© by PSP Volume 29 – No. 07A/2020 pages 6315-6327 Fresenius Environmental Bulletin

EXOGENOUS APPLICATION OF *BACILLUS SUBTILIS* AND H_2O_2 MITIGATED FIRE PEAR BLIGHT BACTERIAL DISEASE INCIDENCE IN CORRELTAED WITH YIELD AND FRUIT QUALITY IMPROVEMENT

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ABSTRACT

Pear fire blight bacterial disease caused by *Erwinia amylovora* as biotic stress is one of the most serious diseases that cause great loss of pear crop in Egypt and over world. *E. amylovora* pathogen was isolated from pear infected plants and molecularly characterized by PCR using specific primer. Koch's postulates applied to assure the pear fire blight disease that causes high defects in the trees growth and yield. The treatments which used in this research to control the disease were *Bacillus subtilis* as abio-agent, Huwa San 25 commercial product of hydrogen peroxide (H_2O_2), oxolinic acid (Starner 20%) and antibiotic streptomycin. *In vitro* bio-agent such as *Bacillus subtilis* was able to inhibit the bacterial growth of *E. amylovora*. However, H_2O_2 did not inhibit the bacterial growth as compared to the starner and streptomycin which showed significant inhibition. Under greenhouse disease symptoms and severity of inoculated pear seedlings and treated with *B. subtilis*, H_2O_2, starner and streptomycin showed significant inhibition and decrease as compared with the control infected only. Under field conditions and natural infection, as a result of application with *B. subtilis*, H_2O_2, starner and streptomycin, disease severity, disease symptoms and electrolyte leakage % were decreased significantly. Interestingly that the vegetative growth such as shoot length and diameter were significantly increased with all treatments. Fruit set, yield and quality were improved compared to the control. At the open field, the application of H_2O_2 gave the best results for the most characteristics followed by *Bacillus subtilis*, starner and streptomycin respectively.

KEYWORDS:
Erwinia amylovora, *Bacillus subtilis*, H_2O_2, Streptomycin, Starner

INTRODUCTION

Pear (*Pyrus communis* L.) is the fifth deciduous fruit cultivated crop by production over the world. The cultivated area in Egypt is 4519 (Ha), with production 71816 tones [1]. Fire blight is the most serious diseases that causes great loss of pome fruits trees especially pears in Egypt and over world. The causative agent of fire blight is *Erwinia amylovora*, the Gram-negative bacteria, a demoralizing disease disturbing species of the family Rosaceae [2]. In 1976, fire blight caused largely damage in pear trees in California, USA, which was determined by 4.7 million dollars. In 1991, a severe eruption in South Western Michigan brings about an evaluated damage of 3.8 million dollars. In Egypt, by 1988 fire blight (infected) damaged about 80% of all cultivated area of pears, resulted in abolition of 50% of all trees. Latterly, fire blight initiated great damages of pear trees that were estimated by 68 million dollars in North- West America, 10 million dollars in one area of New Zealand, and 500,000 trees were devastated in Lebanon and Italy. Meanwhile the finding of fire blight in Morocco in May 2006, the disease extent to most of species of the family Rosaceae producing areas, making serious loss [3]. A temperature of 18 ◦C looks essential for blossom blight epidemics to be happen in vivo and rain and/or weighty dew by the end of warm periods encourage infection actions in the field. Additionally, at 18◦C, the appearance of pathogenicity and extra function genes is improved with higher temperatures [4]. The infection affects all airborne plant structures, making their dieback. Blossoms are the best essential location of infection

for widespread fire blight incidence, afterwards the developing upper sprouts. The pathogen extents from stigmas to the hypanthium in surface moisture and attacks through the nectarthodes. The disease has been accomplished in current periods through danger valuation [5]. It is a multifaceted disease which permits its whole rotation in nearby connotation with the host plant, somewhere it is capable to infect fruits, leafs, shoots and flowers flesh. Diseased plant parts will, in all suitcases, result in gluey drops of ooze, collected of viable bacteria in a polysaccharide matrix, to be formed on the infected plant parts. As a result of suitable weather conditions and susceptible cultivars, sharp development of infection is happen therefore, fire blight has become a main controlling factor for effective family Rosaceae production [6]. Chemical control of this bacterial disease is too difficult, for the reasons that there are limited operative (effective) bactericides registered and even though the most active antibiotic streptomycin also additional antibiotics are not recorded international. Also, antibiotics such as streptomycin, which are very effective compounds, often induce antibiotic resistance in *E. amylovora* and threat the human health [2]. *Bacillus* spp. as bioagent are considered safe, it has high possibility for disease control because they are located everywhere in nature and exhibit high thermal tolerance by forming resistant spores [7]. *Bacillus subtilis* is one of the antagonistic microorganisms used as a bio-control agent against soil-borne and foliar diseases [8], [9]. *Bacillus* spp. has been shown to possess antifungal activity against plant pathogens [10]. *Bacillus megaterium* as a plant growth promoting rhizobacteria plays an important role in controlling plant diseases [11]. Hydrogen peroxide (H_2O_2) one of the most important reactive oxygen species (ROS) in which be used as a safety compound with low concentration to play a pivotal role against plant pathogens such as barley net blotch disease [12], [13], wheat leaf rust [14], [15], barley powdery mildew [16], [17], *Tobacco Mosaic Virus* [18] and cucumber powdery mildew fungus [19]. Low concentration of H_2O_2 was used to control of *Botrytis cinerea* on white pepper Fruits under post-harvest storage [20]. Early increase of H_2O_2 has a essential role in non-host resistance mechanisms in legume and cereal plants to the incompatible pathogens [21]. H_2O_2 is also used to improve seed germination, seedling growth of cabbage and watermelon which growing in soil infected with soil borne diseases [22]. 5-7 mM of H_2O_2 suppresses the necrotic disease symptoms in Tobacco infected with fungal, bacterial and viral pathogens through up-regulation of antioxidants enzymes [22]. Oxolinic acid (Starner) is a commercial resistant inducer compounds used for controlling plant disease [23]. Starner had inhibitory effect to soft rot bacterial on potato caused by *Erwina carotovora* [24]. Oxolinic acid is a quinolone antibiotic, it used for control of bacterial diseases on rice and vegetables such as cabbage, potato, and onion; it is not widely used against fire blight. The efficacy of oxolinic acid against *E. amylovora* was evaluated in 43 orchard experiments in Israel. Changes in the Sensitivity of *E. amylovora* populations to Streptomycin and oxolinic acid also investigated [25]. The aim of the current research is to find out new biological control agents against pear fire blight bacterial disease caused by *Erwinia amylovora* in which safety for the human and environment as well as low cost as compared to the control methods using antibiotic streptomycin and the starner as a bactericidal compound, consequently, improve yield with good fruit quality.

MATERIALS AND METHODS

Plant materials. Infected leaves and flowers of pears were used to isolate *Erwinia amylovora* from pear plants cultivar 'Le conte'. Infected samples showed the typical characteristic symptoms of fire blight bacterial disease. Samples were collected from four Governorates of Egypt namely, El-Gharbia, Kafr El-Sheikh, Dakahlia and Alexandria during the year 2017-2018.

Koch's postulates. Isolation and purification. Isolation was performed from infected leaves and flowers of pear plants on king´s B medium and nutrient sucrose agar medium. Samples were first washed with sterile water then surface sterilized by1% sodium hypochlorite sodium for 5 min., then; samples were washed with sterile distilled water and dried with tissue paper. Samples were cut with sterile scalpel and crushed in sterile mortar with few drops of sterile saline (0.8%) with 5 ml pipette. 50µl of bacterial suspension was streaked onto nutrient sucrose agar (5%) plates. The inoculated plates were incubated at 27^0C for 48hr. Inoculated plates were daily observed for single colony growth and consequently purified. Obtained isolates were kept on 2% glycerol nutrient agar slants, 50% glycerol and lyophilized for using later as well [26].

Pathogenicity test and artificial inoculation. To make pathogenicity assay healthy immature pears fruits were used. Healthy immature pear fruits were washed with sterile water, sterilized with ethanol 70% and dried with tissue paper then cut into slices 1cm in thickness. For each sample 3-5 slices were used. Slices were put in sterile petri dishes up to wet sterile cotton. Every slice was inoculated with 50 µL of *E. amylovora* isolates suspensions (1×10^8 CFU ml). Petri dishes were incubated at 28C for 5 days. Control treatment was treated with ster

TABLE 1
Sequences of specific primer for molecular characterization of *Erwinia amylovora* bacteria the causal agent of pear file blight disease

Primer	Oliogonucleotide sequence (5'-3')	Target gene	Length of amplified fragment	Reference
AmSb1 A	GCTACCAGCAGGGTGAG		1100bp	[27]
AMSb1 B	TCATCACGATGGTGTAG			

ile distilled water. After 5days existence of bacterial oozes was a positive response of *E. amylovora* in contrast to control treatment [2, 27]. Similarly, pear seedlings were inoculated under greenhouse conditions.

Molecular characterization. DNA extraction and PCR analyses. DNA was extracted by i-genomic BYF DNA Extraction mini kit Cat.No.17361. The reaction mixture was 25µl which consisted of 12.5µl of 2X master mix (0.1U/µl Tag polymerase, 500 µM dNTP, 20 mM Tris-HCL (PH8.3), 100mM KCL, 3mM Mgcl2 and stabilizer and enhancer), 10 pmol of each primer, and 2µl of template DNA (50µg/ml). PCR was conducted in an Eppendorf thermal cycler. Amplification was performed in a thermocycler (Applied Bio-Rad, USA). The initial denaturation at 94C for 5 minutes, then the cyclic condition was 35 cycles of denaturation at 94 C for 30sec, primer annealing at 50 C for 1 min and extension at 72 C for 1 min. a final extension at 72 C was given for 10 minutes. The expected DNA fragments were 1100 base pairs in length [27].

Chemical materials and application under field conditions. *Bacillus* subtilis (1×10^6 cfu/ml), was used to evaluate its antibacterial effect against *E. amylovora* the causal agent of pear fire blight bacterial disease using agar well diffusion method [28]. The isolates were obtained from EPCRS Excellence Center, Faculty of Agriculture, Kafrelsheikh University, Egypt. Huwa San 25 commercial product of hydrogen peroxide (H_2O_2) as active compound 24.5-24.9% obtained from International Agronomist for Agricultural Projects Company, 5 E; Salam St., El Ahram, Giza. Egypt was used with dose 3ml/L. Oxolinic acid (Starner 20% WP) was used 1,5 gm/L water and produced from Sumitomo Chemical Limited Company, Japan in which obtained by My Trade company, Egypt. The antibiotic streptomycin was obtained from Egypt Masters Co. (EMC), Dakahlia, Egypt and used with dose 150 ppm.

In vitro evaluation of antibacterial bioagent and compounds. King´s B medium (50°c) was inoculated with 24 hrs broth of *E. amylovora* (10^8 cfu /ml), poured into sterilized Petri –plates (9 cm) and let to harden. 10 mm wells were made in seeded king´s B medium plates by cork-borer and filled with 100µl of 24 hrs broth of isolates. Four replications were made for each treatment. Control treatment was made by adding 100µl of sterilized distilled water in each well, other wells contains the evaluated compound of each plate. Inoculated plates were incubated at 28 C° for 48 hrs and the inhibition zone diameter was measured in mm [29].

Greenhouse and field studies. This study was carried out on seedlings under greenhouse to determine the disease incidence and the ability of treatments against the pathogen to control the disease symptoms. It was also carried out under field condition during two consecutive seasons 2018 and 2019 on five years old Le-Conte pear trees budded on *Pyrus communis* rootstock. Trees were planted in sandy soil in a private orchard at Wady EL-Natron region, EL Behira governorate. Trees were spaced at 4 x 6 m, open-vase shape trained under drip irrigation system and received similar cultural practices adopted in the orchard. Fifteen trees were selected as uniform as possible in growth, productivity and appearance for this study. Five treatments, each one has three replicates, one tree for each replicate. Trees were arranged in a completely randomized block design. Treatments were applied and repeated four times as a foliar application to the runoff using a hand sprayer during March and April of each season. The following spraying treatments were investigated:

1. Control "Water Only"
2. *Bacillus subtilis* at 1×10^6 cfu/ml.
3. Hydrogen Peroxide 25% at (3 ml/L)
4. Starner at (1.5g/L)
5. Streptomycin at (150 ppm)

Fire blight incidence determination. To determine the percentage of fire blight incidence, 100 blossoms, leaf and developed fruitlets were chosen, and the percentage of incidence was calculated according the following equations:

Blossoms fire incidence % = (No. of blossom incidence ÷ Total number of blossom) × 100

Leaves fire incidence % = (No. of leaf incidence ÷ Total number of leaf) × 100

Fruits fire incidence % = (No. of fruit incidence ÷ Total number of fruit) × 100

Electrolyte leakage measurements. Measurements were carried out as described by [14] with some modification. Twenty segments (1 cm 2) of

pear leaves were individually placed into flasks contained each 25 mL deionized water (Milli-Q 50, Millipore, Bedford, Mass., USA). Flasks were shaken for 20 hr at ambient temperature to facilitate electrolyte leakage from injured tissues. Initial electrical conductivity measurements were recorded for each vial using an Acromet AR20 electrical conductivity meter (Fisher Scientific, Chicago, IL). Flasks were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80°C (176°F) for 1 hr to induce cell rupture. Vials were again placed on the Innova 2100 platform shaker for 20 hr at 21°C (70°F). Final conductivity was measured for each flask. Electrolyte leakage Percentage for each bud was calculated as: initial conductivity/final conductivity × 100.

Biochemical assays of antioxidant enzymes. A weight of 0.5 g fresh treated pear leaf material was homogenized at 0-4˚C in 3 ml of 50 mM TRIS buffer (pH 7.8), containing 1 mM EDTA-Na 2 and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4˚C) and the total soluble enzyme activities were measured spectrophotometrically in the supernatant. All measurements were carried out at 25˚C, using the model UV-160A spectrophotometer (Shimadzu, Japan). Polyphenol oxidase (PPO) activity was determined according to the method described by [30]. Peroxidase (POX) activity was measured of the crude enzyme extract according to [31].

Vegetative growth characters. In May of each season, 20 developing shoots of spring cycle were tagged at constant height and at all direction of each tree. During September in both 1st & 2nd seasons, the average shoot length and diameter (cm) were measured by Vernier caliper.

Fruit set percentage and total yield. Four branches from each tree were chosen and marked for recording data of total number of flowers and total number of fruits after fruit set (developed fruitlets), then the final fruit set was calculated after six weeks of petal fall [32] according to the following equation (1).

At harvest during August in both seasons, the total number of fruits per tree was counted and the average fruit number was determined, then the total yield / tree (kg) was calculated.

Fruit physical and chemical characteristics. A sample of 10 fruits per tree from each replicate was randomly collected, then to the certified laboratory (PBHCL, Physiology and Breeding Horticultural Lab, Agriculture Faculty, Kafrelsheikh University) were transported to determine physical and chemical fruit characteristics. Fruit samples were weighted and the average fruit weight for each replicate was calculated. Fruit dimension as length and diameter (cm) were measured by using hand Vernier caliper. Flesh firmness was expressed as (lb/Inch^2) according to [33]. Soluble solids content (SSC) was recorded by digital refractometer. Titratable acidity % (TA) was determined by titration using 0.1 N of NaOH.

Statistical analysis. The data has been processed by excel program (Microsoft office, 2016) and statistically analyzed as a RCBD by analysis of variance (ANOVA) using Statistical Analysis System (CoStat) program. Comparisons among means were made via using the Newly Least Significant Differences (NLSD) test at 0.05 level of probability as mentioned by [34].

RESULTS AND DISCUSSION

Molecular characterization of *E. amylovora*. The bacterial pathogen which causes fire blight of pear was isolated, purified, and Koch´s postulated were applied, then DNA from the pathogen was isolated and PCR technique was conducted using specific primer therefore, the results molecular results proved that the pathogen is: *Erwinia amylovora* based on PCR and specific primer technique (Figure 1).

Effectiveness of antimicrobial compounds on *E. amylovora* inhibition (In Vitro). It was clear that the inhibitory effect of *B. subtilis* on *E. amylovora* was the highest inhibition zone diameters that recorded between all of antimicrobial compounds that used in the experiment at *In Vitro* level. On the other hand, H_2O_2 hydrogen peroxide had no noticeable effect on *E. amylovora* in plates of well diffusion method. Moreover, the antibiotic streptomycin and bactericide (Starner) have high effects on bacterial growth compared to control (Figure 2).

Effect of treatments on the disease symptoms of pear seedlings inoculated with E. amylovora under greenhouse. Pear seedlings artificially inoculated with *E. amylovora*, then treated with the treatments showed that disease symptoms and incidence were suppressed and inhibited. *B. subtilis* extract revealed that the healthiest seedlings followed by H_2O_2, starner and streptomycin (Figure 3).

$$\text{Fruit set \%} = \text{Total number of fruitlets} / \text{Total number of flowers} \times 100 \quad (1)$$

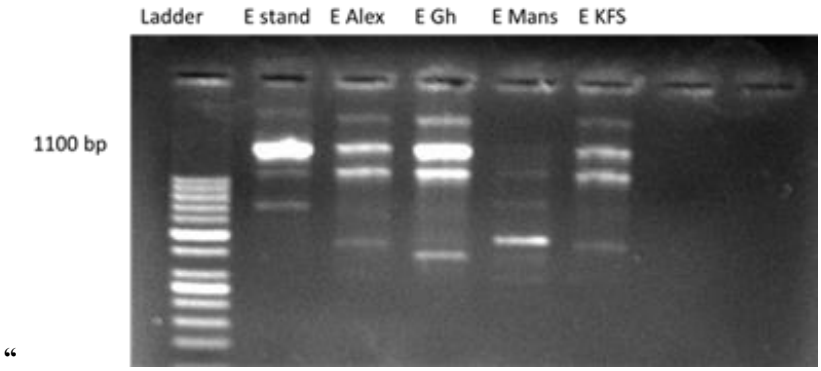

FIGURE 1

Molecular characterization of *E. amylovora* bacteria using PCR technique. Ladder: Marker. Estand: standard isolate of *E. amylovora*. E.Alex: *E. amylovora* isolated from samples obtained from Alexandria Governorate, Egypt. E.Gh: *E. amylovora* isolated from samples obtained from Gharbiyah Governorate, Egypt. E.Mans: *E. amylovora* isolated from samples obtained from Mansoura Governorate, Egypt. E.KFS: *E. amylovora* isolated from samples obtained from Kafr-elshaikh Governorate, Egypt.

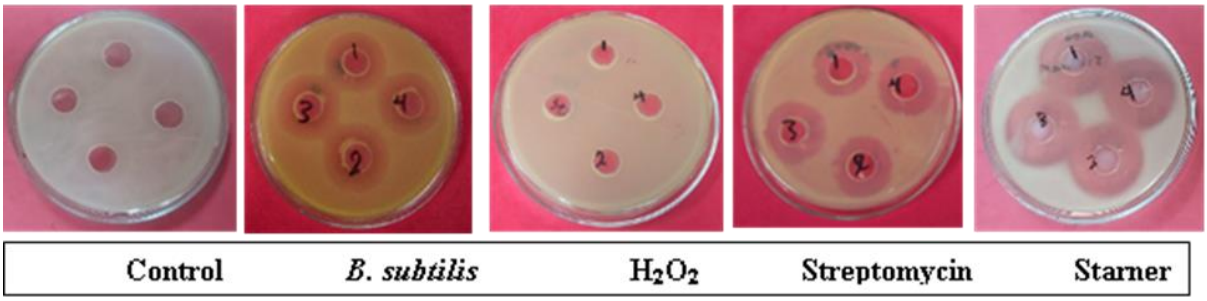

FIGURE 2

Inhibition effect of treatments against *E. amylovora* in vitro. Control: inoculated media with *E. amylovora* only. *B. subtilis*: antagonistic effect of *B. subtilis* against *E. amylovora* . H_2O_2: inoculated media and treated with hydrogen peroxide 3ml/l. Streptomycin: inoculated media and treated with Streptomycin 150ppm. Starner: inoculated media and treated with starner 1.5 gm/L. Inhibition zone was measured mm.

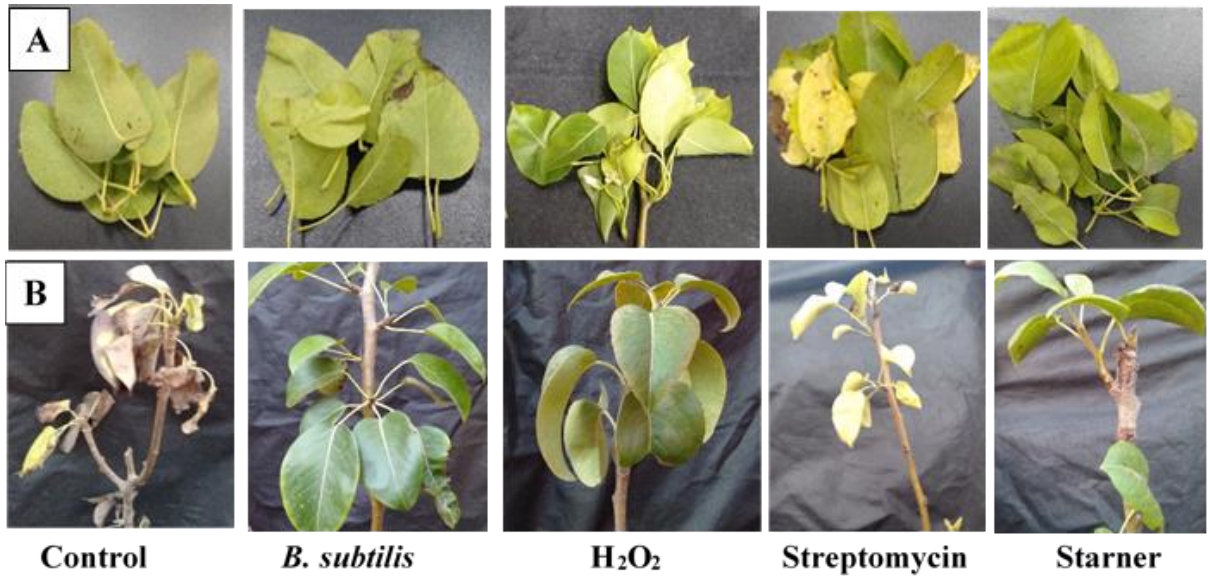

FIGURE 3

Effect of treatments on disease symptoms of inoculated pear seedlings under greenhouse conditions, A: 7 days after treatments, B: 2 weeks after treatments. Control: Pear seedlings were sprayed with water. *B. subtilis*: Pear seedlings were sprayed with *B. subtilis* extract. H_2O_2: Pear seedlings were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear seedlings were sprayed with streptomycin solution 150 ppm. Starner: Pear seedlings were sprayed with starner solution 1.5gm/l.

FIGURE 4

Effect of treatments on disease symptoms of naturally infected pear trees under field conditions 7 days after treatments. Control: Pear trees were sprayed with water. *B. subtilis*: Pear trees were sprayed with *B. subtilis* extract. H_2O_2: Pear trees were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l.

Effect of treatments on the disease symptoms of pear trees infected with *E. amylovora* under field conditions. When pear trees naturally infected with *E. amylovora* were treated with *B. subtilis* extract and hydrogen peroxide H_2O_2 disease symptoms were suppressed and inhibited (Figure 4). Interestingly, similar results were obtained when the trees sprayed with the recommended antibiotic streptomycin and starner (Figure 4).

Effect of treatments on the of fire blight disease incidence under greenhouse. The data presented in Figure 5 revealed that the application of different treatments decreased the incidence percentage of fire blight (number of defected ones/100 even in leaves of pear seedlings compared to control treatment. The *B. subtilis* treatment was the best treatment, where the incidence percentage of fire blight was significantly decreased followed by H_2O_2 application then starner and streptomycin, but in the control treatment the fire blight incidence percentage was increased significantly in leaves.

Effect of different treatments on the percentage of fire blight incidence under field conditions. The data presented in Figure 6 revealed that the application of different treatments decreased the incidence percentage of fire blight (number of defected ones/100 even in flowers or leaves and fruits of pear trees compared to control treatment. The H_2O_2 treatment was the best treatment, where the incidence percentage of fire blight was from 0 to 2% followed by B.S application then starner and streptomycin, but in the control treatment the fire

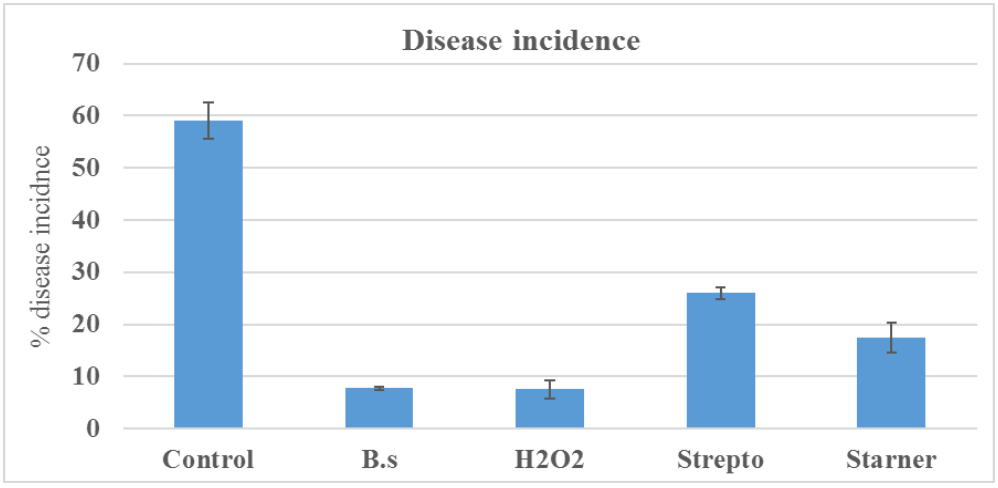

FIGURE 5

Effect of treatments disease incidence of naturally infected pear seedlings under greenhouse conditions 4 weeks after treatments. Control: Pear seedlings were sprayed with water. *B. subtilis*: Pear seedlings were sprayed with *B. subtilis* extract. H_2O_2: Pear seedlings were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear seedlings were sprayed with streptomycin solution 150 ppm. Starner: Pear seedlings were sprayed with starner solution 1.5gm/l.

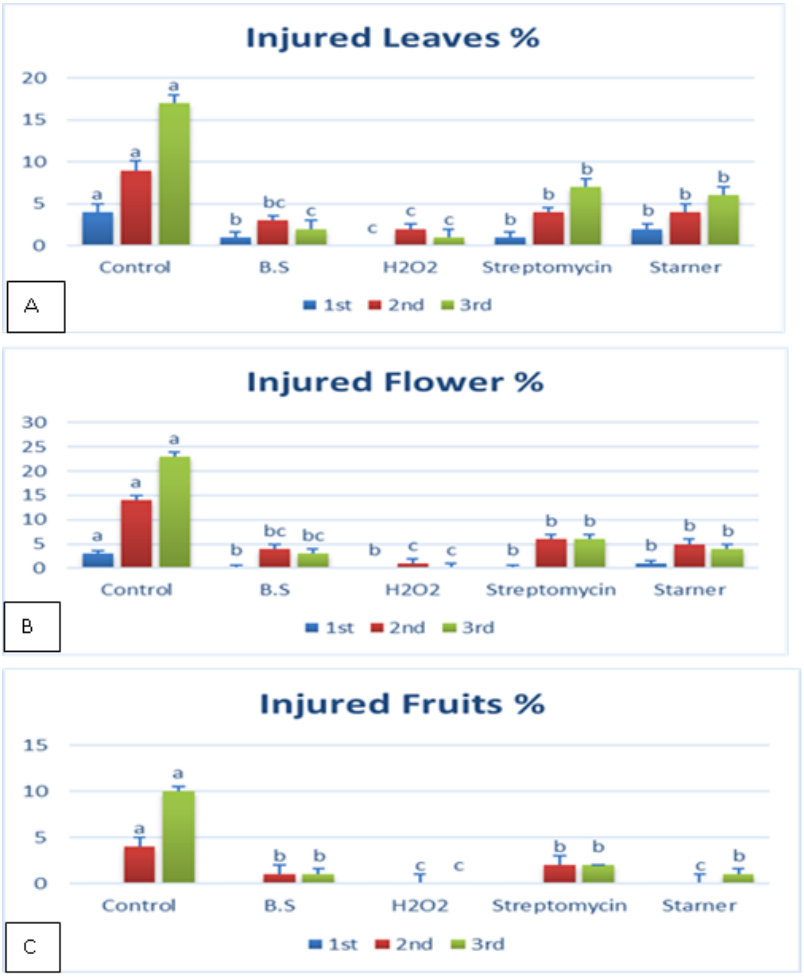

FIGURE 6
Effect of different treatments on the percentage of fire blight incidence for flowers (A), number of defected flowers /100 flowers, were recorded at March 20, April 1 and 10 for 1st, 2nd and 3rd dates respectively. For defected leaves (B), number of defected leaves/100 leaves, were recorded at April 5, 15 and 25 for 1st, 2nd and 3rd dates respectively.

blight incidence percentage was more than 20, 15 and 10% for flowers, leaves and fruits, respectively. After application, the symptoms of fire blight were suppressed.

For defected fruits (C), number of defected fruits/100 fruits, were recorded at April 15 and 25 and May 4, for 1st, 2nd and 3rd dates respectively)) symptoms were recorded under field conditions after 7 days of each application. Control: Pear trees were sprayed with water. *B. subtilis*: Pear trees were sprayed with *B. subtilis* extract. H_2O_2: Pear trees were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l. Columns have the same color and different letters are significantly different.

The inhibition effect of *B. subtilis* against cucumber powdery mildew was similar to the effect of *B. subtilis* on infected squash plants with powdery mildew which obtained with Hafez et al. 2016 [35]. Several of systemic acquired resistant inducers reduce the severity of powdery mildew significantly such as benzothiadiazole (BTH) and salicylic acid (SA) by activation of SA-dependent signaling pathways [36] which activated by *B. subtilis* according to the study of [37]. The highest reduction of disease severity was recorded with *B. subtilis* treatment, this decrease might be related to the important role of *B. subtilis* as plant growth-promoting rhizobacteria (PGPR) that is extensively used to the management of many diseases in many plants [38]. H_2O_2 is the most stable reactive oxygen species (ROS). Similar results were obtained by [39, 40], who found that the application of H_2O_2 reduced the rate of bacterial growth, also it may used as a biocontrol agent for many diseases [41]. The application of B.S is very effective for many diseases as biocontrol agent [42].

Effect of treatments on electrolyte leakage (E.L.). Electrolyte leakage results showed that *B.subtilis* followed by starner bactericide were the highest significant reduction compared with control in which has significant increase of E.L. H_2O_2 comes in third rank followed by streptomycin as it

has the highest increase of E.L. in treatments after control (Figure 7). E.L. has been used to measure the injuries in cell membranes in response to various stresses [43]. In our study, the increase in electrolyte leakage (Figure 3) may be due to that *E. amylovora* is a pathogen which depends on metabolic compounds from the host cells. Application of traetments led to decreased electrolyte leakage in the leaves of pear plants and increased resistance mechanisms [44].

Effect of treatments on activity of antioxidant enzymes of pear trees infected with *E. amylovora*.The results of tested treatments showed significantly increasing of the activity of peroxides (POX) and polyphenol oxidase (PPO). Treatment with *B. subtilis* present in the highest level of POX and PPO activity followed by streptomycin and starner, respectively (Figure 8). The obtained results are in agreement with the results found that plants infected with phytopathogens and treated with bio-agents like *B. subtilis* led to increase the activity of antioxidant enzymes such as polyphenol oxidase and peroxidase [45, 46, 47]. Peroxidases are oxidative enzymes which help in the last step of hydrogen peroxide and lignin formation, from plant factors involved in disease control [48]. Hydrogen peroxide has a key role in inhibiting or killing the pathogens when it accumulated early after the infection consequently later on stimulation the antioxidant enzyme activities in which play thereby a pivotal role of the neutralize the harmful effect of ROS thereby increasing the plant disease resistance against pathogens attacks [49,50]. Additionally, reactive oxygen species are very important signals in abiotic stress such as drought and salinity stress [51-53].

Effect of treatments on yield characters. Data in Table 2 showed that all treatments significantly increased shoot length and diameter compared with control, but the difference among other treatments, was not significant except with streptomycin regarding the shoot length in the both seasons and shoot diameter in 1st season. The highest final fruit set was found with H_2O_2 application followed by *B. subtilis*, starner, stryptomycin and control, respectively. All differences were significant. Data presented in Table 2 revealed that the H_2O_2, *B. subtilis* and Starner significantly increased fruit yield per tree. The fruit yield per tree increased by 40% compared with control. The vegetative growth of pear trees had been improved by H_2O_2 and *B. subtilis* application. Some strains of bacteria produce plant growth stimulator such as indole-3-acetic acid (IAA) and other phytohormones which may increase vegetative growth in many plants [50-53]. H_2O_2 play a main role as ROS which helps the plant to resist the different stresses [54]. The foliar application of H_2O_2 by 5mM improved chlorophyll content and photosynthetic rates of apple trees [55].

The influence of different treatments on chemical and physical fruit properties was shown in Table 3. The most studied characteristics were significantly affected. Regarding the fruit dimensions (length and diameter), the H_2O_2, *B. subtilis* and starner application gave the highest significantly effects then stryptomycin and the control which gave the lowest value. The data in Table 3 revealed that all treatments showed significant effects for fruit weight and SSC compared with control, however, there are no significant differences were noted among other treatments for SSC in the both seasons and all treatments for fruit weight in 2nd season. The highest fruit firmness was noted with starner followed by H_2O_2 and B.S application. Moreover, all applications significantly reduced fruit acidity compared with control for the both seasons. Fruit set, physical and chemical properties and subsequently fruit yield were improved by increasing in vegetative growth which was improved by treatments compared with control. The results are in agreement with Khandaker et al. (2012) [55].

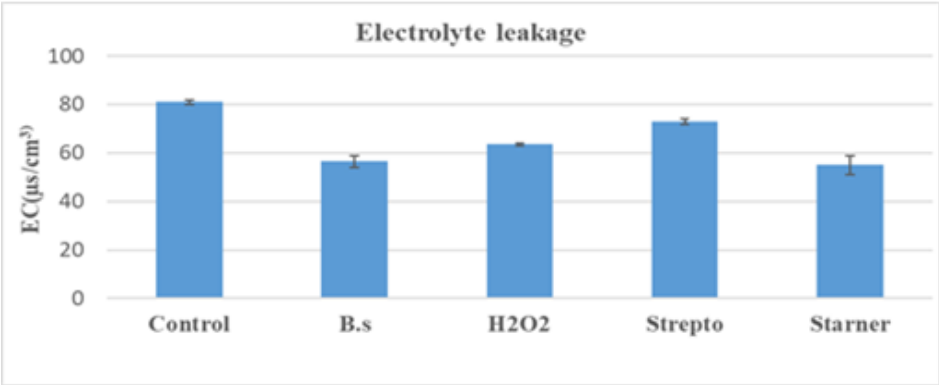

FIGURE 7

Electrolyte leakage of naturally infected pear trees under field conditions 7days after treatments. Control: Pear trees were sprayed with water. *B. subtilis* : Pear trees were sprayed with *B. subtilis* extract . H_2O_2: Pear trees were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l.

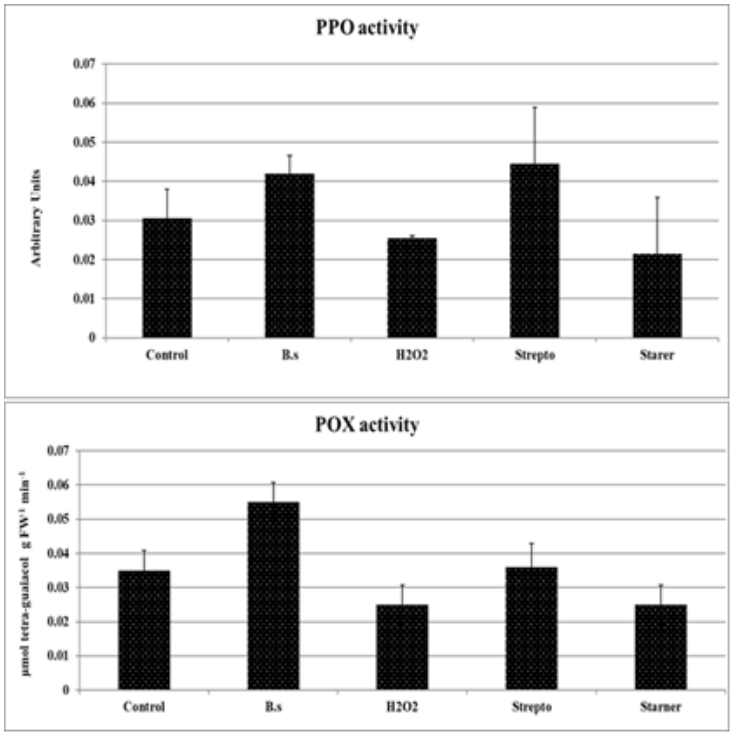

FIGURE 8

Effect of treatments on polyphenol oxidase enzyme (PPO) and peroxidase enzyme (POX) activities in pear infected with *E. amylovora*. Control: Pear trees were sprayed with water. *B. subtilis*: Pear trees were sprayed with *B. subtilis* extract. H_2O_2: Pear trees were sprayed with hydrogen peroxide solution 3ml/l. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l.

TABLE 2

Effect of different treatments on shoot length and diameter, final fruit set and yield per tree during 2018 and 2019 seasons

Treatments	*Shoot length (cm)*	*Shoot diameter (cm)*	*final fruit set (%)*	*Yield/tree (Kg)*
2018 season				
Control	**55.67 c**	**0.82 b**	**3.61 e**	**30.14 c**
B.S	**90.67 a**	**0.93 a**	**5.71 b**	**52.34 a**
H2O2	**89.33 a**	**0.94 a**	**5.92 a**	**52.73 a**
Streptomycin	**82.33 b**	**0.93 a**	**5.16 d**	**47.77 b**
Starner	**89.33 a**	**0.92 a**	**5.48 c**	**50.02 ab**
LSD	**3.05**	**0.021**	**0.147**	**2.639**
2019 season				
Control	**70.0 c**	**0.86 d**	**2.99 e**	**25.03 c**
B.S	**98.67 a**	**0.99 a**	**4.85 b**	**42.48 ab**
H2O2	**99.00 a**	**1.01 a**	**5.19 a**	**47.51 a**
Streptomycin	**91.00 b**	**0.94 b**	**4.44 d**	**38.81 b**
Starner	**101.67 a**	**0.99a**	**4.71 c**	**43.25 ab**
LSD	**3.035**	**0.02**	**0.113**	**5.28**

Values within each column followed by the same letter (s) are not significantly different at 5% level.

TABLE 3
Effect of different treatments on fruit length, diameter, weight, firmness, soluble solid contents (SSC) and acidity during 2018 and 2019 seasons

Treatments	*Fruit length (cm)*	*F. diameter (cm)*	*F. weight (g)*	*Firmness*	*SSC (%)*	*Acidity (%)*
2018 season						
Control	8.01 c	6.50 c	163.17 b	13.02 c	10.87b	0.37 ab
B.S	8.42 ab	6.70 a	187.61 a	14.03 b	11.73a	0.36 bc
H2O2	8.53 a	6.72 a	186.96 a	14.17 b	11.87a	0.33 d
Streptomycin	8.35 b	6.63 b	189.79 a	14.09 b	11.60a	0.38 a
Starner	8.37 b	6.72 a	186.61 a	14.47 a	11.87a	0.35 c
LSD	0.13	0.032	8.31	0.27	0.28	0.017
2019 season						
Control	8.13 e	6.63 c	173.07 a	14.72 c	12.40b	0.29 a
B.S	8.51 c	6.89 a	189.65 a	15.33 a	13.73 a	0.27 b
H2O2	8.63 a	6.92 a	189.28 a	15.32 a	13.73 a	0.25 d
Streptomycin	8.35 d	6.72 b	187.54 a	15.18 b	13.53 a	0.26 c
Starner	8.55 b	6.90 a	186.85 a	15.37 a	13.73 a	0.25 d
LSD	0.026	0.035	21.67	0.114	0.27	0.01

Values within each column followed by the same letter (s) are not significantly different at 5% level.

CONCLUSIONS

It can be concluded that application of bio-agent *B. subtilis* and hydrogen peroxide as compared with antibiotic and starner compound have the potential effect to control fire blight disease in pear seedlings and trees caused by *Erwinia amylovora* the bacterial dangerous disease either under greenhouse or field condition, through reducing electrolyte leakage, disease incidence and symptoms in which correlated with defense-related enzymes (POX and PPO) and elevated. Also, use of bio-agent *B. subtilis* and H_2O_2 led to increase most of morphological and physiological studied characters and improve fruits yield.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the deanship of scientific research at King Khalid University for funding this work through the program of research groups under grant number (R.G.P 1/106/40). Also, the authors extend their appreciation to all members of PPBL and EPCRS excellence center, Fac. of Agric., Kafrelsheikh University.

Funding: The authors extend their appreciation to the deanship of scientific research at King Khalid University for funding this work through the program of research groups under grant number (R.G.P 1/106/40)

Conflicts of Interest: The authors have declared no conflict of interest

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Received: **15.04.2020**
Accepted: **27.04.2020**

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