

Expedition of *Trichoderma* formulations: Production to storage in India-A review

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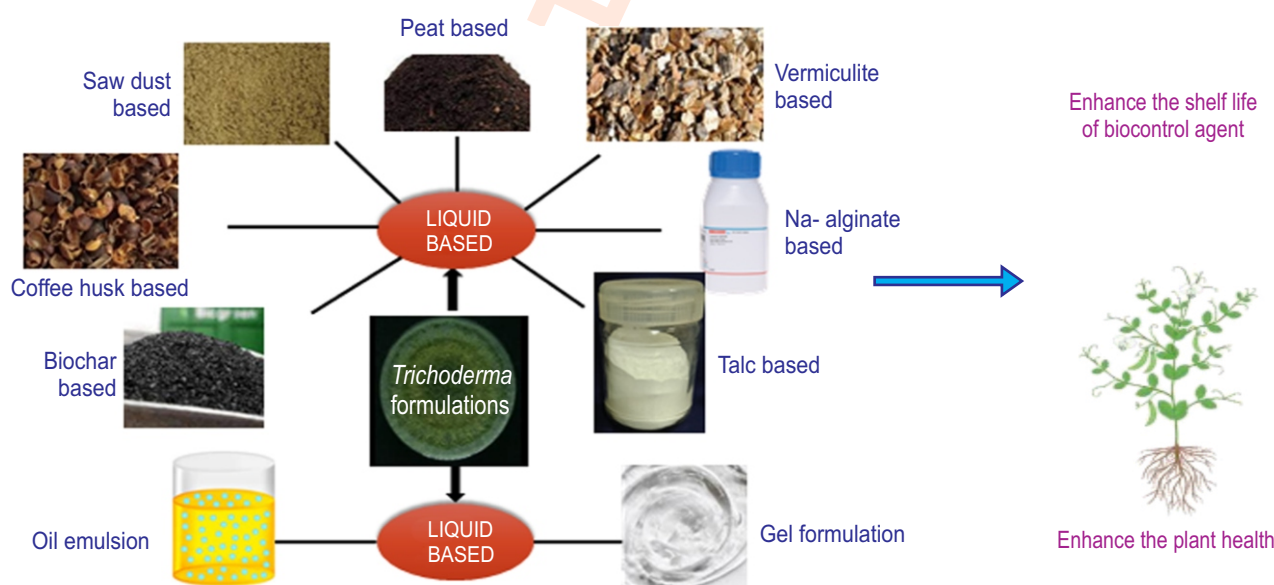
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Abstract

The usage of biopesticides is far less compared to chemical pesticides available in the market. *Trichoderma* is an effective biocontrol agent with a long history of success stories compared to all other available biocontrol agents. Application of *Trichoderma* formulation enhances the plant growth and is more effective in managing diseases of crop plants. In India, several popular products derived from different *Trichoderma* species have found commercial success among organic growers. In India, certain quantity of fruitful produces are from diverse class of *Trichoderma* hence, it must be commercialized. Many small and medium entrepreneurs have recently started producing biocontrol agents for commercial use, which has led to the introduction of numerous biocontrol products in the global market. This paper aims to review and discuss the carrier materials, mass production techniques, target diseases, recommended dose, application methods, storage and shelf life of *Trichoderma* formulations. The main obstacle in the commercialization of bioproducts is reduced shelf life. Therefore, this review will explain the various types of *Trichoderma* formulations in several substrates and commercial viability of the substrates.

Key words: Efficacy, Formulation, Substrates, Shelf life, *Trichoderma*



Introduction

The field of agriculture is always changing and evolving. New farming techniques and biotechnologies are emerging in wake of the widespread use of chemical fertilizers and pesticides in the 20th century, which has contributed to a significant increase yield (Montagu, 2019). Feeding about 9 billion people by 2050 is the next problem to be overcome. Increasing food production, especially plant-based food, while protecting the environment is one of the main concerns worldwide (Godfray, 2014). Currently, nations are working to increase the food production in order to fulfill demand. An increased crop yield is frequently correlated with better farming practices, especially when using more productive varieties that are resistant to common diseases (Liliane and Charles, 2020). Plant diseases can be prevented, mitigated, or controlled using a variety of strategies. Farmers mainly rely on chemical pesticides and fertilizers in addition to proper horticultural and agronomic methods (Fanish, 2013).

However, people's perception on the use of pesticides in agriculture have significantly changed as a result of the environmental damage brought due to overuse and misuse of agrochemicals (Fig. 1). There is an urgent need to ban harmful chemicals from the market as there are severe limits on the use of chemical pesticides today (Sudha et al., 2022). As a result, several researchers studying pest management have concentrated on creating substitutes for artificial chemicals in the management of pests and diseases. Biological controls are a specific kind of alternative among them (Pal and Gardener, 2006). There are plenty of fungal biocontrol organisms like *Ampelomyces quisqualis*, *Anthracozygotea fluoculosa*, *Candida oleophila*, *Coniothyrium minitans*, *Clonostachys rosea*, *Phlebiopsis gigantea*, *Trichoderma virens*, *Trichoderma harzianum*, *Trichoderma polysporum* and *Chaetomium globosum* are present in the environment (Afshan, 2023). Among different antagonist organisms, *Trichoderma* species have been highly efficient as biocontrol agents against a variety of soil-borne phytopathogenic fungus (Ramjegathesh et al., 2016; Palmieri et al., 2022).

Plant disease management is a major challenge in the current scenario of crop production (Sood et al., 2020). The success of biocontrol agent is purely based on the excess sporulation, and support healthy crop growth is the only factor that determines its effectiveness. *Trichoderma* spp. are saprophytic, avirulent, soil-dwelling fungi that use a variety of biocontrol strategies to suppress pathogen growth, including rhizospheric competence, mycoparasitism, and secreting enzymes that break down pathogen cell walls (Zin and Badaluddin, 2020; Joshi et al., 2012). The volatile (VOCs) and non-volatile compounds (NVOCs) are able to suppress the growth of pathogen are produced by *Trichoderma* spp. (Sudha, 2023). *Trichoderma* is a widely recognized source of potential biocontrol agent among commercial fungal antagonists, especially for reducing soil-borne phytopathogens such as *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium oxysporum* etc.

(Thambugala et al., 2020; Rao et al., 2015). Likewise, VOC of *Trichoderma* is also able to control different pathogens like *Lasiodiplodia theobromae*, *Sclerotinia sclerotiorum*, *Fusarium equisetii* etc. (Sudha et al., 2021b) Due to its potency against phytopathogens, the market demand is increasing every year. Among the existing biocontrol agents more than 80 species of *Trichoderma* have been identified that are commonly used in biocontrol agents, including *T. virens*, *T. harzianum* and *T. viride* (de Rezende et al., 2020). Only the latter two species are commercially available in India. Delivery of antagonists in the field requires different carrier-based formulations. Formulations come in a variety of forms; which comprises dry substrate (grains, wettable powders and dust), liquid substrate (water, oil, and emulsions); these typically contain one active component, but occasionally two, and microencapsulation (Mawar et al., 2021). The kind of formulation has a major impact on the microbial inoculants effectiveness and the delivery system that keeps the product fresh for several months and its plant growth promotion (PGR) (Ramalakshmi et al., 2008) / antagonistic action is maintained in every situation (Fig. 2) (Howell, 2003). Both solid and liquid formulations are employed to maintain appropriate amount of viable and active inoculum of *Trichoderma*. These days farmers are adopting organic cultivation, there is a great demand for different formulation of biocontrol agents especially *Trichoderma* formulation (Sudha et al., 2021a). Shelf life of the formulation is of prime importance for commercial use of any biocontrol agent. This review seeks to bring together data on carriers, formulation and the shelf life of formulation which could taken to the field for sustainable crop management. Failure of the developed bioagents may be due to the poor survival rate in soil and this may be a major hindrance to consistent field performance (Sudha et al., 2021a). In addition, the experiments associated with expansion systems for *Trichoderma*, uplifting the success and supportable usage of *Trichoderma* products in the pitch of healthy farming also need to be addressed.

Use of substrates as carrier materials: A variety of low-cost substrates used in numerous studies include neem cake, talc, dried cow dung, fly ash, mushroom compost, peat soil, vegetable, fruit and poultry manure, crop wastes, and farm yard manure, rotten wheat, degraded coffee pulp, sawdust, sorghum grain floor, groundnut shell, wheat bran and molasses (Babu and Pallavi, 2013). The success of biocontrol programs and agricultural sustainability is largely dependent on the development of profitable, widely accepted, and simple-to-prepare formulations (Mustafa et al., 2009).

Types of formulation: *Trichoderma* can be used commercially to improve crop health or control plant diseases, but this requires production of commercial formulations with appropriate carriers that allow *Trichoderma* to survive for a definite period of time. Based on different carrier material formulations can be broadly classified into two types solid based and liquid based.

Solid-based formulation: Agricultural wastes, such as rice bran, sugarcane bagasse, saw dust, corn cob meal, and wheat straw,

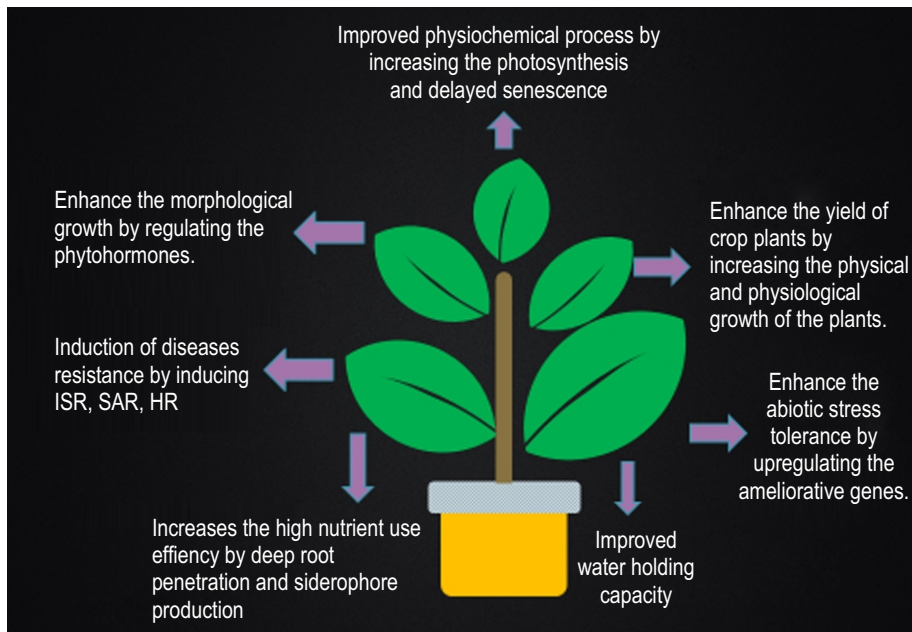


Fig. 1: Impacts of *Trichoderma* on crop plants.



Fig. 2: Salient features of *Trichoderma* formulations.

are utilized either separately or in combination as a substrate or food foundation to aid in the growth of *Trichoderma* (Jin et al., 1992). Although this formulation is enormous and needs a lot of space for preparation, inoculation, storage, drying, and grinding, it is economical for small-scale production. On the other hand, the

extensive manufacturing of microbial formulation with desiccated elements is accomplished by the cost-effective approach of spray drying (Morgan et al., 2006).

Rice bran-based formulation: Rice bran substrates are considered as low cost and they are related to the usage of crop wastes or agro-based wastes as carriers for conidia production, which can support for nutrient and bioagent growth (De la Cruz Quiroz et al., 2015). Sala et al. (2021) reported that temperature and moisture (55–60%) are the main factors influencing spore production (2.0×10^9 spores g^{-1} dry matter) in rice bran formulation. Kobori et al. (2015), reported that substrates, such as wheat bran and rice husk were frequently used, that can be replaced with rice bran or maize, which might be utilized as human feed, or sugarcane bagasse, which has been subjected to further research.

Talc based formulation: Tamil Nadu Agricultural University has created formulations of *Trichoderma viride*, with talc serving as the basic ingredient. *Trichoderma* is cultured in a liquid medium, then mixed with talcum powder and dried in the shade until the moisture level reaches 8% in 3–4 months shelf life. It has become popular in India for its efficacy in managing a range of soil-borne diseases by the application at a dosage of 4 to 5 $g\ kg^{-1}$ seed (Jeyarajan, 2006). *T. harzianum* isolate shows maximum reduction in rot (69.0%), which was followed by foliar spraying of garlic bulb aqueous extract (60.8%) (Zehra et al., 2017). Talc formulation of *Trichoderma* treated seedling plots showed 70–80% decrease in Fusarial wilt in watermelon and a 27–30% increase in fruit yield (Ramesh and Singh, 2017). Sinha et al.

(2018) studied the bioefficacy of talc formulation of *Trichoderma viride* and *T. harzianum* with *Fusarium oxysporum* f. sp. *capsici* causing wilt disease in chilli by 87.5 and 83.93%, Kakvan et al. (2013) tested the efficacy of talc based formulation on damping off disease in surgarbeet caused by *Rhizoctonia solani*, which reduced the diseases incidence upto 69.73% under greenhouse condition. Seed treatment and soil application of *T. harzianum* recorded the lowest incidence of stem rot in cluster bean (4.66%) (Muthukumar et al., 2022). The disease incidence (63.6%) at 50 DAT dramatically decreased by the seedling dip and soil treatment of *T. harzianum* (talc-based) @ 200g per plot. Neem cake fortified with *Trichoderma* talc formulation is recommended to treat stem bleeding of coconut and arecanut as well as coconut basal stem rot (Neeraja et al., 2018).

Formulation based on alginate prills: In a alginate-based *Trichoderma* formulation natural polymer alginate is combined with *Trichoderma*. Alginate is obtained from seaweed. (Bhai, 2020). Sunflower seeds treated with 1% Na-alginate mixture with gum arabic reduced infection in *Macrophomina phaseolina* (Anis et al., 2012).

Press mud-based formulation: A sugarcane waste called press mud is used as a substrate for *Trichoderma* mass multiplication. Application of sulphitated press mud based *T. viride* formulation in calcareous soil increased the production of sugarcane was reported by Ram et al. (2024).

Wheat bran and vermiculite based formulation: The study assessed the usage of inexpensive substrates such as rice, corn, and wheat bran in order to produce *Trichoderma* spores. The best bran was identified as wheat bran, which can be used for spore cultivation because all strains of *Trichoderma* had high spore counts. Cavalcante et al. (2008) found that overall survival and saprophytic development of *Rhizoctonia solani* were examined to be reduced in relation to the efficacy of formulation. Seedlings of melon plants treated with Bentonite–vermiculite formulation of *T. harzianum* was found to reduce the incidence of Fusarium wilt and enhance shoot weight in nursery (Martinez-Medina et al., 2009). Chammem et al. (2021) conducted a study where they found that *T. atroviride* C52 was able to considerably reduce the disease occurrence (>50%) of *F. oxysporum* in *Cucumis melo* plants for eight weeks while remaining stable in a bentonite-vermiculite formulation.

Biochar-based formulations: Biochar is a porous and light material obtained from pyrolysis of biomass. It is composed of carbon and ash, although the precise final composition depends on the type of biomass selected (Li et al., 2018; Garkoti et al., 2014). The survival of spores and viability of *T. viride*, is notably more with the usage of the small particle biochar. Due to easy handling, biochar can be a choice for a dry carrier substance. When applied in fields, it not only increases the spore production of *Trichoderma* spp. and it also increases the soil size to hold water, which lowers soil fertilizer drainage and encourages plant growth. Araujo et al. (2019) showed that applying biochar made

from 1% sewage sludge eradicated *Macrophomina phaseolina* infection *in vitro*.

Coffee husk- based formulation: Spent Coffee Grounds is an organic based amendment and nutrient supplier to enrich the soil which can augment essential micro-and-macro elements (da Silva Delabona et al., 2012). Moreover spent coffee grounds can stimulate respiration and induce microbial activity once incorporated into soil (Oancea et al., 2017). In Karnataka and Kerala, this mixture is frequently used to treat black pepper foot rot caused by *Phytophthora*. SCG sterilized peat substrate was augmented with *T. atroviride* Ta3 and Ta4 decrease the inoculum density of damping off incited by *P. nicotianae*. (Chilosi et al., 2020).

Dry flowable formulation: It is safe for farmers and the environment to use dry flowable/water-dispersible granule formulations (Knowles, 2008). Owing to their granular composition, these preparations do not absorb moisture from the atmosphere. Since encapsulation is essential to improve the formulation type's longevity and efficacy, it is the first step in the manufacturing dry flowable formulations. As previously stated, *Trichoderma* has been encapsulated by using modified alginate microbeads or a dry approach (Jin and Custis, 2011), using chemicals such as citric acid, polyacrylic acid and sodium bicarbonate as a basis for spray-dried flowable system, together with lecithin as a wetting agent and polyvinyl alcohol as a binder (Oancea et al., 2016).

Saw dust-based formulation: Sawdust as a carrier material is highly readily available, naturally able to hold onto a higher degree of non-chemical matter, and has a higher water retaining capacity than other substrates recommended as it (Arora et al., 2001). Compared to other substrates, saw dust was the most acceptable and also examined carrier for bioagent growth. The findings of Huang et al. (2011) suggested that a variety of media containing worm castings and sawdust can enhance crop development. Here, the slow-growing plants can benefit from the medium combination with *Trichoderma*, while the fast-growing crops are affected by the growth medium in addition to 40% worm castings and 60% sawdust, or 60% worm castings and 40% sawdust devoid of *Trichoderma* (Lin et al., 2021). After 25 days of inoculation, the *Trichoderma*-inoculated substrate displayed a spore load of 10.23×10^7 (Saw Dust + Peat Soil + Water, ratio-1:1:2), which is the least amount of spore generation when compared to gram bran and peat soil. (Adan et al., 2015).

Liquid formulation: The composition of liquid formulations for bioagents might differ, nevertheless generally speaking, these consist of mineral or organic oils, polymers, microbial cultures or suspensions combined with water. In the context of microbial inoculants, suspension concentrates are often prepared by employing conventional techniques to introduce dense vigorous ingredients which are free or restrained microbials in an aqueous solution (Gopalakrishnan et al., 2016). Furthermore, items need to be processed with increased sterility since they are more prone

to bacterial contamination in the liquid or gel medium. Due to these factors, dry formulations are most commonly available in markets. In this case, the majority of recent research has been on methods and procedures for formulating the liquid formulation that support spore viability for extended shelf life period (about >24–48 weeks at ambient settings). The freely available liquid and gel formulations are covered below, including those that encapsulate oil and those that encapsulate in natural polymer networks like alginate.

Oil emulsion: *Trichoderma* is combined with an oil carrier, including mineral, vegetable, and coconut oils gives stability to the formulation. The oil carrier serves as a barrier to shield *Trichoderma* formulations from the UV rays and high temperatures. Oil-based formulations have the ability to improve adhesion on plant leaves, seeds and other surfaces. Additionally, they boost the action against plant diseases and allow *Trichoderma* to slowly release into the treated region. Surfactants like vegetable oil (groundnut, etc.) and gasoline distillates (diesel and mineral oil) eliminate bacteria from non-aqueous solvents. When mixed with water, this can produce a constant suspension. Because they need a great application of an emulsifying component (oil-soluble), emulsifiable concentrates quickly form a homogenous emulsion when diluted in water. Batta (2007) created an emulsion formulation of *T. harzianum* that significantly decreased *Botrytis cinerea*-causing post-harvest fruit rot.

Paraffin oil treatment showed the highest CFU count (28×10^8 CFU) at 30 DAS, compared with talc-based cultures (54.06%). From 60 DAI to 180 DAI, there was a notable decrease in spore germination over the entire treatment period. Among the plant pathogenic fungi, *F. oxysporum* showed minimum growth rate (74.44%), followed by *R. solani* in terms of percent growth inhibition on 8th day of infection (Mane et al., 2021). The oil dispersal formulation includes, glucose, soybean oil a dispersing agent, and a physical agent (Mbarga et al., 2014). Herrera et al. (2020) showed that emulsified formulation of *T. asperellum* TV190 in mineral oil (43%) and vegetable oil (63%) had higher spore viability of 28.00×10^8 CFU ml⁻¹ and 18.00×10^8 CFU ml⁻¹ after 180 days.

Gel-based liquid formulations: Typically, semisolid formulations (gel-based) use an oil to soothe condensed *Trichoderma* colonies in a hydrated form. Gelatin polyethylene glycol and starch in 3:1:1 ratio are used to prepare the hydrogel. The maximum activity in gel-based and liquid formulations makes it much more difficult to extend the shelf life than in parched (dry) formulations because of spontaneous germination (Elshafie et al., 2020). Spores and/or mycelia are entrapped within the swollen hydrogel matrices to create novel formulations, or xerogel containing zinc sulphate was physically mixed in it. In the presence of ZnSO₄, *T. harzianum* showed noticeably increased survival up to 150 ppm, after which there was a gradual drop in viability. Due to hydrogel carrier or saturated ZnSO₄, both the compositions (wet and dry) showed extended shelf life with *T. harzianum* up to 180 days deprived of any signs of toxicity. In

comparison to *T. harzianum* and ZnSO₄ alone, or combination of two showed greater bioactivity against the soil-borne fungal pathogen *Rhizoctonia solani* (Sriram et al., 2011).

Shelf life: Effective marketing of a biocontrol agent mainly depends on the product's shelf life. In general an organic food substrates foundation's antagonists must have a prolonged shelf life than an inactive or inanimate food base. *Trichoderma* has a longer shelf-life of more than 18 months in coffee husk. Research conducted on the packing of *T. viride* formulation in polypropylene container of different colors showed that milky white bags with a thickness of 100 microns had the highest population (Sriram et al., 2010). According to Bhat et al. (2009), talc-based formulations have a 180-day shelf life in ambient conditions.

Conidia of *Trichoderma* preserved by using coconut oil as a primary component of the emulsified formulation showed enhanced shelf life (Lei et al., 2022). Rai and Tewari (2016) reported that conidia that are actively growing from different *Trichoderma* species make up about 90% of formulations. Paste and liquid based formulations have a longer shelf life for *Trichoderma* compared to dry formulations. The farmer will be able to guarantee a higher output by applying paste and liquid formulations of biocontrol agents in orchards and farms (Rai and Tewari, 2016). At 25°C, the population of *T. viride* in the charcoal-based formulation decreased to 69.98% on 120th of storage (Kumar et al., 2013). Different types of substrates, their composition and shelf life are tabulated in below Table 1.

Mode of action: Numerous abiotic factors, including soil pH, water retention, temperature, and the presence of heavy metals influence the success rate of *Trichoderma* establishment. *Trichoderma* spp. have the ability to biologically control plant diseases because of their complex interactions with them, which may involve parasitism, the secretion of antibiotics, or competition for nutrients and space. *Trichoderma* spp. induce hydrolytic enzyme production including glucanase, chitinase, and protease during mycoparasitic interactions. They also activate signaling pathways, the most significant of which are MAP kinase, the cAMP pathway, and heterotrimeric G protein. The production of infection structures and the secretion of antifungal metabolites are primarily mediated by G protein and MAPK. The cAMP pathway prevents the growth of harmful fungus by assisting *Trichoderma* mycelium in coiling and conditioning on them. Various formulations of *Trichoderma* spp. have been developed with different carrier materials for their effective impact on crop plants. (Sudha et al., 2019).

Key challenges and future approaches for biopesticides: The field application of *Trichoderma* encapsulated formulation, transmission, documentation of *Trichoderma* spp. for management of leaf diseases (Ramjegathesh et al., 2011), with extended shelf-life, reliability of performance in the field are main challenges. In addition, the registration procedure of *Trichoderma* under CIB&RC, optimization of product and promotion cost should be the focus of future research simultaneously. For

Table 1: Different types of substrates, their composition and shelf life

Substrates	Composition	Shelf Life	References
Talc	Molasses yeast extract (MYE) medium+ glycerol (3%) (V/V) + <i>T. harzianum</i> + Talc powder	Extended the shelf-life for 7 to 12 months	(Sriram et al., 2011)
Oil	<i>T. asperellum</i> + paraffin oil	28.67X10 ⁸ cfu ml ⁻¹ for 30 days	(Patil et al., 2021)
Dextrin	<i>T. harzianum</i> (500 g) + Paraffin oil (500 ml) + CMC (0.2%) + chitosan (0.1%)	4.33 X10 ⁷ cfu g ⁻¹ for six months	(Rai and Tewari, 2016)
Rice powder	Sterilized rice powder + dextrose + talc powder + <i>T. viride</i>	10 X 10 ⁹ up to six months at room temperature	(Rini et al., 2020)
Chitosan-PEG	Chitosan-PEG + <i>T. harzianum</i> spores + glacial acetic acid (0.1%)	Six to seven months 6.0 X 10 ¹⁰ cfu g ⁻¹	(Prasad et al., 2022)
Graphite and silica	<i>B. subtilis</i> and <i>T. harzianum</i> + 5% graphite 80 mesh + 1% silica Nps	Five to six months 6.2 X 10 ¹⁰ cfu g ⁻¹	(Marcelo et al., 2021)
Compost activator with <i>Trichoderma</i>	Fifty grams of soil + rice straw (5 g) + <i>Trichoderma</i> biomass (500 mg)	5.3 X 10 ¹⁰ cfu g ⁻¹ Three months	(Organo et al., 2022)
T2- liquid formulation	Liquid formulation (NIPHM medium) <i>Trichoderma</i> filtrate (250 ml) + water (750 ml) + glycerol (3%)	Nine months 39 X 10 ⁷ cfu g ⁻¹	(Komala et al., 2019)
Wheat flour- kaolin	Wheat flour (80 gm) + kalolin (20 gm) + fermenter biomass (52 ml)	Four months 3.2 X 10 ¹² cfu g ⁻¹	(Ramanujam et al., 2010)
Sodium alginate	<i>Trichoderma</i> suspension + sodium alginate (0.6%) solution+ CaCl ₂ (1.5%)	Uptosix years at room temperature 3 X 10 ¹² cfu g ⁻¹	(Rai et al., 2021)
Banana waste	Banana waste (chopped 5–6 cm length) + rock phosphate	Six months 5.3 X 10 ¹⁰ cfu g ⁻¹	(Thangavelu et al., 2004)
Oil	Glycerol (1%), PVP (1%), Tween 20 (1%) as an emulsifying agent, ZnSO ₄ (0.5%) to increase the shelf-life, coconut oil, and distilled water	Four months 39 X 10 ⁷ cfu g ⁻¹	(Sreenayana and Nakkeeran, 2019)
Vermiculite bentonite	Oat (20 g) + bentonite (50 ml) + vermiculite + <i>T. harzianum</i> + water (60 ml)	3 X 10 ¹² cfu g ⁻¹ Three months	(Martínez-Medina et al., 2009)
Wood pellets	Beech, fir, and chestnut + conidial suspensions of <i>T. atroviride</i> + distilled water + soy flour	Three months 28 X 10 ⁷ cfu g ⁻¹	(Chammem et al., 2021)
Sorghum grain	Partial crushed grain + sugar (1%) solution + distilled water	6.1 X 10 ⁴ cfu g ⁻¹ Two months	(Ananda et al., 2011)
Talc	Talcum powder + CMC + <i>Trichoderma</i> culture	37 X 10 ⁷ cfu g ⁻¹ Four months	(Mohiddin et al., 2017)

instance, finding new strains of *Trichoderma* can support or encourage the utilization of bioenergy or the development of bioproducts (Mishra et al., 2020). The ecological factors including temperature, moisture, pH, nutrients can affect the efficacy of *Trichoderma* (Murphy et al., 2018). Moreover, follow up work has to be carried out during or after the development or delivery of formulation under *in vitro* and *in vivo* conditions.

The present review encompassed the work on *Trichoderma* formulations, shelf life mode of action and their bio efficacy. The genus has many qualities and potential market value among the farming community, especially from organic growers. The formulation has the ability as protectants, growth promoters, environment adaptability and safe to all kinds of living beings. The microbial biocontrol agents have conventionally remained mass-

produced as liquids, wettable powders, and granules for use as soil drenches, foliar sprays, or seed treatments. Globally, the commercialization of biocontrol products is expanding quickly; nevertheless, due to lack of efficient laws, biological products are still gaining traction in the agricultural market slowly. The developed formulation must be tested under protected cultivations and also in various agro-climatic zones. Nonetheless, combining urban technology from a different field with a scant grasp of biological formulations could lead to the creation of more accurate and reliable formulations.

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