



Compatibility of potential biocontrol agents for the development of a microbial consortium against major soil borne pathogens of chickpea

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Abstract

The success of a multi strain microbial consortium depends fundamentally on the compatibility of its constituent biocontrol agents as intra consortium antagonism may reduce overall efficacy. In the present study, mutual compatibility among four biocontrol agents *Trichoderma asperellum* UASR, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR were evaluated in all possible pairwise combinations. The fungal–bacterial and fungal–actinomycete interactions were assessed on potato dextrose agar by the dual culture technique, whereas bacterium–bacterium and bacterium–actinomycete pairs were tested on nutrient agar using the cross-streak method. All the bioagents tested were found to be mutually compatible in every possible pairwise combination with no zone of inhibition, growth suppression or lysis observed at the point of contact in any of the dual culture or cross streak assays. The *T. asperellum* grew unrestricted to the margins of bacterial cultures, *Streptomyces nenu* DS-11 extended into direct contact with each bioagent without suppression, *P. fluorescens* and *B. subtilis* grew freely up to their intersection on nutrient agar. These results establish the biological feasibility of combining all four bioagents into a single, broad spectrum microbial consortium and provide the basis for their subsequent evaluation alone and in combination against major soil borne pathogens of chickpea.

Keywords: Bioagents, chickpea, compatibility, microbial consortium, soil borne pathogens

Introduction

The development of a microbial consortium based biopesticide is a multistep process that begins with the isolation and individual screening of promising biocontrol agents. However, its ultimate success depends on the ability of the selected agents to coexist without exhibiting antagonistic interactions when combined (Poveda and Eugui, 2022) [9]. When biocontrol agents are combined without prior compatibility screening, detrimental interactions such as antibiosis, parasitism or competitive exclusion may nullify the intended synergistic effects of the consortium and in some cases make it less effective than the individual agents applied separately (Poveda and Eugui, 2022) [9]. Compatibility cannot be assumed just because each microbe works well on its own and it cannot be generalized to an entire genus or species. It has to be tested directly for the exact isolates planned to combine because even closely related strains of the same organism can interact very differently with a given partner (Prigigallo *et al.*, 2023) [10]. Recent evaluations of commercial BCA products confirm that antagonistic behaviour among candidate agents is strain and pathogen dependent reinforcing that compatibility testing is a prerequisite step not a formality (Porsche *et al.*, 2026) [8]. Compatibility testing, most commonly performed through the dual culture or cross streak technique therefore constitutes an essential step preceding consortium formulation since only combinations that show no mutual inhibition can be relied upon to deliver a genuinely synergistic effect under field conditions. This screening approach continues to drive consortium assembly across pathosystems: strain selection for *Pseudomonas*–*Bacillus* consortia is now made explicitly on the basis of co-cultivation compatibility (Negrelli *et al.*, 2025) [7].

Trichoderma species combined with *Bacillus* and *Pseudomonas* strains have generally been found to coexist well since the two groups draw on largely distinct antimicrobial mechanisms, fungal cell wall degrading enzymes and mycoparasitism produced by the *Trichoderma* and antibiotics, siderophores and volatile compounds produced by the bacteria, so in majority of the reported combinations neither partner suppresses the other *in vitro* (Poveda and Eugui, 2022) [9]. In chickpea specifically, *Trichoderma* based bioagents have recently been screened for their efficacy against both *Fusarium oxysporum* f. sp. *ciceris* and *Sclerotium rolfsii*, the two major soil borne pathogens of the crop underlining the continued relevance of well characterised compatible antagonists for managing this disease complex (Kumari *et al.*, 2024) [3]. In view of these considerations, the present study was undertaken to assess the *in vitro* compatibility of four potential biocontrol agents, *Trichoderma asperellum* UASR, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR in all possible pairwise combinations so as to identify a compatible set of bioagents suitable for formulation into a consortium against the soil borne pathogens of chickpea.

Material and Methods

The laboratory study was conducted at the Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur during 2025-26. The four biocontrol agents used in the study were *Trichoderma asperellum* UASR strain, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR strain, obtained from the Biocontrol laboratory, Department of Plant Pathology, UAS Raichur.

Compatibility between the fungal antagonist (*T. asperellum*) and each of the bacterial or actinomycete antagonists (*B. subtilis*, *S. nenu* and *P. fluorescens*) was assessed on potato dextrose agar (PDA) using the dual culture technique. A 5 mm mycelial disc of *T. asperellum* was placed at the centre of the plate and the bacterial or actinomycete culture was streaked along both edges of the plate equidistant from the fungal disc. Plates were incubated at 27 ± 1 °C and observed daily for narrowing of the fungal colony margin or thinning of the bacterial/actinomycete streak near the zone of contact either of which would indicate mutual inhibition. Compatibility between pairs of bacterial and actinomycete antagonists (*B. subtilis* × *S. nenu*, *B. subtilis* × *P. fluorescens* and *S. nenu* × *P. fluorescens*) was assessed on nutrient agar (NA) using the cross-streak technique. The two cultures were streaked at right angles to each other and plates were examined for the presence or absence of a zone of inhibition or lysis at the point of intersection of the two streaks. All compatibility tests were replicated thrice. A combination was scored as compatible ('+') when no inhibition zone, growth suppression or lysis was observed at the zone of contact between the two organisms and incompatible ('-') when such inhibition was recorded.

Results and Discussion

All the bioagents tested were found to be mutually compatible in every possible pairwise combination with no

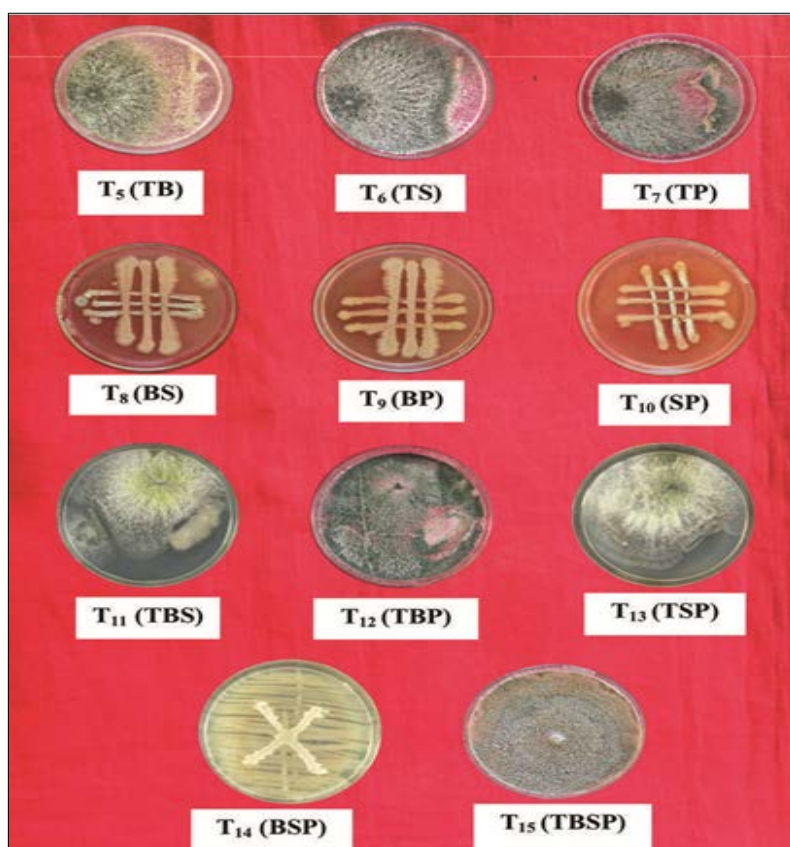
zone of inhibition, growth suppression or lysis observed at the point of contact in any of the dual culture or cross streak assays (Table1, Plate 1a and 1b). In the fungal-bacterial and fungal-actinomycete pairings, *T. asperellum* grew without restriction when paired against both *P. fluorescens* and *B. subtilis* showing no narrowing of its colony margin near either bacterial streak. Correspondingly, both bacterial isolates attained full cell density right up to the margin of the fungal colony with no visible zone of inhibition separating the two organisms. A similar pattern of compatibility was recorded for *Streptomyces nenu* which extended its colony boundary into direct contact with *T. asperellum*, *P. fluorescens* and *B. subtilis* without any accompanying growth retardation indicating that *S. nenu* can coexist with each of the other three bioagents without mutual suppression. In the bacterium-bacterium pairing, *P. fluorescens* streaked against *B. subtilis* on nutrient agar likewise showed no zone of inhibition or lysis at the junction of the two streaks, whether the cultures were streaked horizontally or vertically to one another confirming compatibility between the two bacterial antagonists. Since none of the all pairwise combinations among the four bioagents showed antagonism all were designated compatible ('+') confirming their biological feasibility for co formulation into a single, broad spectrum consortium for management of soil borne phytopathogens.

Table 1: *In vitro* evaluation of compatibility of bioagents

Bio agents	<i>Trichoderma asperellum</i> UASR	<i>Pseudomonas fluorescens</i> UASR	<i>Bacillus subtilis</i> SE76	<i>Streptomyces nenu</i> DS-11
<i>Trichoderma asperellum</i> UASR	+	+	+	+
<i>Pseudomonas fluorescens</i> UASR	+	+	+	+
<i>Bacillus subtilis</i> SE76	+	+	+	+
<i>Streptomyces nenu</i> DS-11	+	+	+	+

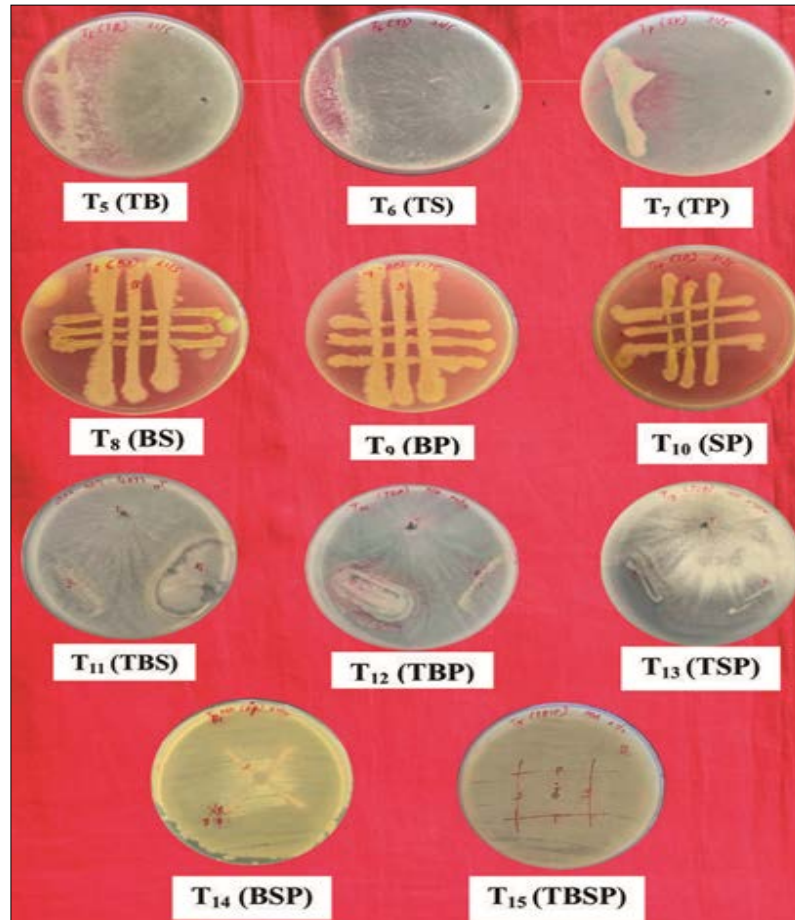
Note: + Indicate compatible (no zone of inhibition)

- Indicate incompatible (no zone of inhibition)



T ₅ : <i>T. asperellum</i> + <i>B. subtilis</i>	T ₁₀ : <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
T ₆ : <i>T. asperellum</i> + <i>Streptomyces</i> sp.	T ₁₁ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp.
T ₇ : <i>T. asperellum</i> + <i>P. fluorescens</i> .	T ₁₂ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>P. fluorescens</i>
T ₈ : <i>B. subtilis</i> + <i>Streptomyces</i> sp	T ₁₃ : <i>T. asperellum</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
T ₉ : <i>B. subtilis</i> + <i>P. fluorescens</i>	T ₁₄ : <i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
	T ₁₅ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>

Plate 1a: Compatibility test among bioagents under *in vitro* conditions (Converse view)



T ₅ : <i>T. asperellum</i> + <i>B. subtilis</i>	T ₁₀ : <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
T ₆ : <i>T. asperellum</i> + <i>Streptomyces</i> sp.	T ₁₁ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp.
T ₇ : <i>T. asperellum</i> + <i>P. fluorescens</i> .	T ₁₂ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>P. fluorescens</i>
T ₈ : <i>B. subtilis</i> + <i>Streptomyces</i> sp	T ₁₃ : <i>T. asperellum</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
T ₉ : <i>B. subtilis</i> + <i>P. fluorescens</i>	T ₁₄ : <i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>
	T ₁₅ : <i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>

Plate 1b: Compatibility test among bioagents under *in vitro* conditions (Inverse view)

These findings are in agreement with earlier reports of compatibility among *Trichoderma*, *Pseudomonas*, *Bacillus* and related biocontrol agents (Srivastava *et al.*, 2010; Jain *et al.*, 2011; Latha *et al.*, 2011) [2, 4, 14]. The results are also consistent with Maina (2012) [5] who reported compatibility among bacterial (*Pseudomonas fluorescens*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) and fungal bioagents (*Trichoderma harzianum* and *T. viride*) along with plant growth promoting rhizobacteria. Rajasekhar *et al.* (2016) [11] similarly reported compatibility between a fungal antagonist (*T. harzianum*), bacterial antagonists (*P. fluorescens* and *B. subtilis*) and *Rhizobium*. Roopadevi (2017) [12] tested the compatibility of *T. harzianum*, *B. subtilis* and *P. fluorescens* in dual culture and concluded that all three bioagents were mutually compatible with no inhibition zones observed between any of the combinations. Harshita *et al.* (2018) [1]

and Manjunath *et al.* (2020) [6] likewise reported that *T. harzianum*, *B. subtilis* and *P. fluorescens* did not inhibit one another. The consistent compatibility recorded across genera in the present study is further supported by recent evidence that *Trichoderma* species combined with *Bacillus* and *Pseudomonas* strains generally draw on largely non overlapping antimicrobial mechanisms allowing the two groups to coexist without triggering mutual antagonism in most reported combinations (Poveda and Eugui, 2022) [9]. This mutual tolerance is likely attributable to the production of distinct metabolites, chitinases, β -1,3-glucanases, surfactin, iturin, fengycin, bacillomycin, phenazines, prodiginines, siderophores and 2,4-diacetylphloroglucinol (DAPG) by each organism, none of which appears to exert an inhibitory effect when co-cultured. Compatibility screening of this kind has increasingly been used as the first

and essential step in developing field effective consortia elsewhere compatibility confirmed four member consortium of *T. afroharzianum*, *P. fluorescens*, *B. licheniformis* and *B. subtilis* achieved substantial suppression of dry root rot in cluster bean (Singh *et al.*, 2024)^[13].

The compatibility test among biocontrol agents is thus an essential prerequisite for developing a microbial consortium based formulation for effective disease management. A compatible combination of biocontrol agents possessing diverse mechanisms of action, mycoparasitism, cell wall degradation, antibiosis, nutrient competition, siderophore mediated iron competition, hydrogen cyanide production and induction of systemic resistance, has consistently been shown to provide greater disease suppression than any individual bioagent used alone. Development of a microbial consortium built on confirmed compatibility among its component organisms can therefore reduce the likelihood of failure that is often associated with reliance on a single antagonist. Since all four selected biocontrol agents, *T. asperellum*, *B. subtilis*, *S. nenu* and *P. fluorescens* were found compatible with one another and showed no inhibitory effect against each other they were considered suitable for formulation into a microbial consortium and were selected for further evaluation of their efficacy alone and in combination against major soil borne pathogens.

Conclusion

All four biocontrol agents evaluated in the present study, *Trichoderma asperellum* UASR, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR were found mutually compatible in every possible pairwise combination with no inhibition, growth suppression or lysis recorded at the zone of contact. This compatibility confirms the biological feasibility of combining all four organisms into a single microbial consortium and formed the basis for their subsequent evaluation against the major soil borne pathogens of chickpea. Compatibility screening of this type should be conducted before formulating any multi organism biocontrol product as it helps to identify combinations capable of producing genuine synergistic effects rather than mutual interference under field conditions.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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