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Review

Journey of *Trichoderma* from Pilot Scale to Mass Production: A Review

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Abstract: *Trichoderma* spp. has the ability to inhibit fungal plant pathogens through several mechanisms like the production of hydrolytic enzymes, mycoparasitism, coiling, and antibiosis and is therefore recommended as a potential and native biocontrol agent for effective control of soil-transmitted diseases. Various species of *Trichoderma*, like *T. virens*, *T. asperellum*, *T. harzianum*, etc., have been explored for their biocontrol activity against phytopathogens. There are different *Trichoderma* species and strains with respect to plant pathogens. Efforts have been made to develop effective and efficient methods, such as microencapsulation use of different polymers, adjuvants, or carriers, to increase the shelf-life and efficacy of *Trichoderma* formulations. The crucial aspects for the success of a biocontrol agent include developing and validating formulations, improvement in shelf-life, cost-effectiveness, easy accessibility, improved delivery systems, broad spectrum in action, robust performance (biocontrol), and integrative strategies for sustainable disease management. This review focuses on recent developments in the isolation, identification, preservation, substrates, consortium, quality control, mass production, delivery methods, field performance, registration, and commercialization of *Trichoderma* formulations for strategic development of next-generation multifunctional biological control formulations.

Keywords: biocontrol; phytopathogen; *Trichoderma*; mycoparasitism; soil-borne; bio-pesticide

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1. Introduction

Biological control of plant diseases has emerged as a promising area in phytopathology. These methods not only minimize reliance on synthetic pesticides but are also comparatively economical, feasible, robust, and sustainable [1,2]. Among the commercial biofungicides/fungal antagonists, *Trichoderma* has been broadly acknowledged as a source of potential biocontrol agent, particularly for lowering soil-borne phytopathogens such as *Fusarium oxysproum*, *Rhizoctonia solani* [3], *Macrophomina phaseolina* [4], *Sclerotinia rolfsii*, and others [5–7]. *Trichoderma* spp. are saprophytic, avirulent, and soil-inhabiting fungi, and they control pathogens with various biocontrol methods such as mycoparasitism [8], retard the pathogen growth by secreting cell-wall-destroying enzymes [9,10], nutrient uptake competition, and rhizospheric competence [2]. Because of its effectiveness against phytopathogens, its market demand is increasing yearly [11].

According to Harman (1991), [12] key points that are required for the production of any biological control system are (1) a potential biocontrol agent, (2) viable propagule with amplified shelf-life, (3) bioprotective delivery mechanisms that may offer the biocontrol agent an advantage to compete against existing microflora, and (4) steady field

performance [13]. As mentioned above, the first and most principal step before mass production of a biocontrol agent is the identification of a robust propagule (hyphae, chlamydospores, and/or conidia) [14]. Once a reliable and efficient biocontrol agent is identified, large-scale production, design, and application ideas need to be performed cautiously for the product's stability during storage and later use. Both liquid and solid formulations are utilized in developing sufficient amounts of viable and active *Trichoderma* inoculums. Conidia and chlamydospores are the preferred propagules for formulations since they can withstand rigorous treatment procedures, whereas hyphae cannot be used because they are not dehydration-resistant [12,15]. Several reports (Table 1) related to the effectiveness of *Trichoderma* strains and their formulations have been published periodically [13]; however, reports concerning advancements in mass production, persistent viability, and related field performance of *Trichoderma* species are fragmentary. Furthermore, the challenges related to the isolation and development methods for elevating the effectiveness and sustainable use of *Trichoderma* formulations in the field of health security and food also need to be addressed. In this review, we intend to fill this gap and unravel the recent developments regarding isolation, identification, preservation, substrates, consortium, quality control, mass production, delivery approaches, field performance, and registration and commercialization of *Trichoderma* formulations.

Table 1. Timeline of historical events in *Trichoderma* research.

Decades	Year	Landmarks in *Trichoderma* Research	References
1700s	1794	In this year, the name *Trichoderma* was introduced.	[16]
1800s	1865	The sexual stage of *Trichoderma viride* (*Hypocrea rufa*) was reported.	[17]
1900s	1932	First evidence that *Trichoderma lignorum* (*Hypocrea virens*) has mycoparasitic and biocontrol abilities.	[18]
	1934	The invention of the first anti-microbial compound (e.g., gliotoxin).	[19]
	1957	Discovery of the effect of light *on T. viride*; syn. *T. reesei*.	[20]
	1972	Demonstration of biocontrol activity of *T. harzianum* against *Sclerotium rolfsii* in field conditions.	[21]
	1979	RUT C30, a carbon catabolite mutant of *T. reesei*, was isolated using ultraviolet (UV) mutagenesis.	[22]
	1983	Cloning of the first *Trichoderma* species (e.g., *T. reesei*).	[23]
	1984	The first international workshop was held on *Trichoderma*.	-
	1985	Papavizas wrote the first review on biology, ecology, and potential for biocontrol by *Trichoderma*.	[15]
	1986	Report on the expression of growth promotion in the root.	[24]
	1987	Successful transformation of *T. reesei*.	[25]
	1989	First registration of commercial formulation (e.g., Binab T).	[26]
	1992	Evidence of cloning of lectin-coated fibers by *Trichoderma* species.	[27]
	1993	*prb1* gene cloning related to mycoparasitism and induced by cell wall.	[28]
	1997	Expression of enhanced plant immunity (ISR) by application of *Trichoderma* spp.	[29]
	1998	Identification of factors that induce genes for mycoparasitism.	[30]
	1999	*Trichoderma* internal colonization of plant roots demonstrated.	[31]

Table 1. *Cont.*

Decades	Year	Landmarks in *Trichoderma* Research	References
2000s	2002	Role of signaling pathways and G protein in mechanism of biocontrol and conidia formation.	[32]
	2003	Role of mitogen-activated protein kinase (MAPK), which negatively regulates conidiation in *T. virens*.	[33,34]
	2004	Identification of photoreceptors (*Brl1* and *Brl2*) in *T. atroviride*.	[35]
	2005	Function of *Trichoderma* MAPK (mitogen-activated protein kinase) in Induce systemic resistance (ISR).	[36]
	2006	Purification of the first true elicitor protein (*Sm1/EplI*) of *Hypocreaatro viridis* on glucose.	[37]
	2006	Cellulases and xylanases regulators identified in *T. reesei* (*XYR1*).	[38]
	2008	First genome sequenced of *Trichoderma* species (e.g., *T. reesei*).	[39]
	2009	Endophytic *Trichoderma* for stress tolerance in plants.	[40]
	2010	Discovery of VELVET protein (Vel1) as a key regulator in biocontrol agents (e.g., *T. virens*) and *Trichoderma* spp. peptide pheromone precursor genes have been identified.	[41]
	2011	Genome comparison of three species of *Trichoderma*.	[42]
	2012	Next-generation sequencing and high-throughput procedures were used for the sequencing of *Trichoderma* spp. (*T. asperellum*, *T. harzianum*, *T. citrinoviride*, *T. longibrachiatum*) genome.	[43]
	2022	Five new *Trichoderma* species were reported.	[44]
	2014–2022	144 *Trichoderma* strains were registered in 40 countries.	[45]

2. *Trichoderma* Serves as a Biocontrol Agent

Trichoderma was initially reported as a biocontrol agent in the 1930s [18,19]. Since the discovery of mycoparasitic activity of *Trichoderma* by Weindling against *Rhizoctonia solani* and other phytopathogens, several other researchers have also obtained positive results with *Trichoderma* isolates as biocontrol agents of plant pathogens [46]. *Trichoderma* continues to hold a significant position among commercial biological control agents (BCAs) in a wide range of crop and disease management, either as a single ingredient or in combination with other ingredients [6,45,47,48]. Up till now, more than 80 species of *Trichoderma* have been reported [13], and among these, *T. harzianum*, *T. virens*, and *T. viride* are frequently deployed biocontrol agents. In India, commercialization of *Trichoderma* is limited to only two species, namely *T. viride* and *T. harzianum* [13]. However, there are ample reports on the effectiveness of *T. virens* and *T. asperellum*, but these are still unregistered under the Central Insecticide Board and Registration Committee (CIBRC) in India. This may be due to several hurdles, such as toxicity assessment, environmental effects of microbes and their formulation, and optimization of technology for mass-scale production [49]. Apart from these, some other constraints include multi-location trials for the purpose of proving its safety, followed by registration. In addition, some other challenges are inconsistent field presentation and low shelf-life of formulation, lack of patent protection, preliminary testing, high registration cost, alertness about the beneficial effect of *Trichoderma* formulations, and training and education shortfalls. Registration also requires good and effective documentation and other confirmations. Moreover, the evaluation process itself (compilation and analysis of data) can be prolonged and costly. In the past, high registration fees were clearly seen as a delay or barrier to BCA market growth, especially for medium and small enterprises that are the chief manufacturers of biocontrol agents. Due to these reasons, there are now diverse products in the market that claim to be plant growth promoters or biofertilizers for managing plant diseases but are not yet registered. The biocontrol activity of *Trichoderma* spp. is represented in Table 2.

However, their safe use cannot be guaranteed without toxicological and efficacy data [50]. Combined with the competitive pesticide industry, BCA companies are finding it hard to generate enough revenue from product sales to justify the registration cost. Unluckily, this has led to some products being removed from the stores. For instance, *T. harzianum* T-39 (Trichodex) was launched in Europe and Israel in 1993 for the control of Botrytis fruit rot through biological methods but was detached from the market. It went bankrupt in 2005 due to low sales and the rising cost of registration. Table 3 contains information on gene identification in *Trichoderma* species.

Table 2. Biocontrol activity of *Trichoderma* spp.

Name of *Trichoderma* Species	Name of Plant Pathogens	Crop Name	Inhibition/Efficacy (%)	Experiment Condition	References
T. harzianum	*Sclerotium rolfsii* and *Rhizoctonia solani*	Ryegrass	42–47	Greenhouse and field condition	[51]
T. harzianum	*Phytophthora cinnamon*	Pine	28.5–37.5	Greenhouse	[52]
T. hamatum and *T. harzianum*	*P. cinnamomi*	Avocado		Greenhouse	[53]
T. viride, *T. virens T. harzianum*, *T. pseudokoningii T. koningii*	*Aspergillus niger*, *Rhizoctonia solani* and *Geotrichum candidum*	*Sapodilla* (*Manilkara zapota* L.)	54–74	Laboratory	[54]
T. harzianum strain Ths97	*F. solani*	Olive trees	25–50	-	[55]
Trichoderma viride 1433 Mutant strains	*Pythium aphanidermatum*	Mustard	85	Lab and field	[56]
Trichoderma spp.	*Pythium aphanidermatum*	Chilli	88.00	Lab and field	[57]
Trichoderma harzianumRifai, *Trichoderma viride*	*Pythium aphanidermatum*	Tobacco	100	Lab and greenhouse	[58]
Trichoderma virens Trichoderma harzianum, *Trichoderma viride*	*Phomopsis vexans*	Brinjal	38–49	Lab	[59]
Trichoderma harzianum T22	*Alternaria alternata*	Sunflower	75	Lab	[60]
T. atrobrunneum	*Armillaria mellea*	Strawberry	91	Field	[61]
Trichoderma harzianum	*Fusarium moniliforme*	Maize	73.33	Lab	[62]
T. viride, *Gliocladium virens*	*Rhizoctonia solani*	Rice	67.94% and 68.62% respectively	Lab	[63]

Table 3. Reports on gene identification in *Trichoderma* species.

S.No.	Identified Genes	*Trichoderma* Species	Function	References
1	*Tvsp1*	*T. virens*	This gene protects cotton seedlings against *Rhizoctonia solani*.	[64,65]
2	*Tag3*	*T. asperellum*	Responsible for glucanase production for cell wall degradation.	[66]
3	*TgaA* and *TgaB*	*T. virens*	Bicontrol efficacy for management of *R. solani* and *Sclerotium rolfsii*.	[67]
4	*ThPG1*	*T. harzianum*	This gene is required for beneficial interaction between *Trichoderma harzianum* and the host.	[68]
5	*ThPRT2*	*T. harzianum*	Mycoparasitism activity for *Botrytis cinerea*.	[69]

Table 3. *Cont.*

S.No.	Identified Genes	*Trichoderma* Species	Function	References
6	*tri5*	*T. brevicompactum IBT40841*	Production of antifungal activity and trichodermin against fungus causing infection in the human body (*Candida* spp. and *Aspergillus fumigatus*).	[70]
7	*erg1*	*T. harzianum* CECT 2413	Ergosterol biosynthetic pathway (EBP).	[71]
8	*TvGST*	*T. virens*	This gene provides enhanced tolerance against cadmium stress.	[72]
9	*TrCCD1*	*T. reesei*	This gene facilitates pigment production and hyphal growth.	[73]
10	*egl1*	*T. longibrachiatum*	Exhibits antagonistic activity against *Pythium ultimum.*	[74]
11	*qid74*	*T. harzianum*	Plant biofertilization and root architecture.	[75]
12	*Taabc2*	*T. atroviride*	ATP binding cassette transporter plays a major role in cell membranes.	[76]
13	*tac1*	*T. virens* IMI 304061	This gene shows mycoparasitism against *S. rolfsii* and *R. solani*.	[77]
14	*TrCCD1*	*T. reesei*	This gene promotes condia formation and elongation of fungal hyphae.	[73]
15	*gluc78 w*	*T. atroviride*	Cell wall disintegartion of *Pythium* and *Rhizoctonia* spp.	[78]
16	*SL41*	*T. harzinum*	Showed mycoparasitic action.	[79]
17	*Taabc2*	*T. atroviride*	This gene is important for biological control of necrotrophs (*B. cinerea* and *R. solani*).	[76]
18	Monooxygenase	*T. hamatum*	This gene shows antagonistic activity against *Sclerotinia sclerotiourum* and *S. cepivorum.*	[80]
19	*Xl1*	*Trichoderma strain Y*	This gene is helpful in hemicellulose breakdown.	[81]
20	*eg1*, β-1,4 glucanase	*T longibrachiatum*	This gene shows antagonistic activity against *Pythium ultimum* in cucumber.	[82]
21	*pacC*	*T. virens*	This gene acts as myctorphy.	[83]
22	*tvhydii1*	*T. reesei*	Important in mycoparasitism and plant-fungus interaction.	[84]
23	*hmgR*	*T. koningii*	These genes inhibit the pathogen *Rhizoctonia solani.*	[85]
24	*Tasx1*	*T. asperellum*	This gene plays a key role in morphological development, mycoparasitism, and antibiosis.	[86]
25	*gpr1*	*T. atroviride*	This gene is required for the stability of cell walls and hyphal growth.	[87]
26	*TvCyt2*	*T. virens*	*Trichoderma*–Arabidopsis interaction	[88]
27	*gluc31*	*T. harzianum*	Mycoparasitism ability and influence cell wall organization.	[89]
28	*ipa-1*	*T. virens*	Antibiosis of *R. solani*.	[90]
29	*TasXyn24.2*, *TasXyn29.4*	*T. asperellum*	Induced resistance and promoted growth in seedlings.	[91]
30	*agl1*	*T. atroviride*	Biological control of plant pathogen.	[92]

3. Biocontrol Properties of *Trichoderma* against Phytopathogens

Trichoderma spp. have the ability to produce metabolites, modulate the plant defense responses, and act as a hyperparasite [93,94]. Moreover, *Trichoderma* strains are effective BCAs because of their high reproductive potential, resilience to harsh environments, efficiency in utilizing nutrients, ability to alter the rhizosphere, aggressiveness

against phytopathogenic fungi, and effectiveness in fostering plant growth and defense mechanisms. Due to these characteristics, *Trichoderma* may be found in all habitats and at high population densities [95]. *Trichoderma* BCAs can even have a beneficial impact on plants by promoting biofertilization, which increases plant development and enhances plant defense systems [96]. *Trichoderma* uses indirect and direct methods to control plant pathogens. The power of these mechanisms in the biocontrol method relies upon the type of *Trichoderma* strain, the antagonized pathogen, including its host, and the ecological situation [96]. The direct mechanism includes mycoparasitism and coiling, whereas the indirect mechanism includes challenges for nutrients and space, systemic acquired resistance, and antibiosis. Among them, mycoparasitism, competition, and antibiosis play a major role in *Trichoderma*-mediated biological control.

3.1. Mycoparasitism

Parasitism is one of the important mechanisms of fungal antagonist, where one fungus parasitizes (mycoparasite) another fungus (host), and this process is known as mycoparasitism. It has been observed that mycoparasitism involves four sequential steps: chemotaxis, recognition, attachment, and wrapping, as well as penetration of the pathogen cell wall and host digestion [97–99]. *Trichoderma* is widely used as a biofungicide against phytopathogens such as *B. cinerea* as well as the soil-borne pathogens *Rhizoctonia*, *Sclerotinia*, *Pythium*, and *Fusarium* spp. [14,100].

Trichoderma species employ several mechanisms to antagonize and mycoparasitize other pathogenic fungi, which includes competing for nutrients [101], releasing antibiotic metabolites, and activities like encircling the host and enlargement of the appressorium-like structure [51,102,103]. The degradation of host tissues containing pathogenic organisms occurs due to the enzymatic breakdown of cell walls facilitated by hydrolytic enzymes (such as chitinase, β-1,3-glucanase, and cellulase) that are synthesized by *Trichoderma* spp. [104]. In *T. atroviride*, the *nag1* gene coding for N-acetylglucosaminidase has a significant effect on chitinase induction, followed by biocontrol [105]. In the parasite interaction between *Trichoderma* and *R. solani*, host-released dispersal factors are responsible for inducing ech42 (encoding endochitinase 42) gene transcription prior to physical touch [30,106]. Lectins present in the host cell wall cause the parasites to cover the hyphae of the host after direct contact [27,100,106].

3.2. Antibiosis

Antibiosis is a phenomenon where one organism is prevented/inhibited by another microorganism through secondary metabolites (SMs). *Trichoderma* spp. synthesizes SMs (pyrone, heterocyclic compounds, terpenoids, polyketides, etc.) [107] and also produces specific low molecular weight compounds/antibiotics for combatting plant pathogens [108]. Antibiosis has been reported to occur during contact among pathogen, plant, and *Trichoderma* spp., which triggers *Trichoderma* to produce antibiotics and SMs to reduce the growth of phytopathogens. More than 180 secondary metabolites have been extracted from *Trichoderma* spp., showing different classes of chemical compounds [109,110]. On the basis of their biosynthetic origin, these compounds can be classified as peptaiboles, polyketides, and terpenes [111]. Many species of *Trichoderma* genus are known to synthesize peptidols that are non-proteinogenic amino acids (α-aminoisobutyric acid, a polypeptide antibiotic with a 500 to 2200 Da molecular weight). These compounds are acetylated at the N-terminus and contain an aminoalcohol at the C-terminus [112].

Different types of *Trichoderma* produce different antibiotics; for example, *T. viride* produces mucortoxins A and B, mucorin, trichophyton, and mucorin. Similarly, mucorin A and B were isolated from *T. mucorin*. *T. harzianum* produces tricholongins BI and BII, while longibrachins and trichokonins were extracted from *T. koningii*. Atroviridines A-C and neoatroviridines A-D have been obtained from *T. atroviride* culture. In addition, other antibiotics and fungicidal compounds have been isolated from *T. harzianum*, *T. koningii*, *T. aureoviride*, *T. virens*, *T. hamatum*, and *T. lignorum* [110].

Growth of soil pathogens such as *Phytophthora solani*, *P. middletonii*, *P. cinnamomi*, *Bipolaris sorokiniana,* and *Fusarium oxysporum* was adversely affected in the vicinity of Koninginin D [113]. Similarly, viridins obtained from *Trichoderma* spp. like *T. viride*, *T. koningii*, and *T. virens* inhibit the spore germination of *Colletotrichum lini*, *Botrytis allii*, *Penicillium expansum*, *Fusarium caeruleum*, *Stachybotry satra*, and *Aspergillus niger* [114]. Harzianic acids derived from *T. harzianum* show antimicrobial activity against *Sclerotinia sclerotiorum*, *R. solani,* and *Pythium irregulare* [115]. It has been found that *Trichoderma* spp. And *Gliocladium* suppressed the growth of various soil-borne plant pathogens (*Fusarium* spp., *Macrophomina*, *Sclerotium rolfsii*, and *Sclerotinia* spp.) [116,117]. Silva et al. (1998) studied the antibiosis mechanism of *Trichoderma* against *Colletotrichum* spp. [118].

3.3. Competition

Limitation and competition for nutrient sources can lead to the natural control of fungal pathogens [119]. *Trichoderma* is a cosmopolitan fungus and is found in all kinds of soils because of its outstanding competitive potential. It can fight with phytopathogens for nutritive sources, such as C, N, and Fe, and also acts as a biological antagonist towards soil-borne pathogens. It is also an aggressive competitor that grows rapidly and quickly colonizes its substrate and controls slow-growing pathogens [120]. *Trichoderma* is more competitive with other microorganisms due to certain abilities, such as a higher growth rate and enhanced aptitude to mobilize and utilize nutrients from soil/substrate [121,122]. Thus, competition for macro and micronutrients plays a key role in the interaction between *Trichoderma*-plant pathogen [123] because *Trichoderma* species compete with bacteria in the rhizosphere of crops for nutrients and sites of infection [124]. Compared to other rhizosphere bacteria, *Trichoderma* shows a better ability to produce and take up nourishment from soil; therefore, the management of certain disease-causing entities, such as *Botrytis cinerea,* using *Trichoderma* through food competition is possible [125]. It is observed that there are four major features of any organism that contribute to its saprophytic ability and inoculum potential: (i) fast germination of fungal propagule, rapid hyphal growth towards nutrients, (ii) production of suitable enzymes for carbon constituents of the host plant, (iii) secretion of growth inhibitor compounds (fungistatic and bacteriostatic), and (iv) tolerance to fungistatic substances produced by competing microorganisms. Antagonistic fungi can compete with the pathogens for food and space by colonizing the normal environment, i.e., plant tissue, rhizosphere, or phyllosphere [126]. It depends on the colonization level of the host plant and acclimatization to the environmental situations in which they are living [127]. In order to successfully compete with other fungal phytopathogens for food and space, *Trichoderma* should exhibit efficient strategies for colonization of the plant and should be plentiful in an area where competition with other microorganisms occurs [126].

Trichoderma spp. produces iron chelating agents and siderophores, which make iron unobtainable for rhizospheric bacteria, which eventually leads to the extinction of the disease. Thus, *Trichoderma* acts as a competitor that helps control plant diseases [128]. Apart from this, due to its ability to colonize the rhizosphere and outcompete for nutrients, *T. harzianum* (T35 Strain) reduces the availability of nutrients and the amount of rhizospheric space available for the fungal wilt agent of watermelon (*Fusarium oxysproum* f.sp. *meloni*) to colonize [129]. Srinivasan et al. (1995) demonstrated the importance of competition between siderophore-producing *Trichoderma* strains and wood decay Basidiomycetes fungi [130].

Mokhtar et al. 2013 studied the interaction between *T. harzianum* and a few fungal species, such as *Alternaria alternata*, *Fusarium acuminatum,* and *A. infectoria*. The results revealed that lack of nutrients caused death of the pathogenic fungi [131]. It has also been found that *Trichoderma* can compete with plant pathogens, including *Colletotrichum* sp., *Botrytis* sp., and *Phytophthora* sp., for complex and simple substrates of carbon [132]. In order to successfully compete with other fungal phytopathogens for food and space, *Trichoderma* should exhibit efficient strategies for colonization of the plant and should be plentiful in an area where competition with other microorganisms occurs [126].

Trichoderma colonization of roots commonly improves nutrient absorption and utilization, crop yield, tolerance to abiotic stressors, and root growth and development [133]. *Trichoderma hamatum* or *Trichoderma koningii* can boost crop production up to 300% after addition in the field. In greenhouse experiments, a substantial increase in yield was reported after treating the seedlings with *Trichoderma* spores [95]. The ability of *Trichoderma* BCAs to produce metabolites that either prevent spore germination (fungistatic), kill cells (antibiosis), or alter the rhizosphere, for example, making the soil acidic, leads to biocontrol that is unsuitable for pathogen proliferation [96]. *Trichoderma* strains quickly proliferate when introduced to the soil because they are inherently resistant to a wide range of hazardous substances, including insecticides, fungicides, and herbicides like DDT [95].

3.4. Production of Antibiotics and Other Antifungal Compounds

It has been shown that the *Trichoderma* species produce a large number of secondary metabolites, about 370 of which are members of several chemical compound classes with potent antagonistic activities [126,134]. Peptaibols and polyketides are the most significant non-volatile and volatile organic compounds (VOCs) produced by the majority of *Trichoderma* strains [2]. The volatile antibiotic 6-phenyl-pyrone (6PAP), responsible for the distinctive coconut scent and the biological control of *F. oxysporum*, is produced by the *T. viride*, *T. harzianum*, and *T. koningii* species [135]. In addition, *T. harzianum* also produces harzianic acid, a tetramic acid that has strong antifungal action as well as the capacity to stimulate plant development and function as a chelator [136].

3.5. Induced Systemic Resistance

Trichoderma can trigger a host plant's defensive mechanism while preventing harmful pathogens from proliferating and growing, and it can also encourage crops to build self-defense mechanisms to gain local or systemic disease resistance [137]. Two methods are used to achieve *Trichoderma*-induced plant disease resistance: first, control the elicitors or elicitors that trigger the plant disease resistance response; and second, release oligosaccharides from the cell-wall-degrading enzymes produced by *Trichoderma* to cause plant resistance [138]. Saravanakumar et al. (2016) found that *Trichoderma* coated corn seeds dramatically increased the peroxidase (POD) and phenylalanine ammonia lyase (PAL) activity, and the plants were resistant to Curvularia leaf spot of corn [139].

4. *Trichoderma* Selective Medium

Isolation and quantitative estimation of *Trichoderma* from the soil is tedious due to fast growth and similar morphology of other existing fungi (competitors) on a routine growth medium. It is isolated from rhizospheric soils using the serial dilution technique. Different types of low-cost and easily procurable culture media have been tested for growth and sporulation in *Trichoderma*, such as carrot agar (CA), oatmeal agar (OMA), potato-dextrose agar (PDA), Czapek's Dox medium (CDA), Rose Bengal chloramphenicol agar (RBCA), etc. However, the *Trichoderma* selective medium (TSM) with low glucose levels is preferred for isolation and quantification of *Trichodema* because it favors its growth while inhibiting the growth of other soil fungi such as *Mucor* and *Rhizopus* spp. [140]. The components of TSM for 1 L media (pH 4) are 0.2 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 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, 0.15 g of Rose Bengal agar (RBA), 20 g of agar-agar, and 0.25 g of chloramphenicol.

5. Methods for Isolation of *Trichoderma*

There are various techniques for the extraction of *Trichoderma* spp. However, serial dilution of samples is one of the most reported methods. This method is very simple, economical, and suitable for dealing with various samples. In this method, the soil sample is collected from the rhizosphere zone of the plants, air-dried, and ground into fine powder. Soil suspension is prepared by mixing ten grams of a dry powdered soil sample in 90 mL

of sterilized water, followed by shaking it for at least 5–10 min on an orbital shaker to make a homogenous solution. Thereafter, dilutions of samples are prepared from 10^{-1} to $10^{-6,}$ and a 100 µL solution is pipetted from the third or fourth diluted test tube and spread evenly on *Trichoderma* selective medium (TSM) using a sterilized 'L-shaped' glass spreader under a laminar air-flow chamber. The inoculated Petri plate is kept in a BOD incubator at 28 ± 1 °C for 4–5 days. After 5 days, each colony that is visible on the plate is considered a single colony-forming unit (cfu). In a report by Gil et al. (2009), rhizosphere soil samples were taken to monitor *Trichoderma* and *Glueomyces* populations with different crops and tillage lines. The best medium for isolating *Trichoderma* and *Gliocladium* spp. was potato dextrose agar + streptomycin + chloramphenicol and rose bengal, and for Actinomyces, Küster medium + cycloheximide and sodium propionate [141].

In the study conducted by Liu and colleagues in 2020, *Trichoderma* was isolated from the rhizosphere soil using a serial dilution technique [142]. Subsequently, these isolates were cultured on a Rose Bengal medium, as described by Zhou et al. (2020) [143]. Following incubation for 48 h, *Trichoderma* colonies were carefully selected and transferred onto a potato dextrose agar (PDA) medium. To ensure the purity of these putative *Trichoderma* colonies, a single spore isolation technique, as outlined by Dou et al. (2019) [144], was employed. This involved transferring a single spore onto a PDA medium, ultimately resulting in the acquisition of a pure *Trichoderma* isolate. In a similar report involving the isolation of *Trichoderma* from rhizosphere soil using a serial dilution technique, the cultures were maintained on PDA at a temperature of 28 °C for 2 to 7 days. Following incubation, specific colonies of *Trichoderma* were selected and transferred onto fresh PDA medium. Further incubation of these selected colonies was performed at 28 °C in a dark environment [145].

6. Identification

Fungi are morphologically, metabolically, ecologically, and phylogenetically diverse [146]. Hence, exact fungal identification is necessary for all fungal investigations and applications, and it enables us to anticipate the beneficial or harmful properties of fungal spp. and their distribution and establish safety measures. Despite their significance in biological control, identifying fungi is still a difficult task, especially for chemists who are not associated with mycologists [147]. There are various challenges in the identification of *Trichoderma* species: (i) problems with fungal species identification using morphology criteria; (ii) potential advantages and limitations of ribosomal genes commonly used for identification of fungal pathogens and their ITS region, the official DNA barcode marker for fungal-level identification; (iii) limited knowledge on the use of NCBI-BLAST to search for DNA barcodes; (iv) extensive curated molecular database of fungal sequences used to increase or replace ITS for species-level identification of certain fungal taxa; and (v) method of constructing phylogenetic trees from DNA sequences to facilitate the identification of fungal species [148].

There are some reports of species misidentification using external features; for example, the name *T. harzianum* was used for a few species [149]. Identification can be obtained using a similarity search tool (TrichoBLAST) and oligonucleotide barcoding (TrichOKEY), both available online at http://www.isth.info/ (accessed on 12 October 2023) [9,150,151]. In addition, phenotypic microarrays can be used to classify new species and allow the analysis of carbon utilization patterns from 96 carbon sources [152]. Chaverri and Samuels (2013) identified endophytic organisms based on habitat preferences and trophic models to understand different groups of radiation types and their potential to be used to develop new biological control strategies [153]. Cellulose acetate electrophoresis can be used to characterize several species with defined isoenzyme structures, indicating that this method can be useful for identifying biochemical differences in some *Trichoderma* species [154]. Ongoing efforts to characterize the diversity and geographic distribution of *T. hypocrea* have resulted in extensive documentation of this genus [153,155]. Currently, the Interna-

tional Subcommittee on *T. hypocrea* provides 104 species properties at the molecular level (http://www.isth.info/biodiversity/index.php, accessed on 12 October 2023).

7. Preservation of *Trichoderma* Cultures

Different isolation and preservation methods have been tried for the *T. viride* fungus. The crosslinking isolation method using 0.5% Tween 20 proved to be the best for isolation and statistics [156]. Various methods have been tried to study the long-term storage and recovery of *T. viride* isolates in the stored conditions. *T. viride* can live for more than 2 years in dry form/weak form, but drying is a crucial step because if it dries too quickly, the fungal spores lose their vigor/pathogenicity, and it will cause the death of the fungal spores, and becomes infected with other bacteria or viruses. In the case of the H_2O preservation method, the culture can be recovered even after 12 months without losing its potency [156,157].

Nowadays, various methods have been optimized to minimize the problems faced by the serial transfer of fungal culture and to extend its life span [158]. Generally, most preservation methods slow down the metabolism of microorganisms by storage at 4 °C and/or mineral oil coating, which restricts the cell's ability to take up oxygen, slows down the metabolic activity, and lengthens the storage time [159]. There are various methods used for the preservation of *Trichoderma* spp., such as oil drop cover, direct freezing of culture, water preservation, and spore suspensions, which are easy and lucrative methods [158]. The main objective of preserving *Trichoderma* culture for the long term is to ensure the viability, physiological, morphological, and genetic integrity (Table 3) of the cultures over time, and safe storage is further required to validate the reliability of strains. The American Type Culture Collection uses a variety of preservation techniques, including soil or oil preservation [160,161], water agar slants, cryopreservation or low temperatures (−20 and −70 °C) [162,163], etc. The cellulose filter paper was used for the preservation of *T. harzinum* and was found to be simple, less costly, less time-consuming, and requires less refrigeration for the storage of the *Trichoderma* isolates. Stocco et al. (2010) and Tucci et al. (2011) tested different methods of preservation, including distilled water, mineral oil, and silica gel for long-term storage of *Trichoderma* [164,165].

8. Estimation of Colony Forming Units (cfu)

Serial dilution, pour plate, and spread plate are standard techniques for CFU estimation of microbes (fungi, bacteria, etc.) [125]. The spread-plate method offers several benefits over the pour-plate method and includes increased flexibility in handling, reducing detrimental effects on temperature-sensitive microbes, preventing aerobic organisms from being trapped in the agar medium, and counting of colony forming units can be performed easily on the surface of the plate [166,167]. Microbial culture is spread on an agar-gelled medium through an L-shaped glass, steel, or plastic spreader. However, the use of a spreader during the standard spread plating method might cause substantial harm to microbial inoculum, which can have an impact on colony-forming units on the surface of the medium [168]. There are alternative approaches that do not involve the use of an inoculum-spreader, known as spotting and tilt spreading (SATS) [168].

9. How to Find a New Bio Control Agent?

Two main techniques are frequently used to find new biocontrol agents. The first strategy entails plant-based or in silico indirect screening of microbial libraries for biocontrol abilities. According to the second method [169,170], organisms are isolated from the environment where the product application is anticipated to take place and then immediately tested for activity. Additionally, the development of NGS (next-generation sequencing) techniques has facilitated a quick shift to a direct functional approach in studies on biological pesticides. Utilizing a variety of bacterial and fungal biofertilizer strains in conjunction with each other can improve plant growth promotion [171,172]. Similar to this, microbes with bio-control abilities can work synergistically with other bio-control agents to exacerbate their resistance to plant diseases [173].

10. Use of Carriers

There are ample reports on the manufacture of *Trichoderma* products for marketing using common synthetic media such as cellulose, glucose, molasses, and soluble starch [174]. However, one of the primary causes of restricted utilization is the high price of these raw materials for the marketable manufacturing of biocontrol agents. Many studies have deployed inexpensive substrates like composted pith of coir and coffee waste [175–177], poultry manure and coffee waste [178], neem cake, degraded coffee pulp, cow dung, and coir pith [179], sorghum grain floor, sawdust, wheat bran, groundnut shell, molasses, farm produced waste, extract of biogas plant, dried cow dung, neem cake, talc, mushroom compost, fly ash, peat soil [180], fruit, vegetable, and crop wastes, poultry manure and FYM [181], rotten wheat grains, sugarcane baggase, fruit juice waste, and vegetable and fruit waste, for mass production of *Trichoderma* species [182]. Various inexpensive and readily available local organic media such as neem cake, coconut husk, and decayed pulp of coffee have been recommended for *Trichoderma* production on a large scale [179]. Mustafa et al. (2009) identified organic fertilizers such as neem cake, vermiculite, and mushroom composts as excellent carriers for *Trichoderma* mass multiplication [183]. Usage of neem cake enriched with a selected isolate of *Trichoderma* talcum powder is suggested for the management of coconut basal stem rot and areca nut and coconut stem bleeding. Over the last few years, due to the lack of high-quality neem cakes in the market and very expensive commercial formulations, farmers need potential biological control agents with pocket-friendly formulations for effective disease control. The production of acceptable, easy-to-prepare, and lucrative formulations has a key role in the accomplishment of biocontrol programs and agricultural sustainability.

11. Pilot Experiments

The formulation consists of an active ingredient in the form of a microbe or microbe-derived products in an appropriate carrier material (sterile or non-sterile) with additives that aid in the stability and protection of the microbial cells during storage, transit, and the target location. Choosing the right formulation is extremely important because it has the potential to make microorganisms successful or fail [184].

Formulation of the bioagents has a significant function in determining the role of application with high performance under field conditions. The majority of the formulation procedure involves combining bioagents with an appropriate carrier and/or additives that ensure ease of distribution, prolonged storage, and low cost. *Trichoderma* is frequently produced on a large scale using solid fermentation. A variety of low-cost cereal grains, including sorghum, millets, and ragi, can be used [185]. The grains are sterilized, hydrated, and inoculated with *Trichoderma* for 10 to 15 days. *Trichoderma* covers the grains with dark green spores, and these grains can be ground into a fine powder and applied to the seed surface, or they can be applied directly to the soil/ enriched FYM before the field application. This method is appropriate for small-scale production in cottage industries or at the level of the individual farmer. However, the drawbacks include lengthy production time and contaminant-prone products.

As aforementioned, formulations are of many types; they include dry products (such as granules, dust, and wettable powders), liquid products (such as emulsions, oil, and water; usually contain one but sometimes two strains of active ingredient), and microencapsulation. The efficacy of microbial inoculants largely depends on the type of formulation and the delivery technology that extends the shelf life for at least a few months, and in all cases, the PGP/antagonistic activity is retained [186]. The production cost also has to be considered and kept to a minimum while developing a microbial formulation. A good formulation should be easy to handle and apply so that it is delivered at the target site, protects the PGP microbes, and enhances its activity from harmful environmental factors under field conditions. A detailed review of different types of formulations, additives used, and PGP/antagonistic microbes used on various crops was reported by Nakkeeran et al. (2005) [187] and Bashan et al. (2014) [184]. It is understood that the major role of a formula-

tion is to provide a more suitable micro-environment that prevents the rapid decline of an introduced PGP microbe in the soil. BCAs can be formulated in many forms, although commercial biocontrol formulations conventionally come only in very few variations: powder, granules, or liquid forms [188].

The formulation of BCAs is a multistep process that begins immediately after the selection of the most promising BCA for the intended effect on a target pathos stem, and it consists of combining viable microorganisms with carriers and adjuvants to produce high-quality preparations of stable shelf-life and proven efficacy. From a technical point of view, this requires in-depth knowledge of the ecology and biology of the BCA, the pathosystem, the environment, and the application niche, along with considerations of the plant growth stage, inoculation techniques, and types of irrigation systems involved in the agri-food system [189].

12. Types of Formulations

As mentioned, several formulations (Figure 1) have been tested for pilot production of *Trichoderma* (Table 4). These can be differentiated based on their efficacy in enhancing the stability of *Trichoderma* cultures.

12.1. Liquid Formulations

Liquid formulations for biological control agents (BCAs) can vary in composition, but they typically involve microbial cultures or suspensions mixed with water, mineral or organic oils, polymers, or a combination of these components. Suspension concentrates, in the context of microbial inoculants, are typically made by introducing solid active ingredients (which can be either free or immobilized microbial cells) into water or an aqueous solution using traditional methods [186]. Liquid formulations for biopesticides are quite common. For instance, *P. fluorescens* was formulated in coconut water supplemented with glycerol or polyvinylpyrrolidone (PVP) as a suspension concentrate [190]. In a study conducted by Mbarga and colleagues in 2014, they formulated an oil dispersion that included soybean oil, an emulsifying-dispersing agent, a structural agent, and glucose, and this formulation contained conidia of *T. asperellum* [191]. In another research reported by Peeran et al. in 2014, water-in-oil emulsions of Ps. *fluorescens* were created using a mixture of water, glycerin, polyethylene glycol, Tween 20, and either coconut, rice bran, or castor oil [192]. Herrera et al. (2020) developed liquid formulations of *T. asperellum* TV190 using emulsified mineral or vegetable oils; this formulation showed greater viability of 37–43% (mineral) and 56–63% (vegetable) compared to the control (8–12%) [193]. A similar investigation was conducted by Patil et al. 2021 to check the efficacy of the liquid formulation of *T. asperellum* against the Fusarium wilt of chickpea [194]. The oil-based formulation was chosen because it maintains the organism in a physiologically dormant state. Three different oils, paraffin oil, soybean oil, and groundnut oil, were used as carrier material for the oil-based liquid formulation of *T. asperellum*. The population density of *T. asperellum* found in paraffin oil was 28.67×10^8 CFU/mL at 30 days, and it decreased to 18.00×10^8 CFU/mL after 180 days.

For the production of liquid formulations, low-cost growth media like sugarcane molasses and brewer's yeast have been used [15]. The benefit of this media is that it increases the production and quality of the biomass by permitting regulation of pH, temperature, and nutrients (among other biological factors), hence reducing contamination [195].

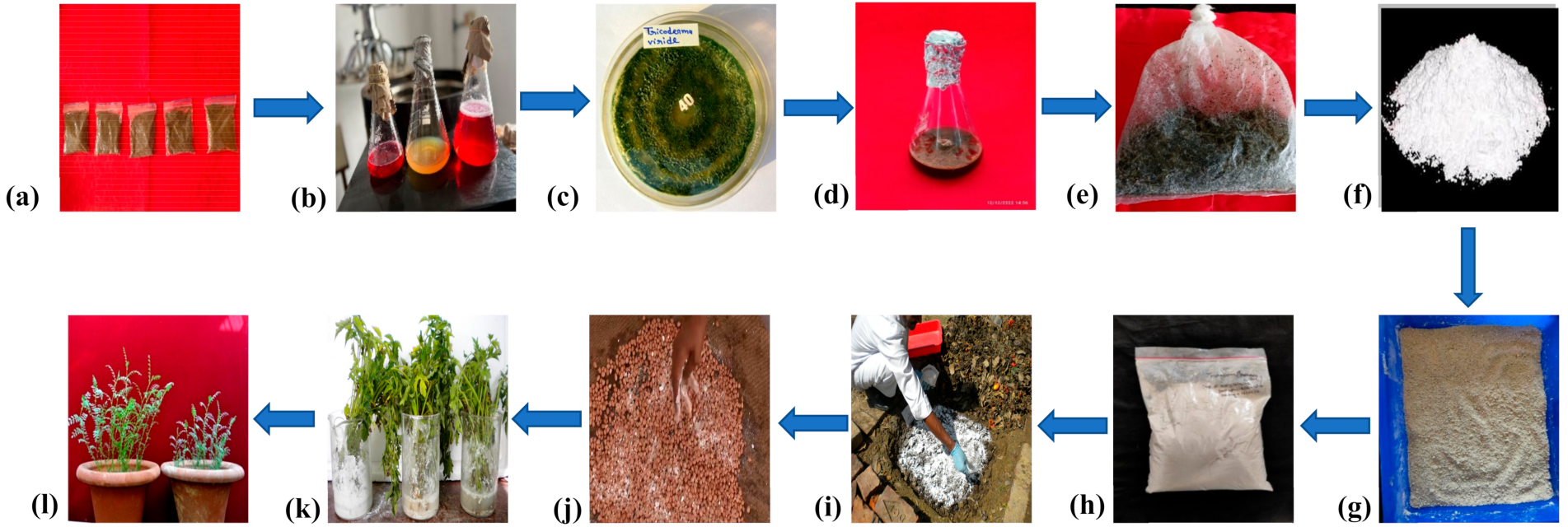

Figure 1. Procedure of *Trichoderma* formulation. (**a**) Rhizospheric soil. (**b**) Selective medium for isolation. (**c**) Pure culture. (**d**) Liquid fermentation. (**e**) Soil fermentation. (**f**) Talcum powder. (**g**) Mixing of *Trichoderma* with Talcum powder. (**h**) Packaging. (**i**) Mixing in FYM. (**j**) Seed priming. (**k**) Seedling treatment. (**l**) Pot application.

12.2. Solid Formulations

Another method to produce *Trichoderma* inoculum is the solid formulation or fermentation. Agricultural wastes, for example, wheat straw, sugarcane bagasse, sawdust, corn cob meal, and rice bran, are used alone or as a combined substrate or food base for the multiplication of *Trichoderma*. To provide a stable product with an extended shelf life, both liquid and solid formulations must be dried [196]. Such formulation is cost-effective for small-scale production, but it is immense since it requires a large area for preparation, inoculation, storage, drying, and milling. However, spray drying (cost-effective) is the technique for the large-scale production of microorganisms, including dried particles [197].

Solid fermentation is often used in the research laboratory in the mass production of *Trichoderma* spp. The fungus is grown in Petri plates, after which spores and other propagules are harvested and processed. The bulk production of biocontrol fungus employing a variety of low-cost agricultural wastes and bioproducts is another way of solid fermentation. Several low-cost cereal grains, including wheat, bajra, and sorghum, are used as a substrate. The grains are soaked in freshwater for 12 h, and extra water is removed the next day. The soaked grains are placed in heat-resistant polypropylene bags and are sterilized in an autoclave. The grains are then inoculated with *Trichoderma* culture and incubated for 10 to 15 days. *Trichoderma* covers the grains with a layer of dark green spores. These grains can either be utilized whole for supplementing FYM for soil treatments or finely ground to treat seeds [198].

12.3. Talc Based Formulation

Trichoderma viride formulations using talc as a base material were developed in India at the Agricultural University of Tamil Nadu for application on seeds and soil and seedlings [199]. The process involves culturing *Trichoderma* in a liquid medium, blending it with talcum powder in a 1:2 ratio, followed by drying it under shade until 8% moisture content. These talc-based formulations of *Trichoderma* can be stored for a period of 3 to 4 months. In India, it has gained popularity for its effectiveness in controlling various soil-borne diseases by treatment of seeds of different crops at a dosing range of 4 to 5 g/kg seed. Numerous non-government companies in India produce huge volumes of talc-based formulations for farmers. The annual requirement of *Trichoderma* in India is nearly 5000 metric tonnes, and this quantity would be sufficient to cover approximately 50% of the country's agricultural land [185].

Pradhan et al. (2022) prepared a talc-based formulation of *T. viride* and tested it against *Fusarium* wilt disease in chickpeas. The biocontrol potential of talc-based formulation was found effective in terms of seed and soil application and for reducing the incidence of wilt in chickpeas [200]. Sundaramoorthy and Balabaskar (2013) also developed the talc-based formulation of *T. harzinaum* to evaluate their biocontrol efficacy against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions [201]. The application of *Trichoderma* showed a minimum incidence (15.33%) of wilt disease.

12.4. Vermiculite–Wheat Bran-Based Formulation

For the preparation of *Trichoderma* vermiculite–wheat bran-based formulation, the fungus was cultivated in a molasses–yeast medium for 240 h. Wheat bran (3.3 g) and vermiculite (100 g) were sterilized for three days at 70 °C in an oven. This substrate was mixed with 14 mL of liquid culture and 17.5 mL of 0.05N HCl and allowed to dry in the shade before packaging [202].

12.5. Oil-Based Formulations

It is a type of liquid formulation where *Trichoderma* is mixed in an oil carrier. The process involves growing *Trichoderma* in a broth medium, followed by mixing it with an appropriate oil carrier. The oil carrier can be mineral oil, vegetable oil, coconut oil, or any other oil that is compatible with *Trichoderma* and provides stability to the formulations. Moreover, it offers several advantages: oil carrier acts as a protective barrier against

unfavorable conditions such as UV radiation and high temperature, which can reduce the efficacy and viability of *Trichoderma* formulations. Additionally, oil-based formulations can enhance the adherence capacity on the surface of seeds, plant leaves, etc. They also facilitate the slow release of *Trichoderma* in the applied area and increase the activity against the plant pathogens. Bacteria are removed with the help of surfactants in non-aqueous solvents such as gasoline distillates (diesel oil and mineral oil) and vegetable oil (peanut, etc.). This can create a stable emulsion when combined with water. When diluted in water, emulsifiable concentrates rapidly create a homogenous emulsion because they need a high concentration of oil-soluble emulsifying component. *Trichoderma* is presently being utilized as an antibiotic. The oil-based mixture should be accepted for foliar application in dry weather and must have a long storage life because the propagules covered with oil can remain alive on the crop plants, even in adverse conditions. Batta (2007) prepared an emulsion formulation of *T. harzianum,* which considerably reduced the postharvest fruit rot caused by *Botrytis cinerea*. The Project Directorate of Biological Control (PDBC) located in India has successfully developed a modified emulsion formulation of *T. harzianum* from local sources, extending its shelf life to 8 months. This innovative formulation has proven to be highly effective in protecting peanuts from soil-borne diseases [203].

12.6. Sodium Alginate Encapsulation of Trichoderma

An alginate-based formulation of *Trichoderma* refers to *Trichoderma* incorporated into an alginate matrix, which is a natural polymer derived from seaweed. *Trichoderma* spores are dissolved in a 1:4 ratio with a solution of 0.6 percent sodium alginate, which is then allowed to drip into a beaker containing 1.5 percent $CaCl_2$ for 30 min. The uniformly sized, 3 mm $CaCl_2$ beads are squeezed through a sterile muslin cloth and allowed to aseptically air dry. The *Trichoderma* beads with sodium alginate coating are placed in plastic bottles with sterile distilled water and maintained at room temperature [204].

12.7. Press Mud-Based Formulation

Press mud is an organic by-product generated from the sugar industry, which can serve as a potential substrate for the cultivation of *Trichoderma* [205]. To carry out the process, a 9-day-old culture of *Trichoderma* cultivated in potato broth medium is blended with 120 kg of mud and kept moist. The mixture is covered with burlap to allow airflow and trap moisture in the shade. The seed culture for the next growth gets ready in 25 days. Before the field application, it is mixed with 8 tons of mud and kept at a low temperature for 8 days.

12.8. Coffee Husk-Based Formulation

Sawant and Sawant (1996) used coffee husk as a substrate for the mass production of *Trichoderma*, which is a by-product of the coffee industry in Karnataka [178]. This formulation has been widely utilized in Karnataka and Kerala in treating *Phytophthora* foot rot of black pepper. Rosane et al. (2008) used several cost-effective solid substrates (rice, corn, and wheat bran) for the cultivation of *Trichoderma* on a large scale and found that wheat bran is one of the best substrates for the successful production of *Trichoderma* spores [206].

12.9. Banana Waste-Based Formulations

Banana waste is also a unique material for the preparation of *Trichoderma*-based solid formulations. Balasubramanian et al. (2008) developed a method for the cultivation of *Trichoderma* using banana waste as a substrate. This method involved urea rock phosphate, banana waste, and a consortium of fungal and bacterial biocontrol agents (*Bacillus polymixa*, *P. sajorcaju*, and *T. viride*) for the preparation of *Trichoderma* formulation [207]. A well is prepared from banana skins, pseudostems, and cores that are cut from 5 to 8 cm long. Five layers of various components are arranged in a prepared pit. One ton of banana waste, 5 kg of urea, 125 kg of rock phosphate, and 1 L of *B. polymixa*, *P. sajorcaju,* and *T. viride* are

all present in each layer. These wastes decompose for 45 days and are cultured to be used later in the field.

12.10. Dry Flowable Formulation

Dry flowable/water-dispersible granule formulations are safe for the environment and farmers [208]. Due to their granular structure, such preparations do not easily absorb moisture from the air. Encapsulation is an initial stage in the production of dry flowable formulations, as it is vital for enhancing the survival and efficacy of the formulation type.

As mentioned, encapsulation techniques have been used to establish encapsulated *trichoderma*. This can be achieved using a dry method [209] or modified alginate microbeads [210]. Another approach involves the development of a spray-dried flowable system based on polyacrylic acid, citric acid, and sodium bicarbonate, with polyvinyl alcohol as a binder and lecithin as a wetting agent [211].

12.11. Sawdust-Based Formulation

The application of sawdust as a carrier material is widely suggested as it is easily procurable and possesses a natural ability to retain a high level of organic matter and water-holding capacity compared to other carrier materials [212–214]. Arora et al. (2008) tested several substrates, and sawdust was found to be the most effective carrier for microbial growth [213].

A one-kilogram mixture consisting of sawdust, soil, and molasses is filled in heat-resistant polybags. These polybags are tightly sealed and sterilized at 15 kg/cm^2 pressure at 121 °C for 15 min. For *T. harzianum*, 10 mg chloramphenicol/kg material is added. Subsequently, the bags containing 1 kg autoclaved mixture were inoculated with a homogenized pure liquid culture of the biocontrol agents (5 mL/bag) using a sterilized needle and syringe. A small hole was made in the polybag to inoculate the culture and then resealed with cello tape to avoid contamination. Inoculated bags are placed in a BOD incubator at 28 ± 2 °C for 10–15 days. Throughout the incubation period, the bags are gently shaken to ensure that the biocontrol agent colonizes the material uniformly [215].

Table 4. Different substrates and their efficacy in *Trichoderma* biomass production.

S.No.	Substrate	Composition/Components	Efficacy	References
1	Cereals (wheat, moong, maize)	Grains (200 g + sugar (1%) + *Trichoderma harzianum*	12.96%	[216]
2	Vermicompost fortified with *Trichoderma*	Vermicompost + cereals + pulses	Reduction of 10.01%incidence of wilt in chili	[216]
3	Wheat seeds-based formulation	Grinded grain + sugar solution (1%) + *Trichoderma harzianum*	38 × 10^7 cfu/g	[217]
4	Cow dung enriched formulation	Decomposed cow dung + *Trichoderma* formulation	37.5 × 10^7 cfu/g	[217]
5	Talc-based formulation	Talcum powder + CMC + *Trichoderma* culture	37 × 10^7 cfu/g	[217]
6	Sorghum grain	Partial crushed grain + sugar (1%) solution + distilled water	6.1 × 10^4 cfu/g	[218]
7	Wood pellets-based formulation	Beech, fir, and chestnut + conidial suspensions of *T. atroviride* + distilled water + soy flour	Growth increase by ten-fold	[219]
8	Vermiculite–wheat bran-based formulation	Vermiculite (100 g) + wheat bran (33 g) + fermented *Trichoderma* biomass (20 g) + 0.05N HCl (175 mL)	-	[202]

Table 4. *Cont.*

S.No.	Substrate	Composition/Components	Efficacy	References
9	Vermiculite bentonite-based formulation	Oat (20 g) + bentonite (50 mL) + vermiculite + *T. harzianum* + water (60 mL)	Maintained cfu after 8 weeks	[220]
10	Coffee husk-based formulation	Coffee fruit skin decomposed with cow dung + poultry manure + *T. harzianum* suspension	9×10^{11} to 3×10^{12} cfu/g substrate	[178]
11	Oil-based formulation	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	More than 180 days	[221]
12	Pesta granules-based formulation	Wheat flour (100 g) + fermenter biomass + sterile water (52 mL)	Viable for a long time	[222]
13	Banana waste-based	Banana waste (chopped 5–6 cm length) + rock phosphate	Six months	[207,223]
14	*Trichoderma* sodium alginate encapsulation	*Trichoderma* suspension + sodium alginate (0.6%) solution + $CaCl_2$ (1.5%)	Viable for more than six years at room temperature	[163]
15	Wheat flour- kaolin	Wheat flour (80 gm) + kalolin (20 gm) + fermenter biomass (52 mL)	Few months	[224]
16	T2- liquid formulation (NIPHM medium)	Liquid formulation (NIPHM medium) *Trichoderma* filtrate (250 mL) + water (750 mL) + glycerol (3%)	-	[225]
17	*Trichoderma*-based compost activator	Fifty grams of soil + rice straw (5 g) + *Trichoderma* biomass (500 mg)	5.3×10^{10} cfu/g	[226]
18	Graphite and silica-based formulation	*B. subtilis* and *T. harzianum* + 5% graphite 80 mesh + 1% silica NPs	35–54% efficacy (in vitro)	[227]
19	Chitosan-PEG based formulation	Chitosan-PEG + *T. harzianum* spores + glacial acetic acid (0.1%)	More than six months	[228]
20	Rice powder-based formulation	Sterilized rice powder + dextrose + talc powder + *Trichoderma viride*	cfu/g 10×10^9 up to six months at room temperature	[229]
21	Dextrin-based formulation	*T. harzianum* filtrate (500 g) + Paraffin oil (500 mL) + CMC (0.2%) + chitosan (0.1%)	Efficacy 26.10%; 4.33×10^7 cfu/g) for six months	[230]
22	Oil-based liquid formulation	*T. asperellum* + paraffin oil	28.67×10^8 cfu/mL for 30 days	[231]
23	Glycerol based formulation	Molasses yeast extract (MYE) medium+ glycerol (3%) (V/V) +*T. harzianum* + Talc powder	Extended the shelf-life for 7 to 12 months	[232]
24	Paste formulation of *T. harzianum*	Starch (10%) + copper sulphate (20 ppm) + *T. harzianum*	11.6×10^{10} cfu/g for 120 days	[233]

13. Exemplary Properties of *Trichoderma* Formulation

For the development of a successful formulation of *Trichoderma*, a product should have the following attributes [234]: (i) formulation should have high rhizosphere competence with other existing microorganisms; (ii) formulation should possess highly competitive saprophytic ability; (iii) formulation should have the potential for disease management and also promotes plant growth; (iv) formulation should be easy to multiply as mass scale and safe to humans and the environment; and (v) formulation should be excellent in terms of action, compatible with other biocontrol agents/biofertilizers and tolerant to stresses such as desiccation, heat, and UV rays (Figure 2).

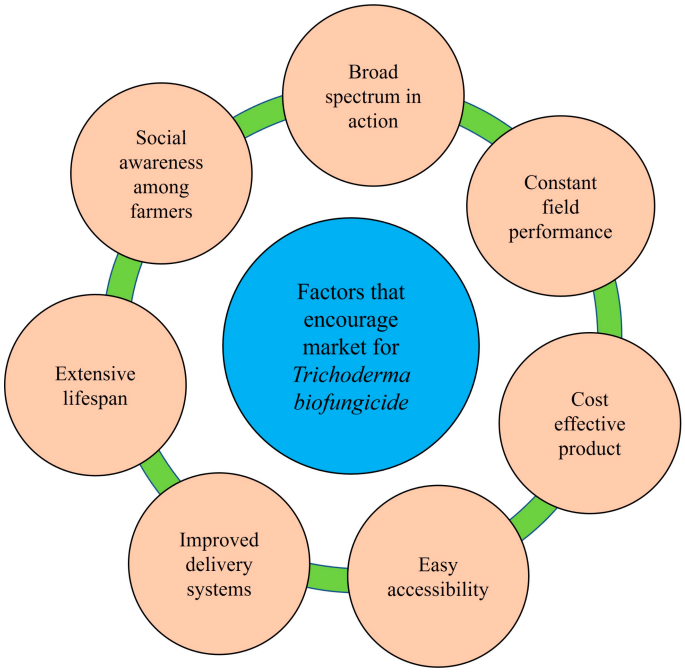

Figure 2. Pictorial representation of factors that encourage a market for the *Trichoderma* bio fungicide.

14. Quality Control Parameters

Before the recommendation of the final product (*Trichoderma* formulation) certain quality parameters are mandatory for successful application. They are: (i) colony forming units (CFUs) of a fresh product should be a minimum of 2×10^6 cfu/g or ml; (ii) shelf life of the formulation should be a minimum of 4–6 months; (iii) the size of talcum powder should be 500 microns, for the development of a formulation; (iv) the final product should be packed in standard white polythene bags; (v) in the final product, moisture should be less than 20%; (vi) additional contamination should not be more than $1 \times 10^4/^{mL}$ or g; and (vii) in the final product, the presence of other pathogens such as *Salmonella*, *Shigella*, and *Vibrio* should be eliminated.

The quality control parameters of any biopesticide established by the Central Insecticides Board (CIB) have certain lacuna with regard to the assessment of a potential biocontrol agent. It is expected that the Department of Agriculture (government) may appoint a central body for inspection of quality assurance, not only with regard to the viability of the propagules in the formulation but also with regard to their efficacy against plant pathogens. The authorized body must regularly assess the quality of the products in terms of these parameters to ensure that the farmers have access to higher-quality products.

15. Methods of Application of *Trichoderma*

Success in controlling plant diseases depends on the distribution method and establishment of *Trichoderma* in the active site. There are many ways to use *Trichoderma*, including seed biopriming, seedling root soaking, foliar spray, soil furrow application, soil soaking application, foliar application, and cloth wrapping.

15.1. Seed Treatment

Seed treatment with *Trichoderma* spp. is one of the precise, simple, and profitable approaches to delivering biocontrol agents for controlling soil and seed-borne pathogens [235]. In this method, a small amount of *Trichoderma* inoculum is applied uniformly on the outer surface of the seed just a few hours before the sowing. This method has been considered promising for effective inoculation of biocontrol agents on crop seeds of different sizes [236–239]. After coating the seed with the inoculum of the antagonist, spores germinate on the surface of the seed and inhabit the roots and rhizosphere of germinated

plantlets [240,241]. However, the effectiveness of *Trichoderma* seed treatment relies on its biocontrol properties and the capacity to multiply quickly in the rhizospheric zone of the seedling. Papavizas (1985) suggested that if the biocontrol inoculum multiplies around the site of application, it probably suppresses the pathogens, causing seed-rot and seedling diseases [15]. Lamichhane et al. (2022) revealed that seed treatment with biological products drastically enhanced the percentage of seedling emergence (91 ± 5%), seed germination (7 ± 6%), disease control (55 ± 1%), plant biomass (53 ± 5%), and crop harvest (21 ± 2%), in comparison to non-treated seeds [242]. The biological potency of *T. viride* talc-based formulation (1% w/w) was found effective against FOC (*Fusarium oxysproum* f.sp *ciceri*), which caused wilt of chickpea and also improved the physiological parameters of the crop [200]. In a study by Couto et al. (2021), wheat seeds were primed with two distinct strains of *Trichoderma*, *T. harzianum*, and *T. asperellum* (2×10^{12} CFU 100 kg^{-1} seed). Couto et al. (2021) revealed that antagonist boosts the percentage of seedling emergence, root and shoot lengths, and shoot dry matter under greenhouse conditions [243]. In another study, three antagonists, namely, T. koningii, T. viride, T. asperelum, and T. reseii, were used as bio slurry for seed treatment of peanut crops. All these three antagonists were found to be effective in various physiological parameters of plants, such as vegetative growth, percentage germination, production, and shoot and root mass of the crop [244]. Thus, multiple studies have demonstrated that *Trichoderma* seed treatment can improve seedling growth and yield in various cereals and vegetables, such as tomato, cucumber, and maize [245,246].

15.2. Seed Biopriming

Biopriming involves the treatment of seed with bioproducts that involves inoculation of seed with beneficial microbes followed by hydration [247]. In this process, the seed is treated with formulations of *Trichoderma* (6–10 gm/kg seed) and then incubated under moist and warm conditions prior to sowing for the emergence of radicals. During this process, germinated *Trichoderma* spores form a protective cover surrounding the seed's coat and defend the seeds from adverse conditions, as well as lead to rapid and uniform germination and high establishment of the crop (good harvest yield and quality) [248,249]. Another study also evaluated the efficacy of *T. asperellum* BHUT8 biopriming on the growth promotion of seedlings. The finding showed that the treated seeds grew faster than the untreated seeds. The treated plant exhibited a considerable increase in various parameters such as length, dry weight, fresh weight (root and shoot), and leaf count, as compared to the control [250]. Rice seeds bioprimed with *Trichoderma* strain exhibited high seed germination, total chlorophyll content, and crop harvest [251]. Degani and Dor (2021) studied the effect of two strains of *T. longibrachiatum* and *T. asperelloides* on the maize seeds and observed that primed seeds showed several positive effects, including improved yield, significant enhancement in growth parameters and quality of crop as well as effective control of *Magnaporthiopsis maydis*, transmitted through soil [252]. In another study, strains of *T. hebeiensis* and *T. erinaceum* were used for priming of rice seeds, and it was found to reduce the time for germination, improve the seedling vigor, leaf chlorophyll content, and the yield by controlling four major rice pathogens *R. solani*, *S. oryzae*, *S. rolfsii*, and *S. delphinii* [253].

15.3. Root Dipping

It is well established that biocontrol agents, especially *Trichoderma* spore or slurry, reduce disease severity and enhance seedling growth in various cereals and vegetables such as rice, brinjal, chili, tomato, and capsicum [254]. *Trichoderma* can be applied to plant roots by dipping them in a *Trichoderma* spore suspension/slurry prior to transplanting, and this has resulted in successful control of diseases in different crops such as rice, chili, cucumber, and tomato [255,256].

15.4. Foliar Spraying/Wound Dressing

The phyllosphere, or the surface of the leaves, experiences fluctuations in humidity, temperature, dew, wind, rain, and radiation, which can affect the water potential of microbes; hence, it is crucial to consider the microclimate and environmental factors when using biocontrol agents for foliar diseases. These factors can also vary between different leaves or parts of a plant, which can affect the concentration of nutrients in the plant and ultimately impact the survival and efficacy of biocontrol agents. Studies have shown that foliar spray of *Trichoderma viride* can reduce linseed blight in controlled conditions [257,258]. Additionally, a mixed formulation of two different species of *Trichoderma*, such as *T. harzianum*, *T. virens*, and *T. hamatum*, *T. viride*, has been observed to be impactful with reasonable shelf life for foliar sprays [259]. Therefore, when selecting biocontrol agents for foliar diseases, it is important to consider their tolerance to environmental factors and their ability to survive and thrive under different microclimates [260].

The ability of *Trichoderma* consortia to establish in the phyllosphere of blue Passiflora has been demonstrated. Through foliar application of a combination of two *Trichoderma* species, namely *T. asperellum* and *T. harzianum*, a synergistic effect was observed. This combination has led to notable enhancements in plant physiological aspects, including increased leaf size and quantity, augmented chloroplast count, and heightened plant antioxidant activity [261]. *Trichoderma* can be sprayed on plants to suppress foliar pathogens and improve plant health. Studies have shown that foliar spraying with *Trichoderma* can ameliorate growth and yield in several plants, including cucumber, pepper, and tomato [262–264]. In another study, foliar spray of *Trichoderma* has been shown to facilitate growth and development in plants as well as resistance to diseases [265]. The effect of *Trichoderma asperellum* foliar spray on tomato plant growth, quantity, and quality was investigated. The results demonstrated that foliar application appreciably enhanced the plant height, leaf area, fruit weight, and total soluble solids in tomatoes [266].

15.5. Seed Material Treatment

For seed material treatment, 8–10 g of *Trichoderma* formulation is suspended in one liter of water, and the suspension is kept for 30 min before the treatment of seed material (before sowing and transplanting) in sugarcane setts, turmeric ginger rhizomes, banana suckers, and potato tubers. The disease-suppressing ability of different strains of *Trichoderma* for the control of 'Panama disease' in bananas (caused by *F. oxysporum* f. sp. *cubense*) was also studied [267]. The results showed that *Trichoderma* spp. considerably reduced disease occurrence and severity in banana plants. The biocontrol capacity of *Trichoderma* spp. against dry rot of potato tubers during storage was also studied. The application of *Trichoderma* spp. to potato tubers before storage significantly retarded the incidence of dry rot [268,269].

15.6. Soil Application

Soil application with *Trichoderma* is a sustainable and effective strategy for improving soil health, promoting plant growth, and suppressing soil-borne plant pathogens. The specific amount of *Trichoderma* formulation, either talcum-based (1–2 kg) or liquid (500–1000 mL), can be added to 25–50 kg FYM. The mixture should be mixed and covered with sugarcane leaves/ jute bag/paddy straw and placed for a few weeks in the shade to allow *Trichoderma* multiplication. Moisture should be maintained for better multiplication, and the mixture should be mixed at an interval of 3–4 days before spreading in the field. It should be applied to the field at least 15 days prior to sowing of the crop. The well-decomposed FYM can be applied in either in-furrow or pots at sowing/transplanting time. This quantity is sufficient for one acre of land. This method of soil application ensures that *Trichoderma* is close to the plant roots, where it can have the most beneficial impact. Jeyalakshmi et al. (2013) revealed that soil application of *T. viride* (2.5 kg/ha) is successful for disease control and significantly enhances crop yield [270]. To assess the bioefficacy of the biocontrol agent, *T. harzianum* was applied to soil as suspension (50 mL/L) to control

root-rot pathogen affecting grapevine in a protected environment. This treatment declined 80% of infection of root-rot disease in grapevine infected by *Macrophomina phaseolina* [271]. The impact of *T. harzianum* on soil health and maize growth was noted. The finding showed that *Trichoderma harzianum* treatment considerably improved soil microbial diversity and increased the abundance of beneficial microorganisms [272,273], for example, nitrogen-fixing and phosphate-solubilizing bacteria (PSB). *Trichoderma*-treated soil also had more soil organic matter content and improved growth and yield of the crop, indicating improved soil function and overall plant performance. Wang et al. (2022) also examined the impact of *Trichoderma harzianum* on soil health as well as an antagonistic effect against *Fusarium* wilt in cucumber planting and showed that the *Trichoderma* treatment significantly retard the risk of Fusarium wilt of cucumber, and improved soil microbial diversity and activity [274]. The *Trichoderma*-treated soil also had higher soil enzyme activity and increased abundance of beneficial microorganisms, indicating enhanced soil health and decreased risk of soil-borne plant pathogens.

The synergism of *Trichoderma* and mycorrhiza consortia (*T. harzianum* and arbuscular mycorrhizae) were tested for soil health, colonization of root, and maize growth promotion in zinc-deficient soils. The finding showed that the combined application of both beneficial fungi (*T. harzianum* and arbuscular mycorrhizae) drastically improved soil health by increasing organic matter, enzyme activity, and microbial diversity in treated soil. *Trichoderma*-treated soil also had higher root colonization and improved maize growth and yield, indicating improved soil health and plant performance [275].

15.7. Cutting/Seedling Root Dip Application

Trichoderma application is a sustainable and environmentally friendly method that promotes plant growth and inhibits the growth of phytopathogens. In this application, 20–25 g of *Trichoderma* sp. (powder form) or 5–10 mL (liquid form) are mixed in 1 L of water for 30 min. The roots and cuttings of seedlings are immersed in the prepared suspension for 1/2 h and then immediately transplanted. This treatment can effectively control soil-borne diseases. A study investigated the impact of *T. harzianum* on growth parameters of olive cuttings. The results showed that *T. harzianum* deployment considerably enhanced the rooting percentage, root length, and shoot length of the cuttings. The *Trichoderma*-treated cuttings also had higher chlorophyll content and lower incidence of fungal pathogens, indicating improved plant health [276].

15.8. Nursery Bed Treatment

Nursery bed treatment with *Trichoderma* is an effective method to promote healthy seedlings by suppressing soil-borne pathogens and improving plant growth. In this method, 500-g *Trichoderma*-talcum-based formulation is mixed with 10–20 kg completely decomposed farm yard manure/vermicast. It should be applied in the evening time, and this quantity is sufficient for a one-acre area. Before its application, appropriate moisture is required for its better multiplication. A study confirmed that *Trichoderma*-enriched compost used as a nursery bed amendment considerably enhanced the yield and growth of tomato seedlings [277]. *Trichoderma*-enriched compost also enhanced the soil fertility and increased the soil enzyme activity, indicating improved soil health. In another study, the effect of nursery bed soil amendment with *Trichoderma* was evaluated on the yield and quality of seedlings in different crops such as brinjal, tomato, and chili. The findings demonstrated that the nursery bed treated with *Trichoderma* greatly enhanced seedling development and decreased the prevalence of soil-borne pathogens. The *Trichoderma* treatment also increased soil enzyme activity and nutrient availability, indicating improved soil health [278]. The biocontrol effect of nursery bed treatment with *Trichoderma* on the tomato seedlings was investigated. The results depicted that the *Trichoderma* treatment significantly improved seedling growth and increased the nutrient content of the seedlings [279]. The seedling also had higher chlorophyll content and reduced the incidence of damping-off disease, indicating improved plant health.

15.9. Soil Drenching

In this method, 1–2 kg *Trichoderma* formulations are suspended in 200 liters of fresh water and directly applied onto the soil around the base of the plant. This method introduces *Trichoderma* into the soil, which protects the crop from infection caused by damping off soil-borne pathogens. During the application, optimum soil moisture is maintained in the field. Studies have shown that soil drenching with *Trichoderma* can improve plant growth and yield in various crops, including maize, tomato, and pepper [280,281].

16. Other Applications of *Trichoderma*

The versatility of *Trichoderma* makes it possible to be widely used in various industries. This fungus provides the opportunity for their use in bioremediation, biodegradation of agricultural waste, wine-making, brewery industries, food additives, textile industry, paper, and pulp industry, and the production of bioethanol from farm waste. *Trichoderma* produces various lytic enzymes that can be used in the animal feed, alcohol, and brewing industries. Cellulase, hemicellulase, and pectinase produced by *Trichoderma* can be used to partially hydrolyze food walls to improve the nutritional value and digestion of food. This increases animal weight and milk production [282]. In another study, it has been reported that the paper industry may use *Trichoderma* for the modification of fiber properties and to reduce the amount of lignin in pulp [282]. Kaczmarek and Jedryczka (2011) have reported the potential of *Trichoderma* to control the pathogen *Leptosphaeria maculans* and *L. biglobosa,* which cause stem canker of *Brassica* [283].

Recently, it has been found that Trichoderma spp. have the ability to metabolize many kinds of pesticides and thus can facilitate bioremediation [284]. Vázquez et al. (2015) revealed that *T. harzianum* strain successfully detoxifies metsulfuron methyl, which is a type of sulfonylurea herbicide [285]. *Trichoderma* spp. are also effective natural decomposers that speed up the breakdown of organic materials. According to Amira et al. (2011), *Trichoderma* spp. considerably accelerate the decomposition of food and oil industry waste [286]. Combined application of *T. harzianum* and *Phanerochaete chrysosporium* in olive pomace compost could successfully break down its cellulose and lignin [287].

17. Major Challenges and Future Strategies on Biopesticides

Biofungicides are recognized as safe because they are target-specific, minimize the risk of ecological pollution, and eliminate the problems of resistance development in pathogens. The desire for safe, disease-free food is driving the popularity of biopesticides. Despite these advantages, the market for biopesticides is in its infancy, particularly in India. These challenges and limitations necessitate better strategies and technical solutions that eliminate the flaws of commercial biopesticides [288]. Biopesticide incorporation in the mainstream of crop production requires a better understanding of mechanisms to enhance their delivery systems against targeted plant pathogens, cost-effective product, consistent field performance, longer shelf-life, easily accessible, social awareness among farmers, and trouble-free registration and regulation policies [289,290] (Figure 2).

18. Constraints in the Development of *Trichoderma*-Based Biopesticide

There are certain constraints in the development of *Trichoderma*-based biopesticide: (i) lack of an appropriate screening protocol for selecting promising *Trichoderma* candidates, (ii) inadequate knowledge regarding the *Trichoderma* ecology and other phytopathogens pathogens, (iii) fermentation technological advancement, coupled with the implementation of large-scale production methods, (iv) inconsistent performance and a short storage life, (v) inadequate patent protection, (vi) registration costs are exorbitant, (vii) training, awareness, and education deficiencies, (viii) an absence of a comprehensive multidisciplinary approach, (ix) technological constraints, and (x) introduction and size of market [291]. Moreover, most of the serious obstacles against the development and commercialization of *Trichoderma*-based biopesticides is their poor shelf-life [292].

19. Future Prospects of *Trichoderma* Application

Trichoderma spp. is utilized as a biopesticide for the management of diseases in various crops. Its main role is not only limited to disease suppression and enhancing plant growth, but this fungus can also be helpful in the decomposition of agricultural residue or remediation of soil toxicity. Every day, increasing awareness of ecology, environment protection, sustainable economy, and interest in substitute sources of energy create a possibility of biofuel production from agricultural waste by the addition of CWDs like cellulases, hemicellulases, ligninolytic, etc., which are produced by *Trichoderma reesei* and this is known as second-generation biofuels. Therefore, *Trichoderma* can be used in various ways: it can be used in controlling plant diseases, in plant growth promotion, enrichment of the compost, as well as for providing an ecofriendly environment for farming systems. However, several new strains of *Trichoderma* have been isolated, but their genome sequencing and characterization have to be performed for further implementation. Furthermore, the application of "omics" technology should be introduced for accurate identification of new or existing species of *Trichoderma.* This technique can also be helpful for the detection of bioactive metabolites at genomic and transcriptomic levels [293]. Apart from this, further studies need to be conducted so as to understand how these biological products influence the environmental microbiome processes. Several reports are present which suggest the role of *Trichoderma* in the remediation of organic solvents [294], heavy metals [295], organo-phosphate insecticide [296], crude fossil oil, benzopyrene and naphthalene [297], Phenanthrene and pyrene [298], cyanide [299], etc., from the fresh water and soil. *T. viride* and *T. asperellum* can remove arsenic by in vitro biovolatilization, and *T. viride* may be successful for in vitro bioremediation of Cd and Pb. *Trichoderma* has been shown to support the uptake and translocation of nitrate and various toxic ions in the rhizosphere, thereby promoting the assimilation of metals and plant extracts [300]. In a study, *T. atroviride* has been reported to enhance the uptake and translocation of Cd, Zn, and Ni in mustard crops, while *T. harzianum* promoted the growth of *Salix fragilis* in soil contaminated with heavy metals [301]. *T. harzianum* can degrade the harmful compound potassium cyanide, promoting root growth and branching in *Pteris* fern, which is known as an arsenic hyperaccumulatory fern [302]. In addition, *Trichoderma* is also capable of biodegrading toxic organic pollutants such as cyanide, nitrate, phenols, polycyclic aromatic hydrocarbons (PAH), dichlorodiphenyltrichloroethane (DDT), and other artificial dyes, endosulfan, pentachlorophenol [2,303]. The immobilized biomass of *Trichoderma* BSCR02 in calcium alginate beads facilitates the biosorption of Cr(VI) [304]. Therefore, *Trichoderma* spp. can be utilized as a low-priced treatment for wastewater and heavy metal-contaminated water. However, it is clear that future bioremediation research will need to combine different approaches, including genetic, molecular, biochemical, ecological, and technological development. For better utilization of beneficial microorganisms, particularly new strains of *Trichoderma*, it is crucial to foster a deeper understanding of their biology. Genetic manipulation, mutagenesis, and fusion of protoplast in *Trichoderma* spp. shall open up new vistas for better performance and fixing the associated problems. Cultivation of *Trichoderma* to treat infected sites could materialize in the near future, as it can be used as an inexpensive biocontrol agent in many parts of the world.

Next-generation bioproducts also create a new option for sustainable agriculture in solving industry and university research problems. For example, identifying novel *Trichoderma* strains can help or promote the production of bioproducts or the use of bioenergy. Increased awareness about eco-friendly farming, where the outcomes should speak to the needs and concerns of farmers and industry partners. Farmers can be ready to implement this approach when there are short-term economic benefits and profits in agriculture [305]. Any biological plan must closely adhere to the aims of integrated disease management (IDM), such as ecofriendly, long viability, and social acceptance. These aims address the needs and challenges of the farmers, suppliers, and customers. Numerous beneficial compounds have already been isolated from *Trichoderma* spp., which is helpful to control plant diseases. Still, these findings could not be converted into active ingredients at the

commercial level. Experts from different fields should be involved in the development of any biopesticide. For example, for the commercialization of a potential biopesticide, an ideal team should be involved in the execution, and the researcher should know the status of the market, the chemistry of the product, analysis, and understanding of plant biology/pathology, microbes, pests, etc. There are several publications on novel features of *Trichoderma* strains. With this large collection, there is a need for additional high-quality genomes and depictions of the many parts of *Trichoderma.* Overall, this inclusive roadmap can provide us with direction for the upcoming production of bioproducts from *Trichoderma*. Simultaneously, future research should be centralized on field application of encapsulated formulation of *Trichoderma*, screening, identification of *Trichoderma* spp. for control of foliar diseases, longer shelf-life, consistency of field performance, risk assessment, which is one of the greatest obstacles in the registration process of *Trichoderma* formulation as well as optimization of the cost of formulation and their marketing.

In a nutshell, future studies should concentrate on the improvement and utilization of *Trichoderma* as an antagonist for disease control in crops. It is formulated for long storage life, performance on a farm, and suitability for unfavorable conditions. The production process in the state can be increased by the cooperation of various industries. Good practices include large-scale agricultural biocontrol technology demonstration, good management, fast registration, identification of suitable organisms for different soils and environments (temperature/low humidity/salt water), and combination of two or more agents to improve performance.

20. Conclusions

Globally, the use of chemical pesticides is severely constrained by rising environmental concerns and the legislation that results from them, necessitating the urgent development of safer and more ecologically friendly solutions. Microbial BCAs have become a potential method for helping plants resist infections. It is now generally acknowledged that formulations are crucial in assisting microbiological BCAs to travel the arduous route between laboratory testing and field application.

Microbial BCAs have traditionally been manufactured as liquids, wettable powders, and granules for use as foliar sprays, soil drenches, or seed treatments. However, combining sophisticated technology from other fields with knowledge of biocontrol formulations (such as spray drying and encapsulation) may result in the creation of more effective and reliable formulations. Technically speaking, this calls for a profound comprehension of the interactions that take place between the BCA, formulation components, and the environment, as well as a comprehension of practical applicability. Recommendations on efficacy evaluation and field testing should be regularly updated while developing sophisticated methodologies for the development and deployment of biocontrol formulations to help provide proof of efficacy and facilitate the commercialization of biopesticides. Commercialization of biocontrol products is steadily growing worldwide, but in the absence of effective regulations, penetration of biological products in the agricultural market is still slow, especially in the European Union, where the registration process is complex and time-consuming. Existing regulations of biopesticides vary from country to country, and requirements for registration are often similar to those of chemical substances. The global harmonization of specific biopesticide regulations would undoubtedly overcome these limitations and encourage the commercialization of microbial-based biopesticides worldwide. To date, the major steps towards this harmonization have been taken by various global agencies such as the Organization for Economic and Cooperative Development, International Organization for Biological Control, and EPPO, but success is limited.

Given that climate change may alter patterns of pest and disease introduction, unification of policymaking procedures is even more essential [306]. It has also been projected that if global warming keeps up, a number of phytopathogens may move to other regions where they would come into contact with new hosts. Similar to animals, plants may become less resistant to disease attacks as a result of climate change [306].

The containment of some pests and illnesses may not be possible in worst-case scenarios when chemicals have been outlawed and/or plants are more sensitive in the absence of efficient microbial-based biopesticides. It is also important to keep in mind that environmental conditions affect, no matter how effective BCAs are. The improvement of BCA manufacturing, formulation, and delivery should be the main focus of future research. In addition, simpler, universal rules and demonstration trials with high-quality products are essential to advancing the commercialization of biopesticides.

Trichoderma spp. is an important biocontrol agent as it can increase crop production through various activities such as plant growth promotion and enhanced nutrient uptake capacity by inhibiting pathogens. Nonetheless, fast research in this field needs to be carefully done to ensure its safe commercialization. Effective production methods are also essential to improve the commercialization of *Trichoderma*. The search for less expensive and alternative substrates, as well as ideal operation parameters to boost conidia production, is also needed. Thus, it is anticipated that consistent research towards highly effective biocontrol formulations for enhanced agricultural production will pay off, displace agricultural pesticides, and ensure sustainable agricultural productivity.

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