Plant Archives Vol. 26, Supplement 1, 2026 pp. 3253-3262 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.435

FORMULATION STRATEGIES TO ENHANCE SHELF LIFE AND BIO-EFFICACY OF *TRICHODERMA ASPERELLUM* FOR SUSTAINABLE MANAGEMENT OF CHICKPEA WILT

R.J. Choudhari[1]*, R.M. Gade[2], M.V. Totawar[1], S.S. Bramhnkar[1], S.T. Ingle[1] and Y.V. Ingle[1]
[1]Department of Plant Pathology. Dr. PDKV, Akola- 444 104 (MS), India
[2] Professor and Former Associate Dean COA, Akola and Associate Dean (Instruction) Dr. PDKV, Akola
*Corresponding author E-mail: choudharirupeshkumar@gmail.com
(Date of Receiving : 26-11-2025; Date of Acceptance : 28-01-2026)

ABSTRACT

Chickpea (*Cicer arietinum* L.) is a major pulse crop in India; however, its productivity is severely affected by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Foc). The present study aimed to enhance the shelf life and efficacy of liquid bioagent formulations for sustainable management of chickpea wilt. Liquid formulations of *Trichoderma asperellum* were developed using basal media supplemented with protective additives such as glycerol, polyvinyl pyrrolidone (PVP), polyethylene glycol (PEG), carboxymethyl cellulose (CMC), trehalose with preservatives and surfactant. Shelf-life evaluation under ambient conditions (28 ± 2 °C) up to 360 days revealed that formulations containing 2% glycerol with 0.1% CMC (C4A1) and 2% glycerol with 10 mM trehalose (C4A2) maintained the highest viable counts of *T. asperellum* (0.87×10^{7} cfu mL^{-1}) after 360 days, whereas controls lost viability within 120 days. *In vitro* assays demonstrated maximum inhibition of *F. oxysporum* f. sp. *ciceri* by *T. asperellum* (71.73%) and *P. fluorescens* (70.01%). Pot culture experiments further confirmed that seed treatment with optimized liquid formulations significantly reduced wilt incidence across different chickpea varieties. The study demonstrated that incorporation of glycerol and carboxymethyl cellulose (CMC) as cell protectants and stabilizers effectively prolonged microbial viability and enhanced bio-efficacy. These findings highlight the potential of such formulations for developing long-shelf-life bioagents and support their application in eco-friendly management of chickpea wilt.
***Keywords*:** Chickpea, *Trichoderma asperellum*, Liquid bioformulation, Shelf life, *Fusarium oxysporum* f. sp. *ciceri*, Biocontrol.

Introduction

Chickpea (*Cicer arietinum* L.), commonly known as Bengal gram, is one of the most important rabbi pulse crops in India, accounting for approximately 30–35% of the total pulse acreage and contributing nearly 38% of national pulse production. Globally, chickpea occupies about 15% of the area under pulses, with India being the largest producer, cultivating approximately 11.2 million hectares and producing 13.98 million tonnes with an average productivity of 1217 kg ha^{-1} (Directorate of Pulse Development, 2021–22; Singh *et al.*, 2014). Chickpea wilt, caused by *Fusarium oxysporum* f. sp. *ciceris*, is one of the most destructive diseases limiting chickpea productivity worldwide. The increasing demand for residue-free and sustainable disease management has promoted the use of biocontrol agents, particularly *Trichoderma* spp., due to their antagonistic activity, plant growth promotion, and environmental safety. However, the large-scale adoption of *Trichoderma*-based bioagents is often constrained by poor shelf life, loss of viability during storage, and inconsistent field performance, especially in liquid formulations.

Earlier studies have demonstrated that formulation strategies play a critical role in maintaining the viability and efficacy of *Trichoderma* spp. Sriram *et al.* (2011) reported that liquid fermentation–based formulations are more susceptible to desiccation stress than solid-state formulations, but supplementation with glycerol effectively reduced water activity and significantly enhanced shelf life. Glycerol-amended formulations of *Trichoderma harzianum* maintained viable populations above 2×10^6 cfu g^{-1} for up to 12 months, showing a positive correlation between microbial viability and water activity. Similarly, Kara and Tozlu (2024) demonstrated that oil- and glycerine-based liquid carriers, particularly neem oil, glycerine, and paraffin oil, sustained high fungal viability for up to 10 months under both ambient and refrigerated storage conditions.

Contrasting findings by Bhai and Anandaraj (2014) indicated that conidial suspensions of *T. harzianum* prepared without cryoprotectants exhibited remarkable long-term stability, retaining viable populations for more than 720 days, highlighting that formulation simplicity can also play a vital role in microbial survival. Supporting these observations, Singh *et al*. (2007) and Nadare *et al*. (2018) reported that substrate type and carrier materials significantly influence population dynamics and shelf life of *Trichoderma* spp., with oil-based and organic carrier formulations generally outperforming conventional talc-based products. Efficient mass production and formulation through liquid fermentation technology under optimized conditions has further been validated by Khan *et al*. (2011), emphasizing its commercial feasibility.

Despite these advances, limited information is available on the combined use of cell protectants and stabilizers such as glycerol and carboxymethyl cellulose (CMC) in enhancing both shelf life and bioefficacy of liquid *Trichoderma asperellum* formulations against chickpea wilt. Therefore, the present study was undertaken to develop and evaluate optimized liquid bioagent formulations of *T. asperellum* with improved storage stability and disease suppression potential, contributing to eco-friendly and sustainable management of chickpea wilt.

Materials and Methods

Procurement of Biocontrol Agents: The biocontrol agent *Trichoderma asperellum* used in the present investigation was procured from the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India.

Preparation of liquid formulations of the bioagent *Trichoderma asperellum* amended with different additives

The effect of storage duration on the shelf life and population dynamics of liquid formulations of *Trichoderma asperellum* was evaluated under laboratory conditions. Liquid bioformulations were prepared using Potato Dextrose Broth as the basal medium and amended with selected additives known for their roles as stabilizers, cell protectants, surfactants, and preservatives. The additives included carboxymethyl cellulose (CMC, 0.1%), polyvinylpyrrolidone (PVP, 2%), polyethylene glycol (PEG, 2%), sodium alginate (SA, 0.1%), glycerol (2%), Alphox (1%), trehalose (10 mM), and a combination of Tween-20 (0.5%) with potassium sorbate (0.5%), selected based on their reported ability to enhance microbial viability during prolonged storage (Anjali *et al*., 2024). A formulation without additives served as the control.

The basal media (250 ml) containing the respective additives were sterilized at 121 °C for 15 min and aseptically inoculated with actively growing cultures of *T. asperellum* at an initial population of 10^7 cells ml^{-1}. The inoculated formulations were incubated at 28 ± 2 °C for 3–5 days to ensure uniform growth and establishment of the bioagent, and subsequently stored under the same laboratory conditions. Periodic observations were recorded at predetermined intervals to assess changes in viable population density and shelf life during storage. This approach enables identification of effective additive-based liquid formulations capable of maintaining high microbial viability, thereby improving the practical applicability and reliability of *T. asperellum* as an eco-friendly bioagent for sustainable crop protection.

Shelf-life Studies on Liquid Formulations of Bioagent

The prepared liquid formulations of fungal bioagents were evaluated for their viability under *in vitro* conditions. Initial population counts were recorded one hour after inoculation, followed by assessments at 24 h and at 30-day intervals up to 360 days of storage. For viability estimation, 1 ml of each formulation was aseptically transferred into 9 ml of sterile distilled water and shaken thoroughly for 3 min to obtain a 10^{-1} dilution. Serial dilutions were subsequently prepared up to 10^{-7} for fungal formulations. An aliquot of 1 ml from the appropriate dilution (10^{-7}) was plated onto Petri plates containing 20 ml of sterilized Potato Dextrose Agar (PDA) medium and gently rotated to ensure uniform distribution of the inoculum. In the case of bacterial formulations, 10 µl of the 10^{-9} dilution was spread evenly on the respective growth medium. All plates were incubated at 25 ± 2 °C for 48 h to allow colony development.

The viable population of fungal bioagents was enumerated using the standard serial dilution and plate count technique as described by Aneja (2003), and results were expressed as colony-forming units (CFU).

$$\text{Number of cfu/ml} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}}$$

Determination of Shelf Life:

The shelf life of the liquid bioagent formulations was assessed by periodically monitoring viable cell populations. Initial viable counts were recorded one hour after inoculation, followed by observations at 24 h and at 30-day intervals thereafter up to 360 days of storage, as described by Dobhal and Hegde (2021).

All treatment combinations were stored at room temperature (28 ± 2 °C). At each sampling interval, the containers were aseptically opened under a laminar airflow cabinet to avoid contamination. Viable cell counts were determined using the standard serial dilution and plating technique, and microbial survival was expressed as colony-forming units per millilitre (CFU ml^{-1}).

***In vitro* antagonism test of Bioagent (*Trichoderma asperellum*) against *Fusarium oxysporum* f. sp. *ciceri* by using Dual culture technique:**

The antagonistic potential of *Trichoderma asperellum* against *Fusarium oxysporum* f. sp. *ciceri* was evaluated using the dual culture technique (Dhingra and Sinclair, 1995). Two most effective *T. asperellum* isolates, selected based on shelf-life studies, were used for the assay. For the antagonism test, 20 ml of sterilized potato dextrose agar (PDA) was aseptically poured into 9 cm diameter Petri plates. Mycelial discs (5 mm) from four-day-old cultures of the antagonist and the pathogen were placed on opposite sides of the plate, approximately 7 cm apart and 1 cm from the periphery. Control plates were inoculated with the pathogen alone. All plates were incubated at 25 ± 2 °C.

Observations were recorded when the pathogen in the control plates completely covered the medium (Morton and Straube, 1955). The extent of antagonism was assessed by measuring the inhibition of radial mycelial growth, and the percentage inhibition over control was calculated using the formula described by Vincent (1927).

$$\text{Per cent Inhibition I} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent reduction in growth of test pathogen.

C = Radial growth (mm) in monoculture check.

T = Radial growth (mm) in dual cultured plates.

Evaluation of Bio-efficacy of Liquid-Based Bioagent (*Trichoderma asperellum*) as Seed Treatment on Wilt of Chickpea under Pot Culture Conditions:

Based on the results of *in vitro* antagonistic assays, *Trichoderma asperellum* was identified as the most effective bioagent and selected for seed treatment studies. Pot culture experiments were conducted under screenhouse conditions following a factorial completely randomized design (FCRD). Plastic pots (50 cm diameter) were filled with sterilized sandy loam soil and artificially inoculated with a two-week-old culture of *Fusarium oxysporum* f. sp. *ciceris* grown on sorghum–sand medium. The pathogen inoculum was incorporated at 2% (w/w), and the soil was allowed to stabilize for five days prior to sowing. Seeds of three chickpea varieties PDKV-Kanak (resistant), Gulak-1 (moderately resistant), and JG-62 (susceptible) were surface sterilized using 1% sodium hypochlorite for 3 min, thoroughly rinsed with sterile distilled water, and air-dried under aseptic conditions. The sterilized seeds were then treated with the selected liquid bioagent formulations as per the experimental treatments. Fifteen seeds were sown per plastic pot (40 cm diameter) containing 5 kg of sterilized soil, with three replications for each treatment. Pots sown with untreated seeds served as the control.

Chickpea wilt incidence was recorded at 30, 60, and 90 days after sowing (DAS). Wilt incidence (%) was calculated using the following formula:

Wilt incidence %

$$\text{Wilt incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Statistical data interpretation

All experimental data were analyzed using the Factorial Completely Randomized Design (FCRD) in OPSTAT software, which was developed and is maintained by Prof. O.P. Sheoran, Department of Mathematics and Statistics, CCS HAU, Hisar, and Dr. Vinay Kumar, Associate Professor, Central University of Haryana, Mahendergarh.

Result and Discussion

Effect of Cell Protectant Polymer Additives with Surfactant and Preservatives on the Shelf Life of Liquid *Trichoderma asperellum* Formulations:

The data presented in Table 1(a) indicate that among the evaluated cell-protectant polymer additives, the formulation containing 2% glycerol (GL) was the most effective in maintaining the viability of *Trichoderma asperellum*. Under ambient storage conditions (28 ± 2 °C), this formulation sustained a population density of 10^7 cfu mL^{-1} for up to 360 days. Although a gradual decline in viable counts was observed over time, the 2% GL treatment consistently recorded significantly higher populations than all other polymer-amended formulations throughout the storage period (24 h to 360 days).

Other polymer additives also enhanced the shelf life of *T. asperellum* compared with the untreated control, which exhibited a rapid decline in viability; however, their effectiveness was comparatively lower than that of the 2% GL formulation. Overall, the results clearly demonstrate that the incorporation of cell-protectant polymers in combination with surfactants and preservatives significantly extended the shelf life of liquid *T. asperellum* formulations for up to one year, with glycerol at 2% emerging as the most efficient additive for sustaining microbial viability.

Sriram *et al.* (2011) reported that liquid fermentation–based formulations of *Trichoderma* spp. are more prone to desiccation than solid-state formulations; however, incorporation of glycerol (3–9%) as an osmoticant significantly reduced water activity and improved formulation stability. Glycerol at 3 and 6% extended the shelf life of talc-based *T. harzianum* formulations up to 7 and 12 months, respectively, maintaining viable populations above 2 × 10^6 cfu g^{-1}, compared to only 4–5 months without glycerol. A positive correlation between water activity and fungal viability was also observed. Similarly, Kara and Tozlu (2024) demonstrated that oil- and glycerine-based liquid carriers, particularly neem oil, glycerine, and paraffin oil, sustained high viability of *T. harzianum* for up to 10 months under both ambient and refrigerated storage. Bhai and Anandaraj (2014) further showed that *T. harzianum* conidia suspended in sterile deionized water retained viability for over 720 days at both 25 ± 1°C and 28–32°C, outperforming formulations containing glycerol, glucose, or DMSO. Collectively, these studies highlight the critical role of formulation components in enhancing the long-term viability and shelf life of *Trichoderma* bioagents.

Effect of adjuvants Combined with surfactant and preservatives on shelf life of liquid formulations of *Trichoderma asperellum*:

The data presented in Table 1(a) indicate that among the adjuvants evaluated, carboxymethyl cellulose (CMC) at 0.1% was the most effective in sustaining the viable population of *Trichoderma asperellum*. Under room temperature storage conditions (28 ± 2 °C), the CMC-amended formulation maintained a population density of 10^7 cfu ml^{-1} throughout the 360-day storage period. Although a marginal decline in the initial population was observed at the end of storage, the reduction was statistically non-significant. Formulations containing other adjuvants also enhanced microbial survival compared to the untreated control; however, viable counts showed a gradual decline over the storage period. Notably, across all observation intervals, ranging from 24 h to 360 days, the population density recorded in the 0.1% CMC treatment remained significantly higher than that observed with other adjuvants.

Overall, while all tested adjuvants contributed to prolonging the shelf life of *T. asperellum*, the incorporation of 0.1% CMC proved to be distinctly superior, followed by trehalose, whereas Alphox was the least effective in maintaining microbial viability. The effective wilt suppression observed with *T. asperellum*-based treatments in the present study is in agreement with the findings of Shriram (2021), who reported that conidial formulations derived from solid substrates exhibit greater stability than liquid fermentation–based products. This enhanced stability was attributed to the presence of nutrients such as chitin, chitosan, and trehalose, which promote conidial germination and establishment. These observations corroborate the consistent efficacy recorded in the present investigation and underscore the importance of stable, nutrient-enriched *Trichoderma* formulations in improving disease suppression.

Similar observations were reported by Zakaria *et al.* (2018), who demonstrated good shelf stability of *Trichoderma* spp. in talc-based formulations, with viable counts declining from 200 × 10^6 to 45 × 10^6 cfu g^{-1} over 120 days of ambient storage. Likewise, Kolombet *et al.* (2008) showed that optimized liquid biomass formulations enhanced the viability and competitiveness of *Trichoderma asperellum*, maintaining biological activity for up to six months at room temperature. Comparable results were reported by Sutthisa *et al.* (2024), where liquid formulations of *T. asperellum* prepared with different protectants retained stable spore populations of approximately 10^7 spores mL^{-1} after 45 days of storage at 28 ± 2 °C.

Interaction Effects of Additives Combinations on the Shelf Life of Liquid *Trichoderma asperellum* bioagent at Room Temperature:

The results on the shelf life of liquid formulations of bioagent *Trichoderma asperellum* with different additives revealed significant variations in population density across the storage period (Table 7-b). At the initial count, all treatments recorded a population density of 17.2-17.9 × 10^7 cfu/ml, indicating no significant variation. However, gradual differences appeared with prolonged storage. After 30 days, formulations containing combinations such as C4-A1 (GL + CMC) and C4-A2 (GL + Trehalose) maintained higher population densities (15.37 and 15.26 × 10^7 cfu/ml, respectively) compared to control (9.60 × 10^7 cfu/ml). By 90 days, viable populations in most amended formulations remained between 10.68-12.26 × 10^7 cfu/ml, whereas the control dropped drastically to 1.37 × 10^7 cfu/ml. A similar trend was noted at 150 days, with treatments like C4-A1 (9.19 × 10^7 cfu/ml), C4-A2 (9.17 × 10^7 cfu/ml), and C3-A2 (8.53 × 10^7 cfu/ml) sustaining superior viability, while the control population was nearly negligible (0.41 × 10^7 cfu/ml). From 180 to 270 days, the population declined progressively in all treatments; however, formulations containing PVP and CMC consistently showed better survival compared to other additives. For instance, C4-A1, C4-A2, and C1-A1 retained 6.10, 6.03, and 6.02 × 10^7 cfu/ml, respectively, at 180 days, while control showed no viable count. At 360 days, although there was a marked decline in all treatments, certain formulations continued to support survival. The highest viable populations were maintained by C4-A1 (0.87 × 10^7 cfu/ml) and C4-A2 (0.87 × 10^7 cfu/ml), followed by C1-A1 (0.80 × 10^7 cfu/ml) and C2-A1 (0.76 × 10^7 cfu/ml). In contrast, the control treatment recorded no survival beyond 120 days. Overall, the results clearly demonstrate that the incorporation of stabilizers such as CMC and Trehalose into Glycerol and PVP-based formulations significantly extended the shelf life of T. asperellum liquid inoculants up to one year, while the untreated control lost viability within 120 days.

Among the different additive combinations tested, glycerol based liquid formulations with CMC (C4-A1) and Trehalose (C4-A2) were most effective in sustaining the shelf life of Trichoderma asperellum, maintaining viable populations up to 360 days. PVP and PEG-based formulations with CMC also supported extended survival, though at comparatively lower densities. In contrast, the control treatment without additives lost viability within 120 days, confirming the vital role of stabilizers in prolonging shelf life.

Bhai and Anandaraj (2014) reported results comparable to the present study while evaluating the shelf life of *Trichoderma harzianum* conidial suspensions prepared with different cryoprotectants. Although glycerol, glucose, CMC and DMSO maintained viability only up to 75 days, suspensions in sterile deionized water showed significantly prolonged survival, retaining viable populations up to 480 days and beyond 720 days at both ambient (28–32 °C) and controlled (25 ± 1 °C) temperatures. Viability was confirmed through in vitro recovery on TSM medium. Similarly, Zakaria *et al.* (2018) reported good shelf stability of *Trichoderma* spp. in talc-based formulations, where viable counts declined gradually from 200 × 10^6 to 45 × 10^6 cfu g^{-1} over 120 days, yet remained at effective levels throughout storage. Nadare *et al.* (2018) further demonstrated that oil-based liquid carriers significantly influence shelf life, with paraffin oil supporting the highest viability of *Trichoderma viride* after six months of storage, followed by soybean and groundnut oils. Overall, these studies highlight the superior stability of liquid and oil-based formulations over conventional solid carriers for enhancing the shelf life of *Trichoderma* bioagents.

***In vitro* Screening of Antagonistic Bioagent against (*Trichoderma asperellum*) *Fusarium oxysporum* f. sp. *cicero*:**

The antagonistic potential of selected bioagents and biofertilizers, shortlisted based on shelf-life and viability studies namely *Trichoderma asperellum* (Ta_1 and Ta_2), *Pseudomonas fluorescens* (Pf_1 and Pf_2), and *Bacillus megaterium* var. *phosphaticum* was evaluated against *Fusarium oxysporum* f. sp. *ciceri* under *in vitro* conditions. Significant differences were observed among treatments with respect to pathogen colony diameter and percent mycelial growth inhibition (Table 3). Among the tested bioagents, *T. asperellum* (Ta_1) exhibited the highest antagonistic activity, recording a minimum mean colony diameter of 25.44 mm and maximum growth inhibition of 71.73%, followed by *P. fluorescens* (Pf_1), which showed a colony diameter of 26.99 mm and 70.01% inhibition. The isolates *T. asperellum* (Ta_2) and *P. fluorescens* (Pf_2) were statistically at par, registering growth inhibition of 68.27% and 69.75%, respectively. In contrast, *B. megaterium* var. *phosphaticum* showed comparatively lower antagonistic efficacy, with a mean colony diameter of 60.33 mm corresponding to 32.97% inhibition. The untreated control recorded the maximum pathogen growth, with a colony diameter of 90.00 mm and no inhibition.

Trichoderma asperellum (Ta_1) emerged as the most effective antagonist against the chickpea wilt pathogen, followed by *Pseudomonas fluorescens* (Pf_1 and Pf_2) and *T. asperellum* (Ta_2), highlighting their strong biocontrol potential under in vitro conditions. Consistent with these results, Ranjana (2024) reported that *Trichoderma* isolates such as PBT13 and PBT3 inhibited over 70% of *Fusarium oxysporum* f. sp. *ciceri* growth, attributing antagonism to mycoparasitism, production of cell-wall degrading enzymes, and antifungal metabolites. The effective suppression of wilt observed in the present study, particularly by *T. asperellum*, corroborates these findings, indicating that enzymatic activity and secondary metabolites play a central role in disease inhibition. Supporting this, Katyayani *et al*. (2020) demonstrated that while chemical fungicides offer only short-term control, *Trichoderma* species, including *T. harzianum*, *T. viride*, and *T. koningii*, exhibited strong mycoparasitic activity and significantly reduced chickpea wilt incidence, further emphasizing the promise of bioagents in sustainable disease management.

Interaction Effect of *Trichoderma asperellum* on Fusarium Wilt of Chickpea Under Pot Condition:

The effect of *Trichoderma asperellum* in combination with different chickpea varieties on Fusarium wilt incidence was evaluated under pot conditions at 30, 60, and 90 days after sowing (DAS). The results (Table 4) revealed significant variation among treatments, indicating a strong interaction between chickpea genotype and bioagent application. In all observation periods, treatments receiving *T. asperellum* showed markedly lower wilt incidence compared to the untreated control.

At 30 DAS, the resistant variety PDKV-Kanak treated with *T. asperellum* (T_1) exhibited complete disease suppression (0.00% wilt incidence), resulting in 100% disease reduction over the control. The moderately resistant variety Gulak-1 (T_2) recorded 3.33% wilt incidence with 85.73% disease reduction, while the susceptible variety JG-62 (T_3) showed 6.66% incidence corresponding to 71.45% reduction. The untreated control (T_4) recorded the highest wilt incidence (23.33%), and differences among treatments were statistically significant.

At 60 DAS, a gradual increase in disease incidence was observed; however, T_1 continued to maintain complete protection with zero wilt incidence. Treatments T_2 and T_3 recorded 6.66% and 10.33% wilt incidence, corresponding to disease reductions of 80.01% and 69.00%, respectively. In contrast, the untreated control exhibited 33.33% wilt incidence. The interaction effect between variety and bioagent remained statistically significant.

By 90 DAS, disease incidence increased further in all treatments except T_1, which consistently recorded 0.00% wilt incidence, confirming the sustained efficacy of *T. asperellum* in the resistant genotype. T_2 and T_3 showed 6.66% and 13.33% wilt incidence, achieving 88.93% and 77.78% disease reduction, respectively. The untreated control recorded maximum disease development with 60.00% wilt incidence. Treatment interactions were significant at this stage as well.

Overall, the combined application of *T. asperellum* with the resistant chickpea variety PDKV-Kanak resulted in complete and sustained suppression of Fusarium wilt throughout the crop growth period, indicating a strong synergistic effect between host resistance and biological control. Treatments involving moderately resistant and susceptible varieties also significantly reduced disease incidence but were comparatively less effective. The gradual increase in wilt incidence in these treatments suggests partial yet sustained protection. The consistently high disease levels in the control confirmed the severe susceptibility of chickpea to Fusarium wilt under untreated conditions. These findings clearly demonstrate that the integration of *T. asperellum* with a resistant chickpea genotype is an effective strategy for Fusarium wilt management and warrants further validation under field conditions.

Similarly, the present findings are in accordance with those of Chohan *et al*. (2024), who reported that *F. oxysporum* infestation markedly reduced chickpea growth under pot conditions, while the application of *T. asperellum* and *T. harzianum* strains, individually or in consortia, effectively mitigated disease impact, resulting in substantially lower DI (22.2% and 11.1%) and DS (86% and 92%) and significant improvements in shoot growth parameters across two years. This further supports the role of Trichoderma species as reliable and effective biocontrol agents for managing chickpea wilt under diverse environmental conditions. Similarly, Dubey (2007) reported that integrating *T. harzianum* with carboxin as a seed treatment significantly improved seed germination and grain yield while reducing wilt incidence by 44.1–60.3%, further supporting the present study where *T. asperellum*–based treatments markedly suppressed Fusarium wilt and enhanced plant health under pot conditions.

Conclusion

The present study demonstrates that incorporation of cell-protectant polymers and stabilizers, particularly 2% glycerol and 0.1% carboxymethyl cellulose (CMC), effectively prolongs the shelf life of liquid *Trichoderma asperellum* formulations up to 360 days while maintaining high microbial viability. Optimized additive combinations, such as glycerol + CMC and glycerol + trehalose, consistently supported superior survival compared to untreated controls. *Trichoderma asperellum* (Ta_1) demonstrated the highest antagonistic potential against *Fusarium oxysporum* f. sp. *ciceri*, effectively suppressing pathogen growth in vitro. Along with *Pseudomonas fluorescens* isolates, it shows strong promise as a biocontrol agent, offering an eco-friendly and sustainable approach for managing chickpea wilt. In vitro and pot culture evaluations confirmed that *T. asperellum*, especially when combined with resistant chickpea varieties like PDKV-Kanak, significantly suppressed Fusarium wilt, achieving complete or near-complete disease reduction. These findings highlight the potential of stable, nutrient-enriched *T. asperellum* formulations as eco-friendly, sustainable biocontrol solutions, offering a practical approach to enhance chickpea productivity and reduce dependence on chemical fungicides.

Table 1: Viable cell count of liquid based bioagent (*Trichoderma asperellum)* amended with additives during storage period of one year at room temperature.

Treatments Details	Population density (×10^7 cfu/ml)												
		STORAGE DAYS											
	Initial count*	**24 hr***	**30***	**60***	**90***	**120***	**150***	**180***	**210***	**240***	**270***	**300***	**330***	**360***
C1- (PVP)	17.56	17.17	14.46	13.06	11.32	8.49	7.51	5.16	3.03	2.06	1.23	1.01	0.67	0.43
C2- (PEG)	17.57	17.13	14.56	13.06	11.29	8.52	7.55	5.25	3.05	2.12	1.27	1.00	0.65	0.39
C3- (SA)	17.51	17.17	14.52	13.04	11.25	8.57	7.45	5.08	3.01	2.06	1.18	0.96	0.61	0.39
C4- (GL)	17.66	17.41	14.98	13.84	11.80	8.87	7.83	5.59	3.35	2.42	1.51	1.28	0.89	0.65
SE(m) ±	**0.10**	**0.024**	**0.019**	**0.017**	**0.027**	**0.026**	**0.023**	**0.019**	**0.025**	**0.023**	**0.023**	**0.019**	**0.024**	**0.027**
CD at 1%	**NS**	**0.09**	**0.07**	**0.07**	**0.11**	**0.10**	**0.09**	**0.07**	**0.10**	**0.09**	**0.09**	**0.07**	**0.10**	**0.11**
A1- (CMC)	17.80	17.42	15.28	14.22	12.22	9.11	8.15	6.04	3.62	2.65	1.75	1.52	1.13	0.80
A2- (TREAHALOSE)	17.48	17.10	14.47	12.93	11.25	8.50	7.43	5.01	2.97	2.02	1.20	0.96	0.61	0.40
A3- (ALPHOX)	17.43	17.14	14.15	12.60	10.77	8.23	7.18	4.77	2.74	1.82	0.95	0.71	0.38	0.20
SE(m) ±	**0.09**	**0.020**	**0.016**	**0.015**	**0.023**	**0.023**	**0.020**	**0.016**	**0.022**	**0.020**	**0.020**	**0.016**	**0.021**	**0.024**
CD at 1%	**NS**	**0.08**	**0.06**	**0.06**	**0.09**	**0.09**	**0.08**	**0.06**	**0.09**	**0.08**	**0.08**	**0.06**	**0.08**	**0.09**

Note- *Means of three replications

Factor 1: - C_1= **PVP 2%** (Polyvinyl Pyrrolidone), C_2= **PEG 2%** (Polyethylene glycol), C_3= SA 0.1% (Sodium alginate), C_4= GL 2% (Glycerol)

Factor 2: - A_1= CMC 0.1% (Carboxy methyl cellulose), A_2= Trehalose 10mM, A_3= Alphox 1%

Common use (Preservatives and Surfactant/emulsifiers): - Potassium sorbate (0.5%) and Tween-20-(0.5%)

Table 2: Interaction effect on shelf life of liquid based bioagent (*Trichoderma asperellum)* amended with additives at room temperature.

Treatments Details		Population density (×10^7 cfu/ml)												
		STORAGE DAYS												
		Initial count*	**24 hrs***	**30***	**60***	**90***	**120***	**150***	**180***	**210***	**240***	**270***	**300***	**330***	**360***
T_1	**C1A1- (PVP+CMC)**	17.67	17.30	15.25	14.22	12.22	9.10	8.13	6.02	3.61	2.64	1.75	1.52	1.12	0.80
T_2	**C1A2-(PVP+TREAHALOSE)**	17.47	17.03	4.10	12.50	11.00	8.17	7.20	4.77	2.76	1.75	0.98	0.76	0.47	0.27
T_3	**C1A3- (PVP+ ALPHOX)**	17.53	17.17	14.04	12.47	10.73	8.20	7.20	4.68	2.72	1.77	0.97	0.74	0.41	0.23
T_4	**C2A1- (PEG+CMC)**	17.87	17.43	15.25	14.19	12.21	9.08	8.12	6.02	3.60	2.62	1.72	1.50	1.12	0.76
T_5	**C2A2- (PEG+TREAHALOSE)**	17.57	17.07	14.19	12.51	10.90	8.13	7.23	4.59	2.73	1.83	1.13	0.77	0.43	0.22
T_6	**C2A3- (PEG+ ALPHOX)**	17.27	16.90	14.24	12.47	10.76	8.33	7.31	5.15	2.80	1.90	0.95	0.73	0.41	0.20
T_7	**C3A1- (SA+CMC)**	17.80	17.43	15.24	14.18	12.19	9.06	8.11	6.01	3.59	2.60	1.69	1.49	1.10	0.75
T_8	**C3A2- (SA+TREAHALOSE)**	17.17	16.83	14.31	12.47	10.89	8.53	7.15	4.64	2.74	1.80	0.93	0.75	0.40	0.23
T_9	**C3A3- (SA+ ALPHOX)**	17.57	17.23	14.01	12.47	10.68	8.11	7.08	4.60	2.70	1.77	0.92	0.63	0.33	0.17
T_{10}	**C4A1- (GL+CMC)**	17.87	17.50	15.37	14.30	12.26	9.19	8.23	6.10	3.67	2.73	1.83	1.57	1.18	0.87
T_{11}	**C4A2- (GL+TREAHALOSE)**	17.73	17.47	15.26	14.24	12.23	9.17	8.13	6.03	3.64	2.68	1.76	1.55	1.13	0.87
T_{12}	**C4A3- (GL+ ALPHOX)**	17.37	17.27	14.30	12.99	10.91	8.27	7.13	4.64	2.74	1.85	0.94	0.73	0.37	0.20
T_{13}	**Control**	17.43	17.00	9.60	4.40	1.37	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE(m) ±		**0.17**	**0.041**	**0.033**	**0.030**	**0.046**	**0.045**	**0.040**	**0.033**	**0.044**	**0.039**	**0.041**	**0.032**	**0.042**	**0.047**
Factor (C X A) CD at 1%		**NS**	**0.16**	**0.13**	**0.12**	**0.18**	**0.18**	**0.16**	**0.13**	**0.17**	**0.16**	**0.16**	**0.13**	**0.17**	**0.19**

*Values are means of three replications.

Note- T_1= PD- Broth + 2% PVP + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_2= = PD- -Broth + 2% PVP + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_3= = PD- Broth + 2% PVP + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_4= = PD- Broth + 2% PEG + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_5= = PD- Broth + 2% PEG + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_6= = PD- Broth + 2% PEG + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_7= = PD- Broth + 0.1 % SA + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_8= = PD- Broth + 0.1 % SA + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_9= = PD- Broth + 0.1 % SA + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_{10}= = PD- Broth + 2 % Glycerol + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_{11}= = PD- Broth + 2 % Glycerol + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_{12}= = PD- Broth + 2 % Glycerol + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, T_{13}= = PD- Broth + Without additives

Table 3 : *In vitro* Screening of Antagonistic Bioagent and Biofertilizers against *Fusarium oxysporum* f. sp. *ciceri*

Treatment details (Fungal and bacterial antagonist)		Mean colony diameter of *Fusarium oxysporum* pv. *ciceri* (mm)	Per cent growth inhibition over control
T_1	*Trichoderma asperellum* (Ta_1)	25.44 (±0.40)	71.73
T_2	*Trichoderma asperellum* (Ta_2)	28.55 (±0.44)	68.27
T_3	*Pseudomonas fluorescens* (Pf_1)	26.99 (±0.48)	70.01
T_4	*Pseudomonas fluorescens* (Pf_2)	27.22 (±0.67)	69.75
T_5	*Bacillus megaterium* var. *Phosphaticum*	60.33 (±0.58)	32.97
T_6	Control	90.00	00.00
SE(m) ±		**0.63**	
CD (P=0.01)		**1.96**	

*Values are means of three replications

Table 4 : Interaction effect of selected bioagent (*Trichoderma asperellum*) on Fusarium wilt of chickpea under pot condition

Treatments Details		*Wilt incidence (30 DAS) %	Disease over control %	*Wilt incidence (60 DAS) %	Disease over control %	*Wilt incidence (90 DAS) %	Disease over control %
T_1	PDKV-Kanak + *Trichoderma asperellum*	0.00 (0.00) **	100	0.00 (0.00) **	100	0.00 (0.00) **	100
T_2	Gulak-1 + *Trichoderma asperellum*	3.33 (1.83) **	85.73	6.66 (2.58) **	80.01	6.66 (2.58) **	88.93
T_3	JG-62+ *Trichoderma asperellum*	6.66 (2.58) **	71.45	10.33 (3.21) **	69.00	13.33 (3.65) **	77.78
T_4	Control (Without treated)	23.33 (4.83) **	00.00	33.33 (5.77) **	00.00	60.00 (7.75) **	00.00
	SE (m) ±	**0.53**		**0.56**		**0.55**	
	Factor (A X B) CD at 1%	**2.08**		**2.22**		**2.18**	

*Values are means of three replications**;** ** Square Root Transform value; DAS- Days after sowing

Note: **T_1**= PDKV-Kanak + *Trichoderma asperellum,* **T_2**= Gulak-1 + *Trichoderma asperellum,* **T_3** = JG-62+ *Trichoderma asperellum,* **T_4**= Control (Without treated)

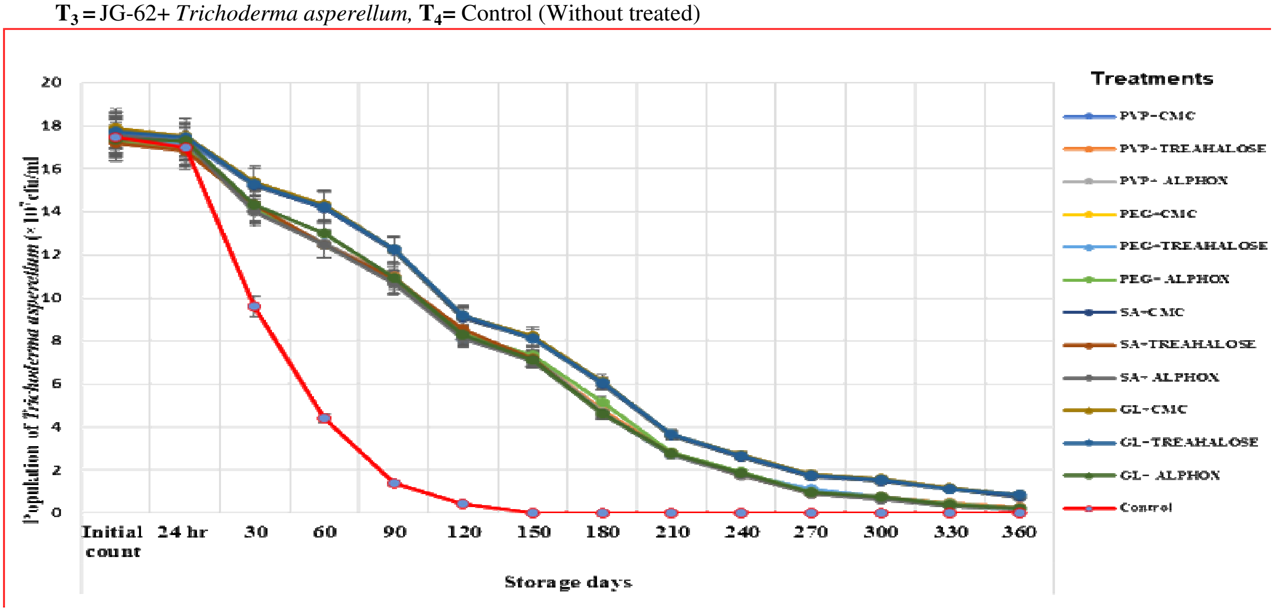

Fig. 1: Shelf life of *Trichoderma asperellum* bioagent amended with additives (One year)

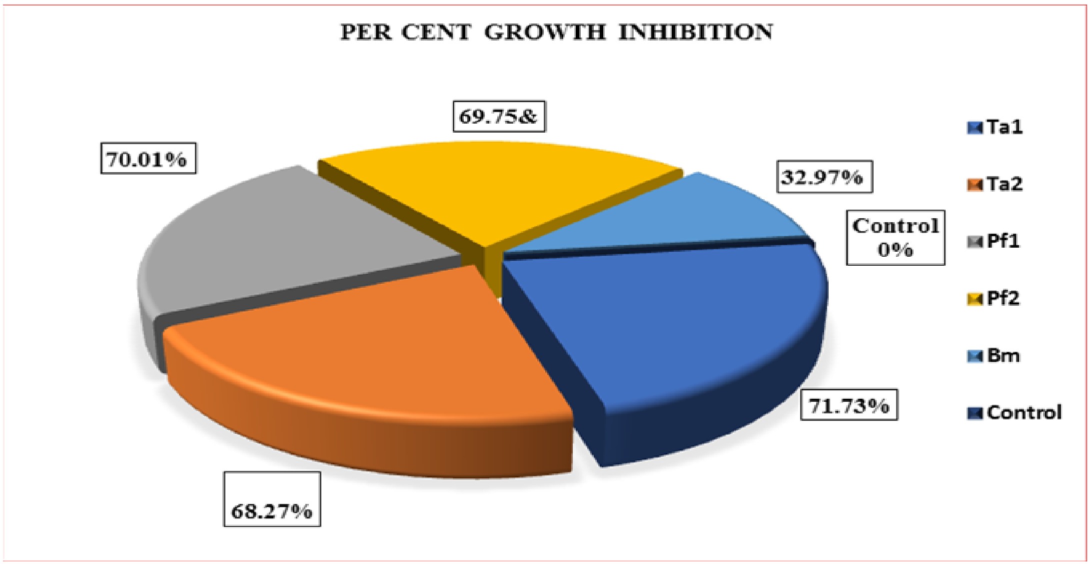

Fig. 2 : *In vitro* growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* by *Trichoderma asperellum* using the dual culture technique

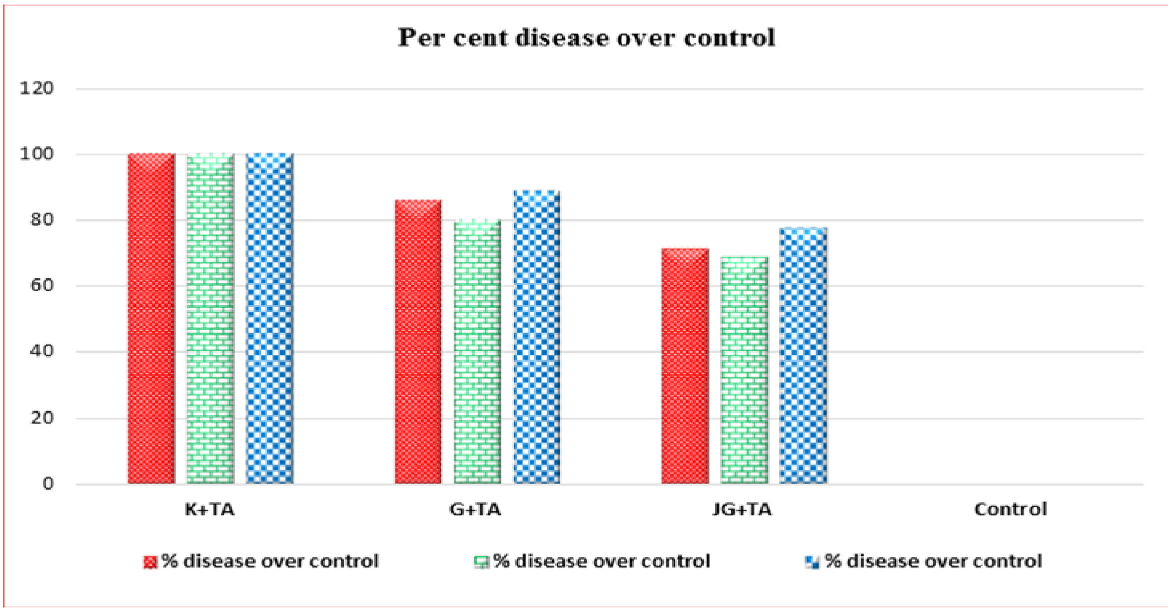

Fig. 3: Percent disease reduction over control by bioagent (*Trichoderma asperellum*) against Fusarium wilt of chickpea under pot condition

Acknowledgement

The authors are thankful to the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, for providing the necessary laboratory and pot culture facilities to carry out the present investigation

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript

References

Aneja, K.R. (2003). Experiments in microbiology, plant pathology and biotechnology. *New Age International*.

Anjali, M.K., Malashree, L. and Anushree, Y.K. (2024). Formulation and Quality Analysis of Yoghurts Fortified with *Mentha spicata, Moringa oleifera* and *Murraya koenigii* Leaf Extracts. *Journal of Advances in Biology & Biotechnology*, **27**(11), 117-127.

Bashan, Y., Holguin, G., de-Bashan, L.E. (2004). Azospirillum-plant relationships: physiological, molecular, agricultural and environmental advances (1997- 2003). *Can J Microbiol* **50**: 521-577.

Bhai, R.S., and Anandaraj, M. (2014). Enhancing shelf life of *Trichoderma harzianum* by conidial storage in sterile deionized water. *J. of Spices & Aromatic Crops*, **23**(2).

Bheemaraya, M.P, Ramesh, S.V. and Kalyan, R. (2011). Influence of temperature and carrier material on shelf life of mass cultured Trichoderma spp. *The Global users can now retrieve the articles from Journal on the repository of*

Commonwealth Agricultural Bureaux International (CABI) on. J.Res. ANGRAU **39**(4)24-29.

Brahmaprakash, G.P., Sahu, P.K., Lavanya, G., Gupta, A., Nair, S.S. and Gangaraddi, V. (2020). Role of additives in improving efficiency of bioformulation for plant growth and development. In *Frontiers in soil and environmental microbiology* (pp. 1-10). CRC Press.

Chohan, S.A., Akbar, M. and Iqbal, U. (2024). Trichoderma based formulations control the wilt disease of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris*, better when inoculated as consortia: findings from pot experiments under field conditions. *PeerJ*, **12**, p.e17835.

Deaker, R, Roughley, R.J. and Kennedy, I.R. (2004). Legume seed inoculation technology-A review. *Soil Biology and Biochemistry*, **36**(8), 1275–1288. https://doi.org/10.1016/j.soilbio.2004.04.009.

Dhingra, O.D. and Sinclair J.B. (1995). Basic Plant Pathology Methods. 2nd Ed. Boca Raton FL, USA. CRC Lewis Publishers.

Directorate of Pulse Development. (2021–22). Pulses development in India: Annual report 2021–22. Ministry of Agriculture and Farmers Welfare, Government of India, Bhopal. *Indian Institute of Pulses Research*.

Dobhal, A. & Hegde, G.M. (2021). *Shelf life study of Pseudomonas fluorescens in liquid formulations. Journal of Farm Sciences*, **34**(01), 84–86.

Dubey, S.C., M. Suresh and B. Singh. (2007). Evaluation of *Trichoderma species* against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biological control*. **40**(1): 118-127.

Hegde, S. V. (2008). Liquid bio-fertilizers in Indian agriculture. *Bio-fertilizer newsletter*: 17-22. https://doi.org/10.1016/j.biotechadv.2004.03.005.

Jimenez-Diaz, R.M., Cirulli, M., Bubici, G., del Mar Jimenez-Gasco, M., Antoniou, P.P. and Tjamos, E.C., (2012). Verticillium wilt, a major threat to olive production: current status and future prospects for its management. *Plant disease*, **96**(3), 304-329.

Kara, Y.E. and E. Tozlu. (2024). Effect of different liquid carriers on the shelf-life and antifungal effectiveness of *Trichoderma harzianum. Journal of Crop Health*. **76**(2): 425-435.

Katyayani K.K.S., Bindal S., Prakash Singh J., Rana M., and Srivastava S. (2020). *In vitro evaluation of Trichoderma spp. against chickpea wilt. International Archive of Applied Sciences and Technology*, **11**(3): 1–4. DOI: 10.15515/iaast.0976-4828.11.3.14.

Khan, S., Bagwan, N. B., Iqbal, M. A. and Tamboli, R.R. (2011). Mass multiplication and shelf life of liquid fermented final product of *Trichoderma viride* in different formulations. *Adv. Biores.* **2**(1): 178-182.

Kolombet, L.V., Zhigletsova, S. K., Kosareva, N. I., Bystrova, E. V., Derbyshev, V. V., Krasnova, S. P. and Schisler, D. (2008). Development of an extended shelf-life, liquid formulation of the biofungicide *Trichoderma asperellum*. *World J. Microbiol. Biotec*., 24(1): 123-131.

Kumari, R., Kumar, V., Arukha, A.P., Rabbee, M.F., Ameen, F. and Koul, B., (2024). Screening of the biocontrol efficacy of potent Trichoderma strains against *Fusarium oxysporum* f. sp. *ciceri* and *Scelrotium rolfsii* causing wilt and collar rot in chickpea. *Microorganisms*, **12**(7), p.1280.

Mahendra, Reddi Kumar, Rajan, C.P. and Sumathi (2022). Shelf life studies on solid and liquid formulations of *Trichoderma harzianum* and *Pseudomonas fluorescens*, *The Pharma Innovation Journal* , **11**(3): 866-873.

Meki, S., Ahmed, S. and Sakhuja, P.K. (2011). Control of chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*) using Trichoderma spp. in Ethiopia. *Archives of Phytopathology and Plant Protection*, **44**(5), pp.432-440.

Morton, D. T. and Straube N.H. (1955). Antagonistic and stimulatory effects of microorganisms *Sclerotium rolfsii*. Phytopath. **45**: 419-420.

Mugnier, J. and Jung, G. (1985). Survival of bacteria and fungi in relation to water activity and the solvent properties of water. *App. Environ. Microbio*. **50**(4), 108–114. https://doi. org/10.1128/aem.50.4.108-114.1985.

Nadare, M., Guldekar, D. D., Potdukhe, S. R. and Parbat, J. M. (2018). Assessment of shelf life of *Trichoderma viride* on different liquid formulations. *Int. J. Curr. Microbiol. App. Sci*. **6**: 2575-2579.

Singh, A., Srivastava, S. and Singh, H. B. (2007). Effect of substrates on growth and shelf life of *Trichoderma harzianum* and its use in biocontrol of diseases. *Biores. Tech*. **98**(2): 470-473.

Singh, N. P., Gupta, S. and Singh, B. B. (2014). Status and prospects of chickpea production in India. *Indian Journal of Pulses Research*. **27**(1): 1–10.

Source: The 22nd edition of AOAC Official Method of Analysis. (2023). is a comprehensive collection of chemical and microbiological methods, including those for nitrogen determination.

Source:https://www.fortunebusinessinsights.com/industry-reports/biofertilizers-market-(2025).

Sriram, S. and Ramanujam, B., (2021). Extension of Shelf Life in Formulations of Fungal Bioagents. In *Biopesticides in Horticultural Crops* (pp. 54-62). CRC Press.

Sriram, S., Roopa, K.P. and Savitha, M.J. (2011). Extended shelf-life of liquid fermentation derived talc formulations of *Trichoderma harzianum* with the addition of glycerol in the production medium. *Crop protection*, **30**(10), 1334-1339.

Sutthisa, W., Hompana, W. and Yutthasin, R. (2024). Enhancing Biocontrol Potential: Development and Efficacy Assessment of a Liquid Formulation of *Trichoderma Asperellum* MSU007 against *Sclerotium Rrolfsii*. *Trends in Sciences*, **21**(4): 7550-7550.

Temprano, F., Albareda, M., Camacho, M., Daza, A., Santamaría, C. and Rodríguez-Navarro, D. N. (2002). Survival of several Rhizobium/Bradyrhizobium strains on different inoculant formulations and inoculated seeds. *International Microbiology*, **5**(1), 81–86. https://doi.org/10.1007/s10123-002-0065-6.

Vincent, J.M. (1927). Distortion of fungal hyphe in presence of certain inhibitor. *Nature*, 159-180.

Zakaria, A. K. M., Rashid, P. M. A. and Bagchi, P. S. (2018). Shelf Life Study of *Trichoderma* spp. in Talc-based Formulation. *evsjv‡k cjøx Dbœqb mgxÿv*, p.27