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Evaluation of Bio-Friendly Formulations from Siderophore-Producing Fluorescent *Pseudomonas* as Biocontrol Agents for the Management of Soil-Borne Fungi, *Fusarium oxysporum* and *Rhizoctonia solani*

Gaber Attia Abo-Zaid [1,*], Ahmed Salah Abdullah [1], Nadia Abdel-Mohsen Soliman [1], Ebaa Ebrahim El-Sharouny [2], Abdulaziz A. Al-Askar [3], Yiming Su [4], Ahmed Abdelkhalek [5,*] and Soraya Abdel-Fattah Sabry [2]

1 Bioprocess Development Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City, Alexandria, Alex 21934, Egypt; abosalahelnazzer@gmail.com (A.S.A.); nsoliman@srtacity.sci.eg (N.A.-M.S.)
2 Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Alex 21526, Egypt; ebaaloulou@yahoo.com (E.E.E.-S.); sabrysoraya@gmail.com (S.A.-F.S.)
3 Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; aalaskara@ksu.edu.sa
4 Utah Water Research Laboratory, Department of Civil and Environmental Engineering, Utah State University, Logan, UT 84341, USA; yiming.su@usu.edu
5 Plant Protection and Biomolecular Diagnosis Department, ALCRI, City of Scientific Research and Technological Applications, New Borg El Arab City, Alexandria 21934, Egypt
* Correspondence: gabozaid@srtacity.sci.eg (G.A.A.-Z.); aabdelkhalek@srtacity.sci.eg (A.A.); Tel.: +20-10-0755-6883 (A.A.)

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Abstract: Secretion of siderophores by *Pseudomonas aeruginosa* F2 and *P. fluorescens* JY3 was evaluated on chrome azurol S (CAS) agar plates and their inhibitory effect was inspected against *Fusarium oxysporum* and *Rhizoctonia solani*. Production of siderophores as biocontrol agents from F2 and JY3 was accomplished in two optimized media. Afterward, cell-free supernatants of the bacterial cultures containing siderophores were used for the preparation of two bio-friendly formulations for the management of *F. oxysporum* and *R. solani* under greenhouse conditions. The investigated bacterial isolates, F2 and JY3, showed antagonistic activity in vitro against *F. oxysporum* and *R. solani* and produced siderophores in optimized media with high efficiency. Colonies of both bacterial isolates were grown exponentially with a constant specific growth rate of 0.07 h^{-1} and 0.27 h^{-1}, correspondingly. Siderophores estimated in 10 µL reached their highest value of 16.95% at 47 h and 19.5% at 48 h for isolate F2 and JY3, respectively. Formulations of siderophore-generating F2 and JY3 reduced damping-off caused by *F. oxysporum* by 40% and 80%, while the reduction percentage of damping-off caused by *R. solani* reached 87.5% and 62.5%, correspondingly. Moreover, both formulations encouraged the growing of wheat plants where the fresh and dry weight of shoots and roots were increased compared to the treatment with each fungus. In conclusion, bio-friendly formulations resulting from this investigation can play an active role in managing soil-borne diseases.

Keywords: siderophores; biocontrol; *Pseudomonas aeruginosa*; *Pseudomonas fluorescens*; bioformulation

1. Introduction

The yield and quality of food crops are primarily reduced by soil-borne fungi, including *Fusarium oxysporum* and *Rhizoctonia solani*. Control of these pathogens is very difficult because they can stay alive in the soil for many years [1]. The accumulation of pesticides used in the management of plant diseases, in addition to industrial pollution reaching agricultural land [2], encouraged scientists to search for environmentally friendly

alternative approaches for controlling plant pathogens [3,4]. Biocontrol is a viable alternative strategy to conventional pesticide usage in the management of plant pathogens, as it offers the potential to mitigate their detrimental impacts without posing risks to human health or the environment [5,6]. *Pseudomonas* is a genus of bacteria, which is utilized as a biocontrol agent. *Pseudomonas* spp. have demonstrated antagonistic properties against various phytopathogens due to their ability to synthesize a range of compounds, such as phenazines, hydrogen cyanide, hydrolytic enzymes, siderophores, and others [7].

Production of siderophores by *Pseudomonas* spp. restricts the availability of iron to potential plant pathogens, thereby impeding their proliferation. Additionally, this process may confer competitive benefits that promote plant growth and development [8]. Production of siderophores by fluorescent pseudomonads is one of their primary mechanisms, which is elaborated in the biocontrol of different plant diseases. Siderophores are defined as specific chelating agents for ferric ions, which are produced by various microorganisms under low iron conditions that have low molecular weights below 1000 Da [9]. Siderophores are secreted by several species of fluorescent pseudomonads, for instance, *P. aeruginosa*, *P. fluorescens,* and *P. syringae* [10,11]. Various plant pathogens are suppressed by siderophore-producing biocontrol agents.

The effect of fluorescent pseudomonads on the production of siderophores is affected by many things, such as the concentration of iron [10], the type and concentration of carbon and nitrogen sources [12], levels of phosphate [13], pH and light [14], degree of aeration [15], temperature [16], and the occurrence of trace elements like magnesium [17], zinc [18], and molybdenum [15]. Various types of soils have been found to contain siderophores, as reported by Powell et al. in 1980. The utilization of microbial siderophores has been observed to augment the absorption of iron by plants. This is attributed to the plant's ability to identify the bacterial ferric–siderophore complex, as reported previously [19–21]. Furthermore, the significance of microbial siderophores in facilitating iron uptake by plants in the presence of metals like nickel and cadmium has been established in research conducted by Burd et al. [22] and Dimkpa et al. [23]. The extent to which bacterial siderophore complexes can fulfill the iron demands of plants is yet to be conclusively determined. The production of siderophores provides a competitive edge to the growth of plants by facilitating the roots colonization by plant growth promoting rhizobacteria (PGPR) and preventing the establishment of other microorganisms in this ecological niche [24].

Siderophores like pyoverdine and pyochelin are produced by PGRB, which may be a mechanism for disease suppression [10]. Pathogens like *F. oxysporum* and *Pythium ultimum*, which are responsible for wilt and root rot disease in crops, need iron for growing, but the siderophores trap Fe near the roots, reducing the available supply [25]. Many scientists believe that pseudomonads capable of producing siderophores could be utilized as biocontrol agents to halt the spread of soil-borne plant diseases. Some examples of these strains are *P. putida* WCS strains [7], *P. fluorescens* CHA0 [26], and *P. syringae* pv. *syringae* strain 22 d/93 [27]. The incidence of wilt diseases in cucumber, radish, and flax, which are caused by *Fusarium* ssp., was effectively mitigated through the application of siderophore-producing *P. putida* as a PGBR [28]. The primary objective of the present study was to examine the synthesis of siderophores by fluorescent pseudomonads. Furthermore, the biocontrol efficacy of fluorescent pseudomonads that produce siderophores was evaluated in a greenhouse setting to mitigate the damping-off incidence attributed to *F. oxysporum* and *R. solani*.

2. Materials and Methods

2.1. Source of Bacterial and Fungal Isolates

The strains utilized in this study were generously supplied by Dr. Gaber Abo-Zaid, namely *P. aeruginosa* F2 (MG210480), which was isolated from the rhizosphere of eggplant (El-Minoufiya governorate) and *P. fluorescens* JY3 (KF922490), which was isolated from the rhizosphere of cotton (El-Mansoura governorate). The soil-borne fungi, specifically *F. oxysporum* and *R. solani*, which were isolated from wheat plants, were provided by the

Plant Pathology Institute at the Agricultural Research Center, Egypt. Following this, an assessment was conducted to determine their pathogenic capabilities.

2.2. Chrome Azurol S (CAS) Agar Plates for Detection of Siderophores Production

CAS agar plates were used to detect siderophore production by *P. aeruginosa* F2 and *P. fluorescens* JY3 [29]. The indicator of siderophores' existence is the conversion of the greenish-blue color of the medium to orange. Spot inoculation of *Pseudomonas* isolates was achieved on the CAS agar plates, incubated for 24 h at 30 °C, and their ability to produce siderophores was demonstrated.

2.3. Antagonistic Activity of P. fluorescens JY3 and P. aeruginosa F2 (In Vitro)

The antagonistic potential of *P. fluorescens* JY3 and *P. aeruginosa* F2 against *R. solani* and *F. oxysporum* was examined per the methodology outlined by Touré et al. [30]. On potato dextrose agar plates, a line of antagonistic isolates was streaked using a loopful of a culture that was 2 days old. The plates were subsequently incubated for 48 h before inoculation with any of the tested fungi. A mycelial disc measuring 5 mm in diameter, derived from a highly active culture of the patterned fungus, was positioned in the central region of the petri plate at a standardized distance from the plate's edge. The plate was then incubated at a temperature of 30 °C for a period ranging from 3 to 7 days. The measurement of the inhibition diameter was conducted to determine the spatial separation between the boundary of antagonistic bacterial progression and the boundary of fungal growth.

2.4. Batch Fermentation in Shake Flask

A solitary colony of *P. aeruginosa* F2 and *P. fluorescens* JY3 was introduced into Luria-Bertani (LB) medium and subjected to overnight incubation at 30 °C with agitation at 200 rpm. A batch fermentation process was conducted in a shake flask utilizing two strains. The inoculum size was maintained at a constant optical density (OD_{600} nm) of 0.3, achieved through inoculation from the pre-culture of LB broth of each strain. The cultivation of *P. aeruginosa* F2 was carried out on a medium optimized for the production of siderophores. The medium was prepared by combining 14.5 g of glucose, 20 mL of glycerol, 4 g of sodium succinate, 1 g of glutamic acid, 0.1 g of urea, 5 g of asparagine, 1 g of $(NH_4)_2SO_4$, 3.5 g of K_2HPO_4, 1 g of MgSO4, 0.5 µM of $FeCl_3$, 1 g of kH_2PO_4, and adding distilled water to reach a final volume of 1000 mL. The pH of the medium was modified to a value of 7. The process of cultivating *P. fluorescens* JY3 was conducted utilizing a medium that had been optimized specifically to enhance siderophore production. The medium was prepared by combining 10 mL of glycerol, 0.5 g of glutamic acid, 1 g of glucose, 3.14 g of sodium succinate, 1 g of asparagine0.1 g of urea, 0.1 g of $(NH_4)_2SO_4$, 6 g of KH_2PO_4, 4 g of K_2HPO_4, 0.1 g of $MgSO_4$, 0.62 µM of $FeCl_3$, and sufficient dH_2O to reach a final volume of 1000 mL. The resulting medium had a pH of 7 [31]. Subsequently, cultures of both strains were incubated at 25 °C while being agitated at a rate of 200 rpm for 48 h. Samples were collected periodically to determine the levels of siderophores, glycerol, glucose, and biomass. Additionally, the specific growth rate, denoted µ, was calculated from the relationship between the natural logarithm of biomass and time in the exponential phase.

2.5. Analytical Procedures

2.5.1. Siderophores Estimation

The quantification of siderophores was assessed utilizing the CAS assay technique as outlined by Schwyn and Neilands [29]. Following this, 10 µL of the culture's supernatant was added to a 0.5 mL CAS test solution and thoroughly stirred. After completely combining, 10 µL of shuttle solution was added, and the resulting mixture was allowed to sit undisturbed at room temperature for a few minutes. The absence of the blue hue is correlated with the existence of siderophores. The measurement of absorbance was conducted at a wavelength of 630 nm, with the media serving as the blank. Siderophore levels are determined by the formula: $\% = [(Ar - As)/Ar] \times 100$. In this formula, Ar

denotes the absorption of the CAS solution in combination with the media and shuttle solution, while As represents the absorption of the CAS solution in combination with the culture supernatant and shuttle solution.

2.5.2. Biomass Estimation

To determine the dry cell weight, a centrifugation process was conducted on a 10 mL sample at a force of 894× *g* for 10 min. The pellet underwent a process of resuspension, washing, and subsequent centrifugation. The pellets were subsequently subjected to an overnight drying process in a dry air oven operating at a temperature of 80 °C. Finally, the pellets were weighted to estimate biomass [32].

2.5.3. Glucose Estimation

An enzymatic colorimetric kit manufactured by Diamond Diagnostics in Egypt was utilized in order to illustrate the glucose levels.

2.5.4. Glycerol Estimation

The level of glycerol was determined using a technique devised by Bok and Demain [33], and the results were calculated.

2.6. Pseudomonas Isolates Producing Siderophores as Biocontrol Agents

2.6.1. Formulation Experiment

Bio-friendly formulations were generated utilizing the cell-free supernatant of *P. aeruginosa* F2 and *P. fluorescens* JY3, both of which are capable of producing siderophores. The utilization of talc powder (TP) was employed as a means of carrying the substance. A quantity of 10 g of carboxymethylcellulose (CMC) was introduced into 400 milliliters of cell-free supernatant to utilize CMC as an adhesive agent. The homogenization of the mixture was achieved by utilizing a vortex mixer. To achieve a pH of 7.0, 15 g of calcium carbonate was incorporated into 1 kg of sterilized TP and thoroughly blended. One kilogram of TP was mixed with 400 mL of cell-free supernatant containing an additive. The formulations underwent shade drying to reduce the humidity level to below 20%. Subsequently, they were placed into polythene bags that had been sterilized with UV light, sealed, and stowed at a temperature of 4 °C until further use, as described in reference [4].

2.6.2. Preparation of Fungal Inoculum

The cultivation of *F. oxysporum* and *R. solani* inoculum was carried out using a medium composed of sorghum, coarse sand, and water in a volumetric proportion of 2:1:2. Upon the completion of the assembly of the components, they were subsequently packaged and subjected to a sterilization process lasting two hours, rendering them suitable for utilization. To inoculate the sterile media, agar discs were collected from the outermost part of a culture that were five days old for each of the fungi being investigated. According to earlier reports, fungal inoculum was obtained for soil infestation after an incubation period of two weeks at a temperature of 30 °C [34].

2.6.3. Soil Infestation

The study entailed the introduction of *F. oxysporum* and *R. solani* inoculum onto the soil surface of separate pots at a concentration of 2% *w*/*w*. A layer of sterilized soil was applied to cover the fungal inoculum. The pots that were impacted by pathogens were irrigated and subsequently left undisturbed for 7 days before being planted.

2.6.4. Greenhouse Experiment

Pots measuring 18 cm in diameter were filled with sterilized soil and subsequently exposed to infestation, following the previously outlined procedure. A total of nine treatments were implemented subsequently. The present study investigated the impact of different formulations on the enhancement of *F. oxysporum* and *R. solani*, alongside a con-

trol group comprising unaffected organisms. The formulations that were examined in this study consisted of the supernatant formulations of *P. aeruginosa* F2 and *P. fluorescens* JY3. Additionally, combinations of these formulations with *F. oxysporum* or *R. solani* were also tested. Each pot was planted with a total of five wheat seeds, and three replicates (pots) were employed for each treatment. The wheat seeds were subjected to seed drench formulations at a rate of 10 g per kilogram of seeds. The utilization of formulations was conducted at a frequency of 3 kg per feddan on two separate instances, specifically at 15 and 30 days following the initiation of seed planting, utilizing the technique of soil drenching. The evaluation of disease incidence was determined by recording the proportion of damping-off, occurring both before and after seedling emergence, at 7 and 21 days following the initiation of planting. This was cited in the reference [4]. The CoStat software was employed to perform data analysis. Analysis of Variance (ANOVA) was used as a statistical method for analyzing the information gathered. The statistical significance of changes between treatments was determined using the Least Significant changes (LSD) method at the $p \leq 0.05$ level.

3. Results

3.1. Detection of Siderophore Production on CAS Agar Plates

The ability of *P. aeruginosa* JY3 and *P. fluorescens* F2 isolates to produce siderophores was confirmed through the utilization of CAS agar plates. A region displaying a color gradient from yellow to orange was detected near the colonies of F2 and JY3 that were not exposed to contamination, suggesting the presence of siderophore production (Figure 1A).

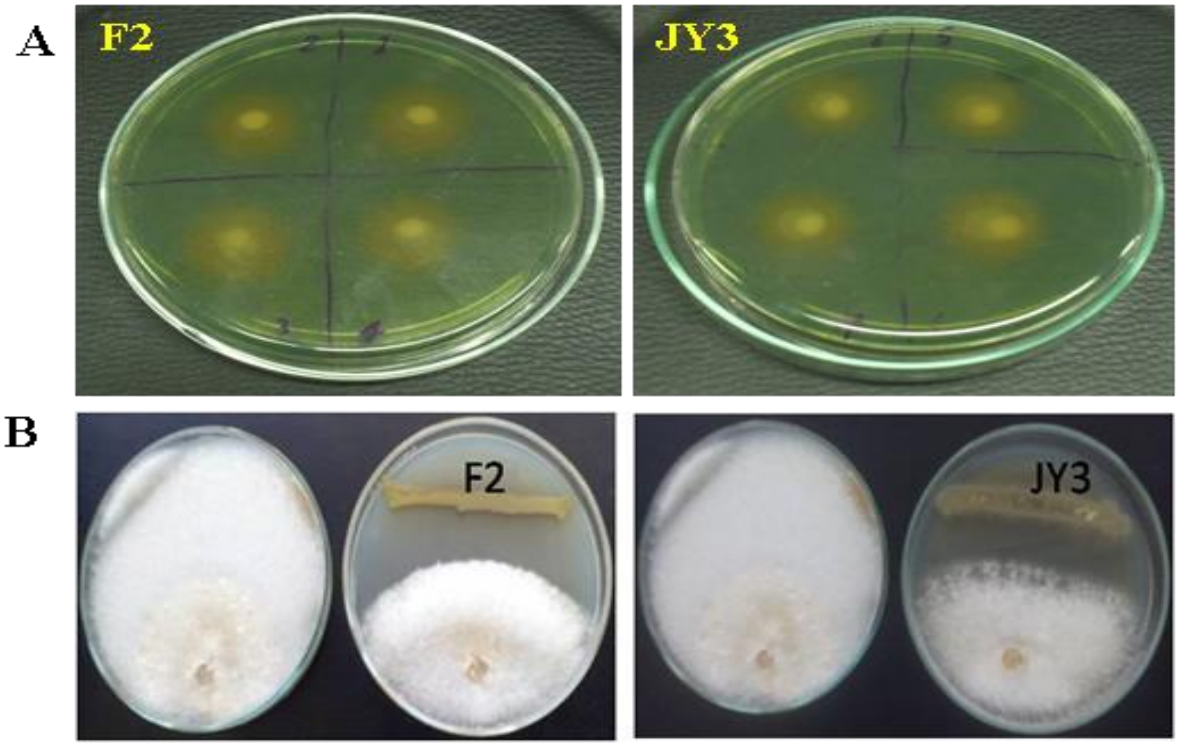

Figure 1. (**A**) Displays the qualitative evaluation of siderophore synthesis on chrome azurol S (CAS) agar plates. The assessment was conducted using spot cultures of *P. aeruginosa* F2 and *P. fluorescens* JY3, with four spots for each isolate; (**B**) illustrates the antagonistic effect of *P. aeruginosa* F2 and *P. fluorescens* JY3 against *F. oxysporum*.

3.2. Antagonistic Activity of P. fluorescens JY3 and P. aeruginosa F2

The results indicate that *Pseudomonas* spp., F2, and JY3 exhibited great activity in suppressing the mycelial growth of *F. oxysporum* and *R. solani* (as depicted in Figure 1B). Reduction of the fungal growth expressed as an inhibition zone or diameter. F2 and JY3

isolates achieved inhibition diameter against *F. oxysporum* equal to 1.7 cm and 1.23 cm, respectively. On the other hand, the inhibition diameter in the case of *R. solani* reached 2.23 cm and 1.07 cm, respectively.

3.3. Batch Fermentation in a Shake Flask

The results depicted in Figure 2 indicate that the cellular mass of *P. aeruginosa* F2 underwent a gradual increase during a lag phase that persisted for approximately 5 h, before entering the logarithmic phase (also known as the exponential phase). During the logarithmic phase, the biomass exhibited a rapid increase with a consistent specific growth rate, denoted as μ, which was calculated to be 0.07 h^{-1} (as illustrated in Figure 3A). At this stage, the yield coefficient $Y_{X/S}$ was determined to be 0.78 g cells per g glycerol, as illustrated in Figure 3B. The concentration of siderophores exhibited an initial value of 9.55% at 39 h, followed by a rapid increase to attain 11.31% at 43 h, and ultimately reached its peak value of 16.95% at 47 h. The maximum biomass recorded during this period was 5.4 g L^{-1}. The concentration of glycerol exhibited a gradual decline, however, it was not entirely depleted, as evidenced by the recorded value of 9.21 g L^{-1} at 48 h. Conversely, the concentration of glucose exhibited a swift decline and was entirely depleted within 48 h. The data presented in Figure 4 indicates that the estimation of siderophore production for *P. fluorescens* JY3 reached 1.95% at 32 h and exhibited a rapid increase, reaching 9.2% after 42 h. The maximum value of siderophore production was observed to be 19.5% at 48 h. At 48 h, the biomass attained a peak level of 4.8 g per liter. The concentration of glycerol exhibited a gradual decrease and ultimately attained a value of 0.65 g L^{-1} after 48 h. The cell mass of *P. fluorescens* JY3 exhibited an exponential increase concerning a specific growth rate, μ, which was calculated to be 0.27 h^{-1} (as depicted in Figure 5A). The yield coefficient $Y_{X/S}$ was determined to be 0.59 g cells per g glycerol, as illustrated in Figure 5B.

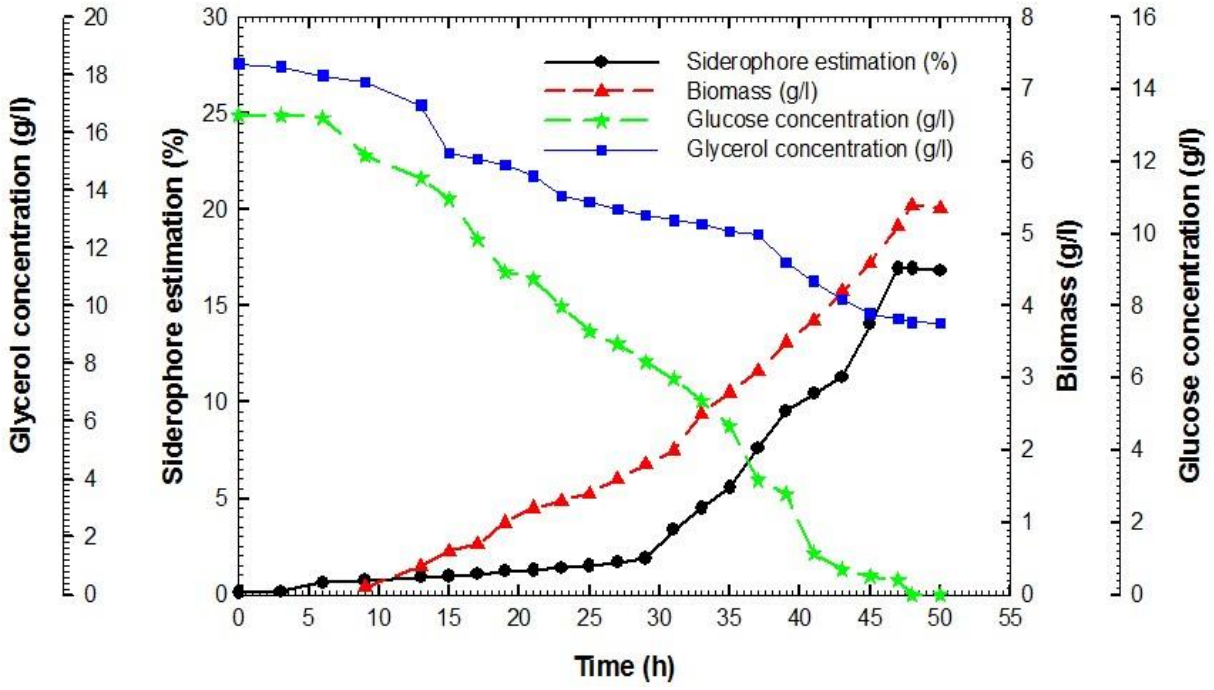

Figure 2. The amount of siderophores, biomass, glucose, and glycerol in the broth of a batch fermentation culture of *Pseudomonas aeruginosa* F2 in a shaking jar as a function of time.

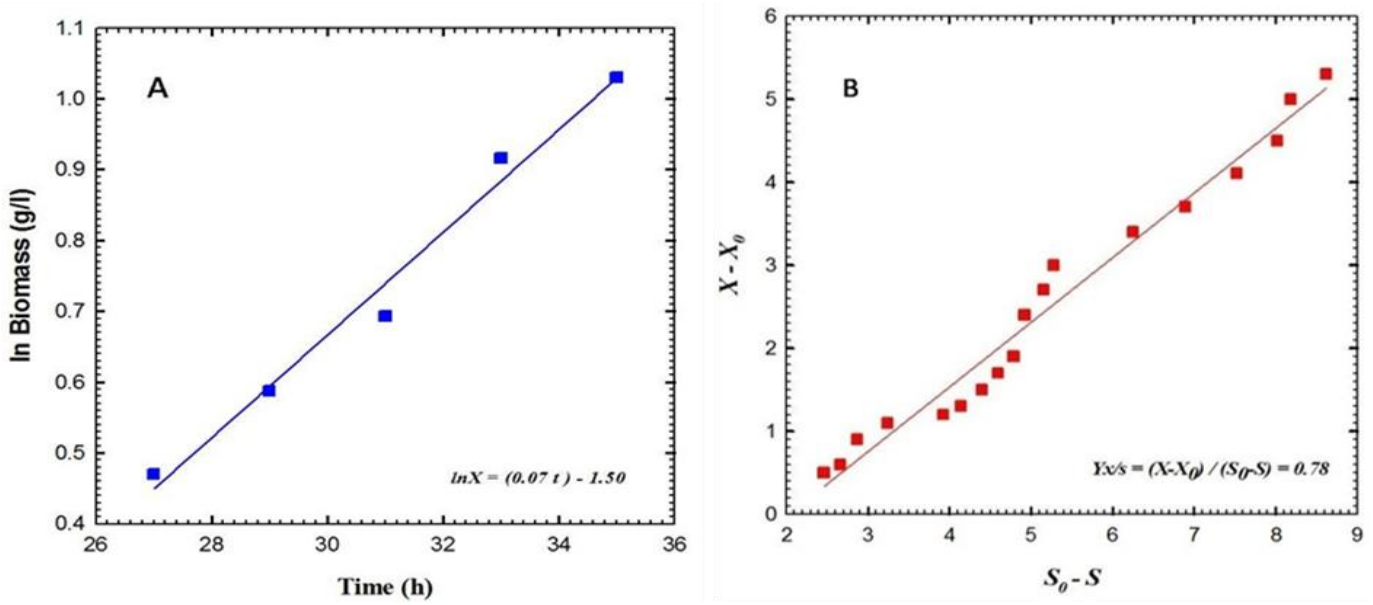

Figure 3. (**A**) Shows the logarithm of the biomass (X) in g L^{-1} as a function of time during the exponential phase of *Pseudomonas aeruginosa* F2's batch fermentation cultivation broth in a shaking flask, and (**B**) shows the yield coefficient on glycerol during this phase.

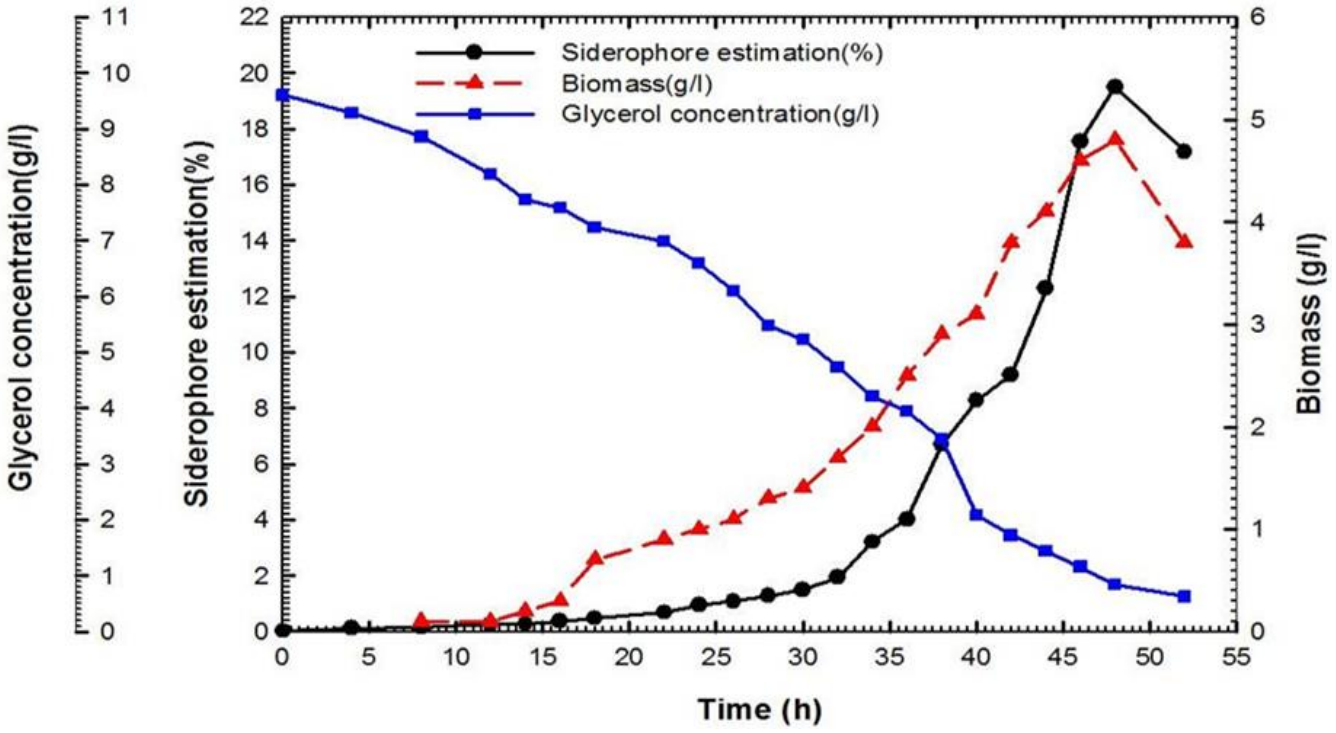

Figure 4. The amount of siderophores, the amount of biomass, and the amount of glycerol in the cultivation broth of *Pseudomonas fluorescens* JY3 as a function of time in a shake jar.

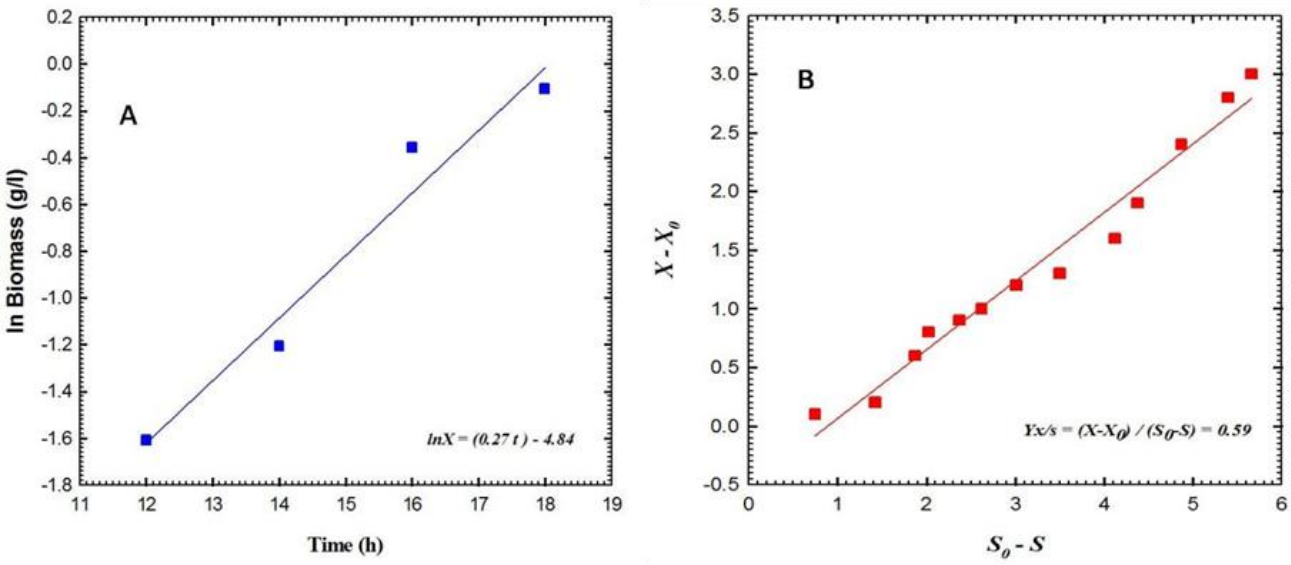

Figure 5. (**A**) Shows the logarithm of the biomass (X) in g L^{-1} as a function of time during the exponential phase of batch fermentation culturing broth of *Pseudomonas fluorescens* JY3 in a shaking flask, and (**B**) shows the yield coefficient on glycerol during this phase.

3.4. Efficacy Evaluation of F2 and JY3 Formulations under Greenhouse Conditions

The efficacy of formulations F2 and JY3, were evaluated against plant pathogens causing damping-off. Results indicated that both formulations were operative in dropping damping-off triggered by *R. solani* and *F. oxysporum* as compared to the control group.

The prevalence of damping-off disease caused by *R. solani* and *F. oxysporum* was documented as 53.3% and 33.3%, respectively. The utilization of F2 and JY3 formulations in soil that was infested with *F. oxysporum* led to a noteworthy decline in the occurrence of damping-off disease, as evidenced by the reduction of values to 20% and 6.67%, respectively. Reduction percentages of 40% and 80% were observed, respectively. The results of the study suggest that using *P. aeruginosa* F2 in soil infested with *R. solani* led to a bigger drop in damping-off disease than using *P. fluorescens* JY3. Comparing *P. aeruginosa* F2 and *P. fluorescens* JY3, damping-off disease was found to happen 6.67% of the time with *P. aeruginosa* F2 and 20% of the time with *P. fluorescens* JY3. According to the data presented in Table 1 and Figure 6, the reduction percentages for F2 and JY3 were found to be 87.50% and 62.50%, respectively.

Both formulations displayed growth-enhancing characteristics on wheat plants that were cultured in soil that was infested with *F. oxysporum* and *R. solani*. This was in contrast to the remaining control treatments, in which each fungus was injected separately. When F2 and JY3 formulations were added to soil with phytopathogenic fungi, the fresh and dry biomass of both above-ground and below-ground plant structures increased significantly. The utilization of the F2 formulation on soil infested with *F. oxysporum* led to a noteworthy augmentation in the mass of newly harvested shoots and roots of wheat flora. The shoot and root fresh weights exhibited a respective increase of 44.50% and 49.39%, ultimately attaining values of 3.73 g and 3.3 g. The utilization of the JY3 formulation resulted in a notable augmentation in the fresh weight of both shoots and roots, exhibiting a respective rise of 45.53% and 51.87%. The shoots and roots achieved fresh weights of 3.80 g and 3.47 g, respectively. On the contrary, the solitary application of the *F. oxysporum* treatment yielded markedly reduced fresh weights of both shoots and roots, measuring at 2.07 g and 1.67 g, respectively. Table 2 presents the aforementioned findings. The study recorded the fresh weight of shoots in treatments that employed formulated F2 and JY3 in soil infested with *R. solani*. The measurements obtained were 3.53 g and 3.23 g, respectively. The observed percentage increase of 72.52% and 69.97% in fresh weight can be attributed to the current treatment, as compared to the treatment involving the sole application of the fungus, which

resulted in a fresh weight of 0.97 g. The application of the aforementioned formulations yielded fresh root weights of 3.20 g and 2.87 g, respectively, representing a percentage increase of 81.25% and 79.09%, respectively, in comparison to the fungus-only treatment, which resulted in a fresh weight of 0.60 g. Table 2 displays the aforementioned results.

Table 1. Effect of *Pseudomonas fluorescens* JY3 and *Pseudomonas aeruginosa* F2 supernatant mixtures on *F. oxysporum* and *Rhizoctonia solani*-caused damping-off of wheat plants.

Treatment	Damping-Off (%)	** *F. oxysporum* Reduction (%)	** *R. solani* Reduction (%)
F. oxysporum (Check)	33.33 * ^{ab}	0	___
R. solani (Check)	53.33 ^{a}	___	0
Control	20 ^{bc}	40	62.50
F2 formulation	0 ^{c}	100	100
JY3 formulation	13.33 ^{bc}	60.01	75
F2 + *F. oxysporum*	20 ^{bc}	40	___
JY3 + *F. oxysporum*	6.67 ^{bc}	80	___
F2 + *R. solani*	6.67 ^{bc}	___	87.50
JY3 + *R. solani*	20 ^{bc}	___	62.50
L.S.D	29.53		

* At the $p \leq 0.05$ level, the means in each column that are followed by the same letter do not vary much from each other. ** Reduction = [check-treatment]/check × 100.

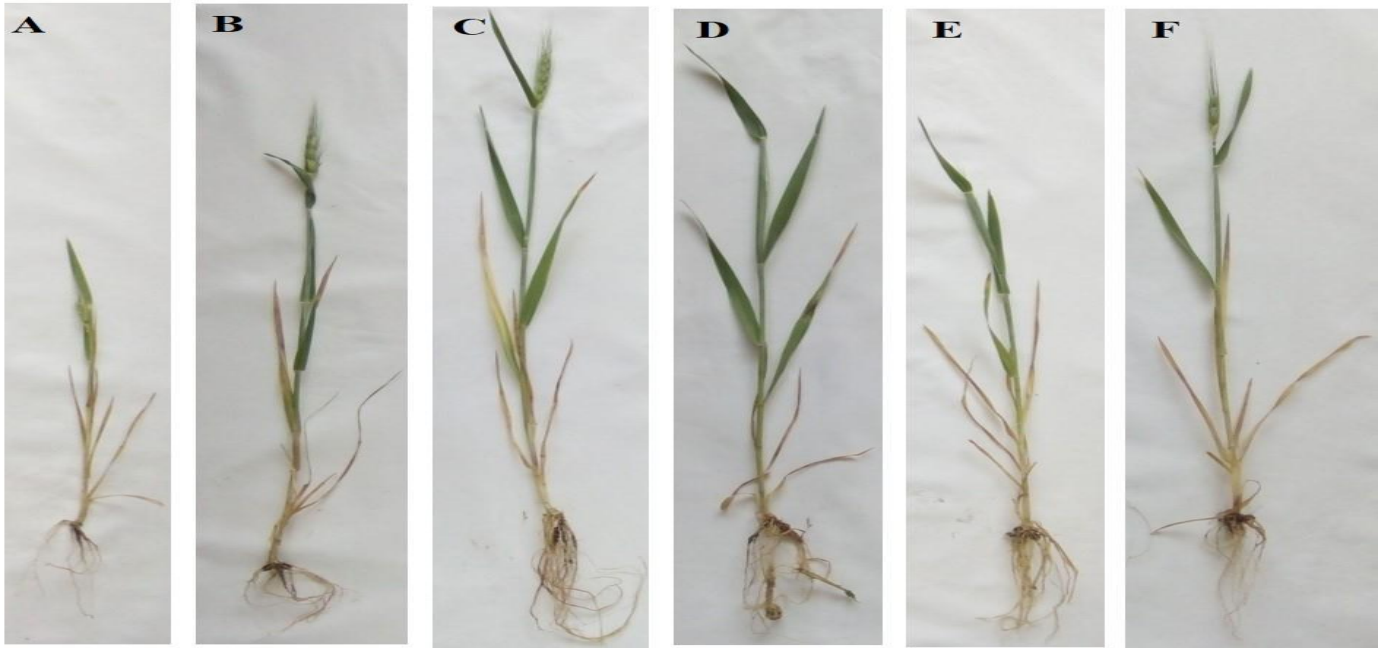

Figure 6. Effects of supernatant formulations of *Pseudomonas fluorescens* JY3 and *Pseudomonas aeruginosa* F2 on wheat plants as biocontrol agents for controlling *Fusarium oxysporum*, from the left, (**A**), *Fusarium oxysporum*; (**B**), Control; (**C**), F2 formulation; (**D**), JY3 formulation; (**E**), F2 formulation + *Fusarium oxysporum*, and (**F**), JY3 formulation + *Fusarium oxysporum*.

Table 2. Effect of *Pseudomonas fluorescens* JY3 and *Pseudomonas aeruginosa* F2 supernatant formulations on fresh weight of wheat stems and roots.

Treatment	Fresh Weight of Shoots (g)	** Increase (%)	** Increase (%)	Fresh Weight of Roots (g)	** Increase (%)	** Increase (%)
F. oxysporum (Check)	2.07 * ^{c}	0	___	1.67 ^{c}	0	___
R. solani (Check)	0.97 ^{d}	___	0	0.60 ^{d}	___	0
Control	3.63 ^{ab}	42.98	73.28	3.23 ^{ab}	48.30	81.42
F2 formulation	4.07 ^{a}	49.14	76.17	3.71 ^{a}	54.99	83.83
JY3 formulation	3.83 ^{ab}	45.95	74.47	3.5 ^{ab}	52.29	82.86
F2 + *F. oxysporum*	3.73 ^{ab}	44.50	___	3.30 ^{ab}	49.39	___
JY3 + *F. oxysporum*	3.80 ^{ab}	45.53	___	3.47 ^{ab}	51.87	___
F2 + *R. solani*	3.53 ^{ab}	___	72.52	3.20 ^{ab}	___	81.25
JY3 + *R. solani*	3.23 ^{b}	___	69.97	2.87 ^{b}	___	79.09
L.S.D	0.65			0.67		

* At the $p \leq 0.05$ level, the means that are followed by the same letter in each column are not very different from each other. ** Increase % = [treatment − check]/treatment × 100.

The results of this study demonstrate that the application of F2 and JY3 formulations in soil infested with *F. oxysporum* and *R. solani* significantly enhanced the biomass of plant shoots. The experimental findings indicated that the application of the aforementioned formulations led to shoot dry weights of 2.73 g, 2.83 g, 2.50 g, and 2.33 g, accompanied by percentage increases of 62.27%, 63.60%, 86.80%, and 85.84%, respectively. On the other hand, the application of *F. oxysporum* and *R. solani* treatments yielded shoot dry weights of merely 1.03 g and 0.33 g, respectively. In contrast, the dry weight of the roots exhibited values of 2.37 g, 2.33 g, 2.03 g, and 1.83 g, indicating a percentage increase of 56.54%, 55.79%, 91.13%, and 90.16%, respectively, in comparison to treatments that employed *F. oxysporum* and *R. solani* in isolation, which registered values of 1.03 g and 0.18 g, respectively (refer to Table 3).

Table 3. The effect of *Pseudomonas fluorescens* JY3 and *Pseudomonas aeruginosa* F2 supernatant formulations on the dry weight of wheat shoots and roots.

Treatment	Dry Weight of Shoots (g)	** Increase (%)	** Increase (%)	Dry Weight of Roots (g)	** Increase (%)	** Increase (%)
F. oxysporum (Check)	1.03 * ^{d}	0	___	1.03 ^{c}	0	___
R. solani (Check)	0.33 ^{e}	___	0	0.18 ^{d}	___	0
Control	2.67 ^{abc}	61.42	87.64	2.1 ^{b}	50.95	91.43
F2 formulation	3.17 ^{a}	67.51	89.59	2.71 ^{a}	61.99	93.36
JY3 formulation	2.93 ^{ab}	64.85	88.74	2.37 ^{ab}	56.54	92.41
F2 + *F. oxysporum*	2.73 ^{abc}	62.27	___	2.37 ^{ab}	56.54	___
JY3 + *F. oxysporum*	2.83 ^{abc}	63.60	___	2.33 ^{ab}	55.79	___
F2 + *R. solani*	2.5 ^{bc}	___	86.80	2.03 ^{b}	___	91.13
JY3 + *R. solani*	2.33 ^{c}	___	85.84	1.83 ^{b}	___	90.16
L.S.D	0.53			0.57		

* At the $p \leq 0.05$ level, the means that are followed by the same letter in each column are not very different from each other. ** Increase % = [treatment − check]/treatment × 100.

4. Discussion

The primary objective of the current investigation was to examine the in vitro antagonistic activity of two *Pseudomonas* isolates that produce siderophores against the growth of root rot fungi, namely *F. oxysporum* and *R. solani*. Isolates F2 and JY3 exhibited great activity in impeding the mycelial progress of the above-mentioned phytopathogenic fungi. The *P. aeruginosa* FP6 strain, which produces siderophores, exhibited antagonistic properties against *R. solani*, as reported in a previous study [35]. The study found that *P. syringae* strain BAF.1 exhibited significant antagonistic effects against *F. oxysporum*, resulting in a 95.24% reduction. This strain was also observed to produce siderophores of the catechol type, with a relatively low molecular weight of 488.59 Da, as reported in a previous study [36]. Based on the findings of Reddy et al. [37], it was observed that fluorescent *Pseudomonas* strains that produce siderophores demonstrated the capacity to inhibit the mycelial growth of *Pyricularia oryzae*, the pathogen responsible for rice blast, as well as *R. solani* AG-1 IA, which is linked to rice sheath blight disease. The study conducted by Mathiyazhagan and colleagues [38] revealed the impact of siderophores on inhibiting the proliferation and sporulation of phytopathogenic fungi. This resulted in various modifications in the morphology of the fungal mycelia due to the lack of iron.

In the current study, the maximum production of siderophores from isolate F2 and JY3 was achieved on optimized production media reported by Abo-Zaid et al. [31]. Glucose as a carbon source was the most effective variable in the production of siderophores from *P. aeruginosa* isolate F2 that was fully consumed compared to glycerol. On the other hand, glycerol was the main variable in the production of siderophores from *P. fluorescens* JY3. Santos et al. [39] observed the change in carbon/nitrogen ratio in the production media of siderophore had no effect. Nevertheless, Ye et al. [36] observed that different carbon/nitrogen ratios affect siderophore production significantly differently.

The present investigation revealed that F2 and JY3 formulations had high proficiency in decreasing wheat damping-off in *F. oxysporum* and *R. solani*-infected soil. Fluorescent *Pseudomonas* spp. have several mechanisms for managing soil-borne diseases. Secretion of siderophores by fluorescent *Pseudomonas* spp. is considered one of the important approaches utilized in biocontrol. It is based on the reduction of the number of ferric ions accessible for plant pathogens in the rhizosphere by chelation of these ions. The presence of low levels of iron in the rhizosphere serves as a stimulus for fluorescent pseudomonads to synthesize siderophores, which exhibit a high affinity for ferric iron. The ferric–siderophore complex resulting from this process is utilized by the producing microorganism through a very specific receptor secreted in its outer-cell membrane. On the other hand, the ferric–siderophore complex is inaccessible to other organisms [40]. This activity could potentially account for the restriction of phytopathogens within the rhizosphere, thereby reducing their capacity to establish themselves on plant roots. The *P. fluorescens* strains SPs9 and SPs20 were found to synthesize siderophores and exhibit significant efficacy in mitigating the incidence of *F. oxysporum*-induced wilt disease in tomato seedlings grown in soil [11]. Furthermore, plant systemic resistance can be accomplished by siderophores of fluorescent pseudomonads. In their study, Leeman et al. [41] documented the induction of plant systemic resistance against *Fusarium* wilt of radish through the production of a siderophore by *P. fluorescens*.

The current investigation provided evidence that the utilization of siderophore-producing F2 and JY3 formulations yielded significant enhancements in the fresh and dry weights of both shoots and roots in wheat plants cultivated in soil infested with *F. oxysporum* and *R. solani*. The results obtained by Arya et al. [11] were consistent with our findings. The previous findings may be attributed to the activation of phytohormones, specifically auxin, cytokinin, and gibberellins, through the use of both isolates. Moreover, it could be associated with the capacity of plants to absorb iron from the microbial Fe–siderophore complex that is produced. In a previous study, it was noted that the introduction of *Pseudomonas* strain GRP3 into *Vigna radiata* plants

resulted in the production of siderophores, leading to an increase in iron levels as well as chlorophyll a and chlorophyll b concentrations [42].

5. Conclusions

The fluorescent *Pseudomonas* spp. isolates F2 and JY3 demonstrated antagonistic activity against soil-dwelling fungi. The utilization of the optimized production media led to the attainment of the highest levels of siderophore production by strains F2 and JY3, with yields of 16.95% and 19.5%, respectively, after a 48 h incubation period. The utilization of fluorescent pseudomonads that possess the ability to produce siderophores has been employed as a means of employing biocontrol agents. This approach has demonstrated significant efficacy in inhibiting the growth of *F. oxysporum* and *R. solani*, as well as reducing the percentage of damping-off caused by these pathogens. Moreover, it was observed that both formulations demonstrated a stimulatory effect on the growth of wheat plants.

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