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Review

Trichoderma: A scientific approach against soil borne pathogens

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The members of the genus *Trichoderma* are widely used as bioagent for the control of phytopathogenic fungi in agriculture sectors. The members of this genus are reproducing asexually by the formation of cyanide and chlamydospore, in wild habitats they reproduce by formation of ascospores. *Trichoderma* species are well known for the production of cell wall degrading enzymes (CWDEs). These CWDEs, play a major role in biocontrol mechanism. We all know that all living organisms are made up of genes that code for a particular function. Similarly, in *Trichoderma*, some genes are responsible for the secretion of these CWDEs. These genes, which aid in the biocontrol action, are called biocontrol genes. These bacterial genes code for a particular enzyme and protein that degrade the pathogen cell wall. These biocontrol genes can be isolated and cloned for large scale commercial production. It has also been found that some of the genes of *Trichoderma* are also helpful in the abiotic and biotic stress. The mechanisms which are employed by *Trichoderma* for the phytopathogenic action are generally included atibiosis, mycoparasistism, competition for nutrients, etc.

Key words: *Trichoderma*, biocontrol, phytopathogen, lytic enzymes, biocontrol mechanisms, biocontrol agent.

INTRODUCTION

In recent times, excessive use of chemical pesticides has posed a threat on the environment. *Trichoderma* based biocontrol agents have better ability to promote plant defense response, promote plant growth and soil remediation. *Trichoderma* species has gained wide acceptance as effective biocontrol agents against several phytopathogens. Micropropagules of *Trichoderma* spp. in the form of conidia are preferred over chlamydospores and mycelia biomass because of its viability and stability in field application (Rosane et al., 2008; Chet, 1987).

The genus *Trichoderma* are commonly found in soils and on decaying wood and vegetable matter. Strains of *Trichoderma* are rarely associated with diseases of living plants, although an aggressive strain of *Trichoderma* causes a significant disease of the commercial mushroom and soil borne pathogens. Samuels (1996) provides a comprehensive review of *Trichoderma* spp. for enzyme production and biological control mechanisms. In *Trichoderma* spp., sexual reproduction not present are believed to be mitotic and clonal. The main

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problem with the nomenclature of *Trichoderma* is pleomorphisim present within the genus. In *Trichoderma*, there are two stages, the sexual stage is called *Hypocrea* (telomorphic) and the asexual stage is called *Trichoderma* (anamorphic). The genus is called *Hypocrea/Trichoderma* (Druzhinina et al., 2011). However, despite these significant advances in our knowledge about this genus, the full taxonomic history of *Trichoderma* is still not complete, and the detailed description of the taxonomic history of *Trichoderma* remains problematic. A refined classification and identification is necessary for predictive indications about ecology.

Trichoderma is widely used for the control of many soil borne plant pathogens. *Trichoderma* spp. are the efficient producer of cell wall degrading enzymes (Srivastava et al., 2014b); some of these enzymes are of commercial importance. Many research workers have proved that *Trichoderma* spp. possess some biocontrol genes that can be isolated and cloned for commercial large scale production (Massart and Jijakli, 2007).

The biococntrol ability of *Trichoderma* is of much importance as it does not acccumulate in food chain and thus do not harm plants, animals and humans. The genes involved in the biocontrol mechanisms of *Trichoderma* are of great importance.

TAXONOMY OF *TRICHODERMA*

Although the genus *Trichoderma* has been known since the 19th century. Its association with *Hypocrea* Fr. was discovered by the Tulasne brothers in 1865; its taxonomy has remained obscure until recent decades. Bisby (1939) thought that the morphological variation could be ascribed to a single species, *Trichoderma viride*. The first serious attempt to morphologically distinguish species, or rather “species aggregate”, was made by Rifai (1969). Some new species subsequently described were keyed out by Domsch et al. (1980). Teleomorph connections were established by means of ascospore isolates by Dingley (1957) and Webster and Rifai (1968). In Japan, Doi (1969a, 1972b) studied a number of telemorphs and described them with cultural and anamorph characters, but no cultures were preserved from that study. After this, no morphological differentiation was given. Bissett (1984, 1991b, c) gave a detailed description of the morphological studies, who distinguished about 21 taxa. These studies have shown that the delimitation of biological species is extremely difficult in this genus on morphological grounds alone.

Apart from morphological studies, there are many other methods that are used in the taxonomy, such as study of secondary metabolites; this has shown a great diversity in this genus (Okuda et al., 1982). Physiological features that are detected by the microtiter plate assay are the useful tools that are used for the identification. Isoenzyme profiles are also used for taxonomic classification (Samuels et al., 1994; Leuchtmann et al., 1996). In modern era molecular techniques, such as sequences of its region of ribosomal DNA and fingerprinting techniques provide the finest resolution of taxonomic entities (Meyer et al., 1992; Fujimori and Okuda, 1994; Muthumeenakshi et al., 1994).

BIOCONTROL GENES OF *TRICHODERMA*

Trichoderma is widely used for the control of many soil borne plant pathogens (Table 1). *Trichoderma* spp. Are efficient producers of cell wall degrading enzymes (Srivastava et al., 2014a), some of which are of commercial importance. Many research workers have proved that *Trichoderma* spp. possess some biocontrol genes that can be isolated and cloned for commercial large scale production (Massart and Jijakli, 2007).

Kuc (2001) has proved that some genes, providing resistance to abiotic and biotic stress are present in *Trichoderma* (Table 2). Mycoparasistism (Figure 1), antibiosis and competition for the nutrients are the main strategies employed by *Trichoderma* for the phytopathogenic action (Janisiewicz and Korsten, 2002). Among the different species tested, *Trichoderma harzianum* was found to be the most promising strain (Gao et al., 2002).

GENOMICS OF *TRICHODERMA*

The genome size of filamentous fungi is very small as it is about 25 to 50 Mb. However, with the advancement of pulse field gel electrophoresis, karyotyping of filamentous fungi is possible. Karyotyping is helpful in the detection, translocations and variations in chromosome numbers. Through pulsed-field gel electrophoresis (PFGE), chromosomal DNA was separated from *Trichoderma (*Gilly and Sands, 1991; Mäntylä et al., 1992; Hayes et al., 1993; Herrera-Estrellaet et al., 1993). The expected genome size and chromosome number of *Trichoderma* ranges from 31 to 39 Mb and from 3 to 7, respectively. From the data obtained through DNA homology, it was found that *T. harzianum* and *Trichoderma veins* are closely related, and it was concluded that they may have the same phylogenetic origin (Herrera-Estrella et al., 1993). On the other hand, Mäntylä et al. (1992) determined molecular karyotypes of various strains of *Trichoderma reesei* that had undergone mutagenesis and screening for the hyper production of cellulase enzyme. The authors found that extensive alteration in the genome organization of these strains occurred.

The first member of the genus sequenced was *T. reesei* (Table 3). This fungus is the first choice, because the genome size of this organism is very small (33 Mb) and has only seven chromosomes. Fungal genomics laboratory of NCSU has expressed sequence tag (EST),

Table 1. *Trichoderma* species and its uses against different plant pathogens.

Plant	Causative agent	*Trichoderma* spp. used
Vigna mungo (Black gram) (Raguchander et al., 1997; Dubey et al., 2012; Mishra et al., 2011)	*Macrophomina phaseolina, Alternaria alternate*	*T. viride, T. harzianum*
Solanum melongena L. (Brinjal) (Jadon, 2009; Balaji and Ahir, 2011)	*Fusarium solani*, *Fusarium oxysporum f. sp*.	*T. viride, T. harzianum*
Cicer arietinum (Chickpea) (Mukherjee et al., 1997; Haware et al., 1999; Pandey et al., 2003; Poddar et al., 2004)	*Fusarium oxysporum, Rhictonia solani*, *Aspergillus niger, Chaetomium* spp.*, Sclerotium rolfsii, Penicillum* spp*. Macropho phaseolina*	*T. harzianum, T. viride*
Capsicum annum L (Chilli), (Rini and Sulochana, 2006; Kapoor, 2008; Vasanthakumari and Shivanna, 2013)	*Sclerotium rolfsii, Fusarium oxysporum, Pythium* spp., *Rhizoctonia solani pseudokoningii* 2013	*T. viride, T. harzianum*
Cocos nucifera L (Coconut) (Karthikeyan et al., 2006)	*Ganoderma lucidum*	*T. harzianum, T. viride*
Coffea arabica L.(Coffee) (Deb et al.,1999)	*Phomopsis thaeae, Glomerella cingulata*	*T. harzianum*
Vigna sinensis(Cowpea) (Pan and Das, 2011)	*Rhictonia solani*	*T. harzianum*
Arachis hypogaea L. (Groundnut) (Biswas and Sen, 2010; Kishore et al., 2001; Rakholiya and Jadeja, 2010; Bagwan, 2011; Sreedevi et al., 2011)	*Thievaliopsis basicola, Sclerotium rolfsii* Sacc, *Aspergillus niger, Rhicotonia solani, Pythium aphanidermatum, Macrophomia phaseolina*	*T. harzianum, T. viride, T longibrachiatum*
Agaricus bisporus (Mushroom) (Rawal et al., 2013)	*Rhizopus stolonifer, Coprinopsis kimurae, Penicillium glabrum, Fusarium oxysporum*	*T. viride*
Cajanus cajan (Pigeon pea) (Hukma and Pandey, 2011)	*Fusarium udum*	*T. viride, T. harzianum*
Solanum lycopersicum (Tomato) ((Sreenivasaprasad and Manibhushanrao, 1990; Dutta and Das, 2002; Jayaraj et al., 2006)	*Fusarium oxysporum f. sp. lycopersici, Pythium aphanidermatum, Rhictonia solani, Sclerotium rolfsii*	*T. harzianum, T. viride,T longibrachiatum, T. virens*
Oryza sativa (Rice) (Chakravarthy et al., 2011; Bhramaramba and Nagamani, 2013; Biswas and Datta, 2013; Gangwar and Sharma, 2013)	*Rhictonia solani, Fusarium* spp	
Capsicum annum L (Capsicum) (Kapoor, 2008)	*Alternaria alternata*	*T. viride T. harzianum*

Table 1. Contd.

Brassica oleracea (Cauliflowe) (Sharma and Sain, 2004, 2005; Ahuja et al., 2012)	*Rhictonia solani, Pythium aphanidermatum*	*T. viride T. harzianum*
Citrus (Kalita et al., 1996; Singh et al., 2000)	*Fusarium solani*	*T. viride T. harzianum*
Gossypium hirsutum (Cotton) (Sreenivasaprasad and Manibhushanrao, 1990; Gaur et al., 2005)	*Rhictonia solani, Sclerotium rolfsii, Pythium aphanidermatum*	*T. viride T. harzianum*
Zingiber officinale (Ginger) (Gupta et al., 2010)	*Pythium aphanidermatum*	*T. harzianum*
Sesamum indicum L (Sesame) (Tamimi and Hadvan, 1985; Sankar and Jeyarajan, 1996; Jeyalakshmi et al., 2013)	*Aspergillus flavus, Curvularia lunata, Pythium notatum, Pythium chrysogenum, Fusarium moniliforme, Fusarium oxysporium , Rhictonia nigricans, Macrophomia phaseolina*	*T. viride T. harzianum*

Table 2. Some biocontrol genes of *Trichoderma* and their function (Srivastava et al., 2014a, b).

Name of gene	Source organism	Function
Tvsp1	*Trichoderma virens*	This gene encodes for serine protease. *Rhizoctonia solani* which affects the cotton seedlings has been controlled biologically by serine protease.
*tri*5	*Trichoderma harzianum*	This gene is responsible for the synthesis of trichothecene which inhibits the protein and DNA synthesis in the cells of the pathogens and inhibits their growth.
TgaA and *TgaB*	*Trichoderma virens*	This gene exhibits antagonist activity against *R. solani* and *Sclerotium rolfsii*
ThPG1	*Trichoderma harzianum*	This gene encodes for endopoly-galacturonase. This enzyme is involved in the cell wall degradation of the pathogens like *R. solani* and *P. ultimum*
Th-Chit	*Trichoderma harzianum*	This gene is responsible for the antifungal activity in transgenic tobacco plant.
tri5	*Trichoderma brevicompactum*	This gene helps in the production of *Trichoderm* in which shows antifungal activity against *S. cerevisiae, Kluyveromyces marxianus, Candida albicans, C. glabrata, C. tropicalis* and *Aspergillus fumigates.*
erg1	*Trichoderma harzianum*	This gene encodes an enzyme named squalene peroxidase, which helps in the synthesis of ergosterol and silencing of this gene provides resistance to terbinafine, an antifungal compound.

Table 2. Contd.

TvGST	*Trichoderma virens*	This gene is responsible for cadmium tolerance
Thkel1	*Trichoderma harzianum*	This gene codes for putative kelch-repeat protein which helps in regulating the glucosidase activity and enhances tolerance to salt and osmotic stresses in *Arabidopsis thaliana* plants
egl1.	*Trichoderma longibrachiatum*	This gene showed biocontrol activity against *P. ultimum* in damping-off of cucumber
qid74	*Trichoderma harzianum* CECT 2413	This gene plays a significant role in cell protection and provide adherence to hydrophobic surfaces that helps the fungus in mycoparasitic activity against *R. solani* pathogen
Taabc2	*Trichoderma atroviride*	This gene has a significant role in ATP binding cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitic activity
tac1	*Trichoderma virens*	This gene has its role in mycoparasitic activity against *R. solani* and *P. ultimum*
TrCCD1	*Trichoderma reesei*	This gene is involved in carotenoid metabolism that helps in the development of conidiospores and hyphal growth in *T. reesei*

Table 3. Summary of some features of the sequenced genomes.

Feature		*T. reesei*	*T. virens*	*T. atroviride*	*T. harzianum*	*T. asperellum*	*T. longibrachiatum*	*T. ctrinoviride*
Genome size (Mb)		34.1	39	36.1	40.98	37.46	32.24	33.48
No of predicted genes		9129	12427	11863	14095	12566	10792	9397
Glycosyl hydrolases	-							
	Chitinases	23	41	34	NA	NA	NA	NA
	Glucanase	15	18	18	NA	NA	NA	NA
Secondary metabolites biosynthesis, transport and catabolisms (KOG)		262	440	349	438	358	253	285
PKS		11	18	18	NA	NA	NA	NA
NRPS		10	28	16	NA	NA	NA	NA
PKS-NRPS		2	4	1	NA	NA	NA	NA
SSCPs		260	319	301	NA	NA	NA	NA
Xenobiotics bidegradation and metabolisms (KEGG)		327	519	453	610	432	232	359
Mating types		MAT1-2	MAT1-2	MAT1-2	MAT1-2	MAT1	MAT1-1	MAT1-2

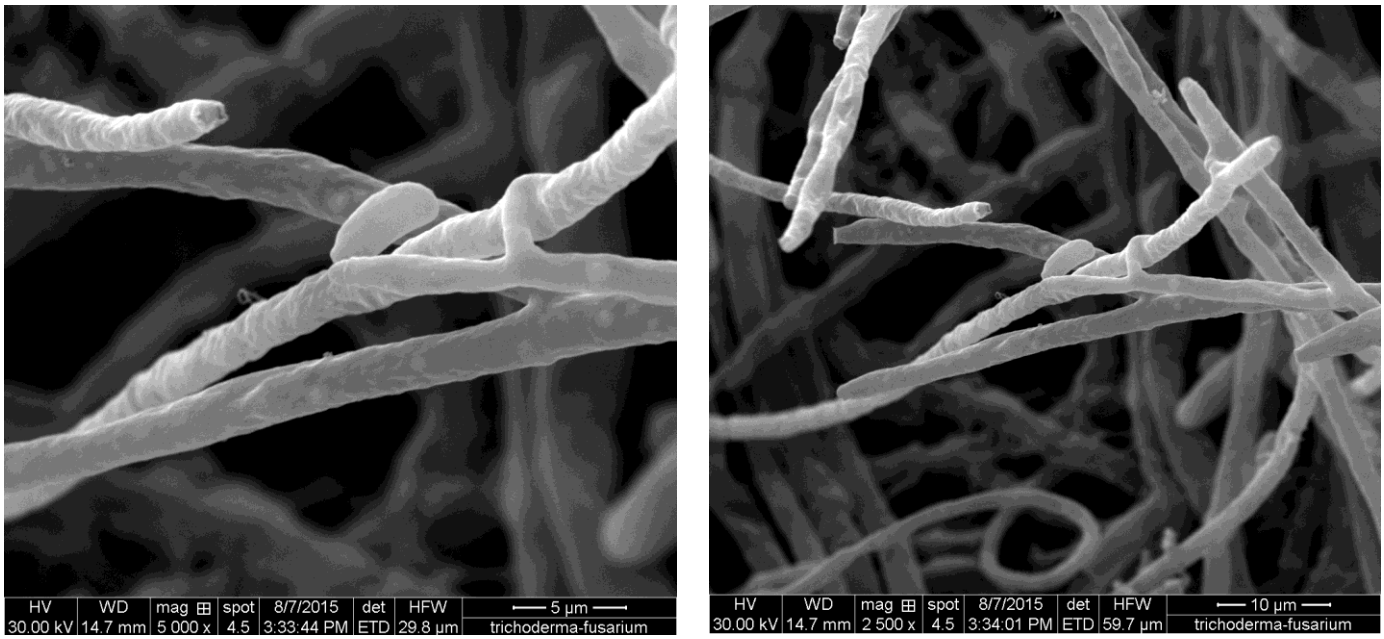

Figure 1. Scanning electron micrograph on mycoparasitism of the *F. oxysporum ciceri* hyphae by the hyphae of *T. harzianum* with pincer shaped structure.

cDNA collection and bacterial artificial chromosome (BAC) libraries for academic researchers. DNA mediated transformations and gene protocols have been developed for the genomic study of *Trichoderma.*

MASS MULTIPLICATION OF *TRICHODERMA*

Grains are cheap, easily available and act as best nutritive media for the mass multiplication of many micro-organisms. Bajra (*Pennisetum typhoides*) grains should be completely soaked in 2% sucrose solution in water for 6 h. After draining out the excess water, the soaked 250 g seeds of bajra should be filled in autoclavable poly-propylene (PP) bags of 30 × 20 cm^2. The PP bags should be plugged with nonabsorbent cotton followed by autoclaving at 15 lbs pressure for 30 min. After autoclaving, the bags should be left for cooling overnight. The next day, the bags should be individually inoculated by using 5 ml stock solution (10^6 to 10^8 CFU/ml) of starter culture grown for 100 days, with syringe. Before inoculation, the place from where the inoculation is to be made should be marked out with a small circle with the help of marker pen. Punctured place of injection of the PP bag must be sealed with cellophane tape. The bags should be incubated at 25 ± 2°C for 15 days in a temperature controlled room. After 15 days of incubation, the contents of the bags should be taken out and kept in hot air oven for drying overnight at 35°C. During the 15 days of incubation visual check every day is essential to ensure detection and elimination of contaminated PP bag(s). Formulation thus prepared should be ground to fine powder, while ensuring that during the process temperature does not go beyond at 35°C. The powdered formulation thus obtained should be mixed with pre-sterilized talc in 1:9 (Trichoderma spore:talc) ratio. Three samples should be taken from each, but lot during production and tested using a standardized method to determine the viability of the active ingredient expressed as colony forming units (CFU). The product thus prepared is ready for packaging at this stage. For storage, the finished product should be stored in vacuum filled plastic bags, covered by paper cartons of different sizes (250, 500 and 1000 g). These packets should then be kept in sealed cartons for transportation purpose.

ADVANTAGES OF *TRICHODERMA*

(1) *Trichoderma* spp. are very useful for fabric detergent, animal feed production, fuel production, alternative to conventional bleaching, effluent treatment, degradation of organochlorine pesticides and biocontrol of crop diseases.
(2) It is a potential bioagent for the management of fungal seed and soil borne pathogens. It is well known for its antagonistic activity against soil borne pathogens, such as *Fusarium, Pythium,* and *Rhizoctonia*.
(3) It is also known to suppress plant parasitic nematodes.
(4) It does not lead to development of resistance in plant pathogens, no phytotoxic effects, do not create any pollution problems as it is eco-friendly, promote plant growth, induces resistance in host, solubilize phosphorus

and micronutrients and hence increase soil fertility.
(5) It significantly minimizes losses due to crop diseases and reduces cost of production, increases yield, quality and profit.
(6) Many *Trichoderma* spp. are of great economic importance producing hydrolytic enzymes, namely, cellulases, chitinases and xylanases, biochemicals and antibiotic products which have been applied to fields, such as food processing and pulp bleaching. In addition, some species produce heterologous proteins and others have been successfully used as biological control agents against a range of phytopathogens.

DISADVANTAGES OF *TRICHODERMA*

There are many advantages associated with the use of *Trichoderma*. However, in addition to their useful properties, there are some disadvantages associated with the use of *Trichoderma*.

(1) Some species of *Trichoderma* pose a threat to the horticultural industry. For example, reduction in mushroom yield by as much as 50% have been attributed to *Trichoderma* infection and hence it is considered as a harmful parasite of mushroom.
(2) It also affects the organ (liver) transplanted in human.
(3) The disease is the major constraint in economical production as it inflicts heavy crop losses.

CONCLUSION AND FUTURE RESEARCH

Chemical based control is very effective, but there are some disadvantages associated with the use of these chemicals. The most dangerous thing with the use of these chemicals is the toxicity which they impart to the soil. That is why today people avoid the use of chemical based fungicide. Biosynthetic design of fungicide has present a new era in the development of fungicide. The genes present in the fungi *Trichoderma* has the ability to enhance host plant's resistance against phytopathogenic fungi.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Ahuja DB, Ahuja, RU, Srinivas P, Singh RV, Malik, M, Sharma P, Bambawale OM (2012). Development of farmer-led integrated management of major pests of cauliflower cultivated in rainy season in India. J. Agri. Sci. 4(2):79-90.
Bagwan NB (2011). Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii, Aspergillus niger* and *Aspergillus flavus*. Int. J. Plant. Prot. 4:107-111.
Balaji LP, Ahir, RR (2011). Evaluation of plant extracts and biocontrol agents against leaf spot disease of brinjal. Indian Phytopathol. 64(4): 378-380.
Bhramaramba S, Nagamani A (2013). Antagonistic *Trichoderma* Isolates to control bakane pathogen of rice. Agric. Sci. Dig. 33(2):104-108.
Bisby GR (1939). *Trichoderma viride* Pers. ex Fr. and notes on *Hypocrea*. Trans. Br. Mycol. Soc. 23:149-168.
Bissett J (1984). A revision of the genus *Trichoderma:* 1. Section *Longibrachiatum,* new section. Can. J. Bot. 62:924-931.
Bissett J (1991a). A revision of the genus *Trichoderma*. II. Infrageneric classification. Can. J. Bot. 69:2357-2372.
Bissett J (1991b). A revision of the genus *Trichoderma*. III. Section *Pachybasium*. Can. J. Bot. 69:2373-2417.
Biswas C, Srivastava SSL and Biswas SK, (2010). Biochemical changes associated with induction of resistance by *Trichoderma* spp. in paddy against brown spot disease. Indian Phytopath. 63(3): 269-272.
Biswas S, Datta M (2013). Evaluation of biological control agents against sheath blight of rice in Tripura. Indian Phytopathol. 66(1):77-80.
Chakravarthy S, Nagamani K, Ratnakumari AR, Bramarambha YS (2011). Antagonistic ability againstRhizoctonia solani and pesticide tolerance of Trichoderma strains. Adv. Environ. Biol. 5(9):2631-2638.
Chet I (1987). *Trichoderma* Application, Mode of Action and Potential as a Biocontrol Agent of Soil-borne Plant Pathogenic Fungi. In: Innovative Approaches to Plant Disease Control, ed. Wiley, New York, pp. 137-160
Deb PR, Deb M, Dutta BK (1999). A preliminary report on the phyllosphere mycoflora of tea and the soil mycofloraof an experimental tea plantation area of Cachar. Indian Phytopathol. 52(2):193-195.
Dingley JM (1957). Life history studies in the genus *Hypocrea* Fr. Trans. R. Soc. N. Z. 84:689-693.
Doi Y (1969). The genus *Hypocrea*and its allies in Japan. Bull. Natl. Sci. Mus. Tokyo 12:693-724.
Doi Y (1972). Revision of the *Hypocreales* with cultural observations. IV. The genus *Hypocrea*and its allies in Japan. Enumeration of the species. Bull. Natl. Sci. Mus. Tokyo 15: 649-751.
Domsch KH, Gams W, Anderson TH (1980). Compendium of Soil Fungi, Vols 1 and 2. Academic Press, London.
Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011). *Trichoderma:* the genomics of opportunistic success. Nat. Rev. Microbiol. 9:749-759 10.1038/nrmicro2637 .
Dubey SC, Patel B (2012). Evaluation of fungal antagonists against *Thanatephorus cucumeris* causing web blight of urd and mung bean. Indian Phytopathol. 54(2):206-209.
Dutta P, Das, BC (2002). Management of collar rot of tomato by *Trichoderma* spp. and chemicals. Indian Phytopathol. 55(2):235-237.
Fujimori F, Okuda T (1994). Application of the random amplified polymorphic DNA using the polymerase chain reaction for efficient elimination of duplicate strains in microbial screening. I. Fungi. J. Antibiot. (Tokyo) 47:173-182.
Gangwar OP, Sharma P (2013). A Growth and survival of *Trichoderma harzianum* and *Pseudomonas fluorescens* on different substrates and their temporal and spatial population dynamics in irrigated rice ecosystem. An. Plant Prot. Sci. 21(1):104-108.
Gao KX, Liu XG, Liu YH, Zhu TB, Wang SL (2002). Potential of *Trichodermaharzianum* and *T. atroviride* to control Botryosphaeriaberengeriana f. sp. piricola, the cause of apple ring rot. J. Phytopathol.150: 271-276.
Gaur RB, Sharma RN, Singh V (2005). Manipulations in the

mycoparasite application techniques against *Rhizoctonia* root rot of cotton. Indian Phytopathol. 58(4):402-409.
Gilly JA, Sands JA (1991). Electrophoretic karyotype of *Trichodermareesei*. Biotechnol. Lett.13:477-482.
Gupta M, Dohroo NP, Gangta V, Shanmugam V (2010). Effect of microbial inoculants on rhizome diseaseand growth parameters of ginger. Indian Phytopathol. 63(4):438-441.
Haware MP, Mukherjee PK, Lenne IM, Jayanthi S, Tripathi HS, Rathi YPS (1999). Integratedbiological-chemical control of *Botrytis gray* mould of chickpea. Indian Phytopathol. 52(2):174-176.
Hayes CK, Harman GE, Woo SL, Gullino ML, Lorito M (1993). Methods for electrophoretic karyotyping of filamentous fungi in the genus *Trichoderma*. Anal. Biochem. 209:176-182.
Herrera-Estrella A, Goldman GH, Van Montagu M, Geremia RA (1993). Electrophoretic karyotype and gene assignment to resolved chromosomes of *Trichoderma* spp. Mol. Microbiol. 7:515-521.
Jadon KS (2009). Eco-friendly management of brinjal collar rot caused by *Sclerotium rolfsiiSacc.* Indian Phytopathol. 62(3):345-347.
Janisiewicz WJ, Korsten L (2002). Biological control of postharvest diseases of fruit. Annu. Rev. Phytopathol. 40:411-441.
Jayaraj J, Radhakrishnan NV, Velazhahan R (2006). Development of formulations of Trichoderma harzianumstrain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum.* Arch. Phytopathol. Plant Prot. 39(1):1-8.
Jeyalakshmi C, Rettinassababady C, Sushma N (2013). Integrated management of sesame diseases. J. Biopestic. 6(1):68-70.
Kalita P, Bora LC and Bhagabati KN (1996). Phylloplane microflora of citrus and their role in management of citruscanker. Indian Phytopathol. 49(3):234-237.
Kapoor AS (2008). Biocontrol potential of *Trichoderma* spp. against important soilborne diseases of vegetable crops. Indian Phytopathol. 61(4):492-498
Karthikeyan M, Radhika K, Bhaskaran R, Mathiyazhagan S, Samiyappan R, Velazhahan R (2006). Rapiddetection of Ganoderma disease of coconut and assessment of inhibition effect of various control measures by immunoassay and PCR. Plant Prot. Sci. 42:49-57.
Kishore GK, Pande S, Rao JN, Podile AR (2001). Biological control of crown rot of groundnut by*Trichoderma harzianum* and *T. viride*. Int. Arachis Newsl. 21:39-40.
Kuc J (2001). Concepts and direction of induced systemic resistance in plants and its application. Eur. J. Plant Pathol. 107:7-12.
Leuchtmann A, Petrini O, Samuels GJ (1996). Isozyme subgroups in *Trichoderma*section *Longibrachiatum*. Mycologia 88:384-394.
Mäntylä AL, Rossi KH, Vanhanen SA, Penttilä M E, Suominen PL, Nevalainen H (1992). Electrophoretic karyotyping of wild-type mutant *Trichoderma longibrachiatum (reesei)* strains. Curr. Genet. 21:471-477.
Massart S, Jijakli HM (2007). Use of molecular techniques to elucidate the mechanisms of action of biocontrol agents: A review. J. Microbiol. Methods 69:229-241.
Meyer W, Morawetz R, Börner T, Kubicek CP (1992). The use of DNA fingerprint analysis in the classification of some species of the *Trichoderma*aggregate. Curr. Genet. 21:27-30.
Mishra DS, Gupta AK, Prajapati, CR, Singh US (2011). Combination of fungal and bacterial antagonistsfor management of root and stem rot disease of soybean. Pak. J. Bot. 43(5):2569-2574.
Mukherjee PK, Haware MP, Raghu K (1997). Induction and evaluation of benomyl-tolerant mutants of *Trichoderma viride* for biological control of *Botrytis grey* mould of chickpea. Indian Phytopathol. 50(4):485-489.
Muthumeenakshi S, Mills PR, Brown AE, Seaby DA (1994). Intraspecific molecular variation among *Trichodermaharzianum*isolates colonizing mushroom compost in the British Isles. Microbiology 140:769-777.
Okuda T, Fujiwara A, Fujiwara M (1982).Correlation between species of *Trichoderma*and production patterns of isonitrile antibiotics. Agric. Biol. Chem. 46:1811-1822.
Pan S, Das A (2011). Control of cowpea (*Vigna sinensis*) root and collar rot (*Rhizoctonia solani*) with someorganic formulations of *Trichoderma harzianum* under field condition. J. Plant Prot. Sci. 3(2): 20-25.
Pandey G, Pandey RK, Pant H (2003). Efficacy of different levels of *Trichoderma viride* against root-knotnematode in chickpea (*Cicer arietinum L*.). An. Plant Prot. Sci. 11:101-103.
Poddar RK, Singh DV, Dubey SC (2004). Integrated application of *Trichoderma harzianum* mutants andcarbendazim to manage chickpea wilt (*Fusarium oxysporum f.sp. ciceris*). Indian J. Agric. Sci. 74(6):346-348.
Raguchander T, Rajappan K, Samiappan R (1997). Evaluating methods of application of biocontrol agent inthe control of mungbean root rot. Indian Phytopathol. 50(2):229-234.
Rakholiya KB, Jadeja KB (2010). Effect of seed treatment of biocontrol agents and chemicals for the management of stem and pod rot of groundnut. Int. J. Plant Prot. 3(2): 276-278.
Rawal P, Sharma P, Singh ND, Joshi A (2013). Evaluation of fungicides, neem bio-formulations andbiocontrol agent for the management of root rot of safed musli caused by *Rhizoctonia solani*. J. Mycol. Plant Pathol. 43(30):297-305.
Rifai MA (1969). A revision of the genus *Trichoderma*. Mycol. Pap. 116: 1-56.
Rini CR, Sulochana KK (2006). Management of seedling rots of chilli (Capsicum annuum L.) using *Trichoderma* spp. and *Pseudomonas fluorescent* (*Pseudomonas fluorescens*). J. Trop. Agric. 44(1-2):79-82.
Rosane SC, Helder LSL, Gustavo ASP, Carlos ATG, Sueli R (2008). Effect of Moisture on Trichoderma Conidia Production on Corn and Wheat Bran by Solid State Fermentation. Food Bioprocess Technol. 1:100-104
Samuels GJ, Petrini O, Manguin S (1994). Morphological and macromolecular characterization of *Hypocrea schweinitzii* and its *Trichoderma* anamorph. Mycologia 86:421-435.
Sankar P, Jeyarajan R (1996). Seed treatment formulation of *Trichoderma* and *Gliocladium* for biologicalcontrol of Macrophomina phaseolina in sesamum. Indian Phytopathol. 49(2):148-151.
Sharma P. and Sain SK (2005). Use of biotic agents and abiotic compounds against damping off of cauliflowercaused by *Pythium aphanidermatum*. Indian Phytopathol. 58(4): 395-401.
Sharma P. and Sain, SK (2004). Induction of systemic resistance in tomato and cauliflower by *Trichoderma* species against stalk rot pathogen (Sclerotinia sclerotiorum). J. Biocontrol 18(1): 21-28
Singh D, Kapur SP, Singh K (2000). Management of citrus scab caused by *Elsinoe fawcettii.* Indian Phytopathol. 53(4):461-467.
Sreedevi B, Charitha Devi M, Saigopal DVR (2011). Induction of defense enzymes in *Trichoderma harzianum*treated groundnut plants against *Macrophomina phaseolina.* J. Biol. Control 25(1):67-73.
Sreenivasaprasad S, Manibhushanrao K (1990). Biocontrol potential of fungal antagonists Gliocladiumvirens and *Trichoderma longibrachiatum*. Z. Pflanzenkr. Pflanzenschutz. 97(6):570- 579.
Srivastava M, Shahid M, Singh A, Kumar V, Pandey S, Srivastava YK (2014**a**). To Develop Strain Specific Molecular Marker for Easy and Proper Identification of Fungal Species Based on Molecular Characters: A Review. J. Mol. Biomark. Diagn. 5:172.
Srivastava M, Shahid M, Pandey S, Singh A, Kumar V, Gupta S, Maurya M (2014**b**). *Trichoderma* Genome to Genomics: A Review. J. Data Mining in Genomics Proteomics 5(3):1000172.
Tamimi KM, Hadvan HA (1985). Biological effect of *Neurospora sitophlla* and *Trichoderma harziaum* on thegrowth of a range of Sesamum wilt causing fungi in vitro. Indian Phytopathol. 38(2):292-296.
Vasanthakumari MM, Shivanna MB (2013). Biological control of anthracnose of chilli with rhizosphere and rhizoplane fungal isolates from grasses. Arch. Phytopathol. Plant Prot. 46(14):1641-1666.
Webster J, Rifai MA (1968). Culture studies on Hypocrea and Trichoderma IV. *Hypocrea pilulifera* sp. nov. Trans. Br. Mycol. Soc. 51:511-514.