ENDOPHYTIC AND EPIPHYTIC MICROBIAL DIVERSITY IN MAJOR TREE SPICES AND THEIR POTENTIAL FOR BIOCONTROL OF FOLIAR PATHOGENS

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Endophytic and epiphytic microbial diversity in major tree spices and their potential for biocontrol of foliar pathogens "** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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Introduction

Introduction

Nutmeg, clove and cinnamon, the major tree spices of Kerala, play an important role in the economic well being of farmers of the State. These spice crops are widely cultivated in the State and are known for their confectory, culinary and pharmaceutical properties. The production and productivity of the crops were almost comparable with that from other parts of the world. In Kerala, net area under cultivation is 1842, 912 and 141 ha respectively in nutmeg, clove and cinnamon (Farm Guide, 2015).

Nutmeg, clove and cinnamon are prone to various diseases. Karunakaran (1981) carried out detailed investigations on the diseases of tree spices and reported the occurrence of 19 diseases affecting nutmeg, clove and cinnamon. Thus, the diseases act as a major constraint in the cultivation of tree spices. To combate this problem , farmers use chemical fungicides. Often use of fungicides, may not give desired effect and will lead to many ecological problems as well as the development of resistant strains of pathogens.

Therefore, it is imperative to develop sustainable disease management strategy with emphasis on reducing the use of chemicals and to search for antagonistic microbes residing in and outside the plants. It is well known that plants growing in humid tropical conditions are rich source of endo and epiphytic microbes that can directly or indirectly offer beneficial effect. Since nutmeg, clove and cinnamon prefer humid condition, there is every possibility of innate association of beneficial microflora. Association of endophytic microbes with nutmeg and cinnamon have been documented by Gary *et al.* 2001 and Soapalun, 2003. Further, the potential of epiphytic and endophytic microflora of cocoa, another crop of humid tropics for the management of *Phytophthora* pod rot have also been established (Bhavani, 2004 and Kurian, 2011).

Hence, search on the endophytic and epiphytic microbiota in nutmeg, clove and cinnamon will throw light on the diversity and possibility of harnessing their biocontrol potential against foliar diseases. Further, studies on the mechanism of antagonism, mutual compatibility and tolerance to plant protection chemicals will help to evolve compatible and sustainable biocontrol agents for large scale use. Hence the present investigation was undertaken to understand the foliar diseases affecting nutmeg, clove and cinnamon in Kerala and to elucidate the potential of epi and endophytic microbes against the diseases of these tree spices, so as to develop a sustainable and ecofriendly management practice.The objectives of present study are as follows

* Isolation of pathogens associated with foliar diseases of nutmeg, clove and cinnamon
* Isolation and enumeration of epiphytic and endophytic microbes of nutmeg, clove and cinnamon
* Evaluation of antagonistic potential of epiphytes and endophytes against the pathogens
* Mutual compatibility of selected antagonists
* Mechanism of action of selected antagonists
* Compatibility of selected antagonists with plant protection chemicals
* Identification of the selected antagonists

Review of literature

2. REVIEW OF LITERATURE

Tree spices *viz*., nutmeg, clove and cinnamon are one among the major spices in Kerala after black pepper, cardamom and ginger which occupy an area of 18,730 ha in India and 41,600 ha in Kerala. They are used as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics and colouring in most parts of the world. They are appreciated for their aroma, flavour and colour and have been in use as preservatives for thousands of years. They act as one of the major source of foreign exchange in India. In India nutmeg is mainly cultivated in Thrissur, Ernakulam and Kottayam districts of Kerala and parts of Tirunelveli and Kanyakumari districts in Tamil Nadu. However, diseases of tree spices, a major constraint in production, have been recognized in various parts of Kerala for the past many years. Sometimes fungal diseases are known to cause heavy mortality of these spice crops. Karunakaran *et al.* (1980) conducted systematic studies on diseases of tree spices and reported 19 foliar diseases. To manage these diseases, farmers, nowadays adopt indiscriminate use of plant protection chemicals which leads to major health hazards. Hence, there is a need to search an alternate tool for the management of these diseases in an ecofriendly manner.

2.1 DISEASES OF TREE SPICES AND SYMPTOMATOLOGY

2.1.1. Nutmeg

Ramakrishnan and Damodaran (1954) observed fruit rot of nutmeg for the first time in India during monsoon season causing considerable damage to the fruits by the pathogen *Diplodia natalensis*. Thereafter, Menon and Remadevi (1967) reported a leaf spot disease of nutmeg caused by *Colletotrichum gloeosporioides* which occurs severely during the monsoon season with minute black spots on leaf lamina surrounded by a yellow halo.

In a study conducted by Karunakaran (1981) on diseases of tree spices in Kerala, reported the prevalence of 19 diseases of nutmeg, clove and cinnamon. Among them *Colletotrichum gloeosporioides* was found common in all the three

spices and he further observed a strain variation in pathogens which are of major concern. Rahman *et al.* (1981) described the symptoms produced by *Cylindrocladium camelliae* causing root rot nutmeg and there by wilting and death of seedlings. In a study on blight of nutmeg, Radhakrishnan, (1986) observed small spots initiating from the edge of the leaf, as the infection proceeds to the entire leaf giving a blighted appearance and he reported the casual organism as *Pestalotiopsis palmarum.* A severe leaf blight of nutmeg was observed by Naseema and Sulochana (1994) causes considerable damage to nutmeg plants and they reported that it was caused by *Pestalotiopsis palmarum.*

Sakaria *et al.* (2000) determined the symptoms of horse hair blight disease of nutmeg caused by *Marasmius equicrinis* and according to him there was a formation of mycelial network on plants causing drying of leaves and branches and thus giving a bird's nest appearance. Severe defoliation of green leaves of nutmeg in major nutmeg growing areas of Kerala during southwest monsoon period was observed by Mathew and Beena (2012). According to her, symptoms initiated as dark brown water soaked lesions on the midrib of the leaves which enlarged and spread along the lateral veins to leaf lamina resulting in blightening, whereas on young leaves black lesions were developed which enlarged in size resulted in rotting of the shoots from tip to downwards.

2.1.2. Clove

Reitsma and Sloof, (1950) elucidated the symptoms of leaf blotch disease of clove which appeared on leaves as minute spots surrounded by chlorotic halo and enlarged gradually to form necrotic patches, where the central region of the patch is dirty brown in colour. Figueiredo *et al*. (1967) observed *Calonectria quinqueseptatum* being perfect stage of *Cylindrocladium quinqueseptatum* causing leaf blotch disease of clove. Karunakaran *et al.* (1980) studied the diseases of tree spices, and described in detail the etiology and symptomatology of leaf spot, twig blight and flower shedding disease of clove caused by *Colletotrichum gloeosporioides*. Joshi and Raut (1992) reported

shedding of mature leaves of young clove trees and reducing plant vigour from Konkan area of Maharashtra which was caused by pathogen *Pestalotia versicolar.* Beena *et al.* (1994) found *Cylindrocladium camelliae* from Malampuzha area of Kerala which affected the seedlings of clove showing dark brown to black region and later spread to cause collar rot and general wilt.

2.1.3. Cinnamon

A new record of *Colletotrichum gloeosporioides* of cinnamon was reported by Karunakaran and Nair (1980) from Kerala and according to them symptoms appeared as small deep brown specks on leaf lamina, which later enlarged and measured 3.15 µm in diameter leading to complete drying of leaf. Bhat *et al.* (1988) observed die back disease in seedlings of cinnamon leading to partial drying of plant from Dharwad area of Karnataka. Similar findings were reported by Prakasam (1991) causing leaf spot of cinnamon in lower Palaney hills of Tamil Nadu and the pathogens was identified as *Colletotrichum capsici.* Rajapakse and Kumara (2007) studied important diseases of cinnamon like leaf spot caused by *Colletotrichum gloeosporioides* and grey leaf spot caused by *Pestalotiopsis cinnamomum* in Sri Lanka. They observed symptoms as small yellow brown, oval or irregular spots, joining together forming necrotic patches where in advanced stage of infection, the major portion of leaf lamina dried up and got blown away by wind producing shot holes.

2.2 CHARACTERS OF PATHOGENS

Among the various diseases studied in nutmeg, clove and cinnamon, few diseases which are endemic and economically important are identified.

2.2.1. Pathogens of nutmeg

2.2.1.1. Colletotrichum leaf spot / Shothole disease of nutmeg

Mordue and Holiday (1971) reported *Colletotrichum* sp. in nutmeg and according to him the most important criterion in identifying the *Colletotrichum* sp.are its setae characters. His findings are in agreement with various workers like

Nair *et al.* (1978) and Karunakaran (1981). Nair *et al*. (1978) observed that the infected leaf samples on isolation yielded black mycelial growth on Potato Dextrose Agar and apart from production of setae, size of the spores are also considered as basic criteria in distinguishing the variation in *Colletotrichum* sp.

2.2.1.2. Phytophthora leaf fall of nutmeg

Prem (1995), Appiah *et al.* (2003) and Bhavani (2004) observed the mycelium of *Phytophthora* as hyaline, coenocytic, measuring 3.22-6.45 µm in width. According to them, the sporangia of *P. palmivora* causing PPR were ellipsoid to ovoid, papillate and caduceus wit L/B ratio 1.2-2.2.

Waterhouse (1974) considered pedicel length of sporangia as a stable character under normal conditions. According to Kaosiri *et al*. (1978), caducous sporangia with short stalks produced by cocoa isolates on carrot agar were typical of *P. palmivora*. According to Waterhouse *et al.* (1983), the size, shape, and length to breadth rate of sporangia are important characters in identification of *Phytophthora* species. Zentmyer (1988) considered sporangial ontogeny as important taxonomic criterion in distinguishing *Phytophthora* sp.

2.2.1.3. Grey blight disease of nutmeg

Fungus initially greyish white colour, mycelium septate and branched. Acervuli of the fungus appear as black minute specks on the culture and size of the conidia ranged from 5.7 to 10.54 µm. According to Pal (2014), morphology of conidia and apical appendages on spores are the characters which helps to distinguish *Pestalotiopsis* sp.

2.2.1.4. Seedling blight of nutmeg

Rhizoctonia solani Kuhn was reported for the first time by Julius Kuhn in 1858 from diseased potato tubers. The vegetative mycelium of *R. solani* is colourless when young but become yellowish or light brown colored as they grow and mature. The mycelium consists of hyphae partitioned into individual cells by a septum containing a dough-nut shaped spore. The hyphae often branch at

right angles and usually possess more than three nuclei per hyphal cell. Under certain conditions the fungus produces brown to black coloured sclerotia (Brown and McCarter, 1976; Anderson, 1982). *R. solani* is the imperfect stage of *Thanetophorus cucumeris* (Carling and Leiner, 1990).

2.2.3. Pathogens of clove and cinnamon

Leaf spot and twig blight disease, leaf blotch disease of clove and *Colletotrichum* leaf spot and grey blight disease of cinnamon are the important diseases noticed.

2.2.3.1. Leaf blotch disease of clove

Sulochana (1980) and Karunakaran (1981) elucidated the fungus isolated from leaf blotch disease of clove and they found it to be slow growing. Hyphae septate and highly branched, conidiophores septate and produced in masses with primary, secondary and tertiary sterigmata having a broom shaped appearance. Conidia straight cylindrical with rounded wounds and 1 to 3 septate and measured from 32.20 to 35.80 x 3.58 to 6.58 µm. According to them, spore size and sterigmata with vesicle taxonomic consideration were used for identification of *Cylindrocladium* sp. *Colletotrichum* leaf spot and grey blight diseases of clove and cinnamon are in tune with the characters of the *Colletotrichum* leaf spot/ shot hole disease and grey blight disease of nutmeg.

2.3 EPIPHYTES AND ENDOPHYTES

2.3.1 Epiphytes

Epiphytes are the beneficial microflora that are present on plants. The aerial parts of plants are the habitat of many epiphytic microflora, which may be harmful or beneficial to the plant. Some of these beneficial microflora may be actively antagonistic thereby protecting the plant from invasion by the harmful organism. But compared to antagonists isolated from soil, aerial antagonists are reported to be less efficient due to obvious reasons inherent in their respective niche. Inspite of this, efforts are being made to exploit the potential of natural epiphytic antagonistic microflora for the management of many plant diseases.

Galindo (1992) also observed abundance of bacteria than the fungi on cocoa pod surface which having the potential to suppress the pathogen *Phytophthora palmivora*. Bhavani (2004) reported the antagonistic nature of epiphytic *Trichoderma* spp. on the cocoa pod surface. Dionisio *et al.* 2008 reported the antagonistic nature of fungal epiphytes isolated from banana for the management of banana crown rot disease.Weininghao *et al*. 2011 observed the antagonistic nature of epiphytes isolated from citrus fruits against the management of blue mold and green mold diseases. Khan *et al.* 2012 also recorded association of epiphytic bacteria with common spices of Bangladesh.

2.3.2. Endophytes

Anton de Bary coined the term endophyte in 1886 to describe microorganisms that colonize internal tissues of stems and leaves. Endophyte is derived from the Greek word 'endon' (within) and 'phyte' (plant). The term endophyte refers to interior colonization of plantsby bacterial or fungal microorganisms. Petrini (1991) first defined endophyte as microorganism living in the plant organization for a certain stage of its life and would not cause disease. Perotti (1926); Hallmann *et al*. (1997) and Azevedo *et al*. (2000) reported that bacteria on roots and in the rhizosphere benefit from root exudates, but some bacteria and fungi are capable of entering the plant as endophytes that do not cause harm and could establish a mutualistic association. Wagenaar and Clardy (2001) identified endophytes as microorganisms growing in the intercellular spaces of higher plants and they are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential.

James and Olivares (1997) stated that all bacteria that colonize the interior of plants, including active and latent pathogens, can be considered to be as endophytes. Kado (1992) and Quispel (1992) suggested that, endophytic bacteria establish endosymbiosis with the plant, whereby the plant receives an ecological benefit from the presence of the symbiont. It is now commonly accepted that each

of the nearly 300,000 existing plant species host, at least one or even several hundred strains of endophytes have been reported (Strobel and Daisy, 2003). Sopalan *et al.* 2003 isoalted the endophytic fungus *Muscudor albus* residing in the cambium tissue of nutmeg tree in Thai Land. Gary *et al.* (2001) also reported the occurrence of *Muscudor albus* in small limbs of *Cinnamomum zeyalanicum*. Zhang *et al.* (2006) and Aly *et al.* (2010) reported the great potential of endophytes as amajor source of biologically active compounds withpromising medicinal or agricultural applications. Kurian (2011) reported the antagonistic nature of bacterial endophytes isolated from cocoa against the management of *Phytophthora palmivora*.

Hatem *et al.* (2013) reported the antagonistic nature *Acremonium* sp as endophytic bioagent in date palm against date palm *Fusarium* wilt

2.3.3 Mode of action of epiphytes and endophytes

Cook and Baker (1983) suggested different mechanism by which the endophytic microbes controlled *Fusarium* wilt of different crops. These mechanisms include production of antifungal compounds, siderophore production, nutrient competition, niche exclusion and induction of systemic resistance. It is possible that several of these mechanisms paly role in biological control exhibited by these organisms. According to Backman *et al.* (1997) the effectiveness of endophytes as biocontrol agents is dependent on many factors. These factors include: host specificity, population dynamics and pattern of host colonization, the ability to move with in the host tissues, and the ability to induce systemic resistance. Production of antimicrobial compounds and mycoparasitism, the feeding on a fungus by another organism are the mechanisms where by *Trichoderma* sp. provides protection to plants against plant pathogens (Chet *et al.*1998; Howell, 2003; Harman *et al.* 2004). A perusal of the literature revealed no reports of epi and endophytes on tree spices.

Production of volatile inhibitory substances by endophytes was studied by (Nejad and Johnson, 2000). They found that, most of the endophytic isolates from

oilseed rape were HCN negative but the isolates produced volatile metabolites which had fungal inhibitory action. Hence they concluded that the endophytes are producing antifungal volatiles other than HCN. Volatile substances such as 2, 3-butanodiol and acetone produced by the bacteria have been reported to be responsible for plant growth promotion (Ryu *et al.,* 2003).

Macllas- Rubaleava *et al.* (2008) first reported production of allelo chemicals with antifungal activity by the newly discovered endophytic fungus *E. gomezpompae* on cocoa. They observed antagonism by the endophyte towards *Phytophthora capsici* and *Phytophthora parasitica* against fungi *Fusarium oxysporum* and *Alternaria solani*.

2.3.3.1. Siderophore production

Findings by Cao *et al.* (2005) indicated the potential of siderophore producing *Streptomyces* endophytes for the biological control of *Fusarium* wilt disease of banana whereas, among the endophytic bacteria from sunflower none of the strains produced siderophores (Forchetti *et al.* 2007). A total of 29 endophytes strains were isolated from the halophyte *Prosopis stombulifera* grown under extremely salinity (Sgroy *et al.,* 2009). However, only one was able to produce siderophores and none of them solublised phosphate. It was observed that, *in vitro* growth of *Xylella fastidiosa sub sp. pauca* was stimulated by the presence of supernatant siderophores of endophytic *Methylobacterium mesophilicum* (Lacava *et al.,* 2008).

Kajula *et al*. (2010) reported siderophore production by endophytic fungi. The siderophore produced *in vitro* was ferricrocin, quqntities ranging between 7.9 to17.6 mgl^{-1}. Only the fungi with antibacterial activity produced ferricrocin and any well known siderophores were not detected in the broths of antioxidant-producing fungi.

2.3.4 Isolation of epiphytes and endophytes

Several workers studied the isolation procedures of epiphytes and endophytes. Gardener *et al.* (1982) and Gagne *et al.* (1987) elucidated the isolation procedures of endophytes which are mainly of two types. According to them in vacuum extraction method the sap is extracted using the vacuum extraction apparatus and spread plate method is used for plating where as homogenization or trituration methods invariably involves surface sterilization followed by homogenization under aseptic condition. The triturate is then serially diluted and placed. Similarly Bell *et al.* (1995) isolated xylem inhabiting bacteria from grape vine by vacuum extraction and homogenization method. They compared the efficiency of two methods. The size of the population varied with the method of extraction: 2.65 X 10^{2} to 3.46 X 10^{3} / ml xylem sap with vacuum method of extraction, 3.83 X 10^{3} to 1.31 X 10^{4} /g xylem tissue with homogenization. Thus trituration or homogenization technique is considered as ideal method for isolation of endophytes because it yields higher number of endophytic bacteria (Hallman *et al.* 1997; Uppala, 2007; Balan, 2009; Kurian, 2011). According to wilson and Carroll, 1994. Collection of leaf samples from different locations helped in the isolation of diverse group of organisms in sufficiently large numbers.

Kado (1992) isolated and characterized the endophytes from the papaya fruits and reported fermentative potential of strains. James (2015) isolated the endophytic bacteria and reported their antagonistic property against bacterial wilt of tomato caused by *Ralstonia solanacearum.*

2.4. MANAGEMENT OF DISEASES

Several workers studied the effect of plant protection chemicals against the leaf spot pathogens. Efforts to control the leaf spot pathogens should be started from very early stage of disease, immediately after the expression of symptom. Nowadays the effect of plant protection chemicals, botanicals and antagonists in the management of diseases has practical importance.

2.4.1. Chemical control

According to Reitsma and Sloof (1950) good control has been achieved against *Cylindrocladium quinqueseptatum* by spraying the seedlings with solution of 1.5 per cent Bordeaux mixture as soon as leaf production, combined with shading to prevent sunburn lesions. Ramachandran *et al.* (1988) studied the sensitivity of 28 *Phytophthora* isolates to ED_{50} value of metalaxyl from black pepper, arecanut, cardamom, nutmeg and cocoa from Kerala. They observed inhibition of growth of *Phytophthora* isolates to metalaxyl. Suharban *et al.* (1990) found that the Difolatan (Captafol) at 0.3 per cent was the most effective for the control of *Colletotrichum gloeosporioides.*

2.4.2. Biological control

Biological control, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. Numerous studies have shown that biological control offers an environmentally friendly alternative to protect plants from soil-borne pathogens (Emmert and Handelsman, 1999; Whipps, 2001; Weller *et al*. 2002). Mechanisms of biological control are diverse. One effective mechanismis the use of antagonist microorganisms such as bacteria, yeast and fungi to control plant disease. Continuous use of chemicals in controlling the diseases is not advisable due to the cost and adverse environmental hazards, besides development of resistance against pathogens. So utilization of fungal / bacterial antagonists are effective to overcome such problems.

2.4.2.1. Fungal antagonists

Though several antagonistic fungi were reported as efficient in the management of plant diseases, *Trichoderma* occupies as pride place. Liu and Baker (1980) reported the genus *Trichoderma* as a potential biocontrol agent against fungal pathogens. The efficacy of *T. harzianum* in reducing PPR has been reported by Galindo (1992) and it was suggested as potential biocontrol agent to include in the integrated disease management of *Phytophthora* pod rot of cocoa.

Gary *et al.* (2001) reported *Muscodor albus* as an endophytic fungus from small limbs of *Cinnamomum zeyalanicum* showing volatile antimicrobial properties. Similarly Sopalun *et al.* (2003) also reported *Muscodor albus*as an endophyte residing in cambium of *Myristica fragrans* a nutmeg tree in Thailand. According to Adedeji, *et al.* (2010) *Trichoderma* spp as biocontrol agents were successfully combined with fungicides there by reducing the frequency of fungicide application from four to one with significant pod rot infection in the field. Hatem *et al.* (2013) reported the antagonistic nature of endophytic fungus *Acremonium kilense* against the *Fusarium* wilt of Date palm caused by *Fusarium oxysporum f. sp*. *albedenis*.

2.4.2.2. Bacterial antagonists

Epiphytic and endophytic microflora comprises resident and transient microorganisms. The main components of microflora were bacteria, yeast and filamentous fungi (Baker and Cook, 1974). Galindo (1992) noticed that epiphytic and endophytic population was the highest during periods of precipitation and high relative humidity and the lowest during dry periods.

The inhibitory effect of *Pseudomonas aeruginosa* to *Phytophthora palmivora* under *in vitro* conditions was reported by Attafuah (1965). Galindo (1992) reported that epiphytic microflora like *P. fluorescens* and *P. aeruginosa*, *Serratia marscens*, *Burkholderia cepacia* and five isolates of *Bacillus* sp against *P. palmivora* and *P.nicotianae* from cocoa rhizosphere based on *in vitro* screening.

Jadhav *et al.* (2009) reported the importance of biological control as a component of integrated disease management of plant pathogens using *P. fluorescens* and *B. subtilis* which was evaluated against *Colletotrichum gloeosporioides* causing leaf spot of clove.

2.4.2.3. Epiphytes and endophytes

Epiphytic and endophytic organisms are ubiquitous in most plant species and influence the host fitness, disease suppression, contaminant degradation and plant growth promotion. They colonize the plant interior, interact more closely with less competition for carbon sources and produce a more protective environment for fixation. Many authors have defined it and some of the relevant definitions are include. Anton de Bary coined the term 'endophyte' in 1886 to describe microorganisms that colonize internal tissues of stems and leaves. Endophyte is derived from the Greek word 'endon' (within) and 'phyte' (plant). The term endophyte refers to interior colonization of plantsby bacterial or fungal microorganisms. Petrini (1991) first defined endophyte as microorganism living in the plant organization for a certain stage of its life and would not cause disease.

Perotti (1926); Hallmann *et al*. (1997) and Azevedo *et al*. (2000) reported that bacteria on roots and in the rhizosphere benefit from root exudates, but some bacteria and fungi are capable of entering the plant as endophytes that do not cause harm and could establish a mutualistic association. Wagenaar and Clardy (2001) identified endophytes as microorganisms growing in the intercellular spaces of higher plants and they are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential.

James and Olivares (1997) stated that all bacteria that colonize the interior of plants, including active and latent pathogens, can be considered to be as endophytes. Kado (1992) and Quispel (1992) suggested that, those bacteria establish endosymbiosis with the plant, whereby the plant receives an ecological benefit from the presence of the symbiont. Zhang *et al.* (2006) and Aly *et al.* (2010) reported the great potential of endophytes as amajor source of biologically active compounds withpromising medicinal or agricultural applications. It isnow commonly accepted that each of the nearly 300,000 existing plant species host at least one or even several hundred strains of endophytes (Strobel and Daisy, 2003).

Strobel *et al.* (2001) recently described endophytic fungus *Muscodor albus* obtained from small limbs *of Cinnamomum zeyalanicum*. This xylariaceous fungus had some inhibitory effect against the test fungi such as *Rhizoctonia solani*, *Phytophthora cinnamon*. Similarly Sopalun *et al.* (2003) reported a new record of *Muscodor albus* as an endophytic fungi residing in cambium of *Myristica fragrans* in Thailand.

2.5. MUTUAL COMPATIBILITY OF EPIPHYTES AND ENDOPHYTES

Recently much importance is given for combined application of the bioagents, as it provided better management against all diseases. It will further provide better management of diseases by way of synergistic effect and multiple mode of action.

Manimala (2003) while studying the management of bacterial wilt of solanaceous vegetables using rhizosphere microbial antagonists reported that they were mutually compatible with each other and no lysis occur at the juncture between the antagonists. The mutual compatibility of PGPR isolates with each other was reported by Vijayaraghavan (2007) while studying plant growth promoting rhizobacteria mediated ISR against bacterial wilt of ginger. James (2015) reported the mutual compatibility of selected endophytic bacterial isolates isolated from tomato against the pathogen *Ralstonia solanacearum* causing bacterial wilt in tomato.

2.6. COMPATIBILITY OF EPIPHYTES AND ENDOPHYTES WITH PLANT PROTECTION CHEMICALS

In any integrated plant disease management programme, its components must be compatible with each other. Otherwise, the desired output of the programme may not be achieved. This is more pertinent when biocontrol agents are used as one of the components in the integrated disease management strategy .

2.6.1. Fungicides

T. harzianum Rifai was tolerant to most of the fungicides and was used for the integrated control of many plant diseases (Henis *et al.,* I979*;* Papavizas and Lewis, 1981; Papavizas, 1982). Papavizas (1985) observed the integrated approach could be successful only if the antagonists were compatible with the fungicides and biopesticides. According to Mukhopadhyay *et al.,* (1986) *T. harzianum* could tolerate fungicides like Metalaxyl (0.1 Per cent) and carbendazim (0.0065 Per cent). Similar results were reported by Mukherjee *et al.,* (1989). Kay and Steward (1994) observed *T. harzianum* C 52 was insensitive to Thiram and Mancozeb.

Krishnamoorthy and Bhaskaran (1994) found that Captan was compatible to *T. viride*, while it had little effect on *T. harzianum.* They also observed in copper oxy chloride poisoned medium *T. harzianum* showed normal growth and sporulation. Mondal *et al.,* (1995) noticed inhibition of mycelial growth of *Trichoderma* sp. to a greater extent with addition of 200 to 500 ppm of Dithane M-45 after three days of incubation. Shanmugham (1996) reported that Bordeaux mixture completely inhibited the growth of *T. viride.*

Sarma and Anandaraj, (1999) observed that copper fungicides were toxic to *Trichoderma* sp. and *Gliocladium virens.* Paciulyte *et al.* (2000) tested the sensitivity of fungicides, copper sulphate and copper oxy chloride and found that both fungicides were active against fungi and it was also found that *Trichoderma* sp. were more sensitive to copper oxy chloride than copper sulphate.

Akbari and Prakhia, (2001) reported that thiram, mancozeb, tridemorph, metalaxyl MZ and fosetyl Al were non inhibitory to *T. harzianum, T. viride and G. virens* at all concentrations tested. Mclean (2001) tested *in vitro* sensitivity of spores of *T. harzianum* to eight fungicides commonly applied to onions and indicated that *T. harzianum* was least sensitive to Pyrocymidone and Captan and most sensitive to mancozeb, tebuconazole and thiram.

Sharma *et al.* (2001) found that among two systemic and six non-systemic fungicides tested, tolerance of *T. harzianum* for metalaxyl was seven times higher than carbendazim. Jeyakumar *et al.* (2003) noticed that *Trichoderma* sp. were compatible with metalaxyl MZ even at 1000 µg/ml. Vijayaraghan and Abraham (2003) reported that Bordeaux mixture at all concentrations completely inhibited the growth of *T. harzianum, T. viride* and *T. longibrachiatum* while, Ridomil MZ, Akomin, Indofil M-45 and Antracol at different concentrations showed varying degrees of inhibition.

Bhavani (2004) studied the biological management of *Phytophthora* pod rot and according to him Akomin and Indofil M-45 were compatible with fungal antagonists like *Trichoderma* sp. at recommended dose of concentrations.

Bhattiprolu (2007) and Madhusudhan *et al.* (2010) found that *T. viride* is compatible with Dithane M-45 and incompatible with carbendazim,hexaconazole, thiophanate methyl and tridemorph. Gaikwad *et al.* (2011) and Ahanger *et al.*(2014) reported that *T. viride* isolates were incompatible with seed dressing fungicides like Captan, Vitavax, carbendazim and found compatible with wettable sulphur, mancozeb and cymoxalin 8% + mancozeb 64% (Curzate) found compatible.

2.6.2. Insecticides

Sharma and Mishra (1995) studied the compatibility of the biocontrol agent *T.harzianum* with aldicarb, phorate and carbofuran which was applied for management of nematodes and mealy bugs in black pepper. The study indicated that these insecticides were less toxic. Jebakumar *et al.* (2000) found that phorate and chlorpyriphos could be safely applied with *T. harzianum*.

T.harzianum strain T-22 was compatible with Diazon, Lindane, Lorsban, Malathion, Methoxychlor and Orthene Sevin. Sushir and Pandey (2001) opined that among four insecticides tested *in vitro*, chlorpyriphos (Durret 20 EC) was

found to be more toxic even at 50 µl ml^{-1} which showed growth inhibition of 55.55 and 57.77 per cent respectively.

Sarma (2003) reported that *Trichoderma* spp. is compatible with potassium phosphonate and chlorpyriphos and therefore indicated their potential for IDM with dual mode of action in suppressing both pathogenic fungi and plant parasitic nematodes.

According to Mathew (2003) *P. fluorescens* was compatible with recommended dose of imidacloprid, Etofenprox, Chlorpyriphos and Triazophos. Vijaraghavan (2003) found that three species of *Trichoderma viz., T. viride, T. harzianum* and *T. longibrachiatum* were incompatible with phorate and carbofuran at all concentrations tested. Monocrotophos and Quinalphos were incompatible with these antagonists and Chlorpyriphos, Endosulfan, Dimethoate and Cypermethrin were showed varying degree of inhibition at different concentrations

Bhavani (2004) reported *T. viride* and *T. harzianum* were found incompatible with endosulfon, Chlorpyriphos and phorate at recommended concentrations in management of insect pests. Gowdar *et al.* (2007) reported that species of *T.harzianum* Rifai exhibited maximum inhibition at 0.75 per cent concentration of imidacloprid and they concluded that it was incompatible

Bhai and Thomas, (2010) reported partial compatibility of *T. harzianum* with insecticides Quinalphos with 55.84 per cent inhibition. Ranganathaswamy *et al.* (2011) evaluated the compatibility of *T. harzianum* and *T.virens* with different insecticides. They reported that *Trichoderma* spp were incompatible with Quinalphos and Chloripyriphos, while dimethoate and endosulfan was found to be least compatible with showing 70 per cent inhibition on radial growth of the fungal isolate.

Materials and Methods

3. Materials and Methods

The present study entitled ″ Endophytic and epiphytic microbial diversity in major tree spices and their potential for biocontrol of foliar pathogens ” was carried out at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2013-15. The experimental materials and methods followed are given below.

3.1 ISOLATION OF PATHOGENS FROM NUTMEG, CLOVE AND CINNAMON

The pathogens causing various foliage diseases of nutmeg, clove and cinnamon were isolated from the infected leaves collected from different locations of Kerala. The diseased leaf samples were brought to the laboratory, washed under tap water and the infected area along with healthy portion were cut in to small bits. The bits were surface sterilized with one per cent sodium hypochlorite solution for one minute followed by washing in three changes of sterile distilled water. The surface sterilized bits were placed on Potato dextrose agar (PDA) medium in Petri dishes and incubated at room temperature (26 ± 2 °C). When the fungal growth was visible, small bits of mycelia were transferred to PDA mediated Petri dishes and the isolates were purified by hyphal tip method. These purified cultures were maintained in PDA slants for further studies. The cultural and morphological characters of the isolates were also studied.

3.2 PATHOGENICITY OF THE ISOLATED ORGANISMS

Pathogenicity of the organisms from nutmeg, clove and cinnamon obtained from different locations were proved by artificial inoculation on detached healthy leaves. For this, healthy leaves were taken, washed with sterile water and 1µm pinpricks were made on the adaxial surface of the leaves. Mycelial disc of 10 mm diameter were taken from seven day old cultures of the respective organisms and placed on the pin pricked area and covered with moistened cotton. The inoculated leaves were incubated in polythene bags, with a pad of cotton wetted with sterile

water in order to provide high humidity. Observations on the development of symptoms were recorded. The organisms were re-isolated from the artificially inoculated leaves and their cultural and morphological character were studied and compared with that of original ones.

3.3 SYMPTOMATOLOGY OF DISEASES OF NUTMEG, CLOVE AND CINNAMON AND IDENTIFICATION OF PATHOGENS

The symptomatology of various foliage disease of nutmeg, clove and cinnamon were studied both under natural and artificial conditions. The pathogens were identified based on cultural and morphological characters growing on the Potato Dextrose Agar medium and for further, confirmation the cultures were sent to NCFT, New Delhi. Morphological characters of mycelia, spore bearing structures and spores were studied by slide culture method.

3.4 ISOLATION AND ENUMERATION OF EPIPHYTIC MICROFLORA FROM NUTMEG, CLOVE AND CINNAMON

Epiphytic microbes were isolated from healthy leaves of nutmeg, clove and cinnamon, collected from infected gardens of different locations of Kerala during the months of June to August 2014 (Table 3.1). Healthy leaves brought to the lab were weighed out to 2 gm each and transferred aseptically to 250 ml conical flask containing 100 ml sterile distilled water. The samples were shaken for 20 min in a shaker so that the propagules present will be separated from the leaf samples. The total epiphytic microflora were quantitatively estimated by serial dilution plate technique. Martins rose Bengal Agar, Nutrient agar, King'S B Agar and Ken knights Agar (Appendix-1) were used for estimating fungi, bacteria, Fluorescent Pseudomonads and actinomycetes at dilutions of 10^{4}, 10^{6} ,10^{6} and 10^{2} respectively. Three replications were maintained and observations on the total population of each microflora were recorded. Further, representative colonies of fungi, bacteria were selected and subcultured and maintained in the respective media for further studies.

Table 3.1. Locations of collections of epiphytic and endophytic microflora of tree spices

Sl.No	Crop	District	Place of collection
1	**Nutmeg**	Thrissur	College of Horticulture Vellanikkara
			Pattikad
		Ernakulam	Aluva and Kalady
		Wayanad	Ambalavayal
2	**Clove**	Thrissur	College of Horticulture Vellanikkara
		Wayanad	Ambalavayal
		Thiruvananthapuram	College of Agriculture Vellayani
3	**Cinnamon**	Thrissur	College of Horticulture Vellanikkara
		Wayanad	Ambalavayal
		Thiruvananthapuram	College of Agriculture Vellayani

3.4.1 Fungi

The single colonies of fungi showing similar colony characters were selected from the dilution plates and transferred to Petri dishes containing PDA. Pure cultures of the isolates were maintained on PDA slants.

3.4.2 Bacteria

Representative bacterial colonies developed in dilution plates were picked and streaked on nutrient agar to get single colonies. The pure cultures were maintained on NA slants.

3.4.3 Fluorescent Pseudomonads

The bacterial colonies developed in the dilution plates on King's B agar were observed under U.V transilluminator for the presence of greenish yellow fluorescent pigment and only such representative fluorescent colonies were selected, they were purified and such cultures were maintained on Kings B slants.

3.4.4 Actinomycetes

No actinomycetes colonies were observed during study

3.5 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROFLORA FROM NUTMEG, CLOVE AND CINNAMON

Endophytic microbes were isolated from healthy leaf samples of nutmeg, clove and cinnamon collected from various locations of Kerala (Table 3.1). Fresh leaf samples brought in separate polythene bags were washed under tap water, and used for isolation within 48 h. of collection.

3.5.1 Surface sterilization

The surface of nutmeg, clove and cinnamon harbor a lot of epiphytes, thorough surface sterilization was needed to eliminate them. The concentration of sterilant and time of exposure were standardized so as to get the maximum number of endophytes with no growth on sterility check. Three different concentrations of sodium hypochlorite *viz.,* one, two and three per cent were tried for three different exposure times *viz.*two, five and ten minutes. Further, three different weights of leaf samples *viz.*0.5gm, 1.0gm and 2.0gm were also tried. Since the isolation from the 2gm of sample after surface sterilization with two per cent sodium hypochlorite for 10 min yielded good number of colonies with no growth in sterility check, it was selected for further studies.

3.5.2 Isolation and enumeration of endophytes

For the isolation of endophytes from healthy leaves of nutmeg, clove and cinnamon, samples were weighed out and exposed to sterilent as described in 3.4.1 followed by washing in three changes of sterile water and blot dried. The leaf bits were then transferred to sterilized mortar containing 8 ml sterile Potassium phosphate buffer (PB 0.1M, pH) washed thoroughly in the buffer. From the final buffer wash, one ml was pipetted out and poured into sterile Petri plate. To this molten and cooled medium was added and this served as a sterility check. If microbial growth was observed in sterility check with in four days, the

isolates obtained from particular samples were discarded. The surface sterilized leaves of nutmeg, clove and cinnamon were triturated (McInroy and Kloepper, 1995) using sterile mortar and pestle with 8 ml of sterile buffer. The triturate was serially diluted in sterile PB up to 10^{7}. The dilution and medium used for enumeration of each group microorganism are furnished in (Table 3.2). One ml of diluted triturate was pipetted in to sterile Petri plate and suitable medium was poured. The plates were incubated at room temperature for various intervals as shown in the Table 3.2

Table 3.2 Dilution and media used for isolation and enumeration of endophytic microflora

Sl.No.	**Organism**	**Dilution**	**Medium**	**Period of incubation (Days)**
1.	Fungi	10^{-4}	Potato dextrose agar (PDA)	2.
2.	Bacteria	10^{-6}	Nutrient agar (NA)	2
3.	Fluorescent Pseudomonads	10^{-6}	King'S B agar	2
4.	Actinomycetes	10^{-1}	Ken Knight'S agar	7

Representative colonies of endophytes based on colony morphology were selected from the dilution plates and transferred to slants to establish pure cultures following standard protocols.

3.6 *IN VITRO* ANTAGONISTIC EFFECT OF EPIPHYTES AND ENDOPHYTES AGAINST THE PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

The *in vitro* antagonistic effect of epiphytes and endophytes towards the pathogens of nutmeg, clove and cinnamon were tested by dual culture method. As

an initial step 118 epiphytic and endophytic isolates obtained from experiments 3.4.1 and 3.4.2 were subjected to preliminary screening to know whether they poses antagonistic properties or not.

3.6.1 Preliminary screening of epiphytic and endophytic microflora against the pathogens of nutmeg, clove and cinnamon

Mycelial disc of the pathogens isolated from the tree spices were taken from seven day old culture on PDA plate and incubated for 2 days. For screening of epiphytic and endophytic bacteria, four isolates were inoculated one each on the four sides of the pathogens as a line of streak one cm away from the periphery of the Petri dish. Similarly for screening of epiphytic and endophytic fungi, the mycelial discs from four isolates were placed one each on four sides of the pathogen at equidistance from the periphery of the Petri dish one cm away from the edge of Petri dish. Plates with pathogen alone served as control. The inoculated plates were incubated at room temperature and observed for inhibition of the pathogen for five days or when there was full growth in the control.

3.7 *IN VITRO* EVALUATION OF ANTAGONISTIC EPIPHYTES AND ENDOPHYTES AGAINST PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

The epiphytes and endophytes which showed antagonistic activity in preliminary screening were further, tested individually to select the most efficient ones.

3.7.1 *In vitro* evaluation of antagonistic epiphytic and endophytic fungi against pathogens of nutmeg, clove and cinnamon

Ninteen fungal isolates were evaluated for their antagonistic potential against the pathogens of nutmeg, clove and cinnamon by dual culture method (Skidmore and Dickson, 1976) in comparison with standard culture of *Trichoderma viride*. The organisms were inoculated on dual cultures after giving due consideration of their growth rate. Mycelial disc of the pathogen from seven

day old culture grown on PDA was placed on one side of the plate and incubated at room temperature (26 ± 2 ° C) for two days. The mycelial disc, (10 mm) of antagonistic fungi were placed on other side of the plate, four cm away from the pathogen and incubated. Three replications were maintained for each isolate. The pathogen grown as monoculture served as control. The plates were observed daily after 24 h of inoculation of antagonists till the pathogen grew and covered the plate kept as control. The per cent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

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

PI = Per cent inhibition, C = Growth of the pathogen in control (mm), T = Growth of the pathogen in dual culture (mm)

Based on the per cent inhibition of mycelial growth of the pathogen, the efficient antagonists were selected for further studies. The nature of antagonistic action of epiphytic fungi against the foliar pathogens of these tree spices was assessed by the method of Purkayastha and Bhattacharya, (1982) and assigned to four categories.

A- Overgrowth : Pathogen overgrown by test organism
B- Homogeneous : Free intermingling of hyphae
C- Cessation of growth : Cessation of the growth at line of contact
D- Aversion : Development of clear inhibition zone

3.7.2 *In vitro* evaluation of antagonistic epiphytic and endophytic bacteria

All the 33 bacterial isolates of epiphytes and endophytes along with the standard culture of *Pseudomonas fluorescens* were evaluated for their antagonistic effect by dual culture method (Utkhede and Rahe, 1983). Mycelial disc (10mm) taken from seven day old culture of the pathogen grown on PDA was placed at the centre PDA mediated Petri dish and incubated for two days. The epiphytic and endophytic bacteria were inoculated as a line of streak on both sides, one cm away from the edge of Petri dish. For each isolate three replications were maintained. Plates with pathogen alone served as control.The plates were incubated at room temperature and observed daily, until the control exhibited full growth of the pathogen. The per cent inhibition was calculated as given in 3.6.1. Based on the per cent inhibition the efficient ones were selected.

3.8 MUTUAL COMPATIBLITY OF SELECTED EPIPHYTES AND ENDOPHYTES

The mutual compatibility of selected epiphytic and endophytic fungal and bacterial isolates including reference cultures of *T. viride* and *P. fluorescens* were tested. In case of bacterial isolates they were streaked perpendicular on nutrient agar medium, three replications were maintained for each bacterial isolate. Similarly fungal isolates were inoculated 2.5 cm away from centre of PDA mediated Petri dish and observed for compatibility, three replications were maintained for each fungal isolate combination. The plates were incubated at room temperature for 48 h.

3.9 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS ON PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

3.9.1 Fungi

The standard dual culture technique was employed to study the mechanism of antagonism of selected fungal antagonists on different pathogens of nutmeg, clove and cinnamon. For this PDA medium was plated on sterile Petri dishes and

allowed to solidify. Mycelial disc of 8mm diameter of various pathogens of nutmeg, clove and cinnamon taken from an actively growing culture were inoculated at one end of the Petri dish 48 h prior to inoculation of the antagonists, which was placed two centimeters away from the pathogen. The plates were incubated at room temperature and observations taken at regular intervals until there was hyphal intermingling. Microscopic observation for hyphal interaction was done by cutting out one sq. cm portion of the intermingling hyphal growth of anatgonists and pathogen which was mounted in cotton blue lactophenol on slides.

3.9.1.1. Antibiosis test for the production of diffusible, nonvolatile inhibitory metabolites

Antibiosis test for production of diffusible, nonvolatile inhibitory metabolite was carried out using cellophane paper method described by Dennis and Webster (1971). Cellophane paper of 9 cm diameter was taken and sterilized in autoclave at 121°C for 15 min and then each sterilized disc was aseptically placed over the PDA inoculated plates. Ten mm discs was taken from the growth of each isolate of endophytes or epiphytes and was placed at the centre of the cellophane paper and incubated for 72 h. After this, the cellophane paper along with adhering antagonists was removed carefully and 8 mm disc of pathogens of nutmeg, clove and cinnamon was immediately placed on the medium at the central position previously occupied by antagonist. The growth of the pathogen was incubated for 48 h up to seven days and the growth was compared with that in control. Three replications were maintained and the per cent inhibition of the pathogen was calculated

3.9.2 Bacteria

The ability of selected epiphytic and endophytic bacterial antagonists and standard culture of *P. fluorescens* were tested for the production of siderophore, hydrogen cyanide (HCN), ammonia production and Indole acetic acid (IAA)

3.9.2.1. Siderophore production

Siderophore production by the bacterial isolates were detected by the method of Kloepper*et al.* (1980). The King's B agar was amended with two concentrations of $FeCl_3$. 6 H_2O at the rate of 1 and 10 mg l^{-1}. The sterilized medium was poured into each Petri dish. The test cultures were streaked on the surface of the medium. Three replications were maintained. The inoculated plates were incubated at room temperature for 48 h. and observed for the production of greenish yellow fluorescent pigment.

3.9.2.2. HCN production

HCN production by bacterial isolates were detected by the method of Baker and Schipper (1987). The King' S B agar was amended with 4.4 gm^{-1} of glycine and sterilized. The sterile medium was poured into dishes and allowed to solidify and the isolates were inoculated. Whatman No.1 filter paper disc (90 mm diameter) was soaked in picric acid solution (2.5 g picric acid + 12.5 g Na_2CO_3 in 1000 ml of water) and placed on the lid of each plate. Three replications were maintained for each isolate. Petri dishes were sealed with parafilm and incubated at room temperature for four days. The uninoculated plate served as control. Observations on colour change of filter paper from deep yellow to orange brown and to red indicates the production of HCN.

3.9.2.3. Ammonia production

Selected epiphytic and endophytic bacterial isolates were tested for their potential for production of ammonia following the method of Dye (1962). The bacterial isolates were grown in 10 ml of peptone water (Appendix 1) and incubated at 30°C for four days. Three replications were maintained for each bacterial isolate. After incubation, 50 µl of Nesseler' s reagent was added to the broth. The change in the colour of the broth from faint yellow to deep yellow or brown colour indicated the production of ammonia. The reaction was scored as nil, low, medium and high in 1-4 scale based on intensity of colour.

3.9.2.4. Antibiosis test for production of volatile inhibitory metabolites

Antibiosis test for volatile metabolite production was carried out by sealed Petri plate technique with slight modification as described by Dennis and Webster (1971). For this, two Petri dish bases of 90 mm diameter were taken and molten cooled PDA poured on both dishes. One dish containing the PDA, was inoculated with fungal antagonist and allowed to grow for three days. However, for bacterial antagonists, lawn of the candidate epiphytic or endophytic isolates were prepared by pour plate method. In the second Petri dish base plated with PDA medium, eight mm disc of seven days old pathogens of nutmeg or clove or cinnamon was placed at centre of the dish and two dishes were sealed together with parafilm and incubated at room temperature in such a way that the antagonist *viz.,* fungus or bacteria lie under the lower dish. This allowed the volatile compounds produced by the antagonists to reach the pathogen growing in the upper dish. Similarly sealed dishes with pathogen inoculated in one plate and no antagonist on the other plate served as control. Three replications were maintained for each isolate. The growth of the pathogen was measured at 48 h interval upto seven days. The per cent inhibition of the pathogen by epiphytes and endophytes was calculated, using the formula given in 3.6.1

3.9.2.5 IAA Production

Bacterial isolates inoculated in five ml of King' S B broth supplemented with L-tryptophan @ 100 µg ml^{-1} (100 µg ml^{-1} L-tryptophan in 50 per cent ethanol), were incubated for 42 h. Growth of the isolates were removed by centrifugation at 5000 rpm for 10 min. One ml aliquot of supernatant was mixed thoroughly with four ml Salkowski' s reagent and allowed to stand for 20 min at room temperature. The absorbance was read at 535 nm. IAA concentration was calculated from the standard curve. The isolates were also scored based on IAA production by following the scale *viz.,* IAA concentration > 5<10 µg ml^{-1} = 1; > 10<25 µg ml^{-} =2; > 25<30 mg 50 ml^{-1} = 3 and > 30 µg ml^{-1} =4.

3.10 COMPATIBILITY OF SELECTED ANTAGONISTS TO PLANT PROTECTION CHEMICALS USED IN NUTMEG, CLOVE AND CINNAMON

The *in vitro* compatibility of the selected fungal and bacterial antagonists to plant protection chemicals commonly used in nutmeg, clove and cinnamon gardens were tested.

3.10.1 Fungicides

The fungicides listed below were used for the *in vitro* evaluation by using poison food technique for fungi, inhibition zone technique for bacteria

Sl. No.	Chemical name	Trade name	Concentrations (per cent)
1	Copper oxychloride 50 % WDP	Fytolan	0.2, 0.3, 0.4
2	Copper hydroxide 77 % WP	Kocide	0.05, 0.1, 0.2
3	Carbendazim 12 % + Mancozeb 64% WP	Saaf	0.5, 1.0, 1.5
4	$CuSO_4$+ Lime+ Water	Bordeaux mixture	0.5, 1.0, 1.5
5	Mancozeb 75 % WP	Indofil-M45 WP	0.2, 0.3, 0.4
6	Cyamoxanil 8% + Mancozeb 64% WP	Curzate M- 8	0.5, 1.0, 1.5
7	Propiconazole 25 EC	Tilt	0.5. 1.0, 1.5
8	Hexaconazole 5EC	Contaf	0.5, 1.0, 1.5
9	Difenoconazole 25 EC	Score	0.5, 1.0, 1.5
10	Carbendazim 50 % WP	Bavistin	0.05, 0.01, 0.15

3.10.2 Insecticides

The insecticides listed below were selected for *in vitro* evaluation by poison food technique for fungi and inhibition zone technique in case of bacteria

Sl.No	Chemical name	Trade name	Concentrations (per cent)
1	Quinalphos 20 EC	Ekalux	0.024, 0.025, 0.026
2	Chlorpyrifos 20 EC	Dursban	0.04, 0.05, 0.06
3	Dimethoate 30 EC	Rogor	0.01, 0.02, 0.03
4	Flubendiamide 480 SC	Fame	0.05. 0.1, 0.2

3.10.2.1 Fungal antagonists

The quantity of fungicides, insecticides needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium, mixed well and poured in sterilized Petri dishes at the rate of 15-20 ml per plate. To avoid contamination, all ten fungicides were exposed to UV light for a period of 30 min before adding it into the medium. After solidification of the medium, mycelial discs of 8mm diameter from actively growing fungal antagonists and *Trichoderma viride* were cut and placed at the centre of the each Petri dish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on the mycelial growth of the fungal antagonists were taken when control plates showed full growth.The per cent inhibition of growth of the antagonists were calculated using the formulae as described in 3.6.1

3.10.2.2 Bacterial antagonists

Sterile filter paper discs of 8 mm diameter were soaked in different concentrations of fungicides and insecticides. The discs were placed at the center of Petri dishes containing the NA medium seeded with 48 h old culture of the

three different isolates of the bacterium. Control consisted of filter paper disc soaked in sterile distilled water.Three replications were maintained. The inoculated plates were incubated at room temperature and the observations on inhibition zone were recorded after 48 h. The per cent inhibition of growth of bacterial antagonists was calculated using the formula given in 3.6.1

3.11 IDENTIFICATION OF EFFICENT EPIPHYTIC AND ENDOPHYTIC ANTAGONISTS

The cultural and morphological characters of fungal isolates were studied for identification. Further, confirmation of the identified fungus was done at National Centre for Fungal Taxonomy (N.C.F.T) New Delhi.

3.11.1 Fungi

A total of four fungi were selected as promising antagonists and they showed more than 60 per cent inhibition of the pathogen. The cultural characters of the antagonists like growth, colony colour, pigmentation were studied in potato dextrose agar medium and morphological characters of the mycelium, spores and spore bearing structures were studied.

3.11.2 Bacteria

The four promising bacterial antagonists selected from nutmeg, clove and cinnamon were characterized based on the cultural morphological and biochemical characters. Cultural characters like Gram reaction, production of fluorescent pigments, growth at 4 ºC and 41ºC, catalase activity, oxidase test, levan production, starch hydrolysis, nitrate reduction, urease activity and arginine hydrolase activity were tested following the procedures suggested by Manual of American Phytopathologists along with the standard cultures of *Pseudomonas fluorescens*. The Hi Assorted TM biochemical test kit for Gram negative rods were also employed for characterization of the endophytic isolates and compared with the interpretation chart given in the manual. Three replications were maintained for each isolate.

3.12 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed,1986). Multiple comparisons among the treatment means was done using DMRT.

Results

4. RESULTS

The results of studies on " Endophytic and epiphytic microbial diversity in major tree spices and their potential for biocontrol of foliar pathogens" carried out at the Department of Plant Pathology, College of Horticulture Vellanikkara during 2013-15 are presented below

4.1 ISOLATION OF PATHOGENS FROM NUTMEG, CLOVE AND CINNAMON

The organisms causing various diseases of nutmeg, clove and cinnamon were isolated from infected leaves collected from different locations of Kerala. The isolates were purified by hyphal tip method and maintained on Potato Dextrose Agar (PDA) slants by periodical subculturing. Pathogenicity of the organisms isolated from the respective host plants was proved by artificial inoculation on detached healthy leaves showed typical symptoms as observed in natural condition and reisolation from artificially inoculated leaves yielded organism having the same characters as the original one.

4.2 SYMPTOMATOLOGY OF DISEASES OF NUTMEG, CLOVE AND CINNAMON AND IDENTIFICATION OF PATHOGENS

Observations on the symptoms developed by different pathogens on nutmeg, clove and cinnamon were recorded from the naturally infected plants and also from the artificially inoculated plants. The pathogens were tentatively identified based on the cultural and morphological characters and also by comparing the characters given in CMI Descriptions of Pathogenic Fungi and Bacteria

4.2.1 Diseases of nutmeg

4.2.1.1 Colletotrichum leaf spot / Shot hole

The symptom developed as minute dark brown circular to irregular sunken lesions with yellow halo on the leaf lamina. Such lesions were seen to develop all over

the lamina but mainly found on the margins. As disease advances, the spots coaelesce and form large lesions with dark dots at the center representing acervuli of the fungus. Later the entire necrotic region got detached and fall off from the healthy portion leaving shot hole symptom (Plate 4.1). At times the infected necrotic region remains intact. Severe incidence on young and half mature leaves leads to leaf fall.

On PDA the fungal colony was fast growing and attained full growth in Petri dish in 6 days. Initially colour of the mycelium was pinkish white but later turned to brown and to black. Acervuli developed within 10 to 12 days. Hypha septate, branched and coloured, acervuli ovoid to saucer shaped with setae scattered on the edge. Conidia single celled, hyaline with round ends and with a size of 11.45 to 23.62 x 3.58 to 5.72 µm. (Plate 4.3). Based on the above characters coupled with pathogenicity on nutmeg, and also confirmation from N.C.F.T New Delhi with ID.NO (6768.15) the organism was identified as *Colletotrichum gloeosporioides*

4.2.1.2 Phytophthora leaf fall

Development of water soaked lesion any where on the leaf lamina was the initial symptom. The lesions were confined along the midrib and veins of leaves (Plate 4.1). Later they enlarged in size, turned dark brown in colour and covered large areas. In some cases, the lesions were seen on the petiole and young stem. Severe infection resulted in extensive defoliation.

The pathogen produced white cottony mycelium and attained 9 cm growth in PDA mediated Petri dish within 5 to 6 days. Hypha branched, hyaline, coenocytic, and 3.2 to 6.45µm wide. Sporangiophore developed from somatic hyphae bearing pear shaped sporangia. Sporangiophores were indeterminate and 40-135 µm in length. Sporangia abundant on PDA, typically pear shaped with small, but prominent papilla, deciduous with short and thick pedicel and 4-5 µm long. The average L/B ratio of sporangia of the isolate ranged from 1.15 to 2.4.

(Plate 4.3) Based on the above characters the organism was identified as *Phytophthora* sp.

4.2.1.3 Grey blight

The grey blight disease on nutmeg was initiated as circular to irregular light brown dots with white center near the margins of the leaf lamina. As the disease advances the spots enlarged in size with greyish white center and dark brown margin which showed a blighted appearance. Sometimes the central papery portion fall off leading to shot-hole symptom.

The fungus was comparatively slow in growth and attained full growth in 10 days in Petri dishes mediated with PDA. The mycelium hyaline, septate with whitish aerial mycelium sparse towards the advancing edges and denser on older parts of the colony. Conidia appeared as conspicuous greenish black spore masses on the centre. Conidia fusiform, 5 celled, central 3 cells darker and basal cells hyaline, with 3 apical hyaline appendages. (Plate 4.3). Based on the above characters the organism was identified as *Pestalotiopsis palmarum.*

4.2.1.4 Seedling blight

The disease initiated as straw coloured small lesions on the foliage of young plants which later coalesce and finally results in blightening (Plate 4.1).

On PDA the fungus produced sparse mycelium in young stage, later became yellowish brown in colour. Hyphae septate, 8-12 µm in diameter and branched at right angles with frequent septation. On prolonged storage of cultures the mycelium gave rise to short swollen and much branched cells eventually produce sclerotia. Sclerotia were round to irregular in shape when young, became dark brown on ageing. Based on the above characters coupled with pathogenicity on nutmeg, organism was identified as *Rhizoctonia solani.*

4.2.1.5 Horse hair blight

Symptoms were characterized by development of hair like black coloured mycelial threads on leaves and young stems. Mycelial threads were attached to the host tissues at random (Plate 4.1). which resulted in drying of the affected leaves and branches. The dried leaves and branches got detached from plant and with the mycelial thread network, sometimes appeared as bird-nest.

The pathogen grew very slowly and took 13 to 15 days to obtain full growth on Petri plates mediated with PDA. The fungus initially produced thick thread like white mycelial growth on the medium. Later this mycelial strands turned to black in colour. The reverse side of the Petri dish showed black pigmentation of colony on medium. Based on above characters the organism was tentatively identified as *Marasmius* sp.

4.2.2 Diseases of Clove

4.2.2.1 Leaf spot and twig blight of clove

Characteristic symptoms of the disease on the leaves developed as small circular to oval, brown specks scattered all over leaf lamina. The specks gradually enlarged and developed in to distinct spots with ashy grey center and dark margin (Plate 4.2). Coalisation of adjacent spots resulted in the formation of necrotic patches. Later the infection spreads to petiole causing defoliation. Infection also extended to young twigs which initially appeared in the form of isolated brownish dots, but later coalesced to form large necrotic areas. In severe cases die back symptom was evident with eventual death of the twigs

Mycelium of the fungus, in culture was greyish white to dark grey, thin and septate. On PDA it showed fast growth and attained full growth in Petri dish in 6 to 7 days. Conidia aseptate, cylindrical, with rounded ends, sometimes slightly ellipsoid with rounded apex and narrow truncated base. Size of the conidia varied from 10.38 to 27.7 x 3.20 to 5.12 µm. On the basis of these

characters the pathogen was tentatively identified as *Colletotrichum gloeosporioides*

4.2.2.2. Grey blight of clove

The disease developed as small light brown dots with white center and the infection initiated from the margins or tip of mature leaves. (Plate 4.2) As these spots enlarged, the center became greyish white and papery and sometimes the entire portion may fall off and showed shot-hole symptom.

The fungus was slow growing and attained full growth in Petri dish mediated with PDA within 14 days. The cultural and morphological characters of the pathogen were same as that described in 4.2.1.3. The size of the conidia was in the range of 5.52 x 9.64 µm.The fungus was tentatively identified as *Pestalotiopsis palmarum.*

4.2.2.3 Leaf blotch disease of clove

Symptoms were characterized by water soaked lesions which develop on leaf lamina. The lesions were seen all over the leaf lamina but mainly on leaf margins and tips. As the disease advances, the lesions enlarged in size and turned dark brown in colour and finally resulted in defoliation (Plate 4.2).

The fungus was slow and it took 13-14 days to attain full growth in PDA mediated Petri dishes. Hyphae septate, highly branched, and light reddish brown in colour. Conidiophores produced in masses with primary, secondary and tertiary sterigmata having broom shaped appearance. The culture sporulate and produced darker reddish brown pigmentation in to the medium. Conidia hyaline, straight, cylindrical with rounded ends and 1 to 3 septate and 32.20 to 35.80 x 3.58 to 6.58 µm in size, sterile filament consisted of vesicle at the tip. (Plate 4.3) Based on these characters and further confirmation from N.C.F.T New Delhi with ID.NO (6766.15) the organism was identified as *Cylindrocladium quinqueseptatum*

4.2.3 Diseases of cinnamon

4.2.3.1 Leaf spot and dieback

The disease developed as small deep brown specks on the leaf lamina. The spots soon enlarged and measured 3-15 mm in diameter which coalesced and resulted in complete drying of leaves (Plate 4.2). The disease may also extended to the stem leading to die back. The central papery portion falls off producing shot-hole symptom. The cultural and morphological characters of the organism were same as that explained in 4.2.2.1. The size of the conidia was in the range of 10.74 to 21.12 X 2.14 to 5.72 µm. (Plate 4.3). Based on the above characters the organism was identified as *Colletotrichum gloeosporioides.*

4.2.3.2 Grey blight disease

Symptoms were characterized as small yellowish brown, oval or irregular spots which later became greyish white with dark brown border.The adjacent patches join together forming necrotic patches (Plate 4.2). In advanced stage of infection major portion of leaf lamina dried up and blown away by wind producing shot-holes. Acervuli of the pathogen was noticed in the affected portion as darker dots.

The general cultural and morphological characters of the organisms were similar to that described in 4.2.1.3 coupled with pathogenicity and further, confirmation from N.C.F.T New Delhi with ID. NO (6767.15) the organism was identified as *Pestalotiopsis palmarum* (Plate 4.3).

PLATE 4.1 DISEASES OF NUTMEG

Colletotrichum leaf spot /
Shot hole disease of nutmeg

Phytophthora Leaf fall of nutmeg

Leaf blight of nutmeg by

Pestalotiopsis palmarum

Blight of nutmeg by *Rhizoctonia solani*

Horse hair blight of nutmeg

PLATE 4.2. DISEASES OF CLOVE AND CINNAMON

Leaf spot and twig blight of clove

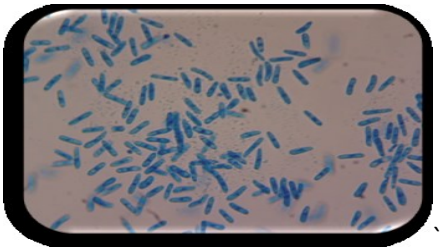
Grey blight of clove

Leaf blotch of clove

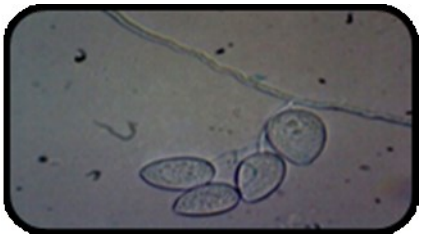
Grey blight of cinnamon

Leaf spot of cinnamon

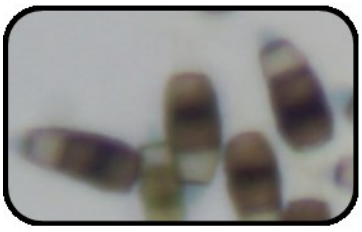
PLATE 4.3 MICROPHOTOGRAPHS OF TREE SPICES PATHOGENS SPORES (400 X)



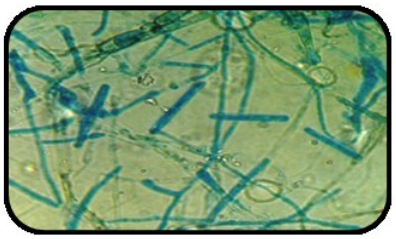
Colletotrichum gloeosporioides – Nutmeg (400X)



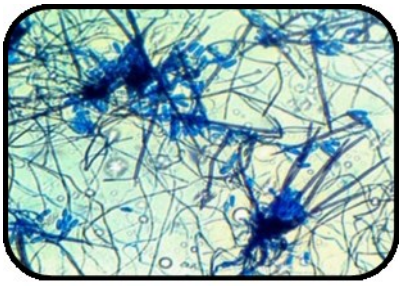
Phytophthora sp. – Nutmeg (400X)



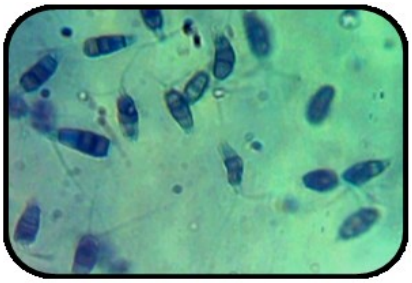
Pestalotiopsis palmarum **-Nutmeg (400 X)**



Cylindrocladium quinqueseptatum**- Clove (400 X)**



Colletotrichum gloeosporioides**- Cinnamon (400 X)**



Pestalotiopsis palmarum**- Cinnamon (400 X)**

4.3 ISOLATION AND ENUMERATION OF EPIPHYTIC AND ENDOPHYTIC MICROFLORA FROM NUTMEG, CLOVE AND CINNAMON

Epiphytic microflora of nutmeg, clove and cinnamon were isolated from the healthy plant samples collected from different areas of Kerala. The total epiphytic microflora *viz.,* fungi, bacteria and fluorescent Pseudomonads were quantitatively estimated by dilution plate method. However, actinomycetes could not be isolated from any of the samples collected.

4.3.1 Nutmeg epiphytes

4.3.1.1 Fungi

The data on the population of epiphytic microbes isolated from different areas are given in Table 4.1. The population of epiphytic fungi varied among the samples collected from different locations which ranged from zero to 7×10^{4} cfu g^{-2} of plant tissue. Fungal population was more in samples collected from Kalady of Ernakulam district (7×10^{4} cfu g^{-2} of leaf tissue) followed by those collected from Vellanikkara of Thrissur district and Ambalavayal area of Wayanad district (6×10^{4}cfu g^{-2} of plant tissue). Samples collected from Pattikad of Thrissur district did not harbour any epiphytic fungi.

4.3.1.2 Bacteria

Epiphytic bacterial population ranged from 7.50 to 27.50×10^{6} g^{-2} plant tissue (Table 4.1). The population was highest in samples collected from Aluva of Ernakulam (27.50×10^{6} cfu g^{-2}) district followed by those from Ambalavayal of Wayanad district (25.70×10^{6} cfu g^{-2}). The minimum population was noticed in samples collected from COA, Vellayani of Thiruvananthapuram district (7.50×10^{6} cfu g^{-2}).

4.3.1.3 Epiphytic fluorescent Pseudomonads

Population of epiphytic fluorescent Pseudomonads also showed variation (Table 4.1). Samples collected from Wayanad district had more number of

fluorescent Pseudomonads (26.50 x 10^6 cfu g^{-2} plant tissue) than that from other districts. It was followed by CoH, Vellanikkara of Thrissur district (24.0 x 10^6 cfu g^{-2}) and the lowest in samples from Kalady of Ernakulam district (5 x 10^6 cfu g^2).

Table 4.1 Population of epiphytes of nutmeg at different locations

Sl.No	District	Location	Fungi(x 10^4 cfu g^{-2})*	Bacteria(x10^6 cfu g^{-2} plant)*	Fluorescent Pseudomonads (x 10^6 cfu g^{-2})*
1	Thrissur	COH Vellanikkara	6	12.0	24.0
		Pattikad	0	8.80	20.0
2	Ernakulam	Aluva	5	27.50	16.30
		Kalady	7	15.0	5.0
3	Wayanad	Ambalavayal	6	25.70	26.50
4	Thiruvananthapuram	COA Vellayani	2	7.50	10.0

*Average of three replications

4.3.2 Nutmeg endophytes

4.3.2.1 Fungi

Population of endophytic microbes isolated from healthy plant samples of nutmeg are given in (Table 4.2). The data revealed that the population of endophytic fungi varied among the samples collected from different locations which ranged from 1 to 11 x 10^4 cfu g^{-2} of plant tissue. The population was highest in samples collected from COA Vellayani of Thiruvananthapuram district (11.0 x 10^4 cfu g^{-2}). The samples from Kalady of Ernakulam and Vellanikkara of Thrissur district also harboured comparatively higher fungal population. But the least population was in samples from Ambalavayal of Wayanad district (1 x 10^4 cfu g^{-2}).

4.3.2.2 Bacteria

The highest number of endophytic bacteria was recorded in samples collected from COA Vellayani of Thiruvananthapuram district (Table 4.2). (20.54 x 10^{6} cfu g^{-2} of plant tissue) followed by those from Vellanikkara of Thrissur (12.5 x 10^{6} cfu g^{-2}).The lowest population was observed in samples taken from Aluva of Ernakulam district (1.50 x 10^{6} cfu g^{-2}).

4.3.2.3 Fluorescent Pseudomonads

Compared to endophytic bacteria, the population of fluorescent Pseudomonads was found more in nutmeg leaf samples and it ranged from 10 to 18.10 x 10^{6} cfu g^{-2} of plant tissue (Table 4.2). The highest number was in samples collected from COA Vellayani of Thiruvananthapuram district and lowest (10 x 10^{6} cfu g^{-2}) in Kalady of Ernakulam district. Samples from Pattikad of Thrissur and Aluva of Ernakulam district harbored a population of 15 x 10^{6} cfu g^{-2} of plant tissue.

Table 4.2 Population of endophytes of nutmeg at different locations

Sl.No	District	Location	Fungi(x 10^{4} cfu g^{-2})*	Bacteria(x10^{6} cfu g^{-2})*	Fluorescent Pseudomonads (x 10^{6} cfu g^{-2})*
1	Thrissur	COH Vellanikkara	6	12.5	11.0
		Pattikad	2	9.0	15.0
2	Ernakulam	Aluva	4	1.50	15.0
		Kalady	8	8.80	10.0
3	Wayanad	Ambalavayal	1	8.50	12.70
4	Thiruvananthapuram	COA Vellayani	11	20.54	18.10

*Average of three replications

4.3.3 Clove epiphytes

4.3.3.1 Fungi

Samples collected from different locations showed variation in the population of epiphytes (Table 4.3). The leaf samples collected from Thrissur district exhibited the highest number of epiphytic fungal population of 4.0 x 10^{4} cfu g^{-2} of plant tissue. However samples collected from COA Vellayani of Thiruvananthapuram district recorded the lowest number of epiphytic fungal population (2 x 10^{4} cfu g^{-2}).

4.3.3.2 Bacteria

Data presented in the (Table 4.3) clearly showed variation in population of epiphytic bacterial population. Leaf samples collected from Thrissur district yielded highest number of epiphytic bacterial population of 24.20 x 10^{6} cfu g^{-2} of plant tissue. Leaf samples from Ambalavayal area of Wayanad district recorded the lowest number of epiphytic bacterial population (13.0 x 10^{6} cfu g^{-2}).

4.3.3.3 Fluorescent Pseudomonads

Results presented in (Table 4.3). exhibited variation in population of epiphytic fluorescent Pseudomonads population. Leaf samples collected from Thrissur recorded the highest number of epiphytic fluorescent Pseudomonads population (20.80 x 10^{6} cfu g^{-2} of plant tissue). However leaf samples collected from Ambalavayal area of Wayanad district recorded the lowest number of (14.0 x 10^{6} cfu g^{-2}) fluorescent Pseudomonads population from plant tissue.

Table 4.3 Population of epiphytes of clove at different locations

Sl.No	District	Location	Fungi(x 10^4 cfu g^{-2})*	Bacteria(x1 0^6 cfu g^{-2})*	Fluorescent Pseudomonads (x 10^6 cfu g^{-2})*
1	Thrissur	COH Vellanikkara	4	24.20	20.80
2	Wayanad	Ambalavayal	3	13.0	14.0
3	Thiruvananthapuram	COA Vellayani	2	18.0	19.50

4.3.4 Clove endophytes

4.3.4.1 Fungi

Results on the population of endophytes isolated from plant tissues of clove are given in Table 4.4 and this data showed variation in endophytic fungal population. Samples collected from Ambalavayal of Wayanad district showed more number of fungi 10.0 x 10^4 cfu g^{-2} of plant tissue compared to those collected from COA Vellayani which yielded 4.0 x 10^4 cfu g^{-2}.

4.3.4.2 Bacteria

Variation in the population of endophytic bacteria was observed in samples collected from different locations (Table 4.4). Highest bacterial population of 27.40 x 10^6 g^{-2} of plant tissue was recorded in samples collected from CoH Vellanikkara. However, leaf samples from Ambalavayal of Wayanad district recorded the least population of 20 x 10^6 cfu g^{-2}.

4.3.4.3 Fluorescent Pseudomonads

The population of endophytic fluorescent Pseudomonads exhibited variations in samples and the data are presented in Table 4.4. The maximum number of fungal population (20.80 x 10^6 cfu g^{-2}) was recorded from CoH Vellanikkara of Thrissur district which was followed by samples collected from

Ambalavayal of Wayanad and the minimum population was recorded from COA Vellayani of Thiruvananthapuram (12.0 x 10 ^{6} cfu g $^{-2}$).

Table 4.4 Population of endophytes of clove at different locations

Sl.No	District	Location	Fungi(x10^{4} cfu g^{-2})*	Bacteria(x10 ^{6} cfu g^{-2})*	Fluorescent Pseudomonads (x 10^{6} cfu g^{-2})*
1	Thrissur	COH Vellanikkara	5	27.40	20.80
2	Wayanad	Ambalavayal	10	20.0	14.0
3	Thiruvananthapuram	COA Vellayani	4	25.0	12.0

*Average of three replications

4.3.5 Cinnamon epiphytes

The epiphytic microbes *viz.,* fungi, bacteria and fluorescent Pseudomonads from cinnamon varied with different locations (Table 4.5).

4.3.5.1 Fungi

The fungal epiphytic population varied with different locations. Samples collected from COH Vellanikkara of Thrissur district recorded the highest number of epiphytic fungal population of 5 x 10 ^{4} cfu g $^{-2}$ of plant tissue and those from Ambalavayal of Wayanad district recorded the lowest value (Table 4.5).

4.3.5.2 Bacteria

Variation in the population of epiphytic bacteria was observed in samples collected from different locations. The highest bacterial population of 30.0 x 10 ^{6} cfu g $^{-2}$ of plant tissue was observed in samples collected from Ambalavayal of Wayanad district where as the lowest population of bacteria (18.5 x 10 ^{6} cfu g $^{-2}$) was recorded from samples collected from COA Vellayani of Thiruvananthapuram district.

4.3.5.3 Fluorescent Pseudomonads

Epiphytic fluorescent Pseudomonads population was also found to vary with different locations (Table 4.5). Samples from Vellayani of Thiruvananthapuram district recorded the highest number of 30.50 x 10^{6} cfu g^{-2} of plant tissue and the lowest was recorded population from COH Vellanikkara area of Thrissur district.

Table 4.5 Population of epiphytes of cinnamon at different locations

Sl.No	District	Location	Fungi(x 10^{4} cfu g^{-2})*	Bacteria(x10^{6} cfu g^{-2})*	Fluorescent Pseudomonads (x 10^{6} cfu g^{-2})*
1	Thrissur	COH Vellanikkara	5	24.0	23.20
2	Wayanad	Ambalavayal	2	30.0	29.20
3	Thiruvananthapuram	COA Vellayani	4	18.5	30.50

*Average of three replication

4.3.6 Cinnamon endophytes

4.3.6.1 Fungi

Data presented in (Table 4.6). exhibited variation in endophytic fungal population of cinnamon. Leaf samples collected from Ambalavayal of Wayanad district recorded the highest number of endophytic fungal population of 10 x 10^{4} cfu g^{-2} of plant tissue. However leaf samples from COA Vellayani of Thiruvananthapuram district recorded the lowest number of endophytic fungi.

4.3.6.2 Bacteria

Results presented in Table 4.6. showed variation in endophytic bacterial population. The highest number of endophytic bacteria (27.40 x 10^{6} cfu g^{-2} of

plant tissue) was recorded from Thrissur district where as the lowest bacterial population was recorded from Ambalavayal of Wayanad district.

4.3.6.3 Fluorescent Pseudomonads

Leaf samples of cinnamon collected from Thrissur district recorded the highest number of endophytic fluorescent Pseudomonads population of 20.80 x 10 ^{6} cfu g $^{-2}$ of plant tissue it was followed by Ambalavayal of Wayanad district (14.0 x 10 ^{6} cfu g $^{-2}$) and the lowest number was recorded from COA Vellayani of Thiruvananthapuram district (12.0 x 10 ^{6} cfu g $^{-2}$).

Table 4.6 Population of endophytes of cinnamon at different locations

Sl.No	District	Location	Fungi(x10^{4} cfu g^{-2})*	Bacteria(x10^{6} cfu g^{-2})*	Fluorescent Pseudomonads (x 10^{6} cfu g^{-2})*
1	Thrissur	COH Vellanikkara	5	27.40	20.80
2	Wayanad	Ambalavayal	10	15.0	14.0
3	Thriuvananthapuram	COA Vellayani	4	20.0	12.0

*Average of three replications

4.4 *IN VITRO* ANTAGONISTIC EFFECT OF EPIPHYTES AND ENDOPHYTES AGAINST THE PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

Evaluation on antagonistic effect of epiphytes and endophytes against the pathogens of nutmeg clove and cinnamon was carried out in *in vitro* condition.

4.4.1 Preliminary screening of epiphytic and endophytic microflora against the pathogens of nutmeg, clove and cinnamon

Isolation of epiphytic and endophytic microorganisms of nutmeg, clove and cinnamon yielded 118 isolates which included 71 epiphytes and 47

endophytes. These were subjected to preliminary screening as described in 3.6.1 in order to evaluate their antagonistic property towards the pathogens of nutmeg, clove and cinnamon. Out of 118 isolates screened 66 isolates did not exhibit any antagonism against any pathogens and 52 isolates showed varying degrees of antagonism which included 32 epiphytes and 20 endophytes. They were further evaluated for their antagonistic property against each pathogen under *in vitro* condition.

4.5 *IN VITRO* EVALUATION OF ANTAGONISTIC EPIPHYTES AND ENDOPHYTES AGAINST PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

4.5.1 Nutmeg epiphytes

4.5.1.1 Epiphytic Fungi

Out of the 11 epiphytic fungal isolates evaluated in the preliminary screening only four were showed antagonistic property against the pathogens of nutmeg. These were further evaluated for the antagonistic property in terms of per cent inhibition against the fungal pathogens of nutmeg namely *Phytophthora* sp., *Colletotrichum gloeosporioides, Pestalotiopsis palmarum* and *Rhizoctonia solani* and their efficiency was compared with the reference culture of *Trichoderma viride* (Table 4.7).

The per cent inhibition on the growth of the pathogen by the antagonist over control was calculated when the growth of pathogen attained 9 cm growth on Petri dish. The four antagonists recorded varying levels of inhibition on the growth of the four pathogens in the range of 20 to 75 per cent and recorded various mechanisms of action. The isolate Nt ep f 1 recorded more than 60 per cent inhibition against all the pathogens tested and it recorded lysis and cessation of growth against *Phytophthora* sp. and *C. gloeosporioides* where as in case of *P. palmarum* and *R. solani*, it recorded aversion type of antagonistic reaction.The isolate Nt ep f 3 showed the lowest inhibition of 20 per cent against *R. solani*

followed by the same isolate against the *Pestalotiopsis palmarum* (25 per cent). The reference culture *T. viride* recorded more than 65 per cent inhibition against all four pathogens.

4.5.1.2 Epiphytic bacteria

Among the 10 epiphytic bacteria only four were antagonistic towards the pathogens and their efficiency was compared with the standard culture of *Pseudomonas fluorescens* (Table 4.8).The bacterial isolates showed 24.44 to 76.25 per cent inhibition on growth of four fungal pathogens. The isolate Ntep b 2 (Na) showed the maximum efficiency and recorded more than 60 per cent inhibition against all the four pathogens. The lowest inhibition of 24.44 was recorded by Nt ep b 4 against *R. solani.*

4.5.1.3 Epiphytic fluorescent Pseudomonads

Out of 10 fluorescent Pseudomonads isolates evaluated in the preliminary screening only three were antagonistic towards the pathogens of nutmeg. These isolates were again evaluated for their antagonistic property against the pathogens along with the standard culture (Table 4.9). The isolates tested exhibited varying levels of inhibition against four pathogens tested which ranged from 25.32 to 52.11 per cent. The highest inhibition of 52.11 per cent was recorded by the isolate Nt ep b 2 against *C. gloeosporioides* which was followed by same isolate against *Phytophthora* sp. recorded 50.44 per cent inhibition. *P. fluorescens* recorded more than 60 per cent inhibition against all four pathogens of nutmeg.

Table 4.7 *In vitro* screening of nutmeg epiphytic fungal isolates against pathogens of nutmeg

Sl.No	Fungal isolate	Per cent inhibition over control*							
		***Phytophthora* sp.**	**AR**	***C. gloeosporioides***	**AR**	***P. palmarum***	**AR**	***R. solani***	**AR**
1	Nt ep f 1	75	LY	70	CS	65	AV	60	AV
2	Nt ep f 2	42.22	LY	35.22	OG	40	CS	46.2	LY
3	Nt ep f 3	37.8	OG	35.66	OG	25	OG	20	CS
4	Nt ep f 4	53.3	LY	50.5	OG	40.22	AV	35.65	CS
5	***T. viride***	74.55	OG	70.55	OG	65.55	CS	72.22	OG

* Mean of three replications

Nt ep f = Nutmeg epiphytic fungus

LY= Lysis; OG = Overgrowth; CS = Cessation of growth; AV = Aversion

AR = Antagonistic reaction

4.8 *In vitro* screening of nutmeg epiphytic bacterial isolates against pathogens of nutmeg

Sl. No	Bacterial isolate	Per cent inhibition over control*			
		***Phytophthora* sp.**	***C. gloeosporioides***	***P. palmarum***	***R. solani***
1	Nt ep b 1	45.44	40.44	37.22	25.55
2	Nt ep b 2	74.45	76.25	65.44	67.22
3	Nt ep b 3	44.33	45.24	40.12	36.15
4	Nt ep b 4	37.71	40.22	35.44	24.44
5	*P. f* (KAU)	64.44	77.14	66.44	75.44

* Mean of three replications

Nt ep b = Nutmeg epiphytic bacteria, *P. f = Pseudomonas fluorescens*

Table 4.9 *In vitro* screening of nutmeg epiphytic fluorescent Pseudomonads isolates against pathogens of nutmeg

Sl.No	Bacterial isolate	Per cent inhibition over control*			
		***Phytophthora* sp.**	***C. gloeosporioides***	***P. palmarum***	***R. solani***
1	Nt ep b 1	34.44	35.22	30.11	25.32
2	Nt ep b 2	50.44	52.11	35.12	30.22
3	Nt ep b 3	44.33	47.15	51.22	35.5
4	*P. f* (KAU)	64.44	77.14	66.44	75.44

*Mean of the three replications

Nt ep b = Nutmeg epiphytic bacteria

P. f = Pseudomonas fluorescens

4.5.2 Nutmeg endophytes

Fifteen fungi, five bacteria and seven fluorescent Pseudomonads of endophytic origin were isolated from nutmeg.

4.5.2.1 Endophytic fungi

Out of 15 endophytic fungi evaluated in the preliminary screening fungal isolates, only six exhibited antagonism towards the pathogens of nutmeg. These six fungal isolates along with the standard culture of *T. viride* were further evaluated in assessing their inhibitory potential against four pathogens of nutmeg. A perusal of the data given in (Table 4.10) revealed that the endophytic fungi exhibited varying levels of inhibition on the growth of the pathogens which was in the range of 24.55 to 77.22 per cent. Among the isolate Nt ed f 2 showed more than 60 per cent inhibition of all pathogens tested and it recorded lysis and cessation of growth against *Phytophthora* sp. and *C. gloeosporioides* and aversion type of reaction against *P. palmarum* and *R.solani*.

The standard culture *T. viride* showed more than 65 per cent inhibition of growth of all four pathogens. All the other endophytic fungi recorded less than 50 per cent reduction on the growth of all four pathogens except Nt ed f 1against *P. palmarum* (50.22 per cent)

4.5.2.2 Endophytic bacteria

Of the five bacterial isolates only three were antagonistic towards the pathogens and they were further evaluated for their efficacy against fungal pathogens of nutmeg in comparison with standard culture *P. fluorescens* (Table 4.11).

All the three bacterial endophytes were not effective (0 Per cent inhibition) against *P. palmarum* and *R. solani* except Nt ed b 1 against *P. palmarum* which recorded 14.11 per cent inhibition. The standard bacterial bio control agent *P. fluorescens* (KAU) recorded more than 64 per cent inhibition against all the four pathogens of nutmeg.

4.5.2.3 Endophytic fluorescent Pseudomonads

Only three Pseudomonads showed antagonistic activity towards the pathogens of nutmeg (Table 4.12). Among them Nt ed b 6 recorded more than 60 per cent inhibition against all the pathogens of nutmeg in the range of 60.24 to 70.22 per cent. The standard culture *P. fluorescens* recorded more than 60 per cent inhibition against all four pathogens.

Table 4.10 *In vitro* evaluation of nutmeg endophytic fungal isolates against pathogens of nutmeg

Fungal isolate	Per cent inhibition over control *							
	***Phytophthora* sp.**	**AR**	***C.gloeosporioides***	**AR**	***P. palmarum***	**AR**	***R. solani***	**AR**
Nt ed f 1	42.22	LY	40.22	CS	50.55	AV	25.55	AV
Nt ed f 2	64.44	LY	71.11	OG	60.12	CS	77.22	LY
Nt ed f 3	37.99	OG	35.22	OG	30.55	OG	25.55	CS
Nt ed f 4	45.55	LY	48.66	OG	32.55	AV	27.55	CS
Nt ed f 5	39.00	AV	35.22	OG	30.20	AV	24.55	LY
Nt ed f 6	44.25	OG	46.54	CS	47.22	LY	35.66	CS
T. viride	74.55	OG	70.55	OG	65.55	CS	72.22	OG

* Mean of three replications Nt ed f = Nutmeg endophytic fungus LY= Lysis; OG = Overgrowth; CS = Cessation of growth; AV= Aversion AR = Antagonistic reaction

Table 4.11 *In vitro* evaluation of nutmeg endophytic bacterial isolates against pathogens of nutmeg

Sl.No	Bacterial isolate	Per cent inhibition over control*			
		***Phytophthora* sp.**	***C. gloeosporioides***	***P. palmarum***	***R. solani***
1	Nt ed b 1	22.55	24.22	14.11	0
2	Nt ed b 2	25.45	23.22	0	0
3	Nt ed b 3	15.22	35.66	0	0
4	*P .f* (KAU)	64.44	77.14	66.44	75.41

*Mean of three replications

Nt ed b = Nutmeg endophytic bacteria

P. f = Pseudomonas fluorescens

Table 4.12 *In vitro* evaluation of nutmeg endophytic fluorescent Pseudomonads isolates against pathogens of nutmeg

Sl.No	Bacterial isolate	Per cent inhibition over control*			
		***Phytophthora* sp.**	***C. gloeosporioides***	***P. palmarum***	***R. solani***
1	Nt ed b 1	50.33	32.22	25.55	30.00
2	Nt ed b 2	15.00	16.00	22.55	28.22
3	Nt ed b 6	64.22	60.24	65.55	70.22
4	*P. f* (KAU)	64.44	77.14	66.44	75.41

*Mean of three replications

Nt ed b = Nutmeg endophytic bacteria on Kings B medium

P. f= Pseudomonas fluorescens

4.5.3 Clove epiphytes

Six fungi, seven bacteria and eight fluorescent Pseudomonads of epiphytic origin were isolated from clove and were evaluated for the antagonistic property during preliminary screening.

4.5.3.1 Epiphytic fungi

Among the six fungal isolates tested in preliminary screening only three exhibited antagonism towards the pathogens of clove. These three isolates were further tested for their antagonistic effect against the pathogens of clove *viz., C. gloeosporioides, P. palmarum* and *C. quinqueseptatum* in comparison with the reference culture of *T. viride.*

Data presented in Table 4.13. showed the varying levels of inhibition against the pathogens which was in range of 35.24 to 46.22 per cent. Among the three fungal epiphytes, Cl ep f 1showed more than 43 per cent inhibition against all three pathogens. The reference culture *T. viride* recorded the highest inhibition of 75.22 per cent against *P. palmarum.*

4.5.3.2 Epiphytic bacteria

Preliminary screening revealed that three out of seven epiphytic bacteria exerted antagonism towards the pathogens of clove. The inhibitory effect was studied in comparison with standard culture of *P. fluorescens.*

The epiphytic bacterial isolate Cl ep b 6 recorded more than 60 per cent inhibition against the all three pathogens of clove tested (Table 4.14). while other isolates showed comparatively less inhibitory effect which ranged from 20.15 to 44.33 per cent. The reference culture of *P. fluorescens* showed the highest inhibition in the growth of pathogen in the range of 64.66 to 75.55 per cent.

4.5.3.3 Fluorescent Pseudomonads

Out of the eight epiphytic fluorescent Pseudomonads tested during preliminary screening, only three bacteria exhibited antagonism towards the

pathogens of clove (Table 4.15). Their percentage inhibition on the growth of pathogens was compared with the standard culture of *P. fluorescens*

The epiphytic fluorescent Pseudomonads were not as effective as standard *P. fluorescens*. The bacterial isolate exhibited per cent inhibition ranging from 20.15 to 47.22 per cent while the standard culture of *P. fluorescens* showed more than 64 per cent inhibition against all three pathogens.

Table 4.13 *In vitro* evaluation of clove epiphytic fungal isolates against pathogens of clove

Sl.No	Fungal isolate	Per cent inhibition over control*					
		C. quinqueseptatum	**AR**	***P. palmarum***	**AR**	***C. gloeosporioides***	**AR**
1	Cl ep f 1	45.22	AV	43.11	LY	46.24	OG
2	Cl ep f 2	46.22	OG	44.24	OG	35.24	AV
3	Cl ep f 3	44.22	AV	40.24	OG	36.22	LY
4	*T. viride*	64.51	OG	75.22	LY	60.44	OG

*Mean of three replications

Cl ep f = Clove epiphytic fungi

AV = Aversion; OG = Over growth; LY = Lysis

AR = Antagonistic reaction

Table 4.14 *In vitro* evaluation of clove epiphytic bacterial isolates against pathogens of clove

Sl.No	Bacterial isolate	Per cent inhibition over control*		
		C. quinqueseptatum	***P. palmarum***	***C. gloeosporioides***
1	Cl ep b 1	44.33	35.22	25.16
2	Cl ep b 2	33.75	30.22	20.15
3	Cl ep b 6	65.44	67.22	60.15
4	*P.f* (KAU)	75.55	64.66	65.66

* Mean of three replications

Cl ep b = Clove epiphytic bacteria

P. f = Pseudomonas fluorescens

Table 4.15 *In vitro* evaluation of clove epiphytic fluorescent Pseudomonads against pathogens of clove

Sl.No	Bacterial isolate	Per cent inhibition over control*		
		C. quinqueseptatum	***P. palmarum***	***C. gloeosporioides***
1	Cl ep b 1	33.75	30.22	20.15
2	Cl ep b 2	34.90	31.25	25.55
3	Cl ep b 6	45.44	47.22	40.15
4	*P. f* (KAU)	75.55	64.66	65.66

* Mean of three replications

Cl ep b = Clove epiphytic bacteria

P. f = Pseudomonas fluorescens

4.5.4 Clove endophytes

Isolation of endophytes from clove yielded four fungi, two bacteria and five fluorescent Pseudomonads.

4.5.4.1 Fungi

Out of the four fungal isolates tested in preliminary screening only two fungi exhibited the antagonism towards the pathogens of clove Table 4.16. Endophytic fungus Cl ed f 2 and *T. viride* were more effective than the others in their antagonistic property with more than 60 per cent inhibition against the pathogens tested. The isolate exhibited over growth type of antagonistic reaction against *C. quinqueseptatum* and *C. gloeosporioides* while lysis in case of *P. palmarum*

4.5.4.2 Bacteria

Two endophytic bacteria tested during preliminary screening exerted varied levels of antagonism towards the pathogens of clove Table 4.17.

The per cent inhibition on the growth of the pathogens of nutmeg by the two endophytic bacteria was in the range of 22.64 to 69.33 per cent. The endophytic bacteria Cl ed b 2 and the standard culture of *P. fluorescens* recorded more than 60 per cent inhibition against all the three pathogens.

4.5.4.3 Fluorescent Pseudomonads

Among the five endophytic fluorescent Pseudomonads only three were antagonistic towards the pathogens of clove.

Compared to the standard culture of *P. fluorescens* with more than 60 per cent inhibition, the antagonistic endophytic fluorescent Pseudomonads were less efficient. They recorded the inhibition on the growth of all three pathogens in the range of 22.22 to 54.33 per cent (Table 4.18).

Table 4.16 *In vitro* evaluation of clove endophytic fungi against pathogens of clove

Sl.No	Fungal isolate	Per cent inhibition over control*					
		C. quinqueseptatum	**AR**	***P. palmarum***	**AR**	***C. gloeosporioides***	**AR**
1	Cl ed f 1	44.24	OG	43.24	OG	40.44	AV
2	Cl ed f 2	66.66	OG	62.22	LY	65.22	OG
3	*T. viride*	64.51	OG	75.22	OG	60.44	OG

* Mean of three replications

Cl ed f = Clove endophytic fungus; AV = Aversion; OG = Over growth; LY= Lysis AR = Antagonistic reaction

Table 4.17 *In vitro* evaluation of clove endophytic bacteria against pathogens of clove

Sl.No	Bacterial isolate	Per cent inhibition over control*		
		C. quinqueseptatum	***P. palmarum***	***C. gloeosporioides***
1	Cl ed b 1	37.33	44.44	22.64
2	Cl ed b 2	69.33	65.44	60.44
3	*P. f* (KAU)	75.55	64.66	65.66

*Mean of three replications

Cl ed b = Clove endophytic bacteria

P. f = Pseudomonas fluorescens

Table 4.18 *In vitro* evaluation of clove endophytic fluorescent Pseudomonads isolates against pathogens of clove

Sl.No	Bacterial isolate	Per cent inhibition over control*		
		C. quinqueseptatum	***P. palmarum***	***C. gloeosporioides***
1	Cl ed b 1	54.33	30.65	22.22
2	Cl ed b 2	43.75	40.56	27.25
3	Cl ed b 3	24.20	36.75	35.75
4	*P. f* (KAU)	75.55	64.66	65.66

* Mean of three replications

Cl ed b = Clove endophytic bacteria

P. f = Pseudomonas fluorescens

4.5.5 Cinnamon epiphytes

A total of 19 epiphytes were isolated from cinnamon which included six epiphytic fungi, five epiphytic bacteria and eight epiphytic Fluorescent Pseudomonads

4.5.5.1 Fungi

Preliminary evaluation of six epiphytic fungal isolates revealed that only three fungi were antagonistic to pathogens of cinnamon. The data of per cent inhibition of two pathogens of cinnamon by antagonistic isolates along with the standard culture *T. viride* are presented in Table 4.19.

After seven days of inoculation of antagonists, all fungal isolates tested were exhibited varying levels of inhibition of the pathogen ranging from 25.44 to 76.55 per cent. Out of the three epiphytic isolates, one isolate *i.e.,* Cn ep f 5 and *T. viride* recorded more than 70 per cent inhibition against the pathogens tested and

it recorded overgrowth type of antagonistic reaction The other isolates *viz.,*Cn ep f 1 and Cn ep f 2 showed inhibition in the range of 25.44 to 35.64 per cent.

4.5.5.2 Bacteria

All the five epiphytic bacterial isolates were antagonistic against the pathogens of cinnamon.They exerted comparatively less inhibition of the pathogens of cinnamon which varied from 20.55 to 48.44 per cent (Table 4.20). However *P. fluorescens* had more antagonistic property with more than 60 per cent inhibition.

4.5.5.3 Fluorescent Pseudomonads

Four out of the eight epiphytic bacterial isolates obtained from cinnamon showed varied levels of antagonism towards the pathogens of cinnamon (Table 4.21). varied from 20.55 to 69.33 per cent. *P. fluorescens* was more effective antagonist which recorded more than 65 per cent inhibition against the two pathogens of cinnamon. However isolate Cn ep b 4 showed more antagonistic property (69.33 per cent) against *P. palmarum* of cinnamon.

Table 4.19 *In vitro* evaluation of epiphytic fungal isolates of cinnamon against pathogens of cinnamon

Sl.No	Fungal isolate	Per cent inhibition over control*			
		P. palmarum	**AR**	***C. gloeosporioides***	**AR**
1	Cn ep f 1	30.55	OG	35.64	LY
2	Cn ep f 2	38.44	AV	25.44	OG
3	Cn ep f 5	76.55	OG	70.64	OG
4	*T. viride*	70.55	OG	75.55	OG

* Mean of three replications ; Cn ep f = Cinnamon epiphytic fungus; AV = Aversion; OG = Over growth; LY = Lysis ; AR = Antagonistic reaction

Table 4.20 *In vitro* evaluation of epiphytic bacterial isolates of cinnamon against pathogens of cinnamon

Sl.No	Bacterial isolate	Per cent inhibition over control*	
		C. gloeosporioides	***P. palmarum***
1	Cn ep b 1	37.33	35.24
2	Cn ep b 2	47.44	25.55
3	Cn ep b 3	48.44	32.55
4	Cn ep b 4	32.55	30.55
5	Cn ep b 5	34.54	20.55
6	*P. f* (KAU)	65.55	75.44

* Mean of three replications; Cn ep b = Cinnamon epiphytic bacteria ; *P. f* = *Pseudomonas fluorescens*

Table 4.21 *In vitro* evaluation of cinnamon epiphytic fluorescent pseudomonads isolates against pathogens of cinnamon

Sl.No	Bacterial isolate	Per cent inhibition over control*	
		P. palmarum	***C. gloeosporioides***
1	Cn ep b 1	47.44	35.24
2	Cn ep b 2	48.44	32.55
3	Cn ep b 3	48.44	20.55
4	Cn ep b 4	69.33	20.55
5	*P. f* (KAU)	65.55	75.44

* Mean of three replications; Cn ep b = Cinnamon epiphytic bacteria; *P. f* = *Pseudomonas fluorescens*

4.5.6 Cinnamon endophytes

Out of 47 endophytes isolated from tree spices three isolates each of fungi, bacteria and fluorescent Pseudomonads were isolated from cinnamon. Among them none of the endophytic bacterial and fluorescent Pseudomonads were antagonistic towards pathogens of cinnamon like *C. gloeosporioides* and *P. palmarum*. However, one out of three endophytic fungi was antagonistic in nature.

4.5.6.1 Fungi

Out of three endophytic fungal isolates only one fungus exerted antagonism towards the pathogens of cinnamon. After seven days of inoculation of antagonists, endophytic fungal isolates showed inhibition of 40.25 and 45.23 per cent against *P. palmarum* and *C. gloeosporioides* respectively (Table 4.22). Standard culture of *T. viride* exhibited sixty and above per cent inhibition against the two pathogens of cinnamon.

4.5.6.2 Bacteria

Among the three bacteria tested during the preliminary screening none of the bacteria was antagonistic to the pathogens of cinnamon.

4.5.6.3 Fluorescent Pseudomonads

During the preliminary screening none of the isolates of fluorescent Pseudomonas recorded antagonism against the cinnamon pathogens.

Table 4.22 *In vitro* evaluation of cinnamon endophytic fungal isolates against pathogens of cinnamon

Sl.No	Bacterial isolate	Per cent inhibition over control*		AR
		P. palmarum	*C. gloeosporioides*	
1	Cn ed f 1	45.33	40.25	OG
2	*T. viride*	65.55	75.44	OG

*Mean of three replications

Cn ed f= Cinnamon endophytic fungus, AR = Antagonistic Reaction, OG = Overgrowth.

4.5.7. *In vitro* evaluation of nutmeg fungal and bacterial antagonists against pathogens of clove and cinnamon

Based on per cent inhibition in *in vitro* evaluation, two fungal and bacterial isolates were selected from nutmeg and further evaluated the antagonistic potential against pathogens of clove and cinnamon. The fungal antagonists Nt ep f 1, Nt ed f 2 and the bacterial antagonists Nt ep b 2, Nt ed b 6 were selected for their study. The data are given in the Table 4.23.

The fungal antagonists recorded more than 60 per cent reduction in the growth of the pathogens of clove and cinnamon. The highest inhibition of 72.44 per cent was showed by Nt ep f 1against *P. palmarum* which was followed by Nt ep f 1against *C. quinqueseptatum* (70.55 per cent) and Nt ed f 2 against *C. gloeosporioides* of clove (70.54 per cent). Similarly the bacterial antagonists also showed more than 60 per cent reduction over all the pathogens of clove and cinnamon. Nt ep b 2 recorded the highest reduction in per cent inhibition over the control against *P. palmarum* (74.44 per cent).The other bacterial isolate Nt ed b 6 recorded highest (64.45) per cent reduction against cinnamon *C. gloeosporioides*.

Table 4.23. ***In vitro*** **evaluation of nutmeg antagonists against the pathogens of clove and cinnamon**

Nutmeg antagonists		Per cent inhibition over control*			
		Pathogens of clove		Pathogens of cinnamon	
		C. quinqueseptatum	*C. gloeosporioides*	*P. palmarum*	*C. gloeosporioides*
Fungal antagonist	**Nt ep f 1**	70.55	65.44	72.44	64.44
	Nt ed f 2	60.44	70.54	66.54	60.54
Bacterial antagonist	**Nt ep b 2**	64.45	65.55	74.44	63.55
	Nt ed b 6	60.54	64.54	60.54	64.45

*Mean of three replications

4.5.8. *In vitro* evaluation of clove fungal and bacterial antagonists against the pathogens of nutmeg and cinnamon.

The fungal antagonists Cl ed f 2 recorded more than 60 per cent and above reduction in the growth of the pathogens of nutmeg, and cinnamon (Table 4.24). The fungal isolate Cl ed f 2 recorded highest per cent reduction (72.56) per cent against *Rhizoctonia solani* of nutmeg followed by *C. gloeosporioides* (70.22 per cent) of nutmeg. It also noticed that the isolate exhibited highest per cent inhibition (65.45) against *P. palmarum* of cinnamon.

The bacterial isolate Cl ep b 6 and Cl ed b 2 recorded 70.45 and 72.44 per cent inhibition over *R. solani* of nutmeg, same isolates recorded less than 60 per cent inhibition over the pathogen *i.e* 55.55 and 50.65 per cent against *Phytophthora* sp. and *C. gloeosporioides* respectively.

4.5.9. *In vitro* evaluation of cinnamon fungal and bacterial antagonists against the pathogens of nutmeg and clove

After *in vitro* evaluation one epiphytic fungus from the isolates of cinnamon was selected and its antagonistic potential against the pathogens of nutmeg and clove was evaluated. Epiphytic and endophytic bacterial isolates of cinnamon were not inhibitory to the pathogens (Table 4.25). The fungal antagonists Cn ep f 5 exhibited more than 60 per cent inhibition on the growth of pathogens of nutmeg and clove.

Table 4. 24. *In vitro* evaluation of clove antagonists against the pathogens of nutmeg and cinnamon

Clove antagonists		Per cent inhibition over control*					
		Pathogens of nutmeg				Pathogens of cinnamon	
		***Phytophthora* sp.**	***C. gloeosporioides***	***P. palmarum***	***R. solani***	***P.* palmarum**	***C. gloeosporioides***
Fungal antagonist	**Cl ed f 2**	64.45	70.22	67.22	72.56	65.45	60.55
Bacterial antagonists	**Cl ep b 6**	65.54	66.75	60.54	70.45	60.54	65.54
	Cl ed b 2	55.55	50.65	65.55	72.44	64.45	60.54

*Mean of three replications

Table 4.25. *In vitro* evaluation of cinnamon antagonists against the pathogens of nutmeg and clove

Sl.NO	Cinnamon Fungal antagonist	Per cent inhibition over control*						
		Pathogens of nutmeg				Pathogens of cinnamon		
		Phytophthora sp.	*C. gloeosporioides*	*P. palmarum*	*R. solani*	*P. palmarum*	*C. gloeosporioides*	*C. quinqueseptatum*
1	Cn ep f 5	65.55	64.56	60.44	75.22	65.45	60.55	60.45

*Mean of three replications

4.6 MUTUAL COMPATIBILITY OF SELECTED EPIPHYTES AND ENDOPHYTES

The mutual compatibility of selected fungal and bacterial epiphytes and endophytes was tested *in vitro* conditions as described in Material and Methods in section 3.8 it was observed that the combinations of bacterial endophytes and epiphytes were compatible with each other and none of the isolate combination showed lysis at the juncture between the antagonists (Plate 4.4). All combinations of epiphytic and endophytic fungal antagonists were found to be compatible with each other except the combination of epiphytic and endophytic fungus (Table 4.26).

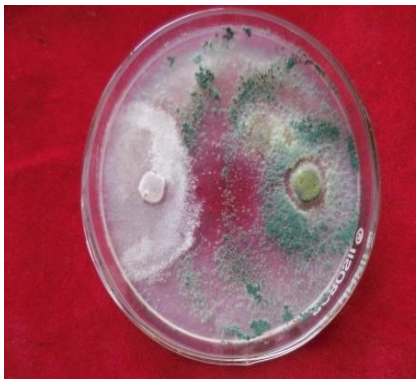
Table.4.26. Mutual compatibility of selected fungal and bacterial antagonists

Sl.No	**Fungal combination**	**Type of reaction**
1	Nt ep f 1 X Nt ed f 2	-
2	Nt ep f 1 X Cl ed f 2	+
3	Nt ed f 2 X Cn ep f 5	+
Sl.No	**Bacterial combination**	**Type of reaction**
1	Nt ep b 2 X Nt ed b 6	+
2	Cl ep b 5 X Cl ed b 2	+
3	Cl ep b 5 X Nt ep b 2	+
4	Cl ep b 5 X Nt ed b 6	+
5	Cl ed b 2 X Nt ed b 6	+

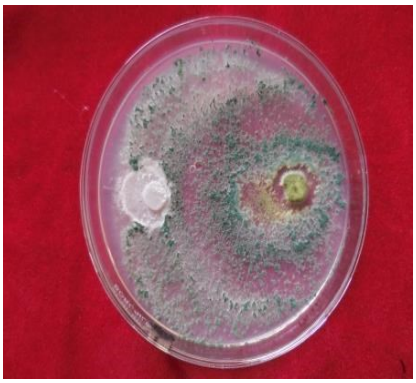
\+ Positive reaction (Compatible)

– Negative reaction (Incompatible)

PLATE 4.4 MUTUAL COMPATIBILITY OF SELECTED ANTAGONISTS



Nt ep f 1 X Cl ed f 2



Nt ed f 2 X Cn ep f 5

Nt ep f 1 X Nt ed f 2

Bacterial isolates

4.7 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS ON PATHOGENS OF NUTMEG, CLOVE AND CINNAMON

4.7.1. Fungal antagonists

The mechanism of action of the selected fungal antagonists *viz.,* Nt ep f 1, Nt ed f 2, Cl ed f 2 and Cn ep f 5 along with the standard culture *T. viride* against the pathogens of nutmeg, clove and cinnamon was studied.

The hyphae of the antagonists along with the pathogens at the meeting point were observed under the microscope. Free intermingling and coiling of hyphae of antagonists and pathogen was noticed which resulted in malformation of hyphae of pathogens. Microscopic observations also revealed the penetration of hyphae of pathogens by their antagonists which resulted in the lysis of hyphae of pathogens. (Plate 4.5)

4.7.1.1. Inhibition of pathogen by diffusible, non-volatile metabolites

Production of non-volatile metabolites by promising epiphytic and endophytic antagonists in comparison with reference cultures (*T. viride* and *P. fluorescens*) was tested by following the cellophane paper method as described in section 3.7.

After 3 days of inoculation in case of *Phytophthora* sp. and *R. solani* of nutmeg, the fungal antagonists Nt ep f 1, recorded per cent inhibition of 62.77. The highest per cent inhibition (72.22) over *R. solani* of nutmeg by fungal antagonists Nt ed f 2 (Table 4.27).

However after 7 days of inoculation in case of *C. gloeosporioides* and *P. palmarum*, the fungal antagonists Nt ep f 1 and Nt ed f 2 recorded per cent inhibition ranging over the pathogens of nutmeg from 55.55 to 65.55. The fungal antagonists Cl ed f 2 and Cn ep f 5 recorded per cent inhibition ranging from 57.55 to 68.55 against pathogens of nutmeg. Cinnamon epiphytic fungus (Cn ep f 5) recorded 67.45 per cent inhibition against *R. solani* of nutmeg (Table 4.28).

Table 4.27. *In vitro* inhibition of pathogens of nutmeg and clove by non-volatile metabolities

Fungal antagonist	Per cent inhibition over control*						
	Pathogens of nutmeg				Pathogens of clove		
	Phytophthora sp.	*C. gloeosporioides*	*P. palmarum*	*R. solani*	*C. gloeosporioides*	*P. palmarum*	*C. quinqueseptatum*
Nt ep f 1	62.77	65.55	60.44	67.77	55.55	58.55	60.44
Nt ed f 2	54.44	60.44	55.55	72.22	64.45	66.55	64.55
Cl ed f 2	55.55	57.55	67.55	65.44	54.55	58.45	60.45
Cn ep f 5	60.55	64.44	68.55	67.22	67.45	65.55	58.54
T. viride	74.55	75.55	70.45	70.55	74.55	75.55	75.55

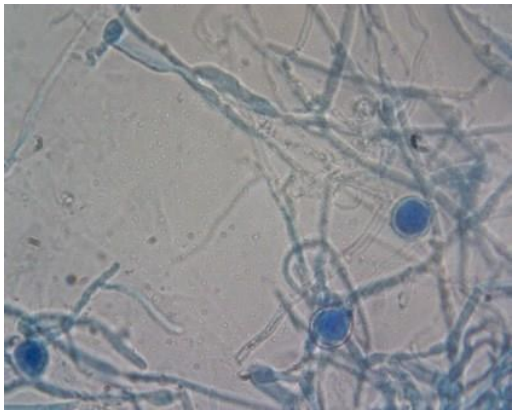
*Mean of three replications

4.28. *In vitro* inhibition of pathogens of cinnamon by non- volatile inhibitory metabolites

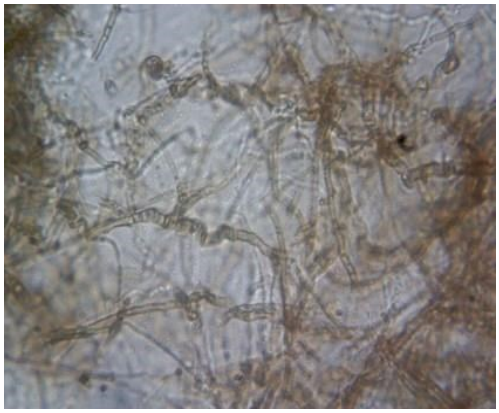
Sl.No	Fungal isolates	Per cent inhibition over control	
		C. gloeosporioides	***P. palmarum***
1	Nt ep f 1	55.45	60.45
2	Nt ed f2	64.55	58.24
3	Cl ed f 2	68.45	59.45
4	Cn ep f 5	57.45	62.22
5	*T. viride*	65.55	74.45

*Mean of three replications

PLATE 4.5 MECHANISM OF ACTION OF FUNGAL ANTAGONISTS WITH *Rhizoctonia solani* OF NUTMEG



Phytophthora cactorum (Coiling)



Trichoderma viride (Coiling)

Trichoderma harzianum (Hyphal disintegration)

After 10 days of inoculation in case of *C. quinqueseptatum* the lowest per cent inhibition (58.54) was recorded by (Cn ep f 5) followed by 60.44 by Nt ep f 1 (Table 4.28). The highest per cent inhibition 64.55 by nutmeg endophytic fungus (Nt ed f 2) against *C.quinqueseptatum.*

4.7.1.2. Inhibition of the pathogen by production of volatile inhibitory metabolites

None of the selected epiphytes and endophytes showed the production of volatile inhibitory metabolites

4.7.2. Bacterial antagonists

The selected potential epiphytic and endophytic bacterial isolates *viz.,* Cl ed b 2, Cl ep b 6, Nt ep b 2 and Nt ed b 6 were subjected to various tests for studying the mechanism of antagonism along with standard culture of *Pseudomonas fluorescens.*

4.7.2.1. Siderophore production

The epiphytic and endophytic bacterial isolates and standard culture of *P. fluorescence* were grown on King' S B medium supplemented with $FeCl_3$ 6 H_2O at different concentrations. All the four bacterial isolates are siderophore negative (Table 4.29).

4.7.2.2. Production of HCN

The potential epiphytic and endophytic bacterial isolates were tested for their ability to produce hydrogen cyanide (HCN). It was observed that none of the isolates produced hydrogen cyanide (Table 4.29).

4.7.2.3. Ammonia Production

Production of ammonia by potential isolates was detected by change in colour of peptone broth media on addition of Nesseler's reagent. The isolates produced varying amount of ammonia as evidenced by colour change of the

medium (Table 4.29). The isolate Nt ep b 2 produced low ammonia as evidenced by the colour change of the medium to yellow and scored as 1 the isolates Nt ed b 6, Cl ed b 2 and Cl ep b 6 produced moderate amount of ammonia since the colour changed to orange these were scored as 3. Standard culture *P. fluorescens* (KAU) produced more ammonia as colour of the medium changed to brownish orange and therefore scored as 4.

4.7.2.4. IAA production

All the isolates, four each of epiphytic and endophytic bacteria and the reference culture *P. fluorescens* produced varying levels of IAA, ranging from 7.5 to 19.1 µg ml^{-1} (Table 4.29). Among the bacterial antagonists the maximum quantity of IAA was produced by Cl ep b 6 (17.1 µg ml^{-1}) followed by Cl ed b 2 (16.5µg ml^{-1}). Nt ed b 6 isolate produced 11.0 µg ml^{-1} followed by Nt ep b 2 isolate produced 7.5 µg ml^{-1}.The reference culture *P. fluorescens* recorded 19.1µg ml^{-1} IAA production.

Table 4.29. Mechanism of action of selected bacterial antagonists

Sl.NO.	Bacterial antagonist	Siderophore production	HCN production	Ammonia production		IAA produced (µg. ml^{-1}) *
				Colour change	Score	
1	Nt ep b 2	_	_	Yellow	1	7.5
2	Nt ed b 6	_	_	Orange	3	11.0
3	Cl ed b 2	_	_	Orange	3	16.5
4	Cl ep b 6	_	_	Orange	3	17.1
5	*P. fluorescens* (KAU)	+	+	Brownish orange	4	19.1

***** Mean of three replications

4.8. COMPATIBILITY OF SELECTED ANTAGONISTS TO PLANT PROTECTION CHEMICALS USED IN NUTMEG, CLOVE AND CINNAMON

Different fungicides and insecticides commonly used in nutmeg, clove and cinnamon gardens were evaluated at three different concentrations to assess their compatibility with the selected four fungal and bacterial antagonists along with the standard cultures of *T.viride* and *P. fluorescens* (KAU).

4.8.1. Compatibility of fungal antagonists with fungicides

4.8.1.1. Nutmeg epiphytic fungus (Nt ep f 1) with fungicides

Ten fungicides *viz.,* Fytolan, Kocide, Saaf, Bordeaux mixture, Indofil M-45, Contaf, Score, Bavistin, Curzate and Tilt each at three different concentrations were evaluated for this study (Table. 4.30). The different fungicides showed varied level of per cent inhibition, on the growth of Nt ep f 1. From the data it was noticed that concentration of fungicides increased, there was a corresponding increase in inhibition. Nt ep f 1 was incompatible with Bordeaux mixture, Saaf, Indofil, Contaf, Score at all concentrations. Bavistin, Curzate and Tilt at higher concentrations recorded cent per cent inhibition. At lower concentrations of Fytolan, Bavistin, Curzate and Tilt recorded per cent inhibition ranging from 65.31 to 85.44.

4.8.1.2. Nutmeg endophytic fungus (Nt ed f 2) with fungicides

Nt ed f 2 was incompatible with fungicides like Bordeaux mixture, Indofil M-45, Contaf and Curzate at all concentrations tested (Table 4.30). Higher concentrations of Score, Bavistin and Tilt recorded cent per cent inhibition of the endophytic fungus. The lowest inhibition of 55.55 per cent was recorded by 0.2 per cent concentration of Fytolan.

4.8.1.3. Clove endophytic fungus with fungicides (Cl ed f 2)

Cl ed f 2 isolate was incompatible with fungicides *viz.,* Bordeaux mixture, Contaf, Score, Bavistin and Tilt at all three concentrations and recorded 100 per

cent inhibition on the fungal antagonist. It is compatible with Indofil M-45 at 0.2 and 0.3 per cent concentration with per cent inhibition of 22.22 and 33.33 (Table 4.30). Cl ed f 2 recorded more than 60 per cent inhibition with Kocide, Fytolan, Score and higher concentrations of Curzate Fig. 4.1.

4.8.1.4. Cinnamon epiphytic fungus with fungicides (Cn ep f 5)

From the data given in (Table 4.31). it was revealed that the isolate was incompatible with fungicides *viz.*, Bordeaux mixture, Contaf, Score, Bavistin, Curzate and Tilt at all three concentrations tested and showed cent per cent inhibition on the growth of the antagonists. Among these fungicides tested Indofil M-45 recorded less inhibition percentage which was in the range of 21.36 to 53.31 per cent Fig 4.2. The copper based fungicides Saaf, Fytolan and Kocide recorded more than 65 per cent inhibition except kocide at 0.05 per cent which recorded 58.88 per cent inhibition on the growth of the antagonist.

4.8.1.5. Trichoderma viride (Standard culture) with fungicides

The fungicides *viz.,* Bavistin, Curzate and Contaf at all concentrations recorded 100 per cent inhibition on the growth of the standard culture *Trichoderma viride* Fig. 4.5. Indofil M-45 at 0.2 per cent concentration recorded lowest inhibition of 19.5 per cent on the growth of the bioagent. Tilt, Score and Kocide at all concentrations showed more than 60 per cent reduction on the growth of *T. viride*.

Table 4.30 Compatibility of selected fungal antagonists with fungicides

Sl. No	Fungicides	Concentration (Per cent)	Nt ep f 1		Nt ed f 2	
			Mean diameter of colony (mm)	PIOC	Mean diameter of colony (mm)	PIOC
1	Fytolan	0.2	31.22(5.53)[b]	65.31	40.00 (6.36)[b]	55.55
		0.3	21.22 (5.52)[b]	76.42	30.22 (5.54)[c]	66.42
		0.4	14.00 (3.67)[e]	84.44	25.00 (5.04)[d]	72.22
2	Kocide	0.05	18.55 (4.36)[d]	79.38	30.66 (5.57)[c]	65.93
		0.1	19.25 (4.44)[d]	78.61	25.55 (5.04)[d]	71.61
		0.15	13.55 (3.74)[e]	84.94	10.00 (3.24)[f]	88.88
3	Saaf	0.5	0 (0.71)[f]	100	35.55 (6.00)[c]	60.55
		1	0 (0.71)[f]	100	25.55 (5.04)[d]	71.61
		1.5	0 (0.71)f	100	30.00 (5.52)[c]	66.66
4	Bordeaux mixture	0.5	0 (0.71)[f]	100	0 (0.71)[g]	100
		1	0 (0.71)[f]	100	0 (0.71)[g]	100
		1.5	0 (0.71)[f]	100	0 (0.71)[g]	100
5	Indofil M-45	0.2	0 (0.71)[f]	100	0 (0.71)[g]	100
		0.3	0 (0.71)[f]	100	0 (0.71)[g]	100
		0.4	0 (0.71)[f]	100	0 (0.71)[g]	100
6	Contaf	0.5	0 (0.71)[f]	100	0 (0.71)[g]	100
		1	0 (0.71)[f]	100	0 (0.71)[g]	100
		1.5	0 (0.71)[f]	100	0 (0.71)[g]	100
7	Score	0.5	0 (0.71)[f]	100	30.22 (5.52)[c]	64.42
		1	0 (0.71)[f]	100	0 (0.71)[g]	100
		1.5	0 (0.71)[f]	100	0 (0.71)[g]	100
8	Bavistin	0.05	30.55 (5.56)[b]	66.05	30.55 (5.56)[c]	66.05
		0.1	20.88 (4.61)[c]	76.8	28.00 (5.53)[c]	68.88
		0.15	0 (0.71)[f]	100	0 (0.71)[g]	100
9	Curzate M-8	0.5	20.88 (4.61)[c]	76.8	0 (0.71)[g]	100
		1	20.45 (4.51)[c]	77.27	0 (0.71)[g]	100
		1.5	0 (0.71)[f]	100	0 (0.71)[g]	100
10	Tilt	0.5	30.55 (5.56)[b]	66.05	22.22 (4.74)[e]	75.31
		1	0 (0.71)[f]	100	0 (0.71)[g]	100
		1.5	0 (0.71)[f]	100	0 (0.71)[g]	100
11	Control	–	90 (9.51)[a]	–	90 (9.51)[a]	

* Mean of three replications, Figures in parenthesis are $\sqrt{x+0.5}$ transformed values, in each column figures followed by same letter

PIOC = Per cent Inhibition Over Control

Contd…

Table 4.31 Compatibility of selected fungal antagonists with fungicides

Sl. No	Fungicides	Cn ep f 5			Cl ed f 2		*Trichoderma viride*	
		Concentration (Per cent)	Mean diameter of colony (mm)	PIOC	Mean diameter of the colony (mm)	PIOC	Mean diameter of colony (mm)	PIOC
1	Fytolan	0.2	30.00 (5.52)^{f}	66.66	40.00 (6.36)^{d}	55.55	54.44 (7.38)^{e}	39.51
		0.3	22.22 (4.74)^{g}	75.31	28.88 (5.42)^{f}	67.91	48.00 (6.96) ^{f}	46.66
		0.4	10.00 (3.24)^{i}	88.88	13.00 (3.67)^{i}	85.55	44.44 (6.67)^{g}	50.62
2	Kocide	0.05	37.00 (6.12)^{e}	58.88	27.00 (5.24)^{g}	70.00	34.44 (5.87) ^{h}	61.73
		0.1	30.00 (5.52)^{f}	66.66	18.00 (4.30)^{h}	80.00	30.00 (5.52) ^{j}	66.66
		0.2	15.55 (4.00)^{h}	82.72	9.55 (3.17)^{e}	89.38	23.55 (4.89) ^{l}	73.83
3	Saaf	0.5	29.00 (5.43)^{f}	67.77	30.55 (5.56)^{f}	66.04	40.00 (6.36)^{e}	55.55
		1	19.00 (4.41)^{e}	78.88	28.00 (5.33)^{g}	68.88	32.22 (5.70)^{i}	64.20
		1.5	15.00 (3.93)^{g}	83.33	15.00 (3.92)^{i}	83.33	25.00 (5.04)^{h}	72.22
4	Bordeaux mixture	0.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		1	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		1.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
5	Indofil M-45	0.2	70.77 (8.80)^{b}	21.36	70.10 (80.40)^{b}	22.11	72.22 (8.51)^{b}	19.75
		0.3	67.77 (8.25)^{c}	24.70	60.00 (7.77)^{c}	33.33	67.00 (8.21)^{c}	25.55
		0.4	40.22 (6.37)^{d}	53.31	30.55 (5.52)^{f}	66.05	60.00 (7.71)^{d}	33.33
6	Contaf	0.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		1	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		1.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
7	Score	0.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	34.44 (5.87)^{h}	61.73
		1	0 (0.71)^{j}	100	0 (0.71)^{j}	100	28.00 (5.33)^{j}	68.88
		1.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	23.00 (4.84)^{l}	74.44
8	Bavistin	0.05	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		0.1	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
		0.15	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
9	Curzate M-8	0.5	0 (0.71)^{j}	100	34.44 (5.90)^{e}	61.73	0 (0.71)^{m}	100
		1	0 (0.71)^{j}	100	26.00 (5.14)^{e}	71.11	0 (0.71)^{m}	100
		1.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	0 (0.71)^{m}	100
10	Tilt	0.5	0 (0.71)^{j}	100	0 (0.71)^{j}	100	25.00 (5.04)^{k}	72.22
		1	0 (0.71)^{j}	100	0 (0.71)^{j}	100	22.22 (4.74)^{i}	75.31
		1.5	0 (0.71)^{j}	100	0(0.71)^{j}	100	20.00 (4.52)^{e}	77.77
11	Control	-	90 (9.51)^{a}	-	90 (9.51)^{a}		90 (9.51)^{a}	-

Fig 4.1. Compatibility of *Trichoderma viride* (Cl ed f 2) with fungicides

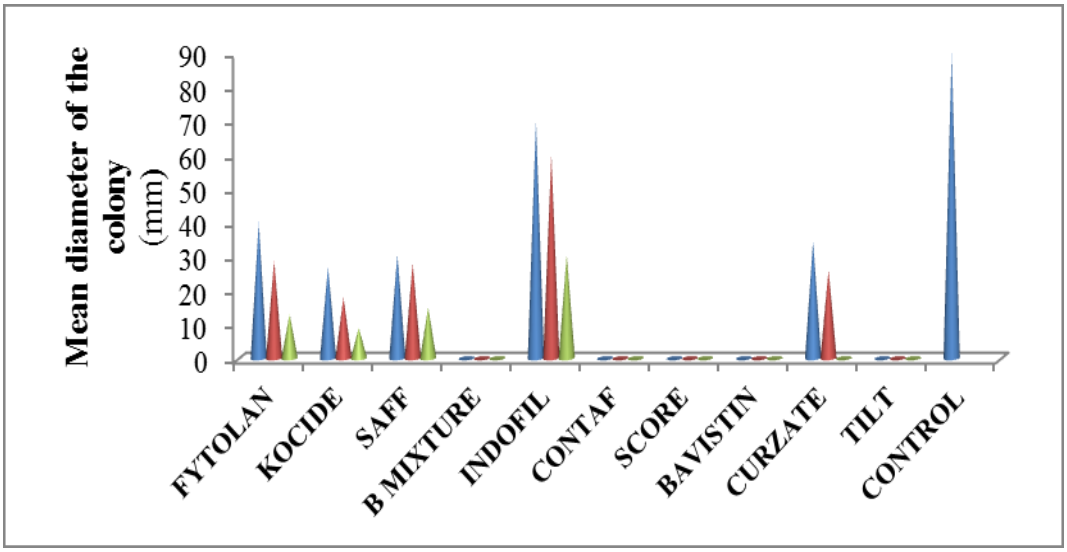
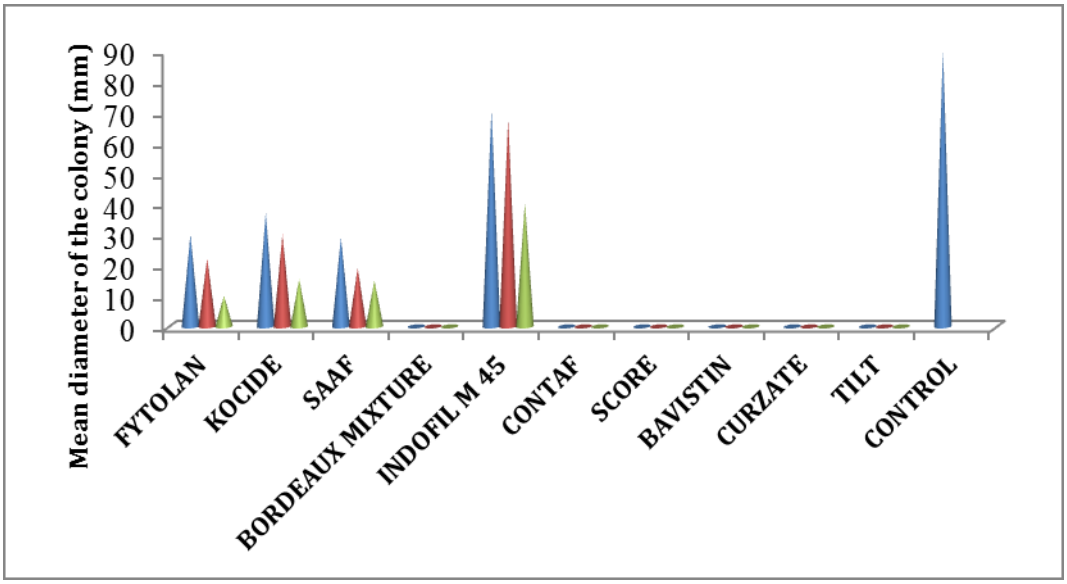


Fig 4.1. Compatibility of *Trichoderma harzianum* (Cn ep f 5) with fungicides



Fytolan (0.2%) Fytolan(0.3%) Fytolan(0.4%) Kocide (0.05%) Kocide (0.1%) Kocide (0.15%) Saaf(0.5%) Saaf (1%) saaf (1.5%) B.mixture (0.5%) B.mixture (0.1%) Indofil M-45 (0.2%) Indofil M-45(0.3%) Indofil M-45 (0.4%) Contaf (0.5%) Contaf (1%) Contaf (1.5%) Score (0.5%) Score (1%) Score (1.5%) Bavistin (0.05%) Bavistin (0.1%) Bavistin (0.15%) Curzate (0.5%) Curzate (1%) Curzate (1.5%) Tilt (0.5%) Tilt (1%) Tilt (1.5%) Control

4.8.2. Compatibility of antagonists with insecticides

The sensitivity of insecticides *viz.,* Ekalux, Chlorpyriphos, Rogor, Fame each at three different concentrations was tested under *in vitro* conditions.

4.8.2.1. Nutmeg epiphytic fungus (Nt ep f 1) with insecticides

The response of the fungal isolate Ntep f 1 to different insecticides differed significantly (Table 4.32). Cent per cent inhibition was noticed at higher concentration of Ekalux, Chlorpyriphos, Rogor and Fame. The lowest inhibition on growth of antagonist was noticed with Rogor at 0.01 per cent concentration (43.44)

4.8.2.2. Nutmeg endophytic fungus (Nt ed f 2) with insecticides

The antagonist (Nt ed f 2) exhibited varying levels of sensitivity to the different insecticides (Table 4. 32). Nt ed f 2 showed incompatibility with the insecticides *viz.,* Rogor and Fame at all three concentrations and at higher concentration of Ekalux and recorded 100 per cent inhibition on growth. Chlorpyriphos at all three concentrations and Ekalux at lower concentrations recorded more than 60 per cent inhibition.

4.8.2.3. Clove endophytic fungus (Cl ed f 2) with insecticides

Data presented in (Table. 4.32). exhibited varying levels of sensitivity of the antagonistic fungus to the different insecticides Fig. 4.3. The fungal antagonist Cl ed f 2 recorded 72.22 to 100 per cent inhibition in growth with Ekalux at all three concentrations tested. Among the other insecticides, the lowest inhibition was recorded by Fame at 0.05 per cent concentration (58.64 per cent) where as in others the reduction in growth was in range of 63.33 to 83.33 per cent over control.

4.8.2.4. Cinnamon epiphytic fungus (Cn ep f 5) with insecticides

All the four insecticides at their higher concentration recorded cent per cent inhibition of the antagonist.In addition to that Ekalux at 0.025 per cent and

Rogor at 0.02 per cent also showed cent per cent inhibition Fig. 4.4. The lowest inhibition of 61.11 per cent was noticed in Fame at 0.05 per cent concentration.

4.8.2.5. Trichoderma viride (Standard culture) with insecticides

Rogor and Ekalux at highest concentration recorded cent per cent inhibition on the growth of the fungus *T. viride*. The other insecticides at different concentrations showed more than 60 per cent inhibition which was in range of 60 to 88.88 per cent Fig.4.6.

Table 4.32.Compatibility of selected fungal antagonists with insecticides

Sl.No	Insecticides	Concentration (per cent)	Nt ep f 1		Nt ed f 2	
			Mean diameter of the colony (mm)	PIOC	Mean diameter of the colony (mm)	PIOC
1	Ekalux	0.024	20.33(4.56)^{e}	74.44	31.00 (5.61)^{c}	65.55
		0.025	20.00 (4.52)^{e}	77.77	24.00 (4.94)^{e}	73.33
		0.026	0 (0.71)^{f}	100	0 (0.71)^{f}	100
2	Chlorpyrifos	0.04	30.5 (5.56)^{c}	66.11	36.00 (6.04)^{b}	60
		0.05	25.0 (5.04)^{d}	72.22	32.00 (5.70)^{c}	64.44
		0.06	0 (0.71)^{f}	100	25.00 (5.04)^{d}	72.22
3	Rogor	0.01	50.9 (7.16)^{b}	43.44	0 (0.71)^{f}	100
		0.02	20.5 (4.58)^{e}	72.22	0 (0.71)^{f}	100
		0.03	0(0.71)^{f}	100	0 (0.71)^{f}	100
4	Fame	0.05	30.00 (5.52)^{c}	66.66	0 (0.71)^{f}	100
		0.1	25.00 (5.04)^{d}	72.22	0 (0.71)^{f}	100
		0.2	0 (0.71)^{f}	100	0 (0.71)^{f}	100
5	Control	-	90 (9.51)^{a}	-	90 (9.51)^{a}	-

* Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are √+0.5 transformed values.
PIOC = Per cent Inhibition Over Control

Table. 4.33. Compatibility selected fungal antagonists with insecticides

Sl.No	Insecticides	Concentration (percent)	Cn ep f 5		Cl ed f 2		*Trichoderma viride*	
			Mean diameter of the colony (mm)	PIOC	Mean diameter of the colony (mm)	PIOC	Mean diameter of the colony (mm)	PIOC
1	Ekalux	0.024	15.00 (3.93)^{f}	83.33	25.00 (5.04)^{g}	72.22	17.00(4.12)^{h}	81.11
		0.025	0 (0.71)^{g}	100	0 (0.71)^{i}	100	10.00(3.24)^{j}	88.88
		0.026	0 (0.71)^{g}	100	0 (0.71)^{i}	100	0 (0.71)^{k}	100
2	Chlorpyrifos	0.04	30.00 (5.52)^{c}	66.66	29.00 (5.43)^{e}	67.77	35.00 (5.95)^{c}	61.11
		0.05	28.00 (5.33)^{d}	68.88	27.00 (5.24)^{f}	70.00	30.00 (5.52)^{d}	66.66
		0.06	0 (0.71)^{g}	100	16.55 (4.12)^{d}	81.61	12.00 (3.53)^{i}	86.66
3	Rogor	0.01	25.00 (5.04)^{e}	72.22	33.00 (5.78)^{d}	63.33	30.55 (5.52)^{d}	66.05
		0.02	0 (0.71)^{g}	100	30.00 (5.52)^{e}	66.66	25.00 (5.04)^{f}	72.22
		0.03	0 (0.71)^{g}	100	27.00 (5.24)^{f}	70.00	0 (0.71)^{k}	100
4	Fame	0.05	35.00 (5.95)^{b}	61.11	37.22 (6.14)^{b}	58.64	36.55 (6.08)^{b}	59.38
		0.1	28.00 (5.33)^{d}	68.88	31.00 (5.61)^{c}	65.55	28.00(5.33)^{e}	68.88
		0.2	0 (0.71)^{g}	100	15.00 (3.93)^{h}	83.33	22.00 (4.74)^{g}	75.55
5	Control	_	90 (9.51)^{a}	_	90 (9.51)^{a}		90 (9.51)^{a}	_

* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are √+0.5 transformed values.
PIOC = Per cent Inhibition Over Control

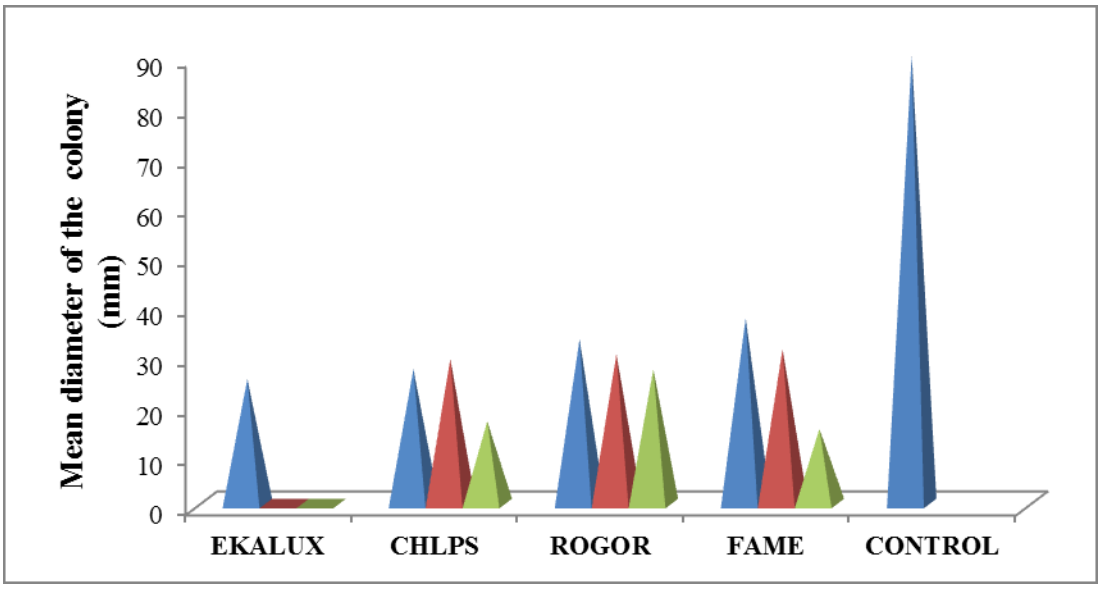


Fig 4.3 Compatibility of *Trichoderma viride* (Cl ed f 2) with insecticides

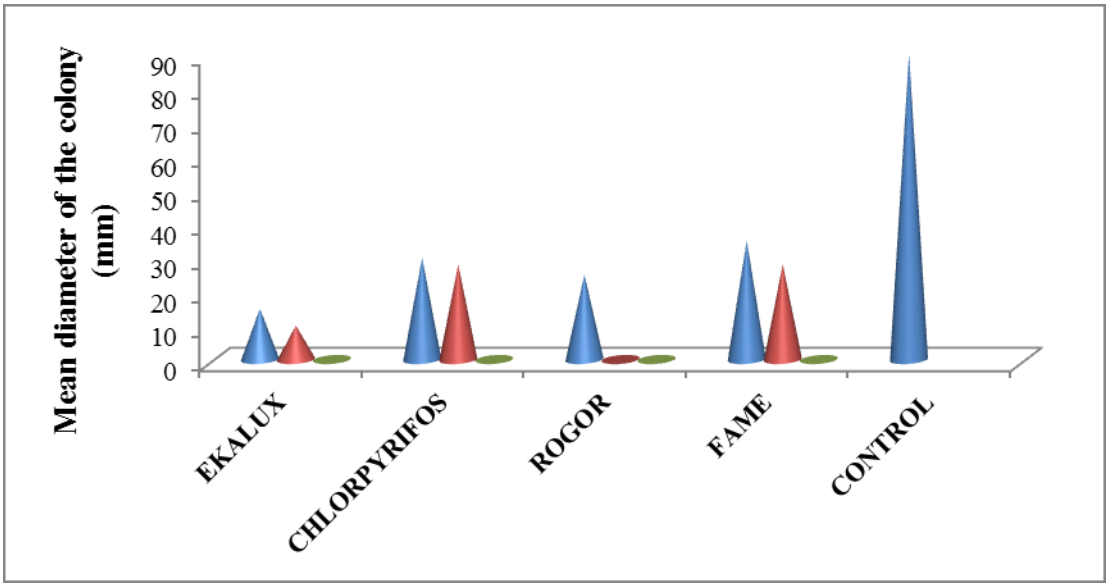


Fig 4.4 Compatibility of *Trichoderma harzianum* (Cn ep f 5) with insecticides

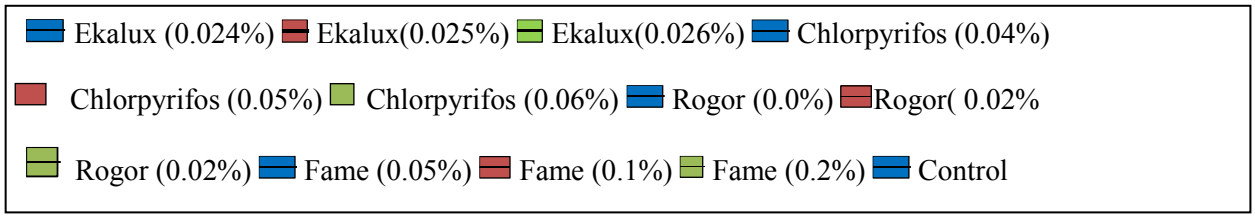
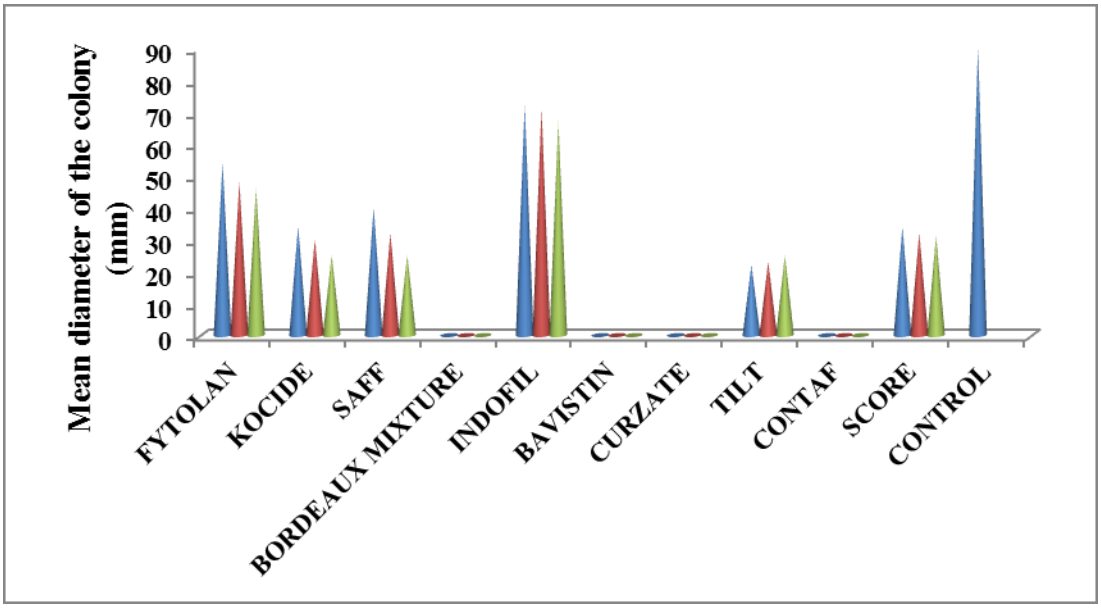


Fig 4.5 Compatibility of standard culture *Trichoderma viride* with fungicides



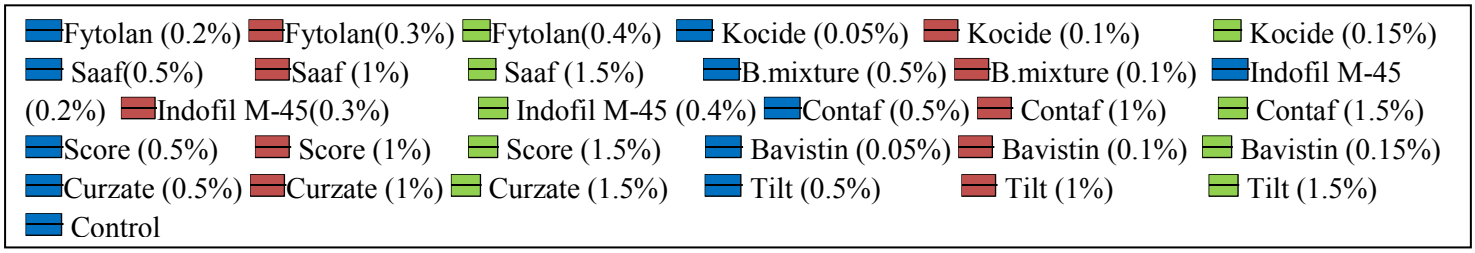
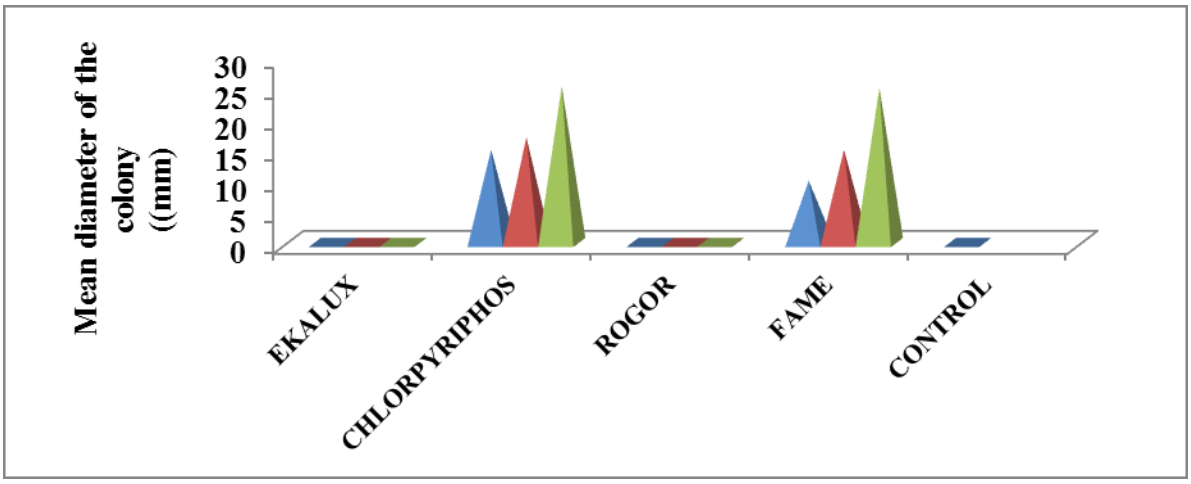
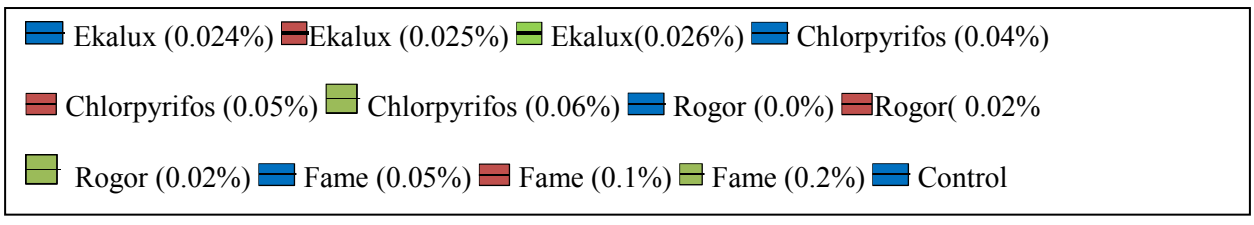


Fig 4.6 Compatibility of standard culture *Trichoderma viride* with insecticides





4.8.3. Bacteria

The bacterial antagonists selected from the *in vitro* evaluation*viz.,*Nt ep b 2, Nt ed b 6, Cl ep b 6 and Cl ed b 2 and standard culture *P. fluorescens* were tested for their compatibility to 10 different fungicides each at three different concentrations.

4.8.3.1. Pseudomonas sp. (*Nt ep b 2) with fungicides*

From data given in (Table 4.34). it was revealed that Bordeaux mixture at all three different concentrations showed higher inhibitory effect on the bacterial antagonist and among them 1.5 per cent concentration of Bordeaux mixture recorded the highest inhibition of 30.22 per cent.

The antagonist was found compatible with Saaf and Tilt at all the three concentrations and Curzate at two lower concentrations on the growth with 0 per cent inhibition. The fungicides like Fytolan, Kocide, Score, Bavistin, Contaf and Indofil M-45 at all three concentrations showed the inhibition on the growth of antagonist in the range of 8.38 and 22.22 per cent.

4.8.3.2. Pseudomonas sp. (Nt ed b 6) with fungicides

The response of isolate *Pseudomonas* sp. (Nt ed b 6) to different fungicides at three different concentrations varied significantly (Table 4.34).The antagonist was found compatible with Indofil M-45, Contaf and Curzate at all the three concentrations and Kocide at two lower concentrations recorded cent per cent inhibition on the growth. The other fungicides recorded the inhibition in the growth of the antagonist in the range of 7.70 to 33.57 per cent.

4.8.3.3. Bacillus sp. *(Cl ep b 6) with fungicides*

Data presented in (Table. 4.34). revealed the response of the isolate to various concentrations of fungicides. The antagonist was found compatibleIndofil M-45 and Curzate at all three concentrations with cent per cent inhibition. The other fungicides

recorded the inhibition in the growth of antagonist in the range of 6.66 to 32.46 per cent.

4.8.3.4. Pseudomonas sp. *(Cl ed b 2) with fungicides*

The response of isolate *Pseudomonas* sp. (Cl ed b 2) to different concentrations of fungicides varied significantly (Table 4.35).The antagonist was found compatible with Indofil M-45, Contaf and Curzate at all the three concentrations and Kocide at two lower concentrations recorded cent per cent inhibition on the growth. The other fungicides recorded the inhibition in the growth of antagonist in the range of 7.7 to 33.57 per cent.

4.8.3.5. *Pseudomonas fluorescens (Standard culture) with fungicides*

The standard culture of *P. fluorescens* (KAU) recorded the compatibility with all three concentrations of Contaf and Curzate and at two different concentrations of Bavistin and at lower concentration of Indofil M-45. In case of other fungicides at different concentrations, the per cent inhibition on the growth of the antagonist vary and was in range of 8.52 to 36.30 per cent. The highest reduction in per cent inhibition (36.30 per cent) was recorded by Bordeaux mixture at 1.5 per cent concentration.

Table 4.34.Compatibility of selected bacterial antagonists with fungicides

Sl. No	Fungicides	Concentration (Per cent)	Nt ep b 2		Nt ed b 6	
			Mean diameter of inhibition zone (mm)	PIOC	Mean diameter of inhibition zone (mm)	PIOC
1	Fytolan	0.2	9.55 (3.17)^{i}	10.61	10.55 (3.32)^{b}	11.72
		0.3	12.00 (3.53)^{g}	13.33	15.22 (3.96)^{f}	16.91
		0.4	15.55 (4.00)^{f}	17.16	18.22 (4.32)^{d}	20.13
2	Kocide	0.05	10.55 (3.32)^{g}	11.72	0 (0.71)^{j}	0
		0.1	15.22(3.96)^{h}	17.22	0 (0.71)^{j}	0
		0.2	20.00 (4.52)^{d}	22.22	9.55 (3.17)^{g}	10.61
3	Saaf	0.5	0 (0.71)^{j}	0	15.22 (3.96)^{e}	16.91
		1	0 (0.71)^{j}	0	17.22 (3.24)^{h}	19.13
		1.5	0 (0.71)^{j}	0	22 (4.74)^{c}	24.44
4	Bordeaux mixture	0.5	22.22 (4.76)^{c}	24.68	22.5 (4.76)^{c}	25
		1	24.00 (4.94)^{b}	26.66	26.55 (5.20)^{b}	29.55
		1.5	27.22 (5.26)^{a}	30.22	30.22 (5.54)^{a}	33.57
5	Indofil M-45	0.2	8.55 (3.08)^{j}	9.55	0 (0.71)^{j}	0
		0.3	10.00 (3.24)^{i}	11.11	0 (0.71)^{j}	0
		0.4	15.22 (3.96)^{f}	16.91	0 (0.71)^{j}	0
6	Bavistin	0.05	15.00 (3.93)^{f}	16.68	7 (2.73)^{j}	7.7
		0.1	17.22 (4.20)^{e}	19.68	12 (3.53)^{g}	13.33
		1.5	18.11 (4.31)^{e}	20.12	15 (3.93)^{f}	16.66
7	Score	0.15	11.55 (3.47)^{g}	12.83	8(2.91)^{i}	18
		1	14 (3.80)^{e}	15.55	10 (3.24)^{h}	11.11
		1.5	15 (3.93)^{e}	16.66	15 (3.93)^{f}	16.66
8	Contaf	0.5	7.55(2.83)^{k}	8.38	0 (0.71)^{j}	0
		1	11 (3.39)^{h}	12.83	0 (0.71)^{j}	0
		1.5	16 (4.06)^{f}	17.77	0 (0.71)^{j}	0
9	Curzate M-8	0.5	0 (0.71)^{j}	0	0 (0.71)^{j}	0
		1	0 (0.71)^{j}	0	0 (0.71)^{j}	0
		1.5	12.55 (3.61)^{j}	13.94	0 (0.71)^{j}	0
10	Tilt	0.5	0 (0.71)^{j}	0	8 (2.91)^{i}	8.88
		1	0 (0.71)^{j}	0	11(3.91)^{f}	12.22
		1.5	0 (0.71)^{j}	0	16.55 (4.12)^{e}	18.38
11	Control	-	0 (0.71)^{j}	0	0 (0.71)^{j}	0

*Mean of three replications

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

Figures in parenthesis are √+0.5 transformed values. PIOC = Per cent Inhibition Over Control (Contd……..**Table.**

4.35. Compatibility of selected bacterial antagonists with fungicides

Sl. No	Fungicides	Concentration (Per cent)	Cl ed b 2		Cl ep b 6		*P. fluorescens (*KAU)	
			Mean diameter of Inhibition zone (mm)	PIOC	Mean diameter of Inhibition zone (mm)	PIOC	Mean diameter of Inhibition zone (mm)	PIOC
1	Fytolan	0.2	10.00 (3.24)^{g}	11.11	14.00 (3.80)^{g}	15.55	20.33(4.56)^{f}	22.59
		0.3	12.55 (3.61)^{e}	13.94	15.00 (3.93)^{f}	16.66	20.67(4.60)^{f}	22.97
		0.4	13.22 (3.70)^{d}	14.68	21.00(3.53)^{h}	23.33	23.00(4.84)^{e}	22.56
2	Kocide	0.05	12.55 (3.61)^{e}	13.94	16.00(4.06)^{e}	17.77	16.67(4.41)^{g}	18.52
		0.1	15.22 (3.96)^{c}	16.91	21.00(4.63)^{c}	23.33	19.00(4.42)^{g}	21.11
		0.15	18.22 (4.32)^{b}	20.24	25.00(5.04)^{b}	27.77	23.67(4.91)^{d}	26.3
3	Saaf	0.5	0 (0.71)^{j}	0	8.00(2.91)^{j}	8.88	10.33(3.29)^{e}	11.44
		0.1	0 (0.71)^{j}	0	15.00(3.93)^{f}	16.66	17.55(4.24)^{h}	19.5
		1.5	0 (0.71)^{j}	0	18.00(4.30)^{d}	20.00	20 (4.52)^{f}	22.22
4	Bordeaux mixture	0.5	8.55 (3.08)^{h}	9.5	24.00(4.94)^{b}	26.00	27.67 (5.30)^{c}	30.74
		1	12.55 (3.61)^{e}	13.74	28.00(5.33)^{a}	31.11	31.00 (5.61)^{b}	34.44
		1.5	19.22 (4.44)^{a}	21.35	29.22(5.45)^{a}	32.46	32.67 (5.76)^{a}	36.3
5	Indofil M-45	0.2	0 (0.71)^{j}	0	0(0.71)^{l}	0	0 (0.71)^{n}	0
		0.3	0 (0.71)^{j}	0	0(0.71)^{l}	0	9 (3.08)^{l}	10
		0.4	0 (0.71)^{j}	0	0(0.71)^{l}	0	12.55 (3.61)^{k}	13.94
6	Bavistin	0.05	0 (0.71)^{j}	0	6.00(2.54)^{k}	6.66	0 (0.71)^{n}	0
		0.1	7 (2.83)^{b}	7.77	9.00(3.08)^{j}	10.00	0 (0.71)^{n}	0
		0.15	10.55 (3.32)^{f}	11.72	15.00(3.93)^{f}	16.66	7.67 (2.86)^{m}	8.52
7	Score	0.5	9.55 (3.17)^{f}	10.61	9.00(3.08)^{j}	10.00	10.55 (3.32)^{k}	11.72
		1	13.55(3.74)^{e}	15.05	10.00(3.24)^{i}	11.11	12.00 (3.23)^{l}	13.33
		1.5	15.22 (3.96)^{c}	17.24	15.00(3.93)^{f}	16.66	18.00 (4.30)^{h}	20
8	Contaf	0.5	8.0 (2.91)^{b}	8.88	7.00(2.73)^{c}	7.77	0 (0.71)^{n}	0
		1	15.0 (3.93)^{d}	16.66	10.00(3.24)^{i}	11.11	0 (0.71)^{n}	0
		1.5	18.0 (4.30)^{c}	20	16.00(4.06)^{e}	17.77	0 (0.71)^{n}	0
9	Curzate M-8	0.5	0 (0.71)^{j}	0	0(0.71)^{l}	0	0 (0.71)^{n}	0
		1	0 (0.71)^{j}	0	0(0.71)^{l}	0	0 (0.71)^{n}	0
		1.5	0 (0.71)^{j}	0	0(0.71)^{l}	0	0 (0.71)^{n}	0
10	Tilt	0.5	0 (0.71)^{j}	0	6.00(2.54)^{k}	6.66	10.55 (3.32)^{d}	11.72
		1	0 (0.71)^{j}	0	8.00(2.94)^{j}	8.88	12.00 (3.80)^{c}	15.55
		1.5	0 (0.71)^{j}	16.66	14.00(3.80)^{f}	15.55	18.00 (3.93)^{c}	16.66
11	Control	-	0 (0.71)^{j}	0	0(0.71)^{l}	0	0 (0.71)^{a}	0

* Mean of three replications

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

Figures in parenthesis are √+0.5 transformed values.

PIOC = Per cent Inhibition Over Control

4.8.4. Insecticides

The *in vitro* sensitivity of four insecticides *viz.,* Ekalux, Chlorrpyriphos, Rogor, Fame each at three concentrations were tested against four epiphytic and endophytic bacterial antagonists.

4.8.4.1. Pseudomonas sp. *(Nt ed b 6) with insecticides*

The response of bacterial isolate recorded variation to different concentrations of fungicides (Table 4.36).The bacterial isolate compatible with Ekalux at all the three concentrations followed by Rogor at 0.01 per cent concentrations with zero per cent inhibition. Other insecticides at various concentrations recorded per cent inhibition ranging from 6.00 to 15.55.

4.8.4.2. Pseudomonas sp. *(Nt ep b 2) with insecticides*

Data presented in the (Table 4.36). clearly indicate the variation in response of bacterial isolate to various concentrations of insecticides. The bacterial isolate found to be compatible with Ekalux at all three concentrations. The other insecticides recorded per cent inhibition ranging from 9.00 to 22.00.

4.8.4.3. Bacillus sp. *(Cl ep b 6) with insecticides*

Bacterial isolate recorded wide range of variation in response to various concentrations of insecticides. The bacterial isolate compatible with Rogor at all the three concentrations tested with zero per cent inhibition and Ekalux at 0.024 per cent concentration with 0 per cent inhibition (Table 4.36).The other insecticides at different concentrations recorded per cent inhibition ranging from 8.00 to 17.55.

4.8.3.4. Pseudomonas sp. *(Cl ed b 2) with insecticides*

Data presented in the (Table 4.37). exhibited the variation in response of bacterial isolate to various concentrations insecticides. The bacterial isolate was

found to be compatible with Ekalux at all three concentrations with zero per cent inhibition. The other insecticides at different concentrations recorded per cent inhibition ranging from 7.77 to 24.44.

4.8.3.5. Pseudomonas fluorescens (Standard culture) with insecticides

The standard culture of *P. fluorescens* (KAU) recorded the compatibility with Ekalux and Rogor at all three concentrations. The other insecticides per cent inhibition ranging from 16.66 to 28.02 per cent.

Table. 4.36. Compatibility of selected bacterial antagonists with insecticides

Sl.No	Insecticides	Nt ed b 6			Nt ep b 2	
		Concentration (Per cent)	Mean diameter of the inhibition zone (mm)	PIOC	Mean diameter of the inhibition zone (mm)	PIOC
1	Ekalux	0.024	0 (0.71)^{h}	0	0 (0.71)^{h}	0
		0.025	0(0.71) ^{h}	0	0 (0.71)^{h}	0
		0.026	0 (0.71)^{h}	0	0 (0.71)^{h}	0
2	Chlorpyriphos	0.04	6.00 (2.54)^{g}	6.66	15 (3.93)^{d}	16.66
		0.05	10.00 (3.24)^{e}	11.11	19 (4.41)^{b}	21.11
		0.06	14.22 (3.83)^{c}	15.8	22 (4.74)^{a}	24.44
3	Rogor	0.01	0 (0.71)^{h}	0	0 (0.71)^{h}	0
		0.02	10.00 (3.24)^{e}	11.11	0 (0.71)^{h}	0
		0.03	15.55 (4.06)^{a}	17.27	10 (3.24)^{f}	11.11
4	Fame	0.05	8.00 (2.91)^{f}	8.88	9 (3.08)^{g}	10.00
		0.1	12.00 (3.53)^{c}	13.33	12 (3.53)^{e}	13.33
		0.2	15.00 (3.93)^{b}	16.66	17 (4.1)^{c}	18.88
5	Control	_	0 (0.71)^{h}	0	0 (0.71)^{h}	0

* Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are √+0.5 transformed values.
PIOC = Per cent Inhibition Over Control

(Contd….

Table 4.37 Compatibility of selected bacterial antagonists with insecticides

*Mean of three replications

Sl.No	Insecticides	Cl ed b 2			Cl ep b 6		*P. fluorescens* (KAU)	
		Concentration (Per cent)	Mean diameter of the inhibition zone (mm)	PIOC	Mean diameter of the inhibition zone (mm)	PIOC	Mean diameter of the inhibition zone (mm)	PIOC
1	Ekalux	0.024	0 (0.71)^{h}	0	0 (0.71)^{g}	0	0 (0.71)^{e}	0
		0.025	0 (0.71)^{h}	0	8 (2.91)^{b}		0 (0.71)^{e}	0
		0.026	0 (0.71)^{h}	0	13 (3.67)^{d}	8.88	0 (0.71)^{e}	0
2	Chlorrpyrifos	0.04	12.22 (3.56)^{f}	13.57	9 (3.08)^{e}	14.44	15 (3.93)^{c}	16.66
		0.05	15 (3.93)^{e}	16.66	14.22 (3.83)^{c}	10.6	17 (4.18)^{b}	18.88
		0.06	20.55 (4.58)^{c}	22.83	17.5 (4.24)^{a}	15.8	25.22(5.07)^{a}	28.02
3	Rogor	0.01	10 (3.24)^{c}	11.11	0 (0.71)^{g}	19.44	0 (0.71)^{e}	0
		0.02	15.55 (4.00)^{e}	17.27	0 (0.71)^{g}	0	0 (0.71)^{e}	0
		0.03	21.22 (4.68)^{b}	23.57	0 (0.71)^{g}	0	0 (0.71)^{e}	0
4	Fame	0.05	7.00 (2.73)^{g}	7.77	9.00 (3.08)^{e}	0	10.00(3.24)^{d}	11.11
		0.1	15.00 (3.93)^{e}	16.66	13 (3.67)^{d}	10.00	15.00(3.93)^{e}	16.66
		0.2	22.00 (4.71)^{a}	24.44	16 (4.06)^{b}	14.44	25.00 (5.04)^{a}	27.77
5	Control	-	0 (0.71)^{g}	0	0 (0.71)^{g}	17.77	0 (0.71)^{e}	0
						0		

In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are √+0.5 transformed values. PIOC = Per cent Inhibition Over Control

Pioc = per cent Inhibition Over Control

4.9. IDENTIFICATION OF EFFICENT EPIPHYTIC AND ENDOPHYTIC ANTAGONISTS

4.9.1. Fungal antagonists

The fungi *viz.,* Nt ed f 2, Nt ep f 1, Cl ed f 2 and Cn ep f 5 was selected as efficient antagonists against the pathogens of nutmeg, clove and cinnamon. The cultural and morphological characters of these antagonists were studied on PDA medium for their mycelial characters comparing that of given in CMI descriptions of Pathogenic Fungi and Bacteria.

4.9.1.1. Nt ed f 2 (Nutmeg endophytic fungus)

Cultures grew rapidly in 12 days, hyphae thin walled, hyaline, conidiogenous cells, phialidic, mostly solitary, conidia produced singly at the tip of phialides and aggregating into slimy heads, ellipsoid to cylindrical with rounded ends. Straight or sometimes slightly curved single celled and hyaline. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T), New Delhi (Id no. 6762.15) the antagonist was identified as *Acremonium kilense.*

4.9.1.2. Nt ep f 1 (Nutmeg epiphytic fungus)

Hyphae irregularly swollen though without characteristic hyphalswellings.Sporangiphore, regular, sporangia abundant on solid media, broadly and regulary ellipsoid or ovoid to obpyriform, (36-50 x 28-35 µm) apex with a hemi spherical papilla with apical thickening with a deciduous pedicel. Cultures usually slightly radiate with uniform slight aerial mycelium. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi, (Id no. 6763.15) the antagonist was identified as *Phytophthora cactorum.*

4.9.1.3. Cl ed f 2 (Clove endophytic fungus 2)

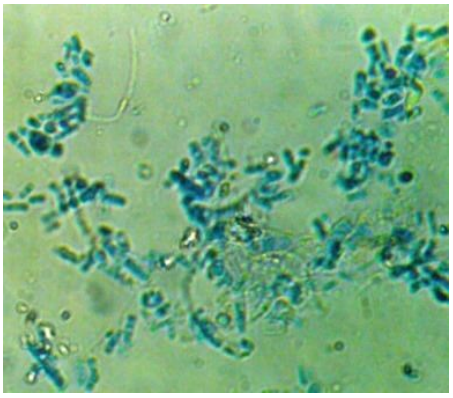
Colonies were dark green, fast growing, formed smooth sparse mycelial mat, which later became hairy and formed loose scanty aerial hyphae. Mycelium hyaline, smooth walled, septate and much branched. Conidiophores arise in compact or loose tuft, main branches produced several side branches in groups of two to three. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with reference (Id no. 6765.15) identified as *Trichoderma viride.*

4.9.1.4. Cn ep f 5 (Cinnamon epiphytic fungus)

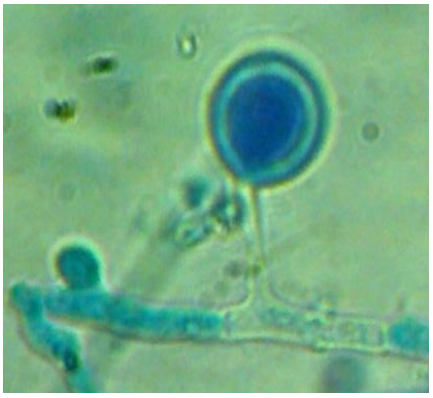
Colonies fast growing white green to bright green with the sporulation in concentric rings as time proceeds. Hyphae hyaline, septate, smooth with 3.84-4.24 µm width. Conidiphores are loose tuft, main branches produced numerous side branches at right angles. Phialides sharp pointed.

Phialospores accumulated at the tip of phialides, subglobose, short obovoid, with truncate base, smooth, pale green with 1.35 µm in size. Based on these characters, and coupled with pathogenicity on cinnamon confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with reference Id no. (6764.15) identified as *Trichoderma harzianum.*

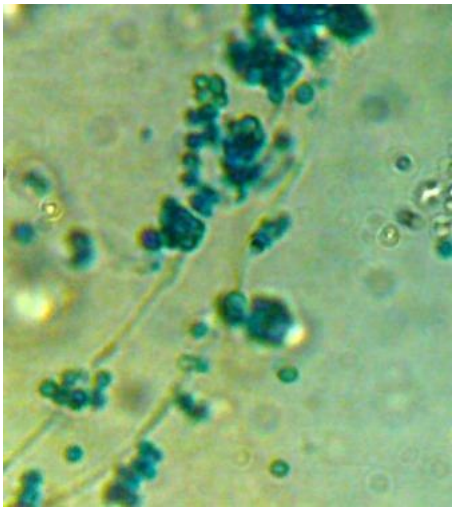
PLATE 4.6. IDENTIFICATION OF ANTAGONISTS



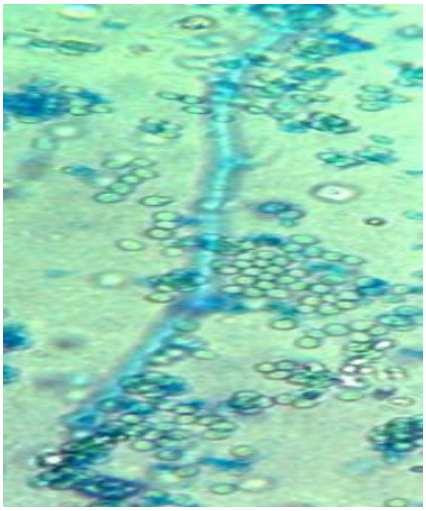
Acremonium kilense



Phytophthora cactorum



Trichoderma viride



Trichoderma harzianum

4.9.2. Bacterial antagonists

4.9.2.1. Characterization of bacterial antagonists

The cultural and biochemical characters of the bacterial isolates Nt ep b 2 = Nutmeg epiphytic bacteria 2, Nt ed b 6 = Nutmeg endophytic bacteria, Cl ep b 6 = Clove epiphytic bacteria, Cl ed b 2 = Clove endophytic bacteria and *P. fluorescens* (KAU) are presented in Table.4. 22.

4.9.2.2. Bacillus sp. (Cl ep b 6)

Colonies were large, irregular, dry, flat and cream coloured with undulated margin, Gram positive, rod shaped, forms endospore. Positive for biochemical tests including for catalase, gelatin liquefication, starch hydrolysis, utilization of citrate, lysine and ornithine, nitrate reduction and utilization of glucose, sucrose, fructose, lactose, and arabinose negative for indole production, denitrification, urease and H_2S production. Based on these tests, the bacteria was tentatively identified as *Bacillus* sp.

4.9.2.3. Pseudomonas sp. (Cl ed b 2, Nt ed b 6, Nt ep b 2)

The isolates were Gram negative short rods, showed growth at 4 ° C, failed to grow at 41°C. The isolate were positive in catalase activity, oxidase reaction, levan production, starch hydrolysis, urease activity, arginine hydrolase and nitrate reduction reaction, Gram negative reaction. Based on the cultural and biochemical characters the isolates were tentatively identified as *Pseudomonas* sp

Table 4.38. Cultural and biochemical characters of the bacterial isolates

Sl.No	Cultural and biochemical characters	Bacterial isolates				
		Cl ed b 2	**Cl ep b 6**	**Nt ep b 2**	**Nt ed b 6**	***P. f* (K)**
1	Gram's reaction	–	+	–	–	–
2	Lysine utilization	–	–	+	–	–
3	Ornithine utilization	–	+	+	–	–
4	Urease production	+	+	+	+	+
5	Phenylalanine deamination	–	–	–	–	–
6	Nitrate reduction	+	+	–	–	–
7	H_2S Production	–	–	–	–	–
8	Glucose utilization	–	–	–	–	+
9	Adonitol utilization	–	–	–	–	+
10	Lactose utilization	–	–	–	–	+
11	Arabinose utilization	–	–	–	–	+
12	Sorbitol utilization	–	–	–	–	–
13	Citrate utilization	+	+	+	+	+

+ Positive reaction - Negative reaction

Discussion

5. DISCUSSION

Nutmeg, clove and cinnamon are the important tree spices cultivated in Kerala fetching regular earning to the farmers. Among these spices, nutmeg is being cultivated by majority of farmers owing to its remunerative prices. One of the constraints in the production and productivity of tree spices is the occurrence of diseases. The humid tropical climatic conditions of the State is conducive for the growth of many fungal pathogens and hence, many of them are endemic in nature. At times, some of the diseases assume serious proportions especially during monsoon periods resulting in heavy crop losses. No resistant or tolerant cultivars are identified so far. So, fungicides are generally used for the management of the diseases. However, use of chemical fungicides not always give a desired effect due to improper method of application and adverse climatic conditions.

For effective ecofriendly management of plant diseases it is desirable to identify and select an agent which not only adversely affects the pathogen but also plays a role in augmenting the resistance mechanism of the host. In this aspect, the role of microbes which are in association with the host plants with innate antagonistic potential and also in triggering the host response against the invading pathogen are important. Rajendran *et al.* (2006) opined that antagonistic endophytic and epiphytic microflora are alternatives to fungicides that can be more reliable ecologically and economically sustainable. Since, different fungal pathogens are known to attack nutmeg, clove and cinnamon in Kerala, a study on the usefulness of antagonistic microbes associated with them will suggest a novel approach in the sustainable management of diseases of these tree spices. Therefore the present study was made to identify the pathogens causing foliar diseases of tree spices and to select potential antagonistic microorganisms with desirable characteristics in the sustainable disease management.

5.1 ISOLATION OF PATHOGENS FROM NUTMEG, CLOVE AND CINNAMON

Surveys were conducted at different locations of Thrissur, Ernakulam, Wayanad, Thiruvananthapuram and Palakkad districts for the collection of diseased specimens of nutmeg, clove and cinnamon. The organisms associated with these disease specimens were isolated. A total of five organisms from nutmeg, three from clove and two from cinnamon were isolated and their pathogenicity established.

5.1.1. Symptomatology of diseases of nutmeg and identification of pathogens

During the survey, five major foliar diseases of nutmeg *viz., Colletotrichum* leaf spot/ shot-hole, *Phytophthora* leaf fall, grey blight, seedling blight and horse hair blight were observed at different locations of Kerala. In *Colletotrichum* leaf spot, small dark brown lesion with yellow halo was observed on leaf lamina where as in *Phytophthora* leaf fall, large dark brown water soaked lesions were developed initiating along the midrib and veins of leaves. The grey blight was observed near the margins of leaf lamina which later developed into lesions with greyish white centre with dark brown margin. *Rhizoctonia solani* was noticed on seedlings causing blight while horse hair blight disease occurred on grown up plants with the presence of hair like fungal growth attached at random leading to defoliation

Among these diseases, *Colletotrichum* leaf spot is endemic in nature and at times assumes epiphytic proportion during rainy season resulting in heavy defoliation. Karunakaran (1981) also reported the occurrence of this disease in nutmeg and other diseases except *Phytophthora* leaf fall. However, from 2012 onwards a leaf fall disease was observed in a severe form in nutmeg from Central Kerala (Mathew and Beena 2012). During the period of study, extensive defoliation in nutmeg incited by *Phytophthora* sp. was observed and it can be considered as an emerging serious disease which requires greater attention.

Further, it was also noticed that the horse hair blight was prevalent in all the places surveyed on grown up plants. Serious occurrence of disease was reported from Ernakulam district by Sakaria *et al.* (2000).

The organisms associated with nutmeg diseases observed during the study were isolated and their pathogenicity established. The cultural and morphological characters of the pathogens were studied in comparison with descriptions given in CMI descriptions of Pathogenic Fungi and Bacteria. Based on the cultural and morphological characters of the pathogens coupled with pathogenicity on nutmeg, these were identified as *Colletotrichum gloeosporioides*, *Phytophthora* sp. *Pestalotiopsis palmarum*, *Rhizoctonia solani* and *Marasmius* sp. inciting leaf spot and shot-hole, *Phytophthora* leaf fall, grey blight, seedling blight, horse hair blight respectively.The results of the study are in line with that of Karunakaran and Nair, 1980; Karunakaran 1981; Sakaria *et al.* 2000 and Mathew and Beena, 2012. However, occurrence of nutmeg seedling blight by *R.solani* has not been reported so far and thus it need further detailed investigations to ascertain the extent of damage caused by the pathogen.

5.1.2. Symptomatology of diseases of clove and identification of pathogens

Leaf spot and twig blight, leaf blotch, grey blight were noticed during the study. Symptomatological studies of these diseases showed the symptoms of leaf spot and twig blight and grey blight were almost similar to that observed in nutmeg. Similarly, the causative agent were identified as *Colletotrichum gloeosporioides* and *Pestalotiopsis palmarum*, based on the cultural and morphological characters and also by that reported earlier (Jose and Paily, 1966; Karunakaran, 1981). The leaf blotch disease was observed in severe form during rainy season resulting in extensive defoliation. The disease was characterized by development of water soaked lesions mainly on the margin and the tip of the leaf. Reddish brown pigmentation during sporulation with typical conidiophores and conidia were observed. Based on cultural and morphological characters studied, the causative agent was identified as *Cylindrocladium quinqueseptatum*.

Extensive damage by this pathogen on clove seedlings were reported by many workers (Wilson *et al.,* 1979; Sulochana, 1980; Beena *et al.*1994 and Jayasinghe and Wijesundhara, 1999)

5.1.3. Symptomatology of diseases of cinnamon and identification of pathogens

Only two foliage diseases were noticed during the survey namely, leaf spot and dieback, and grey blight. The cultural and morphological characters of the pathogens indicated that they belong to the species of *C. gloeosporioides* and *P. palmarum*. Seshadri *et al.* (1972) from Tamil Nadu reported leaf spot disease of this crop incited by *C. cinnamon* but Karunakaran and Nair (1980) reported the organism as *C. gloeosporioides*. Further, detailed investigations by Karunakaran (1981) also revealed that *Colletotrichum gloeosporioides* was the casual agent of leaf spot and dieback disease of cinnamon. He also confirmed the casual agent of grey blight as *Pestalotiopsis palmarum.*

5.2 ISOLATION, ENUMERATION AND EVALUATION OF EPIPHYTIC ANTAGONISTS

It is well known that aerial parts of plants are habitat of many harmful or beneficial microflora. Harmful microflora may inflect damage to the plant during conducive environment. However, among the beneficial ones, some of them may be actively antagonistic by protecting plant from invasion of harmful microbes. But compared to antagonistic microbes from soil, aerial antagonists are reported to be less efficient due to obvious reasons inherent in their respective niche. Inspite of this, efforts are being made to exploit the potential of natural epiphytic antagonistic microflora for the management of many plant diseases. With this aim, epiphytic microflora associated with healthy leaves of nutmeg, clove and cinnamon, collected from heavily infected gardens were enumerated. On the leaf surface, the population of bacteria and fluorescent Pseudomonads was more than that of fungi. The population of epiphytes also varied with the place of collection. Predominance of epiphytic bacteria over fungi on cocoa pod surface was reported

by Galindo (1992) and Bhavani (2004). They attributed the abundance of bacteria on pod surface due to factors favouring the growth of the crops, like high humidity.

It is well known that all the microbes associated with the host surface may not be antagonistic to the pathogen. Therefore, a preliminary screening on the antagonistic nature of epiphytic microbes isolated were done which revealed that out of 71 epiphytic microbes, 32 showed antagonistic reaction against the pathogens tested. Of the 32 microbes, 10 were fungi, 12 bacteria and 10 fluorescent Pseudomonads. A perusal of the literature revealed similar type of studies conducted in other crops also (Bhavani, 2004; Dionisio *et al.,* 2008 and Weininghao *et al*., 2011). Out of the 10 isolates of epiphytic fungi, four were isolated from nutmeg and while three each from clove and cinnamon. These were further evaluated under *in vitro* conditions against the pathogens of the respective host following dual culture method (Dennis and Webster, 1971). Their efficacy was compared with the standard culture of *Trichoderma viride*. Among the four isolates of nutmeg, only one isolate *viz.,* Nt ep f 1 recorded more than 60 per cent inhibition on the growth of all four pathogens of nutmeg. The bacterial isolate Nt ep b 2 exihibited 60 per cent and above inhibition against the pathogens of nutmeg. Not much works have been done of the usefulness of epiphytic microbes for the management of diseases of tree spices. However Khan *et al.* (2012) recorded association of epiphytic bacteria with common spices of Bangladesh. Bhavani (2004) recorded that epiphytic fungi and bacteria of cocoa were antagonistic to *Phytophthora palmivora.*

From the epiphytic organisms isolated from the healthy leaves of clove, nine isolates were selected after the preliminary screening which included 3 isolates each of fungi, bacteria and fluorescent Pseudomonads. On further evaluation against the pathogens of clove, only one *viz.,* Cl ep b 6 was selected as efficient bacterial epiphyte. In case of epiphytes from cinnamon, a total of 12 including fungi, bacteria and fluorescent Pseudomonads were selected after

preliminary screening, but on further evaluation one fungal isolate Cn ep f 5 was selected as efficient antagonistic one.

5.3. ISOLATION, ENUMERATION AND EVALUATION OF ENDOPHYTIC ANTAGONISTS

Leaf samples collected from different locations of nutmeg, clove and cinnamon growing areas of the State were used in isolation of endophytes. Isolation of endophytic microorganisms need elimination of surface contaminants. Hence, the first step was surface sterilization followed by isolation (Araujo *et al.,* 2002). Different kinds of surface sterilization methods have been used by various workers (Bell *et al.,* 1995; Fischer *et al.,* 1992; Shishido *et al.,* 1999; Balan, 2009 and Kurian, 2011). In this study, the sterilization by using two per cent sodium hypochlorite solution for 10 min gave good results.

In the present study endophytic organisms were isolated from leaves of nutmeg, clove and cinnamon. The population of fungal endophytes was less compared to bacteria and fluorescent Pseudomonads but no actinomycetes were obtained. However, there is an earlier report by Fisher *et al.* (1992) that though several fungi and bacteria were present as endophytes, no antagonistic actinomycetes was isolated from maize. Kurian (2011) reported that population of antagonistic bacteria is more as compared with fungi in the cocoa. Recently Hatem *et al.* (2013). reported *Acremonium* sp. as an endophytic bioagent against *Fusarium* wilt of date palm caused by *Fusarium oxysporum f. sp. albedenis.*

A total of 47 endophytic organisms were isolated from nutmeg, clove and cinnamon. As in the case of epiphytes, isolated endophytes were subjected to preliminary screening to locate their antagonistic action. In this screening it was found that, 20 isolates were exerted antagonism against the pathogens tested. Similar line of work was carried out by Sturz *et al.* (1998) who tested the endophytic flora from potato and clover against *R. solani* and found that out of several bacteria tested, 74 per cent showed *in vitro* antibiosis. Kurian (2011) who

reported antagonistic nature of endophytes against *Phytophthora palmivora* of cocoa.

After the preliminary screening, six isolates of endophytic fungi were selected from nutmeg. They were further evaluated along with the standard culture of *T. viride* against the pathogens of nutmeg. Among, them only one isolate *viz.,* Nt ed f 2 recorded more than 60 per cent inhibition against all pathogens. Among, endophytic bacteria and fluorescent Pseudomonads three isolates each were selected after the preliminary screening. On further evaluation along with standard culture of *P. fluorescens* one isolate of fluorescent Pseudomonads *viz.,* Nt ed b 6 was selected as it recorded more than 60 per cent inhibition against all pathogens of nutmeg. Similar line of works like isolation of endophytes and evaluation against pathogens were carried out in nutmeg and cinnamon by earlier workers like Sopalan *et al.* (2003) and Gary *et al.* (2001) respectively

Similarly, further evaluation of endophytes selected from clove and cinnamon was carried out and their efficacy was compared with standard cultures it was found that the endophytes *viz.,* Cl ed f 2 & Cl ed b 2 from clove recorded more than 60 per cent inhibition on the growth of pathogens of clove. None of the endophytes of cinnamon were found efficient against the pathogens of the crop by exerting more than 60 per cent inhibition.

Since the main objective of this study was to identify the most potential isolates of epi and endophytes for the management of foliar diseases of tree spices, the epiphytic and endophytic antagonists selected from a host were evaluated against the pathogens of the other two hosts. The results of the cross inoculation of antagonists from nutmeg against the pathogens of clove and cinnamon resulted in the identification of three antagonists *viz.,* Nt ep f 1, Nt ed f 2 and Nt ep b 2 with more than 60 per cent inhibition on the growth of pathogens of clove and cinnamon.

Similarly the selected antagonists from clove and cinnamon were evaluated against the pathogens of other two hosts. The antagonists *viz.,* Cl ed f 2, Cl ep b 6 , Cl ed b 2, Cn ep f 5 recorded more than 60 per cent inhibition against the growth of different pathogens and hence they were selected as potential antagonists. Thus the study throws light into broad spectrum antagonistic activity of the selected isolates. However, more field oriented studies are essential to locate the most efficient one with long persistent activity.

5.4 MUTUAL COMPATIBILITY OF SELECTED ANTAGONISTS

The epiphytic and endophytic antagonists selected *in vitro* screening were again tested for their mutual compatibility with an idea of developing a consortium for more effective disease suppression. Moreover the antagonistic potential of individual antagonist may sometimes increased when they are combined with another antagonist. This was evaluated and the results revealed that all the epiphytic and endophytic bacterial antagonists were compatible with each other. In the case of fungal antagonists, all the combinations except that of nutmeg epiphytic and endophytic fungi were compatible. There are several reports of biocontrol agents used in combination for plant diseases management and most of these reports showed that combination of antagonists resulted in improved biocontrol efficency (Mazzola *et al.,* 1995 and DeBoar *et al.,* 1997). Balan (2009) reported the successful suppression of *Xanthomonasaxonopodispv. dieffenbachiae* using non pathogenic bacteria and bacterial mixtures. Similarly Vijayaraghavan (2007) and James (2015) reported the mutual compatibility of antagonistic microbes against *Ralstonia* wilt of ginger and tomato respectively.

5.5 MECHANISM OF ANTAGONISM

After two different levels of screening four isolates each of antagonistic fungi and bacteria were selected as the efficient ones against all the pathogens of nutmeg, clove and cinnamon. Their mechanisms of antagonism were studied in detail. Samanta and Dutta, (2004) reported the production ammonia, IAA and HCN plays a significant role in the antagonistic potential of an organism. Several

researchers have studied the production of these substances by endophytes (Nejad and Johnson, 2000; Sturz *et al.,* 1998; Sessitsch *et al.,* 2002; Kuklinsky-Sorbal *et al.,* 2004; Bhavani, 2004; Balan, 2009 and Kurian, 2011).

5.5.1. Mechanism of action of fungal antagonists

Observations on the inhibitory response in dual culture suggested that all four selected fungal antagonists did not produce the same inhibitory effect. Hence, this test was done to compare the promising isolates based on diffusible inhibitory metabolities. All the isolates tested produced inhibitory substances at varying levels which was diffused into the medium through the cellophane and inhibited the growth of pathogens of tree spices. Chet (1990) observed involvement of compounds of microbial origin in the suppression. However, Kurian (2011) noted the production of both volatile and non volatile compound by endophytes of cocoa.

In present study, selected fungal antagonist overgrew the pathogen and showed interactions like coiling, penetration and disintegration of the host hyphae, which ultimately lead to death of the pathogen. Such type of antagonistic reactions reported by Vijayaraghavan (2003) with *Trichoderma* spp. against *Phytophthora* sp. Bhavani (2004) reported similar mechanism of action of antagonistic epiphytes of cocoa against *P. palmivora*.

5.5.2 Mechanism of action of bacterial antagonists

The capacity to produce ammonia is an attribute which is directly related to antagonistic ability of a biocontrol organism. In the present investigation, all the isolates tested produced ammonia. It is not sure whether epiphyte and endophyte can produce volatiles like ammonia in plant (Ryu *et al.,* 2003). Samanta and Dutta (2004) had already proved the role of ammonia production by PGPR in suppressing *S. sclerotium*. The ammonia production by certain endophytic bacteria have been also reported by Balan (2009), Kurian (2011), and James (2015).

HCN is considered as a possible and perhaps frequent mechanism by which bacteria suppress plant pathogens (Ross and Ryder, 1994; Maurhofer *et al.,* 1994). However, it was noticed that all the isolates tested were unable to produce HCN. This result confirmed earlier report by Nejad and Johnson (2000), who found that most of the endophyte isolates from oil seed rape were HCN negative. But they have suggested volatiles other than HCN may be involved in antagonism expressed by endophytic isolates. Kurian (2011), James (2015) also did not observed the cyanogenic nature of the endophytic bacteria used in their study.

Production of phytoharmones as one of the mechanism involved in the growth promotion by endophytes and epiphytes as suggested by Sturz *et al.,* (1998) and Sessitsch *et al.,* (2002). In present study, all isolates produced varying levels of IAA, ranging from 7.5 to 19.1 µg ml^{-1}. According to Mendes *et al.* (2007), IAA production is more prevalent among the bacterial endophytes isolated from aerial parts than those from rhizosphere. Several other workers also reported IAA production by endophytic bacteria in different crops. Kuklinksky-Sobral *et al.* (2004) and Hung *et al*. (2007) reported IAA production by endophytic bacteria from soyabean. Similarly Balan (2009) reported the IAA production of most isolates from anthurium and Kurian (2011) from cocoa.

In present study, none of the endophytic and epiphytic isolates showed siderophore production, which is in contradictory to the earlier findings of Bhavani (2004), Balan (2009) and Kurian (2011), who observed siderophore production by bacterial epiphytes and endophytes. Sgroy *et al.* (2009) reported that the endophytic *B. subtilis* isolate from halophyte *Prosapis srtombulifera* did not produce siderophores, Forchetti *et al.* (2010) and James (2015) also not observed siderophore production by bacterial endophytes isolated from sunflower and tomato respectively.

5.6 COMPATIBILITY STUDIES

In any integrated plant disease management programme, its components must be compatible with each other. Otherwise, the desired output of the

programme may not be achieved. This is more pertinent when biocontrol agents are used as one of the components in the integrated disease management strategy

So in the present study, the compatibility of the selected four fungal antagonists such as Nt ep f 1, Nt ed f 2, Cl ed f 2 and Cn ep f 5 with ten fungicides were evaluated. The nutmeg antagonists Nt ep f 1 and Nt ed f 2 were incompatible with Bordeaux mixture, Indofil M-45, Contaf. Complete inhibition of Nt ep f 1and Nt ed f 2 were also noticed with Score and Curzate respectively.The fungal antagonists from clove and cinnamon *viz.,*Cl ed f 2 and Cn ep f 5 were recorded cent per cent inhibition with Bordeaux mixture, Contaf, Score, Bavistin and Tilt. The isolate Cn ep f 5 was completely inhibited with Curzate. Rest of fungicides recorded varying levels of inhibition towards the fungal antagonists.These findings are in tune with the reports of many workers who studied the compatibility of fungal antagonists with different fungicides. (Mondal *et al.,*1995; Shanmugam,1996; Rajan and sarma, 1997; Sarma and Anadaraj, 1999; Mclean *et al.,* 2001; Vijayaraghavan and Abraham, 2003; Bhavani, 2004; Bhattiprolu, 2007; Madhusudhan *et al.,*2010; Gaikwad *et al.,* 2011; Ahanger *et al.,* 2014). Therefore care should be taken while selecting components in the integrated disease management programme.

The results of the compatibility study of fungal antagonists with insecticides, revealed that in general all insecticides showed varying levels of compatibility. Rogor and Fame at all concentrations inhibited the growth of Nt e d f 2. Ekalux at higher concentration was also inhibitory to all antagonistic fungi. Bhai and Thomas, (2010) conducted similar studies with Quinalphos.

The compatibility of fungicides and insecticides with the selected four bacterial antagonists were also evaluated. The same fungicides tested with fungal antagonists, were used for this study also. On comparing the effect of different fungicides tested it was found that, except Bordeaux mixture and highest concentration of Kocide (0.15 per cent) all other fungicides recorded less than 20 per cent inhibition on the growth of all bacterial antagonists. Hence they may be

considered as incompatible with their antagonists. Elkins and Lindow (1999) found that mancozeb had no determinal effect on *P. fluorescens*. The bacterial antagonists were found to be compatible with Ekalux and Rogor at various concentrations. Therefore of these insecticides could be recommended for the insect control without much adverse effect against the bacterial antagonists. Mathew (2003) reported *P. fluorescens* was compatible with recommended doses of imidacloprid, Chlorpyriphos and Triazophos. So it is evident that there lies in the potential of four biocontol agents to be used along with plant protection chemicals as a control of integrated control packages. However, the performance of these selected bioagents in different tree spices growing areas is to be ascertained before recommending to the farming community as an ecofriendly management practice against the disease.

5.7. IDENTIFICATION OF ANTAGONISTS

5.7.1. Fungal antagonists

The selected epiphytic and endophytic antagonists which were proved as the efficient organisms against the pathogens of nutmeg, clove and cinnamon were identified based on cultural, morphological and biochemical characters. The fungal antagonists *viz.,* Nt ep f 1, Nt ed f 2, Cl ed f 2 and Cn ep f 5 were cultured on PDA medium and cultural and morphological characters were studied in detail. Based on this the isolates were tentatively identified and was confirmed from N.C.F.T, New Delhi. Accordingly the fungal antagonists *viz.,*Nt ep f 1, Nt ed f 2, Cl ed f 2 and Cn ep f 5 were identified as (*Phytophthora cactorum* Leb & Cohn) Schroet. (N.C.F.T.ID.NO. 6763.15), *Acremonium kilense* Grutz (N.C.F.T. ID.NO. 6762.15), *Trichoderma viride* Pers. Ex. S. F. Gray (N.C.F.T. ID.NO. 6765.15) and *Trichoderma harzianum* Rifai. (N.C.F.T. ID.NO. 6764.15) respectively. There are several reports by many workers like (Galindo, 1992; Vijayaraghavan, 2003; Bhavani, 2004 and James, 2015) as *Trichoderma* spp. as epiphytes and endophytes and established their role in antagonistic nature against *Phytophthora* pod rot diseases of cocoa and *Ralstonia* wilt of Tomato respectively. Hatem *et al*.

(2013) reported the antagonistic nature of *Acremonium kilense* against the *Fusarium* wilt of date palm caused by *Fusarium oxysporum f. sp*. *albedenis*.

5.7.2. Bacterial antagonists

The cultural and biochemical characters of bacterial antagonists were studied and based on that the antagonists Cl ep b 6 was identified as *Bacillus* sp. The other three different bacterial antagonists *viz*., Cl ed b 2, Nt ed b 6 and Nt ep b 2 were identified as *Pseudomonas* spp.

Summary

6. SUMMARY

The major constraint faced by tree spices farmers of Kerala is the prevalence of fungal diseases. Antagonistic epiphytes and endophytes are known to possess the beneficial attributes of other biological agents like the capacity to induce systemic resistance and growth promoting ability with additional advantage of systemic existence in plants. Hence, the present investigations are carried out to harness the potential of native epiphytic and endophytic microbes of nutmeg, clove and cinnamon for the management of foliar diseases. The salient findings of the study are summarized below.

1. The various foliar pathogens of nutmeg, clove and cinnamon were isolated and their pathogenicity established. The cultural and morphological characters of the pathogens were studied. *Colletotrichum gloeosporioides*, *Phytophthora* sp. *Pestalotiopsis palmarum*, *Rhizoctonia solani* and *Marasmius* sp from nutmeg, *C. gloeosporioides, P. palmarum*, *Cylindrocladium quinqueseptatum* from clove and *C. gloeosporioides*, *P. palmarum* from cinnamon were isolated and symptomatology of the diseases caused by them were studied.

2. Enumeration of endophytic and epiphytic microorganisms from nutmeg, clove and cinnamon from various areas of State revealed the predominance of bacteria and fluorescent Pseudomonads.

3. Altogether, 118 epiphytes and endophytes from nutmeg, clove and cinnamon were isolated which included 63 epiphytes and 55 endophytes. Out of 118 epiphytic and endophytic isolates screened, 52 were found to possess antagonistic activity against pathogens isolated from the above tree spices.

4. Antagonistic action of these epiphytes and endophytes against pathogens were further studied in comparison with that of reference cultures of *Pseudomonas fluorescens* (KAU) and *Trichoderma viride* (KAU). The study revealed that, only 4 bacterial and 4 fungal cultures showed efficient antagonistic property against the

pathogens of nutmeg, clove and cinnamon and they were selected for further *in vitro* evaluation.

5. The selected epiphytes and endophytes were subjected to various tests for understanding parameters that contributed to their antagonistic potential. All antagonists inhibited growth of the pathogen, produced IAA, ammonia and were negative to HCN and siderophore production. The fungal antagonists were positive to volatile metabolites production and negative to non volatile metabolites production.

6. The compatibility of the selected fungal antagonists, Cl ed f 2, Cn ep f 5, Nt ep f 1 and Nt ed f 2 to ten fungicides were assessed. Bordeaux mixture, Kocide, Fytolan, Saaf, Contaf and Tilt were incompatible.

7. Insecticides, chlorpyriphos, Ekalux and Fame were incompatible with the selected fungal antagonists while Rogor was compatible.

8. The compatibility of fungicides towards the selected bacterial antagonists Nt ep b 2, Nt ed b 6, Cl ep b 6 and Cl ed b 2 were studied. Saff, Curzate, Tilt were compatible with bacterial antagonists. Among copper fungicides, Bordeaux mixture was more inhibitory than Fytolan and Kocide.

9. With regard to insecticides, all concentrations of Ekalux and lower concentration of Rogor were compatible with bacterial antagonists. Fame at higher concentration was incompatible.

10. The fungal antagonists were identified as *Acremonium kilense* (Nt ed f 2), *Phytophthora cactorum* (Nt ep f 1), *Trichoderma viride* (Cl ed f 2) and *Trichoderma harzianum* (Cn ep f 5). Three of the bacterial isolates (Nt ep b 2, N ed b 6, Cl ed b 2) were tentatively identified as *Pseudomonas* spp. while the other one (Cl ep b 6) as *Bacillus* sp.

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Appendices

APPENDIX-1

MEDIA COMPOSITION

(Ingredients per litre)

1. **POTATO DEXTROSE AGAR**

   Potato : 200.0g

   Dextrose : 20.0g

   Agar : 20.0g

2. **MARTIN'S ROSE BENGAL STERPTOMYCIN AGAR**

   Dextrose : 10.0g

   Peptone : 5.0g

   KH_2PO_4 : 1.0g

   $MgSO_4$: 0.5g

   Agar : 20.0g

   Rose Bengal : 0.03g

   Sterptomycin : 30 mg

   Distilled water : 1000 ml

3. **NUTRIENT AGAR MEDIUM**

   Glucose : 5.0g

   Peptone : 5.0g

   Beef extract : 3.0g

   Nacl : 5.0g

   Agar : 20.0g

   p^H : 6.5 to 7.5

   Distilled water : 1000 ml

4. **KING'S 'B MEDIUM**

   Peptone : 20.0g

   Glycerol : 10.0 ml

K_2HPO_4 : 10.0 g

$MgSO_4.7H_2O$: 1.5 g

Agar : 20.0 g

P^H : 7.2

Distilled water : 1000 ml

5. KEN KNIGHT'S AGAR MEDIUM

Dextrose : 1.0 g

KH_2PO_4 : 0.1 g

$NaNO_3$: 0.1 g

KCl : 0.1 g

$MgSO_4$: 0.1 g

Agar : 20.0g

p^H : 7

Distilled water : 1000 ml

6. Peptone water (p^H 7.0)

Peptone : 10.0 g

NaCl : 15.0 g

Distilled water : 1000 ml

Appendix – 2

0.02 M Potassium Phosphate buffer: 7 p H

* Stock solutions:

A- 3.5 g of dibasic K_2 H PO_4 in 1000ml

B- 2.7 g of monobasic KH_2PO_4 in 1000ml

For getting 0.02M Potassium Phosphate buffer of p ^{H} 7, mix 61 ml of solution A with 39 ml of solution B in 100 ml of water

10 Mm Solution Phosphate buffer: pH 6.0

A- 0.2 M solution of monobasic sodium phosphate, it can be obtained by dissolving 27.8 g in 1000ml distilled water

B- 0.2 M solution of dibasic sodium phosphate, it can be obtained by dissolving 71.7 g of Na_2HPO_4 $12H_2O$ IN 1000 ml distilled water

Abstract

ENDOPHYTIC AND EPIPHYTIC MICROBIAL DIVERSITY IN MAJOR TREE SPICES AND THEIR POTENTIAL FOR BIOCONTROL OF FOLIAR PATHOGENS

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ABSTRACT OF THE THESIS

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ABSTRACT

The study on "Endophytic and epiphytic microbial diversity in major tree spices and their potential for biocontrol of foliar pathogens'' was carried out during 2013-2015. The organisms causing various foliar diseases of tree spices were isolated from infected leaves and their pathogenicity established. Based on cultural and morphological characters, pathogens were identified upto the species level.

Endophytes and epiphytes were isolated from leaf samples collected from different locations of Kerala. The population of endo and epiphytic microflora varied among the samples collected from different locations. Bacteria and fluorescent Pseudomonads population were more in number than fungi. A total of 118 endophytes and epiphytes were isolated. Among these, 52 isolates, consisting of 32 epi and 20 endophytes were found to exert antagonism towards pathogens of nutmeg, clove and cinnamon. On further *in vitro* evaluation, eight isolates including four each belonging to fungi and bacteria of epi and endophytic origin were selected as efficient antagonists. Mutual compatibility of the selected antagonists was studied. All the bacterial antagonists were compatible with each other. Among the four fungal antagonists, all except one combination (Nt ep f 1 X Nt ed f 2) were compatible.

For studying the mechanism of antagonism of the selected isolates, they were subjected to various tests like production of ammonia, HCN, IAA, volatile and non volatile metabolites. The bacterial isolates Nt ed b 6, Cl ed b 2 and Cl ep b 6 produced more ammonia. All isolates were negative to HCN while others produced varying levels of IAA. The selected fungal isolates produced non-volatile metabolites inhibitory to the pathogens tested.

Further, the compatibility of selected antagonists with 10 fungicides and four insecticides were studied. In general, the fungal antagonists were incompatible with fungicides like Bordeaux mixture, copper oxy chloride (Fytolan50 WP), carbendazim

(12%) + mancozeb (64%) (Saaf), hexaconazole (Contaf 5 EC), propiconazole (Tilt 25 EC) and insecticides chlorpyriphos (Dursban 20 EC) and quinalphos (Ekalux 20 EC).Others showed varying levels of inhibition.

The bacterial antagonists were compatible with Saaf, Tilt, mancozeb (Indofil M-45 75WP), cyamoxanil (8%) + mancozeb (64%) (Curzate M8) while they were incompatible with BM, copper hydroxide (Kocide 77 WP), Fytolan, Carbendazim (Bavistin 50WP), difenoconazole (Score 25 EC). All concentrations of Ekalux and lower concentration of dimethoate (Rogor 30 EC) were compatible with antagonists while the reverse was with that of chlorpyriphos and flubendiamide (Fame 480 SC).

An attempt has been made to identify the selected epi and endophytic antagonists. The fungal antagonists were identified as *Acremonium kilense* (Nt ed f 2), *Phytophthora cactorum* (Nt ep f 1), *Trichoderma viride* (Cl ed f 2) and *Trichoderma harzianum* (Cn ep f 5). Three of the bacterial isolates (Nt ep b 2, Nt ed b 6, Cl ed b 2) were tentatively identified as *Pseudomonas* spp. while the other one (Cl ep b 6) as *Bacillus* sp.