© by PSP Volume 30– No. 06/2021 pages 6237-6247 Fresenius Environmental Bulletin

THE INFLUENCE OF NANO-COPPER AND SAFETY COMPOUNDS ON VEGETATIVE GROWTH YIELD AND FRUIT QUALITY OF "LE CONTE" PEAR TREES UNDER INFECTION WITH FIRE BLIGHT

Yaser M Hafez[1,*], Abdel-Moety Salama[2], Hatem Kotb[3], Ziad Moussa[4], Nehal Elsaed[1], Elsayed M El-Kady[1], Fahmy A S Hassan[5]

[1]EPCRS Excellence Center, Plant Pathology and Biotechnology Laboratory, Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, 33516-Kafr El-Sheikh, Egypt
[2]Physiology and Breeding of Horticultural Crops Lab., Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, Egypt
[3]Deciduous Fruit Research Department, Horticulture Research Institute, Agriculture Research Centre (ARC), Giza, Egypt
[4]Microbiology Research Department, Soil, Water and Environment Research Institute, Agriculture Research Centre (ARC), Giza, Egypt
[5]Department of Biology, College of Science, Taif University, P.O.Box 11099, Taif 21944, Saudi Arabia

ABSTRACT

Fire blight is the most thoughtful diseases that effects great damage of pome fruits trees particularly pears in Egypt and over world. The causal agent of fire blight is Erwinia amylovora, the Gram-negative bacteria, a disturbing disease troubling species of the family Rosaceae. In this study two extracts were applied which were Hibiscus extract and pomegranate peel extract. Also, Nano cu, oxolinic acid (starner 20%) were evaluated on susceptible pear cultivar (Le-conte pear trees budded on Pyrus communis) and compared with the outcome of streptomycin as standard antibiotic in green house and under field conditions. Under greenhouse disease symptoms and severity of inoculated pear seedlings and treated with plant extracts, nano copper (cu), 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 using with hibiscus extract, pomegranate peel, nano copper(cu), starner and streptomycin, disease severity, disease symptoms and electrolyte leakage % were decreased significantly. Consequently, that the vegetative growth such as chlorophyll content, efficiency of photosynthesis, shoot length and diameter were significantly increased with all treatments. Fruit set, yield and quality were improved compared to the control.

KEYWORDS:
Erwinia amylovora, Hibiscus, pomegranate, Nano cu, Streptomycin, Starner

INTRODUCTION

Pear (Pyrus communis L.), a member of the family Rosaceae, is one of the most important popular and profitable fruits over the world. It is ranked as the fifth deciduous fruit crop globally by production (23.7 million tonnes) [1]. Pear trees, as well most of Rosaceae members, faced by fire blight, most destructive disease produced by the bacterial pathogen Erwinia amylovora and it is an essential infection affecting and signifies an enormous danger to fruit farming in numerous portions of the world. The host Rosaceous plants for Erwinia amylovora are pear, apple, quince, loquat, ornamental and wild plants (cotoneaster, pyracantha, stranvaesia, hawthorn, sorbus). According to EPPO (2012), E. amylovora is presently existing in additional than 50 countries worldwide [2]. The pathogen originate from North America, Fire Blight has in the 1950s been presented to Great Britain and in the 1970s to Northern and Central Europe. The infection has then sustained to extent on the way to Southern and Eastern Europe and further to Central Asia [3]. The causal agent of fire blight is Erwinia amylovora, which naturally settles the nutrient rich stigmata of young flower blossoms. Extensive colonization happens when the pathogen go in the plant via natural openings and wounds, dispersion through the host by the vascular system. Formation of the phytopathogen leads to the progress of infection symptoms and, often, plant decease [4]. primary route of resistance contrary to fire blight often includes traditional management practices, with expensive removal of contaminated trees. often copper-based pesticides and antibiotics as per streptomycin and tetracycline are suggested, while resistance can be a worry. The continuous use of synthetic compounds often copper-based pesticides and antibiotics can induce serious problem due to residual toxicity beside resistance to those compounds. Therefore, to find alternative safe and effective methods to control fire blight, it started in the end of last century. [5] reported that the biological control is to use biological agents on the host plant for the control of disease development. Biocontrol agents such as Bacillus subtilis and H2O2 revealed inhibition activity toward fire blight at level

of in vitro and field conditions ([6] and [7]. [8] tested the inhibitory effect of 24 plant extract against fire blight. They indicated that the most used plant extracts have antibacterial activity for fire blight disease at in vitro level. [9] investigated the antibacterial activities of 33 plant extracts from 11 different plants grown in Turky and they found that those plant extracts had strong effects against many species of Bacteria such as E. coli, P. aeruginosa or K. pneumoniae. In pomegranate fruit peel, nearly 48 phenolic compounds were identified mostly anthocyanins, gallotannins, hydroxycinnamic acids, hydroxybenzoic acids and hydrolysable tannins [10]. High molecular weight ellagitannins (ellagic acid, punicalagin, punicalin and gallagic acid) are the most active tannins in pomegranate peel fruit that have high antioxidant activities. Tannins have a potential to bind proteins, inhibit the enzymes and substrate deprivation of pathogen [11]. The extracts of plants belong the Genus of Hibiscus have high contents of flavonoids, phenolic acids, and polysaccharides that have biological activities against many of pathogens or diseases [12]. Protocatechuic acid which derived from roselle calyx and the aqueous extract of the calyx of Hibiscus sabdariffa inhibited effectively the growth of many bacterial pathogens. In last decades, many reports about the efficacy of plant extracts against human fungal and bacterial diseases had been done, but still to now, no biocontrol agents are commercially used for the control of fire blight of pears.

Many studies had been done in last decades In spite of Biocontrol methods have been intended, containing the usage of the fungus *Aureobasidium pullulans* and bacteria like *Pseudomonas graminis*, *Pantoea agglomerans*, and *Bacillus* spp [13]. Nanomaterials showed an area of attraction due to their exclusive features when connected with their bulk materials, which subsequent their usage in lengthy area of applications and in widespread fields. These features might involve; catalytic properties, thermal characteristics, electrical conductivity, biotic treatments, and optical behavior. They have a beneficial properties for example a special surface energy with a great surface area to size ratio and a hopeful small sizes [14]. 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 plant parts 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.

Plant extracts. ***Preparation of extracts.*** This method was brought off using four different concentrations (10gm /5gm/2.5gml1.25gm) of the ground powder of dried plant (seed –leaves-etc) with 100ml of sterile distilled cold water. Extracts mixtures were mixed with sterile distilled cold water, then water bath was used for 20min at 100c˚. Extracts were filtered through filter paper thereafter sterilized through (o.2µl) porosity mill pore filter. Sterile extracts were put in sterilized bottles in refrigerator for later uses [6].

TABLE 1
Determination of extracts antibacterial activity in vitro using agar well diffusion method

Scientific name	Commercial name	Used part of plant
Thymus vulgaris	Thyme	Leaves
Capsicum annum	Cayenne pepper	Fruits
Zingiber officinale	Ginger	Roots
Allium cepa	Onion	Bulb
Mentha viridis	Spear mint	Leaves
Nigella sativa	Nigella	Seeds
Piper nigrum	Black pepper	Seeds
Cinnamomum cardamomam	Cinnamon	Bark
Syzgium aromaticum	Clove	Seeds
Rhus coriaria	Sumac	Drupes
Majorana hortensis	Marjoram	leaves
Allium sativum	Garlic	Bulb (cloves)
Punica granatum	Pomegranate	Fruit peel
Hibiscus sabdariffa	Rosella/ Hibiscus	Sepals

Oils. Five kind of oils were used in screening the antibacterial effect of oils Rosemary oil, Camphor oil, Garlic oil, olive oil and Nigella oil.

In vitro antibacterial assay. Well diffusion method was used to evaluate the antibacterial activity of extracts with concentrations (10gm/100ml, 5gm/100ml, 2.5gm/100ml, 1.25gm/100ml), nano cu with concentrations (280ppm, 140ppm, 70ppm, 35ppm), Starner 1.5gm/l as recommended, the antibiotic streptomycin 150ppm used as standard while sterile distilled water was as negative control. 2×108 cfu/ml of Erwinia amylovora broth was mixed with molten (45-50˚C) King´s B medium and transferred in petri dishes (90mm) and allowed to solidify under disinfected conditions in laminar airflow chamber. 10mm wells were made in inoculated solidified king´s B medium plates by cork-borer and filled with 100µl of each treatment. Four replications of every treatment were made and the efficiency of treatments were compared with positive control. Inoculated plates were incubated at 28 C˚ for 48 hrs. The inhibition zone diameter was measured in mm and 2mm or more are positive result [15] and [16].

Chemical materials. Hydrogen peroxide 3ml/l, the local and commercial bactericidal Starner 1.5gm/l and nano cu 70ppm all of these chemical compounds were used in this study compared with antibiotic streptomycin 150ppm to screen effective chemical materials against E.amylovora the causal agent of fire blight in vitro.

Seedlings experiment. Two pear seedlings on one years old Le-Conte pear trees used as replicate of each treatment and were inoculated with suspensions of Erwinia amylovora (1 × 106 ml−1) by spraying till runoff. After incubation seedlings were sited in humidity above 80% by covering seedlings with a transparent plastic top for two days at 15˚C with wordy light, the tops were detached then seedlings were incubated moreover for five days at 85% RH, 15˚C and with 16 hrs. light per day. Next, incubated seedlings were sprayed with treatments:

1-Control "Water Only".
2-Hibiscus extract (2.5gm/100ml)
3-Pomegranate extract (2.5gm/100ml)
4-Streptomycin at (150 ppm)
5-Starner at (1.5g/L)
6-nano cu 70ppm.

Avoid connection between leaves of nearby plants. the obligatory temperature and humidity conditions were conserved. [3] and [17]. Disease symptoms and disease severity were measured.

Electrolyte leakage measurements. Measurements were carried out as described by [18] 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 [19]. Peroxidase (POX) activity was measured of the crude enzyme extract according to [20].

Field studies. "Le-Conte" pear trees grown in a private orchard at Wady ELNatron district, Beheira governorate, Egypt, five years old, grown in a sandy soil, spaced at 4x6m, under drip irrigation system, homogenous in growth and received common and recommended horticulture practices for this region, were selected for this investigation during 2018 and 2019 seasons. 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. Trees were randomly assigned to receive one of six treatments; water as control, Streptomycin at 150 ppm, Starner at 1.5g/l, Hibiscus extract at 2.5gm/100ml, Pomegranate extract at 2.5gm/100ml and Cu-nano at 70ppm Treatments were arranged in a completely randomized block design, each treatment has three replicates, one tree for each (totally 18- trees were employed for this study for each season).

Fire blight incidence determination. For determination the percentage of fire blight incidence, 100 blossom, 100 leaf and 100 developed fruitlets were chosen, and the percentage of incidence was calculated according to 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

Vegetative growth characters. In the spring of each season, 20 non –fruiting shoots of spring cycle were tagged at constant height and at all direction of each tree. In September, the average length of tagged shoots was measured by Vernier caliper.

Chlorophyll content (SPAD) and photothynsis efficacy (Fv/Fm). Chlorophyll content (SPAD), was measured as a total green color in the fully expanded and mature of 10 leaves per treatment, by nondestructive chlorophyll content meter (CCM-200 plus- OPTI-SCIENCES). Chlorophyll fluorescence parameter (Fv/Fm) was measured on the abaxial surface of the intact leaves. Modulated fluorescence was measured using a portable chlorophyll fluorimeter (OS-30p, OPTI-SCIENCES). Maximal variable fluorescence (Fv = Fm – F0) and the photochemical efficiency of PSII (Fv/Fm) were measured for dark-adapted leaves (Dewir et al. 2015) with some modification where the portable chlorophyll fluorimeter (OS-30p, OPTI-SCIENCES) is automatically read the Fv/Fm. Ten mature leaves were randomly selected and measurements were made on fully expanded leaves.

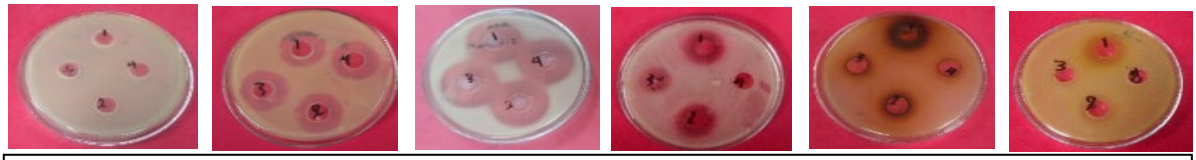
Fruit set percentage and total yield. The final fruit set was calculated six weeks after full bloom stage as a number of persisted fruits per hundred spur and lateral buds [32].

Fruit set % = Number of fruitlets / Number of flowers X 100

At harvest time, the fruits per tree were harvested and weighted, then the total yield/ tree (kg) was calculated.

Fruit Quality Parameters. 10 random fruits were collected from each tree then transported to the laboratory of (PBHCL, Physiology and Breeding Horticultural Lab, Agriculture Faculty, Kafrelsheikh University) to monitor the physical and chemical characteristics. The fruit parameters were measured directly after harvest time in August in both seasons. The average of fruit dimensions (length and diameter (mm) were measured by using hand vernier caliper. Fruit weight (gm) were measured from 10 fruits of each replication. Flesh firmness was expressed as (lb/ Inch2) according to[21]. Soluble solids content (SSC) was recorded by digital refractometer. Titratable acidity % (TA) was determined by titration using 0.1 N of NaOH. The acidity was estimated as malic acid using five milliliters of the fruit juice of each fruit sample and titrated with sodium hydroxide solution of a known normality using phenolphthalein as an indicator (A.O.A.C., 1985).

Experimental Design and Data Analysis. Results of the measured parameters were subjected to computerized statistical analysis using COSTAT package for analysis of variance (ANOVA) and means of treatments were compared using LSD at 0.05 level of possibility using randomized complete blocks design according to [22].



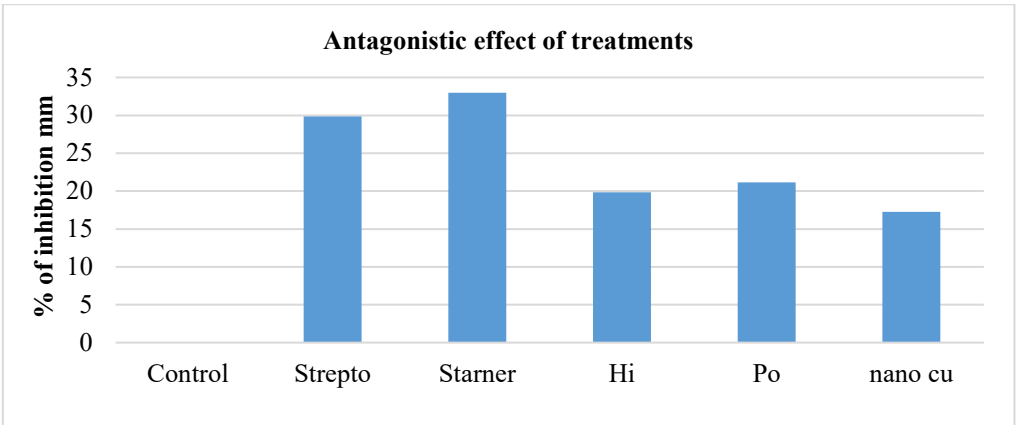


FIGURE 1
Inhibition effect of treatments against Erwinia amylovora in vitro.
Control: inoculated media with E. amylovora. Streptomycin: inoculated media and treated with Streptomycin 150ppm. Starner: inoculated media and treated with starner 1.5 gm/L. Hi: inoculated media and treated with Hibiscus extract 2.5 gm /100ml water. Po: inoculated media and treated with Pomegranate peel extract 2.5 gm /100ml water. nano cu: inoculated media and treated with nano cu 70 ppm. Inhibition zone was measured mm.

RESULTS AND DISCUSSION

Inhibitory effect of antimicrobial agents against E.amylovora (In vitro). In this method five materials were applied compared with control ,streptomycin as recomended antibiotic at concentration 150ppm,starner as local bactericide commonly used in Egypt 1.5gm/l, Hibiscus Sepals extract at four concentrations(10gm/l, 5gm/l, 2.5gm/l and 1.25gm/l) and the third concentration was minimum inhibitory concentration (MIC) that was applied in field experiment, pomegranate fruit peel extract at four concentrations(10gm/l, 5gm/l, 2.5gm/l and 1.25gm/l)and the third concentration was minimum inhibitory concentration (MIC) that was applied in field experiment and finally nano cu at four concentrations (280ppm, 140ppm,70ppm and 35ppm)and the third concentration was minimum inhibitory concentration (MIC) that was applied in field experiment . The most Inhibitory material was starner, streptomycin, pomme extract, Hibiscus extract and nano cu respectively (Figure 1).

Effect of treatments on the disease symptoms of pear seedlings inoculated with E. amylovora under greenhouse. When pear seedlings were artificially inoculated with E.amylovora and treated with treatments it was clear that disease symptoms and incidence were significantly decreased .The most effective treatments were starner and pome extract ,nano cu was in the second place then streptomycin and hibiscus extract (Figure 2,3).

Effectiveness of antimicrobial agents on disease symptoms of pear trees infected with E. amylovora. Similar inhibitory results of starner, nano cu and pomme extract were obtained as they inhibited disease symptoms paradoxically with control treatment and streptomycin (Figure 4).

Effect of treatments on the of fire blight disease incidence under greenhouse. The results of tested treatments showed significantly decrease in the incidence percentage of fire blight (number of defected ones/100 even in leaves of pear seedlings compared to control treatment. The starner treatment was the best treatment, where the incidence percentage of increasing of the activity of fire blight was significantly decreased followed by pome application then nano cu, streptomycin, and hibiscus but in the control treatment the fire blight incidence percentage was increased significantly in leaves (Figure 5).

Effect of treatments on electrolyte leakage (E.L). All treatments were effective in reducing the E.L significantly compared with control. The best treatment was Po followed with starner, nano cu, Hi, streptomycin compared with control. It seems that Po and starner the best treatments however, the streptomycin, Hi and nano cu similarly have less effect as compared with Po and starner (Figure 6). E.L. has been used to measure the injuries in cell membranes in response to various stresses [23]. In our study, the increase in electrolyte leakage (Figure 6) may be due to that E. amylovora is a pathogen which depends on metabolic compounds from the host cells. Application of treatments led to decreased electrolyte leakage in the leaves of pear plants and increased resistance mechanisms [24].

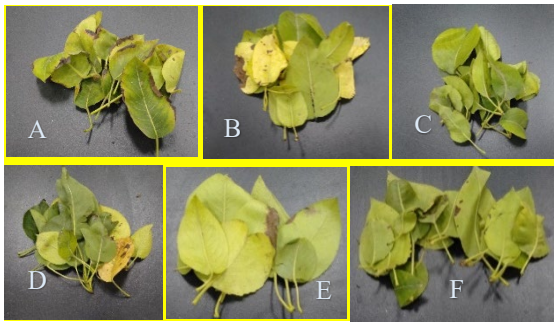


FIGURE 2

Effect of treatments on disease symptoms of inoculated pear seedlings under greenhouse conditions after 2 weeks of treatments.

(A): Pear seedlings were sprayed with water. (B): Pear seedlings were sprayed with streptomycin solution 150ppm. (C): Pear seedlings were sprayed with starner solution 1.5gm/l. (D): Pear seedlings were sprayed with Hibiscus extract 2.5 gm/100 ml water. (E): Pear seedlings were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. (F): Pear seedlings were sprayed with nano cu 70 ppm.

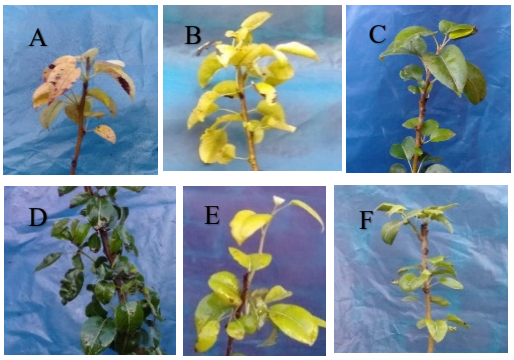


FIGURE 3

Effect of treatments on disease symptoms of inoculated pear seedlings under greenhouse conditions after 4 weeks of treatments.

(A): Pear seedlings were sprayed with water. (B): Pear seedlings were sprayed with streptomycin solution 150ppm. (C): Pear seedlings were sprayed with starner solution 1.5gm/l. (D): Pear seedlings were sprayed with Hibiscus extract 2.5 gm/100 ml water. (E): Pear seedlings were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. (F): Pear seedlings were sprayed with nano cu 70 ppm.

FIGURE 4

Effect of treatments on the disease symptoms under field conditions 7 days after treatments.

Control: Pear trees were sprayed with water. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l. Hi: Pear trees were sprayed with Hibiscus extract 2.5 gm/100 ml water. Po: Pear trees were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. nano cu: Pear trees were sprayed with nano cu 70 ppm.

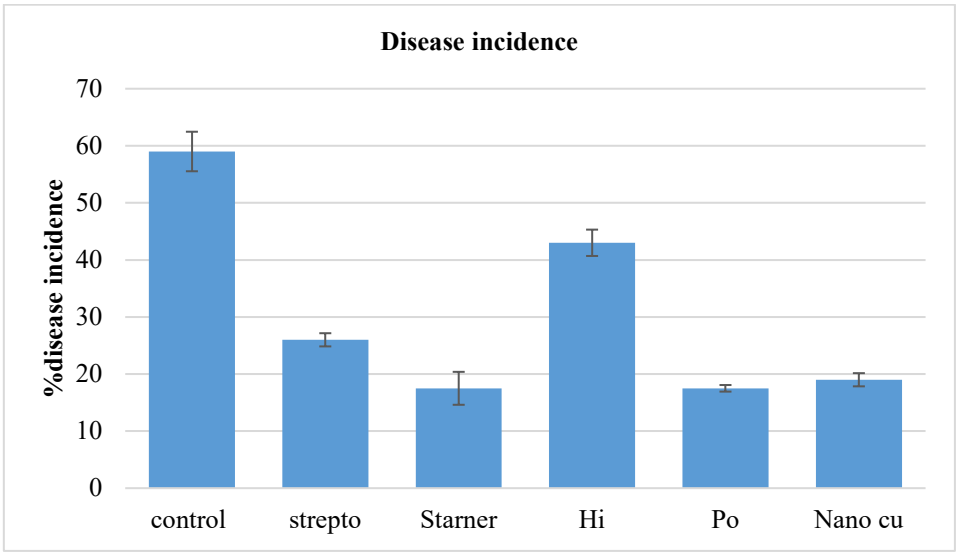


FIGURE 5

Effect of treatments disease incidence of infected pear seedlings under greenhouse conditions 4weeks after treatments.

Control: Pear seedlings were sprayed with water. Streptomycin: Pear seedlings were sprayed with streptomycin solution 150 ppm. Starner: Pear seedlings were sprayed with starner solution 1.5gm/l. Hi: Pear trees were sprayed with Hibiscus extract 2.5 gm/100 ml water. Po: Pear trees were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. nano cu: Pear trees were sprayed with nano cu 70 ppm.

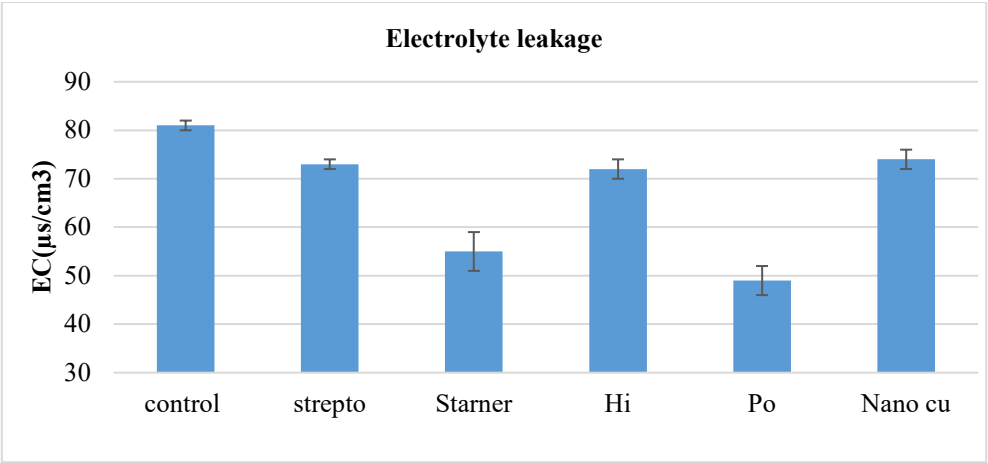


FIGURE 6

Electrolyte leakage under field conditions 7days after treatments.

Control: Pear trees were sprayed with water. Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l. Hi: Pear trees were sprayed with Hibiscus extract 2.5 gm/100 ml water. Po: Pear trees were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. nano cu: Pear trees were sprayed with nano cu 70 ppm.

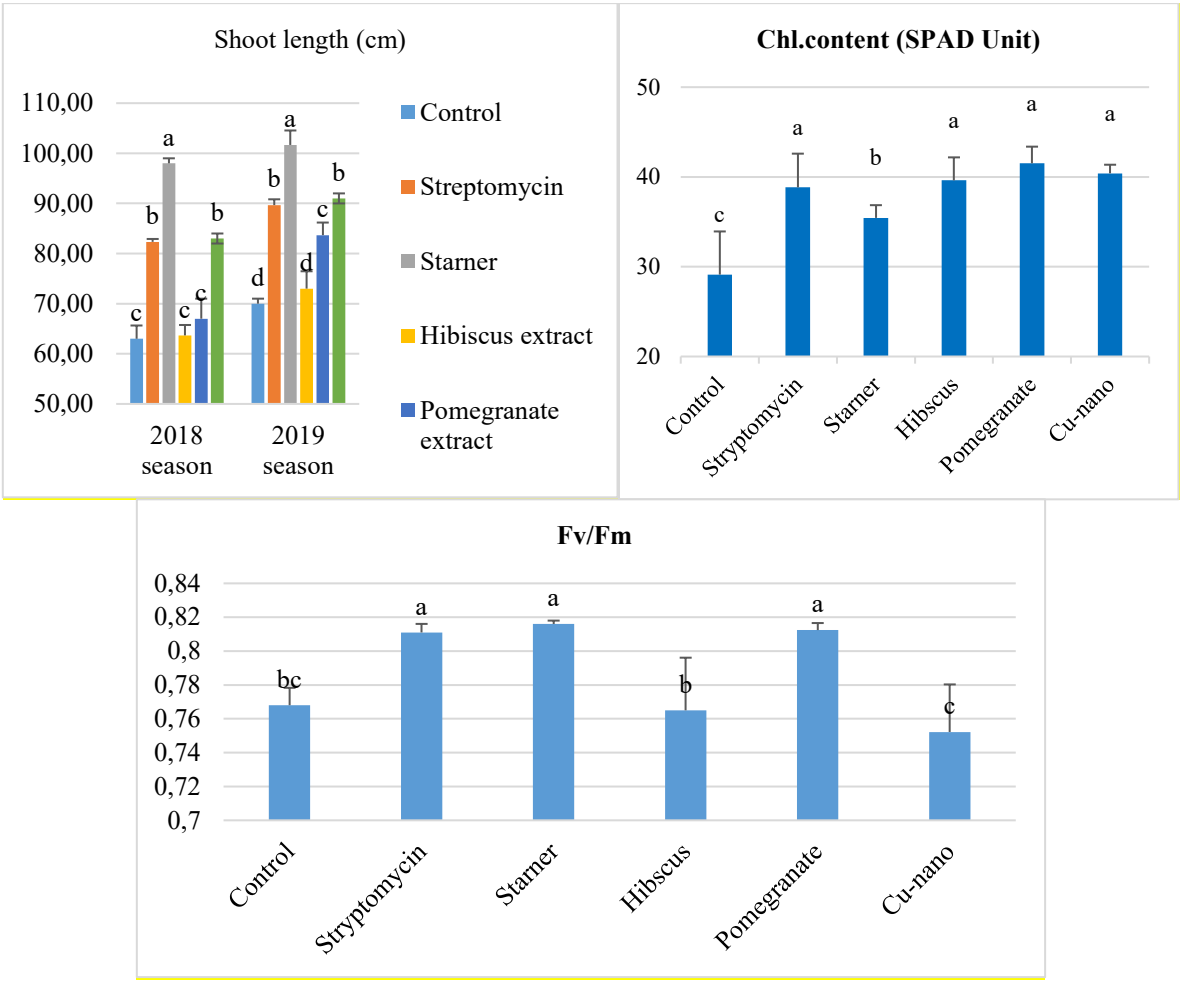


FIGURE 7

Effect of different treatments on shoot length, chlorophyll content (Chl.) and photothynsis efficiency (Fv/Fm) of pear trees. Error bars represent the standard deviation of three replications. Different letters indicate significant differences within different treatments according to Duncan's test (P < 0.05).

Effect of treatments on shoot length, chlorophyll content (Chl.) and photothynsis efficiency (Fv/Fm). Results of tested treatments showed that shoot length, Chl. content and photothynsis efficiency significantly affected (Figure 4). The highest shoot length obtained by application of bactericide starner followed by nano-Cupper (Cu-nano), streptomycin, pomegranate extract, hibiscus extract and control respectively. Chl. content significantly increased by pomegranate and hibiscus extracts, Cu-nano and streptomycin followed by starner compared to control treatment. Photothynsis efficacy (Fv/Fm value) which indicate to a measure of the maximum efficiency of PSII i.e. the quantum efficiency if all PSII centers were open [**25**]. The decrease in this value points out to down or decrease in plant photothynsis efficacy. The Fv/Fm was improved by application of starner, streptomycin and pomegranate extract with no significant differences, followed by hibiscus extract, control and the lowest value was obtained by Cu-nano.

Effect of treatments on fruit set, length, diameter, weight, firmness, yield/tree. Data in figure 5 revealed that the fruit set, physical fruit properties (fruit length, diameter, weight and firmness) and yield/tree significantly affected. The highest fruit set % obtained by application of bactericide starner followed by Cu-nano, streptomycin, pomegranate extract, hibiscus extract and control with lowest value for the both seasons. The treatment of streptomycin gave the highest fruit dimensions (length and diameter) followed by Cu-nano, starner, pomegranate extract, hibiscus extract and control. Similar trend was appeared for fruit weight. starner treatment gave the highest fruit firmness followed by streptomycin, Cu-nano, pomegranate extract, hibiscus extract then control in 1st season, but in the 2nd season the Cu-nano treatment led to the highest fruit firmness followed by starner, streptomycin then hibiscus and pomegranate extracts and at the end control treatment. Regarding the fruit yield/tree, the treatment of starner showed the highest value then Cu-nano, streptomycin, pomegranate extract, hibiscus extract and control treatment respectively.

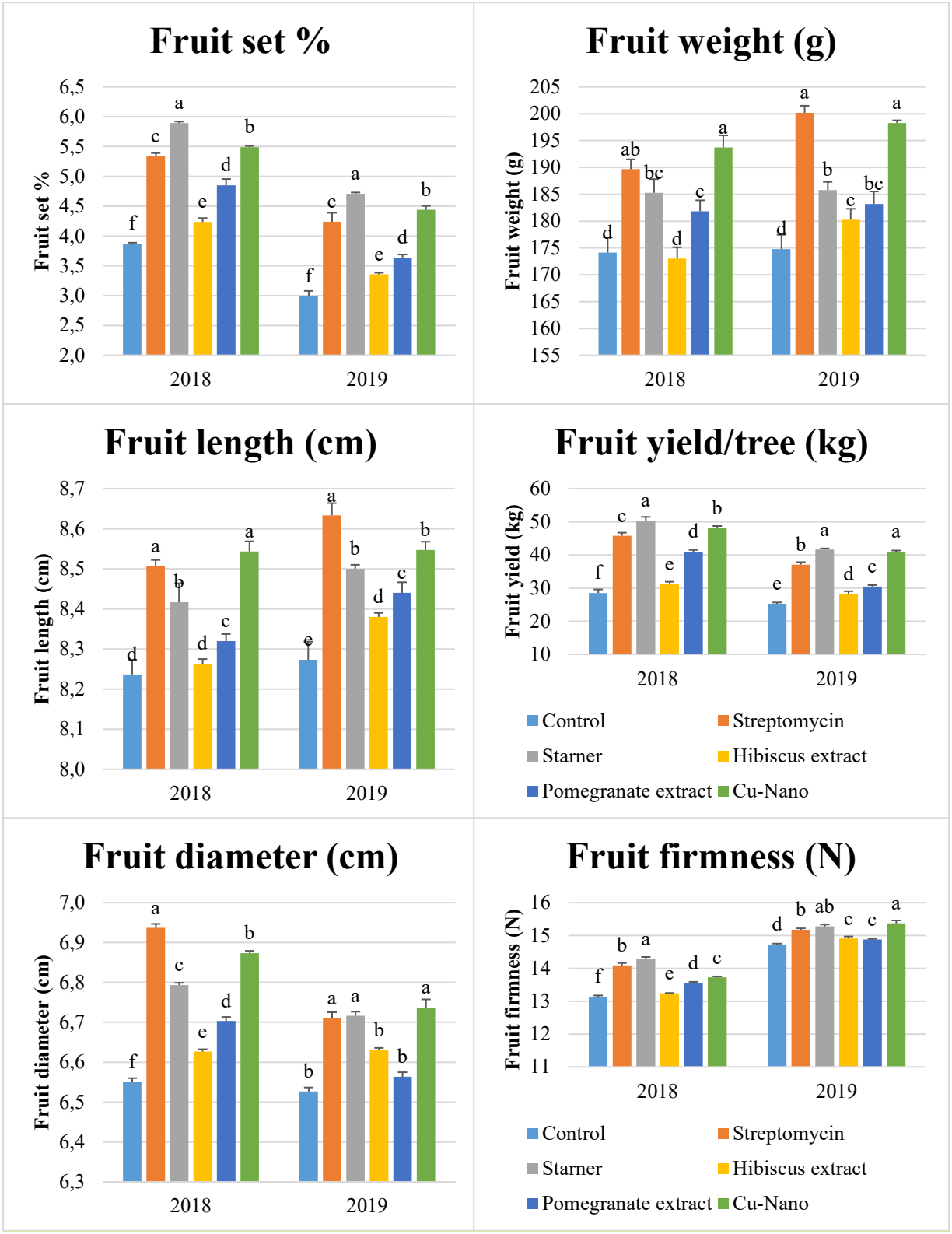


FIGURE 8

Effect of different on fruit set, length, diameter, weight, firmness and yield/tree during 2018 and 2019 seasons. Error bars represent the standard deviation of three replications. Different letters indicate significant differences within different treatments according to Duncan's test (P < 0.05).

Soluble solid contents (SSC) and acidity. Results in Figure 6 revealed that the SSC increased by all treatments compared to control. The Cu-nano application showed the highest SSC value followed by streptomycin, starner, pomegranate extract, hibiscus extract then control treatment where it showed lowest value in 1st season. In 2nd season, SSC revealed

similar trend, but the highest value showed by streptomycin treatment. On the other hand, the lowest fruit acidity showed by streptomycin and Cu-nano treatments followed by pomegranate extract and starner treatments while the highest acid value showed by control and hibiscus extract treatments.

Effect of treatments on activity of antioxidant enzymes peroxidase (pox) and polyphenol oxidase (ppo) of pear trees infected with E. amylovora. The result of tested treatments showed significantly increased of the activity of peroxides POX and polyphenol oxidase PPO. Treatment with streptomycin present in the highest level of POX and PPO activity followed by Pomegranate peel extract and Hibiscus extract, respectively (Figure 10). The obtained results are in agreement with the results found that plants infected with phytopathogens and treated with bio-agents led to increase the activity of antioxidant enzymes such as polyphenol oxidase and peroxidase [26], [27], and [28] Peroxidases are oxidative enzymes which help in the last step of hydrogen peroxide and lignin formation, from plant factors involved in disease control [29]. Hydrogen peroxide

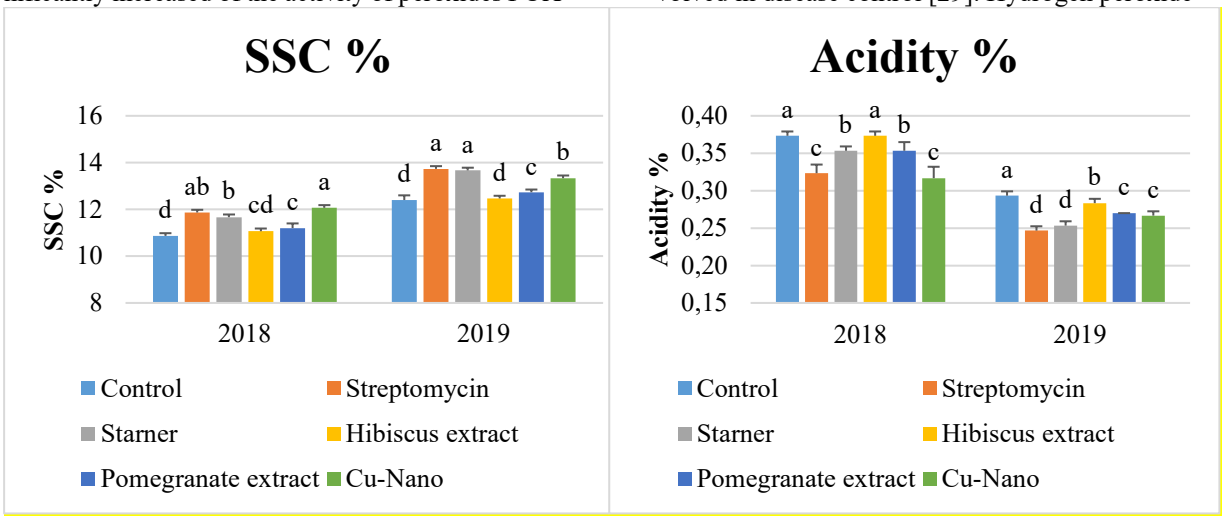


FIGURE 9

Effect of treatments on soluble solid contents (SSC) and acidity of pear fruits during 2018 and 2019 seasons. Error bars represent the standard deviation of three replications. Different letters indicate significant differences within different treatments according to Duncan's test (P < 0.05).

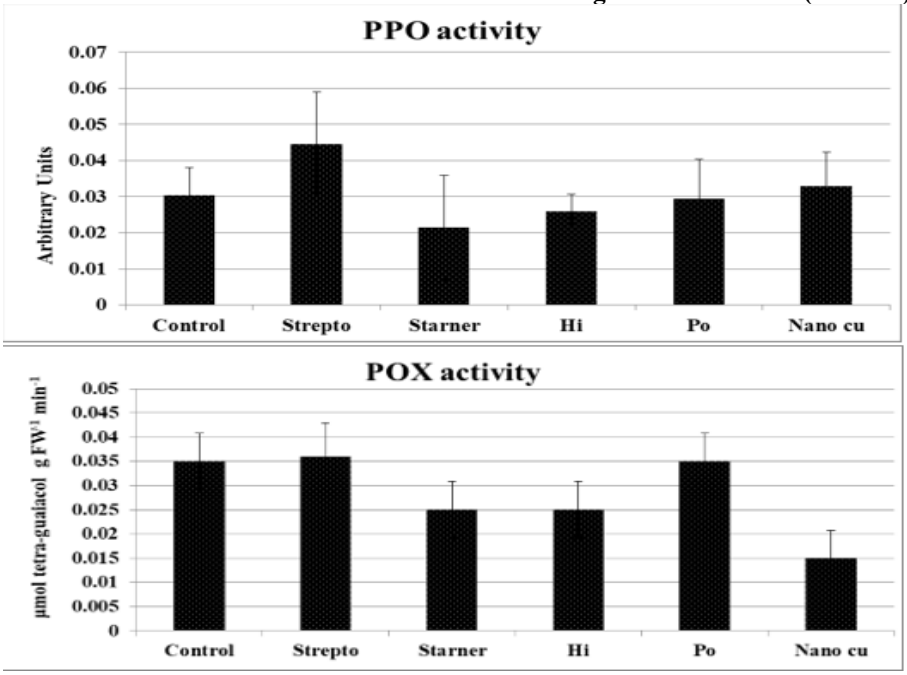


FIGURE 10

Effect of treatments on polyphenol oxidase enzyme (PPO) and peroxidase enzyme (POX) activities in pear infected with E.amylovora .

Streptomycin: Pear trees were sprayed with streptomycin solution 150 ppm. Starner: Pear trees were sprayed with starner solution 1.5gm/l. Hi: Pear trees were sprayed with Hibiscus extract 2.5 gm/100 ml water. Po: Pear trees were sprayed with Pomegranate peel extract 2.5 gm/100 ml water. nano cu: Pear trees were sprayed with nano cu 70 ppm.

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 [30] and [31] Additionally, reactive oxygen species are very important signals in abiotic stress such as drought and salinity stress [32]and [33].

CONCLUSIONS

It can be concluded that application of Pomegranate peel extract and nano cu 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 Pomegranate peel extract and nano cu led to increase most of morphological and physiological studied characters and improve fruits yield.

ACKNOWLEDGEMENTS

The authors acknowledge Taif University Researchers Supporting Project number (TURSP-2020/143), Taif University, Taif, Saudi Arabia. Also, the authors would like to thank colleagues at Plant Pathology and Biotechnology Lab. (Accredited according to ISO/17025) and EPCRS Excellence Centre (Certified according to ISO/9001, ISO/14001 and OHSAS/18001), Department of Agricultural Botany, Faculty of Agriculture, Kafr-Elsheikh University, Kafrelsheikh, Egypt.

REFERENCES

[1] FAO (2018) Faostat: statistical database. Food and agriculture organization of the United Nation, Rome, Italy. Available at http://www.fao.org/faostat/en/#data/QC, (accessed at 31.03.2020)

[2] Doolotkeldieva, T., Bobushova, S., Schuster, C., Konurbaeva, M., Leclerque,A.(2019). Isolation and genetic characterization of Erwinia amylovora bacteria from Kyrgyzstan. Eur. J. Plant Pathol. 155, 677–686.

[3] Doolotkeldieva, T. and Bobusheva, S. (2016). Fire Blight Disease Caused by Erwinia amylovora on Rosaceae Plants in Kyrgyzstan and Biological Agents to Control This Disease. Microbiology. 06(11), 831-851

[4] Smith, D.D.N., Williams, A.N., Verrett, J.N., Bergbusch, N.T., Manning, V., Trippe, K., Stavrinidesa, J. (2019). Resistance to Two Vinylglycine Antibiotic Analogs Is Conferred by Inactivation of Two Separate Amino Acid Transporters in Erwinia amylovora. Journal of Bacteriology. (201), 1-13.

[5] O'Brien, P.A. (2017). Biological control of plant diseases. Australasian Plant Pathol. (46), 293-304.

[6] Arafat, K.H., Hanan, S.A. and Rabab, A.M. (2015). Antibacterial activity of antagonistic bacteria and plant extract on Erwinia amylovora the pathogen of fire blight disease in Egypt. Int. J. Phtopathol. 04(02), 73-79.

[7] Hafez, Y.M., El-Kady, E.M., Moussa, Z., Elsaed, N., Kotb, H., Salama, A., Mazrou, Y. and Makhlouf, A.H. (2020). Exogenous application with Bacillus subtilis and H2O2 mitigated fire pear blight bacterial disease incidence in correlated with yield and fruit quality improvement. Fresen. Environ. Bull. 07(29), 6315-6327.

[8] Mosch, J., Klingauf, F., Zeller, W. (1990). On The Effect Of Plant Extracts Against Fire Blight (Erwinia amylovora).Acta Hortic. (273), 355-361.

[9] Usta, C., Yildrim, A.B. and Turkey, A.U. (2014). Antibacterial and Antitumor Activities of Some Plants Grown in Turkey. Biotechnol. Biotechnol. (28), 306-314.

[10] Akhtar, S., Ismail, T., Fratiernale, P. and Sestili, P. (2015). Pomegranate Peel and Peel Extracts Chemistry And Food features, Food Chemistry. (174), 417-425.

[11] Gurjar, S.M., Ali, S., Akhtar, M. and Singh, S.K. (2012). Efficacy of Plant Extracts In Plant Disease Management Agricultured Science. 3(3), 425-433.

[12] Vasudeva, N., Sharma, S.K. (2008). Biologically Active Compounds From The Genus Hibiscus. Pharmarceutical Biology. 46(3), 145-153.

[13] Leathers, T.D., Saunders, L.P., Bowman, M.J., Price, N.P.J., Bischoff, K.M., Rich, J.O., Skory, C.D., Nunnally, M.S. (2020). Inhibition of Erwinia amylovora by Bacillus nakamurai. Journal of Current Microbiology. 77, 875–881.

[14] El-Batal, A.I., El-Sayyad, G.S., Mosallam, F.M., Fathy, R.M. (2019). Penicillium chrysogenum-Mediated Mycogenic Synthesis of Copper Oxide Nanoparticles Using Gamma Rays for InVitro Antimicrobial Activity Against Some Plant Pathogens. Journal of Cluster Science.31, 79–90.

[15] Singh, R. and Jagtap, G.P. (2017). In vitro evaluation of antibacterial chemicals and bioagents against Ralstonia solanacearum infecting bacterial wilt in ginger .Int. J. Curr. Microbiol. App. Sci. 6(5), 2034-2045.

[16] Chumchalova, J., Stiles, J., Josephsen, J. and Plockova, M. (2004). Characterization and purification of acidocin CH5 a bacteriocin produced by lactobacillus acidophilus CH5. J. of Appl. Micro. 96, 1082-1089.
[17] Ozrenk, K., Balta, F., Çelik, F. (2012). Levels of fire blight (Erwinia amylovora) susceptibility of native apple, pear and quince germplasm from Lake Van Basin, Turkey. Eur. J. Plant Pathol. 132, 229–236.
[18] Omara, R.I., Kamel, S.L., Hafez, Y.M. and Morsy, S.Z. (2015) Role of non-traditional control treatments in inducing resistance against wheat leaf rust caused by Puccinia triticina. Egypt. J. Biol. Pest Cont. 25, 335-344.
[19] Malik, C.P. and Singh, M.B. (1980) Plant Enzymology and Histo Enzymology," Kalyani Publishers, New Delhi. 1-286.
[20] Hammerschmidt, R., Nuckles, E.M., and Kuć, J. (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. Physiol. Plant Pathol. 20, 73-82.
[21] Magness, J.R. and Taylor, G.F. (1982) An improved type of pressure tester for the determination of fruit maturity. USDA Cir. No. 350.
[22] Snedecor, G.W. and Cochran, W.G. (1990) StatisticalMethods, 11th ed. 10 Wa State Univ. press, Ames, Lowa USA.
[23] Viczian, O., Kunstler, A., Hafez, Y.M. and Kiraly, L. (2014) Catalases may Play Different Roles in Influencing Resistance to Virusinduced Hypersensitive Necrosis, Acta Phytopathol. Entomol. Hung. 49, 189-200.
[24] Raj, N.S., Lavanya, S.N., Amruthesh, K.N., Niranjana, S.R., Reddy, M.S., Shetty, H.S. (2012) Histochemical changes induced by PGPR during induction of resistance in pearl millet against downy mildew disease. Biol. Control. 60, 90-102.
[25] Dewir, Y.H., El-Mahrouk, M.L., Al-Shmgani, H.S., Hail, Z., Rihan, H.Z., Teixeira da Silva, J.A. and Michael, P., Fuller, M.P. (2015). Photosynthetic and biochemical characterization of in vitro-derived African violet (Saintpaulia ionantha H. Wendl) plants to ex vitro conditions, Journal of Plant Interactions. 10(1), 101-108.
[26] El-Banna, M.F. and Abdelaal, Kh. A.A. (2018) Response of Strawberry Plants Grown in the Hydroponic System to Pretreatment with H2O2 before Exposure to Salinity Stress. Journal of Plant Production. 9, 989-1001.
[27] Abdelaal, Kh.A.A., EL-Maghraby, L.M., Elansary, H., Hafez, Y.M., Ibrahim, E.I., ElBanna, M., El-Esawi, M. and Elkelish, A. (2020) Treatment of Sweet Pepper with Stress Tolerance-Inducing Compounds Alleviates Salinity Stress Oxidative Damage by Mediating the Physio-Biochemical Activities and Antioxidant Systems Agronomy. 10, 26.
[28] Linglan, M., Chao, L., Chunxiang, Q., Sitao, Y., Jie, L., Fengqing, G. and Fashui, H. (2008) Rubisco activase mRNA expression in spinach: modulation by nanoanatase treatment, Biol. Trace Elem. Res. 122, 168–178.
[29] Prasad, T.N.V.K.V. (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut, J. Plant. Nutr. 35, 905– 927.
[30] Hafez, Y.M., Bayoumi, Y.A. and Shalaby, T.A. (2012) Pivotal role of hydrogen peroxide and biokal on seed germination, seedling growth and soil borne disease of cabbage and triploid watermelon. 6th International Plant Protection Symposium, Debrecen, Hungary, J. Agric. Sci., Acta Agarica Debresensis. Suppl. 161-167.
[31] Omara, R.I., El-Kot, G., Fadel, F.M., Abdelaal, Kh.A.A. and Saleh, E.M. (2019) Efficacy of certain bioagents on patho-physiological characters of wheat plants under wheat leaf rust stress, Physiological and Molecular Plant Pathol. 106, 102-108.
[32] Abdelaal, Kh. A.A., Hafez, Y.M., El Sabagh, A. and Saneok, H. (2017) Ameliorative effects of Abscisic acid and yeast on morphophysiological and yield characteristics of maize plant (Zea mays L.) under water deficit conditions, Fresen. Environ. Bull. 26, 7372-7383.
[33] Smits, T.H.M., Rezzonico, F., Kamber, T., Goesmann, A.C., Ishimaru, A. and Stockwell, V.O. (2010) Complete genome sequence of Pantoea vagans plant-beneficial strain C9-1. Journal of Bacteriology. 192, 6486-6487.

Received: **17.01.2021**
Accepted: **07.02.2021**

CORRESPONDING AUTHOR

Yaser M Hafez
Plant Pathology and Biotechnology Laboratory,
Agricultural Botany Department,
Faculty of Agriculture,
Kafrelsheikh University,
33516 Kafr El-Sheikh – Egypt

e-mail: hafezyasser@gmail.com