Journal of Sohag Agriscience (JSAS) 2022, 7(2):47-59

ISSN 2357-0725
https://jsasj.journals.ekb.eg
JSAS 2022; 7(2): 47-59

Received: 23-06-2022
Accepted: 04-10-2022

Hosny, M.
AsranM. R.
Moharam M.H.A.
Plant Pathology Department
Faculty of Agriculture
Sohag University
Sohag
82524
Egypt

Biological control of potato bacterial wilt disease caused by *Ralstonia solanacearum* using actinomycetes isolates

Hosny, M., Asran, M. R. and Moharam M.H.A.

Abstract

Ralstonia solanacearum is one of the most critical and widespread pathogenic bacteria that causes wilt disease in vegetable crops, including potatoes. *In vitro* study was conducted to screen potential actinobacterial isolates for their antibacterial activity against two isolates (Rs3 and Rs11) of bacterial wilt pathogen, R. *solanacearum*. Soil samples were collected from different localities. From collected soil samples, 45 morphologically distinct actinomycetes isolates were isolated. In the screening test, 7 isolates were selected, showing inhibitory activity against the pathogen. These seven efficient isolates were subjected to the cell-free suspension test. From the cell-free suspension test, only three cell-free suspensions of 3 isolates exhibited inhibitory activity against both pathogenic isolates. Isolate ACT27 exhibited the highest effect on both tested Rs3 and Rs11 pathogenic isolates, followed by ACT7 and ACT9. Whereas isolates ACT20, ACT33, and ACT13 showed the intermediate effect on both tested pathogenic isolates Rs3 and Rs11. Data also indicated that isolate ACT22 exhibited a minor effect on both tested Rs3 and Rs11 pathogenic isolates. Data also indicated that only three cell-free supernatants of isolates (ACT7, ACT9, and ACT27) give an inhibitory effect against both tested R. *solanacearum* pathogenic isolates. They are not varied so much in the degree of their activity. In this study, it can be concluded that actinomycetes isolates have antibacterial activity against this target pathogen. Also, the investigated isolates can be used through detailed further *in vivo* tests.

Keywords:
Potato, actinomycetes, bacterial wilting, *Ralstonia solanacearum*.

Corresponding author:
Hosny, M.
mohamed.hosny@agr.sohag.edu.eg

INTRODUCTION

The bacterial wilt disease of potatoes caused by *Ralstonia solanacearum* causes considerable damage to potatoes and many other crops in tropical, subtropical, and warm regions of the world. It limits the production of many crops, *e.g.*, tomato, eggplant, and pepper. Approximately 450 plant species have been reported as hosts of this pathogen (Grimault *et al.*, 1994; Williamson *et al.*, 2002; Ji *et al.*, 2005; Seleim *et al.*, 2014). During infection, the pathogen can invade plant roots through wounds and multiply in the cortical tissue before invading the xylem elements. In a few hours, the bacteria can spread into the crown and stem through the plant's vascular system (Vasse *et al.*, 1995). As the cell concentration number increases, virulence genes are expressed, and cells become non-motile and secrete exopolysaccharide and pectin-degrading enzymes, leading to plant death (Saile *et al*., 1997). To date, no effective control method has been developed for this wilt disease. However, plant breeding, field sanitation, crop rotation, and use of bactericides have met induce with only limited success due to the high variability of the pathogen, high capacity of the pathogen to survive in diverse environments, and its extensive host range (Lemessa and Zeller, 2007; Nguyen and Ranamukhaarachchi, 2010). Chemical control by soil fumigants, antibiotics, and copper compounds was tried without success (Farag *et al.*, 1982; Hartman and Elphinstone, 1994). Therefore, developing effective biological control agents is crucial for controlling potato bacterial wilt. Biological control increases crop yield suppresses disease and avoids environmental pollution. Streptomycetes are gaining interest in agriculture as plant growth-promoting bacteria and/or biological control agents (Viaene *et al.*, 2016; Dias *et al.*, 2017). The Streptomyces genus comprises Gram-positive bacteria, which show a filamentous form; they can grow in various environments. The strains of this heterogeneous bacterial species differ in biochemical, pathogenic, and genetic characteristics, and no effective control measure has been developed against it yet. The current study was carried out to isolate and identify the potential actinobacterial isolates from different soil samples, and to screen them to determine their biocontrol potential against bacterial wilt pathogen, *R. solanacearum* through *in vitro* conditions.

MATERIALS AND METHODS

Isolation of the causal pathogen of potato bacterial wilt disease

Potato plants and tubers showing typical symptoms of potato brown rot were collected from different localities of Sohag, Assiut, and Minia governorates during the 2015-2016 seasons. Diseased potato parts were washed with tap water several times, about 1 cm^3 infected pieces were cut out from the tubers, surface-sterilized by soaking in 1% sodium hypochlorite for 2 minutes, and rinsed twice in sterilized water for 5 minutes. Samples were homogenized in a sterile mortar and pestle with 5 ml of sterile 0.05 M potassium phosphate buffer and streaked on Petri plates containing Triphenyl tetrazolium chloride (TTC) medium (Kelman, 1954). Plates were incubated at 28 °C for 3 days and then examined for bacterial growth development. The single colony technique was used to obtain a pure culture. A single colony of the isolates was sub-cultured onto nutrient agar (NA) slants and maintained at 4 °C for further studies.

Pathogenicity test

Pathogenicity of obtained 15 isolates of *R. solanacearum*-looking colonies was carried out in a greenhouse by inoculating the susceptible potato cultivar Burna by each isolate during the 2017-2018 season in the greenhouse of Plant Pathology Department, Faculty of Agriculture, Assuit University, Assuit, Egypt. Healthy potato tubers were planted in 30-cm-diameter sterilized pots containing sterile 1:1 soil/sand mixture. Potato tubers were surface disinfection by soaking tubers in 0.5 % sodium hypochlorite for 5 minutes, then rinsed twice in sterilized water directly before planting. Fifteen bacterial isolates were grown on a nutrient agar medium for two days at 28±2 °C. A single colony of the isolates was selected and grown in 250 ml Erlenmeyer flasks containing 100 ml of nutrient sucrose broth and incubated at 28± 2 °C for 48h on a rotary shaker at 150 rpm. The bacterial cell suspension was centrifuged (8 min at 10.000 g), the cells were suspended in sterile distilled water, and an optical density of 0.1 at 600 nm wavelength using a spectrophotometer (model 6405UV/VIS), approximately 10^9 (CFU/ml) was

adjusted. The adjusted bacterial suspensions were used for inoculations (Kelman, 1954).

At six weeks old, potato plants in replicate were inoculated with a suspension of the isolates at the rate of 10^9 CFU/ml with punctures made with a sterile needle in the stem. Inoculation was made at the three to four genuine leaf stages by puncturing the stem at the axils of the third fully expanded leaves from the apex with a needle dipped in the inoculum. Each plant stem was injected with 100ul bacterial suspension. Control plants were treated with 100 µl distilled water. (Winstead and Kleman, 1952). Five replicate pots for each isolate were used. Pots were arranged in a complete randomized block design with five replicates. Control plants were treated similarly by tap water. The plants were grown in the greenhouse under natural temperatures (25±2 °C). Plants were fertilized every 15 days with urea 46% (20 g/pot) and irrigated with water when necessary. The disease incidence (DI) was recorded and calculated according to the scale of (Kempe and Sequeira, 1983) using the following formula:

$$DI = \frac{\sum (R \times T)}{N \times 5} \times 100$$

Where T = Number of plants corresponding to the numerical grade.

N = Total number of plants observed.

R = Disease incidence scale (R= 0, 1, 2, 3, 4 or 5).

5= High score on the incidence scale.

0 = No symptom; 1 = 1-25%, leaves wilted; 2 = 26-50% leaves wilted; 3 = 51-75% leaves wilted; 4 = more than 75%, but less than 100% of leaves wilted; 5 = all leaves wilted and dead

Tomato seedlings bioassay

Tomato bioassay was also recommended by the European Plant Protection Organization (EPPO) for detecting *R. solanacearum* in soil and potato tubers and for pathogenicity testing (Elphinstone *et al.,* 1996). Pathogenicity of fifteen bacterial isolates of *R. solanacearum*-looking colonies was carried out in a greenhouse by inoculating the susceptible tomato cultivar G.S by each isolate during the 2016-2017 season in the greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Bioassay on tomato seedlings was according to (Janse, 1988). All test strains with presumptive *R. solanacearum* colony appearance on TTC medium were inoculated in healthy two-week-old tomato seedlings (cv. G.S) grown in 15 cm sterilized pots containing sterile 1:1 soil/sand mixture. Tomato seedlings' roots were surface disinfection by soaking roots in 0.5 % sodium hypochlorite for 5 minutes, then carefully rinsed twice in sterilized water directly before planting. Fifteen bacterial isolates were grown on a nutrient agar medium for two days at 28±2 °C, suspended in sterile distilled water, and at an optical density of 0.1 at 600 nm wavelength using a spectrophotometer (model 6405UV/VIS), approximately 10^9 (CFU/ml) was adjusted. Seedlings were inoculated with a suspension (10^9 CFU/ml) of each isolate through punctures made in the stem between the two cotyledons by a sterile needle. Inoculation was made at the three to four genuine leaf stages by puncturing the stem at the axils of the third fully expanded leaves from the apex with a needle dipped in the inoculum. Each plant stem was injected with 100 µl bacterial suspension. Control plants were treated with 100 µl distilled water. (Winstead and Kleman, 1952). Five replicate pots for each isolate were used. Seedlings inoculated with sterile water were used as a control. Plants were fertilized every 5 days with NPK (20, 20, 20) and irrigated with water when necessary with avoided wetting the foliage (Williamsson *et al.,* 2002). The experiment was undertaken with a completely randomized design. Seedlings were held in a greenhouse, and the development of typical wilting symptoms was recorded and calculated after inoculation by 14 and 21 days according to (Winstead and Kelman, 1952), using the following formula:

$$\mathbf{I\% = [\sum(n_i \times v_i) \div (V \times N)] \times 100}$$

Where,

I = wilt intensity percentage; $\mathbf{n_i}$ = number of plants with respective disease rating; V= the highest disease rating (= 5); N = the number of plants observed; and $\mathbf{v_i}$ = disease rating as following scale:1= no symptoms; 2= one leaf wilted; 3= two to three leaves wilted; 4= four or more leaves wilted; 5= whole plant wilted

Identification of the causal pathogen of potato bacterial wilt disease

Worldwide the most commonly used method for detecting and identifying *R. solanacearum* has been isolation on TTC medium (Kelman, 1954) because of the relatively low cost and simplicity. Tomato bioassay is also recommended by the European Plant Protection Organization (EPPO) for detecting *R. solanacearum* in soil and potato plants and for pathogenicity testing (Elphinstone *et al.*, 1996). The fifteen isolated bacteria proved to be pathogenic and cause bacterial wilt to tomato and potato plants were identified according to their morphological, cultural, and physiological characteristics recommended by Billing *et al.* (1960), Dye (1968), and Schaad (1992), and Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) and Bergey's Manual of Determinative Bacteriology 9th edition (Holt *et al.*, 1994).

Morphological and physiological characteristics of the causal pathogen

The following morphological, physiological, and biochemical characteristics have tested the shape of cell, motility, the color of colonies, gram staining, gelatin liquefaction, starch hydrolysis, catalase test, esculin hydrolysis, growth at 4 $^{\circ}$C, growth at 40 $^{\circ}$C, H_2S production, levan production, methyl-red test (MR), phenylalanine deaminase, casein hydrolysis, urease and action on carbon compounds also were tested.

Biological control of potato bacterial wilt disease

1-Isolation of actinomycetes from soil samples

Collection of soil samples:

Rhizosphere soil samples were collected from different growing areas of the Sohag and Assuit governorates. Soil samples were taken from a depth of 30 cm surrounding the root system after removing 3 cm topsoil, collected in polythene bags, brought to the laboratory, and stored at 4 ^{o}C for further study. The samples were air-dried for 1 week and sieved through a 2 mm pore. The isolation of actinomycetes was carried out by serial dilution of the soil. One gram of soil sample was diluted to 10^{-2} with sterile water and incubated in an orbital shaker at 28 ^{o}C with shaking at 170 rpm for 30 min and incubated at 50 ^{o}C for 15-20 min. (Matsukawa *et al.*, 2007; Hong *et al.*, 2009). Serial dilutions of the soil were made up to $10^{-7,}$ and 1 ml of each dilution was taken and spread evenly over the surface of two different selective media, SCA (starch-casein nitrate-agar medium) and ISP-2 (International Streptomyces Project-2) medium supplemented with cycloheximide (100 µg/ml) on Petri dishes. Three replicates were used for each dilution. Plates were incubated at 28 ^{o}C for 8-15 days, and actinomycetes colonies were isolated based on traditional morphological criteria. The individual colonies that showed an actinomycetes-like appearance under the light microscope were purified by subculturing on an ISP-2 medium. The isolates were stored in an NGA slant at 4 ^{o}C for further use.

2-Identification of isolated Actinomycetes spp.

As per the standard procedures, selected efficient actinomycetes strains were examined for the colony morphology, growth, pigmentation, cell shape, and gram reaction. The biochemical characterization of selected efficient actinomycetes strains was essentially done per the procedures outlined (Holt *et al.*, 1994).

2-1-Culture morphology

The isolates obtained were first identified using morphology characterization according to Shirling & Gottlieb (1966). The actinomycetes were grown in different ISP medium at 28 ^{o}C for 7 days. Morphological characters such as colony appearance, type of areal hyphae, and growth of vegetative hyphae were observed. The color of spore masses and diffusible pigment production were visually estimated.

2-2-Physiological characterizations of certain actinomycetes isolates

The following physiological and biochemical characteristics were tested: the shape of cell, motility, the color of colonies, gram staining, gelatin liquefaction, starch hydrolysis, catalase test, growth at 4°C, growth at 40°C, casein hydrolysis, and urease as recommended by Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) and Bergey's Manual of Determinative Bacteriology 9th edition (Holt *et al.*, 1994).

2-3-Antagonistic activity screening test of isolated Actinomycetes spp. against *R. solanacearum in vitro*.

Preliminary screening for the isolates' antagonistic activity was done using the streak-plating method (Shomura, T. *et al.*, 1980) on Na medium. Each pure actinomycetes isolate streaked individually on different agar plates in a single line. Then plates were incubated at 32 °C for 5 days to allow the isolates to grow and secrete their metabolites into the medium. After the incubation period, Rs3 and Rs11, the most aggressive isolates of *Ralstonia solanacearum*, were cross-streaked along the line of fully grown isolates. Each streaking was started near the edge of the plates and streaked toward the actinomycetes growth line. Three replicates were used for each treatment. Plates without actinomycetes served as control. The plates were then incubated for 12 hours at 37°C, and the zone of inhibition was measured using a millimeter scale. All the isolated actinomycetes strains that show antagonistic effects against tested *R. solanacearum* isolates in primary screening were tested their supernatants against tested *R. solanacearum* isolates**.**

2-3-Effect of certain actinomycetes supernatant against *Ralstonia solanacearum in vitro*

Antimicrobial activity of certain Actinomycetes bioagent supernatants was tested on the growth of *Ralstonia solanacearum* isolates No. (Rs3 and Rs11) using the agar well diffusion method (Pinchuk *et al.*, 2001). The tested *R. solanacearum* isolates were seeded on an NGA medium. Fifty ml of sterilized NGA agar medium were prepared in Erlenmeyer flasks (100 ml). One ml bacterial suspension of *R. solanacearum* from each tested isolate (3×10 CFU/ml) from 48-hour old cultures was added to the melted medium at 47 °C and mixed gently. The mixture was then poured into sterilized Petri dishes (9 ml in diameter). After mixing, the medium was poured into plates and left for 3 hours until the media became solid, then bored with a sterile cork-borer (diameter = 10 mm). For the preparation of certain isolated actinomycetes supernatant, metabolite preparation from isolates was done according to Składanowski *et al.* (2017). Isolates of Actinobacteria were inoculated in the flask with ISP2 broth and incubated in a rotary shaker at 28 °C for 14 days. After incubation, cell-free supernatants (secondary bioactive metabolites) were prepared by centrifugation at 5000 rpm for 25 minutes and filtration by 0.22 µm pore filter paper to extract the antimicrobial compounds. Then, cell-free supernatants were collected and transferred aseptically into a screw-capped bottle and stored at 4 °C for further assay. Fifty µl from each supernatant were added to the gap of the plate to test their ability to growth inhibition the pathogen. Fifty µl of sterile distilled water was used as a control. To prolong the diffusion of liquid supernatant in an agar medium, the plates were first incubated at 4 °C for 12 hours. Three replicates were used for each treatment. The diameter of the inhibitory zone (mm) was measured after 5 days of incubation at 28 °C.

2-4-Effect of Actinomycetes spp. and their cultural supernatant on controlling potato wilt disease in the open greenhouse

Pot experiments were conducted to evaluate the potential of the actinomycetes isolates (Act7, Act9, and Act27) for their biocontrol efficacy towards certain pathogenic *Ralstonia solanacearum* under greenhouse conditions.

According to in *vitro* studies, the most promising actinomycetes isolates (Act7, Act9, and Act27) and their cell-free supernatants were selected to follow up on their effects under field conditions against *R. solanacearum* isolates (Rs3 and Rs11). The reference Trichoderma asperellum isolate was obtained from the Sohag Plant Pathology Department stock culture. The field trials were carried out in the Experimental Farm of Plant Pathology Dept. Faculty of Agriculture; Assuit Univ. during 2018-2019 and 2019-2020 growing seasons. Healthy potato tubers (Burna cultivar) were planted in 30-cm-diameter sterilized pots containing sterile 1:1 soil/sand mixture. Potato tubers were surface disinfection by soaking tubers in 0.5 % sodium hypochlorite for 5 minutes, then rinsed twice in sterilized water, and then allowed to air-dried directly before planting. With some modifications, plant infection was done using the root irrigation method described by Rado et al. (2015). All pots except healthy control were inoculated 24 hours before treatment, with 50 ml suspension (10^8 CFU/ml) of the pathogen Rs3 and Rs11 isolates. Then, the biocontrol agents were

applied via root irrigation**.** After that, healthy sterilized potato tubers were planted. Fifty ml of ten-day-old culture from each actinomycete/bioagent isolate and Reference isolate *Trichoderma asperellum* were growing on nutrient sucrose broth and incubated at 28± °C for ten days on a rotary shaker at 150 rpm. Then actinomycetes were adjusted to (10^8 CFU/ml). Metabolite preparation from isolates was done as mentioned before, according to Składanowski *et al.* (2017). Actinobacteria isolates were inoculated in a flask with ISP2 broth and incubated in a rotary shaker at 28 °C for 14 days. After incubation, cell-free supernatants (secondary bioactive metabolites) were prepared by centrifugation at 5000 rpm for 25 minutes and filtration by 0.22 µm pore filter paper to extract the antimicrobial compounds. Then, cell-free supernatants were collected and transferred aseptically into a screw-capped bottle and stored at 4 °C for further assay. The inoculum was added around each tuber at the time of planting. Five replicate pots for each tested isolate were used. Pots were arranged in a complete randomized block design with five replicates. Infected control plants were treated with pathogenic isolates only. Healthy control plants were similarly treated with only sterilized water. The plants were grown in the greenhouse under natural temperatures (25±2 °C). Plants were fertilized every 15 days with urea 46% (20 g/pot) and irrigated with water when necessary.

The disease index was recorded, as mentioned before. Disease incidence and biocontrol efficacy percentages were evaluated. Disease incidence (DI) was estimated according to Li *et al.* (2008) with the following formula:

$$\text{Disease incidence \%} = \frac{\text{(Disease index} \times \text{number of diseased plant in this index)}}{\text{(Total number of plants investigated} \times \text{the highest disease index)}} \times 100$$

Biological control efficacy (BCE) was calculated according to Guo *et al*. (2004) as:

$$\text{Biological control efficacy (BCE) (\%)} = [(DC - DT) \times 100 / DC]$$

where, DC = disease incidence of control and DT = disease incidence of the treatment group.

RESULTS AND DISCUSSION

Isolation and characterization of the pathogen

The target bacterial pathogen was isolated from collected samples of potato tubers and was compared based on their fluidity, color, and morphological structures. All isolates produced mummified fluidal of irregular bacterial colonies with a pink color on the TTC agar medium after 48 hours of incubation at 28 °C and were observed for rod shape after being examined under the compound microscope. This observation was confirmed by the observation reported by Kelman (1954). All tested isolates were pathogenic on tomato seedlings and produced typical wilt symptoms. Isolates No. Rs3 and Rs11 exhibited the highest wilt intensity with 96 and 92% wilting after 14 days from inoculation**.** On potato plants, results showed that the fifteen tested isolates were pathogenic and produced wilting symptoms potato plants. They were varied in their virulence. Isolate No. Rs3 gave the highest disease incidence with 100% wilting, followed by isolate No. Rs11 with 96% wilting, respectively**.**

Table (1): Pathogenicity tests of fifteen isolates of *R. solanacearum* on Burna cultivar of potato plants in the greenhouse.

Governorates	**Bacterial isolates No.**	**Disease index %**
Sohag	Rs 1	28
	Rs 2	56
	Rs 3	100
	Rs 4	76
	Rs 5	88
	Rs 6	32
	Rs 7	64
	Rs 8	72
Assuit	Rs 9	88
	Rs 10	36
	Rs 11	96
	Rs 12	60
	Rs 13	72
Minia	Rs 14	24
	Rs 15	64
Control		0.0
L.S.D at 5%		1.66

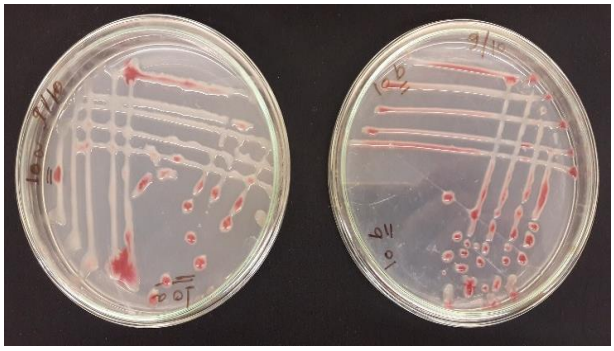

Fig. (1): Isolation of causal pathogen on (TTC) semi-selective medium showing fluidal of irregular bacterial colonies with the pink color.

Fig. (2): Bioassay ontomato seedlings (cv. G.S) after 21 days.

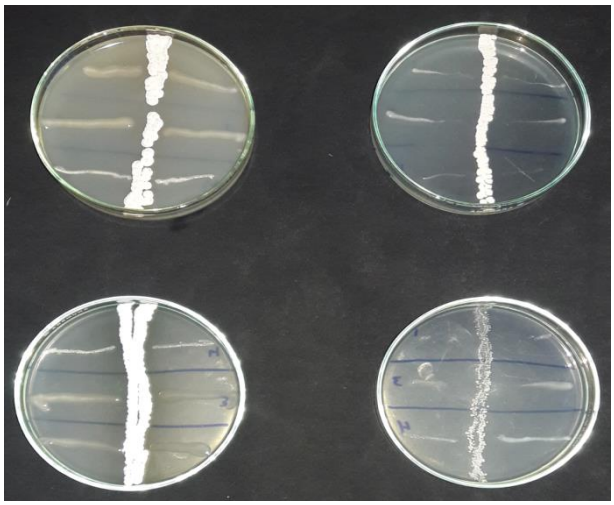

Fig. (3): In vitro antagonism of actinomycetes isolates against R. solanacearum

Isolation and Identification of actinomycetes isolates

Forty-five pure isolates were obtained from soil samples obtained from different localities were examined and then maintained on suitable media for further studies. Identification of actinomycetes isolates was carried out using morphological and physiological characteristics. Data presented in Table (2) indicated that all tested isolates were filamentous-shape, non-motile, sporing, gram-positive, urease negative, no growth at both 4 and 40 ^{o}C isolates hydrolyze starch, couldn't hydrolyze casein, and couldn't liquefy gelatin. Based on the isolated bacteria's morphological, cultural, physiological, and pathological characteristics, it was concluded that all tested isolates could be identified as actinomycetes. A total of 45 isolated actinomycetes were screened for their antibacterial activity against *R. solanacearum* in Na medium using the streak-plating technique (Shomura *et al.*, 1980). Seven actinomycetes isolates showed an inhibitory effect against selected pathogenic *R. solanacearum* isolates. Also, all actinomycetes isolates that showed antagonistic effects against tested *R. solanacearum* isolates in primary screening were tested with their supernatants against *R. solanacearum* isolates.

The antagonistic activity was confirmed by both primary screening (cross-streaking method) and secondary screening using the suitable diffusion method (Pinchuk *et al.*, 2001). Data in Table (3) and Figure (4) indicated that all tested actinomycetes isolates give an inhibitory effect against both tested *R. solanacearum* pathogenic isolates. They varied to some extent in the degree of their activity. Isolate ACT27 exhibited the highest inhibitory effect on both tested Rs3 and Rs11 pathogenic isolates, followed by ACT7 and ACT9. Also, ACT20, ACT33, and ACT13 isolates showed an intermediate effect on both tested Rs3 and Rs11 pathogenic isolates. Data also indicated that isolate ACT22 exhibited a minor effect on both tested Rs3 and Rs11 pathogenic isolates. Data also indicated that only three cell-free supernatants of isolates (ACT7, ACT9, and ACT27) give an inhibitory effect against both tested *R. solanacearum* pathogenic isolates. They are not varied so much in the degree of their activity. ACT27 supernatant exhibited the highest effect on both tested Rs3 and Rs11 pathogenic isolates, followed by ACT9 supernatant and ACT7 supernatant, while cell-free supernatants of isolates (ACT13, ACT20, ACT22, and ACT33) showed no effect on both tested Rs3 and Rs11 pathogenic isolates.

Table (2): Morphological and physiological characteristics of some antagonistic isolates of Actinomycetes.

Isolates	ACT7	ACT9	ACT13	ACT20	ACT22	ACT27	ACT33
Shape of cell	filamentous						
Motility	-	-	-	-	-	-	-
Sporulation	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+
Casein hydrolysis	-	-	-	-	-	-	-
Growth 4°C	-	-	-	-	-	-	-
Growth 40°C	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+
NaCl tolerance	-	-	-	-	-	-	-

+ = positive reaction; - = negative reaction

Table (3): Evaluation of the antibacterial activity of some actinomycetes isolates and their cell-free supernatants against two pathogenic isolates of *R. solanacearum in vitro*:

Actinomycetes isolates	Inhibition zone (mm)		Actinomycetes isolate culture supernatant (100 µl/ well)	Inhibition zone (mm)	
	Rs 3	Rs 11		Rs 3	Rs 11
ACT 7	24	23	ACT 7 supernatant	20	21
ACT 9	22	20	ACT 9 supernatant	22	21
ACT 13	8	11	ACT 13 supernatant	0	0
ACT 20	11	13	ACT 20 supernatant	0	0
ACT 22	4	5	ACT 22 supernatant	0	0
ACT 27	27	29	ACT 27 supernatant	23	22
ACT 33	11	14	ACT 33 supernatant	0	0

The antagonistic activity was confirmed by both primary screening (cross-streaking method) and secondary screening using the suitable diffusion method (Pinchuk *et al.*, 2001). Data in Table (3) and Figure (4) indicated that all tested actinomycetes isolates give an inhibitory effect against both tested *R. solanacearum* pathogenic isolates. They varied to some extent in the degree of their activity. Isolate ACT27 exhibited the highest inhibitory effect on both tested Rs3 and Rs11 pathogenic isolates, followed by ACT7 and ACT9. Also, ACT20, ACT33, and ACT13 isolates showed an intermediate effect on both tested Rs3 and Rs11 pathogenic isolates. Data also indicated that isolate ACT22 exhibited a minor effect on both tested Rs3 and Rs11 pathogenic isolates. Data also indicated that only three cell-free supernatants of isolates (ACT7, ACT9, and ACT27) give an inhibitory effect against both tested *R. solanacearum* pathogenic isolates. They are not varied so much in the degree of their activity. ACT27 supernatant exhibited the highest effect on both tested Rs3 and Rs11 pathogenic isolates, followed by ACT9 supernatant and ACT7 supernatant, while cell-free supernatants of isolates (ACT13, ACT20, ACT22, and ACT33) showed no effect on both tested Rs3 and Rs11 pathogenic isolates.

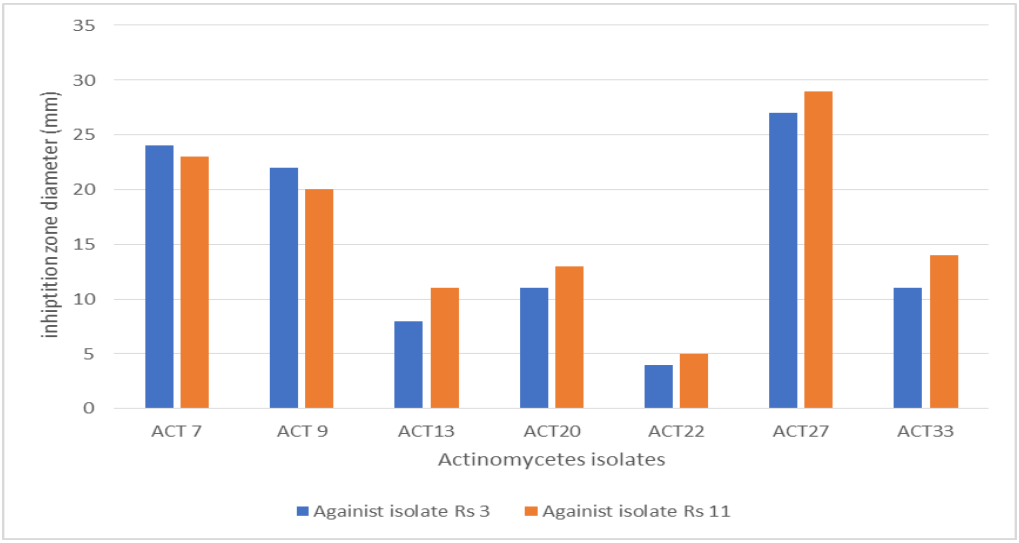

Fig.4. Evaluation of antibacterial activity of certain actinomycetes isolates against two pathogenic isolates of R. solanacearum in vitro.

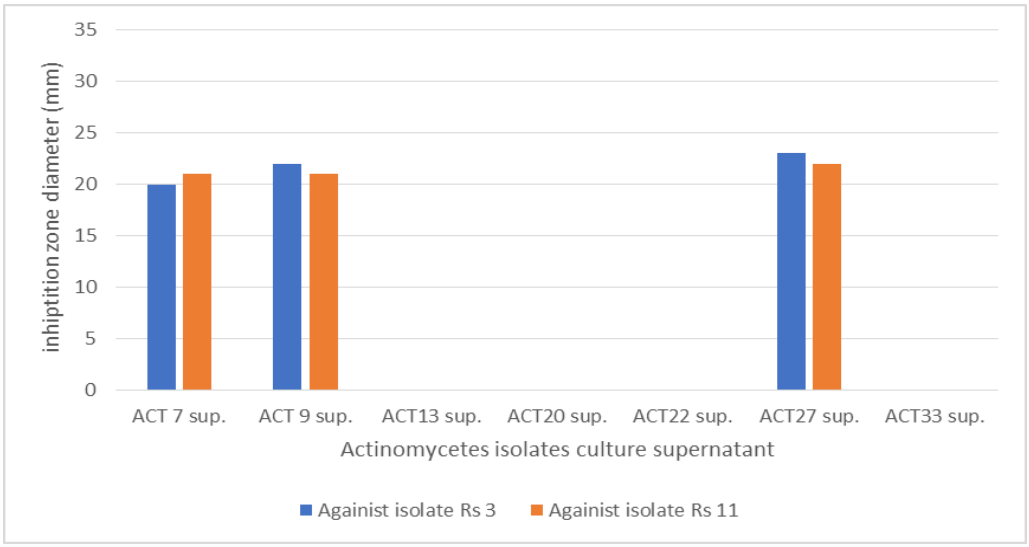

Fig. 5. Evaluation of antibacterial activity of some actinomycetes isolates' supernatant against two pathogenic isolates of R. solanacearum in vitro.

Table (4): Evaluation of the antibacterial activity of tested actinomycetes isolates and their supernatants against two pathogenic isolates of *R. solanacearum* in the greenhouse. Season 2018-2019.

Treatments	Rs 3		Rs 11	
	Disease incidence %	Biological control efficacy %	Disease incidence %	Biological control efficacy %
Act 7	56	44	52	48
Act 9	52	48	44	56
Act 27	56	44	48	52
Act 7 Supernatant	72	28	68	32
Act 9 Supernatant	68	32	64	36
Act 27	72	28	60	40
T. asperellum	56	44	48	52
L.S.D at 5%= 1.79				

Effect of promising actinomycetes isolates, actinomycetes, and isolates' supernatants obtained from previous *in vitro* studies on disease incidence on Burna potato cultivar was tested under greenhouse conditions in the 2018-2019 growing season. Compared with knowing *Trichoderma asperellum* fungal isolate. Data in Table (4) indicated that the treated plants with Act9 isolate exhibited the least disease incidence (52%) in the tested season. Also, it exhibited the highest biological control efficacy percentage (48%), followed by Act7, Act27 isolates, and fungal isolate *T. asperellum* (with 56% incidence percentage and 44% biological control efficacy percentage for all of them), then supernatant of isolate Act9 (with 68% disease incidence percentage and 32% biological control efficacy percentage). Finally, supernatant of Act7 and Act27 isolates caused 72% of disease incidence, whereas both isolates exhibited the highest disease incidence (28%) and the lowest biological control efficacy percentage on tested Rs3 pathogenic isolate. Data also showed that on tested Rs11 pathogenic isolate, isolate Act9 exhibited the least disease incidence percentage (44%) also exhibited the highest biological control efficacy percentage (56%), followed by Act27, fungal isolate *T. asperellum*, Act7, isolate Act27 supernatant, and isolate Act9 supernatant (with 48, 48, 52, 60, 64% of disease incidence, and 52, 52, 48, 40, and 36% biological control efficacy, respectively). Finally, isolate Act7 supernatant with 68% disease incidence, exhibiting the highest and lowest biological control efficacy (32%). Effect of promising actinomycetes isolates, actinomycetes, and isolates' supernatants obtained from previous *in vitro* studies on disease incidence on Burna potato cultivar was tested under greenhouse conditions in the 2019-2020 growing season. Compared with knowing *Trichoderma asperellum* fungal isolate.

Data in Table (5) indicated that in the tested season, treated plants with isolate Act7 exhibited the least disease incidence percentage (48%) and also exhibited the highest biological control efficacy percentage (52%), followed by isolate Act9 (with 52% incidence percentage and 48% biological control efficacy percentage), then isolates Act27, fungal isolate *T. asperellum*, isolate Act7 supernatant, isolate Act27 supernatant (with 56%, 56%, 76%, and 80% disease incidence percentages and 44%, 44%, 24%, and 20% biological control efficacy percentages, respectively). Finally, isolate Act9 supernatant with (88%), which exhibited the highest disease incidence percentage, and (12%), which exhibited the lowest biological control efficacy percentage on tested Rs3 pathogenic isolate.

Data also showed that on tested Rs11 pathogenic isolate, isolate *T. asperellum* exhibited the least disease incidence percentage (44%) also exhibited the highest biological control efficacy percentage (56%), followed by Act7, Act9, Act27, isolate Act7 supernatant, and isolate Act9 supernatant (with 56%, 60%, 60%, 64%, and 64% disease incidence percentages, and 44%, 40%, 40%, 36%, 36% biological control efficacy percentages, respectively). Finally, isolate Act 27 supernatant with (68%), which exhibited the highest disease incidence percentage, and (32%) which exhibited the lowest biological control efficacy percentage.

Table (5): Evaluation of the antibacterial activity of tested actinomycetes isolates and their supernatants against two pathogenic isolates of R. solanacearum in the greenhouse. Season 2019-2020.

Treatments	Rs 3		Rs 11	
	Disease incidence %	Biological control efficacy %	Disease incidence %	Biological control efficacy %
Act 7	48	52	56	44
Act 9	52	48	60	40
Act 27	56	44	60	40
Act 7 Supernatant	76	24	64	36
Act 9 Supernatant	88	12	64	36
Act 27 Supernatant	80	20	68	32
T. asperellum	56	44	44	56
L.S.D at 5%= 1.79				

REFERENCES

Billing, E., Crosse, J., & Garrett, C. (1960). Laboratory diagnosis of fire blight and bacterial blossom blight of pear. Plant Pathology, 9, 19-25.

Dias, M.P., Bastos, M.S., Xavier, V., Cassel, E., Astarita, L., & Santarém, E. (2017). Plant growth and resistance promoted by Streptomyces spp. in tomato. Plant physiology and biochemistry: PPB, 118, 479-493. doi: 10.1016/j.plaphy.2017.07.017.

Dye, D. W. (1968). A taxonomic study of the genus Erwinia. 1-The amylovora group. New Zealand Journal of Science, 11: 59-60.

Elphinstone, J.G., Hennessy, J., Wilson, J.K., Stead, D.E. 1996. Sensitivity of different methods for the detection of Pseudomonas solanacearum (Smith) in potato tuber extracts. EPPO Bulletin 26, 663-678.

Farag, N. S., Lashin, S. M., All-Abdel, R. S., Shatta, H. M., & Seif-Elyazal, A. M. (1982). Antibiotics and control of potato black leg and brown rot diseases. Agricultural Research Reviews, 60, 149–166.

Grimault, V., Anais, G. and Prior, P. (1994) Distribution of Pseudomonas solanacearum in the stem tissues of tomato plants with different levels of resistance to bacterial wilt. Plant Pathology, 43, 663-668.

Hartman, G. L., & Elphinstone, J. G. (1994). Advances in the control of Pseudomonas solanacearum race 1 in major food crops. In A. C. Hayward, & G. L. Hartman (Eds.), Bacterial Wilt: the disease and its causative agent, Pseudomonas solanacearum (pp. 157–178). Wallingford, UK: CAB International.

Holt, J. G., Krieg, N. R., Sneathm, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology 9th ed. Williams and Wilkins, Baltimore, Maryland, U.S.A.

Hong, K., Gao, A., Xie, Q., Gao, H., Zhuang, L., Lin, H., Yu, H., Li, J., Yao, X., Goodfellow, M., Ruan, J., 2009. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Mar. Drugs 7, 24–44.

Janse, J.D. 1988. A detection method for Pseudomonas solanacearum in symptomless potato tubers and some data on its sensitivity and specificity. EPPO Bulletin 18, 343-351.

Ji, P., Momol, M.T., Olson, S.M., Pradhanang, P.M. and Jones, J.B. (2005) Evolution of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. Plant Dis., 89, 497-500.

Kelman, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44, 693-695.

Kempe, J. and Sequeira, L. 1983. Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. Plant Disease, 67: 499-503.

Krieg, N. R. and Holt, J. G. (1984). Bergey's manual of Systematic bacteriology. Vol 1 Williams and Wilkins Company, Baltimore MD., U.S.A.

Lemessa, F. and Zeller, W. (2007) Screening rhizobacteria for biological control of Ralstonia solanacearum in Ethiopia. Biological Control, 42, 336-344.

Matsukawa, E., Nakagawa, Y., Iimura, Y., Hayakawa, M., 2007. Stimulatory effect of indole-3- acetic acid on aerial mycelium formation and antibiotic production in Streptomyces spp. Actinomycetologia 21, 32–39.

Murakoshi, S., & Takahashi, M. (1984). Trials of some control of tomato wilt caused by Pseudomonas solanacearum. Bulletin of the Kanagawa Horticultural Experiment Station, 31, 50–56.

Nguyen, M.T. and Ranamukhaarachchi, S.L. (2010) Soil-Borne antagonists for biological control of bacterial wilt disease caused by Ralstonia solanacearum in tomato and pepper. Journal of Plant Pathology, 92, 395-406.

Pinchuk IV, Bressollier P, Verneuil B, Fenet B, Sorokulova IB, et al. (2001) In vitro anti-Helicobacter pylori activity of the probiotic strain Bacillus subtilis 3 is due to secretion of antibiotics. Antimicrob Agents Chemother 45: 3156-3161.

Saile, E., McGarvey, J., Schell, M. and Denny, T. (1997) Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by *Rastonia solanacearum*. Phytopathology, 87, 1264-1271.

Schaad, N. W. (1992). Laboratory guide for identification of plant pathogen bacteria, 2nd

Ed. International Book Distributing Co., Lucknow, 44-58.

Seleim, M.A., Abo-Elyousr, K.A., Abd-El-Moneem, K.M. and Saead, F.A. (2014) First report of bacterial wilt caused by *Ralstonia solanacearum* biovar 2 race 1 on tomato in Egypt. Plant Pathol. J., 30, 299-303.

Shirling, E.B.T., Gottlieb, D., 1966. Methods for characterization of Streptomyces species 1. Intl. J. System. Bacteriol. 16, 313–340.

Shomura, T. Omoto S. M., Ohba, K. and Ogino H., "SF-1961, a new antibiotic related to bleomycin," Journal of Antibiotics, vol. 33, no. 11, pp. 1243–1248, 1980.

Składanowski, M., Wypij, M., Laskowski, D., Golińska, P., Dahm, H., & Rai, M. (2017). Silver and gold nanoparticles synthesized from Streptomyces sp. isolated from acid forest soil with special reference to its antibacterial activity against pathogens. Journal of Cluster Science, 28(1), 59-79.

Taechowisan, T., Chuaychot, N., Chanaphat, S., Wanbanjob, A., & Tantiwachwutikul, P. (2009). Antagonistic effects of Streptomyces sp. SRM1 on Colletotrichum musae. Biotechnology (faisalabad), 8, 86-92. doi: 10.3923/biotech.2009.86.92.

Vasse, J., Frey, P. and Trigalet, A. (1995) Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by Pseudomonas solanacearum. Mol. Plant-Microbe. Interact. 8, 241-251.

Viaene, T., Langendries, S., Beirinckx, S., Maes, M., & Goormachtig, S. (2016). Streptomyces as a plant's best friend? FEMS microbiology ecology, 92 8. doi: 10.1093/femsec/fiw119.

Williamson, L., Nakaho, K., Hudelson, B., & Allen, C. (2002). Ralstonia solanacearum Race 3, Biovar 2 Strains Isolated from Geranium Are Pathogenic on Potato. Plant disease, 86 9, 987-991.

Winstead, N.N., & Kelman, A. (1952). Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. Phytopathology, 42, 628-634.

Xio, K., Kinkel, L., & Samac, D. (2002). Biological Control of Phytophthora Root Rots on Alfalfa and Soybean with Streptomyces. Biological Control, 23, 285-295. doi: 10.1006/bcon.2001.1015.

الملخص العربي

بكتيريا الرالستونيا سولاناسيرم هي واحدة من أكثر أنواع البكتيريا المسببة للأمراض خطورة وانتشارًا والتي تسبب مرض الذبول البكتيري في محاصيل الخضروات ، بما في ذلك البطاطس. تم اجراء دراسة معملية لفحص النشاط المضاد للبكتيريا والمحتمل وجوده ضد بعض عزلات الاكتينوميستات ضد العزلتين Rs3 و Rs11من مسببات الذبول البكتيري . تم جمع عينات التربة من مناطق مختلفة . ومن عينات التربة التي تم جمعها ، تم عزل 45 عزلة اكتينوميستات تم تعريفها علي أساس مورفولوجي . تم اختيار 7 عزلات أظهرت فاعلية مثبطة ضد البكتيريا الممرضة. كذلك خضعت هذه العزلات السبع الفعالة لاختبار التضاد باستخدام راشح بكتيري خالي من الخلايا. اظهرت نتائج هذا الاختبار أن ثلاثة من هذه الرواشح ناتجة من 3 عزلات أظهرت نشاطًا مثبطًا ضد كل من العزلات الممرضة.حيث أظهرت العزلة ACT 27 ، أعلى تأثير على كل من العزلات الممرضة Rs3 و Rs11 المختبرة ، تليها ACT7 و ACT9 ، بينما أظهرت العزلات ACT20 و ACT33و ACT13 تأثير متوسط على كل من العزلات الممرضة Rs3 و Rs11 المختبرة .كما أشارت البيانات أيضًا إلى أن العزلة ACT22 أظهرت تأثيرًا طفيفًا على كل من العزلات الممرضة Rs3 و Rs11 المختبرة. أشارت البيانات أيضًا إلى أن ثلاثة فقط من الرواشح البكتيرية الخالية من الخلايا للعزلات ACT7و ACT9 و ACT27 أعطت تأثيرًا مثبطًا ضد كل من عزلات بكتيريا الرالستونيا الممرضة. في هذه الدراسة ، يمكن استنتاج أن عزلات الاكتينوميستات المستخدمة لها نشاط مضاد للجراثيم ضد البكتريا الممرضة المستهدفة. أيضا ، كذلك يمكن استخدام هذه العزلات التي تم فحصها في اجراء مزيد من الاختبارات علي النباتات المصابة.