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Research Article

Impact of *Pseudomonas fluorescens* on Field Pathogen: A Commercial Biofungicide Study

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Abstract

To evaluate the efficacy of Ami Sudo Star, comprising *Pseudomonas fluorescens*, as a biocontrol agent for inhibiting mycelial growth of *Fusarium oxysporum* in *Solanum lycopersicum* L. *Pseudomonas fluorescens* was evaluated in vitro against *Fusarium oxysporum* using dual culture and agar well diffusion techniques. Then, using a dose of 1 kg per acre in the field, characteristics including the vigour index, shoot length, root length, and germination percentage were assessed. *Pseudomonas fluorescens* showed zones of inhibition against Fusarium oxysporum in both the dual culture and agar well diffusion techniques, measuring 36.3 ± 4 mm and 39.1 ± 2 mm, respectively, according to the results. Moreover, the treatment of Ami Sudo Star greatly enhanced seedling growth and facilitated plant development as compared to untreated plants. The current study introduced the utilization of Ami Sudo Star in powder formulation, which led to a substantial reduction in the prevalence of *Fusarium oxysporum* as well as noteworthy enhancements in seedling growth and other indicators of plant development.

Keywords: Ami Sudo Star; *Pseudomonas fluorescence; Fusarium oxysporum*; Tomato; Seedling Growth

Introduction

The tomato, or *Solanum lycopersicum* L., represent one of the most widely grown and popular vegetable crops globally. It is a member of *Solanaceae* family and is the second most valuable vegetable product, behind potatoes [1]. The tomato is a climate-adaptable plant that grows well in both dry and temperate climates around the globe. Its high amounts of antioxidants and medicinal qualities contribute to its strong nutritional value and use for human consumption. Interestingly, it has important vitamins like A, C, and E (which make up 95.3% of the water content), as well as trace levels of calcium and niacin, which are important for human metabolism [2]. A model organism with many uses, the tomato plant has been researched extensively in a wide range of scientific fields, such as developmental biology, genetics, abiotic stress responses, disease resistance, and food science, because of its special traits and adaptability [3-5]. Tomatoes are significantly more prone to attacks from pests and diseases compared to other popular crops, making them a special challenge for farmers and gardeners to keep healthy and thriving [6].

Fusarium oxysporum f.sp. lycopersici, the fungus that causes *fusarium* wilt, is a major danger to tomato production globally and causes substantial financial losses. Recent research has demonstrated that in Uttar Pradesh, Fusarium wilt accounts for a reduction of approximately 25.14% to 47.94% in tomato yield [7]. The infection caused by *Fusarium oxysporum f.sp. lycopersici* begins with the germination of soil-borne spores in response to chemosignals from tomato root exudates. Infectious hyphae then invade the rhizosphere, penetrate the epidermis, and multiply intra-or intercellularly in cortex tissues before finally penetrating vascular tissues [8]. Chemical pesticides have been used to prevent *fusarium* wilt disease; nevertheless, it has been shown that these pesticides pollute soil, water, and the air. Researchers have endeavored to figure out environmentally sustainable substitutes for pesticides as a means of controlling plant diseases. It has been shown that biological treatments are practical and efficient substitutes for controlling a variety of plant diseases [9].

Researchers have looked at the possibility of using plant growth-promoting rhizobacteria (PGPR) to fight different diseases. It has been demonstrated that PGPR, when administered in various ways, may colonize plant root systems, enhancing production and growth. To further lessen the severity of illness, PGPR with biocontrol features may be a viable substitute for heavily dosed pesticides used on crops [10].

Many species, such as *Azospirillum*, *Azotobacter*, *Pseudomonads*, *Bacillus*, *Streptomyces*, *Enterobacter*, *Clostridium* and *Burkholderia* are part of the community of plant growth-promoting rhizobacteria (PGPR) [11]. Beneficial microorganisms, particularly *Pseudomonas* species, invade plant roots and offer protection to the plants through the secretion of chemicals that promote plant development, antimicrobial agents, and proteins associated to pathogens [12,13]. *Pseudomonas* species demonstrate their ability to exercise biocontrol by producing a wide variety of antagonistic substances, such as hydrogen cyanide, siderophores, 2,4-diacetylphloroglucinol, phenazines, and biosurfactants. The metabolic chemicals produced by *Pseudomonas fluorescence* species have a role in several biocontrol processes, such as iron competition, systemic resistance induction, and antibiosis [14]. *Pseudomonas spp*. induce systemic resistance in plants, conferring protection on tomato plants against *Fusarium oxysporum* and *Phytophthora infestans* [15]. Therefore, employing *Pseudomonas fluorescens* as a biocontrol agent offers a viable approach for controlling soil-borne infections with the least amount of negative ecological effects along with plant growth promoting traits.

This study was carried out mainly to access the efficacy of *Pseudomonas fluorescens* isolates in the treatment of *Fusarium* wilt in tomato plants and to clarify possible mechanisms of action. To evaluate Ami Sudo Star's efficacy against control groups, field experiments were carried out. The germination %, shoot length, root length, and vigor index were the main variables assessed in this investigation.

Methodology

Research site

Tomato plants exhibiting wilt symptoms were collected from Ucharpi, Mahesana, and the pathogenic agents were isolated from these plants. The experiment was conducted at the Ami experimental farm in Ahmedabad, Gujarat, between 2022 and 2023. Notably, Ami Sudo Star, containing *Pseudomonas fluorescens*, was applied as a biopesticide to agricultural areas to control pests. The experimental design consisted of three replications, employing a completely randomized framework.

Isolation and identification of the pathogen

The affected root and stem segments were initially rinsed with tap water to remove any debris. Subsequently, the areas exhibiting vascular browning were excised into smaller pieces. A 30-second surface sterilization procedure was then performed on these specimens using a 1% solution of sodium hypochlorite ($NaOCl_2$). Following this, the tissues underwent three additional rinses with sterile distilled water to remove any residual sodium hypochlorite solution. After surface sterilization, the pieces were placed on petri plates containing sterilized potato dextrose agar (PDA) and incubated at room temperature (28 ± 2°C) for a duration of five to seven days [16].

Fusarium oxysporum was identified as the pathogen by means of a comparative examination of the cultural and morphological features, which included growth patterns in addition to cultural and morphological aspect.

Evaluation of biocontrol agent against plant pathogen

Dual culture technique

A small disk of *Fusarium oxysporum* culture was placed on a petri dish filled with potato dextrose agar (PDA) medium. Two-day-old culture of *Pseudomonas fluorescens*, a biocontrol agent, was streaked onto the medium, creating a competitive environment where the two microorganisms would interact. The growth of *F. oxysporum* and the measurement of the zone of inhibition (in millimeters) were recorded. A plate without biocontrol agent was considered as control. The significant antagonists were identified by their ability to prevent the pathogen from growing.

Agar well diffusion technique

To evaluate the antagonistic properties of antibacterial metabolites within the culture filtrate of *Pseudomonas fluorescens*, the agar well diffusion method was employed [17]. A seven-day-old culture of *P. fluorescens* was inoculated and incubated in a shaker at 150 rotations per minute (rpm) for two days. Subsequently, 10 milliliters of the culture was centrifuged at 10,000 rpm for 20 minutes. The resulting supernatant was filtered using Whatman No. 1 filter paper to collect the spore suspension.

A nine-day-old *F. oxysporum* culture was centrally injected into petri plates containing solidified potato dextrose agar (PDA) medi-

um. Using a sterile cork borer, equidistant wells were created, and the filtrate was administered to four wells, spaced two centimeters apart from the central *F. oxysporum* disc. DMSO was employed as a control. The plates were then incubated at 28 ± 2°C for seven days to determine the maximum inhibition percentage. The diameter of the inhibitory zone surrounding each well was measured and recorded.

Field application with biopesticides

Individual seeds were submerged in the bioformulation for the entire night during the field testing. The next day, the seedlings were planted in a 28 × 46-meter rectangular area, with three rows per treatment and a 30-centimeter gap between rows within each block. Daily observations began 15 days after seeding and continued thereafter. Row-by-row plant counts were performed to prepare data for further analysis. Every 15 days, the number of dead plants was recorded. After five successive inspections, the number of surviving plants was counted. Further evaluations were conducted to determine the bioagent's effectiveness in reducing the occurrence of *F. oxysporum* in tomato plants.

In the field study, three replications of a predefined dosage of bio-formulation WP were given to infected seedlings at a rate of 1 kg per acre. The group that received the treatment was designated as the treated group, while the group that did not receive the treatment was designated as the Untreated or Control group. In terms of germination percentage, shoot length, root length, and vigor index, different pre-sowing seed treatments produced different results. As part of the experimental procedure, *Fusarium oxysporum* inoculum was added prior to sowing the seeds into the soil. In comparison to the untreated group, plant wilting was closely observed from seedling emergence to crop maturity. Susceptible tomato seedlings were treated with the antagonist *Pseudomonas fluorescence* at the Experimental Farm, and then placed into a 28 × 46-meter wilting area. This study used a randomized block design (RBD) as its experimental methodology, with untreated seeds acting as the untreated group.

Results and Discussion

Isolation and identification of plant pathogen

The features of the colonies varied between the *Fusarium oxysporum* isolates. The isolates' colors varied from white to pinkish-white to pastel pink. Cottony aerial mycelium that was fluffy to moderately fluffy was seen in most isolates.

In-vitro evaluation of biocontrol agent against plant pathogen

Pseudomonas fluorescens considerably inhibited the mycelial development of *Fusarium oxysporum*, according to the results of the laboratory investigations. By using agar well diffusion and dual culture techniques, this biocontrol agent's effectiveness against the previously described plant pathogen was assessed *in vitro*. The results showed zone of inhibition readings of 36.3 ± 4 and 39.1 ± 2, respectively, as presented in Table 1. In earlier research, eight of the twenty *fluorescent Pseudomonas* isolates showed a substantial suppression of the pathogen *Fusarium oxysporum f.sp. udum* development in in-vitro experiments used to evaluate the biocontrol efficiency of the isolates [18]. Using a dual culture technique, six of the 20 isolates of *Pseudomonas fluorescens* were shown to be potent inhibitors of *Fusarium oxysporum f. sp. ciceri* (Foc) [19].

Table 1: Effect of *P. fluorescence* against *F. oxysporum.*

Treatment	Dual culture technique (zone of inhibition in mm)	Agar well diffusion technique (zone of inhibition in mm)
F. oxysporum against *P. fluorescence*	36.3 ± 4	39.1 ± 2
Control	--	--

Effect of biopesticide on field conditions

A field study was conducted to evaluate the effectiveness of biopesticide-treated seeds in terms of germination percentage, root and shoot lengths, and overall vigor index of tomato plants. Observations were made every 15 days, starting from the 15th day after seeding, to assess seed germination and seedling growth. The findings from the field research, including seed treatments for tomato cultivation, revealed significant differences between the Ami Sudo Star-treated group and the control group.

A study examining the effects of inoculated Ami Sudo Star on germination rates and seedling growth revealed that the bioformulation of seeds improved germination and led to noticeably greater growth rates, as seen by increased vigor index values. Moreover, the evaluation of the inhibitory impacts of introduced biopesticide on fungal development revealed that the presence of bacteria in the culture medium significantly impeded the formation of fungal colonies.

Ami Sudo Star considerably enhanced seed germination and seedling growth, as per the Figure 1. These results relate with ear-

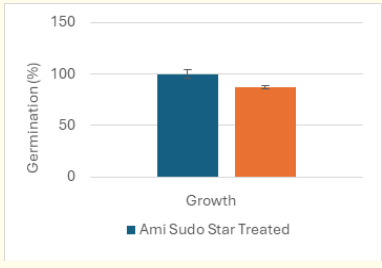

Figure 1: Effect of Ami Sudo Star on % Germination.

lier research showing that all isolates of *Pseudomonas fluorescens* exhibited noteworthy improvements in seed germination percentage, with a notable increase compared to the control (69.16%). Additionally, these isolates minimized seedling mortality at both the pre-emergence and post-emergence stages [20].

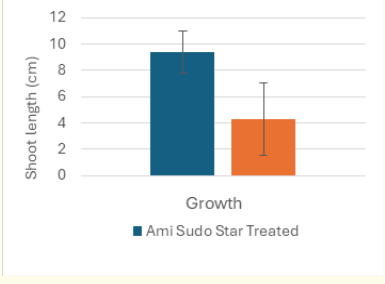

Figure 2: Effect of Ami Sudo Star on Shoot length.

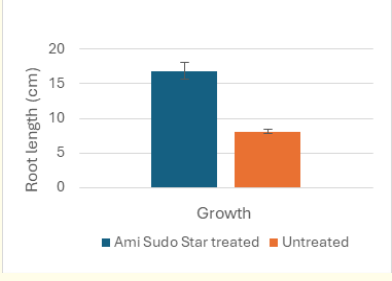

Figure 3: Effect of Ami Sudo Star on Root length.

Following the application of the biocontrol agent, substantial increases were observed in the lengths of both root and shoot tissues compared to the untreated control group, as depicted in Figures 2 and 3.

According to the results shown in Figure 4, an increased vigor index showed that Ami Sudo Star seed inoculation caused noticeably greater growth rates. This analysis implies that *Pseudomonas fluorescens* appears to have growth-promoting properties.

Figure 4: Effect of Ami Sudo Star on Vigour index.

A previous study on watermelon plants revealed that *Pseudomonas* strains have been identified as potential candidates for controlling *Fusarium* wilt and promoting plant growth [21]. In addition to decreasing *Fusarium* growth, prior studies have demonstrated that *fluorescent Pseudomonas* strains that generate siderophores are capable of preventing the mycelial growth of *Pyricularia oryzae*, the agent responsible for rice blast, and *Rhizoctonia solani* AG-1 IA, the agent linked to rice sheath blight disease [22].

One promising approach to make agriculture more sustainable and resistant to stresses is to employ the power of beneficial microorganisms like fungi and bacteria. These beneficial microbes can boost plant growth by producing helpful compounds that stimulate development and health [23].

Conclusion

The powdered formulation of Ami Sudo Star presents a sustainable alternative to synthetic pesticides for the management of plant diseases. In plant disease prevention strategies, *Pseudomonas* species are frequently employed as microbial biocontrol agents. *Pseudomonas fluorescens*, in particular, has been shown to exhibit great potential for promoting seedling growth and inducing susceptibility to pathogens in tomato plants, thereby reducing the incidence of *Fusarium oxysporum*, likely through the production of defense enzymes or direct inhibition of fungal growth. However, it is essential to complement these findings with field studies conducted in a range of environmental conditions.

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