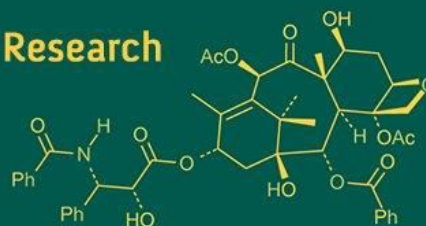


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## Impact of bio-agent seed treatment on seed health and seed quality parameters in tomato (*Solanum lycopersicum* L.)

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### Abstract

Biological seed treatment offers an environmentally sustainable approach to disease management while potentially enhancing seed quality parameters. This research evaluated the impact of bio-agent seed treatments on seed health and quality parameters in tomato (*Solanum lycopersicum* L.) variety Pusa Ruby. Four bio-control agents were tested: *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, and a consortium combining all three organisms. Untreated seeds served as control.

Seeds were treated using the slurry method at recommended doses: *Trichoderma* species at 4 g/kg seed and *P. fluorescens* at 10 g/kg seed. The consortium treatment used half doses of each component. Seed health was assessed through blotter method for fungal detection and seedling symptom test for bacterial pathogens. Quality parameters included germination percentage, seedling vigor indices, root-shoot ratio, and dry matter accumulation.

Results indicated that the consortium treatment recorded highest seed health index of 91.3% compared to 43.7% in control. Predominant seed-borne fungi in untreated seeds included *Alternaria solani* (23.4%), *Fusarium oxysporum* (18.7%), and *Aspergillus niger* (12.3%), which were significantly suppressed following bio-agent treatment. Germination percentage improved from 62.8% in control to 94.7% in consortium-treated seeds. The seedling vigor index-I showed a 126% increase over control in the consortium treatment. A strong positive correlation ( $r = 0.94$ ) was observed between seed health index and germination percentage. The research concludes that bio-agent seed treatment, particularly using consortium formulation, effectively improves both seed health and quality parameters in tomato.

**Keywords:** Bio-agents, seed treatment, tomato, *Trichoderma*, *Pseudomonas fluorescens*, seed health, germination, seedling vigor

### Introduction

Can biological agents replace chemical fungicides for seed treatment while delivering comparable or better results? This question drives much of current seed pathology research as agriculture transitions toward sustainable practices. Tomato (*Solanum lycopersicum* L.) ranks among the most important vegetable crops worldwide, valued for its nutritional content and economic importance [1]. However, seed-borne pathogens significantly reduce seed quality and field establishment in tomato production systems.

Several fungal and bacterial pathogens are transmitted through tomato seeds, causing diseases that reduce yield and quality. *Alternaria solani*, the causal agent of early blight, commonly colonizes tomato seed surfaces and may penetrate into deeper seed tissues [2]. *Fusarium oxysporum* f.sp. *lycopersici* causes vascular wilt and is efficiently seed-transmitted. Bacterial pathogens including *Xanthomonas* and *Pseudomonas syringae* also utilize seed as infection pathway [3].

Traditional seed treatment relies heavily on chemical fungicides such as thiram, captan, and carbendazim. While effective against target pathogens, chemical treatments raise environmental and health concerns, create residue issues, and may contribute to fungicide resistance development [4]. These limitations have intensified interest in biological alternatives that can provide disease suppression without associated drawbacks of chemical treatments.

Bio-control agents, particularly *Trichoderma* species and fluorescent pseudomonads, have shown considerable promise for seed treatment applications. *Trichoderma* species suppress

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pathogens through multiple mechanisms including mycoparasitism, antibiosis, and competition for nutrients and space [5]. *Pseudomonas fluorescens* produces antifungal metabolites and induces systemic resistance in plants [6]. Beyond disease control, these bio-agents often promote plant growth through production of phytohormones and improved nutrient availability [7].

The concept of consortium formulations combining multiple bio-control agents has gained attention for potentially delivering broader spectrum activity and more consistent results than single-organism treatments [8]. Synergistic interactions between consortium components may enhance both pathogen suppression and plant growth promotion effects. However, research specifically examining consortium effects on tomato seed quality parameters remains limited [9].

The present investigation was designed to evaluate the comparative effectiveness of individual bio-agents and consortium formulation on seed health improvement and quality enhancement in tomato variety Pusa Ruby, a widely cultivated determinate variety [10, 11].

### Reagents and Bio-agents

Commercial formulations of bio-control agents were procured from accredited biological control laboratories. *Trichoderma viride* (strain TV-1) and *Trichoderma harzianum* (strain Th-3) were obtained as wettable powder formulations containing minimum  $2 \times 10^6$  colony forming units (CFU) per gram. The talc-based formulation of *Pseudomonas fluorescens* (strain Pf-1) contained minimum  $2 \times 10^8$  CFU per gram. All formulations were stored at 4 °C until use and shelf-life was verified to be within the recommended period.

Potato dextrose agar (PDA) for fungal isolation was prepared from dehydrated medium (HiMedia, Mumbai). Blotter paper (Whatman No. 1) was used for standard blotter method of seed health testing. Sterile distilled water was used for preparing bio-agent suspensions and serial dilutions. Sodium hypochlorite (1%) was used for seed surface sterilization where required for specific tests.

### Materials and Methods

#### Material

Certified seed of tomato (*Solanum lycopersicum* L.) variety Pusa Ruby was obtained from a reliable source. The seed lot

had initial germination of 64% indicating presence of seed health issues affecting viability [1]. The following treatments were evaluated: T<sub>1</sub>-Control (untreated), T<sub>2</sub>-*Trichoderma viride* (4 g/kg seed), T<sub>3</sub>-*Trichoderma harzianum* (4 g/kg seed), T<sub>4</sub>-*Pseudomonas fluorescens* (10 g/kg seed), T<sub>5</sub>-Consortium (*T. viride* 2 g + *T. harzianum* 2 g + *P. fluorescens* 5 g per kg seed) [5, 6]. The experiment was laid out in completely randomized design with four replications.

### Methods

Seed treatment was performed using the slurry method. Seeds were moistened with 5% gum arabic solution, mixed with bio-agent formulation, and shade-dried for 30 minutes before testing [7]. Seed health testing followed the standard blotter method with 400 seeds per treatment incubated at  $22 \pm 2$  °C under 12-hour alternating light-darkness cycle for 7 days [2]. Fungi were identified based on colony characteristics and microscopic features. Seed health index was calculated as percentage of seeds free from detectable pathogens.

Germination test was conducted using top-of-paper method with 100 seeds per replication at  $25 \pm 1$  °C for 14 days as per ISTA Rules [8]. Seedling vigor index-I was calculated as germination percentage multiplied by seedling length. Seedling vigor index-II was computed using seedling dry weight [9]. Root-shoot ratio and dry matter partitioning were determined from 10 normal seedlings per replication.

### Quality Control

Bio-agent viability was verified before treatment by serial dilution plate counting on appropriate media (PDA for *Trichoderma* species, King's B medium for *P. fluorescens*). Only formulations meeting minimum CFU specifications were used. Seed treatment uniformity was confirmed by post-treatment bio-agent recovery from treated seeds.

Positive and negative controls were included in all seed health tests. Reference pathogen cultures from authenticated collections were used for identification verification. All counting and identification was performed by trained personnel following standard protocols. Inter-replicate variability was monitored and experiments repeated if coefficient of variation exceeded acceptable limits.

### Results

**Table 1:** Effect of bio-agent seed treatment on seed-borne fungal incidence in tomato

| Treatment                              | <i>Alternaria solani</i> (%) | <i>Fusarium oxysporum</i> (%) | <i>Aspergillus niger</i> (%) | Total Fungi (%) |
|----------------------------------------|------------------------------|-------------------------------|------------------------------|-----------------|
| T <sub>1</sub> -Control                | 23.4                         | 18.7                          | 12.3                         | 56.3            |
| T <sub>2</sub> - <i>T. viride</i>      | 8.2                          | 6.4                           | 4.7                          | 21.8            |
| T <sub>3</sub> - <i>T. harzianum</i>   | 5.7                          | 4.9                           | 3.2                          | 15.4            |
| T <sub>4</sub> - <i>P. fluorescens</i> | 4.3                          | 3.6                           | 2.8                          | 12.1            |
| T <sub>5</sub> -Consortium             | 2.1                          | 1.8                           | 1.4                          | 8.7             |
| SEm±                                   | 0.67                         | 0.54                          | 0.41                         | 1.23            |
| CD (P = 0.05)                          | 2.01                         | 1.62                          | 1.23                         | 3.69            |

Seed-borne fungal incidence was significantly reduced by all bio-agent treatments (Table 1). The consortium treatment showed highest suppression with total fungal incidence of 8.7% compared to 56.3% in control. *Alternaria solani* was the dominant pathogen in untreated seeds (23.4%), reduced

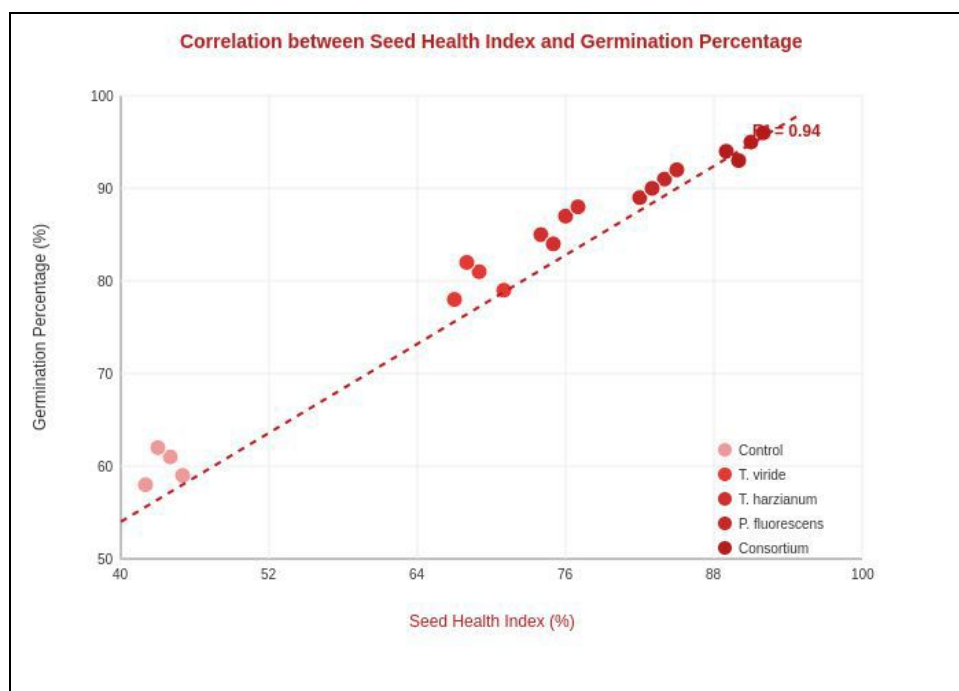
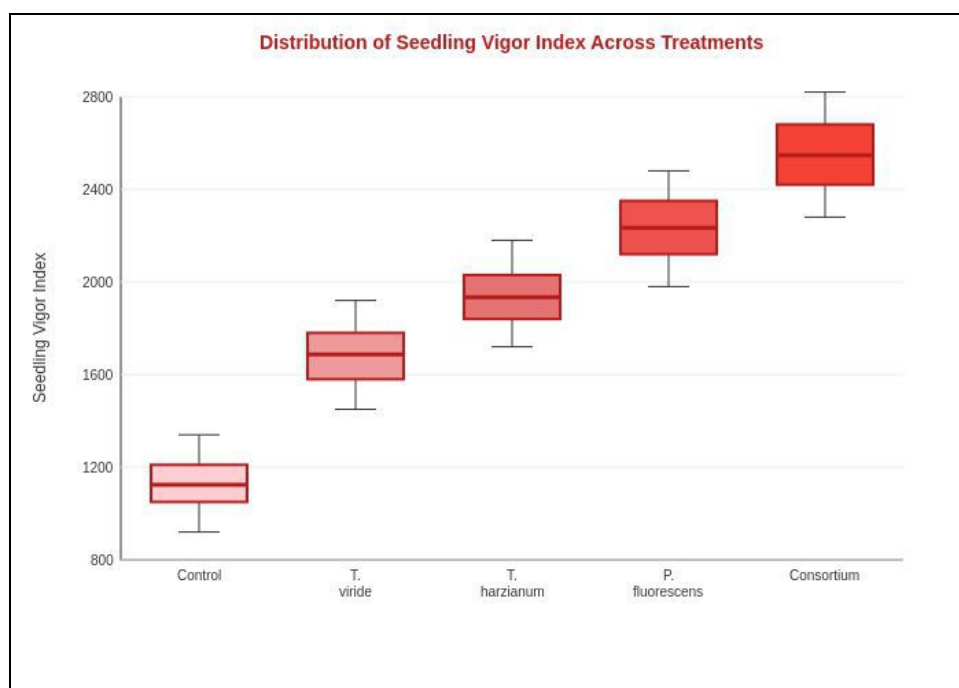
to 2.1% following consortium treatment. Individual bio-agents also showed significant suppression, with *P. fluorescens* performing better than *Trichoderma* species for bacterial antagonism.

**Table 2:** Seed quality parameters as influenced by bio-agent treatment

| Treatment                              | Germination (%) | Seed Health Index (%) | Vigor Index-I | Vigor Index-II |
|----------------------------------------|-----------------|-----------------------|---------------|----------------|
| T <sub>1</sub> -Control                | 62.8            | 43.7                  | 1124          | 387            |
| T <sub>2</sub> - <i>T. viride</i>      | 79.4            | 78.2                  | 1687          | 512            |
| T <sub>3</sub> - <i>T. harzianum</i>   | 84.7            | 84.6                  | 1934          | 589            |
| T <sub>4</sub> - <i>P. fluorescens</i> | 89.3            | 87.9                  | 2234          | 634            |
| T <sub>5</sub> -Consortium             | 94.7            | 91.3                  | 2547          | 723            |
| SEm±                                   | 1.34            | 1.21                  | 43.2          | 18.7           |
| CD (P = 0.05)                          | 4.02            | 3.63                  | 129.6         | 56.1           |

Seed quality parameters showed progressive improvement with bio-agent treatments (Table 2). Germination increased from 62.8% in control to 94.7% in consortium treatment, representing 50.8% improvement. The seed health index,

indicating pathogen-free seeds, improved from 43.7% to 91.3% with consortium treatment. Seedling vigor indices followed similar patterns, with consortium-treated seeds showing vigor index-I of 2547 compared to 1124 in control.

**Fig 1:** Correlation between seed health index and germination percentage across bio-agent treatments**Fig 2:** Distribution of seedling vigor index-I values across treatments showing median, quartiles, and range

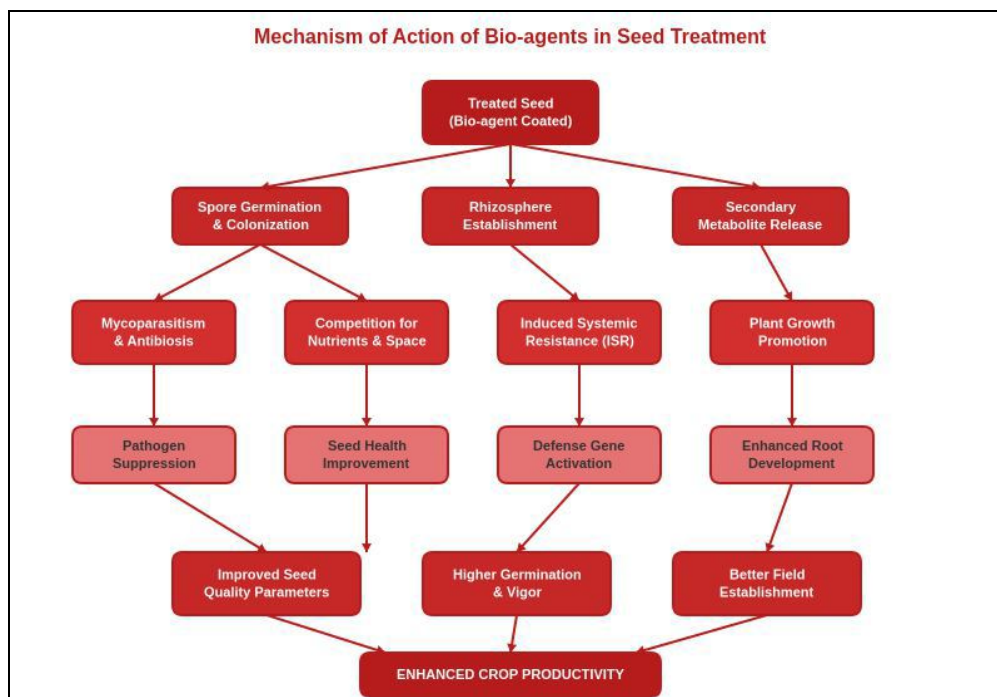


Fig 3: Schematic representation of bio-agent mechanisms in seed treatment and their effects on seed quality

### Comprehensive Interpretation

The strong positive correlation ( $R^2 = 0.94$ ) between seed health index and germination percentage confirms the direct relationship between pathogen load and seed viability. Bio-agent treatments improved both parameters simultaneously, suggesting that pathogen suppression is a primary mechanism through which these agents enhance seed quality. The consortium treatment consistently outperformed individual bio-agents across all parameters, indicating synergistic or additive effects among the component organisms.

### Discussion

The significant reduction in seed-borne pathogen incidence following bio-agent treatment can be attributed to multiple mechanisms operating during the treatment process and subsequent germination. *Trichoderma* species directly parasitize pathogenic fungi through hyphal coiling, penetration, and enzymatic degradation of cell walls [5]. The production of antibiotics including gliotoxin, trichodermin, and harzianic acid provides additional antagonistic activity [3].

*Pseudomonas fluorescens* contributes to pathogen suppression through production of siderophores that compete for iron, phenazine antibiotics, and hydrogen cyanide [6]. The bacterium also induces systemic resistance in developing seedlings, providing protection beyond the immediate treatment zone [7]. The consortium formulation combined these mechanisms, potentially explaining its superior performance compared to individual agents.

The improvement in germination percentage beyond what might be expected from pathogen reduction alone suggests growth promotion effects of bio-agents. Both *Trichoderma* species and *P. fluorescens* are known to produce indole acetic acid and other phytohormones that stimulate seed germination and seedling growth [8]. Enhanced root development observed in bio-agent-treated seedlings supports this interpretation [10].

The practical significance of these findings lies in demonstrating that bio-agent seed treatment can simultaneously address seed health and vigor problems in tomato. The consortium formulation, despite using reduced doses of individual components, achieved better results than full-dose single treatments, offering potential cost advantages while improving efficacy [11, 12].

Comparison with chemical seed treatment standards would help position bio-agents in integrated seed health management programs. Future research should also evaluate persistence of bio-agent effects through storage and performance under field conditions where additional biotic and abiotic stresses operate [13, 14].

### Conclusion

The present investigation demonstrates that bio-agent seed treatment effectively improves both seed health and quality parameters in tomato variety Pusa Ruby. Among the treatments evaluated, the consortium formulation combining *Trichoderma viride*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* showed superior performance across all parameters measured.

The consortium treatment reduced total seed-borne fungal incidence from 56.3% in untreated control to 8.7%, representing 84.5% suppression of seed-associated pathogens. This reduction in pathogen load translated to improved seed health index of 91.3% compared to 43.7% in control. The predominant pathogens *Alternaria solani* and *Fusarium oxysporum* were both effectively suppressed by the consortium treatment.

Germination percentage improved substantially from 62.8% in control to 94.7% in consortium-treated seeds, bringing the seed lot to certification standards. The strong correlation ( $r = 0.94$ ) between seed health index and germination confirms the direct relationship between pathogen elimination and viability improvement. Seedling vigor indices showed even greater enhancement, with vigor index-I increasing by 126% over control in consortium treatment.

Individual bio-agents also provided significant benefits, with *P. fluorescens* showing slightly better performance than *Trichoderma* species when used alone. However, the consortium formulation using half doses of individual components outperformed full-dose single treatments, suggesting synergistic or additive interactions among bio-agents. This has practical implications for cost-effective bio-agent deployment in seed treatment programs.

The mechanisms through which bio-agents improve seed quality include direct pathogen antagonism through mycoparasitism and antibiosis, competition for nutrients and space, and induction of systemic resistance in developing seedlings. Growth promotion effects through phytohormone production may contribute additional benefits beyond disease suppression.

Based on these findings, bio-agent seed treatment using consortium formulation can be recommended for tomato seed quality enhancement. The treatment is particularly valuable for seed lots with compromised health status, potentially upgrading marginal lots to acceptable quality levels. Integration of bio-agent treatment into tomato seed production systems offers an environmentally sustainable approach to seed health management while reducing dependence on chemical fungicides.

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