3$ and 50 ml of 35 % $HClO_4$ in water); the tested fungi were grown in liquid Czapek-Dox containing 0.2% L-tryptophan, and incubated at a 28ºC for 24 hr. After the incubation time cultures were centrifuged (1000 rpm) then 1 ml of the supernatant mixed with 2 ml of the reagent and incubated for 25 min. at room temperature. The optical density was measured using the wave length 530 nm. A standard curve of pure IAA (Sigma-Aldrich) was used as standard to calculated IAA production from tested isolates.

4.2. Chitinase production:

The tested fungi isolates Emericella nidulansand Epicoccum nigrum were cultured in 250 ml conical flasks containing 50 ml of liquid Czapek-Dox for fungal isolates supplemented with 0.2 % sucrose and 0.2% colloidal chitin was prepared according to the method of Campell and Williams (1951). The fungal isolates incubated at 28°Cfor 7 days on a rotary shaker incubator. After the incubation period the cultures were centrifuged at 10000 rpm for 20 min. at 4°C and the supernatant was used as crude enzyme source. A mixture of crude enzyme source (1 ml) and suspension of colloidal chitin (1 ml; 0.1% in 50 mm sodium acetate buffer; pH 5) was incubated at 38°C in a water bath with constant shaking. After 2 hr., the release of N-acetyl-glucosamine in the reaction mixture was estimated by the method of Reissig et al., (1955). The enzyme activity was determined using N-acetyl-glucosamine (Sigma) as the standard. Absorbance was measured at 660 nm using a Milton Roy Spectronic 1201. One unit of Chitinase is defined as the amount of enzyme producing 1 µmol N-acetyl-glucosamine/min in 1 ml of reaction mixture under standard assay conditions. Specific activity was expressed as µg of glucose released / ml /min. 4.3. β-1, 3 glucanase production.

Theisolate were grown in 250 ml conical flasks containing 50 ml of liquid Czapek-Dox both media contained laminarin (0.2%) (From Laminaria digitata; Sigma) Lim *et al*., (1991), incubated at 7 days on a rotary shaker incubator. The cultures then centrifuged as previously mentioned and the resulted supernatant used as crude enzyme source. The reaction mixture was the substrate laminarin (Sigma-Aldrich) (2.5% w/v) in 10 mm ammonium acetate, pH 6.0, and 1 mm DTT. The reaction incubated at room temperature for 24h. Samples were assayed for the release of reducing sugars according to the Smoggy-Nelson method Nelson, (1944) modified by (Naguib 1964 and 1965). Absorbance was measured at 660 nm using a Milton Roy Spectronic 1201. Standard curve of glucose was used as reference

(Lim *et al*., 1991). β 1, 3 glucanase activity was determined as µg of glucose released / ml /min.

5. Antagonistic effect of the isolate on cercospora leaf spot incidence in vivo during the season 2018-2019 Inoculum preparation:

5.1. Preparation of fungal isolates (bio-agent) inoculums:

After the incubation period (7days) the fungal cultures were blinded separately in a partially sterilized electrical blinder (using ethanol 70 %) for 5 min. The fungal suspensions then diluted by sterilized distilled water up to 1000 ml with adding 0.1 ml Tween-80 as described by Esh, (2005) to be ready to spray on the experimental plants.

5.2. Preparation of Cercospora beticola Inoculum:

Colonies of C. beticola 30-days old culture were flooded with 10 ml sterile distilled water and rubbed with a glass rod. One milliliter of this suspension used to inoculate 500 ml conical flasks containing 250 ml of sugar beet leaf broth (SBLB) medium then incubated at 28±2°Cunder a 16-hr photoperiod (fluorescent lights) for 30 days. After incubation cultures were blinded separately in a partial sterilized (by ethanol 70%) electrical blinder for 5 min.

The fungal suspension then diluted by distilled water to reach 3 x 10^4 cfu/ml to spray on the experimental plants. (Vereijssen *et al.*, 2003 and Esh, 2005).

5.3. Inoculation of sugar beet plants:

Sugar beet plants were grown in 30 cm diameter pots filled with 3kg (sand: peat moss: clay soil) (1:1:1).Sugar beet plants variety Kawmera (16 weeks old) were treated by the tested phyllospheric bacteria and fungi 2 times before inoculation with C. beticola in 7 days intervals. One week after the last treatment the conidial suspension 3 x 10^4 cfu/ml ofC. beticola was prepared and atomized on sugar beet leaves from all directions until run off. After inoculation, plants were irrigated and covered with transparent plastic bags to serve as a moist chamber and the greenhouse fog system was kept (fig. 3) running for 5-days. Both procedures were to increase the greenhouse humidity to above 90%. After 5 days, the plastic sheets removed, and plants kept on the bench to allow disease development (Esh, 2005).

The disease severity was calculated according to the discretional Battilani key for disease assessment of cercospora leaf spot disease.

Table (1): Battilani key for disease assessment of cercospora leaf spot disease on sugar beet

Deg.	Description	Deg.	Description
0	Healthy foliage	3	Fully and almost fully grown leaves show several coalesced necrotic areas of 1-2 cm diameter, that don't lead to large necrotic areas..
0.5	A single isolated spot on some leaves.	3.5	Some 2-4 outer leaves show relatively large necrotic areas (20-30%) of the leaf area.
1	50 % of the outer leaves (fully grown or old) show one to a few spots (20) .Coalescence of maximum 2 spots can appear.	4	For the first time some leaves (2-8) show 80 to 100% severity.
1.5	Outer leaves (50% of foliage) show 20 to 200 spots per leaf. Coalescence of maximum 2 spots can appear.	4.5	The entire foliage is strongly affected.
2	Nearly all outer leaves are affected by several spots, still isolated. Coalescence of maximum2 spots can appear.	5	The original foliage is completely destroyed.
2.5	Some (2-4) outer leaves show coalescence of spots to necrotic areas. First spots appear on inner leaves	6	For every week scale, 5 continuous 1.5 is added. This phase shows flushes of growth, which can be affected in turn.

6. Effect of bio-agent application on some compounds related to induce resistance in vivo under greenhouse condition:

6.1. Preparation of enzyme source:

Leaf samples were collected from treated sugar beet plants variety (Kawmera) with the promising bio-control active candidates of phyllospheric fungal isolate two weeks before the artificial infection with Cercospora beticola under greenhouse conditions. Samples were frozen in liquid nitrogen (L-N_2) then grounded in a mortar and pestle to form a fine powder.

One gram of the grounded tissues was mixed with 2 ml of extraction buffer according to Bollag *et al*., (1996). Samples were vortexes and centrifuged at 13000 rpm for 15 min. under 4°C to remove cell debris. The clear supernatant (crude enzyme source) was collected and kept at -80°C for further study. (Soltis and Pamela Soltis, 1990).

6.2. Chitinase production:

The supernatant was used as crude enzyme source. A mixture of crude enzyme source (1 ml) and suspension of colloidal chitin (1 ml; 0.1% in 50 mm sodium acetate buffer; pH 5) was incubated at 38°C

in a water bath with constant shaking. After 2 hr., the release of N-acetyl-glucosamine in the reaction mixture was estimated by the method of Reissig *et al*., (1955). The enzyme activity was determined using N-acetyl-glucosamine (Sigma) as the standard. Absorbance was measured at 660 nm using a Milton Roy Spectronic 1201. One unit of chitinase is defined as the amount of enzyme producing 1 µmol N-acetyl-glucosamine/min in 1 ml of reaction mixture under standard assay conditions. Specific activity was expressed as µg of glucose released / ml /min.

6.3. β-1, 3 glucanase production:

The resulted supernatant used as crude enzyme source. The reaction mixture was the substrate laminarin (Sigma-Aldrich) (2.5% w/v) in 10 mm ammonium acetate, pH 6.0, and 1 mm DTT. The reaction incubated at room temperature for 24h. Samples were assayed for the release of reducing sugars according to the Smoggy-Nelson method Nelson, (1944) modified by (Naguib 1965). Absorbance was measured at 660 nm using a Milton Roy Spectronic 1201. Standard curve of glucose was used as reference (Lim *et al*., 1991). β 1,3 glucanase activity was determined as µg of glucose released / ml /min.

6.4. Determination of peroxidase (PO):

The reaction mixture extraction as described by Malik and Singh, (1990) was contained 0.5 ml phosphate buffer pH 7; 0.2 ml enzyme source; 0.3 ml of 0.05 M pyrogallol; 0.1 ml of 1%(v/v) H_2O_2 and distilled water was added up to obtained 3 ml .The reaction mixture was incubated at 30 ^oC for 5 min. then the reaction stopped by adding 0.5 ml of 5 %(v/v) H_2O_2 (Kar and Mishra, 1976). One unit of peroxidase activity was expressed as the changes in absorbance at 425 nm/ min. / 1 g fresh weight.

6.5. Polyphenol oxidase (PPO):

Samples were extracted according to the method described by Malik and Singh, (1990). The enzyme extract was prepared by grounding 5 g leaves in 0.1 M sodium phosphate buffer pH 7 (2 ml / g fresh weight), then centrifuged at 6000 rpm for 30 min.

Under 4 ^oC , the clear extract was collected, completed to 15 ml volume using phosphate buffer and used as crude enzyme source. The reaction mixture contained 0.2 ml of crude enzyme source, 1 ml of phosphate buffer pH 7; 1 ml of 10^{-3} M catechol and complete with distilled water up to 6 ml.

The reaction was incubated for 30 min. at 30 ^oC. One unite of polyphenol oxidase was expressed as the change in absorbance at 420 nm / 30 min. / 1 g fresh weight (Matta and Dimond, 1963).

7-Effect of bio-agent application on disease incidence under field condition during the season 2019-2020:

The same procedure and sugar beet variety used in the greenhouse experiment was used in the field trails, Sakha Experimental Station, Kafr El-Shaikh. The only difference was that after inoculation with C. beticola the plants were left for the natural environmental conditions without covering the plants with plastic bags after the inoculation.

RESULTS

1-Effect of different methods of duel culture from Emericella nidulans and Epicoccum nigrum isolates on the liner growth of Cercospora beticola (in vitro):

Three methods were used to test the antagonistic activity (bio-control agent activity, BCA) of Conventional streak method, culture filtrate and volatile gases). Data in Fig. (1) Show that, the reaction of the bio- agent and the pathogen differed by the tested methods. In the conventional streak method isolates Emericella nidulans and Epicoccum nigrum significantly inhibited the liner growth of C. beticola by (85 and 28% respectively), while, Emericellanidulans and Epicoccumnigrum in the volatile gases (69.4 and 52.8% respectively) significantly inhibited the liner growth of C. beticola .on the other hand, the method of culture filtrate gave no significant percentage of inhibition of C. beticola growth.

It was found that the results of the Conventional streak method were more accurate and reliable than the other used methods.

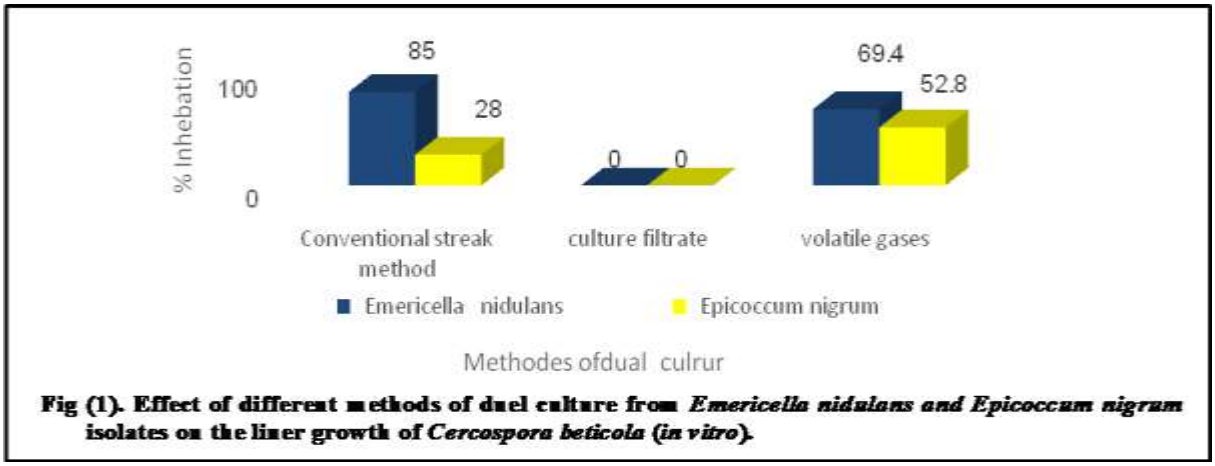

Fig (1). Effect of different methods of duel culture from *Emericella nidulans and Epicoccum nigrum* isolates on the liner growth of *Cercospora beticola* (*in vitro*).

Fig (2) Effect of volatile gases produced by the tested bacterial bio-agents in inverted dual culture plates on the growth of *Cercospora beticola(in vitro*)

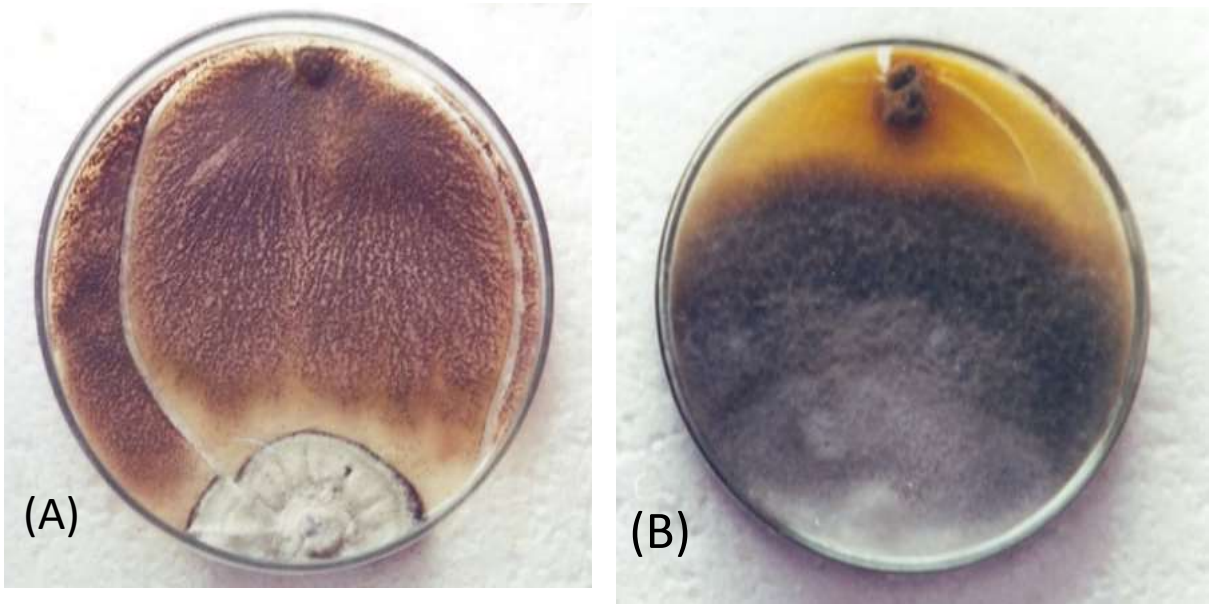

Fig (3) Antagonisticeffectof the isolates on *Cercosporabeticola*growth*invitro* using (A) *Emericella nidulans* (85%) and (B) *Epicoccum nigrum* (28%)

2-Physiological studies on the selected bio-agents in vitro:

Emericella nidulans produced the significant highest IAA amount (110 µg/ml,) while the other isolate (Epicoccum nigrum) didn't produce any IAA.

The obtained results showed that, the tested fungal isolates had the ability to hydrolyze Chitin in the chitin medium (fig.4). The enzyme activity differed significantly between the two fungal isolates. Isolate Emericella nidulans produced chitinase with in the medium amended with the substrate.

It is noticed that, the activity levels of the tested enzyme was significantly higher in the Epicoccum nigrum isolates (83.3 µg of glucose released / ml /min.) compared to Emericella nidulans isolate (78.4 µg of glucose released / ml /min.).

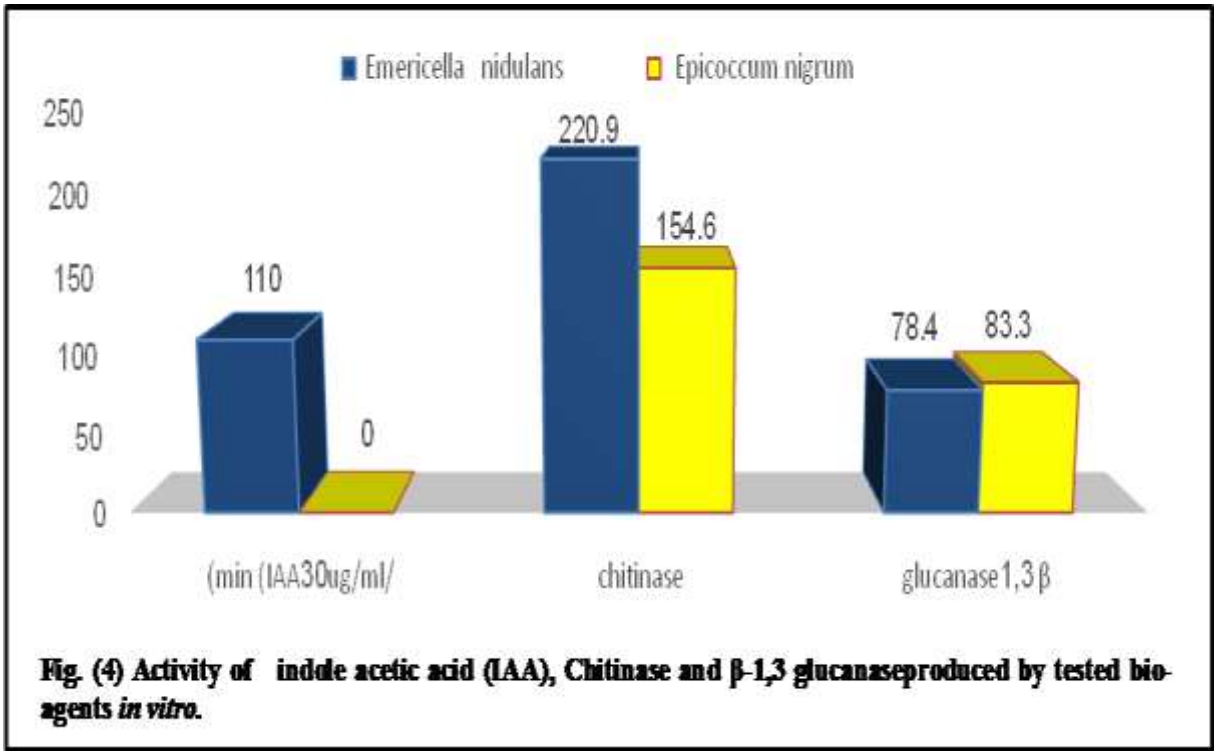

Fig. (4) Activity of indole acetic acid (IAA), Chitinase and β-1,3 glucanaseproduced by tested bio-agents *in vitro*.

3- Effect of bio-agent application on disease incidence in- vivo under greenhouse condition:

3.1. Effect of bio-agents on disease severity under greenhouse condition:

The results presented in Table (2) show that Emericellanidulans and Epicoccumnigrum reduced the disease severity to (1.00 and 2.67 respectively) compared to the untreated control treatment which recorded 4.66. It is worthy to mention that the reduction in disease severity (1.8) caused by the fungicide (Topsin M70) treatment has no significant difference compared to the reduction caused by the bio-agents treatment.

Table (2): Efficiency evaluation of the candidate phyllospheric fungal isolates on decreasing the severity of sugar beet cercospora leaf spot disease on sugar beet variety (Kawmera) when sprayed twice before artificial infection by Cercospora beticola under greenhouse conditions during the season 2018 –2019

Fungal isolates	Disease severity	% of disease inhibition
Emericella nidulans	1.00	78.54
Epicoccum nigrum	2.67	42.70
Topsin M70	1.80	61.37
Infected control	4.66	-
LSD at 0.05	1.7	-

3.2.-Effect of application with the bio-agent on some compounds related to induce resistance in sugar beet plants:

The highest PO activity was recorded by the isolate Epicoccum nigrum (1.49 unite/30 min. /g and 1.5 unite/30 min. /g fresh weight) (Fig 5).

On the other hand, the isolate Epicoccum nigrum recorded the highest PPO activity (0.32 unite/30 min. /g fresh weight.) while isolate Emericella nidulans fresh weight) followed by Emericella nidulans which recorded a lower activity (1.11 unite/30 min. /g fresh weight).

It is worthy to mention that, the untreated healthy and infected control recorded (0.98 recorded the lower activity (0.29 unite/30 min. /g fresh weight). It is worthy to mention that, the untreated healthy and infected control recorded (0.14 and 0.71unite/30 min. /g fresh weight).

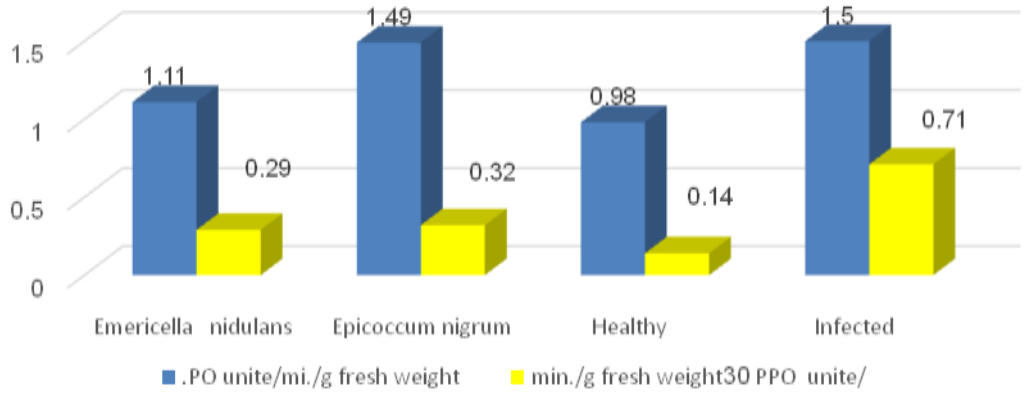

Fig (5) Determination of Polyphenol oxidase (PPO) and Peroxidase (PO) activity in treated sugar beet plants variety (Kawmera) with the candidate phyllospheric fungal isolates two weeks before the Infection with *Cercospora beticola*.

The data illustrated in Fig. (6) Show that, the isolate Epicoccum nigrum recorded the highest β 1, 3 glucanase activity (23.3 µM of glucose released / ml /hr.) while isolate Emericella nidulans recorded the lowest activity (12.2 µM of glucose released/ml/hr) and the untreated healthy and infected control recorded (10.3 and 38.0 µM of glucose released/ml/hr).

While in chitinase activity the isolate of Emericella nidulans recorded the highest chitinase activity (30.5 µM of glucose released / ml /hr).

While Epicoccum nigrum recorded the lower activity (28.0 µM of glucose released / ml /hr.) and the untreated healthy and infected control recorded (13.9 and 52.1 µM of glucose released / ml /hr.).

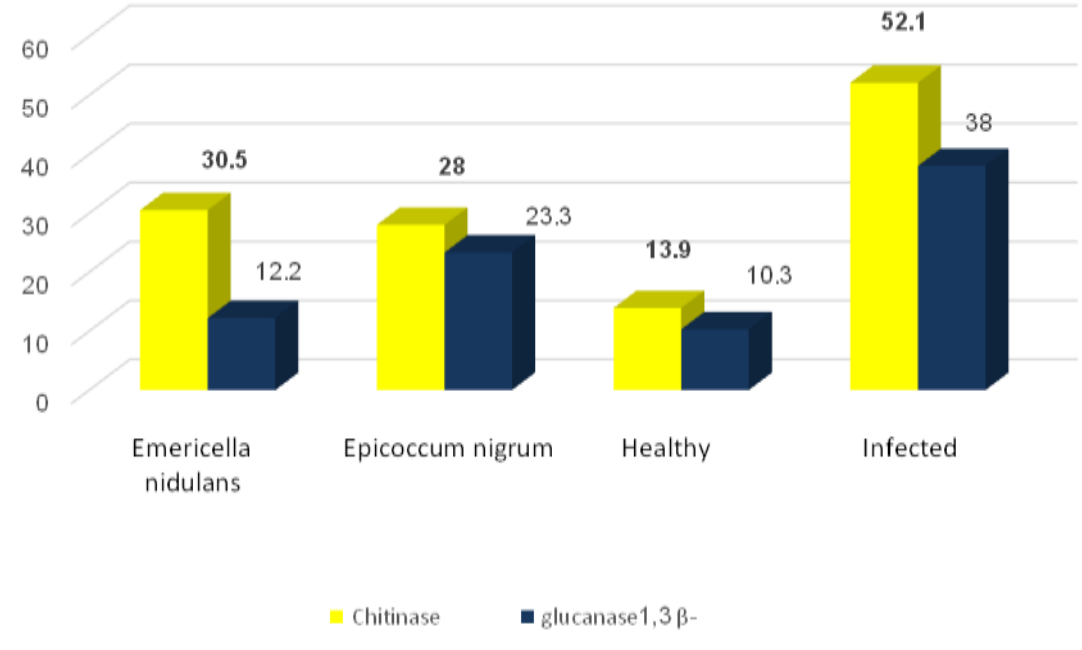

Fig (6) Determination of Chitinase and β1, 3-glucanase activity in treated sugar beet plants variety (Kawmera) with the candidate phyllospheric fungal isolates two weeks before the Infection by Cercospora beticola.

4- Effect of bio-agents on disease severity under field condition:

The field experiment showed a different order in bio-control activity Table (3) and fig (7). The tested Emericellanidulans and Epicoccumnigrum recorded the highest significant increase of disease inhibition (76.47 and 61.76) compared to the infected control treatment. It is worthy to mention that, isolate Emericellanidulans significantly increased the disease inhibition (76.47) compared to the fungicide Topsin M70 treatment which recorded disease severity inhibition of 70.59%.

Table (3): Efficiency evaluation of the candidate phyllospheric fungal isolates on decreasing the severity of sugar beet cercospora leaf spot disease on sugar beet variety (Kawmera) when sprayed twice (two weeks intervals) before artificial infection with Cercospora beticola under field conditions during the season 2019-2020

Fungal Isolates	Disease severity	% of disease inhibition
Emericella nidulans	0.80	76.47
Epicoccum nigrum	1.30	61.76
Topsin M70	1.00	70.59
Infected control	3.40	-
LSD at 0.05	0.35	-

It was noticed that the disease severity in the infected control was higher in the greenhouse experiment (4.6) than in the field experiment (3.4)

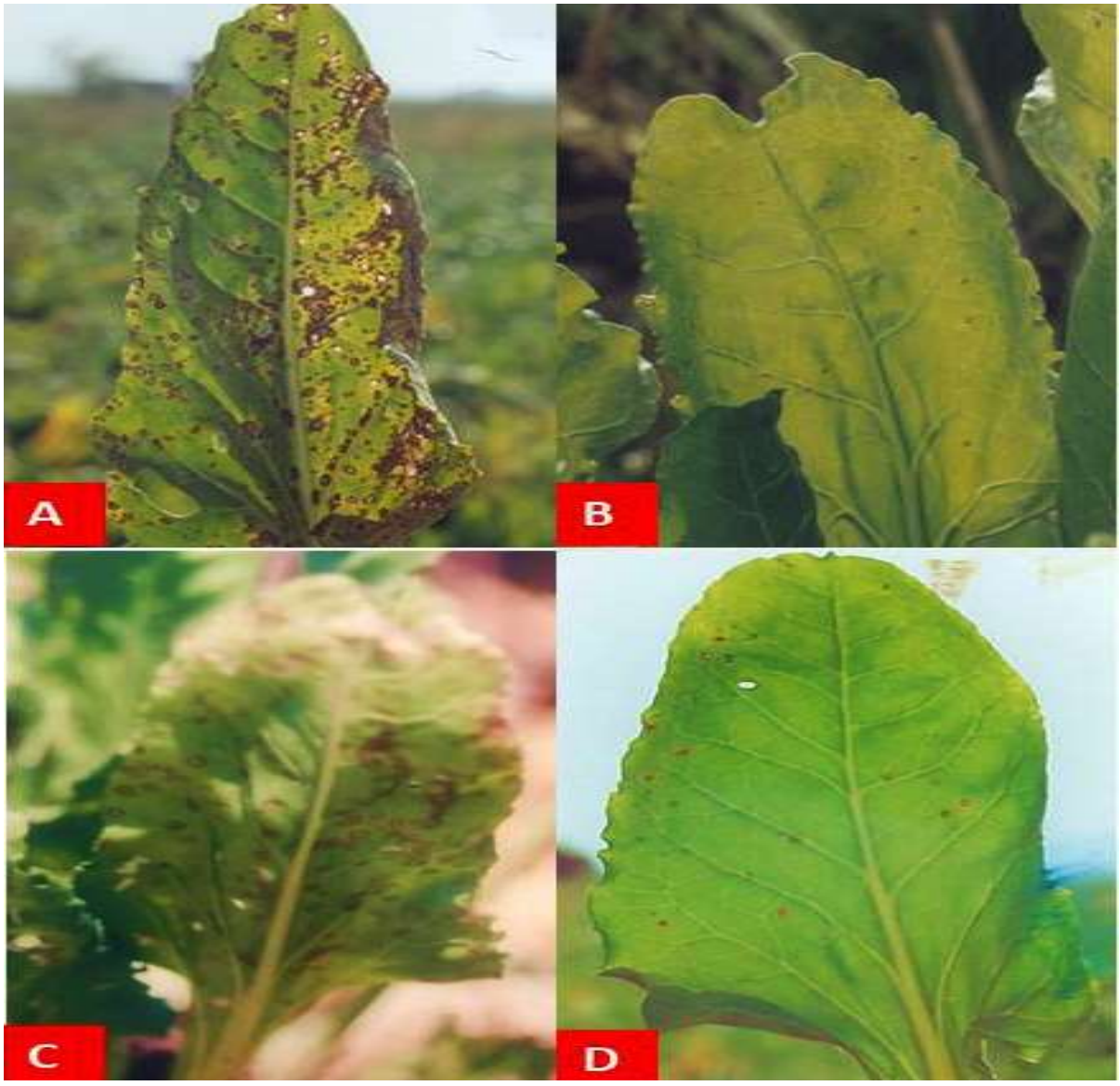

Fig. (7): Different effects of the bio- control active fungal isolates in field experiment, (A) the infected untreated control, B(*Emericella nidulans*), C *(Epicoccum nigrum)* ; and D(Topsin M70)

DISCUSSION

The obtained results from experimenting the different methods of duel culture from Emericella nidulans and Epicoccum nigrum isolates on the liner growth of Cercospora beticola (in vitro) showed a difference in the inhibitory percentage resulted from both methods. These results are in harmony with those reported by Agarry *et al*., (2005) who performed a comparison study on the efficiency of the antagonistic effect measurements in vitro between streak and volatile gases methods. They found a considerable variation in inhibitory activity. The zone of inhibition was more apparent in the streak method than in the other method. Many fungal species are known to emit low concentrations of gaseous substances, especially ones that have distinctiveobnoxious odours, and this has prompted appropriate chemical analyses of the fungal volatiles Bjurman and Kristensson, (1992).

Some of these volatile substances are common to many fungi, whereas others seem to be unique for one species (Schnurer *et al*., 1999 and Rapior *et al*., 2000).The obtained results also showed no significant difference between the disease reduction caused by the tested bio agents and the tested fungicide under field conditions.

These results are in agreement with those reported by Brewer and Larkin, (2005) who found that, some of the biological control treatments reduced disease equally to fungicides.

In the present study Emericella nidulans found to produce a significant IAA amount in vitro while the other isolate (Epicoccum nigrum) didn't produce any IAA. It was reported that the ability of different fungal genera to produce IAA was different. Gusmiaty et al 2019 reported many rhizosphere fungi such as Aspergillus, Trichoderma, Rhizopus, Penicillium, and Fusarium thatcan produce IAA in both in vivo and in vitro conditions.

The tested fungal bioagents were proved to produce chitinases, b-1, 3-glucanases in both in vitro and in vivo studies. Fungal cell wall degradation is typically caused by a range of chitinases, b-1, 3-glucanases and proteases or, in case of hyper-parasites of oomycota, cellulases. Such a necrotrophic hyper-parasitism with invasion of killed host cells is frequently observed by microscopy and electron microscopy (Jeffries, 1995).

Antagonistic activity of selected bio-agents showed a degree of inhibition of C. beticola. The growth inhibition of C. beticola by antagonist fungi is possibly attributed to the secretion of antibiotics by the bio-agent (Upadhyay and Rai,1987) or other inhibitory substances such as geodin, terricin, terric acid, aspergillic acid, dermadin, etc. (Brian *et al*., 1945). The degree of effectiveness varies according to the nature, quality, and quantity of antibiotics/inhibitory substances secreted by the antagonists (Dennis and Webster, 1971a, b and Skidmore and Dickinson, 1976).

Under greenhouse and field conditions, all the tested isolates significantly reduced the CLS severity compared to the untreated control plants. Generally, it was noticed that, the disease severity in the greenhouse was always higher than in the field. Also the bio-control activity of the tested isolates differed in both greenhouse and field experiments, these results in agreement with those reported by Whitney and Duffus, (1986) which reported that, the relative humidity above 90% and temperatures above 15.5 °C are required for Cercospora spore germination. Steinkamp *et al*., (1979) studied the C. beticola infection process determining that C. beticola infection begins by attachment of the conidia to the leaf surface and subsequent germination and entry of a germ tube through stomata during periods of leaf wetness. Rathaiah, (1977) determined that hydrotropism was important in the ability of the germ tube to locate stomata. A higher relative humidity in the stomatal opening as compared to lower ambient levels was correlated with infection through stomates. Stomata do not need to be open to facilitate fungal entry.

Generally in the present study, foliar treatment with fungal isolates increased the activities of various defense enzymes when the plants were inoculated with the pathogen. Romero *et al*., (2003) reported that, fungal bio-control agents against cucurbit powdery mildew performed better under greenhouse conditions of high relative humidity (90–95% RH).

The activity of peroxidase (PO) and polyphenol oxidase (PPO) Ramamoorthy *et al*., (2002) reveled that, Po and PPO declined rapidly in tomato after 4 days of inoculation with Fusarium oxysporum f.sp. lycopersici compared to bacterized plants challenged with the pathogen. PO and PPO catalyze the last step in the biosynthesis of lignin and other oxidized phenols.

On the other hand, The PO activity increased rapidly in cucumber roots treated with P. corrugata challenged with P. aphanidermatum . Enhanced PO activity is very often associated with resistance and lignin production. PO-generated hydrogen peroxide may function as an anti-fungal agent in disease resistance.

Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals that are antimicrobial in nature Reuveni *et al*., 1992. The data obtained from this work would encourage the use of biological control against CLS disease as a eco-friendly alternative to the use of fungicides. nonpathogenic, phyllosphere-colonizing Bacillus mycoides,

REFFRENCE

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الملخص العربى

دور فطري إبيكوكم نيجرام و إميريسيلا نيديولانس في مقاومة مرض التبقع السيركوسبوري فى بنجر السكر

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تم تقييم نشاط المقاومه الحيويه لعزلتين فطريتين معزولتين من علي أسطح أوراق بنجر السكر لاختبار قدرتهما علي مقاومة مرض التبقع السيركوسبوري تحت ظروف المعمل بإستخدام طرق التقييم في الاطباق (طريقة التخطيط التقليدية - تاثير المواد الطياره الناتجه من الفطريات المختبره وتاثير الراشح الناتج منها على الفطر الممرض).

تم دراسة البروتينات التى تؤثر على حدوث المرض بيتا ١،٣جلوكانيز و كايتينيز و كدا المواد التى لها دور محفز للنمو مثل الاندول اسيتك اسيد و ودلك على مستوى العزله فى المعمل. اما على مستوى النبات فى الصوبه فقد تم تقدير كلا من بيتا ١،٣جلوكانيز و كايتينيز و البيروكسيديز والبولى فينول اوكسيديز في النباتات المعاملة و المصابة. .تم ملاحظه إنخفاض فى شدة الاصابه في النباتات المعامله بالفطريات تحت ظروف الصوبه و الحقل مقارنه بالكونترول المعدي بالممرض والسليم ووجد ان تاثير الفطريات المختبره علي مقاومة المرض كان مقارب لتاثير المبيد الفطرى توبسين ام ٧٠.واثبتت النتائج المتحصل عليها ان فطريات سطح الورقه يمكن ان يكون لها دور مهم فى المقاومه الحيويه لمرض التبقع السركسبورى في بنجر السكر.

الكلمات الداله: فطريات الفيلوسفير- التضاد - –الكيتينيز – بيتا ١.٣ جلوكاينيز – سيدروفورز –حامض اندول اسيتك–حامض سلسيليك.