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Evaluation of *Pseudomonas aeruginosa* AL 98 against *Fusarium oxysporum* for Bio-management of Groundnut Wilt

R. R. Rakh[1], L. S. Raut[2], S. M. Dalvi[3], P. B. Nirmal[4] and A. V. Manwar[5]

[1] Department of Microbiology, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) - 431 511, District Parbhani, Maharashtra, India
[2] Department of Microbiology, Sant Tukaram College of Arts and Science, Parbhani - 431 401, Maharashtra, India
[3] Department of Botany, Shri Guru Buddhiswami Mahavidyalaya, Purna (Jn.) - 431 511, District Parbhani, Maharashtra, India
[4] Department of English, Sant Tukaram College of Arts and Science, Parbhani - 431 401, Maharashtra, India
[5] Department of Microbiology, D. S. Mandal's, College of Arts, Commerce and Science, Parbhani - 431 401, Maharashtra, India

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Abstract

Fusarium wilt, caused by *Fusarium oxysporum*, is a serious disease that affects groundnut production worldwide. Currently, managing *Fusarium* wilt is challenging and relies heavily on host resistance and chemical fungicides. However, using biological control methods, such as rhizosphere soil bacteria, can provide a more sustainable and eco-friendly solution for plant disease management. In this study, the biocontrol activity of *Pseudomonas aeruginosa* AL98, a soil-borne bacterium isolated from healthy groundnut plants, was investigated against *F. oxysporum in vitro*. The results showed that *Pseudomonas aeruginosa* AL98 exhibited strong antagonistic activity, inhibiting mycelial growth by 76.00%. The cell-free culture filtrate obtained from this bacterium also showed significant inhibition of *F. oxysporum* growth by 90%. This biocontrol effect was attributed to the production of siderophores and volatile compounds by the *Pseudomonas aeruginosa* AL98. To validate these findings, a pot assay was conducted, which demonstrated a decrease in *Fusarium* wilt incidence in the range 33.33% to 26.67% due to the treatment with *Pseudomonas aeruginosa* AL98 compared to the untreated control. Additionally, the bacterium improved seed germination percentage compared to the control group. These results highlight the potential of *Pseudomonas aeruginosa* AL98 as a biocontrol agent for managing *Fusarium* wilt disease in groundnut.

***Key words*:** *Fusarium wilt, Fusarium oxysporum, Pseudomonas aeruginosa* AL98, Non-volatile diffusible metabolites, Volatile metabolites, Siderophore

Arachis hypogaea L. (Groundnut) is one of the most important crops in the world. Groundnut is an important leguminous oil seed crop, belonging to the family *Fabaceae*. Groundnut is grown on a large scale in almost all the tropical and subtropical countries of the world, including China, India, Nigeria, Sudan, and the USA. The crop was introduced into India by the Portuguese. Especially in India, it is one of the major oil seed crops. In addition to this, being leguminous, groundnuts have the ability to fix atmospheric nitrogen biologically into the soil, which enriches the soil and benefits the succeeding crop. In India, it is one of the most important food and cash crop with a valuable source of all nutrients [1-2]. Groundnut is susceptible to many foliar and soil-borne fungal diseases, including early leaf spot caused by *Cercospora arachidicola* Hori, late leaf spot caused by *Phaeoisariopsis personata* (Bark and Curt.) v. Arx., rust incited by *Puccinia arachidis* Speg., dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid., stem rot incited by *Sclerotium rolfsii* Sacc. and wilt caused by *Fusarium oxysporum*. Among these soil- borne diseases such as dry root rot, stem rot and wilt cause serious losses to the crop, which is extensively grown under rainfed conditions [2].

The groundnut crop is equally vulnerable to soilborne diseases, as both the roots and pods of the plant grow in soil. Diseases caused by soil borne fungal pathogens reduce yields and the quality of the harvested pods. These diseases affect the crop plant until harvest. Pathogens attack all plant parts of groundnut and restrict plant development throughout the growing season, as well as reducing seed quality in post-harvest storage [2-3]. The pathogen *Fusarium oxysporum* that causes the wilt of groundnut was first reported in Tansania by Armstrong *et al.* [4]. *Fusarium wilt* is one of the common diseases of groundnut, caused by the fungus *Fusarium oxysporum* Schlechtend. Emend Snyder and Hans, lead to significant yield losses. The pathogen infects the roots and colonizes the vascular tissue, leading to wilting and finally the death of the plant [5]. The management of *Fusarium* wilt is done mainly through chemical soil fumigation with methyl bromide. Yet, their application can be expensive and have negative impacts on the environment. So, the use of soil fumigants has been banned because of their harmful effects on human health and the environment [6]. Chemical control methods are not only costly and inefficient but also pose potential risks to public health and the environment.

***Correspondence to:** L. S. Raut, E-mail: lasraut76@gmail.com

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On the contrary, biological control, specifically the use of rhizobacteria, offers a promising alternative to chemical control while avoiding the challenges associated with conventional plant management systems. The rhizosphere is the region where soil particles encounter plant roots, constituting a dynamic and highly complex microbial ecosystem [7]. The way plants and microorganisms interact in this environment creates a unique ecosystem where carbon and water cycle, and nutrients and minerals are stored. Studying how plants and microbes work together and the different substances they produce is really fascinating. These metabolites serve various functions, such as acting as energy sources and signaling molecules [8-9]. Rhizobacteria inhabiting the rhizosphere have been shown to synthesize a broad array of beneficial compounds [10]. Several studies have demonstrated that certain rhizobacteria possess antimicrobial properties against causal agents of plant diseases [11-12]. Antifungal rhizobacteria have garnered significant interest for their potential in effectively managing plant diseases through biological control. The *Pseudomonas* genus encompasses over a hundred species [13], with many native to plant rhizosphere, endosphere, and phyllosphere environments, establishing commensal relationships therein. Some *Pseudomonas* strains have found application as plant inoculants due to their ability to mitigate the detrimental effects of specific phytopathogens, thereby promoting plant growth and health [14, 9]. Several *Pseudomonas* species, including *P. aeruginosa*, *P. putida*, *P. chlororaphis*, *P. syringe*, and *P. fluorescens*, are well-recognized for their capacity to enhance plant development and suppress various plant diseases [9], [14-15].

The main goal of this study was to evaluate the efficacy of *Pseudomonas aeruginosa* AL 98 in controlling *Fusarium oxysporum* Schlecht. Emend. Synd and Hans, the fungus responsible for causing *Fusarium* wilt disease in groundnut plants. Keeping this view in mind, the importance of *Pseudomonas spp.* in sustainable agricultural development by controlling the phytopathogen, the present study focused on (i) investigating the interaction between *Pseudomonas aeruginosa* AL 98 and the *Fusarium* wilt pathogen through a confrontation assay. (ii) exploring the in vitro biocontrol mechanism of *Pseudomonas aeruginosa* AL 98 against *Fusarium oxysporum*. (iii) validating the *in vivo* biocontrol potential of *Pseudomonas aeruginosa* AL 98 against *Fusarium oxysporum* using a pot assay.

MATERIALS AND METHODS

Collection and maintenance of Fusarium oxysporum

Fusarium wilt pathogen of groundnut was isolated in earlier studies and was used during this investigation. The pathogen was maintained on PDA slants for subsequent research.

Pseudomonas sp.

Pseudomonas aeruginosa AL 98 is a rhizospheric bacteria isolated in prior studies from soil niche of healthy groundnut plant.

Confrontation assay

Pseudomonas aeruginosa AL 98 was evaluated *in vitro* for its antagonistic activity against *F. oxysporum*. To identify its potential as a bio-control agent, a simple confrontation assay developed by Matarese *et al.* [16] and Kotasthane *et al.* [17] was followed with slight modifications. Instead of PDA, Kings B Agar was used. A PDA disk measuring 5 mm in diameter, obtained from the periphery of a vigorously growing *Fusarium oxysporum* colony, was positioned on a Kings B agar plate. Simultaneously, a loopful of actively growing *Pseudomonas aeruginosa* AL 98 was placed on the opposite side of the fungal disk (2 cm apart) on Kings B Agar plates. Each pairing of antagonist and pathogen was prepared in triplicate. The plates were then kept in an incubator at room temperature, with alternating 12-hour periods of darkness and light, for a duration of 7 days. The percentage of pathogen inhibition by *Pseudomonas* isolates compared to the control was determined by applying the formula developed by Vincent in 1947 [18].

$$Percent\ Inhibition = \frac{Growth\ of\ Pathogen\ in\ Control - Growth\ of\ Pathogen\ test}{Growth\ of\ Pathogen\ in\ Control} \times 100$$

Similarly, from the zone of interaction between the antagonist and FOC in the confrontation assay, the mycelial mats were gently lifted with a needle and put in a drop of cotton blue on a microscopic slide and spread with a needle and observed under a microscope for hyphal interaction.

Illustration of the antagonistic mechanism

An illustration of the antagonistic mechanism of *Pseudomonas aeruginosa* AL 98 was accomplished by testing *Pseudomonas aeruginosa* AL 98 for non-volatile diffusible antibiotic metabolites, volatile compounds and siderophore assay.

Effect of non-volatile diffusible antibiotic metabolites

The effect of diffusible antibiotic metabolites produced by *Pseudomonas aeruginosa AL* 98 was determined by following the methods of Rakh*et al.* [19] with slight modification instead of PDA Plates Kings B Agar Plates.

The Kings B Agar plates, covered with a cellulose nitrate membrane, were inoculated in the center with antagonistic bacterial suspension. After incubation for 48 hours at room temperature, the membrane with the grown bacterial culture was removed, and the plate was inoculated in the center (below the paper) with a 5 mm disk of a pure culture of *Fusarium oxysporum.* The plates were re-incubated at room temperature for 7 days and the growth of the pathogen was measured. The experiment involved conducting a control test using King's B Agar plates that were not inoculated. Instead of using a bacterial suspension, sterile distilled water was added to the plates. The plates were then placed on the cellulose nitrate membrane and incubated further pathogen, *Fusarium oxysporium.* The experiment was run in triplicate. Results were expressed as means of % inhibition of fungal pathogen in the presence and absence of antagonistic bacterial isolate. Percent inhibition was calculated using the following formula [20].

$$Percent\ Inhibition = \frac{Fungal\ Growth\ in\ Control - Fungal\ Growth\ in\ Test}{Fungal\ Growth\ in\ Control} \times 100$$

Effect of volatile metabolites

The *Pseudomonas aeruginosa* AL98 was evaluated in the laboratory to screen out the most efficient one, which inhibits the growth of the pathogens by producing volatile metabolites following the double plate technique described by Raut *et al.* [21].

To detect the secretion of volatile metabolites produced by *Pseudomonas aeruginosa* AL98, 100 µl of an antagonistic bacterial suspension was placed at the center of one-half Petri dish containing Kings B medium, and a 5 mm disk of pure culture of *Fusarium oxysporum* was placed at the center of another Petri dish containing Kings B medium. Both half of the plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension and sealed with cellophane adhesive tape, to isolate and maintain the internal environment and minimize the loss of volatile substances created. Plates were incubated at room

temperature for 6 days and the growth of the pathogen was measured and compared to controls developed in the absence of the antagonist. The experiment was run in quadruplets. Results are expressed as means of inhibition (%) of fungal growth in the presence and absence of bacterial isolate. Percent inhibition was calculated using the following formula [20].

$$Percent\ Inhibition = \frac{Fungal\ Growth\ in\ Control - Fungal\ Growth\ in\ Test}{Fungal\ Growth\ in\ Control} \times 100$$

Quantitative assessment of siderophore production

Siderophore production by *Pseudomonas aeruginosa* AL98 was assessed quantitatively by a liquid CAS assay.

The quantitative siderophore production was assessed using a modified succinate medium as described by Meyer and Abdallah [22]. To initiate the experiment, 0.1 ml of the inoculum of *Pseudomonas aeruginosa* AL98 was inoculated into a 250 ml Erlenmeyer flask containing the Succinate medium. The flask was then placed on a rotary shaker incubator and incubated at room temperature for a duration of 72 hours. The supernatant was harvested after 24, 36, 48, 60, and 72 hours and centrifuged at 10,000 rpm in a cooling centrifuge at 4°C for 10 min to obtain cell free culture filtrate. The method outlined by Payne [23] was employed to evaluate the quantitative siderophore levels in the culture filtrate. In this procedure, 0.5 ml of the cell-free culture filtrate was combined with 0.5 ml of CAS solution. To establish a reference point, an uninoculated succinate medium was prepared. Both the test and reference were read at 630 nm and % siderophore units (% decolorization) in the culture filtrate were calculated.

$$\%\ Siderophore\ Unit = \frac{Absorbance\ of\ Reference\ at\ 630\ nm - Absorbance\ of\ Culture\ filtrate\ at\ 630\ nm}{Absorbance\ of\ Reference\ at\ 630\ nm} \times 100$$

Pot experiment

Multiplication of Fusarium oxysporium inoculum

The fungus, *Fusarium oxysporum*, was multiplied on sand-maize medium [2], [24] with some modification. The medium containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water kg-1 and filled in autoclavable bottles. The bottles were subjected to sterilization under a pressure of 1.4 kg cm-2 for two hours over two alternate days. In the inoculation process, two culture discs of actively growing *F. oxysporum*, each nine millimeters in diameter, were inserted into every bottle. These bottles were then placed in a room with a temperature of 28±2 °C and incubated for a period of 30 days. The purpose of this incubation was to allow the *F. oxysporum* to develop and serve as inoculum for further experiments.

Preparation of sick pots

Plastic pots were filled with a potting mixture (Soil: Sand: FYM @ 1:1:1). The potting mixture was sterilized in an autoclave at 1.4 kg cm $^{-2}$ pressure for two hours on two consecutive days and inoculated with a 5-gm inoculum of *Fusarium oxysporum* multiplied on sand maize medium.

Pot assay for biocontrol activity

Groundnuts were cultivated for over a period of 60 days and data on the emergence of *Fusarium* wilt infection was recorded at 30 and 60 days. Seeds of the groundnut variety TAG24 were used in these pot experiments and seed treatment with a talc-based formulation of the potential bacterial antagonist, *Pseudomonas aeruginosa* AL98 was employed and administered at a rate of 10 g kg^{-1} of the seed, with gum (5 ml kg^{-1}) utilized as an adhesive agent. The treated seeds were spread over clean paper and dried in a cool and shady place. The seeds were sown immediately after drying.

This experiment was conducted in two pots and in triplicate. Pots inoculated with phytopathogen, and the seeds sown in it without biocontrol agent treatment acts as a positive control, whereas the pots inoculated with phytopathogen and the seeds sown in them with biocontrol agent formulation treated acts as test. Each pot was sown with 5 seeds. The pots were watered with tap water as required. The growth parameters like percent seed germination, shoot length, root length, chlorophyll content, and vigour index were recorded after on 30- and 60-day' time intervals.

Treatments	Description
Test	Pot with phytopathogen and the seeds sown in it with treatment of biocontrol agents
Control	Pot with phytopathogen and the seeds sown in it without biocontrol agent treatment (Control)

The percent disease incidence in these areas was calculated using following formula [25]:

$$Percent\ Disease\ Incidence = \frac{\text{Number of Infected Plants}}{\text{Total Number of Plants}} \times 100$$

RESULTS AND DISCUSSION

Fusarium wilt, one of the diseases of groundnut occurring throughout the world and also in groundnut cultivated areas of India, has been controlled by several methods: 1) Cultural methods like crop rotation, hygiene, mulching, avoiding overhead irrigation, use of disease resistant cultivars etc. [26-30]. 2) Physical methods include soil solarization and soil disinfection using heating and steam [26-28] and 3) Chemical methods, as the use of chemical fungicides includes prochloraz, propiconazole, thiabendazole, carbendazim, benomyl, thiophante, fuberidazole and all of the benzimidazoles [26-29].

These alternatives do exhibit certain disadvantages, mainly in regard to their efficiency in disease control (Physical methods) and environmental hazards (Chemical methods) as well as high economical costs [31]. Also, the application of chemical fungicides as the sole method for control of *Fusarium* wilt may indeed result in non-target effects on soil microorganism populations.

A better alternative to chemical fungicides is soil microbes such as *Bacillusspp*., *Pseudomonas spp*., *Trichoderma harzianum, Trichoderma asperellum, Trichoderma koningii, Penicillium spp.* and *Streptomyces griseoviridis* residing in the rhizosphere niches of the plants and having the ability to suppress the pathogens and also stimulate plant growth by the production of phytohormones and/or degradation of complex substrates [26], [32-36]. A number of soil bacterial strains have been exploited for their biocontrol potentials as well as plant growth promotion, particularly the genera *Bacillus* [37], *Pseudomonas* [34], [38], and *Streptomyces* [39]. The adaptive metabolism and diverse production of antifungal compounds make the genus *Pseudomonas* highly effective in [34], [40]. Therefore, the discovery and optimization of choices to control this disease efficiently are necessary. We explored a biological control agent using a bacterial strain, *Pseudomonas aeruginosa* AL98, isolated from healthy rhizospheric niches of groundnut.

Confrontation assay

For sustainable agriculture, *Pseudomonas spp.* played a remarkable role of controlling the phytopathogens and promoting plant growth by secreting growth promoting hormones. In this context, the isolate, *Pseudomonas aeruginosa*

AL98 displayed the highest growth inhibition of *Fusarium oxysporum*76.00 % when co-inoculated on Kings B agar Petri plates in a confrontation assay (Fig 1, Table 1).

Photo Plate 1 Confrontation assay of *Pseudomonas aeruginosa* AL98 against *Fusarium oxysporum* control indicates only *Fusarium oxysporum* inoculated onto Kings B Agar incubated at room temperature for 7 days; Test indicates co-inoculation of *Pseudomonas aeruginosa* AL98 with *Fusarium oxysporum* onto Kings B Agar incubated at room temperature for 7 days

Table 1 Percent inhibition of growth of *Fusarium oxysporum* by *Pseudomonas aeruginosa* AL98 in confrontation assay

Rhizospheric isolate	Fungal growth in test (mm)	Fungal growth in control (mm)	% Inhibition of pathogen
Pseudomonas aeruginosa AL98	19(±0.95)	80(±0.5)	76(±1.18)

± represents standard deviation

In a dual culture confrontation assay conducted by Trivedi *et al.* [40] on Kings B broth between, *Pseudomonas corrugata* and two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*a reduction in biomass of *A. alternata* (93.8%) and *F. oxysporum* (76.9%) was observed. The results obtained are in similar line with previous findings of the antagonistic nature of *Pseudomonas spp.*, documented the antagonistic behavior of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in a laboratory setting [41]. Their study revealed that all six isolates (PF18, PF4, PF20, PF19, PF13, and PF14) exhibited significant efficacy in inhibiting the growth of pathogens in dual culture compared to the control group. Shahzaman *et al.* [42] recorded the effectiveness of thirty isolates isolated from the chickpea rhizosphere against wilt pathogens in dual culture over control. Also, Islam *et al.* [34] screened 35 rhizobacterial isolates for antagonistic activity in dual culture, and isolate BA5 showed the highest antagonistic activity (58.33% mycelial growth inhibition) against Foc.

In the study conducted by Hua *et al.* [35], 14 different fluorescent *Pseudomonas* strains were examined for their effectiveness against *Fusarium oxysporum* f. sp. *niveum*. The results from *in vitro* experiments revealed that WMC16-1-1, WMC16-1-8, and WMC16-2-5 exhibited significant inhibition of the mycelial growth of FON. Muthoni *et al.* [43] isolated Thirty-four *P. fluorescens*, of which 12 isolates produced fluorescence pigment. Five of the 12 strains showed significant antifungal activity ($P<0.05$) against *F. oxysporum* compared to the control. Isolate Pf1 significantly ($P\leq0.001$) inhibited mycelial growth and scored 72.2% compared to control. Similarly, five isolates of *Pseudomonas fluorescens* were evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* by Sahane *et al.* [44]. All isolates suppressed the growth of the test pathogen's mycelium, although none of the *Pseudomonas fluorescens* isolates achieved complete inhibition. Among the isolates, PF5 exhibited the highest effectiveness in inhibiting mycelial growth at a rate of 77.92%, followed by PF2 at 68.61%. The PF4 and PF3 isolates demonstrated 64.58% and 55.27% inhibition of mycelial growth, respectively. Furthermore, an assessment was carried out by Pandey and his colleagues on 20 strains of *Pseudomonas fluorescens* in relation to their effectiveness against *Fusarium oxysporum*. From these 20 strains, six (Pf4, Pf13, Pf14, Pf18, Pf19, and Pf20) were identified as potent inhibitors of *Fusarium oxysporum* f. sp. *ciceri* (Foc) through the use of a dual culture method. Among them, Pf18 demonstrated the highest inhibition of mycelial growth at 80.10%, while Pf7 exhibited the lowest inhibition at 57.20%. It is worth noting that isolates Pf4 (79.80%), Pf13 (72.60%), Pf14 (70.30%), Pf19 (73%), and Pf20 (76.4%) all exhibited mycelia growth inhibition above 70% [45]. 109 bacterial strains assessed for their antifungal properties against Foc. Among these strains, isolate 91 demonstrated the most potent antifungal activity, suppressing 69% of Foc growth in the dual-culture plate experiment [9].

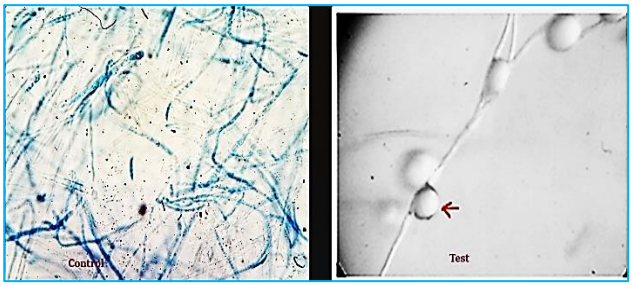

Photo Plate 2 Microscopic observation of zone of interaction between the antagonist and FOC in confrontation assay. Control – Microscopic observation of *Fusarium* mycelium; Test – the swelling indicates the effect of antagonistic, *Pseudomonas aeruginosa* AL98 on the growth of *Fusarium oxysporum* in confrontation assay

When the inhibition zone in the confrontation assay observed under a microscope reveals swelling of *Fusarium oxysporum* mycelium because of metabolites secreted by *Pseudomonas aeruginosa* AL98 (Photo plate 2). All results from these researchers support the fact that *Pseudomonas spp*. plays a remarkably important role in controlling *Fusarium oxysporum*, the causative agent of *Fusarium* wilt disease in different hosts.

Illustration of the antagonistic mechanism

Pseudomonas aeruginosa AL98 produced non-volatile diffusible antibiotic metabolites, volatile compounds and siderophore which significantly contributed to the inhibition of *Fusarium oxysporum*.

Effect of non-volatile diffusible antibiotic metabolites

Pseudomonas aeruginosa AL98 produced non – volatile diffusible antibiotic metabolites, which meaningfully contribute towards inhibition of *Fusarium oxysporum* upto 90% (Photo Plate 3, Table 2). This supports the fact that *Pseudomonas* spp. secretes different non-volatile diffusible antibiotic metabolites, which play a key role in killing the phytopathogens. Islam *et al.* [34] isolated *Pseudomonas aeruginosa*, whose cell – free culture filtrate and ethyl acetate crude extract inhibited the mycelial growth of Foc by 56.66% and 25.0% respectively and maximum fungal biomass reduction (90.20%) was found in King's B broth in shake flask culture.

Pseudomonas fluorescens Pf2 isolate produced non-volatile compounds, which scored the least mycelial growth

(24.0±1.15 mm) compared to control (77.0 ±2.08 mm) [43]. Similarly, Xie *et al.* [9] reported that cell-free fermentation supernatant of *Pseudomonas aeruginosa* isolate 91 demonstrated inhibition of the Foc pathogen when compared to the control. The ethanolic extract of *Pseudomonas aeruginosa* isolate 91, at concentrations of 2 mg/disk, 4 mg/disk, and 6 mg/disk, exhibited inhibition zone diameters of 11 mm, 14 mm, and 17 mm, respectively. While the n-butanol extract, at concentrations of 2 mg/disk, 4 mg/disk, and 6 mg/disk, displayed inhibition zone diameters of 7 mm, 8 mm, and 9 mm, respectively. It is evident from the findings that both ethanolic extracts and n-butanol extracts possess bioactive compounds that have the ability to hinder the growth of Foc. These results support the results obtained for *Pseudomonas aeruginosa* AL98 by us, where non-volatile diffusible metabolites produced do get involved in limiting the growth of *Fusarium oxysporum*.

Photo Plate 3 Effect of non-volatile diffusible antibiotic metabolites produced by *Pseudomonas aeruginosa* AL98 on the growth of *Fusarium oxysporum*. Control – *Fusarium oxysporum* grown on Kings B agar without inoculation of *Pseudomonas aeruginosa* AL98 culture onto cellulose nitrate membrane; Test - *Fusarium oxysporum* inoculated and incubated at room temperature on Kings B agar for 7 days, on which *Pseudomonas aeruginosa* AL98 culture was grown earlier for 48 hr. onto cellulose nitrate membrane

Table 2 Percent inhibition of growth of *Fusarium oxysporum* by non-volatile diffusible metabolite secretion

Rhizospheric isolate	Fungal growth in test (mm)	Fungal growth in control (mm)	% Inhibition of pathogen
Pseudomonas aeruginosa AL98	5(±0.0)	50(±0)	90(±0.0)

± represents standard deviation

Effect of volatile metabolites

Volatile compounds such as ammonia and hydrogen cyanide are produced by several rhizobacteria and are reported to play an important role in biocontrol. *Pseudomonas aeruginosa* AL98 has the ability to secrete volatile metabolites, which helps to limit the growth of *Fusarium oxysporum* (76%) in the double plate technique. Similar, results were obtained by Trivedi *et al.* [40] where *Pseudomonas corrugata* did not exhibit any inhibition zones as a result of producing diffusible antifungal metabolites. However, a decrease in growth ranging from 58% to 49% was observed in both test fungi, *A. alternata* and *F. oxysporum*, when incubated in sealed Petri plates for 120 hours. This reduction in growth was attributed to the production of volatile antifungal metabolites.

Islam *et al.* [34] conducted a study where they found that *Pseudomonas aeruginosa* isolate BA5 produced volatile compounds (VOCs) with antifungal properties. The growth inhibition of Foc in sealed Petri dishes was clearly shown by the 31.11% reduction in radial mycelial growth compared to the control. Similarly, Raza *et al.* [46] demonstrated the biocontrol activities of VOCs produced by *P. fluorescens* WR-1. Recent studies by Kandel *et al.* [47] and Lee *et al.* [37] have also reported the antifungal activities mediated by VOCs. *Pseudomonas aeruginosa* AL98 produced volatile metabolites in double the plate technique, which significantly contributed to the inhibition of *Fusarium oxysporum*. In this study, the volatile metabolites have a drastic impact on the growth of *Fusarium oxysporum* (76 %). These findings are better than the results of other studies.

Photo Plate 4 Effect of volatile metabolites produced by *Pseudomonas aeruginosa* AL98 on Kings B agar, on the growth on *Fusarium oxysporum*; Control – *Fusarium oxysporum* grown onto Kings B agar in double plate technique without bacterial culture; Test – *Fusarium oxysporum* grown onto Kings B agar in double plate technique with bacterial culture

Table 3 Percent inhibition of growth of *Fusarium oxysporum* by volatile diffusible metabolite secretion

Rhizospheric isolate	Fungal growth in test (mm)	Fungal growth in control (mm)	% Inhibition of pathogen
Pseudomonas aeruginosa AL98	19(±0.96)	80(±0.5)	76(±1.29)

± represents standard deviation

Quantitative assessment of siderophore production

A supernatant of succinate broth culture of *Pseudomonas aeruginosa* AL98 was harvested after 12hr, 24hr, 36hr and 48hr, by centrifugation at 10,000 rpm in a cooling centrifuge for 15-20 min. Quantitatively, siderophores in the supernatant were detected as per Payne [23] where 0.5 ml of culture filtrate was mixed with 0.5 ml of CAS solution, instant color change of the CAS reagent from blue to classical golden orange was indicative of siderophore positive test (Photo Plate 5, Fig 1).A reference was prepared using, uninoculated succinate medium. Absorbance of both the test and reference were read at 630 nm and the % siderophore units in the culture filtrate were calculated. The % siderophore units of the culture was calculated after 12, 24, 36 and 48 hr.

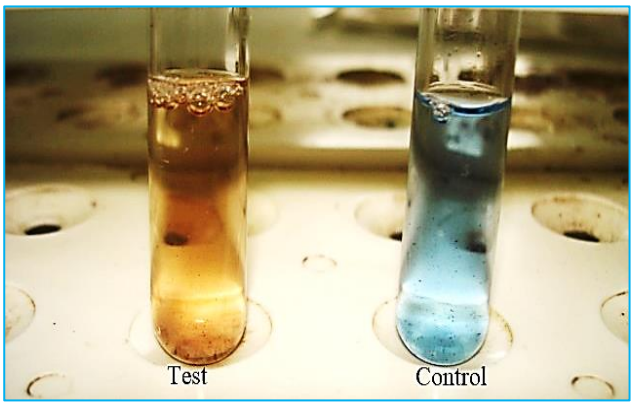

Photo Plate 5 Quantitative assessment of siderophore by liquid CAS assay; Test – Cultural filtrate of *Pseudomonas aeruginosa* AL98 with addition of liquid CAS, turn in blue to golden orange colour indicates siderophore test positive; Control – Succinate medium with liquid CAS, blue colour indicates siderophore test negative

Table 4 Percentage disease incidence in pot assay

Disease incidence		30 Days		60 Days	
		Total number of plants	Number of infected plants	Total number of plants	Number of infected plants
Percent disease incidence	Control	15	10	15	13
			66.67		86.67
Percent disease incidence	Test	15	5	15	4
			33.33		26.67

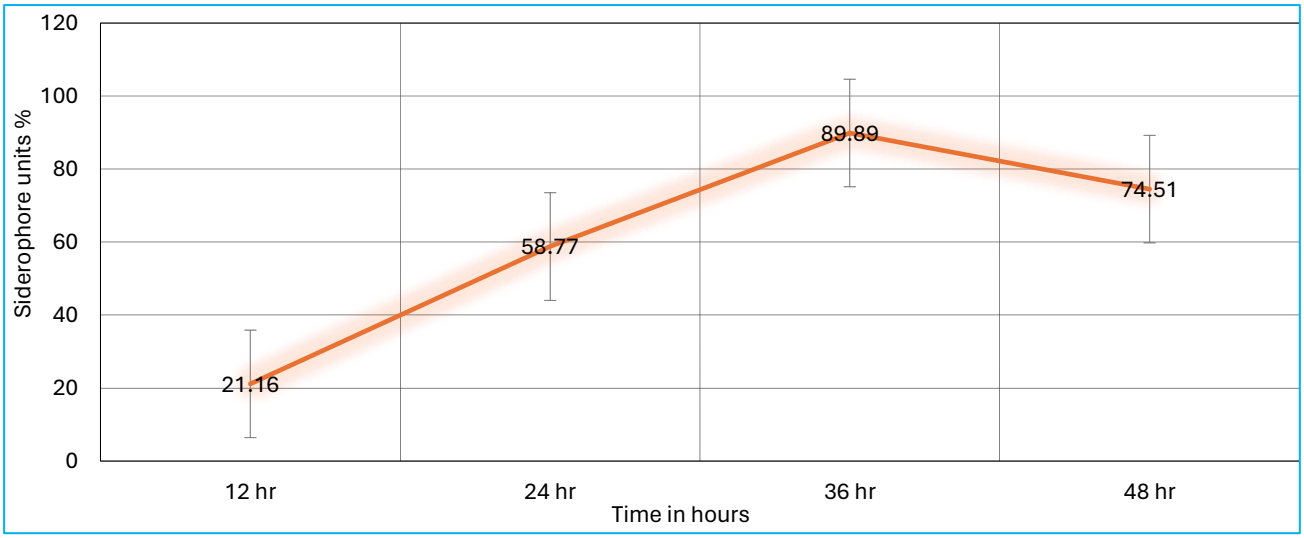

Fig 1 Quantitative assessment of siderophore produced by *Pseudomonas aeruginosa* AL98 by liquid CAS assay

In the time course of siderophore production, maximum siderophore secretion by *Pseudomonas aeruginosa* AL98 (89.89%) was recorded after 36 h.

The role of siderophores in biocontrol has extensively been studied previously [48-49]. Siderophores have the ability to impede the proliferation of soil-dwelling fungi by decreasing the quantity of ferric ions accessible to rhizosphere microorganisms. It has also been stated that colonization of the rhizosphere, production of antibiotics, and antagonistic activity of *P. aeruginosa* are presumably due to the production of siderophores [50]. Yu *et al*. [51] discovered that *Pseudomonas syringae* strain BAF.1 synthesized catechol-species siderophore and demonstrated strong antagonistic activity against *Fusarium oxysporum*, achieving a maximum inhibition rate of 95.24%. Similarly, Islam *et al.* [34] revealed siderophore production by *Pseudomonas aeruginosa*, which was involved in limiting the growth of *Fusarium oxysporum* f. sp. *cucumerinum*. Mrugesh and Trupti [52] characterized the siderophore produced by *Pseudomonas sp*. MT and conclusively proved its involvement in controlling *Fusarium oxysporum* f. sp. *cubense* and *F. oxysporium* f. sp. *ciceris*. All these studies support the involvement of siderophore in controlling the phytopathogen, *Fusarium oxysporum*. *Pseudomonas aeruginosa* AL98 is involved in the production of siderophore, which too contributed towards controlling the *Fusarium oxysporum*.

Pot assay for biocontrol activity

Initial results of *in vitro* experiments revealed that *Pseudomonas aeruginosa* AL98 efficiently controlled *Fusarium oxysporium*. Hence, to further confirm and cross check the results, *In Vivo*, experiments in pots were undertaken with artificially infested soil (sick soil) with the phytopathogens, *Fusarium oxysporium*, causing *Fusarium* wilt. It was revealed that an incidence of the disease symptoms in the sick pot (control) after 60 days of sowing was observed, whereas no symptoms were recorded in the test pots (bacterized seeds) even after 60 days. Also, the overall vigour of the plant was found to be poor in an artificially infested pot as compared to the *Pseudomonads* treated groundnut seeds. The percent of *Fusarium* wilt incidence in control ranges from 66.67 to 86.67 %, while a decrease in disease incidence was recorded due to treatment of *Pseudomonas aeruginosa* AL98 as compared to the untreated control, which ranged from 33.33 to 26.67% in *Fusarium* wilt of groundnut (Photo Plate 6, Table 4). Similarly, treatment with the *Pseudomonads* resulted in an enhancement of % seed germination, shoot length, root length, number of leaves and chlorophyll content of the groundnut in compared to control as shown in (Fig 2).

Photo Plate 6 Pot assay for biocontrol activity of *Pseudomonas aeruginosa* AL98 against *Fusarium oxysporum*, the wilt pathogen of groundnut

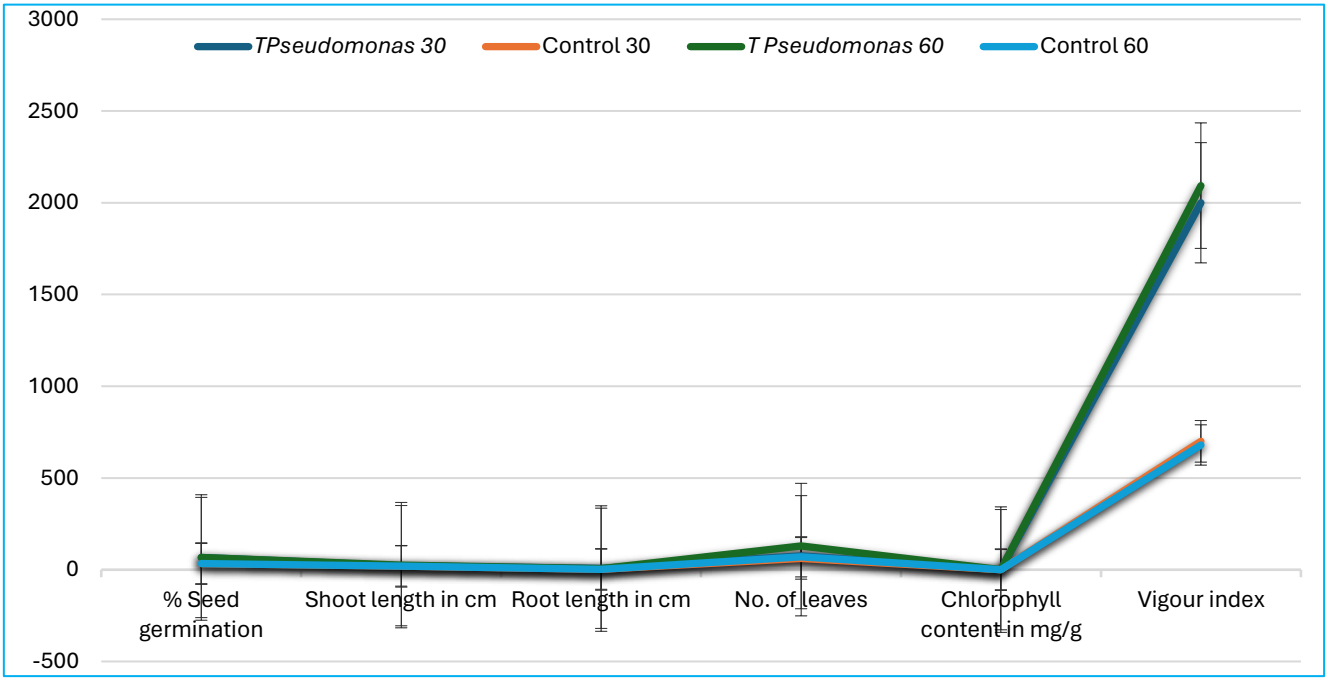

Fig Pot assay for biocontrol activity of *Pseudomonas aeruginosa* AL98 against *Fusarium oxysporum*

Sahane *et al.* [44] recorded that treatment of all isolates of *Pseudomonas fluorescens* significantly improved the percentage seed germination as compared to control (69.16%), with minimizing seedling mortality both at the pre-emergence and post emergence stages. The greatest seed germination percentage was documented in PF5 at 87.49%, with PF2 following closely at 84.16%, then PF4 at 80.83%, and finally PF3 at 79.16%. PF1 gave the least seed germination (77.49%) of chickpeas (JG-62). The least pre and post emergence seedling mortality was found with PF5 followed by seed treatments of PF2, PF4 and PF3.Similarly, Pandey *et al.* [45] reported a reduction in the occurrence of *Fusarium* wilt of chickpeas after treatment with *Pseudomonas fluorescence*. The bacterial antagonist in Pf4 showed a significantly lower wilt incidence (4%) after 30 DAS compared to the control group (24%). However, when compared to the control (48%), isolate Pf14 had the lowest wilt incidence at 60 DAS (14.6%). After 90 DAS, the control had 80% of the wilted plants, whereas isolate Pf18 had the lowest incidence of wilt (30.6%), followed by Pf13 (33.4%), Pf14-20 (34%), Pf4 (34.6%), and Pf19 (39.4%), in that order. The strain Pf18 achieved the highest level of illness control (41.75%) [44-45]. This proves that *Pseudomonas aeruginosa* AL98 would play a key role in the biocontrol and growth promotion of groundnut.

ONCLUSION

An effort has been made to analyze the effect of *Pseudomonas aeruginosa* AL98 with strong antagonistic activity as a biocontrol against the groundnut wilt pathogen *Fusarium oxysporum*. *Pseudomonas aeruginosa* AL98 was a prominent antagonist against the pathogen and was able to produce various antagonistic compounds, including non-volatile diffusible metabolites, siderophores and VOCs, as well as it shows plant growth promotion potentials in vitro. Also, this isolate showed remarkable biocontrol activity in pot assay. The findings suggest that the selected isolate has the potential to be used as a biocontrol agent in the management of *Fusarium* wilt in groundnut. Nevertheless, a field trial is needed to determine the disease suppression efficiency of the *Pseudomonas aeruginosa* AL98 isolate in the natural soil environment. The study suggests that *Pseudomonas aeruginosa* AL98 shows promise as a biocontrol agent for managing *Fusarium wilt* in groundnut plants.

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