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Effects of Plant Growth Promoting Rhizobacteria (PGPR) on *In Vitro* Bread Wheat (*Triticum aestivum* L.) Growth Parameters and Biological Control Mechanisms

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Abstract

Three endemic plants rhizosphere (*Astragalus gombo* Coss. & Dur., *Daucus sahariensis* Murb., *Ononis angustissima* Lam.), were used for actinomycetes isolation. Ninety-three (93) isolates have been screened to evaluate their antagonistic properties against phytopathogenic microorganisms and to determine their biocontrol properties against *Fusarium culmorum*, especially responsible for several cereal diseases like font's seedlings, rust, and burn of ears. Four (04) isolates (D2, D5, D8, and AST1) have been *in vitro* tested to determine PGPR effect and biocontrol characters of bread wheat (*Triticum aestivum* L.), Hidhab (HD) variety cultivated in the Murashigue and Skoog (MS) culture medium. The aim of this study is the evaluation of antagonistic isolates of pathogenic fungi *F. culmorum*, without and within commercial fungicide (Tebuconazole, 60 g/l) solution. Our results showed clearly that these isolates have a significant effect on seed germination and seedling growth. However, results argue that these actinomycetes isolates show a very interesting activity compared to the commercial fungicide. As a result, these bacteria isolates can be used as biocontrol agents against *Fusarium* wilt disease of wheat, which have a beneficial effect on growth parameters.

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Keywords

Bread Wheat, Biological Control, Endemic Plants, *Fusarium culmorum*, PGPR

1. Introduction

Wheat is the most commonly grown crop in the world, representing a major resource for food [1]. Wheat and other small grain cereals may be attacked by a wide range of *Fusarium* spp., so Fusaria have long been recognized as pathogens of many plant species [2]. *F. graminearum* and *F. culmorum* are the predominant species infecting wheat [3]. The economic impact of *Fusarium* damage on wheat has a great importance throughout the world [4]. Pathogenic fungi can survive for a long time in the soil, and then it is difficult to be controlled [5]. To fight against these pathogens via unlimited application of pesticides in soil can cause environmental pollution [6]. Also, the effectiveness of chemical fungicides is often compromised by the emergence of resistant pathogens [7]. Due to the worsening problems in fungal diseases control, further research is needed to identify alternative methods of protecting plants less dependent on chemicals and more respectful to the environment [8]. However, biological control is a promising method that involves antagonistic microorganisms like actinomycetes. These uses are deemed to apply the best form of living cells because of their ability to colonize plant roots, to control microorganisms plant pathogens and spores shaped adapted to stable products formation [9] [10].

Actinomycetes are an important part of the microbial community in soil dispersion surface; it gives the aptitude to colonize the rhizosphere through their antagonists and competitive characters, and their many secondary metabolites production that has chemical structures with different biological activities [11]. More than a thousand secondary metabolites are produced by actinomycetes which made 45% of microbial metabolites [12]. *Streptomyces* genus can produce plant growth regulators such as Indole Acetic Acid (IAA), antibiotics and lytic enzymes as biocontrol agents against *F. culmorum* responsible for various symptoms like damping roots, stems and spikelet fusariosis in many broadleaf and monocotyledons plants such as cereals. These diseases have controlled by synthetic fungicides or chemical compounds. However, chemical control generates enormous negative impacts on the environment, and the repeated use of these chemical molecules causes resistance among these pathogens. This study focuses on the biological ability of actinomycetes to fight against these pathogens. They are often isolated from samples of different ecosystems for producing substances inhibitory activities against plant pathogens, using suitable culture media and then selecting the successful stem through the identification of their *in vitro* antagonistic activity.

2. Materials and Methods

2.1. Plant Material and Actinobacteria Sampling

Seeds of bread wheat (*T. aestivum*), Hidhab cultivars (HD1220/3*Kal/Nac, proven from CIMMYT, selected in agricultural research station of Sétif (Algeria) in 1985), treated and untreated by (Tebuconazole, 60 g/l) and inoculated with actinomycetes isolates of indigenous plants rhizospheric soil (*Ononis angustissima* Lam., *Astragalus gombo* Coss. & Dur. and *Daucus sahariensis* Murb.) taken from Djebel Messaad region at M'sila-State, in southern of Algeria (**Figure 1**). Samples were collected in sterile cylinders, closed tightly and stored in the refrigerator at 4˚C. For each collected sample, 10 g of soil was suspended in 90 ml of physiological water (NaCl, 9 g/l), then incubated in an orbital shaker incubator at 50˚C with shaking for 30 min at 160 rpm. The suspension was serially diluted up to 10.

2.2. Actinomycetes Isolates Recognition

Several solid culture media, such as ISP2, GYEA and GYME (**Table 1**) in addition to streptomycin, penicillin and actidione, antibiotics were used to isolate and maintain actinomycetes isolates from selected soil samples. Culture media autoclaved at 120˚C for 2 h; glassware is sterilized at 180˚C in ventilated oven for 30 min and then microbiological manipulations performed in a laminar flow hood around a Bunsen burner.

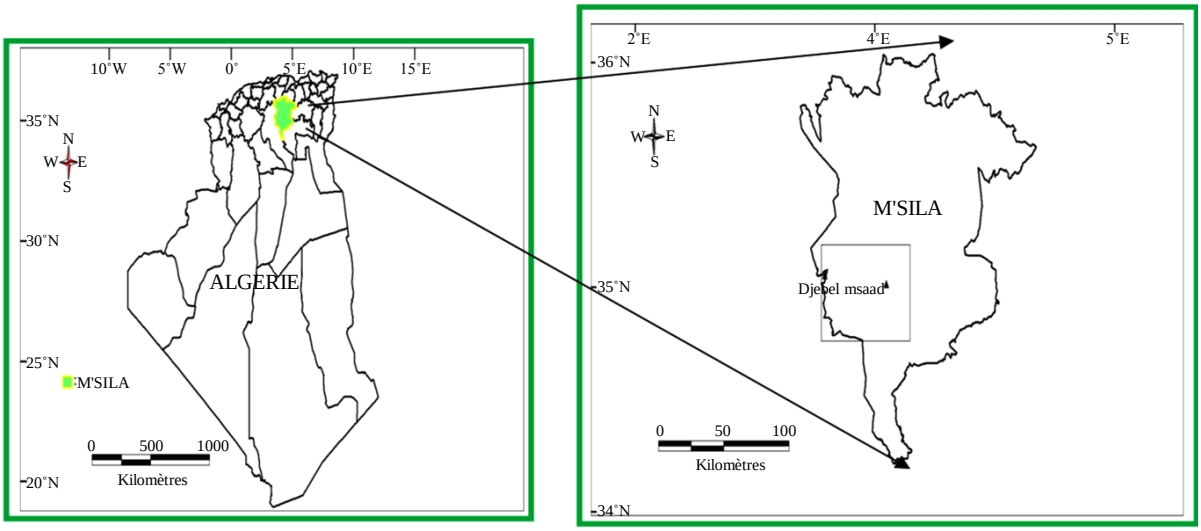
Using the method of Pochon and Tardieux [13] three samples of 100 g to 5 cm of the top soil away from roots have been taken from the rhizosphere of 3 endemic plants zones; then putted in plastic bags and analyzed directly

Daucus sahariensis Murb

Astragalus gombo Coss. & Dur

Ononis angustissima Lam

(a)



(b)

Figure 1. (a) Endemic plants which rhizospheric soil used to isolate actinomycetes; (b) Study geographical area location (Map-Info software, version 8).

Table 1. Media for isolation, seeding, and recognition of actinomycetes isolates.

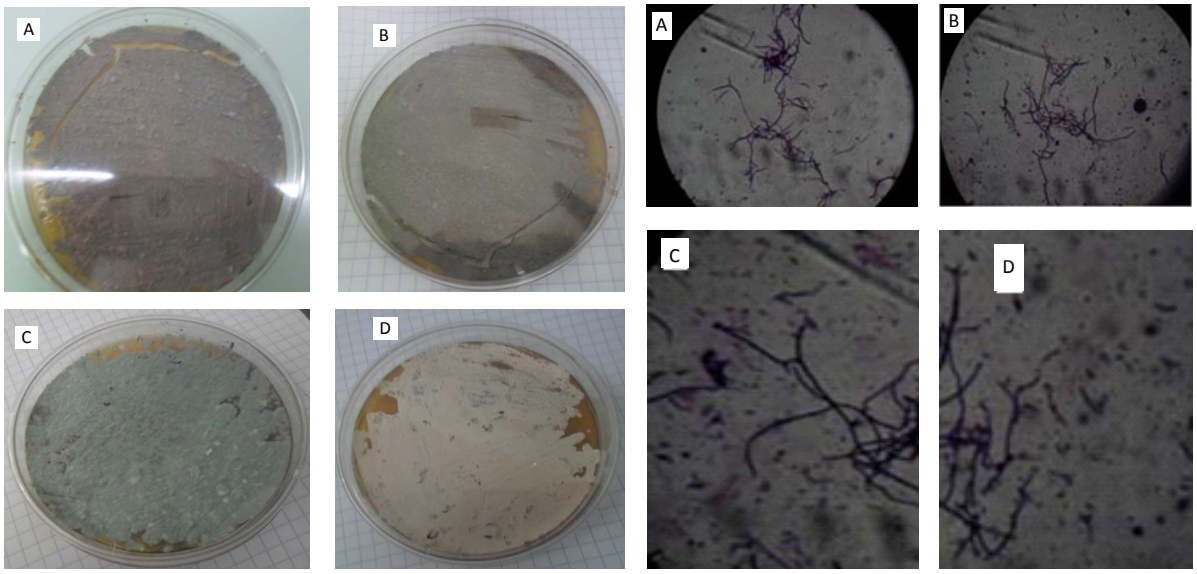
Formula or compounds (ml)	ISP2	GLM	GYEA	PDA
Malt extract (g/l)	10	3	none	/
Yeast extract (g/l)	4	3	10	/
Glucose (g/l)	4	10	10	/
Peptone (g/l)	/	5	/	/
Dextrose (g/l)	/	/	/	20
Infusât de pomme de terre	/	/	/	200
Distilled water	1000	1000	1000	1000
Agar (g/l)	20	20	18	20
pH	7.3	7.2	6.8	5.4

ISP2 = International *Streptomyces* Project (Shirling and Gottlieb 1966), GYME = Gelose Yeast-Malt Extract (Kitouni 2007), GYEA = Glucose-Yeast Extract-Agar (Athalye *et al*. 1981), PDA = Potato dextrose agar.

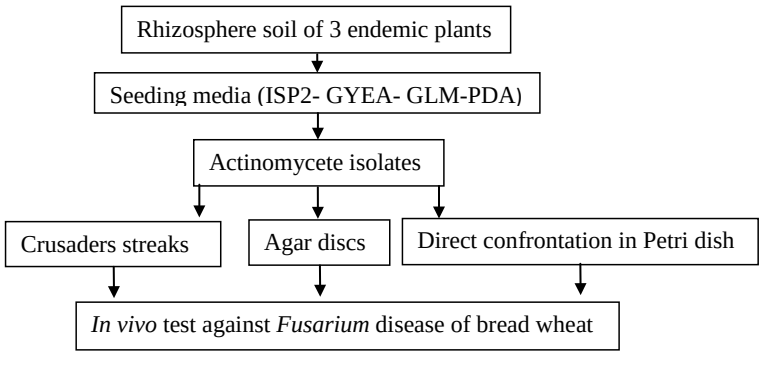
or stored at 4˚C for 6 h. 10 g of each sample was ground in a sterile mortar and then sieved. 5 g were processed by 0.02 g and introduced into a container containing 50 ml of sterile distilled water. After homogenization of solution, a serie of dilutions of 10^{-1} to 10^{-5} was prepared. 0.1 ml from the last three dilutions collected. Then seeded depositing 0.1 ml of each sample corresponding to the dilution 10^{-3}, 10^{-4} and 10^{-5} to the spread surface of the corresponding culture medium (GYME, GYEA, ISP2) in Petri dish. Which were incubated at 28˚C for 7 to 14 days, while the boxes containing only ISP2 backgrounds, GYEA, GYME served as control and incubated in the same previous conditions [14]. Actinomycetes colonies were observed under an optical microscope (×40). They marked by their macroscopic and microscopic appearance of hard colonies; small size with a round shape surrounded by microfilaments (**Figure 2(a)**, **Figure 2(b)**) and then crop diversity was thus carefully analyzed and investigated.

2.3. Purification and Conservation

An aliquot of 0.1 ml of appropriate dilutions was taken and spread evenly over the surface of yeast extract-malt extract agar medium ISP2 (international streptomycetes project) [15], supplemented with 2.5 mg/ml streptomycin and 75 mg/ml amphotericin B to inhibit bacterial and fungal contamination. Plates were incubated at 28˚C and growth development was monitored through 14 days. The isolates maintained on ISP2 medium slants at 4˚C and as a glycerol suspension 20% (v/v) at −20˚C.



(a) (b)



(c)

Figure 2. (a) Macro-morphological observations of actinomycetes isolates in ISP2 medium: *A* = D5 isolate, *B* = Ast1 isolate, *C* = Da8 isolate and *D* = D2 isolate; (b) Micro-morphological observations of actinomycetes isolates in ISP2 medium: *A* = Da8 isolate, *B* = Ast1 isolate, *C* = D5 isolate, *D* = D2 isolate. (c) Antifungal Activity protocol.

2.4. Phenotypic Characteristics of Actinomycetes Isolate

Isolates morphology was examined according to the methods recommended by Shirling and Gottlieb for the International Streptomycetes Project (ISP) [15] and Bergey's Manual of Systematic Bacteriology [16]. Visual observation using light microscopy and Gram-straining performed for further identification. Cultural characteristics of pure isolates in ISP2 media recorded after incubation for 7 to 14 days at 28˚C. Catalase and oxidase activities were determined with 3% (v/v) hydrogen peroxide solution (H_2O_2) and 1% (v/v) tetra-methyl phenylene-di-amine dihydrochloride solution (TMPDADC), respectively. The color of mycelium and soluble pigment were examined in ISP2 medium and determined by comparison with chips color of in the color harmony manual [17]. Growth at pH values (4 - 11), tolerance to NaCl (1% - 12%) and temperature range for growth (5˚C - 50˚C) was examined on ISP2 too.

2.5. Indole Acetic Acid (IAA) Production

The ability of actinobacteria to produce IAA was measured based on the colorimetric method described by Khamna *et al*., [18] and Kaur *et al*., [19] with some modifications. Three discs (6 mm ϕ each one) of growing actinobacteria from yeast malt agar (YMA) were inoculated into 100 ml yeast medium both containing 0.05% L-tryptophan and incubated at 28˚C on a rotary shaker at 160 rpm for seven days. Cultures were harvested and centrifuged at 1000 rpm for 10 min at 4˚C. Their action mixture, which included 2 ml of supernatant and 2 ml of "Salkowski reagent", were incubated at 25˚C for 30 min in the dark. The absorbance of the reaction mixture was measured at 530 nm and the IAA content of the culture filtrate was quantified using a standard curve with known concentrations of pure IAA (Sigma).

2.6. *In Vitro* Biocontrol Assay

Bread wheat (*Triticum aestivum* L.), cultivar (HD), highly susceptible to *Fusarium* head blight (FHB) used in this experiment. Seeds were surface sterilized by immersion in 70% ethanol for 1 min, followed by continuous agitation in a 1% sodium hypochlorite solution (NaOCl) for 5 min and rinsed with sterile distilled water. They were allowed to imbibe water overnight at room temperature. Pre-germinated seeds grown in Petri dishes, which experiments were performed in controlled environmental conditions using plant growth chamber. Seeds germinated in 150 mm diameter plastic Petri dishes each containing 20 seeds on two layers of 125 mm diameter Whitman N˚1 filter paper moistened with 8 ml Murashigue and Skoog (MS) solution [20], in three replicates.

Actives fresh suspensions of actinomycetes isolates, approximately 1 × 10 CFU/ml in 1ml of ISP2 broth with 0.01% Tween-20 and 1 ml of *F. culmorum* approximately 1×10^5 CFU/ml added to the seeds immediately before planting. Two seed lots were used such as seeds inoculated with *F. culmorium* untreated by fungicide Tubeconazole, 60 g/l without actinomycetes isolates and seeds inoculated with *F. culmorum* treated by fungicide Tubeconazole, 60 g/l for 7 days in the presence of actinomycetes isolates. Plants were maintained in a growth room conditions with temperature 24˚C, 16 h light/8h dark photoperiod and relative humidity of 80% and fertilized weekly with MS solution. Germination rate determined after three days after seeding and growth morpho-physiological parameters, such as the number of leaves, leaf area, root length, and chlorophyll content determined after four weeks.

2.7. Actinomycetes Antimicrobial Activities

Antimicrobial activities of actinomycetes isolates were evaluated by cross-striations method on ISP2. It consists in seeding actinomycetes isolates in a single line on the surface of the solid medium. After incubation at 30˚C for seven days, Target isolates were seeded perpendicular to the actinomycetes. Results were obtained after 36 to 48 h by measuring the distance between the edges of inhibition of target strain and actinomycetes isolates. Actinomycetes seeded, on PDA (Potato Dextrose Agar), the device of the Petri dish (away from the edge of the box by 3 mm), with a streak of 6mm wide. These cultures incubated at 28˚C for five days. A disk of 7 mm diameter mushroom cultivation eight days old deposed share of actinomycetes culture. The distance between mushroom and disc edge of the box is 15 mm. For each fungal isolate a control treatment was provided, with disc deposited on PDA medium without actinomycetes. Fungus colonies diameter was measured for all treatments including the control [21]. According to Williams and Willis [22]; Aghighi *et al*., [23], agar discs (6 mm ϕ) were collected from an actinomycetes culture of 14 days then putted into a Petri dish containing (PDA) medium. A

washer (8 mm ϕ) of *F. culmorum* of 10 days old was then deposited in the center of the box and at a distance of 3 cm agar discs away. Petri dishes then incubated at 25˚C for five days. Control sample contains only a washer of *F. culmorum*. Daily reading is done on sample control culture.

Antifungal activity of actinomycetes isolates was underscored by cross streaks method on the ISP2 environment, agar-cylinder method and confrontation method in Petri dish [24]. The target seeds used were filamentous fungi and pathogenic bacteria. The following protocol showed the antifungal activity and biological control against plant diseases by actinomycetes isolates that produce antibiotics (**Figure 2(c)**). Both of 8 treated and untreated seeds of (HD) with the fungicide seeded on MS medium supplemented with 3 ml of *Fusarium* suspension and 3 ml of actinomycetes suspension.

2.8. Antagonistic Actinomycetes Isolate Potential on *Fusarium* Wither of Wheat Seeds and Disease Expression

We have studied actinomycetes action on *Fusarium* mycelium growth to assess the effectiveness of actinomycetes suspensions on Hidhab (HD) variety seeds. Spores of phytopathogenic fungus (*F. colmorum*) obtained by flooding a culture of 14 days on (PDA) medium incubated at 25˚C with 10 ml of sterile distilled water; conidia dislodged by scraping the medium surface with sterile Pasteur pipette. The resulting liquid was filtered through 4 layers of cotton to remove debris from the mycelium. The filtrate obtained containing spores washed twice with sterile distilled water, and spore suspension centrifuged at 1000 rpm for 5 min. Thus, pre-identified isolates of actinomycetes are streaked on Petri dish containing nutrient agar medium and incubated at 30˚C for 24 h. Cans were flooded with 10 m1 of sterile distilled water and scraped with a sterile Pasteur pipette; the recovered suspension homogenized by stirring, then spore concentration estimated using a Malassez cell and adjusted to the required concentration (108 spores/ml) by adding it to sterile distilled water [25]. It noted that we have used in this study the MS medium [20] supplemented with 0.5 g/l of 2.4, Dichlorophenoxyacetic acid. Wheat seeds disinfected by 70% alcohol for 70 seconds, and then they soaked in water for 15 min bleach followed by three successive rinses with sterile distilled water.

2.9. Data Analysis

Measurements related to the enumeration of isolated actinomycetes and their behavior towards the culture medium used and macro-morphological characterizations, and their antagonistic activities mentioned. Data were processed by the analysis of variance, using Crop-Stat 7.2.3 software (2009), and then Newman-Keuls test at 5% probability, is used to compare treatment means.

3. Results

3.1. Seed Germination Percentage

Seed germination capacity of bread wheat (*T. aestivum*), Hidhab variety inoculated by actinomycetes isolates in the presence of *F. culmorum* showed that the percentage of both germinated seeds treated and untreated by commercial fungicide (Tebuconazole, 60 g/l) exceeds 85%, so there is a significant effect of treatment by fungicide. These results, confirm that the treatment by fungicide has a depressive effect on the pathogen and therefore a positive effect on the germination rate (**Figure 3(a)**).

3.2. Morphological and Physiological Growth Parameters

In general, plants are grown from inoculated seeds with actinomycetes isolated and treated with commercial fungicide (Tebuconazole, 60 g/l), the number of leaves is high (6 leaves) compared to seedlings which untreated seeds (2 leaves) (**Figure 3(b)**). However, seedlings treated and untreated with the commercial fungicide and inoculated with D8 and D5 isolates in the presence of (*F. culmorum*) have developed an average leaf area 5.5 cm^2 and 14 cm^2 respectively. While plantlets seeds inoculated with AST1 isolate in the presence of the commercial fungicide, have developed a leaf area (8 cm^2), and in the absence of this fungicide (17 cm^2). For the D2 isolate, leaf area is 18 cm^2 for plantlets treated seeds and 23 cm^2 for those from untreated seeds (**Figure 3(c)**). While root length is 5 to 6.5 cm, for plantlets seed inoculated by D2, D5 and D8 isolates and treated with (Tebuconazole, 60 g/l). Seeds inoculated with AST1 isolate in the absence of fungicide reaches a 3 cm length of roots

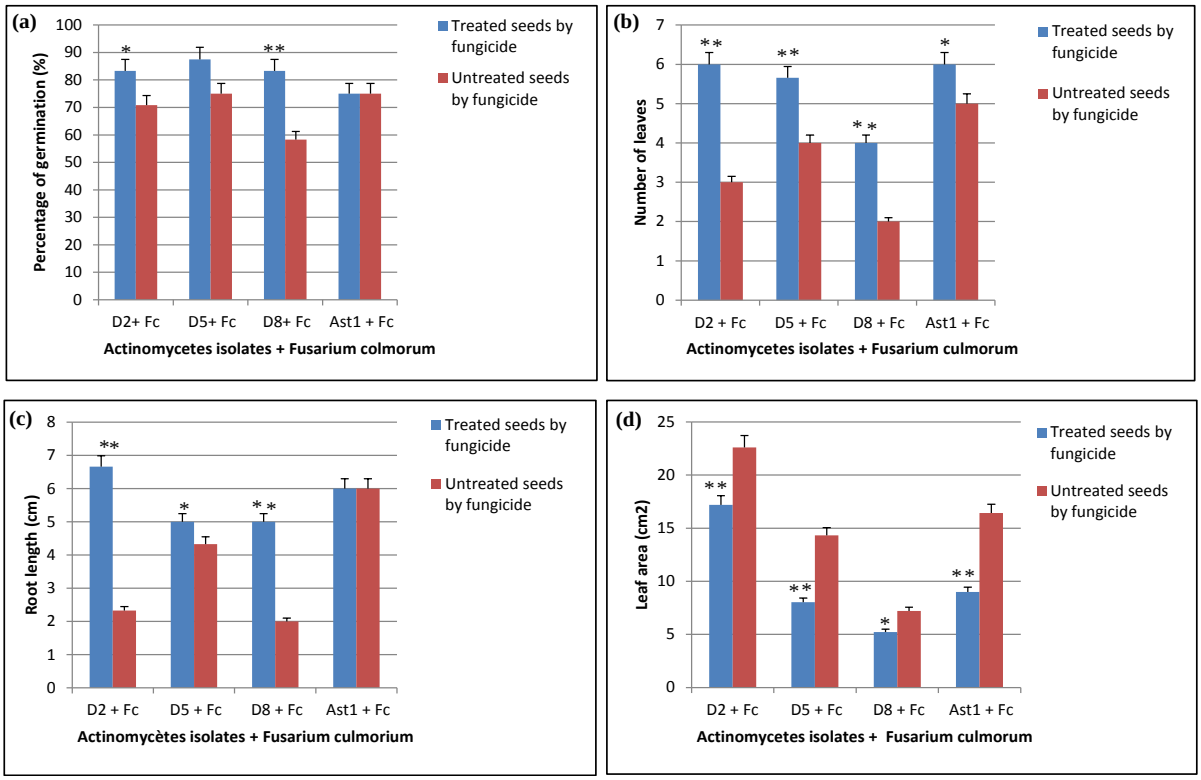


Figure 3. (a) Actinomycetes isolates effects on germination percentage; (b) Leaves number; (c) Roots length and (d) leaf area within *F. colmorum* on treated seeds by fungicide. D2, D5, D8 = *Daucus sahariensis* Murb., isolate number 2, 5 and 8. Ast1 = *Astragalus gombo* Coss. & Dur., isolate number1, Fc = *Fusarium colmorum*). * and ** mean significant and high significant respectively.

(**Figure 3(d)**). The measurements for chlorophyll content of seeds inoculated by D2, D5, D8 and AST1 isolates, shows values SPAD between 0.9 to 1 for seeds treated with (Tebuconazole, 60 g/l) and between 1.1 to 1.2 for untreated seeds by Tebuconazole, 60 g/l (**Figure 4**).

3.3. Biological Control Mechanisms

3.3.1. Biocontrol Agents

Microbial antagonists widely used for the biocontrol of fungal plant diseases. Many species of actinobacteria, particularly those belonging to the genus *Streptomycetes*, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi. Results of this study indicate that seed germination strongly inhibited when inoculated by pathogenic fungi (**Figure 5(a)**). It indicated that (HD) variety seedlings treated with D5, D2, and AST1 isolates have the double character of both stimulation growth and biocontrol agent, (**Figure 5(b)**). It shows that increased index of germination observed from, the wheat seeds indicating the potential of actinomycetes isolates to inhibit pathogenicity and by this means increase germination.

3.3.2. Plant Growth Promoting Hormone Production (PGPHP)

The ability of bacterial strains to produce indole acetic acid **(**IAA) as a plant growth promoting hormone (PGPH) was detected by the development of pink color in ISP2 culture medium after addition of "Salkowski reagent." Among four (4) actinomycetes isolates, two (2) only were able to produce high levels of IAA. Interestingly, D2 and AST1 isolates produced the highest amount of IAA compared to D5 and D8 isolates (**Figure 6**).

4. Discussion

The role of microorganisms in biological control of plants against diseases showed that the use of microorganisms to fight against the enemy culture (bio-pesticides) is a plant protection means respecting the environment.

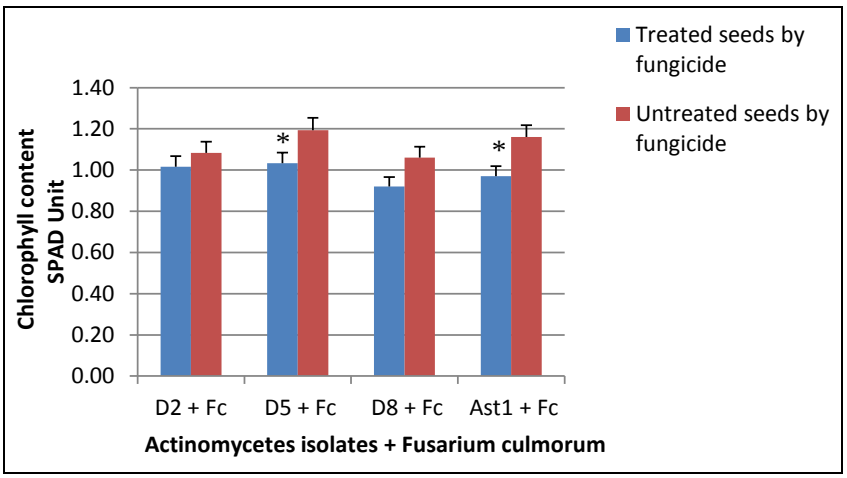


Figure 4. Actinomycetes isolates effects on chlorophyll content within *F. colmorum* on treated seeds by fungicide. * mean significant. D2, D5, D8 = *Daucus sahariensis* Murb., isolate number 2, 5 and 8. Ast1 = *Astragalus gombo* Coss. & Dur., isolate number 1, Fc = *Fusarium colmorum*).

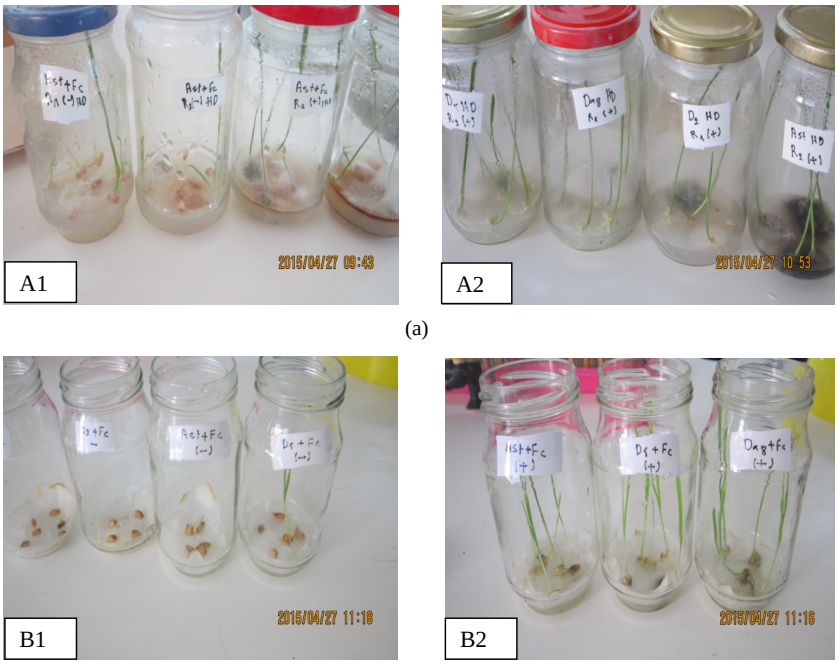


Figure 5. Seeds inoculated with *F. culmorium* untreated (a) and treated (b) by fungicide (Tubeconazole, 60 g/l) in absence of actinomycetes isolates (A1 & B1) and in presence of actinomycetes isolates (A2 & B2).

Yekkour *et al*., [26] applied the biocontrol seedling blight through saharian actinomycetes. Certainly, most parasites, fungi or weeds of the plants have one or more natural microbial enemies, and it will be necessary to know the pathogenic fungi development cycle and antagonist activity conditions. *Streptomycetes* soil produce hydroxamate-type siderophore, could inhibit plant pathogens growth and thus reduce their action by competing for the phenomenon for iron [27]-[29]. Or by stimulation of plant defenses [30]-[33]. Thus, the results obtained in this study on *Fusarium* soft wheat will contribute positively to the improvement of biocontrol process against these diseases. Results analysis of PGPR effects of actinomycetes isolates on growth parameters show that the isolates have a significant effect on the germination rate of the treated and untreated seeds of the same degree by

a fungicide, and then Newman-Keuls test at 5% reached eight homogeneous groups (**Table 2** and **Table 3**). Analysis of variance revealed a very highly significant effect on germination rate for foliage, and significant for leaves number and root length. while the chlorophyll content effect is not significant for all both treated and untreated seeds by fungicide in the presence of actinomycetes isolates, and it is the same for the interaction (fungicide treatment/actinomycetes isolates) (**Table 4**). As against the number of leaves is a differential between treated seeds by a fungicide with a high number of leaves especially for seeds inoculated with D2 and D8 isolates, while for untreated one, leaves number reduced for both isolates compare to AST1isolate. D5 isolate has a high number of leaves for either treated or untreated seeds with fungicide, whereas, this isolate has a beneficial effect on the development of leaf area. Also, analysis results show that D5 isolate has a remarkable influence on the root length. Seeds treatment by actinomycetes isolates from different rhizospheric areas of endemic plants gave important results of PGPR effects, resulting in improved growth parameters studied. However, D5 isolate has a very important effect on growth compared to the fungicide effect. This stimulation resulted mainly from better growth for untreated seeds by fungicide (Tebuconazole, 60 g/l) (**Figure 5(a)**: A1, A2 & **Figure 5(b)**: B1, B2). These results certainly participate in the biocontrol process [34]. Thus, actinomycetes isolates used in this study may be affiliated with (plant growth promoting rhizobacteria (PGPR). Then, it is known that PGPR may also protect plants against phytopathogenic infections. Biocontrol assay of actinomycetes isolates against *F. culmorum* is reflected in germination percentage. That is very important for all seeds inoculated with the four

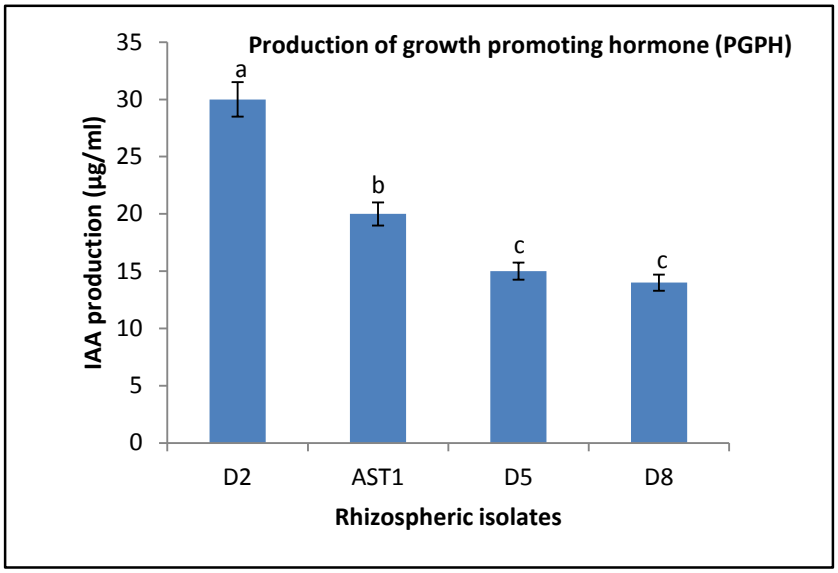


Figure 6. Indole acetic acid (IAA) production by the antagonistic actinomycetes isolates (different lowercase letters a, b and c on top of the histograms indicate significant differences or homogenous groups). D2, D5, D8 = *Daucus sahariensis* Murb., isolate number 2, 5 and 8. Ast1 = *Astragalus gombo* Coss. & Dur., isolate number 1.

Table 2. Measured variables average values of actinomycetes isolates and fungicide used for growth parameters studied.

Growth parameters	Actinomycetes isolated + seeds treated with fungicide			
	D2 + *F.c.*	**D5 + *F.c.***	**D8 + *F.c.***	**AST1 + *F.c.***
Germination (%)	83.33 ± 5.9^{a}	87.5 ± 0^{a}	83.33 ± 5.9^{a}	$75 \pm 0^{a,b}$
Number of leaves	6 ± 0.89^{e}	5.66 ± 0.52^{f}	4 ± 0^{f}	6 ± 0^{e}
Length of roots	6.66 ± 0.52^{e}	5 ± 0^{f}	5 ± 0^{f}	6 ± 0^{e}
Leaf area	17.2 ± 2.5^{d}	8.03 ± 0.85^{e}	5.23 ± 0.23^{f}	9 ± 0.89^{e}
Chlorophyll content	1.02 ± 0.01^{ns}	1.03 ± 0.06^{ns}	0.92 ± 0.04^{ns}	0.97 ± 0.04^{ns}

(D2, D5, D8 = *Daucus sahariensis* Murb., isolate number 2, 5 and 8. Ast1 = *Astragalus gombo* Coss. & Dur., isolate number1, *F.c.* = *Fusarium culmorum*). ns = non significant, lettres (^{a}, ^{b}, ···, ^{f}) = homogenous groups.

Table 3. Measured variables average values of actinomycetes isolates and fungicide used for studied growth parameters.

Growth parameters	Actinomycetes isolated + seeds untreated by fungicide			
	D2 + *F.c.*	**D5 + *F.c.***	**D8 + *F.c.***	**AST1 + *F.c.***
Germination (%)	$70.83 \pm 6.45^{a,b}$	$75 \pm 11.18^{a,b}$	58.33 ± 6.45^{c}	$75 \pm 0^{a,b}$
Number of leaves	3 ± 0^{g}	4 ± 0^{f}	2 ± 0.82^{h}	5 ± 0.82^{f}
Length of roots	2.33 ± 1.03^{h}	4.33 ± 1.03^{f}	2 ± 0^{h}	6 ± 0^{e}
Leaf area	22.6 ± 1.61^{d}	14.33 ± 3.89^{d}	7.2 ± 0.18^{e}	16.43 ± 3.09^{d}
Chlorophyll content	1.08 ± 0.03^{ns}	1.19 ± 0.01^{ns}	1 ± 0.08^{ns}	1.16 ± 0.04^{ns}

D2, D5, D8 = *Daucus sahariensis* Murb., isolate number 2, 5 and 8. Ast1 = *Astragalus gombo* Coss. & Dur., isolate number1, *F.c.* = *Fusarium colmorum*). ns = non significant, lettres (^{a}, ^{b}, ···, ^{h}) = homogenous groups.

Table 4. Parameters signification as a function of actinomycetes inoculation and fungicidal treatment.

	ddl	G %	L N	R L	L A	CC
Act_iso	4	0.001^{***}	0.05^{*}	0.05^{*}	0.01^{**}	0.1ns
F_T	2	0.001^{***}	0.05^{*}	0.05^{*}	0.01^{**}	0.07ns
Act_iso x F_T	8	0.001^{***}	0.05^{*}	0.05^{*}	0.01^{**}	0.1ns

ddl: degree of liberty; ns: no significant ($p > 0.05$); $^{*}\leq 0.05$, $^{**}\leq 0.01$, $^{***}\leq 0.001$: Significant, highly significant and very high significant respectively. G %: Germination percentage, L N: Leaves number, R L: Roots length, L A: Leaf area, CC: Chlorophyll content, Act_iso: Actinomycetes isolates, F_T: fungicide treatment, Act_iso x F_T: Interaction between Actinomycetes isolates x fungicide treatment.

isolates (treated and untreated with fungicide), while, we can confirm that actinomycete isolates had an inhibitory effect against the pathogen, for untreated seeds by (Tebuconazole, 60 g/l) and inoculated into both actinomycetes isolate and *F. culmorium.* They have a high germination rate different leaves number between treated and untreated seeds, which have restricted and highly respectively especially for AST1, D2 and D5 isolates. These results confirm that actinomycetes isolates can reduce *Fusarium* mycelium growth butter than commercial fungicide (Tebuconazole, 60 g/l). Similarly to the parameter of the root length recorded, D2 and AST1 isolates, have a very interesting effectiveness in protecting the root system against *F. culmorum*. The fungicide has a detrimental effect on leaves number increase and root length; while biological control against the pathogen by different actinomycetes isolates especially D5 and D8 is a more best to fight against diseases in bread wheat (HD) variety (**Figure 5(a)**: A1, A2 & **Figure 5(b)**: B1, B2). Production of antifungal substances [35] [36] and phytohormones [35]-[37] by actinomycetes isolates, can increase seed germination and controlling plant pathogenicity [38] [39]. Few or most of these factors attribute an advantage to treated plants. The potential use of actinomycetes isolates as a biocontrol agent has been reviewed previously [38] [39], where inoculation with these microorganisms promoted the growth of plants. Almost all rhizospheric actinomycetes isolate enhanced the agronomic performance of wheat cultivar, Hidhab (HD) by influencing its growth parameters. Khaleeq and Khan [40] reported that actinomycetes isolate significantly improved the use of fungicides which is effective in enhancing germination, emergence and growth as well as in reducing damping-off. Also, accelerated germination is reported to help improve stress resistance and enhance overall plant growth and productivity [41]-[43].

Results obtained for IAA production by actinomycetes isolates showing a positive reaction. It affects plant cell division, extension, and differentiation. Stimulate seeds and tuber germination increase the rate of xylem and root development. Controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light and gravity; affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions [37] [44]. IAA production has a very vital role in the plant growth promotion potential of theses isolates. It should note that one of the possible antifungal mechanisms of D5, D2, and AST1 isolate, may be associated with the production of an antifungal agent, and IAA. Actinomycetes have been reported as biocontrol agents effective against numerous plant pathogens [8] [35] [45]-[48]. So, actinomycetes isolates can also play a role in plant development, especially in root development (**Figure 6**). Growth promoting effects may be related to IAA [18] [49]-[51], and Siderophores production or other antifungal sub-

stances [52]-[54]. The activity against fungal plant pathogens of Actinomycetes attributed by the production of mycolytic enzymes.

5. Conclusion

Antagonistic activities of four actinomycetes isolate from rhizospheric soil of three (3) endemic plants in Algeria, against *F. culmorum* which is a causal agent of Fusarium disease and also biological control mechanisms for (*T. aestivum* L.) variety (HD) seeds, treated and untreated with commercial fungicide (Tebuconazole 60 g/l). The test of PGPR/biocontrol had made a comparison between fungicide action and antagonist's actinomycetes suspensions effects. PGPR effect, in addition to the fungicide, is less effective, and it appeared with the comparison of wheat seeds inoculated only with D5, D8, D2, AST1 isolates suspension that has better results in terms of growth parameters, like germination rate, leaf number, roots length, leaf area and chlorophyll content. Similarly for the bio-control test, the D5 isolate has a strong action against *Fusarium* suspension, but the action of fungicide is very interesting against this pathogen. Depending on the results, we can confirm that the fungicide has an inhibitory effect against *F. culmorum*, but also has a harmful effect on growth parameters. The isolated Actinomycetes play a role in both, protection against pathogenic agents and in improved growth parameters.

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