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REVIEW ARTICLE

A synopsis of *Trichoderma viride* bioformulation: Mass production techniques, methods of farm applications, challenges, limitations and future perspectives

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Abstract

Sustainable agriculture stives to enhance crop productivity while minimizing environmental degradation, emphasizing the need for eco-friendly and effective alternatives to synthetic agrochemicals. Biological control has been proven to be an effective substitute for synthetic chemicals. Among biocontrol agents (BCAs), *Trichoderma viride* has emerged as a well-established biocontrol fungus with multifaceted roles in plant disease management and growth promotion. This review aims to systematically evaluate biology, mechanisms of action, mass production technologies and field application methods to *T. viride*, highlighting its limitations and future research prospects. By consolidating and critically analyzing scattered information, this work seeks to identify knowledge gaps that can guide the development of improved formulations and practical usage strategies. Overall, *T. viride* demonstrates remarkable potential as sustainable bioresource for integrated disease management, improved soil health and environmental stewardship, although its commercial success depends on advances in formulation stability, contamination control and strain selection.

Keywords: antagonism; antibiosis; bioinoculants; biotic stress; induced systemic resistance; plant disease management; *Trichoderma*

Introduction

The overuse of chemical pesticides in modern agriculture has led to severe ecological and health consequences, including soil contamination, resistance development in pathogens and decline of beneficial microorganisms (1, 2). As an environmentally responsible alternative, biological control employs naturally occurring microorganisms to suppress plant pathogens and reduce dependence on synthetic agrochemicals (3, 4). Among the diverse biocontrol agents (BCAs), fungal and bacterial genera such as *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Coniothyrium minitans* and *Trichoderma* species have been widely recognized for their antagonistic and plant growth-promoting abilities (5, 6).

The genus *Trichoderma* comprises of filamentous fungi well known for its rapid growth, adaptability and ability to parasitize or compete with a wide range of phytopathogens (6). It is one of the most studied genera in plant pathology due to its commercial importance as a bio fungicide and biofertiliser. The tenth edition of Ainsworth and Bisby's Dictionary of Fungi classifies *Trichoderma* (teleomorph *Hypocrea*) under Domain: Eukarya; Kingdom: Fungi; Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Hypocreaceae (7).

Trichoderma viride has gained prominence due to its strong mycoparasitic activity, fast colonisation rate, ease of mass production and proven success in managing numerous soil- and seed-borne diseases. In India, *T. viride* dominates the biofungicide market, accounting for over half of the registered microbial products, reflecting both its efficacy and commercial viability (8).

India's National Farmers Policy (2007) emphasizes the promotion of biopesticides as a means to achieve sustainable productivity (9). Despite increasing awareness, biopesticides constitute only about 2 % of India's total pesticide use, compared to 20 - 40 % in developed regions such as Europe and the USA (10). This highlights the urgent need to advance the use of efficient and reliable biocontrol agents like *T. viride* through standardized production and application technologies.

Therefore, this review provides a comprehensive account of *T. viride*, covering its biology and ecology, mechanisms of action as a biological control agent, methods of mass production and

formulation and field application techniques. It also addresses major limitations and future directions, emphasising research gaps in formulation stability, contamination control and molecular strain improvement.

Biology and ecology of *T. viride*

Trichoderma viride is a cosmopolitan filamentous fungus that thrives in diverse ecological habitats, including agricultural soils, decaying organic matter and forest litter (11- 13). It reproduces rapidly through the production of green conidia and exhibits a strong capacity to colonize plant roots and organic substrates (14). Morphologically, *T. viride* colonies are fast-growing and typically green due to abundant sporulation. Optimal growth occurs between 20-28 °C, though it can tolerate a wide temperature range of 6-37 °C (14, 15).

Taxonomically, *T. viride* belongs to Domain: Eukarya; Kingdom: Fungi; Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Hypocreaceae; Genus: *Trichoderma*; Species: *T. viride* Pers. (teleomorph: *Hypocrea rufa*) (16, 17). This species is distinguished by its secretion of hydrolytic enzymes-such as chitinases, glucanases and cellulases-that degrade cell walls of phytopathogens (18, 19).

Ecologically, *T. viride* functions as both a saprophyte and a symbiont. It colonizes rhizospheres, grow rapidly, forming irregular cotton flocks or dense clusters with mostly green surfaces. Temperatures between 20 and 28 °C are excellent for growth, but it may also flourish at 6 or 32 °C. It's a mesophilic fungus that can survive at 37 °C but not at 48 °C (20). For vegetative development, *T. viride* requires a relative humidity of greater than 92 %, while the formation of spores needs a relative humidity of 93 - 95 % (21); consequently, *T. viride* has great viability in wet soil. As a saprophyte *T. viride* can be found on wood, seeds and plant waste (22). *Trichoderma viride*, competes for nutrients and space with other microbes and promotes plant growth by enhancing mineral uptake and inducing systemic resistance (23).

Once applied to soil, this biocontrol agent colonizes the seed - rhizosphere, proliferates on the surfaces, parasitize (coil) pathogens present on the surface of the seed and surround the rhizosphere (24). Plant roots that have been treated with *Trichoderma* have an enhanced capacity to explore the soil and acquire minerals (25). *Trichoderma viride* in the soil assists in the transformation of Fe3+ to Fe2+ in the plant, resulting in increased solubilization and uptake (24, 26). *Trichoderma* inoculation improves the uptake of copper, sodium, zinc and other micronutrients (27). *Trichoderma* strains synthesize acids, including citric acid, glucuronic acid and coumaric acid, which help with the release of the phosphorus ions that are present in most of the soils but are unavailable to plants (28).

However, certain *Trichoderma* strains may act opportunistically, producing metabolites that can suppress other fungi or cause green mold in mushroom cultivation (29-32). Therefore, strain selection and safety assessment are crucial prior to commercialization.

Trichoderma viride as a biological control agent (BCA)

For the first time, *Trichoderma* was described as a biological control agent in the early 1930s (22). The *Trichoderma* species are free-living, cosmopolitan fungi that are found in decomposing organic material, vegetable debris and soils. It is a potent antagonist with the ability to suppress significant plant parasites and is found in practically all soil types (34). It is effective in controlling soil-borne, air-borne and seed-borne plant pathogens (35, 36).

The main biocontrol mechanisms of *Trichoderma* include mycoparasitism, antibiosis and competition for food and space. In mycoparasitism, the fungus coils itself around another pathogen and grows on its surface (37). Then, with the action of lytic enzymes and toxic compounds, it degrades the cell wall of the pathogen and then absorbs the nutrients. The main enzymes responsible for the degradation of the host cell wall are Chitinase and β-glucanases (38). Long-term research has shown that *Trichoderma* has mycoparasitic ability against various phytopathogenic fungi such as *Alternaria alternata, Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum, Pythium* spp., *Phytophthora* spp. and *Fusarium* spp. (39). In antibiosis, *Trichoderma* produces various metabolites and compounds that interfere with the normal functioning of the phytopathogens. These compounds limit the colonisation of the phytopathogens. The effect of these compounds leads to the rupture of the cell wall and the leakage of the cytoplasm of pathogens (38). *Trichoderma viride* produces viridepyronone, which is effective in controlling *Sclerotium rolfsii* (40). Fungus of *Trichoderma* spp. grows very fast and colonises the rhizosphere rapidly. They exhaust nutrients and space, due to which there is very little room for other pathogens to grow. One of the major reasons for the death of pathogenic microorganisms is due to competition for food and space (38). Many strains of the *Trichoderma* genus colonise the roots of plants and encourage the development and growth of plants. *Trichoderma* species have been widely used as bioagents to manage disease and improve plant nutrition and growth. As a bioagent, *Trichoderma* can be a cost-effective and convenient approach. Many studies on *Trichoderma* are currently underway, with the primary focus on its ability to alleviate abiotic stress, but the mechanism is less known that it allows it to modulate multiple abiotic stress elements. On the biochemical side, several biological strategies are being explored to improve and grow *Trichoderma* strains, including genetic engineering and recombinant technology (41). *Trichoderma viride* is environmentally friendly, cost-effective and simple to utilize. It promotes plant growth and can be used in conjunction with other Biofertilizers. There are no residual toxicity and no harm to beneficial soil bacteria and there is no evidence of developing resistance (22, 42) (Fig. 1).

Mass production and formulations of *T. viride*

Trichoderma viride, known for its potent biocontrol properties and ability to enhance plant growth, has garnered significant attention in agricultural and industrial applications. Its mass production is crucial for harnessing its benefits in biological control of seed and soil-borne diseases and bioremediation of soil in sustainable agriculture (21, 43, 44). Various methods of mass production, including dry and solid-state fermentation, have been optimised to enhance the yield and viability of *T. viride* (45).

Understanding the best practices for mass production not only supports the efficient application of this beneficial organism but also contributes to the broader goals of reducing chemical inputs in farming (46). As the demand for eco-friendly agricultural solutions grows, the effective scaling of *T. viride* production becomes increasingly vital (47) (Table 1).

Talc-based formulation

Talc-based formulation of *T. viride* for the seed treatment was developed by the Tamil Nadu Agricultural University (52). Firstly, *Trichoderma* is cultured in a broth medium, then combined in a 1:2 ratio with talc powder and shade dried to an 8 % moisture content. *Trichoderma* talc-based formulations have a shelf life of 3-4 months

Table 1. Different substrates used to produce *Trichoderma viride*

Sl No.	Substrate	References
1	Wheat bran-saw dust modified medium	Parkash and Saikia (48)
2	Sorghum grain	Parkash and Saikia (48)
3	Rice bran	Naeimi et al. (49)
4	FYM	Prasad et al. (50)
5	Spent tea leaf waste	Lima et al. (51)
6	Talc based	Sudha et al. (52)
7	Wheat bran-vermiculite based	Martinez et al. (56)
8	Biochar based	Martinez et al. (56)
9	Alginate beads based	Martinez et al. (56)
10	Press mud-based	Sinha et al. (60)
11	Sorghum grain-based	Singh et al. (64)
12	Sawdust-based	Pandey (66)
13	Coffee husk-based	Nduka et al. (67)
14	Fruit waste-based	Siddiqui et al. (99)
15	Beetroot based	Khandelwal et al. (100)
16	Banana based	Thangavelu and Mustaffa (101)
17	Oil based	Nathan et al. (102)
19	Carrot based	Simon and Anamika (103)
20	Starch industry wastewater	Verma et al. (104)
21	Poultry manure	Asghar and Kataoka (105)
22	Decomposed coconut coir pith	Kumar et al. (106)
23	Spent malt	Gopalakrishnan et al. (107)
24	Groundnut shell medium	Pandya et al. (108)
25	Wheat bran and biogas manure	Bhale (109)

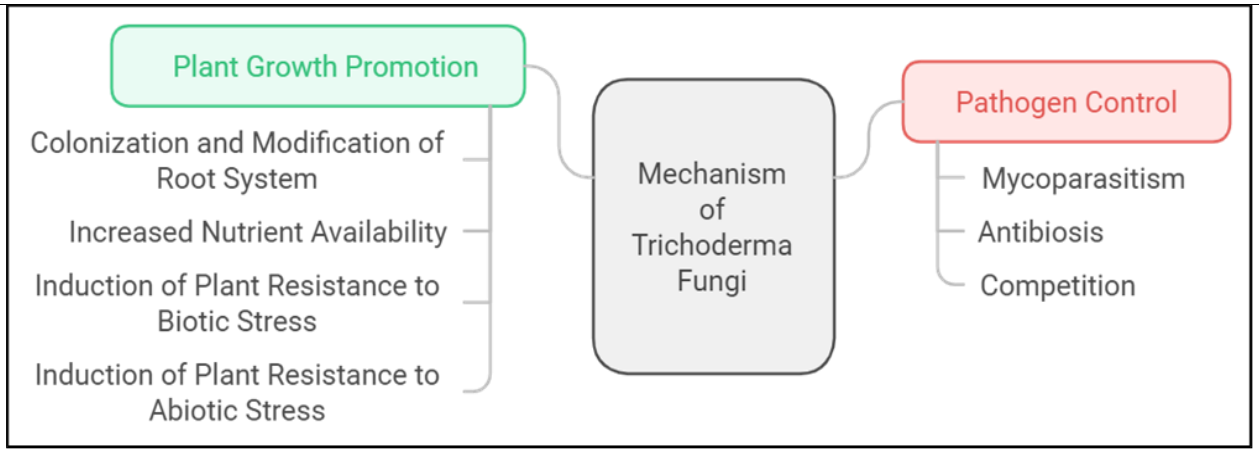

Fig. 1. Flowchart depicting the mechanism of *Trichoderma* as a bioagent.

(53, 54). Seed treatment with talc-based formulation @ 4 - 5 g/kg seed have become increasingly widespread for managing several soil-borne diseases (53, 54). Many private manufacturers produce enormous amounts of talc-based formulations for farmers. As an estimate, to cover 50 % of India's land, 5000 tons of *Trichoderma* are expected to be required each year (54) (Fig. 2).

Wheat bran vermiculite-based formulation

Vermiculite is a phyllosilicate having a high water-holding capacity. It belongs to the group of mica minerals (56). In molasses-yeast medium, *Trichoderma* is grown for 10 days. 33-g wheat bran and 100 g vermiculite are sterilized at 70 °C in an oven for 3 days. This is followed by adding 20 g of fermenter biomass to 0.05 N medium, followed by condensed/whole biomass with Hydrochloric acid (HCL). The material is properly homogenized and dried in the shade (54). A formulation had been created by mixing wheat bran, Vermiculite and dry fermenter-produced biomass from various *Trichoderma* and *Gliocladium* isolates to check the overall survival and growth of *Rhizoctonia solani.* This product was easy to make and did not need sterile conditions. Before use, the dry mix was activated by adding a mild acid (0.05N HCl) and letting it sit for 2-3 days at 23-25 °C, which encouraged the growth of helpful fungal hyphae. When applied to a soilless mix infected with *R. solani*, the activated product effectively controlled damping-off disease in various crops such as pepper, cucumber, eggplant, zinnia and cabbage. Among the 6 tested isolates, *T. hamatum* (TRI-4) and *T. virens* (GI-3) were the most effective, lowering disease levels to those found in healthy controls. In cucumber, even very small amounts (0.13 %) of these products reduced disease, although 0.5 % was necessary for complete protection (57) (Fig. 3).

Press mud (filter cake) based formulation

Press mud, also known as filter cake, is a sugar industry waste that can be used as a starting point for mass *Trichoderma* multiplication (61). During the process, a 9-day-old *Trichoderma* culture was produced in Potato dextrose broth (PDB) and mixed thoroughly into 1.2 quintals of filter cake (54). To keep it moist, water is applied from time to time. Gunny bags are utilized to wrap them to facilitate air circulation and retain humidity under shade. In 25 days, a foundation culture for subsequent growth is prepared (54). Before being applied in the field, the same is completely mixed into 8000 kg of filter cake and incubated for 8 days in the dark (54). This helps to bring 8000 times higher inoculants in the field as compared to prescribed levels of biopesticides, leading to a quick and significant response. Likewise, different chemicals can be employed for a rapid increase of various biological agents at the mass phase (10) (Fig. 4.).

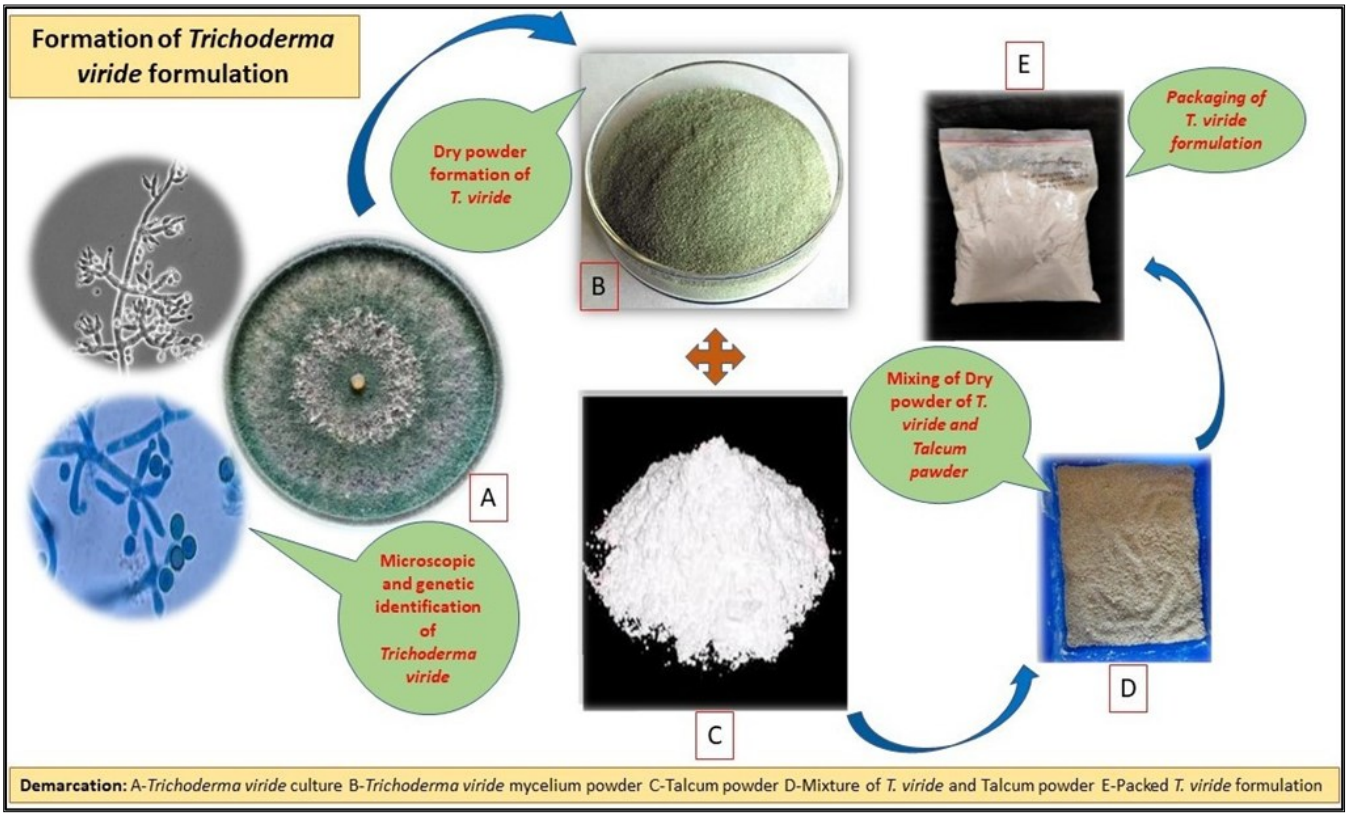

Fig. 2. Talc based formulation of *T. viride.*

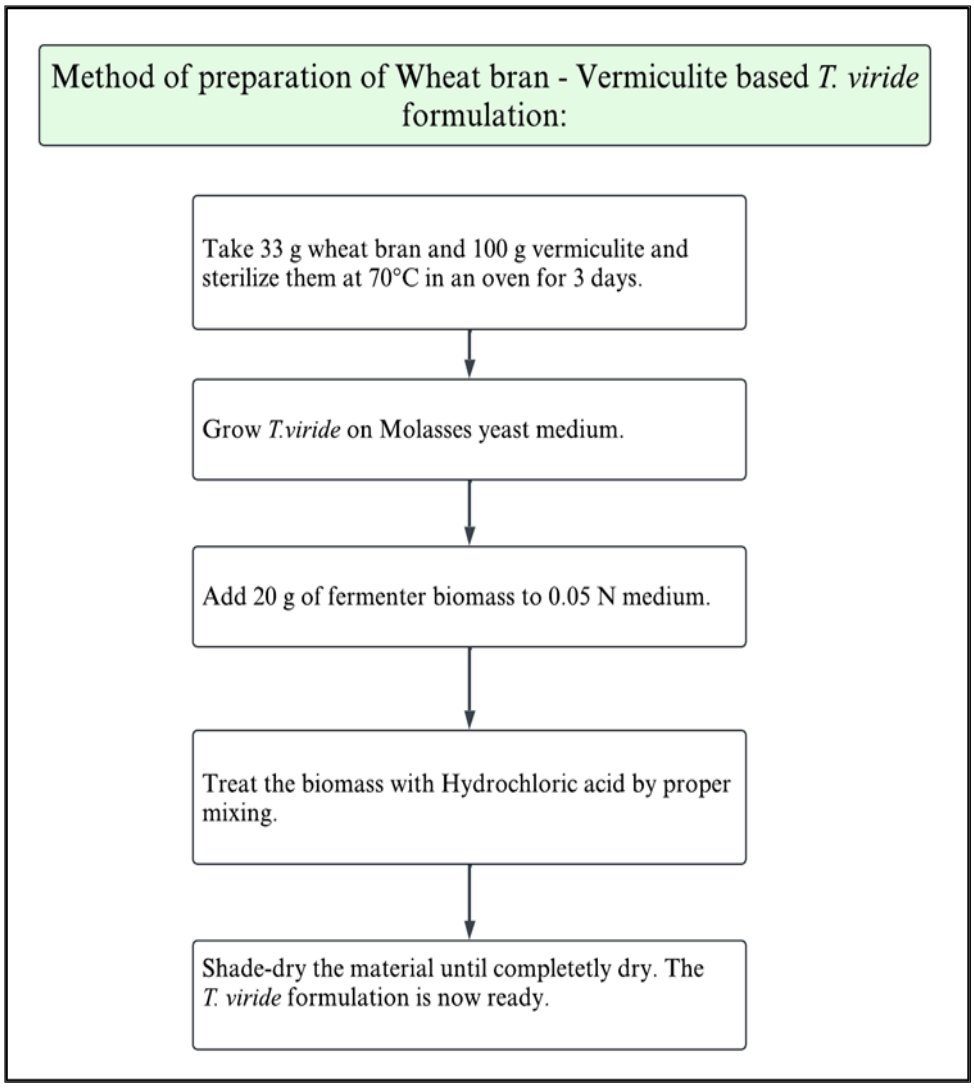

Fig. 3. Wheat bran-vermiculate based formulation of *T. viride.*

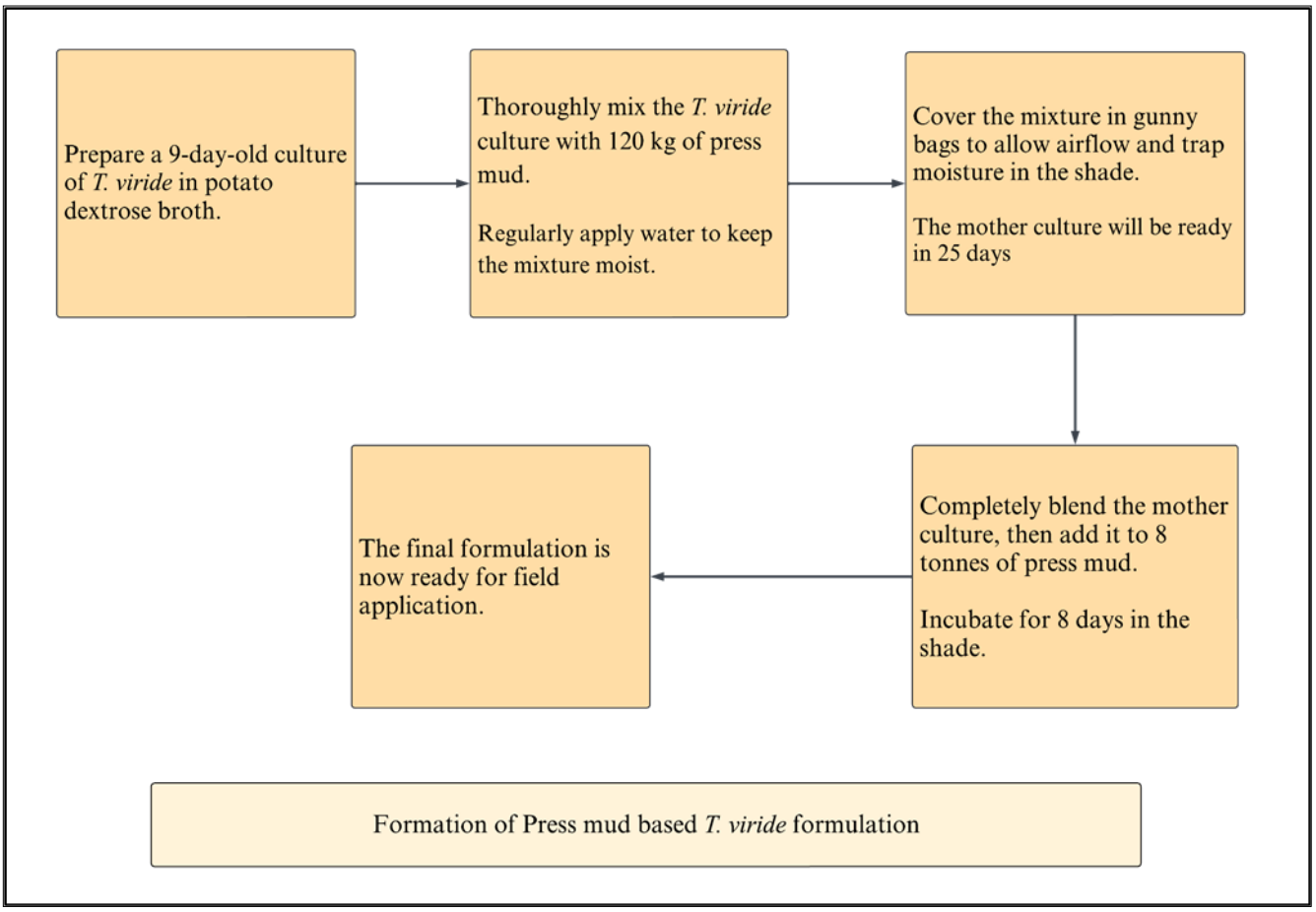

Fig. 4. Press-mud based formulation of *T. viride*.

Biochar-based formulation

Biochar is a lightweight and porous substance produced from heating biomass in the absence of oxygen. Its composition depends on the type of biomass chosen (62). Corn, rice, vegetable peels, wood from agricultural waste and sewage sludge are the most common feed to produce biochar (63). *T. viride* survives better in biochar with a small particle size than in biochar with a large particle size. Biochar boosts *Trichoderma* sporulation increases the water-holding capacity of the soil and reduces fertilizer drainage (56), (Fig. 5).

Sorghum grain-based formulation

Singh and his team in 2014 experimented to evaluate suitable organic substrate for *Trichoderma* formulation to control collar rot disease of cowpeas. The study found that after 7 days of inoculation, the sorghum grain substrate had the maximum population of

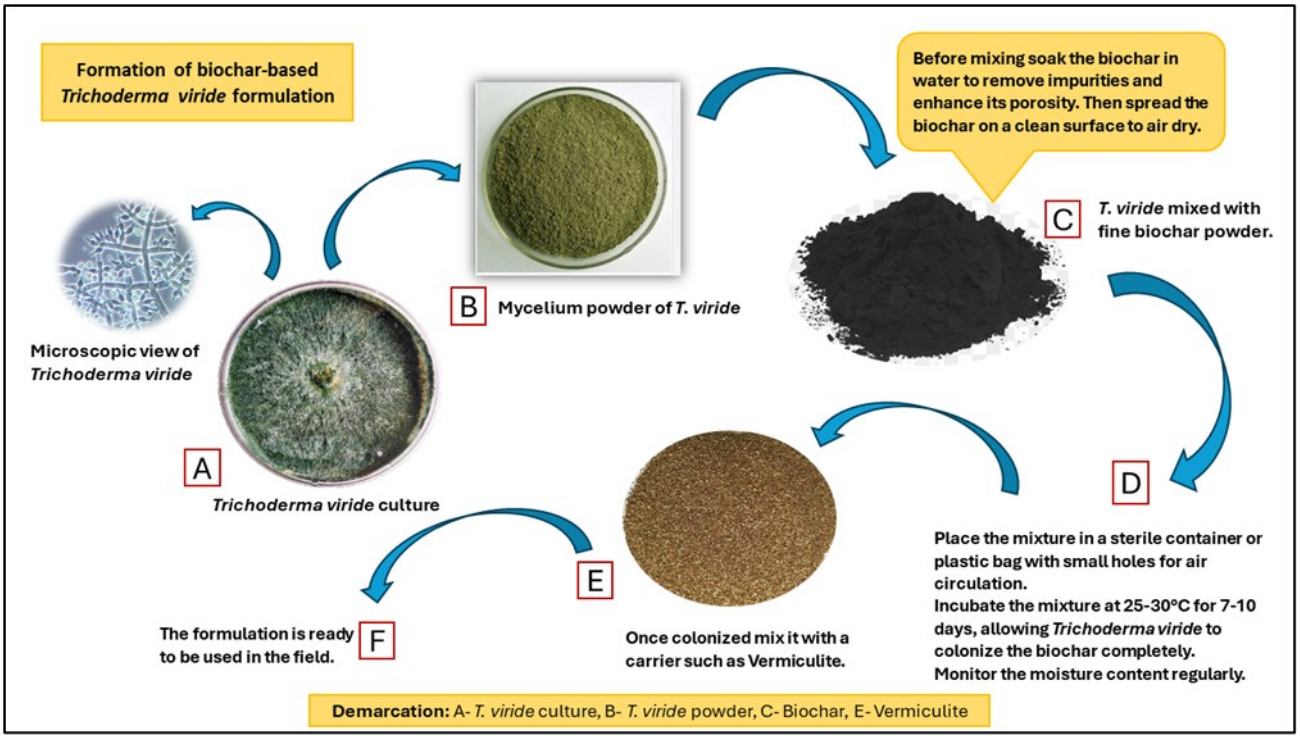

Fig. 5. Biochar-based formulation of *T. viride*.

Trichoderma, followed by wheat grain (64). For the preparation of Sorghum grain-based formulation, take soaked sorghum grains and keep it in autoclavable bags. After filling the bags, a 1.5" PVC pipe is placed on the top of the cover, closed with a cotton plug and tied with a rubber band. The bags are then kept in an autoclave for sterilization at 121 °C and 15 lbs. for 15 min. After sterilization, the grains are cooled at room temperature. After cooling, the sorghum grains are inoculated with 1-2 bits of *Trichoderma* with an inoculation loop or spatula. The inoculated bags are then shaken for proper mixing and are stored at a temperature of 25-30 ^{0}C. The bags are regularly checked for mycelial growth. After mycelium growth (green color), the bags are again shaken for uniform growth and sporulation (65) (Fig. 6, Table. 2).

Coffee husk-based formulation

Trichoderma formulation can also be prepared using coffee husk, a byproduct of the coffee industry (67). The coffee husk was humidified by soaking 50 g of substrate for varying time span (30 min, 1 hr, 2 hr and 3 hr) in 1000 mL of distilled water. Subsequently, the wet substrates were placed inside plastic bags that could be autoclaved for 15 min at 121 ^{0}C. Using a sterile syringe, 1 mL of conidia suspension of *Trichoderma* isolates was added to the autoclaved substrates before they were placed into plastic bags. The plastic bags were perforated to provide for aeration. The inoculated bags were incubated for 10 days at 30 °C (68). This formulation was found to be very efficient in treating black pepper foot rot caused by *Phytophthora* (53, 54) (Fig. 7).

Banana waste-based formulation

In banana waste, (69) recommended a mass multiplication practice for *Trichoderma* spp. The similar banana squander was treated with rock phosphate, urea, *B. polymyxa*, *P. sajor caju* and *T. viride* cultures. A trench of different banana squanders, such as sheath pseudo shoot with heart, is chopped into 5 - 8 cm pieces (54). A trench is prepared, as well as the variety of ingredients are layered in 5 layers. 5 kg of urea, 125 kg of rock phosphate, 1 ton of banana squander along with 1 L of *P. sajor caju*, *B. polymyxa* and *T. viride* broth culture are integrated into every layer (54). Five diverse layers are organized similarly and methodically mixed. Within 45 days, the banana squander decomposes, as well as enriched mass culture is available for field use (54) (Fig. 8.).

Alginate pills-based formulation

Alginate is a non-toxic biodegradable hydrocolloid that in the presence of divalent cations such as calcium forms thermally stable hydrogel beads (56). In one division, sodium alginate (25 g/750 mL) is dissolved in purified water, whereas the food base (50 g/250 mL) is suspended in a different division. As soon as these preparations have cooled, they are autoclaved and blended with biomass. The combination is added to the $CaCl_2$ solution drop via drop, forming sphere-shaped beads that are dried out by air and afterward stored at 5 °C (54). Alginate beads are inexpensive, non-toxic, biodegradable and have a positive effect on the enzymatic activity of the microorganism (56) (Fig. 9).

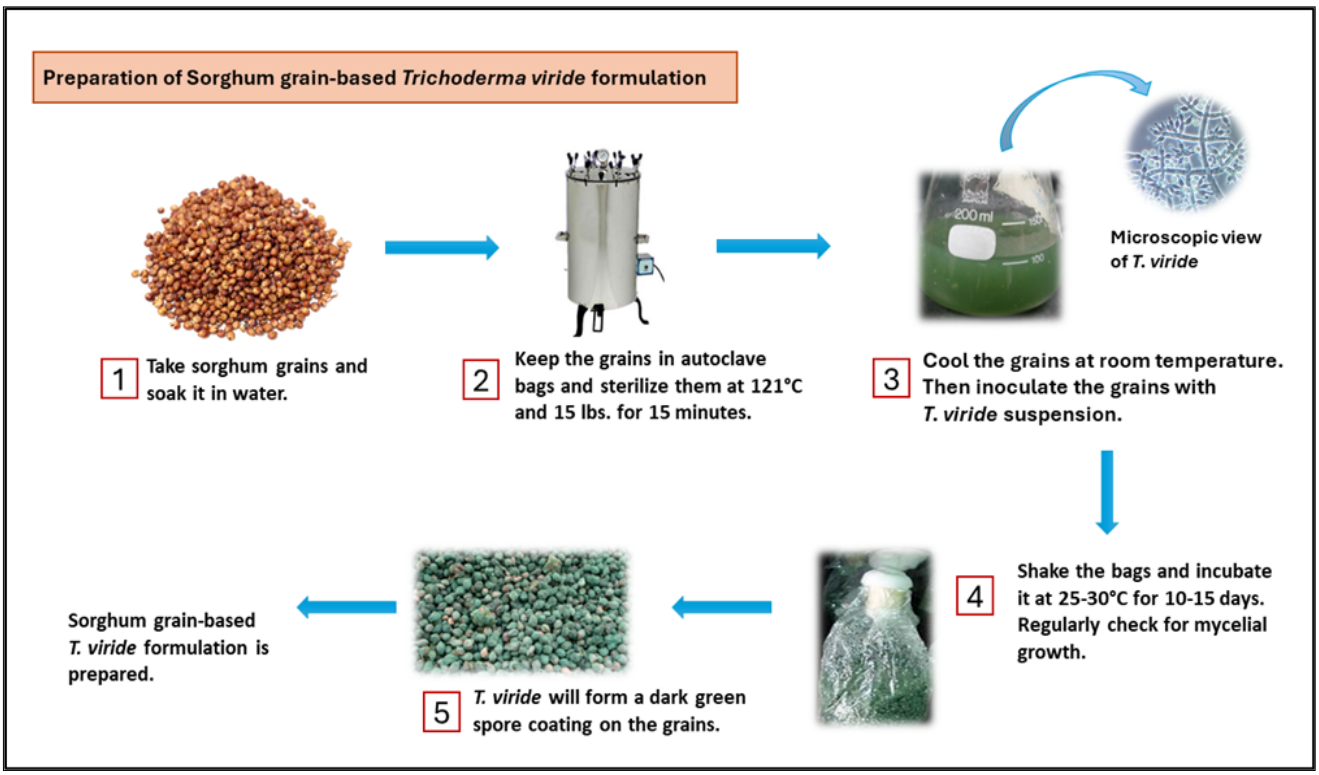

Fig. 6. Sorghum-grain based formulation of *T. viride.*

Table 2. Effect of sorghum-based substrate growth of *Trichoderma viride*

S. No	Substrate	Mixture ratio (grams)	Colonization capacity	No of spores/ gram	Sporulation grade	Reference
1	Rice husk + sorghum	20 + 20	Excellent	1.31 x 10^9	Excellent	Pandey (66)
2	Sawdust + sorghum	20 + 20	Excellent	9.8 x 10^8	Good	
3	Rice bran + sorghum	20 + 20	Excellent	1.15 x 10^9	Excellent	
4	Sorghum grains	40	Excellent	1.06 x 10^{10}	Excellent	

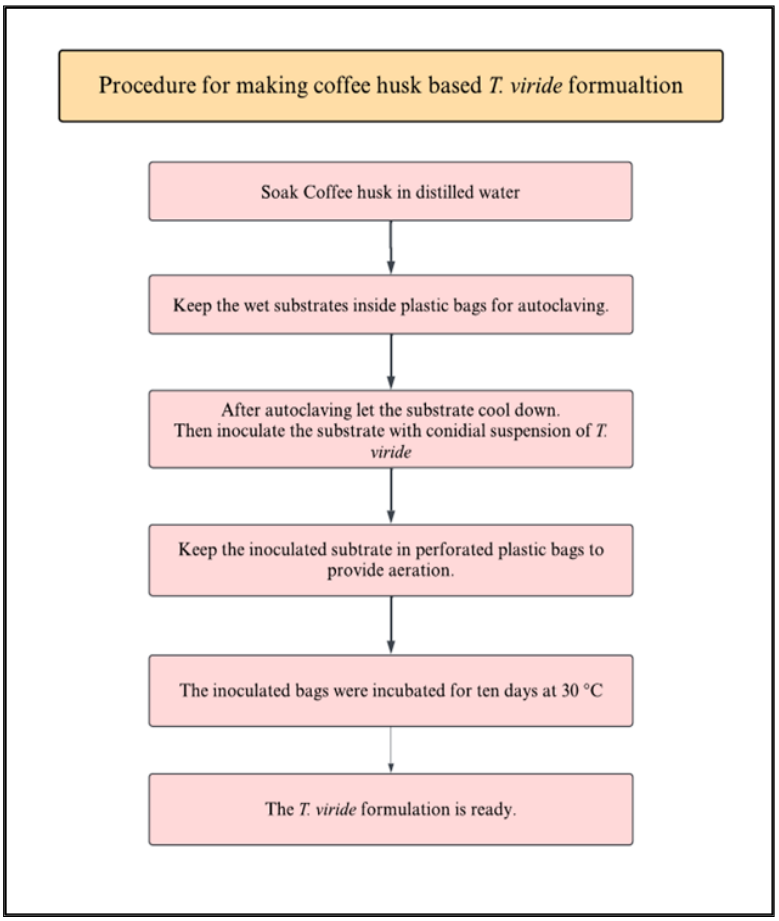

Fig. 7. Coffee-husk based formulation of *T. viride.*

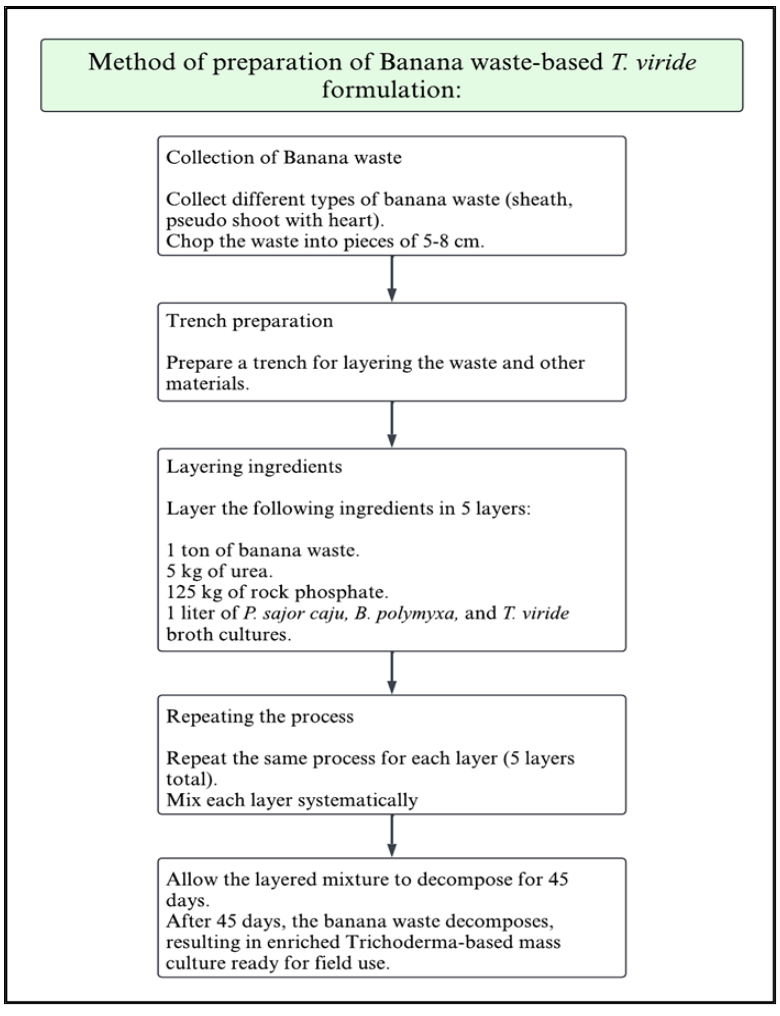

Fig. 8. Banana-waste based formulation of *T. viride.*

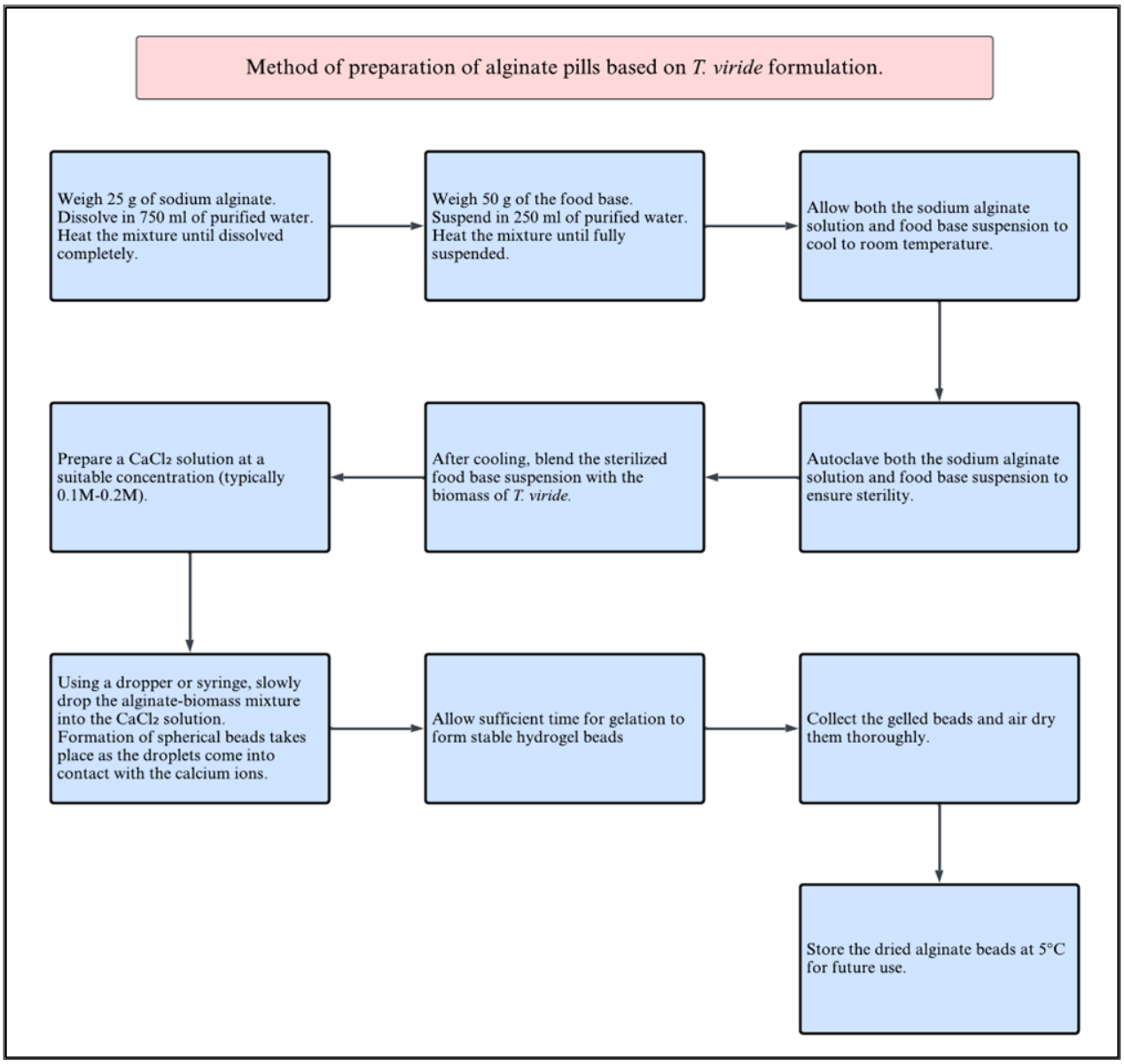

Fig. 9. Alginate-pills based formulation of *T. viride*.

Oil-based formulation

They are prepared by mixing conidia from a liquid-state/solid-state fermentation with a firm emulsion formulation of vegetable oils. Microbial agents are suspended in a water-immiscible solution such as a fuel portion (diesel, mineral oils), or vegetable oils (groundnut, et cetera), with the support of a surface-active agent (53, 54). This can be liquefied in water to form a solid emulsion. To create a uniform emulsion after dilution in water, emulsifiable concentrate needs a high concentration of an oil-soluble emulsifying agent (54). The oil application does not injure crops, humans, fungal spores or animals. *Trichoderma* formulations are also being used as foliar sprays (53, 54). Oil-based formulations must be ideal for foliar spraying in arid weather, along with having a long shelf life. Since the spores are bubbled by oil, which protects them from drying at 5 °C, they can survive for longer on the plant surface, even in arid weather. Reports are on the production of an emulsion formulation of *T. harzianum* to battle *Botrytis cinerea,* which causes apple post-harvest rot (70). A reverse emulsion formulation of *T. harzianum* with an 8-month shelf life was formulated using native constituents at the ex-Project Directorate of Biological Control (PDBC) in India. Moreover, this formulation was found to be effective against soil-borne groundnut diseases (54). Common oils used in the formulation are Canola oil, Neem oil, Paraffin oil and Soybean oil (71).

Comparative significance of *T. viride* formulations

A wide range of *T. viride* formulations has been developed to enhance field performance, stability and adaptability across diverse agro-climatic conditions. As summarized in Table 3, each carrier system offers unique advantages that influence shelf life, ease of production and application efficiency. Talc-based formulations remain the most widely commercialized due to their low cost, simple preparation and suitability for seed treatment (40, 41, 44). Press mud and biochar carriers provide nutrient-rich and moisture-retentive environments that improve spore survival in soil, making them highly effective for field application (47, 50, 58, 61). Grain-based substrates such as sorghum produce exceptionally high conidial yields, supporting vigorous soil or nursery inoculation (66, 68, 70). Advanced alginate bead and oil-based formulations extend shelf life and protect spores under harsh environmental conditions, enabling controlled release and improved foliar performance (72, 74, 78, 80, 83). Together, these formulations offer versatile delivery systems that strengthen the reliability and scalability of *T. viride* in sustainable crop protection (Table 3).

Table 3. Comparative Overview of *Trichoderma viride* formulations

Formulation type	Carrier material	Approx. shelf life	Ease of production	Best use / Application	Remarks	References
Talc-based	Talc powder + $CaCO_3$	3-4 months	Very easy	Seed treatment	Widely commercialized; inexpensive and stable	(40, 41, 44)
Press mud-based	Sugar factory waste	5-6 months	Easy	Soil application	Nutrient-rich; cost-effective carrier with good moisture retention	(47, 50)
Biochar-based	Charred organic biomass	6-8 months	Moderate	Arid and semi-arid soils	Enhances water retention, supports higher spore survival	(58, 61, 63)
Sorghum grain-based	Sterilized sorghum or wheat grains	4-5 months	Easy	Soil or nursery inoculum	Provides high conidial yield and easy nutrient availability	(66, 68, 70)
Alginate bead formulation	Sodium alginate + $CaCl_2$	8-12 months	Moderate	Controlled release, long-term storage	Superior protection against desiccation and UV; best long shelf life	(72, 74, 78)
Oil-based formulation	Vegetable oils (e.g., neem, groundnut, sunflower)	6-10 months	Moderate	Foliar spray and seed coating	Excellent field stability under dry conditions	(80, 83, 86)

Methods of field application of *T. viride*

Methods of field application of *T. viride* include seed treatment, seed biopriming, soil drenching, root dip and foliar spray. These approaches enhance rhizosphere colonization, suppress soil-borne pathogens and promote plant growth (53, 54, 74) (Fig. 10).

Seed treatment

In this technique, seeds are covered with dry powder/dust of *T. viride* just before sowing (72). Crop coating with *Trichoderma* is a successful technique for preventing seed-borne and soil-borne diseases. As these seeds germinate, the *T. viride* present on the surface of the seeds also germinates and colonizes the roots of these seedlings and the rhizosphere (53, 54). Chickpea seeds treated with *Trichoderma* enhanced their vigor and seedling growth (55). Reports in the usage of *Trichoderma* as a seed treatment to control *Helminthosporium* leaf spot disease of the *Chrysalidocarpus lutescens* plant (73). The study concluded that the seeds treated with *Trichoderma* spp. showed reduced disease incidence compared to untreated seeds. The seed treatment also increased seed germination and seedling vigor index (73). Regular wheat seed treatment with the combination of *T. viride, T. harzianum, P. fluorescence, Glicocladum virens* and Vitavax @ 0.125 % (half the recommended dose) also gave good control of loose smut of wheat. However, a single bioagent alone cannot control the disease. The seedling emergence also increased due to the combined use of the bioagents and vitavax (74).

In- situ application of *T. viride;* Seed biopriming

Biopriming is treating seeds with biocontrol agents and incubating them in humid, moist environments before the radicle appears (75). This practice has the potential to do better than simple seed coating in terms of seedling appearance, pace and uniformity. *Trichoderma* conidia sprout on the surface of bio-primed seeds and form a coating outside their surface. Such seeds are extra tolerant of a variety of soil environments (54). Biopriming helps in the use of fewer biocontrol agents on the seed. Seed biopriming is flourishing in brinjal, chickpea, tomato and soybean in Uttarakhand's Terai region (76). Bio-primed seeds of rajma and chickpea in pots and fields, inoculated with microbial strains of *T. asperellum* T42, *Rhizobium* spp. RH4 and *P. fluorescens* OKC, independently and in combination, showed superior germination proportion and enhanced plant establishment in both crops relative to non-bioprimed control plants (77). It was also revealed that combining the microbes enhances seed germination plus increases plant growth more than applying them discretely. In both rajma as well as chickpeas, every combination that included *Trichoderma* was better than others and the triple microbial combination was better than other combinations in respect of seed germination and seedling growth (77) (Fig. 11).

Soil treatment

Soils contain both useful and harmful microbes (78). When we distribute *Trichoderma* spp. to soil, it improves the population dynamics of the fungal antagonists and stops pathogenic microorganisms from colonizing the rhizosphere (4). Biocontrol agents may be supplemented to the soil earlier or after planting to supervise a broad range of soil-borne fungal pathogens (12). *Trichoderma viride* is used alone or in combination with other treatments, which considerably reduce *Colletotrichum falcatum* induced red rot (79). It was 2010 observed that adding *T. viride* to the

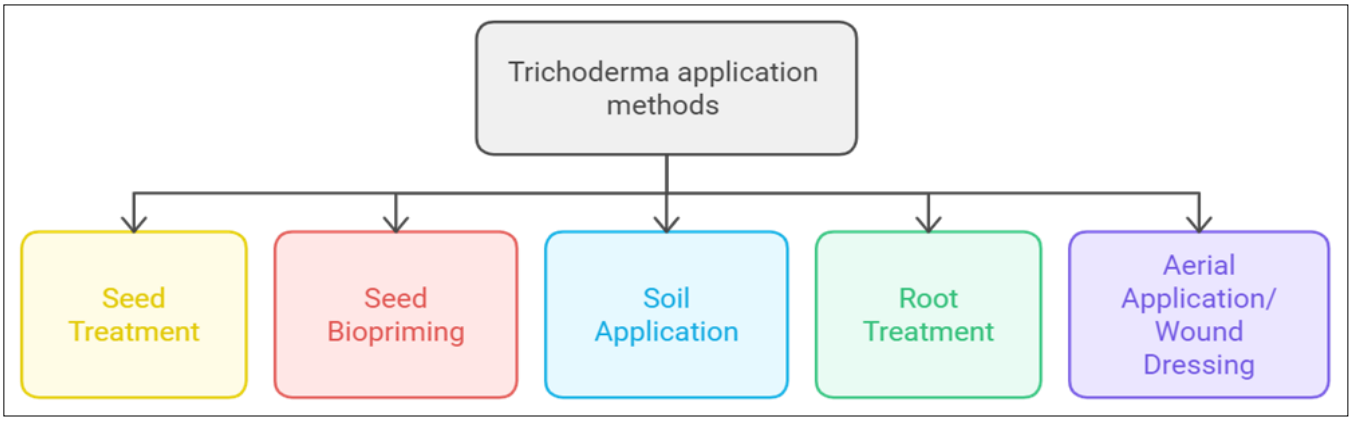

Fig. 10. Methods of field application of *T. viride.*

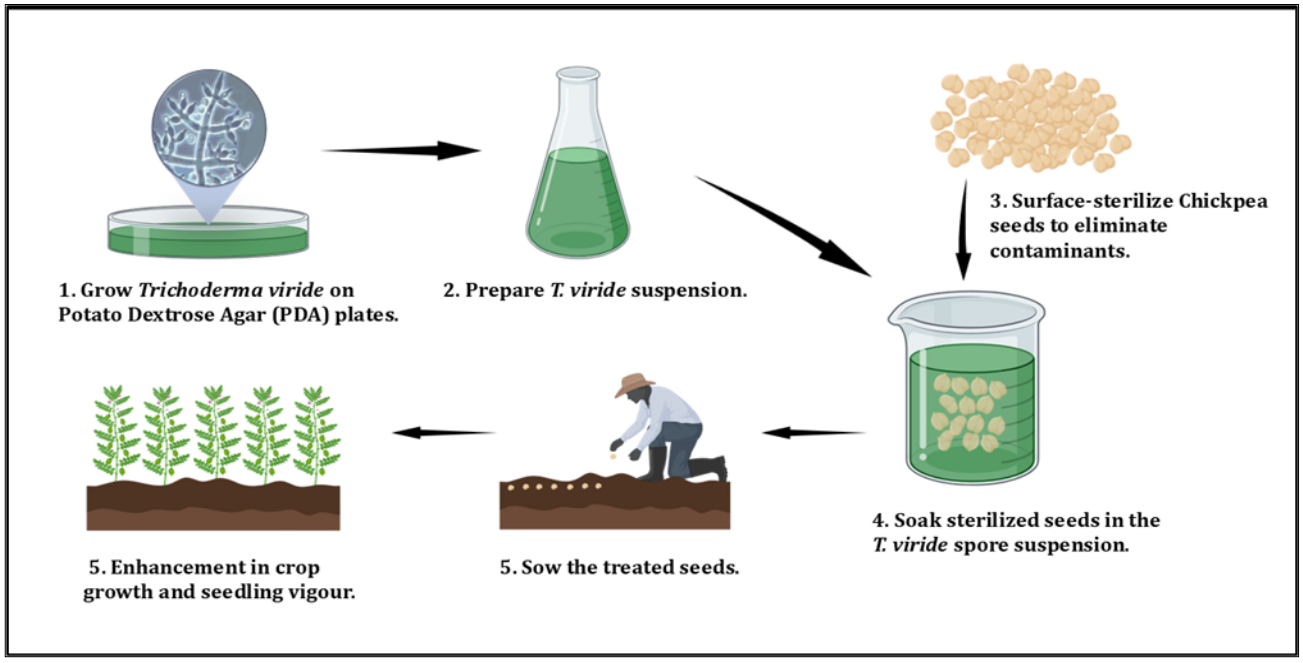

Fig. 11. Graphical representation of seed biopriming with *T. viride*, its field application and impact on plants' growth.

soil was the most efficient way to prevent collar rot, root rot, Jute seedling blight and stem rot disease (80). It was *also* revealed that seed-borne pathogenic fungi *F. oxysporum, F. moniliforme, F. solani, B. theobromae, A. alternata* and *R. solani*, as well as the seedling establishment of *Dalbergia sissoo*, could be regulated by the addition of an organic *Trichoderma* preparation to the soil (81). Since *Trichoderma* colonizes FYM (farmyard manure), it is more appropriate and beneficial to apply colonized FYM to the soil. This is the most proficient way to use *Trichoderma*, principally for the treatment of soil-borne diseases (55).

Root treatment

It is mostly done in the case of transplanted rice and vegetables (82). Seedling roots can be treated using antagonist spore or cell suspension via drenching *Trichoderma* in nursery beds or dipping roots in *Trichoderma* suspension before transplanting (55). Root dipping in an antagonist's suspension boosts seedling growth as well as reducing disease severity in rice, eggplant, chilli, capsicum and tomato (83, 84, 85). Root dips of rice seedlings before transplantation have also been used to decrease sheath blight disease (86). Root-dipped tomato seedlings with *T. viride* showed an increase in plant height, root weight and reduced incidence of root-knot nematode compared to uninoculated plants. High root length was also observed in inoculated plants. On observation, it was found that there were fewer egg masses/galls of root-knot nematode where roots were treated with *T. viride* (87) (Fig. 12.).

Aerial spraying / wound dressing

Trichoderma has been effectively utilized for biological control of decay fungi infecting wounds on shrubs and trees through aerial spraying of plant parts (88). Dissimilarity in microclimates has a key impact on the effectiveness of biocontrol agents for foliar diseases. Temperature, humidity, dew, rain, wind and radiation are all subject to diurnal and nocturnal, cyclic and non-cyclic variations in the phyllo sphere (89). As a product, the water capability of phylloplane microorganisms would be constantly changing. It can also fluctuate on leaves or the canopy's perimeter, as well as on sheltered leaves. In the sheltered, dense region of the plant, relative humidity was higher

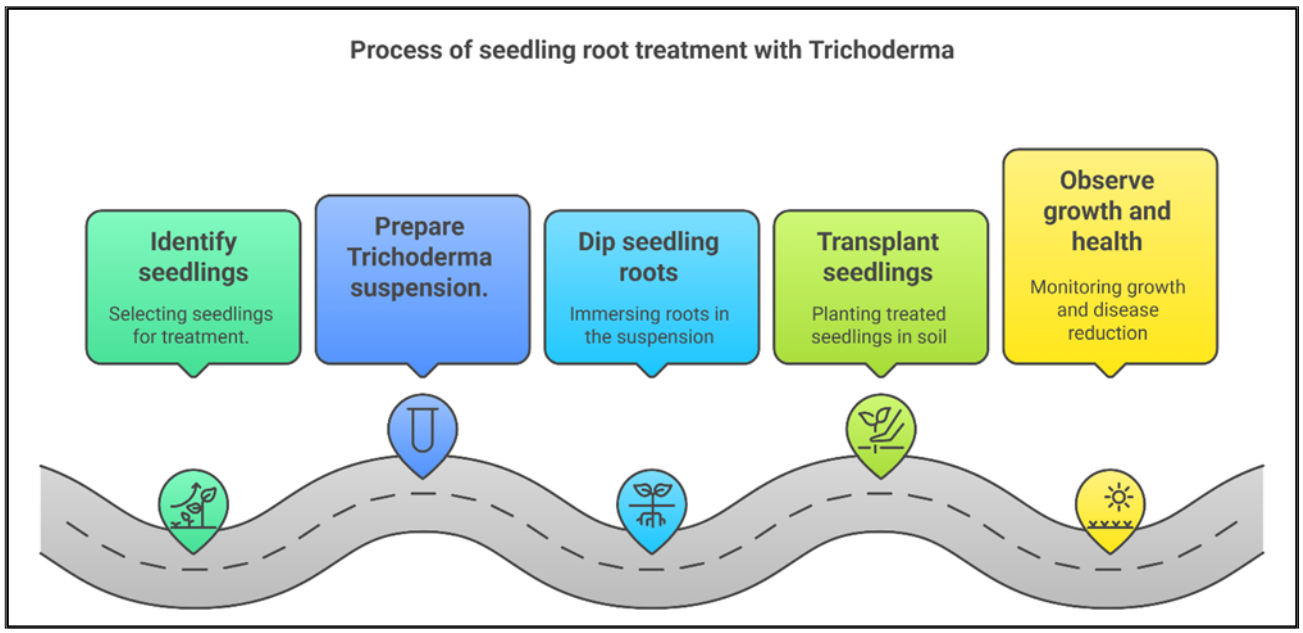

Fig. 12. Seedling root dip treatment with *T.viride*.

than inside the peripheral leaves (54). The deposition of dew is more evident in the middle and on the margins. Hydathodes, lenticels, wounds and stomata exude nutritional amounts of organic acids, sugars and amino acids that differ greatly. In phylloplane, it affects the effectiveness and survival of antagonists (90). For the biocontrol of *Alternaria* leaf spot in *Vicia faba*, a liquid solution of *Trichoderma* was sprayed directly onto the aerial regions of the plant (91). Reports are on the effectiveness of *T. harzianum* and *T. virens* in foliar sprays and talc-based formulations for lowering rice sheath blight disease incidence (58, 59). Field trials in Rajasthan were performed on root rot of groundnuts caused mainly by *Thielaviopsis basicola, S. rolfsii, A. flavus, P. aphanidermatum, R. solani andA. niger*, discovered that *T. harzianum* in powder and liquid bioformulation was successful in managing disease in the field (92). Studies are also on the treatment of scabs of citrus caused by *Elsinoe fawcettii* (93). It was also discovered that spraying of *E. purpurascens* and *T. harzianum* in the field reduced the occurrence of disease by 10 % and 17.8 %, respectively. About the fact that foliar application of *Trichoderma* decreases disease occurrence in the region, due to crop economy and increased dosage, it is not technically feasible. Therefore, crop value must be used to standardize dosage and application frequency, which may be a secure and feasible approach (54).

Challenges and limitations of *T. viride*

Despite its established potential as a biocontrol agent, *Trichoderma viride* faces several challenges during mass production, storage and field application that limit its large-scale adoption.

Viability and shelf life

The most critical limitation lies in maintaining the viability of *T. viride* spores from production to field delivery. The propagules are highly sensitive to fluctuations in temperature, moisture and relative humidity during transport and storage, which reduces their germination and field efficiency (94). Ensuring viable populations at the farmer level remains a challenge, especially under tropical climatic conditions.

Contamination during production

Large-scale fermentation and formulation processes are prone to contamination by bacteria or other fungi that can outcompete *T. viride* (95). Strict aseptic conditions, sterilized substrates and continuous microbiological monitoring are essential to maintain culture purity. Contamination not only affects conidial yield but also shortens shelf life and compromises efficacy.

Environmental dependency

The establishment and activity of *T. viride* in the rhizosphere depend on several ecological parameters such as soil pH, temperature, moisture and organic matter content (94, 96). Optimal conditions for multiplication are often difficult to maintain under field environments, resulting in variable performance. In certain locations, poor colonization limits its suppressive effects against soil-borne pathogens.

Slow mode of action

Compared to synthetic fungicides, *T. viride* operates through biological interactions that require time for colonization and antagonism (95). Consequently, it is less effective when disease incidence is already severe. The biological control process is gradual, often showing delayed symptom reduction compared to chemical treatments.

Safety concerns

Though generally regarded as safe, a few *Trichoderma* species such as *T. aggressivum*, *T. pleurotum* and *T. pleuroticola* are known to cause green mold disease in mushrooms, leading to commercial losses in mushroom cultivation. Furthermore, *T. virens* strain Q produces gliotoxin, a secondary metabolite reported to be immunosuppressive in humans (82). While such risks are minimal in agricultural use, strain-specific safety assessments are recommended before large-scale commercialization.

Formulation and carrier limitations

The physical and chemical stability of the carrier material significantly affects shelf life and field performance. Talc- and press-mud-based formulations have moderate stability, whereas biochar and alginate encapsulations offer longer viability but involve higher production costs. Selection of appropriate carrier materials for target environments remains an active area of research (110).

Regulatory and adoption barriers

Lack of awareness among farmers, absence of standardized quality control across commercial producers and inconsistent regulatory frameworks hinder wider acceptance and market penetration (111).

Addressing these challenges through improved formulation technologies, optimized fermentation, temperature-stable carriers and genomic selection of robust strains will greatly enhance the reliability and scalability of *T. viride* based biocontrol products.

Future perspectives

The growing need for sustainable agriculture and reduced dependency on chemical pesticides calls for intensified research on *T. viride* to improve its efficiency, stability and adaptability under diverse agro-climatic conditions. Globally, *T. viride* and *T. harzianum* remain the most dominant *Trichoderma*-based biopesticides, accounting for a significant share of the commercial biocontrol market (94). In India, *Pseudomonas fluorescens*, *T. viride* and *Bacillus thuringiensis* constitute the majority of registered microbial bioagents (94).

Despite remarkable success, the development of next-generation formulations and strains remains a major frontier. The following thematic areas are central to the future of *T. viride* research and commercialization.

Genomic and omics tools

Advances in genomics, transcriptomics and proteomics will enable the identification of genes linked to biocontrol efficacy, enzyme secretion and abiotic stress tolerance. Whole-genome sequencing of novel *Trichoderma* isolates can reveal molecular determinants of host-pathogen interactions and guide the development of genetically superior strains (56, 82).

Nanocarrier and microencapsulation technologies

Microencapsulation using biodegradable polymers or nanocarriers can significantly enhance shelf life, protect spores from desiccation and ensure controlled release during field application. Techniques such as alginate bead and chitosan coating have shown promise for maintaining spore viability at elevated temperatures (56, 98).

Microbial consortia and compatibility studies

Future efforts should emphasize the development of compatible microbial consortia combining *T. viride* with beneficial bacteria such as *Pseudomonas fluorescens* or *Rhizobium* spp. Such synergistic

formulations can improve nutrient solubilization, root colonization and overall crop resilience under biotic and abiotic stress (94, 97).

Climate-resilient strains

Isolation and breeding of thermotolerant, drought-tolerant and salinity-resistant *T. viride* strains are critical for maintaining biocontrol efficacy in variable agroecological regions. Molecular marker-assisted selection and mutagenesis can help identify robust isolates adaptable to changing climates (98).

Commercial scaling and policy support

To achieve wider adoption, *T. viride* technologies should be supported through national biofertilizer missions, quality control standards and farmer training programs. Development of cost-effective, easy-to-apply formulations will further enhance acceptance among small and marginal farmers (94).

Integrating omics-guided strain improvement, advanced formulation science and policy-driven commercialization will transform *T. viride* from a traditional biocontrol agent into a scientifically optimized, climate-resilient bioresource for sustainable agriculture.

Conclusion

Biological control represents a cornerstone of sustainable crop protection. Among the diverse microbial bioagents, *Trichoderma viride* stands out for its multifaceted benefits- ranging from disease suppression and growth promotion to soil health restoration. Its potential as biofertilizer and biopesticide reduces reliance on synthetic chemicals, thereby mitigating environmental pollution and enhancing long-term soil productivity.

Contamination of cultures during the large-scale production is one of the most recurring problems, particularly when sterile conditions are not maintained. Inadequate drying or improper carrier selection can lead to rapid loss of viability. Storage at >30 ^{0}C often reduces conidial germination; therefore, controlled temperature and moisture are critical to maintain product quality.

Future research should prioritize strain improvement using genomic tools and explore nanocarrier-based formulations to enhance stability and field efficacy, ensuring wider adoption by farmers and industries.

However, the practical success of the *T. viride* depends on overcoming key challenges such as strain stability, contamination management and formulation optimization, continued interdisciplinary research integrating molecular biology, nanotechnology and field-based agronomy will be pivotal in realizing its full potential.

In conclusion, *T. viride* serves as a powerful model organism for developing next-generation bioinoculants that contribute to resilient, eco-friendly and economically viable agricultural systems.

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Authors' contributions

AK and JG contributed to the conceptualization and writing-original draft preparation. AR contributed to figure preparation. AK has contributed to literature collection and tables preparation, SK and DP contributed to the review and editing of the manuscript. All authors read and approved of the final version of manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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