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RESEARCH ARTICLE

In vitro evaluation of biocontrol agents against basal rot of garlic

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Abstract

Basal rot is a soil borne disease caused by *Fusarium oxysporum* f. sp. *cepae.* It is known to affect garlic production throughout the world. Various strategies such as crop rotation and raising of resistant varieties have been tried to limit the damage caused due to this fungus. Biocontrol is another effective option that can be used to control the spread of disease. Biocontrol process is generally harmless to humans, a non-polluting, biodegradable and selective mode of action. Moreover, pathogens cannot develop resistance easily, no harm is caused to other beneficial microorganisms, improve soil health and help in achieving agricultural sustainability. Efficacy of fungal and bacterial bioagents were checked against the basal rot of garlic. The potential of six species of fungal species viz. *Trichoderma harzianum, Trichoderma viridae*, *Trichoderma virens, Trichoderma hamatum,* bacterial species viz. *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *F. oxysporum* under *in vitro* conditions. Dual culture and streak plate method was used evaluation of fungal and bacterial antagonists. Among the fungal antagonists maximum inhibition of mycelial growth was noted in *Trichoderma viridae* (74 %), *Pseudomonas fluorescens* proved to be the most effective bacteria in reducing (30 %) the mycelial growth.

Keywords: basal rot; bioagents; *Fusarium oxysporum* f sp. *cepae*; mycelial growth inhibition; *Trichoderma*

Introduction

Garlic (*Allium sativum* L.) is the second most extensively cultivated crop after onions. It is a member of the Alliaceae family and is produced on a large scale ranging from temperate to subtropical climate areas of the world (1). Garlic cultivation and production have been expanding globally every year. India leads in global garlic production while China is the second largest producer. According to estimates of 2018, about 28.5 million tonnes of garlic were produced throughout the world, of which 26.1 million tonnes were produced in Asia, 0.86 million tonnes in Europe, 0.83 million tonnes in the Americas and 0.73 million tonnes in Africa (1). With a productivity of 8.14 million tonnes per hectare, India produces 391 thousand hectares and 3185 thousand metric tonnes of garlic, respectively (2). Garlic is planted on a large scale in Himachal Pradesh. About 719000 hectares of land is under garlic cultivation giving a yield of 1160000 metric tonnes. The productivity is about 161 tonnes per hectare (2).

Garlic is considered a topical and systemic antibacterial agent. Because of its immense healing power, garlic is sometimes referred to as Russian penicillin (3). *Allium sativum* has high nutritional value, it contains about 65 % of water, 28 % of carbohydrates, 2.3 % organosulfur compounds, 2 % protein, 1.2 % of free amino acids and 1.5 % of fibre. 100 g of raw garlic contains 58.58 g water, 33.06 g carbohydrates and 6.36 g protein. Additionally, the bioactive components such as organic sulphides (4,5), saponins (6), phenolic compounds (7) and polysaccharides (8) present in garlic contribute to its therapeutic effects.

Research indicates that soil borne fungi are one of the most important pathogens that affect garlic. *Fusarium oxysporum* f. sp. *cepae*, *Fusarium moniliforme* var. *subglutnous*, *Fusarium bulbigenum, Fusarium solani* and *Fusarium equiseti* are some of the *Fusarium* species causing significant loss of the crop (9,10). Basal rot, one of the major diseases of garlic, is primarily caused by *Fusarium oxysporum* (11-13). Basal rot caused by *Fusarium* affects the production of garlic throughout the world. *Fusarium* Basal Rot (FBR) is also known as *Fusarium* rot, *Fusarium* wilt or basal plate rot of *Allium* species. It leads to seedling disease known as "damping-off" or "dieback" disease (14). Plant species show susceptibility to disease causing fungus *Fusarium oxysporum*. This fungus is a parasitic saprophyte which can survive for a very long time as klamidiospora. The fungus attacks the roots, particularly the injured roots. The damage can be more if the temperature ranges between 20 °C to 29 °C. The proliferation of fungus increases under rich nitrogen soil conditions (15-20).

Therefore, this study was conducted with the objective of evaluating the effects of various biocontrol agents in control of the pathogen causing basal rot in garlic using *in vitro* studies.

Material and Methods

The present studies were conducted in the laboratory of Department of Plant Pathology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh which is situated at an elevation of 1250 m at 30°51' N latitude and 77°11' E longitude above the mean sea level. During the investigation, the effect of various fungal and bacterial biocontrol agents (*T. harzianum, T. viride, T. virens, T. hamatum, Pseudomonas fluorescens, Bacillus* sp.) on mycelial growth of *Fusarium oxysporum* f. sp. *cepae* was studied by dual culture method and streak plate methods respectively, under *in vitro* conditions (16,17). The experiment was conducted in a completely randomized design with three replications each.

A 5 mm mycelial disc of *F. oxysporum* was placed on one side of the petri plate and antagonists were placed on the opposite side near the periphery of the petri plate. The petri plate was then incubated at 26 ± 2 °C. After 5 days of incubation, mycelial growth of the pathogen and the inhibition zone were measured in treated and control plates. The per cent inhibition of mycelial growth was calculated using the standard procedure and formula (21).

$$I = \frac{C-T}{C} \times 100 \qquad \text{(Eqn. 1)}$$

Where,

I = Inhibition (%)

C = Linear growth in control (mm)

T = Linear growth in treatment (mm)

Results and Discussion

Efficacy of fungal and bacterial bioagents *viz.* 6 unidentified spp. of *Trichoderma; T. harzianum, T. viridae*, *T. virens, T. hamatum, Pseudomonas fluorescens* and *B. subtilis* were evaluated against *F. oxysporum* under *in vitro* conditions by following dual culture and streak plate method for fungal and bacterial antagonists, respectively.

The results revealed that all the antagonists significantly reduced the growth of *F. oxysporum* (Table 1, Fig. 1). In fungal antagonists maximum mycelial growth inhibition was observed in *Trichoderma viridae* (74 %) with minimum mycelial growth of 16 mm followed by *Trichoderma hamatum* (72 %) with minimum mycelial growth of 18 mm. Minimum growth inhibition was observed in *T. virens* (63 %) with maximum mycelial growth of 27 mm. Among bacterial antagonists, minimum mycelial growth inhibition was observed in *Bacillus subtilis* (26 %) with mycelial growth of 64 mm whereas maximum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (30 %) with minimum mycelial growth of 60 mm.

Trichoderma spp. and *Pseudomonas* sp. were screened against *F. oxysporum* f. sp. *cepae* by the dual culture method (16,17). *Trichoderma* isolate was able to control the growth of the basal rot pathogen under *in vitro* conditions by dual culture method (18). Similarly, the antagonists significantly reduced the growth of *F. oxysporum* f. sp. *cepae* either by over growing or by exhibiting inhibition zones (19). Maximum reduction in colony growth was observed in *T. harzianum* (75.92 %) which was significantly superior over all other bioagents tested followed by *T. virens* (74.81 %), *T. koningii* (73.70 %) and *T. viride* (72.59 %), however, *B. subtilis* (57.4 %) and *P. fluorescens* (52.96 %) were least effective in inhibiting mycelial growth of the pathogen (Fig. 2-3). *Bacillus* sp. isolate B4 had the highest inhibition zone (66.16 %), followed by B. subtilis (B5) with a 59.03 % value (20). The results of the present investigation are in conformity with previous studies (21,22). They found that fungal antagonists *T. viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* and the bacterial antagonists *P. fluorescens* and *Bacillus subtilis* were effective against *F. oxysporum* f. sp. *cepae* under *in vitro* conditions. Similarly, *T. harzianum* strain KUEN-1585, *T. viride, T. harzianum, P. putida* and *B. subtilis* inhibited the growth of basal bulb rot pathogen under *in vitro* conditions (23,24). Research indicates that *T. harzianum* to be most effective in controlling growth of *F. oxysporum*.

Table 1. *In vitro* evaluation of biocontrol agents against *Fusarium* spp. associated with basal rot of garlic

Treatments	Mycelial growth (mm)	Mycelial growth inhibition (%)
	Fungal antagonists	
Isolate 1	22	68.00 (60.38)
Isolate 2	20	70.00 (61.91)
Isolate 3	19	71.00 (62.66)
Isolate 4	24	66.00 (58.92)
Isolate 5	25	65.00 (58.21)
Isolate 6	23	67.00 (59.64)
Trichoderma harzianum	20	70.00 (61.93)
Trichoderma viridae	16	74.00 (65.08)
Trichoderma virens	27	63.00 (56.82)
Trichoderma hamatum	18	72.00 (63.45)
	Bacterial antagonists	
Pseudomonas fluorescens	60	30.00 (35.26)
***Bacillus* subtilis**	64	26.00 (32.49)
Control	90	0.00
CD $_{0.05}$	0.08	2.84

Figure in the parentheses are arc sine transformed values

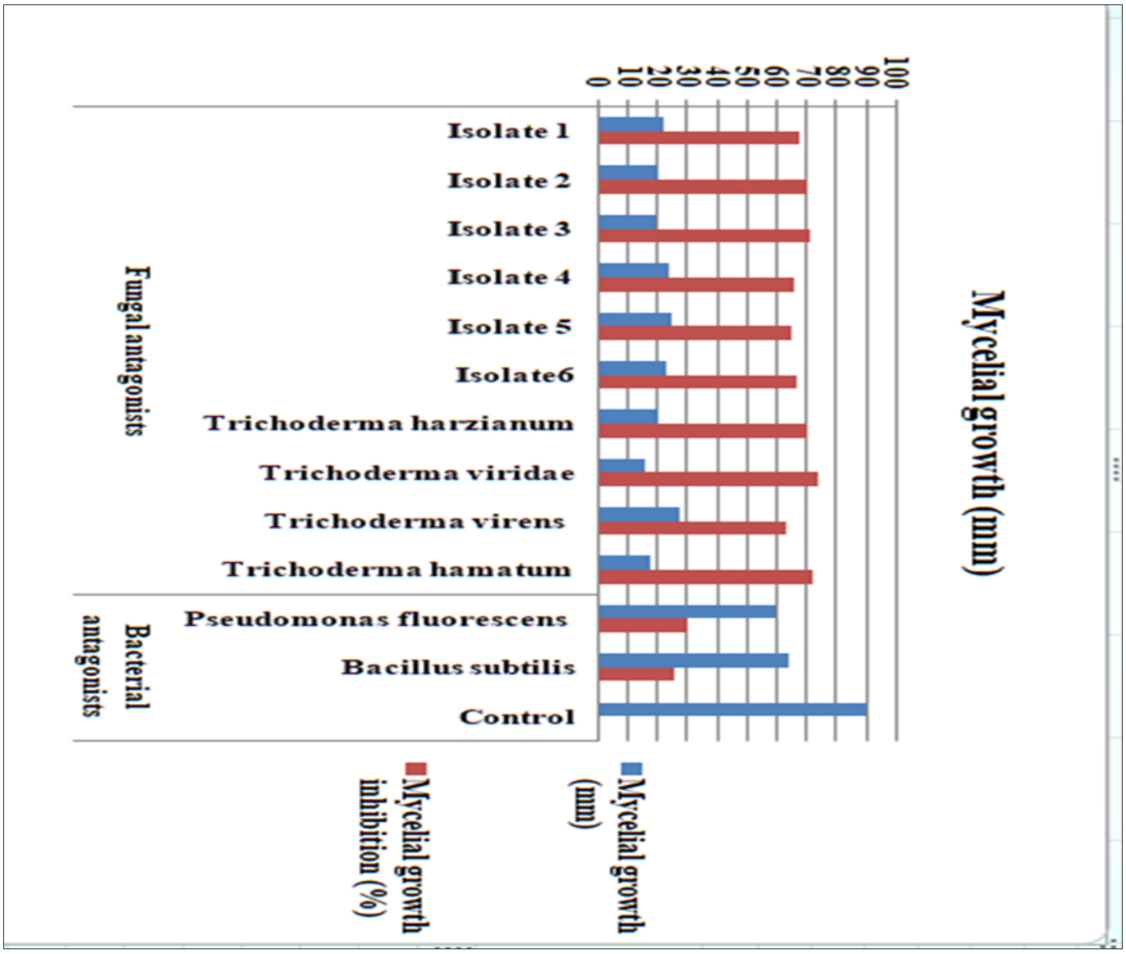

Fig. 1. Mycelial growth (mm) of different biocontrol agents against *Fusarium* spp. associated with basal rot of garlic. 1: Isolate 1; 2: Isolate 2; 3: Isolate 3; 4: Isolate 4; 5: Isolate 5; 6: Isolate 6; 7: *Trichoderma harzianum*; 8: *Trichoderma viridae*; 9: *Trichoderma virens*; 10: *Trichoderma hamatum*; 11: *Pseudomonas fluorescens*; 12: *Bacillus subtilis*; 13: Control

Fig. 2. *In vitro* evaluation of biocontrol agents against *Fusarium* spp. associated with basal rot of garlic (dorsal side). 1: Isolate 1; 2: Isolate 2; 3: Isolate 3; 4: Isolate 4; 5: Isolate 5; 6: Isolate 6; 7: *Trichoderma harzianum*; 8: *Trichoderma viridae*; 9: *Trichoderma virens*; 10: *Trichoderma hamatum*; 11: *Pseudomonas fluorescens*; 12: *Bacillus subtilis*; 13: Control

Fig 3. *In vitro* evaluation of biocontrol agents against *Fusarium* spp. associated with basal rot of garlic (ventral side). 1: Isolate 1; 2: Isolate 2; 3: Isolate 3; 4: Isolate 4; 5: Isolate 5; 6: Isolate 6; 7: *Trichoderma harzianum*; 8: *Trichoderma viridae*; 9: *Trichoderma virens*; 10: *Trichoderma hamatum*; 11: *Pseudomonas fluorescens*; 12: *Bacillus subtilis*; 13: Control

Conclusion

Present study showed that *Trichoderma viridae* (74 %) and *Pseudomonas fluorescens* (30 %) were best proven fungal and bacterial biocontrol agents against *Fusarium* spp. associated with basal rot of garlic respectively. Therefore, it is suggested that these fungal and bacterial antagonists may be used at larger scale to control the basal rot disease in garlic.

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Authors contributions

AK led the writing of the original draft, developed the methodology, performed formal analysis and investigation, contributed to data curation and visualization and secured funding for the study. MG conceptualized the research, provided supervision, contributed to data curation and participated in reviewing and editing the manuscript. SKS co drafted the manuscript, contributed to methodology and visualization and assisted in data curation and editing. SK co drafted the manuscript, contributed to methodology and visualization and assisted in data curation and editing. AS contributed to the conceptual framework, supported methodology development and was involved in data curation. SD participated in data collection and was actively involved in the experimental investigation. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest : Authors do not have any conflict of interest.

Ethical issues: None

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