Chapter 6

SUMMERY
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1) Various sources such as rhizosphere soils of irrigated as well as non irrigated plants, fruit rinds, crop seeds and air were used for the isolation of *Trichoderma* species.

2) Five *Trichoderma* species such as *T. viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* and *T. virens* were isolated.

3) Among these five species, *T. koningii* and *T. pseudokoningii* were isolated and reported first time from Marathwada region of Maharashtra.

4) *Trichoderma* species were isolated mostly from the rhizosphere soil.

5) *Trichoderma viride*, *T. harzianum* and *T. virens* were found to be common.

6) Effect of Carbendazim (50% WP) was completely inhibitory on the growth of *Trichoderma* species.

7) Lower concentration of Mancozeb (75% WP) caused no inhibitory effect on the radial growth and sporulation of *Trichoderma* species while higher concentration of Mancozeb was inhibitory.

8) *Trichoderma* species were resistant to Captan (50% WP) at lower concentration while Captan® 500 ppm was found to be inhibitory.

9) *Trichoderma* species were resistant to Streptocycline and became reduced their radial growth when concentration of antibiotic was increased.

10) *Trichoderma* species exhibited compatibility with the fungicides and can be used in combination with compatible fungicides for integrated disease management of crop plants.

11) For the strain improvement, *Trichoderma* species were treated with mutagenic agent such as Ultra-Violet rays (UV-rays), Ethyl Methyl Sulphonate (EMS), Ethyl Bromide (EBr) and Sodium Azide (SA).

12) Ultra-Violet radiation does not caused inhibitory effect on the radial growth and sporulation of *Trichoderma* species.

13) Treatment of Ethyl Methyl Sulphonate (1%) was not inhibitory to the growth of *Trichoderma* species.

14) Similar kind of resistance by *Trichoderma* species against Ethyl Bromide (1%) was observed.

15) Sodium Azide adversely affected the radial growth and sporulation of *Trichoderma* species.
16) *Trichoderma viride* showed slow growth rate against Sodium Azide (1%); *T. harzianum* grows at Sodium Azide (0.4%) while other *Trichoderma* species were inhibited at Sodium Azide (0.4).

17) Antagonistic nature of *Trichoderma* species were evaluated under *in vitro* condition by applying dual culture method and found that growth of the pathogenic fungi were retarded when their hyphae comes in contact with the antagonist.

18) All *Trichoderma* species inhibited the mycelial growth of *Alternaria alternata* while maximum inhibition performed by *T. viride*. *Trichoderma koningii* was highly antagonistic over *Rhizoctonia solani* followed by *T. viride*. Radial growth of *Aspergillus niger* was highly inhibited by *T. koningii* followed by *T. harzianum*. It was found that antagonistic nature of *Trichoderma* species was moderate on the test fungus.

19) *Geotrichum candidum* was inhibited significantly by *T. viride* followed by *T. pseudokoningii* and *T. virens*. Significant antagonism was exhibited by *T. virens* against *Fusarium oxysporum f. sp. spinacae* followed by *T. harzianum* and *T. pseudokoningii*. Radial growth and sporulation of *Macrophomina phaseolina* was antagonized significantly by the *Trichoderma* species while maximum antagonism performed by *T. viride* followed by *T. harzianum* and *T. pseudokoningii*.

20) *Trichoderma harzianum* showed maximum antagonistic effect on *Pythium* sp followed by *T. virens* and *T. pseudokoningii*. Growth of *Alternaria tenuissima* was inhibited by *T. viride* followed by *T. virens*, *T. harzianum* and *T. pseudokoningii*. Antagonistic nature of *T. viride* against *Fusarium proliferatum* was maximum while *T. pseudokoningii* and *T. koningii* were significantly antagonistic against the test fungus.

21) Among the *Trichoderma* species, *T. viride* was found to be highly antagonistic over the pathogenic fungi.

22) *Trichoderma* species exhibited promoting effect on the seed germination of the crop plants tested in *in vitro* condition. *Trichoderma harzianum* showed maximum effect on the germination of crop plant seeds while other *Trichoderma* species also enhanced the germination rate and found to be more than the control.
23) In *in vitro* condition, effect of *Trichoderma* species on the seedling length was tested and the results indicated that treated seeds produced the superior seedling than the control. Maximum enhancement was achieved by *T. pseudokoningii* and *T. harzianum*.

24) Under *in vivo* condition effect of *Trichoderma* species on germination, seedling length biomass production and vigour index of crop plants were determined and significant increase in the data was obtained.

25) Biomass production and vigour index of wheat (*Triticum aestivum* L.) seedlings were enhanced by *T. virens* while the rate of germination also increased. *Trichoderma viride* showed better results on the paddy (*Oryza sativa* L.) seedlings with respect to germination, biomass production and vigour index. Superior results were exhibited by *T. virens* and *T. pseudokoningii* on the jowar (*Sorghum vulgare* Moench.) seedlings. *Trichoderma viride* and *T. harzianum* were found to be best in case of maize (*Zea mays* L.) seedlings. However, bajra (*Pennisetum typhoides* L.) seedlings performed significantly due to the treatment of *T. harzianum* and *T. vireide*.

26) Treatment of *Trichoderma* species with seeds of pulse crops were found to stimulatory. Germination rate of pigeon pea (*Cajanus cajan* L.) seeds were enhanced by *T. harzianum* and *T. virens*. *Trichoderma harzianum* enhanced the biomass production and seedling vigour index of pigeon pea (*Cajanus cajan* L.). Maximum germination of moth (*Phaseolus aconitifolius* Jacq.) seeds were recorded in treatment of *T. harzianum* while biomass production and vigour index was maximum due to *T. koningii* and *T. harzianum*. Gram (*Cicer aurictininum* L.) seeds were germinated maximum by all *Trichoderma* species while biomass production and seedling vigour index was recorded maximum due to *T. koningii*.

27) Maximum germination and seedling vigour index of groundnut (*Arachis hypogea* L.) was recorded better under the treatment of *T. pseudokoningii* and *T. virens*. Its biomass production was accelerated by *T. koningii*. *Trichoderma virens* was found to be superior in case of soybean (*Glycine max* L.) with respect to germination rate, biomass production and seedling vigour index. Similar trends were shown by *T. harzianum* in case of mustard (*Brassica nigra* L.).
28) *Trichoderma harzianum* showed promoting effect on the germination rate and biomass production of spinach while maximum seedling vigour index was due to the effect of *T. koningii*. Germination rate of shepu (*Anethum graveolens* L.) was better under the treatment of *T. koningii* and *T. virens*, biomass production was maximum by *T. koningii* while seedling vigour index was enhanced due to *T. viride*.

29) *Trichoderma* species are the effective biocontrol agent used for disease management but these also significantly enhanced the growth of crop plants and are responsible for healthy crops and products.

30) Extracellular production of hydrolytic enzymes was the unique feature of *Trichoderma* species that was the main reason of their mycoparasitic nature.

31) Maximum cellulase and lipase activity was exhibited by *T. pseudokoningii* while amylase activity was showed by *T. viride*.

32) Detection of pectinolytic enzyme was carried out and all the *Trichoderma* species performed the enzyme activity. *Trichoderma virens* showed significant pectinolytic activity which was measured in terms of percent loss of viscosity.

33) For the detection of phytotoxic activity of *Trichoderma* species culture filtrate was treated with the seeds of various crop plants and the effects on the seed germination was verified. It was interesting to note that secondary metabolites of *Trichoderma* species does not caused inhibitory effect on seed germination of crop plants.

34) For determining genetic variability of *Trichoderma* species molecular characterization of *T. viride, T. harzianum, T. koningii* and *T. virens* by RAPD analysis was carried out.

35) Five primers viz. OPM-01, OPN-05, OPP-01, OPQ-01 and OPT-04 generated reproducible polymorphism. A total of 36 reproducible and scorable polymorphic bands ranging as low as 100 bp to as high as 2000 bp were generated.

36) All these five primers showed total 27 polymorphic bands and 2 common bands. The data obtained from molecular characterization indicated that all the *Trichoderma* species showed genetic variations.
37) Growth of *Trichoderma* species on the various nutrient media such as Potato Dextrose Agar (PDA) medium, Czepek’s Dox Agar (CZA) medium, Martin’s Rose Bengal Agar (MRBA) medium, *Trichoderma* Selective medium (TSM), Waksman’s medium, Glucose Nitrate (GNA) medium, Richard’s medium and Sabouraud Dextrose Agar medium (SDA) were evaluated.

38) *Trichoderma viride* showed rapid growth on PDA medium and Waksman’s medium. MRBA medium and SDA medium were also good for the growth while on TSM it grows very slowly.

39) Growth of *T. harzianum* on PDA was active as well as it grows better on MRBA and Waksman’s media while poor growth was observed on TSM.

40) Effect of Waksman’s medium on the growth of *T. koningii* was superior while PDA, Richard’s, MRBA, SDA and GNA media were found to be moderate for the growth.

41) Except TSM, *T. pseudokoningii* showed active growth on the various nutrient media.

42) *Trichoderma virens* exhibited rapid growth on Waksman’s PDA, CZA, MRBA, SDA, GNA, and Richard’s media while grows slowly on TSM.

43) Among the various nutrient media PDA and Waksman’s media were found to be effective for the growth of *Trichoderma* species while on *Trichoderma* selective medium (TSM) these grow slowly under controlled manner.

44) *Trichoderma* species were allowed to grown on liquid media such as potato dextrose broth, coconut milk (25% & 50%) and coconut water (25% & 50%). Among these coconut milk (50%) was found to be superior for the growth of *Trichoderma* species.

45) Among the agricultural materials tested as base materials for mass multiplication of *T. viride*, cereal grains showed optimum effect while mass production was recorded significant on the seeds of pulses. Mass multiplication of *T. viride* on agrowaste materials such as rice bran, maize bran, wood chips, combination of saw dust with bran, sugarcane bagasse and waste tea powder were better.
Mass multiplication of *T. harzianum* was significant on moth seeds, gram seeds, maize bran, sugarcane bagasse and waste tea powder was significant while other agricultural and agrowaste materials production was moderate.

Cereal grains and pulse crop seeds were the good base materials for the growth of *T. koningii*. Agrowaste materials such as bran of various crops and their combination with saw dust was also good substrate for the growth of *T. koningii*. Other materials like wood chips, oilcakes, sugarcane bagasse and waste tea powder were showed effective base materials.

*Trichoderma pseudokoningii* showed maximum growth on rice bran, wood chips, bran of various crops and combination of bran and saw dust, bagasse and waste tea powder. However, other agrowaste materials showed moderate growth of *T. pseudokoningii*.

For the mass multiplication of *T. virens*, maize bran, gram husk, sunflower husk and sugarcane bagasse was found to be best substrates while other base materials were optimum.

*Trichoderma* species were grown on the materials those are rich source of carbon and for mass multiplication sufficient quantity of essential nutrients must be needed.

Mass multiplication of *Trichoderma* species in the field is the simple method. The methods are low cost, less time consuming, ecofriendly and safe to handle. Farmers should be aware about the mass multiplication of the bioagent and proper knowledge is needed. Inoculums of the bioagent should be mutant and maintained in the field as well as efforts should be undertaken for increasing the population of bioagents in the field.

Hence, this present research work was investigated and tried for screening of agrowaste materials for mass multiplication of *Trichoderma* species.