

**INVESTIGATIONS ON POTENTIAL *Trichoderma*  
spp. IN KONKAN REGION**

**BY**

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**DEPARTMENT OF PLANT PATHOLOGY  
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DAPOLI- 415 712, DIST. RATNAGIRI (M.S.)**

**JUNE, 2022**

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A thesis submitted to the

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(AGRICULTURAL UNIVERSITY)  
DIST. RATNAGIRI (MAHARASHTRA), INDIA**

*In partial fulfillment of the requirements for the degree of*

**Doctor of Philosophy**

In

**PLANT PATHOLOGY**

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## **C E R T I F I C A T E**

This is to certify that the thesis entitled, "INVESTIGATIONS ON POTENTIAL *Trichoderma* spp. IN KONKAN REGION" submitted to the Faculty of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, Maharashtra State, in the partial fulfilment of the requirements for the degree of *DOCTOR OF PHILOSOPHY* in PLANT PATHOLOGY, embodies the results of a piece of *bona-fide* research carried out by Ms. BARDE PRAVINA SANJAYRAO under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma or published in other form. All the assistance and help received during the course of investigation and the sources of literature have been duly acknowledged by her.

**Place: Dapoli**

**Date:**

**(P. G. Borkar)**

Chairman,

Advisory Committee and

Research Guide

## **DECLARATION OF STUDENT**

I hereby declare that the experimental work and interpretation of the thesis entitled “INVESTIGATIONS ON POTENTIAL *Trichoderma* spp. IN KONKAN REGION” or part of thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived any thesis/ publication of any University or Scientific Organization. Sources and material used and all assistance received during the course of investigation have been duly acknowledged.

**Place : Dapoli**

**(Barde Pravina Sanjayrao)**

**Date :**

**Enrollment No.- ADPD/18/0297**

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Place: Dapoli

(Barde Pravina Sanjayrao)

Date :

Enrollment No. ADPD/18/0297



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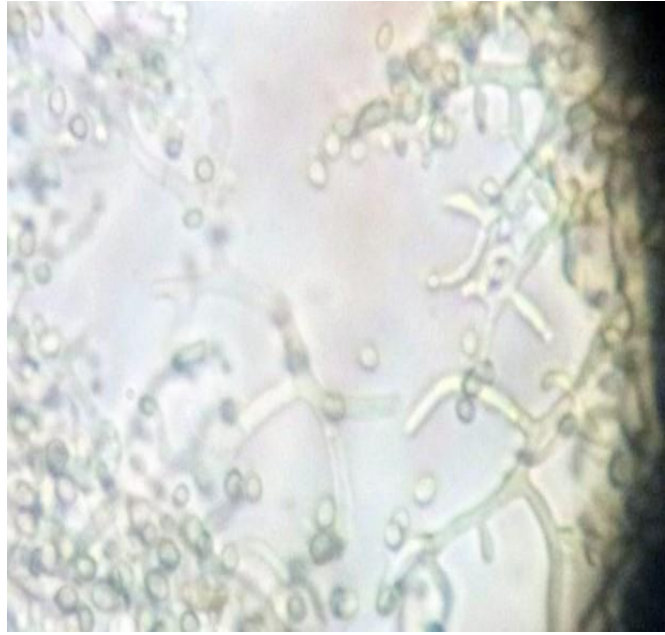
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# ABSTRACT



**DEPARTMENT OF PLANT PATHOLOGY  
COLLEGE OF AGRICULTURE, DAPOLI**

**Title of thesis** : “Investigations on potential *Trichoderma* spp. in Konkan region.”

**Name of the student** : Barde Pravina Sanjayrao

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**Year of award of degree** : 2020-21

**THESIS ABSTRACT**

The present empirical study entitled “Investigations on potential *Trichoderma* spp. in Konkan region” was carried out during 2019-2021 at Department of Plant Pathology, Dr. BSKKV, Dapoli.

*Trichoderma* species are monarch anti-fungal bio-agents in the current era of eco-friendly plant protection because it has the power to recognize, infect and fight pathogenic fungi, insect pests, nematodes and other similar organisms. As a result, in the current investigation, out of sixty seven from various places in Konkan exhibited twenty-seven isolates of *Trichoderma* on TSM agar media.

All the isolates were found effective against *Fusarium* spp., *Rhizoctonia* spp., *Sclerotium* spp., *Colletotrichum* spp. and *Alternaria* spp. when tested by dual culture technique. Among them seven isolates viz., T<sub>11</sub> ((Rhizosphere) - Arecanut- (location) Shrivardhan dist. Raigad), T<sub>3</sub> (Mango- Lanja dist. Ratnagiri), T<sub>23</sub> (Brinjal-Karjat dist. Raigad), T<sub>5</sub> (Rice- Kolambe dist. Ratnagiri), T<sub>14</sub> (Guava- Kelwe dist. Palghar), T<sub>25</sub> (Cabbage- Karjat dist. Raigad) and T<sub>24</sub> (Brinjal- Mahim dist. Palghar) were showed better inhibition. T<sub>11</sub> was the most effective against *Fusarium* spp. (82.22% inhibition), T<sub>14</sub> against *Rhizoctonia* spp. (81.11%), T<sub>23</sub> against *Sclerotium* spp. (86.11%), T<sub>5</sub> against *Colletotrichum* spp. (81.33%), T<sub>25</sub> against *Alternaria* spp. (86.66%). The isolate T<sub>3</sub> ranked second in antagonism against *Sclerotium* spp. (80.54%), third in control of *Fusarium* spp. (79.22%) and *Colletotrichum* spp. (80.22%) and fifth in *Rhizoctonia* spp.

(71.11%) and *Alternaria* spp. (66.11%). As far as the antagonism performance of the isolate T<sub>24</sub> was ranked fourth against *Rhizoctonia* spp. (74.11%) and *Colletotrichum* spp. (78.33%), sixth against *Sclerotium* spp. (42.77%), seventh against *Fusarium* spp. (69.11%) and *Alternaria* spp. (63.66%).

All the isolates recorded above 50 per cent inhibition of all the pathogens except *Sclerotium* spp. In case of *Sclerotium* spp. most of the isolates recorded growth inhibition in the range of 15- 42 per cent.

Among the tested three systemic fungicides, Carbendazim was sensitive to all the isolates of *Trichoderma* and most detrimental with 100 per cent growth inhibition rest of the, 5 isolates were fairly compatible with Sulphur (2500 ppm) while COC and Mancozeb were major inhibitors of mycelial growth.

Based on morphological identification by using compound microscope under 100 x lens with software Micam 2.0 confirmed T<sub>25</sub> as a *Trichoderma* sp. aff. *T. longibrachiatum* Rifai., T<sub>5</sub>, T<sub>14</sub> and T<sub>24</sub> as a *Trichoderma* sp. aff. *T. koningii* Oudem. Morphological study viz., colony characters, colour, hyphal structure, phialides shape, conidial shape and arrangement and measurements of structure- phialide length, conidial diameter, conidial chain length and conidiophores L × B were carried out to identify the species. Beside these molecular characterization were carried out and deposited at NCBI, USA, with their accession numbers (T<sub>11</sub>-*Trichoderma asperellum*- BankIt2546015 NFCCI\_5020 OM471989; T<sub>3</sub>-*Trichoderma harzianum*-BankIt2546015 NFCCI\_5021 OM471990 and T<sub>23</sub>- *Trichoderma asperellum*- BankIt2546015 NFCCI\_5022 OM471991) there by concluded that, 7 indigenous *Trichoderma* isolates have promising antagonistic potential to combat against the five common plant pathogens in Konkan region.

वनस्पती रोगशास्त्र विभाग  
कृषी महाविद्यालय, दापोली

प्रबंध शीर्षक :	“कोंकण भागातील प्रभावी ट्रॅकोडर्मा प्रजातींचा अभ्यास”
विद्यार्थ्यांचे नाव :	कु. प्रविणा संजयराव बरडे
नोंदणी क्रमांक :	एडीपीडी/१८/०२९७
संशोधक मार्गदर्शकाचे नाव आणि पद :	डॉ. प्रमोद ग. बोरकर सहयोगी प्राध्यापक वनस्पती रोगशास्त्र विभाग कृषी महाविद्यालय दापोली -४१५७१२
पदवी पुरस्कार मिळण्याचे वर्ष :	२०२०-२१

प्रबंध सारांश

२०१९-२०२१ या कालावधीत डॉ. बी. एस. के. के. व्ही, दापोली येथील वनस्पती रोगशास्त्र विभागात : “कोंकण भागातील प्रभावी ट्रॅकोडर्मा प्रजातींचा अभ्यास” या विषयावरील अनुभवजन्य अभ्यास करण्यात आला.

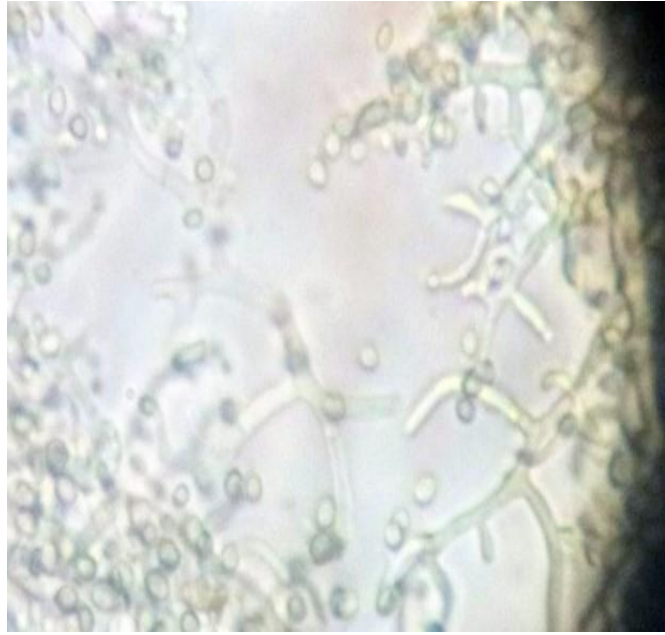
ट्रायकोडर्मा प्रजाती पर्यावरणस्नेही वनस्पती संरक्षणाच्या सध्याच्या युगात बुरशीविरोधी जैव-प्रतिनिधी आहेत कारण त्यात रोगजनक बुरशी, कीटक प्राणी, सूत्रकृमी आणि इतर तत्सम जीव ओळखण्याची, संक्रमित करण्याची आणि लढण्याची शक्ती आहे. परिणामी, सध्याच्या तपासात कोंकणातील विविध ठिकाणच्या साठपैकी सत्तावीस ठिकाणी टीएसएम आगर माध्यमावर ट्रायकोडर्माचे सत्तावीस आयसोलेट्स दाखवण्यात आले. दुहेरी संगोपन तंत्राद्वारे चाचणी केली असता सर्व आयसोलेट्स हे फुसेरियम एसपीपी, रायझोक्टोनिया एसपीपी, स्कलेरोटियम एसपीपी, कोलेटोट्रिचम एसपीपी आणि अल्टरनेरिया एसपीपी विरुद्ध प्रभावी असल्याचे आढळले. त्यामध्ये टी ११ (राइझोस्फीयर) सुपारी- (ठिकाण) श्रीवर्धन जि. रायगड), टी ३ (आंबा- लांजा जि. रत्नागिरी), टी २३ (वांगी-कर्जत जि. रायगड), टी ५ (तांदूळ- कोळंबे जि. रत्नागिरी), टी १४ (पेरू- केळवे जि. पालघर), टी २५ (कोबी- कर्जत जि. रायगड) आणि टी २४ (वांगी- माहीम जि. पालघर) या सात विभागांचा समावेश आहे. टी ११ फुसेरियम एसपीपी (८२.२२%), टी १४ विरुद्ध रायझोक्टोनिया एसपीपी (८१.११%), स्कलेरोटियम एसपीपी (८६.११%), टी ५ विरुद्ध कोलेटोट्रिचम एसपीपी (८१.३३%), टी २५ विरुद्ध अल्टरनेरिया एसपीपी (८६.६६%) सर्वात प्रभावी होते.

आयसोलेट टी ३ स्कलेरोटियम एसपीपी विरुद्ध दुसऱ्या क्रमांकावर आहे. (८०.५४%), फ्युसेरियम एसपीपीच्या नियंत्रणात तिसऱ्या (७९.२२%) आणि कोलेटोट्रिचम एसपीपी (८०.२२ %) आणि रायझोक्टोनिया एसपीपीमध्ये पाचव्या (७१.११%) आणि अल्टरनेरिया एसपीपी (६६.११%),

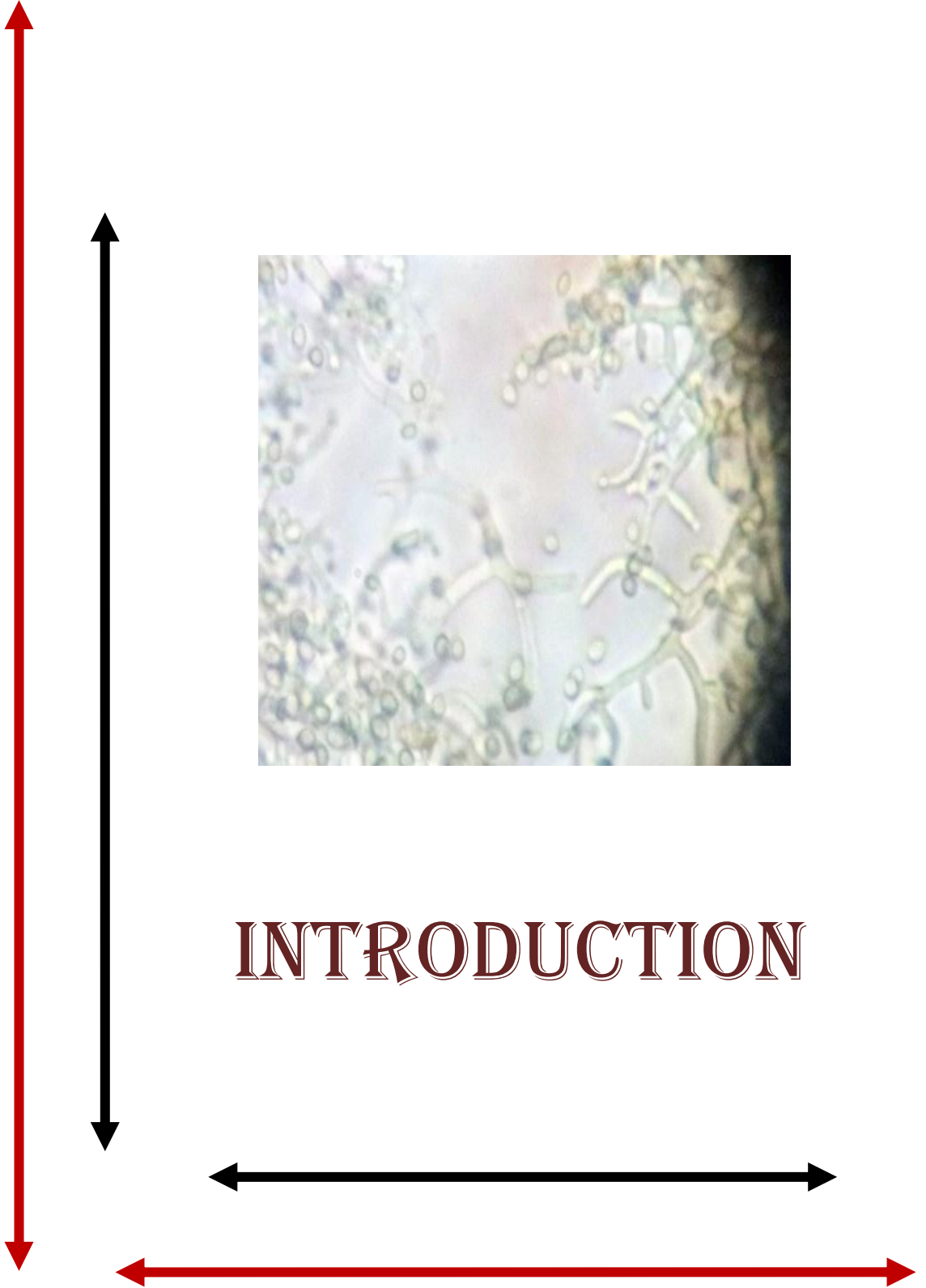


आयसोलेट टी २४ च्या प्रतिस्पर्ध्यांच्या कामगिरीचा विचार केला तर रायझोक्टोनिया एसपीपी (७४.११%) आणि कॉलेटोट्रिचम एसपीपी (७८.३३%), स्कलेरोटियम एसपीपी (४२.७७%), फुसेरियम एसपीपी (६९.११%) विरुद्ध सातवा आणि अल्टरनेरिया एसपीपी (६३.६६%) यांच्याविरुद्ध चौथ्या स्थानावर आहे. स्कलेरोटियम एसपीपी वगळता सर्व रोगजंतूंचा प्रतिबंध ५० टक्क्यांपेक्षा जास्त नोंदविला गेला. स्कलेरोटियम एसपीपीच्या बाबतीत, बहुतेक आयसोलेट्समध्ये १५ ते ४२ टक्क्यांच्या दरम्यान वाढीचा अडथळा नोंदविला गेला.

चाचणी केलेल्या तीन प्रणालीगत बुरशीनाशकांपैकी, कार्बेन्डाझिम ट्रायकोडर्माच्या सर्व आयसोलेट्ससाठी संवेदनशील होते आणि उर्वरित १०० टक्के वाढीस प्रतिबंध करणारे सर्वात हानिकारक होते, ५ आयसोलेट्स सल्फर (२५०० पीपीएम) शी बर्यापैकी सुसंगत होते तर सीओसी आणि मॅन्कोझेब हे मायसेलियल वाढीस प्रमुख अवरोधक होते. मीकॅम २.० या सॉफ्टवेअरसह १०० बाय लेन्सखाली कंपाऊंड मायक्रोस्कोप चा वापर करून मॉर्फोलॉजिकल आयडेंटिफिकेशनच्या आधारे टी २५ ला ट्रायकोडर्मा एसपी एएफएफ ला टी. लोंगीब्राचियाटम रिफाई. तर टी ५, टी १४ आणि टी २४ ट्रायकोडर्मा एसपी एएफएफ ला टी. कोनिंगी औडेम म्हणून पुष्टी दिली. प्रजाती ओळखण्यासाठी वसाहतीची वर्णे, रंग, हायफल रचना, फिआलाइड आकार, कोनिडायल आकार, मांडणी आणि संरचनेचे मोजमाप- फियालाइड लांबी, कोनिडायल व्यास, कोनिडायल साखळी लांबी आणि कोनिडिओफोर्स ची लांबी × रुंदी इत्यादी चा मॉर्फोलॉजिकल अभ्यास करण्यात आला. या आण्विक वैशिष्ट्यांव्यतिरिक्त एनसीबीआय, यूएसए येथे त्यांच्या प्रवेश क्रमांकांसह (टी ११-ट्रायकोडर्मास्पेरेलम-बँकआयटी २५४६०१५ एन एफ सी सी आई \_५०२० ओएम ४७१९८९) जमा केले गेले. टी ३-ट्रायकोडर्मा हर्झियानम-बँकआयटी २५४६०१५ एन एफ सी सी आई \_५०२१ ओएम ४७१९९० आणि टी २३ - ट्रायकोडर्मा एस्पेरेलम- बँकआयटी २५४६०१५ एन एफ सी सी आई \_५०२२ ओएम ४७१९९१) असा निष्कर्ष काढला आहे की, ७ स्वदेशी ट्रायकोडर्मा प्रजातींमध्ये कोकणातील पाच सामान्य वनस्पती रोगजनकांशी लढण्याची आशादायक विरोधी क्षमता आहे.



# INTRODUCTION



## CHAPTER – I INTRODUCTION

Uncontrolled use of chemicals in the form of fertilizers, plant growth regulators, weedicides, fungicides etc. has caused irreparable damage to the crop ecosystem. Resultantly, the present day agriculturists are switching towards organic agriculture which encompasses minimum use of chemicals. Most of the plant diseases, except viral diseases can effectively be managed by using of fungicides, bactericides and nematicides. But, in turn has led to the emergence of new races of plant pathogens which are genetically competent to resist the adverse effect of chemicals recommended for a particular disease. In such circumstances, use of effective bio-control agents against pathogens is an eco-friendly and affordable strategy to manage the diseases of crop plants.

The genus *Trichoderma* belongs to the family Hypocreaceae of order Hypocreales which belongs to the class Sordariomycetes of Phylum Ascomycota in Kingdom Fungi. Asexual reproduction occurs by formation of conidia (Steyaert *et al.*, 2010) and in some species sexual (Teleomorphic) stages (*Hypocrea* spp.) have been reported (Seidl *et al.*, 2009). The members of this genus are omnipresent and predominant in all types of agricultural soils over varied climatic zones. It has potential to colonize the plant parts above and below the ground. Hence they are found in plant litter and soil organic matter. In such eco-friendly era of plant protection, *Trichoderma* species are a king pin anti-fungal bio-agents as they have the ability to recognize, infect and annihilate fungi, insect pests, nematodes and other such organisms.

Among all the fungal bio-agents explored and tested so far for the plant disease management, the antagonistic potential of many members of the genus *Trichoderma* is of par excellence. Apart from their role as an antagonist, they support to enhance the crop growth which boosts up the natural defense system of the crop plants. In early period of bio-control approach, the *Trichoderma* were considered to be effective against soil borne plant pathogens but the recent trends indicate that they are also competent in aerial habitats. As a result about 60-65 per cent bio-pesticides are *Trichoderma* based. Some species have proved to be very efficient decomposers of plant debris.

The term *Trichoderma* has been derived from two words thrix (hair) and derma (skin). It is a group of free living filamentous fungi that reproduce asexually. *Trichoderma* species have been reported from diverse ecological niches all over the world. They are the strong opportunistic invaders, avirulent plant symbionts, resilient competitors as well as myco-parasites (Elad, 2000). They are commonly associated with plant roots, soil and plant debris, forest humus and orchids (Howell, 2003). The root colonization by *Trichoderma* spp. frequently enhanced root growth and development, crop productivity, resistance to a biotic stress and uptake and use of nutrients (Mukhopadhyay, 2005). As a root fungus its association stimulated plant defensive mechanisms induction of resistance metabolism similar to the hypersensitive response (HR), systemic acquired resistance (SAR) and induce systemic resistance (ISR) in plants.

Many members of the genus *Trichoderma* have been playing an important role in integrated disease management (IDM) practices. Some species can also cure a wide range of abiotic stresses such as temperature, salinity and drought and can improve photo-synthetic efficiency, nutrient uptake and nitrogen use efficiency in plants. In the present study, the experiments were carried out to understand the substantial differences between the collected strains from rhizosphere of major crops sown in different pockets of Konkan region. These will also be screened to assess their antagonistic potential against common plant pathogens.

The hot and humid climatic of Konkan are conducive for the growth, multiplication and dissemination of two aerial plant pathogens viz., *Colletotrichum* and *Alternaria* and two universal soil-borne fungi viz., *Rhizoctonia* spp and *Sclerotium* spp. frequently observed on cultivated and wild host seems to be responsible for huge crop losses over the globe.

In Konkan region anthracnose of mango incited by *Colletotrichum gloeosporioides* is one of the most devastating diseases which causes huge losses in pre and post harvest conditions. The pathogen initially infects tender and later the mature leaves as well. As it also has the ability to cause quiescent infection in the tender fruits through the fruit stalk. The actual damage is apparent only on ripen fruits i.e typical, sunken, brownish black spots on rind of the fruits. In severe cases there is blackening of the pulp which the fruit and market value. Apart from mango the host range of this pathogen in the region includes cashew, papaya, areca nut and banana. To cater the need

of farmers for the management of this serious disease through bio-agent is therefore, the need of time.

*Alternaria* spp. are also of common occurrence on solanaceous and cruciferous vegetables and flowering plants like marigold which are cultivated as subsidiary crops in many pockets of Konkan region.

Like most of the soils across the country, Konkan soils are also harbour the remarkable amount of Fusarial inoculum. It is, actually, a seed and soil borne fungus having a wide host range. It is one of the most destructive vascular diseases leading to serious economic losses, especially when grown without crop rotation. (Zitter 1998 ; Martin *et al.* 2006). Brinjal, cowpea, green gram, watermelon and black pepper are the major regional hosts of this pathogen. *Fusarium* being a soil borne pathogen, use of *Trichoderma* for soil application can be the best option for management of wilt diseases caused by this pathogen.

*Sclerotium* spp; is a soil borne pathogen which has enormous potential to cause huge losses in agricultural. It has wide host range encompasses at least 500 species in 100 families. The susceptible and the most common hosts are legumes, crucifers and cucurbits, mostly occurs in the tropics, subtropics, and other warm to temperate regions (Hemanth *et al.*, 2016).

Rice is the major cereal crop of Konkan. At present, sheath blight of rice is an emerging threat in the crop. The pathogens *Rhizoctonia solani* was reported earlier as the causal agent of leaf blight of cardamom. Management of this pathogen by soil application of *Trichoderma* will be worthwhile.

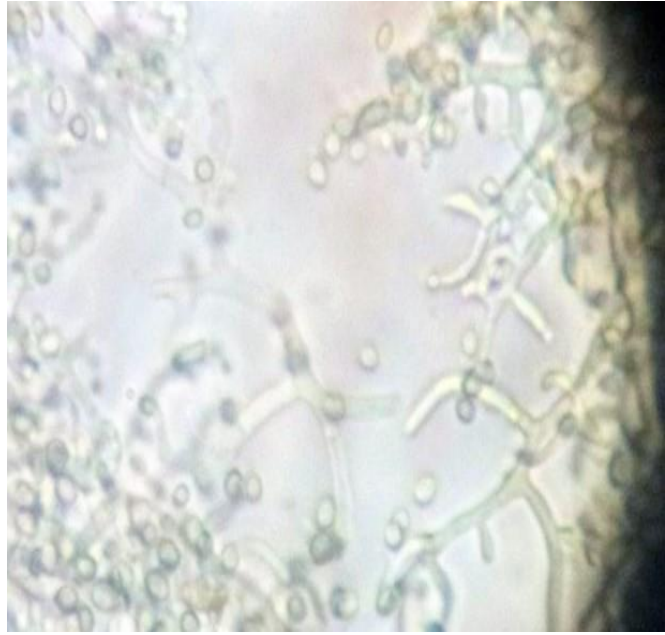
*Trichoderma* species are producers of lytic enzymes which actually lyse the fungal cell wall, enzymes like glucanase, chitinase, xylanase and cellulases (Srivastava *et al.* 2015). A great amount of *Trichoderma* spp. in various soils and coupled with a broad metabolic adaptability, a vigorous colonization of plant rhizosphere and the ability to antagonize and suppress a large number of plant pathogens are direct proof of the role that *Trichoderma* plays in biological control (Papavizas, 1985 and Chet, 1987).

Frequently experienced limitation in use of sole bio-agents for plant disease management is the establishment of the antagonist within a specific period of time under varied weather and field conditions. Therefore, after studying the role of antagonism of local *Trichoderma* isolates it is absolutely necessary to study the use and compatibility of

various strains with commonly used and recommended fungicides. The results of such experiments ascertain the sensitivity of the isolates which in turn enables us to decide the best combination of bio-agent and fungicide in order to plan appropriate IDM strategy.

Considering the importance of bio-agents in present day agriculture, the present study was planned and conducted with the following objectives.

1. Isolation of *Trichoderma* spp. from different soils of Konkan.
2. To test the antagonistic potential of promising isolates against the major plant pathogens in the region.
3. To study the morphological characters and molecular characterization of distinct promising isolates.
4. To test the compatibility of promising isolates with fungicides.



**REVIEW OF  
LITERATURE**



## CHAPTER-II

### REVIEW OF LITERATURE

The present study entitled “Investigations on potential *Trichoderma* spp. in Konkan region” was conducted to isolate, identify and assess the antagonistic ability of native *Trichoderma* species against common fungal pathogens of important crops in Konkan Region of Maharashtra. The taxonomy of *Trichoderma* dates back to Persoon in 1794 but its capability as a mycoparasite was revealed in 20<sup>th</sup> Century when *T. lignorum* was reported as parasite of other soil fungi (Weindling, 1932).

The literature related to the defined objectives of the present study, collected from all the possible sources has been reviewed and presented in this chapter.

#### **2.1. Isolation of *Trichoderma* spp.**

Siven *et al.* (1984) isolated *T. harzianum* from naturally infected soil by *Pythium aphanidermatum* caused and later used for control of damping off of tomato and pepper.

Kim *et al.* (1992) worked on isolation and identification of species of *Trichoderma* antagonistic to soil dwelling pathogens and their activities in the rhizosphere.

Pandey and Upadhyay (2000) isolated *T. harzianum* from pigeon pea rhizosphere and its antagonistic activity was tested against *Fusarium udum*.

Rahman *et al.* (2011) isolated *Trichoderma* species from different habitats such as soil, humus, kitchen waste, and compost from Rajshahi Bangladesh on Rose Bengal Agar. The identified strains were *T. harzianum* (IMI-392432, 392433, 392434); *T. pseudokoningii* (IMI-392431) and *T. virens* (IMI-392430). It was observed that, out of the five strains, *T. harzianum* was common in all the habitats.

In a study conducted by Kumar *et al.* (2011), twelve isolates of *Trichoderma* spp. were attained from different locations of South Andaman. The morphological characters of all the isolates were recorded and the cultural characteristics were examined on four different media viz., OMA, CMDA, PDA and TSM.

Ranganathaswamy *et al.* (2012) obtained two isolates of *Trichoderma* spp., namely *T. virens* (TV9) from citrus orchard and *T. harzianum* (Th4), from cotton ecosystem.



Bharti *et al.* (2016) isolated *T. harzianum* and *T. viride* from mustard leaf and checked their efficacy against *Alternaria* blight of mustard.

Kannangara *et al.* (2017) isolated ten different *Trichoderma* isolates from soil, litter and coir samples collected (but none of them identified from coir) from different locations in Lunuwila area in the North Western Province of Sri Lanka. Among them five were identified as *T. harzianum*, four were identified as *T. viride* and one as *T. polysporum*. All the isolates showed 60 per cent growth inhibition of *Ceratocystis paradoxa* causing stem bleeding on the seventh day of incubation into PDA.

Sekhar *et al.* (2017) isolated ten isolates of *Trichoderma* spp from rhizospheric soil of healthy plants in groundnut field.

Wu *et al.* (2017) isolated *T. asperellum*, a novel strain with high growth rate, high sporulation capacity, and strong inhibitory effects against cucumber *Fusarium* wilt and corn stalk rot, from Foshan, China. The culture was deposited under the name-GDFS1009. The isolate was cultured on rose bengal-agar medium containing 50 µg/ mL streptomycin and 50 µg/ mL chloramphenicol.

Soesanto (2018) isolated *Trichoderma* spp from rhizosphere of ginger, banana, pineapple and shallot.

Ali and Ramadan (2019) isolated *T. harzianum* from commercial formulation obtained from Central Agricultural Pesticide Laboratory and market Plant Guard Zagazig University, Egypt by serial dilution technique on PDA medium and tested the effect of chemical pesticides on radial growth and sporulation of the isolate.

Ashlesha (2019) isolated two isolates of *Trichoderma* from rhizosphere of maize crop cultivated in sandy loam soils in the fields of PAU, Ludhiana. The soil was low in organic carbon; and high in available nitrogen and potassium. The isolates were cultured both on potato dextrose agar and *Trichoderma* selective medium (TSM). Colonies were picked and purified by hyphal tip method and maintained on TSM medium which was identified as *T. harzianum*.

Kumar *et al.* (2019) collected composite soil samples from Kampur and Hardoi (UP) and obtained seven *Trichoderma* isolates. Out of these, two isolates were from chick pea rhizosphere, four from pigeon pea rhizosphere and one from lentils rhizosphere. These isolates were identified as *T. harzianum*; *T. asperellum*; *T. viride*; *T. longibrachiatum*; *T. koningii*; *T. virens* and *T. atroviride*.

Lalngaihawmi and Bhattacharyya (2019) collected soil samples from rhizosphere of different banana cultivars from Assam, Mizoram, Meghalaya and Nagaland. Potato Dextrose Agar (PDA) medium and *Trichoderma* Specific Medium (TSM) were used for isolation.

Naher *et al.* (2019) isolated six different *Trichoderma* species from rhizosphere soils of paddy, banana, oil palm, rubber, vegetables and grass land soils and the species were identified as *T. harzianum*, *T. viride*, *T. koningii*, *T. asperrelum*, and *T. parareesei*.

Yadav *et al.* (2020) isolated 21 *Trichoderma* isolates from banana rhizosphere of wilt suppressive and salt affected soils of Uttar Pradesh and evaluated for their antagonistic potential against *F. oxysporum* f. sp. *cumini* through dual culture assay.

## **2.2. Efficacy of promising isolates against the major plant pathogens in the region.**

*T. harzianum* was an effective biocontrol agent against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* under laboratory conditions (Chet *et al.* 1980; Elad *et al.* 1980).

The ability of *Trichoderma* to produce siderophores that hinder spore germination, kill the cells of plant pathogenic fungi and acidifying rhizosphere soil was demonstrated by Lorito (1994) while testing *Trichoderma harzianum* against *Fusarium oxysporum*. Further it was observed that *T. harzianum* found more effective at low nutrient concentrations.

While testing the efficacy of *Trichoderma* isolates against *C. capsici*, Jayalakshmi *et al.* (1998) reported that *T. viridae*, *T. harzianum* and *T. koningii* inhibited the pathogen mycelium by 51.7, 56.6 and 42.5 per cent.

Goudar and Kulkarni (2000) studied the antagonistic nature of *T. viride*, *T. harzianum*, *Aspergillus niger*, *A. flavus*, *Bacillus subtilis*, *Pseudomonas fluorescense*, *Penicillium* spp. and *Streptomyces* spp. against *F. udum* and found that per cent inhibition was more in *T. viride* (87.03%) followed by *T. harzianum* (85.40%), *P. fluorescense* (81.87%) and *Bacillus subtilis* (72.23%).

Barbosa *et al.* (2001) reported that the growth and sporulation rate of *T. harzianum* was higher in comparison to *C. gloeosporioides* and *C. acutatum* and it gives

a crucial advantage to suppress both the pathogens. Similar results were reported by many researchers (Bhuvaneshwari and Rao, 2001; Freeman *et al.* 2001; Gud, 2001).

Sindhan *et al.* (2002) tested antagonistic activity by using dual culture method of *Trichoderma viride* and *T. harzianum* against *Rhizoctonia bataticolain vitro* and observed that the antagonists inhibited the mycelial growth as well as sclerotial production.

Kaswate *et al.* (2003) revealed that *T. viride* was the most effective in inhibiting various isolates of *Rhizoctonia bataticola* (100%) followed by *B. subtilis* (87.41%) and *P. fluorescens* (73.98%).

Under *in vitro* evaluation of *T. viride* and *T. harzianum* against *Fusarium oxysporum* f. sp. *sesame*, Sangle and Bambawale (2004) reported that *T. viride* and *T. harzianum* reduced the growth (inhibition) of *Fusarium oxysporum* f. sp. *sesame* with 83.18 and 79.54 per cent over control after 7 days of inoculation.

Yadav *et al.* (2005) reported that inhibition of mycelial growth of *F. udum* due to *T. viride* was 70.5 per cent as compared to *T. harzianum* (62.5%) after 7 days of incubation.

Dhar *et al.* (2006) evaluated *T. viride* (TNAU, Coimbatore), *T. harzianum* (GBPUA & T, Pant Nagar) and *Gliocladium virens* (GBPUA & T, Pant Nagar) against ten isolates of *F. udum* obtained from research farm of IIPR, Kanur and observed that after 96 hrs of incubation all the three bio-agents have uniform antagonistic activity against the isolates, where the colony diameter were between 35.5-54.8 mm against *T. viride*, 36.4-54.7 mm against *T. harzianum* and 36.4-57.3 mm against *G. virens*.

Mamatha and Yashoda (2006) demonstrated that, *T. koningii* was more potent than *T. harzianum* against *C. capsici* and *Alternaria alternate* as the former recorded 77.43 per cent and 80 per cent inhibition of *C. capsici* and *A. alternata* respectively while the latter recorded 76.30 per cent and 75.20 per cent inhibition of the test pathogens.

Honmane (2007) found that *T. viride* was the most suppressive with 87.41 per cent inhibition, of *F. moniliforme* causing anthurium wilt and it was followed by *T. harzianum* (86.85%), *T. koningii* (83.33%) and *T. ligorum* (81.87%) and in case of *Colletotrichum gloeosporioides*, maximum per cent inhibition was achieved with *T.*

*viride* (90.74%) followed by *T. koningii* (80.74%), *T. harzianum* (75.19%) and *T. lignorum* (74.63%).

Raul (2007) studied the efficacy of bio-agents against *C. gloeosporioides* inciting leaf spot of cinnamon. The maximum inhibition (86.11%) inhibition was recorded by *T. harzianum* and it was at par with *T. viride* (85.33%)

Gupta *et al.* (2008) studied the effect of three bio-agents viz., *T. viride*, *T. harzianum* and *G. virens* in comparison with the recommended fungicide Mancozeb (0.1%) for the management of anthracnose of bottle gourd. Results revealed that *T. viride* gave maximum mycelial growth inhibition (59.08%) as compared to control.

*Trichoderma* spp. produces secondary metabolites and enhancing antibiotic activity. These secondary metabolites inhibit microbial growth during microbial development and sporulation. The range of secondary metabolites secreted by *Trichoderma* dependent on volatile and nonvolatile antifungal substances (Vinale *et al.* 2008; Vinale *et al.* 2009, Schuhmacher *et al.* 2007).

Jayalakshmi *et al.* (2009) studied that isolates of *Fusarium oxysporum* f. sp. *ciceri* from Junagarh, Jabalpur and Dholi were highly sensitive to *T. viride* with 85 per cent inhibition.

Amin *et al.* (2010) tested six isolates of *Trichoderma* spp. viz., *T. virens* (Ts-1), *T. harzianum* (Th-1), *T. harzianum* (Th-2), *T. viride* (Tv-1), *T. viride* (Tv-2) and *T. viride* (Tv-3) tested against *R. solani* and *Sclerotium rolfsii* wherein *T. viride* (Tv-2) showed maximum inhibition (71.41%) of *R. solani* followed by *T. viride* (Tv-1- 65.71%) and *T. harzianum* (Th-1-60.51%). Same sequence of antagonistic potential was observed in case of *S. Rolfsii*.

Madhusudhan *et al.* (2010) evaluated efficiency of *Trichoderma viride* isolates viz., T2 and T4 against *Fusarium solani* and found effective against *F. solani* with 62.82 per cent inhibition.

Zivkovic *et al.* (2010) tested *in vitro* antagonistic activities of five biocontrol agents like *Trichoderma harzianum*, *Gliocladium roseum*, *Bacillus subtilis*, *Streptomyces noursei* and *Streptomyces natalensis* against *Colletotrichum acutatum* and *C. gloeosporioides*, the causal agents of anthracnose disease in fruit crops and observed that *T. harzianum* inhibited mycelial growth of *Colletotrichum* isolates with mycoparasitism behaviour, observed coiling, penetration, direct contact and parallel growth alongside the

host hyphae and coiled compactly around the hyphae of *C. acutatum* and *C. gloeosporioides*.

Jamwal *et al.* (2011) recorded three bioagents viz., *T. harzianum*, *T. viride* and *Pseudomonas fluorescens* under *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt in tomato and found that the *T. harzianum* showed highest inhibition (77.30%) followed by *T. viride* (75.00%) and *P. fluorescens* (66.15%).

Kumar *et al.* (2011) isolated twelve isolates of *Trichoderma* spp. (TSD1, TWN1, TGN1, TWD1, TND1, TJP1, TWC2, TWC1, TWP1, TCC1, TGD1, TBC1) from the rhizosphere of spice crops cultivated at different locations of South Andaman and revealed that isolates had varied reaction patterns against soil borne pathogen *Sclerotium rolfsii*. The isolates, TND1, TWN1, TWC1, TGD1 and TSD1 were the most effective in per cent inhibition against the test pathogen. Two isolates TGD1 and TWN1 (*T. viride* and *T. harzianum*) showed statistically significant inhibition of mycelial growth (76.3%) over control (80%) against *S. rolfsii* followed by TND1, TBC1, TCC1, TGN1 and TWC2 (*T. erinaceum* (73.3), *T. brevicompactum* (70.8), *T. ovalisopum* (72.5), *H. lixii* (70.8) and *T. harzianum* (72.1%).

Mishra *et al.* (2011) isolated seventeen isolates of *Trichoderma*, from different region of Allahabad district. Among seventeen isolates of *Trichoderma* sp. four isolates were identified as *Trichoderma viride* name as Tr3, Tr8, Tr12 and Tr14. These isolates were tested against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium solani* and *Colletotrichum capsici* of Moong bean (*Vigna radiata*) for antagonism, by using dual culture techniques. The isolate Tr 8 showed 70.00, 68.20, 70.00, 73.30, 69.30 and 70.1 per cent growth inhibition against *R. solani*, *S. rolfsii*, *M. phaseolina*, *A. alternata*, *F. solani* and *C. capsici* respectively and cell free culture filtrate of *T. viride* Tr 8 showed 61.5, 58.32, 63.45, 62.62 per cent radial growth at 10 per cent concentration against *R. solani*, *S. rolfsii*, *M. phaseolina*, *C. capsici* sequentially. Mycelial growth inhibition 100 per cent occurred at 20 per cent concentration.

Sreedevi *et al.* (2011) screened and evaluated the effect of *Trichoderma* spp. for biocontrol of *Macrophomina phaseolina* the causative agent of root rot of groundnut hence, isolated five *Trichoderma* spp. from the rhizosphere soil of healthy groundnut plants, identified using morphological and microscopic characteristics and evaluated for

*in vitro* antifungal activity against *M. phaseolina* with dual culture plate technique and bioassay methods (*in vitro* antibiosis). Among all isolates *T. harzianum* (T<sub>3</sub>) and *T. viride* (T<sub>1</sub>) had maximum antifungal activity against *M. phaseolina* and reduced mycelial growth by 61.10 per cent and 64.40 per cent.

Patel *et al.* (2011) *in-vitro* efficacy eleven different isolates of *Trichoderma* against *Fusarium udum* revealed maximum inhibition (88.77%) of growth due to *Trichoderma* spp. (Sardar krushi nagar) isolate. On the other hand local isolate of *T. harzianum* (L1) was most promising which showed maximum inhibitory effect on mycelial growth (88.69%) of *F. udum*. Soil application of talc based preparation of this potential isolates was proved better to seed treatment. The *in vivo* application of talc based preparation recorded the highest reduction in wilt.

Jat and Agalave (2013) isolated two strains of *Trichoderma* species *i.e.*, *Trichoderma harzianum* and *T. viride* from soil and also pathogenic fungilike *Alternaria alternata*, *Curvularia lunata*, *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium oxysporum* from rhizospheric soil of groundnut, soybean, sesame, sunflower and safflower on PDA medium. In Antagonistic activity of *T. viride* showed highest inhibition against *A. alternata* (68.91%) followed *T. harzianum* (48.33%) whereas it was, 50.00 per cent inhibition against *C. lunata*, *T. viride* showed highest inhibition towards *Fusarium oxysporum* with 50.00 per cent followed by 47.50 *T. harzianum*, *T. harzianum* showed highest inhibition towards *Fusarium moniliforme* with 50.00 per cent followed by *T. viride* 46.42 per cent and *T. harzianum* showed highest inhibition towards *Macrophomina phaseolina* with 48.75 per cent inhibition followed by *T. viride* *i.e.*, 46.34 per cent and hence *Trichoderma* species found to be effective biocontrol against various oilseed-borne pathogenic fungi.

A study conducted by Akinyi (2014) to evaluate virulence efficacy of local isolates *viz.*, *Trichoderma koningii*, *T. asperellum*, *T. atroviride*, *T. reesei*, and *T. harzianum* were isolated from Embu soils to check the ability to inhibit the mycelial growth of *Fusarium oxysporum* f.sp. *phaseoli*, (*in vitro*). It was found that *Trichoderma reesei*, had the highest effect in inhibition of mycelial growth (60.0%) of *Fusarium oxysporum* f. sp. *phaseoli*, followed by *Trichoderma koningii* (55.2%). After coating the maize seeds with the *Trichoderma* inoculums, highest rate of seedling emergence *i.e.*, 84 per cent was also observed. It was concluded that, *Trichoderma reesei* and *Trichoderma*

*koningii* were identical in their biocontrol potential for the use as biological control agents.

Magar *et al.* (2014) studied various isolates of *Trichoderma* from rhizosphere soil of chickpea from Marathwada region and observed that *T. viride* (Parbhani isolate) showed highest mycelial inhibition (66.35%), followed by *T. harzianum* (Beed isolate) (63.75%) and *T. koningii* (Aurangabad isolate) (60.22%) against *Fusarium* wilt.

As per the observations of Bhale and Rajkonda (2015) all *Trichoderma* species inhibited the mycelial growth of *A. alternata* while maximum inhibition showed by *T. harzianum* (90.40%) followed by *T. koningii* (77.70%) inhibition. *T. koningii* was highly antagonistic over *R. solani* with 67.7 per cent inhibition followed by *T. viride* 51.1 per cent inhibition. Significant antagonism was exhibited by *T. virens* (70.00% inhibition) against *Fusarium oxysporum* f. sp. *spinaciae* followed by *T. harzianum* and *T. pseudokoningii* i.e, 66.6 and 62.2 per cent inhibition respectively *M. phaseolina* was antagonized and inhibited significantly by *T. viride* (84.4%) followed by *T. harzianum* (83.3%) and *T. pseudokoningii* (81.1%). It was noticed that the growth of pathogenic fungi was retarded in presence of radial growth and sporulation. Also observed that mycelium of *Trichoderma* species when comes in contact with pathogenic fungi it showed fungistatic effect.

Korat and Priya (2015) tested ten isolates of *Trichoderma* against soil borne pathogens viz. *S. rolfisii*, *Macrophomina phaseolina*, *Fusarium udum*, *F. moliniformae* and *F. oxysporium* f. sp. *lycopersici* employing dual culture technique. *T. harzianum* isolate THO showed maximum per cent inhibition of mycelial growth of *S. rolfisii* (52.68%), *M. phaseolina* (76.67%), *F. udum* (74.44%) and *F. oxysporium* f. sp. *lycopersici* (70.00%) while in the case of *F. moliniformae*, *T. viride* isolate, TVKN showed maximum growth inhibition (73.33%).

Lopez *et al.* (2015) showed that the two species of *Trichoderma* *T. sp2* and *T. sp1* were able to suppress the growth of *S. rolfisii* by 34.92 per cent and 31.44 per cent respectively. Per cent growth inhibition was based on the scale of 2 which indicated moderate inhibition.

Swathi *et al.* (2015) investigated *in vitro* antagonistic potential of two isolates viz., Th4 of *T. harzianum* and Tv5 of *T. virens*, available in the Department of Plant Pathology, Agricultural College, Bapatla, against *S. rolfisii* and studied the effect of their

volatile and nonvolatile metabolites on the growth of *S. rolfsii*, *in vitro*. It was revealed that, *Trichoderma* isolate Th4 showed fast radial growth including parallel lysis and over growth on *Sclerotium rolfsii*. On the otherhand *T. virens* Tv5 exhibited slow radial growth over *S. rolfsii*

In the investigation done by Tapwal *et al.* (2015), two *Trichoderma* species, *T. viride* and *T. harzianum* were screened against five seed borne phytopathogens *viz.*, *Curvularia lunata*, *Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Rhizoctonia solani* by dual culture technique and the efficacy of volatile compounds released by them was evaluated by 'inverted plate method' and both the antagonists showed inhibitory effect on the growth of seed borne phytopathogens. On the third day of inoculation *T. harzianum* showed maximum growth inhibition (34.20%) against *A. alternata* followed by *F. oxysporum* (27.04%), *C. lunata* (25.64%), *C. gloeosporioides* (15.00%) and minimum for *R. solani* (5.10%), while on the other hand *T. viride* showed highest growth inhibition against *C. lunata* (46.79%) followed by *A. alternata* (34.27%), *F. oxysporum* (15.80%), *C. gloeosporioides* (12.50%) and lowest for *R. solani* (1.45%).

*Trichoderma asperellum* (11 isolates), *T. harzianum* (11 isolates), *T. pseudokoningii* (11 isolates) and *T. longibrachiatum* (10 isolates) were used *in vitro* against *Alternaria solani* and *S. sclerotiorum* by Prabhakaran *et al.* (2015). It was observed that all the isolates of the four species of *Trichoderma* were highly antagonistic against the test pathogens. *T. longibrachiatum* isolates T1-06 and T1-07 showed maximum mycelial inhibition of *A. solani* (87.60% and 84.75% respectively). On the other hand *T. pseudokoningii* (Tp-08: inhibition -97.80%) and *T. longibrachiatum* (T<sub>1</sub>-09: inhibition - 93.30%) were the utmost efficient antagonists against *S. sclerotiorum*.

Bharti *et al.* (2016) performed their *in vitro* assay of antagonistic potential of *T. viride* and *T. harzianum* against *Alternaria brassicae* and observed the highest inhibition (55.07%) by *T. harzianum* followed by *T. viride* (51.90%).

Cherkupally *et al.* (2016) evaluated the antagonistic activity of seven *Trichoderma* species, and two *Penicillium* species under *in vitro* conditions against brinjal root rot pathogen (*Macrophomina phaseolina* (Tassi) Goid). Among the seven *Trichoderma* species, *T. harzianum* showed maximum per cent inhibition (77.77%) followed by *T. pseudokoningii* (74.44%); *T. koningi* (72.22%); *T. virens* (70.00%), *T. viride* (70.00%), *T. reesei* (70.00%) and *T. atrovireide* (66.66%).



The isolate of *T.harzianum* and *T.viride*, one isolate of *T. viride* along with three unidentified isolates of *Trichoderma* were evaluated against *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *Sclerotiana sclerotiorum* by Elshahawy *et al.* (2016). The isolate of *T. harzianum* designated as Th2 caused maximum growth reduction (66.7%) of *F. solani* while Th1 was remarkably effective against *F. oxysporum* (58.2%) and against *M. phaseolina* (41.5%). In case of *R. solani*, maximum growth inhibition was obtained by Tvr (51.1%). The isolate Tv1 performed the best (88.9%) against *S. sclerotiorum*.

Khaledi and Taheri (2016), investigated biological control capability of 5 isolates of *T. harzianum* and 6 isolates of *T. viride* against *M. phaseolina*. Among these, the *T. harzianum* isolate designated as T<sub>7</sub> (inhibition-58.7%) and T<sub>14</sub> (inhibition-57.3%) were the most effective.

In a similar experiment carried out by Kumar and Sharma (2016) five isolates of *T. harzianum* and seven isolates of *T. viride* were tested against *Pythium aphanidermatum* and *S. sclerotiorum*. The results revealed that *T. harzianum* isolates were more aggressive as compared to *T.viride*. Isolate Th3 of *T. harzianum* recorded 90.2 per cent inhibition of *S. sclerotiorum*.

Boat *et al.* (2018) conducted a study to evaluate the antagonistic activity of six *Trichoderma* spp against *S. sclerotiorum* responsible for white mold of common bean. The results revealed that the mycelial growth inhibition of *S. sclerotiorum* was ranged between 83.4 and 87.4 per cent. The highest inhibition (87.4%) was recorded by *T. erinaceum* while the lowest inhibition (83.4%) was caused by *T. koningiopsis*.

Kushwaha *et al.* (2018) conducted an experiment wherein antagonistic activity of three *Trichoderma* species viz., *T.viride*, *T. virens* and *T. harzianum* was tested against *Sclerotium rolfisii* under *in vitro* conditions. The results proved that all the three species were effective against the pathogen but *T. harzianum* (inhibition: 63.60%) was the best followed by *T. virens* (51.5%) and *T. viride* (50.85%) after 72 hours of inoculation. The reduction in mycelial growth adversely affected sclerotia formation. *T. viride* (91.31%) showed the highest reduction followed by *T. harzianum* (84.92%) and *T. virens* (84.29%) after 15 days of inoculation.

Parellel results were reported by Priyadharcini *et al.* (2018). The researchers assessed the antagonistic activity of three isolates of *Trichoderma* such as *T. harzianum* (TspT), *Trichoderma* sp. (TspK) and *T. viride* (Tv1) against *S. rolfisii*. In the order of

merit, *T. harzianum* recorded the maximum inhibition (81.27%) followed by unidentified *Trichoderma* (Tspk) 71.91% and *T. viride* (66.29%).

Naher *et al.* (2019) used the six *Trichoderma* species viz., *T. harziaum*, *T. viride*, *T.koningii*, *T. asperellum*, and *T. parareesei* in their experiment to show antagonism against *F. oxysporum*. The highest mycelial growth inhibition of *Fusarium* was seen by *T. parareesei* (91.10%) which was followed by *T. harzianum* (76.09%), *T. asperellum* (74.16%), *T. virens*(73.17%), *T. koningii*(71.40%) and *T. viride* (70.65%).

Similar study conducted by Sreenayana *et al.* (2019) revealed that among the six isolates of *T. virens* assessed, the isolate TR137 recorded the highest i.e; 69.11 per cent mycelial inhibition of *F. oxysporum* f. sp. *cucumerinum* followed by TRI41 (66.89%), TRI15 (62.00%), TNAU-Tad1 (60.89%), TRI 35 (60.22%), TRI36 (56.22%), and TRI44 (54.00%).

Forty *Trichoderma* isolates were collected from East Java, Indonesiaby Yusnawan *et al.* (2019). Amongst these, seven isolates viz, T20A, T19A, TPA1, TAt1, T16A, T15C and T20B were found to be potentially antagonistic to *R. solani* (82.4%) and *Fusarium* spp. (99.60%) mycelial growth inhibition.

Xue *et al.* (2021) obtained 1308 *Trichoderma* strains from three distinct natural habitates such as rhizosphere, above ground plant parts and decaying wood logs. Among these, 49 isolates were found antagonistic to two test pathogens i.e; *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. All the isolates inhibited 85-90 per cent mycelial growth of both the pathogens.

## **2.3. Study of the morphological characters and molecular characterization of distinct promising isolates**

### **2.3.1. Morphological characterization of distinct promising isolates.**

In order to study the morphological characteristics of *Trichoderma* species Grace (2016) cultured the isolates on PDA and Rose Bengal Agar Medium.

The research findings describe the cultural and morphological characters of *T. asperellum* and *T. harzianum* in which he states that, the conidiophores of *T. asperellum* form paired primary branches which are nearly at 90<sup>0</sup> to the main axis. The phialids formed in whorls are normally flask shaped while the solitary phialids usually cylindrical and sharply constricted at the tips. The mycelium is white initially and turns green after conidiation which occurs within 4-5 days. The reverse colony colour is pale yellow. Globose chlamydospores are formed on the hyphal tips within a week's period.

The description of *T. harzianum* states that, the mycelium develops in the form of white concentric rings and speedily covers the top of a solid medium. The margin of the colony is markedly wavy. Though, on PDA medium the mycelium reaches the rim of Petri plate within 4 days, the medium does not support conidiation. Profuse conidiation occurs on Rose Bengal Agar Medium. The pairing of branches and formation of phialids in whorls is similar to *T. asperellum* but the phialids are short, inflated and lateral branches of conidiophores are also short. Conidia are also small in comparison to *T. asperallum*.

Kumar and Sharma (2016) carried out morphological characterization of 5 isolates of *Trichoderma harzianum* and 7 isolates of *T. viride* and observed that the isolates belongs to *T. harzianum* were similar in colony colour, culture smell, mycelial colour, conidiation, conidial wall, conidial colour and conidial shape. Likewise the isolates of *T. viride* displayed definite similarity in colony colour, colony edge, culture smell, conidiophores branching, conidial colour, conidial wall and chlamydospores. Based on morphological characters inter specific differences were performed via cluster analysis grouped the twelve isolates into three major clusters where all the isolates of *T. harzianum* formed a single cluster while *T. viride* were bifurcated into two groups. The clustering was substantiated by similarity index which denoted maximum similarity among *T. harzianum* isolates with only less than 20 per cent variation among them. Comparably the clusters having isolates of *T. viride* had a smaller amount of variation within them.

While working on biocontrol potential of *Trichoderma* isolates against *Ceratocystisparadoxa*, Kannangara *et al.* (2017), studied the colony characters and morphological features of 10 *Trichoderma* isolates which were grouped into three species *T. harzianum*, *T. viride* and *T. polysporum*. Out of 10 isolates 5 were of *T. harzianum*. Their morphological characters comprising the dimensions (40 x magnification) of spores, phialids and mycelium are considered here. The overall spore dimensions (LXB) ranged between 5.1 X 2.5  $\mu\text{m}$ ; phialids 8.25 X 3.58 and mycelial width ranged between 3.70-5.20  $\mu\text{m}$ .

Sekhar *et al.* (2017) obtained ten isolates of *Trichoderma* spp from groundnut rhizosphere soil samples. The isolates were identified upto species level on the basis of morphological characters and colony characters. Out of these 10 isolates 3 were of *T. koningii*; 1 each of *T. harzianum* and *T. aureoviride*; 3 of *T. viride* and 2 of *Trichoderma reeseii*.

The *T. harzianum* colony on PDA was white to light green in colour initially with white, wavy ring like zones on the reverse side of the Petri plate. The colonies grew rapidly, with 7 to 8 cm colony diameter within 5 days. Microscopic examination revealed that conidiophores were highly branched and formed loose tufts, phialides which were short-skittle shaped, bulged in the centre and narrowed at the lower end. The phialids admeasured 7.2–11.2  $\times$  2.5–3.1  $\mu\text{m}$ . Phialospores were subglobose to ovoid with truncate base, smooth walled and measured 2.8–3.2  $\times$  2.5–2.9  $\mu\text{m}$  (Kumar *et al.* 2019).

Naher *et al.* (2019) reported that, the colonies of *T. harzianum* were initially white and gradually changed to yellowish green and finaaly dark green. Conidiophores were fomed in pairs with lateral branches at a right angle to the main axis. Phialids were typically elongated and lageniform. Conidia were subglobose to globose. In case of *T. asperrelum*, the phialideswere solitary or held in whorls of two to three. Conidia were globose, obovoid, dark green and smooth. The phialids of *T. koningii* were also lageniform, while conidiophores were branched and erect.

### **2.3.2. Molecular characterization of *Trichoderma* isolates.**

Detailed identification of common industrially and agriculturally important fungi like *Trichoderma* species is challenging due to the rapid augmentation in microbial taxonomy.

The chromatographic image analysis by high performance liquid chromatography with the help of UV detection of culture extracts were used for the identification of *Trichoderma* strains from water-damaged building materials or indoor dust (Thrane *et al.* 2001). The classes were compared with morphological identification and rDNA sequence data, and in the case of each class all strains had the same identity. With all those three techniques each strain except one was identified as the same species and belonged to *Trichoderma atroviride* (nine strains), *T. viride* (three strains), *T.harzianum* (ten strains), *T. citrinoviride* (twelve strains), and *T.longibrachiatum* (nine strains). One of the odd strain was identified as *T. hamatum* by its morphology and rDNA sequencing, but not by image analysis as there was no reference strains of this species were added and finally concluded that the secondary metabolite profile contains sufficient information for future classification and species identification.

The occurrence and biodiversity of *Trichoderma* spp. from undisturbed soil ecosystems from protected areas in Saudi Arabia were studied by Abd-Elsalame *et al.* (2010). On the basis of its morphology, the seven fungal isolates were identified as *Hypocrea/Trichoderma* species, arising out of section *Trichoderma*, and the species were *H. lixii/T. harzianum* and *H. orientalis/T. longibrachiatum*. In order to confirm the identification of two species of *Trichoderma*, PCR-based markers with primer M13 (core sequence of phage M13) and internal-transcribed spacer sequences of ribosomal DNA were used. By using the TrichOKEY version 2.0 barcode programme and the multi loci likeness search database, TrichoBLAST concluded sequence identification. Sequences from the ribosomal DNA internal-transcribed spacer regions denoted limited variation amid the species and due to this analysis the isolates were split into two groups. Grouping the isolates was mainly based on cluster analysis of their DNA profiles which matched the grouping based on morphological taxonomy. The Molecular data obtained from the analyses of gene sequences played a key role to distinguish phonetically cryptic species in this group as well as to establish phylogenetic relationships.

The characterization of seven isolates of *Trichoderma* using RAPD-PCR procedure was done by Siameto *et al.* (2011) and determined their genetic variability. It was observed that the Jacquard's coefficient of similarity ranged from 0.231 to 0.857 for isolates 055E, 011E, 010E and 015E. The four random primers (203, 230, 220 and 0p13) used in the study, depicted the bands ranging from 350bp to 2000bp. All such intense bands produced summed up to 81. The 7 samples used for DNA polymorphism were

superficially antagonistic to the phyto-pathogens used in the study. Among the seven isolates used for molecular characterization (044E, 011E, 015 E, 051E, 055E, 010E and 029E), in dual culture experiment, the isolate 015E was highly antagonistic (inhibition-88.52%) to *F. oxysporum* f.sp. *lycopersici*; the isolate 029E was extremely antagonistic (inhibition-97.86%) to *F. oxysporum* f.sp. *phaseoli*, isolate 011E was tremendously antagonistic to *F. graminearium* (92.16%) and *Rhizoctonia solani* (90.85%). None of the isolate was able to hinder the growth of *Pythium* sp.

The DNA profiles of *Trichoderma harzianum* isolates were scored and a dendrogram was developed using Squared Euclidean Distance and Clustering on the basis of Ward's method. It was found that in the Dendrogram, all the isolates were patently divided into two major clusters A and B at 20 units. Isolate 051E and 029E covered the extremes of the entire Dendrogram. Genetic dissimilarity ranged from a minimum of 0.143 (between T010 and T015) to a maximum of 0.857 (between 055E and 051E). Isolate 051E, T011, T015, and T010 were assigned to cluster A. Genetic dissimilarity among the entries in this cluster ranged from a minimum of 14.3 per cent (between T015 and T010) to a maximum of 35.7 per cent (between T010 and 051E). The other cluster B comprised of three accessions and here cluster isolate 044E, 055E and 029E were grouped together. The genetic dissimilarity ranged from 33.3 per cent between 055E and 029E to a maximum of 75 per cent between 044E and 029E.

Oskiera *et al.* (2015), collected 104 strains of *Trichoderma* from geographically different locations in Poland and identified them by DNA barcoding, based upon the sequences of internal transcribed spacers 1 and 2 (ITS1 and 2) of the ribosomal RNA gene cluster and on the sequences of translation elongation factor 1 alpha (*tef1*), chitinase 18-5 (*chi18-5*), and RNA polymerase II subunit (*rpb2*) gene fragments. Most of the identified strains were classified as: *T. atroviride* (38%), *T. harzianum* (21%), *T. lentiforme* (9%), *T. virens* (9%), and *T. simmonsii* (6%). Single strains belonged to *T. atrobrunneum*, *T. citrinoviride*, *T. crassum*, *T. gamsii*, *T. hamatum*, *T. spirale*, *T. tomentosum*, and *T. viridescens*.

The study also revealed two strains (*T. pleuroticola* and *T. aggressivum* f. *europaeum*) that were pathogenic to cultivated mushrooms. Four strains *i.e.*, TRS4, TRS29, TRS33, and TRS73 were classified only upto species level as the molecular identification was inconclusive at the species level.

Prabhakaran *et al.* (2015) isolated different isolates of *Trichoderma* from soil samples collected from different region of India. The isolates were confirmed through Internal Transcribed Spacer (ITS) region analysis, by using the region of nuclear ribosomal DNA in phylogenetic analysis at generic and intra-generic levels. The isolates were identified as *T. asperellum* (Ta), *T. harzianum* (Th), *T. pseudokoningii* (Tp) and *T. longibrachiatum* (Tl).

Zhu *et al.* (2017) identified 287 isolates of *Trichoderma* by using morphological and molecular identification techniques. In molecular methods, mostly DNA sequencing and analysis of the 5.8S ribosomal DNA internal transcribed spacer region (ITS1-5.8S-ITS2), part of the nuclear translation elongation factor gene (*TEF1- $\alpha$* ), and the second largest RNA polymerase II subunit (*RPB2*) were used.

Priyadharcini *et al.* (2018), isolated a *Trichoderma* species from *Sclerotium rolfsii* infected soil and confirmed it as *T. harzianum* by using ITS 1 and ITS 4 primers and comparing ITS sequence of the isolate with BLAST sequence in NCBI data base.

Seven different species of *Trichoderma* were identified by Kumar *et al.* (2019), and studied their morphological characteristics, cultural characteristics and molecular identification. In molecular identification of *T. harzianum* isolate Th Azad/CSAU 6796, the observed Locus was KC800922. The isolate had 18S ribosomal RNA gene, partial sequence; internal transcribed spacer (ITS) 1, 5.8 S ribosomal RNA gene, and ITS4, partial sequence, primers used ITS1-AGAGTTTGATCCTGGCTCAG and ITS4-GGTTACCTGTTACGACTT, sequence was 546 bp.

On the other hand *T. asperellum* Tasp (CSAU)-8940 the observed locus was KC800921. The ribosomal RNA, partial sequence ITS were similar to *T. harzianum* and used were ITS1-TCCGTAGGTGAACCTGCGG and ITS2-TCCTCCGCTTATTGATATG including sequence 1200 bp.

Jankar *et al.* (2020), studied molecular variability among the six isolates of *T. viride* collected from different region of Maharashtra by using 16 RAPD primers of OPA (OPA 2, 3, 5, 6, 9,10, 11, 14, 16 and 18) series. The observations of the study revealed that, 78 score able bands were formed out of 10 primers. Among these bands 76 bands were polymorphic and the level of polymorphism was about 97.32 per cent. Further it was concluded that the isolates Tv2 (Pune) and Tv5 (Sangali) were at similitude as they recorded higher value of similarity coefficient (0.400). However, similarity coefficient (0.087) of Tv1 (Akola) and Tv4 (Amravati) isolates with the isolate Tv2 (Pune) was very low.

The isolates collected from banana rhizosphere of wilt suppressive and salt affected soils of Uttar Pradesh were characterized using morphological and molecular methods by Yadav *et al.* (2020). Out the 21 isolates collected, three promising isolates *viz.*, CSR-T-2, CSR-T-3 and CSR-T-4 were identified by molecular methods - sequencing ribosomal RNA using ITS1 and ITS4 universal primers for confirmation of species. The said isolates were identified as CSR-T-2 (*T. koningiopsis*) CSR-T-3 (*T. reesei*) and CSR-T-4 (*T. asperellum*).

To avoid eventual death of the plants due to grapevine trunk disease (GTD) using bio-control agents, Flamand (2021) cultured 29 isolates of *Trichoderma* from grapevine orchards in British Columbia. The molecular analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) and a partial sequence of the translation elongation factor 1-alpha gene of these isolates facilitated the identification of seven species *viz.*, *T. asperelloides*, *T. atroviride*, *T. canadense*, *T. harzianum*, *T. koningii*, *T. tomentosum*, and *T. viticola*.

The survey of the species of the genus *Trichoderma* undertaken by Rodriguez *et al.* (2021), in Cameroon and Ethiopia reported that some species of this genus were endophytes to coffee while some were myco-parasites to coffee rust pathogen *Hemileia vastatrix*. The phylogenetic analysis of all the 94 isolates was done by using a combination of three genes: translation elongation factor-1 $\alpha$  (*tef1*), *rpb2* and *cal* for tabbed isolates. The species recognition was confirmed by using GCPSR criteria supported by morphological and cultural characters.

Silva *et al.* (2021) conducted study on molecular identification and phylogenetic analysis of *Trichoderma* isolates, isolated from leaves of forest trees. During molecular characterization, the DNA regions studied were the genes for the translation elongation factor (*tef1*) and the second largest RNA polymerase subunit (*rpb2*). The sequences of each gene were aligned and the concatenated ones (*tef1 -rpb2*) were compared with most alike *Trichoderma* isolates available in GeneBank for the construction of phylogenetic tree. The study allowed the identification of 14 isolates within three species of *Trichoderma viz.*, *T. orientale* – seven isolates; *T. koningiopsis* – six isolates and *T. longibrachiatum* – one isolate. Remaining isolates could not be identified at the species level. This might have been due to insufficient rate of PCR pass rate.

Xue *et al.* (2021) obtained 1308 *Trichoderma* isolates from the plant rhizospheres, soil, above-ground plants, and decaying wood based natural habitats in



China. Based on the morphological characterization and phylogenetic analysis of the nuclear ribosomal internal transcribed spacer (ITS) and translation elongation factor 1 (tef1), twelve *Trichoderma* strains were identified as *T. asperellum* and one as *T. afroharzianum*.

#### **2.4. Sensitivity of promising *Trichoderma* isolates with fungicides.**

Integrated Diseases Management encompasses cultural, chemical and biological strategies to manage plant diseases. Therefore, use of bioagent friendly chemicals becomes inevitable. This was first proposed by Fisher (1969).

In a study conducted by Bhat and Srivastava (2003) it was revealed that the three triazole group fungicides viz. Hexaconazole, Propiconazole and Penconazole were detrimental to *T. harzianum* strain under study.

Islam *et al.* (2008) observed the effect of fungicides like Carbendazim 50 WP, Copper oxychloride 50 WP, Hexaconazole 5 EC and Propiconazole 25 EC on *Trichoderma* species by poisoned food technique and found that the growth of *Trichoderma* was very much inhibited in presence of Carbendazim and Propiconazole with the recommended dose whereas, normal growth was observed in medium containing Copper oxychloride and Hexaconazole.

Madhavi *et al.* (2008) tested two stable mutants, one each of *Trichoderma viride* (TvM<sub>1</sub>) and *Trichoderma harzianum* (ThM<sub>1</sub>) obtained through gamma radiation for their compatibility with four fungicide in order to fit them in integrated disease management practices for the control of fusarial wilt of chilli. Both the mutants were fairly compatible with carbendazim (0.1%). TvM<sub>1</sub> was also compatible with copper oxychloride (0.15%). However, Mancozeb (0.25) was inhibitory for both the mutants

Madhusudhan *et al.* (2010) found compatibility of *T. viride* isolates viz., T<sub>2</sub> and T<sub>4</sub> with fungicides like Mancozeb (75% WP), Carbendazim (50% WP) Propiconazole (25% EC), Tridemorph (80% EC) and Hexaconazole (5% EC) at five concentrations such as 50, 100, 250, 500 and 1000 ppm and concluded that Mancozeb (75% WP) and Chlorothalonil (75% WP) were compatible with *Trichoderma* upto 250 ppm concentration. There was some mycelial growth in Hexaconazole, Propiconazole and Tridemorph containing medium but Carbendazim (50% WP) totally inhibited the growth at the lowest concentration.

Sarkar *et al.* (2010) evaluated the *in vitro* effect of fungicides, insecticides, and biopesticides commonly used in tea plantations to determine their influence on *T.harzianum*. Seven systemic fungicides and 2 contact fungicides were used at 5, 10, 25, 50, 100, 200 and 300 ppm concentration. Among the systemic fungicides, Hexaconazole recorded cent per cent inhibition at 10 ppm and above concentrations. Copper oxychloride and Copper hydroxide were tolerable upto 100 ppm concentration.

Madhavi *et al.* (2011) evaluated *in vitro* compatibility of *T. viride* with 3 contact and 5 systemic and 1 combination fungicide. Among these Copper oxychloride (50% WP) recorded 62.9 per cent inhibition and Hexaconazole (5% EC) recorded 94.4 per cent inhibition.

In a similar study conducted by Ranganathaswamy *et al.* (2012) eighteen fungicides at different concentrations were used to assess the compatibility of *T. harzianum* and *T. virens* with these fungicides. It was concluded that Sulphur and Mancozeb were less toxic to both the bioagents.

Carbendazim and Mancozeb completely inhibit the mycelial growth of *T. viride* while Copper oxychloride was highly compatible (Tapwal *et al.* 2012).

Saxena *et al.* (2014) analyzed the *in-vitro* effect of some commonly used fungicides, insecticides and herbicides on the mycelial growth of *T. harzianum* strain PBT23. Among fungicides, Captaf, Thiram, Chlorothalonil and Copper hydroxide were found compatible with the test antagonist up to 100 µg a.i. /ml, while Mancozeb up to 250 µg a.i. /ml.

Sneha and Satya (2014) evaluated *in vitro* efficacy of Hexaconazole (5 EC) at different concentrations *i.e.*, 50, 100, 150, 200 ppm against *T. aureoviride* and reported that the fungicide was less toxic (colony diameter – 34 mm) at the lowest concentration only.

The results of a parallel study conducted by Thoudam and Dutta (2014) wherein the compatibility of seven fungicides with *T. atroviride* was observed and revealed that *T. atroviride* was highly compatible with all the test fungicides except Carbendazim. Mancozeb as well as combination of Sulphur and Copper were the most compatible as recorded more than 75 mm colony diameter of the bioagent.

Compatibility of 5 species of *Trichoderma* including *T. harzianum* and *T. koningii* with Mancozeb (75 WP) at 8 concentrations (1000-8000 ppm) was assessed by Bhale

and Rajkonda (2015) and reported that the mycelial growth *T. harzianum* was satisfactory (49 mm) upto 4000 ppm. However, the growth of *T. koningii* was satisfactory (47 mm) only at 1000 ppm.

The study of Dhanya *et al.* (2016), revealed that, *T. viride* was not at all compatible with Carbendazim (50 WP) and Hexaconazole (5 EC) but it was reasonably compatible with COC (50 WP).

The *T. harzianum* strain TCMS-14 was extremely compatible with Sulphur (80 WP) at 2500 ppm concentration but fairly compatible with Mancozeb (75 WP) upto 625 ppm only (Sharma *et al.* 2016).

Kumar *et al.* (2017) reported that Carbendazim was detrimental to *T. asperellum* at the lowest (100 ppm) concentration. The least mycelial inhibition (23.30%) of the bio-agent was recorded at 100 ppm concentration of Mancozeb (75 WP).

Mohamed and Radwan (2017) studied the compatibility of a local strain of *T. harzianum* with six fungicides including Mancozeb (80 WP), COC (50 WP) and Sulphur (80 WP) at seven concentrations such as 1, 5, 10, 50, 100, 500 and 1000 ppm. Wettable sulphur and copper oxychloride proved the most compatible i.e., none of the concentrations tested suppressed the mycelial growth. But Mancozeb was exhibited the minimum inhibition (16.94%) at 1 ppm concentration.

In a study on compatibility of a local strain of *T. harzianum* was assessed with eight contact fungicides at 500, 1000, 1500 and 2000 ppm concentration and ten systemic fungicides at 250, 500, 750 and 1000 ppm concentration. Among contact fungicides COC and Mancozeb were the most compatible with no mycelial inhibition at each concentrations used and it was followed by Sulphur upto 1500 ppm. On the other hand, Carbendazim was deleterious at all the concentrations. The compatibility performance of Thiophanate methyl and Hexaconazole was considerably poor (Sonavane and Venkataravanappa, 2017).

Elshahawy *et al.* (2016) concluded an experiment on compatibility of ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens* and three of *Trichoderma* spp.) which were designated as Th1, Th2, Th3, Tv1, Tv2, Tv3, Tvr, Tsp1, Tsp2 and Tsp3. In all, seven fungicides including Carbendazim, Mancozeb and Thiophanate-methyl were used at nine concentrations *viz.* 50, 100, 200, 300, 400,

500, 600, 700 and 800 ppm. Results revealed that *Trichoderma* spp isolates were compatible with Thiophanate-methyl at all tested concentrations except the isolates Th3 and Tsp2 which exhibited 3.3 per cent and 4.4 per cent inhibition at 800 ppm. Mancozeb inhibited the mycelial growth of Th1 and Th2 at 700 and 800 ppm but rest of the isolates were compatible with it even at 800 ppm. Carbendazim completely inhibited the growth of all the isolates under study.

Dwivedi and Vishunavat (2018) conducted experiment to evaluate compatibility of two *Trichoderma* strains, *T. asperellum* (Th 14) and *T. harzianum* (Th 3) and their mutants ((Th 14 M1, Th14 M2, Th 3 M1, Th3 M2, Th3 M3, Th3 M6) with commonly used fungicides (Captan, Carbendazim and Tebuconazole) *in vitro* at two concentrations, *i.e.* 100 and 250 µg ml<sup>-1</sup> and revealed that the parent strains were fully compatible (100%) with Captan and incompatible with Carbendazim and Tebuconazole. It was found that all the mutants were incompatible with Carbendazim, but compatibility was induced in mutants Th 3 M1 and Th 3 M6 with Tebuconazole at 100 and 250 µg ml<sup>-1</sup> (76.1 and 57.8%; 45.9 and 36.2%, respectively) as compared to their incompatible parent strains. However, mutants Th 14 M2, Th 3 M2 and Th 3M6 exhibited lower compatibility (89.6, 83.3 and 94.4%, respectively) with Captan at 250 µg ml<sup>-1</sup> as compared to their parent strains also found Mutants Th 3 M1 and Th 3 M6 were most promising exhibiting compatibility with all the test fungicides except Carbendazim.

Boat *et al.* (2018) evaluated *in vitro* compatibility of commonly used agrochemicals on the growth of six *Trichoderma* spp. and revealed that effect of fungicides on the growth of *Trichoderma* showed variation and observed no growth of *T. asperellum* (It-13) and *T. erinaceum* (It-58) with Mancozeb as well as *T. asperellum* (It-13) and *T. afroharzianum* (P-8) with Thiophanate methyl.

The results of the study conducted by Kiran *et al.* (2018) revealed that the three tizole group fungicides (Propiconazole, Hexaconazole and Tebuconazole) completely inhibited the growth of two *Trichoderma* species *i.e.*; *T. harzianum* and *T. viride* at even at minimum concentration of 0.05%. Further the sole contact fungicide under study COC also recorded 65.5%, 80.0% and 85.5% inhibition of *T. harzianum* at the three tested concentrations (0.05%, 0.1% and 0.2%).

Most of the workers have reported that Carbendazim completely inhibits the growth of *Trichoderma* species (Kumar *et al.* 2019; Shashikumar *et al.* 2019; and Shrivastava, 2019).

Tomar *et al.* (2018) tested four fungicides *viz.*, Mancozeb, Thiram, Carboxin and Propiconazole at selected concentration (25, 50, 75 and 100 ppm) for their compatibility with *T. harzianum*. It was observed that the Mancozeb was slightly inhibitory at 75 and 100 ppm (inhibition 5.19% and 7.03%, respectively).

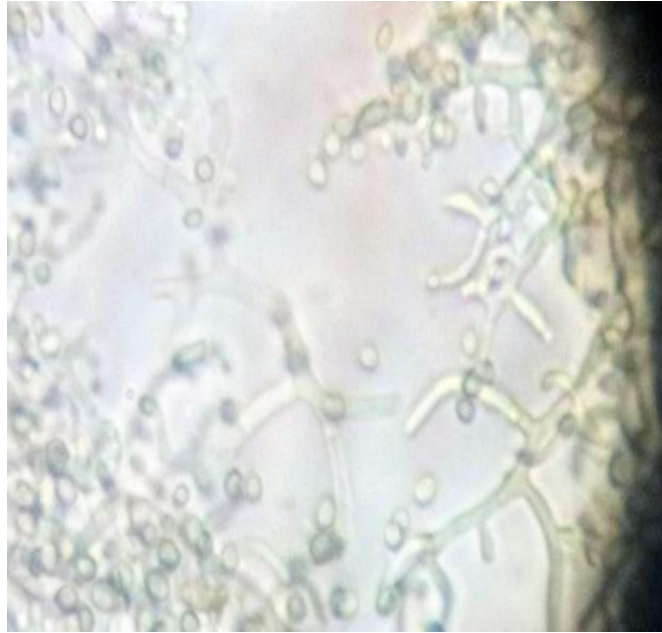
Shashikumar *et al.* (2019) concluded that Mancozeb was the least inhibitory (1.48%) at 0.15% concentration but COC, Chlorothalonil and Carbendazim were detrimental even at the lowest concentration.

Shrivastava (2019) tested the compatibility of *T. harzianum* with carbendazim, Thiophanate methyl, Copper- oxychloride, Mancozeb, and Wettable sulphur each at three concentrations such as 500, 1000 and 1500 ppm. The results ascertained that the mycelial growth of the bio-agent was above 70 per cent in Mancozeb, Wettable Sulphur and COC while Carbendazim and Thiophanate Methyl were absolutely injurious.

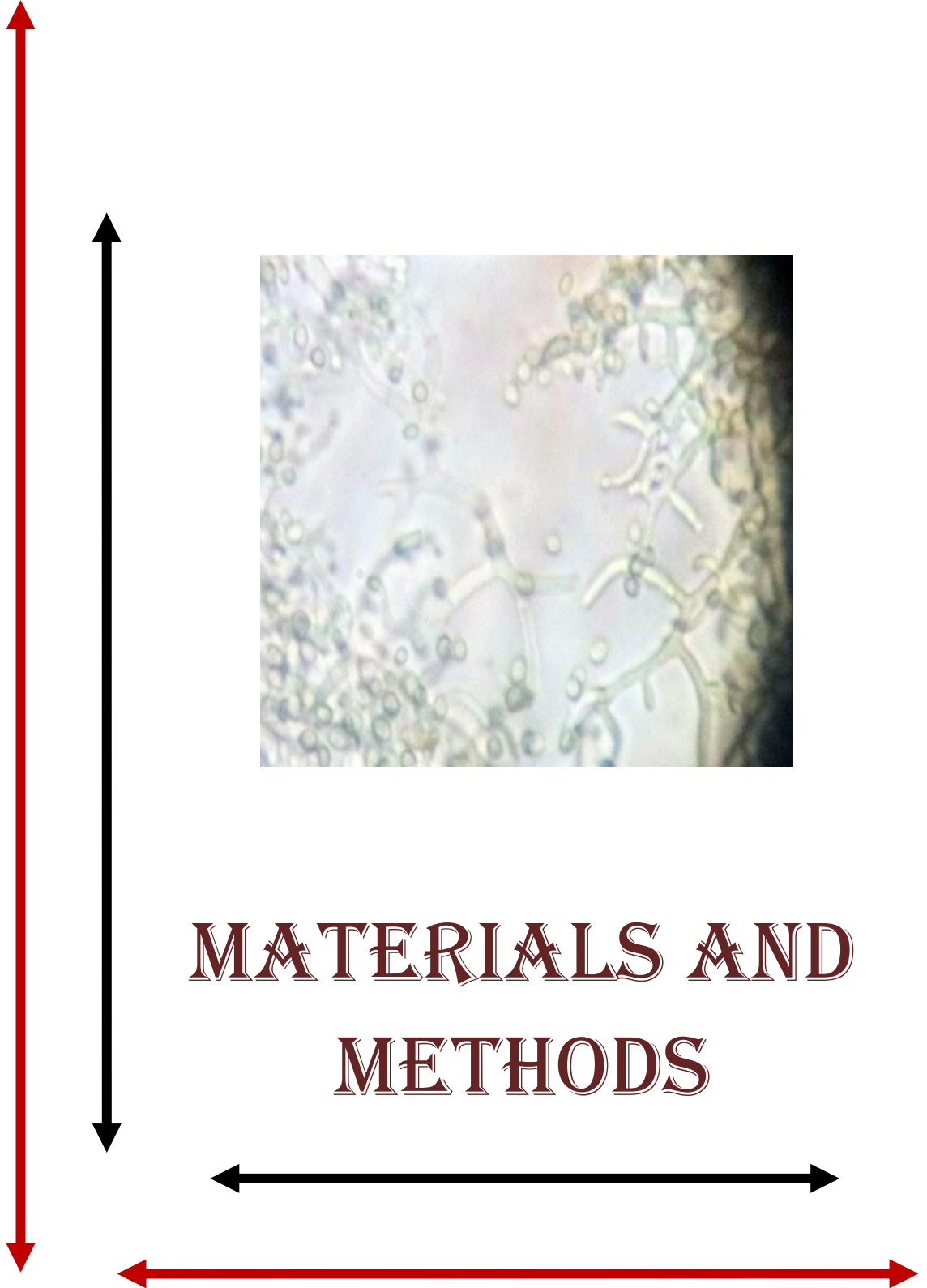
The results of Maheshwary *et al.* (2020) pointed out that, in case of *T. asperellum*, among the four concentrations used (5, 25, 50 and 100 ppm), the mean value of minimum inhibition (7.4%) was in Mancozeb followed by COC (8.8%). Carbendazim did not favor the slightest growth.

Vyas *et al.* (2020) summarized that both COC and Carbendazim were equally hazardous to *T. harzianum*.

Singh *et al.* (2021) stated that Hexaconazole was acutely toxic to *T. harzianum* stain IRRI-1.



**MATERIALS AND  
METHODS**



## **CHAPTER-III**

### **MATERIALS AND METHODS**

All the present study entitled “Investigations on potential *Trichoderma* spp. in Konkan region” were conducted at Post Entry Quarantine Laboratory, Department of Plant Pathology, College of Agriculture, Dapoli, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (MS). The details regarding materials used and methods adopted in the investigations are described under the following headings.

#### **3.1. Material**

Following materials were used for experimentation.

##### **3.1.1. Glasswares and Plastic wares**

During the course of investigation following glass wares and plastic wares were used: glass Petri plate's (Borosil and Schott Duran, Germany), test tubes (Borosil, Germany), conical flasks of 250ml, 500ml and 1000ml (Schott Duran, Germany), funnel (Schott Duran, Germany), beaker (Schott Duran, Germany), glass pipette (Schott Duran, Germany), measuring cylinder (Schott Duran, Germany), cavity slides, cover slip, glass rod, polypropylene Petri plates, and polypropylene centrifuge tube, eppendorf tubes etc.

##### **3.1.2. Equipments**

Standard laboratory equipment's used for different experiments were Autoclave (Equitron, India), BOD incubator (Sanco, India), laminar airflow (Klenzaid, India), student microscope (Olympus, India), stereoscopic binocular (Nikon, India), refrigerator (LG, India), hot air oven (Bio-techniques, India), digital weighing balance centrifuge (Bio fuse, Germany), table top centrifuge (Hawkins, India), Bunsen burner, digital camera (Kodak, India), double distillation unit (JSGW, India), soil sterilization tank (locally made), etc. were used throughout the experiments.

##### **3.1.3. Chemicals**

Essential chemicals for preparation of media, Streptomycin Sulphate, Mercuric chloride, Spirit etc. were obtained from Department of Plant Pathology, College of Agriculture, Dapoli, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (MS).

### **3.1.4. Other materials**

Non-absorbent cotton, muslin cloth, polyethylene bags, cork borer (5 mm), inoculation needle, micropipette, dissection needle, forceps, paper bags, butter paper bags, pencil, permanent marker, cello tapes, Whatman filter paper (4 mm), tags, polyethylene sheets, test tube stand, tray, hand sprayer, wash bottle, thread, wooden sticks, potato, rubber band, scissors, etc were used during study.

## **3.2. Methods**

### **3.2.1. Collection of soil sample**

The Konkan region is divided into two zones such as North Konkan coastal zone comprises Palghar, Thane and Raigad districts and South Konkan Coastal Zone which include Ratnagiri and Sindhudurg Districts. In all 64 samples were collected from different pockets which included Palghar, Karjat and Shriwardhan from North Konkan coastal zone and Dapoli, Bandh-tiware, Wakawali, Harnai, Mandangad, Lanja, Shirgaon, Ratnagiri, Adeli, Hodawade, Kinjawade, Wada, Mangao from South Konkan Coastal Zone. The crops selected for rhizosphere soil sample collection were mango, cashew, banana, guava, sapota, areca nut, coconut, rice, horse gram, lablab bean, Chilli, brinjal, bottle gourd, cabbage, cauliflower, elephant foot yam, groundnut and a flowering plant champak (*Magnolia champaca*). In all the selected fields five spots were chosen and the soil collected from all the spots by standard procedure was mixed to prepare a composite soil sample. Each such sample was stored in a polybag which was labeled with a tag mentioning name of the crop, location and date of collection.

### **3.2.2. Sterilization of glassware and media**

During entire course of investigation glasswaresuch as Petriplates, pipettes, flasks, etc were sterilized in hot air oven for 1 hr before use, whereas distilled water and media were sterilized in an autoclave.

### **3.2.3. Precautions to eliminate contamination**

All isolation work and inoculation of microbial culture was carried out in aseptic conditions under laminar air flow. The laminar flow was sterilized by glowing ultra violet light for ½ hr prior to commencement of work. The working surface of laminar flow and side glasses were surface sterilized with denatured spirit. Moreover, other such necessary care was taken to maintain and carryout work under aseptic condition.



### 3.2.4. Preparation of Potato Dextrose Agar and *Trichoderma* Selective Medium (TSM):

PDA is a routine laboratory medium used for isolation of various microorganisms and therefore the ingredients and preparation procedure is not described here. Serial dilution was carried out on both these media but only the slants of PDA were used for maintenance of the cultures. The ingredients of TSM are mentioned below.

<b><i>Trichoderma</i> Selective Media (TSM)(Elad and Chat, 1983)</b>		
<b>1</b>	Magnesium sulphate heptahydrate (MgSO <sub>4</sub> 7H <sub>2</sub> O)	0.2g
<b>2</b>	Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	0.9g
<b>3</b>	Potassium chloride (KCl),	0.15g
<b>4</b>	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> )	1.0g
<b>5</b>	Glucose	3.0g
<b>6</b>	Chloramphenicol	0.25g
<b>7</b>	Quintozene (pentachloronitrobenzene; Terraclor 75% w.p.,)	0.2g
<b>8</b>	Rose bengal (tetrachlorotetraiodofluorescein),	0.15
<b>9</b>	Metalaxyl	0.3g
<b>10</b>	Agar-agar	20g
<b>11</b>	Distilled water	1000ml

#### **Procedure for TSM medium**

All materials were mixed and dissolved by indirect heating (in boiling water) and volume was made up to one litre. The medium was dispensed in conical flasks which were then plugged with non-absorbent cotton and autoclaved.

### 3.2.5. Isolation and Colony characteristics of *Trichoderma* on PDA and TSM.

In all 64 soil samples collected from different places were subjected to isolation of by serial dilution method. Tubes containing 9 ml distilled water were sterilized in an autoclave at 1.04 kg/ cm<sup>2</sup> for 15 minutes. One gram composite soil sample of respective location was added to 9 ml water in tube under aseptic conditions and homogenised to make 1:10 dilution. Likewise further dilutions up to 10<sup>-9</sup> were obtained by following standard procedure. All the diluted solutions were homogenised and then 1 ml solution of each dilution was poured in sterile Petri plate. Approximately 20 ml sterilized lukewarm PDA or TSM was poured into each plate and the plates were gently rotated. Three replications each of PDA and TSM per dilution were maintained. Such inoculated plates were incubated at ambient temperature for 7 days. The colony characters such as colour,

growth and sporulation were recorded. The pure cultures were maintained in PDA slants for further use.

### 3.2.6. Isolation, Identification and Purification of isolated plant pathogens.

In order to test the antagonistic potential of *Trichoderma* isolates, three common soil borne plant pathogens i.e.; *Fusarium* spp. *Rhizoctonia* spp. ; *Sclerotium* spp. and two airborne plant pathogens such as *Colletotrichum* spp. and *Alternaria* spp. were selected, isolated by following standard procedure and cultured in pure form. The isolates were identified up to genus level on the basis of morphological characters. Their pure cultures were maintained on PDA for further studies.

The sources of collection of the pathogens were as follows.

Sr. no.	Host	Source	Pathogen
1.	Watermelon	Infected soil and roots	<i>Fusarium</i> spp.
2.	Cowpea	Infected soil and roots	<i>Rhizoctonia</i> spp.
3.	Finger millet	Infected soil and roots	<i>Sclerotium</i> spp.
4.	Coriander	Infected leaves	<i>Alternaria</i> spp.
5.	Mango	Infected leaves	<i>Colletotrichum</i> spp.

### 3.2.7. Study of antagonistic activity

Antagonistic potential of *Trichoderma* isolates against the selected pathogens were studied by dual culture technique. After inoculation the plates were incubated at room temperature. Observations on zone of inhibition, colony diameter of the *Trichoderma* isolate and each pathogen was recorded. Three replications were maintained per isolate per pathogen. Plates with sole *Trichoderma* isolate served as control.

### 3.2.8. Morphological and Molecular characterization identification

In order to study the morphology of the *Trichoderma* isolates, microscopic examination was carried out and all the observations were recorded. Microscopy included conidiophore size, shape, branching; position shape and measurement of phialids; spore size, arrangement of spores etc. The recorded observations were compared with information available on standard websites of fungal identification.

The pure cultures of promising isolates were sent to Agharkar Research Institute, Pune for molecular characterization. The protocol is detailed below.

### 3.2.8.1. DNA extraction and PCR amplification:

Liquid culture of the monoconidial isolate was grown in 30 ml Potato Dextrose Broth (PDB) medium and incubated for 7 days at  $25 \pm 1^{\circ}\text{C}$ . Fungal mycelium was harvested aseptically after seven days, and genomic DNA was extracted using modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction protocol (Murray and Thompson 1980). Quantification of DNA was carried out using Eppendorf BioPhotometer<sup>®</sup>D30. The translation elongation factor 1-alpha (*tef1*) [~1300 bp] was amplified by using PCR thermocycler (Eppendorf 5333 Master Cycler Thermal Cycler) to determine the accurate identification of the fungal isolate. EF1-728F [CATCGAGAAGTTCGAGAAGG] and TEF1LLerev [AACTTGCAGGCAATGTGG] primers (Raja *et al.* 2017) were used to amplify the *Tef1* region including the fourth and fifth introns and a significant portion of the last large exon of *Tef1*. The amplified products were checked on 1.5% agarose gel. The sequencing was done at the NFCCI facility, Pune, India. The sequences obtained were checked and edited manually using Chromas Pro software (Technelysium Pty Ltd) and submitted to National Centre for Biotechnology Information (NCBI) as accession:XM\_024901686.1, XM\_024912186.1 and XM\_024901686.1

### 3.2.8.2. Phylogenetic analysis

The phylogenetic tree was constructed using *Tef1* region sequences. The NCBI BLASTn search for *Tef1* sequence similarity was performed using the type database (Altschul *et al.* 1990). The *Tef1* region sequences of type and legitimate strains were acquired from Gen Bank based on the closest similarity of the BLASTn search. MAFFT v 7.0 was used to align the sequences (Kato and Standley 2013). MEGA v 11.0 was used for the phylogenetic study (Kumar *et al.* 2016). During the sequence alignment, gaps and missing data were removed. The phylogenetic tree was built using the Neighbor-Joining method (Saitou and Nei 1987). Bootstrap analysis was performed using 1000 repetitions to calculate the confidence levels for each branch. Bootstrap values less than 50% were not considered.

### 3.2.9. Sensitivity of *Trichoderma* isolates to (Poisoned Food Technique)

Integrated disease management strategies encompass judicious use of all the possible ways and means including bio-agents and chemicals for disease/pathogen control. Sometimes bio-agents and chemicals may be required to be used in combination. In such a situation, the recommended chemicals or their doses may be harmful or most likely to be lethal for bio-control agents. Therefore, it is necessary to understand which chemicals are compatible with the bio-agent.

In this experiment, commonly used 3 systemic and 3 contact fungicides were mixed in the growth medium and the ability of *Trichoderma* isolates to grow in this poisoned medium was studied.

Following chemicals were used for poisoned food technique:

Sr. No.	Common name of fungicides	In ppm %	Trade name of fungicides
1.	Carbendazim	1000	FUNGIGUARD 50 WP
2.	Hexaconazole	500	TATA Contaf PLUS
3.	Thiophenate methyl	500	ROKO
4.	Copper oxychloride	2500	Blitox 50 W
5.	Sulphur	2500	SULTAF
6.	Mancozeb	2500	TATA M-45

The per cent growth inhibition was calculated by the formula

$$PI = \frac{C - T}{C} \times 100$$

Where,

PI - Inhibition percentage

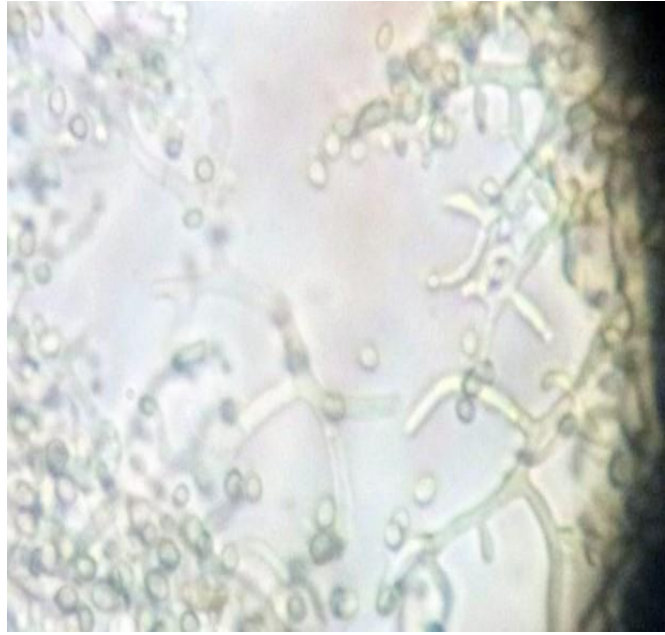
C - Growth in control

T - Growth in treatment

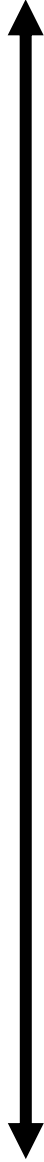
Three replications were maintained per treatment.

### 3.2.10. Statistical analysis

The data of all the experiments were statistically analyzed as per Gomez and Gomez (1984).



**EXPERIMENTAL  
RESULTS**



## CHAPTER-IV

### EXPERIMENTAL RESULTS

The research work on “Investigations on potential *Trichoderma* spp. in Konkan Region” was carried out during 2019-21 in Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

#### **4.1. Isolation of *Trichoderma* spp. from different soils of Konkan:**

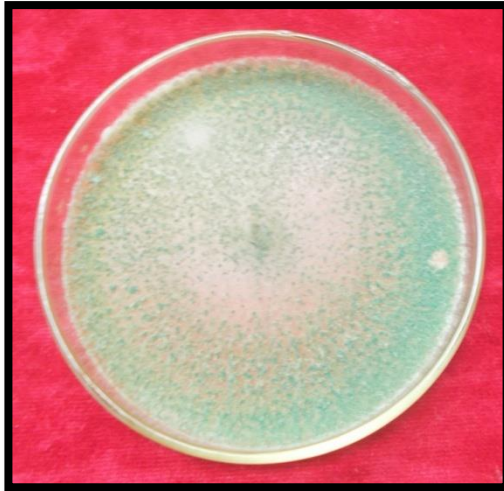
Among the fungal bio-agents explored worldwide, *Trichoderma* species is the unique and prominent group of fungi. The members of this group are not only involved in annihilation of the plant pathogenic fungi but also boost up the resistance mechanism of various crop plants. Due to their presence in all types of soils and varied ecological niches it was thought appropriate to collect, identify and study the antagonistic potential of local strains of *Trichoderma* in the soils of Konkan Region. Accordingly a survey was conducted in five districts of Konkan i.e. Thane, Palghar, Raigad, Ratnagiri, and Sindhudurg to collect soil samples from the rhizosphere of different cultivated crops. The samples were collected from 12-14 cm depth of rhizospheric soil. In all, 67 soil samples were collected out of which 27 samples exhibited presence of *Trichoderma* on both PDA and TSM medium at different dilutions viz.,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$  and  $10^{-9}$ . The details of the 27 pure culture isolates are mentioned below.

**Table1: Details of pure culture isolates**

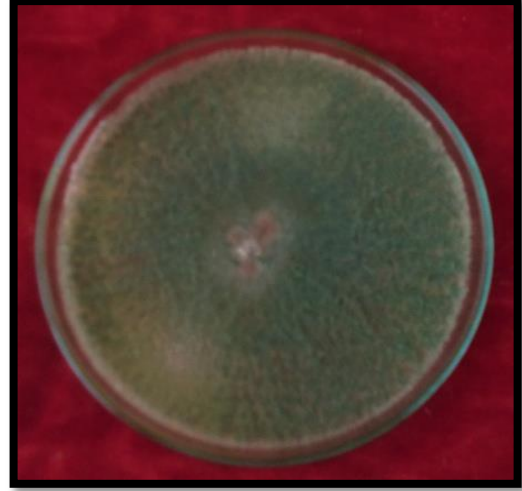
Sr. No	Crop	Location	District	Date of collection	Code
1	Mango	Dapoli	Ratnagiri	20/09/2020	Tmed
2	Mango	Sakhloli	Ratnagiri	24/09/2020	Tamsakh
3	Mango	Lanja	Ratnagiri	28/09/2020	Tmnrj
4	Rice	Kotawade	Ratnagiri	29/09/2020	Tojrr2
5	Rice	Kolambe	Ratnagiri	29/09/2020	Tkorr
6	Rice	Mandangad	Ratnagiri	06/10/2020	Trm
7	Rice	Agave	Ratnagiri	28/09/2020	Tralr
8	Rice	Shirgaon	Ratnagiri	29/09/2020	Tcojrr2
9	Coconut	Dapoli	Ratnagiri	20/09/2020	Tcbfn
10	Coconut	Wakawali	Ratnagiri	26/09/2020	Tcwiki
11	Areca nut	Shrivardhan	Raigad	14/10/2020	Tas
12	Cashew nut	Vengurla	Sindhudurg	20/11/2020	Tcnv
13	Banana	Wakawali	Ratnagiri	24/09/2020	Tbw
14	Guava	Kelwe	Palghar	16/11/2020	Tgpal
15	Sapota	Dahanu	Palghar	18/11/2020	Tsptpal
16	Horse gram	Mandangad	Ratnagiri	06/10/2020	Thm
17	Lablab bean	Harnai	Ratnagiri	22/09/2020	Tlbhar
18	Ground nut	Shirgaon	Ratnagiri	19/10/2020	Tgkh2020s
19	Elephant foot yam	Wakawali	Ratnagiri	24/09/2020	Tefym
20	Chilli	Alibag	Raigad	06/10/2020	Tchal
21	Chilli	Safale	Palghar	17/11/2020	Tchupal
22	Bottle gourd	Unhavare	Ratnagiri	09/10/2020	Tbgu
23	Brinjal	Karjat	Raigad	19/11/2020	Tbk
24	Brinjal	Mahim	Palghar	17/11/2020	Tbpal
25	Cabbage	Karjat	Raigad	21/11/2020	Tckr
26	Cauliflower	Kelwe	Palghar	16/11/2020	Tcaupal
27	Champak (Sonchafa )	Palghar	Palghar	16/11/2020	Tmcpal

The pure cultures of *Trichoderma* isolates obtained from the samples were maintained on PDA slants for further studies.

PLATE I : Twenty-seven *Trichoderma* isolates with code and number



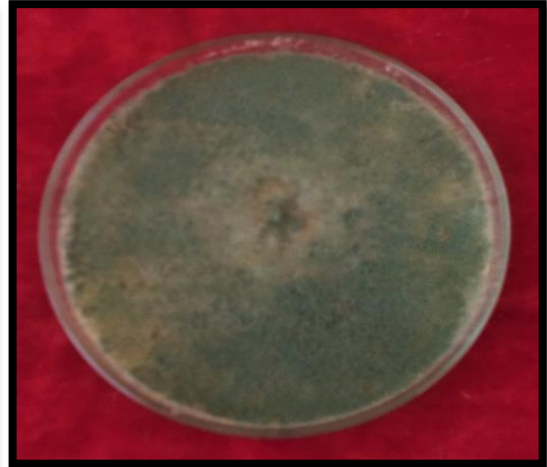
Tmed (T<sub>1</sub>)



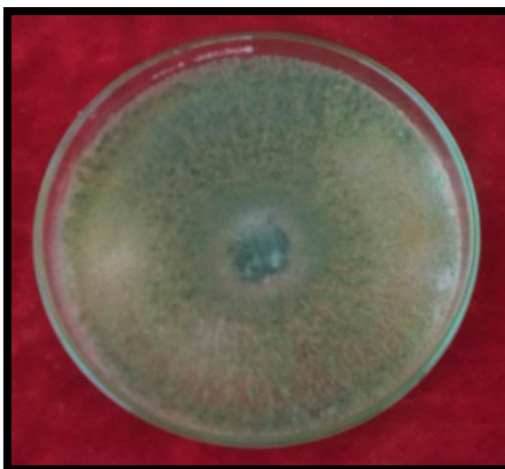
Tamsakh (T<sub>2</sub>)



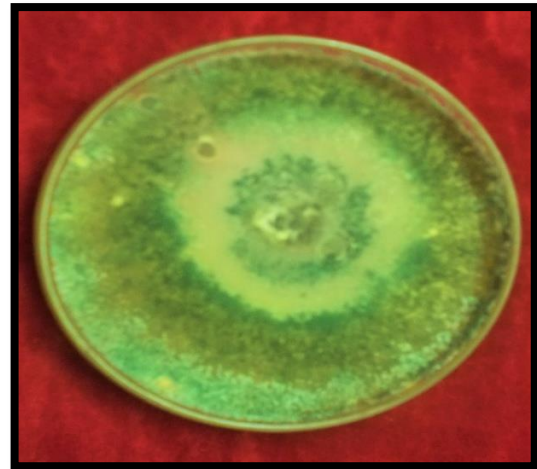
Tmnrj (T<sub>3</sub>)



Tojrr2 (T<sub>4</sub>)



Tkorr (T<sub>5</sub>)



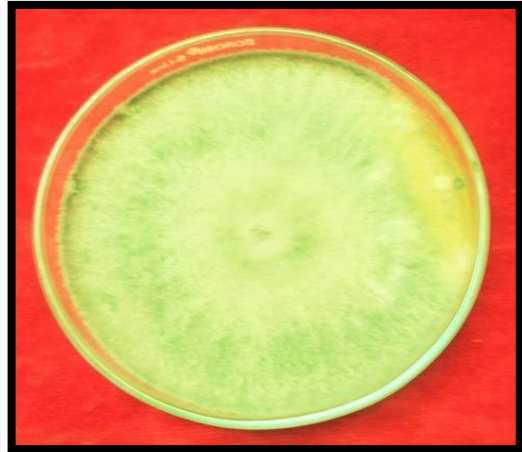
Trm (T<sub>6</sub>)



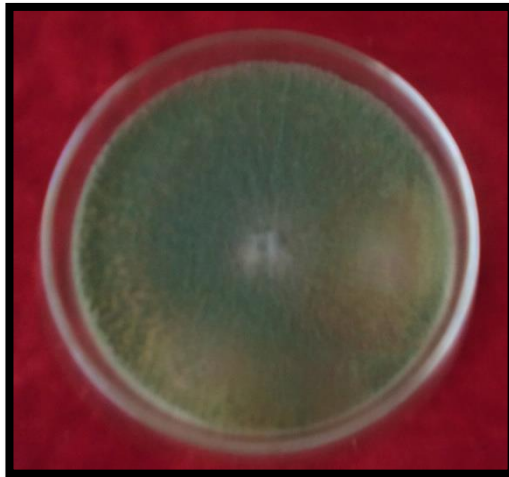
PLATE II



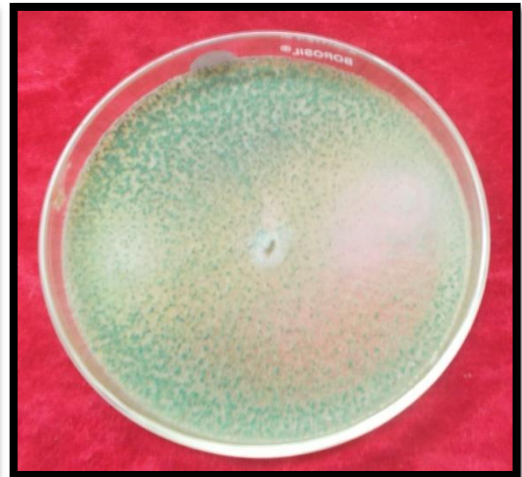
Tralr (T<sub>7</sub>)



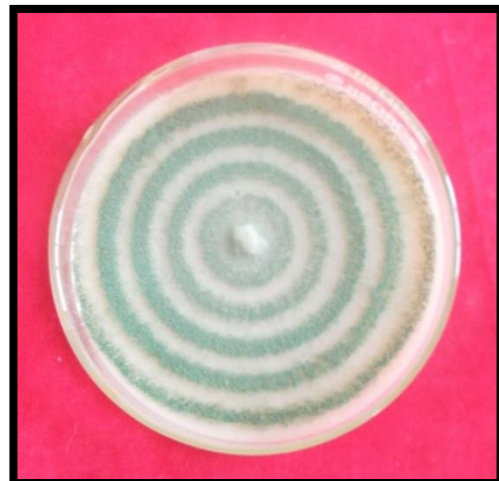
Tcojrr2 (T<sub>8</sub>)



Tcbfn (T<sub>9</sub>)



Tcwki (T<sub>10</sub>)

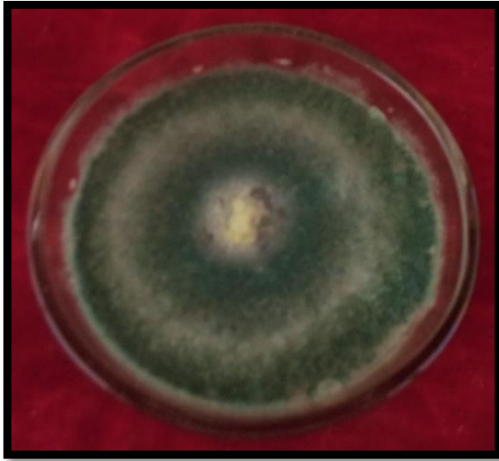


Tas (T<sub>11</sub>)

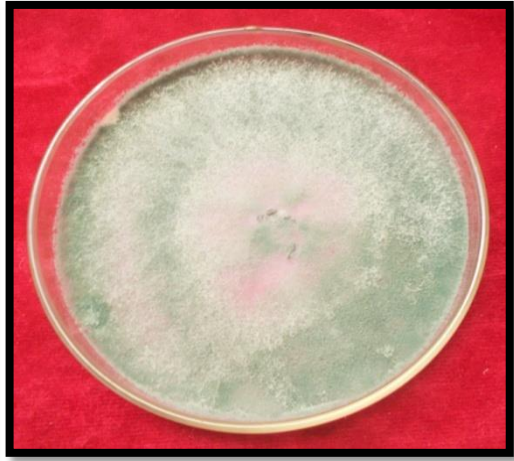


Tcnv (T<sub>12</sub>)

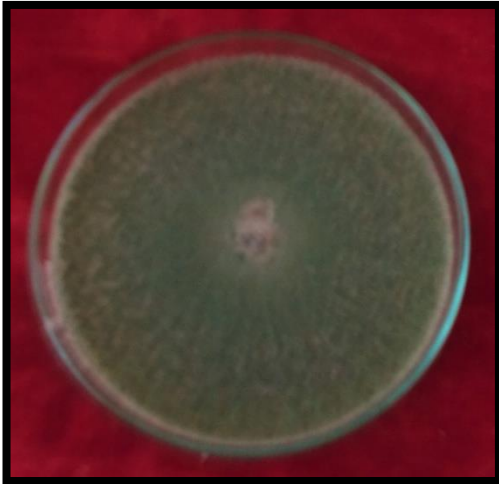
PLATE III



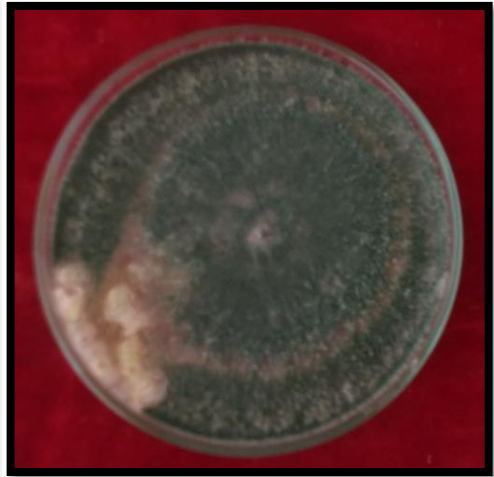
Tbw (T<sub>13</sub>)



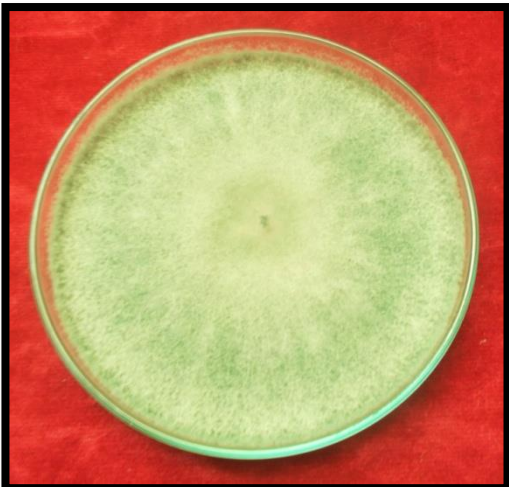
Tgpal (T<sub>14</sub>)



Tsptpal (T<sub>15</sub>)



Thm (T<sub>16</sub>)

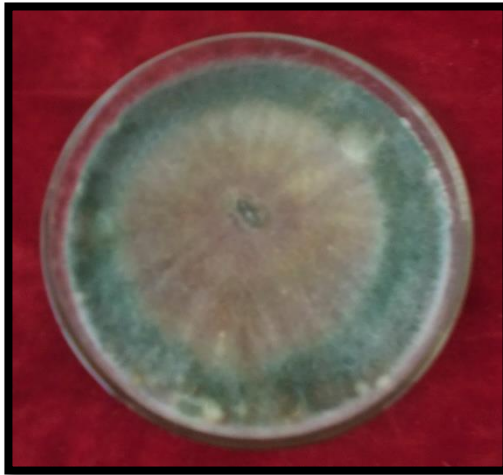


Tlbhar (T<sub>17</sub>)



Tgkh2020s (T<sub>18</sub>)

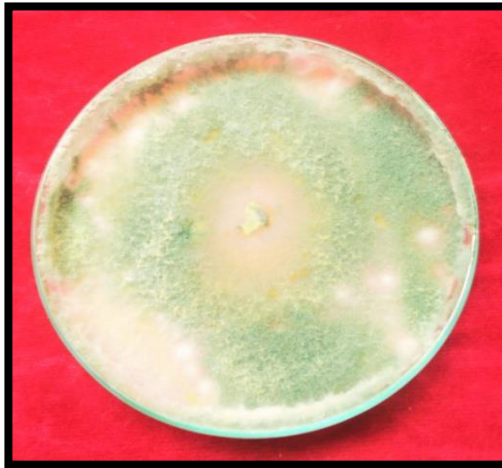
PLATE IV



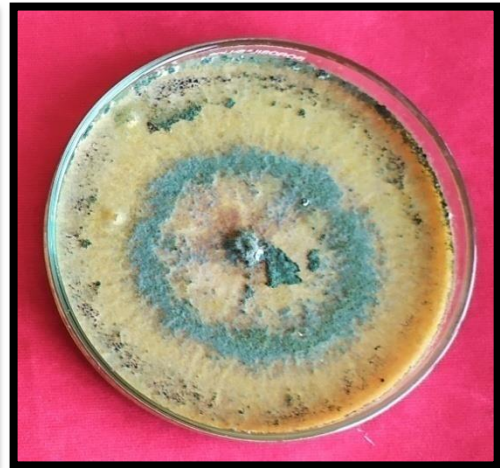
Tefym (T<sub>19</sub>)



Tchal (T<sub>20</sub>)



Tchipal (T<sub>21</sub>)



Tbgu (T<sub>22</sub>)

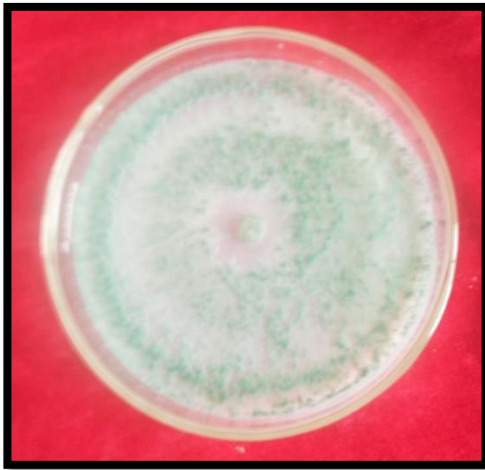


Tbk (T<sub>23</sub>)

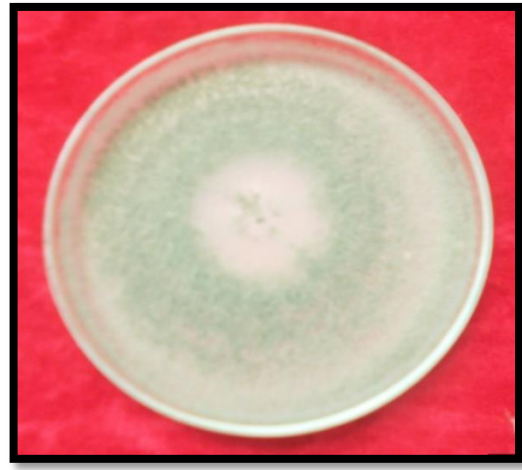


Tbpal (T<sub>24</sub>)

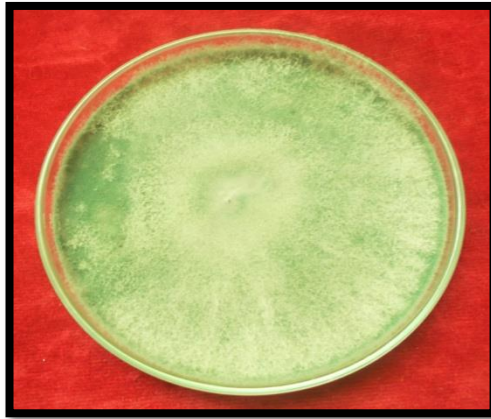
PLATE V



Tckr (T<sub>25</sub>)



Tcaupal (T<sub>26</sub>)



Tmcpal (T<sub>27</sub>)

## 4.2. Antagonistic potential of promising isolates against the selected plant pathogens

### 4.2.1. Isolation and pathogenicity of the major plant pathogens.

The selected common plant pathogens were isolated from the sources mentioned below and their pure cultures were maintained on PDA slants and pathogenicity proved on mentioned host.

Sr. no.	Host	Source of pathogen	Isolated pathogen
1.	Watermelon	Infected roots	<i>Fusarium</i> spp.
2.	Cowpea	Infected roots	<i>Rhizoctonia</i> spp.
3.	Finger millet	Infected roots	<i>Sclerotium</i> spp.
4.	Coriander	Infected leaves	<i>Alternaria</i> spp.
5.	Mango	Infected leaves	<i>Colletotrichum</i> spp.

### 4.2.2. Identification of the pathogens:

The plant pathogens were identified on the basis of colony characters and morphological features described in the following table.

**Table-2: Cultural and morphological characteristics of the pathogens:**

Sr. No.	Colony and morphological characters	Organisms identified as
1.	Fast growing white to pinkish colony, with irregular margin. Mycelium floccose, micro-conidia oval, single celled. Macro-conidia falcate, septate.	<i>Fusarium</i> spp.
2.	Colony with entire margin ash colored initially, turning blackish. Mycelium fluffy, thick. Branching of mycelium at right angle to parent hypha. The sclerotia formed in 7-8 day old culture, which were dark brown to black, round, sometimes irregular in shape.	<i>Rhizoctonia</i> spp.
3.	Colony white initially turning grayish on aging. Colony growth in concentric rings with dark grayish entire margin. Conidia, hyaline, cylindrical with rounded ends, guttulate.	<i>Colletotrichum</i> spp.
4.	Colony slowly growing, dark ash to black coloured, Mycelium fluffy with thick growth. Hyphae septate and irregularly branched. Individual hypha light brown in colour. Conidia light to dark brown, in chain, muriform, with 1 to 2 longitudinal and 3- 10 transverse septa, with tapered apex.	<i>Alternaria</i> spp.
5.	Colony pure white, sparse with long hyphae spreading towards periphery with entire margin. Sclerotia formation within a week of inoculation. Sclerotia dark brown, mostly spherical, discrete in central zone but closely spaced at the periphery of the colony.	<i>Sclerotium</i> spp.

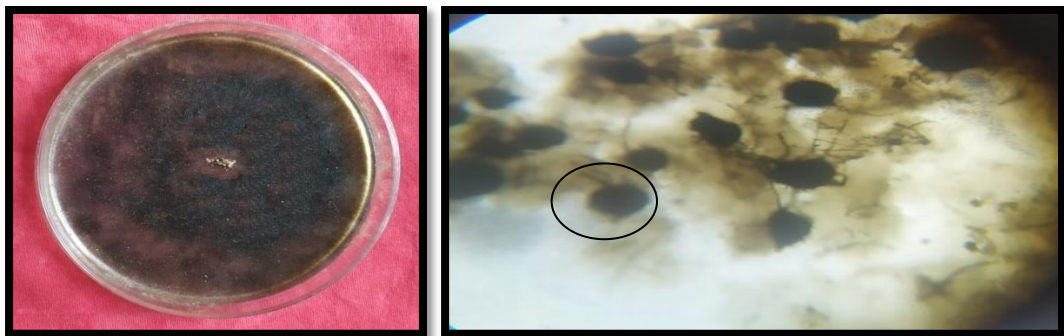
PLATE VI : Pure culture and Microscopic views of isolated common soil borne i.e.; *Fusarium* spp. *Rhizoctonia* spp., *Sclerotium* spp. and two airborne plant pathogens such as *Colletotrichum* spp. and *Alternaria* spp.



Pure culture of *Fusarium* spp. with white to pink pigmentation

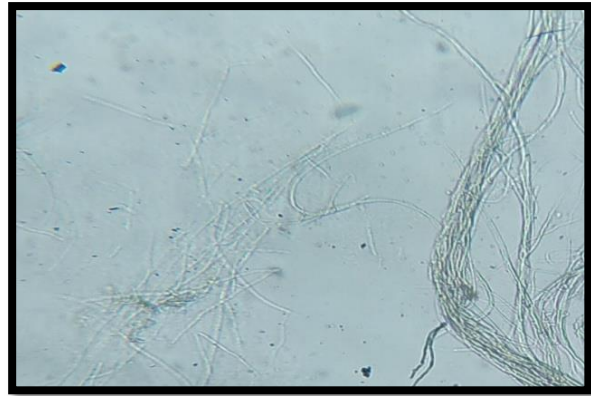


Micro- conidia and macro-conidia



Pure culture of *Rhizoctonia* spp. and sclerotia

PLATE VII



Pure culture of *Sclerotium* spp. with microscopic view of mycelium

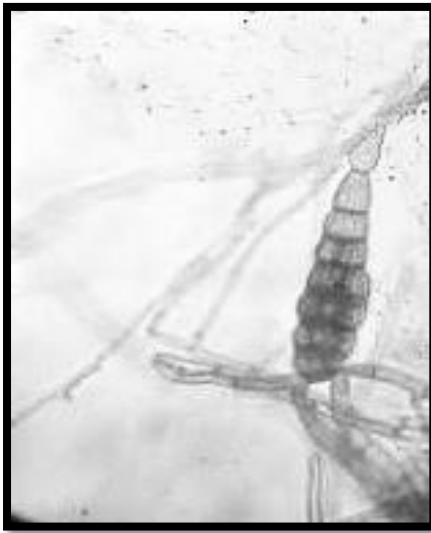


Sclerotia



Pure culture of *Colletotrichum* spp. and conidiophore with conidia

PLATE VIII



Pure culture of *Alternaria* spp. and conidia with longitudinal and transverse septa



#### 4.2.3.1. Antagonistic potential of promising isolates against *Fusarium* spp.

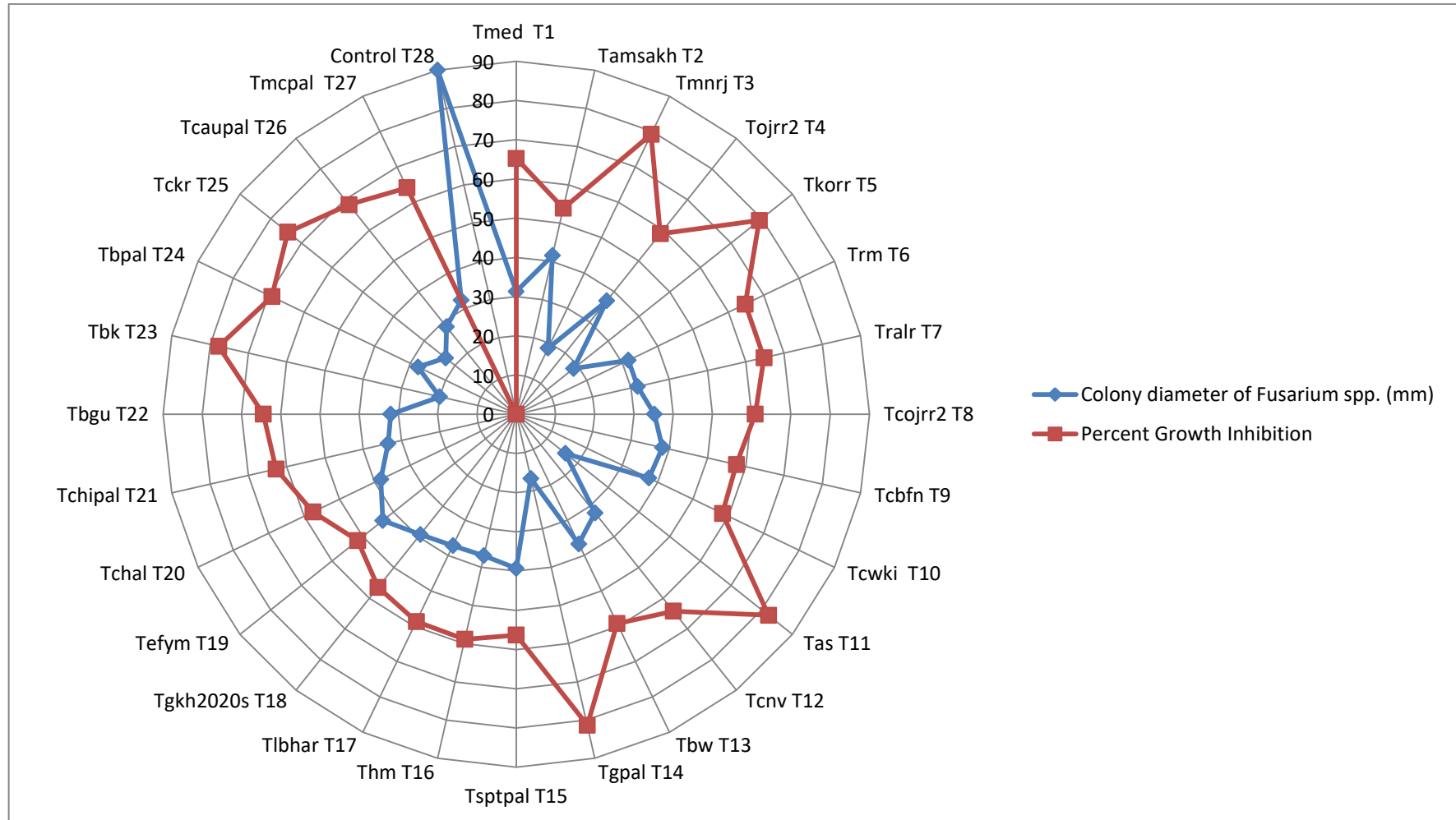
**Table3: *In vitro* efficacy of *Trichoderma* isolates against *Fusarium* spp.**

Isolates code	<i>Trichoderma</i> isolates	Colony diameter (mm)	Percent Growth Inhibition
Tmed	T1	31.30	65.22
Tamsakh	T2	41.50	53.88
Tmnrj	T3	18.70	79.22
Tojrr2	T4	37.00	58.88
Tkorr	T5	18.70	79.22
Trm	T6	31.70	64.77
Tralr	T7	31.70	64.77
Tcojrr2	T8	35.20	60.88
Tcbfn	T9	38.20	57.55
Tcwki	T10	37.50	58.33
Tas	T11	16.00	82.22
Tcnv	T12	32.20	64.22
Tbw	T13	36.70	59.22
Tgpal	T14	16.80	81.33
Tsptpal	T15	39.30	56.33
Thm	T16	37.00	58.88
Tlbhar	T17	37.20	58.66
Tgkh2020s	T18	39.20	56.44
Tefym	T19	43.50	51.66
Tchal	T20	38.30	57.44
Tchupal	T21	33.50	62.77
Tbgu	T22	32.00	64.44
Tbk	T23	20.00	77.77
Tbpal	T24	27.80	69.11
Tckr	T25	23.00	74.44
Tcaupal	T26	28.50	68.33
Tmcpal	T27	32.30	64.11
Control	T28	90.00	00.00
	SE±	0.09	
	CD 1%	0.33	

\*Presented data in table is average of three replications

It is revealed from the data presented in table 3 that all the isolates were effective in inhibiting the growth of *Fusarium* spp. None of the isolates recorded less than 50 per cent inhibition. The maximum inhibition (82.22%) was recorded by the isolate Tas (T<sub>11</sub>-Areca nut -Shriwardhan) and it was significantly superior to rest of the isolates. It was followed by Tgpal (T<sub>14</sub>-Guava -palghar) which recorded 81.33 per cent inhibition of the pathogen. These two isolates were followed by Tmnrj (T<sub>3</sub>- inhibition -79.22%) and Tkorr (T<sub>5</sub>-79.22) which were at par with each other. Among the remaining isolates, Tbk (T<sub>23</sub>-77.77%) was followed by Tckr (T<sub>25</sub>- 74.44%), Tbpal (T<sub>24</sub>-69.11%), Tcaupal (T<sub>26</sub>-68.33%), Tmed (T<sub>1</sub>- 65.22%). The isolates Trm (T<sub>6</sub>- 64.77) and Tralr (T<sub>7</sub>-64.77) were at par with each other. Therefore the isolate Tbgu (T<sub>22</sub>-64.44%) and Tcnv (T<sub>12</sub>- 64.22%) were statistically at par with each other. They were followed by Tmcpal (T<sub>27</sub>- 64.11) and Tchupal (T<sub>21</sub> -62.77) and Tcojrr2 (T<sub>8</sub>-60.88%). Eleven isolates recorded less than 60 per cent inhibition. Tbw (T<sub>13</sub>-recorded 59.22 per cent inhibition while Tojrr2 (T<sub>4</sub>- 58.88) was at par with Thm (T<sub>16</sub>-58.88) and Tlbhar (T<sub>17</sub>- 58.66%). The lowest inhibition (51. 66%) was recorded by T<sub>19</sub>- Tefym.

**Fig. 1: *In vitro* efficacy of *Trichoderma* isolates against *Fusarium* spp.**



#### 4.2.3.2. Antagonistic potential of promising isolates against *Rhizoctonia* spp.

**Table 4: *In vitro* efficacy of *Trichoderma* isolates against *Rhizoctonia* spp**

<b>Isolates code</b>	<b><i>Trichoderma</i> isolates</b>	<b>Colony diameter (mm)</b>	<b>Percent Growth Inhibition</b>
Tmed	T1	36.50	59.44
Tamsakh	T2	33.20	63.11
Tmnrj	T3	26.00	71.11
Tojrr2	T4	32.00	64.44
Tkorr	T5	27.30	69.56
Trm	T6	29.20	67.55
Tralr	T7	26.50	70.55
Tcojrr2	T8	34.00	62.22
Tcbfn	T9	33.20	63.11
Tcwki	T10	33.50	62.77
Tas	T11	22.00	75.55
Tcnv	T12	37.20	58.66
Tbw	T13	35.70	60.33
Tgpal	T14	17.00	81.11
Tsptpal	T15	30.50	66.11
Thm	T16	28.80	68.00
Tlbhar	T17	29.70	67.00
Tgkh2020s	T18	35.70	60.33
Tefym	T19	33.30	63.00
Tchal	T20	33.80	62.44
Tchipal	T21	31.20	65.33
Tbgu	T22	20.00	77.77
Tbk	T23	36.50	59.44
Tbpal	T24	23.30	74.11
Tckr	T25	31.70	64.77
Tcaupal	T26	28.50	68.33
Tmcpal	T27	31.20	65.33
Control	T28	90.00	00.00
	SE ±	0.09	
	Cd 1%	0.33	

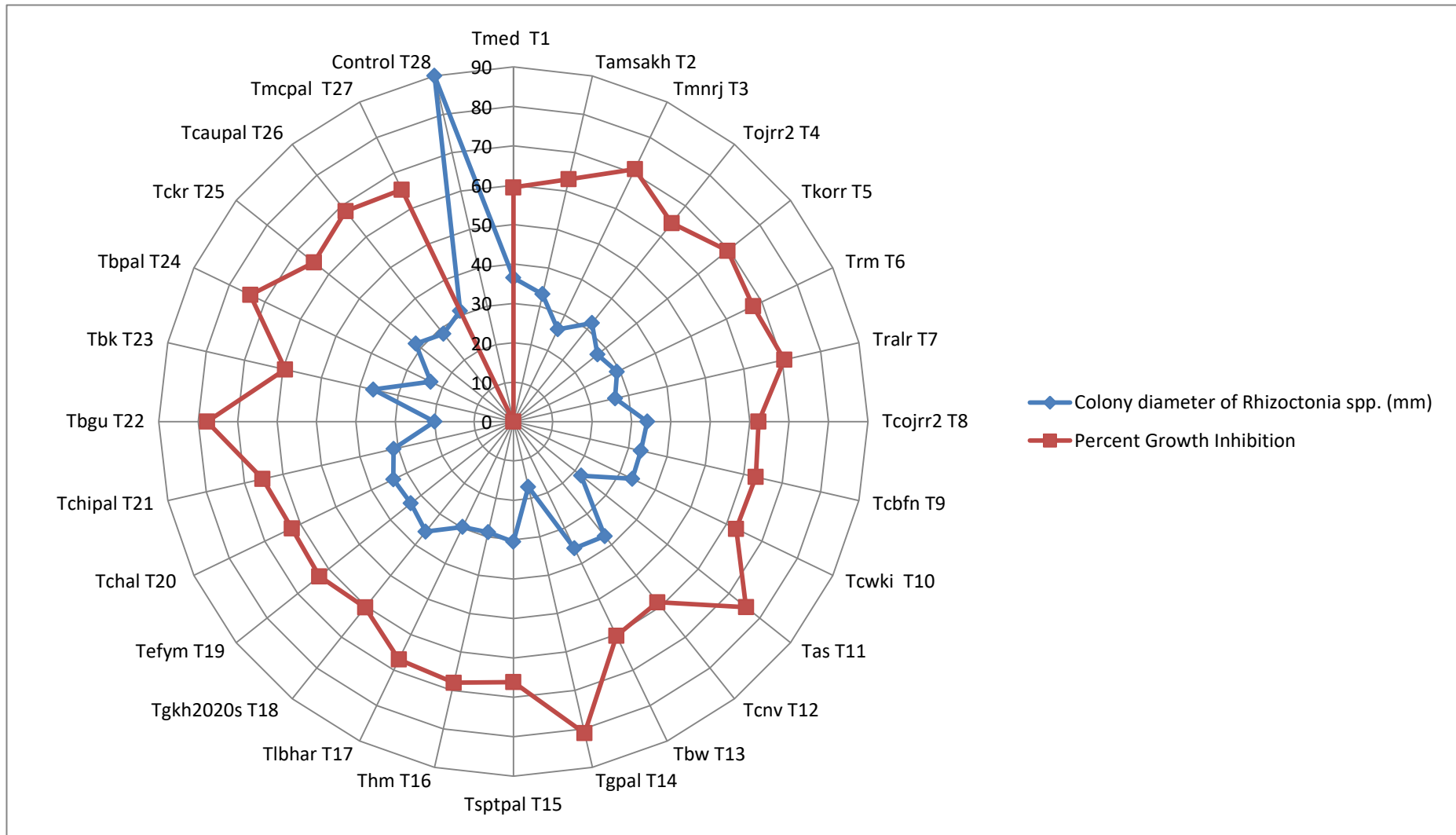
\*Presented data in table is average of three replications

Data depicted in table 4 that, all the isolates were effective in inhibiting the growth of *Rhizoctonia* spp. None of the isolates recorded less the 58 per cent inhibition. The maximum inhibition (81.11%) was recorded by the isolate Tgpal (T<sub>14</sub>-Guava-Palghar) and it was significantly superior to rest of the isolates. It was followed by Tbgu (T<sub>22</sub>-Bottle gourd –Unhavare) which recorded 77.77 per cent inhibition of the pathogen. These isolates were followed by Tas (T<sub>11</sub>- inhibition -75.55%), Tbpal (T<sub>24</sub>-74.11), Tmnrj (T<sub>3</sub>-71.11%) and Tralr (T<sub>7</sub>-70.55%) which were at similitude. Among the remaining isolates, Tkorr (T<sub>5</sub>-69.56%) was followed by Tcaupal (T<sub>26</sub>- 68.33%) and Thm (T<sub>16</sub>-68.00%) which were at par with each other. They were followed by Trm (T<sub>6</sub>-67.55%) and Tlbhar (T<sub>17</sub>-67.00%) which were at par, followed by Tsptpal (T<sub>15</sub>-66.11%). Tmcpal (T<sub>27</sub>-65.33%) and Tchupal (T<sub>21</sub>-65.33%) at par with each other. Tckr (T<sub>25</sub>-64.77%) and Tojrr2 (T<sub>4</sub>-64.44%) were also at par with each other. Remaining ten isolates recorded less than 63 per cent inhibition.

#### **4.2.3.3. Antagonistic potential of promising isolates against *Sclerotium* spp.**

The data in Table-5 revealed that, 27 *Trichoderma* isolates tested against *Sclerotium* spp. among these isolate Tbk (T<sub>23</sub>) showed lowest colony diameter (12.50 mm) which was found in Brinjal at Karjat with 86.11% growth inhibition and it was significantly superior to all treatments. It was followed by Tmnrj (T<sub>3</sub>-Mango –Lanja) which recorded 80.55 per cent inhibition of the pathogen. These isolates were followed by Tas (T<sub>11</sub>-74.22%), Tkorr (T<sub>5</sub>-71.66%) and Tgpal (T<sub>14</sub>-52.55%). Rest of the twenty-two isolates showed below 50 per cent inhibition.

**Fig. 2: *In vitro* efficacy of *Trichoderma* isolates against *Rhizoctonia* spp.**

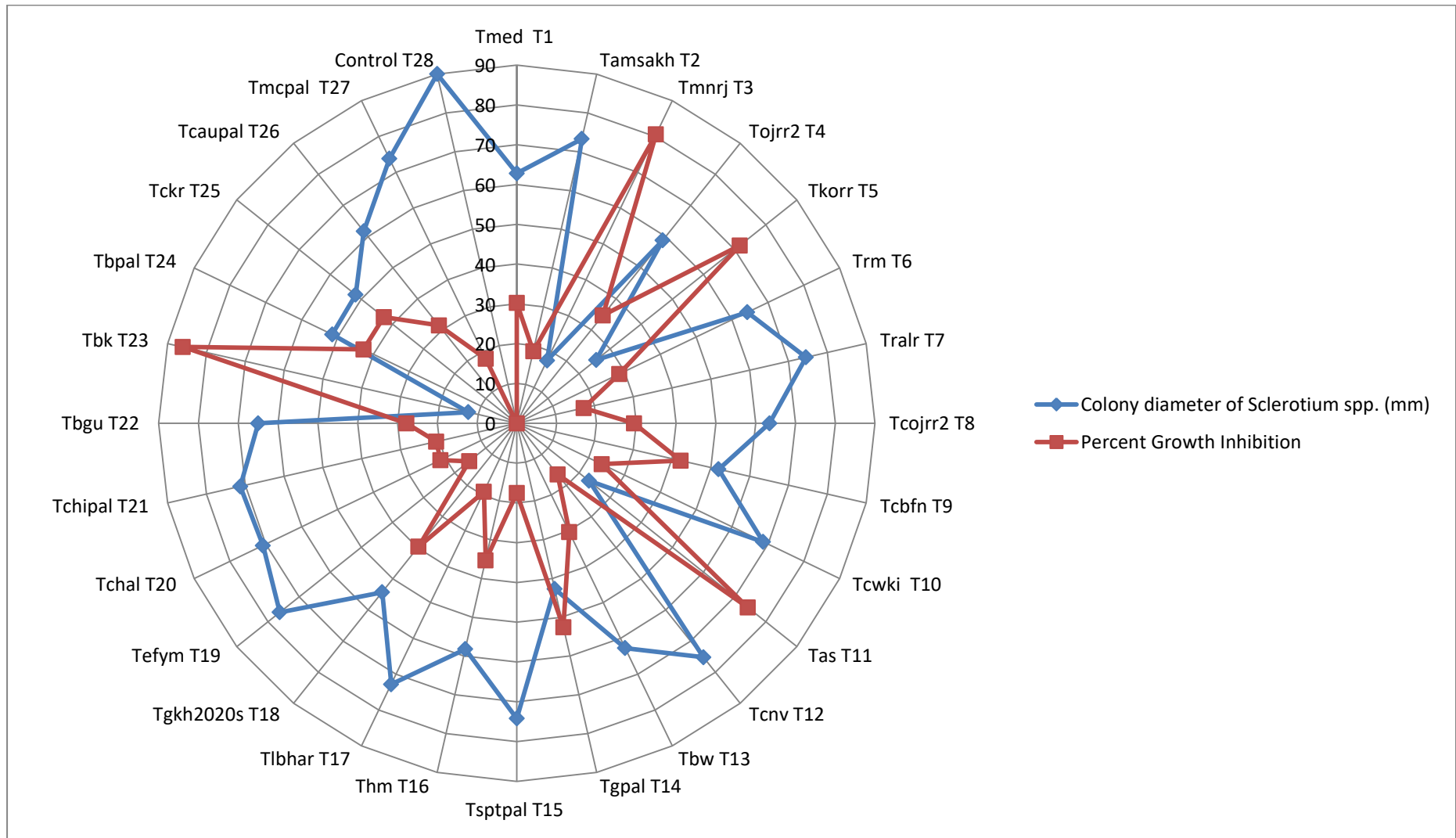


**Table 5: *In vitro* efficacy of *Trichoderma* isolates against *Sclerotium* spp.**

<b>Isolates code</b>	<b><i>Trichoderma</i> isolates</b>	<b>Colony diameter (mm)</b>	<b>Per cent Growth Inhibition</b>
Tmed	T1	62.80	30.22
Tamsakh	T2	73.30	18.55
Tmnrj	T3	17.50	80.55
Tojrr2	T4	58.80	34.66
Tkorr	T5	25.50	71.66
Trm	T6	64.30	28.55
Tralr	T7	74.50	17.22
Tcojrr2	T8	63.50	29.44
Tcbfn	T9	52.00	42.22
Tcwki	T10	68.70	23.66
Tas	T11	23.20	74.22
Tcnv	T12	75.20	16.44
Tbw	T13	62.70	30.33
Tgpal	T14	42.70	52.55
Tsptpal	T15	74.20	17.55
Thm	T16	58.20	35.33
Tlbhar	T17	72.80	19.11
Tgkh2020s	T18	54.30	39.66
Tefym	T19	76.20	15.33
Tchal	T20	70.80	21.33
Tchipal	T21	71.30	20.77
Tbgu	T22	65.00	27.77
Tbk	T23	12.50	86.11
Tbpal	T24	51.50	42.77
Tckr	T25	51.80	42.77
Tcaupal	T26	61.70	31.44
Tmcpal	T27	73.80	18.00
Control	T28	90.00	00.00
	SE ±	0.11	
	Cd 1%	0.43	

\*Presented data in table is average of three replications

**Fig. 3: *In vitro* efficacy of *Trichoderma* isolates against *Sclerotium* spp.**





#### **4.2.3.4. Antagonistic potential of promising isolates against *Colletotrichum* spp.**

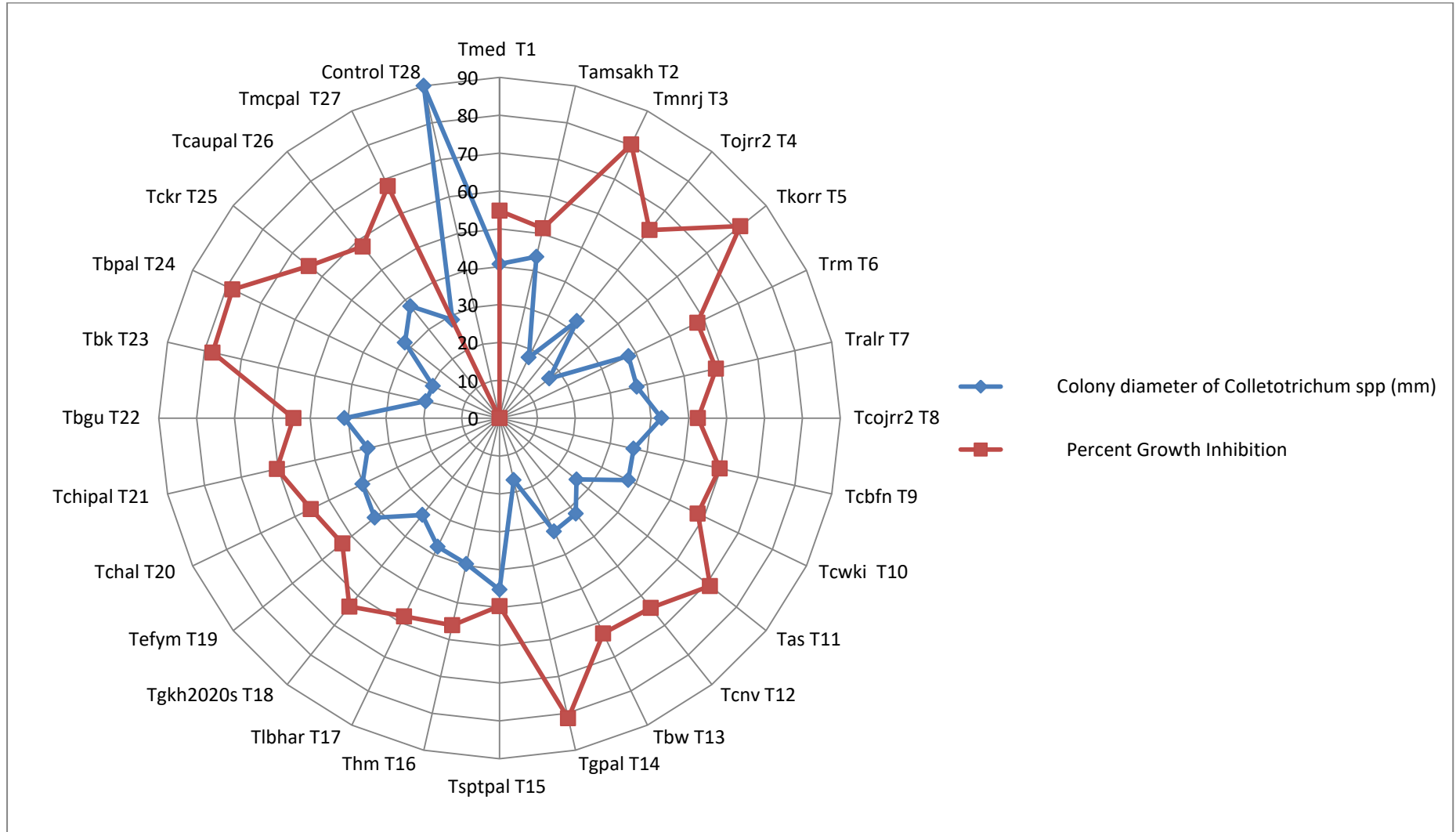
*Trichoderma* isolates were cultured along with *Colletotrichum* spp. in Petri plates and it was found that all the species of *Trichoderma* reduced the mycelial growth of *Colletotrichum* spp. The isolates *i.e.* Tkorr (T<sub>5</sub>-rice) from Ratnagiri and Tgpal (T<sub>14</sub>-guava) from Palghar gave the best results with minimum radial growth of *Colletotrichum* spp. (16.80 mm) and maximum growth inhibition (81.33%). Both these isolates were numerically at par and significantly superior to all the treatments. These isolates were followed by Tmnrj (T<sub>3</sub>-Mango –Lanja-80.22%), Tbpal (T<sub>24</sub>-78.33%), Tbk (T<sub>23</sub>-77.77%), Tas (T<sub>11</sub>-71.11%), Tmcpal (T<sub>27</sub>-68.00%) whereas, Tckr (T<sub>25</sub>-64.44%) and Tcnv (T<sub>12</sub>-64.11%) were at par with each other. Tgkh2020s (T<sub>18</sub>-63.66%), Tojrr2 (T<sub>4</sub>-63.55%) and Tbw (T<sub>13</sub>-63.11%) were also at par to each other and followed by Tchupal (T<sub>21</sub>-60.33%). Remaining fourteen isolates showed below 59.00 per cent inhibition.

**Table 6: *In vitro* efficacy of *Trichoderma* isolates against *Colletotrichum* spp.**

<b>Isolates code</b>	<b><i>Trichoderma</i> isolates</b>	<b>Colony diameter (mm)</b>	<b>Per cent Growth Inhibition</b>
Tmed	T1	40.70	54.77
Tamsakh	T2	43.70	51.44
Tmnrj	T3	17.80	80.22
Tojrr2	T4	32.80	63.55
Tkorr	T5	16.80	81.33
Trm	T6	37.80	58.00
Tralr	T7	37.20	58.66
Tcojrr2	T8	42.80	52.44
Tcbfn	T9	36.30	59.66
Tcwki	T10	37.70	58.11
Tas	T11	26.00	71.11
Tcnv	T12	32.30	64.11
Tbw	T13	33.20	63.11
Tgpal	T14	16.80	81.33
Tsptpal	T15	45.30	49.66
Thm	T16	39.50	56.11
Tlbhar	T17	37.70	58.11
Tgkh2020s	T18	32.70	63.66
Tefym	T19	42.20	53.11
Tchal	T20	40.20	55.33
Tchipal	T21	35.70	60.33
Tbgu	T22	41.00	54.44
Tbk	T23	20.00	77.77
Tbpal	T24	19.50	78.33
Tckr	T25	32.00	64.44
Tcaupal	T26	37.80	58.00
Tmcpal	T27	28.80	68.00
Control	T28	90.00	00.00
	SE±	0.11	
	Cd 1%	0.43	

\*Presented data in table is average of three replications

**Fig. 4: *In vitro* efficacy of *Trichoderma* isolates against *Colletotrichum* spp.**



#### 4.2.3.5. Antagonistic potential of promising isolates against *Alternaria* spp

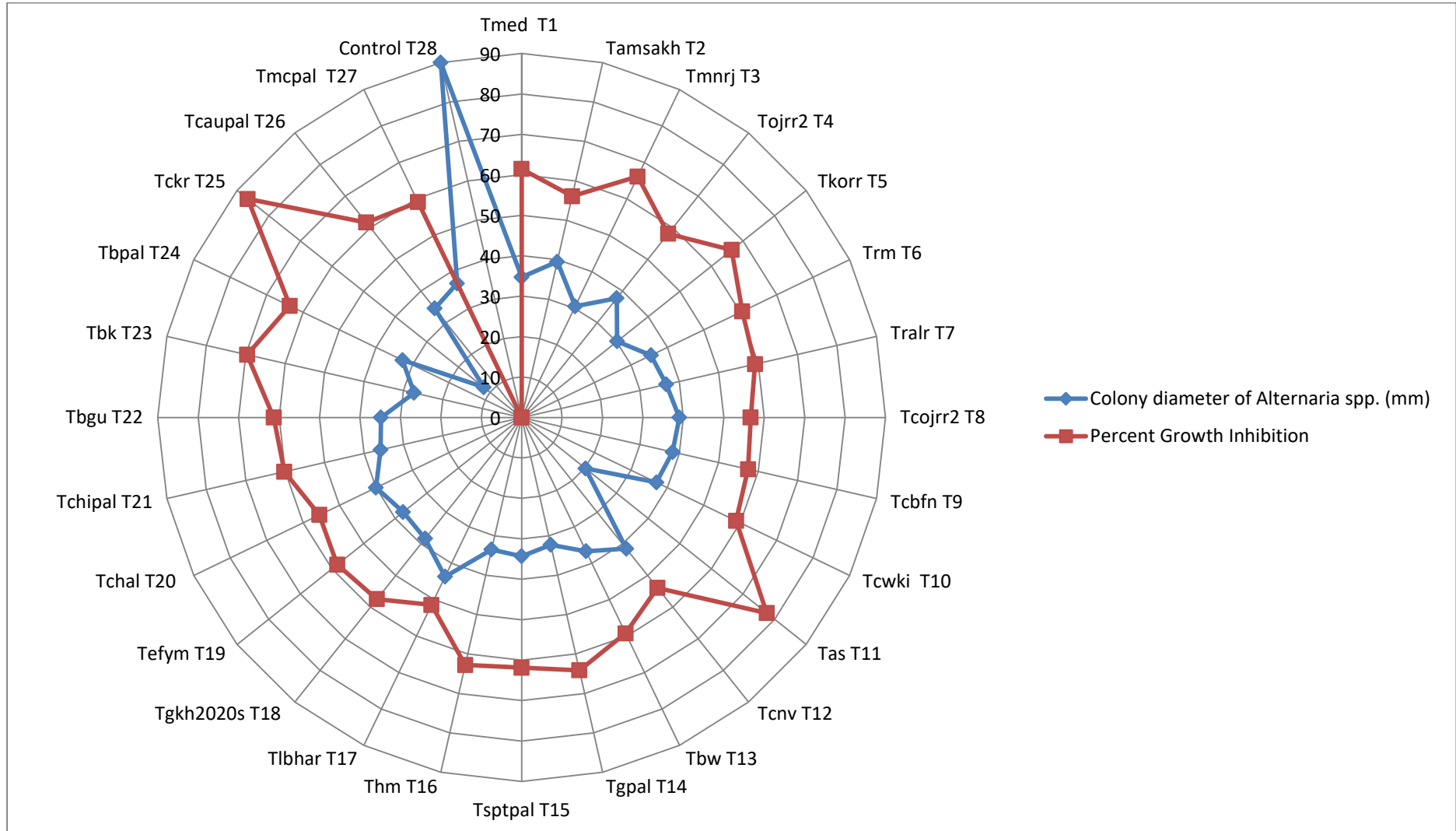
The efficacy of *Trichoderma* isolates against *Alternaria* spp. under *in-vitro* conditions was studied. Data presented in Table 7 revealed that the *Trichoderma* isolate *i.e.* *Tckr* (T<sub>25</sub>-86.66%) was found significantly superior to all treatments against *Alternaria* spp. and followed by *Tas* (T<sub>11</sub>-77.55%), *Tbk* (T<sub>23</sub>-69.66), *Tkorr* (T<sub>5</sub>-66.44) and *Tmnrj* (T<sub>3</sub>-66.11%) were at par to each other, followed by *Tgpal* (T<sub>14</sub>-64.11%), *Tbpal* (T<sub>24</sub>-63.66), *Thm* (T<sub>16</sub>-62.77%). *Tsptpal* (T<sub>15</sub>-61.88%), *Tcaupal* (T<sub>26</sub>-61.66%) and *Tmed* (T<sub>1</sub>-61.44%) were at par to each other. These isolate followed by *Tbgu* (T<sub>22</sub>-61.33%). *Trm* (T<sub>6</sub>-60.55%) and *Tchipal* (T<sub>21</sub>-60.22%) were at par to each other. The isolates *Tralr* (T<sub>7</sub>-59.22), *Tbw* (T<sub>13</sub>-59.22) and *Tmcpal* (T<sub>27</sub>-59.11) were at par with each other. Rest of the seven isolates showed above 50 per cent inhibition.

**Table 7: *In vitro* efficacy of *Trichoderma* isolates against *Alternaria* spp.**

<b>Isolates code</b>	<b><i>Trichoderma</i> isolates</b>	<b>Colony diameter (mm)</b>	<b>Per cent Growth Inhibition</b>
Tmed	T1	34.70	61.44
Tamsakh	T2	39.50	56.11
Tmnrj	T3	30.50	66.11
Tojrr2	T4	37.70	58.11
Tkorr	T5	30.20	66.44
Trm	T6	35.50	60.55
Tralr	T7	36.70	59.22
Tcojrr2	T8	39.00	56.66
Tcbfn	T9	38.30	57.44
Tcwki	T10	37.00	58.88
Tas	T11	20.20	77.55
Tcnv	T12	41.50	53.88
Tbw	T13	36.70	59.22
Tgpal	T14	32.30	64.11
Tsptpal	T15	34.30	61.88
Thm	T16	33.50	62.77
Tlbhar	T17	43.70	51.44
Tgkh2020s	T18	38.30	57.44
Tefym	T19	37.50	58.33
Tchal	T20	40.00	55.55
Tchipal	T21	35.80	60.22
Tbgu	T22	34.80	61.33
Tbk	T23	27.30	69.66
Tbpal	T24	32.70	63.66
Tckr	T25	12.00	86.66
Tcaupal	T26	34.50	61.66
Tmcpal	T27	36.80	59.11
Control	T28	90.00	00.00
	SE $\pm$	0.12	
	Cd 1%	0.44	

\*Presented data in table is average of three replications

**Fig. 5: *In vitro* efficacy of *Trichoderma* isolates against *Alternaria* spp.**



#### 4.2.3.6. Antagonistic potential of promising isolates against five pathogens

It is apparent from the results presented in table 8 that, among all the 27 isolates, 7 isolates Tas (T<sub>11</sub>- areca nut-Shriwardhan), Tgpal (T<sub>14</sub> – guava- kelwe Palghar) Tmnraj (T<sub>3</sub> – mango –Lanja) Tkorrr (T<sub>5</sub>- Rice – Kolambe) Tbk (T<sub>23</sub> – Brinjal – Karjat) Tckr (T<sub>25</sub>- cabbage Karjat) Tbpal (T<sub>24</sub> –Brinjal- Mahim) were very effective against all the five pathogens under study. Out of these, three isolates were from Raigad, 2 from Palghar and 2 from Ratnagiri district of Konkan region. Tas was the most effective against *Fusarium*, Tgpal against *Rhizoctonia*, Tbk against *Sclerotium*, Tkorrr against *Colletotrichum*, Tckr against *Alternaria*. The isolate Tmnraj ranked second in antagonism against *Sclerotium*, third in control of *Fusarium* and *Colletotrichum* and fifth in *Rhizoctonia* and *Alternaria*. As far as the antagonism performance of the isolate Tbpal is concerned it ranked fourth against *Rhizoctonia* and *Colletotrichum*, sixth against *Sclerotium*, seventh against *Fusarium* and *Alternaria*.

All the isolates recorded 50 per cent inhibition of all the pathogens except *Sclerotium*. In case of this pathogen, most of the isolates recorded growth inhibition within a range of 15- 42 per cent and only 5 isolates recorded more than 50 per cent inhibition.

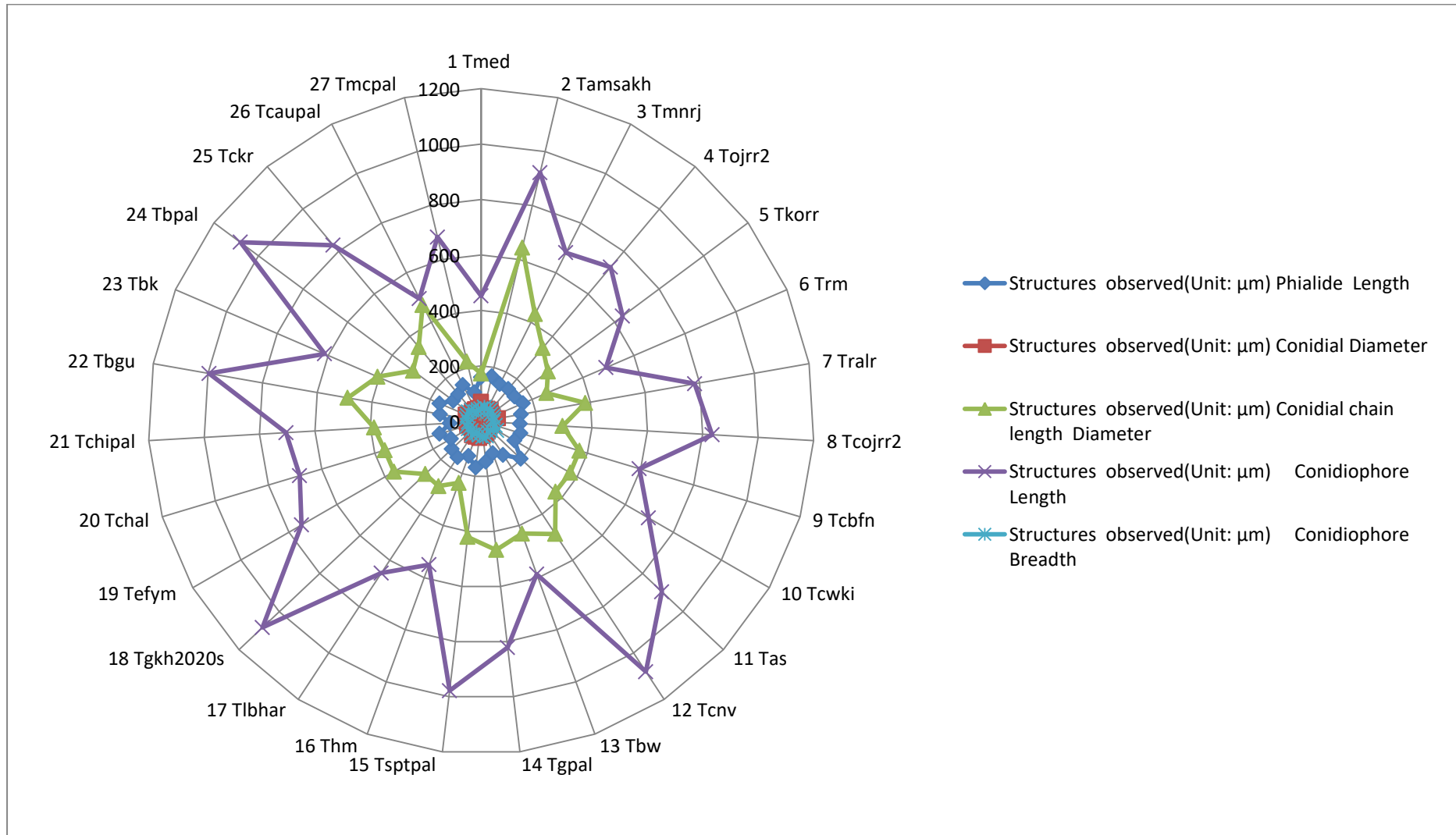
The best seven isolates in the experiment were further investigated for molecular characterization.

**Table 8 : Comparative antagonistic potential of the isolates against five pathogens**

Sr. no	<i>Fusarium spp.</i>		<i>Rhizoctonia spp.</i>		<i>Sclerotium spp</i>		<i>Colletotrichum spp.</i>		<i>Alternaria spp.</i>	
	Isolate code	Inhibition (%)	Isolate code	Inhibition (%)	Isolate code	Inhibition (%)	Isolate code	Inhibition (%)	Isolate code	Inhibition (%)
1	Tas	82.22	Tgpal	81.11	Tbk	86.11	Tkorr	81.33	Tckr	86.66
2	Tgpal	81.33	Tbgu	77.77	Tmnrj	80.55	Tgpal	81.33	Tas	77.55
3	Tmnrj	79.22	Tas	75.55	Tas	74.22	Tmnrj	80.22	Tbk	69.66
4	Tkorr	79.22	Tbpal	74.11	Tkorr	71.66	Tbpal	78.33	Tkorr	66.44
5	Tbk	77.77	Tmnrj	71.11	Tgpal	52.55	Tbk	77.77	Tmnrj	66.11
6	Tckr	74.44	Tralr	70.55	Tbpal	42.77	Tas	71.11	Tgpal	64.11
7	Tbpal	69.11	Tkorr	69.56	Tckr	42.77	Tmcpal	68.00	Tbpal	63.66
8	Tcaupal	68.33	Tcaupal	68.33	Tcbfn	42.22	Tckr	64.44	Thm	62.77
9	Tmed	65.22	Thm	68.00	Tgkh2020s	39.66	Tcnv	64.11	Tsptpal	61.88
10	Trm	64.77	Trm	67.55	Thm	35.33	Tgkh2020s	63.66	Tcaupal	61.66
11	Tralr	64.77	Tlbhar	67.00	Tojrr2	34.66	Tojrr2	63.55	Tmed	61.44
12	Tbgu	64.44	Tsptpal	66.11	Tcaupal	31.44	Tbw	63.11	Tbgu	61.33
13	Tcnv	64.22	Tchipal	65.33	Tbw	30.33	Tchipal	60.33	Trm	60.55
14	Tmcpal	64.11	Tmcpal	65.33	Tmed	30.22	Tcbfn	59.66	Tchipal	60.22
15	Tchipal	62.67	Tckr	64.77	Tcojrr2	29.44	Tralr	58.66	Tralr	59.22
16	Tcojrr2	60.88	Tojrr2	64.44	Trm	28.55	Tcwki	58.11	Tbw	59.22
17	Tbw	59.22	Tamsakh	63.11	Tbgu	27.77	Tlbhar	58.11	Tmcpal	59.11
18	Tojrr2	58.88	Tcbfn	63.11	Tcwki	23.66	Trm	58.00	Tcwki	58.88
19	Thm	58.88	Tefym	63.00	Tchal	21.33	Tcaupal	58.00	Tefym	58.33
20	Tlbhar	58.66	Tcwki	62.77	Tchipal	20.77	Thm	56.11	Tojrr2	58.11
21	Tcwki	58.33	Tchal	62.44	Tlbhar	19.11	Tchal	55.33	Tcbfn	57.44
22	Tcbfn	57.55	Tcojrr2	62.22	Tamsakh	18.55	Tmed	54.77	Tgkh2020s	57.44
23	Tchal	57.44	Tbw	60.33	Tmcpal	18.00	Tbgu	54.44	Tcojrr2	56.66
24	Tgkh2020s	56.44	Tgkh2020s	60.33	Tsptpal	17.55	Tefym	54.11	Tamsakh	56.11
25	Tsptpal	56.33	Tmed	59.44	Tralr	17.22	Tcojrr2	52.44	Tchal	55.55
26	Tamsakh	53.88	Tbk	59.44	Tcnv	16.44	Tamsakh	51.44	Tcnv	53.88
27	Tefym	51.66	Tcnv	58.66	Tefym	15.33	Tsptpal	49.66	Tlbhar	51.44



**Fig. 6: Measurement of morphological structures**



### **4.3. Study of morphological characters and molecular characterization of distinct promising isolates**

#### **4.3.1. Microscopic observations of *Trichoderma* isolates**

The 27 isolates which exhibited mycelial growth in culture were selected for morphological studies. The microscopic examination under 100 X lens revealed morphological variability among the isolates. The details of colony characters are presented in table no 9 and morphological features are presented in table10.

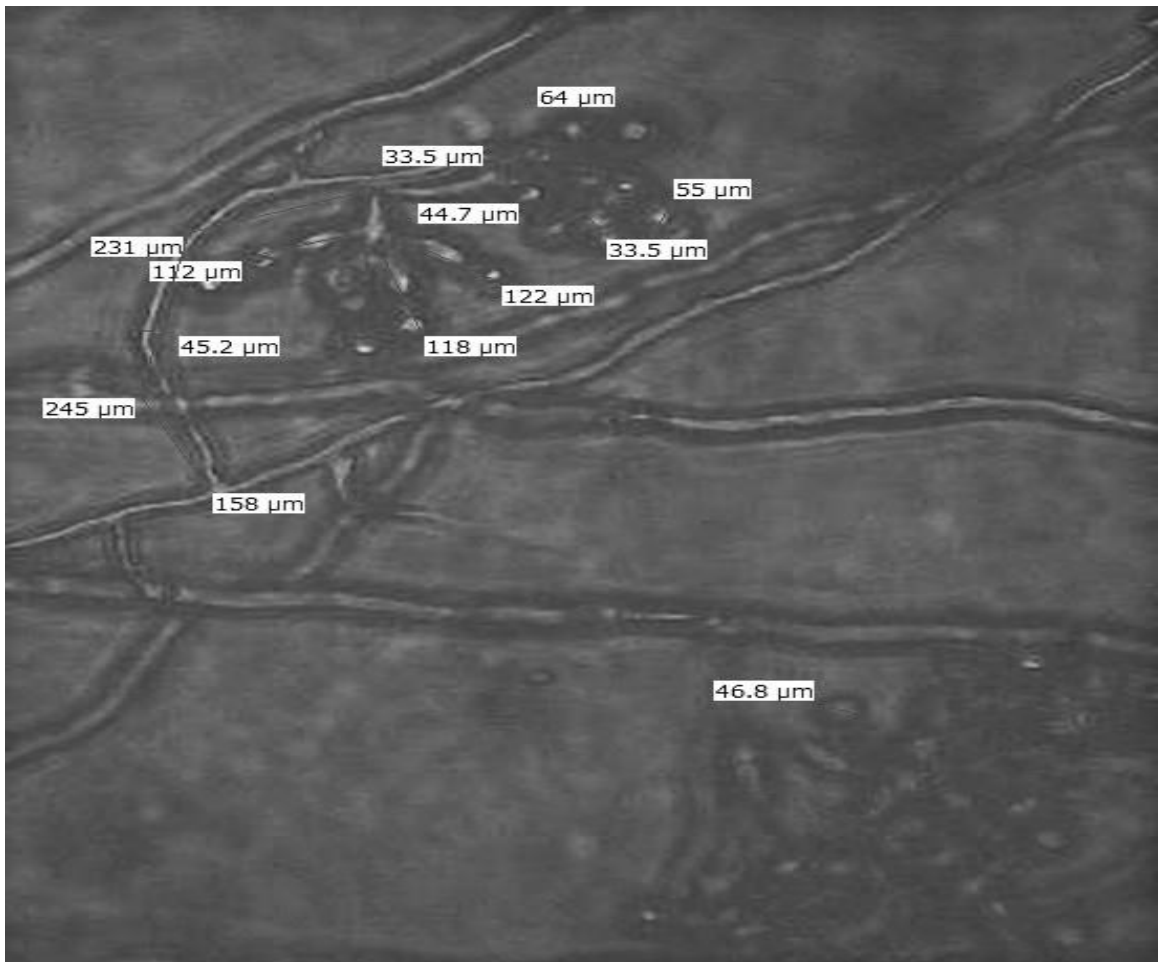
**Table 9: Colony characters of the isolates**

Sr. No	Isolate Code	Colony appearance	Hypha	Phialides	Conidia	Conidia Arrangement
1	Tmed	Yellowish-white to dull green	Tree branches	Ampuliform and Langeniform	Sub-globose	Catenate
2	Tamsakh	Yellow to green	Tree branches	Sigmoid or hooked	Subglobose	Catenate
3	Tmnrj	Green to Dark green	Penicillate	Ampuliform. Held vertically in a whorl at an angle of 90 <sup>0</sup> to the conidiophore, bilateral.	Globose to Subglobose	Catenate
4	Tojrr2	Dark green, light green and white	Penicillate	Langeniform in effuse area and ampuliform in dense	Globose	Catenate
5	Tkorr	Blue green to yellowish green	Penicillate	Arise singly, laterally and appears less lageniform	Globose to Subglobose	Catenate
6	Trm	Green and dark green	Tree branches	Sigmoid or hooked	Globose to subglobose	Catenate
7	Tralr	Dark green and white	Penicillate	Sigmoid or hooked	Globose	Catenate
8	Tcojrr2	Light green, yellowish green and white	Tree branches	Lageniform in effuse area and ampuliform at dense area.	Globose to subglobose	Catenate
9	Tcbfn	Light green and white	Tree branches	Verticillate and less lageniform	Subglobose	Catenate
10	Tcwki	Olive green to dark green	Tree branches	Verticillate more or less lageniform, solitary	Globose	Catenate
11	Tas	Dark green growth including few white aerial mycelia	Penicillate	Produced solitary or in groups, straight to ampulliform to lageniform, variable in shape and size.	Globose to Subglobose	Catenate
12	Tcnv	Light green and white	Tree branches	Sigmoid or hooked	Globose	Catenate
13	Tbw	Light green and white	Penicillate	Sigmoid or hooked	Globose to subglobose	Catenate
14	Tgpal	Blue green to yellowish green	Tree branches	Arise singly, laterally and appear sausage shaped	Globose to Subglobose	Catenate

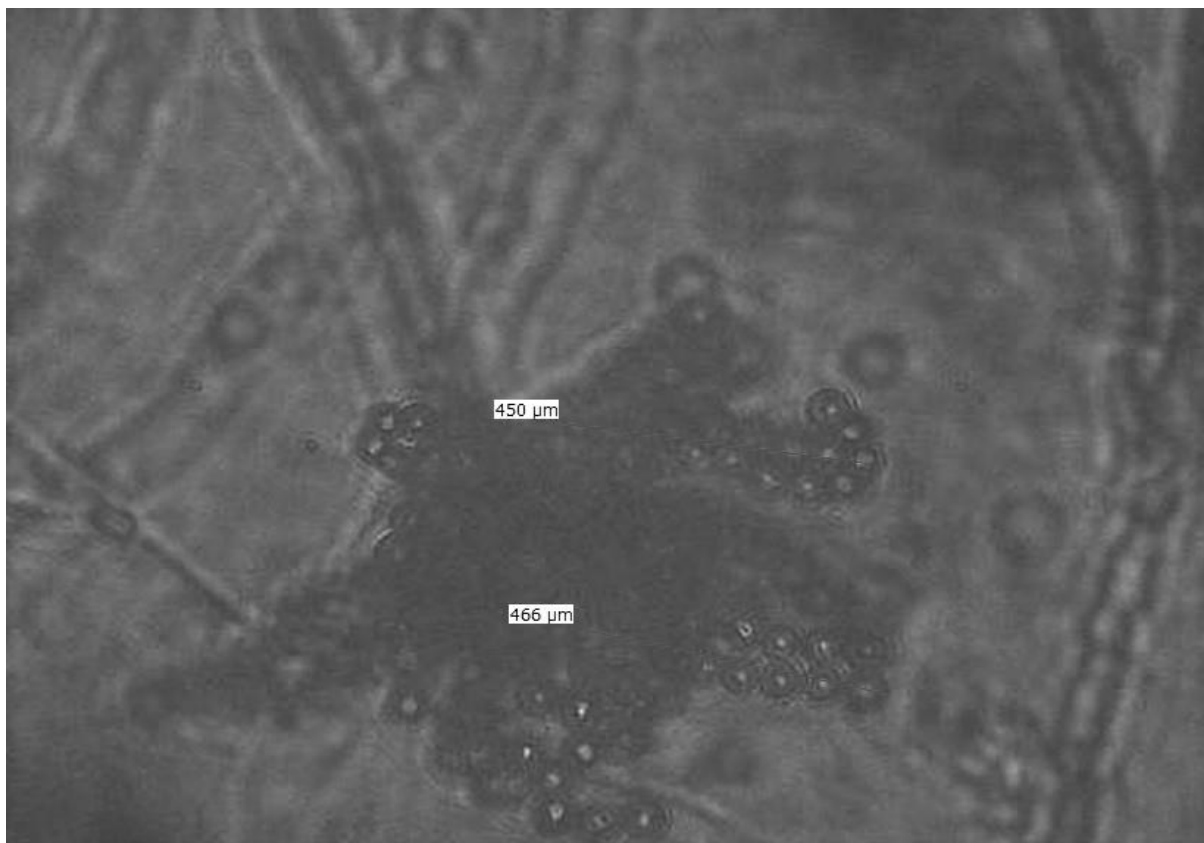
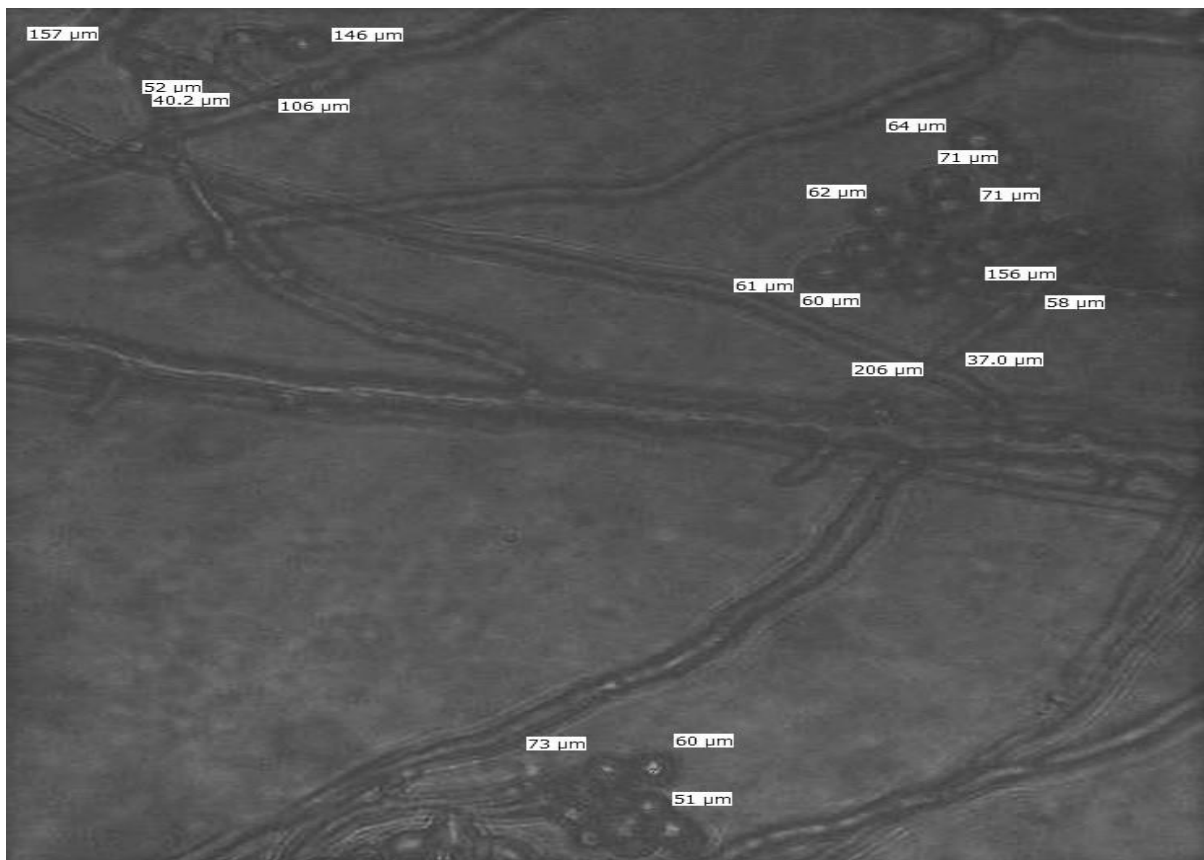
<b>Sr. No</b>	<b>Isolate Code</b>	<b>Colony appearance</b>	<b>Hypha</b>	<b>Phialides</b>	<b>Conidia</b>	<b>Conidia Arrangement</b>
15	Tsptpal	Yellow to green	Tree branches	Sigmoid or hooked	Globose to sub-globose	Catenate
16	Thm	Light green, yellowish green and white	Tree branches	Langeniform in effuse area and ampuliform at dense	Globose	Catenate
17	Tlbhar	Dark green	Penicillate	Nine-pin shape	Globose	Catenate
18	Tgkh2020s	Yellow to green	Tree branches	Sigmoid or hooked	Globose	Catenate
19	Tefym	Dark green	Tree branches	Nine-pin shape	Globose	Catenate
20	Tchal	Olive green to dark green	Penicillate	Less lageniform to subulate divergent phialids	Globose	Catenate
21	Tchupal	Yellow to green	Penicillate	Nine-pin shape	Globose	Catenate
22	Tbgu	Light green and white	Tree branches	Sigmoid or hooked	Subglobose	Catenate
23	Tbk	Dark green growth including few white aerial mycelia	Penicillate	Produced solitary or in groups, straight to ampulliform to lageniform, variable in shaped and size	Globose to Subglobose	Catenate
24	Tbpal	Blue green to yellowish green	Tree branches	Arises singly, laterally and appears nine-pin bowling shaped singly.	Globose to Subglobose	Catenate
25	Tckr	Off-white colonies which later change to grayish green	Penicillate	Arises singly lageniform to subulate divergent phialides	Globose to Subglobose	Catenate
26	Tcaupal	Green to dark green	Penicillate	Sigmoid or hooked	Subglobose	Catenate
27	Tmcpal	Light green and white	Tree branches	Sigmoid or hooked	Globose	Catenate

PLATE IX : Microscopic morphological structures of *Trichoderma* isolates, (under light microscope: 100X lens) observed under microscope like phialides, conidia, conidia arrangement and conidiophores (Unit:  $\mu\text{m}$ )

(T<sub>1</sub>) Tmed

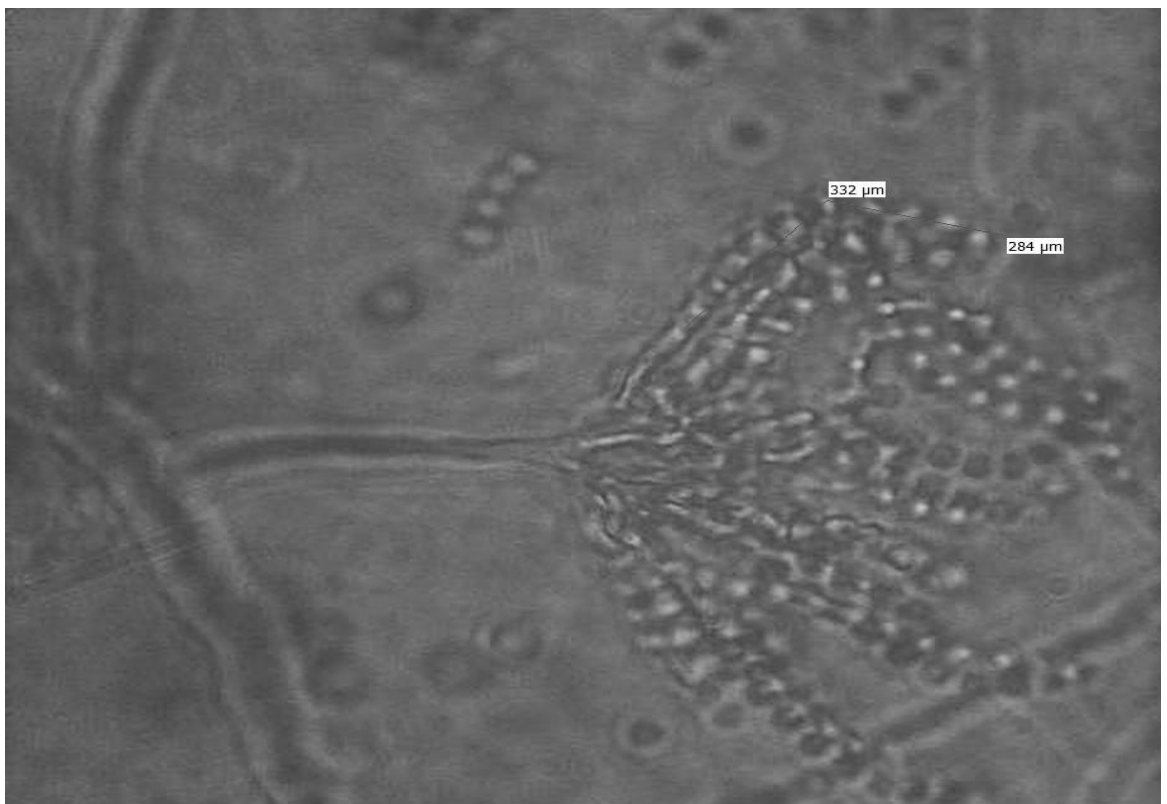
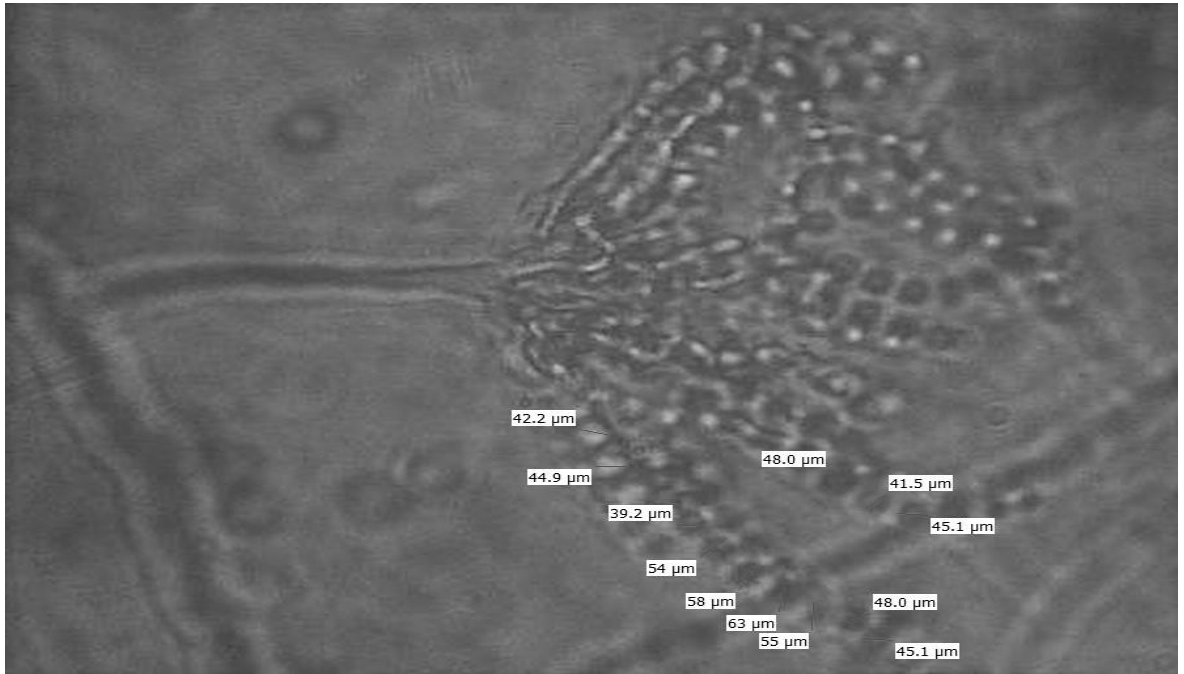


# PLATE X

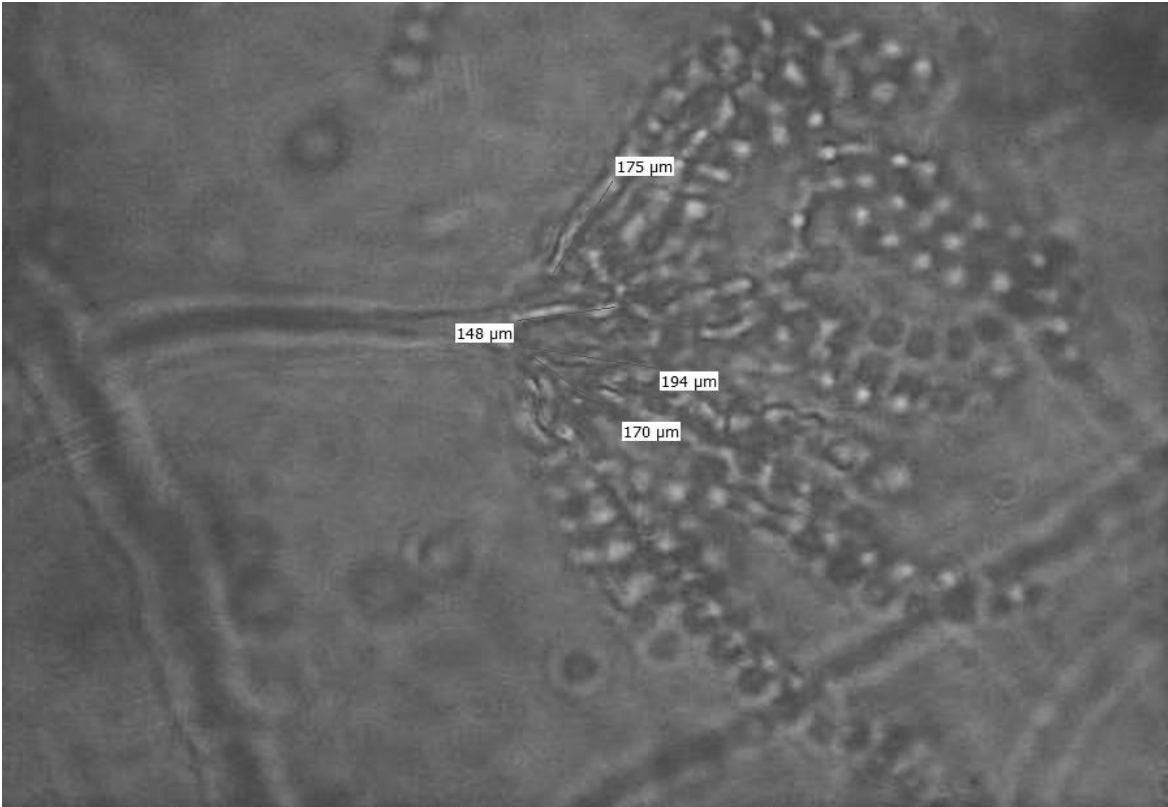
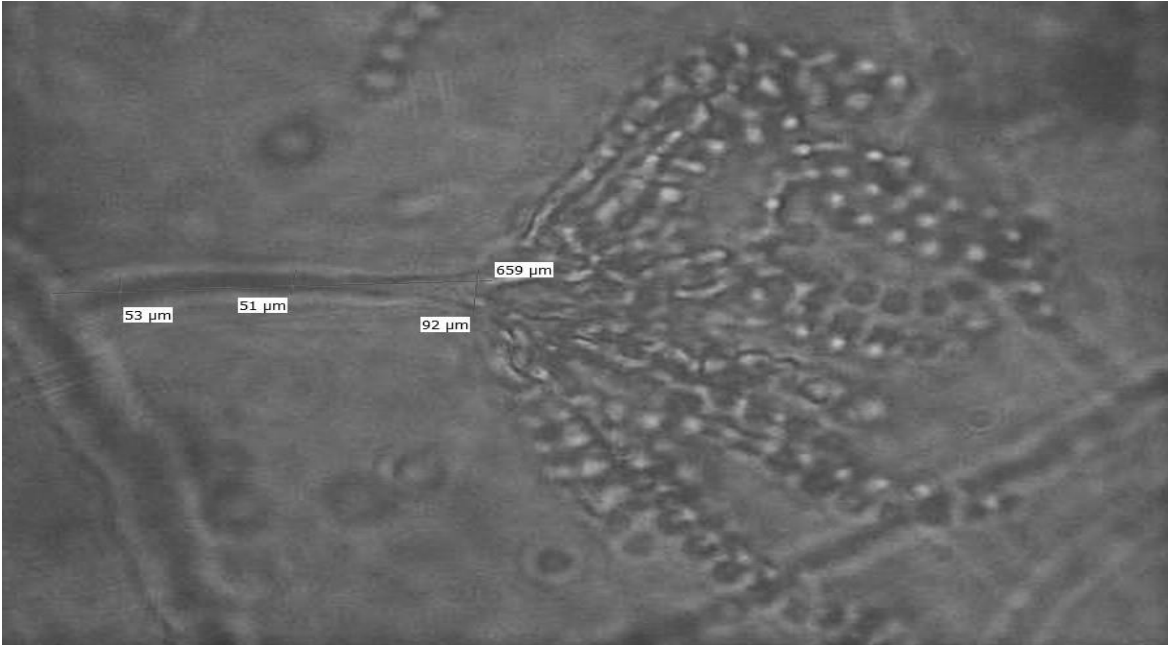


**PLATE XI**

**(T<sub>2</sub>)Tamsakh**

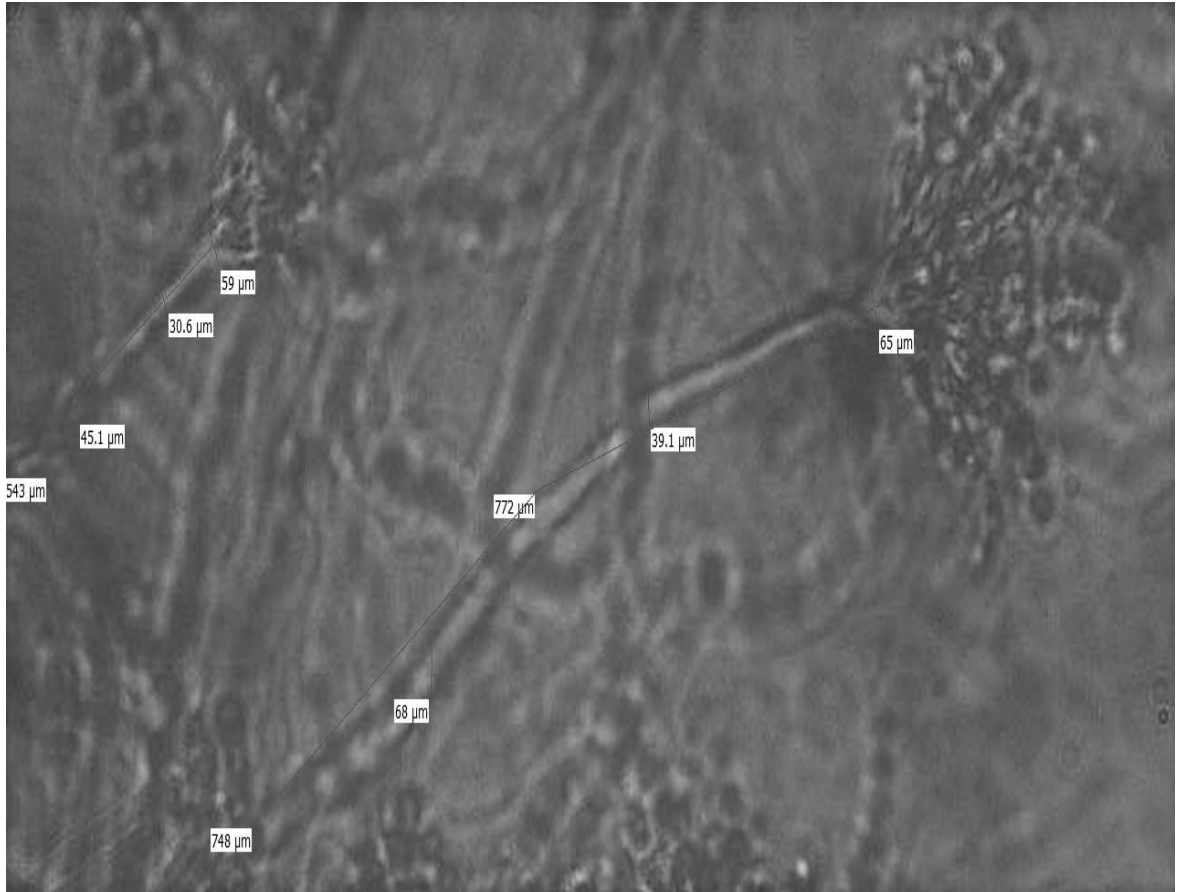


**PLATE XII**



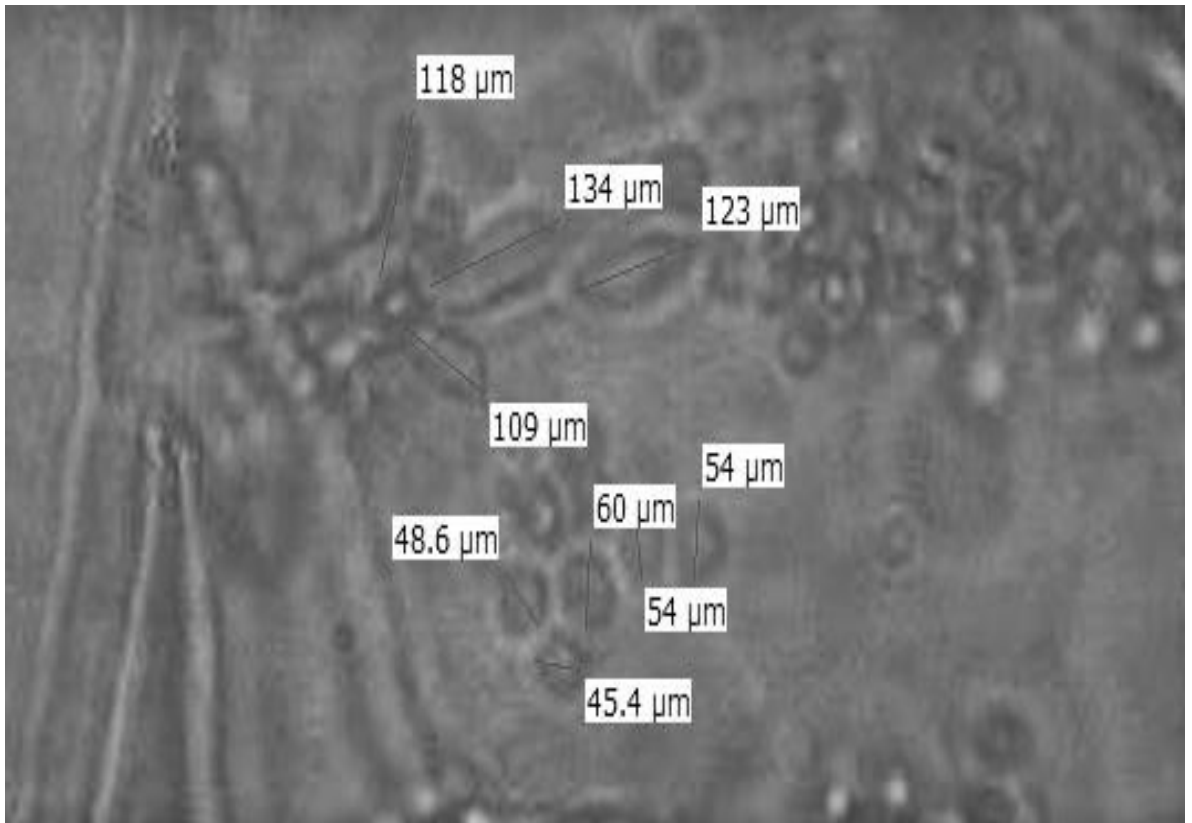
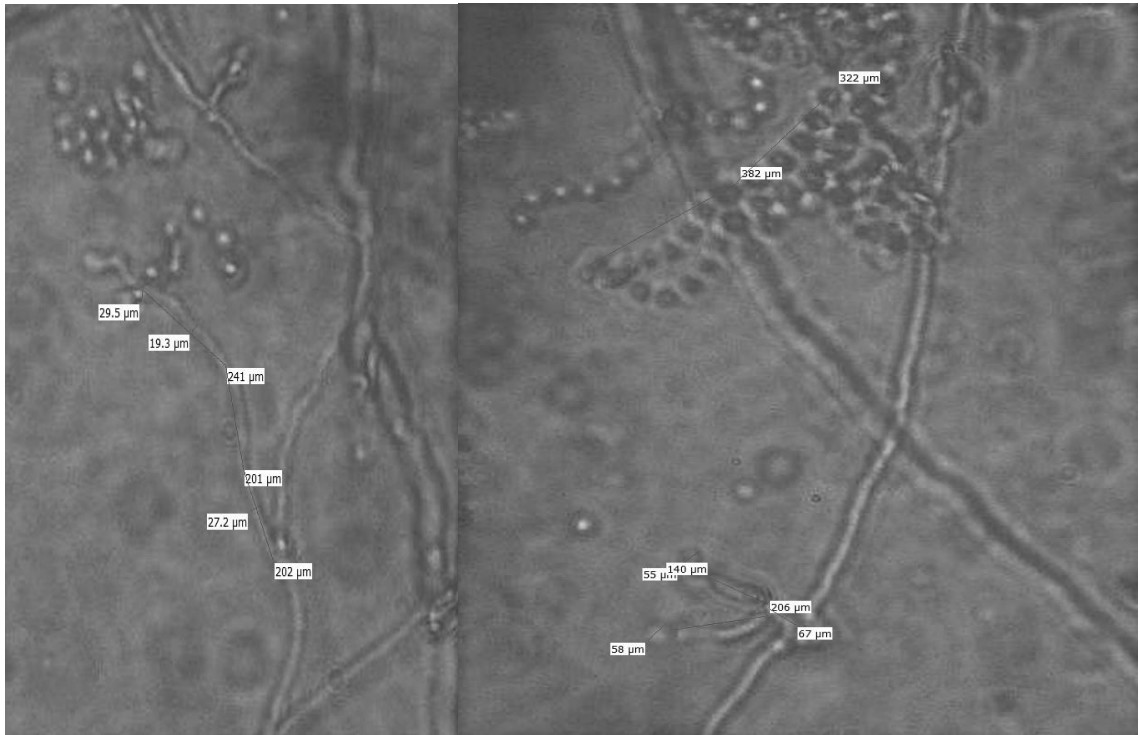


**PLATE XIII**



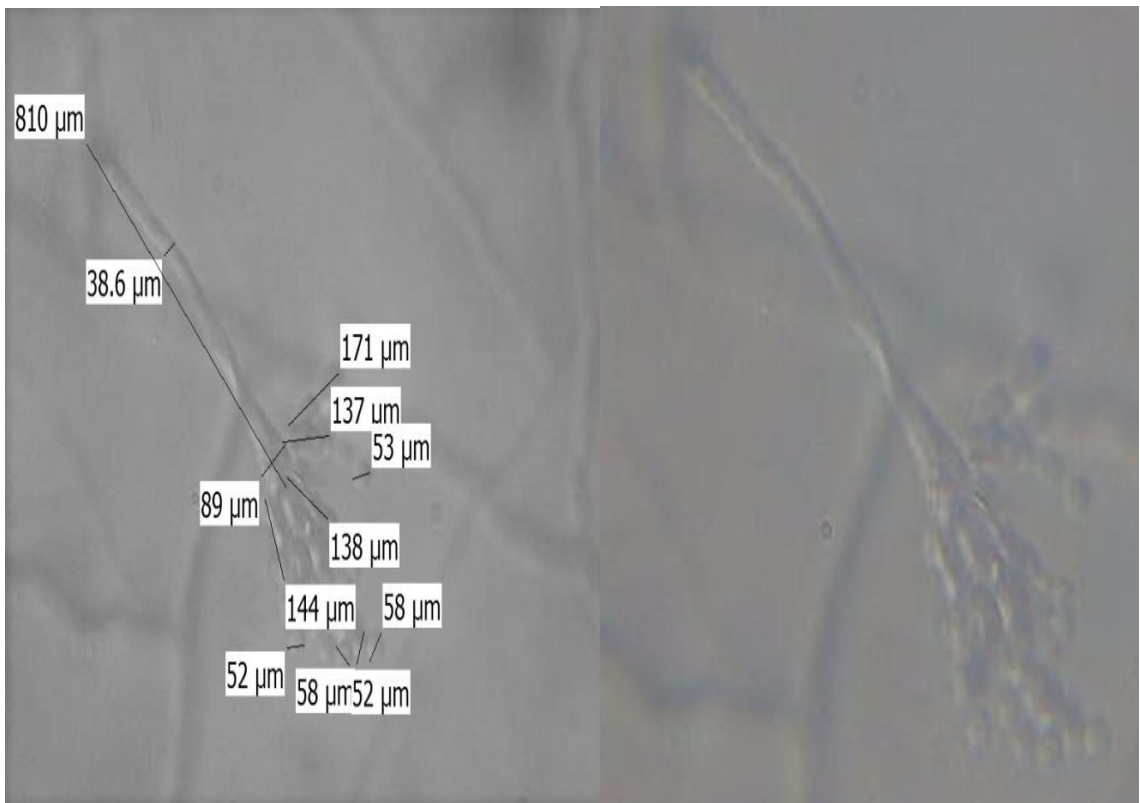
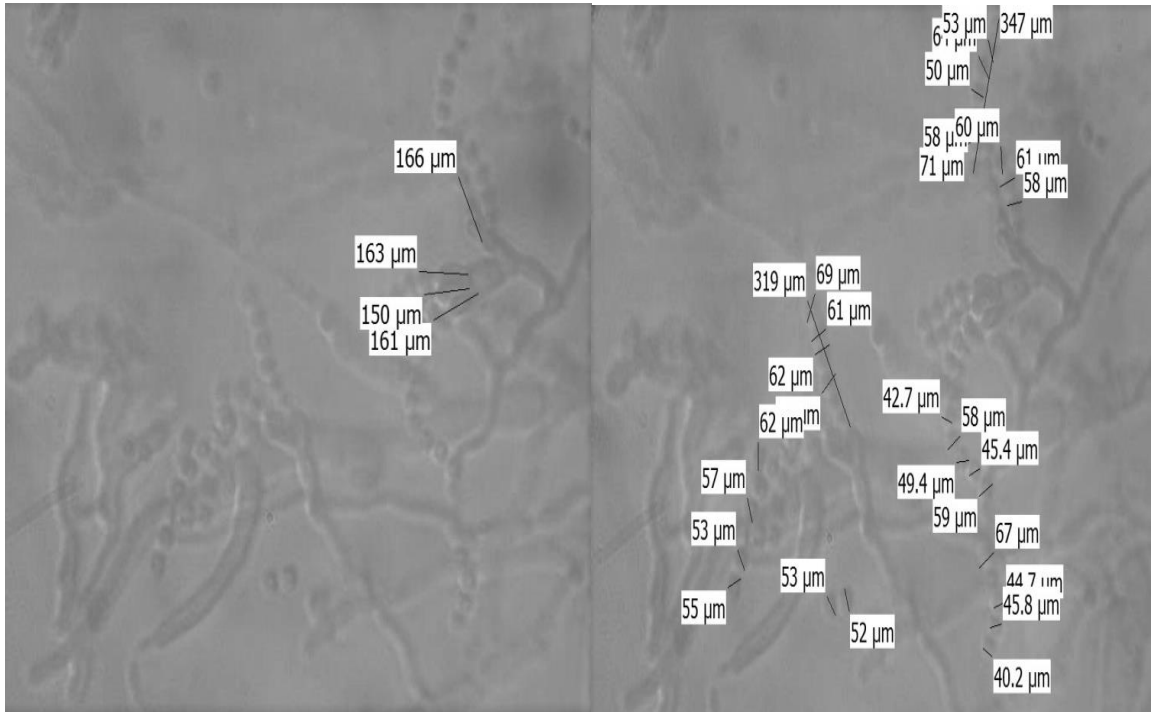
**PLATE XIV**

**(T<sub>3</sub>) Tmnrj**



**PLATE XV**

**(T4) Tojrr2**



# PLATE XVI

(T<sub>5</sub>) Tkorr

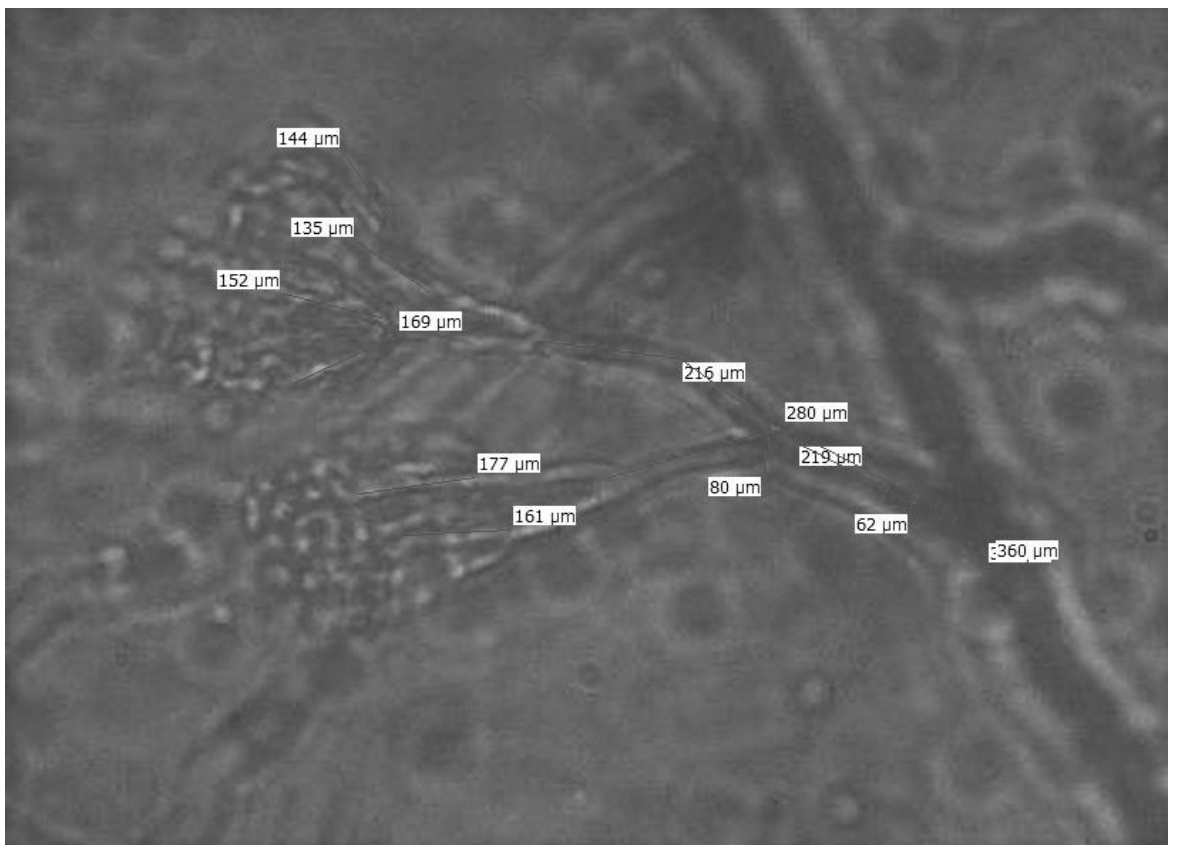
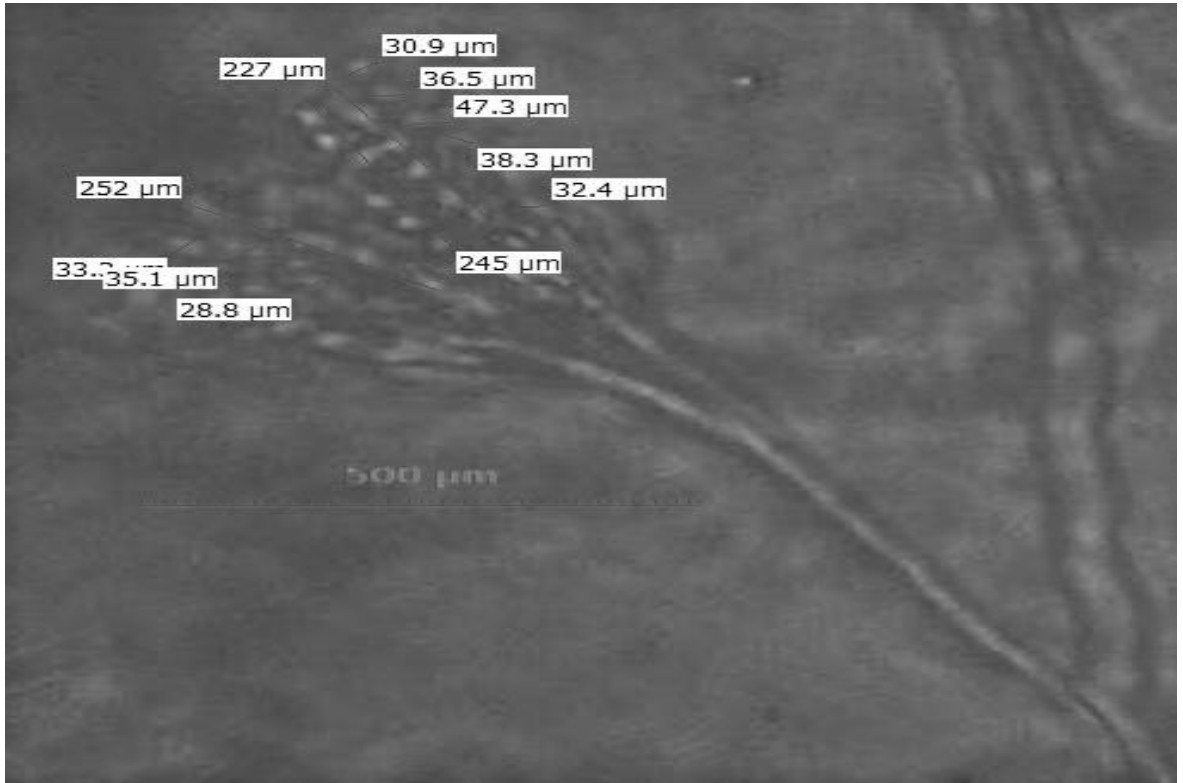
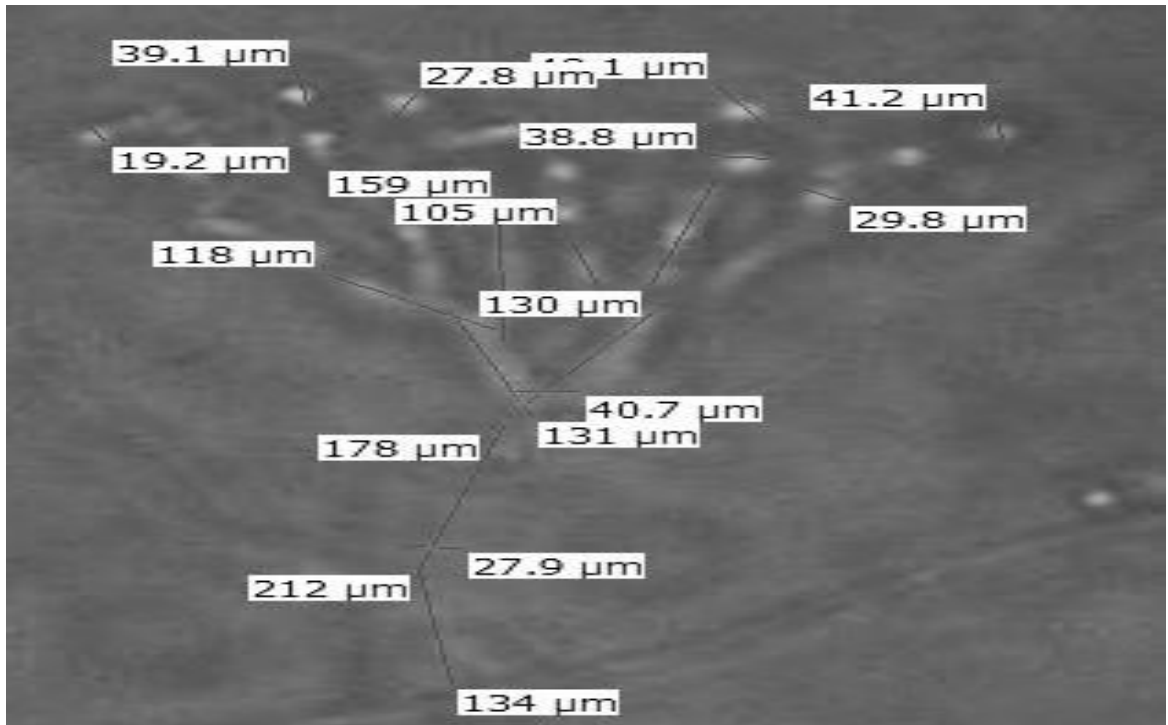


PLATE XVII



**PLATE XVIII**

**(T<sub>6</sub>) Trm**

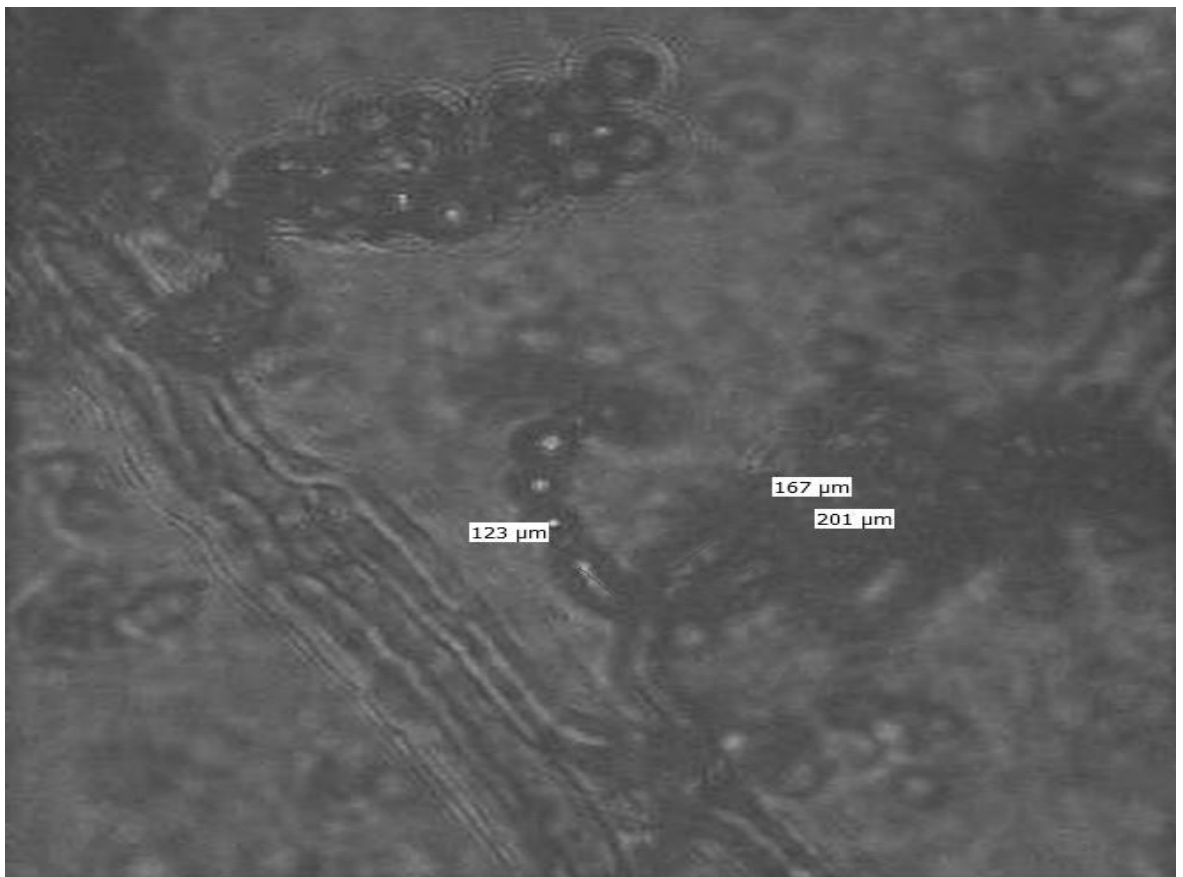
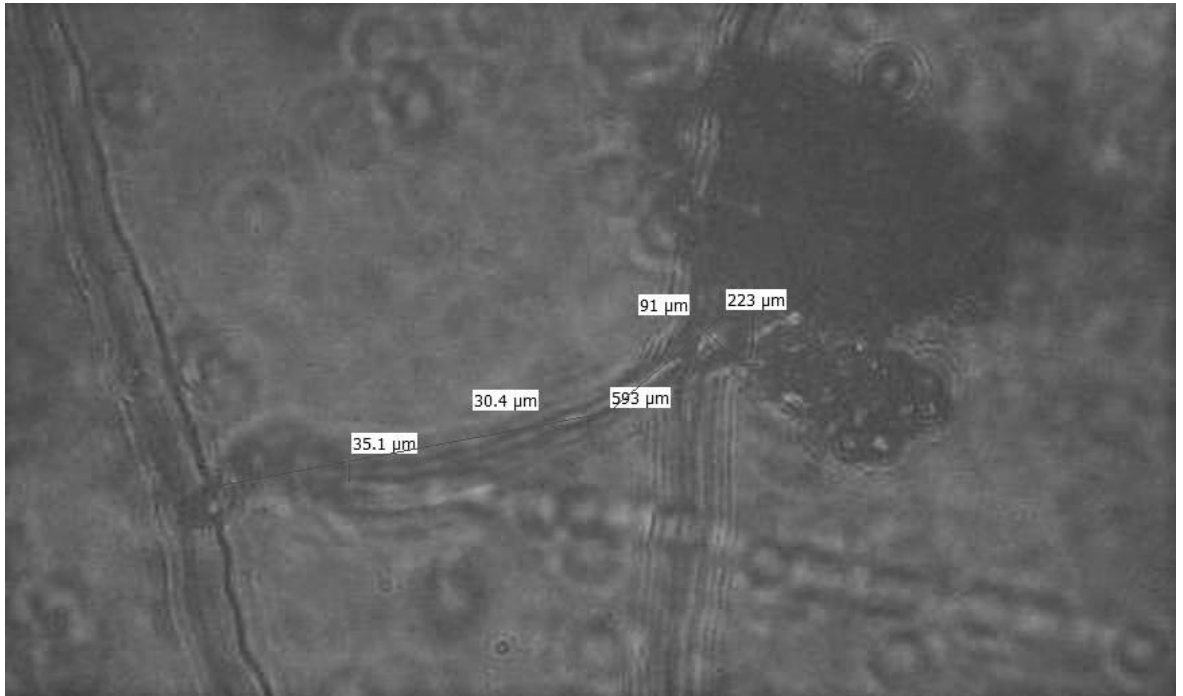
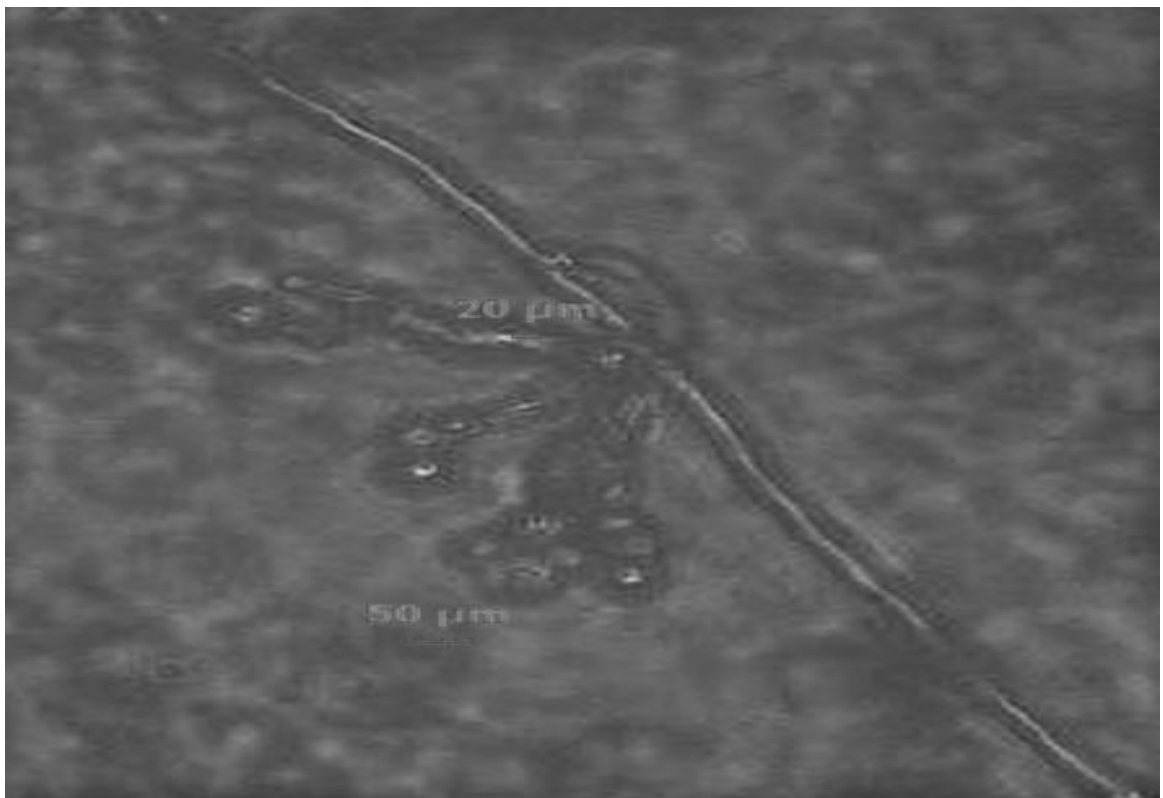
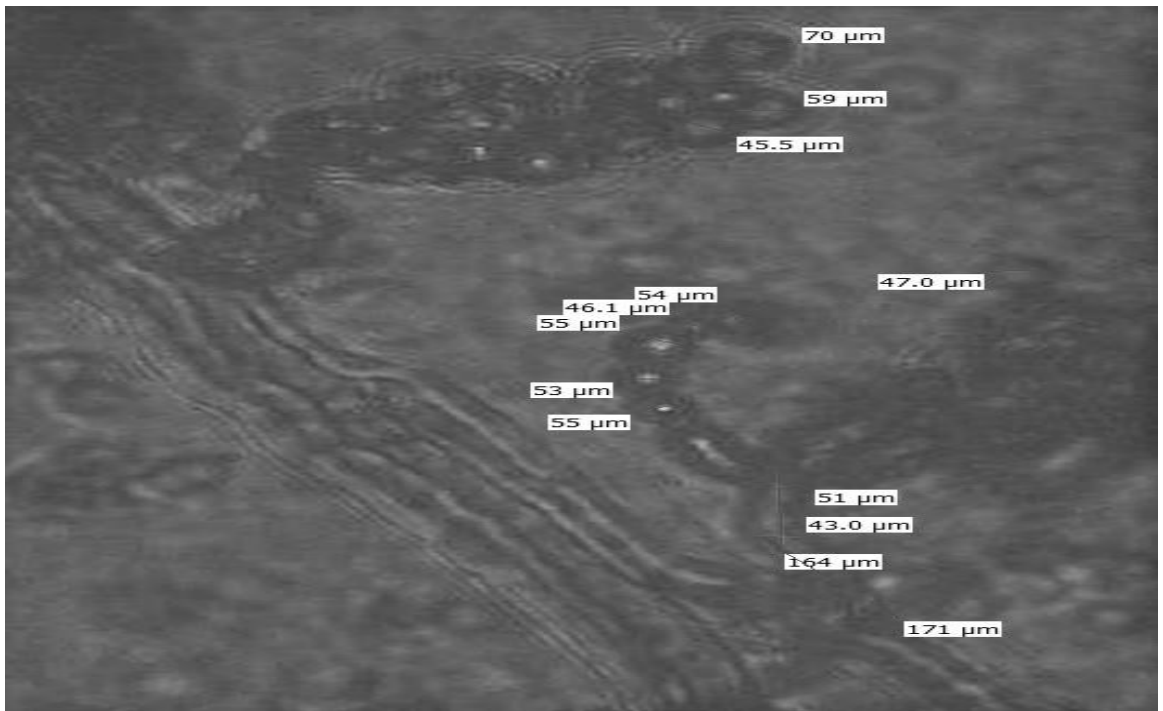


PLATE XIX



**PLATE XX**

**(T<sub>7</sub>) Tralr**

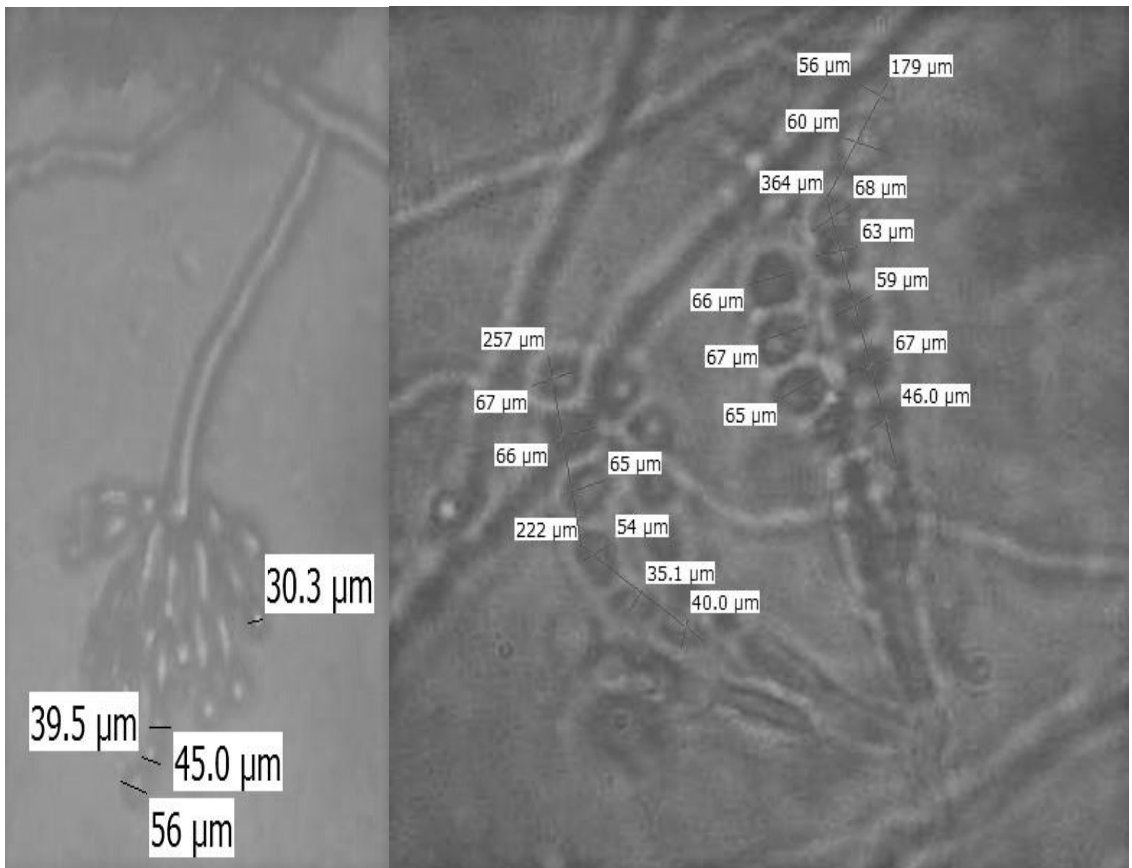
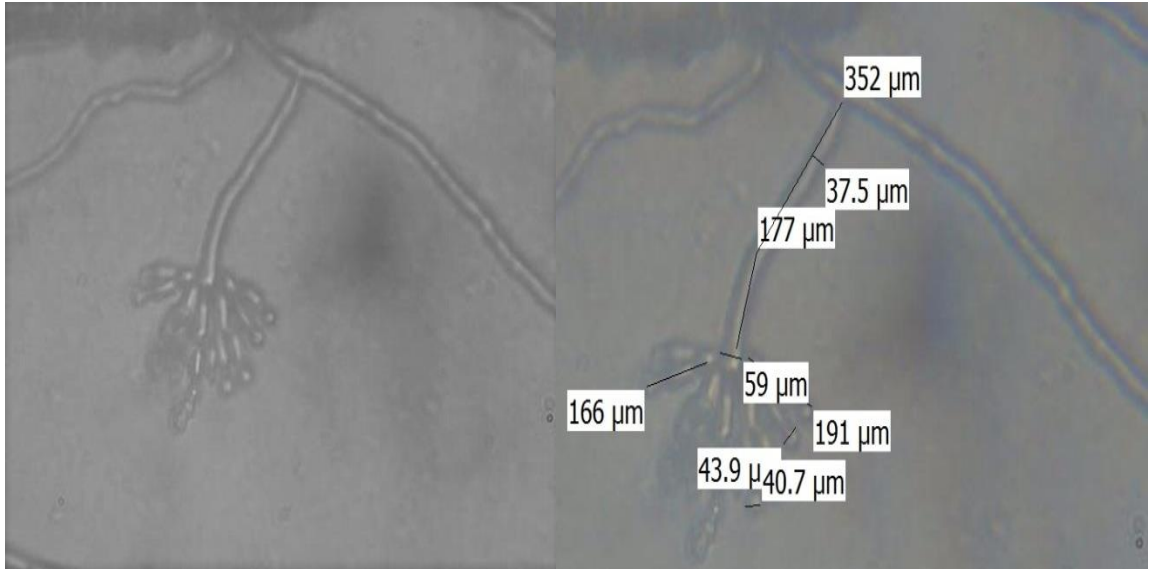




PLATE XXI

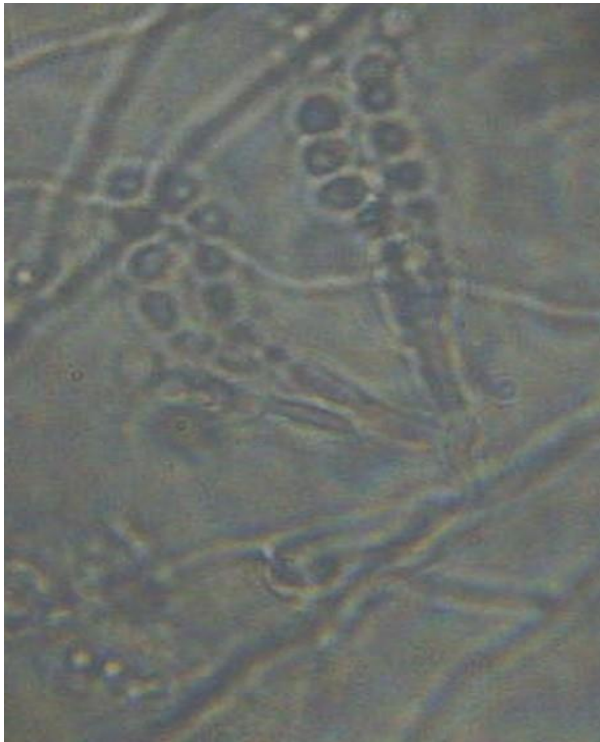
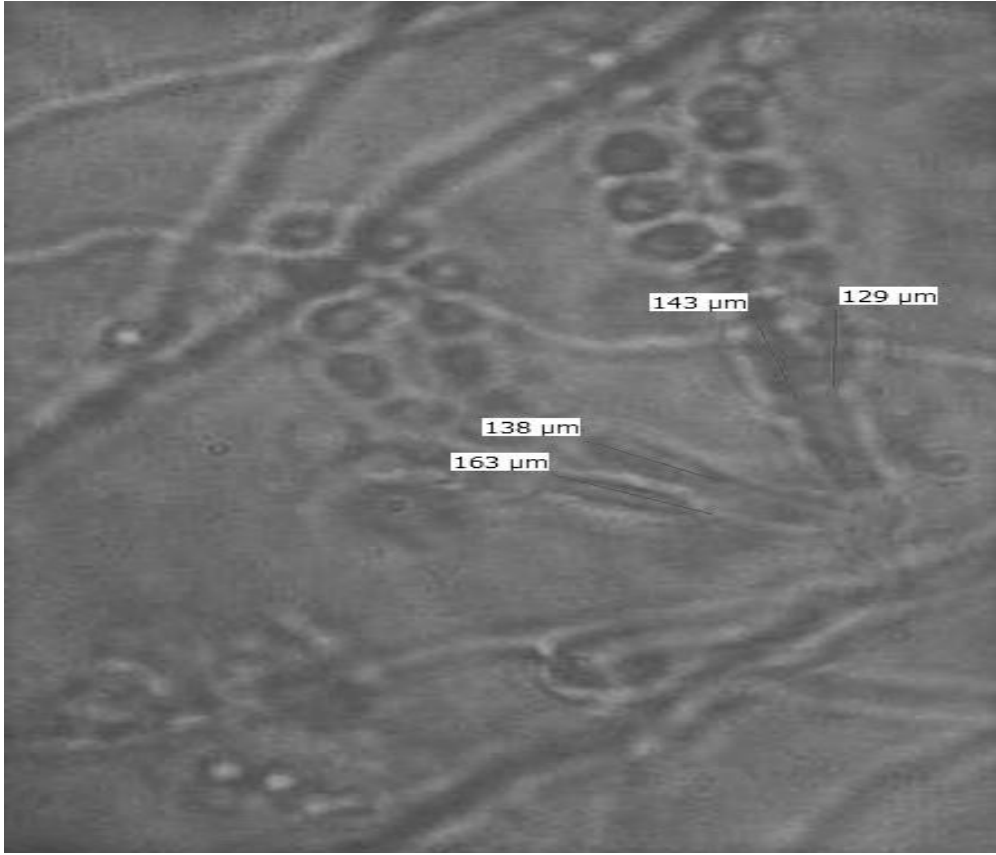
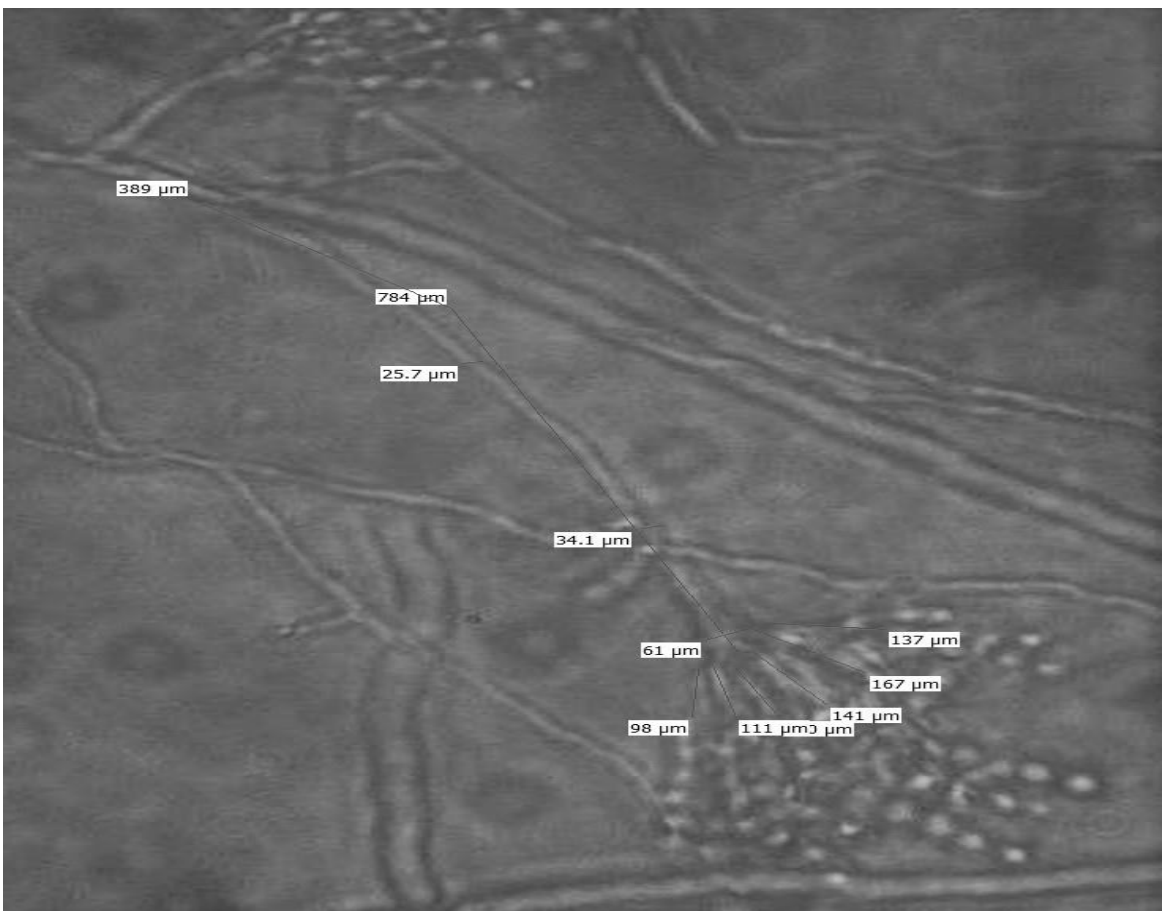
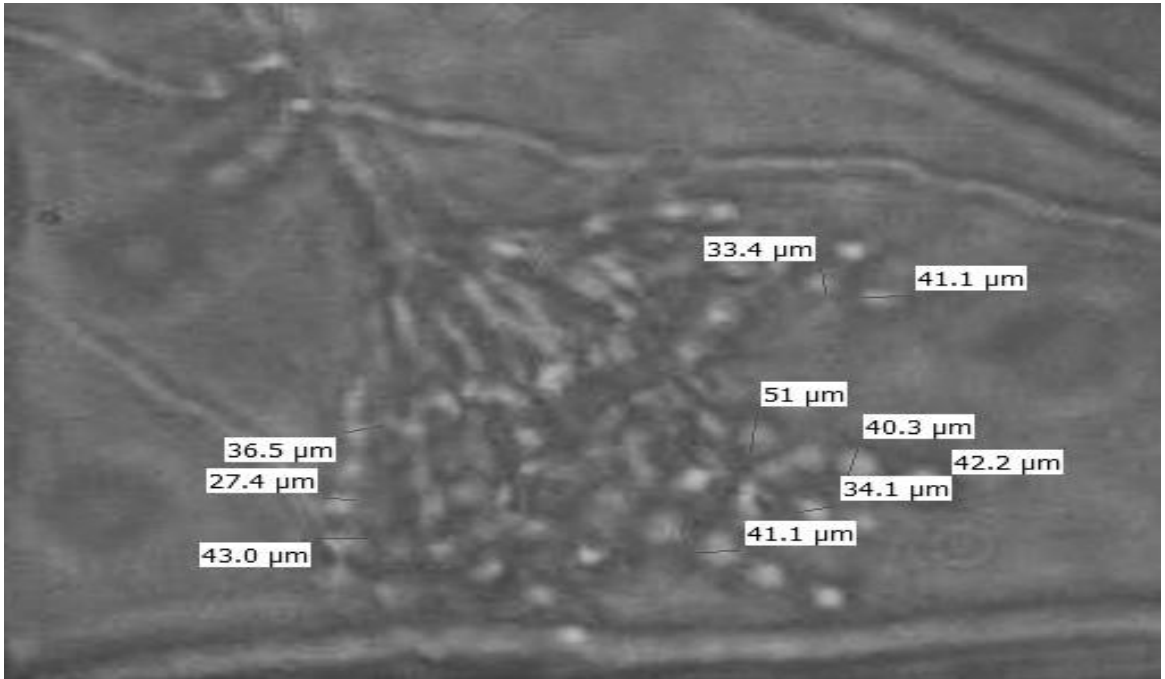
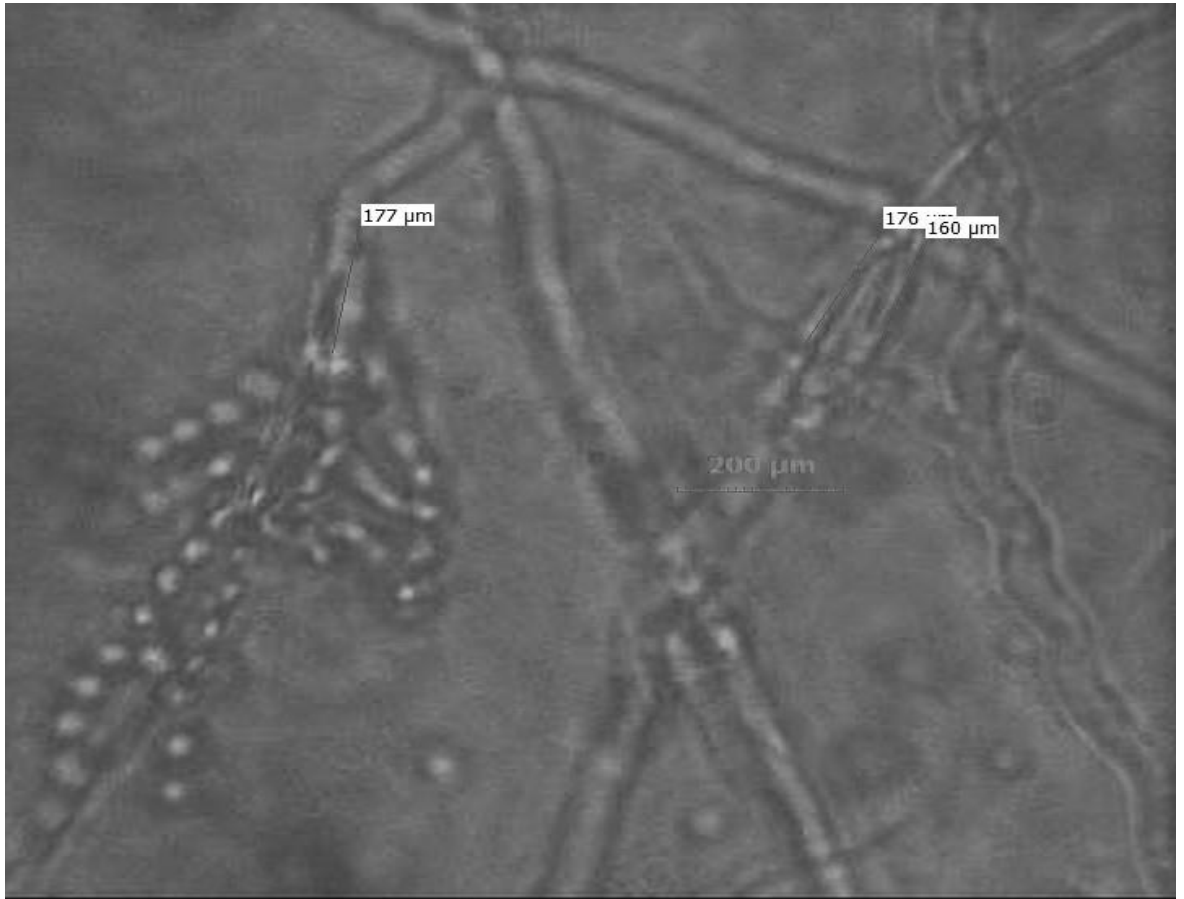


PLATE XXII

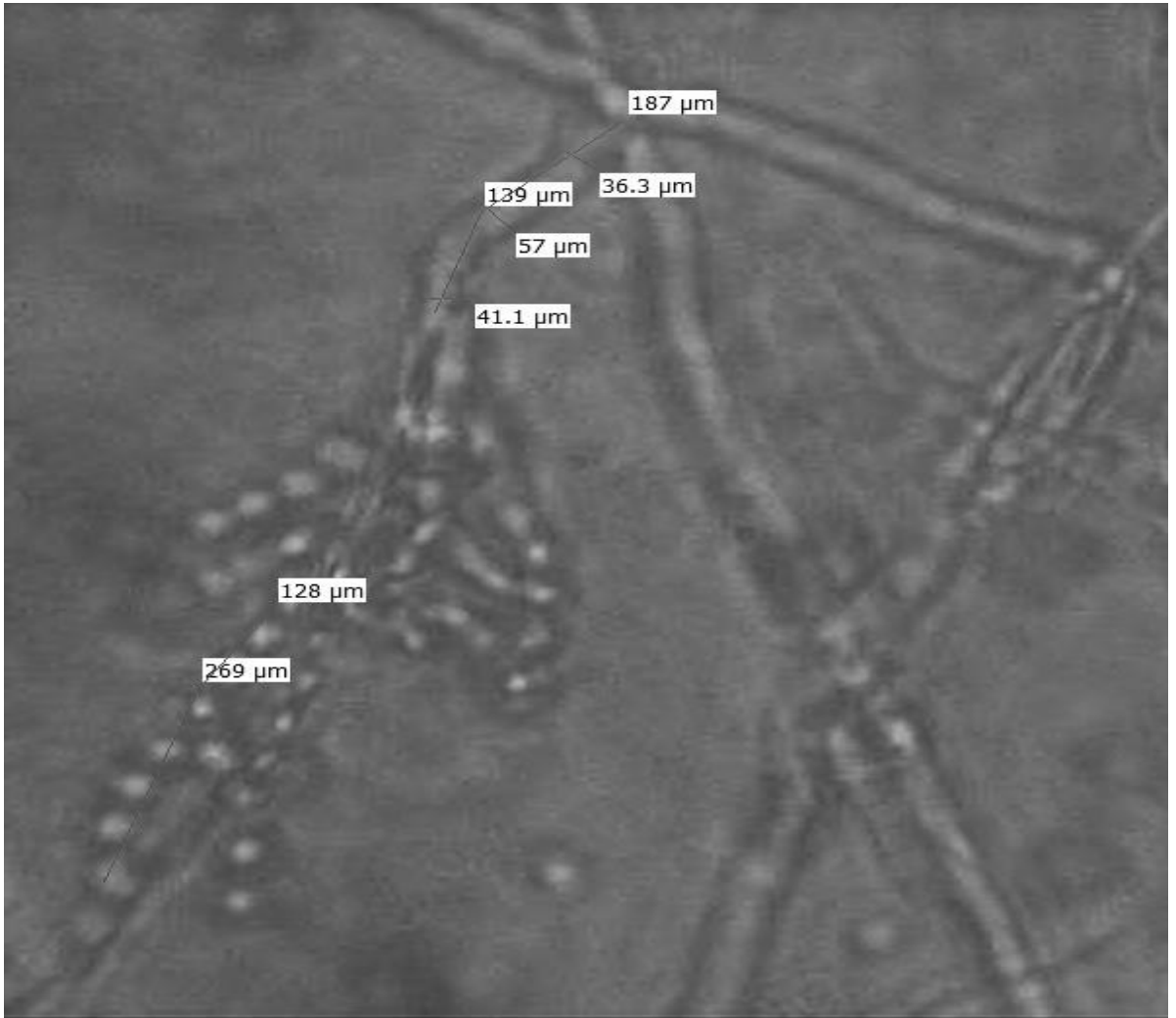
(T<sub>8</sub>) Tcojrr2



**PLATE XXIII**



**PLATE XXIV**



**PLATE XXV**

**(T<sub>9</sub>) Tcbfn**

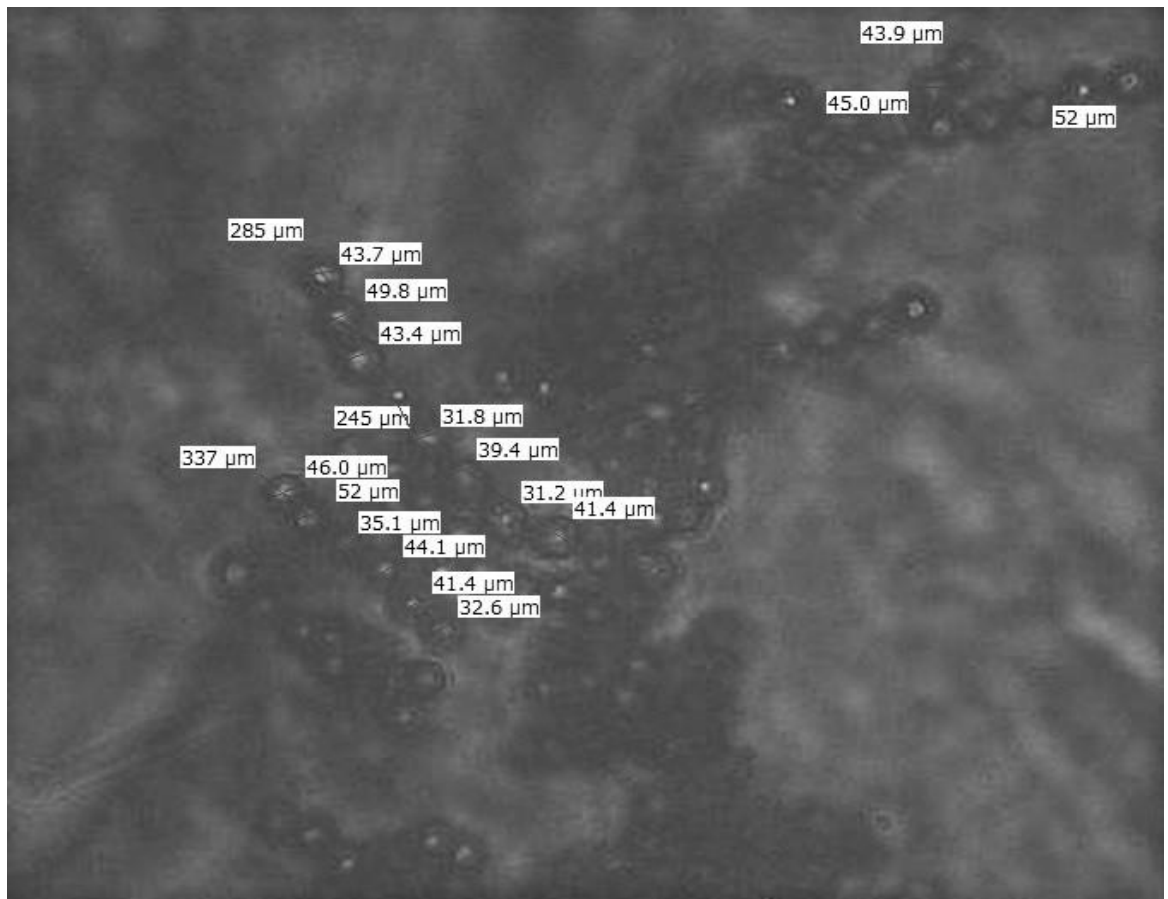
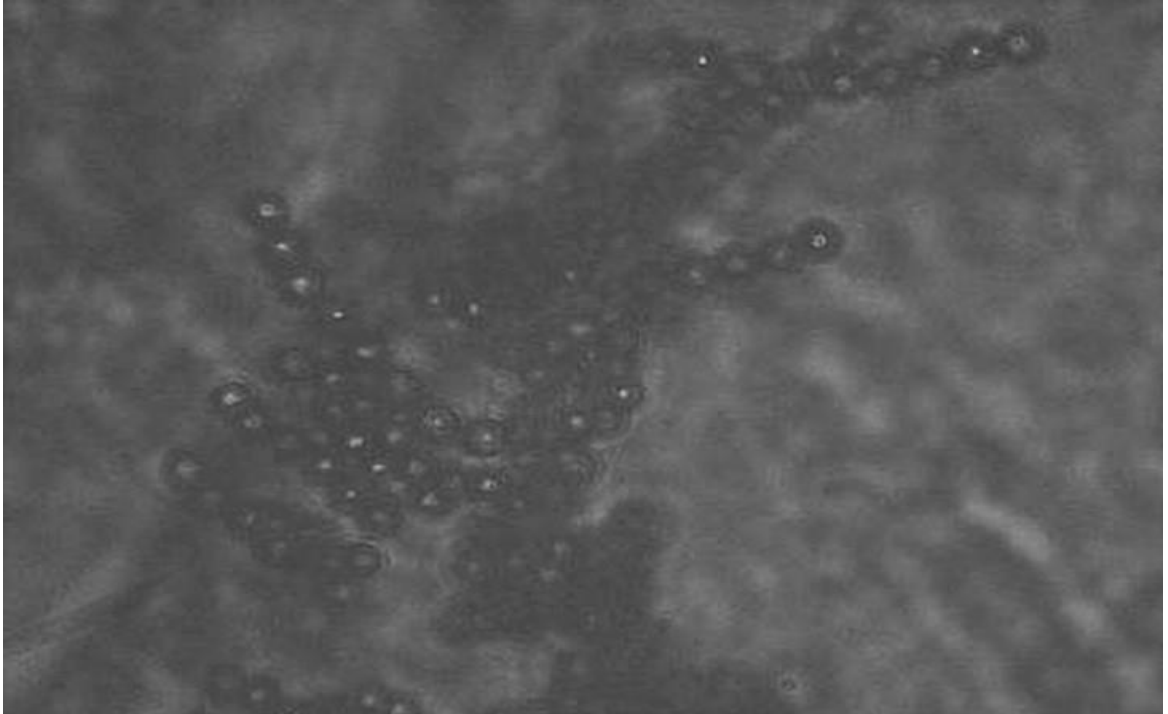
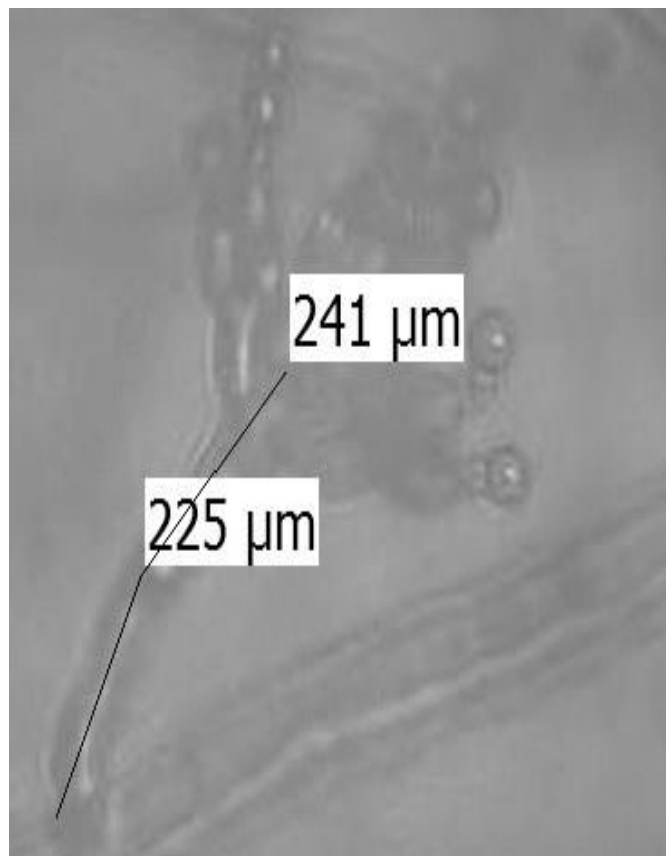
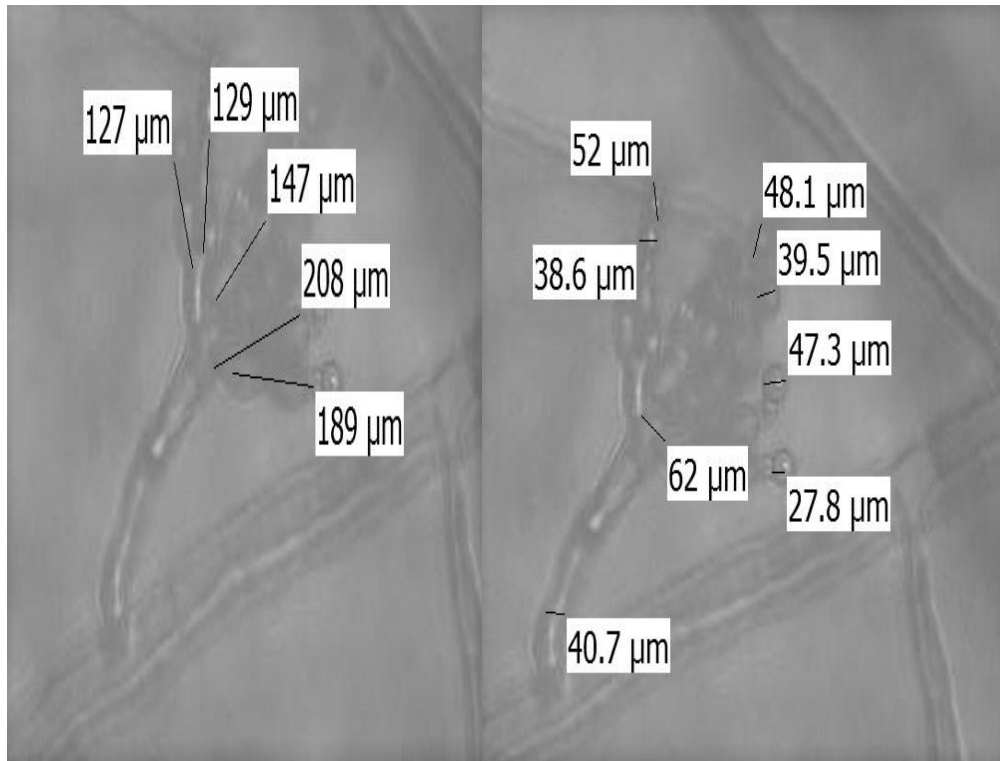


PLATE XXVI



**PLATE XXVII**

**(T<sub>10</sub>) Tcwki**

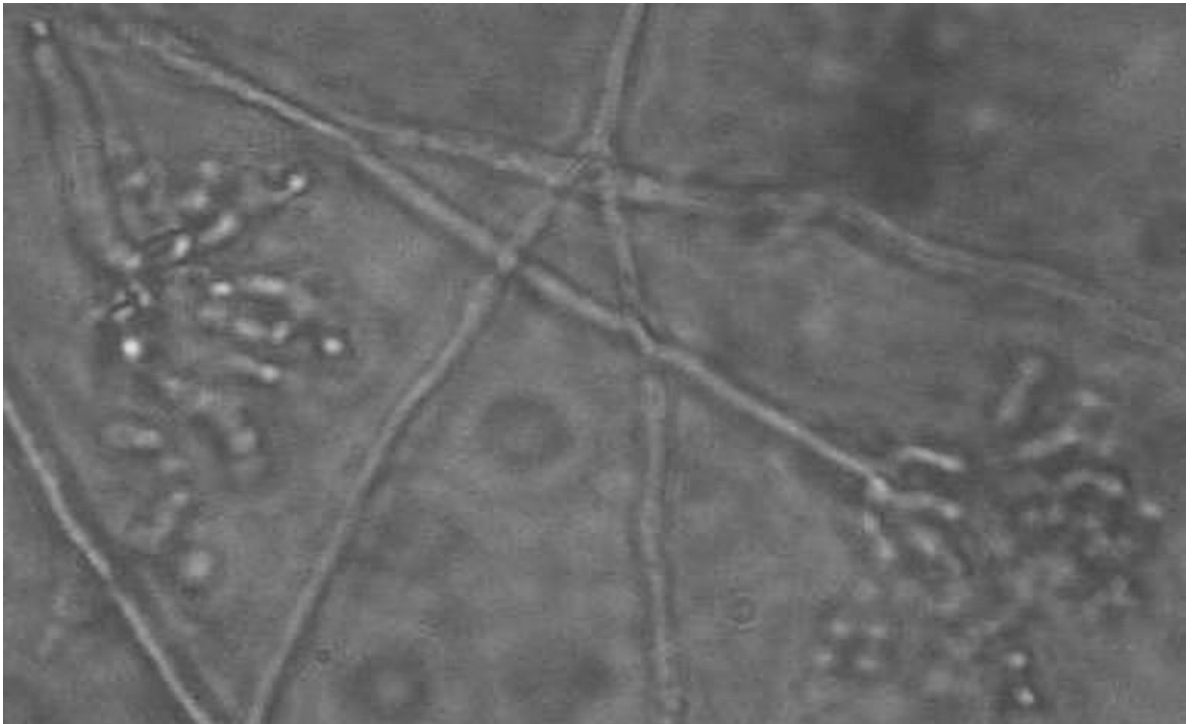
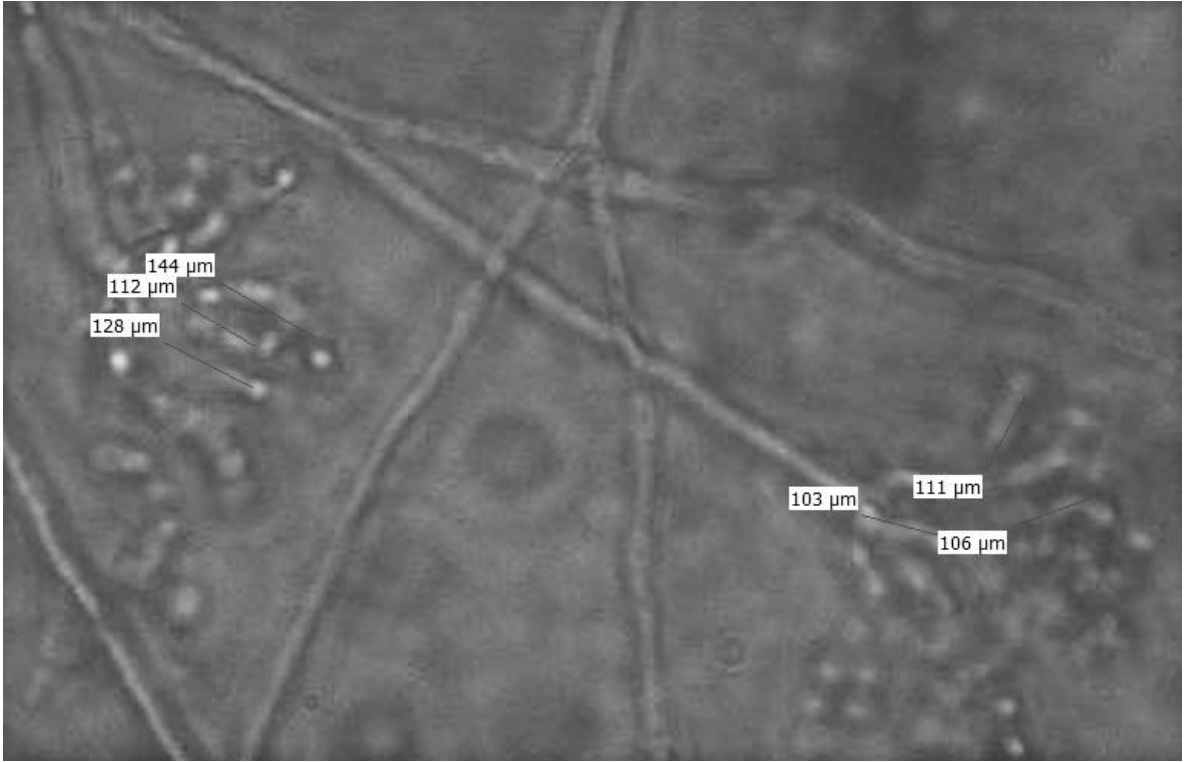


PLATE XXVIII

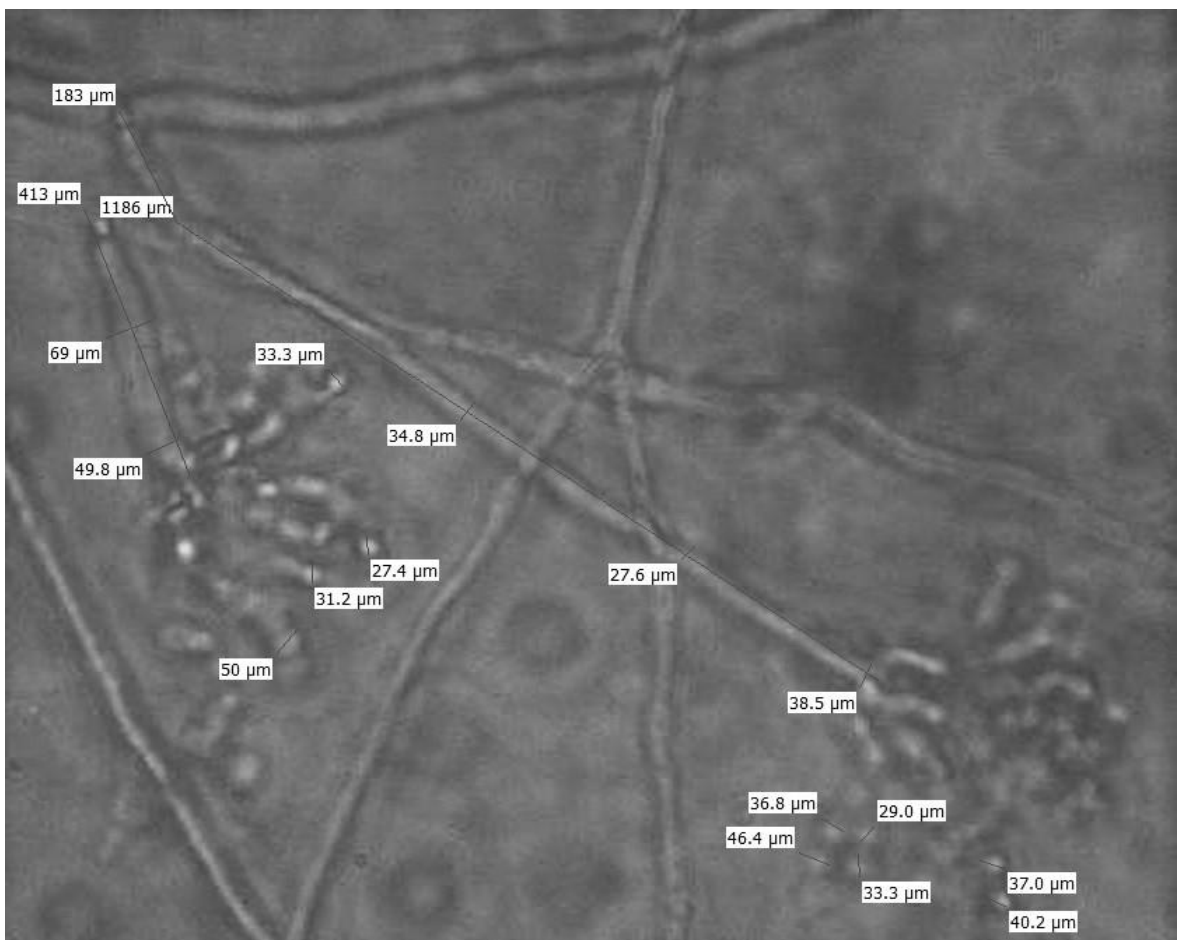
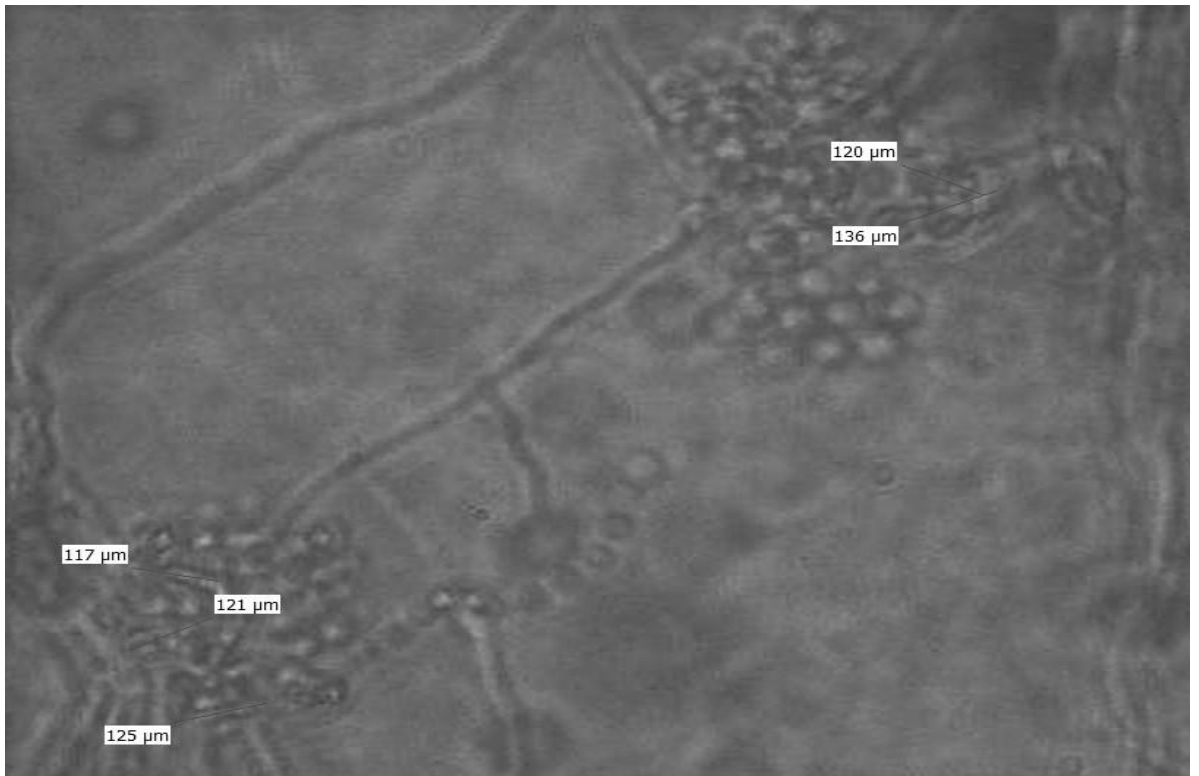




PLATE XXIX

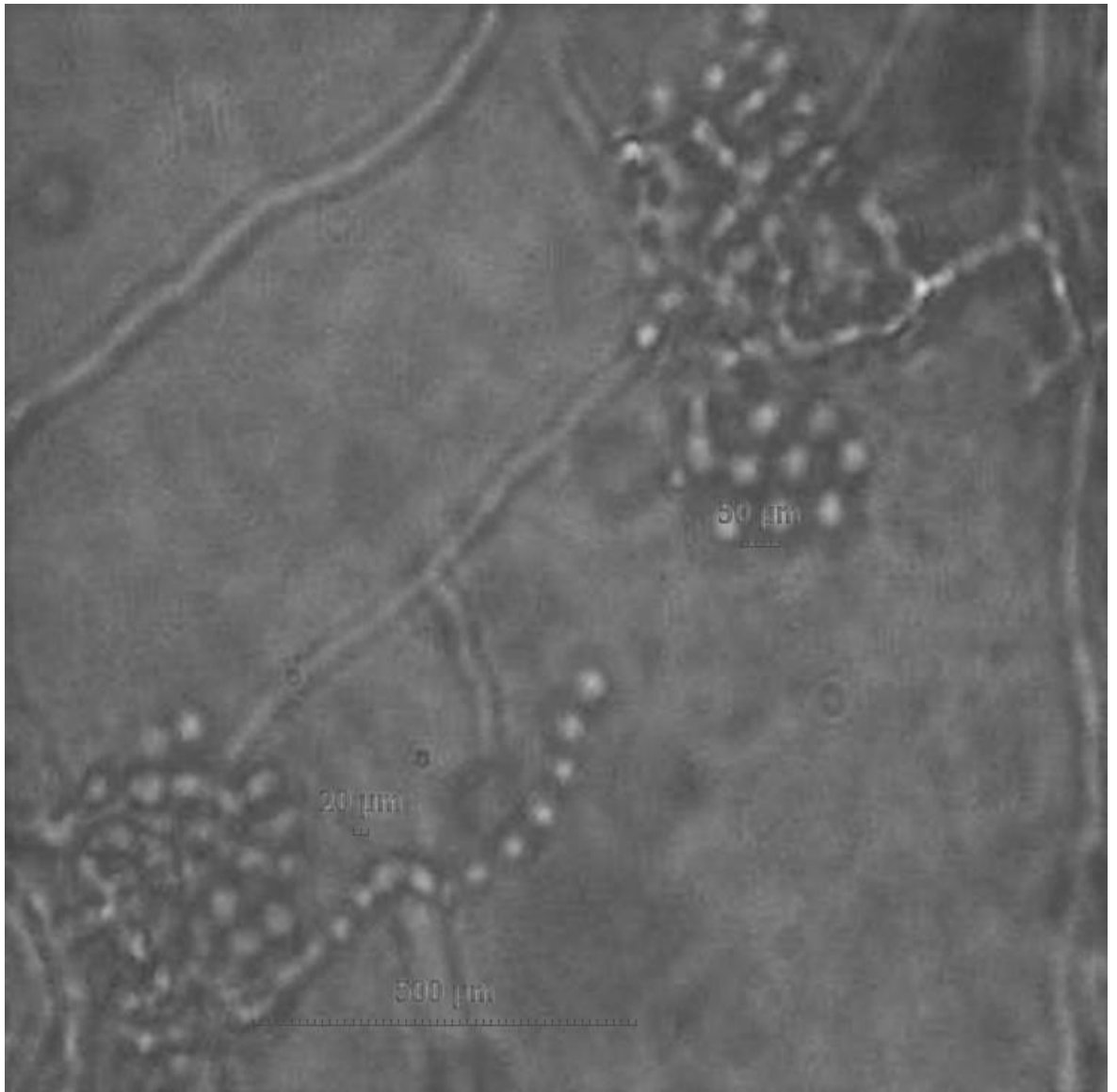


PLATE XXX

(T<sub>11</sub>)Tas

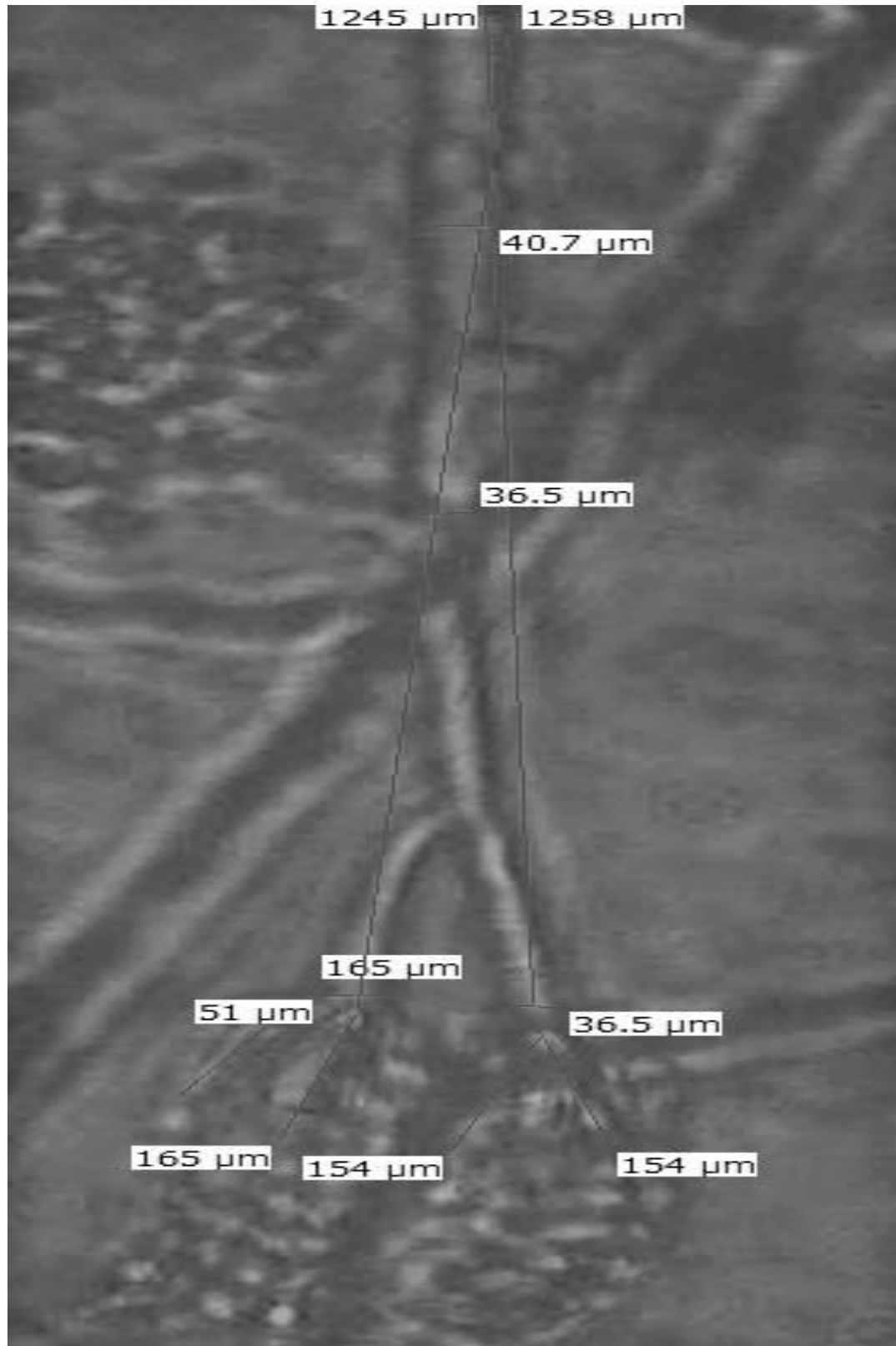


PLATE XXXI

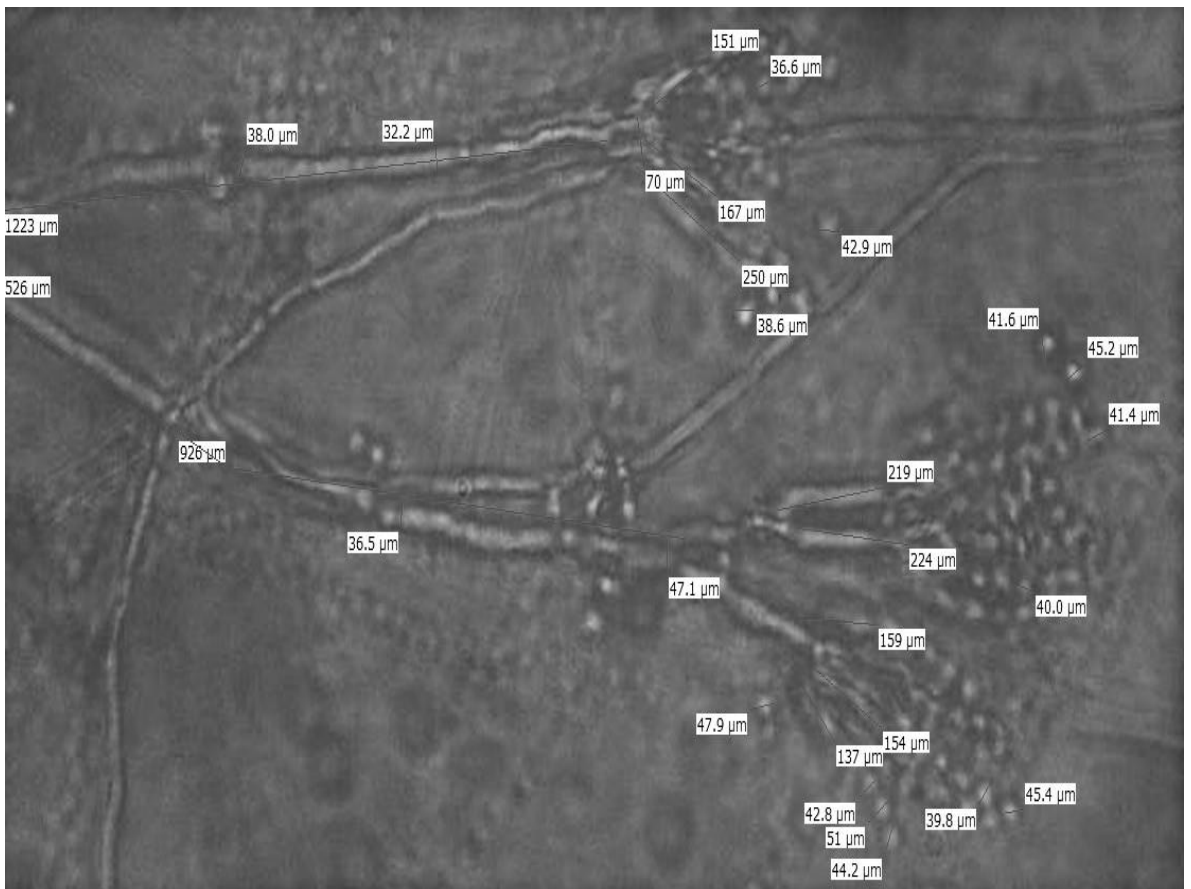
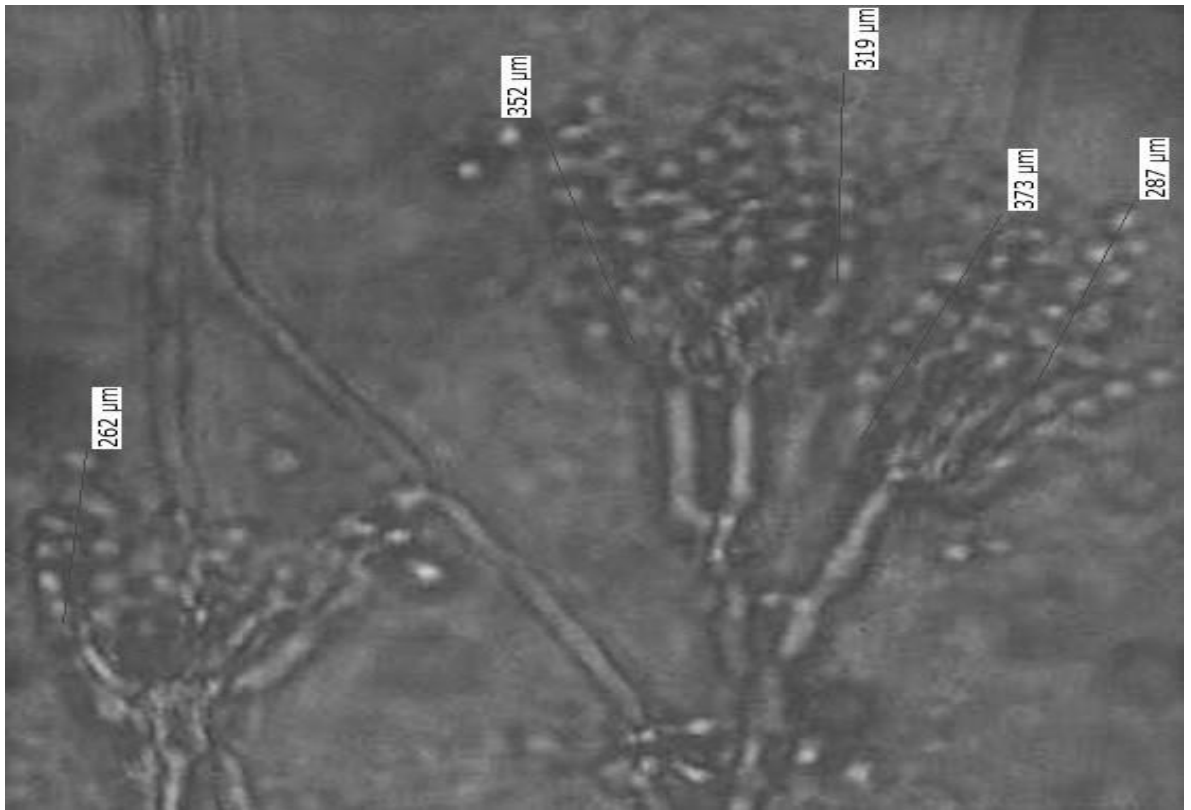


PLATE XXXII

(T<sub>12</sub>)Tcnv

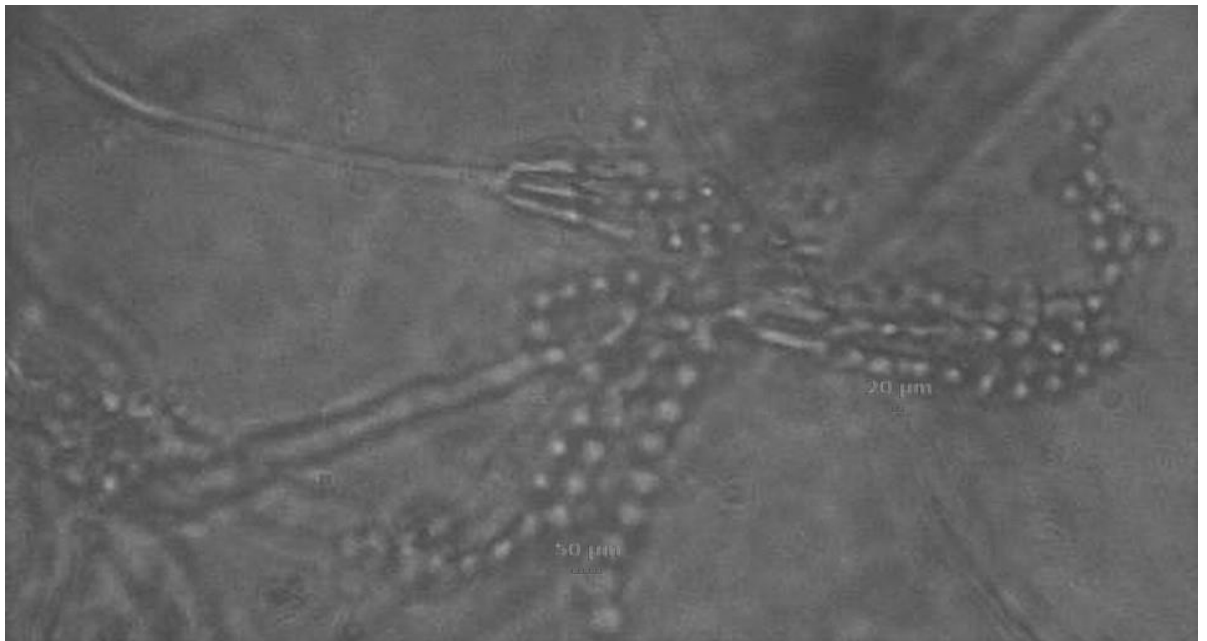
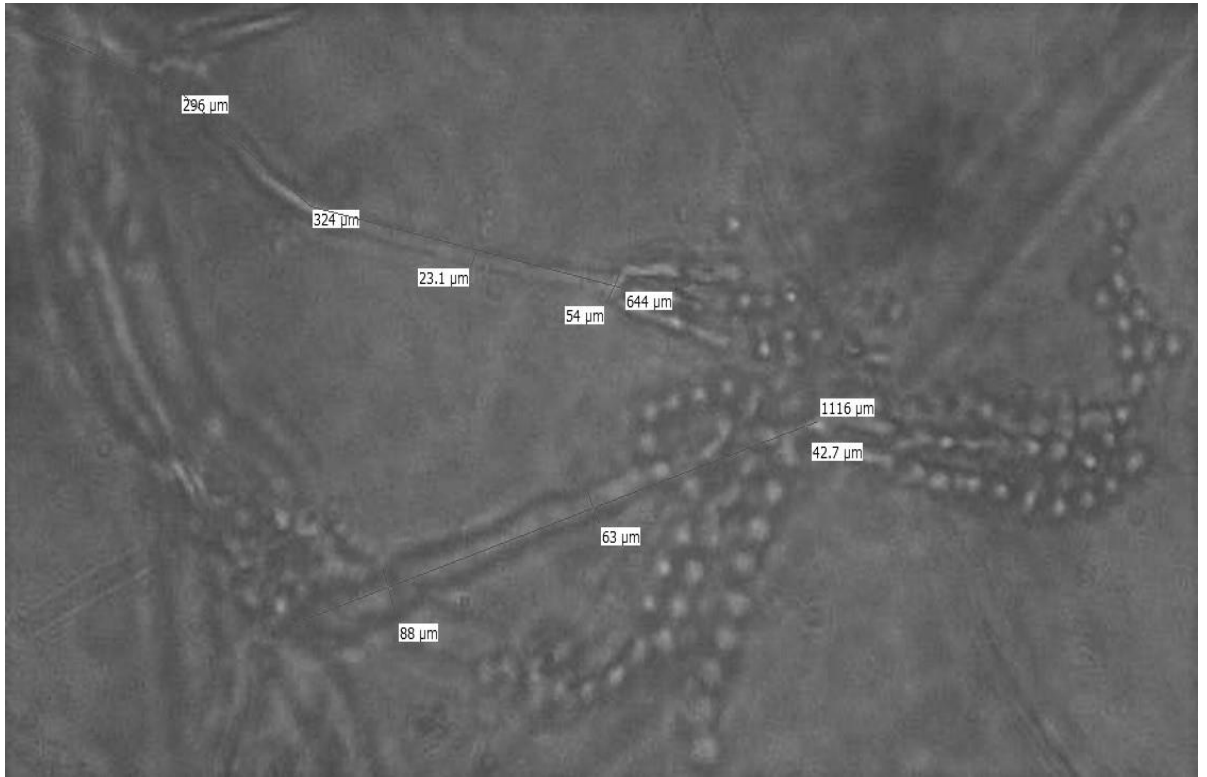
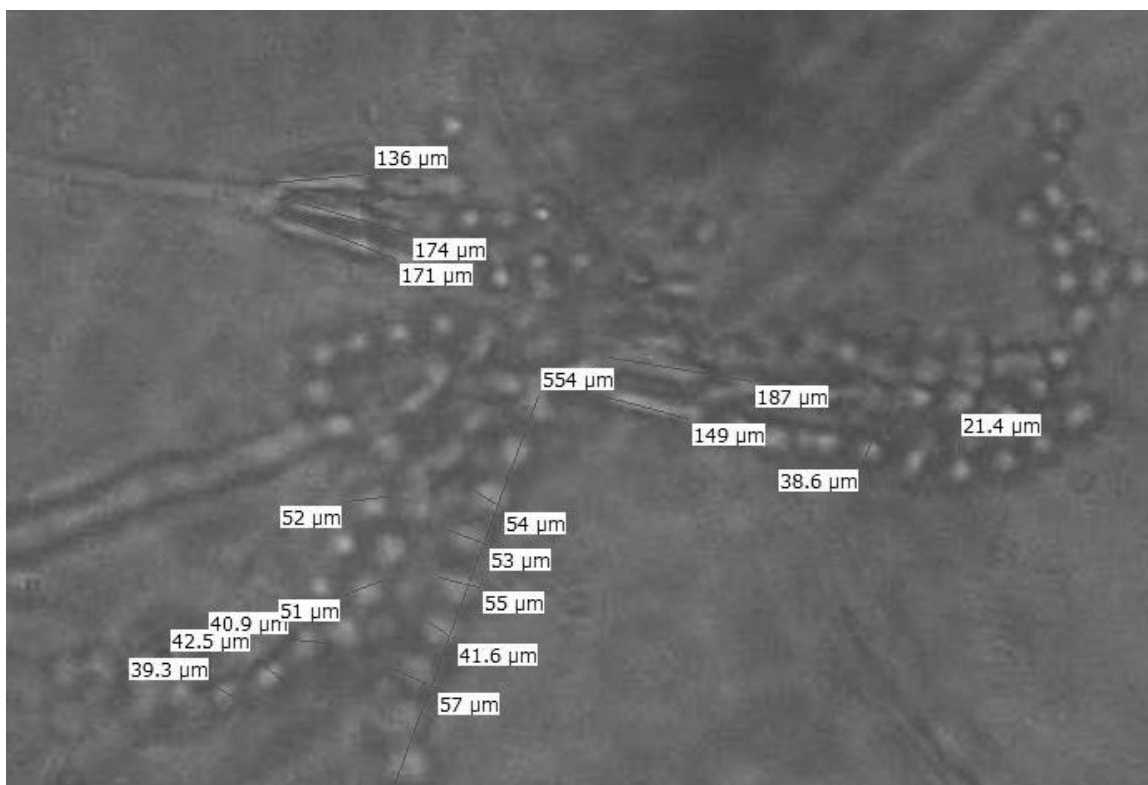


PLATE XXXIII



**PLATE XXXIV**

**(T<sub>13</sub>)Tbw**

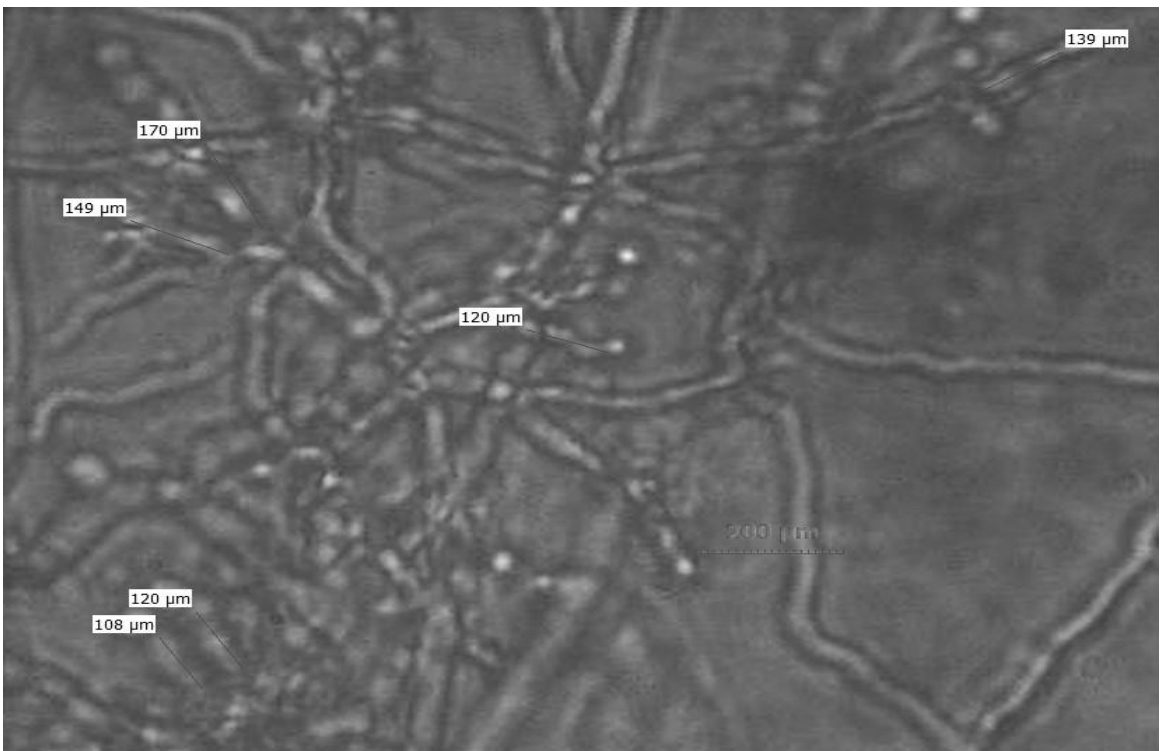
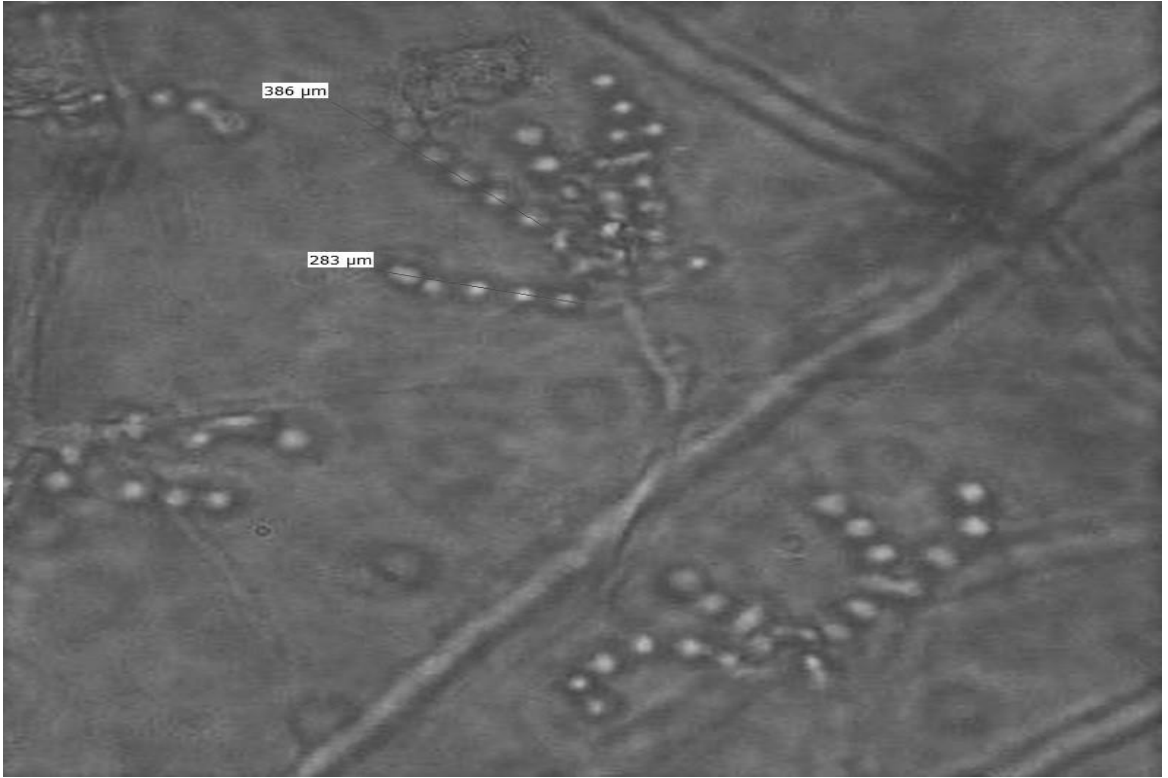
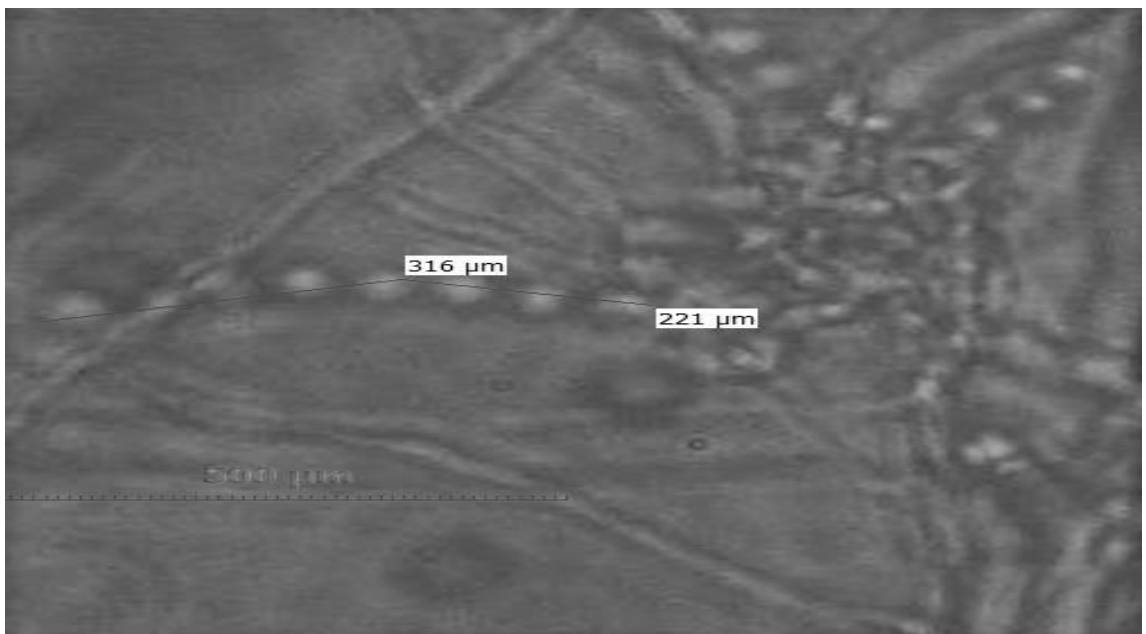
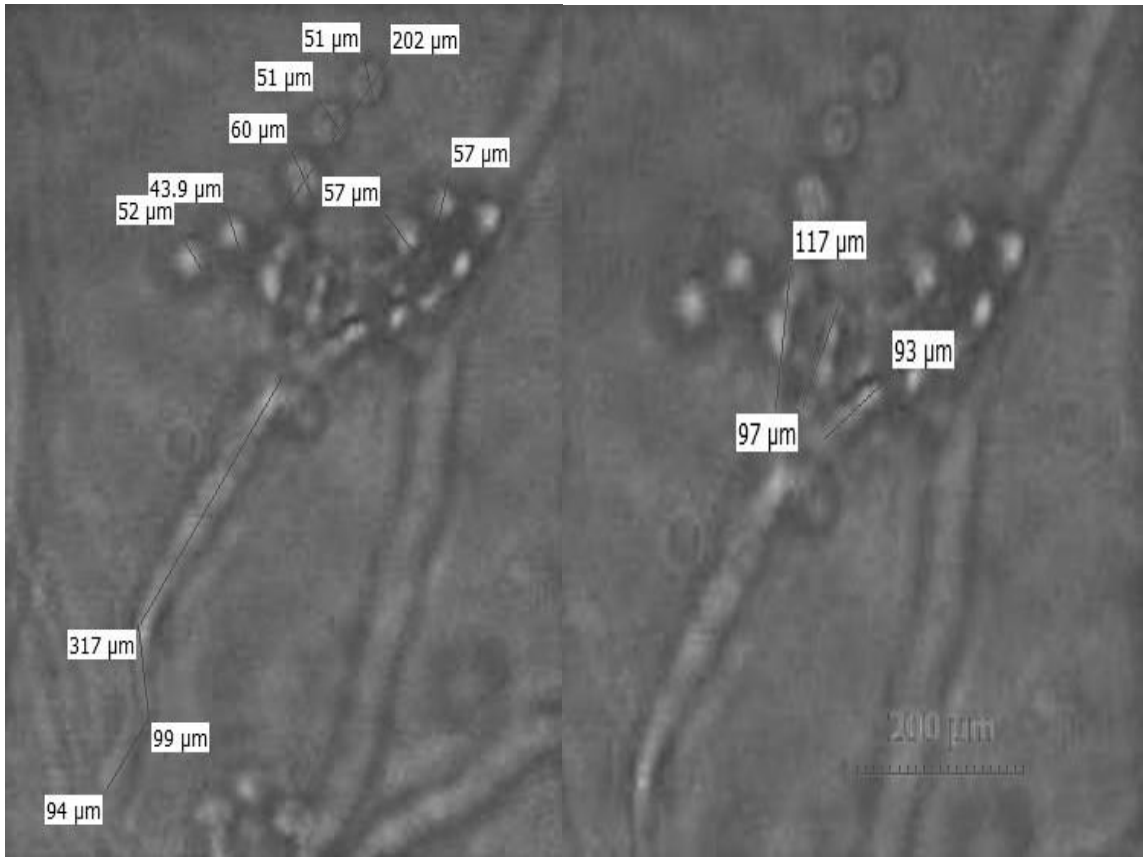


PLATE XXXV



**PLATE XXXVI**

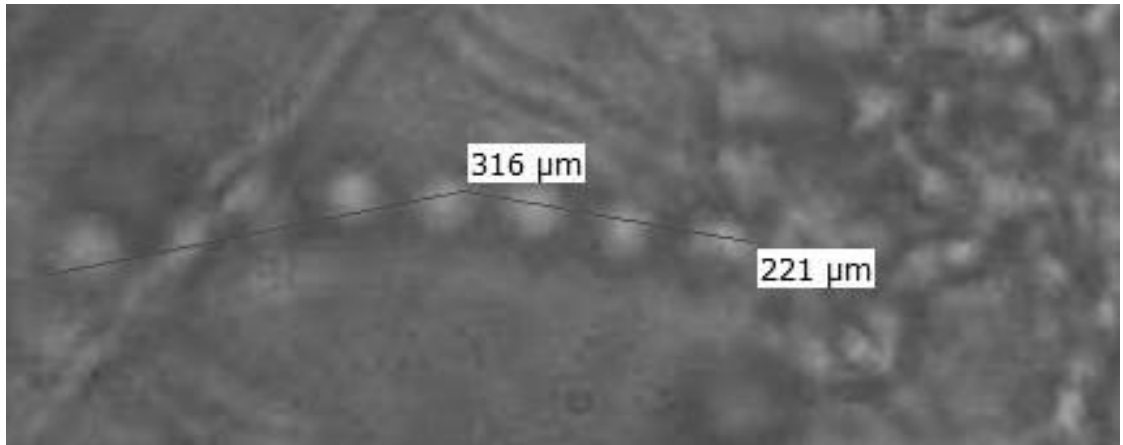




PLATE XXXVII

(T<sub>14</sub>) Tgpal

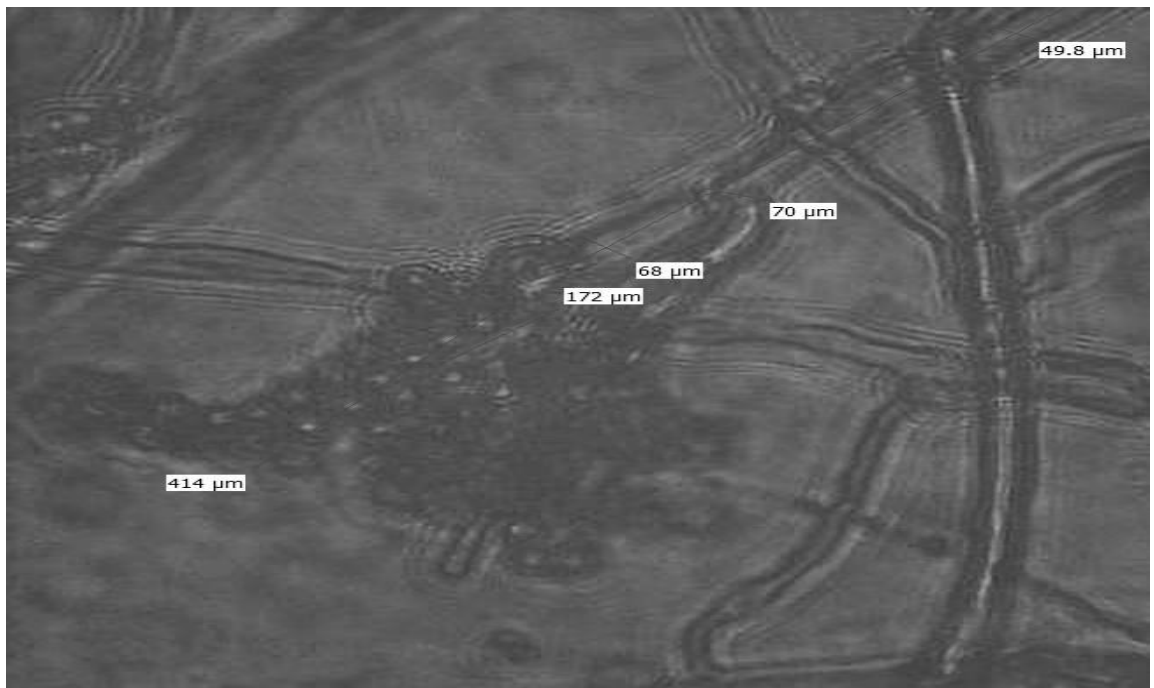
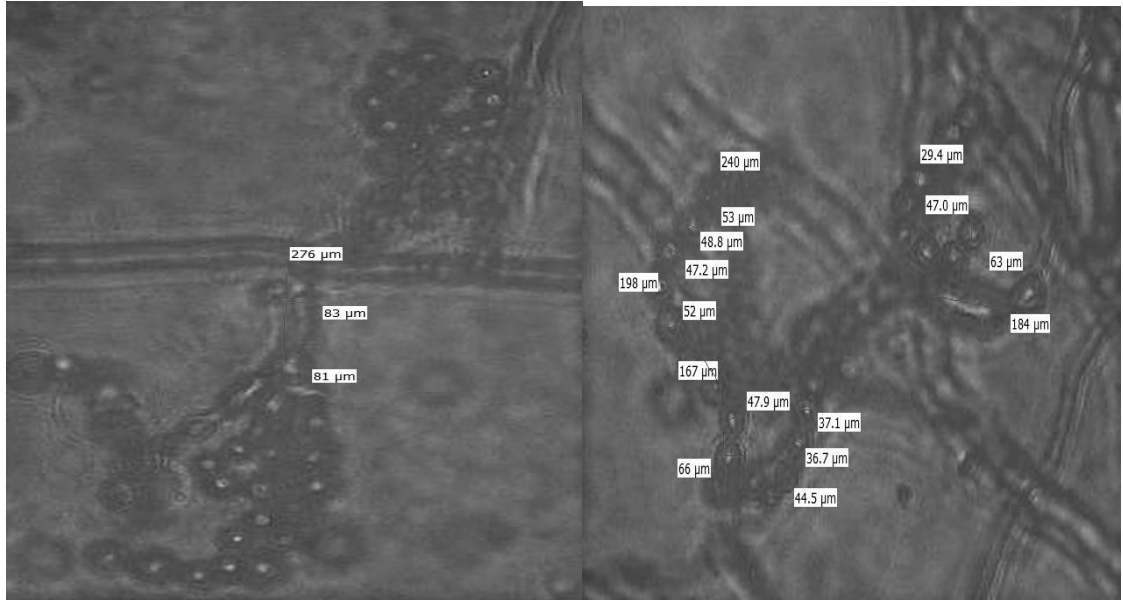
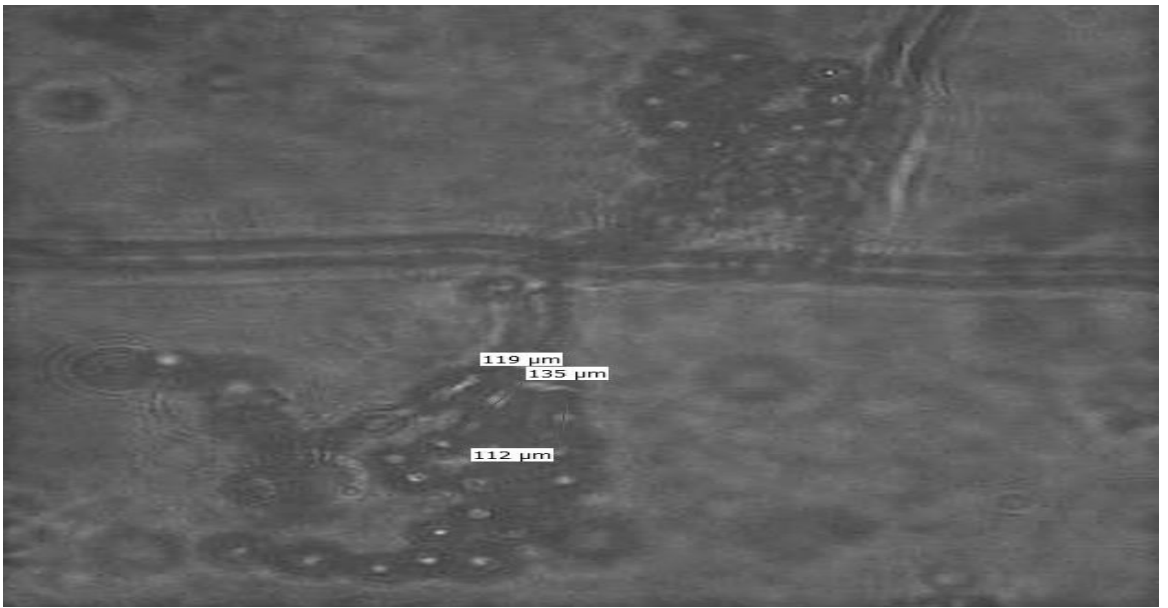
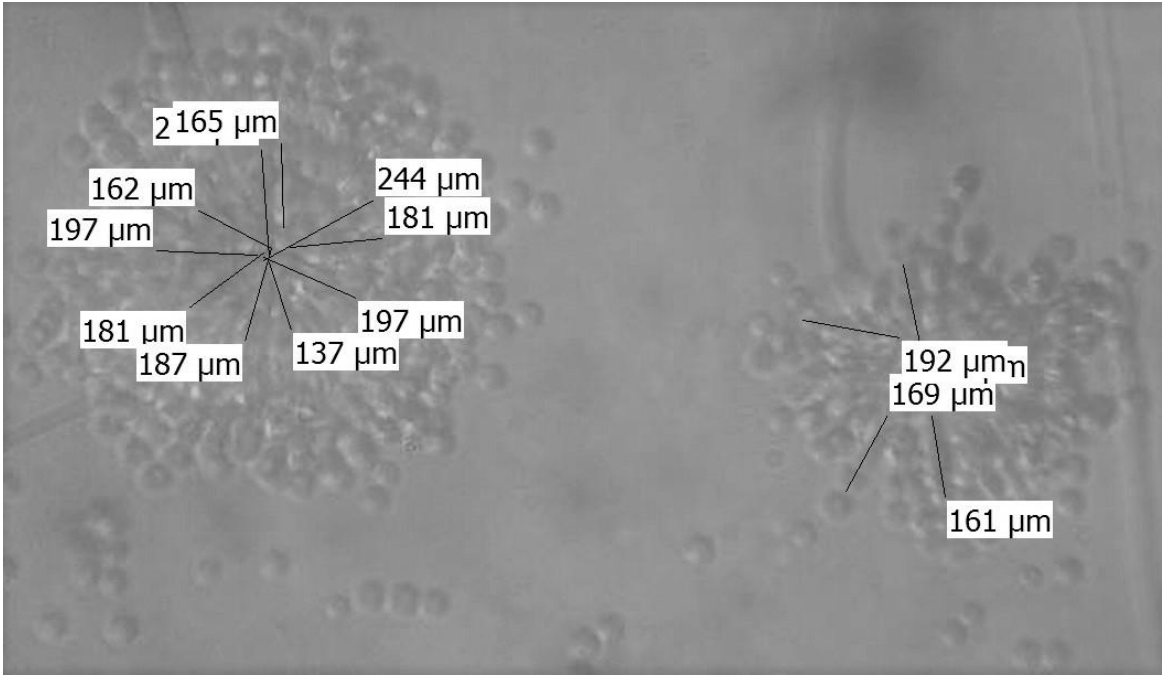


PLATE XXXVIII



**PLATE XXXIX**

**(T<sub>15</sub>) Tsptpal**

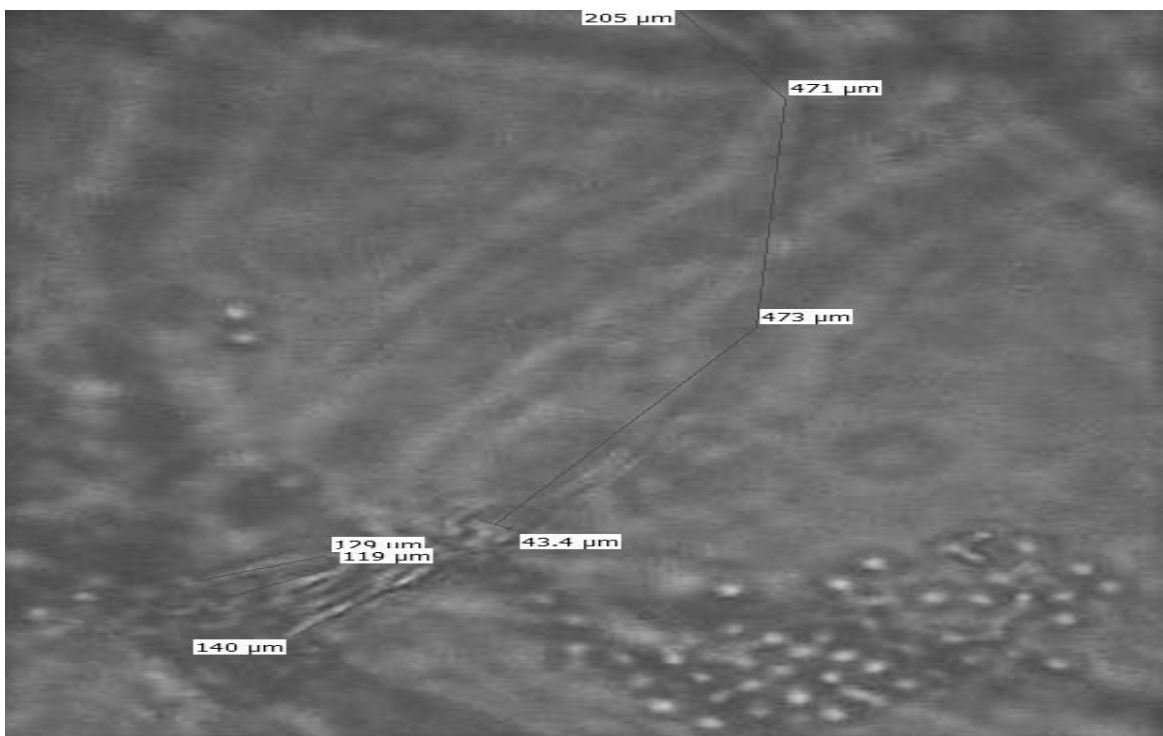
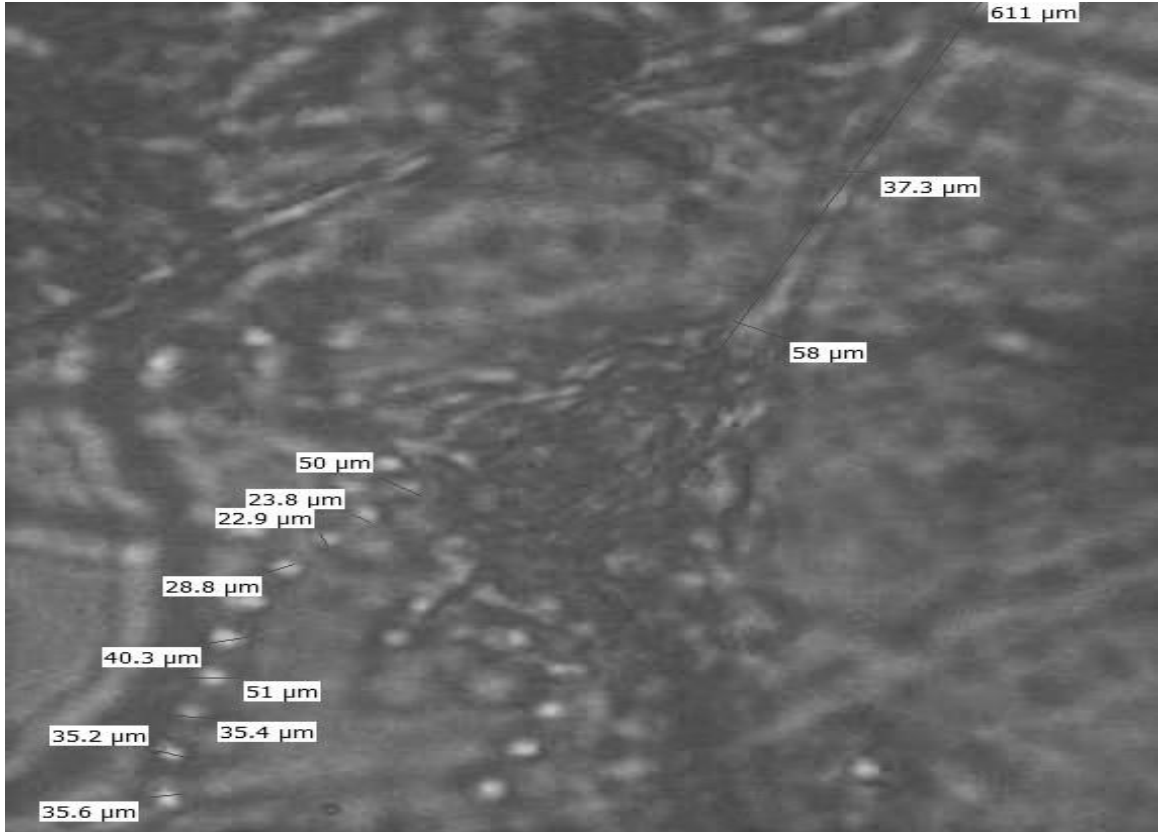
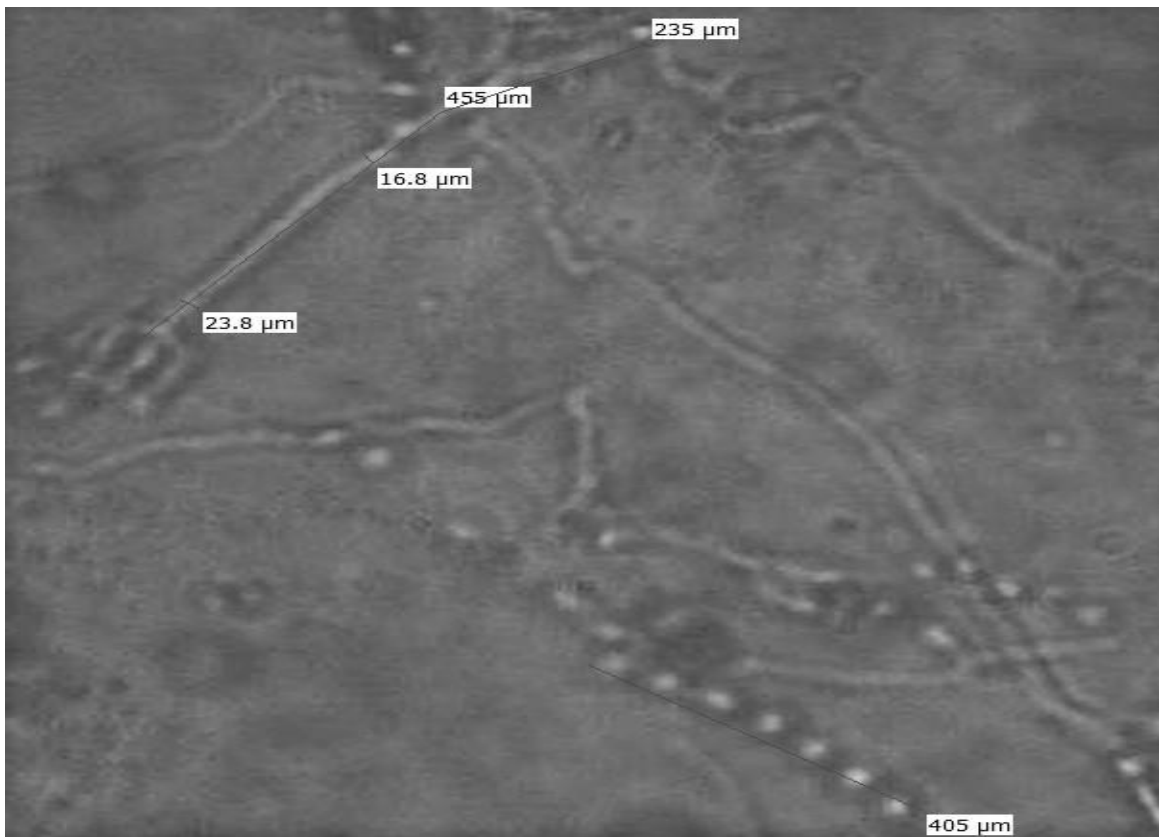
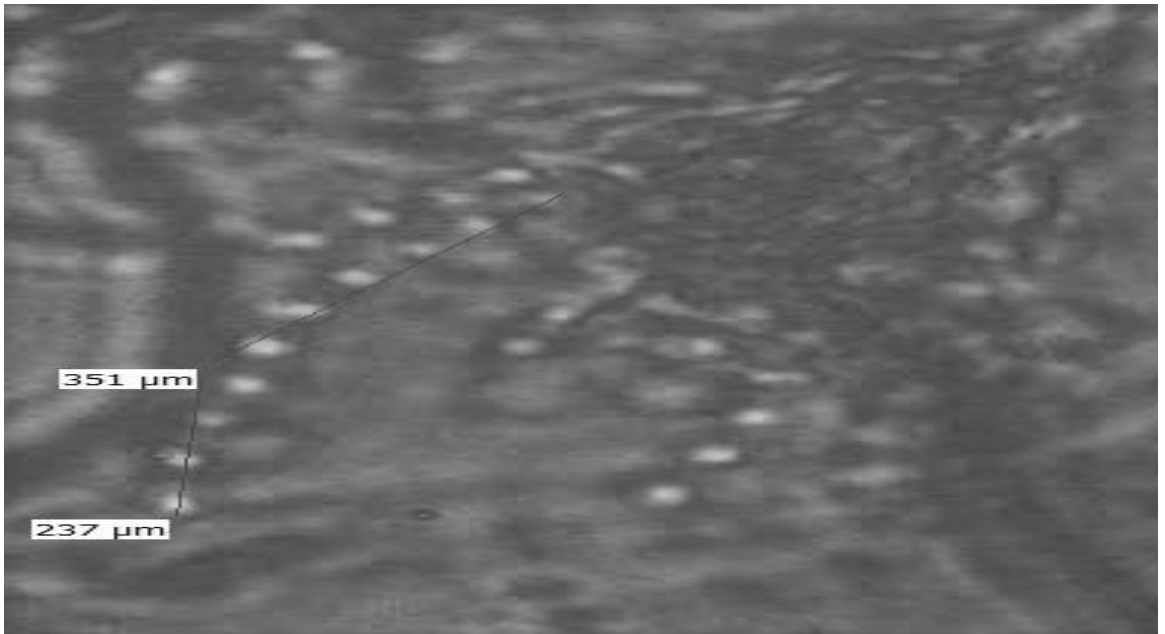


PLATE XL



# PLATE XLI

(T<sub>16</sub>) Thm

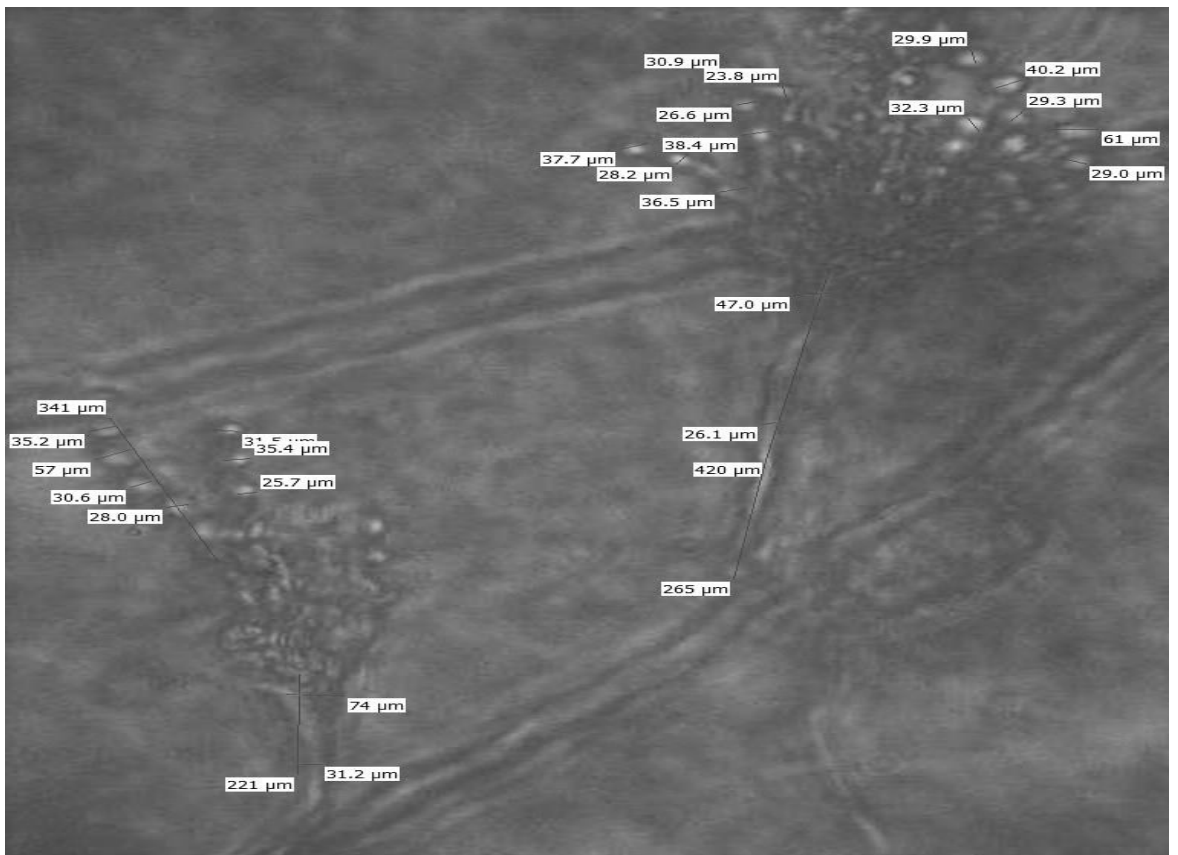
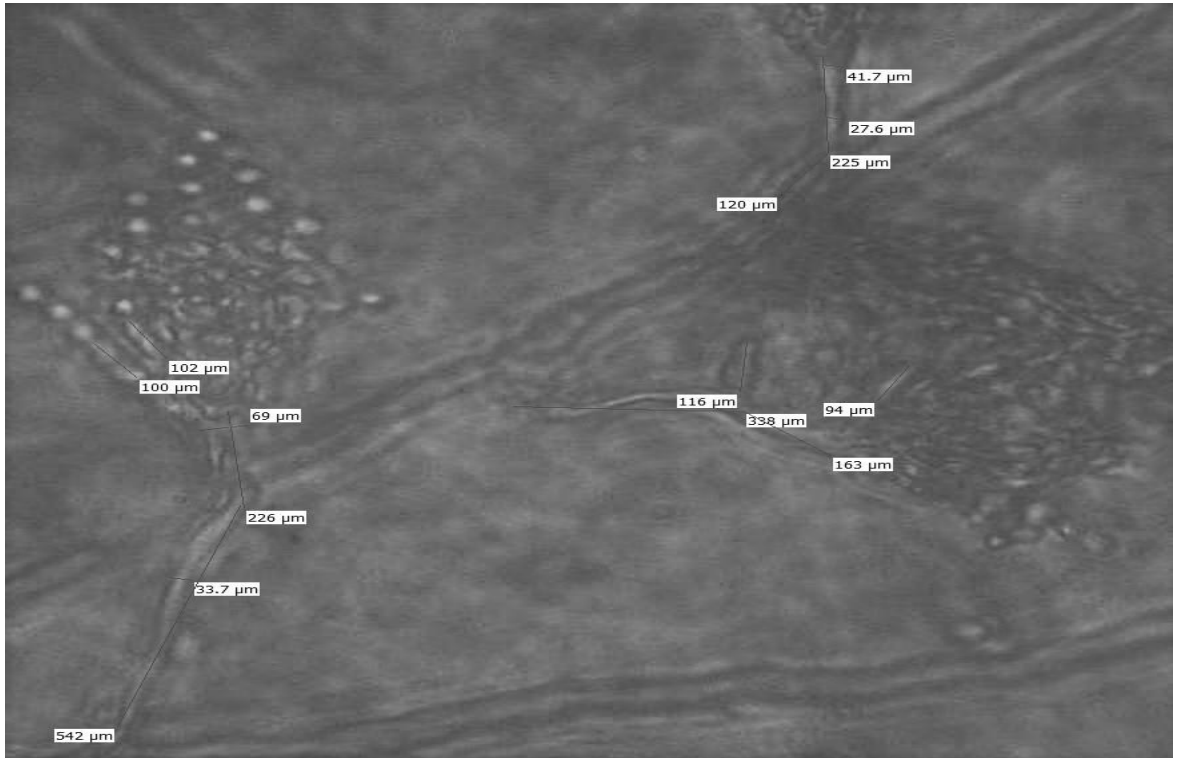


PLATE XLII

(T<sub>17</sub>) Tlbhar

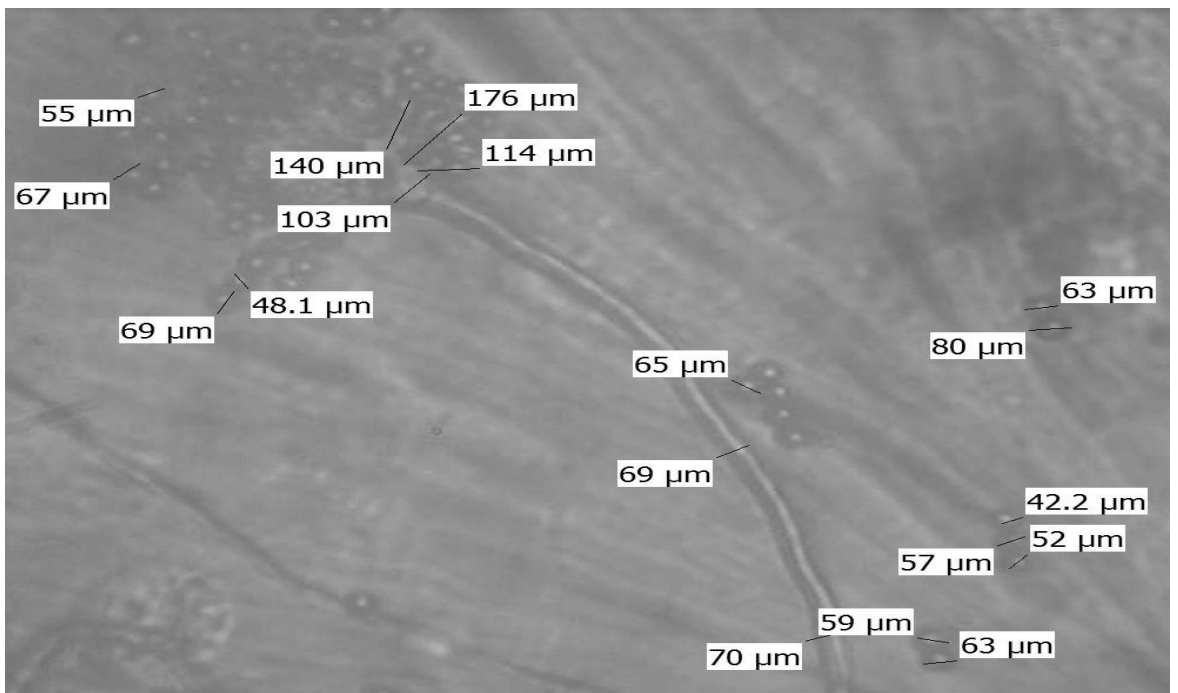
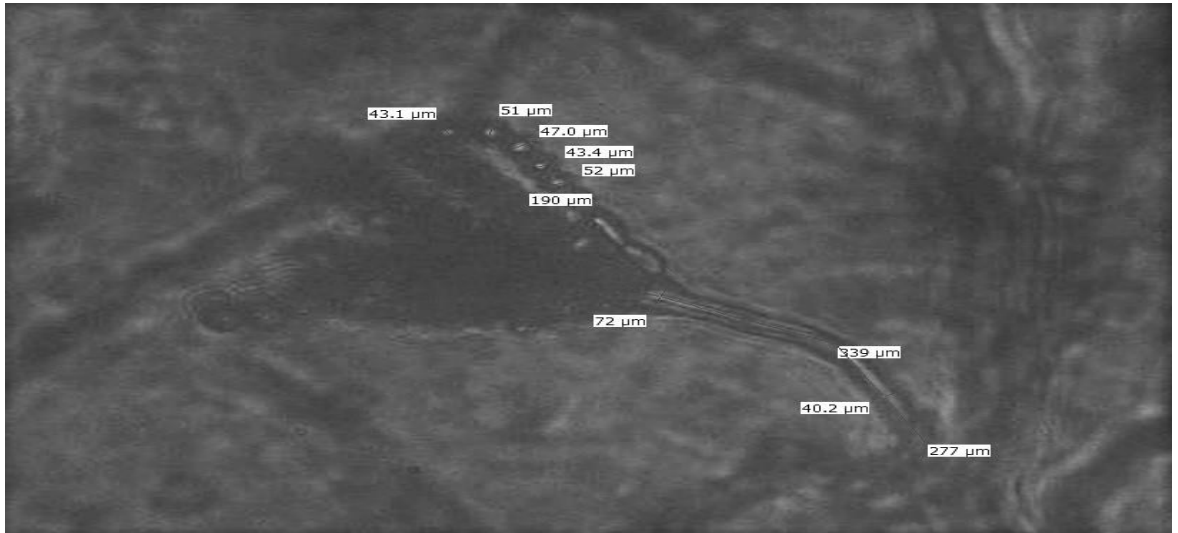


PLATE XLIII

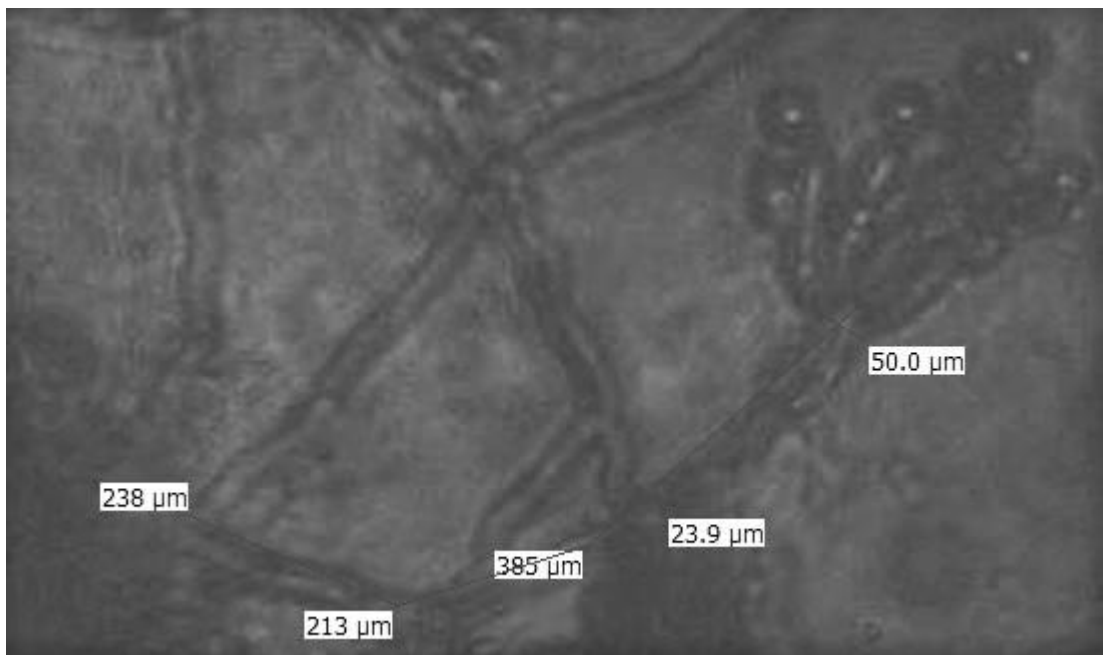
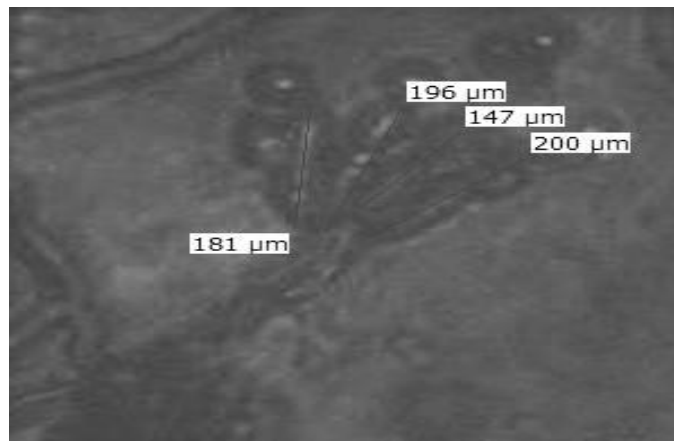
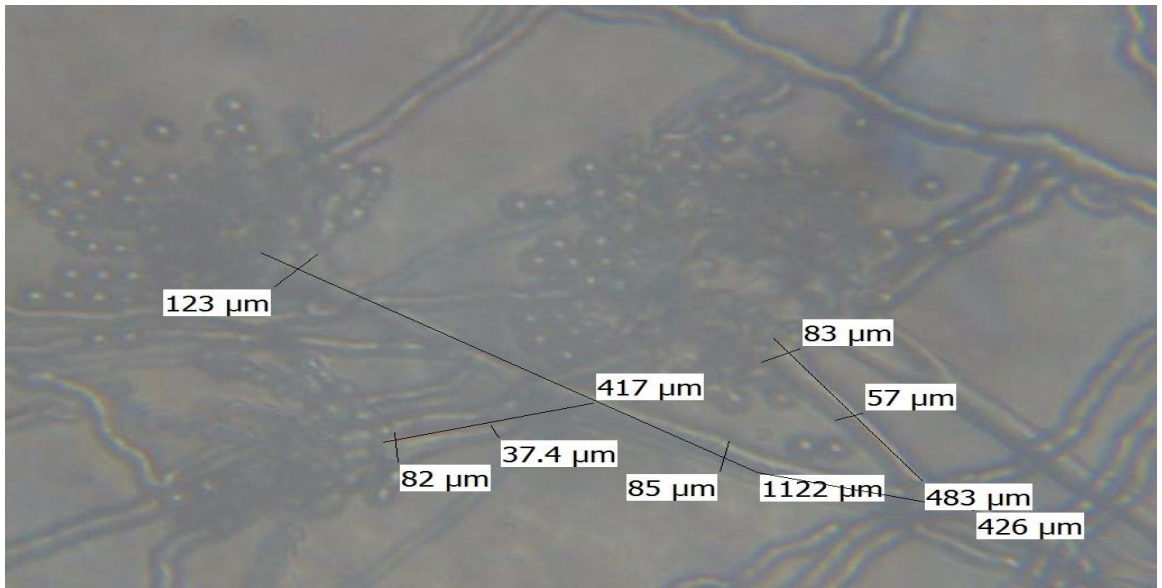


PLATE XLIV

(T<sub>18</sub>) Tgkh2020s

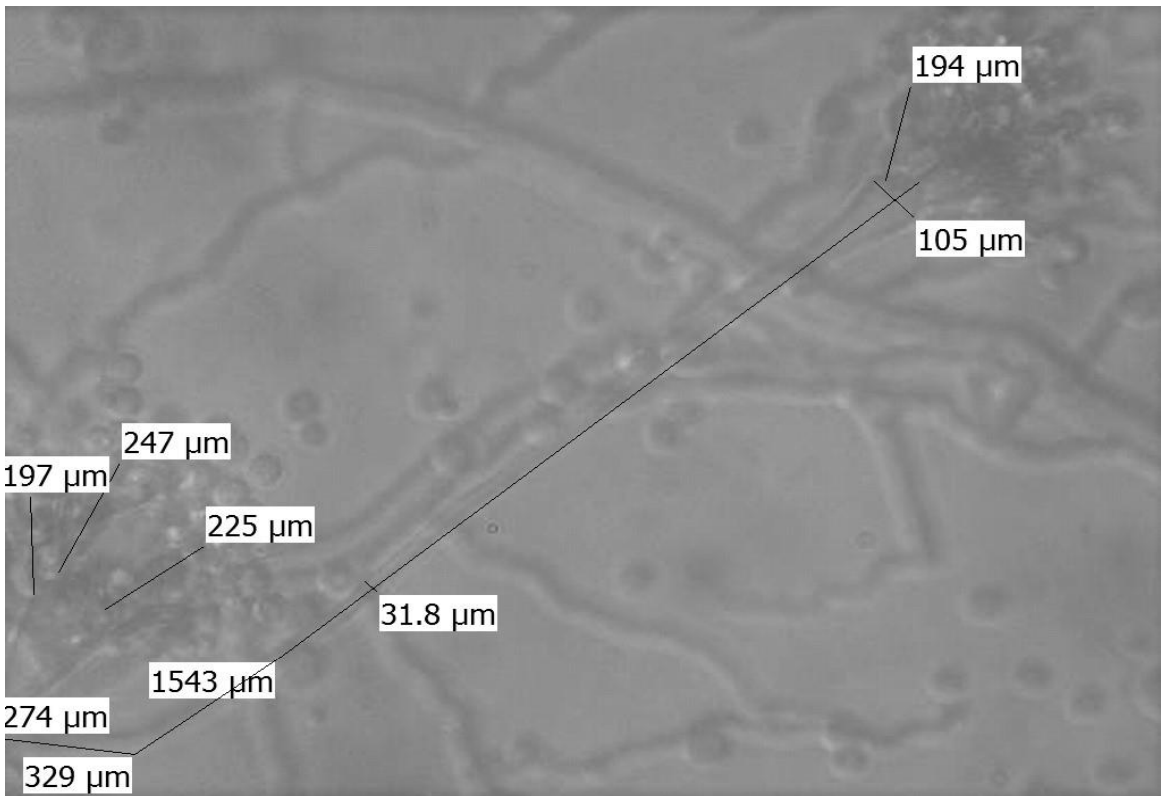
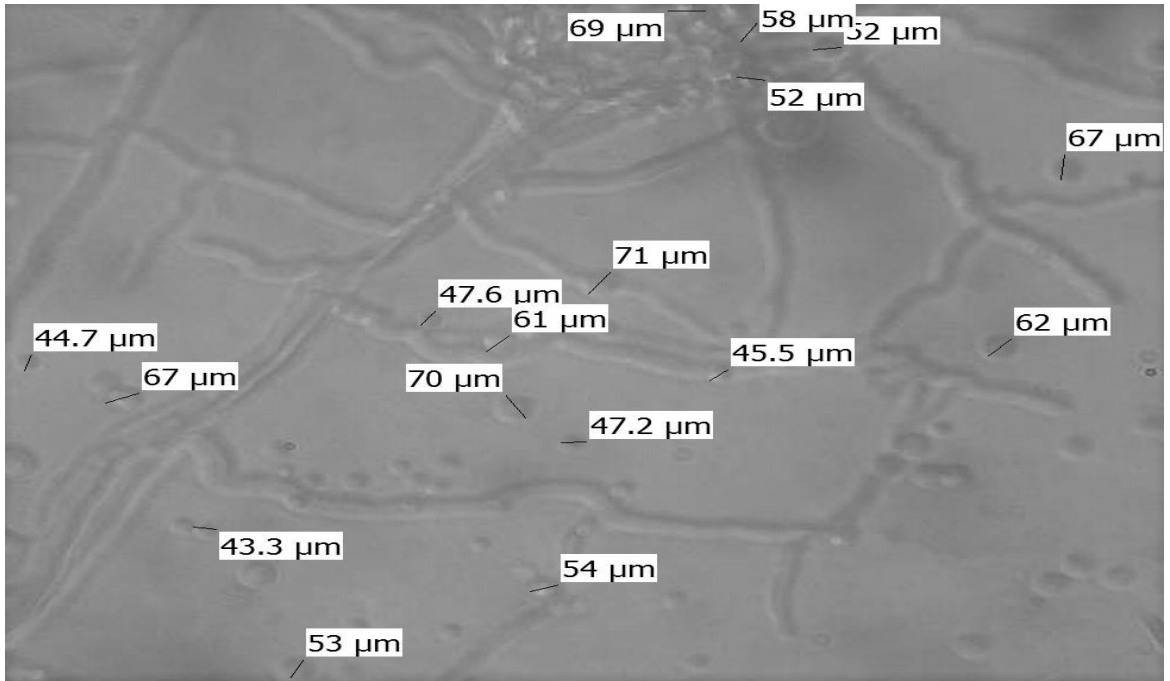
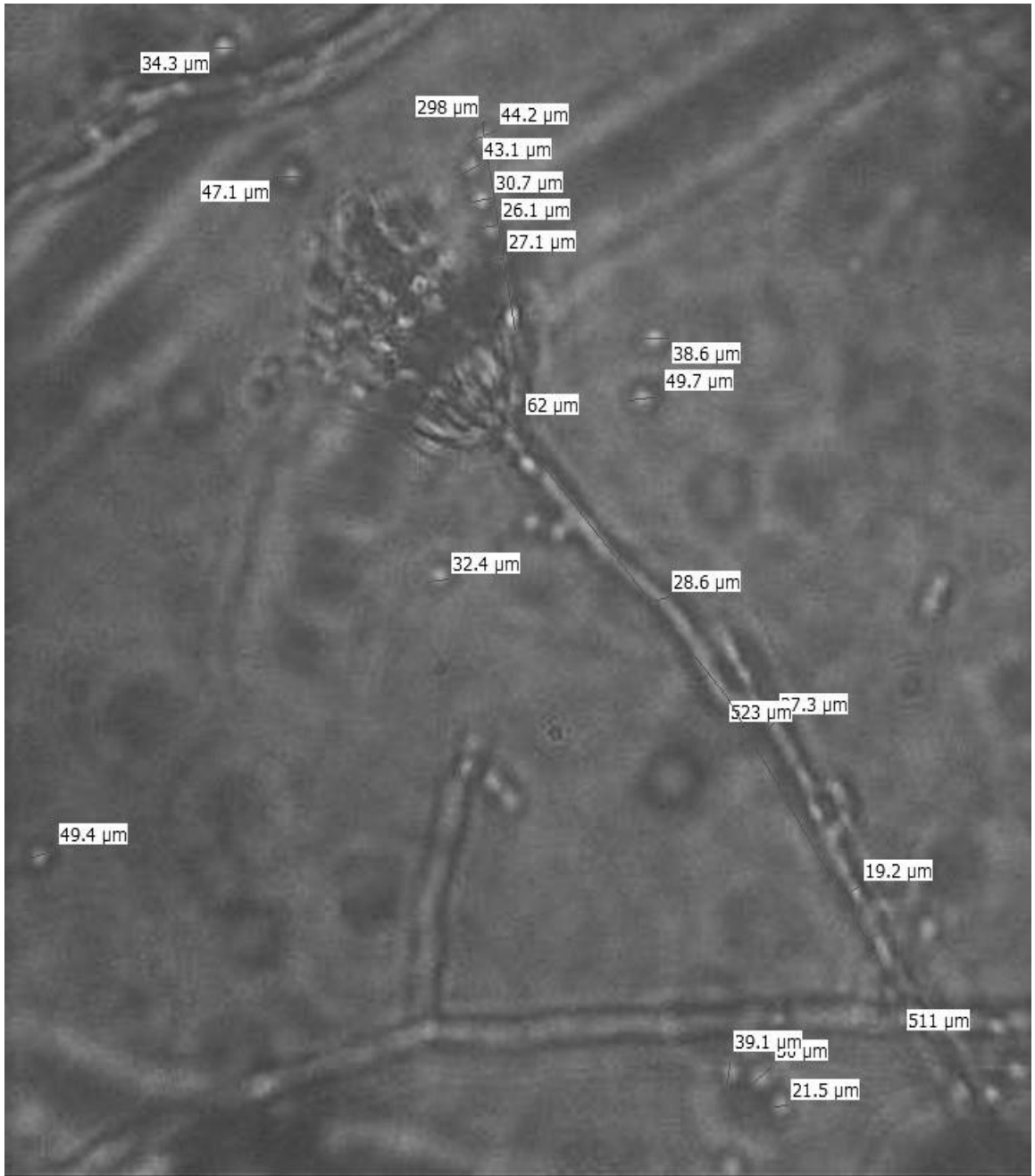


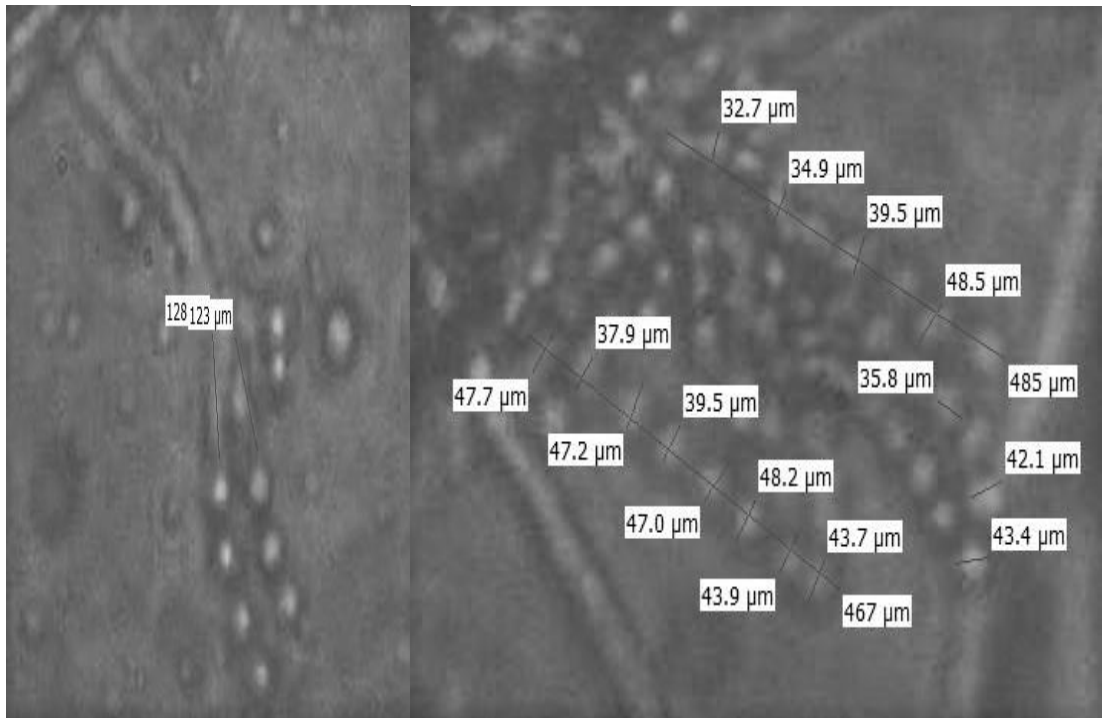
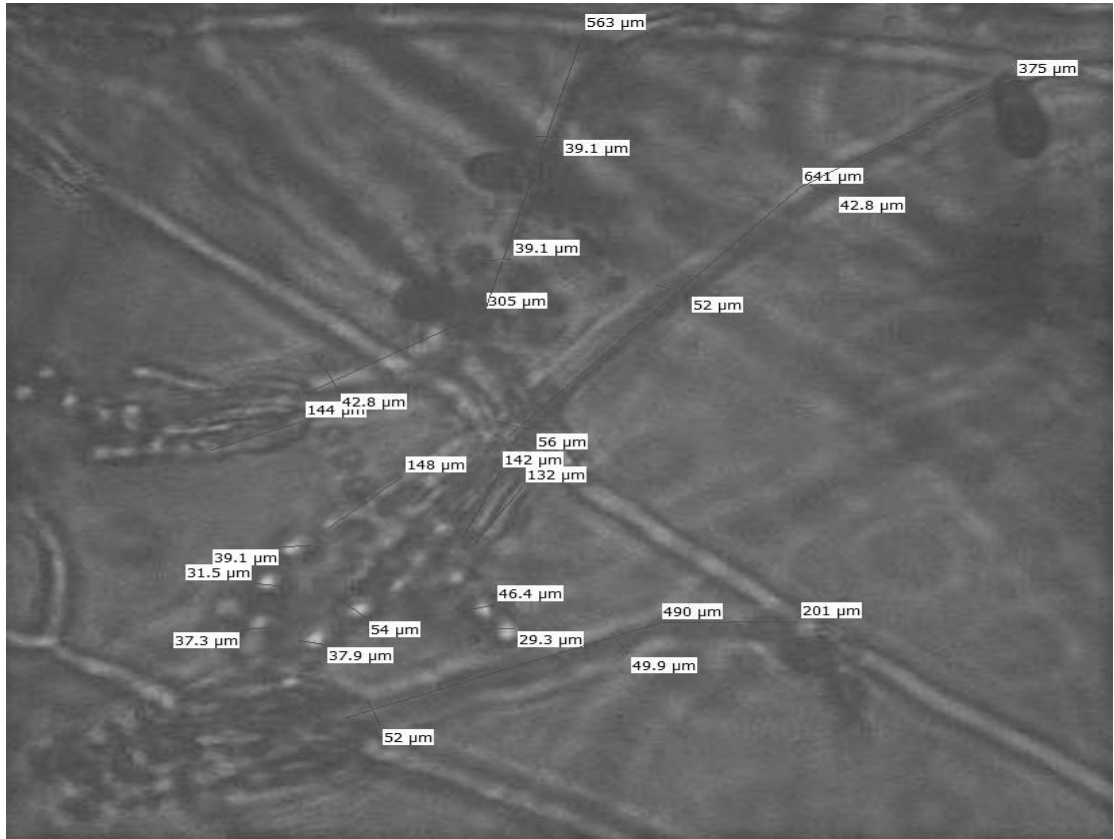


PLATE XLV

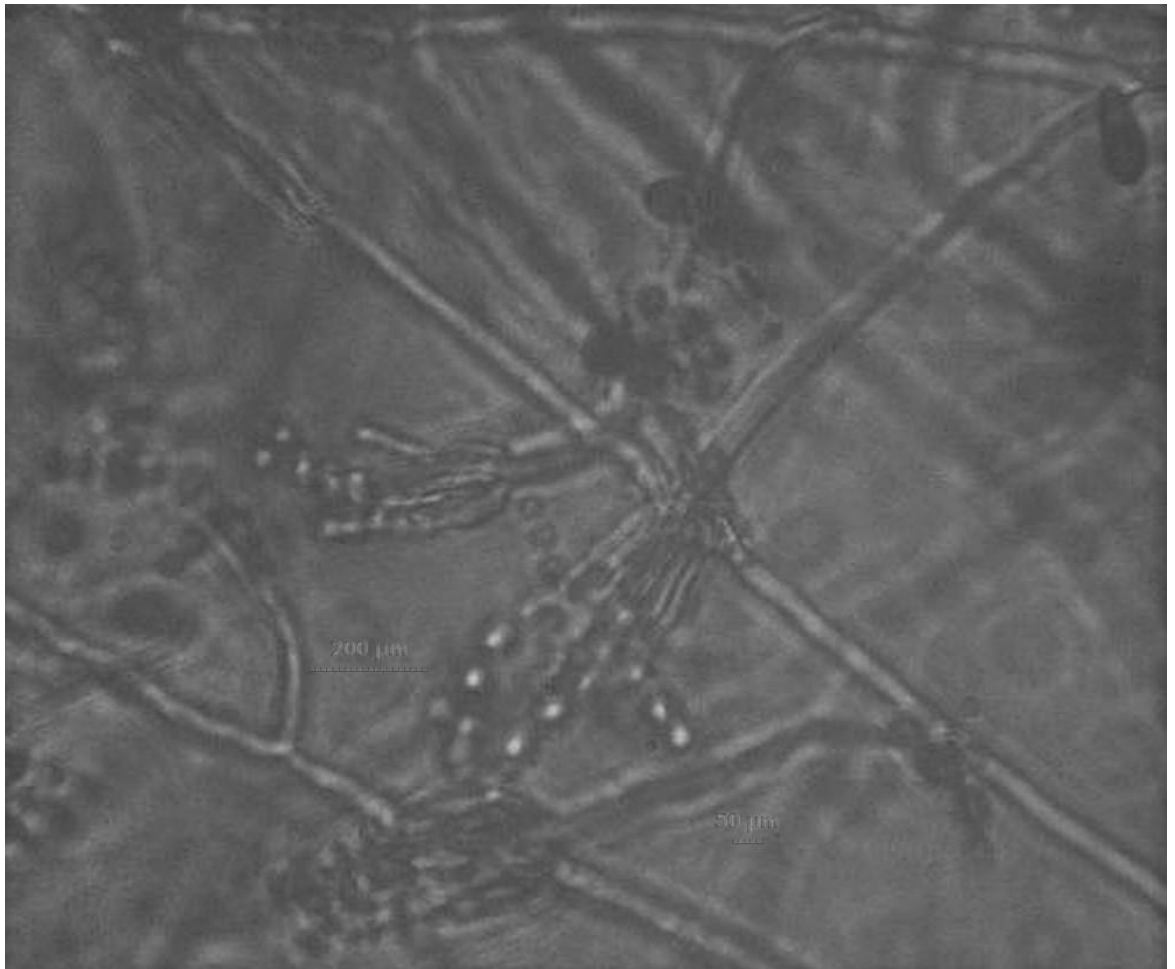


# PLATE XLVI

## (T<sub>19</sub>) Tefym



**PLATE XLVII**



**PLATE XLVIII**

**(T<sub>20</sub>) Tchal**

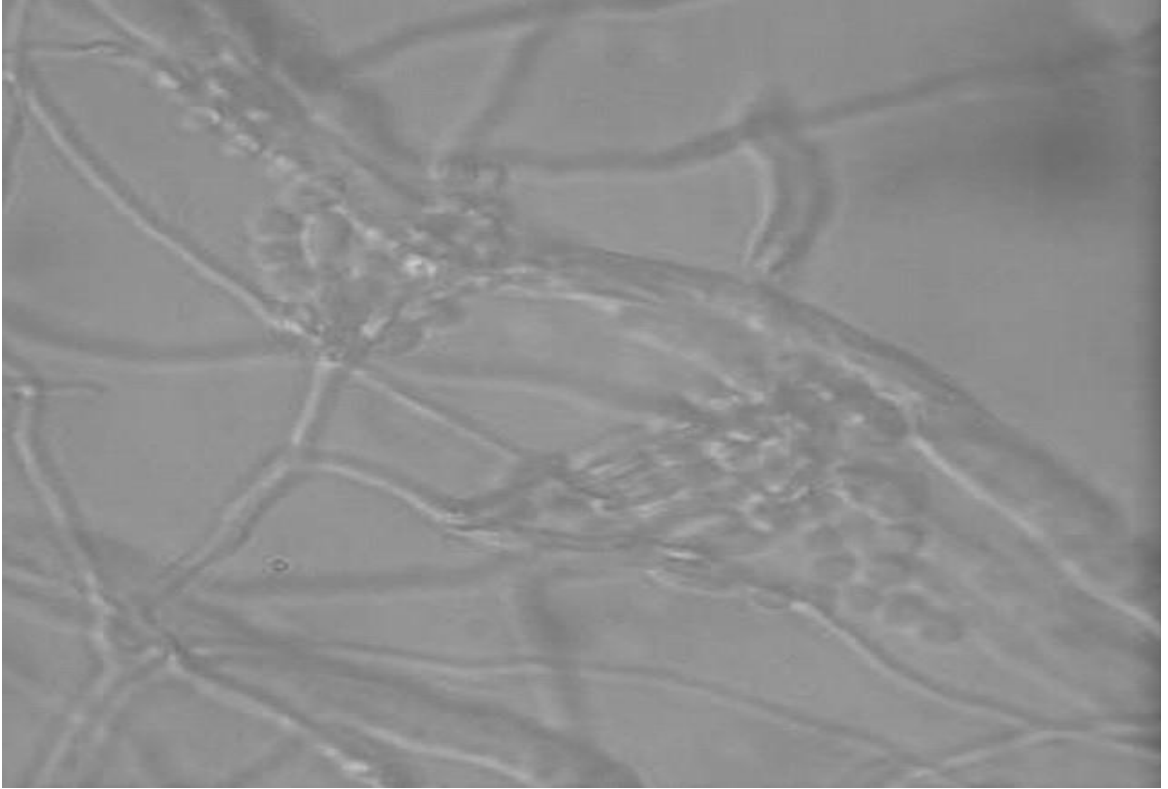
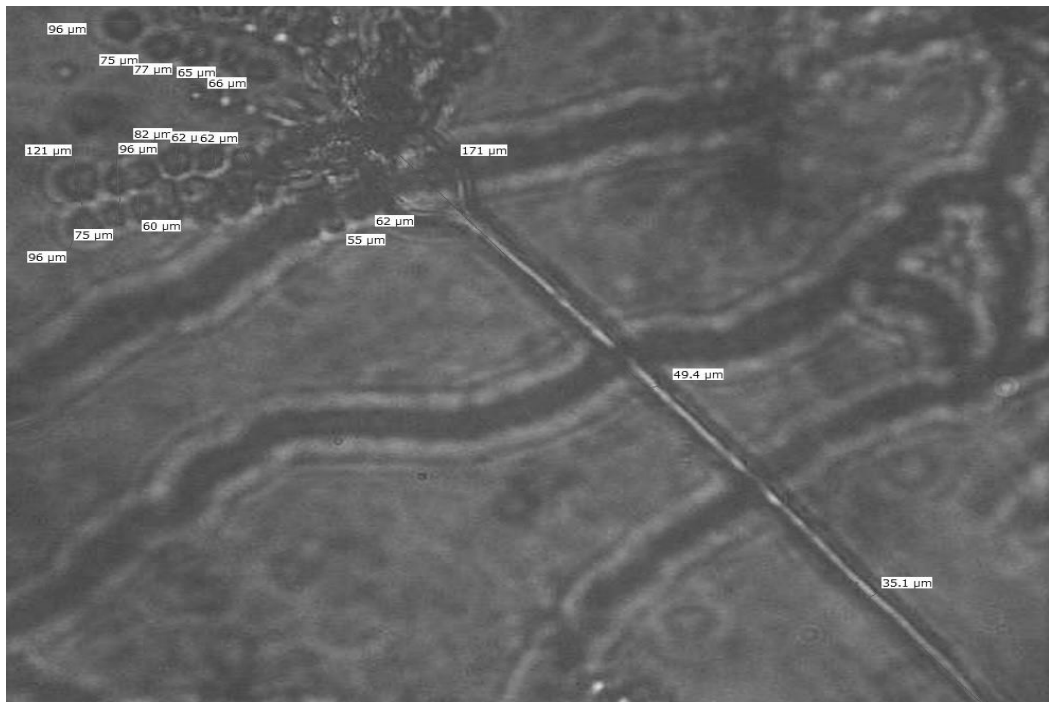
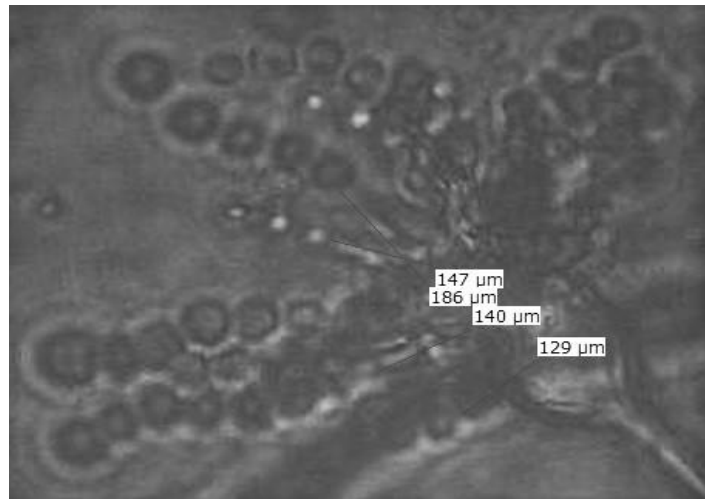
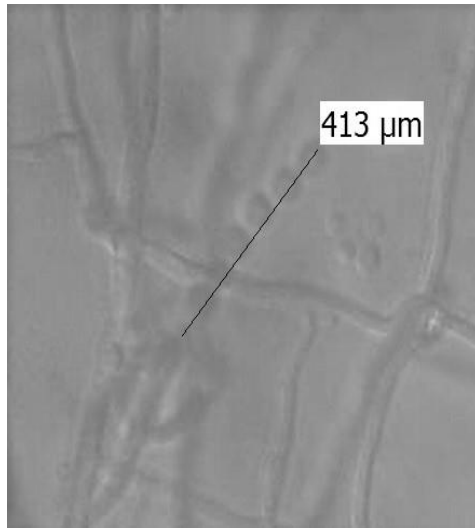
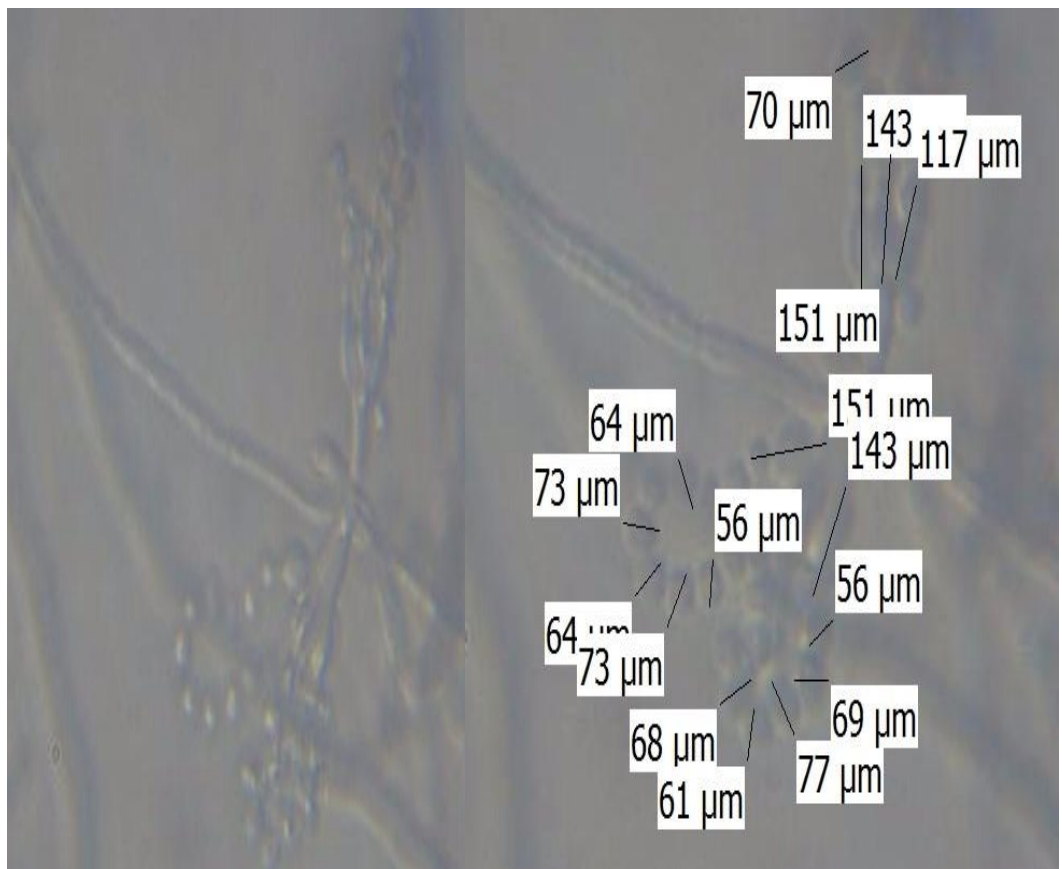
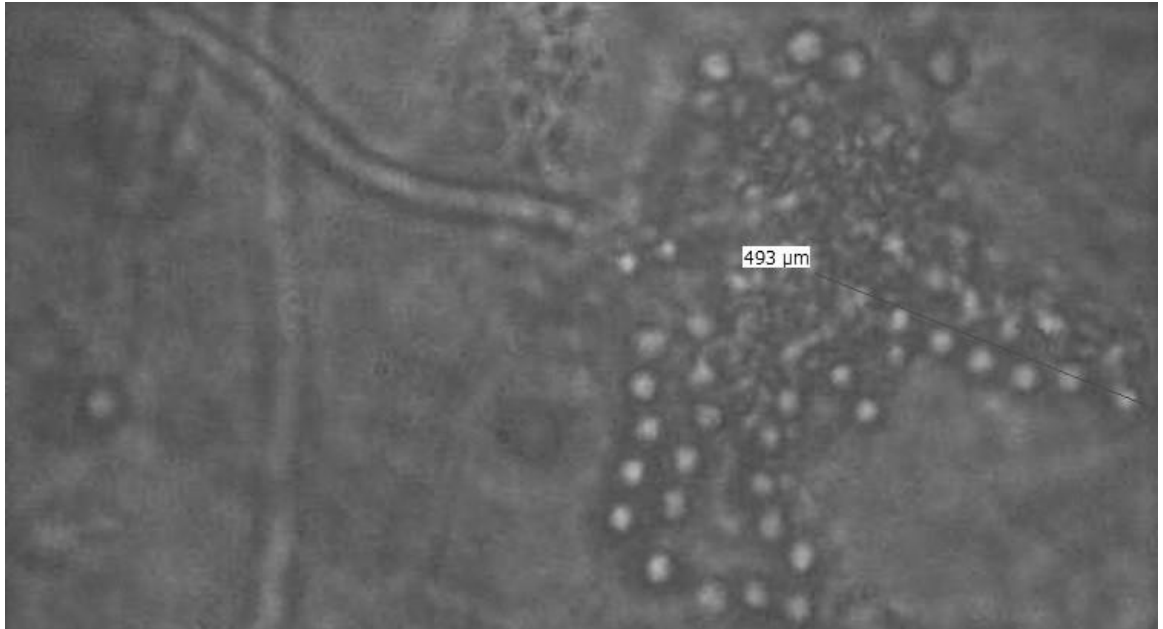


PLATE XLIX

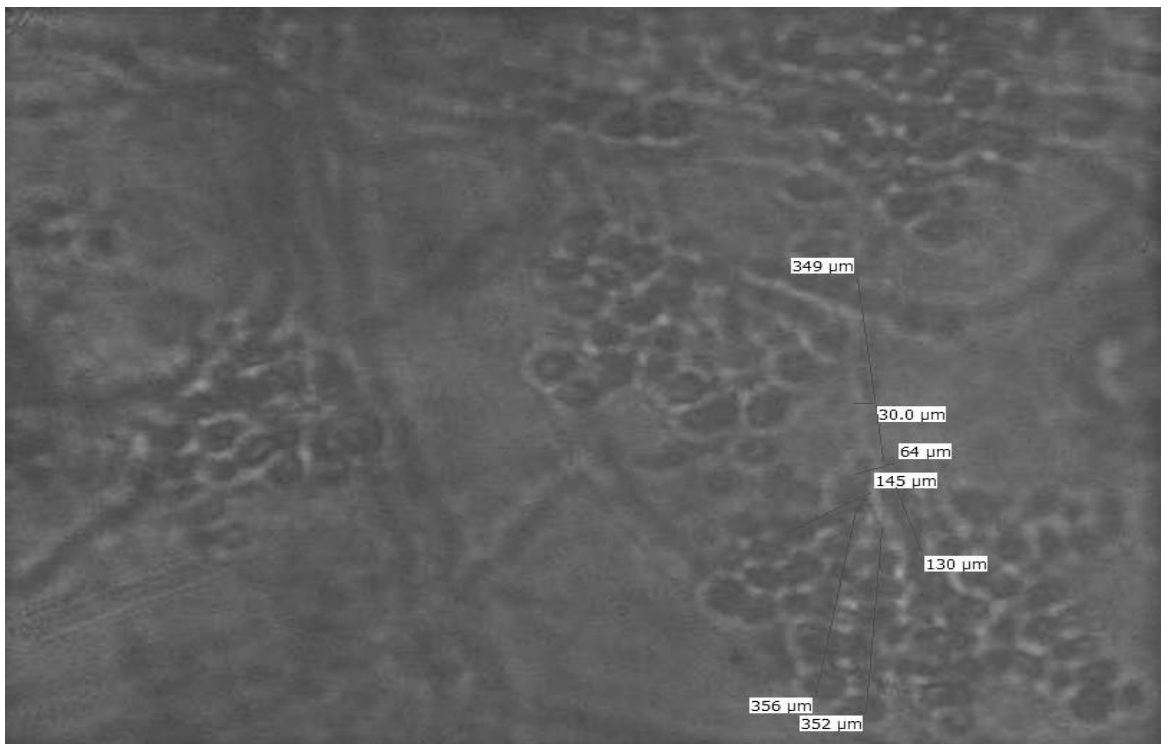
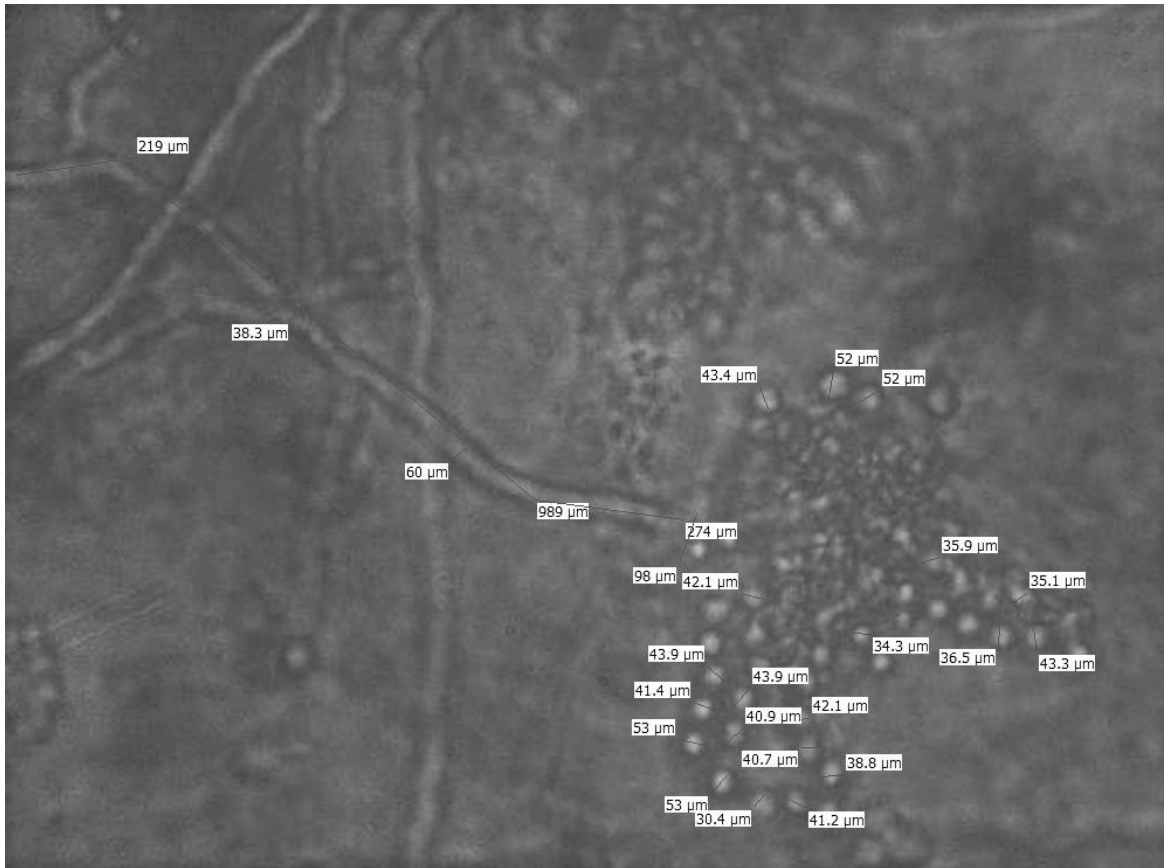


**PLATE L**

**(T<sub>21</sub>) Tchival**

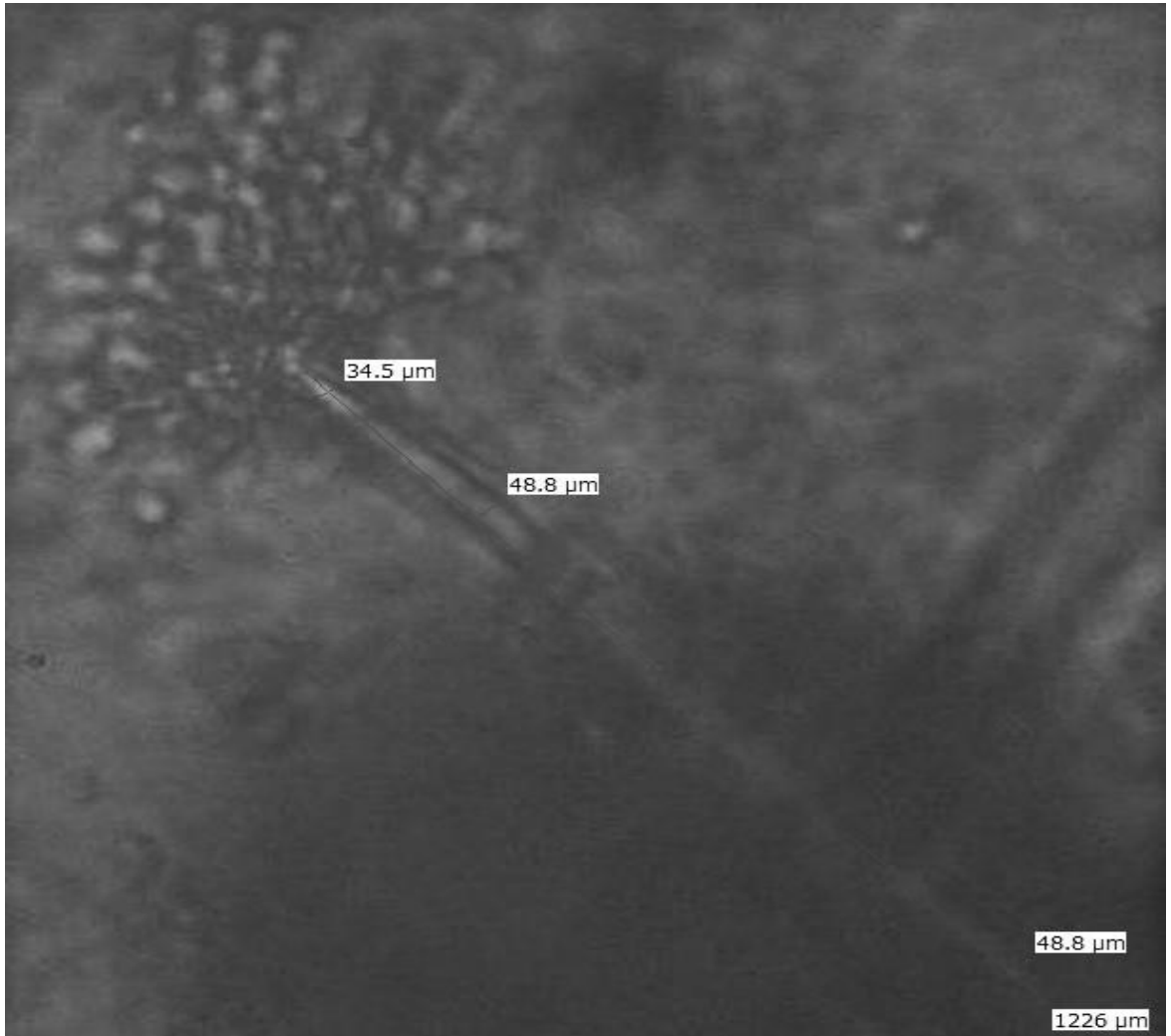


# PLATE LI



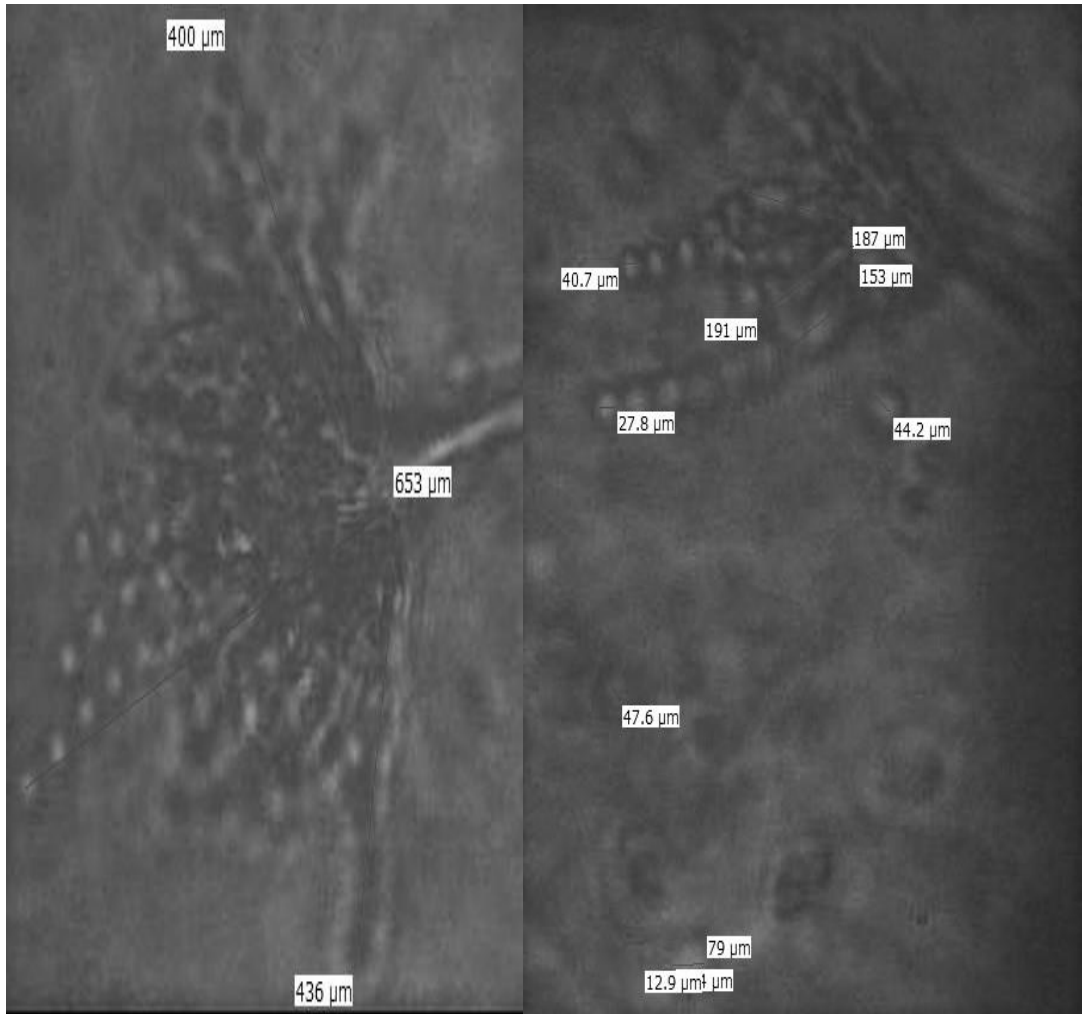
**PLATE LII**

**(T<sub>22</sub>) T<sub>b</sub>g<sub>u</sub>**



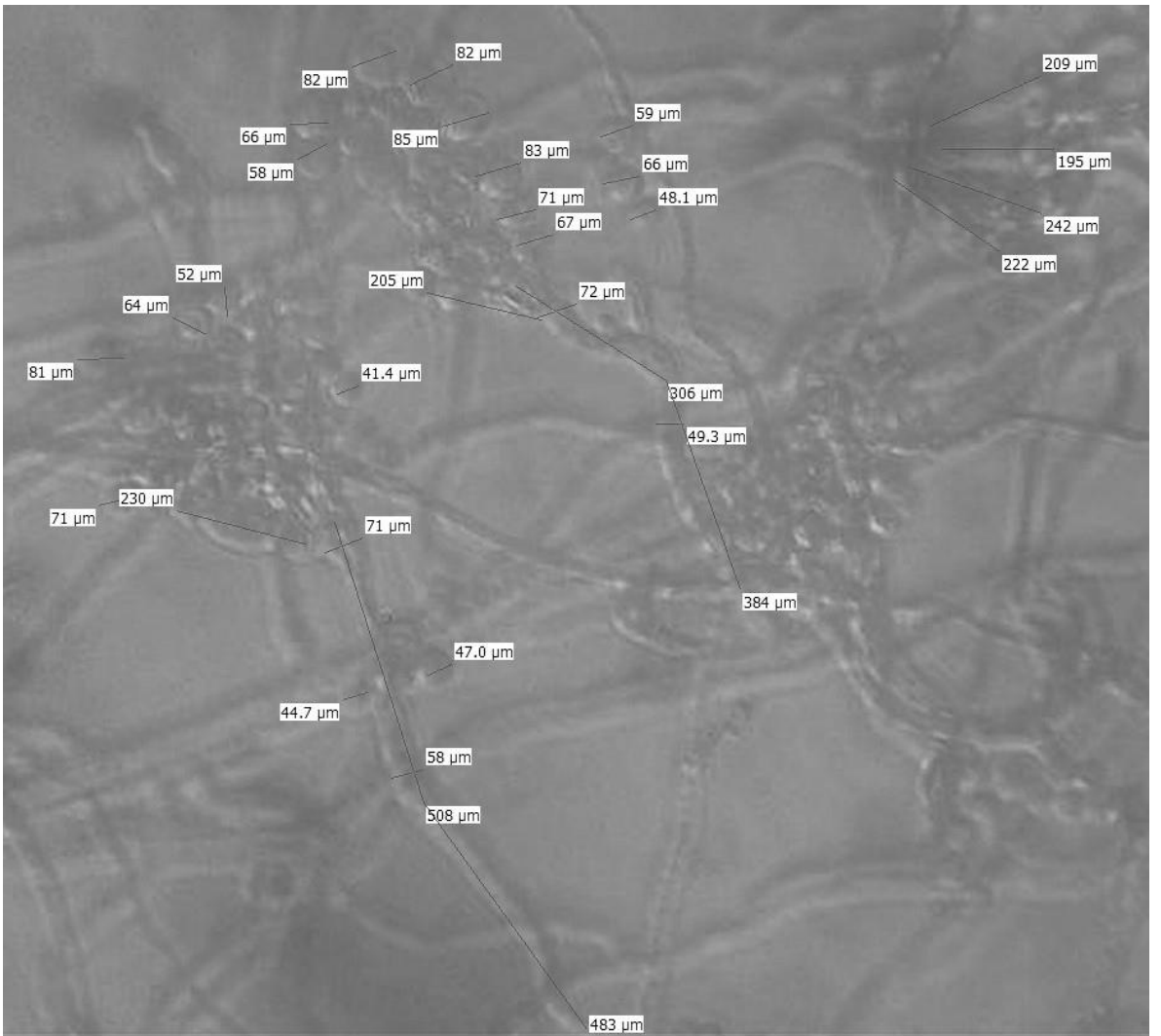
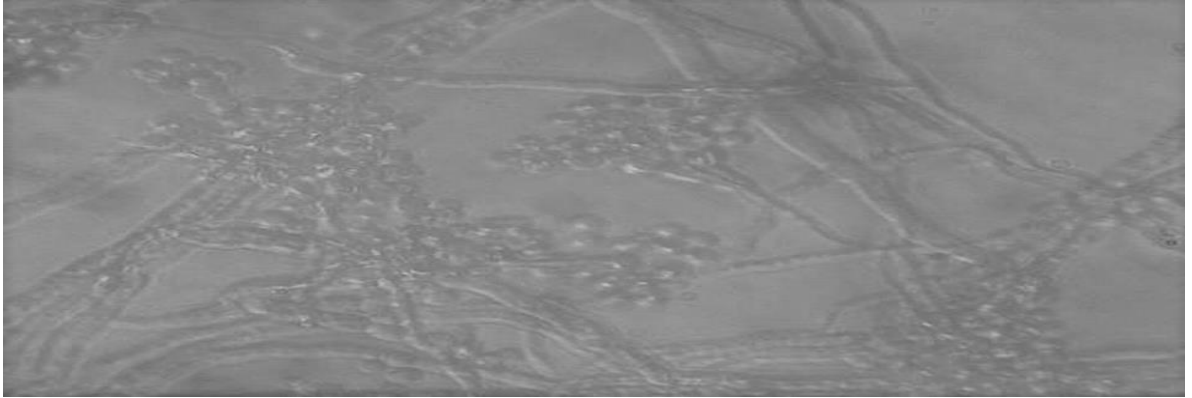


**PLATE LIII**

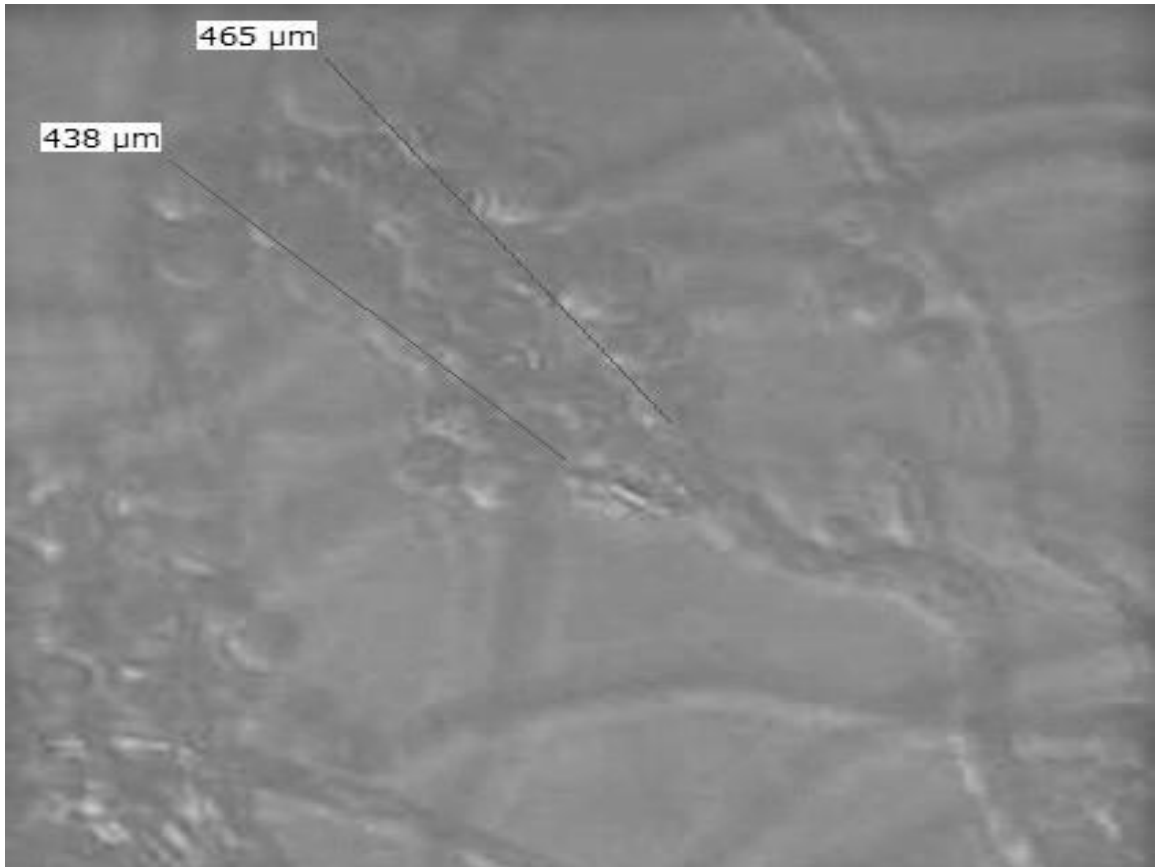


# PLATE LIV

(T<sub>23</sub>) Tbk

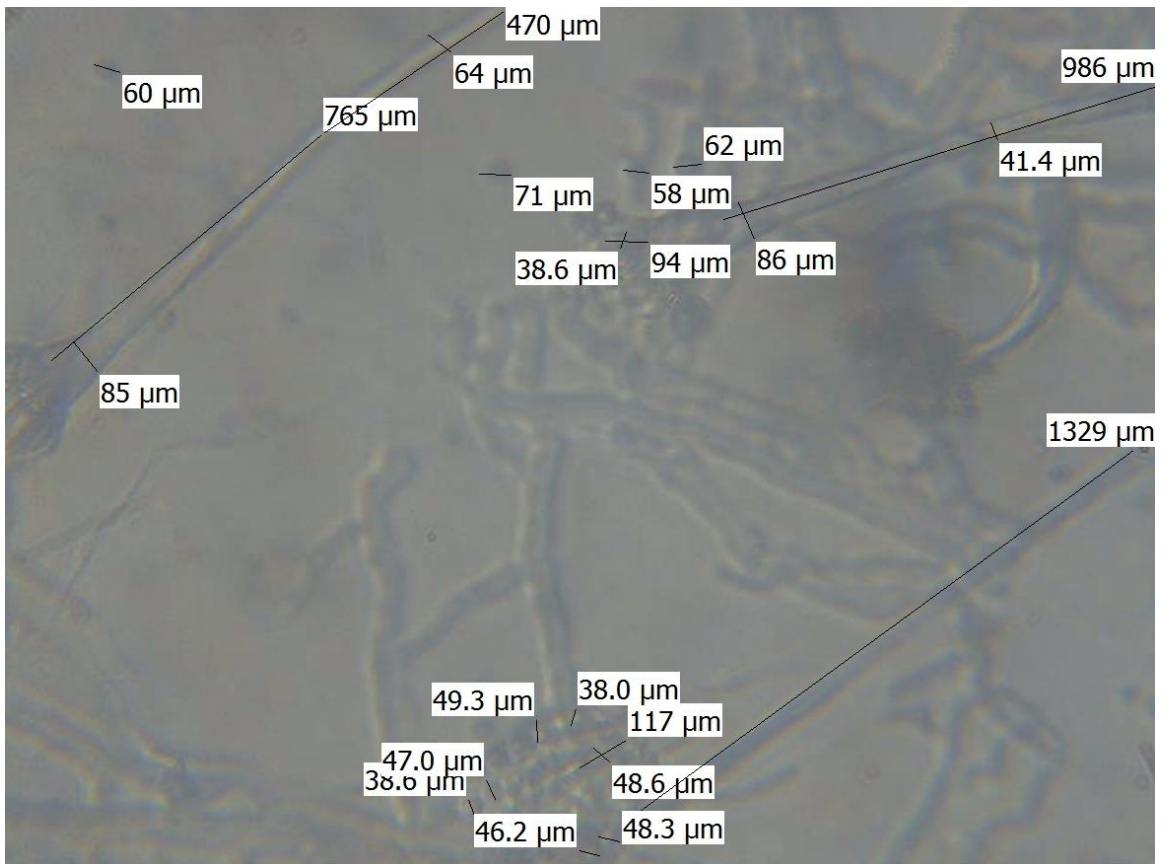
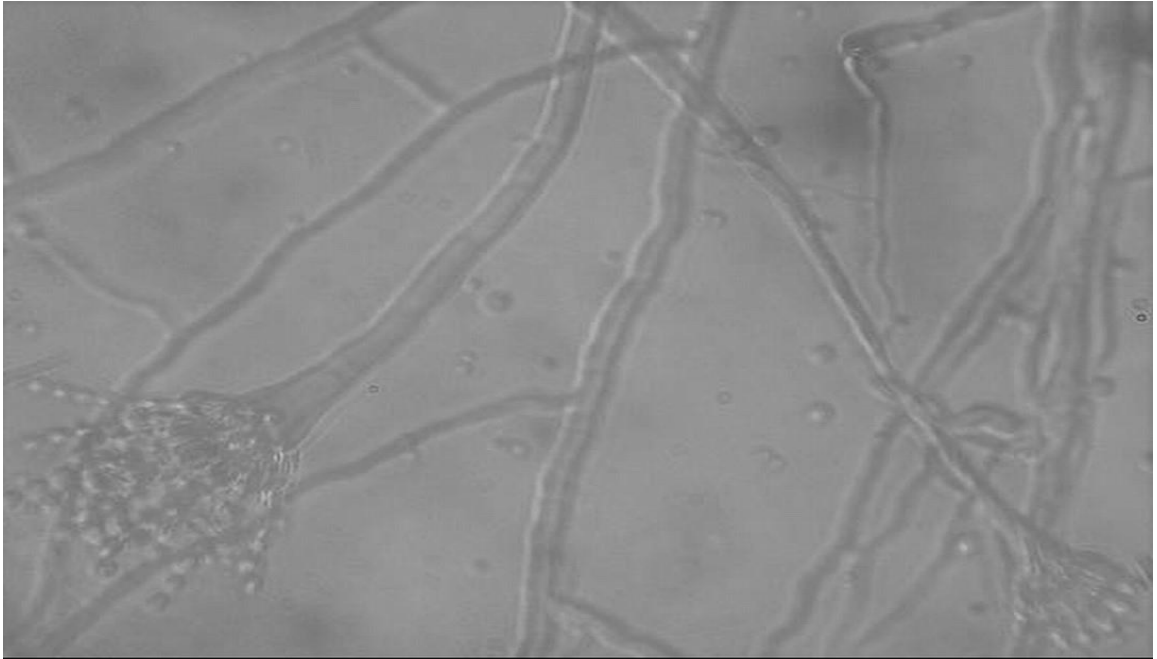


**PLATE LV**



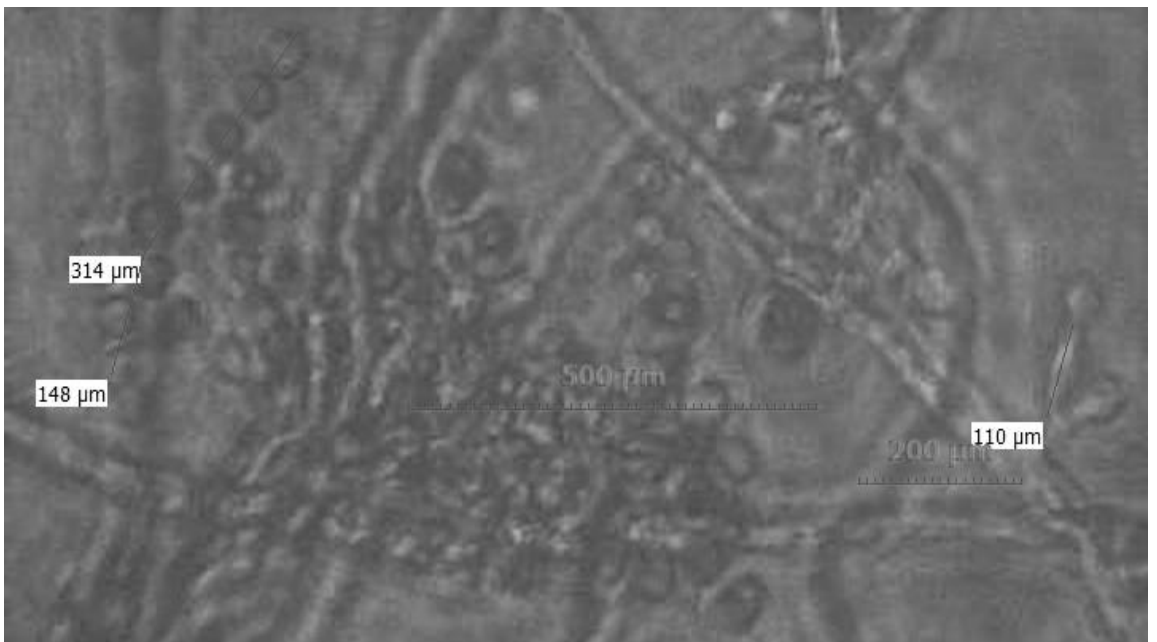
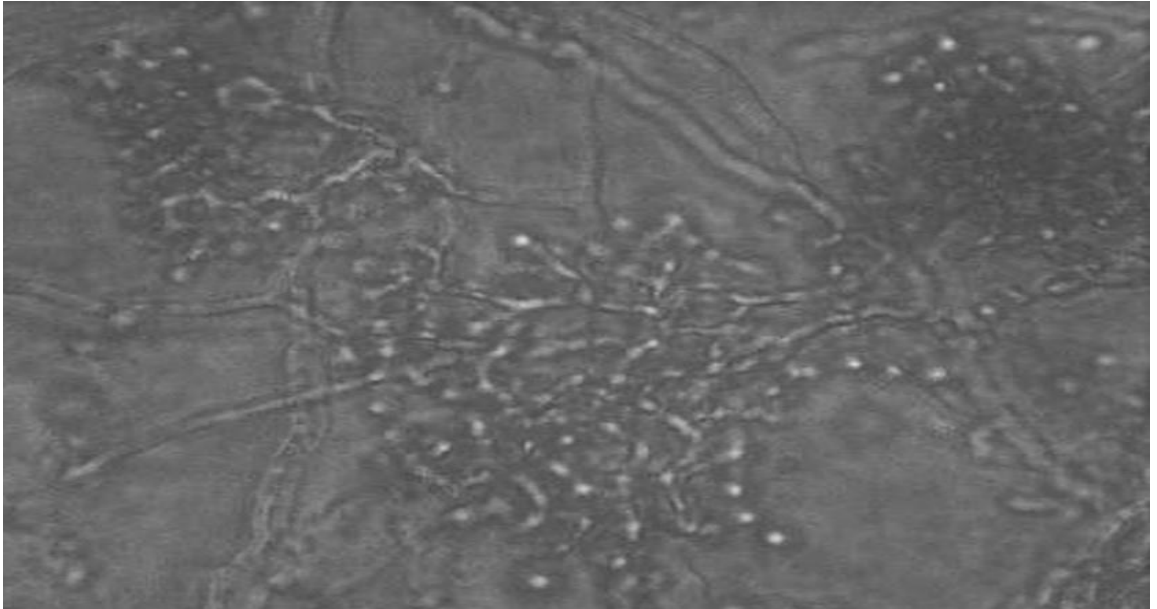
**PLATE LVI**

**(T<sub>24</sub>) Tbpal**



**PLATE LVII**

**(T<sub>25</sub>) Tckr**



# PLATE LVIII

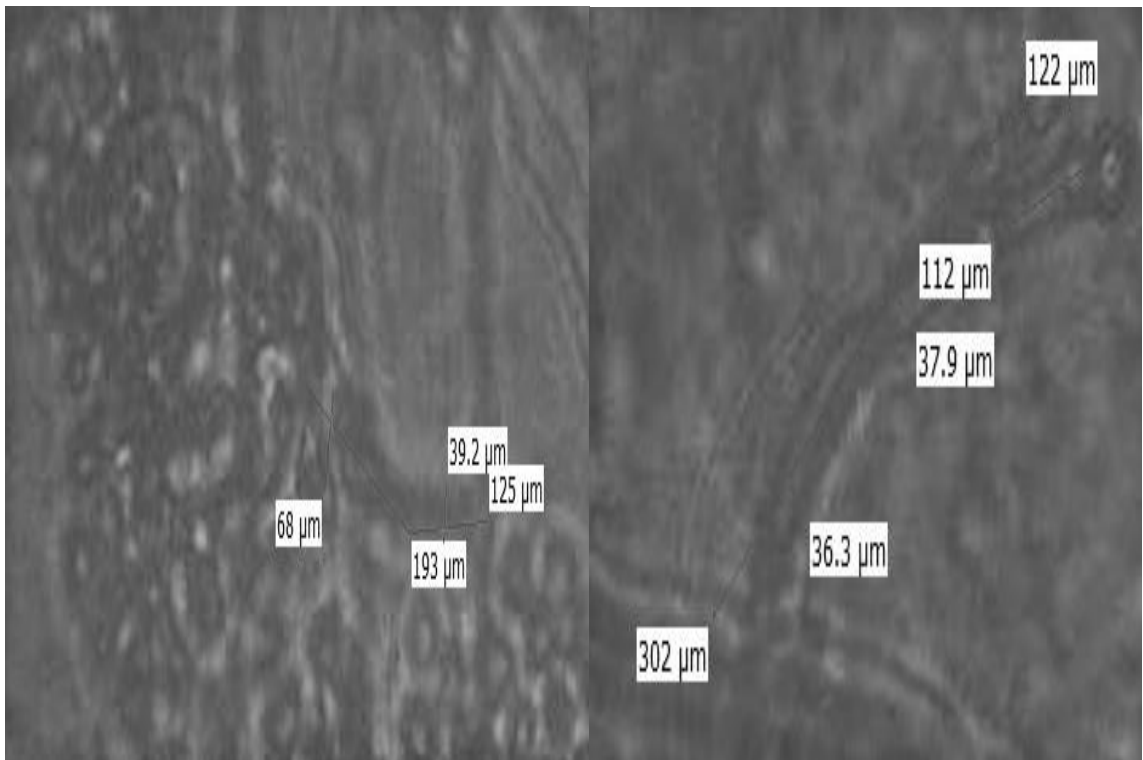
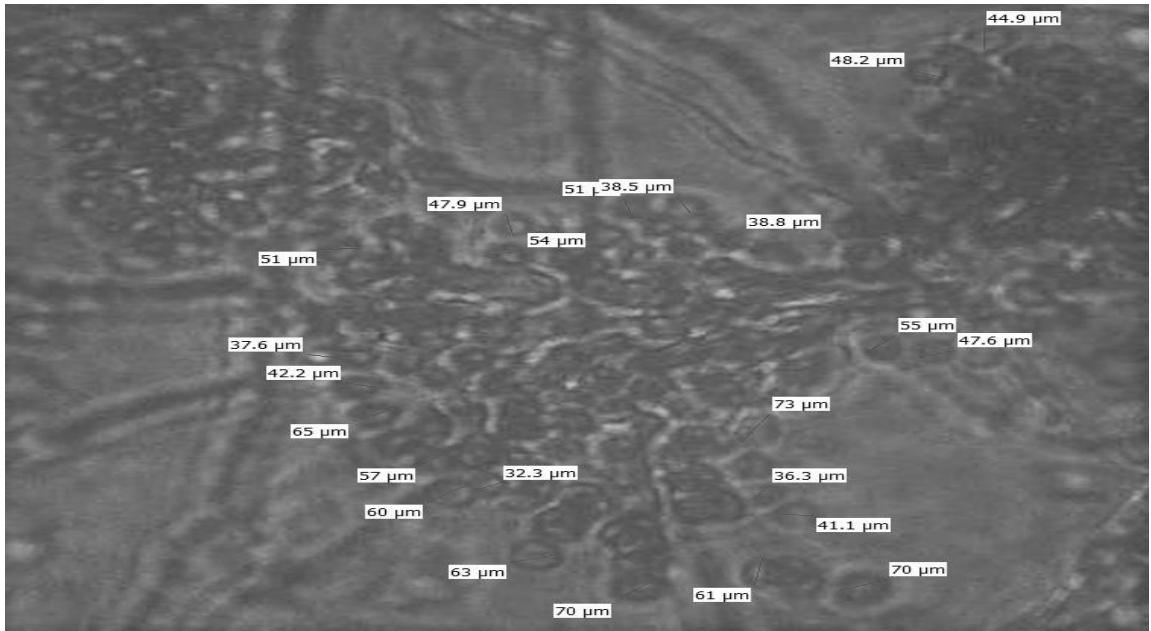


PLATE LIX

(T<sub>26</sub>) Tcaupal

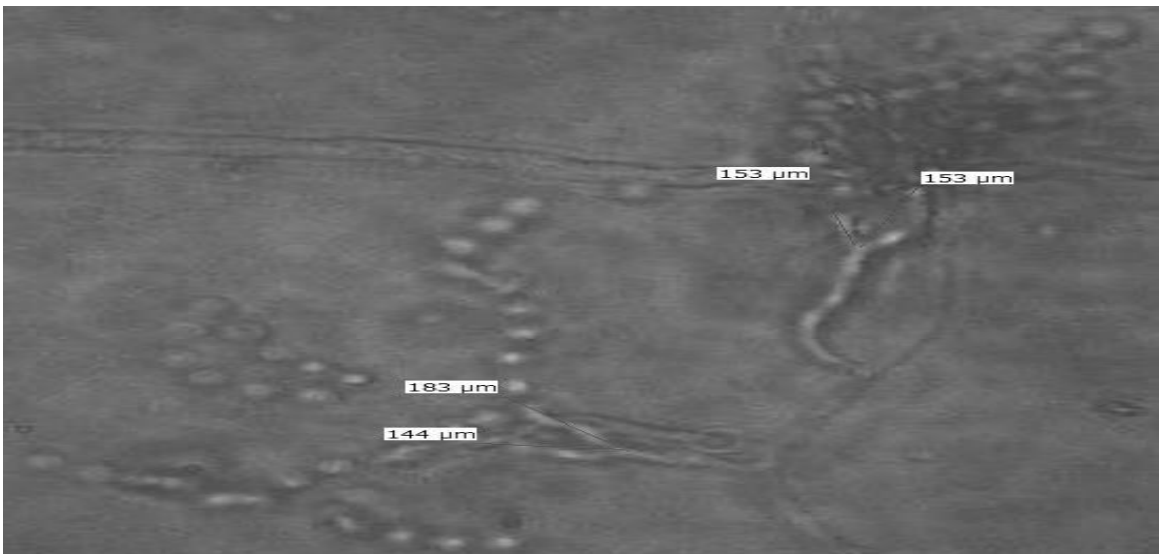
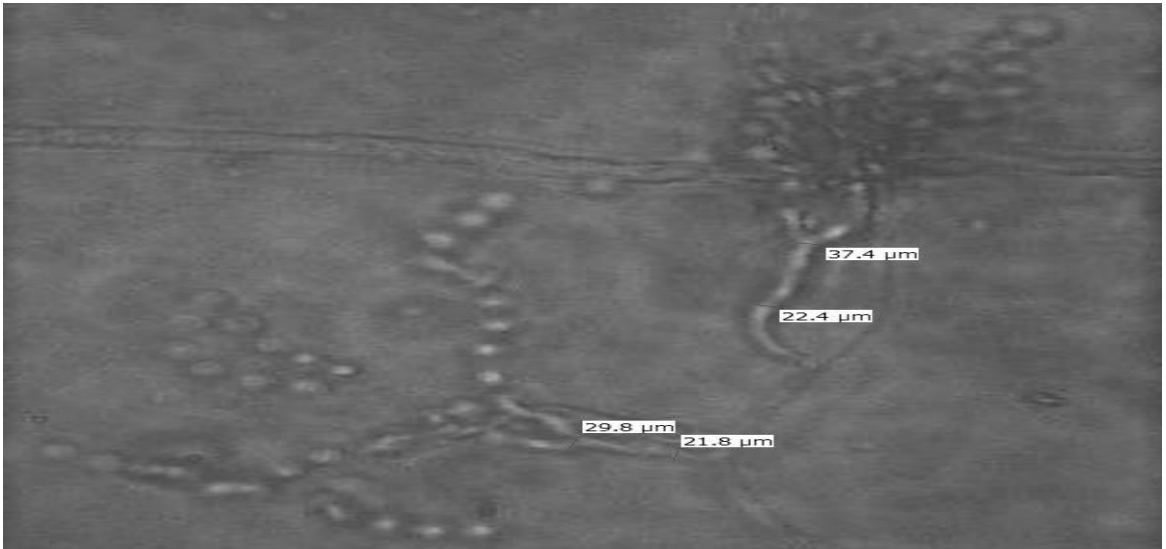
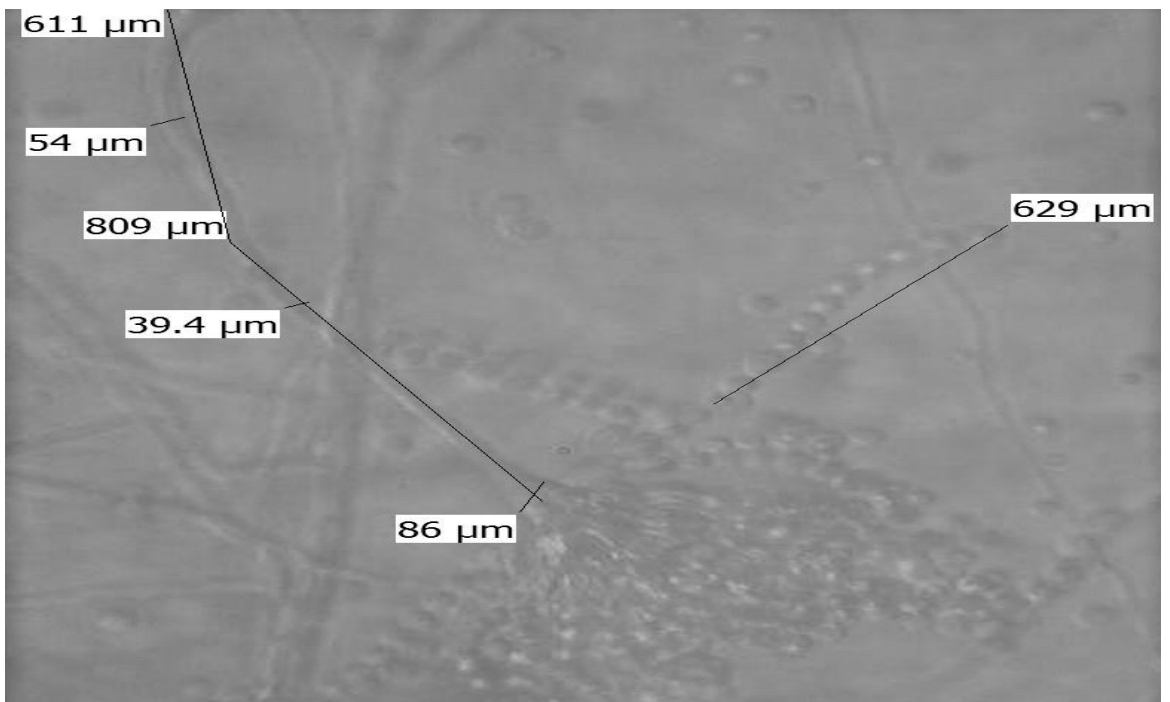
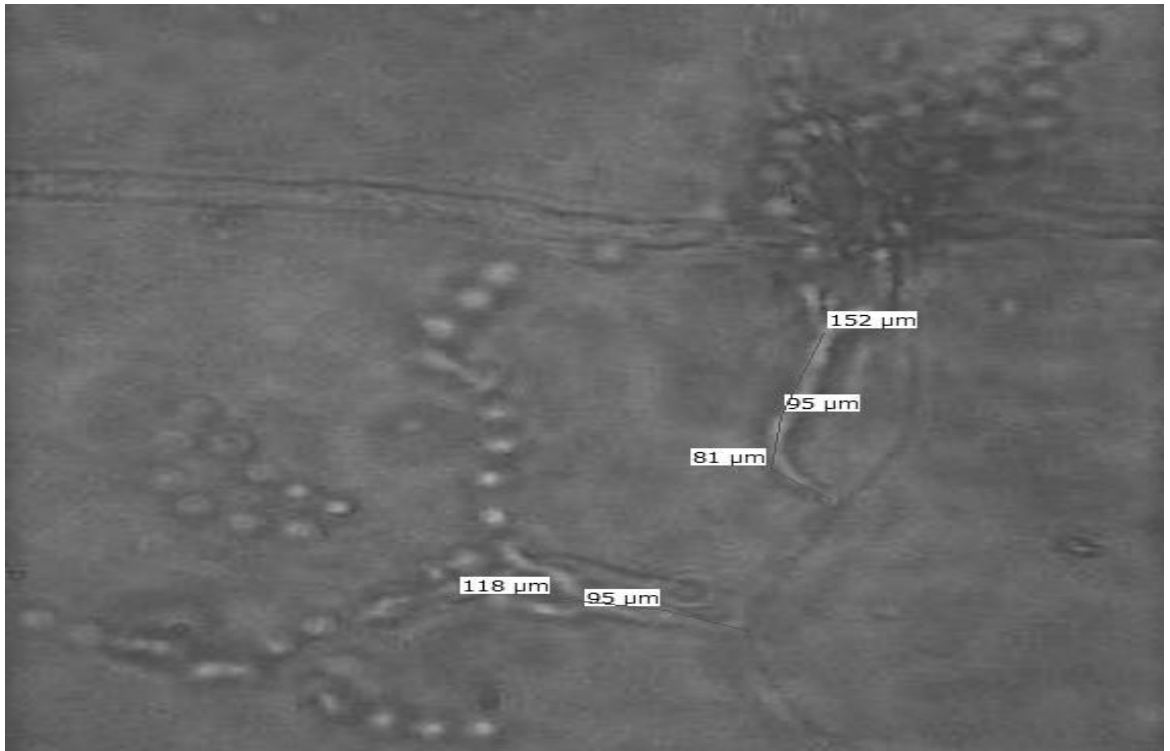


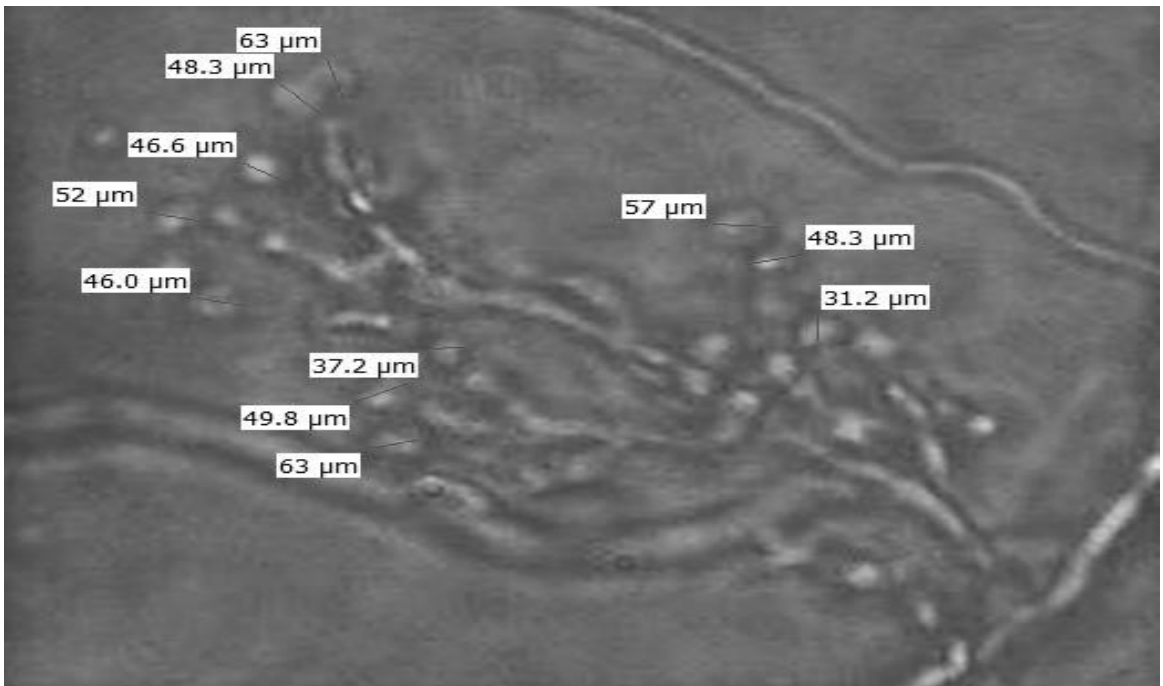
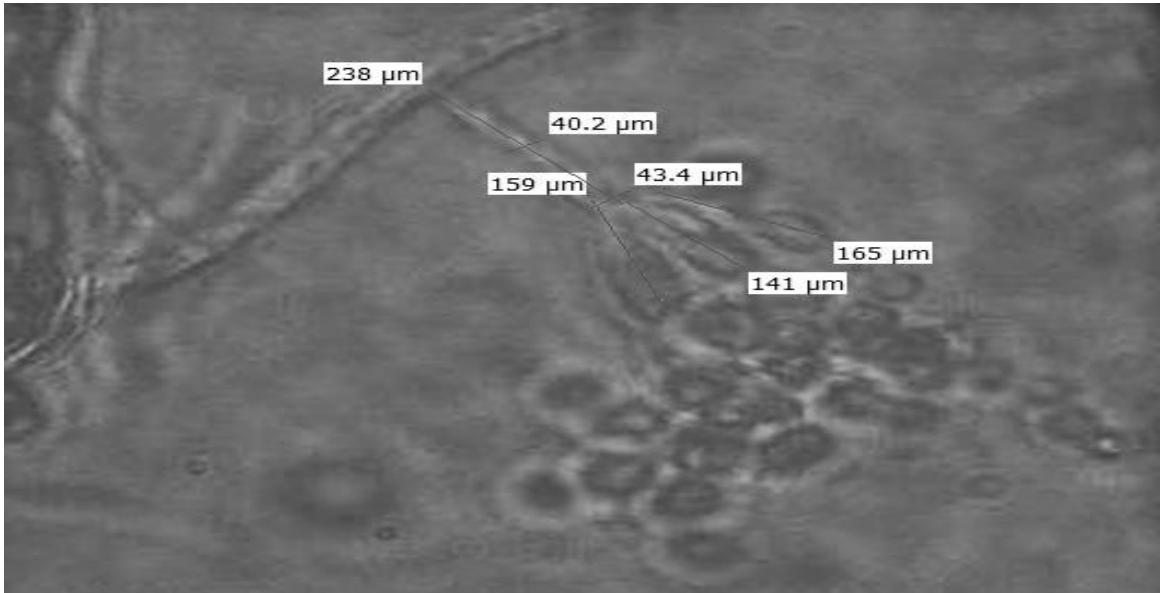
PLATE LX



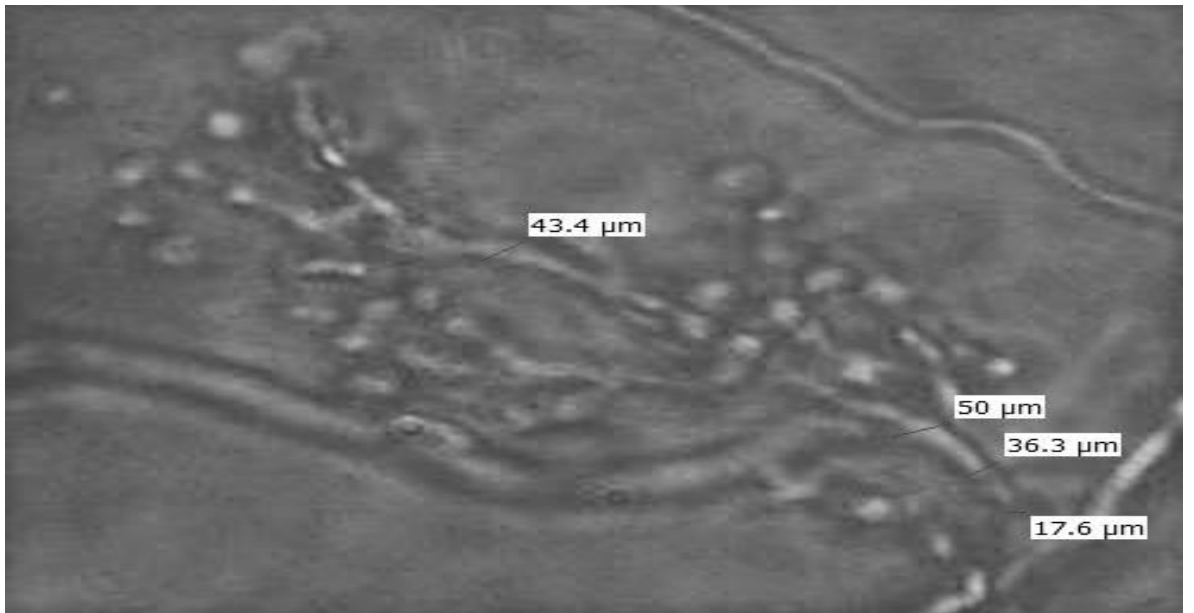
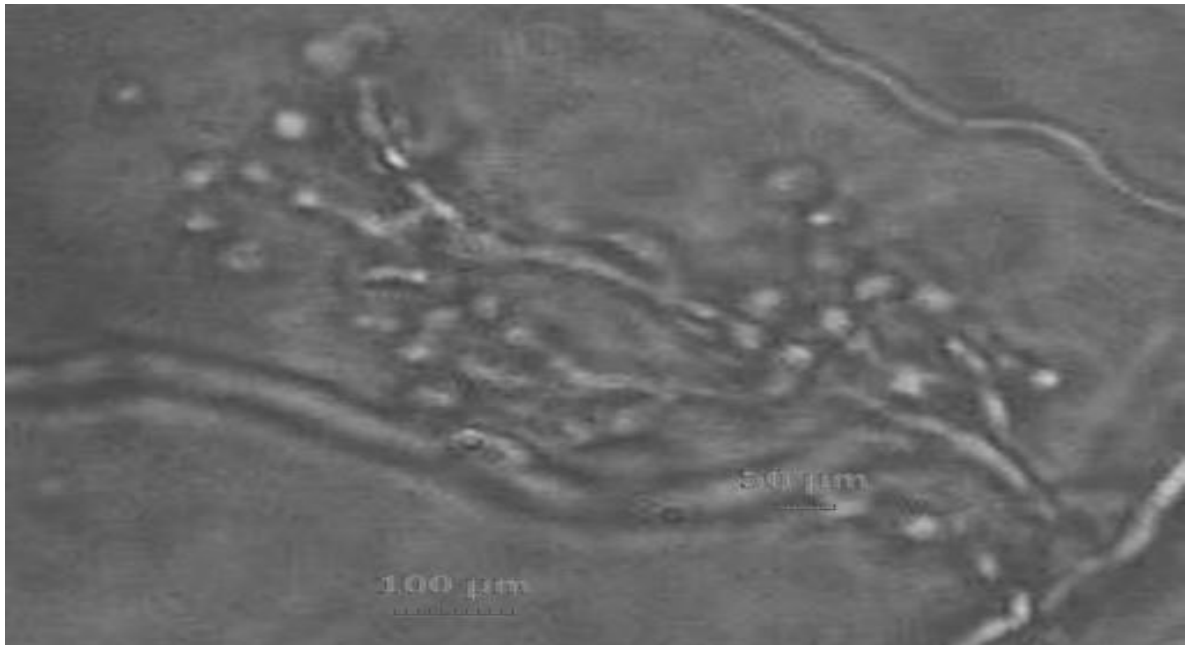


**PLATE LXI**

**(T<sub>27</sub>) Tmcpal**



**PLATE LXII**



The colony colour of isolates under study varied from whitish, off white, yellowish or in different shades of green in the beginning which gradually turned to green or dark green. The hyphal growth of 15 isolates spread out in form of tree branches fashion while it was penicillate in case of rest of the 12 isolates. The shape of phialides was sigmoidor hooked in 10 isolates and in remaining 17 isolates it was ampuliform and or lageniform. Conidia were globose, sub-globose and arrangement was catenate.

**Table 10: Measurement of morphological structures.**

Sr. No	Isolates code	Structures observed (Unit: $\mu\text{m}$ )				
		Phialide Length	Conidial Diameter	Conidial chain length	Conidiophore	
					Length	Breadth
1	Tmed	158.90	70.90	172.40	452.50	44.50
2	Tamsakh	166.80	48.32	644.40	921.80	52.08
3	Tmnrj	150.90	50.64	432.70	680.60	49.69
4	Tojrr2	152.30	54.30	345.40	726.00	53.14
5	Tkorr	150.00	47.18	300.40	635.80	44.70
6	Trm	164.20	46.87	257.20	489.80	47.54
7	Tralr	146.30	62.37	381.60	781.60	45.98
8	Tcojrr2	140.20	39.01	294.30	834.30	43.22
9	Tcbfn	148.70	41.38	370.10	595.40	53.04
10	Tewki	138.60	35.70	371.00	696.90	65.71
11	Tas	196.10	41.36	369.00	895.70	46.66
12	Tenv	144.50	44.40	485.9	1080.70	45.60
13	Tbw	122.60	49.28	430.70	586.60	55.86
14	Tgpal	146.80	53.31	466.90	820.90	57.10
15	Tsptpal	167.20	60.34	419.70	978.20	49.38
16	Thm	132.90	59.20	236.10	550.00	44.75
17	Tlbhar	155.20	59.83	279.60	654.80	58.30
18	Tgkh2020s	144.80	39.25	276.70	1083.5	48.98
19	Tefym	125.30	44.16	362.60	747.70	52.71
20	Tchal	156.30	51.67	362.40	682.80	52.38
21	Tchipal	113.10	51.30	386.70	704.20	45.82
22	Tbgu	150.00	41.99	488.70	995.80	53.35
23	Tbk	163.20	60.45	406.30	614.40	54.00
24	Tbpal	124.50	47.40	305.90	1082.00	56.54
25	Tckr	128.10	50.46	349.50	829.50	52.91
26	Tcaupal	146.50	49.16	469.70	495.40	38.49
27	Tmcpal	107.60	49.62	222.20	683.00	42.73

\*Presented data in table is average of twenty observations

The phialide size ranged from 107.60  $\mu\text{m}$  (Tmcpal) to 196.10  $\mu\text{m}$  (Tas); and conidial size from 35.70  $\mu\text{m}$  (Tcwki) to 70.90  $\mu\text{m}$  (Tmed). The length of conidial chain varied between 172.40  $\mu\text{m}$  (Tmed) to 644.40  $\mu\text{m}$  (Tamsakh) while conidiophore length from 452.50  $\mu\text{m}$  (Tmed) to 1083.5  $\mu\text{m}$  (Tgkh2020s). Conidiophore breadth admeasured between 38.49  $\mu\text{m}$  (Tcaupal) to 65.71  $\mu\text{m}$  (Tcwki).

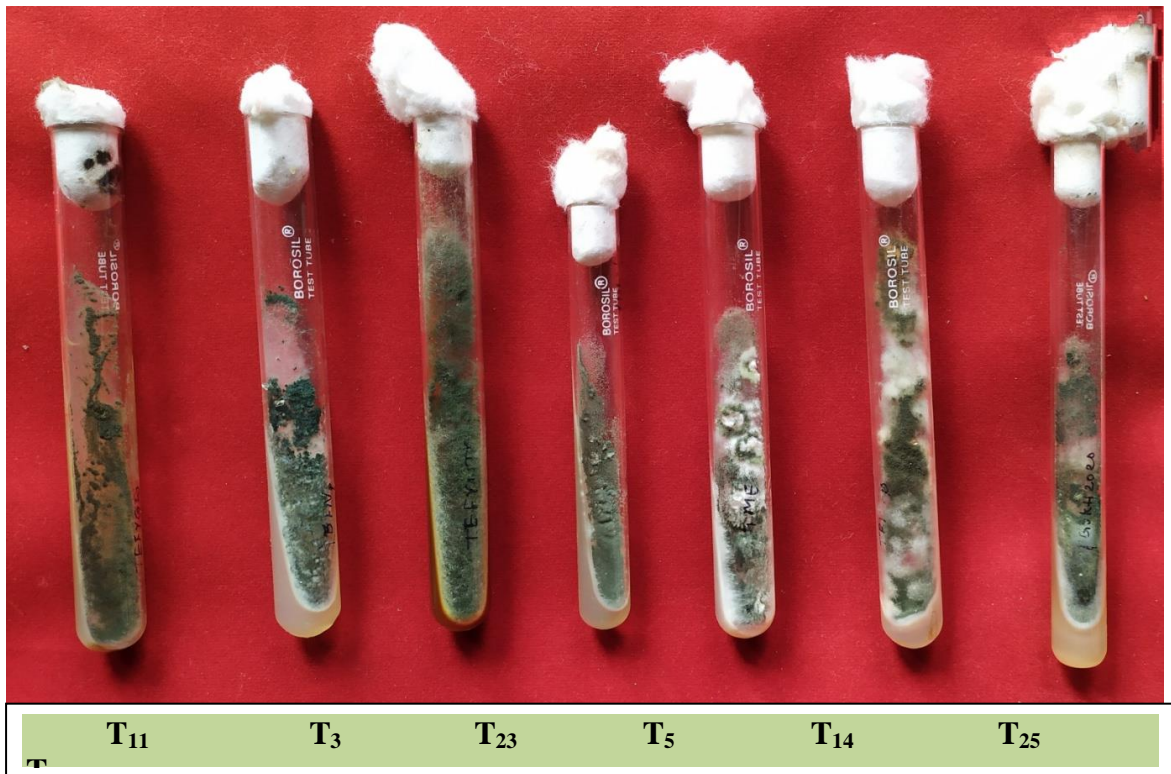
#### **4.4. Compatibility of promising isolates with fungicides.**

Sole use of bio- agents for disease management is a debatable issue. Many a times an appropriate use of bio-agents before and/or after fungicide spray becomes inevitable. Sometimes use of a combination of bio-agent and fungicide also facilitates the management strategy. It is, therefore, necessary to test the compatibility of the bio-agent with the recommended fungicides. With this view the seven promising isolates of *Trichoderma* were cultured in fungicide fortified medium to analyze their compatibility. Three systemic fungicides and three contact fungicides were used in this experiment.

It is evident from the results in the above table that Carbendazim was the most detrimental for all the isolates under study as it completely inhibited the mycelial growth of these isolates. It was followed by Hexaconazole which completely inhibited the mycelium of the isolate Tgpal. The growth inhibition by this fungicide in case of Tkorr was 90 per cent followed by Tbpal (87.77%), Tas (86.11%), Tbk (85.77%), Tckr (84.11%) and that of Tmnrj (63.33%). The third systemic fungicide Thiophanate methyl caused maximum inhibition (86.33) of Tbk, followed by Tgpal (80.22%), Tckr (80.00%), Tkorr (79.77%), Tbpal (75.77%), Tmnrj (68.33%) and Tas (58.00%).

Among the three contact fungicides, sulphur was the most compatible fungicide as the highest inhibition ( 23.11%) recorded by Tkorr, which was subsequently followed by Tbk ( 20.00%), Tckr ( 9. 60%), Tbpal (5.88%), Tgpal (4.22%), Tas (3.66%) and the least inhibition of Tmnrj (0.33%). These results suggest that at a slightly lower concentration this fungicide may not be inhibitory to the test isolates. Copper oxychloride recorded the least inhibition of (43.66%) of Tas and the maximum (84.44%) of Tkorr while mancozeb recorded the least inhibition (59.77%) of Tas and the highest (84.22%) of Tkorr.

PLATE LXIII : Promising isolates of *Trichoderma* spp. viz., Tas (T<sub>11</sub>), Tmrnj (T<sub>3</sub>), Tbk (T<sub>23</sub>), Tkorr (T<sub>5</sub>), Tgpal (T<sub>14</sub>), Tckr (T<sub>25</sub>) and Tbpal (T<sub>24</sub>).



**Table 11: Compatibility of promising isolates with fungicides**

Colony diameter of <i>Trichoderma</i> isolates (mm)															
Tr. No	Fungicide Concentration	<i>Trichoderma</i> isolates													
		1		2		3		4		5		6		7	
		Tas (T <sub>11</sub> )		Tmrj (T <sub>3</sub> )		Tbk (T <sub>23</sub> )		Tkorr (T <sub>5</sub> )		Tgpal (T <sub>14</sub> )		Tckr (T <sub>25</sub> )		Tbpal (T <sub>24</sub> )	
		Colony diameter	Inhibition %	Colony diameter	Inhibition %	Colony diameter	Inhibition %	Colony diameter	Inhibition %	Colony diameter	Inhibition %	Colony diameter	Inhibition %	Colony diameter	Inhibition %
T1	Carbendazim (1000 ppm)	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
T2	Hexaconazole (500 ppm)	12.50	86.11	33.00	63.33	12.80	85.77	9.00	90.00	0.00	100.00	14.30	84.11	11.00	87.77
T3	Thiophenate-methyl (500 ppm)	37.80	58.00	28.50	68.33	12.30	86.33	18.20	79.77	17.80	80.22	18.00	80.00	21.80	75.77
T4	Copper-oxychloride (2500 ppm)	50.70	43.66	28.20	68.66	23.50	73.88	14.00	84.44	21.00	76.66	15.20	83.11	22.20	75.33
T5	Sulphur (2500 ppm)	86.70	3.66	89.70	0.33	72.00	20.00	69.20	23.11	86.20	4.22	81.30	9.60	84.70	5.88
T6	Mancozeb (2500 ppm)	36.20	59.77	19.20	78.66	32.50	63.88	14.20	84.22	20.00	77.77	17.80	80.22	19.20	78.66
T7	Control	90.00	00.00	90.00	00.00	90.00	00.00	90.00	00.00	90.00	00.00	90.00	00.00	90.00	00.00
	Ftest	Sig	-	Sig	-	Sig	-	Sig	-	Sig	-	Sig	-	Sig	-
	SE (m)±	0.12	-	0.08	-	0.10	-	0.19	-	0.04	-	0.08	-	0.11	-
	CD (P=0.01)	0.51	-	0.32	-	0.43	-	0.78	-	0.19	-	0.32	-	0.46	-

\*Presented data in table is average of three replications

**LEGEND**  
**(For Fig. 7)**

<b>Isolate code</b>	<b>Crop rhizosphere</b>	<b>Location</b>
<b>T<sub>11</sub> (Tas)</b>	<b>Areca nut</b>	<b>Shrivardhan dist. Raigad</b>
<b>T<sub>3</sub> (Tmnrj)</b>	<b>Mango</b>	<b>Lanja dist. Ratnagiri</b>
<b>T<sub>23</sub> (Tbk)</b>	<b>Brinjal</b>	<b>Karjat dist. Raigad</b>
<b>T<sub>5</sub> (Tkorr)</b>	<b>Rice</b>	<b>Kolambe dist. Ratnagiri</b>
<b>T<sub>14</sub> (Tgpal)</b>	<b>Guava</b>	<b>Kelwe dist. Palghar</b>
<b>T<sub>25</sub> (Tckr)</b>	<b>Cabbage</b>	<b>Karjat dist. Raigad</b>
<b>T<sub>24</sub> (Tbpal)</b>	<b>Brinjal</b>	<b>Mahim dist. Palghar</b>





**LEGEND**  
**(For Fig. 7)**

<b>Tr. No.</b>	<b>Treatments</b>
<b>T<sub>1</sub></b>	<b>Carbendazim (0.1%) 50% WP</b>
<b>T<sub>2</sub></b>	<b>Hexaconazole (0.05%) 5% SC</b>
<b>T<sub>3</sub></b>	<b>Thiophenate methyl (0.05%) 70% WP</b>
<b>T<sub>4</sub></b>	<b>Copper oxychloride (0.25%) 50% WP</b>
<b>T<sub>5</sub></b>	<b>Sulphur (0.25%) 80% WDG</b>
<b>T<sub>6</sub></b>	<b>Mancozeb (0.25%) 75% WP</b>
<b>T<sub>7</sub></b>	<b>Control</b>

PLATE LXIV : Compatibility of promising isolates with Carbendazim (1000ppm)



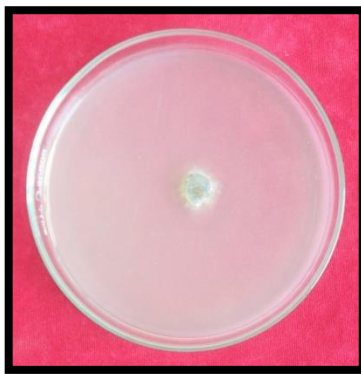
T<sub>11</sub> (100.00 % inhibition)



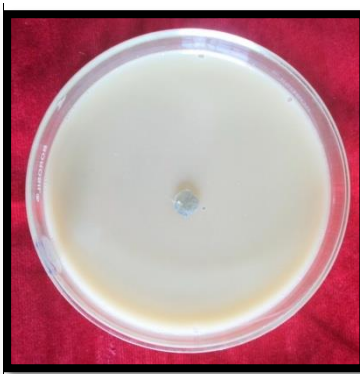
T<sub>3</sub> (100.00 %)



T<sub>23</sub> (100.00 %)



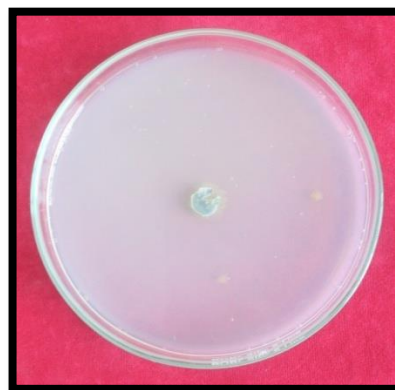
T<sub>5</sub> (100.00%)



T<sub>14</sub> (100.00%)

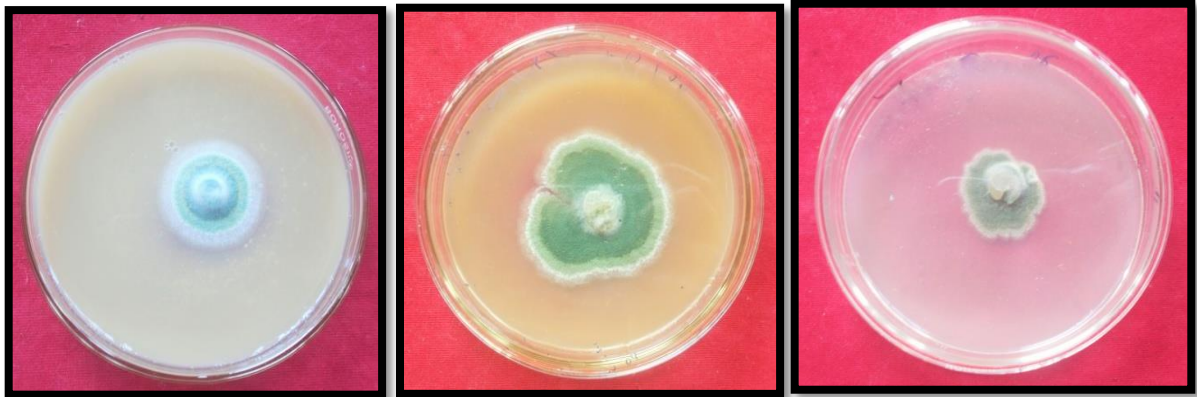


T<sub>25</sub> (100.00%)



T<sub>24</sub> (100.00%)

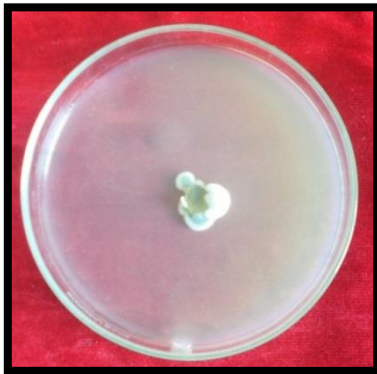
**PLATE LXV : Compatibility of promising isolates with Hexaconazole (500ppm)**



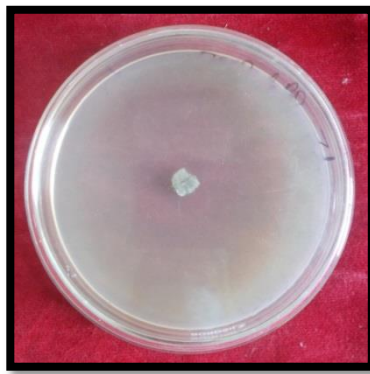
T<sub>11</sub> (86.11%)

T<sub>3</sub> (63.33%)

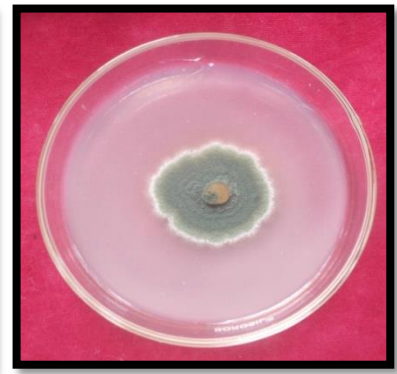
T<sub>23</sub> (85.77%)



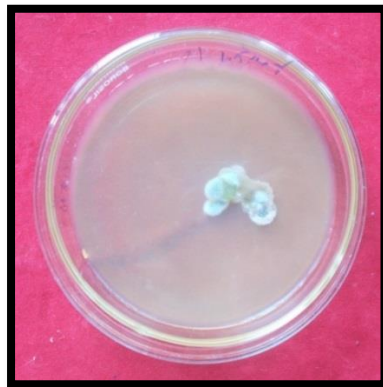
T<sub>5</sub> (90.00%)



T<sub>14</sub> (100.00%)

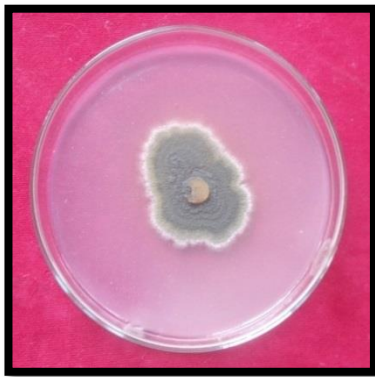


T<sub>25</sub> (84.11%)

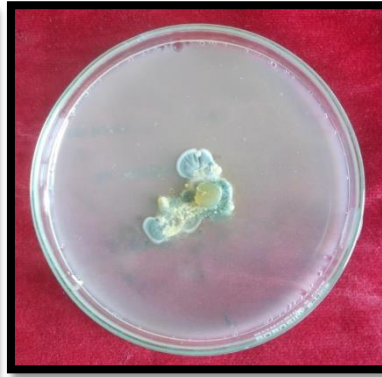


T<sub>24</sub> (87.77%)

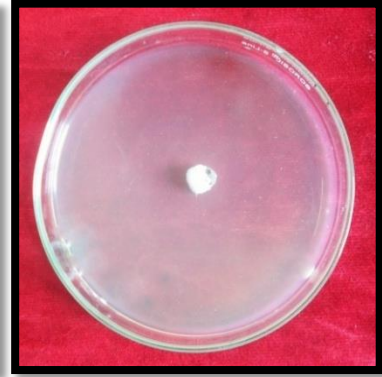
PLATE LXVI : Compatibility of promising isolates with Thiophenate methyl (500ppm)



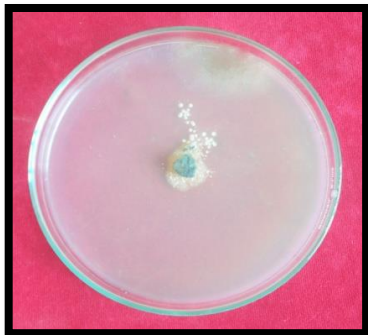
T<sub>11</sub> (58.00%)



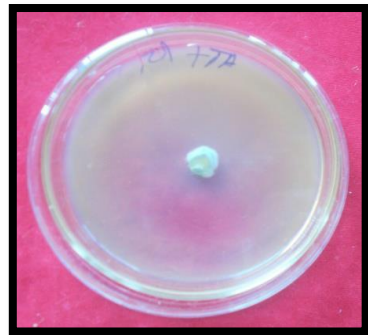
T<sub>3</sub> (68.33%)



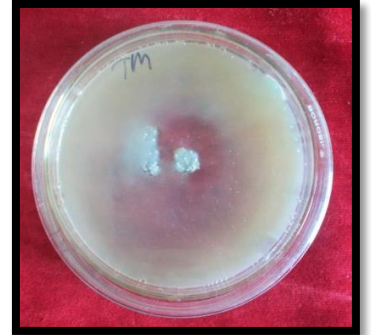
T<sub>23</sub> (86.33%)



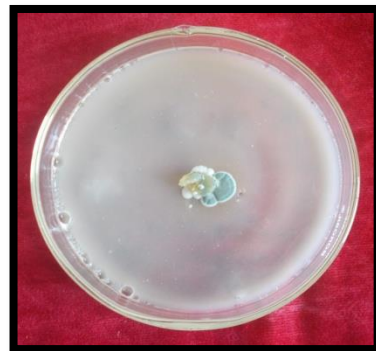
T<sub>5</sub> (79.77%)



T<sub>14</sub> (80.22%)

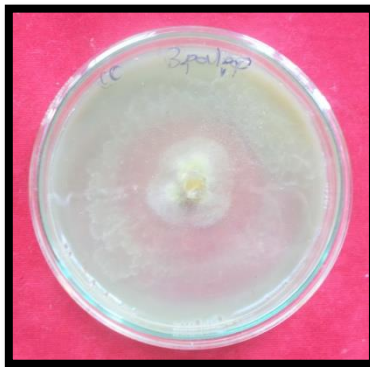


T<sub>25</sub> (80.00%)

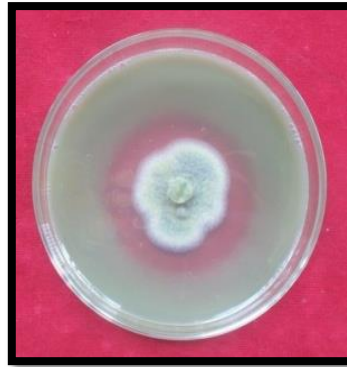


T<sub>24</sub> (75.77%)

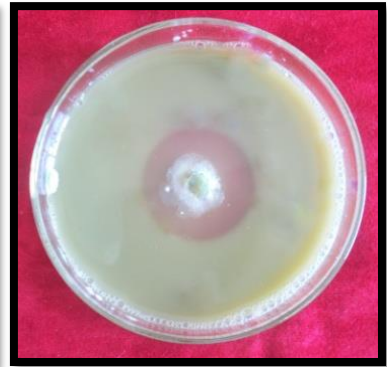
PLATE LXVII : Compatibility of promising isolates with Copper oxychloride (2500ppm)



T<sub>11</sub> (43.66%)



T<sub>3</sub> (68.66%)



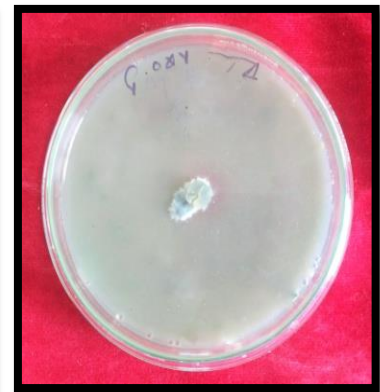
T<sub>23</sub> (73.88%)



T<sub>5</sub> (84.44%)



T<sub>14</sub> (76.66%)



T<sub>25</sub> (83.11%)

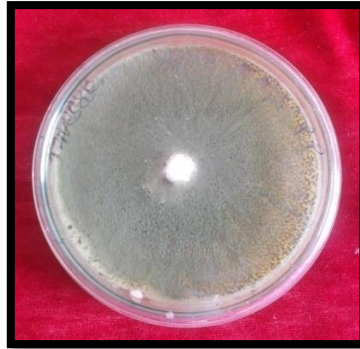


T<sub>24</sub> (75.33%)

**PLATE LXVIII : Compatibility of promising isolates with Sulphur (2500ppm)**



T<sub>11</sub> (3.66%)



T<sub>3</sub> (0.33%)



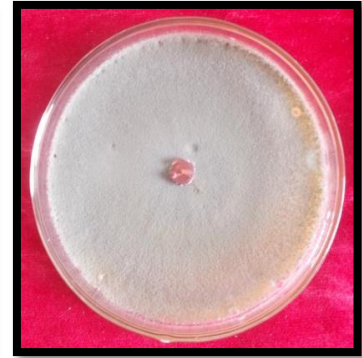
T<sub>23</sub> (20.00%)



T<sub>5</sub> (23.11%)



T<sub>14</sub> (4.22%)

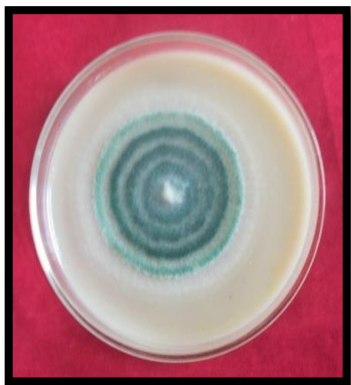


T<sub>25</sub> (9.60%)



T<sub>24</sub> (5.88%)

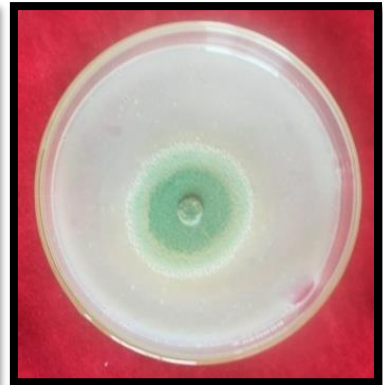
PLATE LXIX : Compatibility of promising isolates with Mancozeb (2500ppm)



T<sub>11</sub> (59.77%)



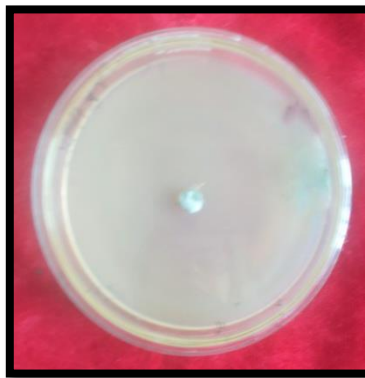
T<sub>3</sub> (78.66%)



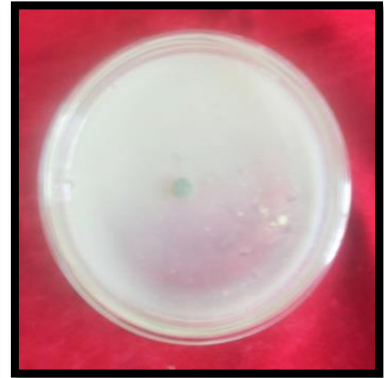
T<sub>23</sub> (63.88%)



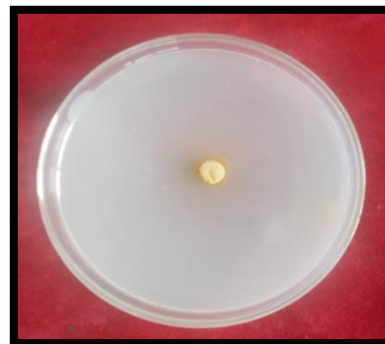
T<sub>5</sub> (84.22%)



T<sub>14</sub> (77.77%)

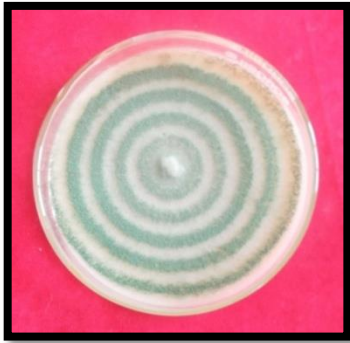


T<sub>25</sub> (80.22%)



T<sub>24</sub> (78.66%)

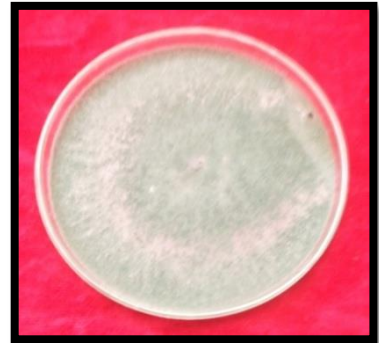
PLATE LXX : Control



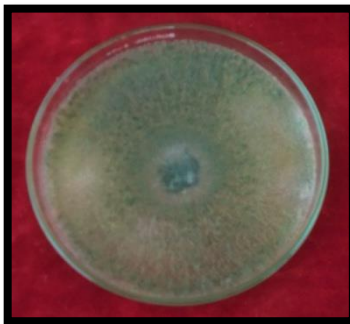
T<sub>11</sub>



T<sub>3</sub>



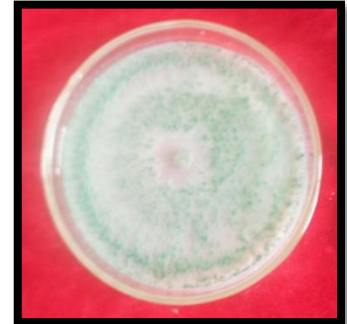
T<sub>23</sub>



T<sub>5</sub>



T<sub>14</sub>



T<sub>25</sub>



T<sub>24</sub>



**LEGEND**  
**(For Fig.7)**

<b>Number of promising isolates</b>	<b>Isolate code</b>	<b>Identified as</b>
<b>T<sub>1</sub></b>	<b>T<sub>11</sub> (Tas)</b>	<i>Trichoderma asperellum</i>
<b>T<sub>2</sub></b>	<b>T<sub>3</sub> (Tmnrj)</b>	<i>Trichoderma harzianum</i>
<b>T<sub>3</sub></b>	<b>T<sub>23</sub> (Tbk)</b>	<i>Trichoderma asperellum</i>
<b>T<sub>4</sub></b>	<b>T<sub>5</sub> (Tkorr)</b>	<i>Trichoderma sp. aff. T. koningii</i>
<b>T<sub>5</sub></b>	<b>T<sub>14</sub> (Tgpal)</b>	<i>Trichoderma sp. aff. T. koningii</i>
<b>T<sub>6</sub></b>	<b>T<sub>25</sub> (Tckr)</b>	<i>Trichoderma sp. aff. T. longibrachiatum</i>
<b>T<sub>7</sub></b>	<b>T<sub>24</sub> (Tbpal)</b>	<i>Trichoderma sp. aff. T. koningii</i>

#### 4.5. Molecular and morphological identification of promising isolates

After the evaluation of antagonistic activity, found seven best isolates of *Trichoderma* which was thought necessary to identify these isolates up to species level. Hence, four isolates were sent for morphological identification and three fast growing isolates were sent for molecular characterization to Agharkar Research Institute, Pune. The results are presented below.

##### 4.5.1. Morphological identification of four isolates (ARI, Pune)

Sr. No.	Culture	Identification Remarks	Family
4.	Tkorr	<i>Trichoderma</i> sp. aff. <i>T. koningii</i> Oudem	Hypocreaceae
5.	Tgpal	<i>Trichoderma</i> sp. aff. <i>T. koningii</i> Oudem.	Hypocreaceae
6.	Tckr	<i>Trichoderma</i> sp. aff. <i>T. longibrachiatum</i> Rifai	Hypocreaceae
7.	Tbpal	<i>Trichoderma</i> sp. aff. <i>T. koningii</i> Oudem	Hypocreaceae

Among the four, three isolates were identified as *T. koningii* Oudem and one was identified as *T. longibrachiatum* Rifai.

##### 4.5.2. Molecular identification of three isolates (ARI, Pune)

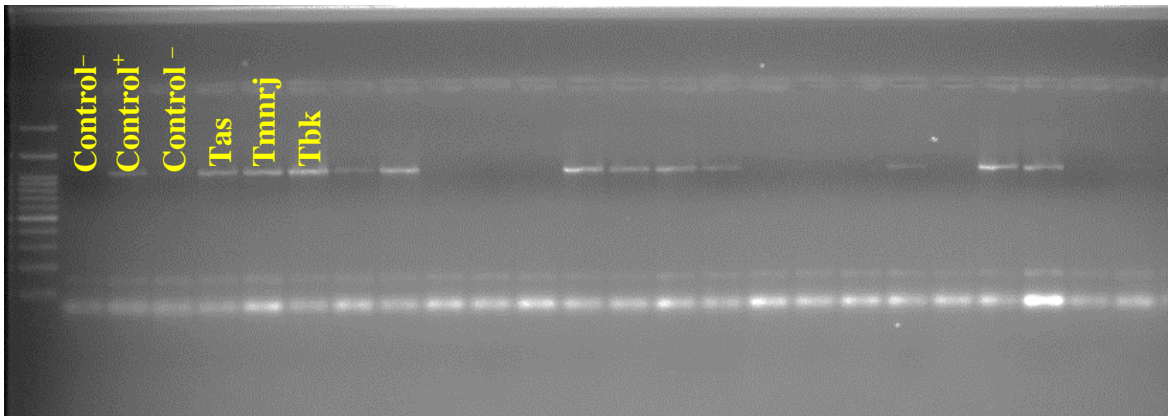
TEF- $\alpha$  gene using primer EF-983F and EF 2118R resulted in amplification of 929bp. The product was purified and sequenced using ABI 3100 automated DNA sequencer and BLAST were done. The results of the sequence-based identification are as given in plate.

##### 4.5.2.1 Molecular identification of Tas

- Cultural characteristics of Tas

1	Culture code	: Tas: <i>Trichoderma asperellum</i> Samuels, Lieckf. & Nirenberg
2	NFCCI Accesssion number	BankIt 2546015 NFCCI_5020 OM471989
3	Substrate/habitat	Rhizospheric soil
4	Macromorphology:	Colonies on PDA at 25±2°C, fast growing, white floccose, sporulating area dark green, pustulate, reverse buff.
5	Micromorphology:	
5.1	Hyphae	Branched septate, smooth walled, subhyaline.
5.2	<i>Conidiophores</i>	Loosely branched, simple, fertile, smooth walled, hyaline
5.3	Chlamydospores	Terminal to intercalary, globose to fusoid, smooth and thick walled, hyaline, upto 10.50 × 8.4 µm.

**PLATE LXXI**



**The amplification of the TEF- $\alpha$  gene using primer EF-983F and EF 2118R on an agarose gel. The ladder used was 2000bp as reference**

<b>5.4</b>	<b>Phialides</b>	Produced solitary or in groups, straight to ampulliform to lageniform, variable in shaped and size, smooth walled, hyaline, 6.7–13.0 × 2.8–3.6 μm.
<b>5.5</b>	<b>Phialospores</b>	Globose to subglobose, oval, smooth walled, hyaline, up to 3.6–4.6 × 2.8–4.3 μm.

- The isolation of genomic DNA has been done from mother culture. TEF- $\alpha$  gene was successfully amplified using primer EF-983 F and EF2118R.
- ABI-Big Dye® Terminator 3.1 Cycle Sequencing kit was used to set up the sequencing PCR. For inconsistency the raw sequence obtained from ABI 3100 automated DNA sequence was manually edited and sequence data was aligned with Altschul *et al.* (1990) sequences as described, which were analyzed to reach identity.
- The tested fungal strain showed 100% sequence similarity with *T. asperellum*. Sequence analyses with NCBI accession number XM\_024901686.1, *T. asperellum* strain CBS 433.97 resulted in following alignment statistics. Alignment statistics: Query Length - 1860, Score - 1772 bits (959), Expect - 0.0, Identities – 959/959 (100%), Gaps-0/959 (0%), Strand-Plus/ Plus.

**Table12: The top five hits upon BLASTn analysis**

<b>Sr. No.</b>	<b>Gene Bank Accession No.</b>	<b>Description</b>	<b>Max score</b>	<b>Query cover</b>	<b>Query coverage</b>	<b>E value</b>	<b>Identity (%)</b>
1	XM_024901686.1	<i>Trichoderma asperellum</i> CBS433.97	1772	1772	99%	0.0	100.00%
2	CP072830.1	<i>Trichoderma asperellum</i> DQ-1chromosome1	1772	1772	100%	0.0	99.90%
3	XM_024548974.1	<i>Trichoderma gamsii</i> (TGAM01_v202318)	1644	1644	99%	0.0	97.70%
4	MK966035.1	<i>Trichoderma</i> sp.isolate TRICHO2	1635	1635	94%	0.0	99.23%
5	KJ634761.1	<i>Trichoderma koningiopsis</i> isolate 7819	1631	1631	97%	0.0	97.98%

#### 4.5.2.2. Molecular identification of Tmnrj

- Cultural characteristics of Tmnrj

<b>1</b>	<b>Culture code</b>	<b>Tmnrj: <i>Trichoderma harzianum</i> Rifai</b>
<b>2</b>	<b>NFCCI Accesssion number</b>	BankIt 2546015 NFCCI_5021 OM471990
<b>3</b>	<b>Substrate/habitat</b>	Rhizospheric soil
<b>4</b>	<b>Macromorphology:</b>	Colonies on PDA at 25±2°C, fast growing, white floccose, sporulating area dark green, forming green pustules, reverse dull yellow.
<b>5</b>	<b>Micromorphology:</b>	
<b>5.1</b>	<b>Hyphae</b>	Branched septate, smooth walled, subhyaline to light olivaceous, pigmented produced in parallel bundles.
<b>5.2</b>	<b><i>Conidiophores</i></b>	Loosely branched, simple, hyaline, smooth walled, septate
<b>5.3</b>	<b>Chlamydospores</b>	Stalked, hyaline, globose to subglobose, smooth walled, upto 9.2 × 8.3 µm.
<b>5.4</b>	<b>Phialides</b>	Produced in form of verticils as well as bilateral, ampulliform, up to 3.7–6.7 × 1.5–2.1µm.
<b>5.5</b>	<b>Phialospores</b>	Produced in gleosporic mass, globose to subglobose, smooth walled, 3.1–2.5 × 3.2-2.1 µm.

- The isolation of genomic DNA has been done from mother culture. TEF- $\alpha$  gene was successfully amplified using primer EF-983F and EF2118R.
- ABI-Big Dye® Terminator 3.1 Cycle Sequencing kit was used to set up the sequencing PCR. For inconsistency the raw sequence obtained from ABI 3100 automated DNA sequence was manually edited and sequence data was aligned with Altschul *et al.* (1990) sequences as described, which were analyzed to reach identity.
- The tested fungal strain showed 98.54% sequence similarity with *Trichoderma harzianum*. Sequence analyses with NCBI accession number XM\_024912186.1, *Trichoderma harzianum* strain CBS 226.95 resulted in following alignment statistics. Alignment statistics: Query Length - 1817, Score - 1694 bits (917), Expect - 0.0, Identities – 945/959 (99%), Gaps-0/959 (0%), Strand-Plus/ Plus

**Table13: The top five hits upon BLASTn analysis**

Sr. No	Gene Bank Accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
1	CP075864.1	<i>Trichoderma simmonsii</i> strain GH-Sj1 chromosome I	1705	1705	100%	0.0	98.75%
2	MT708571.1	<i>Trichoderma</i> sp. REC-2020a isolate T154	1700	1700	100%	0.0	98.64%
3	XM_024912186.1	<i>Trichoderma harzianum</i> CBS 226.95	1694	1694	100%	0.0	98.54%
4	KU933430.1	<i>Trichoderma afroharzianum</i> strain ATCC 20847	1694	1694	99%	0.0	98.74%
5	MT435114.1	<i>Trichoderma lixii</i> culture MUT<ITA>:3171 t	1694	1694	100%	0.0	98.54%

#### 4.5.2.3. Molecular identification of Tbk.

- **Cultural characteristics of Tbk**

<b>1</b>	<b>Culture code</b>	<b>Tbk: <i>Trichoderma asperellum</i> Samuels, Lieckf. &amp; Nirenberg</b>
<b>2</b>	<b>NFCCI Accession number</b>	BankIt2546015 NFCCI_5022 OM471991
<b>3</b>	<b>Substrate/habitat</b>	Rhizospheric soil
<b>4</b>	<b>Macromorphology:</b>	Colonies on PDA at 25±2°C, fast growing, white floccose, sporulating area dark green, pustulate, reverse buff.
<b>5</b>	<b>Micromorphology:</b>	
<b>5.1</b>	<b>Hyphae</b>	Branched septate, smooth walled, subhyaline
<b>5.2</b>	<b>Conidiophores</b>	Loosely branched, simple, fertile, smooth walled hyaline
<b>5.3</b>	<b>Chlamydospores</b>	Terminal to intercalary, globose to fusoid, smooth and thick walled, hyaline, upto 10.50 × 8.4 µm.
<b>5.4</b>	<b>Phialides</b>	Produced solitary or in groups, straight to ampulliform to lageniform, variable in shaped and size, smooth walled, hyaline, 6.7–13.0 × 2.8–3.6 µm.
<b>5.5</b>	<b>Phialospores</b>	Globose to subglobose, oval, smooth walled, hyaline, upto 3.6–4.6 × 2.8–4.3 µm.

- The isolation of genomic DNA has been done from mother culture, TEF- $\alpha$  gene

was effectively amplified using primer EF-983F and EF2118R.

- Used ABI-Big Dye® Terminator 3.1 Cycle Sequencing kit to set up the sequencing PCR. For inconsistency, the raw sequence obtained from ABI 3100 automated DNA sequence was manually edited and sequence data was aligned with Altschul *et al.*, (1990) sequences as described, which were analyzed to reach identity.
- The tested fungal strain showed 100% sequence similarity with *T. asperellum*. Sequence analyses with NCBI accession number XM\_024901686.1, *T. asperellum* strain CBS 433.97 resulted in following alignment statistics.
  - Alignment statistics: Query Length - 1860, Score - 1777 bits (962), Expect - 0.0, Identities –962/962 (100%), Gaps-0/962 (0%), Strand -Plus/ Plus

**Table14: Top hits at BLASTn analysis**

Sr. No	Gene Bank Accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
1	XM_024901686.1	<i>Trichoderma asperellum</i> CBS 433.97	1777	1777	100%	0.0	100.00%
2	CP072830.1	<i>Trichoderma asperellum</i> DQ-1 chromosome 1	1777	1777	100%	0.0	100.00%
3	XM_024548974.1	<i>Trichoderma gamsii</i> (TGAM01_v202318)	1650	1650	99%	0.0	97.71%
4	MK966035.1	<i>Trichoderma</i> sp. isolate TRICHO2	1635	1635	94%	0.0	99.23%
5	XM_014085582.1	<i>Trichoderma atroviride</i> IMI 206040	1633	1633	99%	0.0	97.39%

#### 4.5.3. Sequence Alignments of the Fungal Isolates:

The consensus sequences obtained for a particular isolate were arranged and .txt file were prepared. All these sequences were then loaded in the CLUSTAL OMEGA software and aligned using software MEGA 11. The sequences aligned and the alignment indicated the places with similarities and point nucleotide differences. The analysis of DNA sequences was conducted by Neighbour-joining to assess topology with MEGA11. The species identification and homology between the sequences was identified using BLAST method. The phylogenetic tree was developed using Neighbour-Joining (NJ) method which was tested with Kimura 2-parameter for evolutionary distances in MEGA11. Pairwise distance, transitional/transversional substitutions, and the maximum likelihood substitution matrix were estimated using MEGA 11 software (Table15). Also phylogenetic trees were constructed for each isolate.

**Table 15: Estimates of Evolutionary Divergence between Sequences**

The numbers of base substitutions per site from between sequences are shown. Standard error estimate (s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model [1]. This analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1860 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]

	NFCCI_5021(Tmnrj)_Trichoderma_harzianum	MT435114.1_Trichoderma_lixii_culture_MUT_I TA :3171	XM_024912186.1_Trichoderma_harzianum_CB S 226.95	MT708571.1_Trichoderma_sp_REC-2020a isolate T154	KU933430.1_Trichoderma_afroharzianum_strain ATCC 20847	NFCCI_5020(Tas)_Trichoderma_asperellum	NFCCI_5022(Tbk)_Trichoderma_asperellum	XM_024901686.1_Trichoderma_asperellum_CB S 433.97	MK966035.1_Trichoderma_sp_isolate_TRICH O2	XM_014085582.1_Trichoderma_atroviride_IMI_206040	XM_024548974.1_Trichoderma_gamsii	KJ634761.1_Trichoderma_koningiopsis_isolate 7819
NFCCI_5021(Tmnrj)_Trichoderma_harzianum		0.0045226855	0.0045226855	0.0036620630	0.0036235019	0.0137226173	0.0136744161	0.0136613783	0.0146499155	0.0152355500	0.0152483306	0.0147028063
MT435114.1_Trichoderma_lixii_culture_MUT_I TA :3171	0.0147681706		0.0000000000	0.0026924713	0.0026116280	0.0144098554	0.0143399614	0.0136268903	0.0152278405	0.0183110664	0.0150021329	0.0141738688
XM_024912186.1_Trichoderma_harzianum_CB S 226.95	0.0147681706	0.0000000000		0.0026862427	0.0026116280	0.0144098554	0.0143399614	0.0553983721	0.0152278405	0.0525050270	0.0181408583	0.0141738688
MT708571.1_Trichoderma_sp_REC-2020a isolate T154	0.0136965771	0.0082613292	0.0082427561		0.0009916603	0.0137789136	0.0137194752	0.0131827075	0.0144458125	0.0192022957	0.0153869270	0.0140667426
KU933430.1_Trichoderma_afroharzianum_strain ATCC 20847	0.0127135756	0.0062288493	0.0062288493	0.0010337655		0.0132946010	0.0134571745	0.0133736716	0.0143884163	0.0138000686	0.0144497785	0.0140316064
NFCCI_5020(Tas)_Trichoderma_asperellum	0.0673360136	0.0704526000	0.0704526000	0.0692428516	0.0665743260		0.0000000000	0.0010836191	0.0029791969	0.0075025003	0.0065758062	0.0056277516
NFCCI_5022(Tbk)_Trichoderma_asperellum	0.0671098504	0.0704183672	0.0704183672	0.0692092655	0.0675306860	0.0000000000		0.0000000000	0.0029791969	0.0074426114	0.0064312718	0.0056277516
XM_024901686.1_Trichoderma_asperellum_CB S 433.97	0.0670348002	0.0719637031	0.3083139314	0.0705529040	0.0674961735	0.0010406775	0.0000000000		0.0029791969	0.0383535578	0.0093418216	0.0056277516
MK966035.1_Trichoderma_sp_isolate_TRICH O2	0.0699117682	0.0724095833	0.0724095833	0.0711228957	0.0698757840	0.0077793134	0.0077793134	0.0077793134		0.0080507285	0.0068830126	0.0067320853
XM_014085582.1_Trichoderma_atroviride_IMI_206040	0.0743409683	0.0983380093	0.2919008511	0.1035334054	0.0688708648	0.0288182055	0.0277164589	0.2103824797	0.0318381711		0.0146127188	0.0047505711
XM_024548974.1_Trichoderma_gamsii	0.0768248521	0.0821258491	0.1002162162	0.0843414544	0.0748331207	0.0255870950	0.0244905959	0.0443652358	0.0260102878	0.0724724782		0.0046045646
KJ634761.1_Trichoderma_koningiopsis_isolate 7819	0.0671889075	0.0647278580	0.0647278580	0.0658866312	0.0652145589	0.0206409492	0.0206409492	0.0206409492	0.0252830618	0.0151556474	0.0151742487	



**Table 16: Nucleotide frequencies**

	<b>T (U)</b>	<b>C</b>	<b>A</b>	<b>G</b>	<b>TOTAL</b>
NFCCI5021 (Tmnrj) <i>Trichoderma harzianum</i>	21.1	33.6	20.4	24.9	959
MT435114.1 <i>Trichoderma lixiiculture</i> MUT ITA :3171	21	32.1	21.6	25.3	1350
XM024912186.1 <i>Trichodermaharzianum</i> CBS226.95	21.8	31	23.1	24	1817
MT708571.1 <i>Trichoderma</i> sp. REC-2020a isolate T154	21.2	31.9	21.6	25.3	1344
KU933430.1 <i>Trichoderma afroharzianum</i> strain ATCC20847	21.3	33.6	20.2	24.9	968
NFCCI5020 (Tas) <i>Trichoderma asperellum</i>	21	33.9	20.3	24.8	962
NFCCI5022 (Tbk) <i>Trichoderma asperellum</i>	21.1	33.9	20.1	24.9	962
XM024901686.1 <i>Trichoderma asperellum</i> CBS433.97	22	31	23	24.1	1860
MK966035.1 <i>Trichoderma</i> sp.isolate TRICHO2	21	33.6	20.4	25.1	906
XM014085582.1 <i>Trichoderma atroviride</i> IMI206040	22	30.9	23.1	24	1686
XM024548974.1 <i>Trichoderma gamsii</i>	21.2	31.9	21.5	25.4	1353
KJ634761.1 <i>Trichoderma koningiopsis</i> isolate 7819	20.6	33.9	20.3	25.1	940
Avg.	21.4	32.3	21.6	24.7	1258.9

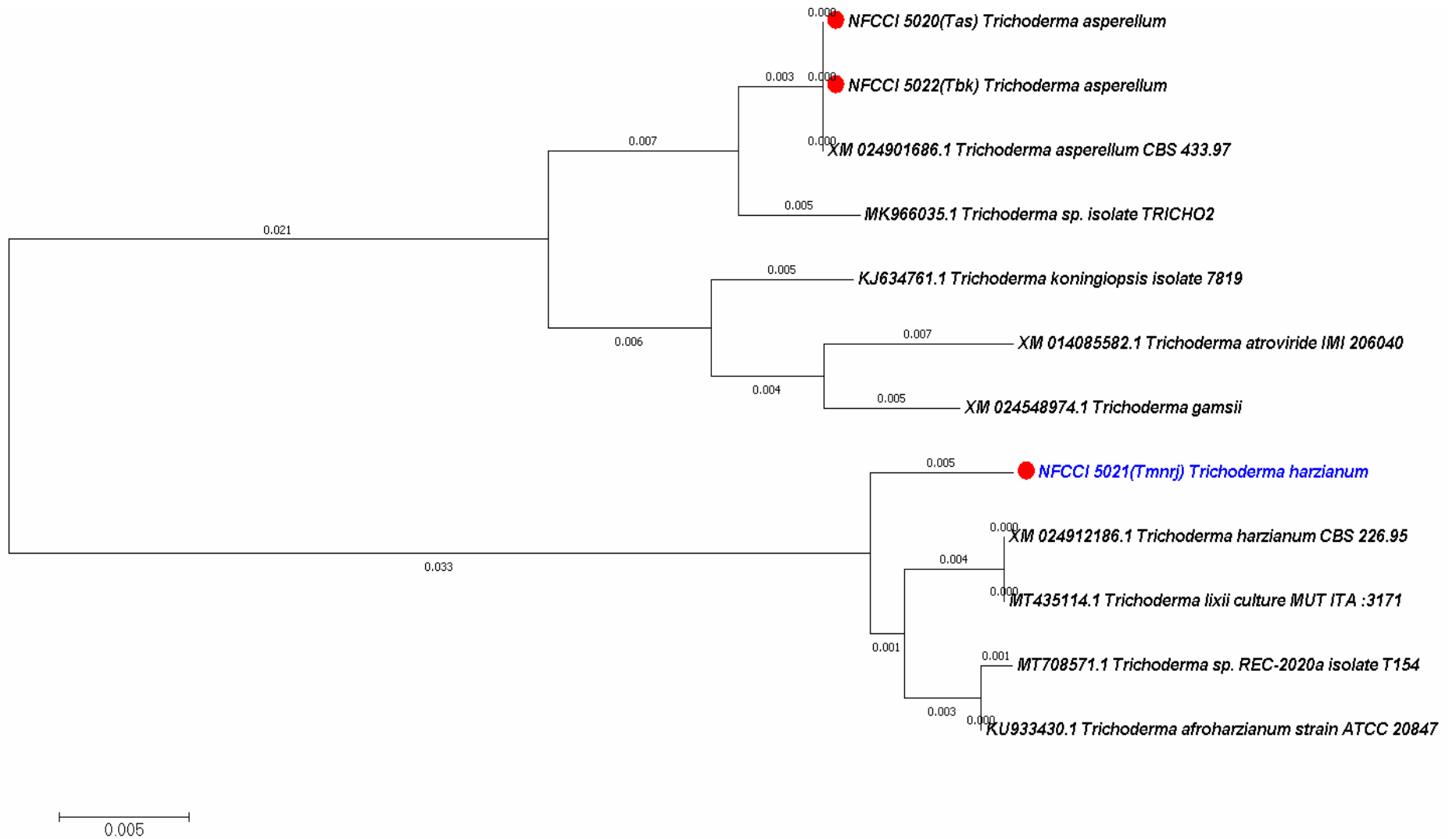
#### 4.5.4. Phylogenetic analysis

As per the Phylogenetic representation which was done using Neighbour Joining Tree method the isolates NFCCI 5020 (Tas) *Trichoderma asperellum* and NFCCI 5022 (Tbk) *T. asperellum* were found to be very closely related as they were seen on the same upper branch of the phylogenetic tree and isolate NFCCI 5021 (Tmnrj) *Trichoderma hazianum* is distinctly related to the other two isolates i. e. NFCCI 5020 (Tas) *T. asperellum* and NFCCI5022 (Tbk) *T. asperellum* as they are found to be on the two different branches of the phylogenetic tree.

Cluster	Sub cluster	Sub-sub cluster	Number of Isolates/ Samples	Isolates/Samples
I	IA	IA (a)	3	NFCCI 5020 (Tas) <i>Trichoderma asperellum</i> ,NFCCI 5022 (Tbk) <i>Trichoderma asperellum</i> , XM024901686.1 <i>Trichoderma asperellum</i> CBS433.97
		IA (b)	1	MK966035.1 <i>Trichoderma sp.</i> isolate TRICHO2
	IB	IB (a)	1	KJ634761.1 <i>Trichoderma koningiopsis</i> . Isolate 7819
		IB (b)	2	XM014085582.1 <i>Trichoderma atroviride</i> IMI206040, XM 024548974.1 <i>Trichoderma gamsii</i>
	II	IIA		1
IIB		IIB (a)	2	XM 024912186.1 <i>Trichoderma hazianum</i> CBS226.95, MT435114.1 <i>Trichoderma lixii</i> culture MUT ITA:3171
		IIB (b)	2	MT708571.1 <i>Trichoderma sp. REC-2020a isolate T154</i> , KU9334 30.1 <i>Trichoderma afroharzianum</i> strain ATCC20847

- ❖ The major cluster-I comprised 7 fungal isolates and nearby relatives, and was further found to be divided into two sub clusters (IA and IB).
- Sub Cluster IA was further sub divided into two sub-sub clusters [IA (a) and IA (b)].
- Sub-sub cluster IA (a) included of three fungal isolates and nearby relatives i.e.

**Fig. 8: Neighbor joining Phylogenetic Tree of samples sequenc**



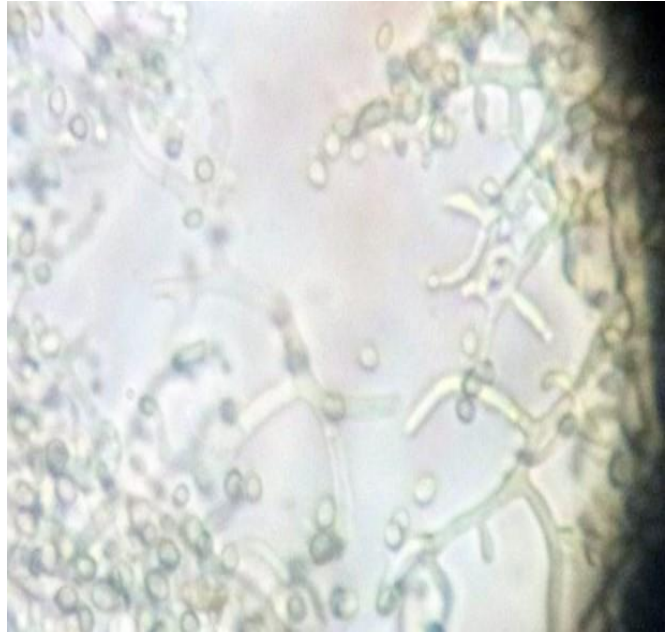
NFCCI 5020 (Tas) *T. asperellum*, NFCCI 5022 (Tbk) *T. asperellum*, XM 024901686.1 *T. asperellum* CBS 433.97.

- Sub-sub cluster IA (b) consisted of one nearby relative i.e. MK966035.1 *Trichoderma sp.* isolate TRICHO2.
- Sub Cluster IB was further sub divided into two sub-sub clusters [IB (a) and IB (b)].
- Sub-sub cluster IB (a) consisted of one nearby relative i.e. KJ634761.1 *T. koningiopsis*. Isolate 7819.
- Sub-sub cluster IB (b) consisted of two fungal isolates and nearby relative's i.e. XM014085582.1 *T. atroviride* IMI 206040, XM 024548974.1 *T. gamsii*.
- ❖ The major cluster-II comprised 5 fungal isolates and nearby relatives, and was further found to be divided into two sub clusters (IIA and IIB).
- Sub Cluster IIA included one fungal isolate i.e. NFCCI 5021 (Tmrj) *T. hazianum*.
- Sub Cluster IIB was further sub divided into two sub-sub clusters [IIB (a) and IIB (b)].
- Sub Cluster IIB (a) included two nearby relatives i.e. XM 024912186.1 *T. hazianum* CBS226.95, MT435114.1 *T. lixii* culture MUTITA:3171.
- Sub Cluster IIB (b) included two nearby relatives i.e. MT 708571.1 *Trichoderma sp.* REC-2020a isolate T154, KU933430.1 *T. afroharzianum* strain ATCC20847.

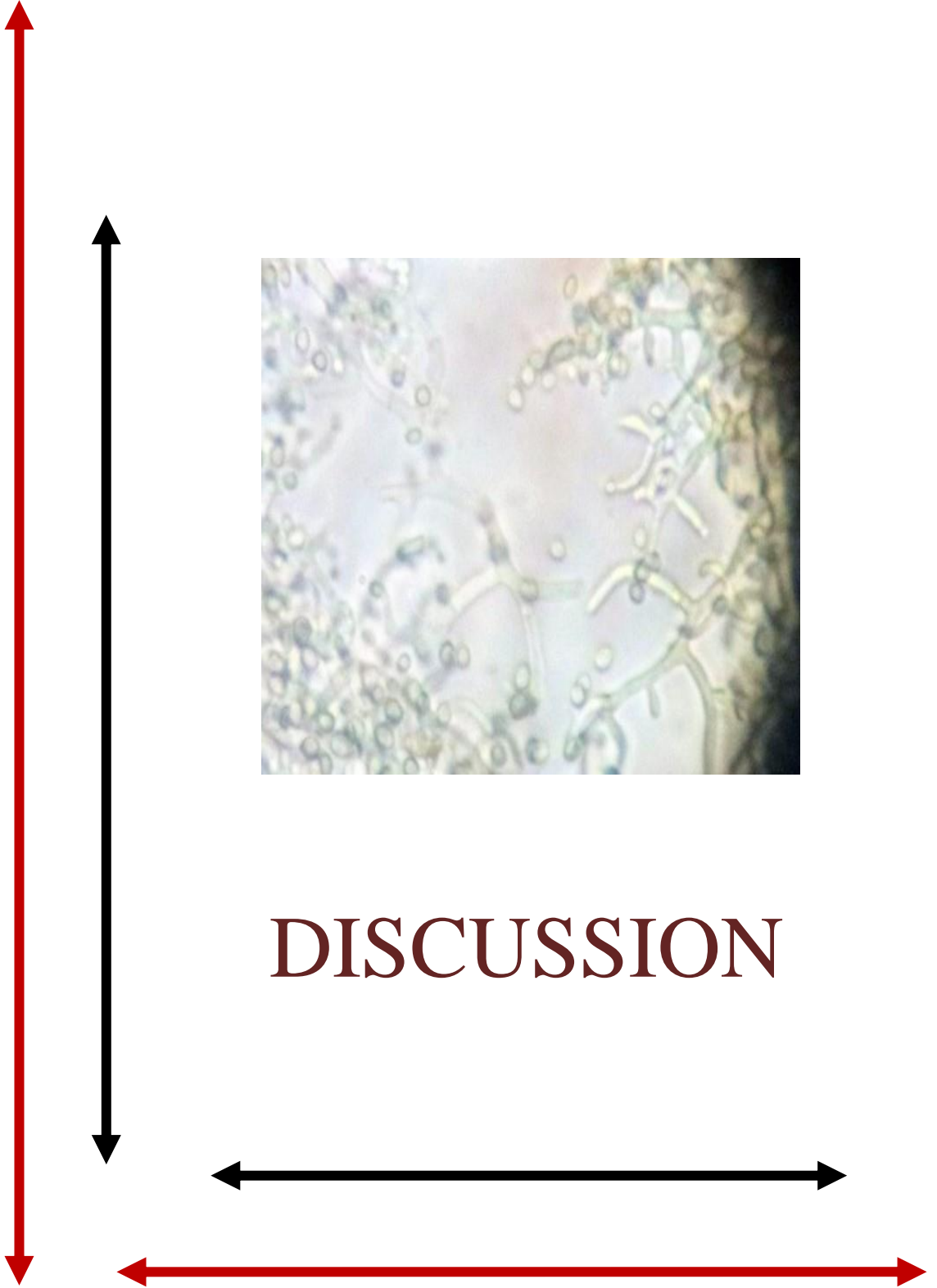
The sequences obtained are deposited at NCBI (<https://www.ncbi.nlm.nih.gov/>) as details below.

**Table 17: Provided Gene Bank accession numbers for nucleotide sequences:**

<b>Sr. No</b>	<b>Isolate code</b>	<b>Identification</b>	<b>NCBI Accession number</b>
1.	Tas	<i>Trichoderma asperellum</i> Samuels, Lieckf. & Nirenberg	BankIt2546015 NFCCI_5020 OM471989
2.	Tmnrj	<i>Trichoderma harzianum</i> Rifai	BankIt2546015 NFCCI_5021 OM471990
3.	Tbk	<i>Trichoderma asperellum</i> Samuels, Lieckf. & Nirenberg	BankIt2546015 NFCCI_5022 OM471991



# DISCUSSION



## CHAPTER-V

### DISCUSSION

It is a well-established fact that among the bio-control agents exploited so far, the members of the genus *Trichoderma* are unique due to their broad spectrum antagonistic potential. In addition to this, they act as an impressive mediator in plant soil relationship. Resultantly the plant health is benefitted many fold. However, it has been experienced by the cultivators that the bio-control agents isolated from one region are not always effective in different soil and climatic conditions in the other region. Under such situation, the present study was a genuine effort to tap the prospective, unexplored *Trichoderma* spp. from *Konkan* region. Out of the 67 soil samples collected from the five districts-Palghar, Thane, Raigad, Ratnagiri, and Sindhudurg of *Konkan*, 27 *Trichoderma* isolates were obtained, which were purified and studied. The samples were collected from the rhizosphere of common perennial, vegetable and other crops in the region such as, Mango, Rice, Cashew-nut, Coconut, Areca-nut, Guava, Sapota, Banana, Groundnut, Horse-gram, Lablab bean, Chilli, Brinjal, Bottle-gourd, Cabbage, Cauliflower, Elephant Foot Yam and a fragrant flowering plant Champaka.

Many workers have isolated the *Trichoderma* from rhizospheric soil of different crops and also from other samples. Pandey and Upadhyay (2000) isolated *T. harzianum* from pigeonpea rhizosphere, Rahman *et al.* (2011) isolated *T. harzianum* (IMI-392432, 392433, 392434); *T. pseudokoningii* (IMI-392431) and *T. Virens* (IMI-392430) from soil, humus, kitchen waste and compost. Kumar *et al.* (2011), obtained twelve isolates of *Trichoderma* spp. from cinnamon, black pepper, clove and nutmeg. Ranganathaswamy *et al.* (2012) isolated two isolates of *Trichoderma* spp., namely *T. virens* (TV9) and *T. harzianum* (Th4), from citrus and cotton rhizosphere. Bharti *et al.* (2016) isolated *T. harzianum* and *T. viride* from mustard leaf, Kannangara *et al.* (2017) isolated ten *Trichoderma* isolates from soil, litter and coir samples out of which, five were *T. harzianum*, four were *T. viride* and one was *T. polysporum*. Sekhar *et al.* (2017) isolated ten isolates of *Trichoderma* spp from rhizospheric soil samples in groundnut field. Soesanto (2018) isolated *Trichoderma* spp. from rhizosphere of ginger, banana, pineapple and shallot. Ashlesha (2019) isolated two isolates of *T. harzianum* from rhizosphere of maize crop cultivated in sandy loam soils. Kumar *et al* (2019) isolated *T. harzianum*; *T. asperellum*; *T. viride*; *T. longibrachiatum*; *T. koningii*; *T. virens* and *T.*

*atroviride* from collected composite soil samples from chick pea, pigeon pea and lentil rhizosphere. Lalngaihawmi and Bhattacharyya (2019) isolated *Trichoderma* from rhizosphere of different banana cultivars while Naher *et al* (2019) isolated *T. harziaum*, *T. viride*, *T.koningii*, *T. asperrelum*, and *T. parareesei* from rhizosphere soils of paddy, banana, oil palm, rubber, vegetables and grass land soils. Yadav *et al*. (2020) isolated 21 *Trichoderma* isolates from banana rhizosphere of wilt suppressive and salt affected soils. The results of present study are in concurrence with the findings of earlier research workers. This confirms that the members of the genus *Trichoderma* can thrive in all types of soils and climatic conditions.

As per morphological identification Tkor (T<sub>5</sub>), Tgpal (T<sub>14</sub>), Tbpal (T<sub>24</sub>) were identified as *Trichoderma koningii* Oudem and Tckr (T<sub>25</sub>) was identified as *T. longibrachiatum* Rifai.

The results of molecular tests identified Tas (T<sub>11</sub>) and Tbk (T<sub>23</sub>) as *T. asperellum* Samuels, Lieckf. & Nirenberg and Tmnrj (T<sub>3</sub>) as *T. harzianum* Rifai.

*T. asperellum* (T<sub>11</sub>) was the most effective against *Fusarium* (82.22 % inhibition), *T. koningii* (T<sub>14</sub>) against *Rhizoctonia* (81.11%), *T.asperellum* (T<sub>23</sub>) against *Sclerotium* (86.11%), *T. koningii* (T<sub>5</sub>) against *Colletotrichum* (81.33%), *T. longibrachiatum* (T<sub>25</sub>) against *Alternaria* (86.66%). The isolate *T. harzianum* (T<sub>3</sub>) ranked second in antagonism against *Sclerotium* (80.54%), third in control of *Fusarium* (79.22%) and *Colletotrichum* (80.22%) and fifth in *Rhizoctonia* (71.11%) and *Alternaria* (66.11%).

*T. asperellum* (T<sub>11</sub>) isolate recorded 82.22 per cent inhibition of *Fusarium* but the isolate *T. asperellum* (T<sub>23</sub>) recorded only 77.77 per cent inhibition against the same pathogen. This difference in the performance of two isolates may be attributed to the difference in the two strains of the same fungus. Komy *et al*. (2015) screened 30 isolates of *T. asperellum* against *F. oxysporum* causing wilt of tomato and reported that 6 isolates recorded the highest inhibition of the pathogen which ranged between 68 and 71 per cent. Among remaining isolates most of the isolates recorded moderate inhibition (61-65%) and seven isolates recorded minimum inhibition (32-36%). The results of present study are in agreement with these results. Naher *et al*. (2019) recorded 74.16 per cent inhibition by *T. asperellum* against *F. oxysporum*. Rai and Maurya (2021) evaluated local strains of *T. asperellum* against *F. oxysporum* f.sp. *lycopersici* and recorded 73.91 per cent inhibition and 64.49 per cent inhibition against *F. oxysporum* f.sp. *cubense*. An



isolate of *T. asperellum* caused 69.50% inhibition of *Rhizoctonia* spp. (Restrepo *et al.* 2022). Asad *et al.* (2014) recorded 74.40 per cent inhibition of *R. solani* by *T. asperellum* after 72 hrs of inoculation. These results are in accordance with the results of present study and this suggests that *T. asperellum* is very effective against *Fusarium* species.

Among the 11 isolates of *T. asperellum* tested by Sharma and Prasad (2018) against *S. sclerotiorum* the isolate T21 recorded 93 per cent inhibition of the pathogen while the same isolate recorded 85.18 % inhibition of *Colletotrichum asianum* causing anthracnose of *Tabernaemontana divericata*.

The two isolates *T. asperellum* obtained in the current research (T<sub>23</sub> and T<sub>11</sub>) were very effective against *C. gloeosporioides* as they recorded 77.77 and 71.11 per cent inhibition respectively. Even though most of the workers have reported more than 50 per cent inhibition potential of *T. asperellum* against many pathogens, Cruz Quiroz *et al.* (2018) reported 14.971 and 22.50 per cent inhibition against of *C. gloeosporioides*. These results are contradictory to the present findings.

*T. asperellum* isolates were very effective against *Alternaria* spp. as the isolate T<sub>11</sub> recorded 75.55 per cent inhibition of *Alternaria* spp. while T<sub>23</sub> recorded 69.66 per cent inhibition of the same pathogen. The results of Pradeep *et al.* (2022) are in congruence with these findings as the reported 73.33per cent inhibition of *A. alternata* with an isolate of *T. asperellum*. But Reddy *et al.* (2018) reported that T4 (*T. asperellum* isolate) was 35.50% effective against the same pathogen. . Their results differ with the present findings.

Among the four morphologically identified isolates three isolates (T<sub>5</sub>, T<sub>14</sub> and T<sub>24</sub>) were of *T. koningii*. The isolate T<sub>14</sub> was superior to other isolates against *Fusarium* spp. (81.33% inhibition) and *Rhizoctonia* (81.11%). The isolate T<sub>5</sub> was superior in case of *Sclerotium* (71.66%) and *Alternaria* (66.44%). It was at par with T<sub>14</sub> (81.33 %) in controlling *Colletotrichum*. The remaining isolate T<sub>24</sub> was inferior two former two isolates in case of all the pathogens under study. Mamtha and Yashoda (2006) recorded 77.43 per cent inhibition of *Colletotrichum* by *T. koningii*. Moreover against *A. alternata* the inhibition was and 80.00 per cent. Honmane (2007) recorded 83.33 per cent inhibition of *F. moniliforme* and 80.74 per cent inhibition of *C. gloeosporioides* with *T. koningii*. As per the results of Febrilia *et al.* (2013), *T. koningii* recorded 83 per cent inhibition *C. gloeosporioides*. These results are in conformity with present findings.

Farah and Nasreen (2013) recorded 32.14, 79.45, 85.32 and 91.09 per cent inhibition of *F. oxysporum*, *A. solani*, *F. solani* and *R. solani* with *T. koningii*. These results are contradictory to present findings in case of *Fusarium*. Bhale and Rajkonda (2015) observed about 50 per cent inhibition of *R. solani* and *F. oxysporum* also 75 per cent reduction in the growth of *A. alternata*. Musheer and Ashraf (2017) noted that *T. koningii* caused 52.46 per cent inhibition of *C. gloeosporioides*.

Rajkonda and Bhale (2018) tested the antagonism of *T. koningii* against the *A. alternata*, *A. tenuissima*, *R. solani*, *F. oxysporum f. sp. spinaceae* and *F. proliferatum*. Inhibition was 61.71 per cent of all the five pathogens.

The results of Reddy *et al.* (2018) suggest that, *T. koningii* was not very effective against *A. alternata* and showed only 24.99 per cent inhibition

Naher *et al.* (2019) recorded 71.40 per cent inhibition of *F. oxysporum* by an isolate of *T. koningii*.

During the present investigation only one isolate of *T. harzianum* was obtained. It caused 80.54 per cent inhibition in the mycelial growth of *Sclerotium* spp. The results of Jana and Mandal (2017) indicated that the three isolates of *T. harzianum* viz. T3, T4 and T11 recorded 52.17, 48.91 and 46.20 per cent inhibition of *S. rolfsii*. It indicates that even though all the isolates were identified as *T. harzianum* their antagonism potential varies depending upon the ability of the isolate to secrete metabolites which are detrimental to the pathogens. So, the antagonistic potential is a genetic character and therefore different isolates of the same bio-control agent perform differently against the same pathogen. *T. harzianum* isolates used by Kushwaha *et al.* (2018) recorded 63.60 per cent inhibition of *S. rolfsii* while Singh *et al.* (2018) recorded 50.67 per cent inhibition of the same pathogen causing collar rot of chickpea. *T. harzianum* isolate TspT recorded 81.27 per cent inhibition of *S. rolfsii*, (Priyadharcini *et al.* 2018). Amin *et al.* (2010) reported that, Th-1 and Th-2 isolates of *T. harzianum* recorded 75.92 and 71.26 per cent inhibition while Kumar *et al.* (2011) recorded 80 per cent and 72.1 per cent inhibition of *S. rolfsii* by TWN1 and TWC2 isolates of *T. harzianum*

Goudar and Kulkarni (2000) recorded 85.40 per cent inhibition of *F. udum* by *T. harzianum*. Jat and Agalave (2013) recorded 47.50 and 50.00 per cent inhibition of *T. harzianum* isolate against *F. oxysporum* and *F. Moniliforme* and 48.33 per cent against *A.alternata*. Elshahawy (2016) noticed that the three isolates of *T. harzianum* (Th1, Th2

and Th3) recorded 59.2, 66.7 and 61.5 per cent inhibition of *F. solani* and 58.2, 52.2 and 56.3 of *F. oxysporum* sequentially. It also observed 38.2, 42.6 and 48.2 per cent inhibition of *R. solani* by these isolates. Sangle and Bambawale (2004) recorded 79.54 per cent inhibition of *F. oxysporum f. sp sesame*. Yadav *et al.* (2005) recorded 62.5 per cent inhibition against *F. udum* with same antagonist. Honmane (2007) recorded 86.85 per cent inhibition against *F. moniliforme*. Honmane (2007) recorded 75.19 per cent inhibition of *C. gloeosporioids* by *T. harzianum*. Raul (2007) recorded 86.11 per cent against the same pathogen while Tapwal *et al.* (2015) recorded the least inhibition *i.e* 15.00 per cent of *C. gloeosporioids*. There are very few reports wherein such a low inhibition of the mycelial growth has been recorded.

Amin *et al.* (2010) reported that isolate Th1 was capable to inhibit the growth of *R. solani* by 60.51 per cent and in another research recorded that 77.81 and 70.25 per cent inhibition by Th-1 and Th-2. Tapwal *et al.* (2015) reported that *T. harzianum* was least effective against *R. solani* (5.10 % inhibition) but it performed moderately against *A. altarnata* (34.20% inhibition).

Many researchers have reported the effective antagonism of *T. longibrachiatum* against different fungal pathogens.

*T. longibrachiatum* was effective against *S. rolfsii* (Shaigan *et al.* 2008; Shewarega *et al.* 2019). It was also effective against *Fusarium*, *Rhizoctonia* (Shewarega *et al.* 2019); *Colletotrichum* (Quiroz *et al.*, 2018) and *Alternaria* (Elyours *et al.* 2013; Prabhakaran *et al.* 2015; and Reddy *et al.* 2018)

The microscopic examination revealed morphological variations in the isolates. As per morphological identification Tkor (T<sub>5</sub>), Tgpal (T<sub>14</sub>), Tbpal (T<sub>24</sub>) were identified as *Trichoderma koningii* Oudem and Tckr (T<sub>25</sub>) was identified as *T. longibrachiatum* Rifai.

The results of molecular tests identified Tas (T<sub>11</sub>) and Tbk (T<sub>23</sub>) as *T. asperellum* Samuels, Lieckf. &Nirenberg while Tmnrj (T<sub>3</sub>) as *T. harzianum* Rifai.

In research findings it was observed that hyphal growth of 7 isolates *viz.*, Tgpal, and Tbpal spread out in form of tree branches fashion while it was penicillate in rest of the isolates *viz.*, Tmnrj, Tkor, Tas and Tckr. The shape of phialides was ampuliform and or lageniform *viz.*, Tmnrj, Tkor, Tas and Tckr in remaining isolate *i.e.*, Tbpal had

nine-pin shape, at last Tgpal showed sausage shaped. Conidia includes category of shapes like sub-globose (Tmed, Tamsakh, Tcbfn, Tbgu and Tcaupal), globose to sub-globose (Tmnrj, Tkorrr, Trm, Tcojrr2, Tas, Tbw, Tgpal, Tsptpal, Tbk, Tbpal and Tckr) and globose (Tojrr2, Tcwki, Tcnv, Thm, Tlbhar, Tgkh2020s, Tefym, Tchal, Tchpal and Tmcpal). Conidia arrangement mostly found catenate.

In the present study, three isolates (T<sub>5</sub>, T<sub>14</sub> and T<sub>24</sub>) were identified as *Trichoderma koningii*. The colony colour of all three isolates was yellowish to blue green. The shape of phialides sequentially was lageniform, sausage shaped and ninepin bowling shaped. The hyphae of T<sub>5</sub> were penicillate while those of the other two isolates were spread out in tree branches fashion. Rest all the characters were at similitude. Conidia were globose and the overall spore diameter of the three isolates ranged from 47.18 to 53.31µm.

Sekhar *et al.* (2017) observed that the colony colour of *T. koningii* isolates varied between pale yellowish, dull green to bluish green. Conidiophores were broad, frequently branching, phialides were lageniform, divergent, terminal phialide more elongated and Conidia were sub cylindrical to narrow ellipsoidal. These results are in agreement with present findings.

Kumar *et al.* (2019) reported that, *T. koningii* colony was whitish, conidiophores were much branched, phialides were narrow at the base, alternate to conical apices, arises singly and laterally and appears nine-pin bowling shaped. These results are in conformity with current results.

Naher *et al.* (2019) stated that, the colony of *T. koningii* blue green to yellowish green, phialids were also lageniform, while conidiophores were branched and erect. These results also concur with present findings.

The colony colour of the two *T. asperellum* isolates (T<sub>11</sub> and T<sub>23</sub>) was dark green. The phialides were formed solitarily or in whorls, ampuliform to lageniform and conidia were globose and catenate.

Grace (2016) observed that the conidiophores of *T. asperellum* form paired primary branches which were nearly at 90<sup>0</sup> to the main axis. The phialids formed in whorls were normally flask shaped. The conclusions of this study are in congruence with present findings.

Kumar *et al.* (2019) reported that the colony of *T. asperellum* was yellowish green, conidiophores were highly branched and phialides aroused singly or in opposite pairs along the branches, phialides were ninepin shape attenuated into long neck, conidia were globose or short obovoid in shape. The results of these researchers are at in conformity with present results.

Colony of *T. asperellum* was dark green to white, conidiophores were symmetrically paired branched, the phialides were solitary or held in whorls of two to three, conidia were globose, obovoid, dark green and smooth (Naher *et al.* 2019)

Rai and Maurya (2021) observed *T. asperellum* colony was dull green and fast growing, conidiophores produce abundantly, intercalary wall thick, phialides were ampulliform 2-3 in groups conidia were globose to oval to cylindrical.

Only one isolate in present study was identified as *T. harzianum*. Colony colour was dark green, phialides ampuliform, in whorls at 90<sup>0</sup> to conidiophore. Hyphae penicillate and conidia were sub-globose.

Grace (2016) reported that in case of *T. harzianum* pairing of branches and formation of phialides in whorls is similar to *T. asperellum* but the phialide are short and inflated. This finding is contradictory to present results.

Kumar and Sharma (2016) studied morphological characters of *T. harzianum* isolates. Colony colour was green to dark green, conidiophores profusely or moderately branched, regular to irregular, phialide position in whorls and solitary and shape was globose, nine-pin and sigmoid or hooked. These results differ in terms of shape of phialides.

In case of *T. harzianum* colony colour was dull yellowish, conidiophore were frequent branching and verticillate. Phialides were ampuliform convergent while conidia were sub globose to obovoid (Sekhar *et al.* 2017). These results are in concurrence with present findings.

Kumar *et al.* (2019) showed least similar results, in isolate, *T. harzianum* colony was white to light green in colour, conidiophores were highly branched and forming loose tufts, where phialides were short-skittle shaped, bulged in the centre and narrowed at the lower end and admeasured 7.2–11.2 × 2.5–3.1 µm. Phialospores were subglobose to ovoid with truncate base.

Naher *et al.* (2019) observed that, the colonies of *T. harzianum* were initially white and gradually changed to yellowish green and finally dark green; conidiophores were formed in pairs with lateral branches at a right angle to the main axis, here phialids were typically elongated and lageniform also conidia were subglobose to globose.

The findings of most of the earlier workers are in concurrence with the morphological characters of *T. harzianum* isolated and identified in present study.

In case of *T. longibrachiatum* colony was yellowish green to lily green, conidiophores were arise from substratum and form irregular tufts or arise from areal hyphae, phialides arises singly or in verticils of 2-3, seen lageniform, apex broadly rounded, size ranges conidia were obovoid to ellipsoidal, dilute green, apex broadly rounded (Kumar *et al.* 2019). Except conidial size all the other morphological features of *T. longibrachiatum* are at similitude with present results.

*In vitro* evaluation of fungicides studies revealed that amongst the systemic fungicides, carbendazim was the most detrimental at 1000 ppm for all the isolates, as it entirely inhibited the mycelial growth of these isolates followed by Hexaconazole which absolutely inhibited the mycelium of the isolate T<sub>14</sub> at 500 ppm. The growth inhibition by this fungicide in case of T<sub>5</sub> (*T. koningii*) was 90 per cent followed by T<sub>24</sub> (*T. koningii*) (87.77%), T<sub>11</sub> (86.11%), T<sub>23</sub> (85.77%), T<sub>25</sub> (*T. longibrachiatum*) (84.11%) and up to T<sub>3</sub> (*T. harzianum.*) (63.33%). The last systemic fungicide *i.e.* Thiophanate methyl caused maximum inhibition (86.33) of T<sub>23</sub> at 500 ppm, followed by T<sub>14</sub> (*T. koningii*-80.22%), T<sub>25</sub> (*T. longibrachiatum*) (80.00%), T<sub>5</sub> (79.77%), T<sub>24</sub> (75.77%), T<sub>3</sub> (*T. harzianum.*) (68.33%) and T<sub>11</sub> (58.00%).

Rest of the three contact fungicides, Sulphur at 2500 ppm was the most compatible fungicide as the highest inhibition recorded by T<sub>5</sub> (23.11%), which was followed by T<sub>23</sub> (20.00%), T<sub>25</sub> (*T. longibrachiatum*) (9.60%), T<sub>24</sub> (5.88%), T<sub>14</sub> (4.22%), T<sub>11</sub> (3.66%) and the least inhibition observed in T<sub>3</sub> (*T. harzianum.*) (0.33%). It was observed that at a slightly lower concentration the fungicide may not be inhibitory to the test isolates.

All the seven isolates under study were the most compatible with Sulphur and to some extent with Copper oxychloride.

Copper oxychloride recorded the slightest inhibition of (43.66 %) of T<sub>11</sub> at 2500 ppm and the maximum (84.44%) of T<sub>5</sub> although Mancozeb recorded the slightest inhibition (59.77%) of T<sub>11</sub> at 2500 ppm and the highest (84.22%) of T<sub>5</sub>.

Bhat and Srivastava (2003) revealed that the Triazole group fungicide Hexaconazole was detrimental to *T. harzianum* strain used in their study.

Islam *et al.* (2008) found that the growth of *Trichoderma* was very much inhibited in presence of Carbendazim 50 WP whereas, normal growth was observed in medium containing Copper oxychloride.

Madhavi *et al.* (2008) tested the compatibility of a mutant of *T. harzianum* (ThM<sub>1</sub>) with Carbendazim (0.1%). The results indicated that the mutant was fairly compatible with Carbendazim but Mancozeb (0.25) was found inhibitory.

The findings of Sarkar *et al.* (2010) revealed that, Hexaconazole recorded cent per cent inhibition of *T. harzianum* at 10 ppm and above concentrations while Copper oxychloride was tolerable upto 100 ppm concentration.

Ranganathaswamy *et al.* (2012) assessed the compatibility of *T. harzianum* with fungicides and concluded that sulphur and Mancozeb were less toxic.

Saxena *et al.* (2014) stated that *T. harzianum* strain PBT23 was compatible with mancozeb up to 250 ppm.

Bhale and Rajkonda (2015) checked the compatibility of *T. harzianum* and *T. koningii* with Mancozeb at 8 different concentrations and revealed that the growth of *T. harzianum* was satisfactory up to 3000 ppm whilst that of *T. koningii* up to 1000 ppm.

The results of the research revealed by Sharma *et al.* (2016) that *T. harzianum* strain TCMS-14 was exceptionally compatible with Sulphur at 2500 ppm where as it was compatible with Mancozeb upto 625 ppm only.

Carbendazim was the most detrimental to *T. asperellum* at 100 ppm where as, Mancozeb with the same concentration recorded the least (23.30 %) mycelial inhibition (Kumar *et al.* 2017).

Mohamed and Radwan (2017) tested the compatibility of a local strain of *T. harzianum* with Copper oxychloride and Sulphur at seven concentrations such as 1, 5, 10, 50, 100, 500 and 1000 ppm. None of the concentration of these two fungicides was

inhibitory to the strain under study but Mancozeb exhibited suppression of mycelial growth at the lowest concentration whereas at total inhibition was recorded at 100ppm

Sonavane and Venkataravanappa (2017) assessed compatibility of a local strain of *T. harzianum* with contact fungicides COC, Sulphur and Mancozeb at 500, 1000, 1500 and 2000 ppm concentrations and systemic fungicides Carbendazim, Thiophanate methyl and Hexaconazole at 250,500, 750 and 1000 ppm concentration. The isolate was compatible with all the three contact fungicides at 2000 ppm. But it was not compatible at all with the three systemic fungicides.

All the three isolates of *T. harzianum* were incompatible with Carbendazim. Isolate Th1 was compatible with Mancozeb up to 600 pm and with Thiophanate methyl up to 500 ppm. The isolate Th2 was compatible with Mancozeb up to 500 and with Thiophanate methyl up to 700 ppm while Th3 was also compatible with Thiophanate methyl up to 700 ppm (Elshahawy *et al.* 2016). Similar results of Carbendazim were reported by Dwivedi and Vishunavat (2018) in case of *T. asperellum* and *T. harzianum*.

Most of the workers have reported that Carbendazim completely inhibits the growth of *Trichoderma* species (Kumar *et al.* 2019; Shashikumar *et al.* 2019; Shrivastava, 2019). Tomar *et al.* (2018) tested Mancozeb at selected concentration (25, 50, 75 and 100 ppm) for its compatibility with *T. harzianum* and observed that the Mancozeb was slightly inhibitory at 75 and 100 ppm (5.19% and 7.03 %, respectively) and Shashikumar *et al.* (2019) concluded that Mancozeb was the least inhibitory (1.48%) at 0.15% concentration.

Copper oxychloride at 2000ppm and Mancozeb at 2000 ppm was safer against *T. harzianum* (Bagwan 2010, Shrivastava, 2019; Maheshwary *et al.* 2020)

Khan and Shahzad (2015) checked the tolerance level of two species *viz.* *T. harzianum* and *T. longibrachiatum* against Topsin- M (thiophanate methyl) and carbendazim, at different concentrations (1, 10, 100, 1000 and 10,000 ppm) and reported that Topsin-M and carbendazim completely suppressed the growth of both species.

Kumar *et al.* (2017) revealed that all the four concentrations (10, 20, 40 and 80 ppm) of Hexaconazole 5% WP were totally detrimental to *T. asperellum*. Singh *et al.* (2021) also stated that *T. harzianum* is highly incompatible with the same fungicide.



Kumar *et al.* (2017) revealed that *T. asperellum* can tolerate Mancozeb up to 100 ppm but the higher concentrations became highly injurious.

The results of the studies revealed that Hexaconazole completely inhibited the growth of *Trichoderma* species (Kiran *et al.* 2018; Singh *et al.* 2021)

Shrivastava (2019) tested the compatibility of *T. harzianum* with Carbendazim, Thiophanate methyl, Mancozeb and wettable Sulphur at 500, 1000 and 1500 ppm concentrations and reported that the mycelial growth of the bio-agent was above 70 per cent in Mancozeb and wettable Sulphur while Carbendazim and Thiophanate methyl were detrimental. These results are contradictory to present findings in terms of Mancozeb.

Maheshwary *et al.* (2020) concluded that COC and Mancozeb at 500 ppm, favour the growth of *T. asperellum* but the higher concentrations (1000, 1500 and 2000) of both the fungicides were slightly injurious to fungus. Carbendazim was inhibitory at 5 ppm.

In the findings of Vyas *et al.* (2020) resulted that both COC and Carbendazim found equally hazardous to *T. harzianum*. These results are in disagreement with present conclusions in context with COC.

The 7 isolates selected for morphological characters and molecular characterization were superficially antagonistic to the phyto-pathogens used in the study. During the molecular identification of the promising fungal strains like Tas (T<sub>11</sub>), Tmnrj (T<sub>3</sub>) and Tbk (T<sub>23</sub>) genomic DNA was isolated in pure form first from the mother culture. Then the TEF- $\alpha$  gene was successfully amplified using primers EF-983F & EF-2118R. Here the sequencing PCR was set up with ABI-Big Dye® Terminator 3.1 Cycle Sequencing Kit. Later the raw sequence obtained from ABI 3100 automated DNA sequence was manually edited for inconsistency. At the end point the sequence data was aligned with publicly available sequences and analyzed to reach identity. Isolate T<sub>11</sub> showed 100 per cent homology match with *T. asperellum* and sequence analyses with NCBI accession number was XM\_024901686.1. Isolate T<sub>3</sub> strain showed 98.54 per cent homology match with *T. harzianum* when went with same identification protocol and sequence analyses with NCBI accession number was XM\_024912186.1, *Trichoderma harzianum* strain CBS 226.95 and T<sub>23</sub> identified with same procedure where homology match 100 per cent with *T. asperellum* and sequence analyses with NCBI accession number XM\_024901686.1, *T. asperellum* strain CBS 433.97. NCBI provided Gene Bank

accession numbers submitted for nucleotide sequences, of these identified isolates were NFCCI\_5020 OM471989, NFCCI\_5021 OM471990 and NFCCI\_5022 OM471991.

The chromatographic image analysis by high performance liquid chromatography with the help of UV detection of culture extracts were used for the identification of *Trichoderma* strains from water-damaged building materials or indoor dust (Thrane *et al.* 2001). The classes were compared with morphological identification and rDNA sequence data, and in the case of each class all strains had the same identity. With all those three techniques each strain except one was identified as the same species and belonged to *T. atroviride* (nine strains), *T. viride* (three strains), *T. harzianum* (10 strains), *T. citrinoviride* (12 strains), and *T. longibrachiatum* (nine strains). One of the odd strain was identified as *T. hamatum* by its morphology and rDNA sequencing, but not by image analysis as there was no reference strains of this species were added and finally concluded that the secondary metabolite profile contains sufficient information for future classification and species identification.

Abd-Elsalame *et al.* (2010) confirmed the identification of two species of *Trichoderma*, PCR-based markers with primer M13 (core sequence of phage M13) and internal-transcribed spacer sequences of ribosomal DNA were used. By using the TrichOKEY version 2.0 barcode programme and the multi loci likeness search database, TrichoBLAST concluded sequence identification. Sequences from the ribosomal DNA internal-transcribed spacer regions denoted limited variation among the species and due to this analysis the isolates were split into two groups. Grouping the isolates was mainly based on cluster analysis of their DNA profiles which matched the grouping based on morphological taxonomy. The Molecular data obtained from the analyses of gene sequences played a key role to distinguish phonetically cryptic species in this group as well as to establish phylogenetic relationships.

The characterization of seven isolates of *Trichoderma* using RAPD-PCR procedure was done by Siameto *et al.* (2011) to determine their genetic variability. It was observed that the Jacquard's coefficient of similarity ranged from 0.231 to 0.857 for isolates 055E, 011E, 010E and 015E. The four random primers (203, 230, 220 and 0p13) were used in the study, depicted the bands ranging from 350bp to 2000bp. All such intense bands produced summed up to 81.

The DNA profiles of *T. harzianum* isolates were scored and a dendrogram was developed using Squared Euclidean Distance and Clustering on the basis of Ward's

method. In the Dendrogram, all the isolates were patently divided into two major clusters A and B at 20 units. Isolate 051E and 029E covered the extremes of the entire Dendrogram. Genetic dissimilarity ranged from a minimum of 0.143 (between T010 and T015) to a maximum of 0.857 (between 055E and 051E). Isolate 051E, T011, T015, and T010 were assigned to cluster A. Genetic dissimilarity among the entries in this cluster ranged from a minimum of 14.3 per cent (between T015 and T010) to a maximum of 35.7 per cent (between T010 and 051E). The other cluster B comprised of three accessions and here cluster isolate 044E, 055E and 029E were grouped together. The genetic dissimilarity ranged from 33.3 per cent between 055E and 029E to a maximum of 75 per cent between 044E and 029E.

Oskiera *et al.* (2015), collected 104 strains of *Trichoderma* from geographically different locations in Poland and identified them by DNA barcoding, based upon the sequences of internal transcribed spacers 1 and 2 (ITS1 and 2) of the ribosomal RNA gene cluster and on the sequences of translation elongation factor 1 alpha (*tef1*), chitinase 18-5 (*chi18-5*), and RNA polymerase II subunit (*rpb2*) gene fragments. Most of the identified strains were classified as: *T. atroviride* (38%), *T. harzianum* (21%), *T. lentiforme* (9%), *T. virens* (9%), and *T. simmonsii* (6%). Single strains belonged to *T. atrobrunneum*, *T. citrinoviride*, *T. crassum*, *T. gamsii*, *T. hamatum*, *T. spirale*, *T. tomentosum*, and *T. viridescens*.

The study also revealed two strains (*T. pleuroticola* and *T. aggressivum* f. *europaeum*) that were pathogenic to cultivated mushrooms. Four strains *i.e.*, TRS4, TRS29, TRS33, and TRS73 were classified only up to species level as the molecular identification was inconclusive at the species level.

Prabhakaran *et al.* (2015) isolated different isolates of *Trichoderma* from soil samples collected from different region of India. The isolates were confirmed through Internal transcribed spacer (ITS) region analysis, by using the region of nuclear ribosomal DNA in phylogenetic analysis at generic and intra-generic levels. The isolates were identified as *T. asperellum* (Ta), *T. harzianum* (Th), *T. pseudokoningii* (Tp) and *T. longibrachiatum* (Tl).

Zhu *et al.* (2017) identified 287 isolates of *Trichoderma* by using morphological and molecular identification techniques. In molecular methods, mostly DNA sequencing and analysis of the 5.8S ribosomal DNA internal transcribed spacer region (ITS1-5.8S-

ITS2), part of the nuclear translation elongation factor gene (*TEF1- $\alpha$* ), and the second largest RNA polymerase II subunit (*RPB2*) were used.

Priyadharcini *et al.* (2018) isolated a *Trichoderma* species and confirmed as *T. harzianum* by using ITS 1 and ITS 4 primers and comparing ITS sequence of the isolate with BLAST sequence in NCBI data base.

In Molecular identification of *T. harzianum* isolate *Th* Azad/CSAU 6796, the observed Locus was KC800922. The isolate had 18S ribosomal RNA gene, partial sequence; internal transcribed spacer (ITS) 1, 5.8 S ribosomal RNA gene, and ITS4, partial sequence, primers used here ITS1-AGAGTTTGATCCTGGCTCAG and ITS4-GGTTACCTGTTACGACTT, sequence was 546 bp. On the other hand *T. asperellum* Tasp (CSAU)-8940 the observed locus was KC800921. The ribosomal RNA, partial sequence ITS were similar to *T. harzianum* and used were ITS1-TCCGTAGGTGAACCTGCGG and ITS2-TCCTCCGCTTATTGATATG including sequence 1200 bp. (Kumar *et al.* 2019).

Jankar *et al.* (2020), studied molecular variability among the six isolates of *T. viride* collected from different region of Maharashtra by using 16 RAPD primers of OPA (OPA 2, 3, 5, 6, 9, 10, 11, 14, 16 and 18) series. The observations of the study revealed that, 78 score able bands were formed out of 10 primers. Among these bands 76 bands were polymorphic and the level of polymorphism was about 97.32%. Further it was concluded that the isolates Tv2 (Pune) and Tv5 (Sangali) were at similitude as they recorded higher value of similarity coefficient (0.400). However, similarity coefficient (0.087) of Tv1 (Akola) and Tv4 (Amravati) isolates with the isolate Tv2 (Pune) was very low.

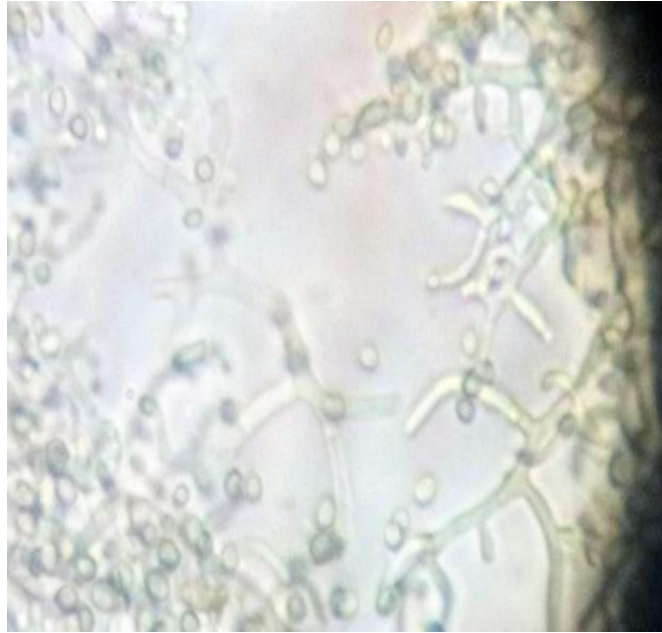
Yadav *et al.* (2020) isolated 21 *Trichoderma* isolates, out of that the 21 isolates collected, three promising isolates *viz.*, CSR-T-2, CSR-T-3 and CSR-T-4 were identified by molecular methods - sequencing ribosomal RNA using ITS1 and ITS4 universal primers for confirmation of species and isolates were identified as CSR-T-2 (*T. koningiopsis*) CSR-T-3 (*T. reesei*) and CSR-T-4 (*T. asperellum*).

Flamand (2021) cultured 29 isolates of *Trichoderma* from grapevine orchards in British Columbia and molecular analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) and a partial sequence of the translation elongation factor 1-alpha gene of these isolates facilitated the identification of

seven species viz., *T. asperelloides*, *T. atroviride*, *T. canadense*, *T. harzianum*, *T. koningii*, *T. tomentosum*, and *T. viticola*. Followed by Rodriguez *et al.* (2021) done phylogenetic analysis of all the 94 isolates by using a combination of three genes: translation elongation factor-1 $\alpha$  (*tef1*), *rpb2* and *cal* for tabbed isolates. The species recognition was confirmed by using GCPSR criteria supported by morphological and cultural characters.

Silva *et al.* (2021) conducted study on molecular identification and phylogenetic analysis of *Trichoderma* isolates. During molecular characterization, the DNA regions studied were the genes for the translation elongation factor (*tef1*) and the second largest RNA polymerase subunit (*rpb2*). The sequences of each gene were aligned and the concatenated ones (*tef1 -rpb2*) were compared with most alike *Trichoderma* isolates available in Gene Bank for the construction of phylogenetic tree. The study allowed the identification of 14 isolates within three species of *Trichoderma* viz., *T. orientale*– seven isolates; *T. koningiopsis*– six isolates and *T. longibrachiatum*– one isolate. Remaining isolates could not be identified upto the species level. This might have been due to insufficient rate of PCR pass rate.

Xue *et al.* (2021) obtained 1308 *Trichoderma* strains and based on the morphological characterization and phylogenetic analysis of the nuclear ribosomal internal transcribed spacer (ITS) and translation elongation factor 1 (*tef1*), twelve *Trichoderma* strains were identified as *T. asperellum* and one as *T. afroharzianum*.



**SUMMARY AND  
CONCLUSION**



## CHAPTER-VI

### SUMMARY AND CONCLUSION

A roving survey was carried out in Konkan region to collect the indigenous promising isolates of *Trichoderma*. In all 67 samples were collected from rhizosphere of different crops in varied pockets of the region. Out of these, axenic cultures of 27 isolates were obtained on TSM. These included 3 isolates from Mango rhizosphere; 5 from Rice; 2 each from coconut Chilli and Brinjal and 1 each from Areca nut, Cashew nut, Banana, Guava, Sapota, Horse gram, Lab-lab bean, Groundnut, Elephant foot yam, Bottle gourd, Cabbage, Cauliflower and Champaka.

The *in vitro* antagonistic potential of all *Trichoderma* isolates was tested against the major devastating plant pathogens in the Konkan region viz., *Fusarium* spp., *Rhizoctonia* spp., *Colletotrichum* spp., *Alternaria* spp. and *Sclerotium* spp. Each one of them showed different antagonistic behavior towards pathogens but the isolates like T<sub>11</sub> (Tas-Areca-nut- Shrivardhan dist. Raigad), T<sub>3</sub> (Tmnrj- Mango- Lanja dist. Ratnagiri), T<sub>23</sub> (Tbk- Brinjal-Karjat dist. Raigad), T<sub>5</sub> (Tkorr- Rice- Kolambe dist. Ratnagiri), T<sub>14</sub> (Tgpal- Guava- Kelwe dist. Palghar), T<sub>25</sub> (Tckr- Cabbage- Karjat dist. Raigad) and T<sub>24</sub> (Tbpal- Brinjal- Mahim dist. Palghar) showed better inhibition. It was found that T<sub>11</sub> ranked first (82.22% inhibition) in antagonism against *Fusarium* spp.; T<sub>14</sub> was the best (81.11% inhibition) against *Rhizoctonia* spp., T<sub>23</sub> (86.11%) against *Sclerotium* spp., T<sub>5</sub> (81.33 %) against *Colletotrichum* spp. and T<sub>25</sub> (86.66%) against *Alternaria* spp. amongst the remaining two isolates, T<sub>3</sub> ranked second against (80.54%) *Sclerotium* spp. and T<sub>24</sub> ranked fourth against *Rhizoctonia* spp. (74.11%) and *Colletotrichum* spp. (78.33%). All these seven isolates performed better against all the pathogens under study and therefore it was thought necessary to understand the identity of these promising isolates up to species level.

Accordingly, first three isolates (T<sub>11</sub>, T<sub>3</sub>, and T<sub>23</sub>) were identified using molecular characterization (T<sub>11</sub>, T<sub>3</sub> and T<sub>23</sub>) and remaining four were subjected to morphological identification (T<sub>5</sub>, T<sub>14</sub>, T<sub>25</sub> and T<sub>24</sub>). The molecular characterization results confirmed T<sub>11</sub> and T<sub>23</sub> as a *T. asperellum* Samuels, Lieckf. & Nirenberg, T<sub>3</sub> as a *T. harzianum* Rifai.

The morphological identification confirmed T<sub>25</sub> as a *Trichoderma* sp. aff. *T. longibrachiatum* Rifai, and T<sub>5</sub>, T<sub>14</sub> and T<sub>24</sub> as a *Trichoderma* sp. aff. *T. koningii* Oudem.

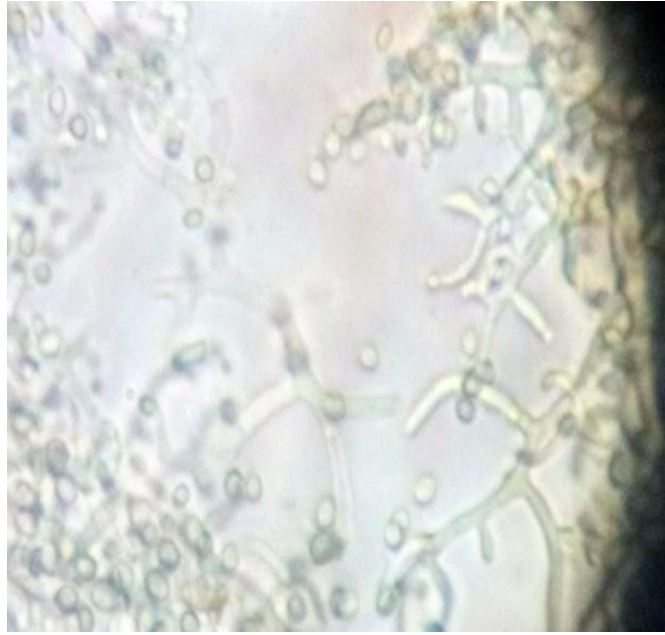
The cultures identified by molecular characterization were deposited at NCBI, USA, and their accession numbers are T<sub>11</sub> - *Trichoderma asperellum* - BankIt2546015 NFCCI\_5020 OM471989; T<sub>3</sub>-*Trichoderma harzianum*- BankIt2546015 NFCCI\_5021 OM471990 and T<sub>23</sub>-*Trichoderma asperellum*- BankIt2546015 NFCCI\_5022 OM471991.

The morphological studies of T<sub>5</sub>, T<sub>14</sub>, T<sub>25</sub> and T<sub>24</sub> under 100X lens with Micam 2.0 software revealed that, phialide length ranged from 124.50 to 150.00 µm and shapes were T<sub>5</sub> -less lageniform, T<sub>14</sub>- sausage shaped, T<sub>24</sub> -nine-pin bowling shaped and T<sub>25</sub>- singly lageniform to subulate divergent phialides., conidiophores length and breadth ranged from 635.80-1082.00 µm × 44.70-57.10 µm. Conidial diameter ranged from 47.18 to 53.31 µm and shape of T<sub>5</sub>, T<sub>24</sub> and T<sub>14</sub> isolates conidia were globose and T<sub>25</sub>- sub-globose. Conidial chain length ranged from 300.40 to 466.90 µm and conidia arrangement was catenate in all isolates, and the features like colony appearance of T<sub>5</sub>, T<sub>14</sub> and T<sub>24</sub> were blue green to yellowish green and T<sub>25</sub>-off white to greyish green, hyphae of T<sub>5</sub> and T<sub>25</sub> were - penicillate, those of T<sub>14</sub> and T<sub>24</sub>- as tree branches. On the basis of above observations, isolates T<sub>5</sub> (Tkorr), T<sub>14</sub> (Tgpal) and T<sub>24</sub> (Tbpal) were identified as *Trichoderma* sp. aff. *T. koningii* Oudem and T<sub>25</sub> (Tckr) were identified as *Trichoderma* sp. aff. *T. longibrachiatum* Rifai. All the isolates were members of the family- *Hypocreaceae*.

*In vitro* efficacy of commonly used fungicides against *Trichoderma* isolates revealed that among the three systemic fungicides, carbendazim was the most detrimental while the other two facilitated meager mycelial growth of all the isolates. Among contact fungicides, 5 isolates were fairly compatible with Sulphur (2500 ppm) whilst COC while Mancozeb were major inhibitor of mycelial growth of the isolates.

On the basis of the results of present study it can be concluded that, among the 27 isolates 7 indigenous *Trichoderma* isolates have promising antagonistic potential to combat against the five common plant pathogens in Konkan region. Hence these need to exploit for commercial formulations/ production.





LITERATURE  
CITED



## LITERATURE CITED

- Abd-Elsalam K. A., I. Almohimeed, M. A. Moslem and A. H. Bahkali. 2010. M13-microsatellite PCR and rDNA sequence markers for identification of *Trichoderma* (Hypocreaceae) species in Saudi Arabian soil. *Genetics and Molecular Research* ISSN: 1676-5680. **9(4)**: 2016-2024.
- Abdullah, F., Ilias, G.N.M and Nelson, M. 2007. Hyperparasitic mechanisms employed by the fungal biocontrol agent in a *Trichoderma-Ganoderma* interaction. In exploring life as a catalyst for technological advancement. Proceedings of the 9th Symposium of the Malaysian Society of Applied Biology, University Sains Malaysia, Malaysia. 107-130.
- Akinyi. O. J. 2014. Characterization and determination of efficacy of local *Trichoderma* isolates as a biocontrol agent (Bca) on *Fusarium wilt* on Beans (*Phaseolus Vulgaris* L). M.Sc. Thesis submitted To Centre for Biotechnology and Bioinformatics. University of Nairobi.
- Ali, A. A. I and M. M. Ramadan. 2019. *In vitro* Integration of *Trichoderma harzianum* with Chemical Pesticides Pertain to different Classes. *J. of Plant Prot. and Path., Mansoura Univ.* Vol. **10** (9), September.
- Altomare, C., Norvell, W. A., Bjorkman, T. and Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plant growth promoting biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied and Environmental Microbiology*. **65**, 2926-2933.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403-410.
- Alvarado - Marchena. L. and W. Rivera - Mendez. 2016. Molecular identification of *Trichoderma* spp. in Garlic and Onion fields and *in vitro* antagonism trials on *Sclerotium cepivorum*. *Rev Bras Cienc Solo*; v40:e0150454.
- Amin F., V.K. Razdan, F. A. Mohiddin, K. A. Bhat, and S. Banday. 2010. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology*. **2(10)**: 38–41 ISSN: 2075-6240.
- Asad S. A. Naeem Ali, A. Hameed, S. A. Khan, R. Ahmad, M. Bilal, M. Shahzad and Ayesha Tabassum. **2014**. Biocontrol efficacy of different isolates of *Trichoderma* against soil born pathogen *Rhizoctonia solani*. *Polish Journal of Microbiology*. ORIGINAL PAPER. Vol. 63, No 1, 95–103.

- Ashlesha, H. Oberoi and P. Kumar. 2019. Rhizosphere *Trichoderma* isolates as potential biocontrol agent for maydis leaf blight pathogen (*Bipolaris maydis*) in fodder maize. *Proc Indian Natn Sci Acad.* **85**. No. 4 December pp. 885-893 O Printed in India. DOI: 10.16943/ptinsa/2019/49607.
- Ashwani Tapwal, Gunjan Thakur and S. Chandra. 2015. *In-vitro* evaluation of *Trichoderma* species against seed borne pathogens. *IJCBS RESEARCH PAPER*. VOL. 1 [ISSUE 10] JANUARY, ISSN:- 2349–2724.
- Atole. D. K, Dr. K. S. Raghuwanshi, Monali N. Pawar and Sanganna S. Kumbhar. 2020. Morphological and cultural studies of *Alternaria alternata* causing leaf spot and fruit rot of chilli. *International Journal of Chemical Studies.* **8(6)**: 1769-1771.
- Bae, H., Roberts, D. P., Lim, H. S., Strem, M. D., Park, S. C., Ryu, C. M., Melnick, R. L. and Bailey, B. A. 2011. Endophytic *Trichoderma* isolates from tropical environments delay deases oset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. *Molecular Plant-microbe Interactions.* **24**, 336-351.
- Bagwan, N. B. 2010. Evaluation of *Trichoderma* compatibility with fungicides, pesticides, organic cakes and botanicals for integrated management of soil borne diseases of soybean [*Glycine max* (L.) Merrill]. *International Journal of Plant Protection.* **3** (2) : 206-209.
- Barbosa, M. A., G. K. Rehn, M. Menezes and L. R. Mariano. 2001. Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzymatic characterization. *Braz. J. of Microbiol.* **32**, 98-104.
- Barnett H. L. and HunterB. B. 1972. Illustrated genera of imperfect fungi. 3<sup>rd</sup> Edition, Burgess Pub.Co., pp.273.
- Beaulieu, R., Lopez-Mondejar, R., Fabio, T., Margarita, R. and Jose, A. P. (2010). qRT-PCR quantification of the biological control agent *Trichoderma harzianum* in peat and compost-based growing media. *Bioresource Technology.* **102**: 2793–2798.
- Bhale U. N and J. N Rajkonda. 2015. Compatibility of Fungicides and Antagonistic Activity of *Trichoderma* spp against plant Pathogens. *Bioscience Methods*, BioPublisher. Vol.6, No.3, 1-9.
- Bharti M., R. Prasad, D. Kumar, S. Kumar and R. Kumar. 2016. Survey and bio-efficacy of bio-agents against *Alternaria brassicae* causing blight disease of Mustard. *Agriways.* **4(2)**: 121-126.

- Bhatt N. M. and L. S. Srivastava. 2003. Evaluation of some fungicides and neem formulations against six soil borne pathogens and three *Trichoderma* spp. *in-vitro*. *Pl. Dis. Res.*, **18**: 56-59.
- Bhuvaneshwari, V. and Subba Rao, M. 2001. Evaluation of *Trichoderma viride* antagonistic to post-harvest pathogens on mango. *Indian phytopath.* 54: 493-494.
- Boat M. A. B, B. Iacomi and F. Fekam Boyom. 2018. Fungicide tolerance and effect of environmental conditions on growth of *Trichoderma* spp. with antagonistic activity against *Sclerotinia sclerotiorum* causing white mold of common bean (*Phaseolus vulgaris*). *International Journal of Innovative Approaches in Agricultural Research*.
- Callaghan S. E, V. I. Puno, A. P. Williams, B. S. Weir, V. Balmas, K. Sengsoulichan, S. Phantavong, T. Keovorlajak, P. Phitsanoukane, P. Xomphouthilath, K. S. Phapmixay, S. Vilavong, E. C. Y. Liew, G. S. Duckitt and L. W. Burgess. 2016. First report of *Fusarium oxysporum* f.sp. *niveum* in the Lao PDR. *Australasian Plant Dis. Notes.* **11**: 9.
- Cherkupally, R., H. Amballa and B. N. Reddy. 2016. *In Vitro* Antagonistic Activity of *Trichoderma* and *Penicillium* species against *Macrophomina phaseolina* (Tassi) Goid. *Scholars Research Library Annals of Biological Research*, ISSN 0976-1233CODEN (USA): ABRNBW. **7(9)**:34-38.
- Chet I. 1987. *Trichoderma* application, mode of action and potential as biocontrol agent of soil borne plant pathogenic fungi. In: I. Chet (ed.). *Innovative Approaches to Plant Disease Control*. Wiley, New York, 137-160.
- Chet, I., Y. Elad and J. Katan. 1980. *Trichoderma harzianum*: Biocontrol agent effective against *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*. *Phytopathology.* 70(2).
- Cruz-Quiroz R. D. L, S. Roussos, R. Rodriguez-Herrera, D. Hernandez-Castillo, and C. N. Aguilar. 2018. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala International Journal of Modern Science.* **4**: 237-243.
- Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma*-II. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.*, **57**: 47-48.
- Dhanya M. K., K. B. Anjumol, M. Murugan and K. B. Deepthy. 2016. Compatibility of *Trichoderma viride* and *Pseudomonas fluorescens* with plant protection

- chemicals and fertilizers in cardamom. *Journal of Tropical Agriculture*. **54**(2): 129-135.
- Dhar, V., S. Mishra and R.G. Chaudhary. 2006. Differential efficacy of bioagents against *Fusarium udum* isolates. *Indian Phytopath.* **59**(3) : 290-299.
- Druzhinina, I. S., Kubicek, C. P., Zelazowska, M. K., Mulaw, T. B. and Bissett, J. (2010). The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. *BMC Evolutionary Biology*. **10**: 94-102.
- Doni, F.; Isahak, A.; Zain, C. R. C. M.; Ariffin, S. M.; Mohamad, W. N. W. and Yusoff, W. M. W. 2014. Formulation of *Trichoderma* sp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. *Springerplus*, 3, 532.
- Dwivedi, M and K. Vishunavat. 2018. Compatibility of *Trichoderma* strains and their mutants with common agrochemicals. *Journal of Pharmacognosy and Phytochemistry*. **7**(5): 2744-2747.
- Elad Y. 2000. Biological control foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*. **19**:709-714.
- Elad Y. and I. Chet. 1983. Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. *Phytoparasitica*. **11**(1): 55-58.
- Elad, Y., I Chet, And J. Katan. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoclonia solani*. *Phytopathology*. **70**:119-121.
- Elmahdi S., J. Kadir, M. T. M. Mohamed, G. Vadamalai and S. Akter. 2015. Isolation, screening and characterization of effective microbes with potential for biological control of *Fusarium* wilt of rock melon. *World Journal of Agricultural Research*. Vol. 3, No. **1**, 11-16.
- Elshahawya. I. E, Karima H. E. Haggagb, and H. A. Khair. 2016. Compatibility of *Trichoderma* spp. with seven chemical fungicides used in the control of soil borne plant pathogens. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. ISSN: 0975-8585. **7**(1):1772-1785.
- Elyousr K. A. M. A, S. I. I. A. Hafez and I. R. A. Rahim. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. *J Phytopathol*. **162**:567–574.
- Fahmi. A. I, R. A. Eissa, Khalil A. E. Halfawi, H. A. Hamza and M. S. Helwa. 2016. Identification of *Trichoderma* spp. by DNA barcode and screening for

cellulolytic activity. *Journal of Microbial & Biochemical Technology*. Volume **8(3)**: 202-209.

Farah S. T and S. Nasreen. 2013. Biocontrol efficacy of *Trichoderma koningii* against some plant pathogenic fungi. *Indian Journal of Research*. Volume : **2**. Issue : 3. March. ISSN - 2250-1991.

Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on plants and plant products in the United States. The American Phytopathological Society press, St. Paul, (Minnesota) USA.

Fisher, F. E. 1969. Chemical control of citrus diseases in Florida. *Plant Disease Reporter*. **53** : 19-22.

Flamand, J. P. 2021. Identification and Characterization of *Trichoderma* species from Vineyards in British Columbia and Studies on Their Potential Use as Biological Control Agents Against the Grapevine Trunk Disease *Botryosphaeria* Dieback. M.Sc. Thesis Submitted to THE UNIVERSITY OF BRITISH COLUMBIA (Okanagan) August.

Flegel, T. W. 1980. Semipermanent microscope slides of microfungi using sticky tape technique, *Canadian Journal of Microbiology*. **26**: 551-553.

Freeman,S.; O. Barbul,; D.R. David; Y. Nitzani; A. Zvebil, and Y. Elad, 2001. *Trichoderma spp.* for biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* in strawberry. *Biological Control of Fungal and Bacterial Palnt Pathogens: IOBC wprs bulletin*. 24(3): 147-150.

Gaikwad. P. A, D. N. Dhutraj and C. V. Ambadkar. 2020. Cultural and Genetic Diversity of *Rhizoctonia bataticola* Isolates Causing Dry Root Rot of Chickpea. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706. Volume **9**, Number 4.

Gams. W and Bisset J. 1998. Morphology and identification of *Trichoderma*. In: *Trichoderma and Gliocladium*. Vol.1 (Harman GE and Kubicck CP, Taylor and Francis Eds), London. pp 3-34.

Grace. K. W. 2016. Determination of the effectiveness of Trianum-P® (*Trichoderma Harzianum*) and Trichotech® (*Trichoderma Asperellum*) in the management of late blight disease of tomatoes. Thesis submitted To The Graduate School, Plant Pathology of Egerton University in November.

Gomez. K. A and A. A. Gomez. 1984. Statistical Procedures for Agricultural Research. Second Edition. Book.

- Goudar, S. B. and S. Kulkarni. 2000. Bioassay of Antagonists against *Fusarium udum* – The causal agent of pigeonpea wilt. *Karnataka J. Agric. Sci.* **13**(1): (64-67).
- Gud, M.B., 2001. Studies on fungal diseases of mango (*Mangifera indica* L.) fruits. M.Sc. (Agri.) Thesis, KKV, Dapoli, Maharashtra, India.
- Gupta, A.; B. Mukand and S. K. Gandhi. 2008. Efficacy of bio-agents and plant extracts against *Colletotrichum lagenarium* causing anthracnose of bottle gourd. *Research on Crops.* **9**(2): 485-489.
- Harman, G. E. 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease.* **84**, 377-393.
- Harman, G. E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology.* **96**, 190-194.
- Harman, G. E. 2011. *Trichoderma*- not just for biocontrol anymore. *Phytoparasitica.* **39**, 103-108.
- Hemanth, G., Kumar P. K. R., Niharika P. S. and kolli, S. K. 2016. Fungicides effect on soil micro flora in Tekkali Mandal, Srikakulam (Dist.). *International Journal of Research and Development in Pharmacy and Life Sci-ences.* **5**(4): 2245-2250.
- Honmane, D. K. 2007. Studies on diseases caused by *Colletotrichum gloeosporioides* penz. and *Fusarium moniliforme* Sheldon. on Anthurium (*Anthurium andreanum* lind.) M.Sc. (Agri.) thesis submitted to Dr. BSKKV., Dapoli.
- Howell C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The History and evolution of current concepts. *Plant Disease.* **87** :4-10.
- Islam, M. S., M. Ali and M. S. Rahman. 2008. *In vitro* studies on the fungicidal effect on *Trichoderma* species in tea Plantation. *Bangladesh J. Agril. Res.* **36** (4) : 677-683.
- Jakubikova, L, V. Farkas, N. Kolarova, M. Nemcovic. 2006. Conidiation of *Trichoderma atroviride* Isolate during Submerged Cultivation in a Laboratory Stirred-Tank Fermenter. *Folia Microbiol.* **51**(3): 209–213.
- Jamwal S., A. Jamwal and V. S. Verma. 2011. Effect of biocontrol agents on wilt management and plant growth of tomato. *Indian phytopath.* **64**(4): 381-382.
- Jana S. C and M. Mandal. 2017. Antagonistic effect of *Trichoderma* isolates on *Sclerotium rolfsii*. *Journal of Experimental Biology and Agricultural Sciences.* Volume – **5**(4). ISSN No. 2320 – 8694.

- Jankar K., Totawar MV and Payal Kose. 2020. Molecular variability among the isolates of *Trichoderma viride*. *International Journal of Chemical Studies*. **8(3)**: 2256-2259.
- Jat J. G and H. R. Agalave. 2013. Antagonistic properties of *Trichoderma* species against oilseed-borne fungi. *Science Research Reporter*. **3(2)**:171-174, Oct. ISSN: 2249-2321.
- Jayalakshmi, C., P. Durairaj, K. Seetharaman and K. Sivaprakasam. 1998. Biocontrol of fruit rot and die-back of chilli using antagonistic microorganisms. *Indian phytopath.* **51(2)** : 180-183.
- Jayalakshmi S. K., S. Raju, S. Usha rani, V. I. Benegii and K. Sreeramulu. 2009. *Trichoderma harzianum* L1 a potential source for lytic enzymes and elicitor of defence responses in chickpea against wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *Australian Journal of Crop Science*. **3**: 44-52.
- Kannangara S., R. M. G. C. S. Dharmarathna and D. L. Jayarathna. 2017. Isolation, Identification and Characterization of *Trichoderma* Species as a Potential Biocontrol Agent against *Ceratocystis paradoxa*. *The journal of agricultural sciences*. Vol. 12, No. 1, January. Pp 57-62.
- Kaswate, N.S., S.S. Shinde and R. R. Rathod. 2003. Effect of biological agents against different isolates of *Rhizoctonia bataticola* (Taub.) Butler *in vitro*. *Indian Phytopath.* **17(2)** : 167-168.
- Katoh, K. and Standley, D. M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, **30**, 772-780. <https://doi.org/10.1093/molbev/mst010>.
- Kavita Yadav, T. Damodaran, Nidhi Kumari, Kakoli Dutt, R. Gopal and M. Muthukumar. 2020. Characterization of *Trichoderma* isolates and assessment of antagonistic potential against *Fusarium oxysporum* f. sp. *cumini*. *Journal of Applied Horticulture*. ISSN: 0972-1045**22(1)**: 38-44.
- Khaledi, N and P. Taheri. 2016. Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. *Journal Of Plant Protection Research*. Vol. **56**, No. 1 DOI: 10.1515/jppr-0004.
- Khan. M. O and S. Shahzad. 2015. Screening of *Trichoderma* species fortolerance to fungicides. *Pak. J. Bot.*, **39(3)**: 945-951.
- Kim, S. I.; Shim, J. O.; Shin, H. S.; Choi, H. J. and Lee, M. W. 1992. Suppressive mechanism of soil-borne disease development and its practical application.



- Isolation and identification of species of *Trichoderma* antagonistic to soil diseases and its activities in the rhizosphere. *Korean Journal of Mycology*. **20(4)**: 337-346.
- Kiran G. V. N. S. M, S. S. Thara and K. R. Jyothi. 2018. Studies on compatibility of biocontrol agents with chemical fungicides for integrated management of *Alternaria* leaf spot of cabbage. *Journal of Pharmacognosy and Phytochemistry*; **7(5)**: 2974-2977.
- Komy M. H. E., A. A. Saleh, A. Eranthodi and Y. Y. Molan. 2015. Characterization of Novel *Trichoderma asperellum* Isolates to Select Effective Biocontrol Agents Against Tomato Fusarium Wilt. *Plant Pathol. J.* **31(1)** : 50-60
- Korat, C.G. and Priya John. 2015. Antagonistic effect of *Trichoderma* against soil borne pathogens. *BIOINFOLET*. **12 (1A)** : 110-112.
- Kumar. A, Rani Devi Bansal, and Y. K. Chelak. 2019. Compatibility of *Trichoderma viride* with fungicides for plant disease management. *International Journal of Pure and Applied Bioscience*. ISSN: 2320 – 7051. **7 (3)**: 44-51.
- Kumar, K, N. Amaresan, S. Bhagat, K. Madhuri and R. C. Srivastava. 2011. Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. *Indian J Microbiol* (Apr–June). **52 (2)**:137–144 DOI 10.1007/s12088-011-0205-3
- Kumar. M. A and Pratibha Sharma. 2016. Morphological characterization of biocontrol isolates of *Trichoderma* to study the correlation between morphological characters and biocontrol efficacy. CC BY 4.0. Published by SciPress Ltd, Switzerland *International Letters of Natural Sciences*. ISSN: 2300-9675, Vol. 55, pp 57-67.
- Kumar R, S. K. Singh, S. Yadav, R. Kumar, A. K. Choubey and A. Kumari. 2018. Compatibility of *Trichoderma viride* with different fungicide and organic cake. *Journal of Pharmacognosy and Phytochemistry*. **7 (2)**: 2398-2401.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol***33**: 1870-1874.
- Kumar. T. V., S. S. Veena, S. Karthikeyan and J. Sreekumar. 2017. Compatibility of *Trichoderma asperellum* with fungicides, insecticides, inorganic fertilizers and bio-pesticides. *Journal of Root Crop.*, Vol. **43** No. **2**, pp. 68-75, Indian Society for Root Crops, ISSN 0378-2409, ISSN 2454-9053 (online).

- Kumar. V, D. K. Verma, A. K. Pandey and Shikha Srivastava. 2019. *Trichoderma* spp.: Identification and characterization for pathogenic control and its potential application. Book chapter published in Research Gate.
- Kushwaha. S. K, S. Kumar and B Chaudhary. 2018. Efficacy of *Trichoderma* against *Sclerotium rolfsii* causing collar rot disease of lentil under in vitro conditions. *Journal of Applied and Natural Science*. **10(1)**: 307 – 312.
- Lalnghaihawmi and A. Bhattacharyya. 2019. Study on the different modes of action of potential *Trichoderma* spp. from Banana Rhizosphere against *Fusarium oxysporum* f.sp. *cubense*. *International Journal of Current Microbiology and Applied Sciences* ISSN: 2319-7706 Volume **8** Number 01 Journal homepage: <http://www.ijcmas.com>
- Lopez. L. L. M. A, R. G. Reyes and D.G. Alvindia. 2015. Evaluation of two species of *Trichoderma* as compost activator and bio-control agents. *Journal of Agricultural Technology*. Vol. **11(2)**: 525-537. Available online <http://www.ijat-aatsea.com>. ISSN 1686-9141.
- Lorito, M., Hayes, C. K., DiPietro, A., Woo, S.L. and G.E, Harman. 1994. Purification, characterization and synergistic activity of a glucan 1, 3-bglucosidase and an N-acetyl-b-glucosaminidase from *Trichoderma harzianum*. *Phytopathology* **84**, 398–405.
- Lorito, M. 2004. Application of *Trichoderma harzianum* (T22) and *Trichoderma atroviride* (P1) as plant growth promoters, and their compatibility with copper oxychloride. *Journal of Zhejiang. University Science*. **30**: 2–8.
- Lorito, M., Woo, S. L., Harman, G. E. and Monte, E. 2010. Translational research on *Trichoderma*: from omics to the field. *Annual Review of Phytopathology*. **48**, 395-417.
- Madhavi. G. B, S. L. Bhattiprolu and V. B. Reddy. 2011. Compatibility of biocontrol agent *Trichoderma viride* with various pesticides. *J. Hortl. Sci.* Vol. **6(1)**:71-73.
- Madhavi. M, C. P. C. Kumar, D. R. Reddy and T. V. K. Singh. 2008. Compatibility of mutant isolates of *Trichoderma* spp. with agrochemicals. *J. BioI. Control*. **22(1)**: 51-55.

- Madhusudhan. P, K. Gopal, V. Haritha. U. R. Sangale and S.V.R.K. Rao. 2010. Compatibility of *Trichoderma Viride* with fungicides and efficiency against *Fusarium solani*. *J. Pl.Dis.Sci.* Vol. **5(1)**: 23 – 26.
- Magar, G. S., S. S. Wagh and D.P. Kuldhar. 2014. Bioefficacy of *Trichoderma* isolates from Marathwada region against *Fusarium oxysporum* f. sp. *ciceri*. *Trends in Biosciences.* **7(18)**: 2728-2730.
- Maheshwary N. P., G. Naik B, A. Chittaragi, M. K. Naik, K. M. Satish and M. S. Nandish. 2020. Compatibility of *Trichoderma asperellum* with fungicides. *The Pharma Innovation Journal.* **9(8)**:136-140.
- Mamatha, G and R. H. Yashoda. 2006. Role of biocontrol agents in management of foliar diseases of turmeric. *International J. Pl. Sciences.* **1(2)**:145-146.
- Mamidyala. S and S. Thirukkurugudi. 2020. Investigating the Effect of the Filamentous Fungus *Trichoderma Reesei* on the Strength of Cracked Concrete. *International Journal of Engineering Research & Technology (IJERT).* ISSN: 2278-0181. Vol. **9** Issue 09, September.
- Manjusha Gaikwad, S. S. Mane, Swati Gawande, Sunita Suryawanshi and S. R. Dalal. 2017. Antagonistic potential of *Trichoderma* against some soil borne plant pathogens. *INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE.* ISSN: 0254-8755 available at [http: www.serialsjournal.com](http://www.serialsjournal.com) Volume **35**, Number 4.
- ManuT. G, A. Nagaraja, T. S. Naik and R. Murali. 2016. Evaluation of integrated approaches for the management of *Sclerotium Rolfsii* causing foot rot disease of finger millet. *Plant Archives.* Vol. **16** No. **1**. pp. 201-204.
- Maruyama. C. R, N. Bilesky-Jose, R. D. Lima and L. F. Fraceto. 2020. Encapsulation of *Trichoderma harzianum* preserves enzymatic activity and enhances the potential for biological control. *Frontiers in Bioengineering and Biotechnology.* [www.frontiersin.org](http://www.frontiersin.org) March. Volume **8**. Article 225.
- Meenakshi Dwivedi and Karuna Vishunavat. 2018. Compatibility of *Trichoderma* strains and their mutants with common agrochemicals. *Journal of Pharmacognosy and Phytochemistry.* **7(5)**: 2744-2747.
- Mishra B. K, R. K. Mishra, R. C. Mishra, A. K. Tiwari, R. S. Yadav and Anupam Dikshit. 2011. Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. Scholars Research Library. *Archives of Applied Science Research.* **3(2)**:361-369.

- Mishra S, P. Mishra, R. Singh<sup>1</sup>, G. Singh and S. K. Sachan. 2019. Compatibility of different systemic and non-systemic fungicides with *Trichoderma viride*. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706. Volume **8** Number **01**: 1005-1010.
- Mohamed N. A and M. A. Radwan. 2017. Impact of pesticides on *Trichoderma harzianum* and on its possible antagonistic activity against *Fusarium oxysporum* under *In vitro* conditions. *Asian J. Agri. & Biol.*; 5(4):291-302.
- Morton, D. T and Stroube N. H. 1955. *Phytopathology*. **45**:419-420.
- Mukhopadhyay A. N. 2005. *Trichoderma* promises and pit falls. Abstract Presented in 2nd Global Conference Plant health Global wealth Nov. 25-29, Udaipur, India. **165**.
- Mukhopadhyay. R and D. Kumar.2020. *Trichoderma*: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. *Egyptian Journal of Biological Pest Control*. **30**:133.
- Murray. M. G and W. F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*. Oct 10; **8(19)**: 4321-4325.
- Musheer, N and S. Ashraf. 2017. Effect of *Trichoderma* Spp. on *Colletotrichum gleosporioides* Penz and Sacc. causal organism of turmeric leaf spot disease. *Trends in Biosciences*.**10(48)**, Print : ISSN 0974-8431, 9605-9608.
- Naher. L, N. Syawani, N. Amieza, A. B. Kamarudin and S. M. R. Karim. 2019. *Trichoderma* species diversity in rhizosphere soils and potential antagonism with *Fusarium oxysporum*. *Biosci. J., Uberlandia*, V. **35**, n. **1**, p. 13-26, Jan./Feb.
- Napitupulu T. P, M. Ilyas, A. Kanti, and I. M. Sudiana. 2019. *In vitro* evaluation of *Trichoderma harzianum* strains for the control of *Fusarium oxysporum* f.sp. *cubense*. *Plant Pathology & Quarantine*. **9(1)**: 152–159 ISSN 2229-2217
- Nishad D., M. Dewangan, A. K. Kurre and Shweta Mishra. 2019. Studies on the compatibility of *Trichoderma viride* and its interaction with different fungicides. *International Journal of Chemical Studies*. **7(6)**: 617-621.
- Nurbailis, A. Djamaan, H. Rahma and Y. Liswarni. 2019. Potential of culture filtrate from *Trichoderma* spp. as biofungicide to *Colletotrichum gloeosporioides* causing anthracnose disease in chilli. *BIODIVERSITAS* ISSN: 1412-033X Volume **20**, Number **10**, October 2019 E-ISSN: 2085-4722 Pages: 2915-2920.

- Oskiera. M, M. Szczech and G. Bartoszewski. 2015. Molecular Identification of *Trichoderma* strains collected to develop Plant Growth-Promoting and Biocontrol Agents. *Journal of Horticultural Research*. Vol. **23(1)**: 75-86.
- Pandey, K. K and Upadhyay, J. P. 2000. Microbial population from rhizosphere and non rhizosphere soil of pigeonpea: Screening for resident antagonist and mode of mycoparasitism. *J. mycol, pl. pathol.* **30(1)**: 7-10.
- Papade. V. V, S. R. Potdukhe, D. R. Navsupe, D. D. Guldekar and A. L. Taral. 2019. Morphological characters of *Colletotrichum gloeosporioides* from various hosts. *International Journal of Chemical Studies*. **7(4)**: 75-78.
- Papavizas G. C. 1985. *Trichoderma* and *Gliocladium* biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology*. **23**: 23-54.
- Patel, S.I., R.L. Patel, A.G. Desai and D.S. Patel. 2011. Biocontrol of *Fusarium udum* Through *Trichoderma*. *Journal of Pharma and Bio Sciences*. **2(4)**: 0975-6299.
- Prameela Devi. T, Deeba Kamil, R. Mehndiratta, N. Prabhakaran and R. S. Toppo.** 2016. Molecular and morphological diversity of *Rhizoctonia bataticola* causing dry root rot disease from India. *Journal of Pure and Applied Microbiology*. DOI: <http://dx.doi.org/10.22207/JPAM.10.4.32>
- Prabhakaran, N, T. Prameeladevi, M. Sathiyabama and D. Kamil. 2015. Screening of different *Trichoderma* species against agriculturally important foliar plant pathogens. *Journal Of Environmental Biology*. ISSN: 0254-8704. Vol. **36**; 191-198.
- Pradeep M, A.A.K. Eraivan, K. Kalpana, M. Shanthi and K. Senthil. 2022. Antagonistic potential of endophytic *Trichoderma asperellum* against *Alternaria alternata* causing Leaf Blight in Watermelon. *Biological Forum – An International Journal*. **14(1)**: 926-931.
- Priyadharcini. M, R. Akila, M. L. Mini, N. Rajinimala and R. Kannan. 2018. Exploration of *Trichoderma* spp. as an effective bio control agents against the Sclerotial wilt caused by *Sclerotium rolfsii* Sacc. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706. Volume **7** Number **(8)**: 1672-1682.
- Quiroz R. D. L. C, S. Roussos, R. R. Herrera, D. H. Castillo and C. O. N. Aguilar. 2018. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala International Journal of Modern Science*. **4**. 237e243.

- Rahman, A., M. F. Begum, M. Rahman, M. A. Bari, G. N. M. Ilias and M. F. Alam. 2011. Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turk J Biol* **35**: 183-194. TUBİTAK doi:10.3906/biy-0905-8.
- Rai, D and S. Maurya. 2021. Assessment of Local Strain of *Trichoderma asperellum* against *Fusarium* spp. *Journal of Plant Pathology & Microbiology*. Vol. **12** Iss. 3. 12:543.
- Raja H. A., A. N. Miller, C. J. Pearce and N. H. Oberlies. 2017. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. *Journal of Natural Products*. **80**, 756–770.
- Rajkonda, J.N and U.N. Bhale. 2018. Evaluation of Phytotoxic Activity and Antagonism of *Trichoderma koningii*. *International Journal of Life Sciences*. Special Issue- **A9** January. 120-124.
- Ranganathaswamy, M, A. K. Patibanda and G. N. Rao. 2012. Evaluation of toxicity of agrochemicals on *Trichoderma* isolates *in vitro*. *Journal of Biological Control*, **26** (4): 391–395.
- Raul, A.J., 2007. Studies on leaf spot of cinnamon (*Cinnamomum verum*). A M.Sc. Thesis submitted to Dr. B.S.K.K.V. Dapoli. (M.S).
- Reddy, M. S. P, Vibha and S. K. Pandey. 2018. Role of root colonizing *Trichoderma* species in management of *Alternaria* leaf blight of asafetida (*Lepidium sativum* L.) caused by *Alternaria alternate*. *Int.J.Curr.Microbiol.App.Sci*. **7**(7): 2544-2561.
- Rekha Rawat and Lakshmi Tewari. 2010. Transmission electron microscopic study of the cytological changes in *Sclerotium rolfsii* parasitized by a biocontrol fungus *Trichoderma* sp. *Mycology*, **1:4**, 237-241, DOI: 10.1080/21501203. 2010. 536172.
- Restrepo D.C, M.I. Dominguez, B. G. Gutiérrez, E. Osorio and K. Sierra. 2022. Biotization of endophytes *Trichoderma asperellum* and *Bacillus subtilis* in *Mentha spicata* Microplants to promote growth, pathogen tolerance and specialized plant metabolites. *Plants*, **11**, 1474.
- Rodriguez M. D. C. H., Harry C. Evans, Lucas M. de Abreu, Davi M. de Macedo, Miraine K. Ndacnou, Kifle B. Bekele and Robert W. Barreto. 2021. New species and records of *Trichoderma* isolated as mycoparasites and endophytes from cultivated and wild coffee in Africa. Scientific reports [www.nature.com/scientificreports](https://www.nature.com/scientificreports). *nature portfolio* **11**:5671.

- Romila Thoudam and B. K. Dutta. 2014. Compatibility of *Trichoderma atroviride* with fungicides against black rot disease of tea: An *in vitro* study. *Journal of International Academic Research for Multidisciplinary*. Impact Factor 1.393, ISSN: 2320-5083, Volume 2, Issue 2, March.
- Sagarika Kannangara, R.M.G.C.S. Dharmarathna and D. L. Jayarathna. 2017. Isolation, identification and characterization of *Trichoderma* species as a potential biocontrol agent against *Ceratocystis paradoxa*. *The Journal of Agricultural Sciences*. Vol. 12, No. 1, January 2017. Pp 51-62.
- Saitou. N and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* Jul; 4(4):406-25. doi: 10.1093/oxfordjournals.molbev.a040454.
- Sangle, U. R. and O. M. Bambawale. 2004. New strains of *Trichoderma* spp. strongly antagonistic against *Fusarium oxysporum* f. sp. *sesame*. *J. Mycol. PL. Pathol.*, 31(4): 107-109.
- Sarkar. S, P. Narayanan, A. Divakaran, A. Balamurugan, and R. Premkumar. 2010. The *in vitro* effect of certain fungicides, insecticides, and biopesticides on mycelial growth in the biocontrol fungus *Trichoderma Harzianum*. *Turk J Biol*. 34: 399-403.
- Saxena, D., A. K. Tewari and D. Rai. 2014. The *in vitro* effect of some commonly used Fungicides, Insecticides and Herbicides for their compatibility with *Trichoderma harzianum* PBT23. *World APPL. Sci. J.*, 31(4) : 444-448.
- Schmitz H. 1930. Poisoned food technique. *Industrial and Engineering Chemistry, Analytical Editon.*; 2:361- 363.
- Schuhmacher R., Stoppacher N. and Zeilinger S. 2007. Peptaibols of *Trichoderma atroviride*: screening, identification and structure elucidation by liquid chromatography-tandem mass spectrometry. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*. A. Méndez\_Vilas (\_Ed\_).
- Seidl,V.,Seibel,C., Kubicek, C.P., and Schmoll, M. 2009. Sexual development in the industrial work horse *Trichoderma reesei*. *Proc. Natl. Acad.Sci.U.S.A.* 106, 13909–13914. doi:10.1073/pnas.0904936106.
- Sekhar Y. C, S. K. Ahammed, T.N.V.K.V. Prasad and R. Sarada Jayalakshmi Devi. 2017. Identification of *Trichoderma* species based on morphological characters isolated from rhizosphere of Groundnut (*Arachis Hypogaea* L). *International*

- Shaigan, S, A. Seraji and S. A. M. Moghaddam. 2008. Identification and investigation on antagonistic effect of *Trichoderma* spp. on tea seedlings white foot and root rot (*Sclerotium rolfsii* Sacc.) *in vitro* condition. *Pakistan Journal of Biological Sciences*. **11(19)**: 2346-2350.
- Sharma, K. S and L. Prasad. 2018. Bioactivity of *Trichoderma asperellum* against *Colletotrichum asianum* and *Sclerotinia sclerotiorum*. *Pesticide Research Journal*. Vol. **30(2)**: 251-255
- Sharma. D, Roopali Sharma and Smita Puri. 2016. Compatibility of biocontrol agents with fungicides. *The Bioscan*. **11(4)**: 2863-2866.
- Shashikumar H. M, S. Koulagi and S. E. Navyashree. 2019. Compatibility of *Trichoderma viride* and *Trichoderma harzianum* with fungicides against soil borne diseases of tomato and cabbage. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706 Volume **8** Number **04** Journal homepage: <http://www.ijcmas.com>
- Shewarega, M, S. T. Ingle, S. S. Mane and P. N. Madavi. 2019. Efficacy of *Trichoderma longibrachiatum* isolates against soil borne pathogens. *Journal of Pharmacognosy and Phytochemistry*. **8(4)**: 1477-1481.
- Shoresh, M., Harman, G.E. and Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual review of Phytopathology*. **48**, 21-43.
- Shrivastava A. 2019. Assessment of compatibility of *Trichoderma* species with different fungicides *in vitro*. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706 Volume **8** Number **02**.
- Silva H. F, E. M. D. Costa, A. M. G. Santos, A. C. T. D. Amaral, R. J. V. D. Oliveira, J. L. Bezerra and E.D.M.N. Luz. 2021. Molecular identification and phylogenetic analysis of *Trichoderma* isolates obtained from woody plants of the semi-arid of Northeast Brazil. *Nova Hedwigia*. Vol. **112**, Issue **3-4**, 485–500.
- Siameto E. N., S. Okoth, N. O. Amugune and N. C. Chege. 2011. Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from embu district, Kenya. *Tropical and Subtropical Agroecosystems*. **13**: 81 – 90.

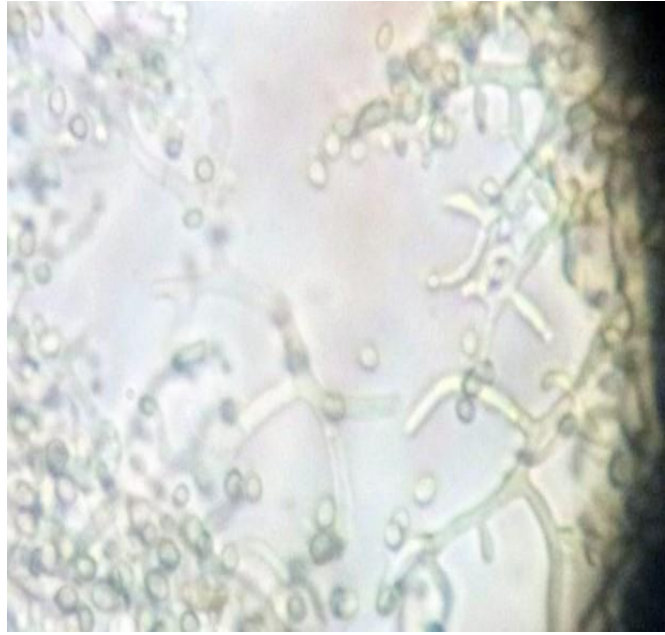


- Sindhan, G. S., I. Hooda and S. S. Karwasra. 2002. Biological control of dry root rot of chickpea caused by *Rhizoctonia bataticola*. *Plant Dis. Res.* **17(1)** : 68-71.
- Singh. M, R. Singh, P. Mishra, R. S. Sengar and U. P. Shahi. 2021. *In-vitro* compatibility of *Trichoderma harzianum* with systemic fungicides. *International Journal of Chemical Studies.* **9(1)**: 2884-2888.
- Singh, U. S., Mishra, D. S., Singh, A., Rohilla, R. and Vishwanath. 2003. Induced resistance present status and future prospects as disease management strategy. In: Koul, O., Dhaliwal, G.S., Marwaha, S.S. and Arora, J.K.(eds) *Biopesticides and pest management, Vol. 1*. Campus Books International, India, pp. 262-302.
- Sivan A., Elad and I. Chet. 1984. Biological control effect of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathology.* **74**: 498-501.
- Smolinska, U, B. Kowalska and M. Oskiera. 2007. The Effectivity of *Trichoderma* strains in the protection of cucumber and lettuce against *Rhizoctonia solani*. *Vegetable Crops Research Bulletin.* Vol. **67**, 81-93. DOI: 10.2478/v10032-007-0033-5.
- Sneha R. and Satya Prasad K.2014. In Vitro Determination of Efficacy of Contaf on the Mycelial Growth of *Fusarium Solani*, *Curvularia Clavata* and *Trichoderma Aureoviride*. *International Research Journal of Biological Sciences.* International Science Congress Association. ISSN 2278-3202. Vol. **3(12)**, 24-28, December.
- Soesanto L., E. Mugiastuti, R. F. Rahayuniati, A. Manan, and R. S. Dewi. 2018. Compatibility test of four *Trichoderma* spp. isolates on several synthetic pesticides. *AGRIVITA Journal of Agricultural Science.* 2018. **40(3)**: 481-489.
- Sonavane. P and Venkataravanappa. 2017. Compatibility studies of *Trichoderma harzianum* isolate with fungicides used against soil borne disease in coorg mandarin-pepper-coffee plantations. *Int.J.Curr.Microbiol.App.Sci.* **6(8)**: 346-354.
- Soriano-Martin, M. L., Porrás-Piedra, A., Porrás-Soriano. 2006. Use of microwaves in the prevention of *Fusarium oxysporum f. sp. melonis* infection during the commercial production of melon plantlets. *Crop Protection.* **25**, 52-57.
- Sreedevi. B., M. Charitha Devi and D. V. R. Saigopal. 2011. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. *Journal of Agricultural Technology.* Vol. **7(3)**: 623-635

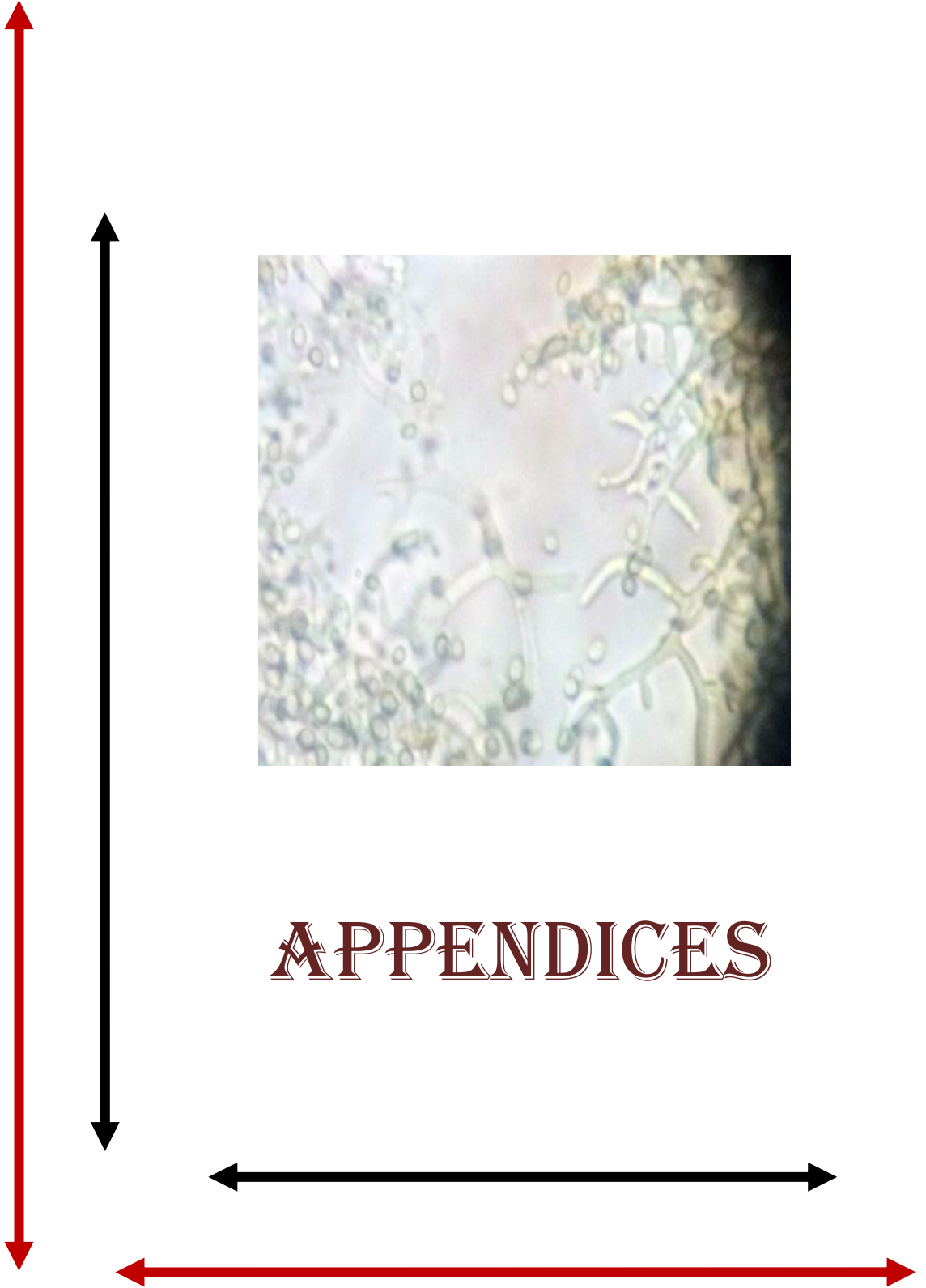
- Sreenayana B, S. Nakkeeran and P. Muthulakshmi 2019. Hyperparasitic interaction of *Trichoderma virens* TRI 37 with *Fusarium Oxysporum* f. sp. *cucumerinum* induce differential display of NVOC against cucumber vascular wilt pathogen. *J Mycol Pl Pathol*. Vol. **49**, No. **3**.
- Srivastava M., M. Shahid, S. Pandey, V. Kumar, A. Singh, S. Trivedi, Y. K. Srivastava and Shivram. 2015. *Trichoderma*: A scientific approach against soil borne pathogens. *Afr. J. Microbiol. Res.* Vol. **9(50)**, pp. 2377-2384.
- Steyaert, J. M., Weld, R. J., Mendoza- Mendoza, A., and Stewart, A. (2010). Reproduction without sex: conidiation in the filamentous fungus *Trichoderma*. *Microbiology* **156**, 2887–2900. doi: 10.1099/mic.0.041715-0.
- Swathi. B, A. K. Patibanda and P. Rani P. 2015. Antagonistic Efficacy of *Trichoderma* Species on *Sclerotium Rolfsi* in Vitro. *IOSR Journal of Agriculture and Veterinary Science* (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume **8**, Issue **7** Ver. I (July.), PP 19-22.
- Tapwal A.,A. Tyagi, G. Thakur and S. Chandra. 2015. *In-vitro* evaluation of *Trichoderma* species against seed borne pathogens. *IJCBS Research Paper* Vol. **1** [Issue **10**] January.
- Tapwal A. [R. Kumar](#), [N. Gautam](#) and [S. Pandey](#). 2012. Compatibility of *Trichoderma viride* for Selected Fungicides and Botanicals. *International Journal of Plant Pathology*. **3**: 89-94.
- Thoudam, R. and B.K. Dutta. 2014. Compatibility of *Trichoderma atroviride* with fungicides against black rot disease of tea: An *in vitro* study. *J. Int. Aca. Res. Multidiscipl.* **2**, 25-33.
- Thrane. Ulf., Sys B. Poulsen, Helgard I. Nirenberg and Elke Lieckfeldt. 2001. Identification of *Trichoderma* strains by image analysis of HPLC chromatograms. *FEMS Microbiology Letters*. **203**. 249-255.
- Tomer A., R. Singh and D. Prasad. 2018. Compatibility *Trichoderma harzianum* with systemic and two non systemic fungicides of *in vitro*. *Asian journal of crop science*. ISSN 1994-7879 DOI: 10.3923/ajcs.174-179.
- Vargas, W. A., crutcher, F.K. and Kenerley, C.M. 2011. Functional characterization of a plant like sucrose transporter from the beneficial fungus *Trichoderma virens*. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. *New phytologist*. **189**, 777-789.

- Vargas, W. A., Wippel, R., Goos, S., Kamper, J. and Sauer, N. 2009. Plant derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. *Plant physiology*. **151**, 792-808.
- Vinale F., Ghisalberti E. L., Sivasithamparam K., Marra R., Ritieni A., Ferracane R., Woo S. and Lorito M. 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. *The Society for Applied Microbiology, Letters in Appl Microbiol.* **48**: 705–711.
- Vinale F., Krishnapillai S., Emilio L., Ghisalberti R. M., Sheridan L. and Woo M. L. 2008. *Trichoderma* plant-pathogen interactions. *Soil Boil. & Biochem.***40**:1-10.
- Vinale F., Marra R., Scala F., Ghisalberti E. L., Lorito M. and Sivasithamparam K. 2006. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *The Society for Applied Microbiology, Letters in A ppl. Microbiol.* **43**: 143–148.
- Vincent, J. M. 1927. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*. **159** : **850**, 232-235.
- Vyas U. M., L. F. Akbari, T. Fartyal, C. Kukadiya and S. Karkar. 2020. Compatibility study of fungal and bacterial bio control agents with agro chemicals. *Journal of Pharmacognosy and Phytochemistry*. **9(5)**: 2132-2135.
- Weindling R. 1932.*Trichodermalignorum* as a Parasite of other Soil Fungi. *Phytopathology*. **22**, 837-845.
- Wu, Q., R. Sun, M. Ni, J. Yu, Y. Li, C. Yu, K. Dou, J. Ren, and J. Chen. 2017. Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. *PLoS ONE*. **12(6)**: e0179957. <https://doi.org/10.1371/journal.pone.0179957>.
- Xue M.,R. Wang,, C. Zhang, W. Wang,F. Zhang,D. Chen,S. Ren,Z. Manman,J. Houand T. Liu. 2021. Screening and Identification of *Trichoderma* Strains isolated from Natural Habitats in China with Potential Agricultural Applications.*Hindawi. BioMed Research International*. Volume, Article ID 7913950, 13 pages.
- Yadav B.C, Gupta R. P and Singh R. V. 2005. Comparative performance of *Trichoderma* spp. as seed dresser and soil application against *Fusarium udum* of pigeonpea. Abstract Presented in 2nd Global Conference Plant health Global wealth Nov. 25-29. Udaipur, India, **195**.

- Yadav K, T. Damodaran, Nidhi Kumari, Kakoli Dutt, Ram Gopal and M. Muthukumar. 2020. Characterization of *Trichoderma* isolates and assessment of antagonistic potential against *Fusarium oxysporum* f. sp. *cumini*. *Journal of Applied Horticulture*. **22(1)**: 38-44.
- Yusnawan. E, A. Inayati Andy. Baliadi. 2019. Isolation of antagonistic fungi from rhizospheres and its biocontrol activity against different isolates of soil borne fungal pathogens infected legumes. *BIODIVERSITAS*. Volume **20**, Number **7**, July ISSN: 1412-033X, E-ISSN: 2085-4722, Pages: 2048-2054.
- Zhang, S.; Gan, Y.; Xu, B. 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front. Plant Sci.* **7**, 1405.
- Zhu, Z.X., Xu, H.X., Zhuang, W.Y. and Li, Y., 2017. Two new green-spored species of *Trichoderma* (Sordariomycetes, Ascomycota) and their phylogenetic positions. *MycKeys*. **26**, 61.
- Zitter, T.A. 1998. Vegetable crops: *Fusarium* diseases of cucurbits fact sheet. Department of plant pathology. Cornell University. NewYork,
- Zivkovic S., S. Stojanovic, Z. Ivanovic, V. Gavrilovic, Tatjana Popovic, And J. Balaz. 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum Acutatum* and *Colletotrichum Gloeosporioides* *Arch. Biol. Sci.*, Belgrade. **62(3)**, 611-623.



# APPENDICES



**APPENDIX – I**  
**ABBREVIATIONS USED**

%	: Per cent
/	: Per
@	: At the rate
<sup>0</sup> C	: Degree centigrade
C.D.	: Critical Difference
cm	: Centimeter
mm	: Millimetre
µm	: Micrometre
Co.	: Company
Conc.	: Concentration
d. f.	: Degree of freedom
Dist.	: District
<i>et al.</i>	: And others
<i>etc.</i>	: Etcetera
Fig.	: Figure
g	: Gram
ha	: Hectare
i.e.	: That is
Kg	: Kilogram
m	: Meter
M.S.S.	: Mean sum of square
mg	: Milligram
<i>Spp.</i>	: Species
mm	: Millimeter
PDA	: Potato Dextrose Agar
RH	: Relative humidity
Pvt.	: Private
S.E.	: Standard error
<i>viz.</i>	: Namely
Min	: Minutes
WP	: Wettable Powder
EC	: Emulsifiable concentrate

WS	: Water soluble
lbs	: Pounds
p.s.i.	: Per square inch
hrs	: Hours
Sig.	: Significant
F-cal	: F calculated
COC	: Copper oxychloride
OMA	: Oat meal agar medium
CMDA	: Corn meal dextrose agar
TEF- $\alpha$	: Translation elongation factor -alpha
IDM	: Integrated diseases management
UV	: Ultraviolet visible light
r-DNA	: Recombinant Deoxyribonucleic acid
RNA	: Ribonucleic acid
ITS	: Internal Transcribed Spacer
BLAST	Basic local alignment search tool
ARI	: Agharkar Research Institute
RBP	: Retinol-binding protein
NCBI	: National center for Biotechnology Information
NFCCI	: National fungal culture collection of India

**APPENDIX – II**  
**STATISTICAL ANALYSIS**

**ANOVA Table: *In vitro* efficacy of *Trichoderma* isolates against *Fusarium* spp**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	27	49.21654	1.892944	83.67174	1.99852	Sig.
Error	56	1.221667	0.022623			
Total	80	50.438				

**ANOVA Table: *In vitro* efficacy of *Trichoderma* isolates against *Rhizoctonia* spp.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	27	147.6957	5.470212	250.7491	1.974421	Sig.
Error	56	1.221667	0.021815			
Total	83	148.917				

**ANOVA Table: *In vitro* efficacy of *Trichoderma* isolates against *Sclerotium* spp.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	27	296.9294	10.99738	281.6403	1.974421	Sig.
Error	56	2.186667	0.039048			
Total	83	299.116				

**ANOVA Table: *In vitro* efficacy of *Trichoderma* isolates against *Colletotrichum* spp.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	27	150.8274	5.586199	144.9952	1.974421	Sig.
Error	56	2.1575	0.038527			
Total	83	152.985				



**ANOVA Table: *In vitro* efficacy of *Trichoderma* isolates against *Alternaria* spp.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	27	121.8023	4.511195	110.2372	1.974421	Sig.
Error	56	2.291667	0.040923			
Total	83	124.094				

**ANOVA Table: *In vitro* efficacy of fungicides- Carbendazim.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	6	210.110	35.01833	788.6166	3.871427	Sig.
Error	14	0.622	0.044405			
Total	20	210.732				

**ANOVA Table: *In vitro* efficacy of fungicides- Hexaconazole.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	6	219.353	36.55881	2132.597	3.871427	Sig.
Error	14	0.240	0.017143			
Total	20	219.593				

**ANOVA Table: *In vitro* efficacy of fungicides- Thiophenate-methyl.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	6	202.865	33.81079	1092.349	3.871427	Sig.
Error	14	0.433	0.030952			
Total	20	203.298				

**ANOVA Table: *In vitro* efficacy of fungicides- Copper-oxchloride.**

Source	DF	SS	MSS	Cal F	Significance F	Result
Treatments	6	213.566	35.59429	344.8581	3.871427	Sig.
Error	14	1.445	0.103214			
Total	20	215.011				

**ANOVA Table: *In vitro* efficacy of fungicides- Sulphur.**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MSS</b>	<b>Cal F</b>	<b>Significance F</b>	<b>Result</b>
Treatments	6	263.833	43.97218	7387.327	3.871427	Sig.
Error	14	0.083	0.005952			
Total	20	263.916				

**ANOVA Table: *In vitro* efficacy of fungicides- Mancozeb.**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MSS</b>	<b>Cal F</b>	<b>Significance F</b>	<b>Result</b>
Treatments	6	233.731	38.95512	2256.71	3.871427	Sig.
Error	14	0.242	0.017262			
Total	20	233.972				

## APPENDIX – III

**Primer:** EF-983F & EF-2118R

### Sequence

>NFCCI\_5020 *Trichoderma asperellum* isolate Tas

GACTGCGCTATCCTGATTATCGCTGCCGGTACTGGTGAGTTCGAGGGCTGGTA  
TCTCCAAGGATGGCCAGACCCGTGAGCACGCTCTGCTCGCCTACACCCTGGG  
TGTCAAGCAGCTCATCGTTGCCATCAACAAGATGGACACTGCCAACTGGGCT  
GAGGCTCGTTACCTTGAGATCATCAAGGAGACCTCCAACTTCATCAAGAAGG  
TCGGCTTCAACCCCAAGACCGTTGCCTTCGTCCCCATCTCCGGTTTCAACGGT  
GACAACATGCTGTCCCCCTCCACCAACTGCCCTGGTACAAGGGCTGGGAGA  
AGGAGACCAAGGCTGGCAAGTCCACCGGTAAGACCCTCCTCGAGGGCCATCG  
ACGCCATTGAGCCCCCAAGCGTCCCACAGACAAGCCCCTCCGTCTGCCCT  
CCAGGACGTCTACAAGATCGGTGGTATCGGAACAGTCCCTGTCGGCCGTATC  
GAGACTGGTGTCTCAAGCCCGGTATGGTCGTACCTTCGCTCCCTCCAACGT  
CACCCTGAAGTCAAGTCCGTGAGATGCACCACGAGCAGCTCGCTGAGGGT  
GTCCCCGGTGACAACGTTGGATTCAACGTCAAGAACGTCTCTGTCAAGGATA  
TCCGCCGTGGTAACGTTGCCGGTGACTCCAAGAACGACCCTCCCATGGGTGC  
CGCTTCTTTCAACGCCAGGTCATTGTTCATGAACCACCCTGGCCAGGTCGGT  
GCCGGTTACGCTCCCGTCCCTCGATTGCCACACTGCCACATTGCCTGCAAGTT  
CTCTGAGCTCCTCGAGAAGATCGACCGCCGTACCGGTAAGGCTACTGAGGCC  
TCCCCCAAGTTCATCAAGTCTGGTGACTCCGCCATCGTCAAGATGGTTCCCTC  
CAAGCCCATGTGCGTTGAGGCTTTCACCGACTACCCTCCCCTGGGTGCTTTCG  
CCGTCCGTGACATGCGTCAAAC

>NFCCI\_5021 *Trichoderma harzianum* isolate Tmnrj

GGCTGACTGCGCCATTCTCATCATTGCCGCCGGTACTGGTGAGTTCGAGGGCT  
GGTATCTCCAAGGATGGCCAGACTCGTGAGCACGCTCTGCTCGCCTACACC  
TGGGTGTCAAGCAGTTATCGTTGCCATCAACAAGATGGACACTGCCAACTG  
GGCCGAGGCTCGTTACCAGGAAATCATCAAGGAGACTTCCAACTTCATCAAG  
AAGGTGCGCTTCAACCCCAAGGCTGTTGCTTTCGTCCCCATCTCCGGTTTCAA  
CGGTGACAACATGCTCCAGCCCTCCACCAACTGCCCTGGTACAAGGGTTGG  
GAGAAGGAGACCAAGGCTGGCAAGTTCACCGGCAAGACCCTCCTTGAGGCC  
ATCGACTCCATCGAGCCCCCAAGCGTCCCACGGACAAGCCCCTCCGTCTTC  
CCCTCCAGGATGTCTACAAGATCGGTGGTATCGGAACAGTTCCCGTCCGGCCG  
TATCGAGACTGGTGTCTCAAGCCCGGTATGGTCGTACCTTCGCTCCCTCCA  
ACGTCACCCTGAAGTCAAGTCCGTGAGATGCACCACGAGCAGCTCACCGA  
GGGTGTTCCCGGTGACAACGTTGGTTTCAACGTCAAGAACGTTTCCGTTAAG  
GAAATTCGCCGTGGTAACGTTGCCGGTGACTCCAAGAACGACCCCCCATGG  
GTGCCGCTTCTTTCACCGCTCAGGTCATCGTCATGAACCACCCTGGCCAGGTC  
GGTGCCGGCTACGCCCCGTTCTTGACTGCCACACTGCCACATTGCCTGCA  
AGTTCGCCGAGCTCCAGGAGAAGATCGACCGCCGTACCGGTAAGGCTACCG  
AGACTGCCCCAAGTTCATCAAGTCCGGTGACTCTGCCATCGTCAAGATGAT  
TCCCTCCAAGCCATGTGCGTTGAGGCTTTCACCGACTACCCTCCCCTGGGTG  
GTTTCGCCGTCCGTGACATGC

>NFCCI\_5022 *Trichoderma asperellum* isolate Tbk

GCTGACTGCGCTATCCTGATTATCGCTGCCGGTACTGGTGAGTTCGAGGGCTG  
GTATCTCCAAGGATGGCCAGACCCGTGAGCACGCTCTGCTCGCCTACACCCT

GGGTGTCAAGCAGCTCATCGTTGCCATCAACAAGATGGACACTGCCAACTGG  
GCTGAGGCTCGTTACCTTGAGATCATCAAGGAGACCTCCAACCTTCATCAAGA  
AGGTCGGCTTCAACCCCAAGACCGTTGCCTTCGTCCCCATCTCCGGTTTCAAC  
GGTGACAACATGCTGTCCCCCTCCACCAACTGCCCTGGTACAAGGGCTGGG  
AGAAGGAGACCAAGGCTGGCAAGTCCACCGGTAAGACCCTCCTCGAGGCCA  
TCGACGCCATTGAGCCCCCAAGCGTCCCACAGACAAGCCCCTCCGTCTGCC  
CCTCCAGGACGTCTACAAGATCGGTGGTATCGGAACAGTCCCTGTGGCCGT  
ATCGAGACTGGTGTCTCAAGCCCGGTATGGTCGTCACCTTCGCTCCCTCCAA  
CGTCACCACTGAAGTCAAGTCCGTGAGATGCACCACGAGCAGCTCGCTGAG  
GGTGTCCCCGGTGACAACGTTGGATTCAACGTCAAGAACGTCTCTGTCAAGG  
ATATCCGCCGTGGTAACGTTGCCGGTGACTCCAAGAACGACCCTCCCATGGG  
TGCCGCTTCTTTCAACGCCAGGTCATTGTCATGAACCACCCTGGCCAGGTCG  
GTGCCGGTTACGCTCCCGTCCCTCGATTGCCACACTGCCACATTGCCTGCAAG  
TTCTCTGAGCTCCTCGAGAAGATCGACCGCCGTACCGGTAAGGCTACTGAGG  
CCTCCCCAAGTTCATCAAGTCTGGTGACTCCGCCATCGTCAAGATGGTTCCC  
TCCAAGCCCATGTGCGTTGAGGCTTTCACCGACTACCCTCCCCTGGGTCGTTT  
CGCCGTCCGTGACATGCGTCA