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RESEARCH ARTICLE

Synthesis and optimization of agro-based solid and liquid formulation for enhanced shelf-life and biomass production of *Trichoderma asperellum*

Munnysha S[1*], Bunker RN[1], Babu SR[2], Mondal K[3], Abhi R[4], Akodiya S[1], Gahlot N[1] & Sharma D[5]

[1]Department of Plant Pathology, Maharana Pratap University of Agriculture and Technology, Udaipur 313 001, India
[2]Department of Entomology, Maharana Pratap University of Agriculture and Technology, Udaipur 313 001, India
[3]Department of Molecular Biology and Biotechnology, Maharana Pratap University of Agriculture and Technology, Udaipur 313 001, India
[4]Department of Nematology, Maharana Pratap University of Agriculture and Technology, Udaipur 313 001, India
[5]Department of Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan 173 230, India

*Correspondence email - munnysha118@gmail.com

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Abstract

Beneficial organism-based bio-pesticides have currently gained high prominence in natural and organic farming systems to ingress and suppress pests and diseases in the realm of modern agriculture. Although conventional bio-pesticides have good track records under laboratory conditions, stability and storage issues are pre-dominantly found when tested under field conditions. Therefore, the preparation and in-field evaluation of precisely structured bio-pesticide formulations are immediately required for agricultural improvement. Herein, the liquid broth media and agro-substrates based *Trichoderma asperellum* suspension culture have been elucidated for the biomass production and shelf-life analysis. Among the 8 different liquid broth medium under the investigation, molasses yeast extract broth was found to have maximum biomass production (20.84 g fresh and 3.14 g dry weight of mycelium; 127.5×10^6 CFU/mL). In contrast, a shelf-life analysis of *T. asperellum* revealed Paraffin oil as the best medium (19×10^6CFU/mL), where a maximum shelf-life (up to 180 days) was achieved with 39.37 % of viability. On solid substrates, a maximum CFU count of 13.33×10^6CFU/g and viability of 15.25 % were observed in shelled maize cob powder. The current findings emphasize the aim of bestowing the different substrates for mass multiplication and viability of *T. asperellum* as a promising antagonist to menace soil borne pathogens and significantly increase disease resistance in plants.

Keywords: biopesticide; disease resistance; formulation; shelf life; soil borne pathogen; *Trichoderma asperellum*

Introduction

Trichoderma is a filamentous and culturable fungus that paves the way for great momentum because of its multiple actions against a copious number of plant pathogens. *Trichoderma* species are useful avirulent plant symbionts that act as biocontrol agents against phytopathogenic fungi. For instance, various mechanisms of competition, antibiotic production, myco-parasitism, and cell wall degrading enzymes not only trigger defense responses in plants but also lead to plant growth promotion (1-4). The eminent *Trichoderma* species acts as an antagonist of phytopathogenic fungi and is significantly harnessed as a prominent biocontrol agent. *Trichoderma* spp. is commonly used as bio-pesticides, bio-fertilizers, and soil amendments to promote plant growth, even under adverse pathogenic conditions. The antagonistic activity of *Trichoderma* against predominant soil borne pathogens has been explored (5-7).

The injudicious use of pesticides has posed several detrimental effects on the environment and human health. They degrade soil fertility, augment resistance to pathogens, and arrest microbial growth. The prime aspects for the revitalization of biopesticides include cost effectiveness, a wide spectrum of action, and poses significantly positive impacts on the environment, which bolster sustainability in agriculture. The use of *Trichoderma* is slowly increasing in the recent years and consequently used as a substitute for chemical pesticides to foster a safe environment (8).

The use of *Trichoderma* spp. are currently been praised in the management of fungal diseases in crop plants exhibiting mycoparasitism against a diverse range of plant pathogens. However, bio-agent formulations with organic amendments and fungicides have been found quiet more effective to suppress the diseases and also enhance host plant resistance (9-11).

The global biopesticide market is booming, with a major share of the various commercial formulations of *Trichoderma* spp. However, the cost of these raw materials and the commercial production of bio-control agents is one of the major hurdles behind the restricted use of these. To overcome this cost limitation, many researchers have revitalized the exploitation of various agricultural and domestic wastes, such as wheat bran-saw dust modified medium, wheat bran, biogas manure, farm yard manure, wheat bran, rice bran, peat soil, tea waste, rice straw corn fiber dry mass, vegetable waste, sewage sludge and compost, to develop an effective formulation and mass multiplication of *Trichoderma* sp. It is crucial to improve disease resistance and ensure widespread adoption of agricultural approaches (12-16).

The liquid state fermentation method of *Trichoderma* is broadly used to produce spores from fungal strains (17). Potato dextrose broth, V8 juice and molasses yeast medium were exploited as the liquid-based substrate for the mass multiplication of *Trichoderma* sp. (18, 19). The shelf-life study of *T. asperellum* was assessed by using various carriers such as paraffin oil, mustard oil, diesel, soybean oil, groundnut oil, sunflower oil and talc powder. Broth, dispersant, suspender and surfactant were also added to the oil-based formulations. The highest viability was observed in the paraffin oil-based formulation (31.2×10^5 CFU/mL) followed by soybean oil (21.5×10^5 CFU/mL) and groundnut oil (19×10^5 CFU/mL) (20-22).

Mass production of *Trichoderma* species were developed worldwide. For instance, *T. harzianum* and *T. asperellum* were fortified with different carriers to assess biomass production. Elicitation of fungal antagonists plays a pivotal role in sustainable agricultural practices while mitigating hazardous pesticides. Notably, the commercial prosperity of these fungal antagonists totally depends on cost-effective formulations attributed to higher shelf-life at the time of application with good coverage and retention after application in the field. Oil holds up as the perfect medium to provide inoculants under viable conditions (23).

An experiment was carried out on shelf-life studies of *T. asperellum* using copious carriers, such as paraffin oil, soybean oil, groundnut oil, potato dextrose broth, and talc powder. Paraffin oil recorded the highest viability of 28×10^8 colony forming units CFU/mL at 30 DAS and it was significantly superior over all other treatments except talc based culture 27.33×10^8 CFU and gradually increased up to 90 DAS and thereafter found to be declined from 120-180 days after inoculation (DAI) in all the treatments. The highest percentage of spore germination was observed in paraffin oil 59.18 % at 30 DAT, followed by talc-based culture 54.06 %. The commercialization of biocontrol products is growing rapidly globally. Nevertheless, biological products are still gaining friction in the agricultural market owing to ineffective legislation. Therefore, it is necessary to test the developed formulation in various agroclimatic zones. However, integrating urban technology from a different sector with a limited understanding of bioformulations could lead to the development of more precise and supportive formulations (24).

Materials and Methods

Collection of bioagent *Trichoderma asperellum*

Pure cultures of *T. asperellum* were collected from the Biopesticide laboratory, Department of Entomology, RCA, MPUAT, Udaipur. Pure culture of Trichoderma spp. was maintained on Trichoderma selective medium (TSM) by the dilution plate method (25). The Trichoderma selective medium (TSM) for the growth and isolation of Trichoderma asperellum was prepared using the following composition: 0.2 g of magnesium sulfate ($MgSO_4$ $7H_2O$), 0.9 g of dipotassium hydrogen phosphate K_2HPO_4, 0.15 g of potassium chloride (KCl), 1.0 g of ammonium nitrate (NH_4NO_3), 3.0 g of glucose, 0.2 g of PCNB, 0.15 g of Rose Bengal agar (RBA), 20 g of agar-agar, 0.25 g of chloramphenicol and 1000 mL of distilled water.

Determination of biomass production of *Trichoderma asperellum* in various liquid formulations and their shelf-life

To determine fresh and dry weight of the mycelium of T. asperellum, four different broth media were tested viz., maltose peptone broth, potato dextrose broth, sabouraud dextrose broth, molasses yeast extract broth. 150 mL of each broth medium were poured into 500 mL conical flask in four replications and sterilized in an autoclave at 1.045 kg cm^2 (15 pounds per square inch) pressure for 20 min. Flasks were inoculated with 5 mm disc of 7-10 days old culture of T. viride with the help of sterilized cork borer and incubated for 15 days at room temperature (27 ± 2 °C). After the incubation period, the average fresh and dry weight of the mycelium of *T. asperellum* was recorded.

To elucidate the maximum sporulation (CFU production ability) of *T. asperellum*, eight different broth media were tested: paraffin oil, glycerol, soybean oil, groundnut oil, sunflower oil, molasses yeast extract, potato dextrose broth, and a marketed formulation (Trichoz-L). Each broth medium (150 mL) was poured into a 500 mL conical flask and sterilized in an autoclave at 1.045 kg cm^2 (15 pounds per square inch) pressure for 20 min. The flasks were inoculated with 5 mm disc of 7-10 days old culture of *T. asperellum* with the help of a sterilized cork-borer and inoculation needle. The flasks were incubated for 15 days at room temperature.

The viability of *T. asperellum* was assessed by calculating the number of colony forming units per millilitre (CFU/mL) of liquid broth media. 1 mL sample was drawn from each broth medium and serial dilutions of $10^{-1}$to 10^{-6}were then made. One mL of suspension was taken from the dilution of $10^{-6}$and transferred to petri plates containing 20 mL sterilized Trichoderma-selective medium and gently shaken to spread evenly. These petri plates were incubated at 27 ± 2 °C for 3 days and CFU were calculated using the following formula.

$$\text{CFU / mL} = \frac{\text{Numbers of colonies per mL plated}}{\text{Dilution factor}} \quad \text{(Eq. 1)}$$

Determination of biomass production of *Trichoderma asperellum* on different agro-based solid substrates and their shelf life

To determine the dry weight of the mycelium of *T. asperellum,* seven different substrates were tested *viz*., shelled maize cob powder, sugarcane bagasse, farm yard manure, vermicompost, spent mushroom compost, neem cake and talc. Each substrate (25 g) was kept in a 90 mm petri plate in four replications and sterilized in an autoclave at 1.045 kg cm^2 (15 pounds per square inch) pressure for 20 min. The plates were inoculated with 5 mm disc of 7-10 days old culture of *T. asperellum* with the help of a sterilized cork borer and incubated for 15 days at room temperature. After the incubation period, the average dry weight of the mycelium of *T. asperellum* on different substrates was recorded.

Results

Evaluation of biomass production of *Trichoderma asperellum* in various liquid and solid formulations

Four liquid broth media viz., maltose peptone broth, potato dextrose broth, sabouraud dextrose broth, and molasses yeast extract were tested for maximum biomass production of *T. asperellum.* Among these, molasses yeast extract broth was found most suitable with highest (20.84 g fresh & 3.14 g dry weight of mycelium) biomass production of *T. asperellum*, followed by potato dextrose broth was found better with 16.71 and 1.23 g fresh and dry weight of mycelium respectively. Sabouraud dextrose broth and maltose peptone broth yielded 10.85 & 0.32 g and 4.68 & 0.29 g of fresh and dry weight of mycelium respectively. The colony forming units (CFU/mL) were recorded with a maximum of 127.5×10^6 CFU/mL in molasses yeast extract broth followed by 102.7×10^6 CFU/mL in potato dextrose broth. Whereas, in sabouraud dextrose broth was recorded 102.2×10^6 CFU/mL and 101.5×10^6 CFU/mL in maltose peptone broth (Table 1 & Fig. 1—4).

Similarly, seven agro-based solid substrates *viz*., sugarcane bagasse, shelled maize cob powder, talc powder, spent mushroom compost, neem cake, vermicompost and farmyard manure were tested for maximum biomass production of *T. asperellum.* Among these, shelled maize cob powder was found most suitable with highest (25.05 g fresh & 0.05 g dry weight of mycelium) biomass production of *T. asperellum*, followed by sugarcane bagasse was found better with 25.04 & 0.04 g fresh & dry weight respectively. The remaining entities, neem cake, farmyard manure, vermicompost, and spent mushroom compost yielded 25.02 & 0.02, 25.04 & 0.04, 25.03 & 0.03 and 25.01 & 0.01 g fresh and dry weight of mycelium, respectively. No *T. asperellum* biomass was obtained from the talc powder. The results of spore counts (CFU/mL) were recorded with maximum 112.5×10^6 CFU/g in shelled maize cob powder followed by 108.7×10^6 CFU/g in sugarcane bagasse. The remaining entities vermicompost, talc powder, neem cake, farmyard manure, and spent mushroom compost was recorded 108.2×10^6, 102×10^6, 101.7×10^6, 101.5×10^6, and 101×10^6 CFU/g respectively (Table 2, Fig. 5—7).

Evaluation of shelf life of *Trichoderma asperellum* in various liquid and solid formulations

The results emphasized that colony forming units (CFU) were between $40.33\text{-}51.66\times10^6$ CFU/mL in different liquid formulations on the first day of formulation preparation. Paraffin oil formulation had maximum viability of 39.37 % with respect to the colony forming units of 46.00×10^6, 42.00×10^6, 40.66×10^6, 40.33×10^6, 36.33×10^6, 35.33×10^6, 29.66×10^6, 27.66×10^6, 25.66×10^6, 25.33×10^6, 24.00×10^6 and 19.00×10^6 CFU/mL population of *T. asperellum* at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180, days after inoculation (DAI). Concurrently, as with passing days there was less reduction in the population of *T. asperellum.* Minimum viability (3.85 %) was observed in potato dextrose broth. Whereas, in marketed formulation (Trichoz-L) 44.00×10^6, 43.00×10^6, 39.33×10^6, 39.00×10^6, 24.33×10^6, 19.66×10^6, 19.66×10^6, 18.66×10^6, 17.66×10^6, 16.66×10^6, 14.66×10^6 and 11.00×10^6 CFU/mL population of *T. asperellum* at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180, days after inoculation (DAI) (Table 3).

Similarly, shelf-life studies of *T. asperellum,* seven different locally available agro-based solid substrates (sugarcane bagasse, shelled maize cob powder, talc powder, spent mushroom compost, neem cake, vermicompost, and farmyard manure) were used. Results emphasized that colony forming units (CFU) were $79.33\text{-}87.66\times10^6$ CFU/g in different solid formulations on the first day of formulation preparation. The shelled maize cob powder-based formulation had a maximum viability of 15.25 % in terms of colony forming units of 121.6×10^6, 123×10^6, 105×10^6, 91.6×10^6, 84.00×10^6, 77.66×10^6, 65.33×10^6, 48.66×10^6, 43.00×10^6, 17.00×10^6, 15.6×10^6 and 13.33×10^6 CFU/g population of *T. asperellum* at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180, days after inoculation (DAI). Whereas, in marketed talc powder formulation 84.33×10^6, 82.66×10^6, 80.66×10^6, 71.33×10^6, 55.33×10^6, 41.66×10^6, 30.33×10^6, 22.33×10^6, 21.33×10^6, 10.6×10^6, 8.66×10^6 and 2.66×10^6 CFU/g population of *T. asperellum* at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180, days after inoculation (DAI) (Table 4).

Table 1. Comparative efficacy of different liquid broth media for biomass and CFU production of *T. asperellum*

S. No.	Treatments	Fresh weight of biomass (g) (15 DAI)**	Dry weight of biomass (g) (15 DAI)*	(CFU×10^6) (15 DAI)*
1	Potato dextrose broth	16.71	1.23	102.75
2	Maltose peptone broth	4.68	0.29	101.5
3	Sabouraud dextrose broth	10.85	0.32	102.25
4	Molasses yeast extract	20.84	3.14	127.5
	SEm±	0.225	0.026	0.625
	CD (*p=0.05*)	0.694	0.079	1.926

*Mean of four replications; DAI** - Days after inoculation

Fig. 1. Comparative efficacy of different broth media in biomass production of T. *asperellum*. (a) Molasses yeast extract broth; (b) PDB-Potato dextrose broth (c) SDB-Sabouraud dextrose broth; (d) MPB-Maltose peptone broth.

Fig. 2. Weight of mycelial mat developed on different broth media after filtered through Whatman No.4 filter paper. (a) Molasses yeast extract broth; (b) PDB-Potato dextrose broth; (c) SDB-Sabouraud dextrose broth; (d) MPB-Maltose peptone broth.

Fig. 3. Dried mat of *T. asperellum* from different broth media. (a) Molasses yeast extract broth; (b) PDB-Potato dextrose broth; (c) SDB-Sabouraud dextrose broth; (d) MPB-Maltose peptone broth.

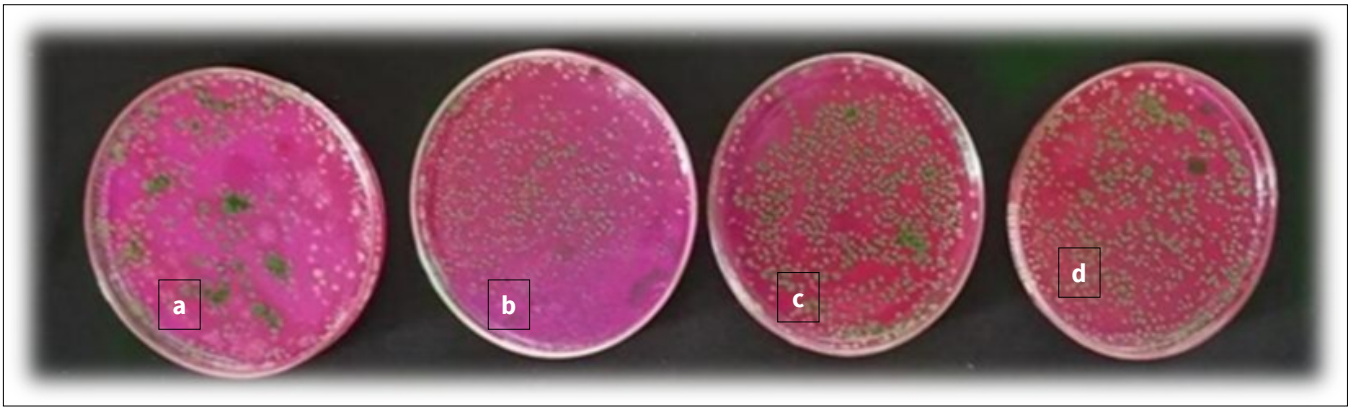

Fig. 4. Number of colony forming units (CFU×10^6) of *T. asperellum* in different broth media. (a) MPB-Maltose peptone broth; (b) SDB-Sabouraud dextrose broth; (c) MYEB-Molasses yeast extract broth; (d) PDB-Potato dextrose broth.

Table 2. Comparative efficacy of different agro-based substrates for biomass and CFU production of *T. asperellum*

S. No.	Treatments	Initial weight of substrate	Fresh weight of substrate (g) (15 DAI)*	Dry weight of mycelium (g) (15 DAI)*	(CFU×10^6/g) (15 DAI)*
1	Shelled maize cob	25	25.05	0.05	112.5
2	Sugarcane bagasse	25	25.04	0.04	108.75
3	Farm yard manure	25	25.04	0.04	101.5
4	Spent mushroom compost	25	25.01	0.01	101
5	Vermi compost	25	25.03	0.03	108.25
6	Neem cake	25	25.02	0.02	101.75
7	Talc powder	25	25	-	102
SE±m			0.003	0.002	0.675
CD at 5 %			0.008	0.005	1.985

Mean of four replications; DAI - Days after inoculation

Fig. 5. Comparative efficacy of *T. asperellum* biomass production on various organic substrates. (a) Maize cob powder; (b) Sugarcane bagasse; (c) Farm yard manure; (d) Vermicompost; (e) Spent mushroom compost waste; (f) Neem cake; (g) Talc powder.

Fig. 6. Number of colony forming units (CFU×10^6) of *T. asperellum* on different organic substrates. (a) Maize cob powder; (b) Sugarcane bagasse; (c) Neem cake; (d) Vermicompost; (e) Farm yard manure; (f) Spent mushroom compost waste; (g) Talc powder.

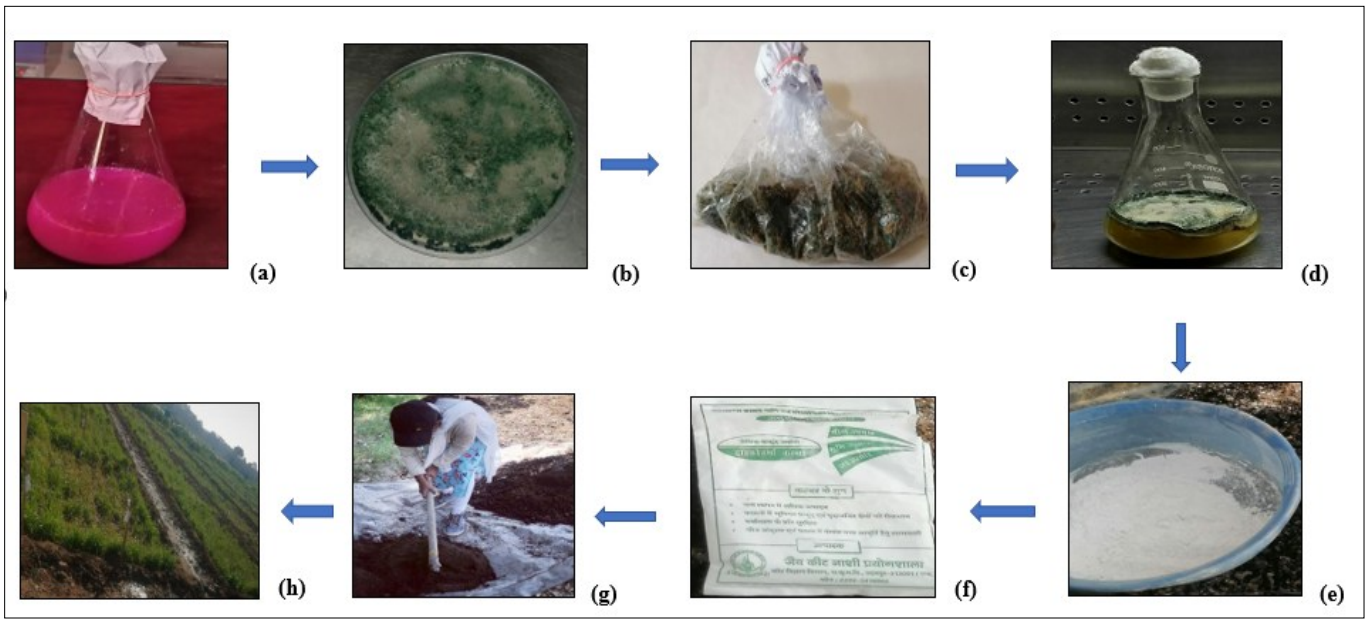

Fig. 7. Procedure of *Trichoderma* formulation. (a) *Trichoderma* selective medium; (b) Pure culture; (c) Solid fermentation; (d) Liquid fermentation; (e) Mixing of *Trichoderma* with Talcum powder; (f) Packaging; (g) Mixing in FYM; (h) Field application.

Table 3. Studies on shelf life of *T. asperellum* (CFU× 10^6/mL)* in different liquid formulations under *in vitro* condition

Treatment	Initial (days)	15** (days)	30 (days)	45 (days)	60 (days)	75 (days)	90 (days)	Viability (%)	105 (days)	120 (days)	135 (days)	150 (days)	165 (days)	180 (days)	Viability (%)
Paraffin oil	48.33	46.00	42.00	40.66	40.33	36.33	35.33	73.05	29.66	27.66	25.66	25.33	24.00	19.00	39.37
Glycerol oil	48.00	44.33	41.66	40.66	39.33	35.00	33.00	68.82	29.00	27.00	25.00	23.66	21.66	17.33	36.13
Potato dextrose broth	51.33	92.66	93.00	73.33	53.66	38.33	26.00	50.33	19.66	16.00	12.33	9.00	6.66	2.00	3.85
Molasses yeast extract broth	51.66	124.66	126.66	80.00	56.33	42.00	29.33	57.13	19.33	18.66	14.33	11.00	7.33	2.66	5.21
Sunflower oil	41.33	40.33	39.66	39.33	37.00	26.33	18.00	45.30	18.00	17.00	15.66	15.33	12.66	8.66	21.13
Groundnut oil	40.33	36.00	34.00	33.00	30.66	26.00	24.00	42.45	19.33	18.66	18.00	16.33	12.33	6.66	17.43
Soybean oil	44.66	43.00	41.33	36.66	36.33	24.66	22.66	50.72	23.00	22.33	21.33	19.66	14.66	9.33	20.94
Marketed formulation (Trichoz-L)	45.66	44.00	43.00	39.33	39.00	24.33	19.66	43.35	19.66	18.66	17.66	16.66	14.66	11.00	24.11
SEm±		1.04	1.03	1.17	1.45	1.54	0.88	2.24	0.47	0.45	0.72	0.58	0.65	0.53	1.15
CD at 5 %		3.04	3.00	3.42	4.25	4.51	2.57	6.56	1.38	1.33	2.11	1.69	1.90	1.56	3.37

*Mean of four replications; **Days after inoculation

Table 4. Studies on shelf life of *T. asperellum* (CFU× 10^6/g)* on different solid formulations under *in vitro* condition

Treatment	Initial (days)	15** (days)	30 (days)	45 (days)	60 (days)	75 (days)	90 (days)	Viability (%)	105 (days)	120 (days)	135 (days)	150 (days)	165 (days)	180 (days)	Viability (%)
Sugarcane bagasse	85.66	118.00	123.00	96.33	80.66	57.66	44.00	51.29	26.33	10.33	10.33	6.66	5.33	1.00	1.53
Shelled maize cob	87.66	121.60	123.00	105.00	91.66	84.00	77.66	90.57	65.33	48.66	43.00	17.00	15.60	13.33	15.25
Farm yard manure	81.66	113.60	115.60	92.33	71.33	51.00	41.00	50.33	32.66	18.66	17.33	9.33	7.33	2.00	2.48
Vermi compost	84.33	118.60	122.00	102.33	89.66	81.00	76.33	88.64	61.66	32.33	30.66	14.66	12.33	6.33	7.56
Spent Mushroom	83.33	105.06	117.00	90.33	77.00	68.00	41.33	49.59	40.33	20.00	19.33	8.33	5.00	1.66	2.02
Neem cake	79.33	113.00	115.00	89.33	74.00	63.66	43.00	54.26	38.33	26.66	26.66	6.66	5.66	1.33	2.12
Talc powder	84.66	84.33	82.66	80.66	71.33	55.33	41.66	49.61	30.33	22.33	21.33	10.60	8.66	2.66	3.15
SEm±		1.51	1.37	1.19	1.41	1.21	1.14	2.01	0.75	0.74	0.57	0.49	0.45	0.51	0.96
CD at 5 %		4.46	4.04	3.52	4.16	3.55	3.36	5.92	2.22	2.18	1.69	1.46	1.34	1.50	2.83

*Mean of four replications; **Days after inoculation

Discussion

Plant disease management is a significant concern in the current crop production scenario, as reported in a study (26). The revitalization of biocontrol agents is mainly based on plant growth promotion, which is dependent solely on excess sporulation. Because of the injudicious use of chemicals, it is imperative to minimize the hazardous use of pesticides in the market quoted in a study (27). There are copious number of fungal bioagents like *Ampelomyces quisqualis, Anthracocystis fluocculosa, Candida oleophila, Coniothyrium minitans, Clonostachys rosea, Phlebiopsis gigantea, Trichoderma virens, Trichoderma harzianum, Trichoderma polysporum* and *Chaetomium globosum* are present in the environment (28). Among the different antagonist organisms, *Trichoderma* species have been highly efficient as biocontrol agents against a variety of soil-borne phytopathogenic fungi. *Trichoderma* species have proven to be very effective bioagents against a wide range of soil-borne plant pathogenic fungi (29). The delivery of antagonists requires multiple carrier-based formulations. Formulations can be different, such as dry substrate (grains, wettable powders, and dust) and liquid substrates (water, oil, and emulsions), which comprise the active component and microencapsulation (30). A study suggested that as more farmers switch to organic farming, there is a significant demand for various biocontrol agent formulations, notably *Trichoderma* formulations (31).

The present investigation revealed that among all the solid based substrates assessed, maximum biomass of *T. asperellum* (25.05 g fresh & 0.04 g dry weight of mycelium) and also significantly higher number of spores were elucidated in shelled maize cob powder up to 30 days after inoculation. Thereafter, the shelf life of *T. asperellum* decreased drastically owing to nutrient exhaustion. In a study, among all the agro-based substrates the maximum spore counts of *T. asperellum* was found in neem cake (446 spores/mL) followed by vermicompost and FYM (32). These results emphasized that the spore count in neem cake drastically declined 60 days after inoculation. Similarly, the present study emphasized that the shelled maize cob powder -based formulation of *T. asperellum* causes a significant rise in biomass production. Shelled maize cob powder outperformed other agro-based substrates in the biomass production of *T. asperellum.* However, explorations of *T. asperellum* exhibited significantly varied shelf life depending on the storage temperature and agro-based medium (33, 34). Similarly, on various liquid broth media tested, the maximum biomass of *T. asperellum* (20.84 g fresh & 3.14 g dry weight of mycelium) and a significantly higher number of spores were observed in the molasses yeast extract broth. In agreement with a study, molasses yeast extract broth and potato dextrose broth yielded maximum biomass after 72 hr of fermentation among the different broth media that were assessed (35). The shelf-life study revealed that there were comparative differences in *T. asperellum* at all -time intervals. The initial population of *T. asperellum* in the first month was the highest (42×10^5CFU/mL) in paraffin oil, which was significantly higher than that in all other treatments. Notably, the application of paraffin oil increases the shelf life of *Trichoderma,* which also acts as a biofungicide (36). *Trichoderma* conidia preserved in oil as the main ingredient in the emulsified formulation showed enhanced viability and shelf life, as reported in a study (37).

The present study has shown that up to 6 months of storage, maximum production of *T. asperellum* of 13.33×10^6 CFU/g was recorded in shelled maize cob powder. Notably, the sustainability of the shelf life of *T. asperellum* on shelled maize cob powder is due to its carbohydrate composition, better particle distribution, and water absorption capacity (38 -42). These findings emphasize that harnessing various media is employed for the mass multiplication and sporulation of *T. asperellum* as a promising antagonist to combat soil borne pathogens.

Conclusion

The luxurious and risky impact of chemical pesticides on agroecosystem has sparked a rise in interest in harnessing bioformulation of *T. asperellum* for combating plant diseases. Future studies should postulate the effectiveness of *Trichoderma* not only in the suppression of pathogens but also in plant growth promotion. It is imperative to augment promising bioagent formulations to uphold sustained agriculture and safeguard global food security.

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Authors' contributions

MS carried out formal analysis, data curation and practical experimental work and drafted the manuscript. BRN carried out research conceptualization, methodology, supervision, design of the experiment. AS and SD participated in the sequence alignment. MK and GN carried out interpretation of the data, cross checking and editing. AR and BSR participated in the proof reading and manuscript preparation.

Compliance with ethical standards

Conflict of interest: The authors have said that there were no conflicting agendas.

Ethical issues: None

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