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Original article

## Efficacy of *Bacillus subtilis*, *Moringa oleifera* seeds extract and potassium bicarbonate on *Cercospora* leaf spot on sugar beet

Mohamed D. Sehsah<sup>a</sup>, Gabr A. El-Kot<sup>b</sup>, Baher A. El-Nogoumy<sup>c</sup>, Mohammed Alorabi<sup>d</sup>,  
Ahmed M. El-Shehawi<sup>d</sup>, Nagwa H. Salama<sup>a</sup>, Amira M. El-Tahan<sup>e,\*</sup>

<sup>a</sup> Maize and Sugar Crops Diseases Research Department, Plant Pathology Research Institution, Agricultural Research Center, Giza, Egypt

<sup>b</sup> Agriculture Botany Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt

<sup>c</sup> Microbiology Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt

<sup>d</sup> Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

<sup>e</sup> Plant Production Department, Arid Lands Cultivation Research Institute, The City of Scientific Research and Technological Applications, SRTA-City, Borg El Arab, Alexandria, Egypt



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### ABSTRACT

*Cercospora* leaf spot caused by *Cercospora beticola* are among the most dangerous plant diseases on sugar beet plants. It causes heavy economic losses, whether on the yield of roots, the percentage of sugar in them, or the quality of sugar produced. In addition to the economic cost caused by chemical control, these chemical pesticides cause an imbalance in the ecosystem and harm the health of humans and animals. In an attempt to search for a safer method than pesticides and environmentally friendly, an evaluation of using biocontrol agents, *Bacillus subtilis* as cell suspension ( $10^8$  cell/ml), was conducted in this study. Seeds extract of *Moringa oleifera* with two concentrations (25 and 50 g/L) and potassium bicarbonate at (5 and 10 g/L (compared to fungicide Montoro 30% EC (Propiconazole 15% + Difenconazole 15%). The evaluation results for twenty-five sugar beet varieties showed a significant discrepancy between these varieties in the extent of their susceptibility to infection with the disease under investigation. *In-Vitro*, *B. subtilis* induced an antagonist to *C. beticola*, and both *M. oleifera* seeds extract and potassium bicarbonate significantly reduced the linear growth of this pathogen. Under field conditions, the treatments used have given positive results in controlling *Cercospora* leaf spots. They significantly decreased the severity of disease and prevented *C. beticola* from creating conidiophores and conidiospores, along with examining their cell walls with the formation of plasmolysis of the fungus cells and reducing both the number and diameter of the spots on the surface leaves; this was demonstrated using a scanning electron microscope (SEM). It is worth noting that the best results obtained were most often when treated with *M. oleifera* seeds extract, followed by potassium bicarbonate, then cell suspension of *B. subtilis*. In addition, the percentage of the content of beet roots from total soluble solids and sucrose has improved significantly due to spraying sugar beet plants with the substances mentioned earlier. These treatments also contributed to a significant improvement in the enzymes polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase.

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### 1. Introduction

Sugar beet (*Beta vulgaris* L., Chenopodiaceae) is one of the most substantial sugar crops worldwide. The cultivated area of the sugar

beet crop amounted to about 584.58 thousand fed in Egypt; the average production of sugar was about 18.7 ton per fed (Bulletin of the Ministry of Agriculture, 2019). Sugar beet crop is ranked as second after sugar cane for the production of sugar. Due to the annual population increase and the increased demand for sugar, it is necessary to take care of growing beets and take care of them against plant diseases that reduce their productivity. Sugar beet is affected by many plant diseases, the paramount of which is the *Cercospora* leaf spot caused by *Cercospora beticola* (Sacc.). This fungal disease is a worldwide (Holtshulte, 2000). It is estimated that the *Cercospora* leaf spot can cause a loss of more than 40% in the sugar yield Rossi et al. (2000). In addition to causing a reduction

\* Corresponding author.

E-mail address: [aeltahan@srtacity.sci.eg](mailto:aeltahan@srtacity.sci.eg) (A.M. El-Tahan).

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in the extraction of sugar from the juice and thus reducing the sugar percentage, the roots of the infested plants are not able to store the sugar in the amount and concentration present in the healthy roots, which causes a significant loss for farmers. At the same time increases impurity concentrations, reduces extractable and root sucrose yields, resulting in higher processing losses Lamey et al. (1996). Breeding varieties tolerant to plant diseases is one of the best means of combating these diseases (Miller et al., 1994; Hassanin et al., 2020). Researchers use four to five genes known in their resistance to *Cercospora* to develop these types. Considering that it is challenging to produce a variety resistant to *Cercospora* and at the same time it is highly productive (Smith and Campbell, 1996). Chemical pesticides such as mancozeb, thiophanate methyl, and fentin hydroxide are the primary method in controlling this disease (Dexter and Luecke, 1999).

Unfortunately, many fungal strains have developed resistance to these pesticides, which led to the reluctance of farmers to use them because they do not give reasonable control in the presence of these strains (Weiland and Smith, 1999). Therefore, there is an urgent need to continue searching for new pesticides with different active ingredients. However, chemical pesticides have severe damage to the ecosystem and public health. It has been proven to kill beneficial organisms and cause many diseases to humans and animals (Seema et al., 2011; Moyo et al., 2012). This led to the need to use alternative means for these chemical pesticides, such as plant extracts and plant oils, such as *M. oleifera* plant extract (El-Mohamedy and Abdallah, 2014; Khedr et al., 2022), or the use of environmentally friendly chemicals such as potassium bicarbonate commercially called Armicarb<sup>®</sup>, copper sulphate, magnesium sulphate, and potassium oxide, (Mann et al., 2004; El-Moghazy et al., 2011; Kaiser et al., 2011; Marku et al., 2014), or the use of microorganisms such as *Trichoderma album* and *B. megaterium*, whether as suspension or as cultural filtrates (Galletti et al., 2008; Hegazi and El-Kot, 2009; Hegazi and El-Kot, 2010; El-Kot and Derbalah, 2011; El-Moghazy et al., 2011; Ashry et al., 2022; El-Saadony et al., 2022). Bio-bicarbonate salt fungicidal activity has been known for at least 80 years (Marloth, 1931).

Many researchers have studied the effect of using some substances in plant disease control on the physiological processes inside plants (El-Tahan et al., 2016; Desoky et al., 2020; Hassan et al., 2021; El-Saadony et al., 2021d). Among the most many functions are the activity of certain enzymes, the most important of which is oxidation enzymes such as polyphenol oxidase, peroxidase, phenylalanine, ammonia-lyase, and other enzymes (Bayoumi and El-Kot, 2010). The scanning electron microscope (SEM) is a leading device in examining and studying fungal growth and other organisms growing on plant parts Jackowiak et al. (2005).

This study evaluates the use of some substances, such as *M. oleifera* seeds extract, potassium bicarbonate, and cell suspension of *B. subtilis* in combating *Cercospora* leaf spot on sugar beet as an alternative to chemical pesticides safer ones, and examined their effect by the scanning electron microscope (SEM), evaluation of 25 sugar beet cultivars against the disease, as well as estimation of oxidative enzymes of peroxidase, polyphenol oxides, and phenylalanine ammonia-lyase

## 2. Materials and methods

### 2.1. Isolation, identifications, and evaluation of sugar beet cultivars as well as treatments

Samples of sugar beet plants showing leaf spot disease symptoms caused by *C. beticola* were collected from Kafrelshiekh governorate. The sugar beet leaves were thoroughly cleaned with tap water, chopped into small pieces, and surface sterilized for three

minutes in a 0.5 sodium hypochlorite solution Alaniz et al. (2011). After being washed several times in sterilized water and blotted between two sterilized filter papers, the extracts were put to Petri dishes containing sugar beet leaf extracts dextrose agar medium (SBLEDA). The fresh sugar beet leaf blades were sliced, and 200 g was boiled in one liter distilled water for 15 min and strained through double layers of cheesecloth. The SBLEDA medium consists of beet leaf extract (100 ml), dextrose (20 g), and agar (15 g). Streptomycin as an antibiotic was added to the media (40 ppm) to avoid bacterial contamination. Plates were incubated at 27 + 2 °C for 3–7 days and examined daily for the occurrence of fungal growth. The growing fungi were examined microscopically and purified using the hyphal tip technique described by Dhingra and Sinclair (1959). Pure cultures of each isolate were maintained on PDA slants at 4 °C for further examination. Identification of the causal fungal pathogen was carried out upon morphological and microscopical characteristics according to (Barnett and Hunter, 1972). A pathogenicity test was carried out in 35 cm diameter pots under greenhouse conditions. Pots were filled with sandy-loam soil (1:2 w/w). Twenty-five sugar beet cultivars (Table 1) were used in a joint experiment between testing the pathogenic ability and assessing the susceptibility of these varieties to infection with *Cercospora* leaf spot. The isolate was grown in liquid CZ-a pek, s medium and incubated at 27 + 3 °C for 15 days to obtain the required inoculate. Ninety-day-old plants were sprayed with 5 × 10<sup>4</sup> spore/ml of each isolate using an atomizer (Crane and Calpouzos, 1984) in four replicates comprising four plants for each. Before inoculation, plants were sprayed with water to make a thin film of water on the leaf surface. Two grams' sucrose and 0.1 ml tween 80 per liter were added to spore suspension to enhance infection. Inoculated plants were kept in a moist polyethylene chamber for seven days. Disease severity % was recorded according to (Shane and Teng, 1992) after 100 days from planting. The used fungicide in this study was Propiconazole 15% + Difenoconazole 15% with a trade name of Montoro 30% EC. This fungicide was applied at its recommended field rate of 1 ml/L. Potassium bicarbonate (KHCO<sub>3</sub>) was used at a rate of 5 and 10 g/L and obtained as technical compounds from Al-Gomhoria Company for Chemicals and Glasses, Cairo, Egypt. The tested microbial bioagent was *B. subtilis*, isolated from healthy sugar beet leaves and identified according to Bergy's Manual of Systematic Bacteriology (1984). *B. subtilis* was applied at 10<sup>8</sup> cell/ml. In addition to one treatment by seed extract of *M. oleifera* at 25 and 50 ml/L. Total soluble solids content (TSS.%) and sucrose % of fresh sugar beet root were determined using a hand refractometer and sacrometer according to (Association of Official Analytical Chemistry, 1990) and (McGinnis, 1982), respectively after harvesting directly.

### 2.2. Evaluation of the tested treatments against *Cercospora beticola* under laboratory conditions

In a completely randomized approach, the four therapies were assessed for their efficacy against *C. beticola*. The effectiveness of the treatments was measured as a percent reduction in linear growth compared to the control treatment. The tested materials' concentrations are presented in (Table 1). By adding the necessary amount of stock solution to 60 ml, volumes of autoclaved PDA media cooled to around 45 °C, the requisite quantities of potassium bicarbonate (KHCO<sub>3</sub>) and Montoro fungicide applications were produced. At the same time, the sterilized seed extract of *M. oleifera* was added to PDA that had been chilled to roughly 45 °C following filter sterilization. For each treatment concentration, including control, four 250 ml flasks were utilized as a replicate. Without adding any treatments, a control treatment was carried out. Each flask was inoculated with a 15-day-old *C. beticola* culture disc (5 mm in diameter). To prevent volatile substances from evap-

**Table 1**  
Reaction of twenty five sugar beet cultivars to *Cercospora* leaf spot disease in two seasons, 2019/2020 and 2020/2021.

	Evaluated sugar beet cultivars	Season 2019/2020		Season 2020/2021	
		Disease incidence%	Disease severity%	Disease incidence%	Disease severity %
1	9 k887	13.00 l	4.33 lm	22 m	7.33 l
2	SMART DJERBA-KWS	31.00 de	10.33 de	25 lm	8.33 jkl
3	SMART JELLA-KWS	27.00 fg	9.00 efg	42 ef	14.00 ef
4	IDIRA-KWS	5.00 n	1.66 op	8 r	2.66 op
5	GREGORIA-KWS	4.00 n	1.33 p	12 op	4.00 n
6	ALLANYA-KWS	23.00 hi	7.66 ghi	29 jk	9.66 ij
7	B 8141	33.00 de	11.00 d	43 ef	14.33 e
8	CARMA	10.00 m	3.33 mn	43 ef	14.33 e
9	POSEIDON	9.00 m	3.00 n	40 fg	13.33 ef
10	MELOOIA	16.00 k	5.33 kl	42 ef	14.00 n
11	FANTAZJA	22.00 i	7.33 hi	11 n	3.66
12	LP 17B4011	45.00 c	15.00 c	17 c	5.66 m
13	FD18B4018	53.00 b	17.66 b	40 ef	13.33 ef
14	FD17B4010	30.00 ef	10.00 def	22 m	7.33 l
15	SV 2173	35.00 d	11.66 d	37 d	12.33 fg
16	MK 4199	15.00 kl	5.00 ij	44 e	14.66 e
17	MK 4200	17.00 jk	5.66 jk	105 a	35.00 a
18	HAMMOND	9.00 m	3.00 n	95 b	31.66 b
19	VANGEUS	20.00 ij	6.66 ij	100 b	33.33 ab
20	SHRB21802	21.00 i	7.00 ij	60 c	20.00 c
21	SI21801	53.00 b	17.66 c	23 m	7.66 kl
22	PINTEA	60.00 a	20.00 a	27 kl	9.00 jk
23	ZEPPEUN	13.00 l	4.33 lm	34 hi	11.33 gh
24	DIPENDRA KWS	26.00 gh	8.66 fgh	32 ij	10.66 hi
25	FRAPPINA KWS	53.00 b	17.66 b	52 d	17.33 d
	LSD (0.01) = 2.997, LSD (0.05) = 2.253	2.997, 2.253	2.149, 1.601	4.946, 3.716	3.007, 2.908

Means designed by the same letter are not significantly difference at the 5% level according to Duncan's test.

orating, Petri dishes were wrapped with parafilm. The dishes were incubated at 22–25 °C until the control treatment reached complete growth (mycelium reaching the dish's edge) after 12 days, at which point the linear growth was calculated. According to Ibrahim et al. (1987), the relative power of *B. subtilis* antibiosis against *C. beticola* was evaluated using the following equation:  $RPA = Z/C$ , where Z represents the diameter of the inhibition zone, and C represents the diameter of the spotted antagonistic isolate.

### 2.3. Evaluation of the tested treatments against *Cercospora beticola* under field conditions

The randomized complete block design with three replicates was used in two seasons (2019/2020) and (2020/2021) at the Research Experimental Farm of the Plant Pathology Research Institute, Sakha Station, Kafrelshiekh, Egypt. Each replication was six rows long and 60 cm wide, with a total length of 900 cm. Each row had 45 hills that were 20 cm apart. All of the recommended culture practices were completed on time. Plants were sprayed three times at ten-day intervals with potassium bicarbonate at 5 and 10 g/L, *M. oleifera* seeds extract at 25 and 50 ml/L, *B. subtilis* at  $10^8$  cell/L, and fungicide Montoro at one ml/L. When illness symptoms were discovered, spraying began (after 90 days of cultivation). As a control, the plot was left untreated. (Shane and Teng, 1992) described a method for assessing disease severity which estimated three times, ten days following every spray.

### 2.4. Determination of polyphenol oxidase, peroxidase and phenylalanine ammonia-lyase enzymes activity:

Enzyme extraction and assay: After 24 h of spraying, leaf samples of each treatment, healthy and infected, were collected for peroxidase and polyphenol oxidase enzyme activity assay. Leaf tissue was ground in a porcelain mortar in 0.1 M sodium phosphate buffer at pH 7.1 (2 m/g leaf tissues) to extract enzymes. Four layers of cheesecloth were used to strain the retrieved tissues. Filtrates

were centrifuged at six °C for 20 min at 3000 rpm. The clear supernatants were collected and considered as crude enzyme extract. The oxidation of pyrogallol to pyrogalline in the presence of hydrogen peroxide was used to test peroxidase activity, as described by Allam et al. (1972). Changes in absorbance at 425 nm were measured every 1 min for up to 4 min to determine peroxidase activity, polyphenol oxidase was determined according to (Maxewill and Bateman, 1967). The changes in absorbance were measured spectrophotometrically at 495 nm and recorded every 1 min for up to 4 min. The Beckman Spectrophotometer Du®7400 was used to take all of the measurements. The enzyme phenylalanine ammonia-lyase (PAL) was measured in acetone powder made from leaves using Zucker's (1968) method of 0.75 g acetone powder suspended in sodium borate buffer, pH 8.8. 0.5 ml enzyme preparation, 1.5 ml borate buffer 0.2 M, pH 8.8, 1 ml % phenylalanine, and 2.5 ml deionized water made up the reaction mixture. As a blank, 1 ml deionized water was used instead of phenylalanine. For one hour, the mixture was incubated at 40 °C. 0.5 ml of 5 N HCL was added to each tube to stop the reaction. Optical density at 290 nm (t-cinnamic acid) per 0.1 g acetone powder was used to measure enzyme activity.

### 2.5. Scanning electron microscopy (SEM) examination of the interaction between *B. subtilis*, seeds extract (50 g/L) of *M. oleifera*, potassium bicarbonate (10 g/L) compared with Montoro fungicide (1 ml/L) and *C. beticola* on sugar beet leaves

The impact of the treatments on the number and diameter of spots on diseased sugar beet leaves and the generation of *C. beticola* conidiospores and spores. Manzali et al. (1993). At the Electron Microscope Unit, Nanotechnology Institute, Kafrelshiekh University, interaction sites (spots) were marked, and disc blocks of 1 cm<sup>2</sup> were excised for SEM using a Jeol Scanning Electron Microscope model JSM-5500lv. The interaction region was fixed with osmium oxide and subsequently dehydrated with a sequence of dilutions of ethyl alcohol and lastly acetone. The samples were

subsequently dried with a critical point drier (EMS 850), coated with gold with a sputter coater (EMS 550), and analyzed with a SEM (Jeol 100cx- 11 ASID-4D).

## 2.6. Statistical analysis

According to (Gomez and Gomez, 1984), statistical analysis was performed, the analysis of variance and the means were further tested using the least significant difference test (LSD).

## 3. Results

### 3.1. Isolation, identification, and evaluation of sugar beet cvs. against *Cercospora beticola*

Isolation trials carried out on diseased sugar beet plants with leaf spots during the sugar beet growing season resulted in the isolation of fifteen fungal isolates. These isolates were identified according to morphological and microscopical characteristics as *C. beticola*. In an experiment, the aim was to evaluate sugar beet varieties for susceptibility to *Cercospora* leaf spot disease. Twenty-five cultivars were artificially infected ninety days after cultivation with  $5 \times 10^4$  spore/ml of *C. beticola*. After a week of infection, the incidence and severity of the diseases were estimated as describe above. The results indicated that the disease severity of *Cercospora* on beet varieties ranged from 1.33 to 20%, and the cultivar Gregoria-kws was the least susceptible to infection in the first season of evaluation. While it ranged between 2.66 and 35% in the second season, and the idira-kws type was the least susceptible to injury (Table 1).

### 3.2. Effect of the used treatments against *Cercospora beticola* under laboratory conditions

*B. subtilis* inhibited the fungal growth as a relative power of antibiosis of *C. beticola*. Potassium bicarbonate at 5 and 10 g/L and seeds extract of *M. oleifera* at 25 and 50 ml/L also significantly inhibited the linear growth of *C. beticola* compared to the fungicide Montoro (Table 2).

### 3.3. Effect of the used treatments against sugar beet leaf spot caused by *Cercospora beticola* under field conditions

The effect of seven treatments, cell suspension ( $10^8$  cell/mL) of *B. subtilis*, Potassium bicarbonate at 5 and 10 g/L, and seeds extract of *M. oleifera* at 25 and 50 ml/L 1 ml/L of fungicide Montoro compared to control treatment (water). These treatments were sprayed three times with ten-day intervals on sugar beet plants aged approximately 90 days and naturally infected (3–5%) with *Cercospora* leaf spots. The disease severity of the *Cercospora* leaf spot was recorded before the first, second, and third spray by 24 hr. for two seasons (2019/2020) and (2020/2021) (Tables 3 and 4). The disease severity of *C. beticola* was significantly reduced due to spraying the used treatments relative to control in both tested seasons. Among the tested treatment, plant extracts of *M. oleifera* at 50 ml/L were the most effective against *C. beticola* in the first season since they reduced disease severity by 68.3%. While Potassium bicarbonate at ten g/L in the second season followed by seeds extract of *M. oleifera* at 50 ml/L. They caused a reduction of disease severity by 66.4 and 63.8% and cell suspension of *B. subtilis*, 64.7% in the first season and 61.6% in the second season.

**Table 2**

Effect of different applied treatments against *Cercospora beticola* growth under laboratory conditions (25–30 °C).

Treatments	Conc.	Linear growth (cm)	Reduction of linear growth %
Potassium bicarbonate	5 g/L	1.6 b	77.14 e
	10 g/L	0.9 bc	87.14 d
Plant extract of <i>Moringa oleifera</i>	25 ml/L	0.8 bc	88.57 c
	50 ml/L	0.5 bc	92.85 b
Fungicide, Propiconazole 15% + Difenconazole 15% (Montoro)	1 ml 1/L	0.0 c	100.00 a
Control (water)	0.00	7.0 a	0.0 f
LSD (0.01, 0.05)		2.054, 1.463	1.141, 1.023

Means designed by the same letter are not significantly difference at the 5% level according to Duncan's test.

### 3.4. Effect of the used treatments on total soluble solids contents% and sucrose% of sugar beet plants

To study the effect of the treatments on some qualities indices of the beetroots, roots content was estimated from total soluble solids and sucrose percentage. Data in (Table 5) showed that total soluble solids content % and sucrose % were significantly increased in all tested treatments relative to control in both tested seasons. Among the tested treatments, Potassium bicarbonate at ten g/L and *M. oleifera* at 50 ml/L were the most effective treatments, followed by cell suspension of *B. subtilis* in both tested seasons, respectively.

### 3.5. Scanning electron microscopy (SEM) examination of the interaction between *B. subtilis*, seeds extract (50 gm/L) of *M. oleifera*, potassium bicarbonate (10 gm/L) compared with Montoro fungicide (1 ml/L), and *C. beticola* on sugar beet leaves

The fungus spots were examined using a scanning electron microscope (SEM) in a study to determine the mechanism in which these treatments affect the growth rate of the fungus that causes *Cercospora* spot disease and the method of its formation for conidiophores and conidiospores, as well as the diameter of infection spots on beet leaves. The scanning electron microscope slides showed that spraying beet leaves with the material under study led to a reduction in the numbers of spots (Fig. 1) and the diameter of the spots (Fig. 2) with different degrees. The tests also revealed the absence of the ability to form conidiophores and conidiospores (Fig. 3), and at the same time, the formation of its appearing in a distorted form (Fig. 3). The slides also indicated the destruction and degradation of fungus conidiophores and conidiospores (Fig. 3) and the presence of blockage of most stomata in the affected areas.

### 3.6. Effect of the used treatments on polyphenol oxidase (PPO), peroxidase (POX) phenylalanine ammonia-lyase (PAL) enzyme activity

One of the essential criteria studied to find out the positive effect of using some treatments in controlling plant pathogens is studying the effect of those treatments on the activity of oxidizing enzymes. Polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase, one of the paramount of these enzymes; therefore, the effect of the activity of the materials used in this study on the activity of these enzymes was estimated for five minutes after each spray, as shown in the following diagrams (Figs. 4, 5 and 6). It is evident through these graphs that there is a common factor between them all, which is the presence of a gradual increase in the activity of these enzymes as a result of treating sugar beet plants with the script of these materials. The most positive of those

**Table 3**  
Disease severity and efficacy of different applied treatments against *Cercospora beticola* in the first season.

Treatments	Conc.	% of reduction of cercospora leaf spot disease severity after						Mean of %R
		1st spray		2nd spray		3rd spray		
		D.S	%R	D.S	%R	D.S	%R	
<i>Bacillus subtilis</i>	10 <sup>8</sup> cell/ml	9 bc	62.5 b	15 bc	62.5 d	20 bc	69.2 c	64.7 d
Potassium bicarbonate	5 g/L	12 b	50.0 e	16 b	60.0 e	22 b	66.2 d	58.7 f
	10 g/L	10 bc	58.3 c	14 bcd	65.0 c	19 bc	75.4 a	66.2 c
Plant extract of <i>Moringa oleifera</i>	25 ml/L	11 b	54.2 d	13 cd	67.5 b	20 bc	69.2 c	63.6 e
	50 ml/L	9 bc	62.5 b	12 d	70.0 a	18 bc	72.3 b	68.3 b
Fungicide, Propiconazole 15% + Difenconazole 15% (Montoro)	1 ml/L	7c	70.8 a	12 d	70.0 a	16 d	75.4 a	72.1 a
Control (water)	0.00	24 a	0.0 f	40 a	0.0 f	65 a	0.0 e	0.0 g
LSD (0.01)		4.681,	0.823,	3.391,	1.804,	4.550,	0.383,	0.660,
LSD (0.05)		3.371	0.594	2.441	1.306	3.280	0.274	0.474

Means designed by the same letter are not significantly difference at the 5% level according to Duncan's test.

**Table 4**  
Disease severity and efficacy of different applied treatments against *Cercospora beticola* in the second season.

Treatments	Conc.	% of reduction of cercospora leaf spot disease severity after						Mean of %R
		1st spray		2nd spray		3rd spray		
		D.S	%R	D.S	%R	D.S	%R	
<i>Bacillus subtilis</i>	10 <sup>8</sup> cell/ml	7 b	61.1 a	15 b	57.1 e	16 b	66.6 c	61.6 d
Potassium bicarbonate	5 g/L	9 b	50.0 c	13 bc	62.9 d	14 bc	70.8 b	61.2 d
	10 g/L	8 b	55.5 b	11 cd	68.6 b	12 c	75.0 a	66.4 b
Plant extract of <i>Moringa oleifera</i>	25 ml/L	9 b	50.0 c	12 bcd	65.7 c	13 bc	72.9 ab	62.9 c
	50 ml/L	8 b	55.5 b	13 bc	62.9 d	13 bc	72.9 ab	63.8 c
Fungicide, Propiconazole 15% + Difenconazole 15% (Montoro)	1 ml/L	7 b	61.1 a	9 d	74.3 a	12 c	75.0 a	70.1 a
Control (water)	0.00	18 a	0.0 d	35 a	0.0f	48 a	0.0 d	0.0 e
LSD (0.01)		4.821,	0.660,	4.610,	0.887,	4.399,	2.107,	0.772,
LSD (0.05)		3.473	0.474	3.326	0.635	3.165	1.517	0.552

Means designed by the same letter are not significantly difference at the 5% level according to Duncan's test.

**Table 5**  
Effect of the used treatments on yields, total soluble solids contents% and sucrose% of sugar beet plants.

Treatments	Conc.	TSS %		Sucrose %	
		1st season	2nd season	1st season	2nd season
<i>Bacillus subtilis</i>	10 <sup>8</sup> cell/ml	23.8 ab	23.2 bc	19.04 a	18.56 abc
Potassium bicarbonate	5 g/L	22.2 c	23.6 b	17.76 b	18.88 ab
	10 g/L	23.2 bc	24.8 a	19.04 a	19.84 a
Plant extract of <i>Moringa oleifera</i>	25 ml/L	23.8 ab	22.4 c	19.04 a	17.92 bc
	50 ml/L	24.6 a	23.8 ab	19.68 a	19.04 ab
Fungicide, Propiconazole 15% + Difenconazole 15% (Montoro)	1 ml/L	23.8 ab	23.2 bc	17.12 bc	16.96 cd
Control (water)	0.00	21.2 d	20.0 d	16.96 c	16.00 d
LSD (0.01, 0.05)		0.951,	1.077,	0.871,	1.934,
		0.686	0.772	0.636	1.390

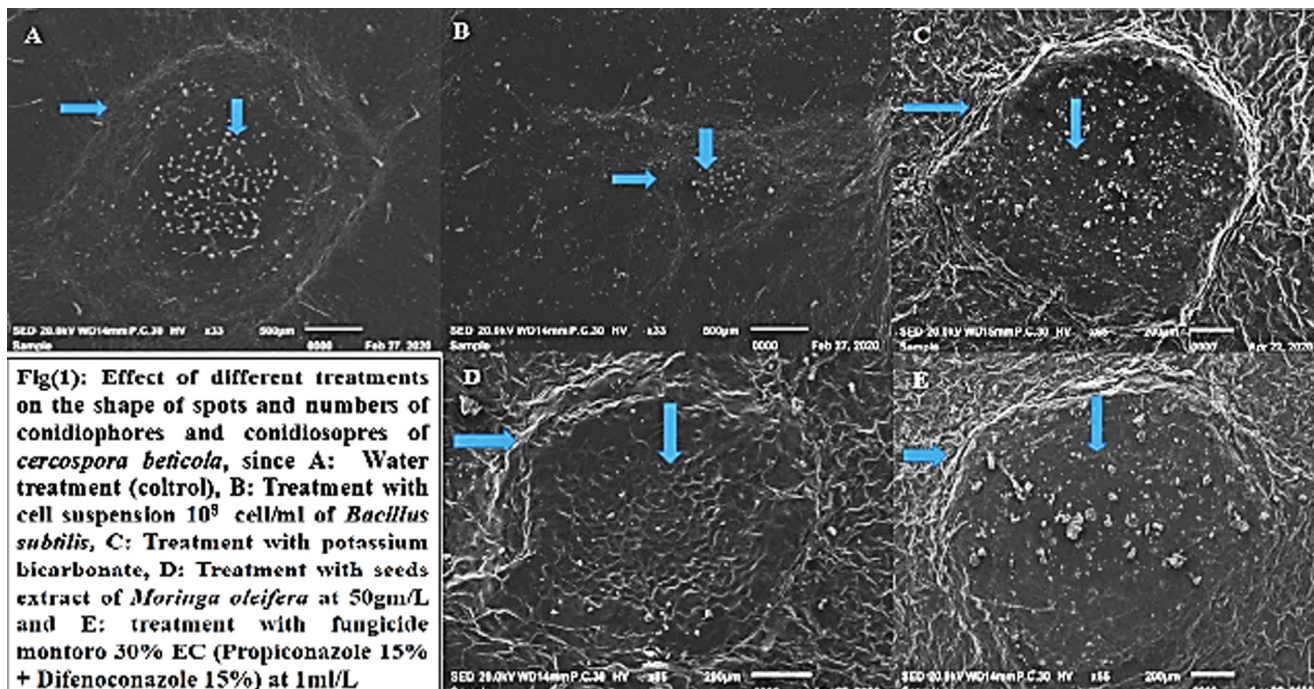
Means designed by the same letter are not significantly difference at the 5% level according to Duncan's test.

treatments in increasing the activity of polyphenol oxidase and peroxidase enzymes was the pesticide Montoro, followed by the extract of *Moringa* seeds, potassium bicarbonate, then the cell suspension of *B. subtilis*. As for the effect of the materials used in this study on the activity of the enzyme phenylalanine ammonia-lyase, the results shown in Fig. 6 show that the best effect was the *Moringa* seeds extract, then the suspension of *B. subtilis*, potassium bicarbonate, then the Montoro pesticide.

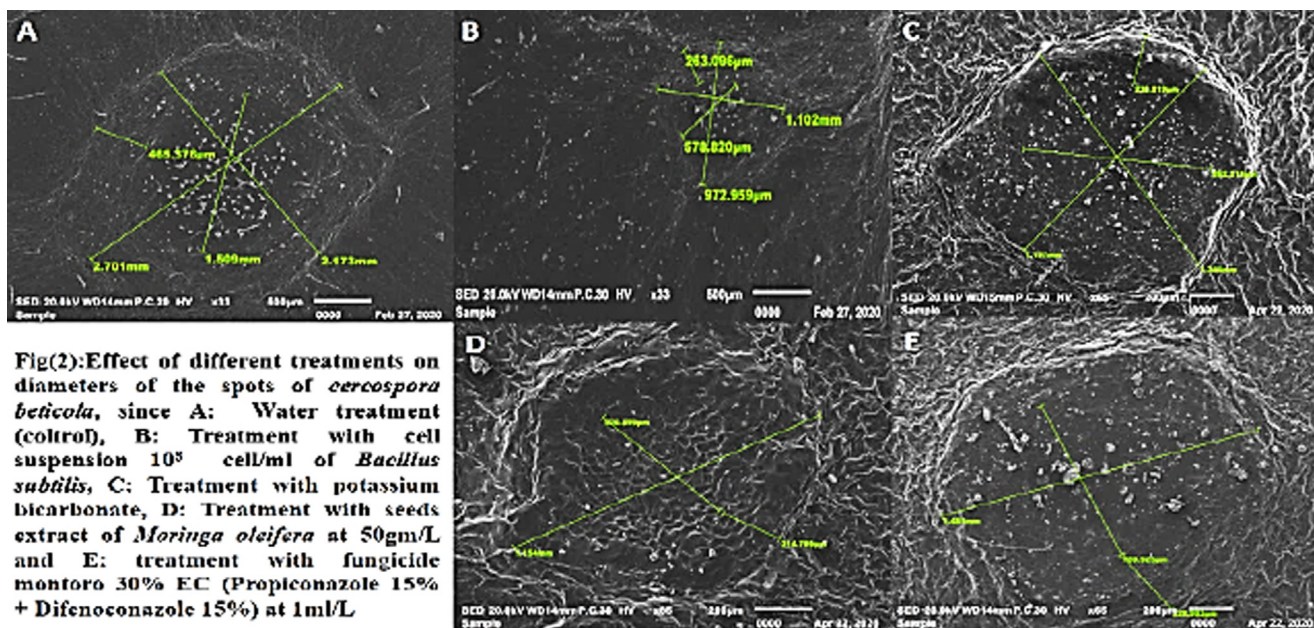
#### 4. Discussion

Many plant diseases affect sugar beet plants; the most important and dangerous is the *Cercospora* leaf spot caused by *C. beticola* (Agrios, 2005). This disease can cause significant losses in root yields and the percentage of sugar in them and make it challenging to extract sugar from the roots and the quality of the produced sugar (Lamey et al., 1996; Rossi et al., 2000; El-Moghazy et al.,

2011). Chemical pesticides are the first choice for farmers in their fight against diseases that afflict their plants. It contains effective substances that kill and destroy the beneficial and harmful microbes significantly, unfortunately. It also eliminates most environmentally friendly insects and microbes, which disrupts the ecosystem and creates the appropriate conditions for producing new physiological strains that are resistant to pesticides and capable of breaking the resistance of plant varieties (2000–2019 (Pesticide Action Network, North America). To avoid or minimize these dangerous drawbacks, researchers are trying to find other safer methods (Saad et al., 2021; El-Ashry et al., 2021). Biological control, whether by using biocontrol agents or essential oils (El-Tarabily et al., 2021; Abd El-Hack et al., 2021), plant extracts (Saad et al., 2021a; El-Saadony et al., 2021; Abdel-Moneim et al., 2022), bioactive peptides (El-Saadony et al., 2021a,b; Saad et al., 2021b), polyphenolic enriched wastes (Saad et al., 2021c), and environmentally friendly chemicals such as amino acids



**Fig. 1.** A: The normal and typical shape of cercospora spots, showing large numbers of conidiophores and conidiospores. And the presence of a defined edge and a large clear halo between the affected and healthy tissue (control treatment). B, C, D and E: The effect of the treatments is shown as it caused extreme minimization in the aura and a clear reduction in the number of fruiting structures of the fungus and may completely disappear in one spot (D and E).

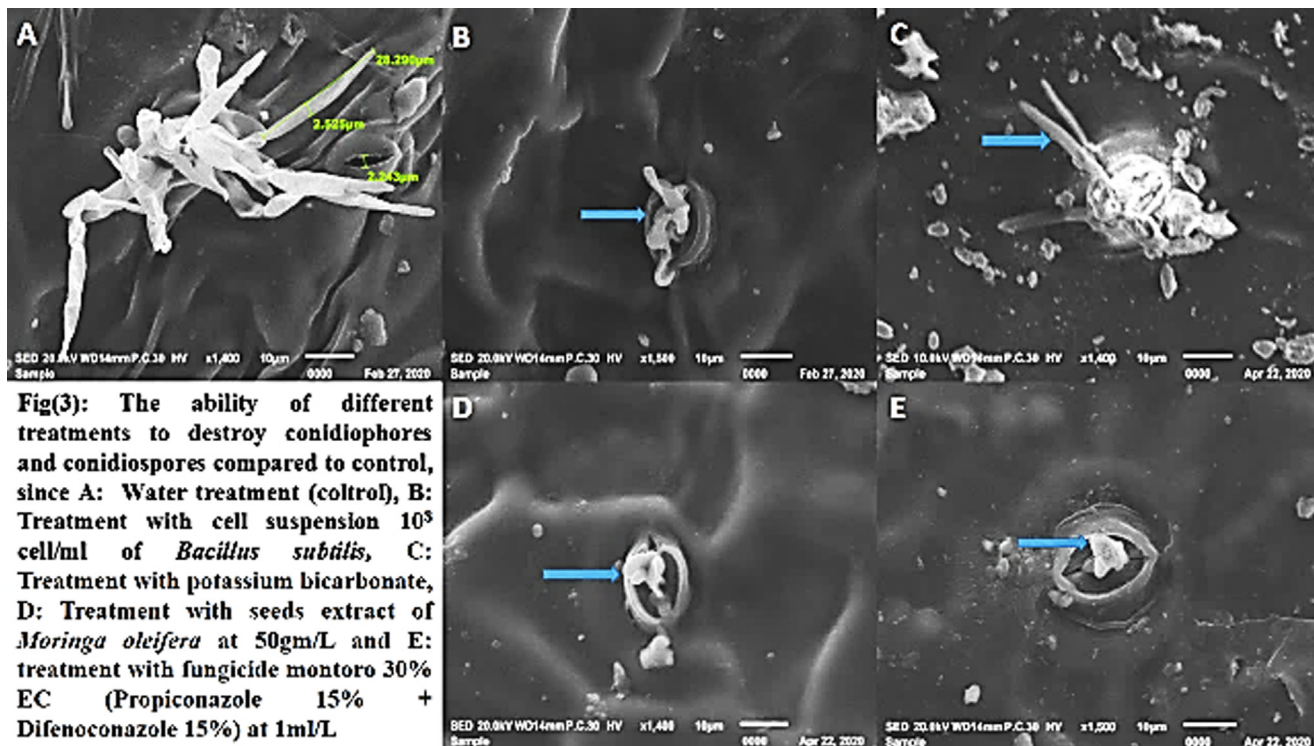


**Fig. 2.** A comparison between the diameters of the spots, as illustrated from the detected measures, therefore, it is noticed that the diameter of the control (A) spot is much larger than the spots of treatments (B) and may be doubled in some treatment (C, D and E). Same for the diameter of the halo.

(Fouda et al., 2022; El-Sobki et al., 2021), is one option that attracts attention vigorously in this field. Therefore, there is a constant need to search for safer and less harmful means of these pesticides (Ezhilarasi et al., 2016).

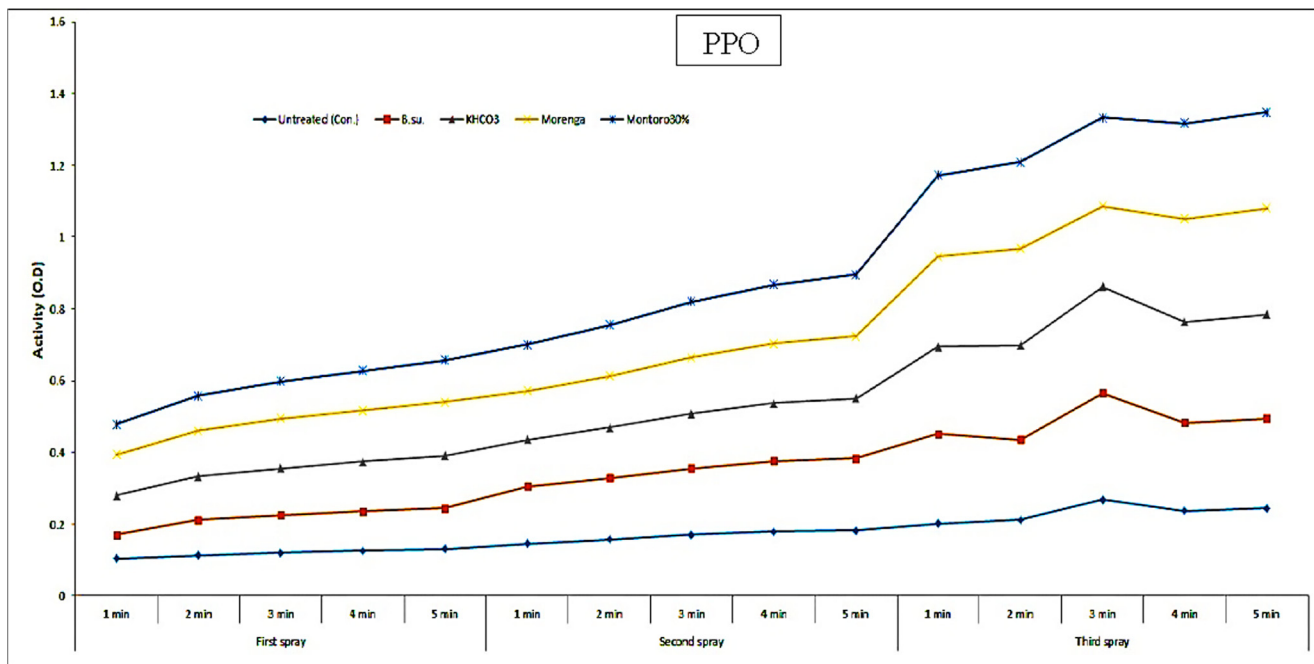
This study was based on evaluating models of these alternatives, where an organism of biocontrol agents, *B. subtilis*, was used in the form of a cell suspension as a type of biological control. *M. oleifera* seeds extract was also present in this study in two different concentrations. In addition to evaluating the use of one of the envi-

ronmentally friendly chemicals that are very beneficial for plants, namely potassium bicarbonate. At the beginning of the study, plant specimens infected with *Cercospora* leaf spots were collected, and the pathogen isolation process was performed on them. Indeed, the pathogen was isolated in pure culture, and it was known as *C. beticola*. Artificial inoculation of this pathogen was performed on twenty-five varieties of sugar beet as a common risk between testing its pathological ability and at the same time assessing the susceptibility of these varieties to infection with *Cercospora* leaf spot.



**Fig(3):** The ability of different treatments to destroy conidiophores and conidiospores compared to control, since A: Water treatment (coltrol), B: Treatment with cell suspension  $10^5$  cell/ml of *Bacillus subtilis*, C: Treatment with potassium bicarbonate, D: Treatment with seeds extract of *Moringa oleifera* at 50gm/L and E: treatment with fungicide montoro 30% EC (Propiconazole 15% + Difenconazole 15%) at 1ml/L

**Fig. 3.** In this figure the direct effect on the fungus is illustrated, and with a simple comparison between the control (A), it's found that spraying plants with different treatments has led to the decomposition of spores and conidiophores (B, C, D and E). At the same time, the fungal structures became worthless and the fungus could not be used to cause new infections.



**Fig. 4.** Effect of Moringa seeds extract, cell suspension of *B. subtilis* and potassium bicarbonate on polyphenol oxidase (PPO) enzyme activity.

This study has proven the ability of this pathogen to cause injury and to show the pathological symptoms characteristic of the disease on all tested varieties with different degrees. Gregoria-kws and Idira-kws cvs were the least susceptible to infection and could be relied upon in future agriculture. Previous studies have shown similar results in this field, as there are four to five genes for resistance to the fungus *C. beticola*. If they are present, the variety

appears resistant to disease more than the variety in which those genes are not present (Smith and Gaskill, 1970). Laboratory *B. subtilis* demonstrated a tremendous ability to inhibit the growth of *C. beticola*, as a large area of inhibition was formed between it and the pathogen. Many researchers have attributed the ability of *B. subtilis* to antagonize and stop the growth of various pathogens to its ability to produce certain antibiotics such as subtilin and mycosubtilin

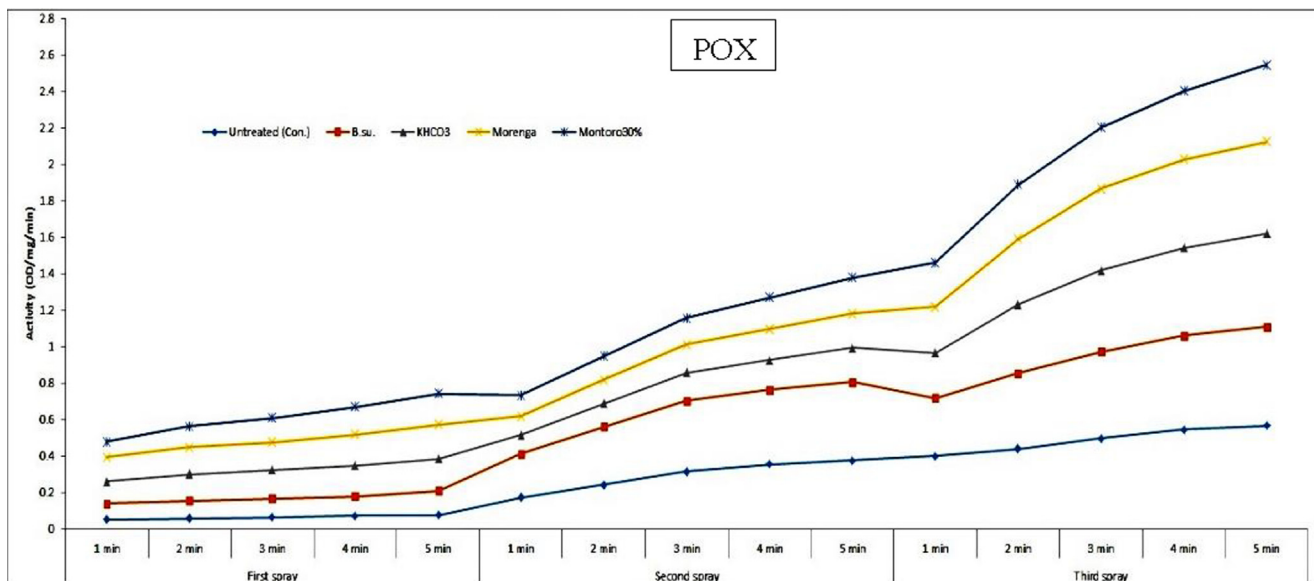


Fig. 5. Effect of Moringa seeds extract, cell suspension of *B. subtilis* and potassium bicarbonate on peroxidase (POX) enzyme activity.

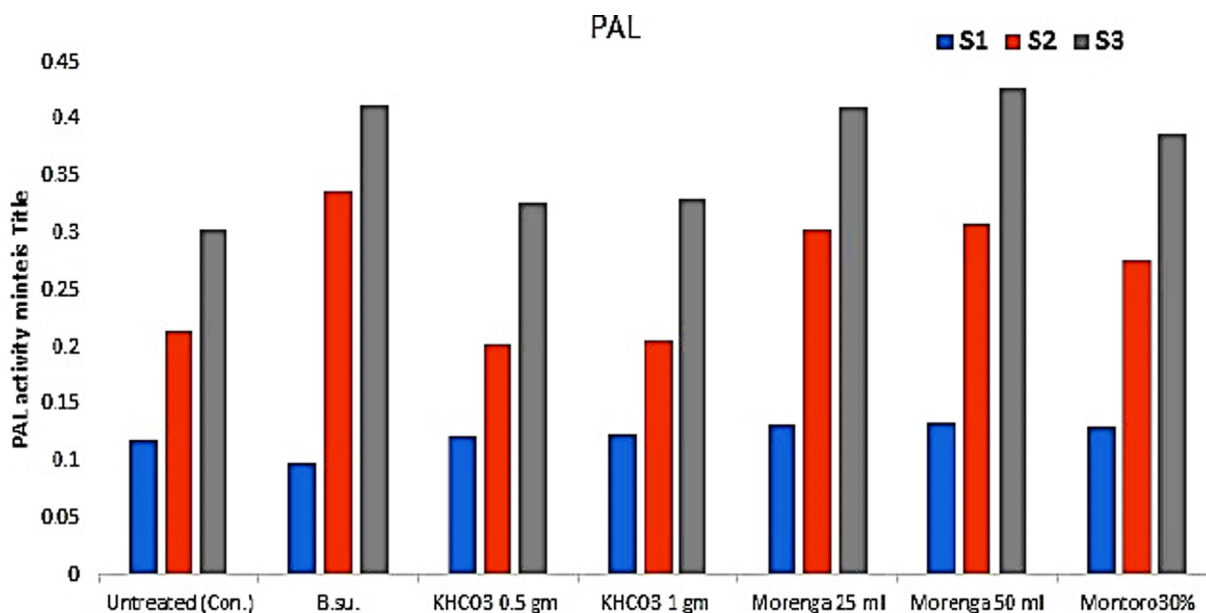


Fig. 6. Effect of Moringa seeds extract, cell suspension of *B. subtilis* and potassium bicarbonate on phenyl alanine ammonia lyase (PAL) enzyme activity.

(Kowall, 1998). Harman et al. (2008) illustrated that another mode of action included the production of enzymes or antibiotics that can inhibit the growth or reduce the competitive ability of other microorganisms. These antibiotics and enzymes are amphiphilic, membrane-active surfactants, and peptide antibiotics with specific antimicrobial potential. Under this study, *B. subtilis* showed a high ability to significantly reduce the disease severity of *Cercospora* leaf spot under field conditions. This ability is due to the rapid spread of bacillus cells on the surface of plant leaves and the prevention of pathogen spores from reaching the natural openings, thus preventing infection. It can also compete heavily for oxygen and nutrients on the surface of the leaves and deprive pathogens of obtaining them and starving them ((Romero et al., 2008). Also, the examination with a scanning electronic microscope (SEM) proved that *B. subtilis* caused the plasmolysis and decomposition of conidiophores and conidiospores of *C. beticola*. This result is in line with the previous explanations for several similar cases with

plant pathogenic fungi, which were mentioned by previous researchers, who indicated that *B. subtilis* secretes fungal cell-degrading enzymes including b-1, 3-glucanase, and protease Antal et al. (2000). Under this research, moringa seeds extract at a concentration of 25 and 50 g per liter has given excellent results, whether under laboratory or field conditions, in the control of *C. beticola*, as it significantly reduced both the linear growth of the fungus and the disease severity of *Cercospora* leaf spot and powdery Mildew. Much previous research has proven that many plant extracts contain many toxins and fungal inhibitors that negatively affect the growth of pathogens. (Siripornvisal and Ngamchawee, 2010; Abdel-Kader et al., 2013; Tabassum and Vidyasagar, 2013; Hadi and Kashefi, 2013). The phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, tannins, triterpenoids, and steroids (Zaffer et al., 2015). The antimicrobial activity of *M. oleifera* was evaluated against *Pasturella multocida*, *Escherichia coli*, *Staphylococcus aureus*, *Fusarium solani*, and *Rhizopus solani* strains



(Jamil et al., 2008) the antimicrobial activity of extract can be improved by combined with other substances (Madubuonu et al., 2019; Matinise et al., 2017; Matinise et al., 2018; Aisida et al., 2019; Aisida et al., 2020). The inhibition zones of growth showed greater sensitivity against the bacterial strains as compared to the fungal strains. Earlier reports have been elucidated on the findings of the antibiotic principle of *M. oleifera* seeds through their purification, elucidation, and antimicrobial properties, and also on the antibiotic substance of the roots of *M. oleifera* (Bowers and Locke, 2000; El-Mohamedy and Abdallah, 2014). Also, Moringa seeds extract contains organic compounds and pigments such as carotenoids, flavonoids, isothiocyanates, niacin, glucosinolates, minerals, and sterols responsible for forming antioxidants. (Bowers and Locke, 2000; Jamil et al., 2008; Dwivedi and Enespa, 2012). Potassium bicarbonate is one of the environmentally friendly chemicals that are very beneficial for plants, which were evaluated for use in this study *In-vitro* against *C. beticola* and *In-vivo* in controlling *Cercospora* leaf spots. Whether laboratory or field, two concentrations of them, five and ten grams per liter, were evaluated. Under this statistical study, potassium bicarbonate had a significant reduction in the linear growth of *Cercospora* despite being less in that than moringa seeds extract. In the field, potassium bicarbonate has achieved good results in combating the two diseases under study. Also, the results of examining samples treated with potassium bicarbonate with a scanning electron microscope (SEM) indicated the occurrence of decomposition of fungal structures and its inability to produce both conidiophore and conidiospores. Previous research indicates the possibility of using potassium bicarbonate to combat certain plant diseases such as scabies, Powdery Mildew and sooty blotch in apples (Ilhan et al., 2006; Jamar et al., 2007; Marku et al., 2014), cucumber powdery mildew Homma et al. (1981), Powdery Mildew and a black spot of roses Horst et al. (1992) and Powdery Mildew in table grapes (Sawant and Sawant, 2008). The mode of action of bicarbonate salts is associated with the perturbation of osmotic pressure, pH, and the bio carbonate/bicarbonate ion balance of sensible fungi. Bicarbonate works by coming into contact with fungi in an aqueous solution and prevents the development of fungal mycelium and spores. Due to its multi-site mode of operation, the risk of developing resistance is believed to be low (Horst et al., 1992; Jamar et al., 2007). Complementing the study and searching for an additional explanation of the positive results of the treatments used to control each of the diseases under study. Some oxidation enzymes, such as polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase, have been estimated, and its action is associated with inducing plants to resist pathogens (El-kazzaz et al., 2015a; El-Kazzaz et al., 2015b; Radjacommaré et al., 2004; Swelum et al., 2020). The results showed that the treatment of sugar beet plants was correlated with a significant improvement in the activity of these enzymes. These results further explain the ability of the treatments to reduce the severity of the pathogen under study. At the same time, improving some qualities of sugar beet, such as total soluble solids content and sucrose percent.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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