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Role of Biocontrol Agents in Management of Corm Rot of Saffron Caused by *Fusarium oxysporum*

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Abstract: Saffron (*Crocus sativus* L.) is considered as one of the most expensive spices. Fusarium corm rot of saffron, caused by *Fusarium oxysporum*, is known to cause severe yield losses worldwide. In the present study, efficacy of biocontrol agents (*Trichoderma asperellum*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus stratosphericus*, *Bacillus pumilus*, and *Bacillus ubtilis*) along with a chemical fungicide, carbendazim, was evaluated for managing the corm rot of saffron. Under in vitro conditions, using dual culture and poison food techniques on potato dextrose agar, *T. asperellum* and carbendazim significantly reduced the mycelial growth of the pathogen *F. oxysporum*, with the inhibition of 62.76 and 60.27%, respectively, compared with control. Under field conditions, dipping of saffron corms in carbendazim and *T. asperellum* exhibited maximum reduction of 82.77 and 77.84%, respectively, in the disease incidence, during the first year of experiment. However, during the second year, maximum reduction in the incidence of corm rot (68.63%) was recorded with the *T. asperellum*. Moreover, the population density of *F. oxysporum* was also significantly reduced by 60% and 80.19% while using *T. asperellum* after 75 and 260 days of sowing of saffron corms, compared to its population before planting of corms. In case of growth promotion traits, such as sprouting and flowering, biocontrol treatments reduced the number of days (average) of sprouting and flower emergence after sowing, compared to control.

Keywords: biocontrol; corm rot; pathogen; saffron

1. Introduction

India is among few countries in the world that are gifted with the commercial cultivation of saffron (*Crocus sativus* L.) on an area of 3674 ha with production and productivity of 9.6 MT and 2.61 kg ha^{-1} during 2015, respectively [1]. Traditionally, this crop is associated with rituals, cuisines, and cosmetic industries [2], and also has numerous health benefits with potent medicinal properties [3–5]. The union territory of Jammu and Kashmir in India has the monopoly to grow saffron, which is mainly confined

to the Kishtwar, Pulwama, Srinagar, and Budgam districts, accounting for 4%, 76%, 6%, and 14%, respectively, of the total area [6]. Throughout the last decade, the productivity of saffron in India has been in continuous decline, due to regional climate variability. such as flood, frequent drought [7], and outbreaks of biotic stresses [8]. The production of saffron is severely affected by different diseases caused by fungi and bacteria [9,10].

Corm rot of saffron is caused by various soil-borne pathogens, which impose serious economic losses and are broadly prevalent throughout the saffron-growing areas of the world [11–18]. Corm rot is of immense importance in the Kishtwar District, an upland valley situated in the northeast corner of Jammu, where saffron is a major source of earnings for the farmers [19]. Saffron is exclusively vegetatively propagated by corms [20] and a perennial crop (four years planting cycle) cultivated under rain-fed conditions [19]. Corm rot incidence and intensity ranged from 4 to 42 and 0.80% to 17.46%, respectively, in Kashmir during 1999–2000 [21], and 59.33% and 35%, respectively, in Kishtwar during 2016 [22]. The disease is primarily characterized by the yellowing and wilting of the shoots [23] that cause the decline in the production of saffron flower and stigma, whereas rotting and decay of corms [24] reduce the production of daughter corms. Among the various plant pathogens associated with corm rot, *Fusarium oxysporum* (Schlecht.) is the most predominant one, occurring worldwide [25,26]. *Fusarium oxysporum* is a soil-borne pathogen causing diseases in many important crops [27]. It survives in the infected corms and in soil, forming mycelium, microconidia, and macroconidia, along with resting structures (chlamydospore), which are resistant to desiccation and unfavorable environmental conditions. The resting structures have the ability to survive in the soil for more than 20 years in the absence of a host in the Fusarium–banana pathosystem [28,29], and are mainly dispersed through infected corms [30] and farming tools. Excessive soil moisture, injuries to the corms due to inter-cultural operations and nematodes [31], poor soil aeration [32], high temperatures [27,33], and poor drainage are the primary factors that predispose the saffron crop to the epidemic of corm rot.

Because of the lack of improved resistant genotypes due to sterile triploid, the disease is mainly managed by chemical fungicides [13,34]. However, their continuous application not only has a deleterious impact on environment, beneficial microflora, and human health [35], but it is also responsible for evolution of resistant races of the Fusarium pathogens [36]. Application of biocontrol agents has emerged as a sustainable alternative strategy for managing soil-borne plant pathogens, as it reduces the disease-producing activity of pathogens through a multi-faceted mechanism [37,38], and, besides this, has no deleterious impact on human health or the environment [39]. Biocontrol may be achieved by introducing a prospective microorganism or using indigenous microorganisms that have potential to delimit the survival and dispersal of the pathogen [25]. Since the crop is export-oriented, the indigenous and putative biocontrol agents were explored for the management of corm rot of saffron caused by *F. oxysporum*, with an aim to test the efficacy of biocontrol agents: (1) in reducing the disease incidence of corm rot; (2) on population of *Fusarium oxysporum*; and (3) on promotion of certain growth parameters.

2. Materials and Methods

2.1. Multiplication of Pathogen

Fusarium oxysporum isolated from the diseased corms collected from Kishtwar were identified by morphological and molecular techniques (GenBank accession no. MT395512). The pathogen was preserved in potato dextrose agar (PDA, HiMedia, Mumbai) slants at −4 °C and sub-cultured every two weeks. For conidia production of *F. oxysporum*, the plugs (5 mm) of actively growing mycelia were aseptically transferred in an Erlenmeyer flask (500 mL) containing 350 mL of potato dextrose broth (PDB, HiMedia, Mumbai) and incubated at 26 ± 2 °C in a Biological Oxygen Demand (BOD) incubator. After twenty days, the flask was vigorously shaken with the addition of sterilized distilled water (50 mL). The colony forming unit (cfu) of *F. oxysporum* culture was assessed by a serial dilution agar plating method [40].

2.2. Multiplication of Biocontrol Agents

The fungal and bacterial biocontrol agents used in these experiments were *Trichoderma asperellum* (Samuels, Liech & Nirenberg) (GenBank accession no MT395682), *Pseudomonas fluorescens* Migula (GenBank accession no KJ194131), *Pseudomonas aeruginosa* (Schroeter) Migula (GenBank accession no. KJ194126), *Pseudomonas putida* Trevisan (GenBank accession no. KJ194130), *Bacillus stratosphericus* Shivaji (GenBank accession no KY423499), *Bacillus pumilus* Meyer and Gottheil (GenBank accession no KY423498), and *Bacillus subtilis* (Ehrenberg) Cohn (GenBank accession no MT395353). All the biocontrol agents were isolated from saffron fields of the Kishtwar District, and properly characterized on the bases of morphology, molecular, and biochemical tests [41,42]. All the selected biocontrol agents were sub-cultured regularly and maintained on their respective media at −4 °C (*Trichoderma asperellum* on potato dextrose agar, *P. fluorescens, P. aeruginosa*, and *P. putida* on King's B agar, and *B. stratosphericus*, *B. pumilus*, and *B. subtilis* on Luria Bertani agar medium). For mass production of these biocontrol agents, *T. asperellum* plugs (5 mm) were transferred in an Erlenmeyer flask (500 mL) containing potato dextrose broth (PDB, HiMedia, Mumbai), *P. fluorescens, P. aeruginosa*, and *P. putida* were transferred in the King's B broth (KB, HiMedia, Mumbai), and *B. stratosphericus*, *B. pumilus*, and *B. subtilis* were transferred in Luria Bertani broth (LB, HiMedia, Mumbai). All the inoculated Erlenmeyer flasks containing the fungal and bacterial biocontrol agents were incubated separately in the BOD at 26 ± 2 °C. After the growth, the flasks were agitated vigorously, mixed with sterilized distilled water (50 mL), and subjected to serial dilution agar plating assay for calculating the concentration of colony forming unit/mL.

2.3. In Vitro Evaluation of Biocontrol Agents

To evaluate the antagonism potential, *T. asperellum*, *P. fluorescens*, *P. aeruginosa*, *P. putida*, *B. stratosphericus*, *B. pumilus*, and *B. subtilis* were tested against *F. oxysporum*, using the dual culture technique [43]. Five mm discs of *T. asperellum* and *F. oxysporum* were taken from the margins of actively growing cultures (seven days old) and transferred to Petri plates (90 mm) containing PDA medium, on opposite sides, one cm away from the periphery of the Petri plate. In case of bacterial antagonists, *P. fluorescens*, *P. aeruginosa*, *P. putida*, *B. stratosphericus*, *B. pumilus*, and *B. subtilis* were streaked individually on one side of the Petri plate containing potato dextrose agar medium and 5 mm disc of *F. oxysporum* was placed at the opposite sides of the streaked Petri plates. Efficacy of carbendazim (100 µg mL^{-1}) was checked by poison food assay [44]. Petri plates containing PDA amended with the desired concentrations of fungicide (100 µg mL^{-1}) were inoculated in the center with a 5 mm disc taken from seven-day-old culture of *F. oxysporum*. Pure culture of *F. oxysporum* was also maintained as a control. The details of treatments are as follows: T_1 = *Bacillus stratosphericus*, T_2 = *B. pumilus*, T_3 = *B. subtilis*, T_4 = *Pseudomonas aeruginosa*, T_5 = *P. putida*, T_6 = *P. fluorescens*, T_7 = *Trichoderma asperellum*, T_8 = carbendazim, and T_9 = control (only *Fusarium oxysporum*). The experiment was laid in a completely randomized design (CRD), with four replications. The plates were incubated at 27 ± 1 °C, and mycelial growth (mm) of *F. oxysporum* was recorded in different treatments, including control, after 15 days. Percentage inhibition of the mycelium was calculated as: inhibition (%) = $((T_1-T_2)/T_1) \times 100$, where T_1 = growth of the *F. oxysporum* in control, and T_2 = growth of the *F. oxysporum* in different treatments (T_1-T_8).

2.4. Management of Corm Rot of Saffron under Field Conditions

A field experiment was conducted from the first week of August 2017 to November 2018, to evaluate biocontrol agents and a chemical fungicide to manage the corm rot of saffron. The studies were conducted in a corm-rot-infested field in Upper Pochhal of Kishtwar District, situated at 75.7667° E and latitude of 33.31° N, and at an altitude of 1638 m above sea level. The field was thoroughly ploughed and properly levelled for the sowing of healthy corms. The experiment was conducted in 3 m^2 plots, each having 18 corms/plot in a randomized block design (RBD) with nine treatments and four

replications. The details of treatments are as follows: T_1 = corm treatment with *Bacillus stratosphericus* at 1×10^9 cfu/mL; T_2 = corm treatment with *B. pumilus* at 1×10^9 cfu/mL; T_3 = corm treatment with *B. subtilis* at 1×10^9 cfu/mL; T_4 = corm treatment with *Pseudomonas aeruginosa* at 1×10^9 cfu/mL; T_5 = corm treatment with *P. putida* at 1×10^9 cfu/mL; T_6 = corm treatment with *P. fluorescens* at 1×10^9 cfu/mL; T_7 = corm treatment with *Trichoderma asperellum* at 1×10^8 cfu/mL; T_8 = corm treatment with carbendazim 50 WP (M/s BASF India Limited, New Delhi) at 0.2%; and T_9 = corm treatment with water (control). Before sowing, 2000 kg of healthy corms (Kishtwar cultivar), each approximately 10 g, were dipped in the suspension of *F. oxysporum* (1×10^7 cfu/mL) for 10 min, and subsequently dipped in the suspensions of different treatments for 30 min. In treatment T_9 (control), the healthy corms were treated with *F. oxysporum* inoculums only. All the treated corms were shade dried for 30 min before sowing. Data regarding disease incidence (%) calculated as (total number of plants infected in selected treatment/total number of plants in selected treatment × 100), disease reduction efficiency (%) calculated as (disease incidence of control − disease incidence of treatment/disease incidence of control × 100) during 2017 (80 days after sowing) and 2018 (430 days after sowing) [45,46], days taken for sprouting (emergence of corm lets/buds), and flowering after sowing during 2017 were recorded.

2.5. Dynamics of Fusarium oxysporum Propagules

For assessing the effects of different treatments on the survival and persistence of the *F. oxysporum* in the treated field, population count was assessed using the Warcup soil plating method [47] on specific Komada modified (KM) medium [48]. Soil samples (250 g) from each treatment plot were collected randomly from the cormosphere, without disturbing the plant, in pre-sterilized polythene bags at different intervals, such as before sowing, 75, and 260 days after sowing. Air-dried soil (0.5 g) was placed in sterilized Petri plates of different treatments, with four replications each, to which 15–20 mL of melted, cooled (45 °C) KM medium was added. Gentle rotation of Petri plates clockwise and anticlockwise was done to ensure uniform mixing of soil with medium, and the plates were incubated at 25 °C in the BOD in an inverted position. After four days of incubation, the plates were observed daily for the emergence of the fungal colonies. Developing colonies were counted visually, and identified based on morphological characters.

2.6. Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) using the statistical package R (1.14.4) [49]. The values of the replication, which included mycelial growth (mm) of the *F. oxysporum*, disease incidence (%), population dynamics of *F. oxysporum* (cfu/g), and emergence of sprouting and flowering (days after sowing) among the treatments were compared using Duncan's new multiple range test (DNMRT) by agricolae package [50]; *p*-values (<0.05) were considered to be statistically significant.

3. Results and Discussion

3.1. In Vitro Evaluation

In the present study, dual plate and poison food assays, we demonstrated the antagonistic effect of different treatments against *F. oxysporum* (Figure 1). *T. asperellum* and carbendazim significantly ($p \leq 0.05$) reduced the radial growth of *F. oxysporum* (24.79 and 26.76 mm, respectively), compared to control. Among the bacterial antagonists, *P. fluorescens* reduced mycelial growth of *F. oxysporum* by 34.02 mm. This was followed by *P. putida, P. aeruginosa*, and *Bacillus subtilis*, reducing mycelial growth of *F. oxysporum* by 43.47, 45.22, and 43.19 mm, respectively, which were statistically similar to each other. The maximum radial growth of *F. oxysporum* (54.70 and 67.30 mm) was recorded by *B. pumilus* and *B. stratosphericus*, respectively.

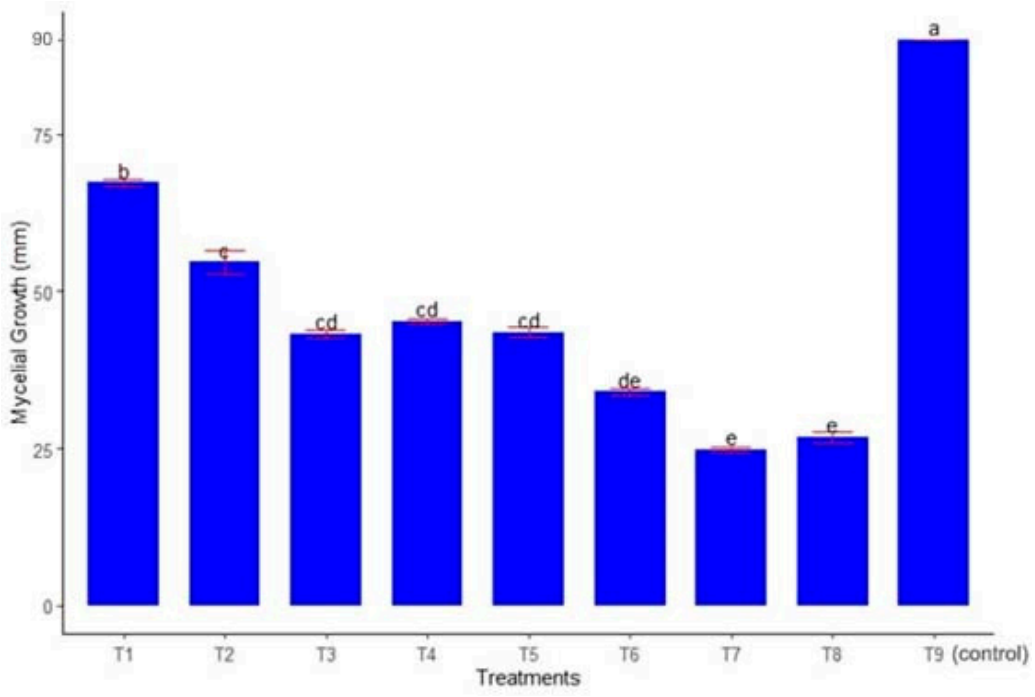

Figure 1. Mycelial growth rate (mm) of *Fusarium oxysporum* treated with biocontrol agents and chemical. Bars represent mean (±standard error) of mycelial growth rate (mm) of *Fusarium oxysporum* treated with T_1 = *Bacillus stratosphericus*; T_2 = *Bacillus pumilus*; T_3 = *Bacillus subtilis*; T_4 = *Pseudomonas aeruginosa*; T_5 = *Pseudomonas putida*; T_6 = *Pseudomonas fluorescens*; T_7 = *Trichoderma asperellum*; T_8 = carbendazim, and T_9 = control (only *Fusarium oxysporum*). Means followed by the same letter do not significantly differ ($p < 0.05$) with DNMRT.

Previous studies have also proved the efficacy of *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus* spp. for the inhibition of radial growth of *F. oxysporum* f. sp. *gladioli* [8,21,51]. Our results are in conformity to those findings. Several other researchers have also reported *Trichoderma viride* and *Trichoderma harzianum* as potential antagonists for inhibiting the growth of several soil- and seed-borne plant pathogens by possessing various mechanisms of action, such as mycoparasitism, competition for nutrients/space, and production of volatile compounds [52,53]. *Bacillus amyloliquefaciens* has been reported to significantly inhibit the radial growth of *F. oxysporum*, causing corm rot of saffron, which may be due to the production of antibiotics, siderophores, and enzymes [25]. *Burkholderia cepacia*, *Bacillus subtilis*, and *P. fluorescens* have also been observed to significantly inhibit the mycelial growth of *F. oxysporum* f. sp. *gladioli*, with the inhibition ranging from 6 to 10 mm [54]. Inhibition of mycelial growth of *F. oxysporum* and *Fusarium solani* reported in this work is similar to the inhibition previously reported for chemical fungicides [34]. Carbendazim is a benzimidazole group of fungicides that interfere with the energy production and cell wall synthesis of fungi [55], and also induce nuclear instability by disturbing the cell division [56].

3.2. Disease Incidences of Corm Rot of Saffron

Biocontrol agents and carbendazim were evaluated for the management of corm rot of saffron during 2017–2019 in a naturally-infested, diseased field (Table 1). In the first year, maximum suppression in disease incidence (82.77%) was recorded when the corms were treated with carbendazim (T_8), which was statistically similar with the *T. asperellum* T_7 (77.84%). During the second year, a maximum disease reduction of 68.63% was observed with *T. asperellum* (T_7), which was statistically similar with *P. fluorescens* (T_6), followed by *P. putida* (T_5), and carbendazim (T_8), having disease reduction of 58.88, 51.67, and 48.24%, respectively. The maximum disease incidence of 83.33% was observed in control (T_9).

Table 1. Field experiment of percentage incidence of corm rot of saffron in different treatments during 2017 and 2018. T_1 = *Bacillus stratosphericus* at 1×10^9 cfu/mL; T_2 = *B. pumilus* at 1×10^9 cfu/mL; T_3 = *B. subtilis* at 1×10^9 cfu/mL; T_4 = *Pseudomonas aeruginosa* at 1×10^9 cfu/mL; T_5 = *P. putida* at 1×10^9 cfu/mL; T_6 = *P. fluorescens* at 1×10^9 cfu/mL; T_7 = *Trichoderma asperellum* at 1×10^8 cfu/mL; T_8 = carbendazim 50 WP at 0.2%; and T_9 = water (control). ± is the standard error of the mean of disease incidence. * Means followed by the same letter do not significantly differ ($p < 0.05$) with DNMRT. Disease incidence = Total number of plants infected in selected treatment/total number of plants in selected treatment × 100. Disease reduction efficacy = Disease incidence of control - disease incidence of treatment/disease incidence of control × 100.

Treatment	Disease Incidence (%)		Disease Reduction Efficacy (%)	
	1st Year	2nd Year	1st Year	2nd Year
T_1	40.27 ± 1.39 ^{ab} *	71.22 ± 3.58 ^{ab}	28.63	14.52
T_2	36.10 ± 3.20 ^{ab}	66.11 ± 0.55 ^{abc}	36.02	20.65
T_3	22.22 ± 0.01 ^{ab}	47.49 ± 0.83 ^{bcd}	60.62	42.99
T_4	37.50 ± 2.77 ^{ab}	50.00 ± 2.04 ^{bcd}	33.54	39.99
T_5	31.00 ± 5.55 ^{ab}	40.27 ± 1.39 ^{cd}	45.06	51.67
T_6	21.00 ± 2.78 ^{ab}	34.50 ± 0.73 ^{d}	62.78	58.88
T_7	12.50 ± 2.77 ^{b}	26.13 ± 1.34 ^{d}	77.84	68.63
T_8	9.72 ± 2.97 ^{b}	43.12 ± 1.41 ^{cd}	82.77	48.24
T_9	56.43 ± 10.84 ^{a}	83.32 ± 2.26 ^{a}		

The present observations are in conformity to the results obtained by earlier researchers, as benzimidazole (carbendazim) fungicides play an important role in the management of soil- and seed-borne diseases by inhibiting DNA synthesis and blocking the nuclear division in pathogenic fungi [57]. Corm rot reduction of 85.49% was recorded by dipping the corm in mancozeb 75 WP (0.3%) + carbendazim 50 WP (0.1%), in saffron-growing areas of Kashmir [58]. Other researchers have also demonstrated that the chemical fungicide (carbendazim) was very effective in managing corm rot of saffron under pot and field conditions [8,59]. However, there is growing concern about the deleterious impacts of synthetic chemicals on the environment, as well as human beings, along with pesticide residues in the product [60]. It has caused the awareness to explore alternative methods, which are eco-friendly and sustainable, for the management of soil-borne diseases. Nowadays, beneficial microbes are widely applied for the management of soil-borne diseases in various agricultural production systems [61]. *Bacillus* spp., due to the advantages of producing heat and desiccation-resistant spores, are reported to significantly reduce the corm rot incidence (40%) of saffron under pot conditions [25], which may be due to the competition for energy, food, and ecological niche or substrate, production of inhibitory allelochemicals, and induction of systemic resistance [62]. *Pseudomonas fluorescens* has also been applied as a potential biocontrol agent for the management of many diseases in agriculture and horticultural crops [63,64]. In the present study, carbendazim, fungal and bacterial biocontrol agents gave better management of corm rot of saffron than the control.

3.3. Dynamics of *F. oxysporum* Propagules

Besides suppressing the incidence of saffron corm rot, different treatments evaluated in the present study also significantly reduced the propagule density of *Fusarium oxysporum* in the soil. The population of *F. oxysporum* was drastically reduced after 75 and 260 days of sowing of saffron crop in all the treatments except control (Table 2). At 75 days after sowing (DAS), a maximum reduction in the population by 60% was recorded in *T. asperellum* (T_7), which was significantly higher than in all other treatments. However, treatments *B. stratosphericus* (T_1), *B. pumilus* (T_2), *B. subtilis* (T_3), *P. aeruginosa* (T_4), *P. putida* (T_5), *P. fluorescens* (T_6), and carbendazim (T_8) were statistically similar in reducing the *F. oxysporum* population by 29.77, 36.38, 35.85, 37.44, 37.77, 40.41, and 44.44%, respectively (Figure 2). At 260 DAS, treatments *T. asperellum* (T_7) and *P. fluorescens* (T_6) were statistically similar in reducing the population of *F. oxysporum* by 80.19 and 68.75%, respectively, followed by *P. aeruginosa* (T_4) and

P. putida (T_5). The minimum reduction in propagules (4.44%) was observed with carbendazim (T_8). The results obtained in the present study are in conformity to the observations made by other researchers. Applications of *T. harzianum*, *Trichoderma hamatum*, and *T. viride* have been reported to reduce the populations of *F. oxysporum* and *Rhizoctonia solani* propagules by 46–56% and 31–44%, respectively, after two months of planting chickpea, whereas the reductions of 36–54% and 15–27% were observed after four months [65]. Bacterial biocontrol agents (*Bacillus* and *Pseudomonas* spp.) gradually become ideal candidates for the biological control, due to their simple nutrition requirements, faster colonization ability, rapid reproduction, endogenous spores, and strong resistance to stress [64]. Carbendazim has been observed to decrease the populations of Fusarium in chickpea by 30–42 and 26–30% at two and four months, respectively, by other researchers also [65]. Even the seed treatment with *Trichoderma* spp. isolates reduced the *F. oxysporum* f. sp. *lycopersici* population under greenhouse and field conditions, and were also responsible for the reductions in tomato wilt incidence [66].

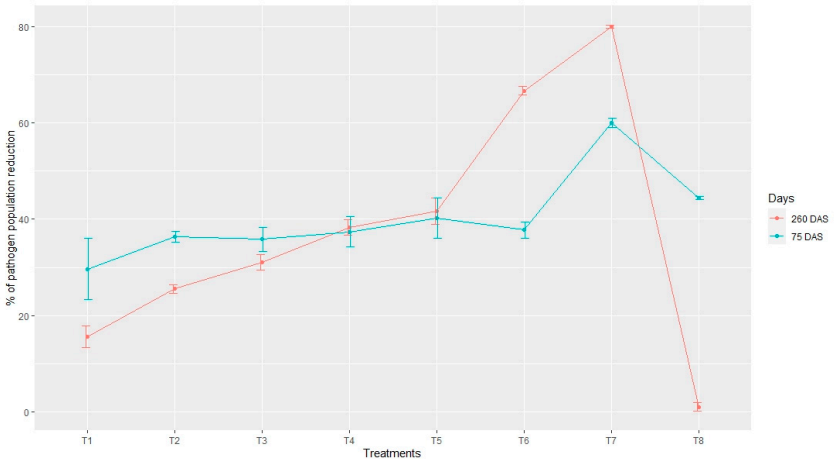

Figure 2. Percentage reduction of population of *Fusarium oxysporum* by treatments T_1 to T_8, compared with control treatment (T_9) at 75 and 260 days after sowing (DAS). T_1 = *Bacillus stratosphericus* at 1×10^9 cfu/mL; T_2 = *B. pumilus* at 1×10^9 cfu/mL; T_3 = *B. subtilis* at 1×10^9 cfu/mL; T_4 = *Pseudomonas aeruginosa* at 1×10^9 cfu/mL; T_5 = *P. putida* at 1×10^9 cfu/mL; T_6 = *P. fluorescens* at 1×10^9 cfu/mL; T_7 = *Trichoderma asperellum* at 1×10^8 cfu/mL; and T_8 = carbendazim 50 WP at 0.2%.

Table 2. Colony forming units (CFUs) recovering of *Fusarium oxysporum* in saffron field at different days after sowing (DAS). T_1 = *Bacillus stratosphericus* at 1×10^9 cfu/mL; T_2 = *B. pumilus* at 1×10^9 cfu/mL; T_3 = *B. subtilis* at 1×10^9 cfu/mL; T_4 = *Pseudomonas aeruginosa* at 1×10^9 cfu/mL; T_5 = *P. putida* at 1×10^9 cfu/mL; T_6 = *P. fluorescens* at 1×10^9 cfu/mL; T_7 = *Trichoderma asperellum* at 1×10^8 cfu/mL; T_8 = carbendazim 50 WP at 0.2%; and T_9 = water (control). ± is the standard error of the mean of *Fusarium oxysporum* (cfu/g). * Means followed by the same letter do not significantly differ ($p < 0.05$) with DNMRT.

***Fusarium oxysporum* (cfu/g)**			
Treatment	**Before Sowing ($\times 10^4$)**	**75 DAS ($\times 10^3$)**	**250 DAS ($\times 10^2$)**
T_1	4.50 ± 0.408 ^{a} *	3.16 ± 0.121 ^{b}	3.80 ± 0.058 ^{c}
T_2	4.70 ± 0.004 ^{a}	2.99 ± 0.023 ^{b}	3.50 ± 0.020 ^{cd}
T_3	4.49 ± 0.298 ^{a}	2.88 ± 0.054 ^{bc}	3.10 ± 0.029 ^{de}
T_4	4.70 ± 0.027 ^{a}	2.94 ± 0.066 ^{bc}	2.90 ± 0.027 ^{e}
T_5	4.50 ± 0.064 ^{a}	2.80 ± 0.007 ^{bc}	2.80 ± 0.033 ^{e}
T_6	4.80 ± 0.057 ^{a}	2.86 ± 0.075 ^{bc}	1.50 ± 0.001 ^{f}
T_7	5.00 ± 0.043 ^{a}	2.00 ± 0.013 ^{c}	0.99 ± 0.002 ^{f}
T_8	4.50 ± 0.001 ^{a}	2.50 ± 0.009 ^{bc}	4.30 ± 0.064 ^{b}
T_9	4.50 ± 0.004 ^{a}	5.49 ± 0.011 ^{a}	5.99 ± 0.004 ^{a}

3.4. Growth Parameters

In our studies, minimum days (average) were taken for sprouting (30, 29, 29, 28, 28, 27, and 26 days) and also for flower emergence (86.25, 81.50, 79.00, 78.25, 78.00, 77.25, and 77.25 days) in T_1 to T_7 treatments, respectively as compared to T_8 and T_9 treatments. Whereas, maximum days (average) taken for sprouting and emergence of flowers (35 and 90 DAS) were observed in control (Figure 3). Other studies have also shown an effect on agronomical variables, whereby the application of beneficial microorganisms (*Azospirillum* spp. + Vesicular-arbuscular mycorrhiza VAM + *Pseudomonas* spp.) increase in the leaf number (34.1), plant height (36.3 cm), and number of flowers (385/plot) in saffron crop, compared to control (21.9, 28.0, and 364 cm, respectively), was observed [67]. Combined application of *Bacillus atrophaeus* (S2BC-2) and *Burkholderia cepacia* (TEPF-Sungal) has also shown reductions in the vascular wilt and corm rot incidences by 48.6 and 46.1%, respectively, resulting in increased spike and corm yield by 58.3 and 27.40%, respectively, in comparison to the control, in gladiolus. This may be due to the activation of defense gene products, such as chitinase and β-1, 3-glucanase [53]. To promote sustainable agriculture, application of plant-growth-promoting fungi (PGPF) and plant-growth-promoting bacteria (PGPB) are widely advocated in a range of different crops for amelioration of biotic and abiotic stresses. Being inhabitants of outer and internal tissues of host, these beneficial microbes promote plant growth by having diverse mechanisms, such as production of useful metabolites and signals, phytohormones, biofertilization, phytostimulation, antibiotic resistance, bioremediation, rhizosphere engineering, lytic enzymes, or increased resistance to osmotic stress and other abiotic factors [64,68].

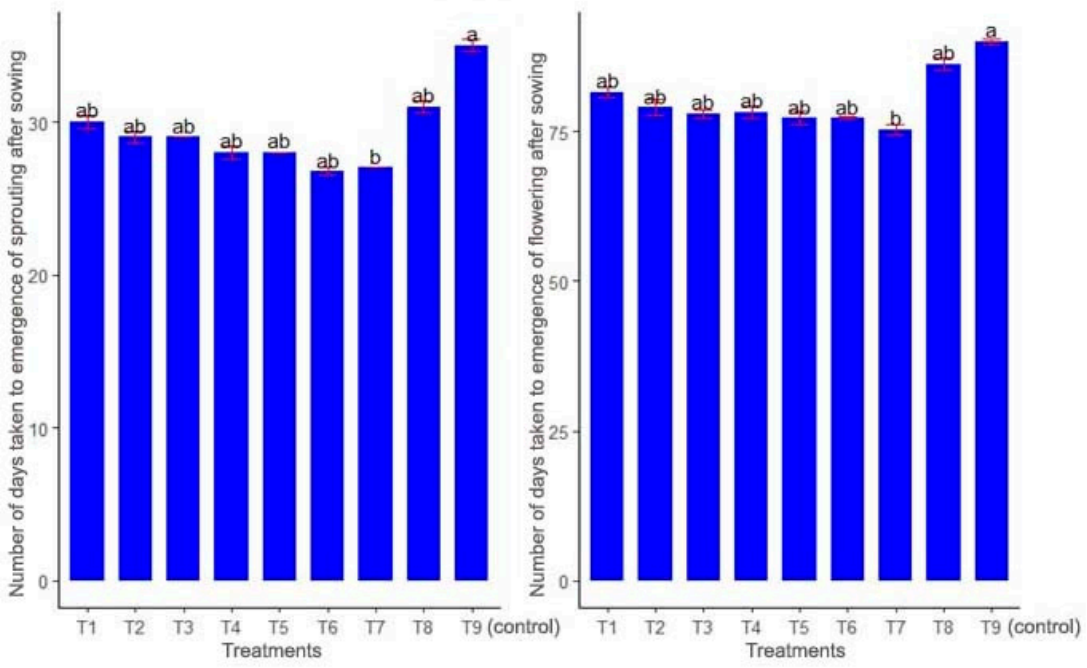

Figure 3. Effect of different treatments on the sprouting and flowering of saffron. Bars represent mean (± standard error) of sprouting and flowering of saffron in T_1 = *Bacillus stratosphericus* at 1×10^9 cfu/mL; T_2 = *B. pumilus* at 1×10^9 cfu/mL; T_3 = *B. subtilis* at 1×10^9 cfu/mL; T_4 = *Pseudomonas aeruginosa* at 1×10^9 cfu/mL; T_5 = *P. putida* at 1×10^9 cfu/mL; T_6 = *P. fluorescens* at 1×10^9 cfu/mL; T_7 = *Trichoderma asperellum* at 1×10^8 cfu/mL; T_8 = carbendazim 50 WP at 0.2%; and T_9 = water (control). Means followed by the same letter do not significantly differ ($p < 0.05$) with DNMRT.

4. Conclusions

The observations recorded in the present studies suggest that carbendazim is useful in managing corm rot of saffron, but in order to minimize the dependence on chemical pesticides in such a valuable

crop, alternative methods, like the use of biocontrol agents, are very essential. It was observed that *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Trichoderma asperellum* successfully managed the corm rot of saffron caused by *Fusarium oxysporum*. These biocontrol agents not only reduced the disease incidence and pathogen population in the soil, but also improved certain growth parameters, such as reducing the days for sprouting and flowering in saffron crop. The persistence of the biocontrol agents in the soil plays a vital role in managing the plant disease, especially in perennial crops. Further research is, however, needed to develop the consortia of putative and native strains and their suitable formulation, along with the elucidation of the mechanisms involved in the growth promotion and disease suppression in saffron. Effects of biocontrol agents in regulation of quality parameters may also be examined in order to increase the eminence of this functional food.

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