CHARACTERIZATION AND MANAGEMENT OF *Ganoderma lucidum* INCITING BASAL STEM ROT OF COCONUT

By

YUNUS C.

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut"** is a bonafide record of research work done by me during the course of research and that the thesis has not been previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 17.08.2012

Yunus C.

(2010-11-121)

Dr. S. Beena

(Major Advisor, Advisory Committee)
Professor,
Department of Plant Pathology,
College of Horticulture
Vellanikkara

CERTIFICATE

Certified that this thesis, entitled **"Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut"** is a record of research work done independently by **Mr. Yunus C. (2010-112-121)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara
17.08.2012

Dr. S. Beena
(Major advisor, Advisory committee)

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Yunus C. (2010-11-121),** a candidate for the degree of **Master of Science in Agriculture,** with major field in **Plant Pathology,** agree that the thesis entitled **"Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut"** may be submitted by **Mr. Yunus C.**, in partial fulfilment of the requirement for the degree.

26/9/12

Dr. S. Beena
Professor
Dept. of Plant Pathology
College of Horticulture
Vellanikkara
(Major Advisor, Advisory Committee)

26/9/12

Dr.T. J. Rehumath Niza
Profeesor & Head
Dept. of Plant Pathology
College of Horticulture
Vellanikkara
(Member, Advisory Committee)

Sheela Paul
26/9/12.

Dr. T. Sheela Paul
Professor
Dept. of Plant Pathology
College of Horticulture
Vellanikkara
(Member, Advisory Committee)

26/9/12

Dr. D. Girija
Professor & Head
Dept. of Agrl. Microbiology
College of Horticulture
Vellanikkara
(Member, Advisory Committee)

Dr. M. Balasundaram
H.O.D Biotechnology
(Retired)
KFRI, Peechi.

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YUNUS C.

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Dedicated to My Guide and My Family

INTRODUCTION

INTRODUCTION

Coconut (*Cocos nucifera* Linn.) belonging to *Arecaceae* is known as 'Kalpavriksha", in view of its enormous contribution to the mankind, every part of the plant is having one or the other uses. It is a major plantation as well as oil seed crop in the tropics of the world with an area and production of 12.5 million hectares, 53.6 billion nuts respectively. India is the third largest coconut producing country with an estimated production of 15729.75 million nuts from 18.94 lakhs hectares with productivity of 8303 nuts/ha (All India final estimate of coconut, 2008-2009). In India, coconut is predominantly cultivated in the southern states *viz.*, Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. Among these, Kerala ranks first in area and production, with an area of 8.5 lakh/ha having production of 57616.36 lakh nuts with a productivity of 6703nuts/ha (All India final estimate of coconut, 2010-2011).

Coconut palm inspite of its hardiness is affected by a large number of diseases, of which bud rot, stem bleeding, basal stem rot and root (wilt) are of economically important. Basal stem rot (BSR) disease caused by *Ganoderma lucidum* (Leys.) Karst is one of the most destructive diseases and a major limiting factor in coconut growing states of India (Sankaran *et al.*, 2005). The incidence of *G.lucidum* in coconut was first recorded by Butler in 1913 from Karnataka state and now it is prevalent in all the major coconut growing states of India. It was reported from Thanjavur district of Tamil Nadu after a cyclone in1952. Hence, BSR is also known as Thanjavur wilt. The other names of the disease are *Ganoderma* disease, bole rot and *Anabe roga* and are known in these names in different states of India (Srinivasulu *et al.*, 2001).

The genus *Ganoderma* has a world wide distribution causing root and stem rots of many plantation crops, including, coconut, rubber, betel nut, tea, cocoa, peaches and pears, grape vine and forest trees like acacia, albizia etc. In forest systems, the pathogen has an ecological role in delignification of woody plants. The bracket shaped fruit body produced on the dead stumps of the tree is the most easily identifying structure associated with the pathogen. The palms and forest trees in the age group of 5-30 years were easily attacked by the pathogen. The symptom first appeared as exudation of reddish brown viscous fluid from the basal portion of the stem and gradually the bleeding patches may extend to 3m upwards as the disease progresses. Under prolonged infection, the bark from the base of the stem peels off. In few cases, just prior to the death of palm, sporophore

of *G.lucidum* appears at the base of the stem or from roots. In the crown region, drooping and drying of leaflets in outer whorls observed. Subsequently the other leaves also drooped down in quick succession leaving the unopened spindle leaf alone. In the final stage of disease, the spindle also breaks and falls off leaving the decapitated stem. Infection spreads to neighbouring palms by root contact if one palm becomes infected. It is a soil borne pathogen and can spread through the soil by root contact. Once established in the field, it is very difficult to eradicate the pathogen.

The basidiomycetous fungus *Ganoderma lucidum* classified under the *Phylum Basidiomycota* in the Kingdom Fungi. The morphological feature of basidiocarps as well as host specificity and geographical distribution has been used in the taxonomy of *Ganoderma* sp. Iyer *et al.* (2004) reported the inhibiting effect of leaf extracts of *Clerodendron infortunatum* (80.7%), *Trichoderma harzianum* (72.2%) and *T. viride* (62%) on the mycelial growth of *G.lucidum* causing basal stem rot of arecanut. Karunanithi *et al.,* (2007) evaluated the effect of 29 plant products and found that 10 per cent leaf extract of *Azadirachta indica* was effective in suppressing the mycelial growth of *G.lucidum in vitro.*

Currently, no cost effective fungicide is available that gives guaranteed control. Development of biological control for basal stem rot disease is accepted as a durable and environment friendly alternate for agrochemicals. Raising intercrops *viz.*, banana, turmeric, cocoa and pineapple increased the yield and were found to be highly profitable to the farmers, *in vitro* studies indicated that banana rhizome extract were highly inhibitory to the growth of *G. lucidum* (Bhaskaran *et al.,* 1988). Treatment of BSR infected palms with rhizosphere bacteria and fungi have been reported to sensitize plants to defend themselves against the pathogen attack by triggering various defence mechanisms including production of phytoalexins, synthesis of phenolics (Chen *et al.,* 2000), accumulation of pathogenesis related proteins (Meena *et al.,* 2000) and deposition of structural barriers (Benhamou *et al.,* 1996).

Recently severe disease incidence in coconut is noticed in several farmers field in Kerala, which resulted in severe yield reduction and death of the palm. Perusal of literature revealed several reports on various aspects of this disease from Tamil Nadu and Karnataka states of India, but only a few from Kerala. Detailed information regarding the character of the pathogen and its management are lacking. Considering the importance of the disease, this project was selected

with an intention to identify and characterize the pathogen and to study the relationship with other crops like arecanut, jack fruit, breadfruit and acacia and also to evolve effective measures for the management of the pathogen.

The research programme entitled "Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut" envisaged the following aspects.

1. Survey and collection of basidiocarps and diseased samples of coconut palm.
2. Isolation and maintenance of the pathogen from different locations.
3. Symptomatology of the disease.
4. Characterization of various isolates of *Ganoderma* sp.
5. Management of the pathogen
    - Isolation of rhizosphere micro-organisms.
    - *In vitro* evaluation of antagonistic property against the pathogen.
    - *In vitro* evaluation of fungicides and phytoextracts against the pathogen.
6. Host range of the isolates of *Ganoderma lucidum*

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Coconut (*Cocos nucifera*) belongs to *Arecaceae*, is an important oil seed as well as plantation crop in India. The coconut palm is found throughout the tropics, where it is interwoven into the lives of the local people. It provides almost all necessities of life-food, drink, oil, medicine, fibre, timber, thatch, mats, fuel and domestic utensils. For good reason, it has been called the "tree of heaven" and "tree of life". In Kerala, it is known as "Kalpavriksha" because of its multifarious uses of every part of it in the commercial sector and therapeutical sector. Coconut palm, inspite of its hardiness is affected by a large number of diseases, of which bud rot, stem bleeding, basal stem rot (BSR) and root (wilt) are of economically important. BSR disease caused by *G. lucidum* (Leys) Karst. is one of the most destructive diseases and is the major limiting factor in coconut production especially in Kerala, Tamil Nadu, Andhra Pradesh and other coconut growing states of India. The literatures reviewed on various aspects pertaining to objectives are given here under.

2.1. OCCURRENCE AND DISTRIBUTION OF THE DISEASE

The pathogen, *Ganoderma lucidum* is ubiquitous in its distribution and causes 'anabe roga' (mushroom disease) on several hosts in temperate and tropical countries. It was first recognised as a pathogen on coconut palm in 1846 (Petch, 1916). The first information published on *Ganoderma* from India dates back to the early 1900s (Lloyd). Butler (1906) reported its importance as a pathogen on cash crops such as coconut, betel nut and other plantations species including *Casuarina, Areca catechu, Dalbergia sissoo* and *Toona ciliate* in the northern states.

In India, BSR disease was first reported on arecanut palm from Karnataka state by Coleman (1911). Two year later, Butler (1913) recorded *G. lucidum* on coconut palm for the first time from the same state. BSR on oil palm was first described during 1915 in the Republic of Congo, West Africa (Wakefield, 1920). Thompson (1931) detected the disease infecting oil palms of over 25 years in Malaysia.

In 1936, Venkatarayan recorded *Ganoderma* diseases of coconut, betel nut, *Cassia siamea* and *Pongamia* from South India. However the gravity of disease problem in forest crops was first published by Bagchee (1945) and later in 1950 Bagchee abd Bakshi reported diseases of forest trees such as *Acacia catechu, D. Sissoo* and *Shorea robusta* in North India.

In Tamil Nadu, it was first reported from Thanjavur district after a cyclone during 1952 and hence it was named as Thanjavur wilt in Tamil Nadu (Vijayan and Natarajan, 1972). Peries (1974) reported basal stem rot disease of coconut in Srilanka caused by *G. boninense*. Rao and Rao (1966) reported that in Andhra Pradesh, the disease was mainly prevalent in lighter soils in the coastal districts than in heavy soils.

Basal stem rot of oil palm has been recorded in Malaysia and Indonesia in South East Republic of Congo in Africa and Papau New Guinea in Oceanid (Turner, 1981). The fungus occurs in India, Pakistan, Srilanka, Burma, Malaysia and also in the tropical and temperate regions of Europe and USA (Govindu *et al.*, 1983) and he also reported that in Karnataka, the disease was widely prevalent in the maiden tract especially in sandy soils of the southern region. Bhaskaran *et al.* (1984) reported that BSR disease was confined to the coastal areas of Tamil Nadu and the incidence of disease ranged from 3.3 - 10.8 per cent with the highest incidence of 10.8 per cent in Thanjavur district followed by Kanyakumari district with 6.3 per cent. Occurrence of BSR of coconut in Kerala was first noted in Palakkad district in 1987 (Wilson *et al.*, 1987) and the disease is now widespread in Kerala, posing a threat to coconut based industries and the livelihood of small scale farmers.

Sengupta *et al.* (1990) observed that basal stem rot of oil palm, which occurs widely in S.E.Asia, was known only from the state of Tripura in India. Kumar and Nambiar (1990) reported that the incidence of BSR in arecanut and was found to be as high as 20-25 per cent. Singh (1991) reported that oil palm planting following coconut, *Ganoderma* infection may become apparent as early as 12-24 months from planting, but more usually when palms were 4-5year old. There after, the incidence could reach 40-50 per cent, by the time of palms were

15 years old. A similar situation was also reported where oil palm was replanted. In oil palm-a high incidence of BSR could be observed after 15 years of planting.

In India, Mehotra *et al.* (1996) reported the red root rot of *Acacia mangium* caused by *Ganoderma* spp. Lee (2000) reported that the species of *Ganoderma* however, have been reported as the most frequent root rot causal agents in *Acacia mangium* in both Malaysia and Indonesia. Rajamannar *et al.* (2000) reported that all the coconut cultivars and hybrids of Kerala were infected by the pathogen, and the surviving plants showed symptoms of the disease. Deepthi *et al.* (2003) reported that coconut palms in row are found infected rather than distantly placed palm. Karthikeyan (2004) reported that the disease incidence was more in 7 to 50 years old palms compared to young palms.

Sankaran *et al.* (2005) conducted extensive surveys in oil palm plantations in Kerala state and could not detect BSR incidence in oil palm. He concluded that the disease might be prevalent in India, but it was unrecorded since sporocarps were absent during the early stages of infection. Samiyappan *et al.* (2006) observed this disease in east coast tall palms and found that palm with age groups of 5 to 30 years were more susceptible than young palms and which showed a disease incidence of 43 % compared to young trees.

2.2. SYMPTOMATOLOGY OF BASAL STEM ROT DISEASE

The symptoms of BSR disease can be seen on the root, stem and crown of infected palms. Different symptoms of disease were described as follows. In 1974, Peries and co workers presented detailed description of the symptomatology of the disease. They observed that the roots were first affected and destroyed and visual symptoms of the disease were similar to those of severe drought. They noticed the poor developments of female flowers and nuts. The nuts became small and distorted when matured and dark brown streaks were also observed on husk. Characteristic reddish brown parallel streaks were developed at the base of the stem, from which exudation of brown, viscous gummy substance was observed.

According to Bhaskaran *et al.* (1982, 1989) and Bhaskaran (1986) the palms and forest trees in the age group of 5-30 years were easily attacked by the pathogen. The fungus first attack the root system and the external symptoms were not clearly visible during very early stage of infection. The symptom first appeared as exudation of reddish brown viscous fluid from the basal portion of the stem and gradually the bleeding patches may extend to 3m upwards as the disease progresses. Production of a dry rot of internal tissue at the base of the stem was characteristic. The stem decays rapidly and resulted in the formation of large cavities and the palms. break off at the base and fell down. Under prolonged infection, the bark from the base of the stem peeled off. In few cases, just prior to the death of palm, sporophore of *G.lucidum* appeared at the base of the stem or from roots. In the crown region, drooping and drying of leaflets in outer whorls observed. Subsequently the other leaves also drooped down in quick succession leaving the unopened spindle leaf alone. In the final stage of disease, the spindle also broken and fell down leaving the decapitated stems in the affected roots, the cortical region turned brown first and the roots became friable and disintegrated. Formation of new roots was progressively reduced. The roots became watery with a distinct smell of alcohol.

In 2002, Srinivasulu and co workers noticed that in advanced stage of infection, the roots became watery with a distinct smell of alcohol. Deepthy *et al.* (2003) reported that the roots of infected coconut palm were the first region infected by the pathogen and observed discolouration and extensive rotting of root system. The roots became watery, exude distinct alcoholic smell and further production of roots was highly reduced. They also observed exudation of reddish brown viscous fluid and browning of internal tissues from the basal portion of the stem. Development of bracket shaped sporophores of *G. lucidum* was also noticed at the base of the stem. The other symptoms they noticed were the yellowing, browning and drooping of outer whorl of leaves around the trunk. Finally the crown blown off leaving decapitated stem. Normal development of flowers and bunches were not observed.

Karunanithi *et al.* (2005) reported that the visual symptoms of BSR disease were generally found in adult coconut palms of more than 10 year old. They observed the initial symptom of the disease as oozing out of reddish brown viscous fluid from the basal portions of the stem near the ground level. They also reported extensive root rotting and discolouration of roots due to the disease incidence and the roots become watery with a distinct alcoholic smell. Production of new roots was also progressively reduced. They also noticed drooping down and drying of leaves, heavy button shedding, stem bleeding and finally death of palm. In some palms, they found the sporophore or fruiting body of *G.lucidum* at the base of the trunk prior to wilting or just after the death of palm. They reported that the time taken from the initial appearance of bleeding patches on the stem to death of palm was about 6-54 months, with an average of 24 months.

2.3. THE GENUS *GANODERMA*

The genus *Ganoderma* has been known for a little over 100 years and was introduced by Finnish mycologist Peter Adolf Karsten, in 1881 (Karsten 1881), with *G.lucidum* (Curtis:Fr.)P.Karst. from England as the type species. The genus belongs to the family *Ganodermataceae* that resides in the order polyporales of the Class *Basidiomycetes*. The family includes eight genera that are distinguished by the unique double walled basidiospores. The genus *Ganoderma* was further sub divided into two subgenera: Subgenus *Ganoderma* based on *G.lucidum* for the laccate species and subgenus *Elfivingia* based on *G.applanatum* for the species with a non-laccate fruiting body (Monocalvo and Ryvarden, 1997). Thus, *G.lucidum* and *G.applanatum* are the two important species complexes in the history and nomenclature of the genus.

The fungus *G.lucidum* was first described under the name *Fomes lucidus* (Leys) Fr. (Butler, 1909). According to Butler and Bisby (1931), the synonyms with *G.lucidum* were *Fomes lucidus*, *Polyporus lucidus*, *G. resinoceum* Boud., *Polystictus egregious* Massee, *G.amboinensis* (Lam.) Pat., *F. Amboinensis* Lam., *P.amboinensis* Ft. Nambiar and Nair (1973) reported that the best medium for growth of *G.lucidum* was Walksman's medium. Basidiocarp production was

observed in saw dust medium (moist saw dust 300g, 10 per cent malt extract and 15ml of biotin, 5ppm) two months after inoculation.

In 1994, Ryvarden reported that more than 250 *Ganoderma* species were described world wide and most of these descriptions have been based on pleomorphic characters. However, uncertaintiy exists about the taxonomic status of many of these species and, the taxonomy of the genus was thus considered to be chaotic and one of the most difficult genera amongst the polypores.

In 2000, Seo and Kirk reported that *G.lucidum* and *G.applanatum* were the two important species complexes and these two were the most poorly understood species of *Ganoderma* and most frequently with misapplied names. Schwarze and Ferner (2003) noticed that when the spores of *Ganoderma* sp. incorporated into the soil it germinated and the hyphae grew over the roots. The fungus simply moved from the roots to the woody trunk tissue where it destroyed the wood affecting the xylem.

Sankaran *et al.* (2005) reported that the identity of most of the species of *Ganoderma* recorded from India had been determined from morphological and cultural characteristics alone and hence many of the names used were incorrect, particularly *G.lucidum* and *G.applanatum*, the names most commonly used in India. He also reported that species of *Ganoderma* which caused root and stem rot disease resulting in losses of crops such as coconut, oil palm, betel nut, rubber, ornamental plants, forest trees and other trees. Pilotti *et al.* (2003) and Pilotti (2005) observed that presence of the fungus could be easily detected when fruiting bodies (basidiocarp) appeared on the stem of a tree and also reported that *Ganoderma* spread primarily through basidiospores.

2.4. ISOLATION OF THE PATHOGEN

During 1987, several workers in India reported that *Ganoderma* cannot be isolated from above ground parts of palm. Two years later, in 1989, Bhaskaran and co-workers isolated *G.lucidum* and *G.applanatum* only from roots of the infected palm even though they attempted to isolate the pathogen from above ground parts and sporocarps. Bhaskaran *et al.* (1991) reported that among

samples of root, bole, bark and cortex taken from affected palms, *G.lucidum* and *G.applanatum* were isolated only from root pieces.

Pilotti *et al.* (2002) found that monokaryotic cultures were obtained by germinating basidiospores of *G.boninense* on water agar with subsequent transfer to PDA. Dikaryotic cultures were isolated from the context of fresh basidioma growing on oil palm and on dead wood. Iyer *et al.* (2004) reported that isolations made from the centre of the fresh fruiting body were found to be better for obtaining pure cultures of *Ganoderma*. They suggested that for the isolation of *Ganoderma* sp. sporophore and diseased root bits were found to be the good sources and the percentage of isolates obtained from sporophore and diseased root bits was 50 and 40 respectively. They could not isolate the pathogen from diseased stem bits.

Naik *et al.* (2008) found that the fungus could be isolated from freshly affected stem piece, root and sporophore by using Walksman's agar medium. Palanna *et al.* (2009) isolated *Ganoderma* sp. from the basidiocarps kept in moist chamber. For this they collected the specimens, surface sterilized and kept in sterilized bags along with wet cotton under room temperature for 8-10 days. The slight mycelial growth observed was transferred to PDA medium. In the same year, Muthelo (2009) isolated the pathogen of BSR by aseptically placing small pieces of basidiocarp tissues on malt extract medium (MEA) supplemented with 0.1g/litre streptomycin sulphate and incubated at 22^0C in the dark for 3-5 days. Fungal colonies obtained were then aseptically transferred to fresh MEA media without streptomycin and incubated for two weeks. In the next year, Mishra and Singh (2010) isolated the collected specimens using tissue culture technique on the Potato dextrose agar (PDA) in the Petri plates and incubated at 24 ± 2^0C for about a week for their growth.

2.5. PATHOGENICITY

In India, BSR disease of coconut was reported as early as 1952, but the isolation of the pathogen involved in the disease and establishing pathogenicity continued to remain as an unsolved problem. Turner (1981) had listed 15 species

of *Ganoderma* which have been recorded as likely pathogens from different parts of the world to be associated with BSR disease and he considered that a single species was unlikely to be the sole cause of the disease in any particular area. Bhaskaran *et al.* (1991) used colonized root pieces as inoculums to prove pathogenicity of *G.lucidum* and *G. applanatum* in coconut and tied to healthy intact roots of healthy palms by means of polythene stripes. They observed root rotting only in palms inoculated with *G.lucidum* at six months after the inoculation. *G. applanatum* was found to colonize the root surface for 8-10cm. They reisolated *G.lucidum* from both cortical tissues and bark of the roots and *G. applanatum* from the bark. The oozing of liquid in the stem and drooping of leaves associated with Thanjavur wilt were not observed in inoculated palms during the six months of the experiments.

Sariah *et al.* (1994) successfully proved the pathogenicity of *Ganoderma boninense* on oil palm seedlings. At Ambagipetta, Andhra Pradesh, *G. applanatum* inoculated coconut seedlings succumbed to infection. At Hirehalli, Karanataka, pathogenicity of *G.lucidum* on coconut was established by Bhaskaran *et al.* (1996). Ganesh Naik (2003) established pathogenicity of *G.lucidum* in three years old Arsikere tall coconut seedlings by root inoculation method. Karthikeyan *et al.* (2007) reported that the pathogenicity and diagnostic methods were standardized for *Ganoderma* disease of coconut. The pathogenicity of *G. lucidum* isolated from coconut was tested using six types of inoculation techniques. Two diagnostic methods, *viz.,* indirect ELISA and PCR were applied for the confirmation of pathogenicity in coconut seedlings.

Kandan *et al.* (2009) tested the pathogenicity of *G.boninenese* on coconut seedlings under green house condition and infection confirmed by using immunological and molecular diagnostic tools. Dessication of older leaves and the emergence of sporophores were observed from pathogen inoculated seedlings, where as a control seedling does not show any pathogenic symptom. They reported that mature sporophores were formed within 10-13 weeks after inoculation.

In 2009, Palanna and co-workers had done a pot culture experiment to prove pathogenicity of *Ganoderma* isolates on coconut seedlings through soil inoculation technique. Nine isolates of *Ganoderma* were mass multiplied separately on sorghum grains in poly bags and were used for soil inoculation by mixing (200g/pot) with the potting mixture at the time of planting. Among the nine isolates, only two isolates were found to be pathogenic. These two isolates caused complete wilting of plants and were reisolated.

2.6. MORPHOLOGICAL CHARACTERS

The taxonomy of Basidiomycetes has traditionally been based on the morphological features of the basidiocarps. Basidiocarps which are the sexual structures in *Ganoderma* and other polypores, grow from a living or more commonly, from a dead trunk or branch of a tree in the form of bracket. One of two types of basidiocarps was produced, depending on the species: a laccate fruiting body with a shiny upper surface or a non-laccate fruiting body with a dull upper surface. Govindu *et al.* (1983) studied the morphological character of mushroom and chlamydospores and observed that the mycelium was aerial, hyaline, thin walled, branched with frequent clamp connections, 1.4-2.9µm in diameter, chlamydospores formed abundantly which were slightly thick walled, terminal or intercalary, ellipsoid, sometimes in chains, 8.8-11.8µm x 3.7-5.9µm in size; cuticular cells from crustose layer hyaline to light brown round to irregular in shape and closely packed. According to Adaskaveg and Gilbertson (1988), the naturally produced basidiocarps of *G.lucidum* showed various morphological characteristics. Perennial, stipitate, usually lateral, sometimes sessile, corky, becoming woody later, usually 10-12 x 10-12 x 3-4 cm, but may grow up to 30cm or more; upper surface is shining, laccate crust ox-blood in colour and smooth. The palisade hyphae were about 40µm long and were impregnated with a dark orange varnishing substance which they secrete. Hymenial surface was whitish or creamish, turning brown later, pores small, round, 90-250µm in diameter. Pore tubes were about 6-7mm long, basidiospores were brown, thick walled, minutely verrucose, truncated at one end and 8.3-10.0 x 5.8-6.7µm in size.

They also observed differences in the basidiospore morphology for different species within this fungal genus. Shin and Seo (1988) reported that fruiting body formation in *G.lucidum*, usually requires three months on saw dust medium. Under dim or dark conditions with poor ventilation, the pileus did not expand and often an abnormal pileus of the 'stag-horn' or 'antler-type' was produced. They also observed fruit bodies of the *G.lucidum* complex showing polymorphic features such as kidney type and antler type with various colours in saw dust bottle culture method. The same observations were made by Stamets (1993a,1993b) and reported that the fruit body formation in *G.lucidum* required three months on saw dust medium and the basidiocarps formed were very sensitive to light and ventilation. The stipe exhibited tropic growth toward light and under dim light or dark conditions with poor ventilation, the pileus did not expand and an abnormal pileus of the 'stag-horn' or 'antler –type' was produced.

Ryvarden (1994) stated that the size of the basidiospores should be used carefully in distinguishing *Ganoderma* sp. He reported that hyphal system in the *Ganodermataceae* was usually trimitic and some occasionally dimitic with the generative hyphae hyaline, thin walled, branched, clamped and septate or not. According to him, clamp connections were difficult to observe in dried specimens but were easily observed in the context of fresh specimens and young parts of fresh hymenium. Hence, it was not important in separating *Ganoderma* sp. because the hyphal system in most species was consistently trimitic and they were also influenced by environmental factors.

Lee (2000) expressed the major disadvantages of using morphology of basidiocarps in species identification of *Ganoderma*. He stated that the basidiocarps appeared seasonally and in fast grown plantations basidiocarps might be absent on diseased trees and identification of the fungus became very difficult or impossible. In the same year Ryvarden reported that basidiocarp morphology should be used with caution due to its plasticity and pleomorphic nature in some species. Seo and Kirk (2000) stated that the morphology of the basidiocarps may differ between the isolates due to different environmental conditions during development. He observed variation among the species on the attachment of the

stipe to the pileus and in their host range. They noticed eccentric, central, imbricate and sessile fruit body production in *Ganoderma* spp.

They also reported that the size of basidiospores of *G.lucidum* obtained from naturally grown specimens from Japan and Korea were measured as 8.5-11 x 6.5-8.5µm (average 10.1 x 7.5µm) and 8.5-13 x 5.5-7µm (average of 10.4 x 6.6 µm), respectively with mean spore indexes of 1.62 and 1.58 respectively. Rajendran (2006) described the cultural and morphological characters of sporophore of *Ganoderma*. He reported that the sporophore appeared as small, white buttons of fungal tissues in the early stages that developed rapidly into the bracket shaped mature sporophore. The young sporophore was white or yellow, where as the matured one was with light to dark brown upper surface and slight margin and a shiny lacquered finish. The under surface of the fruiting body was whitish in colour and had numerous minute pores. He also observed the presence of bark beetle *Xyloborus perforans* in most of the diseased palms which tunnelled into the stem in large numbers pouring out powdery mass from inside the stem.

According to Muthelo (2009), the colour of the pileus surface varied from deep red, laccate, non laccate and yellow to white. The laccate character of the pileus was not considered as an important criterion in the segregation of the species within the genus but it has been widely employed and used as an identification aid. He observed variation in the context colour which varied from white to deep brown and changed with the age of the fruit body or upon drying.

Srivastava *et al.* (2010) noticed that badidiospores of *Ganoderma lucidum* were brown and ovate, with a truncate to rounded apex, and an eccentric hilar appendix on a rounded base. They observed double wall with inter-wall pillars separating the two walls in the basidiospores. The apex contained a germ canal from which germ tubes were observed to emerge. They also measured the size of basidiospores of *G.lucidum* and found that it varied from 0.2-0.3µm with a spore index of 1.5.

2.7. CULTURAL CHARACTERISTICS

Studies on cultural characteristics are very important in species identification. Nambiar and Nair (1973) reported that the best medium for growth of *G.lucidum* was Walksman's medium. Basidiocarp production was observed in saw dust medium two months after inoculation.

Adaskaveg and Gilbertson (1986) stated that in addition to basidiocarp morphology, cultural characteristics such as chlamydospore production, growth rate and thermophily have been used to differentiate various species of *Ganoderma*.

Seo (1987) noticed the colony characters of *Ganoderma* sp. and reported that colony was white to pale yellow, even, felty to floccose at the optimum temperature on PDA medium. In the next year Shin and Seo (1988) reported that the colony of *Ganoderma* became more yellowish when exposed to light. They observed that the hyphal growth of most isolates was 2-4mm/day on PDA but chlamydospore forming isolates grew faster than those isolates which did not produce chlamydospores. They also noticed various features of the colonies such as sectoring, pigmentation, formation of fruit body primordial and atypical fruiting structures which formed basidia and basidiospores without basidiocarp formation.

In 1989, Adaskaveg and Gilbertson also made the same observations and reported that the colony of *Ganoderma* was white to pale yellow and even, felty to floccose at the optimum temperature on Potato dextrose agar medium. They observed the formation of aberrant fruiting bodies of *G.lucidum* with basidiospores on agar media *in vitro*. The basidiospores were formed on red, laccate, coral like fruit bodies. These fruit bodies might be aberrant because of similarity in their appearance and in their ability to form basidiospore. They also reported that *Ganoderma* cultures grew at different optimum temperatures depending on the species and these species could produce various hyphal structures in culture such as generative hyphae with clamp connections, fibre or skeletal hyphae, stag-horn hyphae, cuticular cells and vesicles, and hyphal rosettes as well as chlamydospores.

Seo *et al.* (1995) observed that some isolates produced fruiting body like primordial (FBP) on agar medium but they were not developed into mature fruit bodies during the 30 days of cultivation. They reported that higher rate of ventilation was required for aberrant fruiting body structure (AFS) formation under *in vitro* condition but FBP could be formed under conditions of lower ventilation. They observed that the chlamydospore formation was absent in those cultures where AFS and FBP were present. Chlamydospore forming isolates formed neither AFS nor FBP under any of the conditions examined.

Mishra and Singh (2010) examined the growth rate of *G.lucidum* on four media *viz.*, malt extract Agar, potato dextrose agar, oat meal agar and yeast extract agar. Among the four media tested, malt extract agar showed the highest growth rate (7.5mm/day) followed by potato dextrose agar (6.2mm/day).

Veena and Panda (2010) attempted to use locally available sawdust, wood chips, sorghum and wheat grains in combination with chalk powder ($CaCO_3$) and gypsum ($CaSO_4$) for the production of *G. lucidum* spawn. Due to its higher growth rate, shorter spawn run period, negligible contamination and significantly higher biological efficiency, sorghum proved to be the ideal material for *Ganoderma* spawn production.

Geetha (2011) carried out a test to find out the feasibility of *G. lucidum* cultivation in Kerala using the locally available cheaper substrates *viz.*, paddy chaff, rubber saw dust, sugarcane bagasse and paddy straw by artificial log and natural log method. Out of the two methods, artificial log method resulted in faster spawn, earliness in sporophore production and higher yield compared to natural log method.

2.8. EFFECT OF TEMPERATURE, pH AND LIGHT ON DISEASE INCIDENCE

Rao and Rao (1966) reported that the disease incidence was low in heavy soils in Andhra Pradesh due to retention of more moisture besides the presence of high population of antagonistic micro-flora. According to Ramasami *et al.* (1977), presence of hard pan formation in the sub soil impedes root

penetration, which in turn predisposes the coconut palms to infection. Ramapandu *et al.* (1981) noticed that lesser disease spread during rainy season was attributed to the adverse effect of high soil moisture on the pathogen due to heavy rainfall. Lewin *et al.* (1983) noticed a positive correlation between mean maximum soil temperature and number of bleeding palms.

Shin and Seo (1989a) reported that the aberrant fruiting body formation was induced by light with ventilation from the white mycelial colony stage of *Ganoderma* sp. Shin and Seo (1989b) reported that the growth of mycelium was suppressed by light. But Stamet in 1993 reported that primordium formation, pileus differentiation and tropic growth of the stipe of *G. lucidum* were affected positively by light.

According to Bhaskaran *et al.* (1985) disease incidence of BSR was more between March and August in the endemic areas of Tamil Nadu. The disease incidence was directly related with mean maximum soil temperature and it was not related with minimum temperature, rainfall and relative humidity. Srinivasulu *et al.* (2003) reported that number of rainy days and rain had a negative relationship with the spread of basal stem rot disease in coconut .

Karthikeyan *et al.* (2006) observed that the disease incidence was more from the months of February to August coincided with high mean temperature and soil temperature. Correlation of the incidence of disease with weather factors was worked out and found that the minimum air temperature, maximum air temperature and soil temperature were positively correlated with disease incidence, while rainfall, relative humidity and soil moisture were negatively correlated. Among the weather factors, maximum air temperature, soil temperature and soil moisture significantly influence the development of the disease (Kandan *et al.*, 2006). BSR was generally observed in sandy soils in coastal areas where coconut was grown under rainfed conditions and also in neglected plantations. Soil moisture stress experienced during summer months, water stagnation during rainy season, presence of old infected stumps in the garden and non-adoption of recommended cultural practices were found to favour the spread of the disease.

Mishra and Singh (2010) reported that the fungus showed better growth at temperature range of 25-30^{0}C. Increasing the temperature to 35^{0}C or above resulted in decreased average growth rate. They observed good growth of the fungus at pH 5 and 6. The fungus showed growth at pH 4 and even at pH 7 but on the alkaline side, there was a sudden drop.

Karunanithi *et al.* (2002) observed that the per cent pore space was more (20%) and bulk density was less (1.73 g/cc) in soils of BSR affected garden compared to healthy gardens (pore space of 17.66% and bulk density of 1.8g/cc). The infected soil had higher pH (6.5-7.9) and electrical conductivity (12.6dsm-1) compared to healthy soils (pH 6.2-7 and EC 9.4dsm-1). Srinivasulu *et al.* (2003) recorded complete inhibition of spread of the disease in coconut by water stagnation and reported that disease incidence was maximum (62.5 per cent) in coconut palms raise in sandy soils and red soils while negligible (1.21%) incidence was observed in black soils and on paddy bunds.

2.9. TOXIN PRODUCTION BY *GANODERMA* SPP.

Kimura *et al.* (1973) noticed the importance of toxic metabolites of the pathogen in pathogenesis. Sakthivel and Gnanamanickam (1986) described that visible disease symptoms of BSR disease appeared at a very late stage of infection when already more than half of the root tissue has been decayed, leaving no chance for the grower to cure the infected palms. In 1986, Vidhyasekaran and co-workers reported that the virulence of the pathogen should be correlated with the ability to produce the toxin.

Vidhyasekaran *et al.* (1992) reported that inorder to prove the involvement of toxin in disease development; the toxin should be able to produce typical symptoms to the disease. Song *et al.* (1993) explained that, to prove the involvement of toxin in disease development, the toxin should exhibit the same host specificity as that of the pathogen. Hemalatha *et al.* (1999) reported that potential use of crude toxin preparation in *in vitro* screening for disease resistance in plants provided circumstantial evidence that the culture fluid may contain

phytotoxic principle which could act as a pathogenic factor *in planta.* Yoshida *et al.* (2000) graded the reaction of leaves to different concentration of toxin.

Karthikeyan *et al.* (2008) reported that an extra cellular, hydrophilus, thermostable phytotoxin was purified to homogeneity from culture fluids of *G. lucidum.* Toxicity was evaluated with detached leaf sheath and electrolyte leakage bioassays. They observed that the purified phytotoxin induced visible symptoms of the disease, when applied to coconut leaves, fronds and roots even at low concentration. They characterized the toxin and found that it was glycoprotein with carbohydrate as the major component. The importance of the carbohydrate moiety for toxic activity was indicated by inactivation of toxic compounds after periodates oxidation. They also proved that the non host specificity of toxin could infest monocots and dicots, but the sensitivity was significantly higher in coconut, oil palm and arecanut than other crops

2.10. DISEASE MANAGEMENT

The current methods of control of *Ganoderma* diseases of perennial crops are aimed at either delaying the progress of infection, or prolonging the productive life of trees. Curative methods are neither practical nor economically feasible in infected trees. The three main methods of control are cultural, chemical and biological. Uses of antagonists, phytoextracts and chemicals in the management of a disease have practical importance. So many workers have conducted studies on these aspects.

2.10.1. Bio-control agents

Bio control can be defined as reduction of inoculums or disease producing activity of a pathogen accomplished by or through one or more organisms other than man (Cook and Baker, 1983). Today antagonistic interactions have been exploited in the area of biological control of plant pathogens. Potential agents for bio-control activity are rhizosphere competent fungi and bacteria which in addition to their antagonistic activity are capable of inducing growth response by either controlling minor pathogens or producing growth stimulating factors (Cook and Weller, 1986).

To date there is no adequate control for BSR in field. Cultural control techniques have little effect on the control of BSR, because the pathogen can survive in the soil for several years. Chemical control has not been effective and long lasting, even though *in vitro* screening has identified several chemicals that are effective against *Ganoderma* sp. The effective use of chemical control treatment of *Ganoderma* infected palms is limited by the fact that both visibly infected and subclinical palms may harbour established infections by the time treatment is applied. Additional difficulties may occur in the effective placement of fungicides as lesions are frequently very large in size. As lesions are most commonly found at the stem base, high pressure injection of fungicide frequently results in the passage of chemical straight into the soil.

Garret (1955) reported that *Trichoderma viride* and *Streptomyces* spp. were antagonistic to *G.lucidum*. Gunasekaran *et al.* (1986) reported that *T.harzianum* and *T.hamatum* were found to be antagonistic to *G.lucidum* and application of neem cake encouraged the saprophytic soil microflora especially *Trichoderma* spp. in coconut basins and was effective in the control of *Ganoderma* disease. According to Claydon *et al.* (1987) *Trichoderma* strains produce a variety of volatile and non volatile toxic metabolites. Of these, some were considered to be antibiotics as they could inhibit the growth of other micro-organism without physical contact between the fungi. The best known antifungal metabolites produced by isolates of this genus was the coconut scented 6-n-pentyl-2H-pyran-2-ome (PPT).

Shukla and Uniyal (1989) reported that saprophytes can be used to compete against *Ganoderma* to reduce its opportunity for colonizing oil palm roots. In the same year Dharmaputra *et al.* noticed the antagonistic activity of *Penicillium* against *G.boninense*. They reported that *P. citrinum, T. harzianum* (isolate BIO- 1 and BIO-2), and *T. viride* isolated from oil palm plantation in North Sumatra were antagonistic to *G. boninense. T. harzianum* (BIO-1) was the most potential antagonist against the pathogen. *P. citrinum* produced an antibiotic substance that diffused into the medium, while *Trichoderma* causes lyses of the pathogens hyphae.

Bhaskaran (1990) reported that the soil application of *Trichoderma harzianum* at 500g inoculums along with various organic manures *viz.*, green leaves (50kg/palm) or farm yard manure (50kg/palm) or neem cake (5kg/palm) significantly reduced the BSR intensity and increase the nut yield. Khara and Hadwan (1990) reported that the fungal antagonists that have shown inhibition in dual culture studies were grown on PD broth to test the effect of the culture filtrates on *Ganoderma* by food poisoning technique. Kumar and Nambiar (1990) mass multiplied *Trichoderma* in rice bran and neem cake media(1:1) which was found to suppress *Ganoderma* under *in vitro* condition and utilised for testing its efficacy in controlling *Ganoderma* under field conditions. The palms affected by *Ganoderma* were inoculated with the bio agent as soil amendment. Out of four palms, two palms which were in advanced stage have dried up completely within one month of application and the other two have not shown any change in their stand.

Abdullah *et al.* (1999) reported that *T. harzianum* was more effective as an antagonistic agent against *G. boninense* than *T. longibrachiatum* and *T. virense*. Widyastuti *et al.* (1999) showed in paired cultures that a *Trichoderma* isolate which was highly effective against one isolate of a pathogen could have minimal effect on other isolates of this pathogen. This might be related to the high pathogen-*Trichoderma* specifity of antagonistic mechanisms due to antibiosis. Bhaskaran (2000) reported that soil application of bio control agents prevented the entry of *G. lucidum* in the vascular tissue by strengthening of cell wall structures and accumulation of phenolic substances.

Wafaa (2002) reported that different bio control agents could be used for the control of diseases including bacteria, fungi and actinomycetes. He observed different mechanisms by which bio control agents control other microorganisms and stated that most of the bio control agents applied only one of these four mechanisms but sometimes employed more than one *viz.*, direct competition with the target organism, antibiosis, and parasitism of the target organism and induced resistance of the host plant.

According to Perello *et al.* (2003), non pathogenic rhizosphere colonizing *Trichoderma* spp. suppress the soil borne pathogens by diversified mechanisms

viz., production of wide range of broad spectrum antifungal metabolites, mycoparasitism, competition with the pathogen for nutrient and for occupation of the infection court, induced resistance, production of protease and fungal cell wall degrading enzymes.

Widyastuti *et al.* (2003) described that same isolate of *Trichoderma* exhibited different types of fungal inhibitions depending on the particular species of fungal pathogens with which it was interacted. In the same year, when he tested the antagonistic efficiency of three isolates of *Trichoderma* against *Ganoderma* sp., *Trichoderma reesei* was the most effective mycoparasite interacted with *Ganoderma* isolates, followed by *T.koningi* and *T.harzianum.* Next year Karunanithi *et al.* (2004) reported that the combined application of 50g each of *T.viride* and *P. fluorescens* per palm reduced the disease severity of BSR in coconut.

Susanto *et al.* (2005) reported that strains of *T. harzianum* were commercialised because they exert strong competitive effects for space and nutrients and more importantly they produce toxins against phytopathogenic species and made them good bio control agents. Karthikeyan *et al.* (2005) reported that application of *Trichoderma viride* and *Pseudomonas fluorescens* talc formulations at the rate of 200g each/palm in combination with 50kg FYM was found effective against the disease.

In 2006, Widyastuti reported that when three *Trichoderma* isolates previously shown to have high antagonistic ability (*T.koningi, T.reesei, T.harzianum*) were tested against isolates of *Ganoderma* collected from different tree species, it was found that these *Trichoderma* isolates showed great variation in their level of antagonism towards the different *Ganoderma* isolates. In another test he explained that among the 120 isolates of *Trichoderma* spp. tested against the pathogenic fungi, the basic types of antagonistic behaviours *viz.*, antibiosis, mycoparasitism and competition were recorded and the most effective in inhibiting the root rot pathogens tested was mycoparasitism.

Srinivasulu *et al.* (2006) studied the antagonistic efficacy of *Trichoderma* spp. *viz., T. viride, T.harzianum* and *T.hamatum* and a bacterial strain of

Pseudomonas fluorescence against the pathogens *viz.*, *G.lucidum* and *G. applanatum*. From the results it was revealed that the mycelial growth of coconut pathogens were highly inhibited by *T.viride* by more than 80% and *P.fluorescense* completely inhibited the growth of both the species of *Ganoderma*. All the three species of *Trichoderma* were found to be very effective in producing specific volatile metabolites against *Ganoderma* sp. The volatile metabolites of *P. fluorescence* completely inhibited *G. applanatum* and *G. lucidum* but non-volatile metabolites were not effective. Application of 50g of talc formulation of *T.viride* or *T.harzianum* or *T. hamatum* with combination of 5kg neem cake/palm//year in basins around the palm was found to be effective in the management of BSR.

Srinivasulu *et al.* (2006) reported that the mycelial growth of *G.lucidum* and *G.applanatum* was suppressed when exposed to 0, 15 and 25 days old cultures of all the *Trichoderma* spp. They showed a positive correlation between an increase in age of the antagonistic culture of all isolates of *Trichoderma* spp. before being exposed to the BSR pathogen with per cent inhibition of the pathogens. Of all the *Trichoderma* spp., *T.viride* was found to be very effective against *G.applanatum* and *G.lucidum* with maximum inhibition of 48.42% and 42.4% respectively. In non-volatile metabolites at 100% concentrations, considerable inhibition on mycelial growth of *G.applanatum* (48%) and *G.lucidum* (57%) was observed in inhibition by *T. Viride*

Izzati and Abdullah (2008) tested the conidial suspension of *T. harzianum* against *Ganoderma* infection in oil palm seedlings in glass house condition to determine the effectiveness of the fungus as bio-control agents. The highest efficacy of control was achieved by treatment right after artificial infection; the total number of infected plants was reduced to give the lowest disease severity index (DSI) value of 5% compared to the infected and non treated control that had the highest DSI of 70%. They reported that once the external symptoms appeared, the infection was already too severe and a bio control agent could not control the pathogen during this stage.

Srinivasulu *et al.* (2008) reported that growth of *Trichoderma* spp. was found to be maximum and continuous on PDA while the growth was restricted

and was in discontinuous fashion on malt extract medium. Seven day old culture of *Trichoderma* was grown faster on PDA and covered total plate (90mm) after 3 days. Where as 15 day old culture of *Trichoderma* covered 90mm only after 7 days at room temperature (30^0C). No difference in mycelial growth of *Trichoderma* on PDA was observed when exposed to total light, alternate light and darkness and complete darkness. In the same year, Srinivasulu *et al.* also reported that in dual culture technique, *T.viride*, *T.harzianum* and *T.hamatum* were found to inhibit the mycelial growth of *G.applanatum* and *G.lucidum* on PDA under *in vitro* conditions. Among the three species of *Trichoderma* tested, maximum suppression was noted with *T.harzianum* to an extent of 72% in *G.applanatum*, 75% in *G.lucidum* over control.

2.10.2. Phytoextracts

Beye (1978) reported the usefulness of constituents of higher plants as a possible alternative source to pesticides on account of their non-phytotoxic, more systemic, easily biodegradable and host metabolism stimulatory nature. *In vitro* studies done by Bhaskaran *et al.* (1988) indicated that banana rhizome extract and tephrosia root extract were highly inhibitory to the growth of *G. lucidum.* In 1993 Bhaskaran *et al.* tested several plant extracts against *G.lucidum* and found out that banana rhizome extract inhibited the growth to an extent of 86 per cent.

According to Karthikeyan and Bhaskaran (2001) growing intercrops in basal stem rot affected coconut plantations reduced the incidence of the disease and banana was found to be most effective and intercropping in diseased plantations increased the coconut yield. The population of fungi, actinomycetes and the antagonistic organism *viz.*, *Trichoderma* spp. increased significantly in soils of intercropped coconut. The population of bacterial bio-fertilizers *Phosphobacteria*, *Azotobacter* and *Azospirillum* were also higher in intercropped plots.

Iyer *et al.* (2004) screened standard aqueous leaf extracts of 43 plant species against *G. lucidum* under *in vitro* conditions by poisoned food technique. They observed complete inhibition on the growth of the pathogen by the extract of

Allium sativum. Fresh leaf extract of *Peperomia pellucida* exerted 91.6% inhibition followed by that of *Clerodendron infortunatum* (80.7%) and *Musa paradisiaca* (43.45%) at 96 hr.

Karunanithi *et al.* (2007) evaluated 29 plant products *in vitro* and *in vivo* for the management of BSR of coconut. Leaf extracts of *Pongamia glabra*, *Azadirachta indica* and *Prosopis juliflora* (10 per cent) were effective in suppressing the mycelial growth of *Ganoderma lucidum in vitro*. In field conditions, these plant products recorded lesser disease index of BSR compared to the control but all the plant products increased the population of fungi and bacteria in soil.

2.10.3. Chemicals

A perusal of the literature revealed few reports on the *in vitro* and *in vivo* studies with fungicides on basal stem rot disease of coconut. In 1979, Anbalagan reported the efficacy of aureofungin and tridemorph in arresting the growth of *G.lucidum*. Satyanarayana *et al.*(1985) found aureofungin 1.5g + $CuSO_4$ one gram in 5 litre of water (AF), carboxin stem injection 500mg in 5ml of water (CAR) and AF + CAR reduced the linear spread of the disease significantly when compared to control. In the same year Bhaskaran *et al.* reported that soil drenching with 40 litre of one per cent Bordeaux mixture and stem injection of aureofungin two gram and one gram of $CuSO_4$ in 100ml of water thrice at quarterly intervals reduced the disease intensity and increased the yield of nuts.

Sindha Mathar and Balasubramaniam (1987) reported the efficacy of tridemorph in arresting the growth of *G. lucidum*. Field trials conducted at Veppankulam in Tamil Nadu revealed that root feeding of aureofungin or tridemorph along with neem cake application was found effective in disease control (Anon, 1997). Next year, field trial conducted at Palghat by CPCRI, Kasargod showed that in tridemorph and aureofungin sol treated palms, the disease was less.

Nambiar *et al.* (1992) reported that root treatment with tridemorph at 2ml in 100ml water or aureofungin 2g + $CuSO_4$ one gram in 100ml water was

effective against *Ganoderma* disease. According to Bhaskaran (1993), root treatment with tridemorph (2ml/100ml) at quarterly intervals for one year combined with application of 5Kg neem cake per palm per year controlled BSR of coconut effectively.

Bhaskaran *et al.* (1996) reported that root feeding of aureofungin or tridemorph along with soil application of neem cake was found superior in controlling the disease. Naik (2001) did experiment to control coconut basal stem rot caused by *G.lucidum* using two systemic fungicides *viz.*, tridemorph and hexaconazole. These were given as root feeding, soil drenching and root feeding + soil drenching at quarterly interval. He used seven treatment combinations and all the treatments were found quite effective in controlling the disease. The lowest disease index was achieved with tridemorph root feeding (2%) + soil drenching (0.3%), followed by hexaconazole root feeding (1%) + soil drenching (0.2%), soil drenching with tridemorph (0.3%) and hexaconazole (0.25) compared to root feeding alone. In the same year, Naik and Venkatesh (2001) conducted experiment using 3% kitazin root feeding + 5Kg neem cake and 2% tridemorph root feeding + 0.1% soil drenching + 5kg neem cake, 1.3% aureofungin (root feeding) + 1% BM + neem cake to control BSR caused by *G. lucidum* and reported that the lowest disease index was achieved with aureofungin (root feeding) (1.3 %) and BM (1%) soil drenching at quarterly interval day with soil application of neem cake at 5 Kg /day/ yr.

Karunanithi *et al.* (2005) reported that root feeding of 2ml of tridemorph in 100 ml of water thrice at quarterly interval combined with soil drenching of 40 litres of one per cent Bordeaux mixture was effective for the management of the disease. Sankaran *et al.*(2005) reported that in India, application of fungicides to control BSR of coconut and other palms often done in combination with soil amendments such as neem cake (*Azadirachta indica*) or farm yard manure. Chemicals commonly used include aureofungin, carbendazim, carboxin, copper oxychloride, hexaconazole and tridemorph. Palanna *et al.* (2009) reported that tridemorph (0.1%) and hexaconazole (0.1%) were found to completely inhibit both *G. applanatum* and *G. lucidum* under *in vitro* condition.

2.11. HOST RANGE OF *GANODERMA LUCIDUM*

Host specificity has been used to circumscribe *Ganoderma* taxa. *Ganoderma* has got a wide host range attacking a variety of palms and several forest, avenue and fruit trees. Butler (1906) reported the importance of *Ganoderma* as a pathogen on cash crops such as coconut, betelnut and other plantation species including *Casuarina*, *Areca catechu*, *Dalbergia sissoo* and *Toona ciliata* in north-eastern states of India. These reports were followed by the report by Venkatarayan, who recorded *Ganoderma* diseases of coconut, betelnut, *Cassia siamea* and *Pongamia* from South India in 1936. Bagchee (1945) reported diseases of forest trees *viz.*, *Acacia catechu*, *D.sissoo* and *Shorea robusta* in North India. According to Khara (1993) in Ludhiana, 13.3% of *D. sissoo*, 5.5% of *Leucaena leucocephala*, 6% of *Delonix regia*, 4% of eucalyptus hybrids and 2% of pear trees were severely affected by *Ganoderma lucidum.* According to Naidu *et al.* (1966), hosts belonging to 19 families, 36 genera and 48 species have been reported to be affected by *Ganoderma lucidum.*

Deepthi *et al.* (2003) reported that *Ganoderma* spp. were found to infect different crops *viz.*, coconut, arecanut, mahagoni, *Araucaria*, etc. In 2005 Sankaran *et al.* reported that a total of 144 host plants were affected by *Ganoderma* sp. Among the members of *Ganoderma* complex, *G. lucidum* had the widest host range of 91 host plants, the majority of which are forest trees. *G. applanatum* has the next widest range with 39 host plants. They found out new host plants of *Ganoderma lucidum viz.*, *Roystonea regia, Peltophorum pterocarpum, Parkia biglandulosa, Paraserianthes falcataria, Vateria indica* L. and *Borassus* sp.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study on the "Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the year 2010-12. The details of materials used and the techniques adopted for the investigation are described below.

3.1. SURVEY AND COLLECTION OF DISEASED SAMPLES OF COCONUT PALM

Purposive sampling surveys were conducted in coconut gardens of northern, central and southern parts of Kerala for the occurrence of basal stem rot disease and for the collection of diseased samples and basidiocarps of the pathogen. The locations surveyed are given in Table 1.

Table 1. Locations surveyed for the collection of diseased samples and basidiocarps

Sl No.	Region	Location
1	Southern region	Instructional farm, College of Agriculture, Vellayani
		Farmer's field, Trivandrum
		Farmer's field, Kalavoor
		Farmer's field, Alapuzha
		Farmer's field, Muttom
2	Central region	Farmer's field, Mannuthy
		Farmer's field, Vettikkal
		Farmer's field, Madakathara
		Farmer's field, Chirakacode
		College of Horticulture, Vellanikkara
		Farmer's field, Nadathara
		Farmer's field, Karuvankallu
		Farmer's field, Vengara
		Farmer's field, Calicut-1

		Farmer's field, Chevayoor
		Farmer's field,Calicut-2
		Farmer's field, Karathode
		Farmer's field, Mudikkode
3	Northern region	Farmer's field, Kannur
		Farmer's field, Iritty
		Farmer's field, Kasargod

From these locations specimens of stem and roots of diseased coconut palm and basidiocarps of the pathogen were collected and brought to the laboratory for isolation of the pathogen. During the survey, symptomatology of the disease was also been studied.

3.2. ISOLATION OF PATHOGEN

The isolation of pathogen associated with stem and roots of infected coconut palm and basidiocarps collected from various locations was carried out on Potato Dextrose Agar (PDA) medium.

3.2.1. Diseased stem and root

The diseased specimens of stem and roots collected from various locations were brought to laboratory, washed well under tap water to remove dust particles and cut into small bits. These bits were surface sterilized with one per cent sodium hypochlorite solution and then washed three times with sterile water and transferred to sterile Petri dishes containing solidified PDA medium. The dishes were incubated at room temperature (26 ± 2°C) and observed for the growth of pathogen from next day onwards. The pathogen grown on medium was purified, sub cultured periodically and maintained on PDA medium for further investigation.

3.2.2. Basidiocarp

Basidiocarps collected from different locations were brought to the laboratory and isolated the pathogen on PDA medium by adopting two different methods.

3.2.2.1. Direct isolation by tissue segment method

The basidiocarps collected from diseased palm were washed well under tap water to remove dirt particles. Tissues from inner part of basidiocarp at the junction where pileus attach to the stipe portion were taken with the help of forceps and surface sterilized with one per cent sodium hypochlorite solution and washed thrice in sterile water and transferred to sterile Petri dishes plated with PDA medium.

3.2.2.2. Moist chamber method

Isolation of pathogen from basidiocarp was done by moist chamber method described by Palanna *et al.* (2009). The collected specimens of basidiocarps were surface sterilized with 70 per cent ethyl alcohol and kept in sterilized polythene bags by providing high humidity with moistened cotton and were incubated at room temperature. The mycelial growth developed on the surface of the basidiocarp was transferred aseptically to sterile mediated Petri dishes.

The dishes were incubated at room temperature (26 ± 2°C) and observed for the growth of pathogen. The isolates obtained from different locations were purified and maintained on PDA slants by periodical sub culturing.

3.3. *In vitro* TESTS FOR THE CONFIRMATION OF PURE CULTURE OF *Ganoderma* sp.

Mycelial growth and basidiocarp formation by the pathogen on saw dust-rice bran substrate was used as a test for the confirmation of pure culture

of different isolates of *Ganoderma*. The sawdust-rice bran substrate was prepared by using the ingredients given below (Geetha, 2011).

Rubber saw dust	- 80%
Rice bran	- 20%
Sugar	- 2%
$CaCo_3$	- 2%
Moisture content	- 50-60%

Rubber saw dust was soaked in water treated with carbendazim (75ppm) and formaldehyde (50ppm) for 18 h. The excess water was drained off and spread over a clean surface for drying. The moisture content was reduced to optimum level and was used for substrate preparation. To prepare the substrate all ingredients were weighed and mixed well with the sterilized saw dust. The substrate was heaped for 3 days, turned it and again heaped for another 3 days. Then transferred this mixture into $¼^{th}$ of 250 ml conical flasks and autoclaved for two hours. Upon cooling the substrate was inoculated separately with mycelium of different isolates of pathogen and incubated in dark at room temperature (28 ± 2°C). Three replications were maintained for each isolate. Observations on the number of days taken for complete colonization, exudates production and development of fruiting bodies were taken.

3.4. PATHOGENICITY TEST

The pathogenicity of different isolates of the pathogen was studied by artificial inoculation on one year old healthy coconut seedlings under *in planta* conditions. Two types of inocula *viz.*, mycelial growth of the different isolates of pathogen on PDA medium and spawn of *Ganoderma* sp. on saw dust- rice bran substrate were used for inoculation. Four replications were maintained for each inoculum.

3.4.1. Preparation of inoculums

3.4.1.1. Mycelial growth on PDA medium

Mycelial growth of different isolates of pathogen on PDA medium was used as an inoculum for artificial inoculation. For that 10mm diameter disc of 7 day old growth of different isolates of pathogen was cut and transferred separately into sterile Petri dishes mediated with PDA medium. All the Petri dishes were incubated at room temperature (26 ± 2°C). Mycelial growth obtained on 5 DAI was used for the artificial inoculation.

3.4.1.2. Saw dust- rice bran substrate with mycelial growth of pathogen

Rubber saw dust-rice bran substrate was prepared as mentioned in 3.3. and was used as inoculum for pathogenicity test.

3.4.2. Method of inoculation

The above mentioned two inocula were used for the artificial inoculation on coconut seedlings. The inoculation was given at basal part of the stem and in soil. One year old coconut seedlings were used for the pathogenicity test.

3.4.2.1. Stem inoculation

Mycelial growth of different isolates of pathogen grown on PDA medium was used as inoculum for the stem inoculation. For this coconut seedlings having one year old were procured from nursery and were planted in black coloured polythene bag containing fumigated potting mixture. The potting mixture was sterilized by fumigating with formaldehyde and water in the ratio 1:15 and covered for 4 days. Then the polythene cover was removed and kept in open condition for ten days to remove excess formaldehyde. With

the help of a sharp razor blade or knife a small cut was made on the stem of coconut seedling at the base near to the soil surface and removed the bark. Then inoculated with mycelium of different isolates of pathogen separately on the injured portion of the stem and moisture was provided with the help of wetted cotton. The plants were observed for the symptom expression. Sterilized plastic sheets were placed over the soil surface to prevent contamination. The plants inoculated with PDA alone served as control.

3.4.2.2. Soil inoculation

Mycelial growth of different isolates of pathogen on saw dust-rice bran medium prepared as mentioned on 3.3 was used for the soil inoculation.

3.5. SYMPTOMATOLOGY

3.5.1. Symptomatology under natural condition

Symptoms developed on the leaves, stems, roots and flowers under natural conditions were recorded during the survey at various locations.

3.5.2. Symptomatology under artificial condition

To study the symptomatology under artificial condition the different isolates were inoculated artificially on coconut seedlings and the symptoms developed were recorded.

3.6. CHARACTERIZATION OF DIFFERENT ISOLATES OF PATHOGEN

The cultural characters of different isolates of the pathogen collected from different locations were studied to find out the variations in characters existing among them.

3.6.1. Cultural characters of different isolates of the pathogen

The cultural characters of the different isolates such as colour, texture, mycelial type, growth rate, presence of exudation, time taken for colour change in medium, origin of colour change in mycelium, presence of aberrant fruiting bodies etc. were recorded on four different media *viz*., Potato Dextrose Agar (PDA), Czapek's (Dox) Agar (CDA), Richard's Agar (RM) and Soil Extract Agar (SEA) media. (Appendix.1)

3.6.2. Effect of different media on growth rate of different isolates of pathogen

The growth rate of different isolates of the pathogen was studied on different media *viz*., PDA, CDA, RA and SEA. Using cork borer 10mm diameter disc of seven day old fungal growth was cut and transferred to the centre of mediated sterile Petri dishes and incubated at room temperature (26±2°C). Five replications were kept for each isolate and for each medium. Radial growth of all the isolates were taken every day till the growth of any one isolate in any one medium fully covered the Petri dish. The cultural characters of each isolate on all media were also recorded as mentioned in 3.6.1.

3.7. MORPHOLOGICAL CHARACTERS OF BASIDIOCARPS, BASIDIOSPORES AND HYPHAE OF DIFFERENT ISOLATES OF PATHOGEN

To study the morphological characters of different isolates of the pathogen and basidiospores, permanent slides of the different isolates of pathogen were prepared. Microscopic observations on nature of hyphae, colour, shape and size of basidiospores, structure of hyphal system of basidiocarp, presence of chlamydospores were recorded. Photomicrographs of hyphal system of basidiocarps and basidiospores of the pathogen were also

made. Morphological characters of basidiocarps of various isolates of pathogen collected from different locations were studied. Macro morphological features *viz*., colour and size of stipe and pileus, attachment pattern of stipe to pileus, margin and surface pattern of pileus, shape of basidiocarp, presence of ornamentation etc. were recorded.

3.8. EFFECT OF TEMPERATURE, LIGHT AND pH ON THE GROWTH RATE OF DIFFERENT ISOLATES OF PATHOGEN

An *in vitro* evaluation on the effect of temperature, light and pH on the growth rate of different isolates of the pathogen was carried out on the best medium selected from the experiment described in 3.6.2.

3.8.1. Effect of temperature

The effect of temperature on the growth rate of different isolates of pathogen was tested at 25, 30, 35 and 40°C. Using cork borer 10mm diameter disc of 7 day old fungal growth was cut and transferred to the centre of mediated Petri dish and incubated at four different temperatures in BOD incubator. Three replications were kept for each isolate. The daily observations on the radial growth of mycelium of each isolate were recorded till the Petri dish attained 90mm growth.

3.8.2. Effect of pH

The effect of pH on the growth rate of different isolates of pathogen was tested on the best medium with pH 5, 6, 7 and 8. The medium was adjusted to acidic and alkaline pH by using 1 N HCl and 1N Sodium hydroxide solution respectively. pH meter and pH paper were used for testing the pH of the medium. Three replications were kept for each pH. Mycelial discs of 10mm diameter were cut from the actively growing cultures of different isolates of pathogen and placed at the centre of the mediated Petri dishes and incubated at

room temperature (26±2°C). The daily observations on the radial growth of mycelium of each isolate were recorded till the Petri dishes attained full growth of 90mm.

3.8.3. Effect of light

The influence of light on the growth rate of different isolates of pathogen was studied on the best medium. Petri dishes mediated with PDA were inoculated at the centre with 10mm disc of mycelial growth cut from an actively growing culture of the pathogen. The inoculated plates were kept in light and darkness. For providing full darkness dishes were covered with black paper. The daily observations on the radial growth of mycelium of each isolate were recorded till the Petri dishes attained full growth.

3.9. MANAGEMENT OF THE PATHOGEN INCITING BASAL STEM ROT

An *in vitro* evaluation on the effectiveness of antagonistic micro-organisms of rhizosphere soil, phytoextracts and fungicides was carried out for the management of pathogen causing basal stem rot of coconut.

3.9.1. Isolation of rhizosphere microorganisms

Fungal and bacterial micro-organisms were isolated from the rhizosphere soil of healthy coconut palm adjacent to the infected ones by serial dilution technique (Johnson and Curl, 1972). The rhizosphere soil from healthy coconut palm was collected from the same locations of northern, central and southern regions of Kerala from where the basidiocarps of pathogen was obtained for this study.

Dry soil of 10g was transferred to 90ml of sterilized water in 250 ml conical flask and shake well for 10 minutes in a shaker (10^{-1}dilution). The serial dilutions upto 10^{-7} were prepared from this dilution. The fungal micro-organisms were isolated on PDA medium by spread plate method. Transferred

1ml from 10^{-3} and 10^{-4} dilutions separately to sterilized Petri dishes mediated with PDA medium and spread uniformly with a sterilized spreader. Petri dishes were incubated at room temperature and observed for the development of fungal colony. Next day onwards, the observations on total number of fungal colonies and number of colonies showing the same characters were recorded. The colonies showing the same cultural characters were transferred to PDA slants. These isolates were purified, sub cultured, and maintained as pure culture for further work.

The bacteria were isolated by spread plating 1 ml of 10^{-6} dilution of soil on solidified Nutrient agar and Kings B medium, with the help of a sterilized spreader. The plates were incubated at room temperature for 48 to 72 hours. After incubation period, the bacterial colonies were examined and representative isolates of bacteria were maintained on slants of nutrient agar medium for further studies.

3.9.2. *In vitro* screening of fungal isolates

A total of 39 fungal isolates obtained from rhizosphere soil were preliminarly screened for their antagonistic efficiency against different isolates of pathogen by dual culture method outlined by Skidmore and Dickinson (1976) under *in vitro* condition on PDA medium. With the help of cork borer 10mm diameter mycelial growth from actively grown culture of different isolates of pathogen was transferred separately to the centre of PDA mediated Petri dishes. Mycelial disc of 10mm diameter of each fungal isolate was transferred at 2cm away from the pathogen. In each Petri dish disc of four fungal isolates were screened against the pathogen. Three replications were maintained for each treatment, the pathogen and antagonist grown as monoculture served as control. All the plates were incubated at room temperature and were examined for the antagonistic activity. The measurements on the radial growth of pathogen and the antagonists were taken daily till the control plate showed full growth. Those isolates showing antagonistic properties were selected for the further studies.

3.9.3. *In vitro* screening of bacterial isolates

A preliminary screening on the antagonistic efficiency of 28 bacterial isolates obtained from rhizosphere soil of healthy coconut palm was done by dual culture technique (Dennis and Webster, 1971). With the help of cork borer 10mm mycelial growth of different isolates of pathogen was cut from an actively grown culture and transferred to the centre of PDA mediated Petri dishes. In each Petri dish four numbers of bacterial isolates were streaked at one centimeter from the disc of pathogen for screening the antagonistic efficiency. Three replications were maintained and Petri dish inoculated with pathogen alone served as control. All the plates were incubated at room temperature for 48-72 hours and the bacterial isolates showing antagonistic property were selected for further studies.

3.9.4. *In vitro* evaluation of fungal antagonists against the pathogen

The fungal isolates which recorded the antagonistic property during preliminary screening were used for further evaluation under *in vitro* condition against the pathogen by dual culture method. The antagonistic property of these isolates was compared with the reference cultures of bio control agents viz., *Trichoderma viride and Trichoderma harzianum.* Mycelial disc of 10mm diameter was cut from actively growing cultures of the pathogen and placed at the centre of one half of the Petri dish . Similarly the 10mm disc of fungal antagonist was transferred and placed at the centre of the other half the same Petri dish. Three replications were maintained for each treatment and the pathogen and antagonist grown as monoculture served as control. The plates were examined for the antagonistic activity and the measurements on the radial growth of pathogen and the antagonist were taken daily till the control plates attained full growth.

Per cent inhibition of growth of pathogen over control was calculated by the formula suggested by Vincent (1927)

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

PI= Per cent inhibition on growth of test pathogen

C= Radial growth of pathogen (mm) in control

T= Radial growth of pathogen (mm) in treatment

The nature of reaction of the antagonist on the pathogen was studied by following the method given by Purkayastha and Bhattacharya (1982)

Types of reaction

Homogenous (H) = free intermingling of hyphae

Overgrowth (O) = pathogen overgrown by antagonists

Cessation of growth (C) = cessation of growth at line of contact

Aversion (A) = development of clear zone of inhibition

3.9.5. *In vitro* evaluation of bacterial antagonist against the pathogen

The bacterial isolates which recorded the antagonistic property were selected after preliminary screening and were further evaluated for their antagonistic activity against the pathogen by dual culture technique. The antagonistic property of these isolates was evaluated in comparison with reference biocontrol agent *Pseudomonas fluorescens* on PDA medium. The bacterial antagonist was streaked at one side of the PDA mediated Petri dish (1cm from the edge of the plate) and then mycelial disc of 10mm diameter of the pathogen was placed 1cm from the edge on the opposite side in the Petri dish perpendicular to the bacterial streak. Petri dishes inoculated with pathogen alone were also maintained which served as control. All the plates were incubated at room temperature and observations on growth of pathogen were taken at regular interval. Three replications were maintained for each isolate of pathogen. The per cent inhibition of mycelial growth of pathogen over control was calculated by the method given in 3.9.4.

3.9.6. Identification of effective fungal antagonists

Cultural characters of the effective fungal antagonists were studied for the identification. The colony characters such as colour, nature of mycelial growth, growth rate, time taken for sporulation, colour of spores were studied on PDA medium. Photomicrographs of the efficient antagonists were made. Based on the cultural characters, tentative identification of these antagonistic fungal isolate was carried out. For the further identification, the pure cultures of the antagonists were sent to NCFT, New Delhi for identification upto species level.

3.9.7. *In vitro* evaluation of phytoextracts against the pathogen

An *in vitro* evaluation on the fungicidal efficiency of four different phytoextracts *viz.*, *Clerodendron aculeatum, Azadirachta indica, Chromolaena odorata* and *Musa* sp. (extract of sheath, leaves & rhizome together of Nendran) was carried out on PDA medium by poisoned food technique (Webster and Dennis, 1971).

Table 2. Phytoextracts used for *in vitro* evaluation against *Ganoderma* sp.

Sl. no.	Phytoextracts	Concentration (%)
1	*Azadirachta indica*	20
2	*Azadirachta indica*	10
3	*Chromolaena odorata*	10
4	*Clerodendron aculeatum*	10
5	*Musa* sp.(extract of sheath, leaves& rhizome)	10

To prepare cent per cent concentration of each phytoextract 100g leaves of *Clerodendron*, *Azadirachta*, *Chromolaena* and *Musa* sp. (sheath, leaves & rhizome together) were taken separately, washed well with tap water to remove dirt particles and washed in sterile water. The samples were disinfected with 70 % ethyl alcohol and then exposed to UV light for one hour by keeping it upside down at every 15 minutes. The extract was prepared by macerating the material

using sterilized pestle and mortar with 100 ml of sterile water under aseptic condition and filtered through clean and sterilized muslin cloth to remove coarse particles. Required quantity of the standard extract was taken from cent per cent concentration and was added to molten PDA medium at 45°C, mixed well and then poured into sterile Petri dishes. Mycelial disc of 10mm diameter cut from the actively growing culture of different isolates of pathogen was transferred to the centre of each Petri dish. The plates were incubated at room temperature and measurements on the radial growth of isolates of pathogen were taken till full growth occurred in control plates. Three replications were maintained for each treatment and plates without phytoextracts served as control. The per cent inhibition on growth of the pathogen over the control was calculated by using the formula mentioned in 3.9.4.

3.9.8. *In vitro* evaluation of fungicides against the pathogen

An *in vitro* evaluation of five different fungicides against the pathogen was carried out on PDA medium by poisoned food technique (Zentmeyer, 1995). The details of fungicides used for the evaluation is given below.

Table 3. Fungicides used for *in vitro* evaluation against the pathogen

Sl. no.	Chemical	Trade name	Concentration (%)
1	Aureofungin	Aureofungin sol 46.15% SP	0.03
2	Carbendazim	Bavistin 50% WP	0.1
3	Flusilazole	Nustar 25 SC	0.2
4	Hexaconazole	Contaf 5% EC	0.2
5	Iprobenphos	Kitazin P 48 EC	0.2

Hundred milliliter of PDA medium was sterilized in 250 ml conical flask. The required concentration of each fungicide was mixed separately with the medium sterilized in conical flask, shaked well and poured into sterilized Petri plates at 20 ml per plate. Mycelial discs of ten mm diameter were cut from the actively growing culture of different isolates of the pathogen and placed at the centre of each Petri dish containing the poisoned medium. Three

replications were maintained for each fungicide and medium without fungicide served as control. All the dishes were incubated at room temperature at 26 ± 2°C. The radial growth of pathogen was recorded up to and when the control plates were fully covered with the growth of pathogen. The per cent inhibition of growth of the pathogen was calculated by the formula mentioned in 3.9.4.

3.10. STUDIES ON THE ANTAGONISTIC MECHANISMS OF FUNGAL ANTAGONISTS AGAINST THE PATHOGEN

3.10.1. Mycoparasitism

The mechanisms of antagonism of the selected fungal antagonists identified as *Trichoderma* spp. against the pathogen were studied by dual culture technique on plane agar medium. Sterilized cellophane disc of 90mm diameter was placed in the Petri dish mediated with plane agar with the help of sterilized forceps. Agar discs of 10mm containing the mycelium of pathogen taken from an actively growing culture was inoculated at the centre of one half of the Petri plate 48 hrs prior to inoculation with culture disc of antagonists. Agar disc containing the growth of antagonist was placed at the centre of the other half of the Petri dish. The plates were incubated at room temp and observations were taken at regular intervals until the hyphal growth of the pathogen and antagonists met at the centre.

Microscopic observations on the hyphal interactions between the pathogen and *Trichoderma spp.* was made by transferring a bit of cellophane containing the hyphal growth of both the organisms to a clean slide mounted with cotton blue-lactophenol stain. Photomicrographs of the type of interaction were also made.

3.10.2. Effect of volatile metabolites of *Trichoderma* spp. against the pathogen

The isolates of *Trichoderma spp.* were tested for their ability to produce volatile metabolites to inhibit the growth of the pathogen by inverted plate technique given by Dennis and Webster (1971a) on PDA medium. Transferred a disc of 10mm diameter cut from the actively growing culture of the pathogen to the centre of Petri plate containing sterilized PDA medium. Similarly transferred a disc of 10mm size cut from the actively growing culture of *Trichoderma spp.* to another Petri dish of same size with sterilized PDA medium. Then inverted the plate containing the disc of pathogen on the plate containing disc of *Trichoderma* spp. These plates were sealed with parafilm under aspetic condition. Petri plates without *Trichoderma* spp. served as control. Colony diameter in each treatment was recorded and per cent inhibition was calculated by the same method as given in 3.9.4.

3.10.3. Effect of non-volatile metabolites of *Trichoderma* spp. against the pathogen

The effect of non-volatile metabolites of *Trichoderma* spp. on the growth of the pathogen was studied by two methods *viz.*, culture filtrate method and cellophane paper method (Dennis and Webster, 1971b)

3.10.3.1. Culture filtrate method

The effect of culture filtrates of different isolates of *Trichoderma* spp. on the growth of pathogen was studied by culture filtrate method (Dennis and Webster, 1971b). Fifty milliliter of potato dextrose broth was sterilized in 100ml conical flask and was inoculated with a 5mm mycelial disc of the *Trichoderma* spp. cut from the edge of four day old culture. The inoculated flasks were incubated at 28 ± 2°C till full growth and colour change occurred in the medium (Plate 15).

The broth culture was filtered through Whatman No.1 filter paper and the filtrate was collected in a flask. The culture filtrate was mixed with molten double strength PDA medium in 1: 1proportion. The medium was autoclaved and used to test the effect of non-volatile metabolite of *Trichoderma* spp. The molten double strength PDA medium was poured into Petri plates and after solidification the Petri plates were carefully inoculated with 10mm discs of the test pathogen cut from actively growing culture. Petri plates without amended with culture filtrate served as control and the plates were incubated at room temperature (26 ± 2°C). Three replications were maintained for each treatment. Daily observations on radial growth of mycelium were recorded. The per cent inhibition on the mycelial growth of the pathogen was calculated by the formula mentioned in 3.9.4.

3.10.3.2. Cellophane paper method

The sterilized Petri dishes were mediated with 20ml of molten sterilized PDA medium. A circular disc of sterilized cellophane of same size of the Petri dish was placed over the solidified PDA medium in the Petri plate. From the selected isolates of *Trichoderma* spp. 10mm discs were cut and placed separately on the centre of the cellophane. All the plates were incubated at room temperature for the growth of *Trichoderma* spp. After the incubation period, the cellophane along with the disc of *Trichoderma* spp. was removed and each dish was separately inoculated with different isolates of the pathogen by transferring mycelial disc of 10mm diameter from the actively growing culture. All the Petri dishes were incubated at room temperature and the radial growth of the pathogen was recorded. Petri plates without pre inoculation with antagonists served as control. Three replications were maintained for each treatment. The per cent inhibition on the mycelial growth of different isolates of pathogen was calculated by the formula given in 3.9.4.

3.11. EFFECT OF CULTURE EXUDATE OF *GANODERMA* ON COCONUT LEAVES

Among the different isolates of pathogen production of toxin was noticed in the culture of isolates *viz.,* GM and GVe and toxicity of these exudates were tested on leaves of coconut seedlings. The leaves were inoculated with the exudate after giving pin pricks and the inoculated area was covered with moistened cotton to give humidity and observed for the symptom expression from next day onwards.

3.12. HOST RANGE

The host range of different isolates of pathogen was studied by inoculating the virulent isolates selected from three different regions of Kerala on selected crops *viz.*, arecanut, jackfruit, bread fruit and acacia. Seedlings of the crops were raised in polythene bags containing sterilized potting mixture. The plants were inoculated with five days old cultures of different isolates of pathogen separately at the basal part of the stem after giving injury. The inoculum was covered with a thin layer of moistened cotton. Plants were also inoculated with soil application of the inoculums grown on saw dust-rice bran substrate as described in 3.3. The entire plants were covered with moistened polythene cover to maintain humidity. The inoculated plants were maintained under controlled condition inside a net house and were observed for the symptom expression.

3.14. STATISTICAL ANALYSIS

Analysis of variance was done on the data collected using statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatments were done by using DMRT (Duncan's Multiple Range Test).

RESULTS

4. RESULTS

The present investigation was carried out for the characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut. Studies were conducted on the cultural, morphological and pathogenic characters of the different isolates of the pathogen, symptomatology of the disease, host range and effective management of the pathogen using biocontrol agents, phytoextracts and selected fungicides. The results of experiments are presented below.

4.1 SURVEY AND COLLECTION OF DISEASED SAMPLES OF COCONUT PALM

Purposive sampling surveys were conducted in 21 different coconut gardens of northern, central and southern regions of Kerala. From these regions, specimens of coconut palm infected with basal stem rot were collected and the pathogen was isolated.

4.2 ISOLATION AND MAINTENANCE OF DIFFERENT ISOLATES OF *Ganoderma* sp.

Isolation of *Ganoderma* sp. from stem and roots of diseased coconut palm and from basidiocarps collected from different locations was carried out on Potato dextrose agar (PDA) medium by two different methods. A total of 14 isolates of *Ganoderma* sp. were obtained from 21 locations. Four isolates of *Ganoderma* sp. from southern region, eight isolates from central region and two isolates from northern region of Kerala were obtained. The initiation of the mycelial growth of the pathogen was observed on third days after inoculation in Petri dishes in which the isolation of pathogen was carried out by tissue segment method directly from the basidiocarps. Growth of the pathogen was observed on six days after incubation from the basidiocarps isolated by using moisture chamber method. All the 14 isolates were purified and sub-cultured at frequent intervals.

4.3. *In vitro* EVALUATION FOR THE CONFIRMATION OF CULTURES OF DIFFERENT ISOLATES OF *Ganoderma* sp.

For the confirmation of cultures of different isolates of pathogen obtained from basidiocarps, an *in vitro* study was carried out in saw dust-rice bran medium. The different isolates of pathogen were inoculated on saw dust-rice bran medium in 250ml conical flask and incubated at room temperature. The cultures were observed daily for the growth of mycelium and basidiocarp formation. Those isolates which produced fruiting body and showed similar cultural characters in conical flask were identified as pure cultures of the pathogen and were selected for further studies.

All the 14 isolates of *Ganoderma* sp. took 10-30 days to complete full growth in conical flask mediated with saw dust- rice bran medium. Two isolates from Chevayoor and Kalavoor *viz*., GCe and GA respectively did not show full growth of mycelium in conical flask. Exudate production was observed in isolates *viz*., GM, GVe, GC, GMa, GK and GKa. Among these, very high exudates production was noticed in GVe and GMa. Initially the colour of mycelium in all the isolates was white and later turned to pale yellow in isolates *viz*., GV, GT, GM and brown in GVe, GC, GMa, GK and GKa. Colour change in media was noticed in GM and GMa. Fruiting body development was noticed in eight isolates of *Ganoderma* sp. *viz*., GV, GT, GM, GVe, GC, GMa, GK and GKa and took 14-40 days for its development. The colour and shape of fruiting body developed in conical flask were found vary with different isolates. Semicircular shaped fruiting body was noticed in isolates from Mannuthy (GM) and Chirakacode (GC), tubular in isolates from Vellayani (GV) and Trivandrum (GT), button shaped in isolate from Kannur (GK) and Kasaragod (GKa) and kidney shaped in the isolate from Vettikal (GVe). The results are given in Table 4.

All these eight isolates of pathogen which showed cultural characters similar to that observed in cultural growth on PDA medium and fruiting body production in the saw dust- rice bran medium were selected for further studies and other isolates were discarded (Plate 1). List of isolates of *Ganoderma* sp. selected for further studies are given in Table 5. (Plate 2)

Table 4. *In vitro* evaluation for the confirmation of cultures of different isolates of *Ganoderma* sp.

Sl No.	Characters		Isolates of *Ganoderma* sp.						
			GV	GT	GM	GVe	GC	GMa	GK
1	Time taken for complete mycelial growth (DAI)		10	10	15	15	12	15	13
2	Colour change	Mycelium	White to pale yellow	White to pale yellow	White to yellow	White to yellowish brown	White to pale brown	White to dark brown	White to pale brown
		Medium	-	-	Dark brown	-	Brown	Brown	Dark brown
3	Presence of exudation		-	-	+	++++	++	++++	++
4	Fruiting body	Development(DAI)	40	14	30	23	30	23	31
		Colour	Yellow with white margin	Yellowish white	Brown with yellowish white margin	White margin with grey centre	Pale brown with white margin	White	Yellowish
		Shape	Tubular	Tubular	Semicircular	Kidney shaped	Semicircular	Button shaped	Button shaped

++++ : Very high ++ : Medium + : Very less - : Nil DAI- Days after incubation

GV : Isolate of *Ganoderma* sp.-Vellayani
GT: Isolate of *Ganoderma* sp.-Trivandrum
GM : Isolate of *Ganoderma* sp.-Mannuthy
GVe: Isolate of *Ganoderma* sp.-Vettikal
GC : Isolate of *Ganoderma* sp.-Madakathara
GMa : Isolate of *Ganoderma* sp.-Madakathara
GK : Isolate of *Ganoderma* sp.-Kannur

Contd.....

Sl No.	Characters		Isolates of *Ganoderma* spp.						
			GKa	GMLP	GHC	GCa	GKO	GCe	GA
1	Time taken for complete mycelial growth (DAI)		10	15	20	30	14	#	#
2	Colour change	Mycelium	White to dirty brown	White	White	White	White	White	White
		Medium	yes	-	-	-	-	-	-
3	Presence of exudation		+	-	-	-	+	-	-
4	Fruiting body	Development (DAI)	20	-	-	-	-	-	-
		Colour	Brown with white margin	-	-	-	-	-	-
		Shape	Button shaped	-	-	-	-	-	-

- : Nil + : Very less DAI: Days after incubation # : Incomplete mycelial growth

GKa : Isolate of *Ganoderma* sp.-Kasargod

GMLP : Isolate of *Ganoderma* sp.- Malappuram

GHC : Isolate of *Ganoderma* sp.- Horticulture campus ,Vellanikkara

GCa : Isolate of *Ganoderma* sp.-Calicut

GKO : Isolate of *Ganoderma* sp.-Kottayam

GCe : Isolate of *Ganoderma* sp. -Chevayoor

GA : Isolate of *Ganoderma* sp.-Alapuzha(Kalavoor)

Plate 1. Development of aberrant fruiting bodies in saw dust-rice bran medium

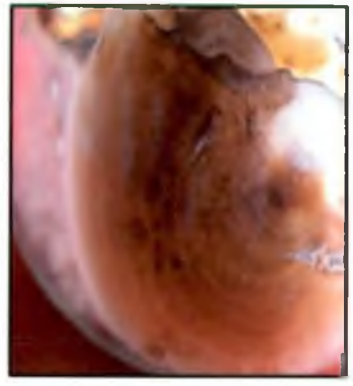
a.*Ganoderma* sp.-Vellayani

b. *Ganoderma* sp.- Trivandrum

c. *Ganoderma* sp.-Mannuthy

d. *Ganoderma* sp.-Vettikal

e. *Ganoderma* sp.-Madakathara



f. *Ganoderma* sp.-Chirakacode

g.*Ganoderma* sp.-Kannur

h. Ganoderma sp.-Kasaragod

Table 5. Isolates of *Ganoderma* sp. selected for further studies

Sl. No.	Region	Location	Isolates
1	Southern	Instructional farm, COA, Vellayani	GV
		Farmer's field, Trivandrum	GT
2	Central	Farmer's field, Vettikkal	GVe
		Farmer's field, Mannuthy	GM
		Farmer's field, Chirakacode	GC
		Farmer's field, Madakathara	GMa
3	Northern	Farmer's field, Kannur	GK
		Farmer's field, Kasaragod	GKa

4.4. PATHOGENICITY TEST

The pathogenicity of the eight different isolates of *Ganoderma* sp. obtained from different locations of Kerala was tested by artificial inoculation on one year old coconut seedlings under *in planta* condition. The inoculation was done at the basal part of the stem and in soil with actively growing mycelium of the pathogen and with spawn of the pathogen multiplied on saw dust- rice bran substrate respectively. Observation on the symptoms expression revealed that none of the seedling inoculated separately with different isolates of pathogen showed any symptoms of the disease at one month after inoculation (MAI). Later all seedlings except those inoculated with the isolate from Trivandrum (GT) showed yellowing symptom on outer leaves.

The seedlings inoculated with the isolates of pathogen from Vellayani (GV) showed yellowing of outer leaves at 2 MAI and one seedling showed drying up of the outer leaves. Later it completely dried up. The other three seedlings inoculated with the isolate GV also showed drying symptom at 6 MAI.

All the seedlings inoculated with the isolate GM from Mannuthy showed yellowing and wilting of leaves and brown discolouration at the base of the stem around the inoculated area. Lesions were developed at the base of stem which

Plate 2. Basidiocarps of selected isolates of *Ganoderma* sp.

a. *Ganoderma* sp.-Vellayani

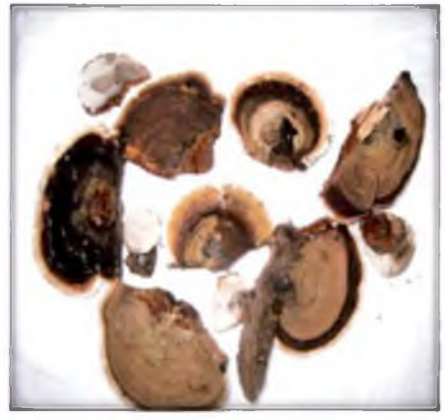
b. *Ganoderma* sp.-Trivandrum

c. *Ganoderma* sp. Mannuthy

d. *Ganoderma* sp. Vettikal

e. *Ganoderma* sp.- Chirakacode

f. *Ganoderma* sp.- Madakathara



g. *Ganoderma* sp. Kannur

h. *Ganoderma* sp.- Kasaragod

later enlarged and observed mycelial growth at the inoculated area and the seedlings were wilted completely at 5MAI.

Similarly the plants inoculated with the isolate from Vettikal (GVe) showed initial symptoms of yellowing of leaves at 2 MAI and complete wilting of one seedling was observed at 5MAI and gradually the other seedlings were also showed wilting symptoms. Development of brown lesions at the base of stem was also noticed in all seedlings inoculated with this isolate (GVe). In the case of seedlings inoculated with the isolates GMa and GC, yellowing of leaves was noticed at 2 MAI and wilting of leaves of all seedlings inoculated with the isolate was observed at 4MAI.

The seedlings inoculated with the isolate GK showed yellowing of leaves at 2MAI. Later drying up of leaves was observed at 4 MAI, and the seedlings wilted and broken at the basal portion. At the inoculated area rotting of tissue was observed. The isolate from Kasaragod (GKa) also showed the yellowing and wilting of seedlings at 2MAI.

Seedlings inoculated with the isolate GT from Trivandrum did not produce any symptoms on the seedlings. The symptom of BSR that is oozing out of exudates was not observed in any seedling. Development of basidiocarp was observed in one seedling inoculated with the isolate GV from Vellayani(Plate 3d). The seedlings maintained as control, did not show any symptom and remained as healthy seedlings, during the period of observation. Reisolation of the pathogen was carried out from the basidiocarp produced by the isolate GV from Vellayani and the cultural characters were compared with the original culture of the isolate. Reisolation could not yield mycelial growth in the case of other seven isolates.

4.5. SYMPTOMATOLOGY OF THE DISEASE

Symptomatology of the BSR disease was studied in detail under natural and artificial conditions.

4.5.1. Symptomatology under natural conditions

Symptoms developed under natural condition were recorded during the survey conducted for the collection of diseased specimens and basidiocarps. In the three regions of Kerala, the palms infected with BSR disease were noticed and the symptoms exhibited by these diseased palms were almost same. Difference in the symptom expression was not observed. The external visible symptoms were yellowing of outer whorl of leaves, gradual drying and drooping of leaves around the trunk leaving the central spindle leaf in up right position (Plate 3). Heavy button shedding was also noticed at the early stage of infection.

In advanced stages of infection, the crown was detached and blown off leaving the decapitated trunk. Exudation of reddish brown viscous fluid from the basal portion of the stem was also noticed in most of the coconut gardens surveyed. In certain areas, bleeding patches were noticed on the trunk near the ground level. Formation of reddish brown discolouration and cracks were also observed on the bark (Plate 3a).

Coconut palms in advanced stages of infection were also noticed during the survey. The decapitated stem became shrivelled and dried up. In certain areas, only the stumps of the dried up palms were observed. Basidiocarp formation was noticed in all areas surveyed. Small sized to large sporocarps were found on the basal portion of the diseased palms as well as on the stumps of the dried up palms (Plate 3b).

4.5.2. Symptomatology under artificial condition

To study the symptomatology of basal stem rot of coconut, one year old coconut seedlings were inoculated separately with eight different isolates of *Ganoderma* sp. obtained from different locations. No symptom development was noticed in any seedlings up to two month after inoculation (MAI). Seedlings

Plate 3. Symptomatology of basal stem rot of coconut under natural conditions

a. Stem bleeding

b. Basidiocarp formation

c. Yellowing and drooping of leaves

d. Decapitated stem

e. Breaking of basal stem

inoculated with all isolates of pathogen except Trivandrum isolate (GT) showed yellowing of outer leaves at 2MAI (Plate 4b). Brown discolouration on the basal part of the stem was noticed in seedlings inoculated with GK isolates of pathogen. Later, yellowed leaves were gradually dried up, complete drying and drooping of leaves were observed at 6 MAI (Plate 4c). Basidiocarp formation was noticed only in one seedling inoculated with the isolate of pathogen obtained from Vellayani (GV) at 5MAI (Plate 4d).

4.6. CHARACTERIZATION OF DIFFERENT ISOLATES OF PATHOGEN

The cultural and morphological characters of different isolates of the pathogen were studied on four different media *viz.*, Potato dextrose agar, Czapek's (Dox) agar, Richard's agar and soil extract agar media.

4.6.1. Cultural characters

The cultural characters *viz.*, colour, texture, mycelial type, rate of growth, number of days required to change the colour of mycelium, point of origin of colour change in mycelium, colour change in medium, production of exudates and presence of aberrant fruiting bodies in the colony were studied on four different media.

4.6.2. Growth rate of different isolates of pathogen on different media

Growth rate of different isolates of *Ganoderma* sp. was studied on four different media mentioned in 4.6. The colony diameter was recorded daily till the colony completely covered 90mm growth in Petri dish. The results are presented in Tables 6 to 9.

4.6.2.1. Growth rate on PDA medium

The data on the growth rate presented in Table 6 revealed that even though there was variation in growth rate, all the eight isolates produced good growth on PDA medium and visible mycelial growth was observed from second day onwards. The isolates from different locations showed variation in the growth rate

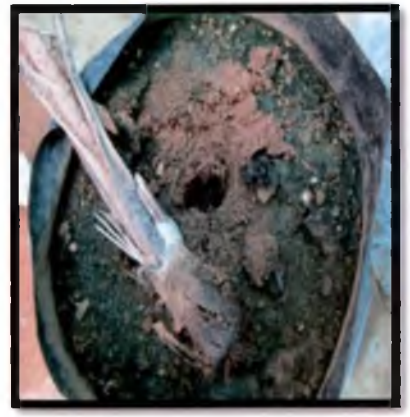
Plate 4. Symptomatology of *Ganoderma* infection under artificial condition

a. Inoculated coconut seedlings

b. Yellowing symptom

c. Drying and wilting of plants

d. Sporophore development- initial and mature stage



e. Breaking at basal portion

Table 6. Growth rate of different isolates of *Ganoderma* sp. on PDA medium

Sl No.	Isolates	Colony diameter in mm*														
		Days after incubation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	GV	18.0	24.1	36.2	53.1	73.0	81.0	90.0								
2	GT	11.0	12.1	15.4	20.1	24.3	34.2	39.6	46.5	56.4	61.1	67.1	71.3	79.6	84.3	90.0
3	GM	17.1	23.9	37.0	50.3	63.1	79.0	90.0								
4	GVe	13.2	18.0	25.2	32.2	43.1	52.0	61.2	70.0	79.1	87.0	90.0				
5	GMa	18.0	26.2	40.1	58.1	80.0	90.0									
6	GC	14.3	17.2	25.2	30.0	43.1	54.2	64.1	77.1	87.0	90.0					
7	GK	12.0	15.1	22.1	29.1	41.3	55.1	70.2	81.1	88.0	90.0					
8	GKa	12.1	17.3	24.2	37.1	49.2	60.0	71.3	82.0	90.0						

* Mean of five replication

GV : *Ganoderma* sp.-Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp. - Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

and the time taken for full growth in Petri dish which ranged from 6 to 15 days. Among the eight isolates, isolate from Madakathara (GMa) took minimum time (6 days) to complete 90mm growth in Petri dish, followed by isolate from Vellayani (GV) and Mannuthy (GM) which took 7 days for the complete growth. The isolate GKa and GVe took 7 and 11 days respectively to complete full growth in Petri dish. The isolates *viz.*, GC and GK took ten days where as the isolate from Trivandrum (GT) took the maximum time of 15 days to complete the full growth in Petri dish (Plate 5).

4.6.2.2 Growth rate on Czapek's (Dox) Agar medium

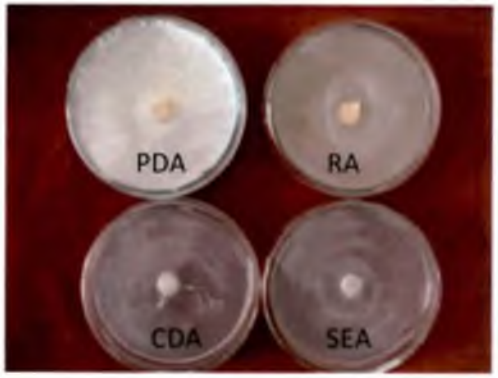
The data presented in Table 7, on the growth rate of different isolates of *Ganoderma* sp. on Czapek's (Dox) agar medium revealed that there was variation in growth rate of isolates from different locations and visible mycelial growth was observed from second day onwards

The isolates GM and GK took minimum of seven days to attain full growth in Petri dish. Isolate from Kasaragod (GKa) took 8 days and it was followed by the isolate from Vettikal (GVe) which took 9 days to complete full growth of 90mm in Petri dish. Among the remaining isolates of *Ganoderma* sp., the isolates from Madakathara (GMa), Chirakacode (GC) and Vellayani (GV) took 10, 12 and 13 days respectively to fill 90 mm growth in dish. Isolate from Trivandrum (GT) took maximum days of 20 to complete full growth to cover the Petri dish (Plate 5).

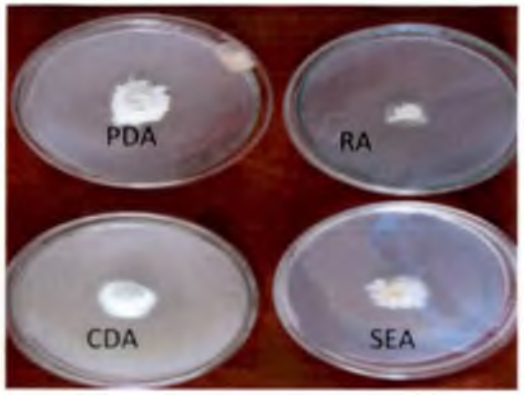
4.6.2.3. Growth rate on Richard's Agar medium

The data presented in Table 8 revealed that there was variation in growth rate of isolates from different locations. Among the eight isolates, three isolates *viz.*, GV, GM and GVe recorded the minimum time of 12 days. Where as the isolates from Kasaragod (GC) and Madakathara (GMa) took 13 and 16 days respectively to complete growth in Petri dish. The isolates *viz.*, GC and GK completed the 90mm growth on the same day (17 days). The isolate GT from Trivandrum could attain only 48.1mm diameter of growth even on 20^{th} DAI and failed to produce full growth in Petri dish on this medium (Plate 5).

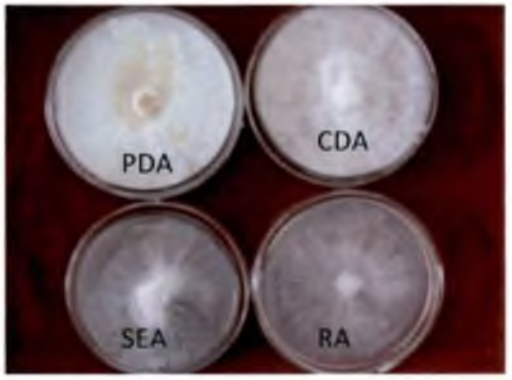
Plate 5. Cultural characters and growth rate of *Ganoderma* sp. on different media



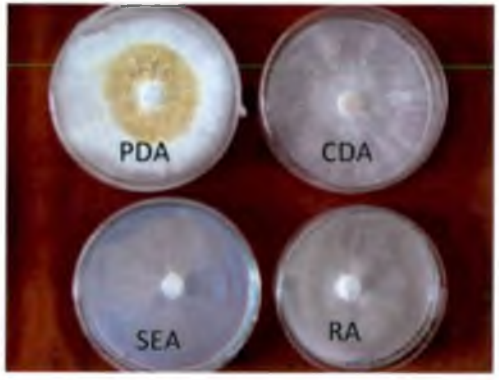
a. ***Ganoderma*** **sp.- Vellayani**



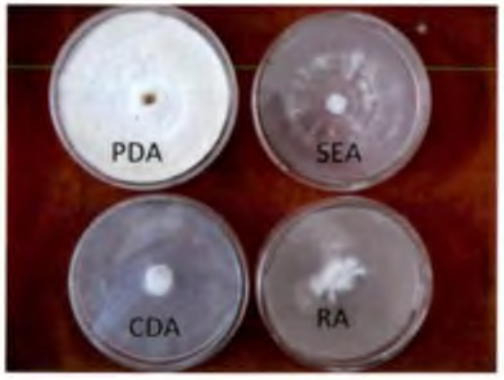
b. ***Ganoderma*** **sp.- Trivandrum**



c. ***Ganoderma*** **sp.-Mannuthy**



d. ***Ganoderma*** **sp.- Vettikal**



e. ***Ganoderma*** **sp.-Madakathara**

f. ***Ganoderma*** **sp.- Chirakacode**

Table 7. Growth rate of different isolates of *Ganoderma* sp. on Czapek's (Dox) agar medium

Si No	Isolates	Colony diameter in mm*																			
		Days after incubation																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	**GV**	18.0	21.3	30.9	40.0	48.1	58.2	65.0	70.1	77.0	83.3	86.0	88.1	90.0							
2	**GT**	11.1	11.6	12.1	15.2	17.1	20.2	24.3	28.8	34.9	40.2	48.3	55.6	60.8	67.4	72.5	77.6	80.5	85.3	88.2	90.0
3	**GM**	17.1	21.0	35.0	48.9	64.1	79.9	90.0													
4	**GVe**	14.0	18.0	25.1	34.8	48.1	62.0	76.1	88.0	90.0											
5	**GMa**	20.1	23.2	28.2	40.2	50.1	62.0	71.1	77.2	85.3	90.0										
6	**GC**	11.0	13.0	17.2	28.0	40.0	58.9	63.2	67.1	76.1	82.3	88.0	90.0								
7	**GK**	17.2	23.1	35.0	54.1	65.2	78.4	90.0													
8	**GKa**	13.2	22.0	30.1	39.1	45.3	65.1	79.2	90.0												

* Mean of five replication

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

Table 8. Growth rate of different isolates of *Ganoderma* sp. on Richard's agar medium

Sl	Isolates	Colony diameter in mm*																			
		Days after incubation																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	GV	12.0	15.1	17.1	22.0	29.9	34.1	43.0	44.9	64.8	74.9	85.0	90.0								
2	GT	10.0	11.0	11.8	13.0	14.1	15.1	17.0	17.9	21.9	24.4	27.0	29.0	30.8	31.9	42.4	43.9	45.0	46.2	47.8	48.1
3	GM	10.0	13.1	17.0	27.0	28.1	36.0	46.0	57.1	68.0	78.1	85.0	90.0								
4	GVe	12.1	14.0	15.0	23.1	31.0	42.1	54.0	63.1	81.0	84.0	88.0	90.0								
5	GMa	11.0	11.1	13.1	16.0	20.0	33.2	34.2	40.0	46.1	55.2	64.1	75.0	79.0	83.1	87.2	90.0				
6	GC	10.0	11.0	13.0	13.1	14.1	15.2	17.0	25.1	32.0	38.9	46.0	53.0	61.9	68.0	76.1	83.0	90.0			
7	GK	10.0	11.0	13.0	13.1	14.1	15.2	17.0	25.1	32.0	38.9	46.0	53.0	61.9	68.0	76.1	83.0	90.0			
8	GKa	14.1	19.0	24.1	29.0	34.0	40.1	47.9	56.1	62.9	72.0	80.0	85.0	90.0							

* Mean of five replication

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa – *Ganoderma* sp.- Kasargod

4.6.2.4. Growth rate on Soil Extract Agar medium

The data on the growth rate of different isolates of *Ganoderma* sp. on soil extract agar medium was presented in Table 9. There was slight variation in growth rate of isolates from different locations and it ranged from 12 to 17 days. The isolate GKa took minimum of 12 days where as GVe and GC took 14 days to complete the 90mm growth. The two isolates *viz*., GV and GM completed the 90 mm growth on the same day (15days) where as the isolate from GMa and GK took 17 days. The isolate GT recorded only 16mm diameter growth on 17 DAI and failed to grow even after prolonged incubation (Plate 5).

4.6.3. Colony characters of different isolates of pathogen on different media

4.6.3.1. Potato dextrose agar medium

All the isolates of *Ganoderma* sp. produced white coloured mycelial growth on Potato dextrose agar medium (PDA) (Table.10). A smooth textured colony was observed in all isolates except in GT and GMa, which showed a rough textured colony (Plate 6a). Among the eight isolates, except GV and GK, all others showed thick mycelial growth. All the isolates showed even growth except GT, which showed uneven growth (Plate 6a). Isolates like GM, GVe, GMa and GKa were having a raised and floccose growth but GV and GT showed compact and felty growth. A longitudinal fissure at the centre with a concentric ring was observed only on the colony of the Chirakacode isolate (GC).

Variation in the colour of mycelium and time taken for the initiation of colour change was also observed. The isolates GT, GM and GC obtained from Trivandrum, Mannuthy and Chirakacode respectively recorded a yellow colour to the mycelium and all other isolates showed a yellowish brown colour.

The number of days required for the colour change varied from 5 to 14 days in different isolates. Except GT, GVe and GKa, all other isolates recorded the colour change on 10 DAI where as GVe and GKa took 5 and 11 days respectively for the colour change. Among the eight isolates, GT took the maximum days (14 days) for the colour change of the mycelium. In all the isolates

Table 9. **Growth rate of different isolates of *Ganoderma* sp. on Soil extract agar medium**

Si No	Isolates	Colony diameter in mm																
		Days after incubation																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	GV	16.0	18.0	24.1	27.9	32.1	38.1	44.1	47.0	50.2	57.1	65.4	74.2	80.3	86.1	90.0		
2	GT	11.1	11.2	12.1	12.4	14.0	14.9	15.3	15.3	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
3	GM	18.0	22.1	26.9	38.1	44.8	53.2	59.0	64.1	70.3	75.1	80.0	84.1	87.0	89.0	90.0		
4	GVe	14.0	17	25	30	39	45	55	62	72	76	80	86	89	90.0			
5	GMa	16.1	19	25	30	37	43	49	61	64	69	74	78	81	82	86	88	90.0
6	GC	12.9	16	21	26	33	41	50	57	63	69	75	80	86	90.0			
7	GK	16.1	19	25	30	37	43	49	61	64	69	74	78	81	82	86	88	90.0
8	GKa	12.0	18.9	24.1	30.9	38.1	49.0	60.2	69.9	75.1	85.0	88.0	90.0					

* Mean of five replication

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

Table 10. Cultural characters of different isolates of *Ganoderma* sp. on Potato dextrose agar medium

SI No.	Isolates	Colony Characters			Colour change of mycelium			Colour change of Medium	Production of exudates	Aberrant fruiting bodies
		Colour	Texture	Mycelial type	DAI	Origin	Mycelium			
1	GV	White	Smooth	Thin, compact, felty and even	10	Centre	Yellowish brown	Brown	-	Absent
2	GT	White	Rough	Thick,compact &felty, uneven	14	Centre	Yellow	-	+	Present
3	GM	White	Smooth	Thick, raised, even &floccose	10	Centre	Yellow	Yellowish brown	+++	Absent
4	GVe	White	Smooth	Thick,even, raised &floccose	5	Centre	Yellowish brown	Brown	++++	Absent
5	GMa	White	Rough	Thick, even raised& floccose	10	Periphery	Yellowish brown	-	+	Absent
6	GC	White	Smooth	Thick, felty to floccose, longitudinal fissure at centre with a concentric ring	10	Centre	Yellow	Yellow	+	Absent
7	GK	White	Smooth	Thin, even	10	Centre	Yellowish brown	Yellow	+	Absent
8	GKa	White	Smooth	Thick, even and raised	11	Centre	Yellowish brown	Yellow	++	Absent

DAI : Days after incubation ++++ Very high +++ High ++ Medium + Low - Absent

GV : *Ganoderma* sp.- Vellayani GT : *Ganoderma* sp.- Trivandrum GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal GMa : *Ganoderma* sp.- Madakathara GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur GKa : *Ganoderma* sp.- Kasargod

except GMa, colour change originated from the centre and spread towards periphery of the colony but in GMa, it was initiated from periphery to centre.

Production of exudate from the culture of different isolates of pathogen was observed. Among the eight isolates, all except GV produced exudate on the surface of the culture. GM and GVe recorded high and very high exudate production respectively where as GT, GC and GK recorded low exudate production. The isolate from Kasaragod (GKa) recorded medium exudate production (Plate 6e).

All isolates, except the isolates from Trivandrum (GT) and Madakathara (GMa) recorded prominent colour change of PDA medium due to their growth. The isolates from Vellayani (GV) and Vettikal (GVe) produced a brown discolouration to the medium where as yellow colour development was observed in GC, GK and GKa. The isolate GM recorded a yellowish brown discolouation to the medium (Plate 6d). Among the eight different isolates, the isolate GT produced the aberrant fruiting body in the culture (Plate 6f).

4.6.3.2. Czapek's (Dox) Agar medium

White coloured and smooth textured mycelial growth of all the eight isolates of *Ganoderma* sp. was observed on CDA medium (Table.11). Thick mycelial band and uneven growth were recorded by the isolates, GT and GC. where as the isolates, GM and GMa recorded uneven growth which was thick at centre and thin towards periphery. The isolates, GV and GK also showed an uneven mycelial growth but it was very thin in GV and thick in GK. The isolates, GKa and GVe recorded thin and even type mycelial growth.

From the data it was observed that no isolates produced colour change in mycelium and medium and aberrant fruiting bodies in CDA medium. Very low quantity of exudate production was observed in three isolates *viz.*, GM, GVe and GMa.

Table 11. Cultural characters of different isolates of *Ganoderma* sp. on Czapeks (dox) agar medium

SI No.:	Isolates	Colony Characters			Colour change				Production of exudates	Aberrant fruiting bodies
		Colour	Texture	Mycelial type	DAI	Origin	Mycelium	Medium		
1	**GV**	White	Smooth	Very thin, filamentous and uneven	-	-	-	-	-	Absent
2	**GT**	White	Smooth	Thick mycelial band & uneven	-	-	-	-	-	Absent
3	**GM**	White	Smooth	Thick at centre & thin towards periphery	-	-	-	-	+	Absent
4	**GVe**	White	Smooth	Very thin and even	-	-	-	-	+	Absent
5	**GMa**	White	Smooth	Thick at centre and thin towards periphery, uneven	-	-	-	-	+	Absent
6	**GC**	White	Smooth	Thick mycelial band and uneven	-	-	-	-		Absent
7	**GK**	White	Smooth	Thick and uneven	-	-	-	-	-	Absent
8	**GKa**	White	Smooth	Thin & even	-	-	-	-	-	Absent

DAI : Days after incubation ++++ Very high +++ High ++ Medium + Low - Absent

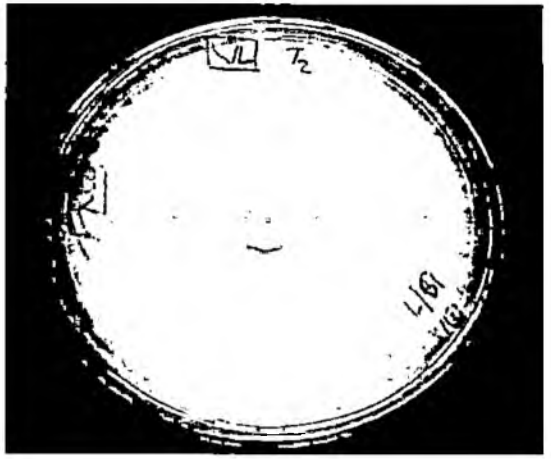
GV : *Ganoderma* sp.- Vellayani GT : *Ganoderma* sp.- Trivandrum GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal GMa : *Ganoderma* sp.- Madakathara GC : *Ganoderma* sp.- Chirakacode

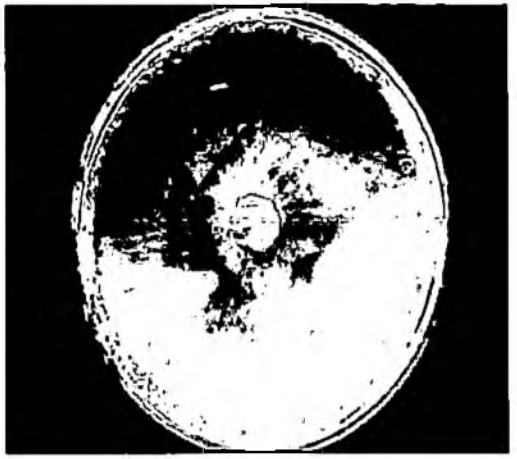
GK : *Ganoderma* sp.- Kannur GKa : *Ganoderma* sp.- Kasargod

Plate 6. Cultural characters of *Ganoderma* sp. on Potato dextrose agar medium

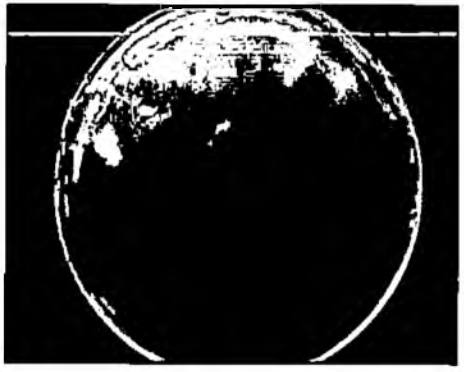
a.Rough uneven colony



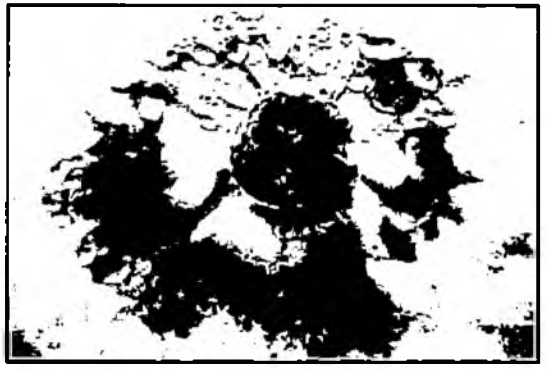
b.Smooth even colony



c. Colour change in mycelium



d. Colour change in medium



e. Exudate production

f. Development of aberrant fruiting body

4.6.3.3. Richard's Agar medium

The colour and texture of the colony recorded by all the eight isolates of *Ganoderma* sp. were white and smooth respectively on RA medium (Table.12). All isolates except GT showed thin and even type mycelial growth where as the isolates from Mannuthy (GM) and Madakathara (GMa) produced thick and uneven type mycelial growth. Filamentous band type mycelium was observed in isolate from Trivandrum (GT).

Colour change of medium and mycelium were observed only in two isolates *viz.*, GM and GMa. Both the isolates showed a pale brown discolouration to the medium, where as a colour change of an yellowish brown and pale brown was observed at the centre of the colony produced by GM and GMa respectively.

The colour change in the mycelium of GM and GMa was observed on 15 and 23 DAI respectively and the same isolates recorded a low production of exudates on the culture. Aberrant fruiting body production was absent in all isolates grown on Richard's agar medium.

4.6.3.4. Colony characters on Soil Extract Agar medium

All eight isolates of the pathogen recorded white coloured and smooth textured mycelial growth on SEA (Table.13). Mycelial growth was thin in all the isolates and even growth was observed in GV and GT where as filamentous band was observed in the isolate GC. Among the eight isolates grown on soil extract agar medium, only one isolate *viz.*, GVe recorded the colour change of mycelium and medium and production of low quantity of exudate in the culture. The colour change of mycelium was started from centre on 6 DAI. The data showed absence of fruiting body production in all isolates grown in this medium.

4.7. MORPHOLOGICAL CHARACTERS OF BASIDIOCARP, BASIDIOSPORES AND HYPHAE OF DIFFERENT ISOLATES OF PATHOGEN.

The morphological characters of basidiocarp such as colour, shape and size of its parts, attachment pattern of stipe and pileus, presence of ornamentation,

Table 12.Cultural characters of different isolates of *Ganoderma* sp. on Richard's agar medium

SI No.	Isolates	Colony Characters			Colour change				Production of exudates	Aberrant fruiting bodies
		Colour	Texture	Mycelial type	DAI	Origin	Mycelium	Medium		
1	**GV**	White	Smooth	Very thin ,even	-	-	-	-	-	Absent
2	**GT**	White	Smooth	Filamentous band	-	-	-	-	-	Absent
3	**GM**	White	Smooth	Thick at centre and towards periphery it is thin	15	Centre	Yellowish brown	Pale brown	+	Absent
4	**GVe**	White	Smooth	Very thin	-	-	-	-		Absent
5	**GMa**	White	Smooth	Thick & uneven	23	Centre	Pale brown	Pale brown	+	Absent
6	**GC**	White	Smooth	Thin	-	-	-	-	-	Absent
7	**GK**	White	Smooth	Thin and even	-	-	-	-		Absent
8	**GKa**	White	Smooth	Thin ,even &compact	-	-	-	-	-	Absent

DAI : Days after incubation ++++ Very high +++ High ++ Medium + Low - Absent

GV : *Ganoderma* sp.- Vellayani GT : *Ganoderma* sp.- Trivandrum GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal GMa : *Ganoderma* sp.- Madakathara GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur GKa : *Ganoderma* sp.- Kasargod

Table 13. Cultural characters of different isolates of *Ganoderma* sp. on Soil extract agar medium

SI No.	Isolates	Colony Characters			Colour change				Productionof exudates	Aberrant fruiting bodies
		Colour	Texture	Mycelial type	DAI	Origin	Mycelium	Medium		
1	GV	White	Smooth	Very thin & even	-	-	-	-	-	Absent
2	GT	White	Smooth	Thin, even & compact	-	-	-	-	-	Absent
3	GM	White	Smooth	Very thin	-	-	-	-	-	Absent
4	GVe	White	Smooth	Very thin	6	Centre	Yellow	Brown	+	Absent
5	GMa	White	Smooth	Very thin	-	-	-	-	-	Absent
6	GC	White	Smooth	Thin, filamentous band	-	-	-	-	-	Absent
7	GK	White	Smooth	Thin	-	-	-	-	-	Absent
8	GKa	White	Smooth	Thin	-	-	-	-	-	Absent

DAI : Days after incubation ++++ Very high +++ High ++ Medium + Low - Absent

GV : *Ganoderma* sp.- Vellayani GT : *Ganoderma* sp.- Trivandrum GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal GMa : *Ganoderma* sp.- Madakathara GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur GKa : *Ganoderma* sp.- Kasargod

method of formation of basidiocarp and pattern of margin of pileus were studied in detail (Plate 7). The descriptions of morphological characters and macroscopic measurements of various parts of basidiocarp and basidiospores (Plate 8) are given in Table 14. Variation in the characters of basidiocarps of the pathogen obtained from different locations was studied. The hyphal system of the basidiocarp was studied and observed trimitic basidiocarp with generative hyphae hyaline, thin walled, branched, septate and clamped. Reddish brown skeletal hyphae and hyaline binding hyphae were also noticed (Plate 9). The vegetative hyphae of different isolates showed no variation. The hyphae were hyaline, thin walled, septate and branched. Clamp connections were observed but not in frequent intervals.

The cultural and morphological characters of basidiocarp, basidiospores and mycelium of the eight different isolates of pathogen were compared with the characters given in CMI Descriptions of Pathogenic Fungi and Bacteria and in the book " Mushrooms" by Roger Phillips and were identified as *Ganoderma lucidum* (Leys.) Karst.

4.8. EFFECT OF TEMPERATURE, LIGHT AND pH ON THE GROWTH RATE OF DIFFERENT ISOLATES OF PATHOGEN

4.8.1. Effect of temperature on the growth rate of different isolates of pathogen

The effect of four different temperatures *viz*., 25, 30, 35 and 40^{0}C on the radial growth of different isolates of the pathogen was studied and the data are presented in Table 15 (Fig 1).

4.8.1.1. Isolate of pathogen from Vellayani (GV)

The isolate GV from Vellayani showed difference among the the treatments on the growth rate. Among the four temperatures tested, the minimum days (5days) taken by the isolate to complete full growth in Petri dish was at 30^o C. It was followed by 25 and 35^{o}C, which recorded 8 days for completing full

Plate 7. Morphological characters of basidiocarp

a. Singly formed basidiocarp

b. Clustered basidiocarp

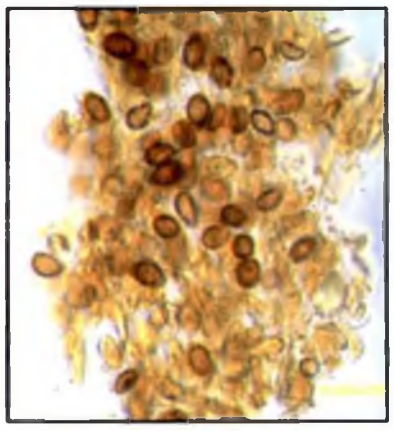
d. Ornamentation

e. Pore surface

c. Parallel and divided attachment

f. Flesh and pore tube

Plate 8. Microphotographs of basidiospores



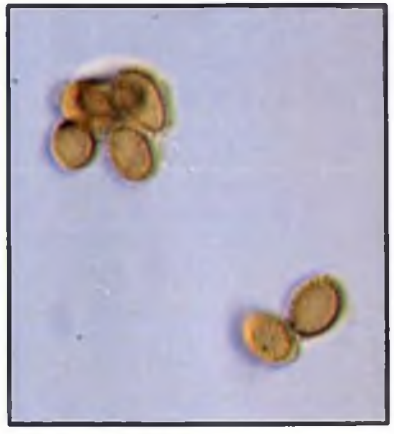
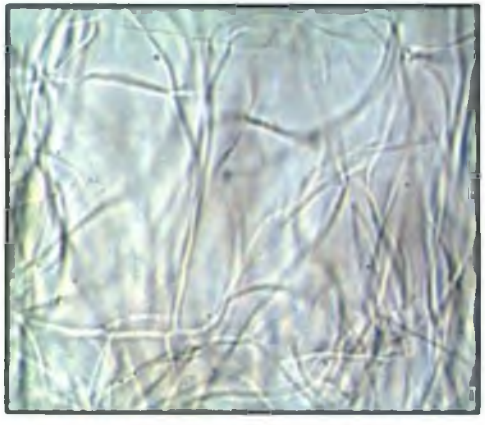


Plate 9. Hyphal system of basidiocarp



a. Generative hyphae (1000X)

b. Skeletal hyphae (1000X)

c. Binding hyphae (1000X)

Table 14. Morphological characters of basidiocarp and basidiospore of different isolates of *Ganoderma* sp.

Sl No.	Isolate	Location	Characters of basidiocarp and basidiospores
1	GV	Vellayani	Basidiocarp stipitate, semicircular, ornamentation absent, formed in cluster, pileus pale brown, shallow waved pileus surface, smooth and yellowish brown margin, creamy white pore surface, 4x5cm size, 3-6 mm flesh thicknes, 1-3mm pore length, 250-288 x 168-190µm pore diameter, stipe reddish brown, 5cm in length, attached laterally to pileus Basidiospore brown, ovoid, truncate to round apex, double walled with inter-wall pillars separating two walls, 7.3-8.4 x 4.7-5.6µm size with 1.41 spore index
2	GT	Trivandrum	Basidiocarp stipitate, semicircular, ornamentation absent, formed singly, pileus shiny,yellowish red, smooth and white to yellowish brown margin, creamy white pore surface, pileus surface shallow waved with 1 to 3 concentric zones, 4 x 3.5cm size, 2-4mm flesh thickness, 2-4mm pore length, 145-162 x 129-155µm pore diameter, shiny and reddish brown stipe, 3-5cm length, parallel and divided attachment pattern of stipe to pileus Basidiospores brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 8.5-13 x 5.5-7.0 µm in size wit 1.57 spore index
3	GM	Mannuthy	Basidiocarp stipitate, triangular to conical, ornamentation present, formed in clusters, pileus shiny and brown, white and smooth margin, creamy white to pale brown pore surface, waved surface with shallow concentric zones, 17 x 12cm size, 10mm flesh thickness, 1-3mm pore length, 139-201 x 122-172µm pore diameter, stipe brown, 8cm length, attached laterally to pileus. Basidiospores brown, ellipsoid to ovoid, truncated apex, double walled with interwall pillars separating two walls, 8.3-11.1 x 4.7-6.2µm size with 1.68 spore index

Contd.....

Sl No.	Isolate	Location	Character of basidiocarp and basidiospores
4	GVe	Vettikal	Basidiocarp sessile, semicircular, ornamentation absent , formed singly, reddish brown pileus, waved brown margin, creamy white to dark brown pore surface, pileus surface waved with 3-4 concentric zones, 11 x7 cm size, 5-8mm flesh thickness, 2-5mm pore length, 180-201 x 153-159µm pore diameter. Basidiospore brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 7.5 x 6.5µm size with 1.15 spore index.
5	GMa	Madakathara	Basidiocarp stipitate, conical, ornamentation absent, formed in single, shiny reddish brown pileus, yellowish white and waved margin, creamy white pore surface, pileus surface shallow waved with three deep concentric zones and 6-9 shallow concentric zones, 10 x 8 cm size, 6mm flesh thickness, 3mm pore length, 162-180 x 122-153µm pore diameter, reddish brown stipe laterally attached to pileus. Basidiospore brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 6.2-8.9 x 4.8-6.2µm size with 1.41 spore index.
6	GC	Chirakacode	Basidiocarp sessile, circular, ornamentation present, formed singly, clay brown pileus, white and waved margin, creamy white pore surface 11 x 10 cm size, 6mm flesh thickness, 3-10mm pore length, 254-305 x 167-178µm pore diameter, , pileus surface waved with 3-5 concentric zones. Basidiospores brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 4.9-8.4 x 4.5-5.7µm size with 1.39 spore index

Contd....

Sl No.	Isolate	Location	Character of basidiocarp and basidiospores
7	**GK**	Kannur	Basidiocarp stipitate, semicircular, ornamentation absent, formed singly, pileus shiny dark brown, pale brown with white tinged smooth margin, white pore surface, 9.5 x 6cm size, 2-4mm pore length, 194-200 x 171-185µm pore diameter, brown stipe, 2cm length, 7mm flesh thickness, stipe laterally attached to pileus Basidiospores brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 4.8-8.4 x 4.7-5.6µm size with 1.39 spore index
8	**GKa**	Kasargod	Basidiocarp stipitate, semicircular, ornamentation present, formed singly, pileus shiny, pale brown to dark brown, white and smooth to shallow waved margin, surface is waved with 2-3 concentric zones, 3.9-6.5 x 4.4-6.5 cm size, 6-8 mm flesh thickness, 2mm pore length, 168-194 x 158-171 µm pore diameter, stipe dark brown, 2.3 cm length, attached laterally to pileus. Basidiospore brown and ovate, truncated apex, double walled with inter-wall pillars separating two walls, 8.1-10.9 x 4.5-5.8 µm size with 1.7 spore index.

Table 15. Effect of temperature on growth rate of different isolates of pathogen

Isolate	Temp (°C)	Colony diameter in mm *																	
		Days after incubation																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
GV	25	12.1	15.2	31.2	44.1	56.3	71.3	86.3	90											
	30	15.3	25.3	45.6	61.0	90[a]														
	35	12.0	14.0	30.3	46.0	62.6	78.0	84.0	90											
	40	11.6	13.3	15.3	17.0	20.0	23.0	23.0	24,0											
GT	25	10.0	12.0	16.0	20.3	25.2	38.0	53.0	71.6	84.0	90.0									
	30	11.0	12.1	15.4	20.1	24.3	34.2	39.6	46.5	56.4	61.1	67.1	71.3	79.6	84.3	90.0				
	35	10.9	11.8	12.6	13.2	13.9	15.0	16.4	17.6	18.9	22.1	25.2	28.7	31.5	34.1	37.2	40.8	43.5	48.2	52.2
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0												
GM	25	12.3	17.3	27.6	40.3	54.3	71.3	87.3	90.0											
	30	12.1	18.3	31.0	43.6	67.6	81.3	90.0												
	35	10.6	12.3	37.6	51.6	68.6	84.0	90.0												
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0											
GVe	25	11.5	18.9	29.4	39.8	52.1	66.4	78.1	90											
	30	12.1	19.0	31.2	41.0	64.1	87.2	90.0												
	35	11.1	12.9	29.6	36.3	68.6	86.0	90.0												
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0											
GMa	25	11.3	17.3	26.6	35.1	43.3	69.6	87.3	90.0											
	30	12.3	19.0	27.0	46.6	68.3	88	90.0												
	35	11.1	13.0	43.3	59.6	76.3	90.0													
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0											

Contd.....

Isolate	Temp (°C)	Colony diameter in mm *										
		Days after incubation										
		1	2	3	4	5	6	7	8	9	10	11
GC	25	12.1	18.2	27.6	36.6	51.1	67.3	82.1	90.0			
	30	12.9	17.8	27.8	37.1	50.9	63.7	82.1	90.0			
	35	11.8	14.0	20.3	26.0	30.6	40.0	52.0	63.6	77.3	85.6	90.0
	40	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	
GK	25	11.1	16.4	24.1	30.8	42.5	59.4	70.5	83.7	88	90.0	
	30	13.0	17.6	23.6	31.6	52.0	72.2	85.6	90.0			
	35	10.1	12.8	3.6	51.1	58.0	74.3	86.0	90.0			
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0		
GKa	25	12.1	16.3	27.2	34.3	45.6	57.6	69.0	80.6	88.0	90.0	
	30	13.1	17.0	27.6	38.6	52.0	64.6	76.1	86.2	90.0		
	35	10.2	12.0	20.0	29.7	36.4	47.9	60.3	73.8	84.2	90.0	
	40	10.0	10.0	10.0	10.0	10.0	10.0	10.0				

* Mean of five replication

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp.- Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

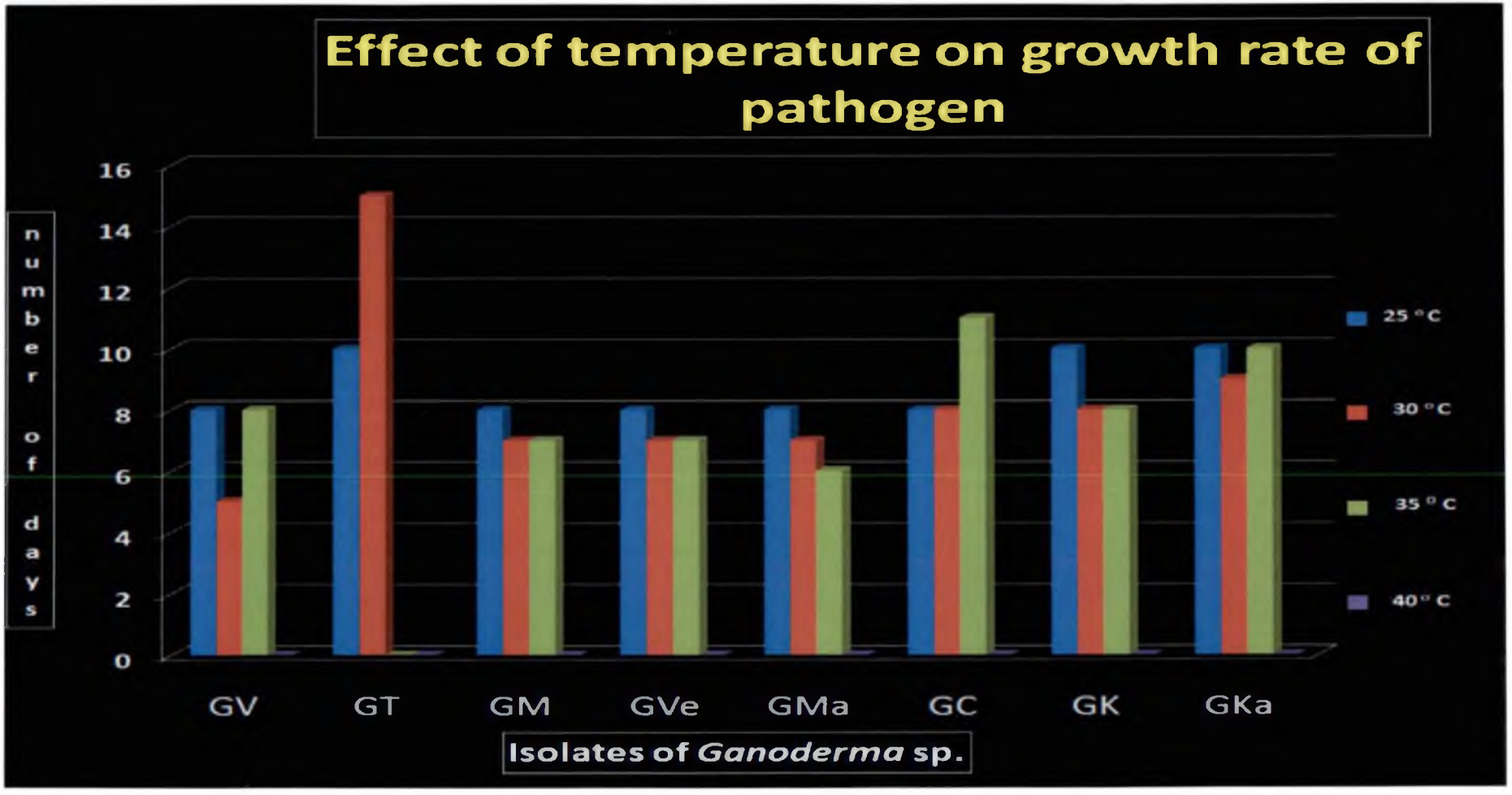


Fig 1. Effect of temperature on growth rate of different isolates of *Ganoderma* spp.

growth in Petri dish. This isolate recorded a slight growth at 40°C and on 8th day it attained 24 mm growth and further growth of this isolate was absent.

4.8.1.2. Isolate of pathogen fromTrivandrum (GT)

The growth rate of *Ganoderma* isolate from Trivandrum showed difference in different treatments. Among the four treatments the maximum growth rate was observed at 25°C and completed 90mm growth in Petri dish on 10th day. It was followed by 30°C, which took 15 days for the complete growth. Full growth of the isolate was not recorded at 35°C which could complete only 52.2mm growth even at 17th day of incubation. No growth was observed at 40° C.

4.8.1.3. Isolate of pathogen from Mannuthy (GM)

Among the four treatments, the isolate showed fast growth rate at 30°C and 35°C and recorded 7 days to complete full growth in Petri dish. It was followed by the growth at 25°C and took 8 days to complete full growth. No growth of the isolate was recorded in Petri dishes maintained at 40°C.

4.8.1.4. Isolate of pathogen fromVettikal (GVe)

From the data on the growth rate of the isolate from Vettikal (GVe) at various temperatures are given in Table 15. It was observed that maximum growth rate was in Petri dishes maintained at 30°C and 35°C which recorded on 7 DAI. It was followed by 25°C which took 8 days to complete full growth in Petri dish. Mycelial growth was not observed in Petri dishes kept at 40°C.

4.8.1.5. Isolate of pathogen from Madakathara (GMa)

The data on the growth rate of the isolate GMa from Madakathara revealed that there was variation in the growth rate under different temperatures. Maximum growth rate of the isolate was recorded in Petri dishes maintained at 35°C (6days) followed by 30°C, which took 7 days to complete the full growth. At 25°C, the isolate took 8 days to complete 90mm growth in Petri dish. No growth rate of the isolate was recorded at 40° C.

4.8.1.6. Isolate of pathogen from Chirakakode (GC)

The isolate from Chirakakode (GC) took the minimum days of 8 days to complete the full growth in Petri dishes at 25 and 30^{0}C. The isolate kept at 35°C took 11 days to attain 90mm growth where as no growth was recorded at 40°C.

4.8.1.7. Isolate of pathogen from Kannur (GK)

The data on the growth rate of the isolate from Kannur (GK) on various temperatures revealed that there was no mycelial growth in Petri dishes exposed to 40°C. Among the remaining three treatments, maximum growth rate (8days) was observed in Petri plates maintained at 30 and 35°C. At 25°C, the isolate took 10 days to complete full growth in Petri dish.

4.8.1.8. Isolate of pathogen from Kasaragod (GKa)

From the data given in Table 15, it was observed that maximum growth rate of the isolate was recorded at 30°C, which took 9 days where as at 25 and 35°C, 10 days were taken to complete full growth in Petri dishes. Mycelial growth was not observed in Petri dishes maintained at 40°C.

4.8.2. Effect of light on growth rate

The results on effect of light on the growth rate of different isolates of pathogen are presented in Table 16 (Fig 2). There is slight difference in the growth rate of different isolates of pathogen exposed to light and darkness. The isolate of *Ganoderma* sp. from Vellayani (GV), Mannuthy (GM) and Kannur (GK) did not show any difference in the growth rate under light and dark condition. The isolates GV and GM took 7 days where as GK took 10 days to complete full growth in Petri dish. The isolates from Trivandrum (GT), Vettikal (GVe), Chirakacode (GC) and Kasaragod (GKa) recorded more time under light condition to cover full growth in Petri dish than in darkness. Among these, the isolate from Kasaragod (GKa) showed fast growth rate and recorded 8 days under dark to complete full growth. It was followed by the isolates from Vettikal and Chirakacode which took 9 days for full growth. The isolate GT took maximum

Table 16. Effect of light and dark on growth rate of different isolates of pathogen

Sl No.	Isolates		Colony diameter in mm*																		
			Days after incubation																		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	GV	L	17.6	24.0	36.0	52.6	72.6	81.1	90.0													
		D	14.0	19.6	31.0	46.6	65.6	87.6	90.0													
2	GT	L	11.1	11.6	12.1	15.2	17.1	20.2	24.3	28.8	34.9	40.2	48.3	55.6	60.8	67.4	72.5	77.6	80.5	85.3	88.2	90.0
		D	11.1	11.6	13.6	16.0	18.6	32.0	45.0	50.3	56.3	61.0	65.3	72.1	79.0	85.6	90.0					
3	GM	L	17.1	24.7	373	49.5	63.9	79.8	90.0													
		D	12.1	19.8	35.6	49.6	64.7	85.8	90.0													
4	GVe	L	12.6	17.6	25.0	32.3	43.3	51.6	61.0	69.6	79.3	86.6	90.0									
		D	12.0	17.1	23.1	32.2	40.3	55.9	70.9	82.3	90.0											
5	GMa	L	18.0	26.1	39.6	58.3	80.3	90.0														
		D	16.3	27.0	40.3	1.0	68.3	74.3	90.0													
6	GC	L	14.9	17.3	24.9	31.1	43.8	55.7	64.8	75.9	86.4	90.0										
		D	13.6	21.6	33.3	42.3	61.6	78.3	84.3	87.0	90.0											
7	GK	L	12.0	15.0	22.3	28.6	41.3	55.3	69.3	81.0	86.5	90.0										
		D	11.8	14.6	21.9	28.0	39.8	54.6	69.0	78.1	86.2	90.0										
8	GKa	L	11.5	17.8	23.6	36.3	47.0	60.3	71.3	82.3	90.0											
		D	10.1	14.6	27.0	40.3	52.6	68.0	7.3	90.0												

* Mean of five replication

L- Light D- Dark

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.- Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GC : *Ganoderma* sp.- Chirakacode

GMa : *Ganoderma* sp.-Madakathara

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

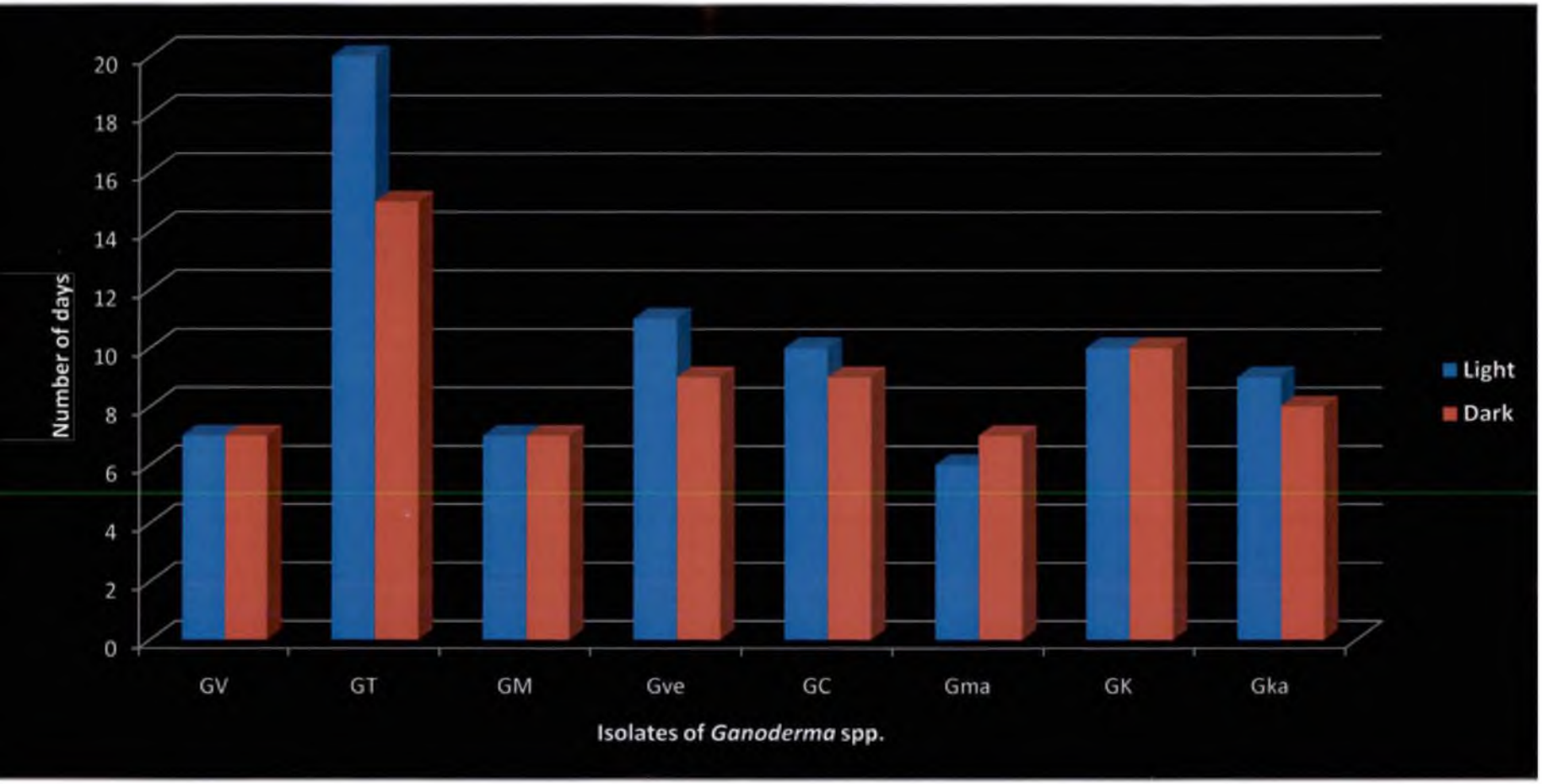


Fig 2. Effect of dark and light on growth rate of different isolates of *Ganoderma* spp.

time of 15 days under dark condition to attain full growth in Petri plates. Among the eight isolates, the isolates GMa recorded fastest growth rate under light and completed the full growth of 90mm diameter at 6 DAI.

4.8.3 Effect of pH on growth rate of different isolates of pathogen

The effect of pH on the growth rate of different isolates of pathogen was tested on PDA medium with pH 5, 6, 7 & 8. The daily observations on the radial growth of mycelium of each isolate were recorded till the Petri dishes attained 90mm growth. The data are presented in Table 17(Fig 3).

4.8.3.1. Isolate of pathogen from Vellayani (GV)

The data given in Table 17, showed difference in the growth rate of isolate from Vellayani (GV) on PDA medium with different pH levels. Among the four pH level tested the isolate GV took the minimum time (6 days) in pH 7 to complete full growth in Petri dish. It was followed by pH 5 and 6 where it takes 7 days to complete full growth. On the medium with pH 8, the isolate took maximum time (9 days) to attain 90 mm growth in Petri dish.

4.8.3.2. Isolate of pathogen from Trivandrum (GT).

The data presented in Table17 on the growth rate of the isolate GT from Trivandrum on PDA medium at various pH levels, revealed that there was slight variation in growth rate of the pathogen. At pH 7, the isolate took the minimum time (15days) to complete the 90mm growth in Petri dish. At pH 5 and 6 it recorded 16 days to complete the full growth. At pH 8, the pathogen took maximum time of 19 days to complete the full growth.

4.8.3.3. Isolate of pathogen from Mannuthy (GM)

From the data furnished in Table 17, it was evident that the minimum times taken by the isolate GM to complete the full growth in Petri dish was recorded at pH 6 and 7 (6days) and at pH 5 (7 days). The isolate took 8 days at pH 8 to complete full growth in Petri dish.

4.8.3.4. Isolate of pathogen from Vettikkal (GVe)

The data presented in Table 17, on the growth rate of the pathogen on PDA medium at four levels of pH recorded variation in growth rate of the isolate, GVe. The maximum growth rate of the isolate was noticed at pH 7, which took 8 days to complete full growth and was followed by pH 8 with 9 days to complete full growth. The maximum time of 11 days was recorded at pH 5 and 6 to attain 90mm growth in Petri dish.

4.8.3.5. Isolate of pathogen from Madakathara (GMa)

From the data, it was found that Petri dishes mediated with PDA medium at pH 5, 6 and 7 took 6 days to complete 90mm growth in Petri dishes. At pH 8, the pathogen took maximum time of 9 days to complete the full growth in Petri dish.

4.8.3.6. Isolate of pathogen from Chirakacode (GC)

The data on the growth rate of the isolate GC recorded difference in completing full growth on Petri dishes at various pH levels. Among the pH levels, the maximum growth rate was recorded at pH 5 (5 days) to complete the 90 mm growth in Petri dish and was followed by 7 days growth rate at pH 6. At pH 7 and 8 the pathogen took 10 and 11 days respectively to complete full growth.

4.8.3.7. Isolate of pathogen from Kannur (GK)

Variation in growth rate of isolate (GK) was observed in medium with various pH levels. Among the four pH levels tested the pH 6 and 7 recorded the minimum time of 9 days where as pH 5 took 10 days to complete full growth in Petri dish. The growth of the isolate at pH 8 recorded a maximum time of 12 days to complete the 90mm growth in Petri dish

4.8.3.8. Isolate of pathogen from Kasaragod (GK)

From the data given in the Table 17, it was observed that at pH 5 and 6, the pathogen took minimum time of 8 days to complete the full growth in 90 mm

Table 17. Effect of pH on growth rate of different isolates of pathogen

Isolate	pH	Colony diameter in mm*																	
		Days after incubation																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
GV	5	13.0	18.3	32.0	47.5	63.0	79.3	90.0												
	6	13.0	17.1	27.2	37.9	64.2	88.0	90.0												
	7	12.9	18.0	33.1	45.8	69.1	90.0													
	8	12.5	20.1	27.3	36.1	54.9	68.1	80.8	88.0	90.0										
GT	5	10.2	13.0	19.8	23.8	29.6	40.2	46.9	51.4	59.2	65.2	69.8	73.5	79.6	83.2	88.0	90.0			
	6	10.0	13.2	21.0	25.0	35.9	47.2	60.0	64.2	67.1	70.0	74.1	78.0	81.0	84.1	88.0	90.0			
	7	12.0	14.1	19.2	24.1	36.1	50.9	64.1	66.0	70.2	74.1	77.0	80.2	83.2	85.1	90.0				
	8	14.0	16.1	18.1	20.1	27.9	33.1	38.0	49.1	59.9	70.0	72.0	74.0	77.0	79.1	81.0	83.2	86.1	88.0	90.0
GM	5	10.5	17.0	33.3	45.8	60.8	75.2	90.0												
	6	10.0	21.1	38.3	54.2	71.9	90.0													
	7	15.0	21.2	36.1	51.8	71.1	90.0													
	8	15.1	22.0	29.1	41.3	58.4	72.1	85.0	90.0											
GVe	5	13.1	17.6	25.8	31.9	42.4	50.1	61.0	69.2	76.9	85.0	90.0								
	6	13.0	18.1	25.2	32.3	43.1	52.4	61.4	70.0	79.1	87.0	90.0								
	7	14.2	21.2	32.4	43.5	56.8	69.5	81.2	90.0											
	8	13.0	18.1	22.2	30.1	43.9	58.1	68.8	81.0	90.0										
GMa	5	11.0	19.8	38.9	63.1	76.5	90.0													
	6	11.0	22.2	40.4	68.1	80.4	90.0													
	7	14.9	20.0	39.1	58.0	74.3	90.0													
	8	12.1	16.0	20.1	29.2	49.3	66.4	83.2	88.1	90.0										

Contd.....

Isoloate	pH	Colony diameter in mm*											
		Days after incubation											
		1	2	3	4	5	6	7	8	9	10	11	12
GC	5	13.9	19.0	47.0	70.1	90.0							
	6	14.0	20.1	30.3	40.8	58.2	76.3	90.0					
	7	14.0	19.8	29.0	37.1	49.0	60.2	72.3	80.0	85.0	90.0		
	8	13.1	18.1	22.3	27.2	34.1	44.3	54.2	65.8	77.1	88.0		
GK	5	12.0	14.2	19.1	26.9	39.8	51.6	63.4	73.2	82.6	90.0		
	6	12.1	13.0	18.3	23.5	38.0	53.1	68.2	83.1	90.0			
	7	12.0	17.1	23.0	29.2	42.0	56.1	70.2	84.0	90.0			
	8	11.6	13.1	19.5	25.4	34.2	44.1	50.9	59.8	66.8	75.9	86.2	90.0
GKa	5	13.9	20.0	31.9	43.2	56.0	69.0	83.1	90.0				
	6	14.0	20.3	32.1	45.0	58.2	71.1	84.0	90.0				
	7	13.1	18.2	28.1	40.0	53.2	61.0	73.2	84.3	90.0			
	8	14.2	17.0	21.2	25.2	34.1	44.0	53.3	65.4	78.0	90.0		

* Mean of five replication

GV : *Ganoderma* sp.- Vellayani

GT : *Ganoderma* sp.- Trivandrum

GM : *Ganoderma* sp.-Mannuthy

GVe : *Ganoderma* sp.- Vettikal

GMa : *Ganoderma* sp.- Madakathara

GC : *Ganoderma* sp.-Chirakacode

GK : *Ganoderma* sp.- Kannur

GKa : *Ganoderma* sp.- Kasargod

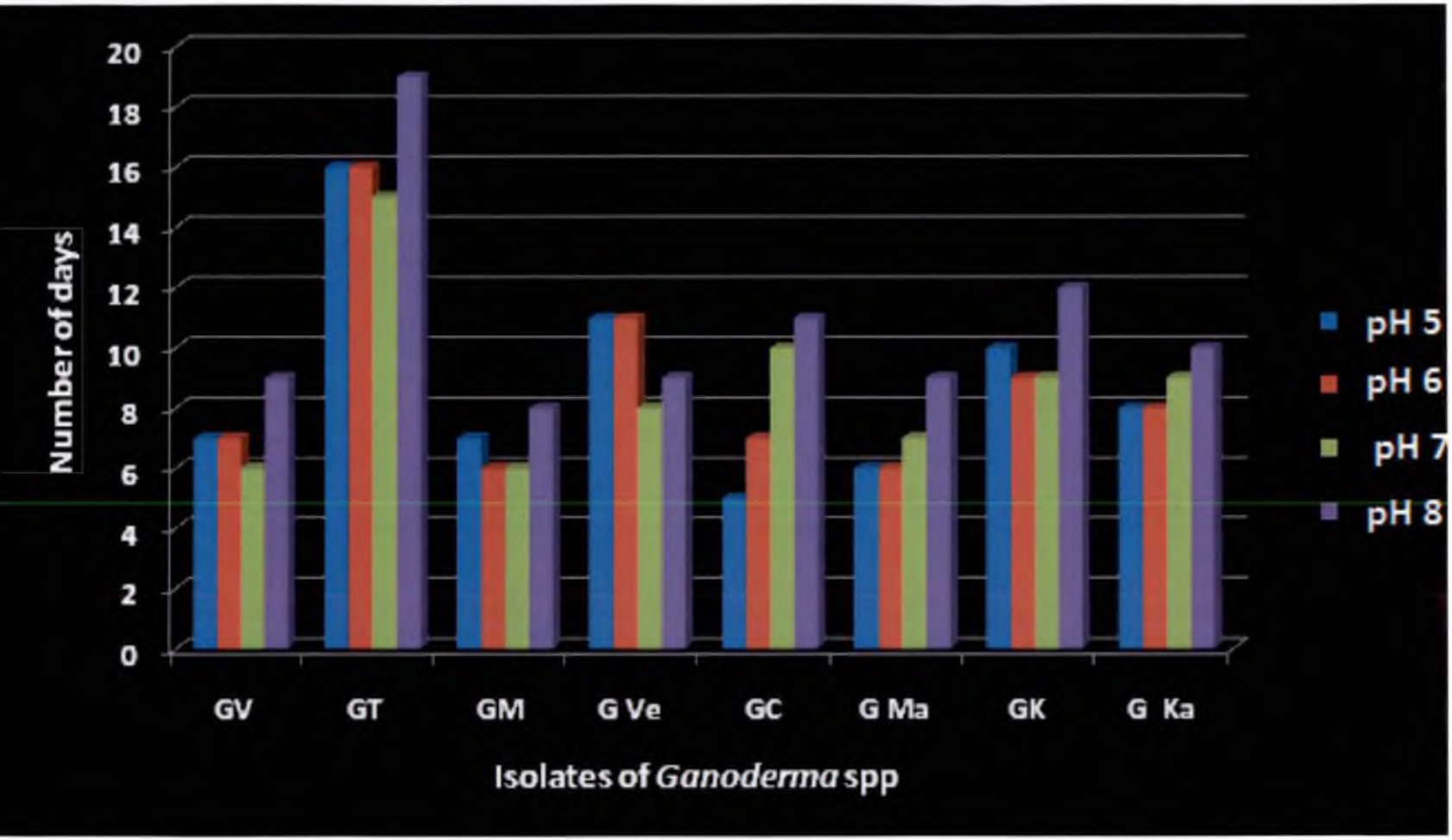


Fig 3. Effect of pH on growth rate of different isolates of *Ganoderma* spp.

Petri dish. At pH 7, 9 days were taken where as in pH 8, the pathogen took 10 days to complete the full growth in Petri dish.

4.9. MANAGEMENT OF PATHOGEN INCITING BASAL STEM ROT

An *in vitro* evaluation on the effectiveness of antagonistic micro-organisms, phytoextracts and fungicides was conducted for the management of pathogen causing basal stem rot of coconut.

4.9.1. Isolation of rhizosphere microflora

Antagonistic micro-organisms were isolated from the rhizosphere region of healthy coconut palm near to BSR infected palm from three regions of Kerala *viz.*, southern, central and northern regions. Rhizosphere soil samples were collected from different locations and antagonists were isolated by serial dilution technique using PDA medium. For the isolation of fungal and bacterial antagonists, a dilution of 10^{-4} and 10^{-6} were used respectively. From the eight different locations a total of 39 fungal isolates and 28 bacterial isolates (Table 18) were obtained.

Table 18. Isolation of antagonistic organisms from rhizosphere soils

Sl no.	Location	No. of colonies		Name of isolate
		Fungi $x10^4$	Bacteria $x10^6$	Fungi
1	Vellayani	4	6	*Trichoderma* sp. *Penicillium* sp.
2	Trivandrum	2	4	*Apergillus flavus*
3	Mannuthy	9	4	*Curvularia* sp. *Trichoderma* sp. *Aspergillus* sp. *Penicillium* sp. Unknown
4	Vettikal	5	3	*Penicillium* sp. *Trichoderma* sp.
5	Chirakacode	3	5	*Penicillium* sp

				Unknown
6	Madakathara	7	2	*Aspergillus niger* *Aspergillus flavus*
7	Kannur	5	2	*Trichoderma* sp. *Aspergillus* sp. Unknown
8	Kasaragod	4	2	*Aspergillus* sp. Unknown
	Total	39	28	

A preliminary screening of these isolates was carried out to evaluate the antagonistic property by dual culture technique. From the preliminary screening eight fungal isolates and three bacterial isolates which showed inhibition on the growth of pathogen were selected for further screening. Tentative identification of the selected antagonists from the preliminary screening was carried out by microscopic observations on the spore, hyphal characters and by studying the cultural characters of the isolates. The identification of the isolates and characters studied are given in the Table 19.

4.9.2. *In vitro* evaluation on the antagonistic efficiency of selected fungal and bacterial isolates against different isolates of the pathogen

The inhibitory effect of eight fungal and three bacterial isolates selected from the preliminary screening was evaluated against different isolates of pathogen under *in vitro* condition. The efficiency of these isolates were compared with the reference culture of fungal antagonists *viz.*, *T. viride* and *T. harzianum* and bacterial antagonist *P. fluorescens*. The results are presented in the Table 20 to 27.

4.9.2.1. Evaluation of antagonistic efficiency of fungal isolates

The antagonistic efficiency of eight selected fungal isolates from the preliminary test was carried out under *in vitro* condition by dual culture technique (Fig 4, Plate 10).

Table. 19. Cultural characters of antagonistic microorganisms

Sl No.	Antagonistic organism	Colony characters
1	*Aspergillus flavus*	Colony fast growing, mycelium mostly submerged, heavily sporulating, conidial heads yellow to green becoming brownish in age, conidiophore and conidia green, biseriate sterigmata
2	*Aspergillus niger*	Fast growing colony, white mycelium, heavily sporulating in black, conidial heads large and globose, conidia coloured and smooth walled conidiophores, colourless biseriate sterigmata
3	*Curvularia* sp.	Hyphae branched, septate and brown coloured, conidiophores erect, conidia produced singly and acrogenously at the tip, 3-4 septate, one of the central cells distinctly larger and darker than the terminal cells.
4	FA-5	Colony slow growing, white, submerged mycelial growth, mycelium hyaline, non-septate thin walled, colourless sporangium like structures, culture have alcoholic smell
5	FA-7	White slow growing colonies, hyphae hyaline, non-septate
6	*Trichoderma* sp. from Vellayani	Fast growing colony, smooth surface, pale green spores, mycelium hyaline, smooth, septate and branched
7	*Trichoderma* sp. from Mannuthy	Fast growing, smooth surface, become hairy dark green, mycelium hyaline, smooth, septate and branched, conidiophores arranged in loose tuft, phialides in false whorls beneath each terminal phialides, usually more than 2-3 phialides
8	*Trichoderma* sp. from Kannur	Fast growing colony, smooth surface, spore pale green, mycelium hyaline, smooth and branched

4.9.2.1.1. Isolate of *Ganoderma* sp. from Vellayani (GV)

The data showed significant difference among treatments on their inhibitory effect against this isolate of pathogen (Table 20). Among the ten antagonists except *A. niger* and *A. flavus*, all other isolates recorded more than 50 per cent inhibition on the growth of pathogen over control. *Trichoderma* sp. from Kannur recorded the highest inhibition of 74.1 per cent on the growth of pathogen over control followed by 66.6 per cent inhibition by the reference culture of *T. harzianum*. The unidentified fungal isolate (FA-5) recorded 64.4 per cent inhibition over control. The lowest inhibition (44.4 %) on pathogen growth was recorded by *A.niger and A.flavus*.

The observations on mechanism of antagonism revealed that among *Trichoderma* spp., cessation of growth was observed in reference culture *T.viride* and *Trichoderma* sp. from Mannuthy and over growth was observed in reference culture of *T.harzianum* and *Trichoderma sp.* from Vellayani. *Trichoderma* sp. from Kannur showed aversion of growth and produced an inhibition of zone with yellow halo in between the pathogen and antagonist. Among the other antagonists except *Curvularia*, cessation of the pathogen growth was observed. In treatment with *Curvularia* sp. free intermingling of hyphae of both antagonist and pathogen was observed.

4.9.2.1.2. Isolate of *Ganoderma* sp. from Trivandrum (GT)

The data furnished in Table 21 revealed that all the antagonists were found very effective in inhibiting the radial growth of the isolate GT of pathogen and recorded cent per cent inhibition on the growth over control. The growth of GT was very slow compared to the growth of antagonists in the dual culture, so the antagonists over grew the pathogen from the third day onwards and complete over growth was observed within a week.

4.9.2.1.3. Isolate of *Ganoderma* sp. from Mannuthy (GM)

From the data given in Table 22, it was evident that there was significant difference in the antagonistic efficiency of different antagonists against the isolate

Table 20. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GV)

SL No.	Isolate		Mycelial growth in mm* Days after incubation								PIOC	Mechanism of antagonism
			2		4		6		7			
			T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	20.1	10.0	45.0	35.2	50.0	43.0	50.0	43.0	44.4^{g}	Cessation of growth
		M	20.1	10.0	50.1	32.1	80.1	90.0	90.0	90.0		
2	*Aspergillus niger*	D	21.1	10.0	50.5	38.1	51.0	40.0	50.0	41.0	44.4^{g}	Cessation of growth
		M	20.1	10.0	50.1	37.1	80.1	90.0	90.0	90.0		
3	Curvularia sp.	D	17.1	25.2	45.1	47.0	45.1	49.0	45.0	49.0	50.0^{f}	Homogenous
		M	24.1	36.2	50.2	85.1	72.5	90.0	90.0	90.0		
4	FA-5	D	16.1	20.0	35.2	45.0	32.0	58.0	32.0	58.0	64.4^{c}	Cessation of growth
		M	20.0	24.0	50.0	63.1	80.0	90.0	90.0	90.0		
5	FA -7	D	29.0	18.0	45.1	45.2	45.0	49.3	45.0	60.0	50.0^{f}	Cessation of growth
		M	20.1	23.9	50.1	62.3	80.1	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	23.6	10.0	41.0	66.0	37.6	76.3	35.0	80.0	61.1^{d}	overgrowth
		M	28.1	10.0	52.1	62.0	81.9	90.0	90.0	90.0		
7	*Trichoderma* sp. from Mannuthy	D	35.0	10.0	40.0	50.0	40.0	50.0	40.0	90.0	55.5^{e}	Cessation of growth
		M	25.5	10.0	45.1	85.0	80.3	90.0	90.0	90.0		
8	*Trichoderma* sp. from Kannur	D	19.7	10.0	44.9	50	35.6	72.5	29.3	75.0	74.1^{a}	Aversion
		M	25.5	10.0	45.1	90.0	80.3	90.0	90.0	90.0		
9	*T.harzianum*	D	19.7	10.0	44.6	48.2	35.2	55.0	30	60.0	66.6^{b}	Overgrowth
		M	25.5	10.0	45.1	90.0	80.2	90.0	90	90.0		
10	*T.viride*	D	21.0	10.0	47.0	43.1	34.5	65.0	34.0	65.0	62.2^{d}	Cessation of growth
		M	25.5	10.0	45.1	90.0	81.0	90.0	90.0	90.0		

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

Table 21. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GT)

Sl No.	Isolate		Mycelial growth in mm* Days after incubation												PIOC	Mechanism of antagonism
			2		6		8		10		12		13			
			T	A	T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	10.0	10.0	15.0	75.0	15.0	75.0	0.0	90.0	0.0	90.0	0.0	90.0	100	Over growth
		M	11.0	10.0	24.0	90.0	39.9	90.0	55.2	90.0	82.0	90.0	90.0	90.0		
2	*Aspergillus niger*	D	10.0	10.0	16.1	70.0	16.0	75.0	12.0	81.0	0	90.0	0	90.0	100	Over growth
		M	11.0	10.0	24.1	90.0	39.9	90.0	55.2	90.0	82.0	90.0	90.0	90.0		
3	*Curvularia sp.*	D	12.0	10.0	18.0	70.2	15.1	85.3	0	90.0	0	90.0	0	90.0	100	Over growth
		M	11.0	36.2	24.0	90	40	90	55.2	90	82.0	90.0	90.0	90.0		
4	Unknown-5	D	11.0	20.1	16.3	62.0	16.0	80.0	10.0	85.0	0	90.0	0	90.0	100	Over growth
		M	11.0	10.0	24.0	62.3	40.0	90.0	55.2	90.0	82.0	90.0	90.0	90.0		
5	Unknown-7	D	10.0	20.3	16.0	60.0	15.0	80.0	0	90.0	0	90.0	0	90.0	100	Over growth
		M	10.0	23.9	24.0	62.3	39.9	90.0	55.2	90.0	82.0	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	10.0	10.0	15.0	75.0	0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	100	Over growth
		M	11.0	10.0	24.0	90.0	39.0	90.0	55.2	90.0	82.0	90.0	90.0	90.0		
7	*Trichoderma* sp. from Mannuthy	D	10.0	10.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	100	Over growth
		M	11.0	10.0	30.1	90.0	47.9	90.0	63.2	90.0	82.1	90.0	90.0	90.0		
8	*Trichoderma* sp. from -Kannur	D	10.0	10.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	100	Over growth
		M	10	10.0	30.0	90.0	48.0	90.0	63.0	90.0	82.0	90.0	90.0	90.0		
9	*T.harzianum*	D	11.0	10.0	0.0	90.0	090	0.0	90.0	0.0	90	0	0	90.0	100	Over growth
		M	11.0	10.0	24.0	90.0	39.0	90.0	56.2	90.0	82.0	90.0	90.0	90.0		
10	*T.viride*	D	12.0	10.0	0.0.	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	90.0	100	Over growth
		M	11.0	10.0	24.0	90.0	39.0	90.0	56.2	90.0	82.0	90.0	90.0	90.0		

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

GM. Among the 10 antagonists, *Trichoderma* sp. from Mannuthy recorded cent per cent inhibition on the growth which was followed by the reference culture, *T. harzianum* and *T. viride* which recorded 86.6 and 77.7 per cent inhibition over control respectively. The antagonists *viz.*, FA-5 and FA-7 inhibited the radial growth of pathogen by 66.6 per cent. The per cent inhibitions of growth recorded by other antagonists were *A.niger* (64.3%), *Trichoderma* sp.–Kannur (61.6%), *Trichoderma* sp.-Vellayani (56.3%), *Curvularia* sp. (55.5%) and *A.flavus* (49.8%).

The observation on the mechanism of antagonism showed that antagonists *viz.*, *A.flavus*, *A.niger* and *Trichoderma* sp. from Vellayani arrest the mycelial growth of the pathogen where as in *Curvularia* sp., free intermingling of hyphae between the antagonist and pathogen was observed. In all isolates of *Trichoderma* spp. except *Trichoderma* sp. from Vellayani and the unidentified isolates, FA-5 and FA-7, overgrowth of the antagonists on the pathogen was observed.

4.9.2.1.4. Isolate of *Ganoderma* sp. from Vettikal (GVe)

The data on the inhibitory effect of different antagonists on the radial growth of the isolate of *Ganoderma* from Vettikal (GVe) are given in Table 23. The statistical analysis of the data showed significant difference among the treatments in their inhibitory effect on the growth of pathogen. All the isolates of *Trichoderma* sp. and the two reference cultures recorded an inhibition of more than 80 per cent on the growth of pathogen over control. *Trichoderma* sp. from Mannuthy and reference culture *T.viride* recorded cent per cent inhibition on the growth of pathogen and were followed by 88.3 per cent inhibition recorded by *Trichoderma* sp. from Kannur. *A. niger* and unidentified isolate (FA-5) showed inhibition on the growth of pathogen by 77.7 per cent over control. The per cent inhibitions recorded by other antagonistic isolates were 55.5 per cent by *Curvularia* sp., 52.2 per cent by FA-7 and 50 per cent by *A. flavus.*

The observations on the mechanism of antagonism showed that all the isolates of *Trichoderma* spp., *A.niger* and unidentified isolate FA-5 over grew on the mycelial growth of the pathogen and showed inhibition on the growth of pathogen. Among the other antagonists, FA-7 and *A. flavus* showed cessation of

Table 22. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GM)

Sl No.	Isolate		Mycelial growth in mm* Days after incubation												PIOC	Mechanism of antagonism
			2		4		6		7		8		9			
			T	A	T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	14.1	10.0	43.1	35.2	45.0	43.1	45.1	44.9	45.1	44.9	45.1	44.9	49.8^{i}	Cessation of growth
		M	15.2	10.0	45.3	32.0	73.1	90.0	83.0	90.0	84.5	90.0	90.0	90.0		
2	*Aspergillus niger*	D	14.8	10.0	30.0	38.0	30.0	60.0	30.0	60.0	32.1	58.9	32.1	57.9	64.3^{e}	Cessation of growth
		M	15.2	10.0	45.3	37.1	73.1	90.0	83.0	90.0	84.5	90.0	90.0	90.0		
3	*Curvularia* sp.	D	13.1	24.9	33.1	52.2	40.1	55.1	40.0	55.1	40.0	55.3	40.0	55.5	55.5^{h}	Homogenous
		M	18.2	36.2	44.9	85.1	70.2	90.0	80.2	90.0	85.1	90.0	90.0	90.0		
4	FA-5	D	15.4	19.7	45.8	45.2	45.0	45.0	43.1	54.1	35.2	60.1	30.0	62.3	66.6^{d}	Over growth
		M	16.1	23.9	45.3	62.1	70.2	90.0	82.5	90.0	87.0	90.0	90.0	90.0		
5	FA-7	D	16.1	20.2	43.1	45.2	44.0	46.0	40.0	53.0	35.1	61.1	30.0	70.0	66.6^{d}	Over growth
		M	15.2	23.9	45.3	62.3	73.1	90.0	83.0	90.0	84.5	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	23.1	10.0	41.6	58.3	40.0	60.0	39.3	60.7	31.1	60.0			56.3^{g}	Cessation of growth
		M	22.2	10.0	58.3	62.0	78.9	90.0	90.0	90.0	90.0	90.0				
7	*Trichoderma* sp. from Mannuthy	D	18.0	10.0	35.1	64.6	20.0	82.6	0	90.0	0	90.0			100^{a}	Over growth
		M	18.3	10.0	38.1	85.0	73.8	90.0	80.2	90.0	90.0	90.0				
8	*Trichoderma* sp. from Kannur	D	18.1	10.0	38.5	62.5	40.0	77.1	40.0	90.0	35.0	90.0			61.1^{f}	Over growth
		M	18.3	10.0	38.1	90.0	73.8	90.0	80.2	90.0	90	90.0				
9	*T.harzianum*	D	25.8	10.0	36.0	64.0	18.5	70.0	14.3	90.0	12.0	90.0			86.6^{b}	Over growth
		M	33.1	10.0	62.9	90.0	80.2	90.0	85.1	90.0	90.0	90.0				
10	*T.viride*	D	15.0	10.0	37.5	43.0	40.0	55.2	20.0	70.0					77.7^{c}	Over growth
		M	22.0	10.0	58.3	90.0	78.9	90.0	90.0	90.0						

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

Table 23. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GVe)

Sl No.	Isolate		Mycelial growth in mm * Days after incubation										PIOC	Mechanism of antagonism
			2		4		6		7		8			
			T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	17.2	19.9	40.4	33.6	45.0	45.0	45.0	45.0			50.0^{h}	Cessation of growth
		M	23.2	32.2	58.2	90.0	85.0	90.0	90.0	90.0				
2	*Aspergillus niger*	D	18.0	17.8	35.0	50.0	20.0	60.0	20.0	60.0			77.7^{e}	Over growth
		M	23.2	37.1	58.2	90.0	85.0	90.0	90.0	90.0				
3	*Curvularia* sp.	D	20.0	20.1	35.1	40.2	40.0	45.0	40.0	50.0			55.5^{f}	Homogenous
		M	20.0	36.2	50.1	85.1	78.0	90.0	90.0	90.0				
4	FA-5	D	20.9	20.1	44.9	40.2	30.1	59.9	20.0	70.0			77.7^{e}	Over growth
		M	23.2	23.9	58.2	62.3	85.0	90.0	90.0	90.0				
5	FA-7	D	15.2	20.3	40.2	45.3	41.1	52.2	43.0	56.0			52.2^{g}	Cessation of growth
		M	24.1	23.9	58.2	62.3	8.0	90.0	90.0	90.0				
6	*Trichoderma* sp. from Vellayani	D	21.1	10.0	42.5	52.5	31.1	60.1	23.2	67.2	15.2	74.9	83.1^{d}	Over growth
		M	23.1	10.0	47.5	62.0	76.3	90.0	82.5	90.0	90.0	90.0		
7	*Trichoderma* sp. from Mannuthy	D	20.2	10.0	37.5	57.5	25.0	64.2	18.0	68.0	0	90.0	100^{a}	Over growth
		M	23.1	10.0	47.5	85.0	76.3	90.0	82.5	90.0	90.0	90.0		
8	*Trichoderma* sp. from Kannur	D	20.2	10.0	38.0	53.2	25.2	65.2	22.5	70.3	10.5	90.0	88.3^{b}	Over growth
		M	23.1	10.0	47.5	90.0	76.3	90.0	82.5	90.0	90.0	90.0		
9	*T.harzianum*	D	20.5	10.0	40.2	55.2	28.0	65.0	22.0	68.0	15.0	75.0	83.3^{c}	Over growth
		M	23.1	10.0	61.5	90.0	81.3	90.0	90.0	90.0	90.0	90.0		
10	*T.viride*	D	19.1	10.0	40.2	55.0	35.0	70.1	22.1	75.0	0	90.0	100^{a}	Over growth
		M	23.1	10.0	47.5	90.0	76.3	90.0	82.5	90.0	90.0	90.0		

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

pathogen growth where as *Curvularia* sp. showed homogenous growth with the pathogen.

4.9.2.1.5. Isolate of *Ganoderma* sp. from Chirakacode (GC)

The data (Table 24) revealed that all the isolates of *Trichoderma* spp. and the reference cultures recorded cent per cent inhibition on the growth of the pathogen over control. Among the remaining antagonists the highest inhibition of 83.4 per cent on the growth of pathogen was recorded by unidentified isolate FA-5. *A. niger* and unidentified FA-7 inhibited the mycelial growth of pathogen by 75.5 per cent and they were found on par with each other. The lowest inhibition on the growth of pathogen was noticed in *Curvularia* sp. by 61.1 per cent over control.

In all the isolates of *Trichoderma* spp., *A. niger*, unidentified fungal isolates *viz.*, FA-5 and FA-7, it was noticed an over growth on the mycelial growth of pathogen as mechanism of antagonism. Cessation of pathogen growth was observed in plates inoculated with *A. flavus* and *Curvularia* sp. showed free intermingling of hyphae with the pathogen.

4.9.2.1.6. Isolate of *Ganoderma* sp. from Madakathara (GMa)

The data given in Table 25 showed statistical difference among the treatments in their antagonistic efficiency and all antagonists recorded more than 50 per cent inhibition on the growth of the isolate from Madakathara. *Trichoderma* sp. from Kannur and the reference culture, *T. harzianum* recorded cent per cent inhibition on the growth of pathogen over control. Among the remaining antagonists maximum inhibition on the growth of pathogen was recorded by *Trichoderma* sp. from Mannuthy (77.7 %) followed by FA-7 (72.2 %). Among the 10 antagonists, lowest inhibition on the growth of pathogen over control was recorded in *A. flavus* (55.5 %).

In all the isolates of *Trichoderma* sp. and unidentified isolates *viz.*, FA-5 and FA-7, over growth on the mycelial growth of pathogen was observed as mechanism of antagonism. *A. flavus* and *A. niger* exhibited cessation on the

Table.24. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GC)

Sl No.	Isolate		Mycelial growth in mm * Days after incubation												PIOC	Mechanism of antagonism
			2		4		6		8		10		11			
			T	A	T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	12.0	19.9	18.5	37.9	27.1	46.7	30.0	53.0	30.0	55.0			66.6^{d}	Cessation of growth
		M	12.4	32.1	29.2	90.0	50.5	90.0	73.1	90.0	90.0	90.0				
2	*Aspergillus niger*	D	12.4	17.8	25.1	35.2	29.6	50.4	28.0	55.1	22.0	59.0			75.5^{c}	Over growth
		M	12.4	37.1	29.2	90.0	50.5	90.0	73.1	90.0	90.0	90.0				
3	*Curvularia* sp.	D	17.0	30.1	40.0	53.0	40.0	55.0	35.0	60.0	35.0	60.0	35.0	60.0	61.1^{e}	Homogenous
		M	14.0	36.2	29.1	85.1	48.1	90.0	67.1	90.0	75.2	90.0	90.0	90.0		
4	FA-5	D	13.3	19.1	38.1	45.2	39.0	50.9	35.1	60.9	14.9	75.1			83.4^{b}	Over growth
		M	12.4	24.1	29.2	62.3	50.5	90.0	72.5	90.0	90.0	90.0				
5	FA-7	D	15.1	20.1	30.2	46.3	35.0	56.1	29.1	61.9	22.0	70.0			75.5^{c}	Over growth
		M	12.4	23.9	29.2	62.3	50.5	90.0	72.5	90.0	90.0	90.0				
6	*Trichoderma* sp. from Vellayani	D	15.7	10.0	25.5	62.5	15.0	90.0	0	90.0	0	90.0			100^{a}	Over growth
		M	16.0	10.0	33.9	62.0	56.8	90.0	80.1	90.0	90.0	90.0				
7	*Trichoderma* sp. from Mannuthy	D	21.0	10.0	26.0	62.0	23.0	77.0	0	90.0	0	90.0			100^{a}	Over growth
		M	16.0	10.0	33.9	85.0	56.8	90.0	80.1	90.0	90.0	90.0				
8	*Trichoderma* sp. from Kannur	D	12.0	10.0	25.5	62.5	0	90.0	0	90.0	0	90.0			100^{a}	Over growth
		M	16.0	10.0	33.9	90.0	56.8	90.0	80.1	90.0	90.0	90.0				
9	*T.harzianum*	D	12.5	10.0	25.5	57.5	25.0	65.0	15.0	75.0	0	90.0			100^{a}	Over growth
		M	16.0	10.0	33.9	90.0	56.8	90.0	80.1	90.0	90.0	90.0				
10	*T.viride*	D	13.0	10.0	27.1	55.0	30.0	90.0	0	90.0	0	90.0			100^{a}	Over growth
		M	16.0	10.0	33.9	90.0	56.8	90.0	80.1	90.0	90.0	90.0				

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

Table.25. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GMa)

Sl No.	Isolate		Mycelial growth in mm * Days after incubation										PIOC	Mechanism of antagonism
			2		4		6		7		8			
			T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	12.3	10.0	26.1	30.5	40.0	50.0	40.0	50.0	40.0	50.0	55.5^{i}	Cessation of growth
		M	12.3	10	27.1	32.1	68.3	90.0	86.2	90.0	90.0	90.0		
2	*Aspergillus niger*	D	12.2	17.8	26.3	35.1	39.4	50.5	36.8	53.4	36.0	54.0	60.0^{h}	Cessation of growth
		M	12.3	10.0	27.1	37.1	68.3	90.0	86.2	90.0	90.0	90.0		
3	*Curvularia* sp.	D	12.2	30.1	27.0	53.0	34.0	57.1	33.0	58.0	31.0	59.1	65.5^{g}	Homogenous
		M	12.4	36.2	27.1	85.1	68.3	90.0	86.2	90.0	90.0	90.0		
4	FA-5	D	12.1	19.1	26.8	48.3	34.1	58.0	30.0	60.1	25.1	64.9	72.2^{c}	Over growth
		M	12.3	24.1	27.1	64.1	68.3	90.0	86.2	90.0	90.0	90.0		
5	FA-7	D	12.1	20.1	26.9	48.9	34.0	57.1	30.6	59.4	27.1	63.0	69.8^{e}	Over growth
		M	12.3	23.9	27.1	62.3	68.3	90.0	86.2	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	15.5	10.0	36.5	56.5	39.0	70.3	37.5	75.0	27.0	80.0	70.0^{d}	Over growth
		M	16.1	10.0	49.5	62.0	71.3	90.0	80.4	90.0	90.0	90.0		
7	*Trichoderma* sp. from Mannuthy	D	35.0	10.0	40.0	47.0	35.0	65.0	28.0	72.0	20.0	80.0	77.7^{b}	Over growth
		M	26.3	10.0	49.5	85.0	71.3	90.0	80.4	90.0	90.0	90.0		
8	*Trichoderma* sp. from Kannur	D	35.0	10.0	43.0	53.0	20.0	70.0	0	90.0	0	90.0	100^{a}	Over growth
		M	16.1	10.0	49.5	90.0	71.3	90.0	80.4	90.0	90.0	90.0		
9	*T.harzianum*	D	15.7	10.0	26.5	50.5	28.0	62.5	25.0	68.0	0.0	90.0	100^{a}	Over growth
		M	16.1	10.0	49.5	90.0	71.3	90.0	80.4	90.0	90.0	90.0		
10	*T.viride*	D	16.0	10.0	27.0	53.0	35.0	58.0	32.0	60.0	29.1	64.5	67.6^{f}	Over growth
		M	16.1	10.0	49.5	90.0	71.3	90.0	80.4	90.0	90.0	90.0		

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

growth of pathogen and free intermingling of pathogen and antagonist was observed in plates inoculated with *Curvularia* sp.

4.9.2.1.7. Isolate of *Ganoderma* sp. from Kannur (GK)

The data showed significant difference among treatments on their inhibitory effect against the isolate of pathogen (GK) (Table 26) and all antagonists recorded more than 50 per cent inhibition on the growth of pathogen. Among the ten antagonists tested, *A. niger*, *Trichoderma* sp from Vellayani and the reference culture *T.harzianum* recorded cent per cent inhibition on the radial growth of pathogen over control. Among the other treatments, maximum inhibition of 80 per cent was recorded by the antagonist FA-5. It was followed by *Trichoderma* sp. from Mannuthy, where it recorded 79.8 per cent inhibition over control. The inhibition percentage of other antagonists were FA-7 (76.7 %), *Trichoderma* sp. from Kannur (72.2 %), reference culture *T.viride* (68.6 %) and *Curvularia sp.* (55.5 %). The lowest per cent inhibition was observed in the isolate *A. flavus* (50 %).

All antagonists except *A. flavus* and *Curvularia* sp. recorded over growth on the mycelial growth of the pathogen as mechanism of antagonism. *A. flavus* and *Curvularia* sp. showed cessation of growth and homogenous growth respectively with the pathogen.

4.9.2.1.8. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

The data on the inhibitory effect of different antagonists on isolate of *Ganoderma* from Kasaragod (GKa) in dual culture technique are given in Table 27.The statistical analysis of data showed significant difference among the treatments on their inhibitory effect on the growth of pathogen. *Trichoderma* sp. from Kannur and the reference culture *T. viride* completely inhibited the growth of isolate of *Ganoderma* from Kasaragod. Among the other antagonists, FA-5 recorded highest inhibition of the pathogen (82.6 %) over control and it was followed by FA-7, *Trichoderma* sp. from Mannuthy and reference culture *T. harzianum* with inhibition of 77.7, 76.6 and 72.3 per cent respectively. The lowest inhibition (50 %) was observed in *A. niger*.

Table.26. Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GK)

Sl No.	Isolate		Mycelial growth in mm Days after inoculation										PIOC	Mechanism of antagonism
			2		4		6		8		10			
			T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	14.0	10.0	45.0	32.0	45.0	45.0	45.0	45.0	45.0	45.0	50^{h}	Cessation of growth
		M	12.1	10.0	22.3	32.1	55.2	90.0	75.2	90.0	90.0	90.0		
2	*Aspergillus niger*	D	13.2	10.0	35.0	38.0	29.0	62.0	0	90.0	0.0	90.0	100^{a}	Over growth
		M	12.1	10.0	22.3	37.2	55.2	90.0	78.2	90.0	90.0	90.0		
3	*Curvularia* sp.	D	12.0	26.0	20.0	47.0	33.0	55.1	38.9	60.0	40.0	60.0	55.5^{g}	Homogenous
		M	12.1	36.2	22.3	85.1	55.2	90.0	75.2	90.0	90.0	90.0		
4	FA-5	D	14.1	20.1	38.1	50.3	35.0	55.3	29.5	61.5	18.0	72.0	80.0^{b}	Over growth
		M	12.1	25.0	22.3	62.3	55.2	90.0	75.2	90.0	90.0	90.0		
5	FA-7	D	10.0	20.0	19.2	45.0	32.0	58.1	27.6	63.0	20.9	69.0	76.7^{d}	Over growth
		M	12.1	24.0	22.1	62.0	55.2	90.0	75.2	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	17.0	10.0	29.3	63.3	0	90.0					100^{a}	Over growth
		M	23.1	10.0	50.1	62.0	90.0	90.0						
7	*Trichoderma* sp. from Mannuthy	D	16.9	10.0	35.1	54.9	18.1	72.9					79.8^{c}	Over growth
		M	23.1	10.0	60.1	85.0	90.0	90.0						
8	*Trichoderma* sp. from Kannur	D	16.9	10.0	33.3	65.1	25.0	73.0					72.2^{e}	Over growth
		M	23.1	10.0	60.1	90.0	90	90.0						
9	*T.harzianum*	D	16.9	10.0	24.0	68.1	0	90.0					100^{a}	Over growth
		M	23.1	10.0	60.1	90.0	90.0	90.0						
10	*T.viride*	D	16.9	10.0	33.3	55.0	28.2	60.0					68.6^{f}	Over growth
		M	23.1	10.0	60.1	90.0	90.0	90.0						

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

Table.27 Antagonistic efficiency of selected fungal antagonists against *Ganoderma* sp. (GKa)

Sl No.	Isolate		Mycelial growth in mm * Days after incubation										PIOC	Mechanism of antagonism
			2		4		8		9		10			
			T	A	T	A	T	A	T	A	T	A		
1	*Aspergillus flavus*	D	17.0	10.0	40.0	31.9	35.0	40.0	35.0	40.0			61.1^{g}	Cessation of growth
		M	18.9	10.0	46.1	32.1	85.6	90.0	90.0	90.0				
2	*Aspergillus niger*	D	15.0	10.0	40.0	38.	45.0	45.0	45.0	45.0	45.0	45.0	50.0^{i}	Cessation of growth
		M	18.3	10.0	44.6	37.1	83.1	90.0	89.0	90.0	90.0	90.0		
3	*Curvularia* sp.	D	17.0	30.1	30.1	50.3	39.0	55.0	39.0	56.0	39.0	56.0	56.6^{h}	Homogenous
		M	16.3	36.2	36.1	85.1	73.0	90.0	84.1	90.0	90.0	90.0		
4	FA-5	D	18.0	19.9	39.8	43.4	34.3	62.3	30.3	70.2	15.6	80.1	82.6^{b}	Over growth
		M	18.3	24.9	44.6	62.3	81.2	90.0	89.1	90.0	90.0	90.0		
5	FA-7	D	17.0	18.0	45.1	487	30.1	60.2	27.0	63.0	20.0	68.0	77.7^{c}	Over growth
		M	18.3	23.9	44.6	62.3	83.1	90.0	89.0	90.0	90.0	90.0		
6	*Trichoderma* sp. from Vellayani	D	17.2	10.0	33.2	60.0	30.0	72.0	26.0	75.0			71.1^{f}	Over growth
		M	18.1	10.0	44.0	62.0	84.0	90.0	90.0	90.0				
7	*Trichoderma* sp. from Mannuthy	D	18.0	10.0	42.1	50.0	35.6	63.2	21	76.0			76.6^{d}	Over growth
		M	18.3	10.0	45.0	85.0	81.2	90.0	90.0	90.0				
8	*Trichoderma* sp. from Kannur	D	17.1	10.0	38.1	72.5	0.0	90.0	O.0	90.0			100^{a}	Over growth
		M	18.0	10.0	44.1	90.0	88.7	90.0	90.0	90.0				
9	*T.harzianum*	D	19.8	10.0	39.9	55.0	31.0	65.0	24.9	70.0			72.3^{e}	Over growth
		M	20.0	10.0	44.2	90.0	82.9	90.0	90.0	90.0				
10	*T.viride*	D	15.0	10.0	36.1	40.0	17.2	65.3	0.0	90.0			100^{a}	Over growth
		M	19.1	10.0	44.1	90.0	84.1	90.0	90.0	90.0				

* Mean of three replication PIOC- Per cent inhibition over control D- Dual culture M- Mono culture T- Test organism
A- Antagonist FA-5: Unknown fungal antagonist. FA-7: Unknown fungal antagonist
In each column, figures followed by same letter do not differ significantly according to DMRT

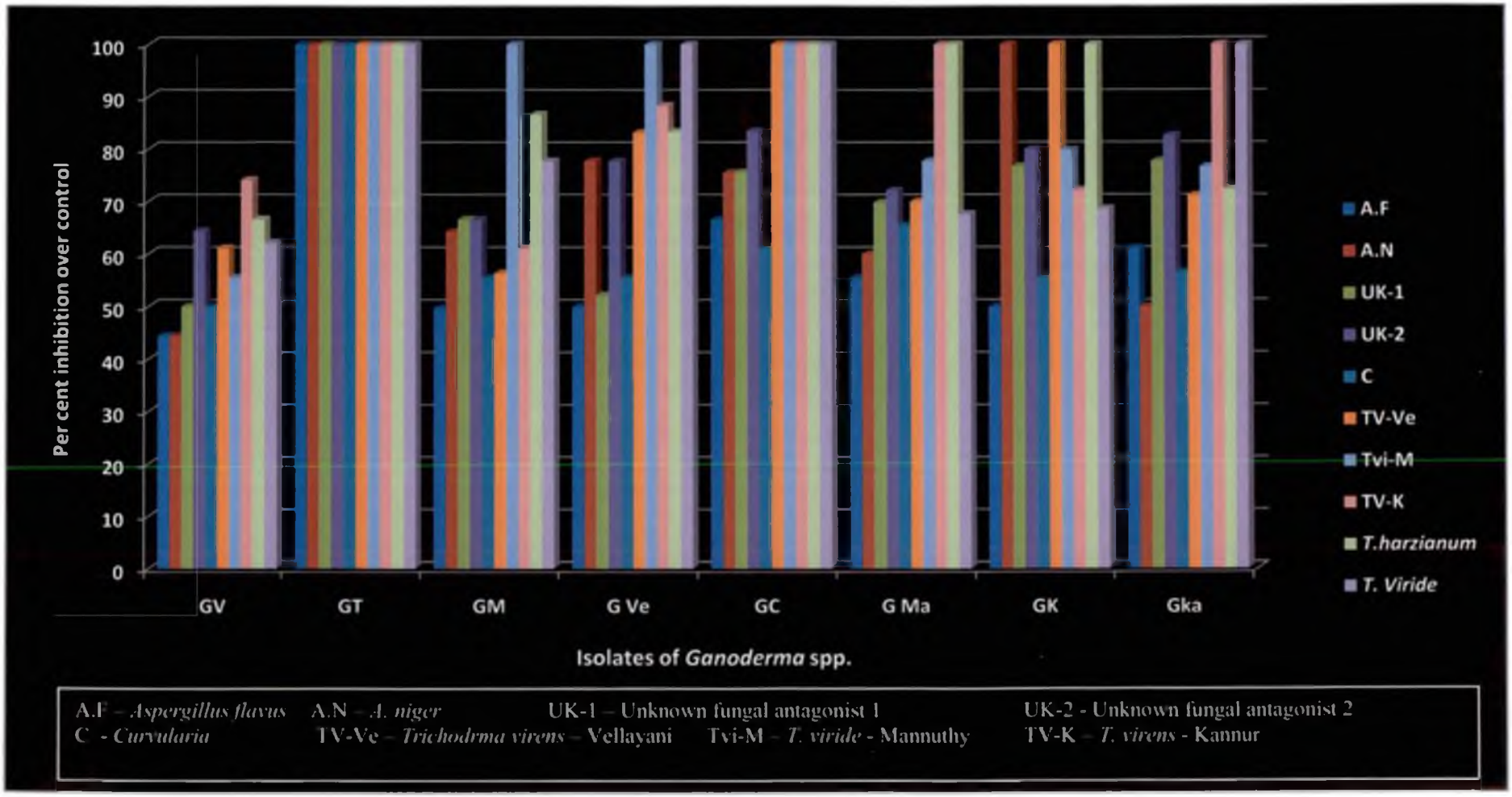
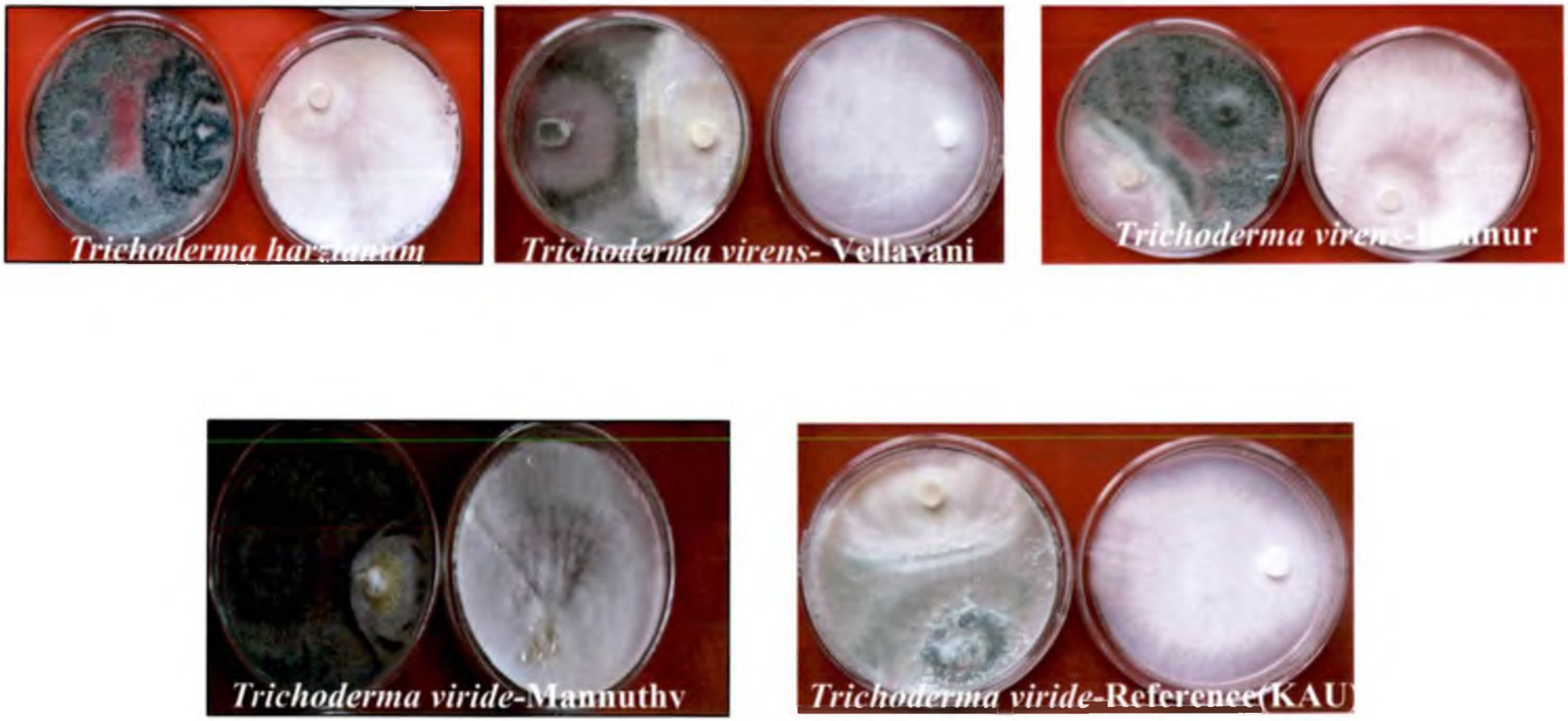


Fig 4. *In vitro* evaluation of fungal antagonists against different isolates of *Ganoderma* spp.

Plate 10. ***In vitro*** **evaluation of** ***Trichoderma*** **spp. against** ***Ganoderma lucidum***



The observations on mechanism of antagonism showed that all the isolates of *Trichoderma* and unidentified isolatates *viz.*, FA-5 and FA-7 over grew the mycelium of the pathogen. *A. flavus* and *A. niger* exhibited cessation on the growth of pathogen. Free intermingling of pathogen and antagonist was observed in plates inoculated with *Curvularia* sp.

4.9.2.2. Identification of selected fungal antagonists

From the above *in vitro* evaluation, *Trichoderma* sp. obtained from Vellayani, Mannuthy and Kannur were selected as effective biocontrol agent against the pathogen (Plate 11). The other antagonist viz., *A. flavus, A. niger, Curvularia* sp. and two isolates of unknown fungal antagonists were not used for further works. The cultural characters of the three isolates of *Trichoderma* sp. were studied on PDA medium in comparison with the reference cultures *viz.*, *T.viride* and *T.harzianum* and the results are given in Table 28.

Observations on age of culture on colony growth rate, light on growth rate, sporulation and metabolite production were recorded.

4.9.2.2.1. Age of culture on growth rate

To know the effect of age of the antagonist on their growth rate, 10mm disc were cut from 2 days, 10 days and 20 days old cultures of *Trichoderma* spp. and inoculated on PDA mediated Petri plates. From the data it was observed that all isolates of *Trichoderma* spp. having 2 days and 10 days old except that from Vellayani and Mannuthy completed 90mm growth on Petri dish on 2 DAI where as the culture disc taken from 20 days old culture of all isolates completed full growth on Petri dish on 4 DAI.

4.9.2.2.2. Effect of light on growth rate

The PDA mediated plates inoculated with different *Trichoderma* spp. were kept at complete light, alternating light and darkness and at complete darkness to know the effect of light on the growth rate of *Trichoderma*. The *Trichoderma* sp. from Vettikal and the reference cultures *viz.*, T.*viride* and *T.harzianum* took 2 days and *Trichoderma* sp. from Vellayani and Mannuthy took 3days to complete

Table 28. Cultural characters of selected *Trichoderma* spp.

Sl No.	Characters			DAI	Radial growth of various *Trichoderma* spp. (mm)				
					TVi-Ve	TV-M	TVi-K	TV	TH
1	Growth rate on PDA	Age of culture disc used for inoculation (Days old)	2	1	33.3	40.0	40.0	69.0	55.0
				2	62.1	85.0	90.0	90.0	90.0
				3	90.0	90.0	90.0	90.0	90.0
			10	1	33.3	40.0	40.0	69.0	55.0
				2	61.5	80.0	90.0	90.0	90.0
				3	90.0	90.0	90.0	90.0	90.0
			20	1	21.5	26.6	33	24.8	32.3
				2	43.6	51.0	59.8	51.3	56.1
				3	68.9	75.0	74.5	69.2	72.5
				4	90.0	90.0	90.0	90.0	90.0
	Effect of light on growth rate	Complete light		2	62.1	85.0	90.0	90.0	90.0
				3	90.0	90.0	90.0	90.0	90.0
		Alternating light (12h)& darkness(12h)		3	90.0	90.0	90.0	90.0	90.0
		Complete darkness		3	90.0	90.0	90.0	90.0	90.0
7	Sporulation	Days taken for initiation of sporulation			2	3	2	3	2
		Complete sporulation(Days)			4	4	4	4	4
8	Colour of spore in culture				Dark green	Pale green	Dark green	Light green	Dark green
9	Metabolite production in PDA				Yes	No	No	Yes	No
10	Colour change in broth culture				Ligtht reddish yellow	Yellow	Yellow	Reddish brown	Yellow

DAI: - Days after incubation
TVi-Ve:-*T.virens* from Vellayani
TV-M:-*T.viride* from Mannuthy
TVi-K: *T.virens* from Kannur
TV-*T.viride* (Reference culture)
TH-*T.harzianum* (Reference culture)

full growth of 90mm in Petri dish under complete light. Under aiternate light and darkness and in complete darkness, all the *Trichoderma* spp. including the reference cultures took 3 days to complete full growth of 90mm in Petri dish.

4.9.2.2.3. Sporulation

The time taken for sporulation of different isolates of *Trichoderma* spp.are given in Table 28. Among the five isolates of *Trichoderma* sp. except reference culture *T.viride* and *Trichoderma* sp. from Mannuthy, sporulation was noticed from second day onwards where as in reference culture of *T.viride* and *T.viride* from Mannuthy sporulation was observed on third day onwards. But in all isolates of *Trichoderma* complete sporulation was noticed in fourth day after incubation.

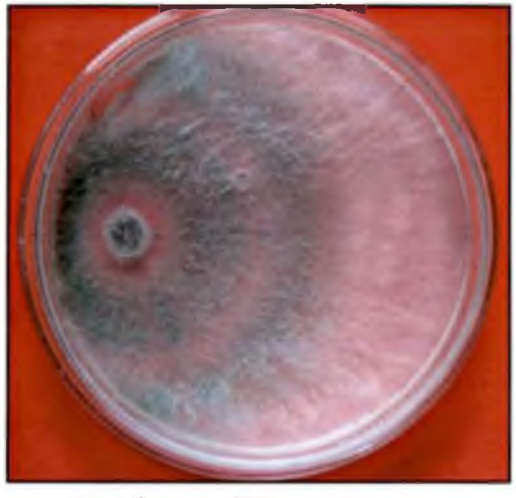
Colour of spores of *Trichoderma* sp. was dark green in three isolates of *Trichoderma viz.*, *Trichoderma* sp. from Vellayani, *Trichoderma* sp. from Kannur and reference culture of *T.harzianum,* where as in other two isolates light green coloured spores were observed .

4.9.2.2.4. Metabolite production

Among the five isolates of *Trichoderma* spp., metabolite production was recorded only in *Trichoderma* sp. from Vellayani and reference culture of *T.viride*. During incubation, colour change in broth culture was recorded. Light reddish yellow colour change observed in the Potato dextrose broth cultured with *Trichoderma* sp. from Vellayani where as reddish brown colour was observed in broth cultured with reference culture of *T. viride*. In the remaining isolates of *Trichoderma*, colour of the broth was yellow.

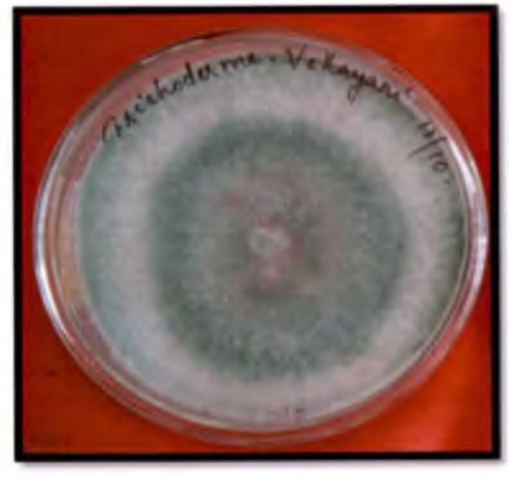
The three selected cultures of *Trichoderma* sp. were identified tentatively by comparing the characters given in "Manual of an identification of plant pathogenic and biocontrol fungi of agricultural importance". The identification was further confirmed by NCFT, New Delhi. The details of identification are given below (Plate 11 and 12).

Plate 11. Selected *Trichoderma* spp. from rhizosphere soil



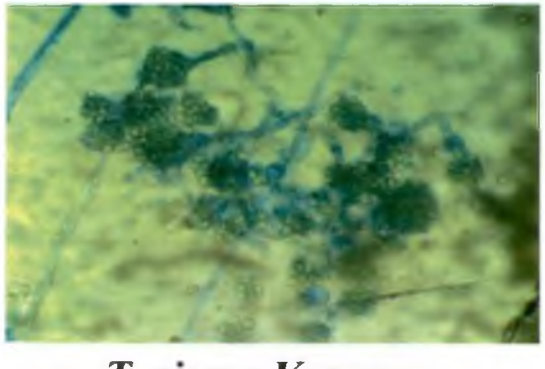
a. *T. virens*-Kannur

b. *T. viride*- Mannuthy

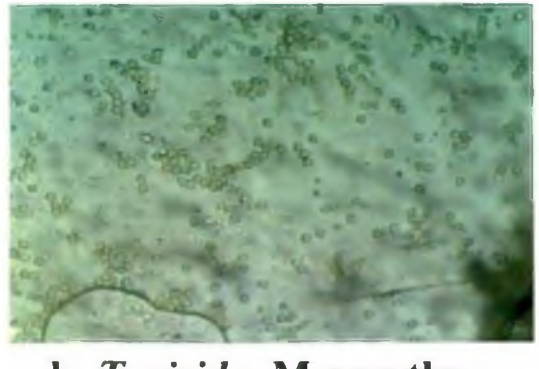


c. *T. Virens*- Vellayani

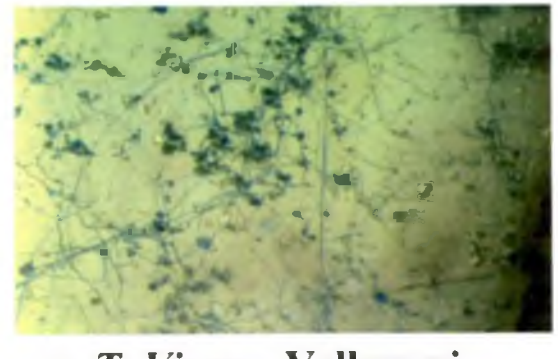
Plate 12. Microphotographs of selected *Trichoderma* spp.



a. *T. virens*-Kannur



b. *T. viride*- Mannuthy



c. *T. Virens*- Vellayani

SI No.	Name of isolate	NCFT ID No.	Identification
1	*Trichoderma* from Vellayani	4602.11	*Trichoderma virens*
2	*Trichoderma* from Mannuthy	4601.11	*Trichoderma viride*
3	*Trichoderma* from Kannur	4603.11	*Trichoderma virens*

4.9.2.3. Evaluation of antagonistic efficiency of bacterial antagonists

An *in vitro* evaluation on the antagonistic efficiency of bacterial antagonists selected from the preliminary screening against different isolates of pathogen was carried out along with the reference culture *Pseudomonas fluorescens* (Plate 13). The results are given Table 29 (Fig 5).

4.9.2.2.1. Effect of bacterial antagonist, BA-1 against the pathogen

From the data given in Table 29, it was revealed that the antagonistic efficiency of bacterial antagonist, BA-1 against the pathogen ranged from 29.1-64.3 per cent. Among the eight isolates of *Ganoderma* sp. the highest inhibition on the radial growth was observed in *Ganoderma* isolate from Trivandrum (64.3 %) and the lowest inhibition of 29.1 per cent was recorded in isolate from Madakatahra (GMa). The isolates of *Ganoderma* sp. obtained from Trivandrum and Chirakacode recorded more than 50 per cent inhibition on the growth over control and which recorded 64.3 and 50.1 per cent respectively.

4.9.2.2.2. Effect of bacterial antagonist, BA-2 against the pathogen

The bacterial antagonist BA-2 recorded the maximum inhibition of 58.6 per cent on the growth of *Ganoderma* isolate from Kannur (GK) over control and it was followed by 52 per cent which was noticed in the isolate of pathogen from Trivandram (GT). The bacterial antagonist showed less than 50 per cent inhibition on the growth of other isolates of pathogen.

4.9.2.2.3. Effect of bacterial antagonist, BA-3 against the pathogen

The data on the antagonistic efficiency of the bacterial isolate BA-3 showed that the maximum inhibition on the growth of the pathogen was recorded

Table 29: Per cent inhibition on radial growth of isolates of *Ganoderma* sp. by the selected bacterial antagonists

Sl No.	Bacterial isolates	Percent inhibition over control*							
		GV	GT	GM	GVe	GMa	GC	GK	GKa
1	**BA-1**	44.3^{b}	64.3^{a}	42.0^{b}	41.1^{a}	29.1^{c}	50.1^{a}	38.8^{c}	34.8^{c}
2	**BA-2**	49.7^{a}	52.0^{b}	43.2^{b}	39.8^{a}	43.0^{a}	38.5^{c}	58.6^{b}	49.7^{b}
3	**BA-3**	46.5^{b}	49.8^{c}	45.6^{a}	40.0^{a}	28.0^{c}	51.0^{a}	66.5^{a}	50.5^{a}
4	**PF**	33.0^{c}	27.5^{d}	34.3^{c}	33.1^{b}	35.4^{b}	40.6^{b}	27.5^{d}	33.2^{d}

* Mean of three replication

PF : *Pseudomonas fluorescens*

BA-1: Bacterial antagonist-1

BA-2: Bacterial antagonist-2

BA-3: Bacterial antagonist-3

GV : *Ganoderma lucidum*–Vellayani

GT : *G. lucidum* -Trivandrum

GM : *G. lucidum* –Mannuthy

GVe: *G. lucidum* - Vettikkal

GMa: *G. lucidum* –Madakathara

GC : *G. lucidum* - Chirakacode

GK : *G. lucidum* – Kannur

GKa: *G. lucidum* - Kasaragod

In each column, figures followed by same letter do not differ significantly according to DMRT

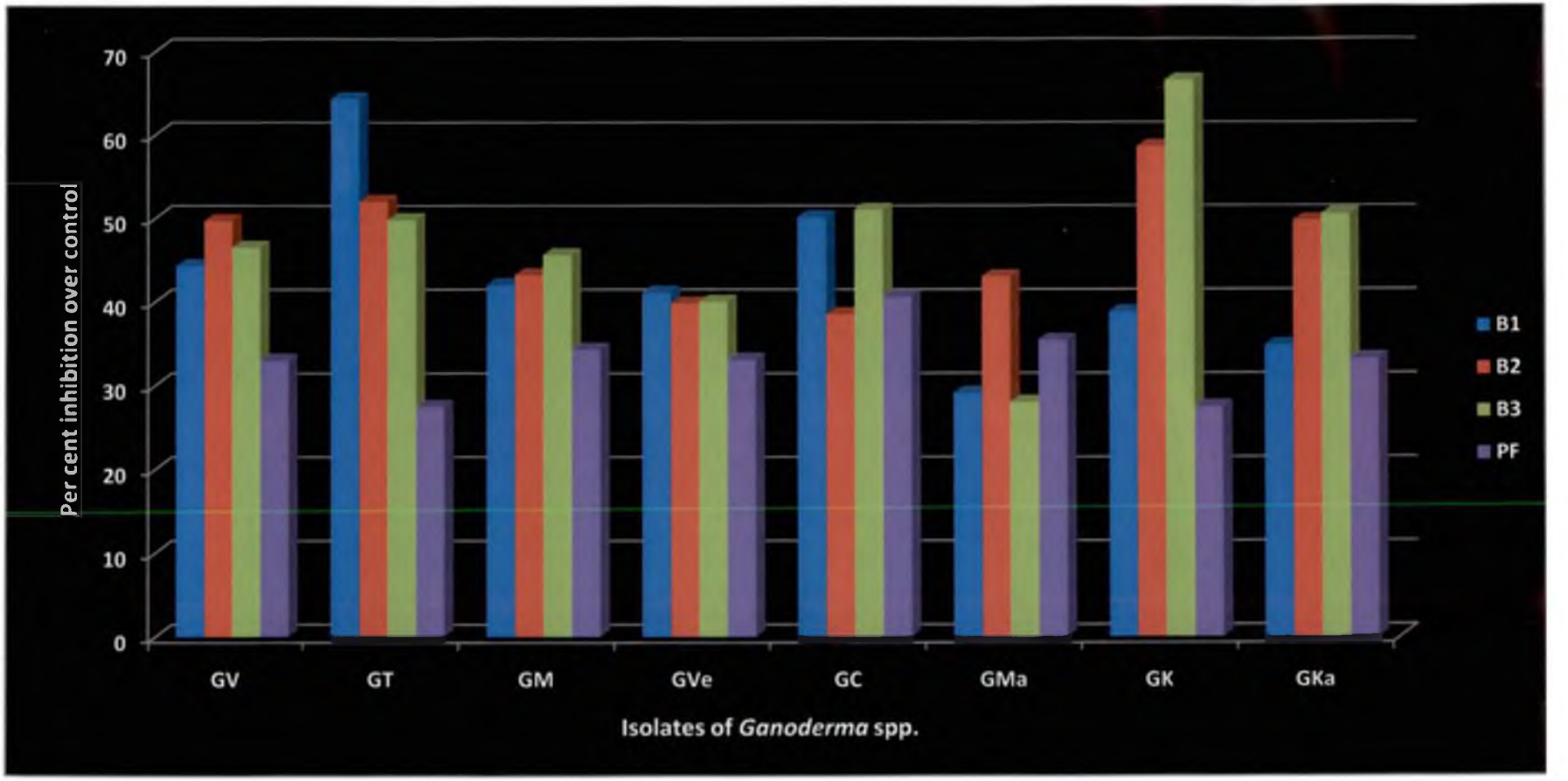
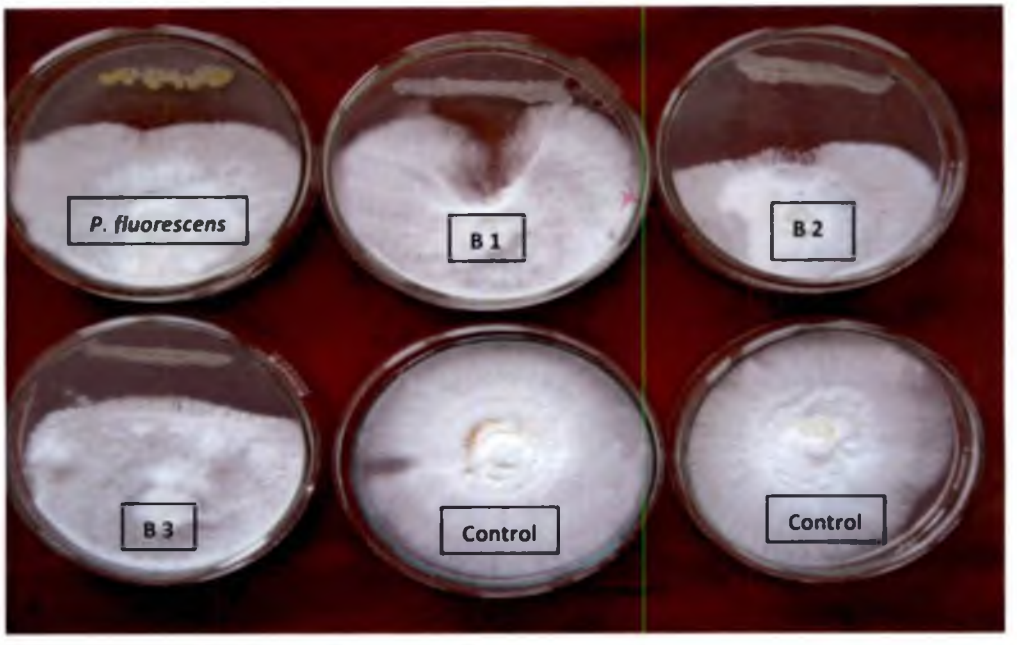


Fig 5. *In vitro* evaluation of bacterial antagonists against different isolates of *Ganoderma* spp.

Plate 13. ***In vitro*** **evaluation of bacterial antagonists against** ***G. lucidum***



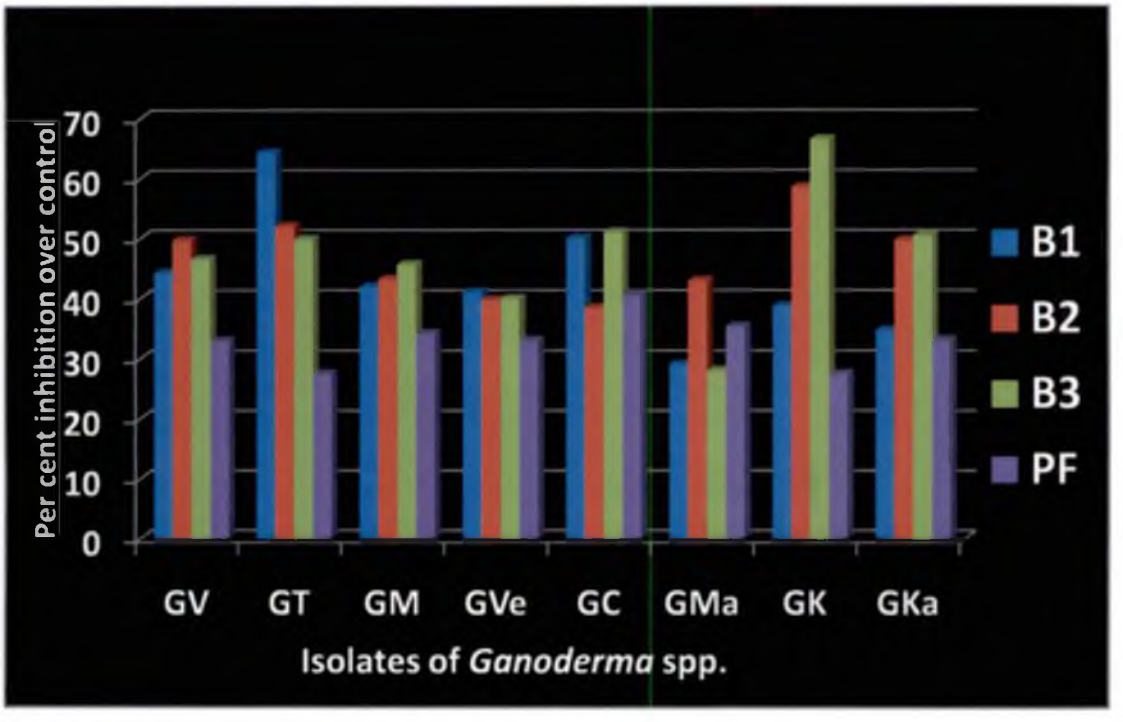


Fig.5. ***In vitro*** **evaluation of bacterial antagonists against different isolates of** ***Ganoderma*** **spp.**

in the isolate of pathogen from Kannur (66.5 %). It was followed by the isolates of pathogen from Chirakacode (51 %) and Kasaragod (50.5 %). The minimum inhibition was recorded in the isolate of pathogen from Madakathara (28 %) over control.

4.9.2.2.4. Effect of reference culture, *P. fluorescens* against the pathogen

The reference culture *P. fluorescens* recorded the inhibition percentage in the range of 27.5 to 40.6 on the radial growth of different isolates of pathogen. The reference culture *P. fluorescens* recorded less than 50 per cent inhibition on the growth of eight different isolates of pathogen and among them the highest inhibition was recorded against the isolate GC (40.6 %)

4.9.3. Mechanism of antagonism of selected fungal antagonists against the pathogen

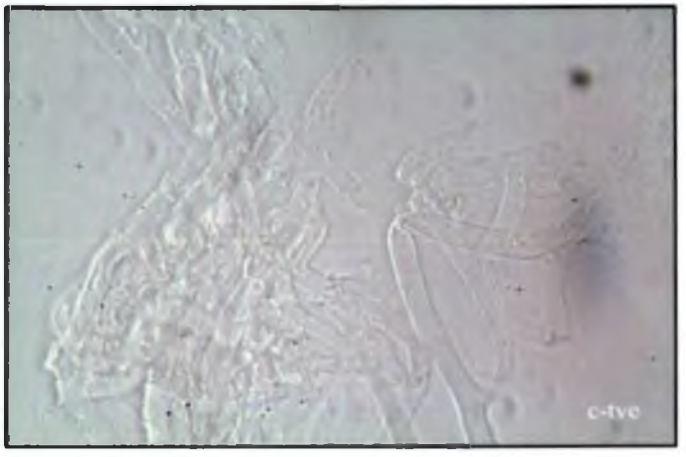
The mechanisms of antagonism of selected fungal antagonist *viz.*, two isolates of *T. virens*, one isolate of *T.virtde* were studied under *in vitro* condition on comparison with the reference cultures. The results are presented in Table 30 to 32.

4.9.3.1. Mycoparasitism

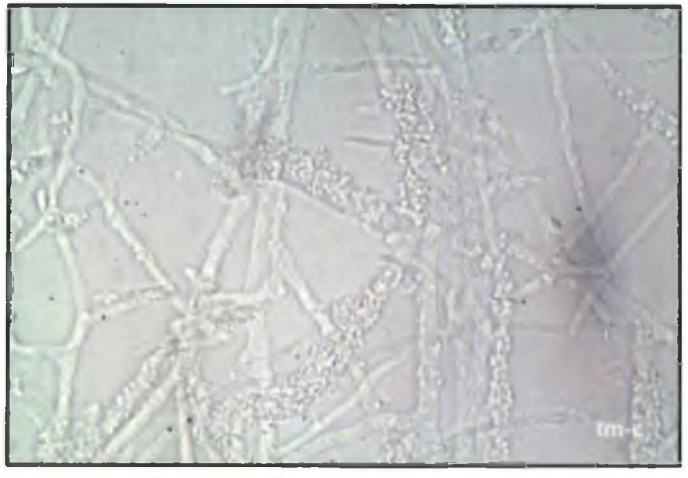
The mycoparasitism of the fungal antagonists was studied by cellophane paper method on plane agar medium. The microscopic observations on the hyphal interactions between the pathogen and the antagonists revealed coiling of hyphae of pathogen with hyphae of the antagonist (Plate 14a). It was observed in the three selected antagonists *viz.*, two isolates of *T.virens* and one isolate of *T.viride*. The hyphae of antagonists freely intermingled with hyphae of pathogen, then it coiled around the hyphae of pathogen and disintegration of hyphae were observed (Plate 14c). Hyphal malformation was also noticed during the microscopic observations (Plate 14b).

Plate 14. Mechanism of antagonism of *Trichoderma* spp. against *Ganoderma lucidum*

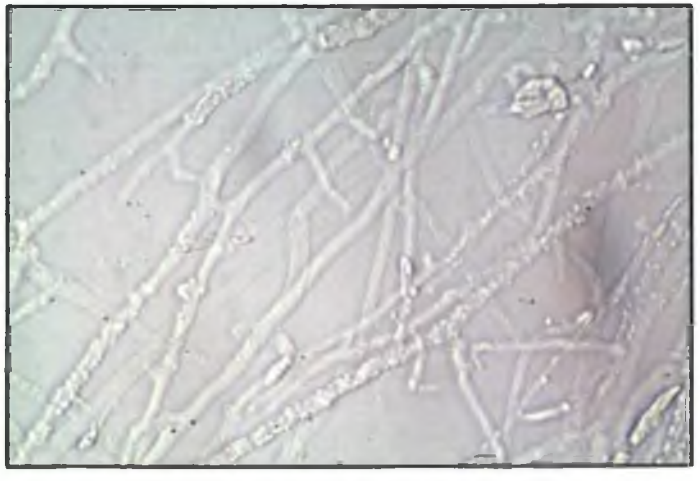
a. Coiling of hyphae (1000X)



b. Malformation (1000X)



c .Lysis (1000X)



d. Penetration (1000X)

4.9.3.2. Effect of non-volatile metabolites of *Trichoderma* spp. on different isolates of *Ganoderma* sp. by culture filtrate method

The inhibitory effect of non-volatile metabolites produced by the selected isolates of *Trichoderma* spp. and reference cultures against different isolates of BSR pathogen of coconut was studied under *in vitro* condition by culture filtrate method (Plate 15). Reference cultures viz., *T. harzinum* and *T. viride* were used for the study. The results are presented in Table 30a to 30h (Fig 6).

4.9.3.2.1. Isolate of *Ganoderma* sp. from Vellayani (GV)

The data showed significant difference among treatments on their inhibitory effect of non-volatile metabolites against the isolate of pathogen from Vellayani (Table 30a). Maximum inhibition of growth was observed in treatment with reference culture of *T. harzianum* (44.4 %) followed by *T.virens* from Vellayani (16.6 %). Treatment with *T.viride* from Mannuthy showed inhibition of 13.3 per cent and reference culture of *T. viride* recorded 5.5 per cent of inhibition of fungal growth over control. Among the five treatments *T.virens* from Kannur did not inhibit the mycelial growth of the pathogen.

4.9.3.2.2 Isolate of *Ganoderma* sp. from Trivandrum (GT)

From the data given in Table 30b, it was observed that the maximum inhibition on growth of the pathogen was recorded by the reference culture, *T. harzianum* (38.4 %). It was followed by the reference culture *T. viride* (34 %). Isolates of *T.viride* from Mannuthy recorded 25.4 per cent inhibition on growth of the pathogen over control. *T.virens* from Kannur and *T.virens* from Vellayani were on par with each other and gave 21.6 and 20.7 per cent inhibition on the growth of pathogen over control respectively.

4.9.3.2.3. Isolate of *Ganoderma* sp. from Mannuthy (GM)

From the data furnished in Table 30c, statistical difference among the treatments in their efficiency to inhibit the growth of pathogen was observed. Maximum inhibition on the growth of pathogen was noticed in reference culture of *T. harzianum* (23.3 %) and was followed by *T.virens* from Kannur (17.7 %). In

Plate 15. Production of culture filtrate by *Trichoderma* spp. in potato dextrose broth

Plate 16. *In vitro* evaluation of non-volatile metabolites against *Ganoderma lucidum*

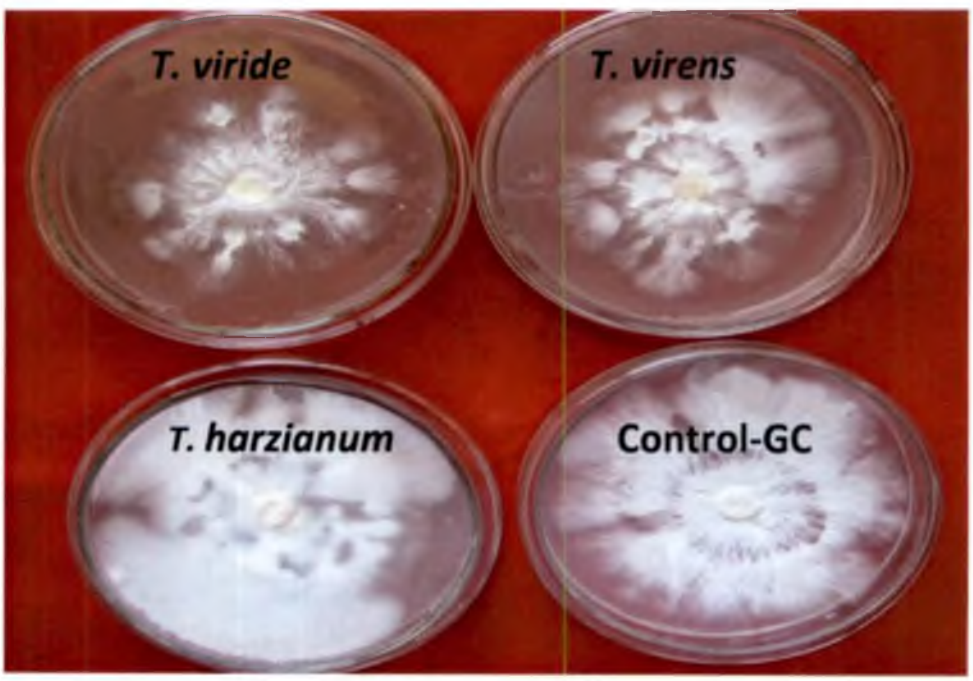


Table30. Effect of non volatile metabolite of *Trichoderma* spp. on different isolates of *Ganoderma* sp. by culture filtrate method

a. Isolate of *Ganoderma* sp. from Vellayani (GV)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI			PIOC
		2	4	6	
1	*T.virens*-Vellayani	20.0	47.0	75.0	16.6[b]
2	*T.viride*-Mannuthy	19.2	49.8	78.0	13.3[c]
3	*T.virens*-Kannur	21.5	56.0	90.0	0
4	*T.harzianum*	16.5	35.3	50.2	44.4[a]
5	*T.viride*	17.5	55.0	85.0	5.5[d]
6	Control	26.5	59.1	90.0	

b. Isolate of *Ganoderma* sp. from Trivandrum (GT)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI							PIOC
		2	4	6	8	10	12	13	
1	*T.virens*-Vellayani	10.9	13.2	21.9	32.3	49.5	65.4	71.3	20.7[d]
2	*T.viride*-Mannuthy	10.6	14.0	21.8	31.9	48.0	62.1.	67.1	25.4[c]
3	*T.virens*-Kannur	10.4	14.1	21.9	31.0	49.1	64.2	70.5	21.6[d]
4	*T.harzianum*	10.3	13.2	19.1	28.2	39.8	49.2	55.4	38.4[a]
5	*T.viride*	10.4	13.6	20.1	29.4	40.5	53.1	59.4	34.0[b]
6	Control	11.2	15.1	24.3	39.0	56.2	82.0	90.0	

c. Isolate of *Ganoderma* sp. from Mannuthy (GM)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI				PIOC
		2	4	6	8	
1	*T.virens*-Vellayani	18.5	39.5	70.0	90.0	0.0
2	*T.Viride*-Mannuthy	18.1	38.6	67.2	79.5	11.6[d]
3	*T.virens*-Kannur	15.0	32.0	53.5	74.0	17.7[b]
4	*T.harzianum*	16.0	29.0	52.5	69.0	23.3[a]
5	*T.viride*	18.0	39.0	66.5	76.5	15.0[c]
6	Control	20.3	41.5	72.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

the remaining treatments, 15 per cent of inhibition on mycelial growth over control was recorded by the reference culture of *T. viride*. The isolate, *T.virens* from Mannuthy gave 11.6 per cent inhibition on growth over control. Among the treatments, *T.virens* from Vellayani was the least effective and did not show inhibition on the growth of pathogen.

4.9.3.2.4. Isolate of *Ganoderma* sp. from Vettikal (GVc)

The effectiveness of nonvolatile metabolites produced by various isolates of *Trichoderma* spp. on the growth of isolates of *Ganoderma* from Vettikal (GVe) is given in Table 30d. The data revealed that all the treatments were less effective against the pathogen and recorded less than 25 per cent inhibition on the growth of pathogen. Among the treatments, maximum inhibition of 21.8 per cent over control was noticed in reference culture of T. *harzianum* followed by an inhibition of 15.4 per cent recorded by *T.virens* from Kannur. In *T.virens* from Vellayani an inhibition of 9.3 per cent over control was recorded and the lowest inhibition was noticed in treatment with culture filtrate of reference culture of *T. viride*, which recorded 5.4 per cent inhibition over control.

4.9.3.2.5. Isolate of *Ganoderma* sp. from Madakathara (GMa)

The data (Table 30e) revealed that *T.virens* obtained from Vellayani showed highest inhibition of 22.2 per cent over control compared with other treatments. Remaining treatments recorded less than 10 per cent inhibition on growth of isolate of *Ganoderma* sp. (GMa) over control. The isolates of *Trichoderma viz., T.viride* from Mannuthy and reference culture of *T. viride* didn't inhibit the growth of the isolate, GMa.

4.9.3.2.6. Isolate of *Ganoderma* sp. from Chirakakode (GC)

From the data furnished in Table 30f statistical difference among the treatments in their efficiency against the growth of GC was observed (Plate 16). Non –volatile metabolite produced by the selected antagonist, *T.virens* from Vellayani recorded highest inhibition of 47.2 per cent on growth of pathogen over control. It was followed by *T.virens* from Kannur which recorded 44.4 per cent

d. Isolate of *Ganoderma* sp. from Vettikal (GVe)

Sl no.	Isolates	Growth rate of *Ganoderma*(mm)*				PIOC
		DAI				
		2	4	6	7	
1	*T.virens*-Vellayani	14.0	34.0	70.0	81.6	9.3[d]
2	*T.viride*-Mannuthy	15.2	36.5	70.6	80.0	11.1[c]
3	*T.virens*-Kannur	13.0	29.0	65.0	76.1	15.4[b]
4	*T.harzianum*	14.5	30.0	59.0	70.3	21.8[a]
5	*T.viride*	15.0	42.5	76.2	85.1	5.4[e]
6	Control	20.6	44.0	78.6	90.0	

e. Isolate of *Ganoderma* sp. from Madakathara (GMa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*			PIOC
		DAI			
		2	4	6	
1	*T.virens*-Vellayani	20.0	40.6	70.0	22.2[a]
2	*T.viride*-Mannuthy	21.1	49.8	90.0	0
3	*T.virens*-Kannur	19.0	48.5	82.0	8.86[b]
4	*T.harzianum*	16.0	50.0	89.0	1.1[c]
5	*T.viride*	20.5	50.2	90.0	0
6	Control	20.8	52.6	90.0	

f. Isolate of *Ganoderma* sp. from Chirakacode (GC)

Sl no.	Isolates	Growth rate of *Ganoderma*(mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	15.0	24.5	35.0	47.5	47.2[a]
2	*T.viride*-Mannuthy	15.6	24.2	39.9	62.1	31.0[c]
3	*T.virens*-Kannur	15.0	24.0	37.0	50.0	44.4[b]
4	*T.harzianum*	15.5	29.0	45.0	67.5	25.0[d]
5	*T.viride*	17.0	36.0	55.0	75.5	16.1[e]
6	Control	21.8	37.2	59.5	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

inhibition over control. *T.viride* from Mannuthy recorded 31 per cent inhibition over control followed by the reference culture *T. harzianum*, which exhibited 25 per cent inhibition on the growth of pathogen over control. Among the five treatments, lowest inhibition (16.1 per cent) on the growth of pathogen over control was recorded by reference culture of *T.viride*.

4.9.3.2.7. Isolate of *Ganoderma* sp. from Kannur (GK)

The data on the inhibitory effect of non-volatile metabolites of different isolates of *Trichoderma* spp. against the *Ganoderma* isolate from Kannur (GK) are given in Table 30g. The statistical analysis of data showed significant difference among the treatments. Maximum inhibition on the growth was noticed in treatment with reference culture of *T. harzianum*, which was 27.1 per cent over control. The reference culture of *T. viride* exhibited 22.1 per cent inhibition and was on par with *T.virens* obtained from Vellayani which recorded 22.2 per cent inhibition over control. Among the treatments the lowest inhibition was noticed in *T.virens* from Kannur, which recorded 8.8 per cent inhibition over control.

4.9.3.2.8. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

From the data furnished in Table 30h, statistical differences among the treatments in their efficiency against the growth of the isolate of pathogen from Kasaragod (GKa) were observed. Non–volatile metabolites produced by *T.virens* from Kannur recorded the maximum inhibition of 28.6 per cent on growth of pathogen over control. It was followed by the reference culture of *T. harzianum* which recorded 20.7 per cent inhibition over control. The reference culture *T. viride* recorded 19.7 per cent inhibition and which was followed by *T.viride* from Mannuthy, which exhibited 18.2 per cent inhibition on the growth of pathogen over control. Among the five treatments, the lowest inhibition on the growth of pathogen over control was recorded by *T.virens* from Vellayani, which was 15.2 per cent.

g. Isolate of *Ganoderma* sp. from Kannur (GK)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	15.5	28.5	50.0	70.0	22.2[b]
2	*T.Viride*-Mannuthy	14.9	26.2	50.2	72.3	19.6[c]
3	*T.virens*-Kannur	15.0	28.1	52.1	82.1	8.8[d]
4	*T.harzianum*	14.5	24.6	49.0	65.6	27.1[a]
5	*T.viride*	13.5	24.1	40.0	70.1	22.1[b]
6	Control	16.0	29.5	59.5	90.0	

h. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	14.0	26.0	50.0	76.3	15.2[e]
2	*T.Viride*-Mannuthy	15.1	31.2	51.3	73.6	18.2[d]
3	*T.virens*-Kannur	14.0	26.0	43.0	64.2	28.6[a]
4	*T.harzianum*	13.0	28.5	50.0	71.3	20.7[b]
5	*T.viride*	15.0	30.0	50.0	72.2	19.7[c]
6	Control	21.0	40.5	66.2	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

i. Effect of non volatile metabolites of *Trichoderma* spp. against different isolates of *Ganoderma* sp. by culture filtrate method

Sl no.	Isolates	Percent inhibition over control							
		GV	GT	GM	GVe	GMa	GC	GK	GKa
1	*T.virens*-Vellayani	16.6	20.7	0	9.3	22.2	47.2	22.2	15.2
2	*T.viride*-Mannuthy	13.3	25.4	11.6	11.1	0	31.0	19.6	18.2
3	*T.virens*-Kannur	0	21.6	17.7	15.4	8.86	44.4	8.8	28.6
4	*T.harzianum*	44.4	38.4	23.3	21.8	1.1	25.0	27.1	20.7
5	*T.viride*	5.5	34.0	15.0	5.4	0	16.1	22.1	19.7

GV : *Ganoderma lucidum*–Vellayani GT : *G. lucidum* -Trivandrum
GM : *G. lucidum* –Mannuthy GVe: *G. lucidum* - Vettikkal
GMa: *G. lucidum* –Madakathara GC : *G. lucidum* - Chirakacode
GK : *G. lucidum* – Kannur GKa: *G. lucidum* - Kasaragod

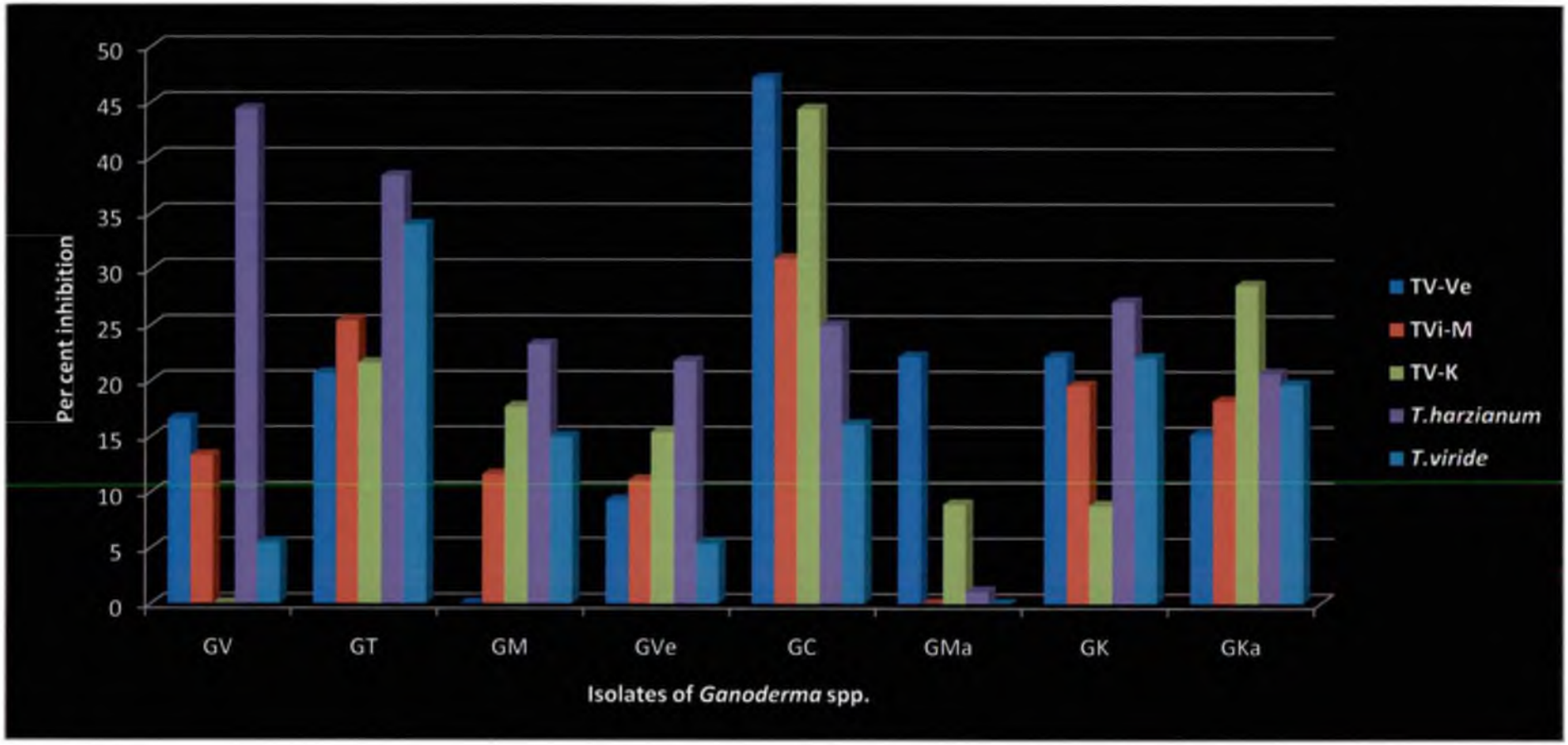


Fig 6. Effect of non volatile metabolite of *Trichoderma* spp. on isolates of *Ganoderma* spp. by culture filtrate method

4.9.3.3. Effect of non-volatile metabolites of *Trichoderma* spp. against various isolates of *Ganoderma* sp. by cellophane method

The inhibitory effect of non-volatile metabolites produced by the selected isolates of *Trichoderma* spp. and reference cultures against different isolates of BSR pathogen of coconut was studied under *in vitro condition* by cellophane method. Reference cultures *viz.*, *T. harzinum* and *T. viride* were used for the study. The results are presented in Table 31a to 31h (Fig 7).

4.9.3.3.1. Isolate of *Ganoderma* sp. from Vellayani (GV)

The inhibitory efficacy of non–volatile metabolites of different *Trichoderma* spp. on the growth of *Ganoderma* isolate from Vellayani (GV) is given in Table 31a. From the data, it was evident that the treatments differed significantly in their efficiency and recorded more than 60 per cent inhibition on growth of the pathogen over control. Among the five *Trichoderma* spp. tested, the reference culture *T. harzianum* recorded the maximum inhibition of 82 per cent followed by the reference culture *T. viride*, which recorded 80 per cent inhibition on the growth of pathogen over control. *T.viride* from Mannuthy recorded the lowest inhibition (64.8 %) over control.

4.9.3.3.2. Isolate of *Ganoderma* sp. from Trivandrum (GT)

The data given in Table 31b revealed that all the five treatments recorded more than 60 per cent inhibition on the growth of pathogen over control. Among the treatments, the reference culture, *T. harzianum* recorded the maximum inhibition of 74.2 per cent over control. Treatment *viz.*, the reference culture *T. viride* and *T.viride* from Mannuthy were on par, which showed 71.5 and 71 per cent inhibition over control respectively. The lowest inhibition on pathogen was recorded by *T.virens* from Vellayani (69.7%), but it was on par with *T.virens* from Kannur, which recorded an inhibition of 69.8 per cent over control.

4.9.3.3.3. Isolate of *Ganoderma* sp. from Mannuthy (GM)

From the data given in Table 31c, it was found that the treatments differed significantly in their inhibiting efficiency and recorded more than 60 per cent

Table 31. Effect of non volatile metabolite of *Trichoderma* spp. on different isolates of *Ganoderma* sp. by cellophane method

a. Isolate of *Ganoderma* sp. from Vellayani (GV)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI			PIOC
		2	4	6	
1	*T.virens*-Vellayani	11.0	13.0	21.0	76.6[c]
2	*T.viride*-Mannuthy	14.3	18.6	31.6	64.8[e]
3	*T.virens*-Kannur	13.2	15.0	29.0	67.7[d]
4	*T.harzianum*	13.0	12.0	16.0	82.0[a]
5	*T.viride*	12.0	15.0	18.5	80.0[b]
6	Control	26.5	59.1	90.0	

b. Isolate of *Ganoderma* sp. from Trivandrum (GT)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI							PIOC
		2	4	6	8	10	12	13	
1	*T.virens*-Vellayani	10.1	12.9	15.8	19.3	21.3	24.3	27.2	69.7[c]
2	*T.viride*-Mannuthy	10.0	10.6	14.5	16.2	18.6	24.2	26.1	71.0[b]
3	*T.virens*-Kannur	10.2	11.4	14.3	16.9	18.1	24.5	27.1	69.8[c]
4	*T.harzianum*	10.0	10.9	13.6	15.9	16.5	21.9	23.2	74.2[a]
5	*T.viride*	10.0	11.1	13.8	15.8	18.0	22.6	25.6	71.5[b]
6	Control	11.2	15.1	24.3	39.0	56.2	82.0	90.0	

c. Isolate of *Ganoderma* sp. from Mannuthy (GM)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI				PIOC
		2	4	6	8	
1	*T.virens*-Vellayani	14.0	18.1	23.4	27.6	69.6[d]
2	*T.viride*-Mannuthy	16.4	20.3	24.2	28.6	68.2[e]
3	*T.virens*-Kannur	15.1	19.2	21.6	25.9	71.3[c]
4	*T.harzianum*	11.5	14.1	16.2	22.3	75.2[a]
5	*T.viride*	12.1	15.4	17.3	23.6	73.7[b]
6	Control	20.3	41.5	72.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

inhibition on growth and control. Reference culture *T. harzianum* showed the maximum inhibition of 75.2 per cent over control followed by *T. viride* (73.7%). The Selected antagonist *T.virens* from Kannur recorded 71.3 per cent inhibition on the growth of pathogen over control. *T.virens* from Vellayani inhibited the growth of GM by 69.6 per cent and the minimum per cent inhibition of 68.2 per cent was recorded by *T.viride* from Mannuthy.

4.9.3.3.4. Isolate of *Ganoderma* sp. from Vettikkal (GVe)

The data showed significant difference among the treatments on their efficacy to inhibit the growth of isolate of *Ganoderma* sp. (GVe) (Table 31d). The reference cultures *viz*., *T. harzianum* and *T. viride* recorded 53.7 and 44.5 per cent inhibition over control respectively. The reference culture *T. viride* was on par with *T.virens* from Vellayani which recorded inhibition over control (44.4 %). Among the five treatments, the lowest inhibitory effect of non-volatile metabolite on the growth of pathogen over control was recorded in *T.virens* from Kannur (41.8 %). *T.viride* from Mannuthy recorded 48.1 per cent inhibition on growth of pathogen over control.

4.9.3.3.5. Isolate of *Ganoderma* sp. from Madakathara (GMa)

The effects of non-voloatile metabolite of Trichoderma spp. against the growth of Ganoderma isolate from Madakathara (GMa) are given in the Table 31e. From the data, statistical difference among the treatments in their inhibitory efficiency against the growth of GMa was observed. All the five treatments recorded more than 60 per cent inhibition on the growth of pathogen over control. Among them, *T. harzianum* recorded the maximum inhibition (67.5 %) over control followed by *T.virense* from Kannur (65.5 %) and Vellayani (65 %). The inhibitory effect of the reference culture, *T. viride* recorded the lowest inhibition of 63.2 per cent over control.

4.9.3.3.6. Isolate of *Ganoderma* sp. from Chirakakode (GC)

From the data furnished in Table 31f, statistical difference among the treatments in their efficiency against the growth of GC was observed. Non-volatile

d. Isolate of *Ganoderma* sp. from Vettikal (GVe)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	7	
1	*T.virens*-Vellayani	16.2	22.4	42.1	50.0	44.4[c]
2	*T.viride*-Mannuthy	14.1	20.0	36.8	46.7	48.1[b]
3	*T.virens*-Kannur	14.9	21.3	41.6	52.3	41.8[d]
4	*T.harzianum*	15.2	22.3	35.8	41.6	53.7[a]
5	*T.viride*	15.4	23.6	43.5	49.9	44.5[c]
6	Control	20.6	44.0	78.6	90.0	

e. Isolate of *Ganoderma* sp. from Madakathara (GMa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*			PIOC
		DAI			
		2	4	6	
1	*T.virens*-Vellayani	12.1	19.8	34.5	65.0[c]
2	*T.viride*-Mannuthy	14.6	21.9	32.6	63.7[d]
3	*T.virens*-Kannur	14.5	22.5	31.0	65.5[b]
4	*T.harzianum*	11.2	18.1	29.2	67.5[a]
5	*T.viride*	13.8	21.9	33.1	63.2[e]
6	Control	20.8	52.6	90.0	

f. Isolate of *Ganoderma* sp. from Chirakacode (GC)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	17.1	24.6	41.9	58.7	34.7[b]
2	*T.Viride*-Mannuthy	17.2	24.9	42.3	59.8	33.5[b]
3	*T.virens*-Kannur	16.8	25.9	39.8	57.1	36.5[b]
4	*T.harzianum*	16.2	24.3	35.6	45.0	50.0[a]
5	*T.viride*	16.1	24.0	39.8	56.8	36.8[b]
6	Control	21.8	37.2	59.5	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

metabolites produced by the reference culture of *T. harzianum* recorded maximum inhibition of 50 per cent on growth of pathogen over control. It was followed by the reference culture of *T. viride*, which recorded 36.8 per cent inhibition over control and was on par with the three isolates of *Trichoderma* spp. *viz.*, *T. virens* Kannur (36.5 %) and Vellayani (34.7 %) and *T.viride* from Mannuthy (33.5 %). The lowest inhibition on the growth of pathogen over control was recorded by *T. viride* from Mannuthy (33.5 %).

4.9.3.3.7. Isolate of *Ganoderma* sp. from Kannur (GK)

The data on the inhibitory effect of non-volatile metabolite of *Trichoderma* spp. on the isolate of *Ganoderma* from Kannur (GK) are given in Table 31g. Statistically significant difference among the treatments in their efficiency against the growth of GK was observed. Among the five treatments, inhibition efficiency of selected antagonists *T.virens* from Vellayani (49 %), reference culture of *T.harzianum* (48.7 %) and *T.virens* from Kannur (48.3 %) over control were on par with each other. Inhibitory effect of reference culture of *T.viride* was on par with the selected antagonist of *T.virens* from Kannur. Among the five treatments, lowest inhibition on the growth of pathogen over control was recorded by the selected antagonist *T.viride* from Mannuthy (46.8 %) but it was on par with reference culture of *T.viride.*

4.9.3.3.8. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

The inhibitory efficacy of non-volatile metabolite of different *Trichoderma* spp. on the growth of *Ganoderma* isolate from Kasaragod (GKa) is given in Table 31h. From the data, it is evident that the treatments differed significantly in their efficiency and recorded more than 50 per cent inhibition on growth of the pathogen over control. Among the five *Trichoderma* spp. tested, the selected antagonist of *T.virens* from Kannur recorded the maximum inhibition (61.6 %) on the growth of GKa and it was on par with the reference culture of *T. harzianum*, which recorded 61.3 per cent inhibition over control. In remaining treatments reference culture of *T. viride* recorded an inhibition of 59.2 per cent over control

g. Isolate of *Ganoderma* sp. from Kannur (GK)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI				PIOC
		2	4	6	8	
1	*T.virens*-Vellayani	11.1	19.8	35.4	45.9	49.0[a]
2	*T.viride*-Mannuthy	13.9	21.9	39.9	47.8	46.8[c]
3	*T.virens*-Kannur	13.1	20.6	37.6	46.5	48.3[ab]
4	*T.harzianum*	11.3	18.7	35.0	46.1	48.7[a]
5	*T.viride*	12.6	19.0	36.3	47.2	47.5[bc]
6	Control	16.0	29.5	59.5	90.0	

h. Isolate *Ganoderma* sp. from Kasaragod (GKa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)* DAI				PIOC
		2	4	6	8	
1	*T.virens*-Vellayani	12.0	16.5	23.4	38.2	57.5[c]
2	*T.viride*-Mannuthy	12.3	16.8	24.2	39.6	56.0[d]
3	*T.virens*-Kannur	12.4	17.6	25.2	42.5	61.6[a]
4	*T.harzianum*	12.6	17.8	23.9	34.8	61.3[a]
5	*T.viride*	12.7	18.1	26.1	36.7	59.2[b]
	Control	21.0	40.5	66.2	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

i. Effect of non volatile metabolites of *Trichoderma* spp. against different isolates of *Ganoderma* sp. by cellophane paper method

Sl No	Isolates	Percent inhibition over control							
		GV	GT	GM	GVe	GMa	GC	GK	GKa
1	*T.virens*-Vellayani	76.6	69.7	69.6	44.4	65.0	34.7	49.0	57.5
2	*T.viride*-Mannuthy	64.8	71.0	68.2	48.1	63.7	33.5	46.8	56.0
3	*T.virens*-Kannur	67.7	69.8	71.3	41.8	65.5	36.5	48.3	61.6
4	*T.harzianum*	82.0	74.2	75.2	53.7	67.5	5.0	48.7	61.3
5	*T.viride*	80.0	71.5	73.7	44.5	63.2	36.8	47.5	59.2

GV : *Ganoderma lucidum* –Vellayani GT : *G. lucidum* -Trivandrum
GM : *G. lucidum* – Mannuthy GVe: *G. lucidum* - Vettikkal
GMa: *G. lucidum* – Madakathara GC : *G. lucidum* - Chirakacode
GK : *G. lucidum* – Kannur GKa: *G. lucidum* - Kasaragod

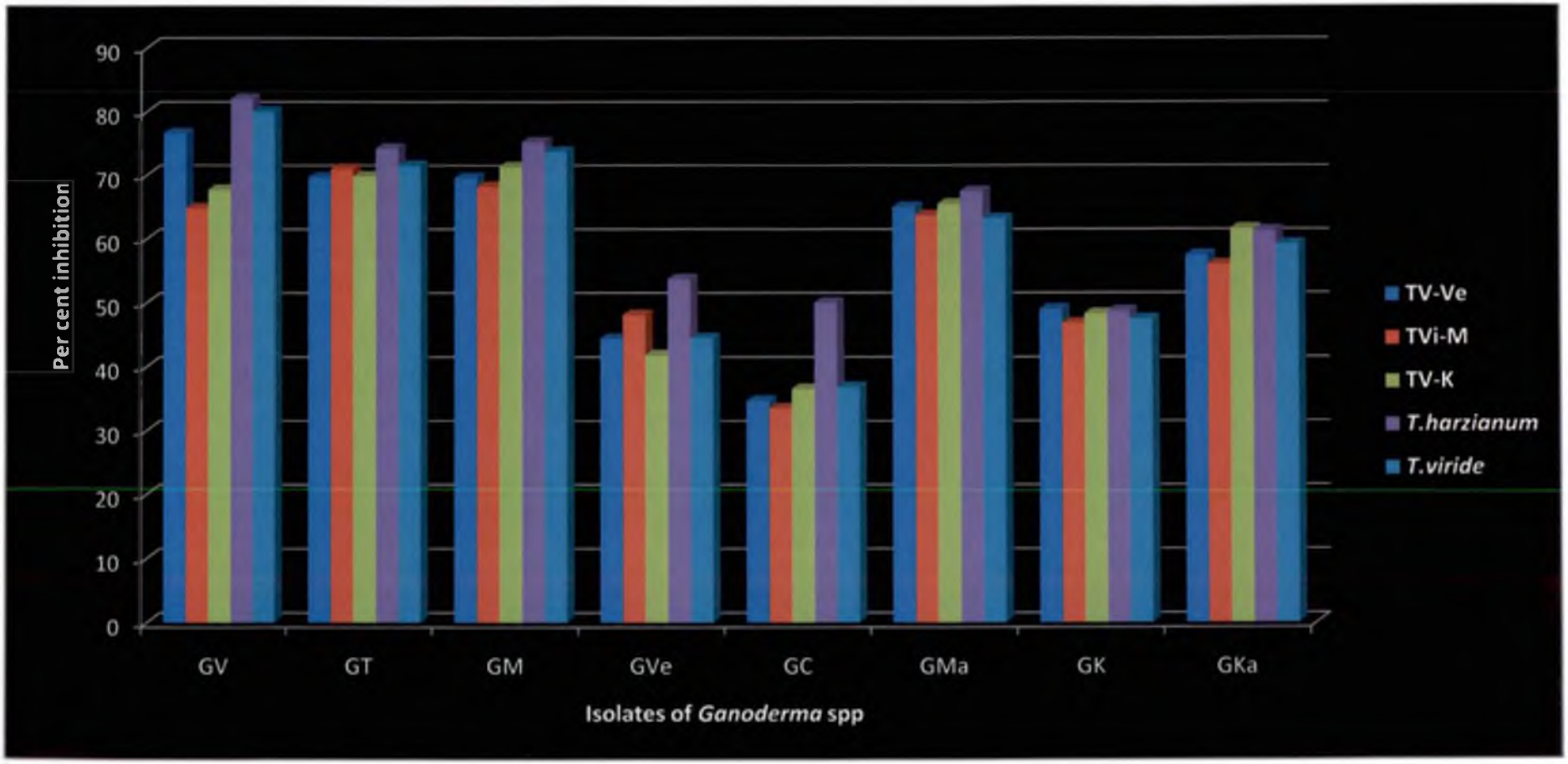


Fig 7. Effect of non volatile metabolites of *Trichoderma* spp. on isolates of *Ganoderma* spp. by cellophane method

followed by *T.virens* from Vellayani (57.5 %). Among the five treatments, *T.viride* from Mannuthy was the least effective (56 %) against GKa.

4.9.3.4. Effect of volatile metabolites of *Trichoderma* spp. on different isolates of *Ganoderma* sp.

The inhibitory effect of volatile metabolites produced by the selected *Trichoderma* spp. against the different isolates of pathogen was studied under *in vitro* condition by inverted plate method. The antagonistic property of these antagonists was compared with the reference cultures of *T. viride* and *T. harzianum* and the results are presented in Table 32a to 32h (Fig 8).

4.9.3.4.1. Isolate of *Ganoderma* sp. from Vellayani (GV)

From the data given in Table 32a it was found that the treatments differed significantly with each other in their efficiency in inhibiting the growth of *Ganoderma* sp. from Vellayani (GV). Among the five treatments, maximum inhibition of 11.1 per cent over control was recorded by the reference culture *T. harzianum* followed by an inhibition of 8.2 per cent over control by *T.virens* obtained from Kannur. Volatile metabolites produced by *T.virens* from Vellayani and *T.viride* from Mannuthy showed 5.5 and 5.7 per cent inhibition respectively. The reference culture of *T. viride* didn't show inhibition on the radial growth of pathogen.

4.9.3.4.2. Isolate of *Ganoderma* sp. from Trivandrum (GT)

The data showed significant difference on the antagonistic efficiency of different treatments against the isolate of the pathogen (Table 32b). The reference culture, *T. viride* recorded maximum inhibition of 25.8 per cent on the growth of pathogen which was followed by the reference culture of *T.harzianum*, which recorded 22.1 per cent over control. Among the remaining treatments, *T.virense* from Vellayani showed the maximum inhibition of 21 per cent followed by *T.viride* from Mannuthy which recorded 19.8 per cent inhibition over control. Among the five treatments, the selected antagonist of *T.virens* from Kannur recorded the lowest inhibition (16.4 %) on the growth of pathogen over control.

Table 32. Effect of volatile metabolite of *Trichoderma* spp. on dfferent isolates of *Ganoderma* sp.

a. Isolate of *Ganoderma* sp. from Vellayani (GV)

Sl no.	Isolates	Growth rate of *Ganoderma*(mm)*			PIOC
		DAI			
		2	4	6	
1	*T.virens*-Vellayani	22.1	52.0	85.0	5.5[d]
2	*T.viride*-Mannuthy	23.5	58.9	84.8	5.7[c]
3	*T.virens*-Kannur	23.1	57.5	82.6	8.22[b]
4	*T.harzianum*	19.7	50.0	80.0	11.1[a]
5	*T.viride*	21.8	60.0	90.0	0.0
6	Control				

b. Isolate of *Ganoderma* sp. from Trivandrum(GT)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*							PIOC
		DAI							
		2	4	6	8	10	12	13	
1	*T.virens*-Vellayani	10.1	13.5	21.0	32.5	46.7	65.3	71.1	21.0[c]
2	*T.viride*-Mannuthy	10.3	13.1	21.2	31.1	46.3	66.1	72.1	19.8[d]
3	*T.virens*-Kannur	10.4	13.5	21.3	31.5	48.2	67.1	75.2	16.4[e]
4	*T.harzianum*	10.3	13.1	20.5	29.5	43.2	63.2	70.1	22.1[b]
5	*T.viride*	10.3	12.9	19.8	28.5	40.2	59.9	66.7	25.8[a]
6	Control	11.2	15.1	24.3	39.0	56.2	82.0	90.0	

c. Isolate of *Ganoderma* sp. from Mannuthy (GM)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	7	
1	*T.virens*-Vellayani	18.3	40.0	67.0	74.5	17.2[b]
2	*T.viride*-Mannuthy	19.6	42.8	73.8	83.5	7.2[e]
3	*T.virens*-Kannur	18.9	43.0	74.0	80.0	11.1[d]
4	*T.harzianum*	19.1	45.0	60.0	67.2	25.3[a]
5	*T.viride*	18.1	43.0	71.0	76.25	15.3[c]
6	Control	20.3	41.5	72.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

4.9.3.4.3. Isolate of *Ganoderma* sp. from Mannuthy (GM)

The data showed (Table 32c) statistical difference among the treatments in their efficiency against the growth of the isolate of pathogen. Among the five treatments maximum inhibition (25.3 %) was recorded by the reference culture of *T. harzianum* followed by the selected antagonist, *T.virens* from Vellayani (17.2%). The reference culture of *T. viride* recorded 15.3 per cent inhibition over control. Volatile metabolites produced by *T.virens* from Kannur inhibited the growth of pathogen by 11.1 per cent over control. Among the five treatments, the lowest inhibition of 7.2 per cent was noticed in *T.viride* from Mannuthy.

4.9.3.4.4. Isolate of *Ganoderma* sp. from Vettikal (GVe)

The data (Table 32d) showed significant difference among treatments on their inhibitory effect against the isolate of *Ganoderma* sp. from Vettikal (GVe). The reference culture, *T. harzianum* recorded the maximum inhibition (30.5 %) on the growth of pathogen which was followed by the reference culture, *T. viride* showed 16.1 per cent inhibition on the growth over control. Among the selected isolates of *Trichoderma* sp., the isolate from Vellayani (TVe) recorded the maximum inhibition of 13 per cent which was followed by *T.virens* from Kannur (11.6 %) and the minimum inhibition on the growth of the isolate of pathogen was obtained in *T.viride* from Mannuthy (5.2 %).

4.9.3.4.5. Isolate of *Ganoderma* sp. from Madakathara (GMa)

The data showed significant difference among the treatments on their inhibitory effect of volatile metabolites against the pathogen (Table.32e) All the treatments recorded less than 50 per cent inhibition on the growth of pathogen over control. Among the treatments, the reference culture of *T. harzianum* was superior to others in its inhibitory effect and recorded 38.8 per cent inhibition on the growth of pathogen over control. It was followed with 33.3 per cent inhibition by the reference culture of *T. viride*. An inhibition of 30 per cent over control was noticed in *T. virens* from Kannur. Statistically, *T.virens* from Vellayani and *T.viride* from Mannuthy were on par with each other in their efficacy to inhibit the

d. Isolate of *Ganoderma* sp. from Vettikal (GVe)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	7	
1	*T.virens*-Vellayani	18.1	45.0	73.0	78.3	13.0[c]
2	*T.viride*-Mannuthy	18.5	46.2	75.8	85.3	5.2[e]
3	*T.virens*-Kannur	17.2	46.0	70.0	72.5	11.6[d]
4	*T.harzianum*	17.9	42.0	65.0	62.5	30.5[a]
5	*T.viride*	16.3	40.0	66.0	75.5	16.1[b]
6	Control	20.6	44.0	78.6	90.0	

e. Isolate of *Ganoderma* sp. from Madakathara (GMa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*			PIOC
		Days after inoculation			
		2	4	6	
1	*T.virens*-Vellayani	19.4	42.0	70.0	22.2[d]
2	*T.viride*-Mannuthy	17.9	41.2	69.8	22.4[d]
3	*T.virens*-Kannur	18.7	41.0	63.0	30.0[c]
4	*T.harzianum*	16.9	40.0	55.0	38.8[a]
5	*T.viride*	18.4	40.0	60.0	33.3[b]
6	Control	20.8	52.6	90	

f. Isolate of *Ganoderma* sp. from Chirakacode (GC)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	20.3	33.0	63.0	88.0	2.2[d]
2	*T.viride*-Mannuthy	19.9	33.3	53.2	78.2	13.1[b]
3	*T.virens*-Kannur	21.0	36.5	56.8	79.8	11.3[c]
4	*T.harzianum*	17.8	26.0	55.0	76.3	15.2[a]
5	*T.viride*	21.7	37.0	59.3	79.2	12.0[bc]
6	Control	21.8	37.2	59.5	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

growth of pathogen and recorded 22.2 and 22.4 per cent inhibition over control respectively.

4.9.3.4.6. Isolate of *Ganoderma* sp. from Chirakakode (GC)

The data on the inhibitory effect of volatile metabolites produced by different isolates of *Trichoderma* on *Ganoderma* isolate from Chirakakode are given in Table 32f and the statistical analysis of data showed significant difference among the treatments. The reference culture of *T. harzianum* recorded the maximum inhibition of 15.2 per cent on the growth of pathogen over control and it was followed *T.viride* from Mannuthy (13.1 %) and *T.viride* (12 %) and these two were on par with each other. *T.virens* from Kannur recorded 11.3 per cent inhibition on the growth of pathogen over control and was on par with *T. viride*. Among the treatments, volatile metabolites *Trichoderma virens* from Vellayani recorded the lowest inhibition on the growth over control (2.2 %).

4.9.3.4.7. Isolate of *Ganoderma* sp. from Kannur (GK)

From the data furnished in Table 32g, statistical difference among the treatments in their efficiency against the growth of pathogen was observed. Maximum inhibition of 26.4 per cent was recorded by the reference culture of *T. harzianum*. But it was on par with reference culture *T. viride* which recorded 25.7 per cent inhibition on growth over control. Among the selected antagonists, *T.virens* from Kannur recorded 16.3 per cent inhibition and was followed by *T.viride* from Mannuthy which showed 6.1 per cent inhibition over control. *T.virens* from Vellayani did not show inhibition on growth of the isolate of *Ganoderma* from Kannur.

4.9.3.4.8. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

The data (Table 32h) revealed that reference culture of *T.viride* showed the maximum inhibition of 56.6 per cent on the growth of pathogen over control and was found statistically superior than other treatments. The selected antagonists *viz*., *T.virens* from Vellayani and Kannur recorded 38.8 and 28.8 per cent inhibition over control respectively. *T.viride* from Mannuthy showed on inhibition

g. Isolate of *Ganoderma* sp. from Kannur (GK)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	8	
1	*T.virens*-Vellayani	15.2	42.0	70.0	90.0	0
2	*T.viride*-Mannuthy	15.1	41.2	63.1	84.5	6.1[c]
3	*T.virens*-Kannur	15.1	41.0.	63.0	75.3	16.3[b]
4	*T.harzianum*	14.8	40.0	55.0	66.2	26.4[a]
5	*T.viride*	14.7	40.0	60.0	66.8	25.7[a]
6	Control	16.0	29.5	59.5	90.0	

h. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

Sl no.	Isolates	Growth rate of *Ganoderma* (mm)*				PIOC
		DAI				
		2	4	6	7	
1	*T.virens*-Vellayani	14.0	24.0	40.0	55.0	38.8[b]
2	*T.viride*-Mannuthy	16.1	39.0	61.0	72.5	19.4[d]
3	*T.virens*-Kannur	19.1	38.0	61.0	64.0	28.8[c]
4	*T.harzianum*	19.7	43.0	59.1	80.0	11.1[e]
5	*T.viride*	16.2	30.3	35.0	39.0	56.6[a]
6	Control	21.0	40.5	66.2	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

i. Effect of volatile metabolites of *Trichoderma* spp. against different isolates of *Ganoderma* sp. by inverted plate method

Sl No	Isolates	Percent inhibition over control							
		GV	GT	GM	GVe	GMa	GC	GK	GKa
1	*T.virens*-Vellayani	5.5	21.0	17.2	13.0	22.2	2.2	0	38.8
2	*T.viride*-Mannuthy	5.7	19.8	7.2	5.2	22.4	13.1	6.1	19.4
3	*T.virens*-Kannur	8.2	16.4	11.1	11.6	30.0	11.3	16.3	28.8
4	*T.harzianum*	11.1	22.1	25.3	30.5	38.8	15.2	26.4	11.1
5	*T.viride*	0	25.8	15.3	16.1	33.3	12.0	25.7	56.6

GV : *Ganoderma lucidum*–Vellayani
GM : *G. lucidum* –Mannuthy
GMa: *G. lucidum* –Madakathara
GK : *G. lucidum* – Kannur
GT : *G. lucidum* -Trivandrum
GVe: *G. lucidum* - Vettikkal
GC : *G. lucidum* - Chirakacode
GKa: *G. lucidum* - Kasaragod

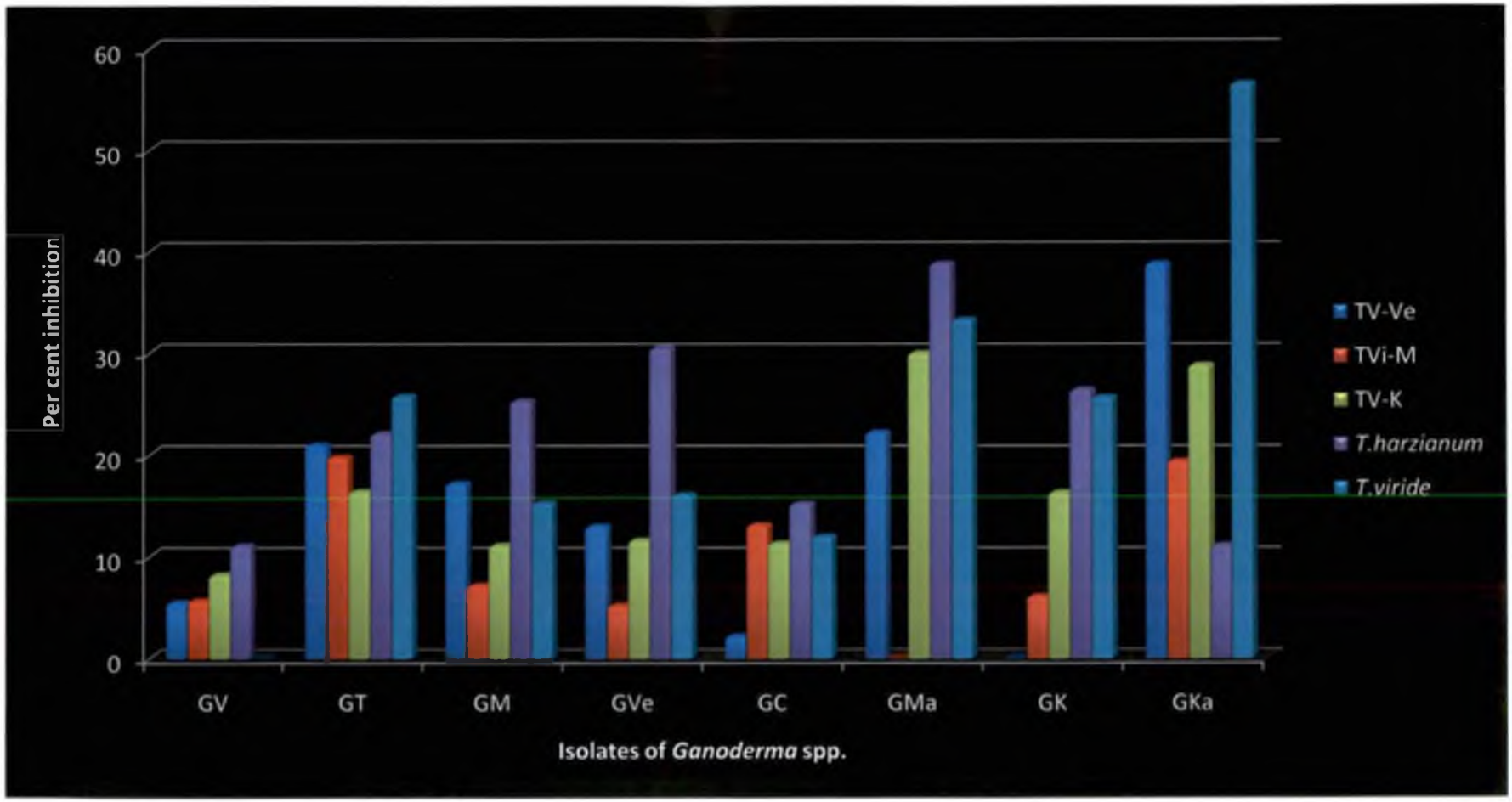


Fig 8. Effect of volatile metabolites of *Trichoderma* spp. on isolates of *Ganoderma* spp. by inverted plate method

of 19.4 per cent on the growth of pathogen over control. Among all the treatments, the reference culture of *T. harzianum* showed the lowest inhibition of the pathogen (11.1 %) over control.

4.9.4. *In vitro* evaluation of phytoextracts against different isolates of *Ganoderma* sp.

An *in vitro* evaluation on the fungicidal effect of four phytoextracts *viz.*, *Azadirachta indica* (10 and 20%), *Clerodendron aculeatum* (10%), *Chromolaena odorata* (10%) *and Musa* sp. (extract of sheath, leaves and rhizome together) at 10 per cent on the radial growth of different isolates of pathogen of BSR over control was studied (Fig 9, Plate 17). The resulted are presented in Table 33a to 33h.

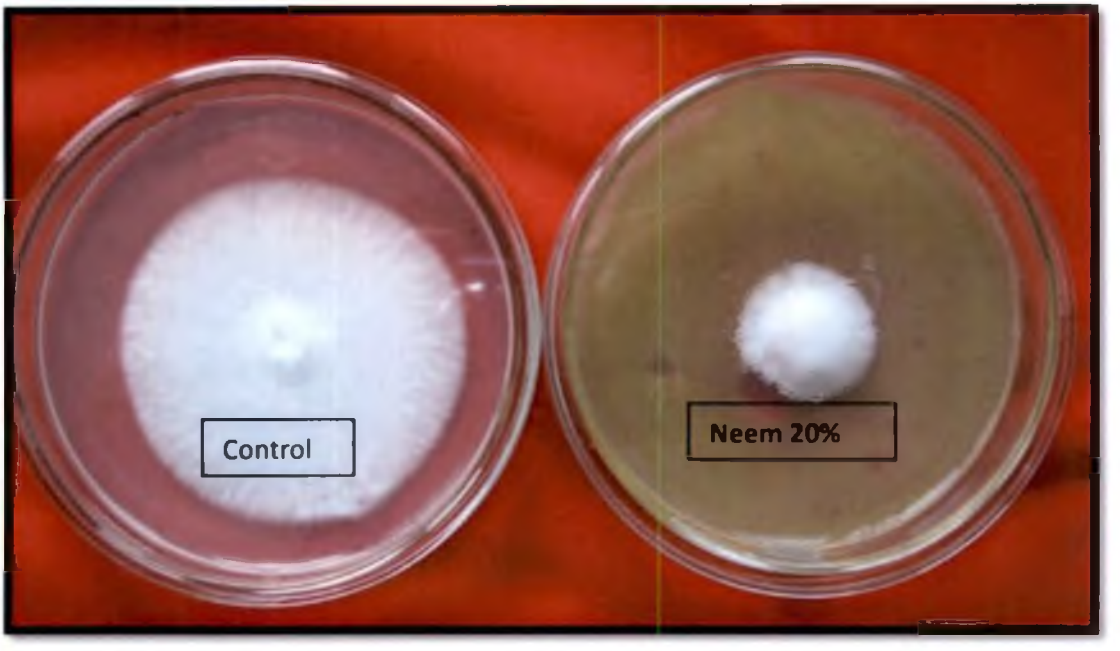
4.9.4.1. Isolate of *Ganoderma* sp. from Vellayani (GV)

From the data furnished in Table 33a, statistical difference among the treatments in their inhibiting efficiency against the growth of isolate of *Ganoderma* from Vellayani (GV) was observed. *Azadirachta indica* at 20 per cent recorded the maximum inhibition (61.8 %) on the growth of the pathogen followed by *Azadirachta indica at* 10% (42.2 %). Among the other phytoextracts, *Musa* sp and *Clerodendron aculeatum* showed 24.8 per cent and 12.6 per cent over control respectively. *Chromolaena odorata* at 10 per cent concentration recorded the lowest inhibition over control (6.6 %).

4.9.4.2. Isolate of *Ganoderma* sp. from Trivandrum (GT)

The data on the inhibitory effect of different phytoextracts on the isolate of *Ganoderma* from Trivandrum (GT) showed significant difference among the treatments (Table 33b). The maximum inhibition of 39.7 per cent over control was recorded by *Azadirachta indica* at 20 per cent concentration. It was on par with *Musa* sp. (37.6 %) at 10 per cent concentration. The lowest inhibition on growth of the pathogen was noticed at 10 per cent concentration of *Chromolaena odorata*, which recorded only 11.2 per cent inhibition over control.

Plate 17. ***In vitro*** **evaluation of phytoextracts against different isolates of** ***Ganoderma lucidum***



a. Effect of neem (20%) against ***Ganoderma*** **sp**

b. Effect of ***Musa*** **sp. (10%) against** ***Ganoderma*** **sp.**

Table 33: Effect of phytoextracts against different isolates of *Ganoderma* sp.

a. Isolate of *Ganoderma* sp. from Vellayani (GV)

Sl No.	Phytoextracts	Concent-rations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIOC
			2	3	4	5	6	
1	*Azadirachta indica*	10	14.0	23.0	35.1	46.2	52.0	42.2[b]
		20	0.0	19.1	24.6	30.2	34.3	61.8[a]
2	*Chromolaena odorata*	10	14.5	33.8	46.5	64.3	84.0	6.6[e]
3	*Clerodendron aculeatum*	10	21.0	35.1	48.1	61.3	78.6	12.6[d]
4	*Musa* Sp.	10	21.6	33.1	44.6	57.3	67.6	24.8[c]
5	Control		25.3	43.3	61.3	81.6	90.0	

b. Isolate of *Ganoderma* sp. from Trivandrum(GT)

Sl No.	Phytoextracts	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIOC
			2	8	10	12	13	
1	*Azadirachta indica*	10	10.5	33.1	46.2	62.9	70.1	22.1[c]
		20	10.1	29.6	37.1	47.1	54.2	39.7[a]
2	*Chromolaena odorata*	10	11.1	36.3	51.2	69.9	79.9	11.2[e]
3	*Clerodendron aculeatum*	10	10.9	39.1	53.5	64.8	78.0	13.3[d]
4	*Musa* Sp.	10	10.0	22.1	30.2	45.9	56.1	37.6[b]
	Control	10	11.6	39.0	56.2	82.0	90.0	

c. Isolate of *Ganoderma* sp. from Mannuthy (GM)

Sl No.	Phytoextracts	Concent-rations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIOC
			2	4	5	6	7	
1	*Azadirachta indica*	10	15.0	39.6	52.5	61.6	70.3	21.8[c]
		20	11.1	24.8	39.9	51.2	63.1	29.8[b]
2	*Chromolaena odorata*	10	14.0	37.1	55.8	74.1	85.0	5.5[d]
3	*Clerodendron aculeatum*	10	17.0	51.6	70.2	90.0	90.0	0.0
4	*Musa* Sp.	10	13.6	21.0	27.3	38.3	48.3	46.3[a]
5	Control	10	15.5	55.3	67.0	84.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control
DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

4.9.4.3. Isolate of *Ganoderma* sp. from Mannuthy (GM)

The data (Table 33c) revealed that all the phytoextracts showed less than 50 per cent inhibition on the growth of the isolate of *Ganoderma* sp. from Mannuthy. Among the treatments maximum inhibition was recorded by Musa sp at 10 per cent concentration (46.3 %) followed by *Azadirachta indica* (29.8 %) at 20 per cent concentration. *Clerodendron aculeatum* at 10 per cent did not show any inhibition on the growth of pathogen.

4.9.4.4. Isolate of *Ganoderma* sp. from Vettikal (GVe)

From the data given in Table 33d, it was found that all the treatments showed least efficiency against the growth of the pathogen. Among the treatments, maximum inhibition (18.8 %) was observed in Azadiracta at 20 per cent concentration over control. But it was on par with Musa sp (18 %) inhibition at 10 per cent concentration. Phytoextracts *viz.*, *Azadirachta indica* and *Chromolaena odorata* at10 per cent concentration didn't show any inhibition on the growth of pathogen over control. *Clerodendron aculeatum* at 10 per cent concentration recorded the least inhibition on the growth (1.88 %) over control.

4.9.4.5. Isolate of *Ganoderma* sp. from Madakathara (GMa)

The inhibitory efficacy of different phytoextracts on the growth of *Ganoderma* isolate from Madakathara (GMa) is given in Table 33e. From the data, it was found that the treatments differed significantly with each other in their efficiency in inhibiting the growth of GMa. Among the four phytoextracts tested, *Azadirachta indica* at 20 per cent concentration showed the maximum inhibition of 76.5 per cent on the growth of pathogen over control and it was followed by *Azadirachta indica* (55.2 %) at 10 per cent concentration. *Musa* sp. at 10 per cent recorded 38.3 per cent inhibition over control and the lowest inhibition (6.1 %) was recorded by *Chromolaena odorata* at 10 per cent concentration.

4.9.4.6. Isolate of *Ganoderma* sp. from Chirakakode (GC)

From the data given in Table 33f, it was observed that the treatments differed significantly with each other in their efficiency in inhibiting the growth of

d. Isolate of *Ganoderma* sp. from Vettikal (GVe)

Sl No.	Phytoextracts	Concent-rations (per cent)	Growth rate of *Ganoderma* (mm)*					PIOC
			DAI					
			2	4	5	6	7	
1	*Azadirachta indica*	10	19.8	45.1	69.7	81.2	90.0	0.0
		20	15.7	39.1	51.3	62.1	73.0	18.8
2	*Chromolaena odorata*	10	19.0	49.1	74.3	86.2	90.0	0.0
3	*Clerodendron aculeatum*	10	20.1	49.8	66.1	77.2	88.3	1.88
4	*Musa* Sp.	10	18.5	34.0	44.2	58.5	73.8	18.0
5	Control	10	23.2	58.2	78.3	85.0	90.0	

e. Isolate of *Ganoderma* sp. from Madakathara (GMa)

Sl No.	Phytoextracts	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)*				PIOC
			DAI				
			2	3	4	5	
1	*Azadirachta indica*	10	0.0	19.0	28.5	40.3	55.2[b]
		20	0.0	13.2	16.2	21.1	76.5[a]
2	*Chromolaena odorata*	10	23.9	44.8	63.9	84.5	6.1[d]
3	*Clerodendron aculeatum*	10	26.0	49.2	71.1	90.0	0.0
4	*Musa* Sp.	10	18.6	29.7	40.8	55.5	38.3[c]
5	Control	10	26.3	47.3	68.3	90.0	

f. Isolate of *Ganoderma* sp. from Chirakacode (GC)

Sl No	Phytoextracts	Concent-rations (per cent)	Growth rate of *Ganoderma* (mm)*					PIOC
			DAI					
			2	4	5	6	7	
1	*Azadirachta indica*	10	0.0	18.0	26.1	32.2	38.9	56.7[b]
		20	0.0	13.0	17.2	20.2	23.9	73.4[a]
2	*Chromolaena odorata*	10	15.1	37.1	66.1	77.0	90.0	0.0
3	*Clerodendron aculeatum*	10	17.2	39.0	52.1	74.0	90.0	0.0
4	*Musa* Sp.	10	16.0	29.3	37.0	47.6	57.3	36.3[c]
5	Control	10	18.6	37.0	50.3	73.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

the pathogen. Among the four phytoextracts tested, *Chromolaena odorata* and *Clerodendron aculeatum* at 10 per cent concentration did not inhibit the growth of pathogen. In the remaining treatments, the maximum inhibition on the growth of pathogen was recorded by *Azadirachta indica* (73.4 %) at 20 per cent concentration and it was followed by *Azadirachta indica* at 10 per cent concentration, which recorded 56.7 per cent inhibition over control. *Musa* sp. at 10 per cent concentration recorded the least inhibition 36.3 per cent over control.

4.9.4.7. Isolate of *Ganoderma* sp. from Kannur (GK)

The data (Table 33g) revealed that all the phytoextracts except *Azadirachta indica* at 20 per cent concentration, recorded less than 50 per cent inhibition on the growth of pathogen over control. *Azadirachta indica* at 20 per cent concentration showed 66.6 per cent inhibition on the growth of pathogen over control. It was followed by *Azadirachta indica* at 10 per cent concentration, which shows 47.6 per cent inhibition on pathogen over control. Among the four phytoextracts, *Clerodendron aculeatum* at 10 per cent didn't show any inhibition on the radial growth of pathogen. *Chromolaena odorata* at 10 per cent concentration recorded the lowest inhibition of 12.3 per cent on the growth of pathogen over control.

4.9.4.8. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

The data on the evaluation of phytoextracts against the growth of isolate of *Ganoderma* from Kasaragod (GKa) are given in Table 33h. All treatments were exhibited less than 50 per cent inhibition of pathogen over control. Among the four phytoextracts, *Clerodendron aculeatum* did not show any inhibition on the growth of pathogen. In the remaining treatments, the lowest inhibition on growth of the pathogen was noticed at 10 per cent concentration of *Azadirachta indica* (10 %) and highest inhibition was recorded by *Musa* sp. (35.5 %) at10 per cent concentration over control.

g. Isolate of *Ganoderma* sp. from Kannur (GK)

Sl No	Phytoextracts	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIOC
			2	4	5	6	7	
1	*Azadirachta indica*	10	11.0	25.1	32.7	39.9	47.1	47.6[b]
		20	0.0	16.6	20.8	24.1	30.0	66.6[a]
2	*Chromolaena odorata*	10	19.0	39.5	51.8	63.9	78.9	12.3[d]
3	*Clerodendron aculeatum*	10	20.0	45.6	69.0	90.0	90.0	0.0
4	*Musa* Sp.	10	14.0	34.5	47.5	62.5	75.0	16.6[c]
5	Control	10	20.6	48.8	68.2	89.0	90.0	

h. Isolate of *Ganoderma* sp. from Kasaragod (GKa)

Sl No	Phytoextracts	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIRG
			2	4	6	8	9	
1	*Azadirachta indica*	10	15.2	34.9	55.6	75.0	81.0	10.0[d]
		20	12.0	25.2	40.1	54.0	62.1	31.0[b]
2	*Chromolaena odorata*	10	14.0	25.1	50.1	68.7	78.4	12.8[c]
3	*Clerodendron aculeatum*	10	17.9	41.9	69.8	90.0	90.0	0.0
4	*Musa* Sp.	10	18.6	40.8	38.3	51.1	58.0	35.5[a]
5	Control	10	18.3	46.1	70.9	85.6	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

In each column, figures followed by same letter do not differ significantly according to DMRT

i. *In vitro* evaluation of phytoextracts against different isolates of *Ganoderma* sp.

Sl No	Phytoextracts	Concentration (%)	Percent inhibition over control							
			GV	GT	GM	GVe	GC	GMa	GK	GKa
1	*Azadirachta indica*	10	42.2	22.1	21.8	0.0	56.7	55.2	47.6	10.0
2	*Azadirachta indica*	20	61.8	39.7	29.8	18.8	73.4	76.5	66.6	31.0
3	*Chromolaena odorata*	10	6.6	11.2	5.5	0.0	0.0	6.1	12.3	12.8
4	*Clerodendron aculeatum*	10	12.6	13.3	0.0	1.8	0.0	0.0	0.0	12.8
5	*Musa* Sp.	10	24.8	37.6	46.3	18.0	36.3	38.3	16.6	35.5

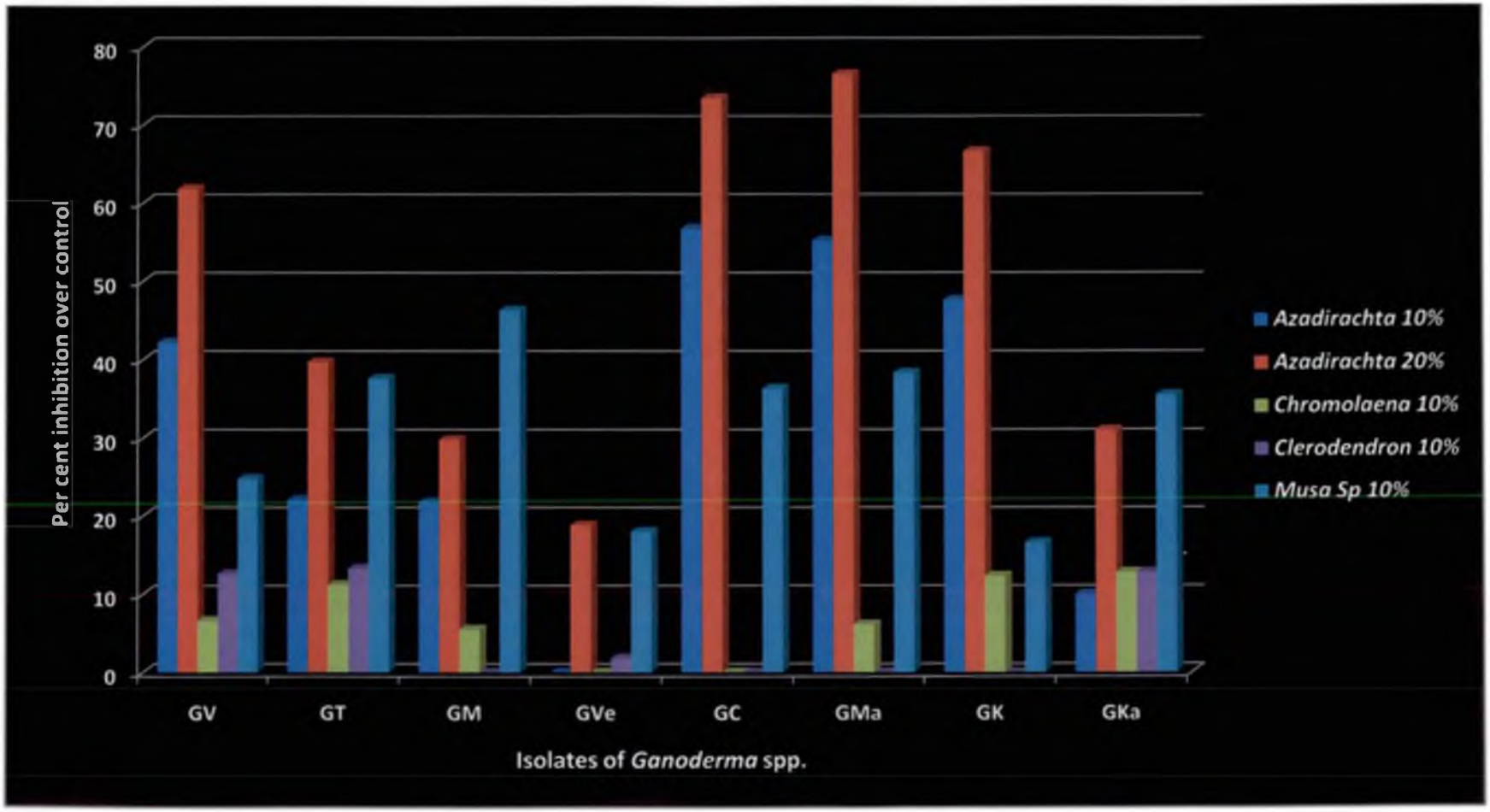


Fig 9. *In vitro* evaluation of phytoextracts against different isolates of *Ganoderma* spp.

4.9.5. Effect of fungicides on different isolates of *Ganoderma* sp.

An *in vitro* evaluation of five different fungicides against the pathogen of BSR was carried out on PDA medium by poisoned food technique. The results are given in Table 34a to 34h (Fig 10).

From the data given in table 34a to 34h, it was revealed that the fungicide *viz*., flusilazole, hexaconazole and iprobenphos at 0.2 per cent concentration recorded cent per cent inhibition on the growth of isolates of *Ganoderma* sp. from different locations. The remaining two fungicides *viz*., aureofungin (0.03 %) and carbendazim 0.1 per cent were statistically analyzed and significant difference was observed among the treatments on their fungicidal efficiency. The inhibitory efficiency of aureofungin at 0.03 per cent concentration against different isolates of pathogen ranged from 28.8 to 71 per cent. Maximum inhibition of 71 per cent by aureofungin was noticed against the isolate of *Ganoderma* sp. from Trivandrum and minimum inhibition of 28.8 per cent was recorded in isolates of *Ganoderma* sp. from Mannuthy. More than 50 per cent inhibition was recorded against the isolate of *Ganoderma* sp. from Madakathara and Chirakacode by aureofungin at 0.03 concentrations. Treatments with carbendazim at 0.1 per cent concentration against all the isolates of *Ganoderma* sp. gave more than 70 per cent inhibition on the growth over control. Inhibitory efficiency of carbendazim (0.1 %) ranged from 74.3 to 100 per cent. Cent per cent inhibition on the growth over control was recorded in the isolate of pathogen from Madakathara and the lowest inhibition was noticed in the isolate of pathogen from Kasaragod. All other isolates of pathogen, except the isolates from Mannuthy and Vettikal, recorded more than 80 per cent inhibition on the growth over control (Plate 18).

4.10. TOXICITY OF CULTURE EXUDATE OF DIFFERENT ISOLATES OF *Ganoderma* sp.

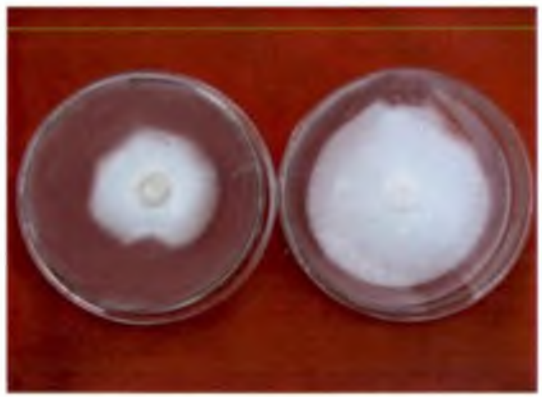
Production of metabolites on the surface of culture was noticed in three isolates of pathogen viz., GM, GVe and GKa obtained from Madakathara, Vettikal and Kasaragod respectively. Toxicity of the exudates was tested by inoculating separately on the leaves of coconut seedlings. On 2 DAI, the initiation

Plate 18. ***In vitro*** **evaluation of fungicides against** ***Ganoderma lucidum***

a. Hexaconazole

b. Flusilazole

c. Iprobenphos



d. Aureofungin

e. Carbendazim

Table 34: Effect of fungicides on different isolates of *Ganoderma* sp.

a. Isolate of *Ganoderma* from Vellayani (GV)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI					PIOC
			2	3	4	5	6	
1	Aureofungin	0.03	17.8	26.1	35.1	44.2	51.6	42.6
2	Carbendazim	0.1	10.8	11.3	11.9	12.4	13.2	85.3
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		25.3	43.3	61.3	81.6	90.0	

b. Isolate of *Ganoderma* from Trivandrum (GT)

SI No.	Fungicide	Concent-rations (per cent)	Growth rate of *Ganoderma* (mm)* DAI							PIOC
			2	4	6	8	10	12	13	
1	Aureofungin	0.03	10.3	11.6	14.2	17.1	21.3	24.0	26.1	71.0
2	Carbendazim	0.1	10	10.1	10.5	10.9	11.1	11.5	12.0	86.6
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		11.2	15.1	24.3	39.0	56.2	82.0	90.0	

C. Isolate of *Ganoderma* from Mannuthy (GM)

SI No	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI						PIOC
			2	3	4	5	6	7	
1	Aureofungin sol	0.03	11.2	20.3	30.4	41.2	51.5	64.1	28.8
2	Carbendazim	0.1	10.0	10.6	11.7	13.2	17.1	21.0	76.6
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		15.5	32.2	55.3	67.0	84.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

d. Isolate of *Ganoderma* from Vettikal (GVe)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI						PIOC
			2	3	4	5	6	7	
1	Aureofungin	0.03	14.1	26.8	33.6	41.2	48.2	52.6	41.5
2	Carbendazim	0.1	0.0	13.1	16.5	16.5	18.1	19.6	78.2
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		23.2	45.1	58.2	78.3	85.0	90.0	

e. Isolate of *Ganoderma* from Madakathara (GMa)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* DAI				PIOC
			2	3	4	5	
1	Aureofungin	0.03	15.0	23.2	33.0	40.9	54.5
2	Carbendazim	0.1	0.0	0.0	0.0	0.0	100.0
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	100.0
6	Control		26.3	47.3	68.3	90.0	

f. Isolate of *Ganoderma* from Chirakacode (GC)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)* Days after inoculation					PIOC
			2	4	5	6	7	
1	Aureofungin	0.03	13.1	21.1	26.2	30.5	34.6	61.5
2	Carbendazim	0.1	10.0	11.9	12.0	12.5	13.0	85.5
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		18.6	37.0	50.3	73.0	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

g. Isolate of *Ganoderma* from Kannur (GK)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)*						PIOC
			DAI						
			2	3	4	5	6	7	
1	Aureofungin sol	0.03	12.0	17.3	24.3	29.5	37.5	48.0	46.6
2	Carbendazim	0.1	11.1	13.5	15.5	16.0	16.7	17.3	80.7
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6	Control		20.6		48.8	68.2	89.0	90.0	

h. Isolate of *Ganoderma* from Kasaragod (GKa)

SI No.	Fungicide	Concentrations (per cent)	Growth rate of *Ganoderma* (mm)*						PIOC
			DAI						
			2	4	6	7	8	9	
1	Aureofungin	0.03	11.1	20.1	31.5	38.2	44.3	49.9	44.5
2	Carbendazim	0.1	10.0	10.6	17.6	19.2	20.0	23.1	74.3
3	Flusilazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	Hexaconazole	0.2	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	Iprobenphos	0.2	0	0	0	0	0	0	100.0
6	Control		18.3	46.1	70.9	78.2	85.6	90.0	

* Mean of three replication PIOC – Per cent inhibition over control

DAI- Days after incubation

i. *In vitro* evaluation of fungicides against different isolates of *Ganoderma* spp.

l o	Fungicide	Concent-rations (%)	Percent inhibition over control							
			GV	GT	GM	GVe	GMa	GC	GK	GKa
	Aureofungin sol	0.03	42.6	71.0	28.8	41.5	54.5	61.5	46.6	44.5
	Carbendazim	0.1	85.3	86.6	76.6	78.2	100.0	85.5	80.7	74.3
	Flusilazole	0.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Hexaconazole	0.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Iprobenphos	0.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

GV : *Ganoderma lucidum*–Vellayani GT : *G. lucidum* -Trivandrum
GM : *G. lucidum* –Mannuthy GVe: *G. lucidum* - Vettikkal
GMa: *G. lucidum* –Madakathara GC : *G. lucidum* - Chirakacode
GK : *G. lucidum* – Kannur GKa: *G. lucidum* - Kasaragod

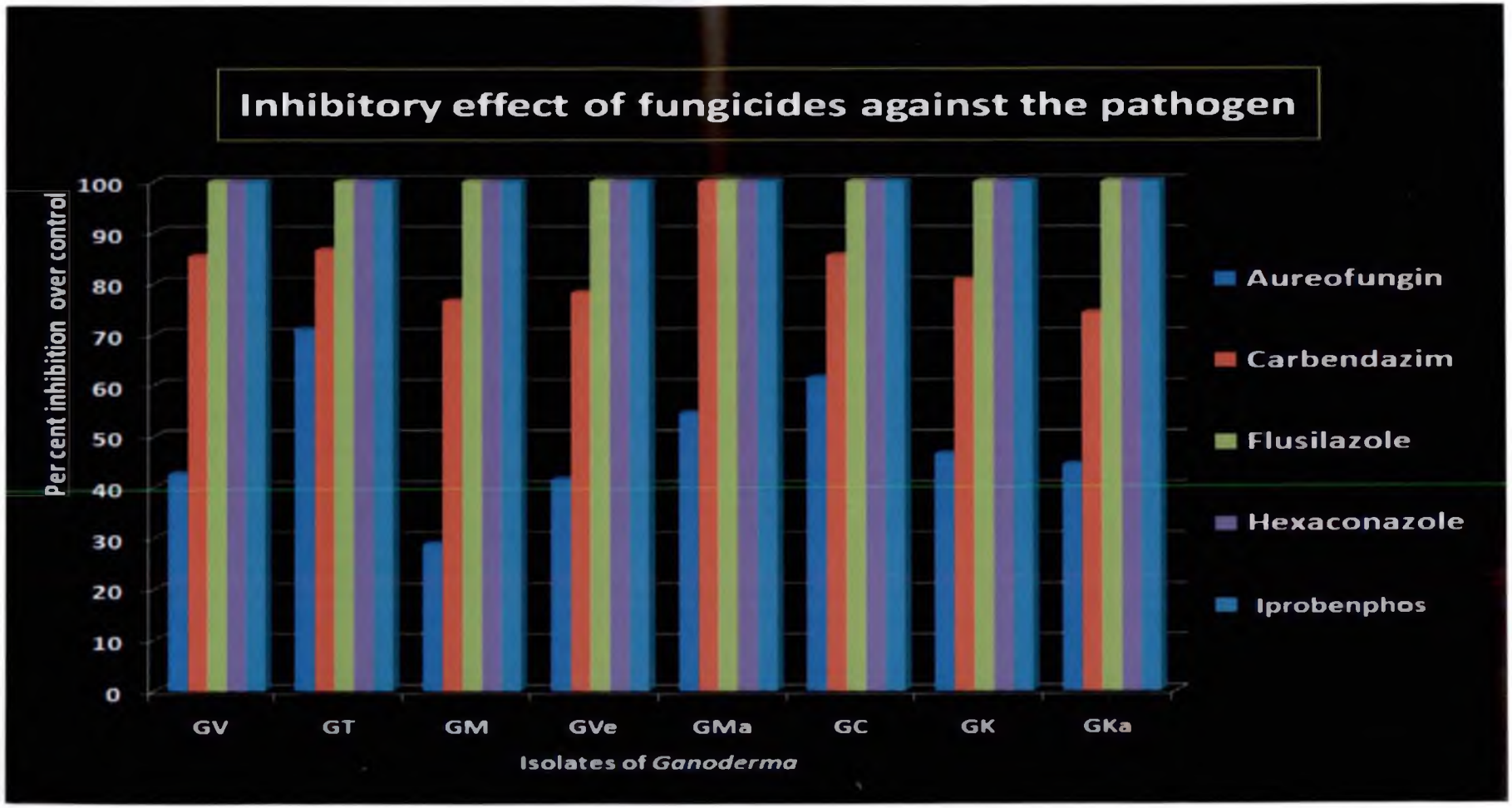


Fig 10. *In vitro* evaluation of fungicide against different isolates of *Ganoderma* spp.

of infection was noticed on all inoculated leaves and it appeared as dark brown to black lesion. Gradually the lesion enlarged in size with grey centre and dark brown margin with yellow halo. The exudates obtained from the isolate GKa showed large sized elongated lesion with a size of 30 x 11mm where as the exudates from the other isolates *viz.*, GVe and GMa produced 23 x 18mm sized lesion (Plate 19).

4.11. HOST RANGE

An *in planta* study was conducted to know the host range of *Ganoderma lucidum* isolated from coconut palms infected with BSR disease. The pure culture of the most virulent isolate selected from each region *viz.*, southern, central and northern was used for inoculation on one year old seedlings of selected crops *viz.*, arecanut, breadfruit, acacia and jackfruit. The plants were inoculated with mycelial growth of the isolates after giving injury at the basal part of the stem along with soil application of inoculums grown on saw dust-rice bran medium.

In breadfruit, yellowing of leaves was noticed in 2 weeks after inoculation and later the leaves were dried up and the plants wilted in 4 weeks after inoculation. The seedlings maintained as control were free of infection. The acacia seedlings inoculated separately with the three isolates of pathogen showed wilting of plants at one month after inoculation in jack fruit, inoculated seedlings with the three isolates of pathogen caused yellowing and drooping of leaves one month after inoculation. Brown lesions were developed at the point of inoculation. Complete wilting of all the seedlings were observed at 50 days after inoculation. Arecanut seedlings inoculated with the three isolates of pathogen showed yellowing and drooping of leaves at 30 days after inoculation.

The seedlings of the selected crops maintained as control did not show any symptoms and were found free of infection. Difference in the growth rate of control seedlings with the inoculated seedlings was observed in all seedlings of selected crops (Plate 20).

Plate 19 . Toxic effect of culture exudates of *Ganoderma lucidum* on coconut leaves

Plate 20 . Host range of *Ganoderma lucidum*

a. Jackfruit

b. Bread fruit

c. Arecanut

DISCUSSION

5. DISCUSSION

Coconut is an important oil seed as well as plantation crop cultivated through out the humid tropics. In India, most of the acreage under coconut palm lies in the four southern states *viz.*, Kerala, Karnataka, Tamil Nadu and Andhra Pradesh. The major part of the production comes from small and marginal holdings. Coconut palms are affected by many pests and diseases. Among them, basal stem rot (BSR) disease caused by *Ganoderma lucidum* has been recognized as a serious disease of coconut, causing severe economic losses during the past several years and continues to do so. This polypore fungus also causes white rot of hardwoods, conifers and palms. It preferably attacks old and declining trees and decay the dead wood and stumps but it causes severe disease in plantation and forest trees (Bhaskaran *et al.*, 1989; Lattiffah *et al.*, 2002). Basal stem rot in coconut is a serious disease in India and in severely infected areas, incidence as high as 80 per cent was recorded (Ramadoss, 1991). It has a wide host range infecting cultivated crops *viz.*, coconut, arecanut, breadfruit, jack fruit, mango etc. A total of 144 hosts of *Ganoderma* have been reported from India and the major pathogens being *G.lucidum* and *G.applanatum* (Sankaran *et al.*, 2005). Identification has largely been made from morphological and cultural characters. Various management practices like management of soil moisture regime, organic manure amendments, use of fungicides and management through bio control agents were tried by many workers. However, these management practices were found effective only when the disease was detected in the early stages.

In Kerala, severe disease incidence in coconut is noticed in many farmers field, which resulted in severe yield reduction and death of the palm. *Ganoderma* infection is also noticed in other crops like arecanut, jackfruit, bread fruit, acacia, eucalyptus, pongamia, etc. A decline in productivity and death of trees are the main economic impacts due to *Ganoderma* disease. Perusal of literature revealed several reports on the research work on various aspects of pathogen and disease management from Tamil Nadu and Karnataka states of India, but only a few reports from Kerala. Considering the importance of the disease, this project is selected and attempts were made to study the cultural, morphological and

pathogenic characters of the organism, symptomatology of the disease, host range and effective management of the pathogen by using selected fungicides, biocontrol agents and phytoextracts. The results obtained on the above aspects are discussed in detail below.

To find out the occurrence of BSR disease caused by *Ganoderma lucidum* and for the collection of diseased specimens, purposive sampling surveys were conducted in coconut gardens of northern, central and southern parts of Kerala. During the survey, BSR infected crops *viz.*, coconut, arecanut, divi divi, gulmohar, rubber, mango, albhizia, acacia and jack fruit were noticed. A total of 21 numbers of basidiocarps were collected either from diseased palms or from the stumps of these diseased coconut palms of different locations. In coconut, symptoms of disease were noticed on leaves, stem and roots. Sporocarps formation was observed in some palms near the basal part of the trees. Sporocarps and diseased specimens of stem and roots were collected from different locations for the isolation of pathogen associated with the diseased specimens.

The collected basidiocarps, stem and roots were brought to laboratory and isolated on PDA medium by adopting two methods. In the first method, isolation was carried out by tissue segment method and the tissues from the basidiocarp, infected roots and stems were used for the isolation. In the second method, the collected specimens *viz.*, basidiocarps, diseased stem and roots were surface sterilized and kept in moisture chamber to produce mycelial growth of the pathogen. Fluffy white mycelial growth was observed on the surface of basidiocarp. This mycelial growth was aseptically transferred to the PDA medium. By these two methods a total of 14 isolates of *Ganoderma* sp. were isolated from basidiocarps collected from the 21 locations surveyed. Among the 14 isolates, four isolates from southern region, eight from central and 2 from northern region were obtained. Isolation of pathogen from diseased stem and root did not yield any mycelial growth on PDA medium. Iyer *et al.* (2004) reported that isolation made from the centre of the fresh fruiting body were found better for obtaining pure culture of *Ganoderma* sp. and they could not isolate the pathogen from diseased stem bits. But Palanna *et al.* (2009) isolated *Ganoderma* sp. from the

basidiocarp kept in the moist chamber. Later Muthelo (2009) and Mishra and Singh (2010) also reported the isolation of *Ganoderma* sp. from basidiocarps. So the reports from earlier workers support the present result.

The pathogen *Ganoderma* spp., a basidiomycetous fungus, will not produce spores in the cultures. They produce basidiospores only in the basidiocarp. So observation on the basidiocarp formation by the isolate is a reliable and easy method to find out the pure culture of the organism. In order to confirm the pure cultures of different isolates of pathogen obtained from basidiocarps, an *in vitro* study was carried out in saw dust-rice bran medium in 250 ml conical flasks. Out of the 14 isolates only eight isolates produced fruiting body and showed similar cultural characters in this medium. The fruiting bodies showed variation in their colour and shape. The colours vary from yellowish white to brown with yellowish white margin. Tubular/ semi circular/ kidney shaped /button shaped fruiting bodies were produced by different isolates. Fruiting body formation in saw dust medium with various shapes was observed by earlier workers. They reported that the development of basidiocarp was very sensitive to light and ventilation. The stipe exhibited tropic growth towards light. Under dim light or dark condition, the pileus did not expand and normally an abnormal pileus of stag-horn or antler-type was produced (Shin and Stamets, 1998; Seo, 1993). In this study also, pileus with typical shape of the basidiocarp of the pathogen was not observed. This might be due to the poor ventilation and lack of bright light to this culture. Isolates which produced fruiting body in saw dust-rice bran medium were selected and named as GV, GT, GM, GVe, GC, GMa and GK based on the name of locations from where it was isolated. These eight isolates were selected for further studies and others were discarded. Exudates production was observed in six isolates of pathogen *viz.*, GM, GVe, GC, GMa, GK and GKa and among them, high exudate production was noticed in GVe and GMa.

Pathogenicity of these eight isolates of *Ganoderma* sp. was tested by artificial inoculation on one year old healthy coconut seedlings. Among these isolates, the pathogen obtained from Trivandrum (GT) did not cause infection on seedlings and symptom expression was not observed. Where as in all other seven

isolates, yellowing of the outer leaves of the seedlings was observed from two month after inoculation (MAI) and by the end of six months, wilting and drying up of the leaves were observed. Basidiocarp formation was noticed only in one seedling inoculated with the isolate from Vellayani (GV). Pathogen was reisolated from the basidiocarp developed on seedling inoculated with GV. The cultural and morphological characters were compared with the original culture of that isolate. In the case of other isolates, re-isolation from the inoculated area could not yield mycelial growth. According to Koch's postulates, the artificially inoculated healthy plants should produce all the typical symptoms of the disease. But, in this study all the symptoms of basal stem rot disease were not developed in the inoculated seedlings. The important symptom of the disease like stem bleeding was not seen, but symptoms on the leaves like yellowing, drying and drooping of leaves were observed at least in one seedling inoculated with each isolate. Earlier, there were several reports on the test of pathogenicity of *Ganoderma* sp. in coconut. Bhaskaran *et al.* (1991) reported that there were no stem bleeding and drooping of leaves in artificially inoculated palms during six months of experiment. Palanna *et al.* (2009) tested pathogenicity of nine isolates of *Ganoderma* sp. in coconut seedlings and proved the pathogenicity of two isolates. Karthikeyan *et al.*, 2007 and Kandan *et al.*, 2009 confirmed the pathogenicity of the organism by the immunological and molecular diagnosis.

The symptom expression by the host is an important stage in the disease cycle and the successful infection results in the appearance of symptoms. The visible and detectable changes in the infected plant make up the symptoms of the disease. The symptoms developed in the infected coconut palms were studied in detail under natural conditions. Exudation of reddish brown viscous fluid from the basal portions of the stem in the affected palm was noticed. In some palms bleeding patches spread upwards. The sporophore initially appears as small, white buttons of fungal tissues that develop rapidly into the bracket shaped mature sporocarp. In all the infected palms drooping and drying of the outer whorls of leaflets were observed and later the spindle also broken and fell down leaving the decapitated stem. Earlier workers also reported the same type of symptoms of BSR diseases by *G. lucidum* on different crops (Bhaskaran *et al.*, 1989; Rethinam,

1984; Srinivasulu *et al.*, 2002; Jayalakshmi and Hameed Khan 2003; Karunanithi *et al.*, 2005 Sankaran *et al.*, 2005; Samiyappan *et al.*, 2006).

In artificially inoculated seedlings, symptoms on leaves and on bark at the base of stem were observed. Yellowing of lower leaves appeared as first symptom and was observed in all the seedlings except those inoculated with the isolate GT. It was observed around 2MAI in all the seedlings and by the end of 6MAI, wilting and drying of the leaves was observed. Sporophore development was observed only in one coconut seedling inoculated with *Ganoderma* isolate GV. Complete drying of plants was observed in seedlings inoculated with GV and GVe. In these seedlings, stem broken at the point of inoculation. Oozing out of exudates is one of the important identifying symptoms of BSR in the field. But it was not observed in the artificially inoculated seedlings. Earlier workers also reported the same type of symptom development by *Ganoderma* sp., on artificially inoculated coconut seedling. (Karthikeyan *et al.*, 2007; Kandan *et al.*, 2009; Palanna *et al.*, 2009). In the present study drooping of outer whorl of leaves was observed in all seedlings which are an important symptom of Thanjavur wilt. Contrary to this observation, Bhaskaran *et al.* in 1991 reported that they could not observe the drooping symptom of Thanjavur wilt in inoculated seedlings of coconut.

Detailed study on the phenotypic characters of the eight different isolates of *Ganoderma* sp. was done to find out any variability existing among the different isolates. This includes the study on cultural and morphological characters of the pathogen, which are the most important criteria for the correct identification of the plant pathogens. The cultural characters of the eight isolates were studied on four different media *viz.*, PDA, Czapek's (DOX) agar, Richard's agar and Soil extract agar. In all the four media, white coloured mycelial growth was produced by all the eight isolates. Slight variation was observed in the texture and mycelial type. A smooth textured mycelial growth was observed in all isolates which grew on four media except GT and GMa which showed a rough textured colony on PDA. Among the eight isolates, except GV and GK, in all others the mycelium was thick on PDA medium where as GV and GK showed thin mycelial growth.

Mycelium of GV was compact, felty and even in all the media except Czapek's (DOX) agar medium where it was filamentous and uneven. The isolate, GT showed compact, felty and uneven mycelium in all media other than soil extract agar where it produced even mycelial type. The isolates *viz.*, GT, GM, GVe, GMa and GKa recorded even, raised and floccose mycelial growth on PDA. On Czapek's (DOX) agar medium, all isolates except GVe and GKa, gave uneven type of mycelial growth. All the eight isolates of *Ganoderma* sp. except GMa recorded even type of mycelial growth on Richard's agar and soil extract agar medium. Many workers reported white to pale yellow coloured smooth, even, felty to floccose nature of the colony characters of *Ganoderma* sp. The present observations on cultural characters of *Ganoderma* sp. is in conformity with the earlier reports (Adaskaveg and Gilbertson, 1987; 1989; Seo, 1987; Seo and Kirk, 2000).

Variation was also observed among the isolates in number of days required for the colour change in mycelium and it ranged from 5-23 DAI. Potato dextrose agar medium recorded minimum days for the colour change of mycelium (5-14 DAI). Isolate from Vettikkal (GVe) recorded colour change in mycelium on 5 DAI and GT took the maximum period of 14 days. In the remaining three media, colour change in mycelium was not observed except GT and GMa on Richard's agar medium and GVe on soil extract agar medium. In all isolates of *Ganoderma* sp., colour change on mycelium originated from the centre of the culture except GMa, in which it was observed from periphery to centre, and this colour of the mycelium changed from white to yellow or yellowish brown. Shin and Seo (1988) reported that this colony became more yellowish when exposed to light. Similar type of colony characters of *Ganoderma* sp. have been reported earlier by Adaskaveg and Gilbertson in 1987, 1989; Seo 1987.

Variations in the production of culture exudate, colour change of medium and aberrant fruiting bodies were observed among these isolates. On PDA medium, all the isolates except GV produced exudates in the culture and among them GM and GVe recorded high and very high exudate production respectively. On comparing the four media, exudates production was found high on PDA

medium. This isolates of *Ganoderma* sp. *viz.,* GV, GT, GC, GK and GKa did not show the production of exudate in Czepek's (Dox), Richard's agar & soil extract agar media. Among the eight isolates, the aberrant fruiting body production was recorded only by the isolate GT on PDA medium. Chlamydospore formation was observed in all isolates of *Ganoderma* sp. except the isolate; GT. Earlier workers reported that *G. lucidum* occasionally produced aberrant fruiting bodies with basidiospores on agar media. Seo *et al.* (1995) observed that some isolates produced fruiting body like primordial (FBP) in medium and they observed chlamydospore formation in those culture where FBP were absent. (Banerjee and Sarkar, 1956; Adaskaveg and Gilbertson, 1986; 1989; Shin and Seo, 1988). In this study also, the aberrant fruiting body formation was observed only in the isolate GT on PDA medium in which chlamydospore formation was absent. So this observation is supportive to the earlier reports.

The growth rate of different isolates of *G. lucidum* was studied in the four different media *viz.*, PDA, Czapek's (DOX) agar, Richard's agar and soil extract agar medium. It was observed that, all isolates showed highest growth rate on PDA medium compared to other three media. Among the four media tested, low growth rate of isolates was noticed in soil extract agar medium, in which the isolates took 12-20 days for the completion of full growth. Potato Dextrose Agar medium was found to be the best one for supporting the fast growth of the pathogen, which recorded the highest growth rate of 13.3mm of the isolate GMa. More over, from the observations made on the cultural characters of these isolates the colour change of the mycelium and medium and production of exudates were observed on PDA medium. Hence PDA medium was selected as the most suitable culture medium for the growth of *G. lucidum*. These findings are supportive to the reports of some earlier workers (Seo, 1987; Shin and Seo, 1988; Adaskaveg and Gilbertson, 1989). But, Mishra and Singh (2010) reported that malt extract agar medium showed the highest rate (7.5mm per day) of *G. lucidum* and was followed by PDA medium (6.2 mm per day).

Perusal of literature revealed that *Ganoderma* disease of coconut was influenced by weather factors. The report showed that incidence of disease was

observed between February and August and highest incidence occurred between March and June when soil temperature was the highest. So an *in vitro* investigation was carried out to find out the effect of various factors such as temperature, pH and light on growth rate of eight isolates of *Ganoderma* sp. from different locations. It was found that temperature had an influence on the growth rate of different isolates. From the results, it was observed that all the isolates of pathogen prefer 30 to 35^{0}C for the fast growth on PDA medium except GT which recorded the fast growth at 25^{0}C. The different isolates took 5-10 days to complete 90 mm growth in Petri dishes. The isolates GV and GKa showed fast growth at 30^{0}C where as GM, GVe and GK took minimum days at 30 and 35^{0}C to complete full growth on Petri dishes. Another important result of this study was that, when temperature increased from 35 to 40^{0}C, none of the isolates except GV and GC showed mycelial growth on medium. In the case of GV and GC, a slight growth of mycelium was observed at 40^{0}C was recorded. So from these results, it is observed that temperature had an influence on the growth rate on the pathogen and from the statistical analysis of data, it was concluded that the isolates of pathogen preferred a temperature range of 30 to 35^{0}C. This result is in supportive to the observation of Karthikeyan *et al.* (2006) and they reported that maximum air temperature, soil temperature and soil moisture were significantly highly influence the development of disease. The report given by Mishra and Singh (2010) was contradictory to this result who reported that better growth of *G. lucidum* was noticed at temperature range of 25-30^{0}C and by increasing the temperature to 35^{0}C and above resulted in decreased average growth rate.

Light also has an influence on the development of plant diseases as it affect the survival of infective propagule of the pathogen, incubation period, secondary inoculums production and some times development of symptom. In this context, the influence of light on the growth of isolates of *Ganoderma* was evaluated and found that in all isolates except GT and GVe, the growth rate was almost same under full light and full darkness. Isolates from Trivandrum (GT), Vettikal (GVe), Chirakacode (GC) and Kasargod (GKa) recorded fast growth rate under complete darkness and took 1 to 5 days more under full light to complete 90mm growth in Petri dishes. Among the eight isolates, only one isolate, GMa

showed fast growth rate under light than in darkness. Perusal of literatures revealed no reports on the effect of light on mycelial growth of *Ganoderma* spp. But there are many reports on the influence of light on primordium formation, pileus differentiation and tropic growth of the stipe of *G. lucidum* and were affected positively by light (Shin and Seo; 1989; Stamets, 1993).

The fungus, *Ganoderma lucidum* is a soil inhabitant and survives in soil. Soil pH is an important factor which determines its growth and survival in soil. They may tolerate the pH range in which their hosts normally grow. Hence a study was done to find out the effect of pH on the growth of isolates of *Ganoderma* sp. Slight acidic to neutral pH was found favourable for the maximum growth of different isolates of the pathogen. Among the eight isolates, GV, GT and GVe recorded fast growth rate at pH 7, whereas the isolates GM, GK, GKa and GMa showed fast growth rate at pH 6 and 7. The pH 5 was found favourable for the isolate GC and also for GMa. For all the isolates, the pH 8 was found unfavourable for the growth and took more time to complete full growth in Petri dishes. So from these results, it was concluded that the pathogen, *G. lucidum* require neutral to acidic pH (5 to 7) for their growth and multiplication. Early reports by many workers also showed variation in the growth rate at different pH levels. Triratana *et al.* (1991) and Rai, (2003) reported that acidic pH favoured the mycelial growth of the fungus. Mishra and Singh (2010) observed good growth of *G.lucidum* at pH 5 and 6 but they noticed sudden drop in the growth at alkaline side.

The identification and taxonomy of Basidiomyectous fungi has traditionally been based on the morphological features of the basidiocarps. The characters of basidiocarp may vary in different fungi. Basidiocarps are the sexual structure in *Ganoderma* sp. and other polypores which formed from a living or more commonly, from a dead trunk or branch of tree in the form of bracket. The naturally produced basidiocarps of *G. lucidium* showed various morphological characteristics. Similarly difference in the morphological characters of basidiocarps of different species of the pathogen was reported. Observations on the morphological characters of basidiocarps of various isolates of *Ganoderma* sp.

showed a slight variation in the size, shape and colour of its parts, attachment pattern of stipe to pileus, presence of ornamentation, method of formation of basidiocarp and pattern of margin of pileus. Basidiocarp stipitate in all isolates except GC and GVe in which they were sessile. Among the eight specimens of basidiocarps, five were semicircular in shape (GV, GT, GVe, GK and GKa), one was circular (GC) and two were conical (GM and GMa). All of them formed singly except GV and GM, yellowish red, pale brown or reddish brown coloured pileus with smooth to wavy margin and creamy white to brown coloured pore surface were observed. The surface of pileus was deeply waved with or without concentric zones, 2.6 -17.0 x 4.4 -12.0 cm size, 1-10 mm pore length, 139-254 x 122-190µm pore diameter, 2-10mm flesh thickness, parallel to lateral attachment of stipe to pileus were recorded.

Basidiospore were brown, ovate to ellipsoidal, echinulate, truncated apex, double walled with inter wall pillars separating two walls. The size of these basidiospores showed variation in the range of 4.8-13 x 4.5-7.0µm with a spore index of 1.15-1.7. Observations were also made on the hyphal system of basidiocarps. Even though there were difference in the colour, shape and ornamentation of the basidiocarp, the hyphal system in all the isolates was found same. It was trimitic, with generative hypha hyaline, thin walled, branched, septate and clamped, skeletal hypha reddish brown, pigmented, binding hypha hyaline and branched. These observations on the morphological characteristics of basidiocarp and basidiospores are in favour to the reports of earlier workers (Govindu *et al.*, 1983; Seo and Kirk, 2000; Muthelo, 2009; Srivastava *et al.*, 2010).

Based on the cultural characters of the different isolates and morphological characters of basidiocarp, basidiospores and mycelium, all the eight isolates were identified as *Ganoderma lucidum* (Leys) Karst. The identification of most of the species of *Ganoderma* from India had been made based on cultural and morphological characteristics alone and hence many of the names used were incorrect particularly *G. lucidium* and *G. applanatum* (Sankaran *et al.*, 2005). *G lucidium* and *G. applanatum* are the two important species amplexes in the history

and nomenclature of the genus (Monocalvo and Ryvarden, 1997). Leelavathy and Ganesh (2000) studied polyporales of Kerala and recorded the occurrence of only two species *viz., G. australe* and *G. lucidum* from the state. There are several contradictions in the literature and these clearly indicate the confusion over species identification in *G. lucidum.*

The second objective of the present study was the management of *G. lucidum* causing BSR disease in coconut. The cultural, chemical and biological method is important practice for the management of any disease. Now a days, more importance is given to biological methods of management of the pathogen. In this study the effectiveness of native antagonist organisms along with the reference cultures of fungal and bacterial antagonists, phytoextracts and fungicides were evaluated against the pathogen under *in vitro* condition. At first, antagonistic microorganisms were isolated from the rhizosphere soil of healthy coconut palm near to BSR infected palm from three regions of Kerala *viz.*, southern, central and northern regions. From the eight different locations, a total of 39 fungal isolates and 28 bacterial isolates were obtained. After preliminary screening, eight fungal and three bacterial isolates which showed inhibition on the growth of pathogen were selected. Based on the cultural characters the selected fungal antagonists were tentatively identified as *Aspergillus flavus, Aspergillus niger, Curvularia* sp.,and *Trichoderma* sp. from three locations. The two isolates of antagonistic fungi were not identified and named as FA-5 and FA-7. The three isolates of *Trichoderma sp.* were further identified up to species level by NCFT, New Delhi, as *Trichoderma virens* from Vellayani and Kannur, *Trichoderma viride* from Mannuthy. The antagonist efficiency of the eight selected fungal isolates against the pathogen was compared with the reference culture of fungal antagoinst *viz., Trichoderma viride and T. harzianum.* From this result it was revealed that the different isolates of *Trichoderma* sp., including reference culture recorded more than 60 per cent inhibition on the growth of all isolates of pathogen. Among them cent per cent inhibition of the isolates of pathogen *viz.*, GT and GC was observed by the three selected *Trichoderma* sp. and the reference culture. More over cent per cent inhibition of GM, GVe by *T. viride* from Mannuthy, GMa and GKa by *Trichoderma virens* from Kannur, GK by *T. virens*

from Vellayani, GVe and GKa by reference culture *T.viride*, GMa and GKa by *T. harzianum* was recorded. Among these eight fungal antagonists except *Trichoderma* spp., only *Aspergillus niger* recorded cent per cent inhibition of the isolate GK of pathogen. Hundred per cent inhibition on the growth of GT was recorded by all the fungal antagonists which might be due to the slow growth rate of that isolate of pathogen.

Per cent inhibition on the growth of different isolates of the pathogen due to the selected bacterial antagonists and reference culture of *Pseudomonas fluorescens* were evaluated. From this data it was observed that, the selected bacterial antagonists and the reference culture of *P. fluorescens* recorded less than 50 per cent inhibition on the growth except in cases of few isolates of the pathogen. The highest inhibition of 66.5 per cent was observed against the isolate GK by the antagonist BA-3 and it was followed by GT which recorded 64.3 per cent inhibition. The antagonist BA -3 showed more than 50 per cent inhibition only against three isolates, BA-1 against two isolates and BA-2 against one isolate of the pathogen. Even the reference culture, *P. fluorescens* recorded less than 50 percent inhibition against all the isolates of the pathogen. So from these results, it was observed that the selected bacterial antagonist were not much effective in inhibiting the pathogen compared to fungal antagonists and hence further studies in the bacterial antagonists were not carried out. Srinivasulu *et al.* (2006) reported that the volatile metabolites of *P. fluorescens* completely inhibited *G.applanatum* and *G.lucidum* but non volatile metabolites were not effective. This might be the reason for the less effectiveness of the reference culture against the isolates of pathogen. Hence the results were supportive to the reports of earlier workers (Srinivasulu *et al.*, 2006; Rajendran *et al.*, 2007; Rahamath bivi *et al.*, 2010).

The cultural characters of the selected three isolates of *Trichoderma* spp. were studied on PDA medium. From this result it was observed that *Trichoderma* spp. showed slow growth rate on increasing the age of the culture. It was observed in the selected as well as the reference culture of *Trichoderma* spp. No difference in mycelial growth of *Trichoderma* spp. on PDA was observed when exposed to complete light and darkness and complete darkness. Metabolite production was

observed in *T. virens* from Vellayani and in reference culture *T. viride*. This result is in favour of the report of Srinivasulu *et al.* (2008) where they observed fast growth rate of young culture of *Trichoderma* spp. compared to old culture.

After the selection of efficient antagonists a detailed study on the mechanisms of antagonism was carried out. Microscopic observations on the mycoparasitism of *Trichoderma* spp. revealed coiling of hyphae of pathogen with hyphae of the antagonists. Disintegration and malformation of hyphae of pathogen were also noticed.

The antagonist, *Trichoderma* spp. is known to produce non volatile and volatile metabolites which are inhibitory to most of the plant pathogens. The inhibiting effect of non volatile metabolites produced by the selected *Trichoderma* spp. was studied by culture filtrate method and cellophane paper method. On comparing the two methods, per cent inhibition on the growth of pathogen recorded was less than 50 per cent in culture filtrate method where as, except GVe, GC and GK in all other isolates of pathogen, more than 50 per cent inhibition was recorded by all the isolates of *Trichoderma* spp. The cellophane paper method was found best for evaluating the effect of non volatile metabolite of antagonistic organisms. The reference culture *T. harzianum* recorded the highest inhibition on the growth of all isolates of pathogen except GK in the range of 48.7 to 82 per cent. *T. viride* from Vellayani recorded the highest inhibition of 49 per cent on the isolate GK. The effect of non volatile metabolite of *Trichoderma* sp. was reported by many workers. (Srinivasulu *et al.*, 2006; Claydon *et al.*, 1987; Izzati and Abdullah 2008).

The efficacy of volatile metabolites produced by various isolates of *Trichoderma* spp. was tested against the different isolates of *G. lucidum* by inverted plate method. From the data it was revealed that inhibitory effect of volatile metabolites produced by *Trichoderma* spp. were least effective against the *Ganoderma* isolates while comparing it with the effect of non volatile metabolites. All isolates of *Trichoderma* spp. recorded less than 40 per cent inhibition on the growth of all isolates of pathogen except GKa. Among the five *Trichoderma* sp. tested, the reference culture of *T.viride* showed maximum inhibition of 56.6 per

cent on the growth of GKa followed by *T. harzianum* and *T.viride* from Vellayani which recorded 38.8 per cent inhibition of the isolates, GMa and GKa respectively. The isolates of pathogen *viz.,* GK and GV showed zero per cent inhibition when exposed to volatile metabolites of *T.virens*-Vellayani and *T.viride* (reference culture). But workers like Pandey and Uapadhyay (1997) and Srinivasulu *et al.* (2008) reported the effectiveness of volatile metabolites by *Trichoderma* species *in vitro* against *Ganoderma* spp.

Another important aspect studied in the management of the pathogen was the evaluation of phytoextracts against the pathogen. Phytoextracts *viz., Azadirachta indica* (10 and 20%), *Chromolaena odorata* (10%) *Clerodendron aculeatum* (10 %) and *Musa* sp. (10 %) were used against the pathogen. Among them maximum inhibition was noticed by *A. indica* at 20 per cent concentration against all isolates except GM and GKa. The highest inhibition on the growth of GM and GKa was recorded by the extract of *Musa* sp. *Azadirachta indica* at 10 per cent concentration was not effective against all the isolates. It recorded no inhibition on the growing GVe and less than 25 per cent inhibition was noticed in the growth of GT, GM and GKa. When the concentration was increased to 20 per cent, it showed more than 50 per cent inhibition on antagonist of GV, GC, GMa and GK. The 10 per cent extracts of *Chromolaena odorata and Clerodendron aculeatum* were not effective against different isolates of *G. lucidum,* which recorded the maximum inhibition of 12.8 per cent over control. The extract of leaves, sheath and rhizome together of *Musa* spp. at 10 per cent concentration recorded 18.0 to 46.3 per cent inhibition on the growth of different isolates of pathogen. The inhibiting effect of banana rhizome against *G. lucidum* was reported by Bhaskaran *et al.* (1993) and Karthikeyan and Bhaskaran. (2001). Karthikeyan *et al.* (2007) reported the inhibiting effect of 10 per cent concentration of leaf extract of *Azadirachta indica* in suppressing the mycelial growth of *G. lucidium.* So the result of this study is in line with the early reports. The early reports showed the effectiveness of banana in containing the BSR disease. Intercropping within diseased plantation increased the coconut yield. This might be due to the increased population of fungi, actinomycetes and the antagonistic organisms *viz., Trichoderma* spp. etc. in soil of intercropped coconut.

Next aspect of management studied was the evaluation of fungicides against the pathogen by poisoned food technique. Fungicides *viz.*, aureofungin (0.03%), carbendazim (0.1%), hexaconazole, iprobenphos and flusilazole (0.2%) were used. From the data, it was revealed that fungicide *viz.*, flusilazole, hexaconazole and iprobenphos at 0.2 per cent concentration recorded cent per cent inhibition on the growth of all the eight isolates of *Ganoderma* sp. obtained from different locations. The data on the effect of remaining two fungicides *viz.*, aureofungin (0.03%) and carbendazim (0.1%) were statistically analysed and significant difference among them was observed. Inhibitory efficiency of aureofungin against different isolates of pathogen ranged from 28.8-71 per cent and that of carbendazim (0.1%) ranged from 74.3-100 per cent. On comparing two fungicides, carbendazim 0.1 per cent was more effective than aureofungin. Earlier many workers reported the inhibiting efficiency of these fungicides used for *in vitro* and field experiments. (Bhaskaran *et al.*, 1996; Naik *et al.*, 2008; Srinivasulu *et al.*, 2002, 2008; Naik and Venkatesh, 2001). Palanna *et al.*, (2009) reported that tridemorph and heaxaconazole at 0.1 per cent concentration were very effective for the complete inhibition of *G. lucidium* and *G. applanatum* under *in vitro* condition.

The last part of the investigation was the study of the host range of the pathogen causing basal stem rot of coconut. For this the most virulent isolate of pathogen from each region were used to inoculate on selective hosts *viz.*, arecanut, bread fruit, acacia and jack fruit. All the inoculated plants showed yellowing and drooping of leaves about one month after inoculation. Complete wilting of seedlings of bread fruit, acacia and jack fruit were observed after one to one and half month after the inoculation. For arecanut yellowing and drooping of leaves were observed. The other typical symptoms caused by *G. lucidum* on mature trees were not observed in these seedlings. Sporocarp formation was not observed. The seedlings maintained as control in case of all the four selected crops were found free of infection did not show any symptoms. There were several earlier reports which showed a wide host range of this pathogen attacking a variety of palms, forest trees and fruit plants. (Naidu *et al.*, 1966; Bakshi *et al.*, 1972; Venkatarayan, 1936; Govindu *et al.*, 1983). The root rots and wilts of *Acacia*

caused by *Ganoderma* sp. became a serious problem through out Kerala where ever the tree is grown (Sankaran *et al*; 2005)

Summing up the results of the study discussed so far, it may be concluded that the occurrence of basal stem rot disease of coconut was observed through out Kerala and the isolation of pathogen from basidiocarps yielded eight isolates of *Ganoderma* spp. which produced fruiting body in rubber saw dust rice bran substrate. The pathogenicity of eight isolates of *Ganoderma* sp. was tested and observed yellowing, drying and drooping of leaves of coconut seedlings inoculated with all isolates except GT. Basidiocarp formation was noticed only in one seedling inoculated with the isolate GV and reisolation of pathogen was done from this basidiocarp.

Under field condition the typical symptom of BSR disease was observed in all surveyed areas but all the typical symptoms of disease were not observed under artificial condition. All the isolates of pathogen produced white mycelial growth on PDA, Czapek's (DOX) agar, Richard's agar and soil extract agar media, but variation in texture, mycelial type, and colour change of mycelium, exudates production and formation of aberrant fruiting body were observed. PDA was found to be best medium for the growth of pathogen. The pathogen preferred a temperature range of 30-35^0 C and neutral to acid pH of 5-7 for the growth. Not much variation in growth rate was observed under light and darkness.

Morphological characters of basidiocarp, basidiospore and mycelium of all eight isolates were studied and based on the observations the eight isolates of pathogen were identified as *Ganoderma lucidum* (Leys) Karst.

Regarding the *in vitro* management of the pathogen, two isolates of *T. virens* and one isolate of *T. viride* were selected and were equally effective with the reference culture, *T. viride* and *T. harzianum* in inhibiting the growth of pathogen. Mycoparasitism and production of non volatile metabolites were found to be the mechanisms exhibited by these selected *Trichoderma* spp. Among the phytoextracts *Azadirachta indica* at 20 per cent concentration was found most effective and was followed by *Musa* sp. at 10 per cent concentration to inhibit the

growth of pathogen. The *in vitro* evaluation of fungicides showed that flusilazole, hexaconazole and iprobenphos at 0.2 per cent concentration were the most effective and recorded cent per cent inhibition on the growth of all isolates of pathogen. The study on the host range of *G. lucidum* revealed that the seedlings of arecanut, breadfruit, acacia and jack fruit showed yellowing and drooping of leaves and finally wilting of all seedlings.

The present investigation on characterization and management of *G. lucidum* inciting basal stem rot of coconut enriched our knowledge in various aspects of this disease, especially on the etiology and symptomatology of the disease, morphological and cultural characters of the pathogen, management of pathogen using bio agents, phytoextracts and fungicides and the host range of the pathogen.

SUMMARY

Summary

The present investigation on "Characterization and management *Ganoderma lucidum* inciting basal stem rot of coconut" was carried out in the Department of plant pathology, College of Horticulture, Vellanikkara to study the various aspects of the disease particularly the cultural, morphological and pathogenic characters of the pathogen, symptomatology of the disease, host range and effective management of pathogen using bio control agent, phytoextracts and selected fungicides.

Purposive sampling surveys were conducted in coconut gardens of northern, central and southern parts of Kerala. The diseased roots and stems and basidiocarps were collected from 21 different locations, and the pathogen was isolated on PDA medium by tissue segment and moist chamber method. By these two methods a total of 14 isolates of *Ganoderma* sp. were isolated from basidiocarps collected from the 21 locations surveyed. Among the 14 isolates, four isolates from southern region, eight from central and 2 from northern region were obtained. Isolation of pathogen from diseased stem and root did not yield any mycelial growth on PDA medium.

In order to identify the pure cultures of different isolates of pathogen, an *in vitro* study was carried out in saw dust-rice bran medium. Out of the 14 isolates only eight isolates produced fruiting body and showed similar cultural characters in this medium. The colour of the fruiting body varies from yellowish white to brown with yellowish white margin. Tubular/ semi circular/ kidney shaped /button shaped fruiting bodies were produced by different isolates. Observations on the number of days taken for complete colonization, exudates production and development of fruiting bodies were taken. Isolates which produced fruiting body in saw dust-rice bran medium were selected and named as GV, GT, GM, GVe, GC, GMa and GK based on the name of locations from where it was isolated.

Pathogenicity of these eight isolates of *Ganoderma* sp. was tested by artificial inoculation on one year old healthy coconut seedlings. Observed

yellowing, drying and drooping of leaves of coconut seedlings inoculated with all isolates except GT. Basidiocarp formation was noticed only in one seedling inoculated with the isolate GV and reisolation of pathogen was done from this basidiocarp.

The symptoms developed in the infected coconut palms were studied in detail under natural and artificial conditions. Exudation of reddish brown viscous fluid from the basal portions of the stem and yellowing, drooping and drying of the outer whorls of leaflets were observed under natural conditions. Later the spindle also broken and leaving the decapitated stem. In artificially inoculated seedlings, symptoms on leaves and on bark at the base of the stem were noticed. Sporophore development was observed only in GV inoculated seedlings. Oozing out of exudation was not observed in artificially inoculated seedlings.

Detailed study on the phenotypic characters of the eight different isolates of *Ganoderma* sp. was done to find out any variability existing among the different isolates. This includes the study on cultural and morphological characters of the pathogen, which are the most important criteria for the correct identification of the plant pathogens. The cultural characters of the eight isolates were studied on four different media *viz.*, PDA, Czapek's (DOX) agar, Richard's agar and soil extract agar. In all the four media, white coloured mycelial growth was produced by all the eight isolates but variation in texture, mycelial type, and colour change of mycelium, exudates production and formation of aberrant fruiting body were observed. PDA was found to be the best medium for the growth of pathogen.

Variation was also observed among the isolates in number of days required for the colour change in mycelium and it ranged from 5-23 DAI. PDA medium recorded minimum days for the colour change of mycelium (5-14 DAI).Variation in the production of culture exudates, colour change of medium and aberrant fruiting bodies were observed among these isolates. On PDA medium, all the isolates except GV produced exudates in the culture and among them GM and GVe recorded high and very high exudate production respectively. On comparing the four media, exudates production was found high on PDA medium. Among the eight isolates, the aberrant fruiting body production was

recorded only by the isolate GT on PDA medium. Chlamydospore formation was observed in all isolates of *Ganoderma* sp. except the isolate, GT.

The growth rate of different isolates of *G. lucidum* was studied in the four different media *viz.*, PDA, Czapek's (DOX) agar, Richard's agar and Soil extract agar medium. It was observed that, all isolates showed highest growth rate on PDA medium compared to other three media. Among the four media tested, low growth rate of isolates was noticed in soil extract agar medium, in which the isolates took 12-20 days for the completion of full growth.

An *in vitro* investigation was carried out to find out the effect of various factors such as temperature, pH and light on growth rate of eight isolates of *Ganoderma* sp. from different locations. It was found that temperature had an influence on the growth rate of different isolates. All the isolates of pathogen prefer 30 to 35^{0}C for the fast growth on PDA medium except GT which recorded the fast growth at 25^{0}C. Another important result of this study was that, when temperature increased from 35 to 40^{0}C, none of the isolates except GV and GC showed mycelial growth on medium. In the case of GV and GC, a slight growth of mycelium was observed at 40^{0}C was recorded.

The influence of light on the growth of isolates of *Ganoderma* was evaluated and found that in all isolates except GT and GVe, the growth rate was almost same under light and dark. Isolates from Vettikal (GVe), Trivandrum (GT), recorded fast growth rate under complete darkness and took 2 and 5 days more under full light respectively to complete 90mm growth in Petri dishes. Among the eight isolates, only one isolate GMa showed fast growth rate under light than in darkness.

An *in vitro* study was conducted to know the effect of pH on the growth of isolates of *Ganoderma* sp. Slight acidic to neutral pH (5-7) was found favourable for the maximum growth of different isolates of the pathogen.

The identification and taxonomy of *Basidiomycetous* fungi has traditionally been based on the morphological features of the basidiocarps. The characters of basidiocarp may vary in different fungi. Observations on the

morphological characters of basidiocarps of various isolates of *Ganoderma* sp. showed a slight variation in the size, shape and colour of its parts, attachment pattern of stipe to pileus, presence of ornamentation, method of formation of basidiocarp and pattern of margin of pileus. Basidiocarp stipitate in all isolates except GC and GVe in which they were sessile. Among the eight specimens of basidiocarps, five were semicircular in shape (GV, GT, GVe, GK and GKa), one was circular (GC) and two were conical (GM and GMa). All of them formed singly except GV and GM, yellowish red, pale brown or reddish brown coloured pileus with smooth to waved margin and creamy white to brown coloured pore surface were observed. The surface of pileus was deeply waved with or with out concentric zones, 4.4-12.0 x 2.6-17.0 cm size, 1-10 mm pore length 139-254 x 122 -190 µm pore diameter, 2-10mm flesh thickness, parallel to lateral attachment of stipe to pileus were recorded.

Basidiospores were brown, ovate to ellipsoidal, truncated apex,echinulate, double walled with inter wall pillars separating two walls. The size of these basidiospores showed variation in the range of 4.8-13 x 4.5-7.0µm with a spore index of 1.15-1.7. Observations were also made on the hyphal system of basidiocarps. Even though there were difference in the colour, shape and ornamentation of the basidiocarp, the hyphal system in all the isolates was found same. It was trimitic, with generative hyphae hyaline, thin walled, branched, septate and clamped. Reddish brown pigmented skeletal hyphae and colourless binding hyphae were noticed.

Based on the cultural characters of the different isolates and morphological characters of basidiocarp, basidiospores and mycelium, all the eight isolates were identified as *Ganoderma lucidum* (Leys) Karst.

In this study the effectiveness of native antagonist organisms along with the reference cultures of fungal and bacterial antagonists, phytoextracts and fungicides were evaluated against the pathogen under *in vitro* condition. At first, antagonistic microorganisms were isolated from the rhizosphere soil of healthy coconut palm near to BSR infected palm from three regions of Kerala *viz.*, southern, central and northern regions. From the eight different locations, a total

of 39 fungal isolates and 28 bacterial isolates were obtained. After preliminary screening, eight fungal and three bacterial isolates which showed inhibition on the growth of pathogen were selected. Based on the cultural characters the selected fungal antagonists were tentatively identified as *Aspergillus flavus, Aspergillus niger, Curvularia* sp. *and Trichoderma* sp. The two isolates of antagonistic fungi could not identified and named as FA-5 and FA-7. The three isolates of *Trichoderma sp.* were further identified up to species level by NCFT, New Delhi, as *Trichoderma virens* from Vellayani & Kannur, *T. viride* from Mannuthy. The antagonist efficiency of the eight selected fungal isolates against the pathogen was compared with the reference culture of fungal antagonist *viz., T. viride* and *T. harzianum.*

Isolates of *Trichoderma* sp. including reference culture recorded more than 60 per cent inhibition on the growth of all isolates of pathogen. Among them cent per cent inhibition of the isolates of pathogen *viz.*, GT and GC was observed by the three selected *Trichoderma* sp. and the reference culture. Among the eight fungal antagonists except *Trichoderma* spp., only *Aspergillus niger* recorded cent per cent inhibition of the isolate GK of pathogen.

Per cent inhibition on the growth of different isolates of the pathogen due to the selected bacterial antagonists and reference culture of *P. fluorescens* were evaluated and recorded less than 50 percent inhibition on the growth except in cases of few isolates of the pathogen. So from the results, it was concluded that the selected bacterial antagonist were not much effective in inhibiting the pathogen compared to fungal antagonists and hence further studies in the bacterial antagonists were not carried out.

The cultural characters of the selected three isolates of *Trichoderma* spp. were studied on PDA medium. From this result it was observed that *Trichoderma* spp. showed slow growth rate on increasing the age of the culture. It was observed in the selected as well as the reference culture of *Trichoderma* spp. No difference in mycelial growth of *Trichoderma* spp. on PDA was observed when exposed to complete light, alternate light and darkness and complete darkness. Metabolite

production was observed in *T. virens* from Vellayani and in reference culture, *T viride*.

After the selection of efficient antagonists a detailed study on the mechanisms of antagonism was carried out. Microscopic observations on the mycoparasitism of *Trichoderma* spp. revealed coiling of hyphae of pathogen with hyphae of the antagonists. Disintegration and malformation of hyphae of pathogen were also noticed.

The inhibitory effect of non volatile metabolites produced by the selected *Trichoderma* spp. was studied by culture filtrate method and cellophane method. On comparing the two methods, the cellophane paper method was found best for evaluating the effect of non volatile metabolite of antagonistic organisms. The efficacy of volatile metabolites produced by various isolates of *Trichoderma spp.* was tested against the different isolates of *G.lucidum* by inverted plate method. But the inhibitory effect of volatile metabolites was least effective against the *Ganoderma* isolates while comparing it with the effect of non volatile metabolites.

Another important aspect studied in the management of the pathogen was the evaluation of phytoextracts against the pathogen. Phytoextracts *viz., Azadirachta indica* (10, 20 per cent), *Chromolaena odorata* (10 per cent), *Clerodendron aculeatum* (10 per cent) and *Musa* spp. (10 per cent) were used against the pathogen. Among them maximum inhibition was noticed by *A. indica* at 20 per cent concentration against all isolates except GM and GKa. The highest inhibition on the growth of GM and GKa was recorded by the extract of *Musa* sp. *Azadirachta indica* at 10 percent concentration was not effective against all the isolates. It recorded no inhibition on the growth of GVe and less than 25 per cent inhibition on the growth of GT, GM and GKa. When the concentration was increased to 20 per cent, it showed more than 50 per cent inhibition against GV, GC, GMa and GK.

The next aspect studied for the management of pathogen was the evaluation of fungicides against the pathogen by poisoned food technique.

Fungicides *viz.*, aureofungin (0.03 per cent), carbendazim (0.1 per cent), hexaconazole, flusilazole and iprobenphos (0.2 per cent) were used. Fungicide *viz.*, flusilazole, hexaconazole and iprobenphos at 0.2 per cent concentration recorded cent per cent inhibition on the growth of all the eight isolates of *Ganoderma* sp. obtained from different locations. The remaining two fungicides *viz.*, aureofungin (0.03 per cent) and carbendazim (0.1 per cent) were statistically analysed and significant difference among them was observed. Inhibitory efficiency of aureofungin against different isolates of pathogen ranged from 28.8 – 71 per cent and that of carbendazim (0.1 per cent) ranged from 74.3 -100 per cent.

The last part of the investigation was the study on the host range of pathogen causing basal stem rot of coconut. For this the most virulent isolate of pathogen from each region were used to inoculate on the selected hosts *viz.*, areca nut, bread fruit, acacia and jack fruit. All the inoculated plants showed yellowing and drooping of leaves about one month after inoculation. Complete wilting of seedlings of bread fruit, acacia and jack fruit were observed on one to one and half month after the inoculation. For arecanut, yellowing and drooping of leaves were observed. The other typical symptoms caused by *Ganoderma lucidum* on mature trees were not observed in these seedlings. Sporocarps formation was not observed.

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*Originals not seen

APPENDICES

Appendix-1

MEDIA COMPOSITION

1. POTATO DEXTROSE AGAR

Potato	: 200g
Dextrose	: 20.0g
Agar	: 20.0g
Distilled water	: 1000ml

2. CZAPEK'S (DOX) AGAR

Sucrose	: 30.00g
Sodium nitrate	: 2.00g
Dipotassium phosphate	: 1.00g
Magnesium sulphate	: 0.50g
Potassium chloride	: 0.50g
Ferrous sulphate	: 0.01g
Agar	: 20.00g
Distilled water	: 1000ml

3. RICHARD'S AGAR

Potassium nitrate	: 10.00g
Potassium dihydrogen phosphate	: 5.00g
Magnesium sulphate	: 2.50g
Ferric chloride	: 0.02g
Sucrose	: 50.0g
Agar	: 20.00g
Distilled water	: 1000ml
pH	: 6.6-7.2

4. SOIL EXTRACT AGAR

Soil extract	: 100ml
Glucose	: 1.0g
Dipotassium phosphate	: 0.5g
Agar	: 20g
Tap water	: 900ml
pH	: 7-7.2

5. KING'S B MEDIUM

Peptone	: 20g
Glycerol	: 10ml
K2HPO4	: 10g
MgSO4.7H2O	: 1.5g
Agar	: 20g
Distilled water	: 1000ml
pH	: 7.2-7.4

6. NUTRIENT AGAR MEDIUM

Beef extract	: 1g
Peptone	: 5g
Sodium chloride	: 5g
Agar	: 15g
Distilled water	: 1000ml

CHARACTERIZATION AND MANAGEMENT OF *Ganoderma lucidum* INCITING BASAL STEM ROT OF COCONUT

By

YUNUS C.

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ABSTRACT OF THE THESIS

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Department of Plant Pathology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR-680 656

KERALA, INDIA

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ABSTRACT

ABSTRACT

The present study on " Characterization and management of *Ganoderma lucidum* inciting basal stem rot of coconut " was undertaken in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2010-2012 with an aim to isolate the pathogen associated with the disease and to study the cultural, morphological and pathogenic characters of different isolates of the pathogen, symptomatology of the disease, host range and effective management of the pathogen using bio-control agents, phytoextracts and selected fungicides.

Purposive sampling surveys were conducted and the occurrence of basal stem rot disease of coconut was observed through out Kerala. The isolation of pathogen from basidiocarps yielded eight isolates of *Ganoderma* sp. which produced fruiting body in saw dust- rice bran substrate. The pathogenicity of these isolates was tested and observed yellowing, drying and drooping of leaves of coconut seedlings inoculated with all isolates except the isolate GT- from Trivandrum. Basidiocarp formation was noticed only in one seedling inoculated with the isolate GV from Vellayani and reisolation of pathogen was done from this basidiocarp.

Symptomatology of the disease under natural and artificial conditions was studied. Under field condition the typical symptom of BSR disease *viz.*, yellowing and drooping of leaves, stem bleeding and basidiocarp formaton were observed in all surveyed areas but all the typical symptoms of disease were not observed under artificial condition. The cultural characters of all the isolates of pathogen were studied on four media *viz.*, Potato dextrose agar, Czapek's (DOX) agar, Richard's agar and Soil extract agar media. All isolates produced white mycelial growth on all media but variations in texture, mycelial type, and colour change of mycelium, exudates production and formation of aberrant fruiting body were observed. PDA was found to be the best medium for the growth of pathogen in which all isolates recorded highest growth rate. The pathogen preferred a temperature range of 30-35^{0}C and neutral to acid pH of 5-7 for the growth. Slight variation in growth rate was observed under light and darkness.

Basidiocarps showed variations in the morphological characters and were stipitate in all isolates except GC from Chirakkacode and GVe from Vettikkal, semicircular to conical shaped, yellowish red to reddish brown with smooth to waved margin, creamy white to brown pore surface, 4.4 – 12.0 x 2.6-17.0 cm size, 1-10 mm pore length, 139- 254 x 122 – 190 µm pore diameter and 2-10 mm flesh thickness. Basidiospores were brown, ovate to ellipsoidal, truncated apex, double walled with inter wall pillars separating two walls. The size of these basidiospores showed variation in the range of 4.8-13 x 4.5-7.0µm with a spore index of 1.15-1.7. It was trimitic, with generative hyphae hyaline, thin walled, branched, septate and clamped. Reddish brown pigmented skeletal hyphae and colourless binding hyphae were noticed. Based on these observations the eight isolates of the pathogen were identified as *Ganoderma lucidum* (Leys) Karst.

Regarding the *in vitro* management of the pathogen, two isolates of *T. virens* and one isolate of *T. viride* were isolated from rhizophere soil and were proved equally effective with the reference culture, *T. viride* and *T. harzianum* in inhibiting the growth of pathogen. Mycoparasitism and production of non volatile metabolites were found to be the mechanisms exhibited by the selected *Trichoderma* spp. The bacterial antagonists obtained from rhizosphere soil and the reference culture *P. fluorescens* recorded less than 50 percent inhibition on the growth except in cases of few isolates of the pathogen. It was observed that the selected bacterial antagonists were not much effective in inhibiting the pathogen compared to fungal antagonists.

Among the phytoextracts, *Azadirachta indica* at 20 per cent concentration was found the most effective and recorded more than 50 percent inhibition on the growth of pathogen over control. It was followed by *Musa* sp. at 10 per cent concentration. The *in vitro* evaluation of fungicides showed that flusilazole, hexaconazole and iprobenphos at 0.2 per cent concentration were the most effective and recorded cent per cent inhibition on the growth of all isolates of pathogen. The study on the host range of *G. lucidium* revealed that the seedlings of arecanut, breadfruit, acacia and jack fruit showed yellowing and drooping of leaves and finally wilting of all the seedlings were observed.