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 The Open Mycology Journal, **2014,** *8,* **(Suppl-1, M3) 58-70**

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Trichoderma- Foliar Pathogen Interactions

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Abstract: Among the group of ubiquitous soil inhabiting fungi, *Trichoderma* species are of considerable commercial importance due to their ability to suppress many plant pathogenic fungi. They have been widely studied and employed for management of root and seedling diseases of many crops since decades. In recent years they are also being utilized for minimising diseases of foliar plant parts, especially to supress grey mold caused due to infections of *Botrytis cinerea* and powdery mildews on a number of crops. They also express potential to control downy mildews and have improved the shelf life of grapes. Out of the eighty-nine species of *Trichoderma*, a number of species exhibit the bio-control activity. The destruction of host hyphae and protoplasm by the various lytic enzymes and toxic volatile and non-volatile compounds produced by these species are the major mechanism of bio-control; apart from deactivation of pathogen enzymes and induction of systemic resistance in host plants. However, significant differences in antagonistic potential among isolates even from the same species from the same niche is observed; and hence selection of a strain with wide host range and environment adaptability becomes a key issue for sustainable biological control.

Keywords: *Botrytis cinerea*, *Colletotrichum gloeosporioides*, fungicide, induced systemic resistance, mechanism, *Plasmopara viticola*, powdery mildew, *Trichoderma*.

1. THE GENUS *TRICHODERMA*

Trichoderma species are ubiquitous soil inhabiting fungi and are the anamorph stage of the genus *Hypocrea*. They are classified under the fungal division Ascomycota; order, Hypocreales; family, Hypocreaceae; genus, *Trichoderma*. Eighty-nine species of *Trichoderma* have been named based on molecular phylogenetic analyses [1]. *Trichoderma* species have the ability to suppress fungi belonging to many other genera, including some of those which are pathogenic to crop plants. This unique ability of *Trichoderma* is due to the production of extracellular lytic enzymes, non-volatile and volatile toxic metabolites, high competitive saprophytic ability, high proliferation rate, etc. as well as induction of systemic resistance in host plants. They are also the most studied and exploited group of biocontrol agents as they are present in most of the soils worldwide, easy to isolate, identify, multiply (Figs. **1** to **6**) and considered safe for plants and animals. Though *Trichoderma* spp. are used mainly as soil inoculants for the control of root diseases, their ability to control some of the foliar diseases like gray mold, powdery mildews and postharvest decays on different crop species are also well documented.

2. THE FOLIAR ENVIRONMENT

Unlike the rhizosphere, which is buffered in the biological, chemical and physical soil environment, the phyllosphere and the fructosphere are exposed to the harsh gaseous atmosphere and faces sharp fluctuations in temperature and surface wetness, vapour water pressure deficit, gases, air pollutants, wind, radiation, etc. This makes the foliar environment very different from the soil environment and the epiphytic microbial populations differ substantially from the rhizosphere populations. The rhizosphere is highly suitable for proliferation and activities of diverse microfloral species comprises of bacteria, filamentous fungi, actinomycetes, protozoa and algae. In contrast, the phyllosphere is colonised predominantly by bacteria and then yeasts, while the filamentous fungal species may be present mainly as spores [2]. These populations keep changing as a result of fluctuations in the physical, chemical and nutritional environment of the phyllosphere.

The phyllosphere is, thus, considered a difficult site for biological control as the applied biocontrol agent should be able to establish, proliferate and retain its antagonistic potential in the harsher and frequently fluctuating foliar environment. These environmental effects are minimized in the controlled conditions of poly houses and many studies on biological control of fruit and leaf diseases have focused in polyhouse grown crops. However, research has shown that it is possible to achieve a significant level of biological control in field, too, as in the case of gray mold of grapes in vineyards. In this article an attempt has been made to review the work on the interactions of *Trichoderma* with foliar pathogens.

3. CONTROL OF FOLIAR DISEASES BY *TRICHODERMA*

3.1. *Botrytis* Blight or Gray Mold Disease

Gray mold is caused by the ubiquitous, necrotrophic fungus *Botrytis cinerea*. It attacks leaves, flowers and fruits

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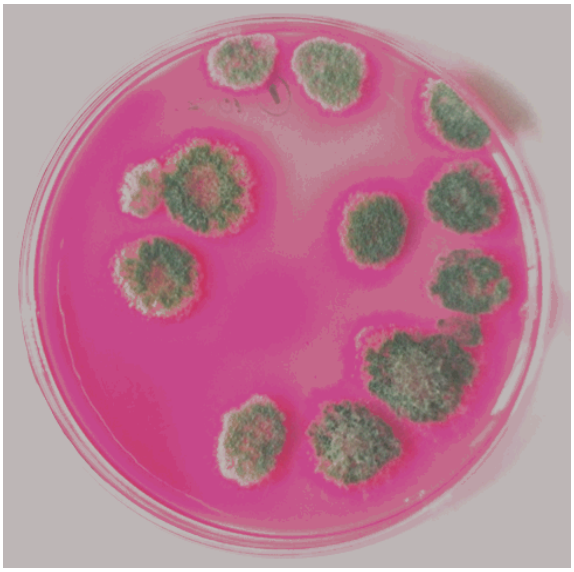

Fig. (1). *Trichoderma* colonies growing on semi-selective medium.

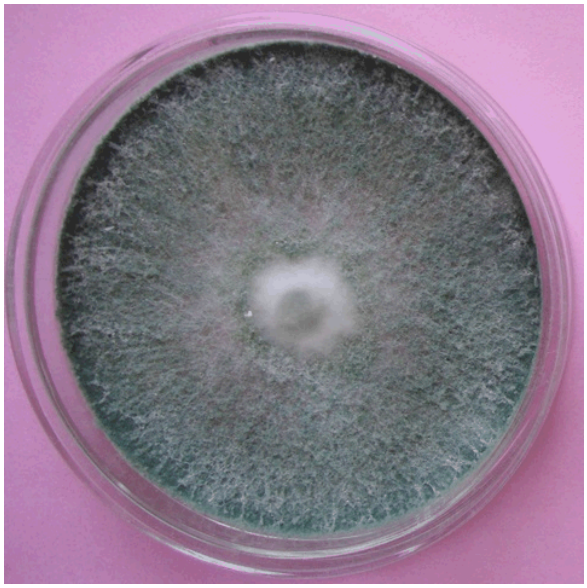

Fig. (2). *Trichoderma* growth on potato dextrose agar medium.

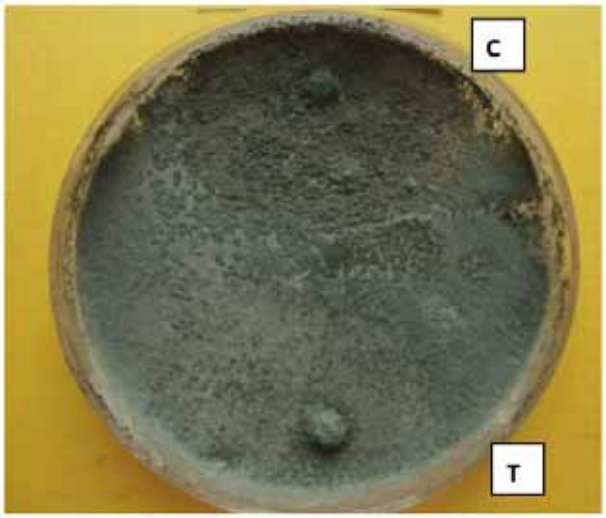

Fig. (3). *T. koningii* (T) overgrowing on *Colletotrichum gloeosporioides* (C) colony.

Fig. (4). Phialids and conidia of *T. koningi.*

Fig. (5). Multiplication of *Trichoderma* on composted sterilized coffee husk in pp bags.

The photo used in Fig. (**5**) is by the same author which was used in a symposium poster presented in National symposium on Horticultural Biotechnology, Bangalore, 28-30th October 1996.

The title of the poster was: Sawant, Indu S. and S.D. Sawant. 1996. Commercial production of *T. harzianum* on coffee wastes- prospects and problems.

New Agriculturist: http://www.new-ag.info/en/focus/focusItem.php?a=1178

Fig. (6). Multiplication of *Trichoderma* on potato dextrose broth in used brandy bottles.

of many field and poly house grown crops like grape, strawberry, tomato, cucumber, beans, onion, rose, gerbera, begonia, carnation, chrysanthemum, geranium, marigold, petunia, etc. Among the different species and isolates of *Trichoderma* evaluated worldwide for control of foliar diseases, one of the successful examples is of *T. harzianum* Rifai strain T39, which was initially selected and commercialized for the control of *Botrytis* diseases [3]. *T. harzianum* strain T39, was isolated from the natural surface microflora of cucumber fruit [4]. It is thought that this strain acts by competing with the pathogen for nutrients and space on the plant surface and thus inhibits its pathogenicity process. Subsequent studies have shown that it also induces local and systemic resistance in plants, and suppresses enzymes involved in pathogenesis. The commercial product is available under the name Trichodex WP (Makhteshim Chemical Works) containing fungal mycelium and conidia at minimum $1x10^9$ per gram cfu (colony forming units) of T39 isolate [5]. The product has been tested successfully on various crops for control of grey mold, powdery mildew, leaf spots etc.

Probably the most extensive evaluation of the *T. harzianum* T39 formulation was done for control of grey mold disease of grapes. The product was evaluated under diverse commercial conditions in one hundred and thirty three field studies conducted on thirty four grape cultivars in nineteen countries over a seven year period [6]. Overall results showed that T39 could provide 36.3% control of grey mold as compared to 52.3% control obtained by chemical treatments [6]. *Trichoderma* could establish well on grape berries as seen by the high populations of 4.5×10^5 cfu per berry on treated grapes as compared to 400 to 2000 on untreated grapes [7]. As *Botrytis* mainly infects grapes during flowering and then remains as latent infection till near veraison; five *Trichoderma* applications throughout bloom and fruit development or two late season applications were sufficient to control the disease [8]. It was also possible to minimize the overwintering inoculum of *B. cinerea* by late season spray applications of selected cold tolerant strains of *Trichoderma* species [9].

Trichoderma has also been used for the biocontrol of *Botrytis cinerea* on strawberries [10, 11]. However as strawberry flowering is a continuous process, frequent applications of T39 at 2 day intervals were needed which was not economical. A novel method of dissemination of *Trichoderma* to strawberry flowers was devised utilizing bees which regularly visit the strawberry flowers for collection of pollen. The *Trichoderma* spores were kept near the exit passage in the bee boxes in such a way that the legs and other body parts of the bees get dusted by the spores [12, 13]. Each bee carried about 1×10^5 cfu of *T. harzianum* on its body parts of which about half were carried on its legs; which got deposited on the strawberry flowers when the bees visited them for collection of nectar. This method of delivery deposited less number of cfus on the flowers than when *T. harzianum* was applied as spray, but it resulted in better disease control. These plots yielded more than even those plots which were treated with fungicides. The bees could effectively disseminate *T. harzianum* upto a distance of 200 m [13]. Similarly, *T. harzianum* T39 (Trichodex) at 0.2 g per l could also effectively control gray mold in greenhouse grown tomato and cucumber [14].

These studies have brought out the commercial feasibility of biological control of grey mold, with *Trichoderma* applications alone or in alternation with fungicide applications. This method would considerably minimize the use of chemical fungicides in fruits and may help to increase food safety.

3.2. Powdery Mildews

In cucumber, *T. harzianum* T39 (25% powder of Trichodex) at 0.2% concentration applied as foliar spray or as soil drench as a 100-ml dose in a 10 l container, could provide effective control of powdery mildew [15]. The disease control was better when the preparation was applied as soil drench than as spray, indicating that T39 induces systemic resistance in cucumber against powdery mildew apart from direct parasitism or antimicrobial action on the pathogen. The authors showed that the biocontrol agent gave more than 75% to almost cent percent disease control on young leaves but its biocontrol efficacy declined as the leaves grew older indicating that the age of the leaf has an impact on disease control efficacy of T39. Further, the control was more effective under low disease pressure conditions as compared to under high disease pressure conditions. Studies have also indicated that epiphytic *Trichoderma* spp. isolated from dogwood plants growing in wild, where the incidence of the disease was low, had the ability to reduce the severity of powdery mildew on dogwood under pot test conditions [16]. In another study it was shown that different species of *Trichoderma* viz. *T. viride, T. harzianum, T. hamatum, T. longiforum* and *T. koningi* could effectively control powdery mildew on cluster bean [17].

The ability of culture filtrates of *T. harzianum* and *T. viride* to control powdery mildew disease in mulberry by more than 60%, on par to that provided by carbendazim, was shown to be due to its ability to inhibit conidial germination of the pathogen *Phyllactinia corylea* by almost 50% [18]. In squash also application of culture filtrates of *T. viride* and *T. harzianum*, either alone or mixed with fungicide in equal proportions, could provide more than 60% control of powdery mildew under field conditions in two year trials [19]. The disease control provided by the mixture was also significantly higher than that obtained when the fungicide was used alone.

These studies indicate to the possibility of inducing the defense mechanism of the plants against powdery mildew disease by soil application of efficient strains of *Trichoderma* and of further control of the disease by foliar applications, which should be sufficiently effective under low disease pressure conditions. When the disease pressures are high and if the disease is not controlled by the above bio-control method, the *Trichoderma* sprays can be alternated with or used in combination with safe fungicides disease, as practiced for grey mold control.

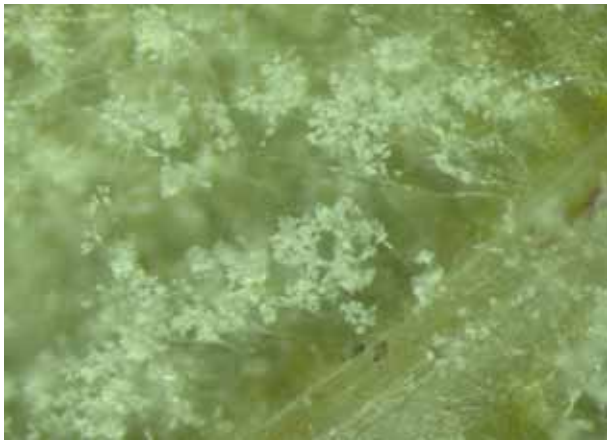

Fig. (7). Abundant sporangial growth of *P. viticola* on naturally infected grape leaves.

Fig. (8). Suppression of sporangial growth of *P. viticola* by *Trichoderma*.

Fig. (9). Colonisation of necrotic leaf areas by *Trichoderma.*

3.3. Downy Mildew

Two to three preventive sprays of *T. harzianum* T39 could also protect grapevines against downy mildew infections in a greenhouse [20]. The antagonist did not inhibit sporangial germination or parasitize *P. viticola,* the pathogen of downy mildew. But when T39 was selectively applied on few leaves or on leaves on one side of the plant, the treated as well as the untreated leaves showed resistance to the disease, indicating that T39 acts by eliciting an induced systemic reaction in grapevines against downy mildew as was observed in cucumber against powdery mildew disease [15]. Transcriptional analysis revealed that T39 influenced the expression of genes involved in recognition of the pathogen as well as those which regulate/trigger the plant defense mechanisms [21].

In ongoing studies at this Centre, it was observed that *Trichoderma* species could suppress growth of *Plasmopara viticola* and *Erysiphe necator* on naturally infected grape leaves (Figs. **7-9**) (Sawant *et al*., unpublished data). Grape downy mildew pathogen *P. viticola* infects grapevines

Fig. (10). Reduction of anthracnose infection on grape leaves by *Trichoderma.* Treated leaf (right) and untreated control (left).

through the stomata and post-infection the sporangiophores also emerge though the stomata. In grapevine leaves, the stomata are present only on the lower (abaxial) leaf surface, thus the fungicide applications, especially those with contact action alone are not very effective as the sprays do not completely cover the entire lower leaf surface. On the other hand *Trichoderma* can grow and proliferate on healthy leaf surfaces, and it sporulates profusely on the downy mildew infected chlorotic/necrotic leaf areas. These spores will be easily dispersed by air currents and rain water to the inner canopy, too, and provide protection to that part of the foliage which could not be covered by the fungicide spray.

Further studies are required to identify *Trichoderma* isolates which will be capable of eliciting an ISR effect in grapevines against downy mildew as well as directly inhibiting or parasitizing the pathogen. Such antagonistic *Trichoderma* isolates may also be used in integration with safer fungicides to enhance the disease control in vineyards.

3.4. Anthracnose

Antagonism and parasitism of many *Colletotrichum* species by different species of *Trichoderma* and their potential use for biological control of anthracnose disease is reported in various crops [22, 23] including potential for control of *C. gloeosporioides* on brambutan [24] and grapes [25]. *Trichoderma* species could also control anthracnose infections on grapevine foliage (Fig. **10**) caused by *C. gloeosporioides* in vineyard [26].

3.5. Other Foliar Diseases

In tomato, *T. harzianum* T39 could effectively control leaf mold (*Cladosporium fulvum*) in tomato and white mold (*Sclerotinia sclerotiorum*) in cucumber growing in greenhouses [14]. Postharvest treatment with *T. harzianum* (TrH 40) could control stem end rot, anthracnose and brown spot of rambutan fruits [24]. Further, the colour and post-harvest quality of the fruits was better in the *T. harzianum* treated fruits. Control of *Phytophthora* induced black pod disease of cocoa was achieved by fortnightly sprays of *T. asperellum* isolated from soil [27]. Interestingly, isolates which provided better disease control in the beginning of the season were not so effective at the end of the season and *vice versa*. *Cladosporium herbarum* which is responsible for causing verrucose of passion fruit could be parasitized by *T. harzianum, T. viride* and *T. koningii* [28]. These isolates also produced toxic volatile and non-volatile metabolites as well as cellulose, pectinase and amylase enzymes.

3.6. Postharvest Diseases

Pre-harvest spray application of *T. harzianum* 5R was found effective for management of postharvest decay in table grapes. Two sprays at 5×10^6 spores/ml applied at 20 and 2 days before harvest could prevent postharvest decay in grapes stored for short durations at ambient temperatures [29]. There was less decay in *T. harzianum* 5R treated grapes than in non-treated grapes which were stored with or without sodium metabisulphite (available commercially as grape guard sheets, which are a source of in-package sulphur dioxide generation) (Fig. **11**). Furthermore, grapes treated with *T. harzianum* 5R retained their freshness for longer duration as compared to non-treated grapes.

Similarly, the shelf-life of table grapes which were stored for long duration at low temperature was enhanced by two pre-harvest sprays of *T. harzianum* 5R at 1×10^6 spores/ml [30]. The grapes treated with 5R could be safely packed with a lower than recommended dose of sodium metabisulphite per box without fear of increased decay associated with the lower dose of the generator sheet. Further, in these grapes the sulphur-dioxide injury observed at the recommended dose of the sheet was also not seen. The mechanism by which *T. harzianum* 5R could delay the browning of rachis and pedicles and maintain berry freshness (Fig. **12**) enhancing the shelf life needs to be studied.

An antagonistic isolate of *T. harzianum* (TrH 40) isolated from rambutan orchards could effectively reduce the severity of postharvest stem end rot, anthracnose and brown spots caused by *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* in this crop [31]. Similarly, an antagonistic *T. asperellum* isolate was identified for control of black rot disease in pineapples caused by *Thielaviopsis paradoxa* [32].

In another study, it was shown that the shelf-life of various kinds of fresh fruits is reduced due to postharvest decay caused by fungal pathogens like *Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum* and postharvest treatment with a *T. harzianum* formulation could minimize the decay and enhance the shelf-life [33].

Fig. (11). Control of postharvest decay by *Trichoderma* in grapes stored for short duration at ambient temperatures.
The photo used in Fig. (**11**) is by the same author which was used in the following book chapter.
Sawant, S. D. and Sawant, Indu S. 2003. Enhancement in shelf life and quality of grapes by effective management of plant diseases and other cultural practices - success story of Maharashtra. In. Singh D.P (Ed.) Implications of Plant Diseases on Produce Quality. Kalyani Pubishers, Ludhiana. pp. 33-41 (198p).

Fig. (12). Control of postharvest decay by *Trichoderma* in grapes stored for long duration at zero degree temperature.
The photo used in Fig. (**12**) is by the same author which was used in:
"4.1 Mass multiplication of *Trichoderma harzianum* 5R. In. Commercialiazation of Horticultural Technologies. ICAR, Krishi Bhavan, New Delhi. p. 36.

3.7. Improved Plant Growth

Trichoderma species also have beneficial effects on plants. Application of *T. hamatum* 382 (T382) to potting mixture increased plant vigour of both susceptible and highly susceptible cultivars of rhododendron, even though it did not suppress *Phytophthora* die-back in the highly susceptible cultivars [34].

4. MECHANISM OF BIOCONTROL

The mechanisms involved in biocontrol of foliar pathogens by *Trichoderma* species are the same as those involved in the control of root pathogens i.e. mycoparasitism, antibiosis and competition for space and nutrients, induction of systemic resistance, etc. It is possible that more than one mechanism is involved in a host-antagonist-pathogen interaction. For example, the suppression of gray mold by *T. harzianum* T39 was related to the competition of resources, inhibition of enzymes involved in pathogenesis, and induced systemic resistance [35], but antibiosis and mycoparasitism were not involved in the biocontrol process.

4.1. Mycoparasitism

Species of *Trichoderma* produce a number of enzymes which hydrolyse the polysaccharides, cellulose, β-glucans and chitin present in the cell walls of the plant pathogenic fungi [36] and aid the mycoparasitise to penetrate the cells. These enzymes include both endo- and exo-chitinases which are effective against the fungi containing chitin in their cell walls. Most of these enzymes are induced in the presence of substrates. Further, the levels of enzymes viz. chitinase, N-acetylglucosaminidase, β-1,3-glucanase, protease, cellulose, endoglucanase, glucosidase, amylase were shown to be increased in presence of substrates [37]. *T. harzianum* isolate 1051 produced a novel protease which was biologically active against *Crinipellis perniciosa*, the causal agent of witches' broom [38].

4.2. Antimicrobial Chemicals

Trichoderma species are producers of various volatile and non-volatile antimicrobial compounds which are active against a range of microorganisms [39]. Though the inhibitory effect against many root pathogens is well

documented, there are a few reports on their effect on foliar pathogens. The volatile metabolites produced by isolates of *T. harzianum*, *T. virens*, *T. viride*, *T. reesei* and *T. saturnisporum* were inhibitory to *Colletotrichum capsici* [40, 41]. In another study, all thirty four *Trichoderma* isolates produced volatile metabolites which were toxic to *C. gloeosporioide* [26]. These isolates belonged to seven *Trichoderma* species viz. *T. harzianum*, *T. viride*, *T. koningii*, *T. pseudokoningii*, *T. hamatum*, *T. asperellum* and *T. lectea* and were collected from different geographical locations of India.

4.3. Suppression of Enzymes Produced by the Pathogen

Some isolates of *Trichoderma*, including *T. harzianum* T39 have the ability to deactivate or minimize the activities of enzymes involved in pathogenesis by *B. cinerea* viz. endo-and exo-polygalacturonases, pectin methyl esterase, pectate lyase, and cutin esterase, etc [42-44]. However, it had no effect on the production of carboxymethyl esterase and carboxymethyl cellulose [42-44] by breaking them into peptide chains or constituent amino acids.

A *T. harzianum* isolate could also reduce the secretion of endo-polygalacturonase by *Alternaria alternata* [45]. Interestingly, the authors observed that the secretion of the enzyme was also slightly inhibited in the presence of GA_3, IAA or BAP bio-regulators.

4.4. Supression of Sporulation or Conidial Germination of Pathogen

Trichoderma spp. produce chitinolytic enzymes which may inhibit the infection process at pre-infection stage, e.g. endochitinase and chitobiosidase inhibited the spore germination and germ tube elongation of fungi which contain chitin in their cell wall, viz. *Uncinula necator*, *Botrytis cinerea*, *Ustilago avenae*, *Fusarium solani*, but did not inhibit sporangial germination of *Pythium ultimum*, which has no chitin in its walls [46]. Similarly, a low level of inhibition in spore germination was observed in fungi which contain a low level of chitin in their cell walls such as the yeast *Saccharomyces cerevisiae*. Isolates of *Trichoderma* and *Gliocladium* can also suppress sporulation of *B. cinerea* on strawberry [47] and would help in reducing inoculum load.

4.5. Induced Systemic Resistance

Trichoderma isolates are also known to induce systemic resistance (ISR) in plants against fungal diseases. Apart from parasitism, *T. harzianum* T39 was also shown to be an elicitor of systemic resistance in plants as soil applications of live spores in the root zone or even the application of dead cells could provide reduction in foliar diseases including powdery mildew [35]. Further, even dead cells of *T. harzianum* T39 were partially effective in controlling gray mold disease. However, in grape leaves only the spray application of *T. harzianum* T39 elicited a lateral and acropetal induced systemic reaction against *P. viticola* and root drenching was ineffective in inducing systemic resistance [20]. The authors had observed the ISR effects on the leaves present on the same shoot as those where the treatments were applied, but had not studied as the effects on leaves present on other shoots of the grapevine. The response decreased as the interval between application of *Trichoderma* and the pathogen challenge was increased.

They further observed that treatment with *T. harzianum* T39 increased the expression of defense-related genes [48] which induced resistance in the plants. However, this effect was more pronounced in tissues closer to the site of antagonist application than in tissues which were distant, and this was reflected in a higher disease control near the site of application as compared sites which were distant. Interestingly, priming of defense gene expression was greater in plants treated with *T. harzianum* T39 than in those plants which were treated with the chemical ISR inducer, benzothiadiazole. Further, benzothiadiazole adversely affected the grapevine vegetative growth parameters and also chlorophyll content; while no such adverse effects were seen in plants treated with T39. In contrast to the activation of salicylic acid plant defense pathway by the chemical inducer, T39 induced the jasmonic acid plant defense pathway.

Addition of conidia of *T. hamatum* 382 (T382) in the form of dry powder to the potting mixture to a final count of 2×10^5 cfu per g dry weight of the mixture, minimized the *Botrytis* infections on greenhouse grown begonia plants [49]. The reduction in blight severity in T382 treatment was at par to the reduction observed in plants treated with chlorothalonil at weekly intervals. Further, the vegetative growth and over all marketability of T382 inoculated plants was higher as compared to the uninoculated control or chlorothalonil treaed plants. Some other studies suggest that the ISR effect is more apparent in compost amended medium, probably it supports proliferation of the introduced organism; and in cultivars which are not highly susceptible to the disease [34], even though it may induce other effects like enhanced plant vigour in the highly susceptible cultivar, too.

Further, *Phytophthora* infections in roots and crowns of cucumber were significantly reduced by adding T382 into the potting mixture [50]. The ISR is attributed to an increase in the activities of the defense enzymes, peroxidase and chitinase in the leaves of cucumber [51].

4.6. Competition for Resources

T. harzianum T39 suppression of gray mold was proposed to be due to the antagonist competing with the pathogen for resources which resulted in delayed germination of conidia and subsequent growth of the germ tube. Furthermore, timely application of *Trichoderma* allowed it to colonize the senescing floral tissues before *Botrytis* and helped to reduce infections [52]. One of the successful examples of biological control in above ground plant parts is the control of silver leaf disease by application of *Trichoderma* to the pruning wounds through pruning shears [53] which are able to parasitize the tissues before the pathogen can attack.

Fig. (13). Efficacy of *T. harzianum* 5R in controlling anthracnose infection caused by carbendazim resistant strain of *C. gloeosporioides* on leaves first treated with the fungicide/5R and then inoculated with the pathogen (L); or first inoculated with the pathogen and then treated with the fungicide/5R (R).

5. INTEGRATION WITH FUNGICIDES

i. For Management of Diseases

T. harzianum could safely be used in alternation with diethofencarb plus carbendazim, or as tank mix with iprodione in the table and wine grape vineyards providing 64-68% reduction in postharvest rot caused by *Botrytis cinerea* [7]. *Trichoderma* applications could be used effectively to substitute few fungicide applications, or it could be used with half-rates of iprodione, thus decreasing overall fungicide use in vineyards [8].

The chitinolytic and glucanolytic enzymes produced by *Trichoderma harzianum* enhanced the antifungal properties of fungitoxic compounds viz. gliotoxin, flusilazole, miconazole, captan and benomyl as evident by *Botrytis cinerea* spore germination bioassay [54]. The enhancement was synergistic as the EC_{50} of the mixtures were much lower than that of the enzymes or the fungitoxic compounds alone. As would be expected, the level of synergism appeared to be higher when the enzymes were combined with compounds acting on the membrane structure as compared with those compounds having multiple or cytoplasmic sites of action.

ii. For Management of Fungicide Resistant Strains of Pathogens

A *T. harzianum* isolate could parasitize carbendazim resistant isolates of *C. gloeosporioides* and could suppress development of symptoms in artificially inoculated Thompson Seedless leaves [55]. The supression was on par to that obtained by application of 10000 ppm carbendazim (Fig. **13**). Integration of efficient isolates of *Trichoderma* in an IPM programme may prove useful in management of fungicide resistant strains of pathogens.

6. ESTABLISHMENT – POST INOCULATION

The establishment and proliferation of *Trichoderma* on foliage will vary according to the plant species, prevailing environmental condition and the *Trichoderma* isolate. The populations will also be affected by the age of the leaves and nutrient status of the plants [56]. Other factors which may affect the populations are interactions of *Trichoderma* with the dominant leaf micro-flora, the type of formulation used and other agricultural interventions. The proliferation of the introduced *Trichoderma* spp. on the phyllosphere results in a change in the biological equilibrium existing on the plant surfaces due to competition for nutrients or space. This competition results in an increase or decrease in the natural populations of bacteria, yeasts and filamentous fungi, pre-existing on the phylloplane [57] and would affect the survival of the introduced *Trichoderma* spp. Temperature and RH requirements of the particular *Trichoderma* isolate would also affect its survival. Fungicide residues on leaves also affect the survival and proliferation of *Trichoderma* isolates (Sawant *et al.*, unpublished data).

Thus, different post-application population dynamics on leaf and fruits have been reported by different workers. The populations of *T. harzianum* on grapes treated at 0.5 to 1.0 g/1 were 4.5×10^5 per grape berry compared with 400–2000 per berry on untreated bunches [7]. Under the tropical climate of India, two foliar applications of an aqueous suspension of *T. harzianum* 5R containing 1×10^6 spores/ml applied at 20 and 2 days before harvest on grapes, resulted in about 120 cfu/berry [30]. When the same isolate was applied on grape foliage as an aqueous suspension containing 5×10^6 spores/ml, the initial counts were about 1×10^5 per cm^2, which reduced to 4000 cfu per cm^2 after 5 days of application and further to 260 cfu per cm^2 after 15 days of application [26]. In an interesting study on survival of four *Trichoderma*

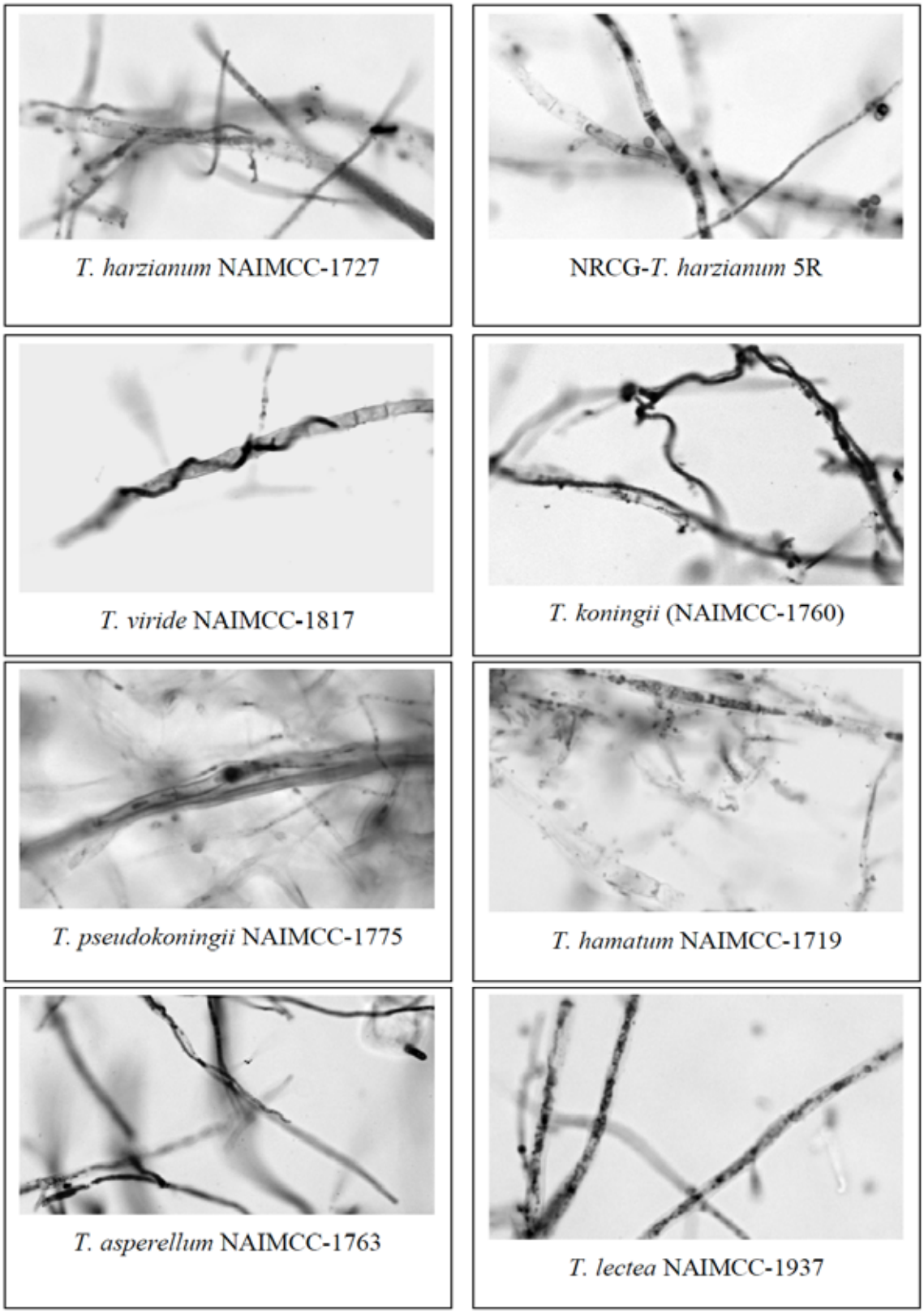

Fig. (14). Parasitism of *Colletotrichum gloeosporioides* by different species of *Trichoderma.*

isolates with different biological properties against two pathogens of strawberry, it was shown that the isolate which could parasitize both *C. acutatum* and *B. cinerea* had better survival than others which either did not mycoparasitise or parasitized only one of the isolates [58]. However, instead of a mix, when they were applied separately, the populations of all four declined sharply by day three. The populations of *T. harzianum* T39 on treated cucumber leaves were 70, 55

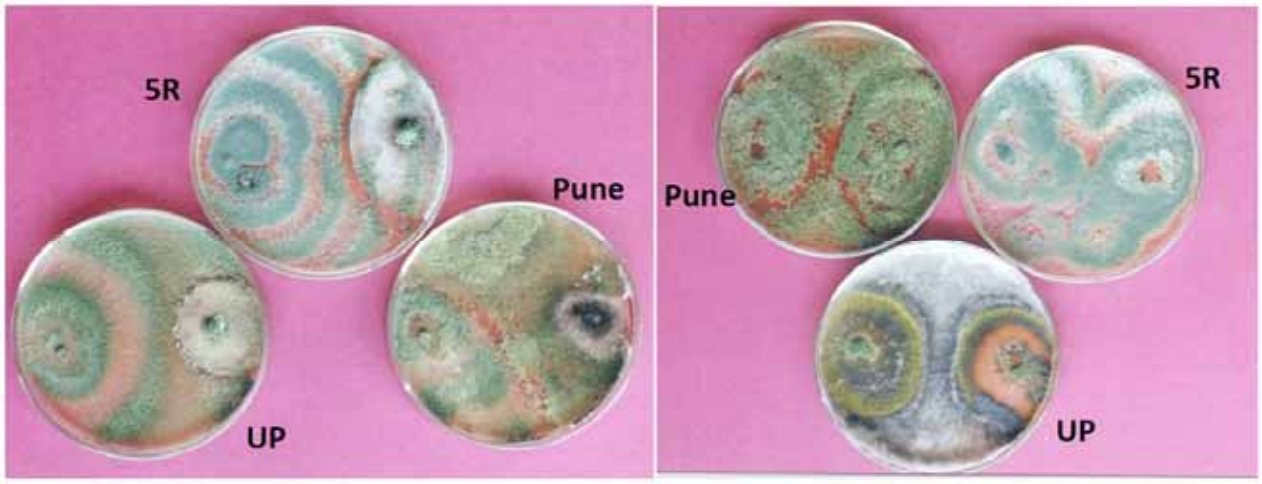

Fig. (15). Overgrowth of three isolates of *Trichoderma* on (**a**) *Alternaria alternata* and (**b**) *Botryodiplodia theobromae.* Note isolate 5R has overgrown both the fungi, while Pune isolate has overgrown *B. theobromae* but not *A. alternata*, while UP isolate has overgrown *A. alternata* but not *B. theobromae.*

and 50 cfu/cm^2 of leaf after 0, 2 and 6 days of application at rate of 0.2% of formulation containing 10^9cfu/g [15].

On the contrary, the isolate *T. atroviride* C65 applied as foliar spray at bud burst could survive on leaves and flowers/fruits of kiwi fruits up till harvest, a period of more than 3 months [59]. It also spread to unsprayed leaves and fruits upto 3 m away, dispersed either by insects or by wind currents.

7. ISOLATE VARIATIONS

Different *Trichoderma* species are reported as antagonists of a particular pathogen species, eg. all the 34 isolates belonging to seven *Trichoderma* species could cause lysis of *C. gloeosporioides mycelium* (Fig. **14**); but variations among isolates from the same species were reported [26]. Variation in antagonism of any one isolate to different pathogen species are also observed, e.g. three *Trichoderma* isolates viz. 5R, Pune and UP when tested against two pathogens of grapes, isolate 5R overgrew and lysed mycelium of both pathogens, while Pune isolate has overgrown and lysed *B. theobromae* but not *A. alternata*, and UP isolate has overgrown and lysed *A. alternata* but not *B. theobromae* (Fig. **15**). Thus systematic search for isolates with wide host range is essential when dealing with crops where more than one pathogen needs to be controlled simultaneously. One study has shown that isolates belonging to the taxonomic section *Trichoderma* sect. *Pachybasium* produce chemicals inhibitory to fungi and bacteria, while isolates belonging to *T*. sect. *Longibrachiatum* were more inhibitory to yeasts [60]. This kind of information can guide search for potential antagonistic isolates.

8. FUTURE CHALLENGES

Commercialization of the selected efficient isolates is the biggest challenge. The large numbers of research laboratories around the world have, through their painstaking research, identified a number of isolates which can be successfully utilized for control of a specific disease on a target crop in that particular geographical area. In most cases, these isolates have not been tested for their efficiency of, i) disease control under different agro-climatic conditions and over a number of years, ii) the same pathogen on different crops cultivated under different agroecosystems; iii) control of multiple disease on the same crop; iv) integration with fungicides etc. This has resulted in a large number of isolates which, though, highly efficient under the experimental conditions, may be limited in their use on a commercial scale. Inter-institutional collaborations for wide scale evaluation of these isolates is the immediate need for identifying isolates with wide applicability. The most efficient isolate(s) can be developed into commercial products which will overcome many of the issues associated with small scale production.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

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Received: August 01, 2013 Revised: December 13, 2013 Accepted: May 26, 2014

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