Evaluation of some essential oils and plant extracts in management of post harvest fungal diseases of kiwifruit (*Actinidia deliciosa*)

Thesis submitted for the
Degree of Doctor of Philosophy in Botany

By

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राजीव गान्धी विश्वविद्यालय

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CERTIFICATE

This is to certify that the thesis entitled "**Evaluation of some essential oils and plant extracts in management of post-harvest fungal diseases of kiwifruit (*Actinidia deliciosa*)**" submitted for the degree of Doctor of Philosophy in Botany to Rajiv Gandhi University, Itanagar, embodies the record of original research work carried out by **Ms. Habung Yami** under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree whatsoever either by her or by anyone else. The thesis is forwarded for examination of the degree of Doctor of Philosophy in Botany of the Rajiv Gandhi University.

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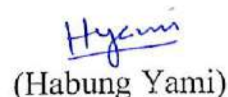

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(Habung Yami)

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INTRODUCTION

Postharvest losses of fruits and vegetables is a serious problem, because the values of fresh product significantly increase while passing from the farm to the consumers table and due to overpopulation the demand for fruits and vegetables increases in the world. Enyiukwu *et. al.*, (2014) stated that most important losses in agricultural production which involve the greatest costs on the farm economy occur postharvest. It is estimated that worldwide between 10 to 40% losses of agricultural produce occur are postharvest. Losses are more severe in developing than developed nations of the world. Several species of fungi and in some cases bacteria participate in postharvest deterioration and rots of tubers and agroproduce. These include species of *Aspergillus, Botrytis, Fusarium, Colletotrichum, Macrophomina, Penicillium* and *Rhizopus* amongst several others.

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* Hayward] is a climacteric and susceptible fruit to fungal decays in postharvest stage. After harvest, fruit rot diseases cause a severe loss of kiwifruit during cold storage, transportation, marketing, and in retail stores (Koh *et. al.*, 2003). Many fungi are associated with post harvest fruit rots of kiwifruit (Pennycook 1985; Hawthorne *et. al.*,1982). Botrytis gray mold rot caused by *Botrytis cinerea* is the most important and can directly invade the fruit or enter through wounds. Kiwifruit become much more susceptible to Botrytis (and other fungi) as they soften. Other fungal pathogens *Phomopsis mali*, Botryosphaeria *dothidea* and *Diaporthe actinidiae* have also been reported to cause post harvest fruit rots of kiwifruit (Lee *et. al.*, 2001; Koh *et. al.*, 2003). Post harvest application of dicarboximide fungicides immediately after harvest has been shown to reduce the incidence of rots to very low levels. However, this option is increasingly unacceptable due to public concern about and legislation on pesticide residues in fruit.

Fresh fruits and vegetables are considered as an important component of a healthy balanced diet. Fruits are excellent source of dietary fiber, vitamins, carbohydrates and antioxidants and are highly perishable products especially during the post-harvest phase, when considerable losses due to microbiological diseases, disorders, transpiration and senescence can occur. Fruits are perishable

by nature and require protection from spoilage during their storage and distribution to give them desired shelf life. Because fruits are now often sold in areas of the world far remote from their production sites, the need for quality as well as extended shelf life for these has also expanded. Approaching towards the target of second green revolution in India there is an urgent need to unearth new strategies to control post harvest pathogens, so that we can increase the production of fruits and vegetables by improving their shelf life.

Post harvest and storage pathogens of kiwifruit most often have been controlled by use of synthetic fungicides (Eckert and Ogawa 1988; Wurms *et. al.*, 1999). In the recent years, pesticide residues on horticultural products, especially the fungicides used in postharvest stage, are of major concerns to the horticultural industry. Also, the increase in consumer's awareness about hazards of pesticide residues on fresh products and their demand to non-residue products, human health, and environmental pollution are considered. Additionally, the reduction in efficacy of fungicides and consequently development of resistant strains of fungi causes that agriculture researches assay new methods and substances to develop effective and safer alternatives to agrochemicals.

Biologically active plant extracts, including essential oils, represent rich potential sources of alternative and perhaps environmentally more acceptable disease management compounds. Besides, higher plants also contain a wide spectrum of secondary substances viz. phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols. Plant diversity serves the humankind as renewable natural resources for a variety of biologically active chemicals. These chemicals bear a variety of properties viz. antibacterial, antifungal, antiviral, anthelmintic, anticancer, sedative, laxative, cardiotonic, diuretic and others (Parajuli *et. al.*, 1998). Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. The secondary metabolites performs defensive role in plant from their invaders. The factors that affect biochemical profiles and secondary metabolite production in plant include physiological, genetics, and environmental variables. Secondary metabolites content is also subject to time of harvesting, storage, drying, extraction and processing for final experiment. The preservative nature of some plant extracts has been known for centuries and there has been renewed interest in

the antimicrobial properties of extracts from aromatic plants (Hiremath *et. al.*, 1996, Kapoor, 1997). Active constituents of the medicinal and aromatic plants have been found to be less phytotoxic, more systemic and easily biodegradable (Fawcett and Spencer 1970).

Many plant extracts have potential as natural antimicrobial agents that can be applied to agricultural produces, foods and pharmaceutics (Horburg, 1998; Maoz and Neeman, 1998) because they contain a phytochemical that exhibits antimicrobial and cytotoxic effects on microorganisms (Feldberg *et. al., 1988).* Plant extracts contain a wide range of bioactive secondary metabolites which include alkaloids, flavonoids, tannins, saponins, phenols, phlobatannins quinones, lecitins, polyphenols, glycosides, terpenoids, polypeptides and steroids (Edeoga *et al.*, 2005; Shukla *et al.*, 2012; Enyiukwu and Awurum, 2013). These bioactive groups of natural products have been given as the reason for their inhibitive roles against pathogens in ethnobotany, drug application and plant health management (Okwu and Njoku, 2009; Enyiukwu *et al.*, 2013). Kumar & Tripathi (1991) mentioned that extracts of *Eupartorium cannabinum* completely inhibited the mycelial growth of *Pythium debaryanum, R. solani* and *Sclerotium rolfsii.* Gatto *et. al.*, (2011) reported *in vitro* and *in vivo* activity of extracts obtained from nine wild edible herbaceous species (*Borago officinalis, Orobanche crenata, Plantago coronopus, P. lanceolata, Sanguisorba minor, Silene vulgaris, Sonchus asper, Sonchus oleraceus,* and *Taraxacum officinale*) against some important postharvest pathogens, i.e. *Botrytis cinerea, Monilinia laxa, Penicillium digitatum, P. expansum, P. italicum, Aspergillus carbonarius,* and *A. niger.* Ikeura *et. al.*, (2011) evaluated sixteen plants (garlic, clove, dokudami, kumasasa, dandelion, kusagi, yomogi, ginkgo, marigold, lavender, thyme, hot pepper, ginger and lemon basil) by means of solvent extraction with either dichloromethane or diethyl ether screened against *P. expansum* for antifungal activity. Similarly, *Rhizopus stolonifer* and *Fusarium oxysporium* amongst other pathogens were also reported inhibited by extracts from *Chromolaena odorantum, Azachirachta indica, Vernonia amygdalina* and *Tridax procumbens* in eggplants and tomato (Ijato *et. al.*, 2011). Lakshmi *et. al.*, (2014) studied anti fungal activity of 10 plant extracts against the fungal disease (*Cercospora, Sphaceolema, Certocystis fimbriata, Collitotrichum gleosporides, Fusarium solani,* and *Fusarium oxysporum*) of pomegranate using agar well

diffusion method. They found the Hexane, Choloform, Methanol, extracts of 10 plant extracts exhibited varying degrees of inhibition activity against the fungal pathogens of Pomegranate.

Essential oils or volatile oils are very complex mixture of compounds whose constituents of the oils are mainly monoterpenes and sesquiterpenes. Generally, the action of essential oils is the result of the combined effect of both their active and inactive compounds. These inactive compounds might influence resorption, rate of reactions and bioavailability of the active compounds. Until recently, essential oils have been studied most from the viewpoint of their flavor and fragrance only for flavoring foods, drinks and other goods. Actually, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey 2001). Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance (Goubran & Holmes 1993). The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (Wijesekara *et. al.*, 1997).

It has long been recognized that some essential oils have antimicrobial properties (Boyle 1955) and these have been reviewed in the past (Shelef, 1983; Nychas, 1995) as have the antimicrobial properties of spices (Shelef, 1983) but the relatively recent enhancement of interest in 'green' consumerism has lead to a renewal of scientific interest in these substances (Tuley, 1996). Besides antibacterial properties (Mourey and Canillac, 2002; Rasooli and Razzaghi 2004; Rasooli and Owlia, 2005), EOs or their components have been shown to exhibit antiviral (Bishop, 1995), antimycotic (Mari *et al.*, 2003), anti oxidative (Gachkar *et al.*, 2006; Yadegarinia *et al.*, 2006; Bektas *et al.*, 2007), antitoxigenic (Akgul *et al.*, 1991; Juglal *et al.*, 2002; Ultee and Smid, 2001), antiparasitic (Pandey *et al.*, 2000; Pessoa *et al.*, 2002), and insecticidal (Karpouhtsis *et al.*, 1998) properties.

Furthermore, Juglal *et al.*, (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxin producing moulds and noted that, clove, cinnamon and oregano oils were able to prevent the growth of *Aspergillus parasiticus* and *F. moniliforme*. Thyme oil proved to be extremely effective as a fumigant as well as a contact fungicide against a range of the economically

significant fungi *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea* and *Erysiphe graminis* (Alefyah & Avice 1997). Essential oils of cinnamon and clove contain compounds such as cinnamaldehyde and eugenol, respectively, which have been successfully tested on fresh fruits such as mandarin, kiwi and rambutan to control post-harvest diseases caused by fungi (Arras 1988, Thanassoulopoulos and Yanna 1997, Sivakumar *et al.*, 2002). Siripornvisal (2010) evaluated essential oil extracted from mature seeds of ajowan (*Trachyspermum ammi* Lin.) against three strains of *Fusarium oxysporum*, *F. oxysporum* f.sp. *lycopersici*, *F. oxysporum* f.sp. *cubense* and *F. oxysporum* f.sp. *capsici* the wilt pathogen of tomato, banana and chili respectively. The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) were 240 and 480 μg·mL-1, respectively. Nabigol and Morshedi (2011) evaluated the antifungal potential of *Thymus danensism* and *Thymus carmanicus* against four pathogenic fungi (*Rhizopus stolonifer*, *Penicillium digitatum*, *Aspergillus niger* and *Botrytis cinerea*) which can reduce the shelf life of strawberry fruit. Complete minimum inhibitory concentration (inhibition 100%; MIC) of both oils against *B. cinerea*, and *R. stolonifer* were 300 μl/L. The MIC of *T. danensis* and *carmanicus* against *P. digitatum* and *A. niger* was 600 μl/L. Nosrati *et al.*, (2011) studied antifungal activity of 1, 3 and 5 μl of the essential oil of spearmint plants on the mycelia growth of *Fusarium oxysporum f. sp. radicis- cucumernum*. All amounts of the essential oil restricted significantly the mycelia growth of the pathogen with maximum activity detected for samples treated with 5 μl of essential oil. Mohammadi and Aminifard (2013) determine the antifungal effects of the fennel essential oil against fungal pathogen *Botrytis cinerea* the causal agent of grey mould disease of tomato fruit under *in vitro* and *in vivo* conditions. They found remarkable inhibitory effect of oil at different concentrations and under *in vivo* condition reported to increase the shelf life of tomato fruits. Mohapatra *et al.*, (2014) evaluated biocontrol potentials of three essential oils against the pathogens *Aspergillus, Mucor, Rhizopus, Geotrichum* and *Fusarium* causing rapid spoilage. All the oil displayed antifungal activity in varying degrees. Among the oils, carrot oil was found to be most effective in inhibiting all the pathogens.

Although a lot of studies were carried out to examine the antifungal activity of essential oils under *in vitro* conditions (Rasooli and Owlia 2005; Yahyazadeh *et al.* 2008; Abdolahi *et al.* 2010), a few work were conducted to

evaluate a possible use of antifungal property of essential oils to preservation of fruits and vegetables in postharvest stage. Thanassoulopoulos and Yanna (1997) screened antifungal activity of essential oils from origanum, sweet basil and thyme against gray mold rot on inoculated kiwifruits with *B. cinerea*. Tripathi *et al.* (2008) investigated the antifungal property of twenty six essential oils against *B. cinerea* under *in vitro* conditions and found a total growth inhibition by using extracts from *Chenopodium ambrosioides, Eucalyptus citriodora, Eupatorium, Cannabinum, Lawsonia inermis, Ocimum canum, O. gratissimum, O. sanctum, Prunus persica, Zingiber cassumunad* and *Z. officinale*. On the other hand, essential oils from *P. persica, O. sanctum* and *Z. officinale* increased the storage life of oil treated grapes 4, 5 and 6 days, respectively. Additionally, vapor of essential oils from peppermint and sweet basil against *Monilina fruticola, Rhizopus stolonifer* and *Aspergillus niger* showed a good antifungal activity under *in vitro* and *in vivo* conditions, reducing decay and maintaining quality parameters of peach fruits after prolonged storage (Ziedan and Farag 2008).

The modes by which microorganisms are inhibited by essential oils and their chemical compounds seem to be involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven *et al.*, 1994). Reports indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim *et al.* 1995).

With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post harvest diseases (Hartmans *et al.*, 1995; Bang, 1995). The general antifungal activity of essential oils is well documented (Deans and Ritchie, 1987; Reuveni *et.al.*, 1984; Tripathi and Shukla, 2007) and there have been some studies on the effects of essential oils on post harvest pathogens (Bishop and Thornton, 1997; Anthony *et al*, 2003; Tripathi *et al.*, 2008). Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more acceptable disease management compounds. With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post-harvest diseases. The advantage of essential oils is their bioactivity in a vapour

phase, a characteristic that makes them attractive as possible fumigants for stored product protection. Perusal of literature reveals that a work was conducted to evaluate a possible use of antifungal property of essential oils for preservation of kiwifruits in postharvest stage (Shirzat *et al.*, 2011) but so far studies have not yet been carried out to control the post harvest diseases of kiwifruit by using essential oils and plant extracts.

Based on the merits of exploitation of plant products for their biological activities, the proposed work has been framed to evaluate the potency of some essential oil and plant extracts of commonly occurring higher plants in the management of post harvest rotting of kiwi fruits with the following objectives.

Objectives

- Isolation and identification of post harvest fungal pathogens of kiwifruits.
- Extraction of essential oils from locally available angiospermic taxa and subsequently Screening against the post harvest fungal pathogens of kiwifruits
- Plant extracts preparation in different organic solvents from some angiospermic taxa and Screening against the post harvest pathogens of kiwifruits.
- *In vivo* efficacy evaluation of the effective essential oils and the plant extracts for the enhancement of shelf life of kiwifruit.
- Organoleptic tests of the treated fruits.

REVIEW OF LITERATURE

After harvest, fruit rot diseases cause a severe loss of kiwifruit during cold storage, transportation, marketing, and in retail stores (Koh *et al.*, 2003). Many fungi are associated with postharvest fruit rots of kiwifruit (Pennycook, 1985; Hawthorne *et al.*, 1982). Usually the fruits with external symptoms have internal symptoms inside the postharvest diseased fruits. However, a great amount of fruit which seemed to be healthy in appearance turned out to be decayed after the skin of the fruit was peeled back (Koh *et al.*, 2003). The gap between the percentage of kiwifruit showing internal and external symptoms often leads to the underestimation of the importance of post harvest fruit rots of kiwifruit, and the necessity of controlling the diseases is neglected. Postharvest pathogen of kiwifruit in New Zealand, nine fungi were isolated from postharvest fruit rots of kiwifruit (Hawthorne *et. al.,* 1982). Pennycook (1985) reviewed field rot, storage rot, and ripe rot as the three main fungal fruit rots of kiwifruit in New Zealand. In the United States, botrytis rot, surface mold (*Alternaria* rot), *Dothiorella* rot, *Phoma* rot, *Phomopsis* rot, Sclerotinia rot, *Mucor* rot, blue mold, and buckshot rot were reported to occur in kiwifruit (Kader 1992; Michailides and Elmer 2000). Among these fungi, *Phomopsis* sp. and *Botryosphaeria* sp. were reported to be major causal organisms of postharvest fruit rots of kiwifruit (Chung 1997; Lee *et al.*, 2001; Koh *et al.,* 2003). Fruit rot of kiwifruit caused by *Phomopsis* sp. is called stem-end rot (Beraha 1970). Somer and Beraha (1975) reported its telemorph state as *Diaporthe actinidiae*. In Korea, its anamorph and telemorph states were also reported in kiwifruit, respectively (Lee *et. al.,* 2001; Koh *et al.,* 2003). Fruit rot of kiwifruit caused by *Botryosphaeria dothidea*, which is called ripe rot (Pennycook 1981).

Observation of Koh *et al.*, (2003) on collecting 1600 random fruit from 16 orchards located in three major kiwifruit-cultivating provinces of Korea, Jeonnam, Gyeongnam, and Jeju showed that incidence of postharvest fruit rots was examined after cool storage at 1°C for 1 month followed by ripening at room temperature. The overall mean disease incidence of postharvest fruit rots was 32%. However, the incidence varied with the orchards harvested and ranged from 5% to 68%. Usually *B. dothidea* and *D. actinidiae* cause postharvest fruit rots

during ripening, but *B. cinerea* can initiate gray mold during storage, even at low temperatures. The initial symptom of gray mold began to appear after 4 weeks of cold storage and up to 32% of postharvest fruit rots were caused by *B. cinerea* in New Zealand (Pennycook 1985). Hawthorne *et al.*, (1982) also reported that *B. dothidea* and *D. actinidiae* were isolated more frequently than other fungi from the diseased kiwifruit. Thus, distributions or frequencies of the post harvest fruit rot pathogens of kiwifruit were quite different, not only from country to country but also from region to region. Similarly, considerable variation in gray mold incidence occurred among growing seasons, regions, and individual kiwifruit orchards within a region (Pennycook 1985; Pyke *et al.*, 1994). Ripe rot caused by *B. dothidea* is usually found in overripe fruit (Koh *et al.*, 2003). Clear external symptoms of ripe rot are sometimes absent on the surface of fruit, but a portion of the fruit surface collapses Symptoms of stem-end rot caused by *D. actinidiae* appear at the stem-end of the fruit as it ripens (Lee *et al,*. 2001).

2.1. Chemical control of pathogen

Fungicidal control of storage rots in kiwifruit has been reported by Hawthorne and Reid (1982) and Eckert and Ogawa (1988). Since post harvest fruit rot pathogens infect kiwifruit during the entire growing season, and more so during the rainy season (July and August) in Korea, intensive application of fungicides just before or after the rainy season could effectively control the *Phomopsis* rot (Chung 1997). Benomyl WP and thiophanate-methyl WP, registered as preventive fungicides against postharvest fruit rots, have usually been sprayed more than 5–6 times at 10-day intervals (Koh *et al.*, 2003). However, Koh *et al,*. (2003) also selected tebuconazole WP, iprodione WP, and flusilazole WP as alternative fungicides which could be substituted for benomyl WP and thiophanate-methyl WP, based on laboratory and field trials. Trials using δ-decalactone, δ-dodecalactone and β-ionone have shown some of these natural products to be effective for inhibiting Botrytis rots in kiwifruit (Ward *et al.*, 1996). Some work has been carried out by workers (Bishop and Thornton 1997, Anthony *et al.*, 2003, Tripathi *et al.*, 2008) for post harvest diseases management of different fruits using essential oils.

A perusal of literature shows that several plants have been found to possess pronounced fungitoxic activity against different fruit rotting fungi.

Aqueous or organic extracts of plants and essential oils have been reported time to time to demonstrate pronounced fungitoxic activity.

2.2. Fungitoxicity of Essential Oils

In many plants antimicrobial power lies with the essential oil or volatile fractions. A large number of essential oils have been screened for their antifungal activity against different fungi by different workers. Avadhoot and verma, (1978) reported that essential oil of *Lantana camara* var. *aculeate* inhibit the growth of pathogenic fungi *Curvularia lunata*. Sharma *et al.* (1978) screened essential oils of *Ageratum conyzoides, Feronia elephantum* and *Blumea membranacea* against some storage fungi and found strong fungicidal activity of *B.membranacea* oil. *Ageratum conyzoides* oil was tested against *colletotrichum capsici* and *penicillium italicum* (Chandra and Dixit, 1981). Dubey et al. (1983) demonstrated the efficacy of essential oil of *Ocimum canum* and *Citrus medica* as volatile fungitoxicant in protection of some spices against their post-harvest fungal deterioration. Chandra (1984) reported the essential oil of *Ageratum conyzoides* to protect oranges from blue mould rot infection. Leaves of 12 plant species *Abutilon indicum, Amaranthus spinosus, Callistemon lanceolatus, Cinnamomum camphora, Cymbopogon martini, Euphorbia hirta, Jatropha curcas, Mussaenda fondosa, Ranuculus sceleratus, Santalum album, Zingiber officinale, Zinnia elegans* were tested for their volatile toxicity against *Fusarium oxysporum.* Nature of toxicity of oil was found to be fungistatic. Tripathi *et al.* (1985) screened oil of *Ocimum gratissimum* against fungi *Alternaria alternata, Colletotrichum capsici* and *Sclerotium rolfsi* and found the oil to be effective at 250, 500, and 50ppm respectively. Asthana *et al.* (1986) screened essential oil of *Schinus molle* against *Alternaria alternata ,Aspergillus flavus* and *Penicillium italicum*. They found that the effective concentration of oil varied from 200-900ppm and exhibited narrow range of activity. *Seseli indicum* oil was tested against thirty one fungi and the oil was found to be fungistatic at 800µg/ml against *Fusarium oxysporum, Aspergillus flavus, A. niger* and the oil showed broad range of activity (Chaturvedi and Tripathi, 1989). *Cymbopogon martini* showed fungicidal nature at its MIC of 1500ppm with broad fungitoxic spectrum (Srivastava *et al.*, 1990).

Gangradel *et al.* (1991) recorded essential oil of leaf of *cymbopogon martini* var *motia*, seeds of *Pimpinella anisum* and *Vetiveria zizonoides* having strong activity against *Fusarium oxysporum, Aspergillus flavus, A.niger* and

penicillium species.Essential oil of *pelargonium gravelens* exhibited *in vitro* antifungal activity against *Colletotrichum gleoesporiordes* that causes anthracnose in fruits like mango, citrus and papaya (Chandravadana and Nidiry,1994). The oil isolated from the fruits of *Litsea cubeba* was tested *in vitro* for its activity at different concentrations against *Alternaria alternate, Fusarium moniliforme, Fusarium solani* and *Aspergillus niger* (Gogoi *et al*., 1997). Using *in vitro* test, 21 essential oils were tested for effect on Conidium germination, germtube elongation and mycelia growth of *B.cinerea (*Antonov *et al., 1997).* Extracts from 345 plants and 49 essential oils were evaluated for their antifungal activity against *B. cinerea.* Among 345 plant extracts analyzed, 13 showed high levels of antifungal activity, with species of *Allium* and *Capsicum* predominating. Among the 49 essential oils tested, palmarosa (*Cymbopogon martini*), red thyme (*Thymus zygis*), cinnamon leaf (*Cinnamomum zeylanicum*), and clove buds (*Eugenia caryophyllata*) demonstrated the most antifungal activity against *B. cinerea* (Wilson,*et al. 1997*). Deena and Thoppil (2000) reported the essential oil of *Lantana camara* remarkably inhibited the growth of most of tested bacteria and fungi. Sharma and Trivedi (2002) screened fifteen leaf extracts against root–knot nematode, *meloidogyne incognita* and Wilt fungus *F. oxysporum.* Out of which *Datura stramonium* and *Calotropis procera* showed maximum antifungal activity against *Fusarium oxysporum* f.sp. *cumini*. Curini *et al.,* (2002) studied essential oils of *Erigeron canadensis L.* and *Myrtus communis L. in vitro* as growth inhibitors against phytopathogenic fungi *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc. and *Colletotrichum lindemuthianum* (Sacc. & Magn.*)* Briosi & Cav. Both showed weak fungicidal activity, except the essential oil of *M. communis* that exerted a 60% growth inhibition against *R. solani* at a dose of 1600 ppm. While, at the same dose, only the hyphal morphology of *C. lindemuthianum* was affected by the essential oil of *Erigeron canadensis.* Carvacrol and cinnamaldehyde were very effective at reducing the viable count of the natural flora on kiwifruit when used at 0.15 µl/ml in dipping solution, but less effective on honeydew melon. It is possible that this difference has to do with the difference in pH between the fruits; the pH of kiwifruit was 3.2–3.6 and of the melon 5.4–5.5 (Roller and Seedhar 2002). Abeywickrama *et al*., (2003) screened *Cymbopogon nardus* (L.), *Ocimum basilicum* (L.), *Eucalyptus citriodora* Hook and *Elettaria Cardamomum* (Maton) oils using as fumigant bioassay against banana fruit

pathogens and were fungistatic at concentration between 0.03-0.66% (v/v) and fungicidal at concentration between 0.05 -0.66 % (v/v). The essential oil of *Cymbopogon flexuosus* against dominant Postharvest fungal pathogen, oil showed potent bioactivity with MIC of fungicidal action was found to be 0.2µl/ml to 0.5µl/ml.(Shashi *et al.,* 2003). Essential oils of seven, Moroccan Labiates' were chemically analyzed and evaluated for their *in vitro* antifungal activity against *Botrytis cinerea* (Chebli, *et al.*, 2003). Among them, *Origanum compactum* and *Thymus glandulosus* greatly inhibited the growth of the mycelium and the inhibition of *Botrytis cinerea* was 100% for both oils at 100 ppm.

Anthony et *al.* (2004) reported *Cymbopogon nardus* and *Ocimum basilicum* oils showed fungicidal activity against crown rot pathogens of banana fruit at concentration between 0.2 -0.6 % (v/v) in a poisoned food bioassays. Kulakiotu *et al.,* (2004) reported the potential of volatile of 'Isabella' grapes(*vitis labrusca*) to control gray mold (*B.cinerea*) on ' Hayward' Kiwifruit (*Actinidea deliciosa*). The 'Isabella' volatiles limited the incidence of infection by reducing both the inoculum density and the activity of the pathogen. The study showed the potential for successful biological control of *B. cinerea* on kiwifruit by volatiles from 'Isabella' grapes. Nine plant volatiles were tested for their activity *in vitro* and *in vivo* against *penicillium expansum,* the cause of blue mould of pear and reported that *trans*-2-hexenal, carvacrol, *trans*-cinnamaldehyde and citral gave consistent fungicidal activity against *P. expansum* (Neri *et al.*, 2006). Sharma and Tripathi (2006) screened the essential oil extracted from the epicarp of *Citrus sinensis* exhibited absolute fungitoxicity against the 10 post-harvest pathogens and the oil was fungicidal at 700ppm (mg/l) to 1000ppm (mg/l) range. Tripathi *et al*., (2007) studied pharmacological trials with essential oils of *Mentha arvensis, Ocimum canum* and *Zingiber officinale* and found to exhibit pronounced fungitoxic effects on several types of fungi which are harmful to some crops. Tzortzakis, and Economakis, (2007) screened Lemongrass *(Cympopogon citratus* L.) oil against *B. Cinerea, Collectorium coccodes, Cladosporium herbarum, Rhizopus Stolonifer* and *A. niger*. The Oil-enrichment resulted in significant ($P<0.05$) reduction on subsequent colony development for the examined pathogens. Fungal spore production inhibited up to 70% at 25 ppm of lemongrass oil concentration whereas at 5000ppm concentration fungal sporulation was completely retarded. And Pawar and Thaker (2007) evaluated the effect of 75

different essential oils against *Fusarium oxysporum* and *Alternaria porri*. The most active essential oils found were those of Lemongrass, clove, cinnamon bark, cinnamon leaf, cassia, fennel, basil and evening primrose were the most active among them. Sharma *et, al*., (2007) extracted twenty essential oils from various aromatic plants for screening their antifungal activity *in vitro* against the soil-borne pathogens viz. *Rhizoctonia bataticola* and *Sclerotium rolfsii* by poisoned food technique and reported that the oil extracted from rhizome of *Acorus calamus* was most effective. Dubey et, al., (2007) reported that the essential oil of *Eupatorium cannabinum* was fungitoxic in nature against the tested fungi causing mango-rotting. The oil also enhanced the shelf life of mango fruits by protecting from fungal rotting when tested as a fumigant. Essential oil of *C. cyminum* and *E. citriodora* showed strong suppression on the development of Botrytis lesion on apple fruit infected by *B. cinerea*. The incidences of disease were reduced by increasing dosages of the applied essential oil (Lee *et al*., (2007)). Antifungal activities of plant essential oils were tested against five phytopathogenic fungi such as *B. cinerea, C. gloeosporioides, F. oxysporum, P. ultimum* and *R. solani*. Among 39 essential oils *Origanum vulgare* oil alone inhibited all of the phytopathogenic fungi tested. Both *Cuminum cyminum* and *Eucalyptus citriodora* oil displayed *in vitro* antifungal activities against four phytopathogenic fungi except for *Colletotrichum gloeosporioides*. The essential oil of *Thymus vulgaris* suppressed the mycelia growth of *C. gloeosporioides, F. oxysporum* and *R. solani* and that of *Cymbopogon citratus* was active to only *F. oxysporum* (Lee *et al*.,(2007)). Ziedan and Farrag (2008) evaluated peppermint and sweet basil volatile oils exracted from leaves and tested as a natural biocide to control postharvest decay of peach. *In vitro,* vapour crude oils of peppermint and sweet basil was found to be antifungal against fungal pathogen of peach fruit. Tripathi *et al*., (2008) tested *Hyptis suaveolens* L. (poit.) essential oil *in vitro* on the growth and morphogenesis of *Fusarium oxysporum* f.sp. *Gladioli* (Massey) snyder & Hansen, which causes *Fusarium* corm rot and yellows in various susceptible cultivars of gladiolus, which was found to be fungicidal at nature. Tripathi *et al.* (2008) investigated the antifungal property of twenty six essential oils against *B. cinerea* under *in vitro* conditions and found a total growth inhibition by using extracts from *Chenopodium ambrosioides, Eucalyptus citriodora, Eupatorium, Cannabinum, Lawsonia inermis, Ocimum canum, O. gratissimum, O. sanctum,*

Prunus persica, Zingiber cassumunad and *Z. officinale*. Hadizadeh et al (2009) studied the antifungal activity of some medicinal plants of Iran viz., *Urtica dioica L., Thymus vulgaris L., Eucalyptus spp., Ruta graveolens L.* and *Achillea millefolium L.* against *Alternaria alternata* on tomato and found Both the nettle and the thyme oils exhibited antifungal activity against *A. alternata.* The thyme oil exhibited a lower degree of inhibition ranges between 68.5 and 74.8% at 1500 and 2000 ppm respectively. Spore germination and germ tube elongation of the pathogens in potato dextrose broth was strongly reduced at 1500 ppm of the nettle oil. Chutia *et al.* (2009) evaluated the essential oil of *Citrus reticulata* Blanco peel against five plant pathogenic fungi viz *Alternaria alternate (*Aa), *Rhizoctonia Solani* (Rs), *Curvalaria Lunata* (Cl, *Fusarium oxysporum* (Fo) and *Helminthosporium oryzae* (Ho). The Minimum inhibitory concentration (MIC) for Aa, Rs and Cl was 0.2 ml/100 ml whereas >0.2 ml/100 ml for Fo and Ho in PF technique. Fungal sporulation was also completely inhibited at 2 ml/100 ml of the oil except for Cl and Ho, which was only 0.5%′ (±0.5) and 0.25% (±0.25) respectively as compared to control. Barrera – Necha *et al.* (2009) tested 10 essential oils for their fungicidal activity against *Fusarium oxysporum* isolated from gladiolus corm rots. A significant antifungal effect was observed with *Cinnamomum zeylanicum, Thymus vulgaris* and *Syzygium aromaticum* oils which had total inhibition at 100,150, 200, 250 and 300ppm. *Teloxysioides, Mentha piperita* and *Citrus aurantifolia* oils exhibited a dose dependent inhibition on mycelia growth to increase the dose of 100 at 300ppm. Siripornvisal *et al.*, (2009) studied the essential oils derived from four medicinal plants were evaluated compared for their antifungal efficacies against *B. cinerea*, an aggressive postharvest pathogen on a wide range of fruit. Vapors of clove oil, cinnamon oil and lemongrass oil exhibited strong inhibitory effects on *B. cinerea*, with a MIQ (minimal inhibitory quantity) equal to 15 μL The results of the nature of this inhibition of these oils indicated that clove oil, cinnamon oil and lemongrass oil all exhibited fungicidal effect on the pathogen, while galingale oil exhibited some fungistatic properties. The insecticidal properties of *Litsea cubeba* were evaluated under laboratory conditions. The result showed that the oil of *L. cubeba* strongly repelled *Sitophilus zeamais* and *Tribolium castaneum* even at low concentration (Koko *et al.*,(2009)). Vagelas *et al.*, (2009) investigated the effect of sterilized filtered and non sterilized olive oil mill wastewater(OMW) as tested *in vitro* and

in vivo against *B. cinerea* and strawberry and pepper fruits infected with the pathogen. The result revealed that the filter sterilized olive OMW inhibits the growth of *B. cinerea* mycelium *in vitro* and olive OMW significantly decreased fungus mold formation on the tested fruits. And further the fungicidal activity of a new compound 1 and 2 a known monoterpene and the essential oil from *Litsea cubeba* against *B.cinerea, A.mali,* and *F.oxysporum,* were evaluated using the mycelia growth rate test The preliminary bioassay results showed that 1 and 2 have good fungicidal activities against *Sclerotinia sclerotiorum, Thanatephorus cucumeris, Pseudocer-cospora musae* and *Colletotrichum gloeosporioides* at the concentration of 588 and 272 μM, and the essential oil has good fungicidal activities against *T. cucumeris* and *S. sclerotiorum*, with IC_{50} values of 115.58 and 151.25 μg/mL, repectively (Yang *et al.*,(2010).

Siripornvisal (2010) evaluated essential oil extracted from mature seeds of ajowan (*Trachyspermum ammi* Lin.) against three strains of *Fusarium oxysporum, F. oxysporum* f.sp. *lycopersici, F. oxysporum* f.sp. *cubense* and *F. oxysporum* f.sp. *capsici* the wilt pathogen of tomato, banana and chili respectively. The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) were 240 and 480 μg·mL-1, respectively. The effects of ajowan oil on the biomass production and sporulation of the test fungi indicate that it has significant retardation effect on the biomass production and sporulation of the fungi. Kumar *et, al.*, (2010) studied the efficacy of *Ocimum sanctum* essential oil and its major component, eugenol against the fungi causing biodeterioration of food stuffs during storage. Out of 20 Eos evaluated during antifungal screening tested fungi, the EO of *O. sanctum* exhibited absolute fungitoxicity at 1.0 μl ml^{-1} while *A.calamus*, and *A. conyzoides* showed remarkable antifungal activity where as EOs of *T. erecta* and *L. camara* showed moderate antifungal property. Tariq *et al.*, (2010) used Pakistanian *Acorus calamus* and further studied its importance and implementation as a biopesticides. They proved that the essential oil of the *Acorus calamus* is safe as compared to the other commercial pesticides. This essential oil can be safely used in the agriculture as well as in health sector. The essential oil of *A. calamus* has been tested on the cuts and wounds and found that it was more effective as compared to the other oils. Soylu, et al. (2010) investigated antifungal activities of essential oils obtained from aerial parts of aromatic plants, such as origanum (*Origanum syriacum* L. var. bevanii), lavender

(*Lavandula stoechas* L. var. stoechas) and rosemary (*Rosmarinus* officinalis L.), against *Botrytis cinerea.* They showed that complete growth inhibition of pathogen by essential oil of lavender and rosemary.

Nabigol and Morshedi (2011) evaluated the antifungal potential of *Thymus danensism* and *Thymus carmanicus* against four pathogenic fungi (*Rhizopus stolonifer, Penicillium digitatum, Aspergillus niger* and *Botrytis cinerea*) which can reduce the shelf life of strawberry fruit. Complete minimum inhibitory concentration (inhibition 100%; MIC) of both oils against *B. cinerea,* and *R. stolonifer* were 300 µl/L. The MIC of *T. danensis* and *carmanicus* against *P. digitatum* and *A. niger* was 600 µl/L. The minimum fungicidal concentration (MFC) of the oils of *T. Danensis* and *T. carmanicus* against *B. cinerea, and R. stolonifer* was 1200 µl/L whereas a fungicidal effect was not observed on *P. digitatum* and *A.niger* even at high concentration. Nosrati *et al.,* (2011) studied antifungal activity of 1, 3 and 5 µl of the essential oil of spearmint plants on the mycelia growth of *Fusarium oxysporum f. sp. radicis- cucumernum.* All amounts of the essential oil restricted significantly the mycelia growth of the pathogen with maximum activity detected for samples treated with 5 µl of essential oil. The inhibitory effect of the essences was affected by the amount of the essential oil and the incubation time of the sample. Habib *et al.,* (2011) evaluated essential oil of Thymus *vulgaris* L., *Carum copticum* L., *Foe*niculum *vulgare* L. and *Satureja hortensis* L. under *in vivo* condition for antifungal activity against *B. cineria* on kiwifruits. Antifungal activity of essential oils showed that with the increase of their concentrations the antifungal activity was increased, but no significant differences were observed. The quality parameters such as total soluble solids (TSS), titrable acidity (TA) and vitamin C reduced in fruits treated with essential oil. Weight loss and firmness values were not affected by essential oil treatment and essential oil treated kiwifruits showed off-flavor in compare to control. Shirzad *et. al.,* (2011) assessed effect of essential oils obtained from thyme (*Thymus vulgaris* L.), ajowan (*Carum copticum* L.), fennel (*Foeniculum vulgare* L.) and summer savory (*Satureja hortensis* L.) under *in vivo* condition for antifungal activity against *Botrytis cinerea* on kiwifruits. Mohammadi and Aminifard (2012) studied effect of four essential oils (anise, ammi, ziziphora and cinnamon) and five concentrations (0, 200, 400, 600 and 800 µL.L-1).Results of *in vitro* experiment showed that all of used essential oils at all applied concentrations

inhibited grey mould growth. These entire essential oils in concentration 800 µL.L-1 were without germination spores of grey mould. The essential oils application significantly decreased weight loss percentage and increased life storage fruits. Also, essential oils positively affected on postharvest quality factors including total soluble solids, titrable acidity, anthocyanin, carbohydrate content and pH value. It was observed that treated fruits with ammi essential oil at concentration 800 µL.L-1had the highest total soluble solids; titrable acidity, anthocyanin, and carbohydrate content and it had the lowest decay and acidity.

Shahi *et. al.*, (2012) reported that *in vitro* Cumin oil *(Cuminum cyminum L.)* showed potent bioactivity against dominant post harvest fungal pathogens of apple fruits. The minimum bioactive concentrations with fungicidal action of the oil was found to be 1.2 ml ml-1 for *Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum capsici, C. falcatum, Fusarium cerealis, F. culmorum, Gloeosporium fructigenum, Penicillium digitatum, Penicillium expansum, P. italicum, P. implicatum, P. minioluteum,* 1.4 ml ml-1 for *Aspergillus flavus, A. fumigatus, A. niger, A. parasiticus, Curvularia lunata, Fusarium oxysporum, F. udum, Penicillium variable, Helminthosporium oryzae, H. maydis, Phoma violacea,* and 1.6 ml ml-1 for *Rhizopus nigricans*. The oil exhibited potency against heavy doses (30 mycelial discs, each of 5 mm in diameter) of inoculum at 2.0 ml ml-1 concentrations. The bioactivity of the oil was thermostable up to 1000C and lasted upto 48 months. The oil preparation did not exhibit any phytotoxic effect on the fruit skin (epicarp) of *Malus pumilo* up to 40 ml ml-1 concentrations. *In vivo* trials of the oil as a fungicidal spray on *Malus pumilo* for checking the rotting of fruits, it showed that 35 ml ml-1 concentration controls 100% infection by pre-inoculation treatment, while in post-inoculation treatment, 45 ml ml-1 concentration of fungicidal spray were required for the 100% control of rotting. Mohammadi and Aminifard (2013) determine the antifungal effects of the fennel essential oil against fungal pathogen *Botrytis cinerea* the causal agent of grey mould disease of tomato fruit under *in vitro* and *in vivo* conditions. They found remarkable inhibitory effect of oil at different concentrations and under *in vivo* condition reported to increase the shelf life of tomato fruits. Freire *et. al.*, (2013) evaluated the *in vitro* effect of castor bean oil (*Ricinus communis*), and its constituents, on the mycelial growth and spore germination of *Lasiodiplodia theobromae*, suggesting an alternative to chemical

control during the post-harvest. Vitoratos *et. al.*, (2013) studied *in vitro* and *in vivo* activity of essential oil obtained from oregano (*Origanum vulgare* L. ssp. *hirtum*), thyme (*Thymus vulgaris* L.) and lemon (*Citrus limon* L.) plants, against some important postharvest pathogens (*Botrytis cinerea, Penicillium italicum* and *P. digitatum*). *In vitro* experiments indicated that *P. italicum* did not show any mycelium growth in presence of thyme essential oils at 0.13 μl/ml concentration. Moreover, *B. cinerea* did not show any mycelium growth in presence of lemon and oregano essential oils at concentration of 17μl/ml and 0.02μl/ml, respectively. Moreover, the essential oils from three species were effective in reducing the spore germination. The *in vivo* experiments confirmed the strong efficacy shown in vitro by essential oils.

Enyiukwu *et. al.*, (2014) stated that most important losses in agricultural production which involve the greatest costs on the farm economy occur postharvest. It is estimated that worldwide between 10 and 40% losses of agricultural produce occur postharvest. Losses are more severe in developing than developed nations of the world. Several species of fungi and in some cases bacteria participate in postharvest deterioration and rots of tubers and agroproduce. These include species of *Aspergillus, Fusarium, Colletotrichum, Macrophomina, Penicillium* and *Rhizopus* amongst several others. Mohapatra et al (2014) evaluated biocontrol potentials of three essential oils against the pathogens *Aspergillus, Mucor, Rhizopus, Geotrichum* and *Fusarium* causing rapid spoilage. All the oil displayed antifungal activity in varying degrees. Among the oils, carrot oil was found to be most effective in inhibiting all the pathogens. Francesco and Mari (2014) stated that the use of biocontrol agents (BCAs) to control postharvest fruit diseases is still constrained by the lack of high levels of disease control required in the postharvest phase (more than 95%). Their inconsistent activity is one of the main factors preventing their routine application on fruit after harvest. Therefore, to overcome this issue, integrated strategies were explored and continue to be one of the fields most investigated in postharvest fruit disease control, in order to achieve maximum effectiveness. The combination of BCAs with physical and chemical treatments, including fungicides at low doses, resulted in an increase of BCAs effectiveness against fungal diseases such as *Pencillium* spp., *Monilinia* spp., *Botrytis cinerea*, etc. Since the integration of different treatments could benefit from their additive or synergic effects and improve the

efficacy of each single method. Behdani *et. al.*, (2014) observed antifungal activity of 13 species of medicinal plants essential oil against apple gray mold through pour plate method and production of volatile compounds. The results showed that essential oils of Anise, Cumin, Caraway, Ammin, Pennyroyal, Thyme and Cinnamon in pour plate method at all concentrations of 250, 500, 750 microliters per liter, have a significant effect against *Botrytis cinerea* on PDA culture.By the volatile compounds production method, essence of Cumin, , Ammin, Pennyroyal, Dill, Cinnamon, Anise and Caraway showed the most inhibitory effect and the others somewhat illustrated fungistatic power. Abdallah (2014) reported inhibitory effect *in vitro* of tested essential oils on mycelium growth was higher than on conidium germination of *F. semitectum*. Complete inhibition of fungal growth and spore germination was recorded at the concentration of 1.0% of tested cinnamon and bitter almond oils. Under storage conditions, artificially inoculated bananas showed reduction in both crown rot disease incidence and severity when treated with cinnamon, thyme bitter and sweet almond oils. Complete reduction (100.0%) of crown rot disease incidence of banana fruits was recorded at the concentration of 4.0% of applied cinnamon and thyme oils, followed by sweet almond and bitter almond oils which they reduced disease incidence by 87.1 and 78.7%, respectively.

2.3. Fungitoxicity of Higher Plant Extracts

Anselme (1959) reported reduction of infection cause by *Botrylis cinerea* on flax when treated with chloroform extract of *Lupinus* sps at 1:1000 dilution. Ischenko (1961) found inhibition of spore germination of *Venturia inaequalis* from the leaf extract of three varieties of apple. Abdullaeva (1962) found seed extract of *Allium sativum* and *A. cepa* to exhibit volatile toxicity against *Fusarium oxysporum, Rhizoctonia solani* and *Verticillium dehalae.* The extracts of *Canna indica L., Convolvulus arvensis L., Ipomoea palmata Forsk., Cenchrus catharticus Delite., Mentha piperita L., Prosopsis spicigera L. (Mant), Allium cepa L., A. sativum L., Lawsonia inermis L., Argemone mexicana L., Datura stramonium Jim. and Clerodendron inerme Gaertn* completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower (Sheikh and Agnihotri 1972). Kumar *et al.* (1979) reported aqueous extract of onion, garlic, *Kalnchoe* sp., *Parthenium* sp., cotton and *Phaseolus antropurpureus* to completely inhibit the spore

germination of *Drechslera rostrata, Fusarium oxysporum* and *Alternaria alternata.*

Chandra and Dikshit (1981) screened leaf extracts of some plant species and found *Ageratum conyzoides* to inhibit the growth of *Colletotrichum capsici* and *Penicillium italicum.* Pandey *et al.* (1982) screened extracts of seeds of thirty two plants and found soybean, lentil, *Leonotis nepetaefolia, Paspalum scrobiculatum* and *Peltophorum pterocarpum* to exhibit absolute toxicity against *Alternaria alternata* and *Aspergillus niger.* Saxena and Tripathi (1985) tested leaves of fifteen aromatic plants and found *Cuminum cyminum, Ocimum sanctum, Angelica* sp. and *Lantana camara* to be highly effective against *Aspergillus niger, Mucor mucedo* and *Alternaria alternate.* Dixit *et al.* (1986) screened seedling extracts of forty taxa belonging to twenty two families against spore germination of *Botrytis cinerea* and *Colletotrichum gloeosporioides* and found 100 per cent inhibition with the extracts of *Abrus precatorius, Carum copticum* and radish. Jagdish and Reddy (1986) screened some plant extracts and found aqueous extracts of *Eucalyptus globulus, Punica granatum, Lawsonia inermis and Datura stramonium* to be effective against fruit rot of lemon caused by *Colletotrichum gloeosporiodes* and *Botryodiplodia theobromae.* A systematic screening of medicinal plants viz. *Allium cepa, Argemone Mexicana, Calotropis procera, Datura metel, Ocimum sanctum, Rauvolfia serpentine, Tagetes erecta, Catharanthus roseus* and *Lochnera resea* were carried out and their fungitoxic principles were determined and further antifungal activity was seen against *Alternaria alternata* exhibiting significant inhibitory effect (Srivastava and Srivastava, 1988). Misra *et al.* (1988) found leaf extract of *Ageratum conyzoides* and *Chrysanthemum* sp. to exhibit strong activity against *Aspergillus sydoui.* Shell extract of groundnut inhibited spore germination of *Alternaria alternata* and *Curvularia spicata (*Kaliachelvan and Mahadevan, 1988). Naidu (1988) found leaf extract or *Codiaeum variegatum* to be active against *Alternaria alternate* and *Fusarium oxysporum.* Eswaramurthy *et al.* (1989) found extracts of neem, *Acacia, Prosopis,* and *Ipomoea* sp. to completely inhibit mycelia growth of *Sarocladium oryzae* and *Fusarium oxysporum.* Yasmeen and Saxena (1990) also observed that the extracts prepared from the leaves of *Lawsonia alba*, roots of *Datura stramonium* and inflorescence of *Mentha piperita* had fungitoxic activities against *A. brassicae* isolated from cauliflower leaves. Leaf extracts of *Clerodendron* and

C. fragrans brought about considerable reduction in growth and sporulation of various fruit rot causing fungi to varying extent (Sharma and Verma, 1991). Ethanolic extracts of 50 plants were tested against 7 fungi known to cause fruit rots (Mohamed *et al.*, 1994). Srivastava *et al.*, (2001) isolated insecticidal activity of myristicin , a 1,3- benzodionole, from the hexane fraction of alcoholic extract of fruit bearing inflorescence of *piper mullesua.* They evaluated both the contact toxicity and antimetabolic activity against lepidopterous insect pest *Spilarctia obliqua.* Topical application of myristicin caused significant toxicity towards 4th instar larvae as compared to post-ingestive effect of the compound. The water extracts from the weed species (*Ageratum conyzoides, Oxalis corniculata, Phyllanthus debilis, Vernonia cinerea* and *Desmodium trifolium*) were assayed for their antifungal activity against some plant pathogenic fungi. The extract from *Ageraum conyzoides* inhibited the mycelia growth of *Rhizoctonia solani, Aspergillus niger* and *Phomopsis theae*. The extract from *Oxalis corniculata* was active against *A. niger* while *Phyllanthus debilis* suppressed the growth of *P. theae*.(Iqbal *et. al*., (2001)).

Hur et al., (2002) evaluated antifungal activity of cold- tolerant *Eucalyptus darlympleana* against postharvest pathogens of kiwifruits- *Botrytis cinerea, Botryosphaeria dothidea,* and *Diaporthe actinidiae*. Methanol extract of the tree showed strong antagonistic activity against all the three pathogens. And aerial mycelium growth and spore germination of the pathogens were strongly inhibited by the phenolic compound. Matos and Barreiro (2004) evaluated safety use of bioactive products of plant origin for the control of postharvest fungal diseases of Portuguese “Rocha” pear and found *O. vulgare* and caprilic acid have potentialities to treat Portuguese “Rocha” pear infected with *B. cinerea* and *P. expansum.* Maximum *in vitro* inhibition of mycelial growth and spore germination of *A. solani* was observed with (5-10%) dried root extracts of Acorus calamus. Extracts from *Prosopis julifera* (10% leaf) and garlic (5% bulb) gave moderate levels of inhibition. Palmarosa oil (0.1 and 0.05%), Neem 60 EC (3%), Neem oil (3%) and *Madhuca indica* oil (3%) also inhibited mycelial growth. Among the antagonists, *Bacillus subtilis, Trichoderma viride* and *Gliocladium virens* also inhibited mycelial growth of *A. solani* (Vadivel and Ebenezar, 2006).

Montes-Belmount and Prados-Ligero (2006) reported from studies in Cameroun, that the combination of extracts showed that the effect of each plant could be

modified by the reactions of the complex mixture of plant compounds in the extracts. For instance in studies on Onion rot (*Sclerotium rolfsii*) a mixture of extracts of *Piper nigrum* and clove (*Syzygium aromanticum*) resulted in enhanced and synergistic inhibition effect against the causal pathogen while all combinations involving clove and other extracts resulted in single fungicidal (unity) effect. However, they noted that all combinations of other extracts with allspice (*Pimerta dioica*) showed antagonistic influences and negative inhibition effects.

Begum *et, al*.,(2007) evaluated ethanolic extract of 40 higher plants representing 23 families for antifungal activity against some phytopathogenic fungi. *Acorus calamus* and *piper betel* were the two most active plants showing potent antifungal activity. The rhizome extract of *A. calamus* exhibited highest antifungal activity inhibiting the mycelial growth completely (100%) against all the 6 test pathogens. *P. betel* exhibited more than 50% inhibition against most of the test fungi. The neem leaf extract showed high efficacy to inhibit the radial growth of *A. solani* (43.3 and 26.7% respectively at 0.1% and 0.01%) (Sharma *et al*., 2007). The antimicrobial potential of seventy-seven extracts from twenty-four plants was evaluated against eight bacteria and four pathogenic fungi, using microbroth dilution assay. Water extracts of *Acacia nilotica, Justicia zelanica, Lantana camara* and *Saraca asoca* exhibited good activity against all the bacteria tested and the MIC was recorded in range of 9.375-37.5 μg/ml and 75.0-300.0 μg/ml against the bacterial and fungal pathogens, respectively (Dabur, R *et al* (2007). Amadioha and Markson (2007) found extracts of *Piper nigrum*, *Ageratum conyzoides* and *A.melegueta* to significantly arrest the mycelial growth and biomass development of *Botrydiplodia acerina* causal agent of rot of cassava *in vivo*. A related study demonstrated that extracts and powder formulations of *Cassia alata and Dennettia tripetala* effectively checked the growth, development and spread of *Sclerotium rolfsii* induced rot of corms of cocoyams (Nwachukwu and Osuji, 2008). Ubulua and Oti (2008) reported that ethanol extracts of *A. sativum* and *Landolphia owerrence* exhibited broad-spectrum activity against fungal pathogens associated with cassava rot. The combination of the extracts *A. sativum* and *Garcimia kola* was found to demonstrate remarkable inhibition of the assayed pathogens after 16 days in storage with only 2% rot; while that of *A. sativum and L. owerrence* roots exhibited little or no activity. Ojeda-Contreras *et*

al., (2008) conducted experiments both *in vitro* and *in vivo* to test the effect of caffeic acid phenethyl ester (CAPE; a resinous component of propolis, collected by bees having positive effects on human health) on *A. alternata* infected tomato fruits. The results of *in vitro* experiment revealed that 64 and 100µm of CAPE reduced colony size by 30%. In case of *in vivo*, 50 and 100µm of CAPE reduced severity of infection better than the fungicide Captan (R) without any effect on respiratory rate, ethylene production, weight etc. Hence, they concluded that CAPE controls *A. alternata* infection better than a commercial fungicide without negative effects on tomato fruit ripening and fruit quality. Coetzee, G *et al.*, (2008) evaluated green tea extracts from indigenous South African rooibos (*Aspalathus linearis*) and honey bush (*Cyclopia* species) as potential antifungal agent against the plant pathogen *B. cinerea* and found that when applied 10mg/ml, the tea extracts stimulated biomass production in *B. cinerea* by more than 3-fold after 24 hrs. However, when applied 100mg/ml, the *A. linearis* and *C. genistoides* extracts reduced spore germination of *B. cinerea* by 33.3% and 16.7% respectively. Prasad *et al*., (2008) reported that aqueous extracts of *Samanea saman* showed inhibitory activity against *E. coli, S. aureus* and *C. albicans.* 5mg/ml inhibited E. coli but slight higher concentration of 10mg/ml needed to inhibit *S. aureus* and *C. albicans*. Raghavendra, *et al*., (2008) evaluated aqueous and different solvent exracts and isolated constituents (alkaloids) of leaves of *samanea saman* were assayed for antibacterial activity by cup diffusion method against three phytopathogic and 14 human pathogenic bacteria. Aqueous and methanol exract showed significant antibacterial activity against all pathovars. Balestra, G.M *et al* (2008) reported, *in vitro* and *in vivo*, aqueous extracts from *Allium sativum* and *Ficus carica* fruits reduced the survival and the damages caused by bacterial pathogens of kiwifruit and tomato plants. Ok oh, *et al.*, (2008) studied antifungal activity of natural substrances from *Eucalyptus darlympleana, E.globules, E.gunnii, E.unigera* against postharvest pathogens of kiwifruits, *Botrystis cinerae, Botryosphaeria dothidea,* and *Diaporthe actinidiae* to screen effective natural substances as an alternative to chemical fungicides. Methanol extracts of *Eucalyptus* leaves exhibited strong antifungal activity against *B. dothidea, B. cinerea* and *D. actinidae*. Strength and spectrum of antifungal activity of the extracts varied in *Eucalyptus* tree species and in fungal species.

Mvuemba *et. al.*, (2009) found that cinnamon, ginger and nutmeg significantly inhibited the mycelial growth of *Aspergillus niger* (Ascomycota), *Fusarium sambucinum* (Ascomycota), *Pythium sulcatum* (Oomycota) or *Rhizopus stolonifer* (Zygomycota), whereas horseradish extract did not lead to the inhibition of any microorganism at the tested concentration. Among the most effective extracts, 0.05 g mL-1 of cinnamon extract completely inhibited *A. niger* and *P. sulcatum*, and 0.10 g mL-1 of cinnamon extract completely inhibited *F. sambucinum*. A concentration of 0.05 g mL-1 of ginger extract also caused 100% inhibition of *P. sulcatum*. *In vivo*, cinnamon extract significantly reduced lesions of potato dry rot and carrot cavity spot, and ginger extract reduced lesions of carrot cavity spot. Hasan *et al.*, (2009) conducted a study to determine the antibacterial and antifungal activities of *Polygonum hydropiper* (L.) root extract with chloroform against both bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram-positive (*Bacillus subtilis, Bacillus megaterium, Stapphylococcus aureus* and *Enterobacter aerogenes*) and four gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella sonnei*) bacteria. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 16 to 64 μg/ml. The antifungal activities were found strong against six fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Rizopus oryzae* and *Tricophyton rubrum*). Devi *et. al*., (2009) studied the antimicrobial and antifungal activity of *Acorus calamus* rhizome and leaf. In this study they used petroleum ether, chloroform, hexane, and ethyl acetate extract of rhizome and leaves and found that ethyl acetate was highly effective in antifungal and anti yeast activity.

Abdul and Waheeta (2010) screened six organic solvent extract of *Artemisia nilagirica* for the potential antimicrobial activity against phytopathogens and clinically important standard reference bacterial strains. All the extracts showed antibacterial activity against the tested strains. Of all, methanol and hexane extracts showed high inhibition against clinical and phytopathogens, respectively. The results also indicate the presence of major phytochemical derivatives in the *A. nilagirica* extracts. Singh *et al.*, (2010) showed the antifungal activity using methanolic extract of *Acorus calamus*. It involves the number of inducible defence mechanism against many diseases or

pathogens. Majority of them are induced but after recognition they become constitutive and non specific. Results easily established antifungal potential of methanolic extracts of *Acorus calamus*.

The *In vitro* fungitoxic potential of *Tagetes erectus* L. was scrutinized against *Ascochyta rabiei,* the causal agent of chickpea blight disease. All the employed concentrations of both flower and shoot extracts significantly suppressed the growth of target fungal pathogen. There was 4-35% and 55- 73% reduction in colony diameter of *A. rabiei* due to different concentrations of aqueous flower and shoot extracts of *T. erectus* and 12-50% and 4-42% due to different concentrations of methanolic flower and shoot extracts of *T. erectus,* respectively, (Safique *et. al.*, 2011). Bark extract of *Bauhinia purpurea* was phytochemically analyzed and evaluated for antimicrobial and antioxidant activities. Among different solvent extracts, aqueous extract exhibited a broad spectrum of antimicrobial activity and showed strong antibacterial activity against Gram positive bacterial strains like *Bacillus subtilis, Staphylococcus aureus* and Gram negative strains like *Escherichia coli* and *Klebsiella pneumonia* and antifungal activity against *Candida albicans.* While methanolic extract showed moderate to strong antibacterial activity against *B. subtilis, E. coli* and *K. pneumonia,* the extracts of hexane, chloroform and ethyl acetate did not show any anti bacterial or antifungal activity against the tested fungal and bacterial strain (Avinash *et. al.*, 2011). Vivek *et al.*, (2011) investigated antioxidant capacity and antimicrobial activities of various extracts of *Michelia champaca* linn flowers. The antioxidant activity of extract increases with increase in amount of extract (5-20mg). The crude extract exhibited high anti candidal activity on *Candida albicans.* Antifungal activity of ethanolic extracts of medicinal plants viz. *Azadirachta indica, Jatropha gossypifolia, Lantana camara, Tridax procumbens, Calotropis procera,* and *Datura stramonium* were evaluated against Fusarium oxysporum involved in wilt diseases of Zingiber officinales. Results showed the ethanolic extract of *Azadirachta indica* leaf, exhibit maximum toxicity against the test fungus inhibiting the mycelia growth up to 80%, followed by *Ocimum sanctum* 70%. And *T.procumbens* and *L. camara* moderately inhibiting up to60% and 52% respectively, (Shrivastava et al., 2011). According to Taiga (2011) 40% aqueous extract of *Nicotinia tabacum* completely inhibited yam rot development (*Rhizopus stolonifer*) *in vivo*. Gatto et al (2011) reported *in vitro* and *in vivo* activity of

extracts obtained from nine wild edible herbaceous species (*Borago officinalis, Orobanche crenata, Plantago coronopus, P. lanceolata, Sanguisorba minor, Silene vulgaris, Sonchus asper, Sonchus oleraceus, and Taraxacum officinale*) against some important postharvest pathogens, i.e. *Botrytis cinerea, Monilinia laxa, Penicillium digitatum, P. expansum, P. italicum, Aspergillus carbonarius,* and *A. niger.* Phenolic composition of all extracts was evaluated by HPLC. Several derivatives of caffeic acid, of the flavones apigenin and luteolin, and of the flavonols kaempferol and quercetin, were identified. Extracts from *S. minor* and *O. crenata* showed the highest efficacy in all the trials. In particular, S. minor completely inhibited *in vitro* the conidial germination of *M. laxa, P. digitatum, P. italicum,* and *A. niger* and strongly reduced those of *B. cinerea; O. crenata* extract showed a lower but still significant reduction of conidial germination on all the tested fungi.

Ikeura *et al*., (2011) evaluated sixteen plants (garlic, clove, dokudami, kumasasa, dandelion, kusagi, yomogi, ginkgo, marigold, lavender, thyme, hot pepper, ginger and lemon basil) by means of solvent extraction with either dichloromethane or diethyl ether screened against *P. expansum* for antifungal activity. Similarly, *Rhizopus stolonifer* and *Fusarium oxysporium* amongst other pathogens were also reported inhibited by extracts from *Chromolaena odorantum, Azachirachta indica, Vernonia amygdalina* and *Tridax procumbens* in eggplants and tomato (Ijato *et. al*., 2011).

Phytopesticides from *Afromomium meleguata* and *Zingiber officinale* reportedly inhibited *Penicillium digitatum, Mucor piriformis, Aspergillus niger* and *Heminsthoporium solani* causal agents involved with soft rot of tomato (Chiejina and Ukeh, 2012; Ukeh and Chiejina, 2012). Bhardwaj (2012) in like manner, reported from a study conducted in India that seed extracts of *Dedonia viscosa* also showed strong inhibition of *Fusarium solani* and combination of leaf extracts of *Acacia catechi* and *Lawsonia alba* resulted in efficient enhancement of their antifungal activities over the individual extracts of each plant material against the mycelial growth of the pathogenic organism in potato. Gujar and Talwankar (2012) showed that extractants have profound effects on activity of extracts. They remarked that inhibition of *Ocimum sanitum, O. basilcum* and *Lantana camara* against the storage pathogens *A. flavus, A. niger, R. solani* and *R. bataticola* was solvent-dependent being highest in alcohol, followed by acetone

and least in water. Green plants provide a reservoir of effective chemicals that can be exploited against phyto-parasites. Gujar and Talwankar (2012) tested six different plants viz. *Azadirachta indica, Aloe vera, Ocimum sanctum, Ocimum basilicum, Lantana camara* and Asparagus. These plants showed the antifungal activity against the *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani, Rhizoctonia bataticola. Azadirachta indica* and *Aloe vera* appeared significantly the most effective and suppressed the radial mycelial growth of Aspergillus and Rhizoctonia species. Whereas *Ocimum sanctum* exhibited maximum inhibition (90-95%), against *Aspergillus niger*. However *Ocimum basilicum* and *Lantana camara* exhibited moderate type of inhibition against all tested pathogen and Asparagus shows least potential of inhibition against all tested pathogen. Onyeani and Osunlaja (2012) investigated the effect of aqueous and alcohol extract of *Anonna squamosa, Azadirachta indica* and *Vernonia amygdalina* in the control of fruit anthracnose disease of mango. The result of the investigation revealed that there was a significant reduction in the development and severity of anthracnose lesions on mango fruits treated after harvest with aqueous and alcohol extracts of the three indigenous plants.

Ademe *et. al.*, (2013) evaluated antifungal activities of nineteen plant extracts against *Colletotrichum gloeosporioides* under *in vitro* and anthracnose caused by *Colletotrichum gloeosporioides*, on papaya (*Carica papaya* L.) during storage. Ethyl acetate extracts of *Lantana camara* resulted in the highest inhibition (with inhibition zone of 35.3 mm) and showed strong activity against *C. gloeosporioides*. Inhibition levels of spore germination that reached 88.7, 85.8, 85.1 and 84.6% were recorded over the control by extracts of *Lantana camara, Lantana viburnoides, Echinops sp.* and *Ruta chalepensis*. Four aqueous extracts were evaluated for control of anthracnose under *in vivo* for 14 days, and *Echinops sp.* (25%) was found to be most effective in the reduction of disease development and maintaining the overall quality of papaya fruit. The antifungal potential of extracts from seven Cameroonian plants was evaluated against *Phythopthora infestans*, pathogen of late blight disease of potato and tomato. Essential oils, hot water, cold water and ethanol extracts were obtained from *Ageratum conyzoides, Bidens pilosa, Callistemon citrinus, Cymbopogon citratus, Erigeron floribundus, Ocimum gratissimum* and *Tephrosia vogelii*. They found that the essential oils exhibited the best control of the pathogen, followed by ethanol extracts: total

inhibition of pathogens growth was obtained with essential oils of *C. citratus* at 300 ppm, *O. gratissimum* at 400 ppm, and *C. citrinus* at 5000 ppm. The ethanol extracts of *A. conyzoides* and *C. citrinus* totally inhibited the pathogen at 5000 ppm, and that of *O. gratissimum* at 10000 ppm, (Hubert *et. al.*, 2013).

Lakshmi *et. al.*, (2014) studied anti fungal activity of 10 plant extracts against the fungal disease (*Cercospora, Sphaceolema, Certocystis fimbriata, Collitotrichum gleosporides, Fusarium solani,* and *Fusarium oxysporum*) of pomegranate using agar well diffusion method. The Hexane, Choloform, Methanol, extracts of 10 plant extracts exhibited varying degrees of inhibition activity against the fungal pathogens of Pomegranate. Among the all plant extracts 9 plants shows low activity while *Tribulus terristris* methanol extract shows high activity inhibition zones. Gawade *et. al.*, (2014) tested Methanol and petroleum ether extracts of seeds of *Aegle marmelos* against anthracnose causing plant fungal pathogen *Colletotrichum acutatum.* The antifungal potential of same extracts was also tested against three fungal strains, *Metarrhizium anisopliae*, *Trichoderma harzianum* and *Penicillium* spp. Both methanol and petroleum ether extracts showed antifungal activity at tested concentrations (1:1, 1:2, and 1:3). Activity exhibited by methanol extract was greater compared to petroleum ether extract. Sattar *et. al.*, (2014) made evaluation of antifungal activity against *P. expansum* from four plants (Garlic, Clove, Dodonaea (Sanatha) and Polygonium) by means of solvent extraction with methanol. For *in vivo* experiment conidial suspension (100 µL) was applied to each injury site on apple fruits. Lesion diameter of the treated fruits was observed daily for 8 days. The antifungal activity of *Polygonium* was found highly effective than other three. In these results, methanol was found to be an appropriate solvent for use in extracting active compounds from plants presenting antifungal activity against *P. expansum.* Methanol extracts of *Polygonium* at 50 µL/wound was the most effective for inhibiting mycelial growth of the fungus.

A survey of literature reveals that much investigation have not been carried out on the treatment of post harvest diseases of kiwifruit with botanical products such as essential oils and plant extracts. Therefore it was thought desirable to study the impact of some essential oils and plant extract to control the post harvest diseases of kiwifruit.

CHAPTER - 3

MATERIALS AND METHODS

3.1. Isolation and identification of post harvest fungal pathogens of kiwifruits

Isolation of post harvest pathogens of kiwifruits were carried out from infected fruits on rose Bengal agar and potato dextrose agar medium (Johnson and Curl, 1972). Infected kiwifruits were randomly collected from market. Fruits were surface sterilized by 4% sodium hypochlorite and then by 75% alcohol and finally with sterilized distilled water. Small pieces of fruit were cut and placed in the petriplates containing sterilized medium and incubated at 27°C for 7-10 days. Identification of fungal pathogens was done on the basis of morphological, cultural and microscopic characteristics as detailed in available literature (Barnett and Hunter 1972, Domsch *et al.*, 1980).

3.2. Culture media and inoculums

To culture the isolated fungal pathogens Potato Dextrose Agar (PDA) medium and Peptone Dextrose Rose Bengal Agar medium were used. Potato dextrose agar medium (39 gm of Hi–PDA medium dissolved in 1000 ml of distilled water) medium was used throughout the investigation. The medium was autoclaved and cooled to 40°C ±2°C. Thirty milligram of streptomycin was added to it and mixed thoroughly so as to prevent bacterial contamination. Similarly Peptone Dextrose Rose Bengal Agar (31.55 gm of Hi-RBA medium dissolved in 1000 ml of distilled water) medium was prepared to maintain the fungal culture.

3.3. Plant material collection

Plants were collected from different parts of Arunachal Pradesh during the study period. Identification of plants was done by the plant taxonomist in the Department of Botany, Rajiv Gandhi University, Itanagar as well as by the Scientist from Regional Centre of Botanical Survey of India at Itanagar. Herbarium was preserved and voucher specimens were deposited in the department.

3. 4. Extraction of essential oils

Extraction of essential oils was carried out from some locally available larger number of angiospermic taxa namely *Acorus calamus, Ageratum conyzoides, Artimesia nilogerica, Erigeron canadensis, Eupatorium odoratum, Litsea cubeba, Mesua ferrae, Mikania cordata, Piper mullesua and Pogostemon cablin* etc. Subsequently on getting results potent 5-10 plants were taken for detailed study. An amount of 250 gm of fresh leaves of each plant were cut separately into small pieces and were thoroughly washed with sterilized water. The volatile fractions were isolated by hydro distillation through Clevenger's apparatus. Leaves of the plants were used for extraction of essential oils except in case of *Litsea cubeba, Acorus calamus* and *Mesua ferrae* where fruits, rhizome and flowers were respectively used for the oil extraction. The isolated fractions of plant parts exhibited two distinct layers an upper oily layer and the lower aqueous layer. Both the layers were separated and the essential oils were stored in clean glass vials after removing water traces with the help of capillary tubes and anhydrous sodium sulphate (Guenther, 1972).

3.5. Screening of essential oils for fungitoxicity

Fungitoxic activities of the essential oils were tested by the poisoned food technique of Grover and Moore (1962) and Perrucci *et al.,* (1994). Potato dextrose agar medium (39 gm of Hi–PDA medium dissolved in 1000 ml of distilled water) was used throughout the investigation. The medium was autoclaved and cooled to 40°C ±2°C. Thirty milligram of streptomycin was added to it and mixed thoroughly so as to prevent bacterial contamination. A requisite amount of the oil was dissolved separately in 0.5ml of 0.01 percent of aqueous solution of Tween –80 in presterilized Petri plates (7cm. diam.). While using Tween-80 as solvent care was taken in designing the experiments to evaluate the true effect of essential oils on the pathogenic fungi. PDA medium (9.5 ml) was pipetted to each Petri plate and was mixed so as to obtain the requisite concentrations viz. 5000ppm, 1000ppm, 500ppm, 250ppm and 125ppm. For control sets, requisite amount of sterilized water in place of the oil was added to the medium.

Discs of test fungi (5 mm diam) were cut with the help of sterilized cork borer from the periphery of a seven day old culture and were inoculated aseptically to the center of each Petriplate of treatment and control sets. The petriplate were incubated at 27± 1°C for six days in incubation chamber. Measurement of colony diameters of the test fungus in treatment and control sets were measured in mutually perpendicular directions and were recorded in terms of percent mycelial inhibition using the following formula

$$\text{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

Where dc =mean colony diameter of control sets

dt = mean colony diameter of treatment sets

3.6. Standardization of essential oils through fungitoxic properties

The standardization of essential oils was done through fungitoxic properties viz. minimum inhibitory concentration, nature of toxicity (Thompson, 1989), effect of increased inoculum density on toxicity of the oils (Molyar and Pattisapu, 1987), effect of storage and temperature on fungitoxicity of the oils, comparison of efficacy of the oils with some prevalent synthetic fungicides and fungitoxic spectrum against various fruit rotting fungi.

3.6.1. Minimum inhibitory concentration (MIC)

To find out the minimum inhibitory concentration at which the oil showed absolute fungitoxicity (complete inhibition of growth of test fungi), experiments were carried out by the usual poisoned food technique. Different concentrations of the oils were prepared by dissolving separately their requisite amount in 0.5 ml of 0.01 per cent of aqueous solution of Tween-80 and then mixing with 9.5 ml potato dextrose agar medium. The medium of control sets contained requisite amount of sterilized water dissolved in 0.5 ml Tween-80 in place of oils. As usual the prepared plates were inoculated upside down aseptically with the assay disc of the test fungi to the center of petriplate of treatment and control sets. The petriplates were incubated at 27± 1° C for six days in BOD incubator. Diameters of fungal colony of treatment and control sets were measured in mutually perpendicular directions on the seventh day and percentage inhibition calculated.

3.6.2. Nature of toxicity

Nature of toxicity (funtgistatic / fungicidal) of essential oils against the fungi was determined as suggested by Thompson (1989). Requisite amount of the oil was dissolved separately in 0.5 ml of 0.01 per cent of aqueous solution of Tween-80 and mixed with 9.5 ml potato dextrose agar medium to get final concentrations. Sterilized water was used in control sets in place of the oils. The plates were inoculated upside down aseptically with fungal disc (5mm diam.) taken from the periphery of a seven day old culture of the test fungi and were incubated for six days at 27± 1°C. On seventh day the inhibited discs were taken out from the plates, washed with sterilized water and reinoculated aseptically to plates containing fresh potato dextrose agar medium. The revival of the growth of the fungal discs was observed and the per cent inhibition of growth of the test fungi were calculated on the seventh day with respect to control sets.

3.6.3. Effect of increased inoculums density on toxicity of the oils

The effect on increased inoculums density of the fungus fungitoxity of the oils was studied following Moleyar and Pattisapu (1987). Requisite amount of the oil was dissolved separately in 0.5 ml of 0.01 per cent of aqueous solution of Tween-80 and mixed with 9.5 ml potato dextrose agar medium to get final concentration of 1000ppm for *Litsea cubeba* against *Penicillium expansum* and *Fusarium oxysporum* and 5000ppm against *Botrytis cineria* and 500ppm for *Acorus calamus* against *Fusarium oxysporum* and *Borytis cineria* and 1000ppm against *Penicillium expansum*. Five sets for each oil were thus prepared and were inoculated separately by the assay discs (5 mm in diam.) of test fungus in geometric progression of two *i.e.* 1, 2, 4, 8, and 16. The approximate number of spores of each set was recorded with the help of haemocytometer by introducing the similar number of fungal discs in distilled water. For control set equal quantity of sterile water dissolved in 0.5ml Tween-80 was mixed with the liquid potato medium. All the sets were incubated for six days at 27± 1°C and observations were recorded on the seventh day.

3.6.4. Effect of Storage and Temperature on fungitoxicity of the oil

The effect of storage and temperature on the fungitoxicity of the oils was observed so as to know the shelf life and thermostability of the fungitoxicity of the oil.

Effect of Storage

To determine the effect of storage, the oils stored separately in air tight specimen tubes at room temperature were subjected to fungitoxic testing at their respective minimum inhibitory concentrations at a regular interval of one month by the usual poisoned food technique. The observations were recorded and percentage inhibition of growth was recorded.

Effect of temperature

To study the effect of temperature, three lots of each oils, each containing 2 ml of the oil, were kept separately in air tight glass vials and were treated at different temperatures *viz.* 5°C, 30°and 121°C for three hours. The oil of each tube was tested for fungi toxicity at their respective minimum inhibitory concentrations by the usual poisoned food technique and percentage inhibition of growth of test fungus was recorded.

3.6.5. Comparison of efficacy of the oils with some prevalent synthetic fungicides

The efficacy of the oils was compared with some available standard synthetic fungicides. Active principles of two fungicides viz Captaf 50% and Bavistin were compared with the oils by the usual poisoned food technique. The requisite amount of fungicides and the oils of *Litsea cubeba, Acorus calamus, Artimisea nilogerica* and *Ageratum conyzoides* was dissolved separately in 0.5ml of 0.01% Tween-80 and then mixed with potato dextrose agar medium to obtain different concentration viz.500ppm, 1000ppm, 2000ppm, 3000ppm, and 5000ppm. For control requisite amount of Tween-80 was used in place of fungicides/oils. The plates were incubated for six days at 27°C±1°C and percentage of growth of test fungus was recorded.

3.6.6. Fungitoxic Spectrum

The range of fungitoxicity of the oils was determined at their toxic and hypertoxic concentrations *i.e.* at 500ppm and 1000ppm for *Acorus calamus, Ageratum*

conyzoides, Artemisia nilagirica and *Litsea cubeba* against four available fungi (mostly storage fungi) by the usual poisoned food technique using potato dextrose agar medium.

3.7. Preparation of plant extracts

Plant extract were prepared using leaves/fruit/rhizome of different locally available angiospermic taxa namely *Ageratum conyzoides, Artimesia nilogrica, Erigeron canadensis, Mesua ferra* and *Piper mullesua* etc. were evaluated for their fungitoxic activity against post harvest pathogens of kiwifruits. Extraction of plant parts was done separately in different organic solvents viz. ether, benzene, chloroform, ethyl acetate, methanol and absolute alcohol. Twenty gm of fresh plant leaves were taken for each extraction in ratio of 1:1 (W/V). Twenty gm of fresh plant parts of each plant thoroughly washed with running tap water and finally with sterile distilled water, were kept for shade dry for few days. It was then ground to powder using a grinder. Twenty ml of each solvent was added separately to the powder (1:1 w/v) in conical flask and was left overnight at room temperature. The extracts were filtered separately through Whatman's filter paper No. 1. The filtrates were evaporated at about 80°C temperatures in a water bath for two hours to evaporate the solvent, and remaining filtrates were assayed separately against the test fungi.

3.8. Screening of plant extracts against pathogens

The extracts were assayed against the post harvest fungal pathogens of kiwi fruits by the Modified paper disc technique (Conner and Beachat, 1984). Potato dextrose agar medium was used for culture of fungi. Two ml of the filtrate of each sample was impregnated separately to assay disc by repeated addition and evaporation of solvent with the help of hair drier. The assay discs (19 mm diam.) were prepared using Whatman's filter paper no.1. For control discs were impregnated similarly with the same amount of requisite solvents.

The assay discs were aseptically transferred to the center of the sterilized petriplates (7cm diam) containing 10 ml potato dextrose agar medium per plate. A mycelial disc (5mm diam) cut from the periphery of the seven day old culture of the test fungus were aseptically inoculated upside down to the center of each assay

disc in treated as well as in control sets. The plates were incubated at 27± 1° C for six days and observations were recorded seventh day onwards.

3.9. *In vivo* efficacy evaluation of the effective essential oils and the plant extracts

Practical applicability of the isolated essential oils and plant extracts were tested on the kiwifruits. Dip, impregnated wrappers and fumigation methods was used for evaluation of their efficacy against fruit rotting fungi.

3.9.1. *In vivo* efficacy of the essential oils in enhancement of storage period of kiwifruit

3.9.1.1 Dip method:

The procedure was followed as recommended by Vyas and Singh (1977) with some modification. The requisite amount of the oils were dissolved in Tween 80 and finally the volume was made up to 1 litre by adding sterilized distilled water to get desired concentration of the oil depending on their MIC. To evaluate *in vivo* efficacy of essential oils the selected one were used on the basis of screening results. Dipping of kiwifruits in essential oils of *L.cubeba, A.calamus, A.conyzoides,* and *A.nilogerica* at their respective MIC for about 20min was done in order to find out *in vivo* efficacy of the oils in control of the post harvest decay of kiwifruits. Fresh kiwifruits were washed in running water; surface disinfested with 0.1 per cent sodium hypochlorite solution and then washed in sterilized distilled water. The fruits of treatment set were then dipped in the essential oil of *L.cubeba* at MIC 1000ppm against test fungus *Botrytis cinerea,* and at 1000ppm against *Fusarium oxysporum* and *Penicillium expansum, A.calamus* oil at 500ppm against *P.expansum, F.oxysprum* and *B.cinerea, A.nilogerica* oil at 5000ppm against *P.expansum* and *B.cinerea* and *A.conydoides* oil at 1000ppm against *P.expansum, F.oxysporum* and *B.cinerea* for about 20min, while those of control sets were simply dipped in sterile distilled water. The fruits were inoculated by 1ml of standard spore suspension of *P.expansum, F.oxysporum* and *B.cinerea.* for fruit inoculation spore from a seven day-old culture were suspended in sterile distilled water.

Fruits were wounded by puncturing them with a pin on different sides of the kiwifruits. Each wound site was then inoculated by spraying with 50µl of spore suspension (10^5spore/ml) of *P. expansum, F. oxysporum* and *B. cinerea.* The inoculated fruits were kept in plastic container (five fruit per container). The fruits of each set were incubated at 27± 1° C and were observed for the initiation of rotting. Five kiwifruits were taken in each control and treatment sets and the experiment was repeated twice.

3.9.1.2 Impregnated wrapper treatment:

In this method the procedure reported by Luepschen (1964) with some modification was followed. Tissue papers were impregnated with essential oils. Requisite concentrations of oils *viz.*5000ppm, 1000ppm, 500ppm were made in Tween –80 which were applied on the tissue paper to get the wrappers. The treated tissue papers were air dried and cut into 20X.25 cm for fruit wraps. Fresh kiwifruits were washed in running water; surface disinfested with 0.1 per cent sodium hypochlorite and then washed in sterilized distilled water. The fruits were inoculated by 1ml of standard spore suspension of *P.expansum, F.oxysporum* and *B.cinerea.* For fruit inoculation spore from a seven day-old culture were suspended in sterile distilled water.

Fruits were wounded by puncturing them with a pin on different sides of the kiwifruits. Each wound site was then inoculated by spraying with 50µl of spore suspension (10^5spore/ml) of *P.expansum, F.oxysporum* and *B.cinerea.* The inoculated fruits were wrapped with impregnated wrappers. Simultaneously control A (inoculated unwrapped), B (uninoculated wrapped), C (uninoculated unwrapped) were maintained. The fruits will be stored at room temperature as 25°C. Observation for infection percentage (Ogava *et. al.,* 1963) and infection grade were recorded.

3.9.1.3 Fumigation:

The fruits were treated separately with the active essential oils of *L.cubeba, A.calamus, A.nilogerica* and *A.conyzoides* to find out efficacy of the oils against postharvest fruit rotting fungi *P.expansum, F.oxysporum* and *B.cinerea* of kiwifruit. The fruits were treated with the oils following techniques as suggested

by Chandra (1984) and Sharma and Yadav (1996). Mature and healthly kiwifruits were used for the experiment. The kiwifruits of control as well as of treatment sets were washed in running water and were surface sterilized with 0.1% sodiumhypochlorite solution and were then washed with distilled water. The kiwifruits were inoculated by 1ml of standard spore suspension of *P.expansum, F.oxysporum* and *B.cinerea*. For fruit inoculation from a seven day-old culture were suspended in sterile distilled water.

Kiwifruits were wounded by puncturing them with a pin on different sides of the kiwifruits. Each wound site was then inoculated by spraying with 50µl of spore suspension (10^5spore/ml) of *P.expansum, F.oxysporum* and *B.cinerea*. The inoculated fruits were kept in desiccators (5 fruits per desiccators). In treatment sets, the requisite amount of essential oils was introduced separately in the desiccators by soaking in cotton pieces so as to get their concentrations of *L.cubeba* oil at 5000ppm and 1000ppm, *A.calamus* oil at 1000ppm and 500ppm, *A.nilogerica* oil at 5000ppm and *A.conyzoides* oil at 5000ppm and 1000ppm (at their MIC value). The initiations of rotting of the fruits were observed. Three replicates were kept for treatment and control sets.

3.9.2. *In vivo* efficacy of the plant extract in enhancement of storage period of kiwifruit

3.9.2.1. Dip method:

The procedures were followed as recommended by Vyas and Singh (1977) with some modification. To test the *in vivo* applicability of plant extracts aqueous solution of selected plants were used on the basis of screening results. Dipping of kiwifruits for about 20min in the aqueous extracts of leaves of *Piper mullesua, Samanea saman, Ageratum conyzoides* and rhizome of *Acorus calamus* (1:1 w/v) was done in order to find out *in vivo* efficacy of the extract in control of the post-harvest decay of kiwifruit. Fresh kiwifruits were washed in running water, surface disinfested with 0.1 per cent sodiumhypochlorite solution and then washed in sterilized distilled water. The fruits of treatment set were then dipped in the plant extracts of *P.mullesua, S.saman, A.conyzoides* and *A.calamus* for about 20min, while those of control sets were simply dipped in sterile distilled water. The fruits were inoculated by 1ml of standard spore suspension of *P.expansum,*

F.oxysporum and *B.cinerea.* for fruit inoculation spore from a seven day-old culture were suspended in sterile distilled water.

Fruits were wounded by puncturing them with a pin on different sides of the kiwifruits. Each wound site was then inoculated by spraying with 50µl of spore suspension (10^5spore/ml) of *p.expansum, F.oxysporum* and *B.cinerea.* The inoculated fruits were kept in plastic container (five fruit per container). The fruits of each set were incubated at 27± 1° C and were observed for the initiation of rotting. Five kiwifruits were taken in each control and treatment sets and the experiment was repeated twice.

3.9.2.2. Impregnated wrapper treatment:

In this method the procedure reported by Lüepschen (1964) with some modification were followed. Tissue paper were impregnated with plant extracts of leaves of *Piper mullesua, Samanea saman, Ageratum conyzoides* and rhizome of *Acorus calamus* (1:1 w/v) to prepare the wrappers. The treated tissue papers were air dried and cut into 20X 25 cm for fruit wraps. Fresh kiwifruits were washed in running water, surface disinfested with 0.1 per cent sodiumhypochlorite and then washed in sterilized distilled water. The fruits were inoculated by 1ml of standard spore suspension of *P.expansum, F.oxysporum* and *B.cinerea.* For fruit inoculation spore from a seven day-old culture were suspended in sterile distilled water.

Fruits were wounded by puncturing them with a pin on different sides of the kiwifruits. Each wound site was then inoculated by spraying with 50µl of spore suspension (10^5spore/ml) of *P.expansum, F.oxysporum* and *B.cinerea.* The inoculated fruits were wrapped with impregnated wrappers. Simultaneously control A (inoculated unwrapped), B (uninoculated wrapped), C (uninoculated unwrapped) were maintained. The fruits were stored at room temperature as 25°C. Observation for infection percentage (Ogava *et. al.,* 1963) and infection grade were recorded.

3.10. Organoleptic tests (Sensory evaluation)

Fruit taste and firmness were assessed organoleptically by a taste panel. Sensory evaluation were carried out with fruits of the sets in which percentage

rotting was nil. Tests were carried out on 20th day of storage on the fruits stored at room temperature and in cold storage as recommended by Petersen et al. (1998). Evaluation of firmness and flavour of the fruits were conducted by six judges using Hedonic scale. Fresh, mature, healthy fruits from local market were used as standard. Numerical flavour ratings of 4 (equal to standard), 3(below standard but with no off flavour), 2(below standard and with definite off flavour) and 1(unacceptable) were assigned for the judgments and mean scores were calculated for each treatment.

RESULTS

4.1 Collection of plants and essential oil extraction

Various plant species were collected from the adjoining area to extract the essential oil.

Table 4.1: List of plants collected for essential oil extraction

Sl. No.	Name of plants	Family	Parts used
1	*Abroma augusta*	Sterculiaceae	Leaves
2	*Acorus calamus*	Acoraceae	Rhizome
3	*Adhatoda vesica*	Acanthaceae	Leaves
4	*Adrographis paniculata*	Acanthaceae	Leaves
25	*Ageratum conyzoides*	Asteraceae	Aerial parts
6	*Altingia excelsa*	Hemamelidaceae	Leaves
7	*Allium fistulosum*	Amaryllidaceae	Leaves
8	*Artemisia nilagirica*	Asteraceae	Leaves
9	*Azadirachta melia*	Meliaceae	Leaves
10	*Bauhinia purpurea*	Fabaceae	Flowers
11	*Boehmeria macrophylla*	Urticaceae	Leaves
12	*Callicarpa arborea*	Verbenaceae	Twig
13	*Cassia alata*	Fabaceae	Leaves
14	*Cassia tora*	Fabaceae	Leaves
15	*Cardamine trichocarpa*	brassicaceae	Whole plant
16	*Cissampelos pareira*	Menispermaceae.	Leaves
17	*Crotolaria juncea*	Fabaceae	Leaves
18	*Eclipta alba*	Asteraceae	Aerial parts
19	*Elaeocarpus sphaericus*	Elaeocarpaceae	Leaves
20	*Erigeron canadensis*	Asteraceae	Leaves
21	*Eupathorium odoratum*	Asteraceae	Leaves
22	*Euphorbia hirta*	Asteraceae	Aerial parts
23	*Gynura crepidioides*	Asteraceae	Leaves
24	*Houttuynia cordata*	Saururaceae	Whole plant
25	*Ipomoea quamocit*	Convulvulaceae	Leaves
26	*Juticia gendarnssa*	Acantheceae	Leaves
27	*Lantana camara*	Verbenaceae	Leaves
28	*Lagerstroemia*	Lythraceae	Leaves
29	*Leucas aspara*	lamiaceae	Aerial parts
30	*Litsea cubeba*	Lauraceae	Fruits
31	*Melastoma malabathrium*	Melastomataceae	Leaves
32	*Mesua ferrae*	Guttiferae	Flower
33	*Mikania cordata*	Asteraceae	Aerial parts
34	*Plantago major*	Plantaginaceae	Leaves
35	*Piper betle*	Piperaceae	Leaves
36	*Piper mullesua*	Piperaceae	Twig
37	*Piper brachystachyum*	Piperaceae	Twig
38	*Pogostemon cablin*	Lamiaceae	Leaves
39	*Polygonum capitatum*	Polygonaceae	Aerial parts

40	*Polygonum hydropiper*	Polygonaceae	Aerial parts

Among the collected plants fourteen species were recorded to yield the essential oil (Table 4.2). Higher amount of oil was extracted from *Pogostemon cablin* and lower from *Houttuynia cordata* and *Polygonum hydropiper.* On the basis of easy oil extraction and yield only ten plants were selected for evaluation against different fungi.

Table 4.2: List of plants which were used for extraction of essential oils

Sl . N0.	Name of plants	Family	Parts used	oil amount (ml)/ 100g
1	*Acorus calamus*	Acoraceae	Rhizome	7.0
2	*Ageratum conyzoides*	Asteraceae	Aerial parts	0.7
3	*Artemisia nilagirica*	Asteraceae	Leaves	0.4
4	*Eupatorium odoratum*	Asteraceae	Leaves	0.4
5	*Erigeron Canadensis*	Asteraceae	Leaves	0.4
6	*Houttuynia cordata*	Saururaceae	Whole plant	0.2
7	*Litsea cubeba*	Lauraceae	Fruits	1.7
8	*Litsea sp.*	Lauraceae	Fruits	0.5
9	*Mesua ferrae*	Guttiferae	Flower	0.4
10	*Mikania cordata*	Asteraceae	Aerial parts	0.4
11	*Piper mullesua*	Piperaceae	Twig	0.4
12	*Piper pedicellatum*	Piperaceae	Twig	0.3
13	*Pogostemon cablin*	Lamiaceae	Leaves	10.0
14	*Polygonum hydropiper*	Polygonaceae	Aerial parts	0.2

4.2. Evaluation of Essential Oils against Different Fungi

Essential oils extracted from different plant species were evaluated to visualize their effect on the growth of four phytopathogenic fungi of kiwifruit viz. *Penicillium expansum, Fusarium oxysporum, Botrytis cinerea* and *Alternaria alternata* following Poisoned food technique method. Different concentration of oil such as 125ppm, 250ppm, 500ppm, 1000ppm and 5000ppm were taken to evaluate the effect. Simultaneously, a control was also maintained by inoculating culture disc on the medium without adding any oil.

4.2.1. *Acorus calamus*

Essential oil of *A. calamus* was found effective against the growth of all tested fungi. In case of *P. expansum* and *F. oxysporum* 100% inhibition of growth was

recorded at 5000 and 1000ppm concentration by essential oil of *A. calamus*. At 500ppm concentration also 100% inhibition was found up to 9th day of growth in *P. expansum* and 7th day on *F. oxysporum*. But growth was observed during subsequent period of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* also the effect of oil was significant inhibitory. At 5000, 1000 and 500ppm concentration 100% inhibition was recorded. At 250ppm also in case of *A. alternata* it restricts 100% up to 7th day and after that slight growth was noticed during subsequent period of incubation.

Essential oil of *A. calamus* inhibited the growth of all four phytopathogenic fungi, at 5000, 1000 and 500ppm concentration and at lower level i.e 125 and 250ppm concentration of oil colony growth was recorded. But it always remains lesser than the control.

4.2.2. *Ageratum conyzoides*

Essential oil of *A. conyzoides* was also effective on the growth of all tested fungi at higher concentration 5000 and 1000ppm. In case of *P. expansum, F. oxysporum and B. cinerea* 100% or complete inhibition of growth of fungus colony was recorded at 5000 and 1000ppm concentration even after 15 days of inoculation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* also the effect of essential oil on growth was observed. By and large in comparison to the control growth of fungus at all concentration of essential oil was lesser.

Essential oil of *A. conyzoides* significantly inhibits the growth of all the three fungi (*P. expansum, F. oxysporum and B. cinerea*) at 5000 and 1000ppm concentration. In case of *A. alternata* impact was not remarkable. Increase in diameter of colony was always remained lesser than control one. The effect of essential oil was corresponding to their concentration.

4.2.3. *Artemisia nilagirica*

Essential oil of *A. nilagirica* inhibits the growth of all phytopathogenic fungi at higher concentration. In case of *P. expansum* at 5000ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* there was 100% inhibition up to 11th day at 5000ppm concentration of oil. However, slight growth was seen on subsequent period of

incubation. But in case of *F. oxysporum* even at 5000ppm concentration of oil slight growth was recorded. Increase in diameter of fungus colony was recorded at lower concentration of oil however it always remains lesser than control.

Table 4.3: Effect of various plant based essential oils at different concentrations (ppm) on the colony growth (cm) of *Alternaria alternata*. ±S.E

Period	*Acorus calamus*					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.10±0.00	2.60±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.65±0.05	3.20±0.10
9th	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.00	2.05±0.05	4.90±0.10
11th	0.00±0.00	0.00±0.00	0.00±0.00	0.90±0.40	2.60±0.00	5.60±0.10
13th	0.00±0.00	0.00±0.00	0.00±0.00	2.05±0.25	3.15±0.05	6.00±0.10
15th	0.00±0.00	0.00±0.00	0.00±0.00	2.45±0.25	3.80±0.00	6.20±0.10
	Ageratum conyzoides					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.80±0.00	1.00±0.00	1.15±0.05	1.75±0.05	2.40±0.2	2.60±0.00
7th	1.00±0.00	1.35±0.05	1.70±0.10	2.40±0.10	3.45±0.25	3.20±0.10
9th	1.30±0.00	1.75±0.05	2.45±0.15	3.05±0.15	4.65±0.25	4.90±0.10
11th	1.50±0.10	2.25±0.05	3.20±0.10	3.40±0.40	5.30±0.30	5.60±0.10
13th	1.65±0.15	2.65±0.05	4.00±0.40	3.85±0.65	5.90±0.10	6.00±0.10
15th	1.85±0.25	3.05±0.05	4.55±0.45	4.45±0.55	6.10±0.10	6.20±0.10
	Artemisia nilogirica					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.60±0.10	2.60±0.20	3.35±0.05	3.90±0.10	2.60±0.00
7th	0.00±0.00	1.65±0.15	3.25±0.55	4.05±0.05	4.50±0.10	3.20±0.10
9th	0.00±0.00	1.95±0.05	3.25±0.55	4.15±0.05	5.15±0.15	4.90±0.10
11th	0.00±0.00	2.05±0.05	3.25±0.55	5.00±0.10	5.25±0.25	5.60±0.10
13th	0.85±0.05	2.10±0.10	3.30±0.50	5.00±0.10	5.60±0.20	6.00±0.10
15th	0.85±0.05	2.10±0.10	3.30±0.50	5.00±0.10	5.60±0.20	6.20±0.10
	Erigeron Canadensis					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	3.50±0.50	4.05±0.25	4.15±0.05	4.20±0.10	4.25±0.05	2.60±0.00
7th	3.75±0.75	4.45±0.55	4.70±0.10	4.75±0.15	5.20±0.40	3.20±0.10
9th	4.05±0.85	4.70±0.80	4.95±0.25	4.95±0.05	6.10±0.40	4.90±0.10
11th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	5.60±0.10
13th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	6.00±0.10
15th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	6.20±0.10
	Eupatorium odoratum					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.10±0.10	1.65±0.15	2.15±0.15	2.60±0.10	2.90±0.10	2.60±0.00
7th	1.60±0.10	2.05±0.05	2.65±0.15	3.25±0.25	3.70±0.20	3.20±0.10
9th	2.10±0.10	2.40±0.10	3.10±0.10	4.25±0.15	4.45±0.05	4.90±0.10

11th	2.10±0.10	2.40±0.10	3.90±0.10	4.25±0.15	5.25±0.45	5.60±0.10
13th	2.60±0.10	3.25±0.25	4.70±0.30	4.50±0.10	5.25±0.45	6.00±0.10
15th	2.65±0.15	3.80±0.20	4.70±0.30	4.50±0.10	5.35±0.35	6.20±0.10

	Litsea cubeba					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.80±0.00	1.10±0.60	2.20±0.10	2.65±0.15	2.60±0.00
7th	0.00±0.00	0.80±0.00	2.90±0.50	2.65±0.15	3.80±0.00	3.20±0.10
9th	0.00±0.00	1.20±0.10	3.05±0.45	3.65±0.55	4.50±0.30	4.90±0.10
11th	0.00±0.00	1.50±0.10	3.05±0.45	3.65±0.55	4.50±0.30	5.60±0.10
13th	0.00±0.00	1.90±0.05	3.05±0.45	3.65±0.55	4.50±0.30	6.00±0.10
15th	0.00±0.00	1.90±0.05	3.05±0.45	3.65±0.55	4.50±0.30	6.20±0.10
	Mesua ferrae					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.35±0.15	1.50±0.10	1.30±0.10	1.50±0.00	1.55±0.05	2.60±0.00
7th	1.85±0.15	2.15±0.15	1.85±0.15	2.00±0.10	2.00±0.10	3.20±0.10
9th	2.25±0.15	2.60±0.10	2.40±0.10	2.65±0.05	2.55±0.05	4.90±0.10
11th	2.55±0.25	2.90±0.10	2.85±0.05	3.05±0.05	2.95±0.05	5.60±0.10
13th	2.85±0.25	3.20±0.10	3.30±0.00	3.50±0.00	3.40±0.10	6.00±0.10
15th	3.25±0.25	3.55±0.25	3.90±0.10	4.10±0.10	4.20±0.00	6.20±0.10
	Mikania cordata					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.90±0.10	2.10±0.10	2.25±0.05	2.35±0.05	2.50±0.10	2.60±0.00
7th	2.20±0.10	2.65±0.15	2.95±0.35	3.35±0.05	3.35±0.35	3.20±0.10
9th	3.10±0.20	3.30±0.40	4.00±1.31	4.05±0.15	5.20±0.00	4.90±0.10
11th	3.40±0.20	3.70±0.50	4.15±1.45	5.20±0.00	5.55±0.05	5.60±0.10
13th	3.55±0.05	4.10±0.70	4.15±1.45	5.65±0.05	5.80±0.10	6.00±0.10
15th	3.70±0.20	4.10±0.70	4.15±1.45	5.65±0.05	5.80±0.10	6.20±0.10
	Piper mullesua					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.35±0.05	1.30±0.00	1.40±0.00	1.40±0.00	1.55±0.05	2.60±0.00
7th	1.85±0.05	1.80±0.00	1.90±0.00	1.90±0.00	2.20±0.30	3.20±0.10
9th	2.35±0.05	2.00±0.20	2.40±0.00	2.40±0.00	2.75±0.25	4.90±0.10
11th	2.85±0.05	2.30±0.50	2.90±0.10	2.90±0.10	3.10±0.20	5.60±0.10
13th	3.10±0.10	2.50±0.70	3.20±0.10	3.20±0.10	3.40±0.10	6.00±0.10
15th	3.60±0.20	3.15±0.75	3.75±0.05	3.80±0.10	3.80±0.10	6.20±0.10
	Pogostemon cablin					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.55±0.05	0.70±0.00	0.80±0.00	1.55±0.05	2.35±0.15	2.60±0.00
7th	0.85±0.05	0.80±0.10	1.25±0.05	1.95±0.05	2.6±0.00	3.20±0.10
9th	0.85±0.05	1.00±0.00	1.55±0.05	2.30±0.10	2.85±0.05	4.90±0.10
11th	1.10±0.10	1.40±0.10	1.65±0.05	2.70±0.20	2.85±0.05	5.60±0.10
13th	1.10±0.10	1.40±0.10	1.90±0.10	2.70±0.20	3.10±0.10	6.00±0.10
15th	1.25±0.05	1.90±0.10	2.10±0.10	2.70±0.05	3.10±0.10	6.20±0.10

Table 4.4: Effect of various plant based essential oils at different concentrations (ppm) on the colony growth (cm) of *Botrytis cinerea*. ±S.E

Period	***Acorus calamus***					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	1.65±1.50	3.05±2.80	4.50±0.10
7th	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.30	4.25±0.65	5.90±0.00
9th	0.00±0.00	0.00±0.00	0.00±0.00	2.85±0.35	4.70±0.80	6.50±0.10
11th	0.00±0.00	0.00±0.00	0.00±0.00	4.25±0.45	4.85±0.85	6.80±0.10
13th	0.00±0.00	0.00±0.00	0.00±0.00	4.60±0.30	5.10±0.60	6.80±0.10
15th	0.00±0.00	0.00±0.00	0.00±0.00	4.90±0.00	5.10±0.60	7.00±0.10
	Ageratum conyzoides					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.40±0.40	1.35±0.45	2.25±0.05	4.50±0.10
7th	0.00±0.00	0.00±0.00	0.50±0.50	2.10±0.60	2.50±0.10	5.90±0.00
9th	0.00±0.00	0.00±0.00	0.60±0.60	2.25±0.45	2.70±0.10	6.50±0.10
11th	0.00±0.00	0.00±0.00	0.65±0.65	2.25±0.45	2.90±0.10	6.80±0.10
13th	0.00±0.00	0.00±0.00	1.35±0.65	2.50±0.60	3.00±0.00	6.80±0.10
15th	0.00±0.00	0.00±0.00	1.50±0.55	2.65±0.75	3.20±0.10	7.00±0.10
	Artemisia nilogirica					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.60±0.20	2.6±0.25	3.35±0.05	3.90±0.00	4.50±0.10
7th	0.00±0.00	1.65±0.15	3.25±0.6	4.05±0.20	4.50±0.15	5.90±0.00
9th	0.00±0.00	1.95±0.05	3.25±0.6	4.15±0.10	5.15±0.25	6.50±0.10
11th	0.00±0.00	2.05±0.05	3.25±0.6	5.00±0.30	5.25±0.20	6.80±0.10
13th	0.85±0.15	2.10±0.10	3.3±0.55	5.00±0.30	5.60±0.20	6.80±0.10
15th	0.85±0.15	2.10±0.60	3.3±0.65	5.00±0.30	5.60±0.20	7.00±0.10
	Erigeron Canadensis					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	3.65±0.65	4.05±0.25	4.30±0.20	3.95±0.15	3.95±0.65	4.50±0.10
7th	3.85±0.85	4.70±0.80	4.70±0.10	5.25±0.45	4.10±0.80	5.90±0.00
9th	4.05±0.85	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.50±0.10
11th	4.10±0.90	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.80±0.10
13th	4.10±0.90	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.80±0.10
15th	4.10±0.90	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	7.00±0.10
	Eupatorium odoratum					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	3.60±0.00	3.90±0.10	3.70±0.20	4.05±0.05	4.45±0.05	4.50±0.10
7th	5.40±0.10	4.20±0.20	4.75±0.35	4.10±0.00	5.15±0.55	5.90±0.00
9th	5.80±0.00	4.55±0.05	4.90±0.50	4.50±0.10	5.55±0.75	6.50±0.10
11th	6.20±0.10	4.70±0.20	4.95±0.45	4.50±0.10	5.65±0.75	6.80±0.10
13th	6.20±0.10	4.75±0.15	4.95±0.45	4.50±0.10	5.70±0.80	6.80±0.10
15th	6.20±0.10	4.75±0.15	4.95±0.45	4.50±0.10	5.70±0.80	7.00±0.10

	Litsea cubeba					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	2.25±0.25	3.35±0.15	4.25±0.15	4.50±0.10
7th	0.00±0.00	0.00±0.00	4.90±0.30	5.75±0.25	5.05±0.95	5.90±0.00
9th	0.00±0.00	0.00±0.00	5.60±0.40	5.90±0.10	5.25±1.05	6.50±0.10
11th	0.00±0.00	0.00±0.00	6.10±0.10	6.65±0.35	7.00±0.00	6.80±0.10
13th	0.00±0.00	0.00±0.00	6.20±0.20	6.75±0.25	7.00±0.00	6.80±0.10
15th	0.00±0.00	0.00±0.00	6.20±0.20	6.75±0.25	7.00±0.00	7.00±0.10
	Mesua ferrae					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.70±0.10	2.65±0.15	3.20±0.00	3.70±0.10	3.85±0.25	4.50±0.10
7th	2.50±0.00	4.25±0.15	4.75±0.05	5.10±0.10	5.35±0.25	5.90±0.00
9th	2.90±0.30	5.30±0.10	5.80±0.20	5.45±0.25	5.40±0.20	6.50±0.10
11th	3.05±0.45	5.45±0.05	6.10±0.10	5.60±0.10	5.40±0.20	6.80±0.10
13th	3.05±0.45	5.45±0.05	6.10±0.10	6.20±0.10	6.00±0.20	6.80±0.10
15th	3.05±0.45	5.45±0.05	6.10±0.10	6.20±0.10	6.30±0.20	7.00±0.10
	Mikania cordata					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.55±0.05	2.60±0.10	2.90±0.00	3.05±0.05	3.05±0.15	4.50±0.10
7th	2.20±0.30	3.50±0.50	4.05±0.15	4.35±0.05	4.00±0.40	5.90±0.00
9th	3.30±1.30	5.50±0.30	5.55±0.45	6.40±0.00	4.10±0.50	6.50±0.10
11th	3.50±1.50	5.70±0.10	5.90±0.20	6.40±0.00	4.15±0.45	6.80±0.10
13th	3.65±1.65	5.85±0.15	5.90±0.20	6.40±0.00	4.15±0.45	6.80±0.10
15th	3.90±1.90	5.90±0.10	5.90±0.20	6.40±0.00	4.15±0.45	7.00±0.10
	Piper mullesua					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.65±0.15	2.25±0.35	2.35±0.25	3.75±0.05	3.55±0.55	4.50±0.10
7th	1.95±0.40	2.85±0.65	2.50±0.10	4.25±0.05	3.85±0.85	5.90±0.00
9th	2.30±0.50	3.20±1.00	2.65±0.15	4.45±0.15	3.95±0.85	6.50±0.10
11th	2.30±0.50	3.75±1.45	2.65±0.15	4.45±0.15	3.95±0.85	6.80±0.10
13th	2.30±0.50	4.00±1.60	2.65±0.15	4.45±0.15	3.95±0.85	6.80±0.10
15th	2.30±0.50	4.00±1.60	2.65±0.15	4.45±0.15	3.95±0.85	7.00±0.10
	Pogostemon cablin					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.50±0.00	1.30±0.10	1.65±0.15	2.40±0.00	2.95±0.00	4.50±0.10
7th	1.80±0.00	2.05±0.25	2.70±0.20	3.90±0.00	4.40±0.10	5.90±0.00
9th	2.90±0.70	3.10±1.30	5.15±0.15	4.30±0.10	4.60±0.10	6.50±0.10
11th	3.10±0.90	3.45±1.65	5.45±0.45	4.30±0.10	4.60±0.10	6.80±0.10
13th	3.20±1.00	3.85±2.05	5.55±0.55	4.35±0.15	4.60±0.10	6.80±0.10
15th	3.20±1.00	4.05±2.25	5.55±0.55	4.35±0.15	4.60±0.10	7.00±0.10

Table 4.5: Effect of various plant based essential oils at different concentrations (ppm) on the colony growth (cm) of *Fusarium oxysporum*. ±S.E

Period	*Acorus calamus*					
Days	5000ppm	1000ppm	500ppm	250ppm	125ppm	Control
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.90±0.40	1.65±0.15	4.00±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	1.75±0.25	2.50±0.00	4.50±0.10
9th	0.00±0.00	0.00±0.00	0.75±0.25	2.35±0.25	3.30±0.20	4.70±0.10
11th	0.00±0.00	0.00±0.00	1.45±0.05	3.2±0.30	4.15±0.35	5.40±0.10
13th	0.00±0.00	0.00±0.00	2.05±0.05	4.15±0.15	5.20±0.40	6.30±0.10
15th	0.00±0.00	0.00±0.00	2.65±0.05	5.05±0.25	5.95±0.25	7.00±0.10
	Ageratum conyzoides					
Days	5000ppm	1000ppm	500ppm	250ppm	125ppm	Control
5th	0.00±0.00	0.00±0.00	0.75±0.05	1.30±0.30	2.45±0.05	4.00±0.00
7th	0.00±0.00	0.00±0.00	0.75±0.05	1.30±0.30	3.25±0.15	4.50±0.10
9th	0.00±0.00	0.00±0.00	1.60±0.10	1.70±0.10	3.90±0.50	4.70±0.10
11th	0.00±0.00	0.00±0.00	2.05±0.05	2.25±0.05	4.20±0.80	5.40±0.10
13th	0.00±0.00	0.00±0.00	2.45±0.05	2.80±0.00	4.60±1.20	6.30±0.10
15th	0.00±0.00	0.00±0.00	2.60±0.20	3.25±0.05	4.60±1.20	7.00±0.10
	Artemisia nilogirica					
Days	5000ppm	1000ppm	500ppm	250ppm	125ppm	Control
5th	1.10±0.10	1.60±0.10	1.75±0.05	2.00±0.00	2.10±0.10	4.00±0.00
7th	1.10±0.10	1.95±0.15	2.15±0.05	2.35±0.05	2.50±0.10	4.50±0.10
9th	1.65±0.05	2.35±0.05	2.45±0.05	2.75±0.05	2.90±0.10	4.70±0.10
11th	1.90±0.10	2.65±0.15	2.90±0.00	2.75±0.05	2.90±0.10	5.40±0.10
13th	2.10±0.10	2.95±0.05	3.35±0.05	3.50±0.00	3.60±0.10	6.30±0.10
15th	2.45±0.05	2.95±0.05	3.35±0.05	4.00±0.00	4.25±0.25	7.00±0.10
	Erigeron Canadensis					
Days	5000ppm	1000ppm	500ppm	250ppm	125ppm	Control
5th	0.85±0.05	1.10±0.10	1.45±0.05	2.10±0.10	2.25±0.25	4.00±0.00
7th	0.85±0.05	1.10±0.10	1.90±0.10	2.50±0.10	2.80±0.20	4.50±0.10
9th	1.15±0.05	1.60±0.10	2.20±0.10	2.95±0.05	3.30±0.30	4.70±0.10
11th	1.50±0.10	1.90±0.10	2.55±0.05	3.50±0.10	3.65±0.15	5.40±0.10
13th	1.75±0.05	2.20±0.10	2.95±0.05	3.90±0.10	4.05±0.15	6.30±0.10
15th	2.05±0.05	2.45±0.05	3.25±0.25	4.25±0.05	4.50±0.10	7.00±0.10
	Eupatorium odoratum					
Days	5000ppm	1000ppm	500ppm	250ppm	125ppm	Control
5th	0.85±0.05	0.85±0.05	1.60±0.20	2.45±0.05	3.05±0.45	4.00±0.00
7th	0.85±0.05	1.10±0.10	2.05±0.15	3.00±0.00	3.50±0.50	4.50±0.10
9th	1.60±0.10	1.70±0.10	2.40±0.10	3.65±0.05	3.90±0.40	4.70±0.10
11th	1.95±0.05	2.05±0.05	2.80±0.10	4.00±0.10	4.20±0.40	5.40±0.10
13th	2.35±0.05	2.50±0.10	3.25±0.15	4.25±0.05	4.55±0.35	6.30±0.10
15th	2.35±0.05	2.50±0.10	3.65±0.15	4.25±0.05	4.55±0.35	7.00±0.10

Period	*Litsea cubeba*					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	2.45±0.05	3.30±0.10	3.10±0.10	4.00±0.00
7th	0.00±0.00	1.40±0.00	4.20±1.00	5.00±1.00	3.55±0.45	4.50±0.10
9th	0.00±0.00	1.60±0.00	4.75±1.25	5.00±1.00	3.75±0.45	4.70±0.10
11th	0.00±0.00	1.90±0.10	5.25±0.75	5.00±1.00	3.85±0.45	5.40±0.10
13th	0.00±0.00	2.00±0.00	5.85±0.15	5.05±0.95	3.95±0.45	6.30±0.10
15th	0.00±0.00	2.20±0.10	6.00±0.00	5.20±1.00	4.10±0.40	7.00±0.10
	Mesua ferrae					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	2.55±0.05	2.05±0.05	2.10±0.10	2.20±0.00	2.15±0.05	4.00±0.00
7th	2.55±0.05	2.35±0.05	2.45±0.05	2.45±0.05	2.45±0.05	4.50±0.10
9th	2.90±0.10	2.65±0.05	2.85±0.05	2.95±0.05	2.95±0.05	4.70±0.10
11th	2.90±0.10	2.90±0.10	3.15±0.05	3.25±0.05	3.15±0.05	5.40±0.10
13th	3.30±0.20	3.40±0.20	3.55±0.05	3.65±0.05	3.85±0.05	6.30±0.10
15th	3.35±0.15	3.40±0.20	3.55±0.05	4.05±0.05	4.20±0.10	7.00±0.10
	Mikania cordata					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	2.80±0.00	3.50±0.05	3.50±0.05	3.00±0.00	2.90±0.10	4.00±0.00
7th	4.20±0.00	4.50±0.00	4.65±0.05	4.60±0.00	3.75±0.65	4.50±0.10
9th	6.50±0.05	5.80±0.30	6.50±0.00	6.25±0.25	4.10±0.90	4.70±0.10
11th	6.85±0.05	6.00±0.50	6.75±0.05	6.50±0.50	4.85±1.65	5.40±0.10
13th	6.95±0.05	6.15±0.65	6.90±0.00	6.50±0.50	5.15±1.85	6.30±0.10
15th	6.95±0.05	6.15±0.65	7.00±0.00	6.50±0.50	5.15±1.85	7.00±0.10
	Piper mullesua					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.15±0.05	1.45±0.05	1.40±0.10	1.75±0.05	2.20±0.20	4.00±0.00
7th	1.45±0.05	1.75±0.05	1.75±0.05	2.30±0.20	2.75±0.05	4.50±0.10
9th	1.75±0.05	2.20±0.20	2.35±0.25	2.95±0.05	3.30±0.10	4.70±0.10
11th	2.15±0.20	2.65±0.15	2.75±0.15	3.45±0.05	3.80±0.10	5.40±0.10
13th	2.50±0.10	3.20±0.10	3.40±0.10	3.85±0.05	4.50±0.10	6.30±0.10
15th	3.20±0.20	3.70±0.10	3.90±0.10	4.30±0.10	4.70±0.10	7.00±0.10
	Pogostemon cablin					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.45±0.05	1.40±0.00	1.30±0.00	2.45±0.05	2.75±0.05	4.00±0.00
7th	2.05±0.05	2.15±0.05	2.95±0.05	3.50±0.30	4.05±0.05	4.50±0.10
9th	3.15±0.15	3.20±0.50	4.55±0.55	4.60±1.40	4.70±0.20	4.70±0.10
11th	3.70±0.20	3.55±0.85	4.70±0.70	4.85±1.65	4.85±0.35	5.40±0.10
13th	4.20±0.20	3.95±1.25	4.85±0.85	5.00±1.80	5.00±0.50	6.30±0.10
15th	4.55±0.15	4.25±1.55	4.85±0.85	5.00±1.80	5.00±0.50	7.00±0.10

Table 4.6: Effect of various plant based essential oils at different concentrations (ppm) on the colony growth (cm) of *Penicillium expansum*. ±S.E

Period	*Acorus calamus*					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.00	1.20±0.10	3.60±0.10
7th	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	1.95±0.05	4.50±0.10
9th	0.00±0.00	0.00±0.00	0.00±0.00	1.45±0.05	2.65±0.05	5.00±0.10
11th	0.00±0.00	0.00±0.00	0.50±0.00	1.95±0.05	3.15±0.15	5.50±0.10
13th	0.00±0.00	0.00±0.00	0.75±0.05	1.95±0.05	3.85±0.05	5.80±0.10
15th	0.00±0.00	0.00±0.00	0.75±0.05	2.15±0.15	4.15±0.15	6.00±0.10
	Ageratum conyzoides					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	1.35±0.15	2.15±0.15	1.95±0.15	3.60±0.10
7th	0.00±0.00	0.00±0.00	1.70±0.10	2.60±0.20	2.15±0.35	4.50±0.10
9th	0.00±0.00	0.00±0.00	1.95±0.35	2.80±0.40	2.15±0.35	5.00±0.10
11th	0.00±0.00	0.00±0.00	2.05±0.45	3.00±0.60	2.40±0.60	5.50±0.10
13th	0.00±0.00	0.00±0.00	2.15±0.55	3.25±0.75	2.40±0.60	5.80±0.10
15th	0.00±0.00	0.00±0.00	2.35±0.75	3.50±1.00	2.40±0.60	6.00±0.10
	Artemisia nilogirica					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	1.05±0.05	1.20±0.10	3.40±0.20	3.75±0.05	3.60±0.10
7th	0.00±0.00	1.65±0.15	2.05±0.05	3.80±0.20	4.00±0.00	4.50±0.10
9th	0.00±0.00	1.90±0.10	2.55±0.25	4.10±0.10	4.35±0.05	5.00±0.10
11th	0.00±0.00	1.95±0.05	2.55±0.25	4.10±0.10	5.05±0.05	5.50±0.10
13th	0.00±0.00	2.05±0.05	2.75±0.25	4.35±0.15	5.05±0.05	5.80±0.10
15th	0.00±0.00	2.05±0.05	2.75±0.25	4.35±0.15	5.15±0.05	6.00±0.10
	Erigeron Canadensis					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.55±0.05	1.65±0.05	2.00±0.10	2.30±0.10	3.05±0.05	3.60±0.10
7th	1.85±0.05	1.95±0.05	2.35±0.05	2.55±0.05	3.65±0.65	4.50±0.10
9th	2.20±0.10	2.25±0.05	2.75±0.05	2.90±0.10	3.95±0.75	5.00±0.10
11th	2.60±0.10	2.65±0.05	3.05±0.05	2.90±0.10	4.25±0.75	5.50±0.10
13th	3.05±0.05	3.00±0.10	3.05±0.05	3.15±0.15	4.50±0.50	5.80±0.10
15th	3.05±0.05	3.05±0.05	3.25±0.05	3.15±0.15	4.50±0.50	6.00±0.10
	Eupatorium odoratum					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.20±0.00	1.75±0.05	1.85±0.05	1.95±0.05	2.05±0.05	3.60±0.10
7th	1.70±0.00	2.05±0.05	2.10±0.10	2.45±0.05	2.55±0.05	4.50±0.10
9th	2.20±0.00	2.35±0.05	2.45±0.05	2.85±0.05	2.95±0.05	5.00±0.10
11th	2.45±0.05	2.65±0.05	2.75±0.05	3.15±0.05	3.35±0.05	5.50±0.10
13th	2.45±0.05	2.65±0.05	2.75±0.05	3.45±0.05	3.60±0.10	5.80±0.10
15th	2.45±0.05	2.65±0.05	3.05±0.05	3.45±0.05	3.60±0.10	6.00±0.10

Period	*Litsea cubeba*					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	1.45±0.25	2.25±0.15	1.95±0.35	3.60±0.10
7th	0.00±0.00	0.00±0.00	2.35±0.25	2.5±0.40	2.55±0.85	4.50±0.10
9th	0.00±0.00	0.25±0.00	2.80±0.60	3.35±0.15	2.55±0.85	5.00±0.10
11th	0.00±0.00	0.50±0.00	3.15±0.75	3.65±0.45	2.55±0.85	5.50±0.10
13th	0.00±0.00	0.65±0.05	3.50±0.90	4.00±0.70	2.60±0.80	5.80±0.10
15th	0.00±0.00	0.70±0.00	4.05±0.55	4.05±0.75	2.70±0.80	6.00±0.10
	Mesua ferrae					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.50±0.10	1.80±0.10	2.40±0.40	2.45±0.05	2.80±0.20	3.60±0.10
7th	1.90±0.10	2.10±0.10	2.75±0.25	2.65±0.05	2.95±0.05	4.50±0.10
9th	2.25±0.15	2.55±0.05	2.75±0.25	3.05±0.05	3.40±0.10	5.00±0.10
11th	2.55±0.25	2.90±0.10	3.00±0.30	3.50±0.00	4.20±0.00	5.50±0.10
13th	2.80±0.20	3.20±0.10	3.25±0.25	4.10±0.10	4.35±0.15	5.80±0.10
15th	3.25±0.25	3.50±0.20	3.45±0.05	4.10±0.10	4.45±0.25	6.00±0.10
	Mikania cordata					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	1.65±0.05	1.9±0.1	2.05±0.05	2.25±0.05	2.35±0.05	3.60±0.10
7th	2.25±0.05	2.45±0.35	2.95±0.35	2.95±0.35	3.35±0.05	4.50±0.10
9th	3.1±0.2	3.25±0.65	3.65±1.05	4±1.3	5.2±0	5.00±0.10
11th	3.4±0.2	3.4±0.8	3.8±1.2	4.15±1.45	5.55±0.05	5.50±0.10
13th	3.55±0.05	3.7±1.1	3.85±1.25	4.15±1.45	5.8±0.1	5.80±0.10
15th	3.7±0.2	3.7±1.1	3.9±1.3	4.15±1.45	5.8±0.1	6.00±0.10
	Piper mullesua					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th±	1.45±0.05	1.90±0.10	2.50±0.30	2.95±0.05	3.55±0.05	3.60±0.10
7th±	1.95±0.35	2.50±0.30	2.70±0.30	3.05±0.55	4.25±0.05	4.50±0.10
9th±	2.30±0.50	2.85±0.65	2.75±0.25	3.85±0.65	4.45±0.15	5.00±0.10
11th ±	2.30±0.50	3.05±0.75	3.15±0.35	3.85±0.65	4.55±0.05	5.50±0.10
13th±	2.40±0.40	3.30±0.90	3.15±0.35	3.85±0.65	4.55±0.05	5.80±0.10
15th±	2.40±0.40	3.30±0.90	3.15±0.50	4.00±0.80	4.55±0.05	6.00±0.10
	Pogostemon cablin					
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.45±0.05	0.50±0.00	0.50±0.00	1.15±0.05	2.05±0.15	3.60±0.10
7th	0.75±0.05	0.80±0.10	0.95±0.05	1.75±0.05	2.30±0.00	4.50±0.10
9th	0.75±0.05	1.00±0.00	1.30±0.10	2.35±0.45	2.35±0.05	5.00±0.10
11th	0.75±0.05	1.00±0.00	1.40±0.20	2.35±0.45	2.35±0.05	5.50±0.10
13th	0.75±0.05	1.00±0.00	1.55±0.35	2.35±0.45	2.35±0.05	5.80±0.10
15th	0.75±0.05	1.00±0.00	1.70±0.50	2.35±0.45	2.35±0.05	6.00±0.10

4.2.4. *Eupatorium odoratum*

Essential oil of *E. odoratum* was also inhibitory against the four phytopathogenic fungi. In case of *P. expansum, F. oxysporum* and *A.alternata* at

higher concentration of oil during their initial days of incubation the inhibition was remarkable, but during subsequent period of incubation growth of fungus colony was recorded. However, it always remains lesser than control. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. In case of *B. cinerea* also growth of colony diameter of fungus was found at 5000ppm concentration oil. Colony diameter of *B.cinerea* was higher than other three phytopathogenic fungi.

4.2.5. *Erigeron canadensis*

Essential oil of *E. canadensis* also inhibits the growth of phytopathogenic fungi. In case of *P. expansum* and *F. oxysporum* at higher concentration of oil inhibition was drastic. However, at 5000ppm also growth of fungus colony was recorded. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. While in case of *A. alternata* and *B. cinerea* at 5000ppm concentration of fungus colony was reduced initially but during subsequent period of incubation growth of colony increases but it always remain lesser than the control. At lower level of concentration of oil at 125ppm the growth of fungus colony was almost similar to the control. There was not much effect on growth of fungus colony by *E. canadensis* oil.

4.2.6. *Litsea cubeba*

Essential oil of *Litsea cubeba* inhibited the growth of all phytopathogenic fungi. In case of *P. expansum*, 100% or complete inhibition in growth was recorded at 5000ppm concentration, but at 1000ppm concentration growth was inhibited up to 7th day and during subsequent period of incubation slight increase in colony diameter was observed. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *F. oxysporum* and *A. alternata* the effect of oil was also found to be sever and 100% inhibition was observed at 5000ppm concentration and at 1000ppm slight growth was seen. In case of *B. cinerea*, effect of *L. cubeba* essential oil was quite remarkable, at 5000 and 1000ppm concentration 100% inhibition was recorded even after 15 days of incubation.

Essential oil of *L. cubeba* shows drastic effect on the growth of fungus colony at higher concentration 5000 and 1000ppm. At lower level of concentration *i,e* 125, 250, and 500ppm of oil colony growth was recorded for all the four

phytopathogenic fungi but it always remain lesser than the control. The effect of essential oil was corresponding to their concentration.

4.2.7. *Mesua ferrea*

Essential oil of *M. ferrea* also inhibits the growth of four phytopathogenic fungi. In all four tested fungi effect of *M. ferrea* oil was quite similar. At 5000ppm concentration also growth of fungal colony was noticed. at initial days there was sever inhibition but during their subsequent period of incubation growth of fungus colony was recorded for all four fungi at all concentration of oil. However, the growth of fungus colony always remains lesser than the control. The decrease in colony diameter in growth of fungus colony was corresponding to their concentration of oil. Thus, effect of *M. ferrea* oil on all four phytopathogenic fungi was not so effective.

4.2.8. *Mikania cordata*

Essential oil of *M. cordata* inhibits the growth of all four phytopathogenic fungi. Hundred percent of inhibition in growth of tested fungus was not found at any concentration of oil. However, it was observed that growth was reduced on increasing the concentration in case of all the tested phytopathogenic fungi. Comparatively *P. expansum, A. alternata* and *B. cinerea* growth was effected more than the *F. oxysporum*. At the end of study period growth of *F. oxysporum* at lower concentration of treatment was similar to control.

4.2.9. *Piper mullesua*

Essential oil of *P. mullesua* also inhibits the growth of four phytopathogenic fungi. In case of all four fungi i.e. *P.expansum, F. oxysporum, B.cinerea* and *A. alternata* the effect of oil was quite similar. At initial days of growth there was remarkable inhibition. In case of *P. expansum* percent inhibition ranges between 11 and 60 while in case of *F.oxysporum* it was 28.5 to 71%. Growth of *A. alternata* was inhibited 38 to 62% at different concentration of oil. Effect of oil on growth of *B. cinerea* was similar to *A. alternata*. In general growth of tested fungi was noticed at all concentration of oil treatment. The effect of essential oil was corresponding to their concentration.

4.2.10. *Pogostemon cablin*

Essential oil of *P. cablin* was also found to inhibit the growth of all four phytopathogenic fungi. In case of *P. expansum* and *A. alternata* at 5000 and 1000ppm concentration inhibition in growth was almost 90%. At 500ppm also the

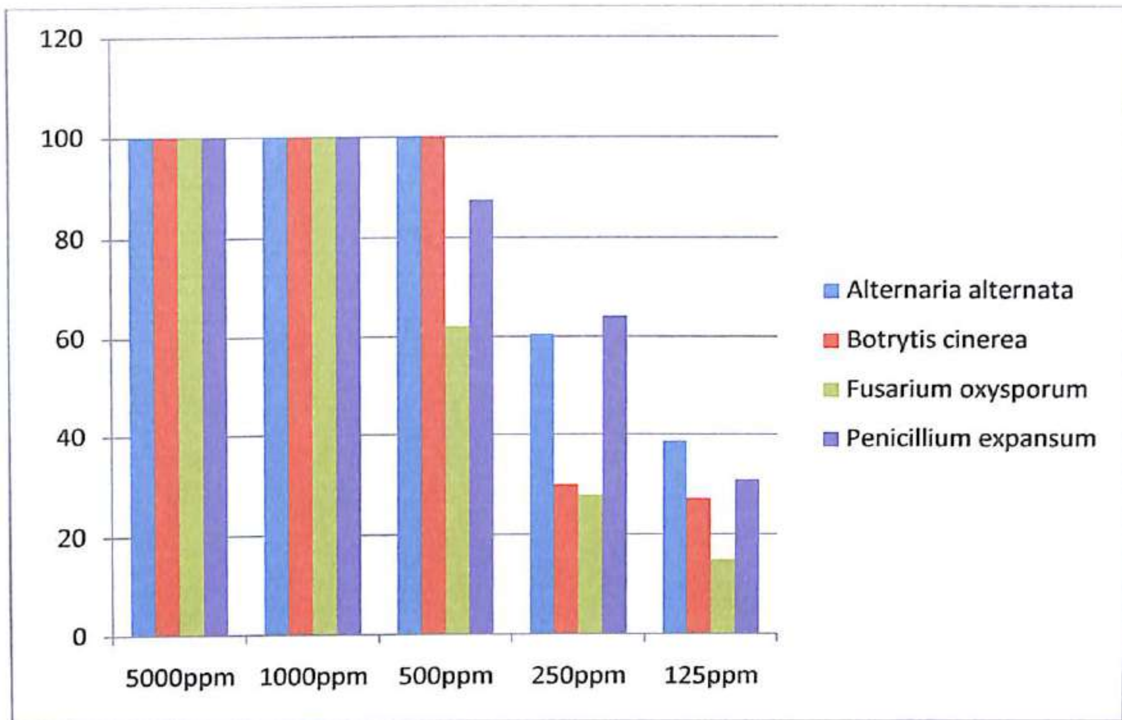

Figure 4.1: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Acorus calamus* oil.

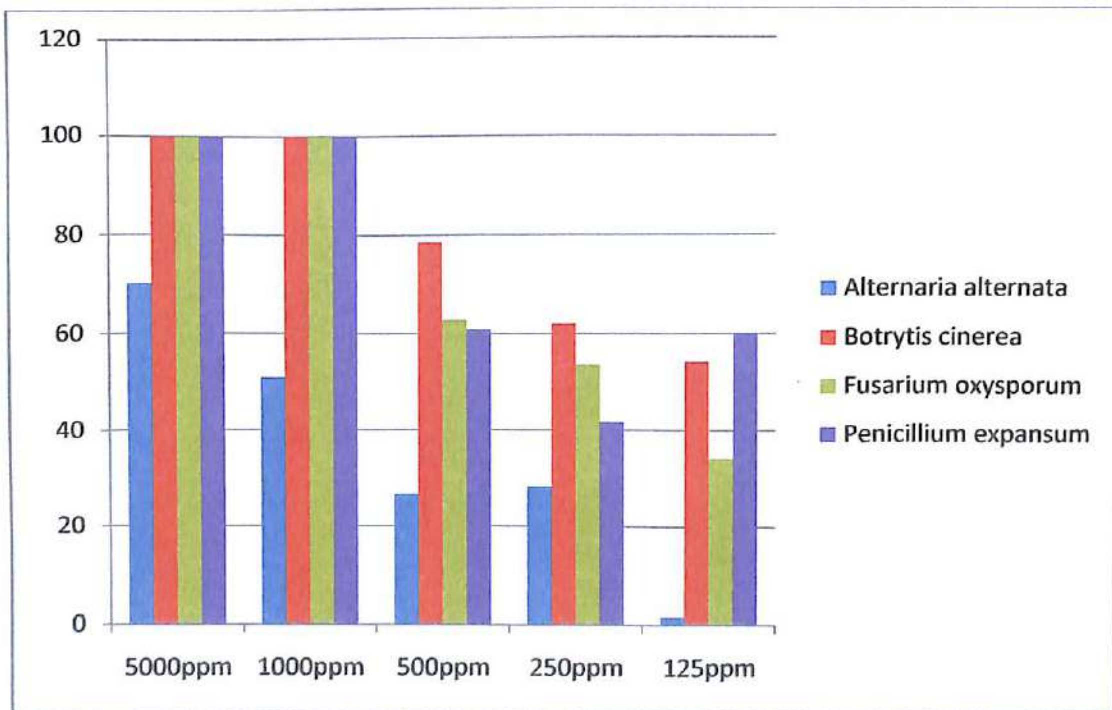

Figure 4.2: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Ageratum conyzoides* oil.

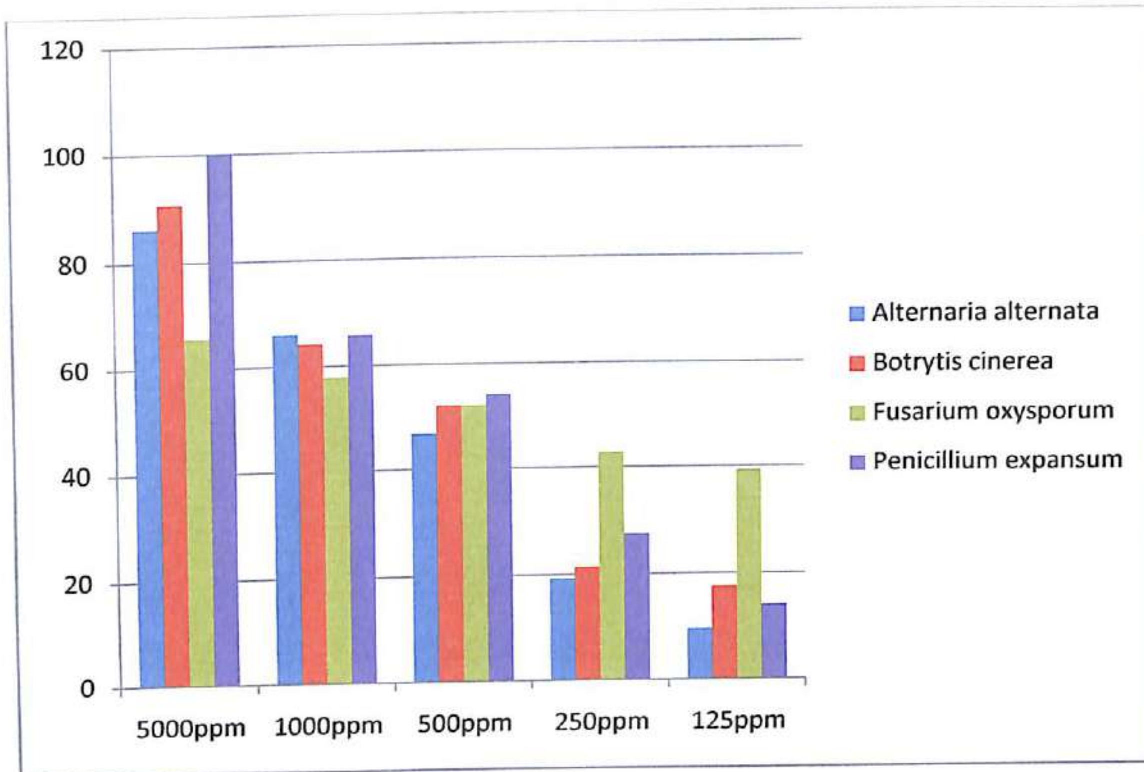

Figure 4.3: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Artimesea nilogerica* oil.

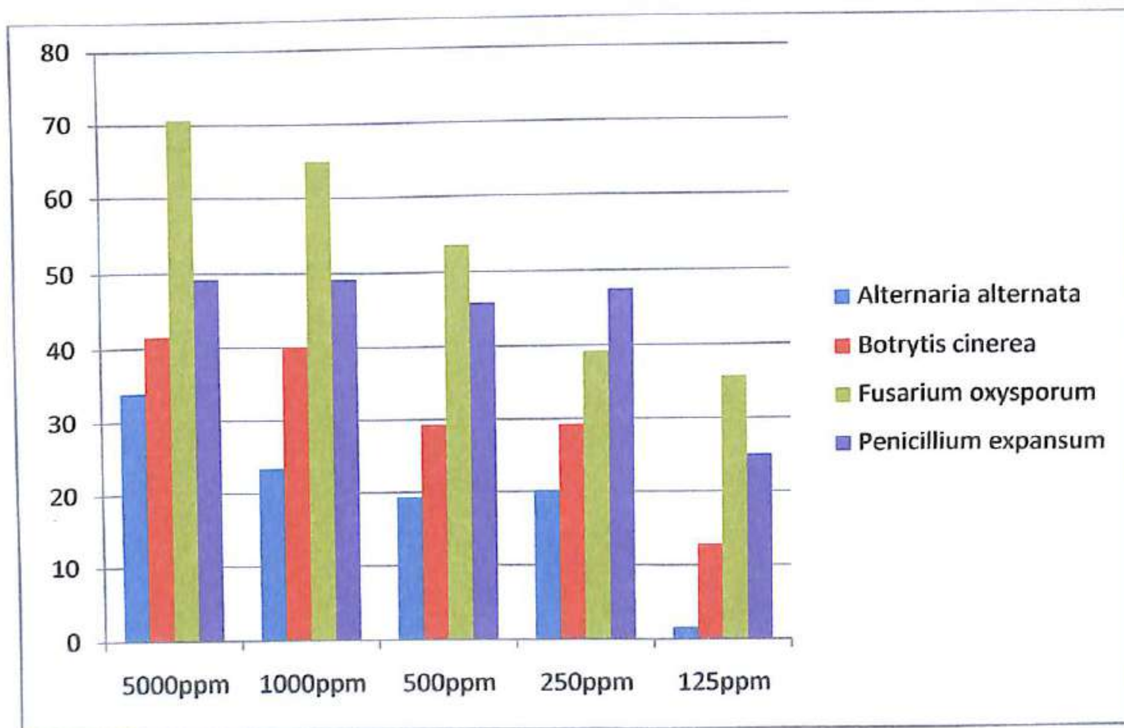

Figure 4.4: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Erigeron canadensis* oil.

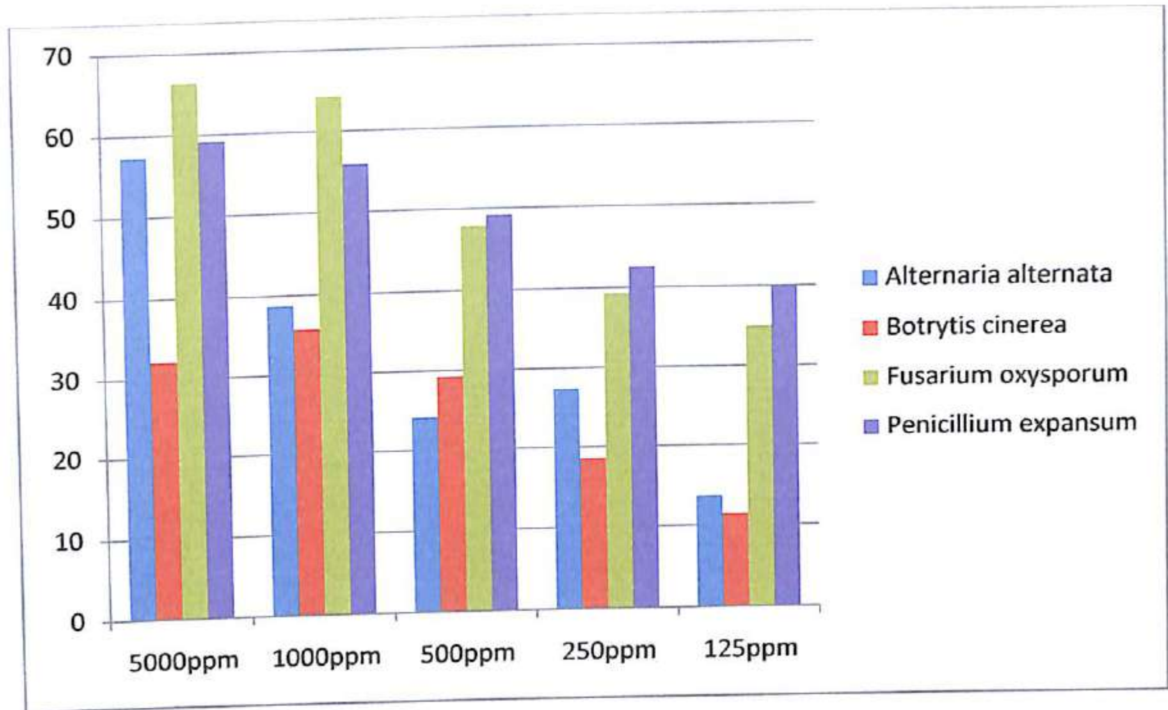

Figure 4.5: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Epatorium odoratum* oil.

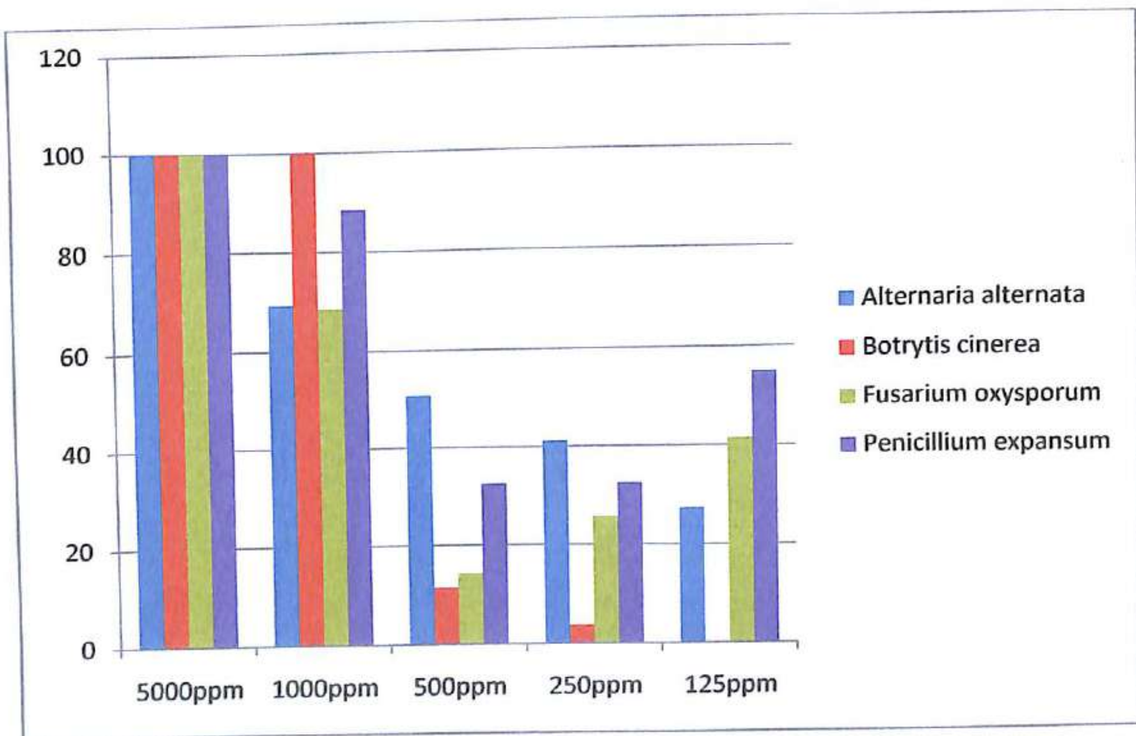

Figure 4.6: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Litsea cubeba* oil.

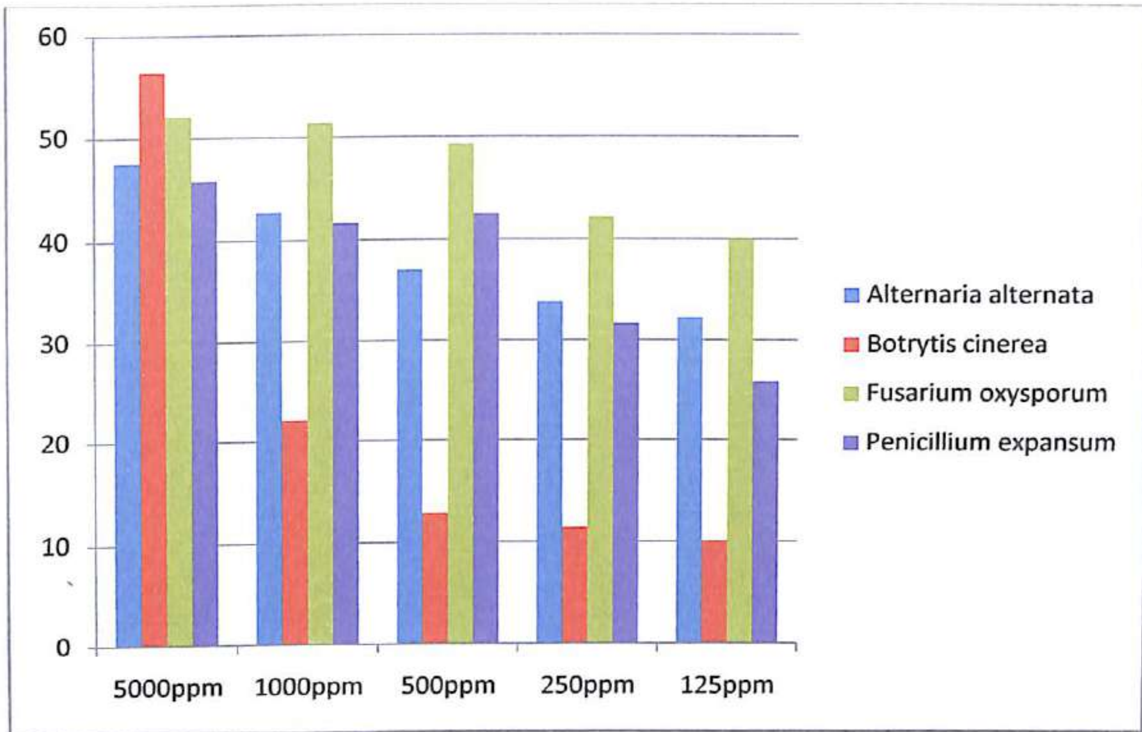

Figure 4.7: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Mesua ferrae* oil.

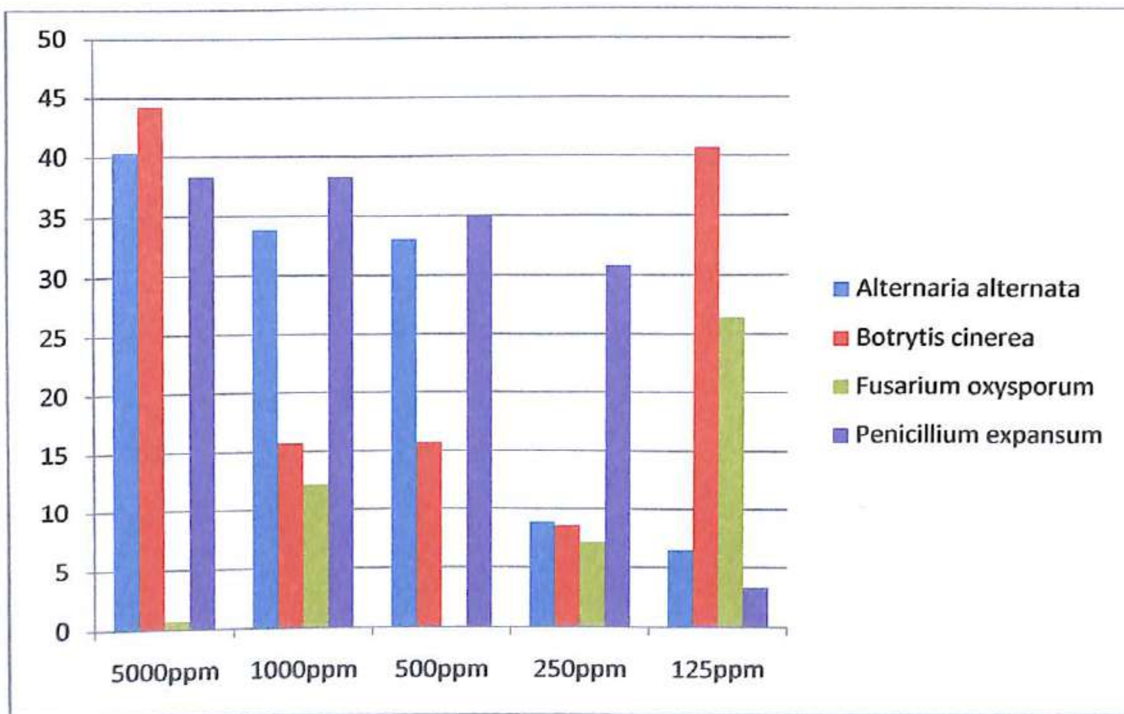

Figure 4.8: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Mikania cordata* oil.

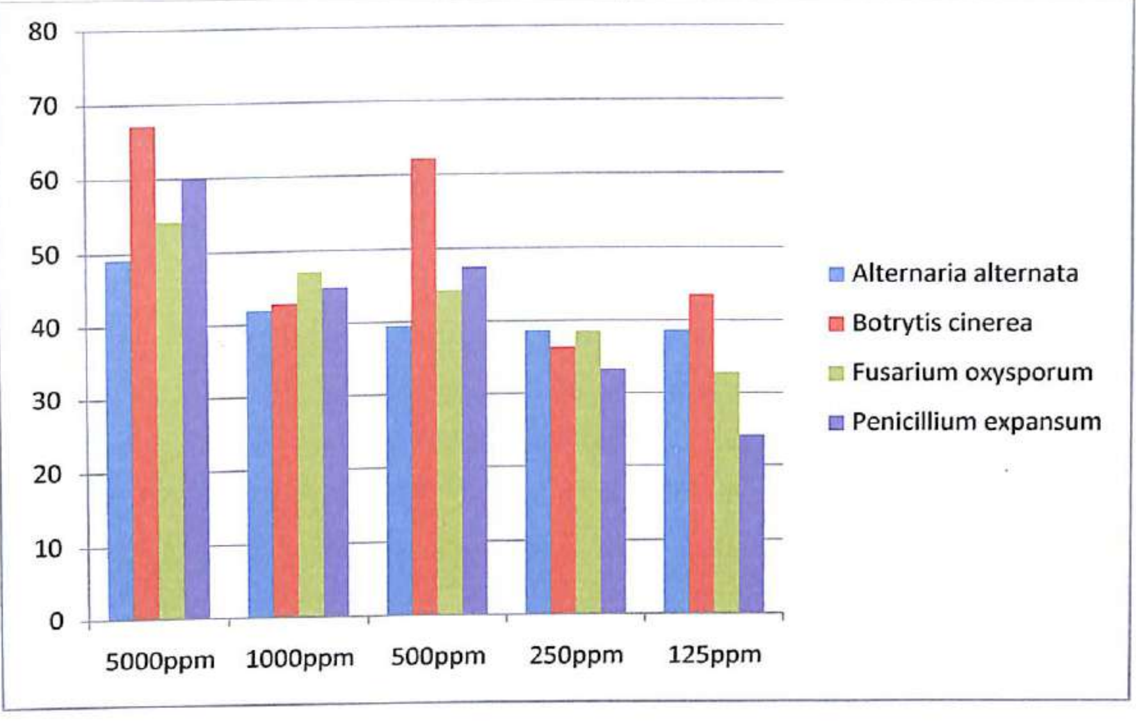

Figure 4.9: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Piper mullesua* oil.

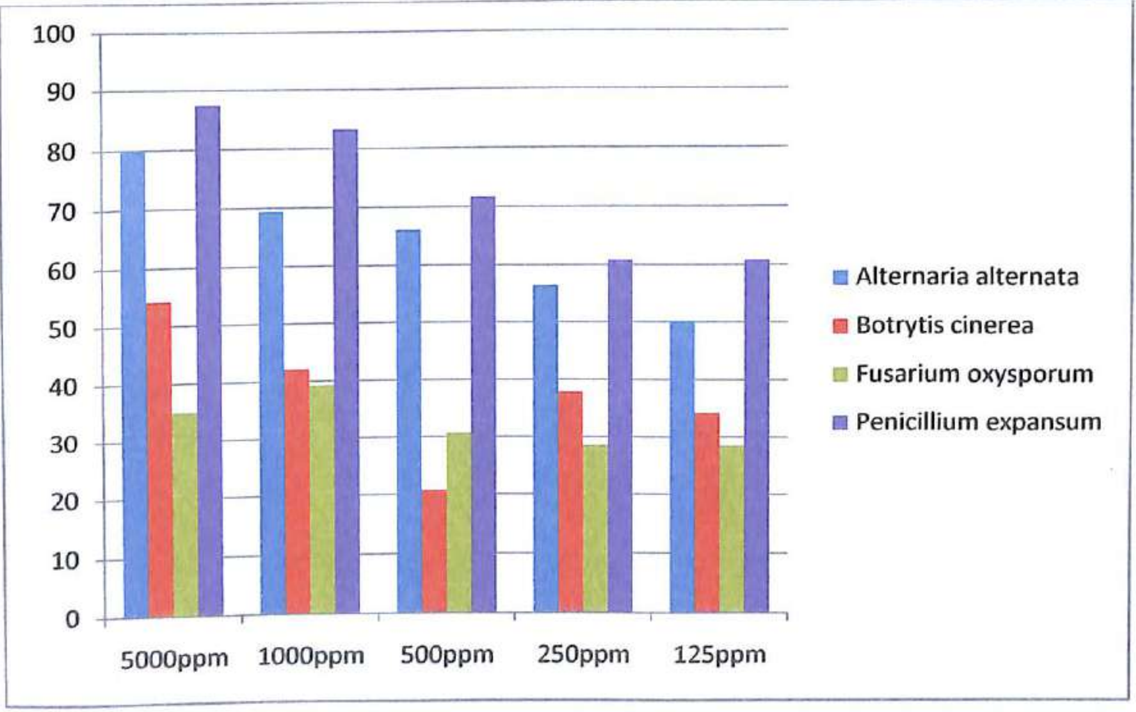

Figure 4.10: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Pogostemon cablin* oil.

growth was negligible. In case of *F. oxysporum* and *B. cinerea* during initial period of incubation growth was restricted but after 9th day of incubation enhancement in growth was recorded and difference was approximately between 19 to 37 percent. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. At lower concentration of oil i.e. 125, 250 and 500ppm colony growth of fungus always remain lesser than the control.

4.3. Minimum Inhibitory concentration (MIC)

Inhibitory evaluation of essential oils against phytopathogenic fungi showed the effective results by only 4 plant species. Essential oil of *Acorus calamus* was found fungi toxic at 250ppm for *A. alternata* and 500ppm for rest of the three fungi. EO of *Ageratum conyzoides* was inhibitory at 1000ppm for *B. cinerea, F. oxysporum and P. expansum* but it was not found effective against *A. alternata. Artemisia nilagirica* EO was found fungitoxic at 5000ppm concentration against *A. alternata B. cinerea, and P. expansum. Litsea cubeba* EO inhibited growth of test fungus at 1000ppm and 5000ppm. Therefore considering fungi toxic property only four plant oils were selected out of 10 evaluated ones. Concentration for MIC was taken below 5000 ppm only.

Table 4.7: Minimum inhibitory concentration of essential oils against pathogenic fungi

MIC of oils against fungi				
Essential oils of plants	**Phytopathogenic fungi**			
	A. alternata	***B. cinerea***	***F. oxysporum***	***P. expansum***
Acorus calamus	250ppm	500ppm	500ppm	500ppm
Ageratum conyzoides	Higher Conc.	1000ppm	1000ppm	1000ppm
Artemisia nilagerica	5000ppm	5000ppm	Higher Conc.	5000ppm
Litsea cubeba	5000ppm	1000ppm	1000ppm	1000ppm
Fungicides				
Bavistin	>5000ppm	>5000ppm	>5000ppm	>5000ppm
Caftaf 50%	>5000ppm	5000ppm	>5000ppm	>5000ppm

4.4 Storage and Temperature effect on toxicity of oil

Essential oils extracted from different plants were stored for a period of 730 days. Stored oils were tested for their fungi toxic effect at the interval of 30, 360 and 730 days respectively. Results of fungi toxic nature are given in table 4.8. It

was noticed that oil of *Acorus calamus* remains effective even after 730 days on using 500ppm concentration.

Table 4.8: Effect of storage on fungi toxicity of oils in relation to Percent inhibition in growth of test fungus

storage Period (days)	*Acorus calamus* oil (500ppm) (%)	*Ageratum conyzoides* oil (1000ppm) (%)	*Artemisia nilagirica* oil (5000ppm) (%)	*Litsea cubeba* oil (1000ppm) (%)
30	100	100	100	100
360	100	100	100	100
730	100	100	100	100

Similarly oils of *Ageratum conyzoides, Artemisia nilagirica* and *Litsea cubeba* were found inhibitory at 1000, 5000 and 1000ppm concentrations respectively. Results showed that quality of oils was deteriorated even after storage of such a long period.

Table 4.9: Effect of temperature on fungi toxicity of oils in relation to Percent inhibition in growth of test fungus

Temperature (°C)	*Acorus calamus* oil (500ppm) (%)	*Ageratum conyzoides* oil (1000ppm) (%)	*Artemisia nilagirica* oil (5000ppm) (%)	*Litsea cubeba* oil (1000ppm) (%)
5	100	100	100	100
30	100	100	100	100
121	100	100	100	100

Results on exposing of essential oils at different temperature are given in table 4.9. Fungi toxic effect of oils was not found altered even oil were incubated at 5, 30 and 121 ° C of temperature. Oils were found 100% inhibitory at the tested concentration.

4.5 Efficacy comparison of oil with fungicides

In case of oil at 5000 ppm and in some cases lower than that inhibited the growth of fungi whereas fungicides could not affected. Except in case of *Artemisia nilagirica* all the plant based selected essential oil 100% inhibited the growth of fungi either at 1000ppm or 500ppm. In case of *Artemisia nilagirica*

5000ppm concentration was found to inhibit the growth of phytopathogenic fungi 100%. Different concentrations of Bavistin were used and even at 5000ppm concentration growth of phytopathogenic fungi was recorded. Another fungicide Captaf was found to inhibit the growth of phytopathogenic fungi at higher concentrations. At 5000ppm concentration growth of *B. cinerea* was completely inhibited. Captaf was also recorded to affect the growth of *Fusarium oxysporum, Alternaria alternata* and *Penicillium expansum* at 5000ppm concentration. Effect of captaf on the growth of phytopathogenic fungi was proportional to the concentration. At lower concentration growth was more compare to the higher concentration. Effect of captaf was less on *F. oxysporum* in comparison to other phytopathogenic fungi. More severly affected was *B. cinerea* and *P. expansum.* Overall growth of fungi was affected by the fungicides but comparatively essential oils showed more inhibitory effect than mentioned fungicides.

Table 4.10: Effect of Bavistin fungicide on percent inhibition of growth of pathogenic fungi using different concentrations

A. alternata					
Days	**5000ppm**	**3000ppm**	**2000ppm**	**1000ppm**	**500ppm**
5th	15.38	15.38	03.84	00.00	00.00
7th	06.25	00.00	00.00	00.00	00.00
9th	32.65	18.36	10.20	06.12	00.00
11th	35.71	19.64	10.71	07.14	00.00
13th	40.00	16.66	08.33	05.00	03.33
B. cinerea					
Days	5000ppm	3000ppm	2000ppm	1000ppm	500ppm
5th	88.88	88.88	77.77	71.11	60.00
7th	88.13	83.05	77.96	74.57	61.01
9th	76.92	76.92	69.23	69.23	60.00
11th	73.52	69.11	67.64	61.76	55.88
13th	70.58	64.70	58.82	55.88	48.52
F. oxysporum					
Days	5000ppm	3000ppm	2000ppm	1000ppm	500ppm
5th	45.00	35.00	30.00	27.50	25.00
7th	20.00	17.77	04.44	04.44	00.00
9th	23.40	14.89	00.00	00.00	00.00
11th	27.77	16.66	00.00	00.00	00.00
13th	34.92	25.39	00.00	00.00	00.00
P. expansum					
Days	5000ppm	3000ppm	2000ppm	1000ppm	500ppm

5th	36.11	33.33	33.33	27.77	27.77
7th	40.00	24.44	22.22	20.00	17.77
9th	40.00	20.00	18.00	16.00	12.00
11th	45.45	21.81	16.36	12.72	10.90
13th	48.27	22.41	17.24	13.79	08.62

Table 4.11: Effect of Captaf 50% fungicide on percent inhibition of growth of pathogenic fungi using different concentrations

A. alternata					
Days	**5000ppm**	**3000ppm**	**2000ppm**	**1000ppm**	**500ppm**
5th	80.76	80.76	73.07	65.38	61.53
7th	81.25	75.00	68.75	59.37	56.25
9th	85.71	83.67	75.51	69.38	65.30
11th	83.92	82.14	89.13	67.85	66.07
13th	73.33	73.33	58.33	48.33	46.66
B. cinerea					
Days	**5000ppm**	**3000ppm**	**2000ppm**	**1000ppm**	**500ppm**
5th	100	88.88	88.88	84.44	77.77
7th	100	89.83	88.13	83.05	79.66
9th	100	90.76	87.69	81.53	80.00
11th	100	88.23	86.76	80.88	77.94
13th	100	86.76	83.82	77.94	72.05
F. oxysporum					
Days	**5000ppm**	**3000ppm**	**2000ppm**	**1000ppm**	**500ppm**
5th	75.00	62.50	50.00	47.50	42.50
7th	64.44	55.55	48.88	48.88	44.44
9th	57.44	46.80	40.42	36.17	27.65
11th	50.00	42.59	38.88	33.33	25.92
13th	31.74	11.11	11.11	04.76	00.00
P. expansum					
Days	**5000ppm**	**3000ppm**	**2000ppm**	**1000ppm**	**500ppm**
5th	86.11	80.55	77.77	72.22	66.66
7th	80.00	77.77	77.77	73.33	66.66
9th	80.00	76.00	76.00	70.00	64.00
11th	80.00	72.72	74.54	67.27	63.63
13th	65.51	56.89	55.17	50.00	48.27

Table 4.12: Toxicity nature of Essential oils on phytopathogenic fungi

Essential oils	*Alternaria alternata*	*Botrytis cineria*	*Fusarium oxysporum*	*Penicillium expasum*
Acorus calamus	Fungicidal at 500ppm	Fungicidal at 500ppm	Fungicidal at 1000ppm	Fungicidal at 500ppm
Ageratum conyzoides	–	Fungicidal at 1000ppm	Fungicidal at 1000ppm	Fungicidal at 5000ppm
Artemisia nilogirica	Fungistatic	Fungistatic	–	Fungicidal at 5000ppm
Litsea cubeba	Fungicidal at 5000ppm	Fungicidal at 1000ppm	Fungicidal at 1000ppm	Fungicidal at 5000ppm

Table 4.13: Effect of Increased Inoculums Density of pathogenic fungi on the Fungitoxicity of oils

Alternaria alternata									
No. of Fungal Discs	Approximate No. of Spores	Growth of the Test Fungus							
		Acorus calamus oil		*Ageratum conyzoides* Oil		*Artemisia nilagirica* Oil		*Litsea cubeba* Oil	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
1	2358×10^3	-	+	+	+	-	+	-	+
2	47174×10^3	-	+	+	+	-	+	-	+
4	94348×10^3	-	+	+	+	-	+	-	+
8	188696×10^3	-	+	+	+	-	+	-	+
16	377392×10^3	-	+	+	+	-	+	-	+
Botrytis cineria									
No. of Fungal Discs	Approximate No. of Spores	Growth of the Test Fungus							
		Acorus calamus oil		Ageratum conyzoides Oil		Artemisia nilagirica Oil		Litsea cubeba Oil	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
1	2358×10^3	-	+	-	+	-	+	-	+
2	47174×10^3	-	+	-	+	-	+	-	+
4	94348×10^3	-	+	-	+	-	+	-	+
8	188696×10^3	-	+	-	+	-	+	-	+
16	377392×10^3	-	+	-	+	-	+	-	+
Fusarium oxysporum									
No. of Fungal Discs	Approximate No. of Spores	Growth of the Test Fungus							
		Acorus calamus oil		Ageratum conyzoides Oil		Artemisia nilagirica Oil		Litsea cubeba Oil	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
1	2358×10^3	-	+	-	+	+	+	-	+
2	47174×10^3	-	+	-	+	+	+	-	+
4	94348×10^3	-	+	-	+	+	+	-	+
8	188696×10^3	-	+	-	+	+	+	-	+

16	377392 × 10³	-	+	-	+	+	+	-	+
Penicillium expansum									
No. of Fungal Discs	Approximate No. of Spores	Growth of the Test Fungus							
		Acorus calamus oil		Ageratum conyzoides Oil		Artemisia nilagirica Oil		Litsea cubeba Oil	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
1	2358 × 10³	-	+	-	+	-	+	-	+
2	47174 × 10³	-	+	-	+	-	+	-	+
4	94348 × 10³	-	+	-	+	-	+	-	+
8	188696 × 10³	-	+	-	+	-	+	-	+
16	377392 × 10³	-	+	-	+	-	+	-	+

-indicates no growth of test fungus
+indicate growth of test fungus

Table 4.14: Fungitoxic spectrum of plant based essential oils on percent inhibition of growth of different pathogenic fungi

Acorus calamus								
	Aspergillus niger		*Cladosporium herborum*		*Periconia micrspinosa*		*Rhizopus oryzae*	
Days	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**
5th	100	100	100	100	100	100	100	100
7th	100	100	100	100	100	100	100	100
9th	100	100	100	100	100	100	100	100
11th	100	100	100	100	100	100	100	100
13th	100	100	100	100	100	100	100	100
15th	100	100	100	100	100	100	100	100
Ageratum conyzoides								
	Aspergillus niger		*Cladosporium herborum*		*Periconia micrspinosa*		*Rhizopus oryzae*	
Days	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**	**1000ppm**	**500ppm**
5th	100	100	100	100	100	100	100	100
7th	100	100	100	100	100	100	100	100
9th	47.36	21.05	100	100	100	100	100	100
11th	55.55	22.22	100	100	100	52.38	100	63.63
13th	56.14	38.59	100	100	100	50.00	100	50.00
15th	53.84	44.61	100	100	100	49.15	100	55.88

Artemisia nilagirica								
Aspergillus niger			*Cladosporium herborum*		*Periconia micrspinosa*		*Rhizopus oryzae*	
Days	1000ppm	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm	500ppm
5th	100	100	100	100	100	50.00	100	100
7th	100	20.00	100	100	46.42	35.71	100	100
9th	47.36	34.21	100	100	44.44	30.55	100	100
11th	44.44	33.33	100	55.00	42.85	28.57	100	81.81
13th	47.36	47.36	100	43.18	40.00	34.00	100	75.00
15th	46.15	46.15	100	45.45	40.67	37.28	100	70.58
Litsea cubeba								
Aspergillus niger			*Cladosporium herborum*		*Periconia micrspinosa*		*Rhizopus oryzae*	
Days	1000ppm	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm	500ppm
5th	100	100	100	100	100	100	100	100
7th	100	100	100	100	100	100	100	100
9th	100	100	100	100	100	100	100	100
11th	100	100	100	100	100	100	100	100
13th	100	100	100	100	100	100	100	100
15th	100	100	100	100	100	100	100	100

4.6 Nature of toxicity, increased inoculums density and fungitoxic spectrum

Acorus calamus, Ageratum conyzoides and *Litsea cubeba* oils was found fungicidal for all the phytopathogenic fungi. *Artemisia nilogirica* oil was fungistatic in nature only for *P. expansum* it was fungicidal in nature. Results related to effect of increased inoculums density of pathogenic fungi on the fungitoxicity of oils are given in table 4.13. In case of *Alternaria alternata* on increasing the inoculums density fungi toxicity of *Acorus calamus, Artemisia nilagirica* and *Litsea cubeba* oils was not affected. But *Ageratum conyzoides* oil was found decreasing toxicity on increasing the inoculums density. Essential oils were found cent percent inhibitory on *Botrytis cineria* at all density of the fungus. Similar results were obtained in case of *Fusarium oxysporum* except in case of *Artemisia nilagirica* oil which was not found affecting the growth of fungi at any concentration of fungal spores. In case of *Penicillium expansum* also fungi toxicity of oils was not altered due to variation in inoculums density of pathogens.

The fungitoxic spectrum of the plant based essential oils was studied by applying against a number of other fungi. Results showed that essential oil of *Acorus calamus* was fungicidal and cent-percent inhibitory for fungi like *Aspergillus niger, Cladosporium herbarum, Rhizopus oryzae.* Fungi *Periconia microspinosa* was found to grow at 500ppm concentration but at 1000ppm concentration colony growth was completely stopped. Essential oil of *Ageratum conyzoides* were recorded fungi static in nature. It stopped the growth of fungi during initial period of incubation up to 11th days but after that slight growth was occurred in petriplates. Essential oil of *Artemisia nilogirica* was also found in fungi static nature for the fungi and after initial inhibition growth started after nine days of incubation. *Litsea cubeba* oils were found fungicidal for all the phytopathogenic fungi and growth of fungi was completely stunted.

4.7. Evaluation of plant extracts against different phytopathogenic fungi

A number of plant species were taken to evaluate their effect on the growth of four phytopathogenic fungi of kiwifruit viz. *A. alternata B. cinerea, F. oxysporum,* and *P. expansum* following modified paper disc technique (Conner and Beachat, 1984). Plant extracts were prepared using different organic solvents namely ether, benzene, chloroform, ethyl acetate, methanol and absolute alcohol. Simultaneously, a control was also maintained by similarly impregnating with the same amount of requisite respective solvent.

Table 4.15: List of plants which were screened for aqueous extract fungitoxicity

Sl. No.	Name of plants	Family	Parts used	Remarks
1	*Abroma augusta*	Sterculiaceae	Leaves	-
2	*Acorus calamus*	Acoraceae	Rhizome	++++++
3	*Adhatoda vesica*	Acanthaceae	Leaves	-
4	*Adrographis paniculata*	Acanthaceae	Leaves	-
5	*Ageratum conyzoides*	Asteraceae	Aerial parts	++++++
6	*Altingia excelsa*	Hemamelidaceae	Leaves	-
7	*Allium fistulosum*	Amaryllidaceae	Leaves	-
8	*Artemisia nilagirica*	Asteraceae	Leaves	++++++
9	*Azadirachta melia*	Meliaceae	Leaves	++
10	*Bauhinia purpurea*	Fabaceae	flowers	++++++
11	*Boehmeria macrophylla*	Urticaceae	Leaves	-
12	*Callicarpa arborea*	Verbenaceae	Twig	++++++
13	*Cassia alata*	Fabaceae	Leaves	-

14	*Cassia tora*	Fabaceae	Leaves	-
15	*Cardamine trichocarpa*	brassicaceae	Whole plant	-
16	*Cissampelos pareira*	Menispermaceae .	Leaves	-
17	*Crotolaria juncea*	Fabaceae	Leaves	-
18	*Eclipta alba*	Asteraceae	Aerial parts	-
19	*Elaeocarpus sphaericus*	Elaeocarpaceae	Leaves	++++++
20	*Erigeron Canadensis*	Asteraceae	Leaves	++++
21	*Eupatorium odoratum*	Asteraceae	Leaves	++++++
22	*Euphorbia hirta*	Asteraceae	Aerial parts	-
23	*Gynura crepidioides*	Asteraceae	Leaves	-
24	*Houttuynia cordata*	Saururaceae	Whole plant	++
25	*Ipomoea quamocit*	Convulvulaceae	Leaves	-
26	*Juticia gendarnssa*	Acantheceae	Leaves	-
27	*Lantana camara*	Verbenaceae	Leaves	+++++
28	*Lagerstroemia*	Lythraceae	Leaves	-
29	*Leucas aspara*	lamiaceae	Aerial parts	++
30	*Litsea cubeba*	Lauraceae	Fruits	++++++
31	*Melastoma malabathrium*	Melastomataceae	Leaves	-
32	*Mesua ferrae*	Guttiferae	Flower	+++
33	*Michelia champaca*	Magnoliaceae	Fruit	+++++
34	*Mikania cordata*	Asteraceae	Aerial parts	+++++
35	*Plantago major*	Plantaginaceae	leaves	-
36	*Piper betle*	Piperaceae	leaves	-
37	*Piper mullesua*	Piperaceae	Twig	++++++
38	*Piper brachystachyum*	Piperaceae	Twig	++
39	*Pogostemon cablin*	Lamiaceae	Leaves	++++
40	*Polygonum capitatum*	Polygonaceae	Aerial parts	-
41	*Polygonum hydropiper*	Polygonaceae	Aerial parts	++++++
42	*Rubia cordifolia*	Rubiaceae	Whole plant	-
43	*Samanea saman*	Fabaceae	Leaves	++++++
44	*Scoparia dulcis*	Plantaginaceae	Twig	-
45	*Schefflera venulosa*	Araliaceae	Leaves	-
46	*Solanum indicum*	Solanaceae	Fruit	++
47	*Solanum spirale*	Solanaceae	Fruit	+++++
48	*Sonchus arvensis*	Asteraceae	Aerial parts	+++++
49	*Spilanthus oleraceae*	Asteraceae	Aerial parts	++
50	*Tabernaemontana divaricata*	Apocynaceae	Leaves	-
51	*Tagetes erectus*	Asteraceae	Flower	+++++
52	*Terminalia arjuna*	Comretaceae	Fruit	-
53	*Taxus baccata*	Taxaceae	Leaves(spin)	+++++
54	*Urena lobata*	Malvaceae	Leaves	-
55	*Vitex nigundo*	Lamiaceae	Leaves	-
56	*Zanthoxylum oxyphyllum*	Rutaceae	Fruits	+++++
57	*Zanthoxylum sp.*	Rutaceae	Leaves	++

Table 4.16: List of plant species selected for various solvent extract fungitoxicity

Sl. No.	Name of plants	Family	Parts used	Remarks
1.	*Acorus calamus*	Acoraceae	Rhizome	++++++
2.	*Ageratum conyzoides*	Asteraceae	Aerial parts	++++++
3.	*Artemisia nilagirica*	Asteraceae	Leaves	++++++
4.	*Bauhinia purpurea*	Fabaceae	flowers	++++++
5.	*Callicarpa arborea*	Verbenaceae	Twig	++++++
6.	*Elaeocarpus sphaericus*	Elaeocarpaceae	Leaves	++++++
7.	*Eupatorium odoratum*	Asteraceae	Leaves	++++++
8.	*Lantana camara*	Verbenaceae	Leaves	+++++
9.	*Litsea cubeba*	Lauraceae	Fruits	++++++
10.	*Michelia champaca*	Magnoliaceae	Fruit	+++++
11.	*Mikania cordata*	Asteraceae	Aerial parts	+++++
12.	*Piper mullesua*	Piperaceae	Twig	++++++
13.	*Polygonum hydropiper*	Polygonaceae	Aerial parts	++++++
14.	*Samanea saman*	Fabaceae	Leaves	++++++
15.	Solanum spirale	Solanaceae	Fruit	+++++
16.	*Tagetes erectus*	Asteraceae	Flower	+++++
17.	*Taxus baccata*	Taxaceae	Leaves(spin)	+++++
18.	*Zanthoxyllum oxyphyllum*	Rutaceae	Fruits	+++++

Total numbers of 57 plant species were collected to screen for their inhibitory effect on the fungi. Among the screened plant species eighteen were found inhibiting growth of fungi. On the basis of extracts effect on the tested fungi 18 plants were selected for further study.

Table 4.17: Effect of *Acorus calamus* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00

11th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
18th	0.50±0.00	0.50±0.00	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

4.7.1. *Acorus calamus*

Plant extract of *A.calamus* was found to have significant effect on the growth of all the four phytopathogenic fungi. It was noticed that extract in ethyl acetate, methanol and alcohol was not quite effective against *Fusarium oxysporum*. But extract in other solvent was recorded inhibitory. Phytopathogenic fungi *Alternaria alternata, Botrytis cineria and Penicillium expansum* growth was significantly affected in all the solvent extracts. Overall restriction of phytopathogenic fungi was 100%. Growth of phytopathogenic fungi was found completely inhibited that indicate fungicidal nature of the plant extract. In case of *Fusarium oxysporum* growth was very poor showing fungistatic nature of the extract.

4.7.2. *Ageratum conyzoides*

Plant extract of *A. conyzoides* shows significant inhibitory effect on the growth of phytopathogenic fungi. In case of *P. expansum* and *B. cinerea* growth of fungal colony was found to be inhibited drastically. Methanol and benzene extracts of *A. conyzoides* inhibited 100% growth of *B. cinerea* up to 7th day of incubation.

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However, in other solvent extracts both *P. expansum* and *B. cinerea* had slightly more colonial growth than the control. Growth of *F. oxysporum* was inhibited 35 to 74% under different solvent plant extract treatment. Among the extracts methanol was found quite effective in reducing the growth. In case of *A. alternata* benzene extract inhibit 79 to 86% growth at different days interval of incubation. In general all the extracts reduced growth in range of 52 to 81%.

4.7.3 *Artemisia nilagirica*

In general plant extract of *A. nilagirica* was found to have moderate effect on the growth of phytopathogenic fungi. Plant extract in ethyl acetate, methanol and absolute alcohol was found to inhibit growth of *P. expansum* from 60 to 75%. Extract in ether did not show much effect on the growth of *P. expansum*. In case of F. *oxysporum, B. cinerea* and *A. alternata* solvent extract of methanol, chloroform were found inhibitory for the growth. Extract prepared using other solvents were also noticed to restrict the growth of phytopathogenic fungi. Range of inhibition was between 13 and 50%. Plant extract in all solvents was recorded to inhibit the growth of phytopathogenic fungi. Phytopathogenic fungi grown in media amended with plant extract have always poor growth as compare to the control.

Table 4.18: Effect of *Ageratum conyzoides* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.15± 0.05	0.90±0.10	0.95±0.15	1.25±0.15	1.05±0.05	0.80±0.20
7th	1.35±0.15	1.05±0.05	1.15±0.25	1.65±0.05	1.25±0.15	0.95±0.25
9th	1.90±0.00	1.10±0.10	1.45±0.45	2.25±0.15	1.70±0.20	1.00±0.20
11th	1.90.±0.00	1.15±0.15	1.85±0.05	2.60±0.30	1.75±0.15	1.10±0.10
13th	1.90±0.00	1.15±0.15	1.85±0.05	2.70±0.30	2.10±0.50	1.10±0.10
15th	1.90±0.00	1.15±0.15	2.00±0.10	2.70±0.30	2.35±0.55	1.10±0.10
18th	1.90±0.00	1.15±0.15	2.00±0.10	2.70±0.30	2.45±0.65	1.10±0.10
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.35±0.15	1.30±0.10	1.45±1.45	1.95±0.25	2.00±0.20	1.40±0.20
7th	2.45±0.15	1.90±0.30	2.10±0.70	2.75±0.25	2.75±0.05	2.50±0.90
9th	2.65±0.05	2.20±0.60	2.25±0.75	3.65±0.65	3.45±0.05	3.10±1.10
11th	2.75±0.15	2.20±0.60	2.35±0.75	4.85±1.85	3.45±0.05	3.20±1.20

13th	2.75±0.15	2.20±0.60	2.35±0.75	5.00±2.00	3.45±0.05	3.25±1.25
15th	2.75±0.15	2.20±0.60	2.35±0.75	5.10±2.10	3.45±0.05	3.25±1.25
18th	2.75±1.15	2.20±0.60	2.35±0.75	5.10±2.10	3.45±0.05	3.25±1.25
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.50±0.00	0.00±0.00	0.25±0.25	0.25±0.25	0.65±0.15	0.00±0.00
7th	0.50±0.00	0.00±0.00	0.50±0.50	0.25±0.25	0.65±0.15	0.00±0.00
9th	0.70±0.00	0.25±0.25	1.45±0.15	0.75±0.25	0.95±0.05	0.25±0.25
11th	1.00±0.20	1.00±0.50	2.95±1.35	1.00±0.50	1.15±0.05	0.25±0.25
13th	1.10±0.10	2.00±1.40	2.95±1.35	1.00±0.50	1.15±0.05	0.25±0.25
15th	1.20±0.10	2.20±1.40	2.95±1.35	1.00±0.50	1.15±0.05	0.25±0.25
18th	1.30±0.00	2.20±1.40	2.95±2.20	1.00±0.50	1.15±0.05	0.25±0.25
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.40±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.35±0.05	0.50±0.00
7th	1.90±0.10	1.60±0.20	1.90±0.10	1.50±0.00	1.80±0.20	0.85±0.05
9th	2.20±0.20	1.70±0.05	2.10±0.10	1.50±0.00	2.05±0.15	1.05±0.05
11th	2.50±0.30	2.10±0.30	2.35±0.15	1.50±0.00	2.55±0.05	1.15±0.05
13th	2.75±0.45	2.25±0.45	2.50±0.20	1.50±0.00	2.40±0.00	1.35±0.05
15th	3.05±0.55	2.25±0.45	2.55±0.15	1.50±0.00	2.40±0.00	1.45±0.05
18th	3.55±0.55	2.30±0.40	2.55±0.15	1.50±0.00	2.40±0.00	1.45±0.05

Table 4.19: Effect of *Artemisia nilagirica* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium *expansum*						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.60±0.10	1.45±0.05	1.60±0.00	1.75±0.15	1.45±0.05	1.40±0.00
7th	2.40±0.10	2.65±0.45	1.60±0.00	2.90±0.40	2.40±0.00	2.10±0.70
9th	2.50±0.10	2.10±0.50	1.60±0.00	3.05±0.45	2.40±0.00	2.45±0.45
11th	2.55±0.15	2.15±0.55	1.60±0.00	3.05±0.45	2.40±0.00	2.45±0.45
13th	2.65±0.15	2.15±0.55	1.60±0.00	3.05±0.45	2.40±0.00	2.45±0.45
15th	2.65±0.15	2.15±0.55	1.60±0.00	3.05±0.45	2.40±0.00	2.45±0.45
18th	2.65±0.15	2.15±0.55	1.60±0.00	3.05±0.45	2.40±0.00	2.45±0.45
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.30±0.60	2.45±0.35	3.25±0.35	3.55±0.25	2.95±0.35	3.35±0.25
7th	4.40±1.70	2.55±0.35	3.35±0.45	3.60±0.30	3.10±0.40	3.70±0.60
9th	5.00±2.30	2.65±0.35	3.55±0.65	3.95±0.45	3.45±0.65	3.75±0.55
11th	5.10±2.40	2.70±0.30	3.55±0.65	4.00±0.50	3.45±0.65	3.75±0.55
13th	5.10±2.40	2.70±0.30	3.65±0.65	4.05±0.55	3.45±0.65	3.80±0.50
15th	5.10±2.40	2.70±0.30	3.65±0.65	4.05±0.55	3.45±0.65	3.80±0.50
18th	5.10±2.40	2.70±0.30	3.65±0.65	4.05±0.55	3.45±0.65	3.80±0.50

Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	4.60±0.00	3.30±0.00	3.60±0.10	3.85±0.25	2.40±0.30	3.90±0.10
7th	5.40±0.20	3.55±0.05	3.60±0.10	3.85±0.25	3.45±0.45	3.90±0.10
9th	5.40±0.20	3.60±0.10	3.90±0.20	4.00±0.40	3.45±0.45	4.05±0.25
11th	5.40±0.20	3.60±0.10	4.05±0.35	4.10±0.50	3.45±0.45	4.05±0.25
13th	5.40±0.20	3.60±0.10	4.05±0.35	4.10±0.50	3.50±0.40	4.15±0.35
15th	5.40±0.20	3.60±0.10	4.05±0.35	4.10±0.50	3.50±0.40	4.15±0.35
18th	5.40±0.20	3.60±0.10	4.05±0.35	4.10±0.50	3.50±0.40	4.15±0.35
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.00±0.10	2.10±0.10	2.00±0.00	2.30±0.10	2.20±0.10	2.00±0.10
7th	2.40±0.10	2.55±0.05	2.35±0.05	2.65±0.05	2.40±0.10	2.55±0.05
9th	2.65±0.05	2.75±0.05	2.95±0.05	2.95±0.05	2.85±0.25	2.75±0.05
11th	2.65±0.05	3.00±0.10	2.95±0.05	3.55±0.15	2.85±0.25	2.75±0.05
13th	3.00±0.10	3.35±0.05	3.65±0.05	3.85±0.15	3.35±0.05	3.85±0.05
15th	3.55±0.05	3.60±0.10	3.90±0.10	3.85±0.15	3.35±0.05	3.85±0.05
18th	3.95±0.05	3.60±0.10	3.90±0.10	3.85±0.15	3.35±0.05	3.85±0.05

4.7.4 *Bauhinia purpurea*

Plant extract of *B. purpurea* was also effective in reducing the colony growth of tested fungus. The growth of *P. expansum* and *F. oxysporum* was not affected by the extract prepared in ethyl acetate, methanol and absolute alcohol. However, growth of *B. cinerea* and *A. alternata* extract with above mentioned solvent found to reduce the growth from 18 to 50%. Extracts prepared with solvents chloroform and benzene did not have any remarkable inhibitory effect on the growth of all tested fungi.

Table 4.20: Effect of *Bauhinia purpurea* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.55±0.05	1.95±0.05	1.50±0.00	1.50±0.00	1.45±0.05	1.70±0.10
7th	2.70±0.00	2.85±0.05	2.70±0.00	2.75±0.05	2.40±0.30	2.90±0.10
9th	3.50±0.00	3.80±0.00	3.55±0.05	3.50±0.00	3.05±0.55	3.55±0.25
11th	4.35±0.05	4.55±0.05	4.35±0.05	4.35±0.05	3.75±0.75	3.90±0.40
13th	5.15±0.05	5.25±0.05	5.15±0.05	5.00±0.10	4.45±0.75	4.30±0.60
15th	6.30±0.00	6.50±0.00	6.45±0.15	6.20±0.15	5.40±0.90	4.70±0.70
18th	6.30±0.00	6.50±0.00	6.45±0.15	6.20±0.15	5.40±0.90	4.70±0.70

Fusarium oxysporum						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	2.35±0.05	2.55±0.05	2.55±0.05	2.55±0.05	2.65±0.05	2.90±0.10
7th	3.50±0.00	3.80±0.00	3.80±0.10	3.65±0.05	3.85±0.05	3.90±0.10
9th	4.80±0.00	5.15±0.05	5.15±0.15	4.9±0.10	5.10±0.00	5.05±0.15
11th	5.95±0.05	6.35±0.05	6.15±0.05	6.00±0.40	6.25±0.05	5.75±0.05
13th	7.20±0.10	7.50±0.00	7.40±0.10	7.05±0.45	7.45±0.05	6.45±0.05
15th	8.00±0.00	8.50±0.00	8.25±0.25	7.50±0.50	8.50±0.00	8.70±0.10
18th	8.00±0.00	8.50±0.00	8.25±0.25	7.50±0.50	8.50±0.00	8.70±0.10
Botrytis cineria						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	1.00±0.00	1.50±0.00	1.00±0.00	1.70±0.70	1.60±0.10	1.00±0.00
7th	1.50±0.00	2.55±0.05	1.70±0.40	1.95±0.45	2.75±0.25	3.65±0.35
9th	1.85±0.05	3.10±0.20	2.65±0.15	1.95±0.45	3.00±0.20	4.50±0.50
11th	2.45±0.05	3.70±0.40	3.40±0.10	2.35±0.55	3.70±0.10	4.85±0.35
13th	3.10±0.00	4.25±0.65	4.20±0.20	2.70±0.70	4.20±0.20	5.25±0.25
15th	4.85±0.05	5.15±1.55	5.60±0.10	3.50±1.00	5.15±0.15	5.25±0.25
18th	4.85±0.05	5.15±1.55	5.60±0.10	3.50±1.00	5.15±0.15	5.25±0.25
Alternaria alternata						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	2.10±0.00	2.30±0.00	2.25±0.05	1.95±0.05	2.2±0.10	2.05±0.15
7th	2.60±0.10	2.75±0.05	2.75±0.05	2.45±0.05	2.8±0.10	2.55±0.15
9th	3.05±0.15	3.15±0.05	3.35±0.05	2.80±0.20	3.35±0.25	2.85±0.35
11th	3.80±0.10	3.85±0.05	3.85±0.05	3.10±0.40	3.95±0.15	3.25±0.45
13th	4.50±0.20	4.65±0.05	4.65±0.05	3.45±0.65	4.55±0.25	3.65±0.65
15th	5.50±0.00	5.65±0.05	5.55±0.05	4.10±1.10	5.65±0.15	4.35±0.65
18th	5.50±0.00	5.65±0.05	5.55±0.05	4.10±1.10	5.65±0.15	4.35±0.65

4.7.5 *Calicarpa arborea*

The plant extract of *C. arborea* was found to effect growth of phytopathogenic fungi significantly. Plant extract in methanol, chloroform and ethyl acetate was recorded to restrict growth of *P. expansum, F. oxysporum*, and *B. cinerea* up to 50%.

Table 4.21: Effect of *calicarpa arborea* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	1.50±0.30	1.30±0.20	1.55±0.05	1.45±0.25	1.30±0.00	1.15±0.05
7th	2.15±0.15	1.80±0.70	1.70±0.10	2.65±0.15	2.00±0.10	1.95±0.15
9th	2.20±0.20	2.05±0.95	1.80±0.10	2.95±0.25	2.30±0.40	2.25±0.05
11th	2.25±0.25	3.10±2.00	2.15±0.15	3.25±0.35	2.55±0.65	2.45±0.15

13th	2.25±0.25	3.90±1.90	2.15±0.15	3.25±0.35	2.95±1.05	2.50±0.20
15th	2.25±0.25	3.90±1.90	2.15±0.15	3.25±0.35	2.95±1.05	2.50±0.20
18th	2.25±0.25	3.90±1.90	2.15±0.15	3.25±0.35	2.95±1.05	2.50±0.20
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.95±0.35	2.25±0.05	2.05±0.05	1.70±0.00	2.35±0.05	2.45±0.05
7th	3.30±0.50	3.70±0.40	2.05±0.05	3.65±0.35	4.00±0.00	4.10±0.30
9th	3.35±0.45	4.15±0.35	2.05±0.05	3.85±0.45	4.55±0.05	4.30±0.40
11th	3.45±0.45	4.85±0.55	2.10±0.10	4.05±0.55	4.90±0.10	4.45±0.45
13th	3.65±0.35	4.85±0.55	2.15±0.15	4.05±0.55	4.95±0.05	4.50±1.05
15th	3.65±0.35	5.00±0.50	2.15±0.15	4.05±0.55	4.95±0.05	4.55±0.55
18th	3.65±0.35	5.10±0.50	2.15±0.15	4.05±0.55	5.00±0.00	4.65±0.65
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.50±0.00	1.70±0.30	2.10±0.50	1.55±0.35	1.50±0.20	1.15±0.05
7th	2.90±0.10	2.10±0.10	2.40±0.50	2.35±0.75	3.05±0.05	2.70±0.70
9th	3.05±0.15	2.70±0.10	2.75±0.25	2.65±0.75	3.65±0.15	3.45±0.95
11th	3.25±0.15	3.15±0.05	3.30±0.20	2.85±0.85	4.15±0.35	4.05±1.05
13th	3.30±0.10	3.35±0.05	3.30±0.20	2.85±0.85	4.80±0.30	4.55±1.05
15th	3.30±0.10	3.40±0.00	3.30±0.20	2.85±0.85	5.05±0.45	4.60±1.00
18th	3.30±0.10	3.50±0.00	3.30±0.20	2.85±0.85	5.35±0.65	4.65±0.95
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.25±0.15	1.25±0.15	1.35±0.15	1.20±0.10	1.00±0.00	1.10±0.10
7th	2.00±0.20	2.00±0.00	2.40±0.40	2.45±0.05	2.15±0.25	1.95±0.25
9th	2.10±0.20	2.00±0.00	3.00±0.50	3.30±0.50	2.20±0.20	2.35±0.65
11th	2.40±0.30	2.00±0.00	3.90±1.10	4.00±1.00	2.25±0.15	3.00±1.00
13th	2.40±0.30	2.00±0.00	4.30±1.50	4.45±1.35	2.25±0.15	4.10±1.10
15th	2.40±0.30	2.00±0.00	4.30±1.50	4.45±1.35	2.25±0.15	4.10±1.10
18th	2.40±0.30	2.00±0.00	4.30±1.50	4.55±1.35	2.30±0.10	4.10±1.10

The colony growth of *A. alternata* was found affected remarkably at the end of study period and inhibition was between 70 to 73% in case of ethyl acetate, methanol and chloroform extract. Plant extract in other solvent could inhibit growth of fungi in range of 21 to 50%. Inhibitory impact on the colony growth was consistent. Results indicate that effect was till the end of study period and it could be fungitoxic in nature. As such all the solvent extracts had detrimental effect on the colony growth of the phytopathogenic fungi.

Table 4.22: Effect of *Elaeocarpus sphaericus plant* extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.95±0.55	1.70±1.70	3.00±0.10	3.85±0.25	3.00±0.30	2.90±0.30
7th	3.20±0.80	1.70±1.70	3.15±0.15	4.45±0.65	3.15±0.45	2.95±0.35
9th	3.50±1.10	1.70±1.70	3.45±0.05	4.95±1.05	3.20±0.50	3.15±0.55
11th	3.50±1.10	1.70±1.70	3.45±0.05	4.95±1.05	3.20±0.50	3.15±0.55
13th	3.85±1.45	1.70±1.70	3.45±0.05	5.15±1.15	3.25±0.55	3.15±0.55
15th	3.85±1.45	1.70±1.70	3.45±0.05	5.15±1.15	3.25±0.55	3.15±0.55
18th	3.85±1.45	1.70±1.70	3.45±0.05	5.15±1.15	3.25±0.55	3.15±0.55
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.90±1.10	3.35±0.35	2.25±2.25	6.45±0.05	6.65±0.05	6.75±0.15
7th	4.10±1.30	4.90±0.40	2.50±2.50	6.80±0.40	6.80±0.20	8.25±0.25
9th	4.25±1.45	6.35±1.35	2.85±2.35	6.80±0.40	6.85±0.25	9.00±0.00
11th	4.25±1.45	6.50±1.50	2.85±2.35	6.80±0.40	8.05±0.05	9.00±0.00
13th	4.25±1.45	6.75±1.75	3.00±2.50	6.80±0.40	9.00±0.00	9.00±0.00
15th	4.25±1.45	6.75±1.75	3.00±2.50	6.80±0.40	9.00±0.00	9.00±0.00
18th	4.25±1.45	6.75±1.75	3.00±2.50	6.80±0.40	9.00±0.00	9.00±0.00
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.25±0.25	0.00±0.00	0.00±0.00	6.50±0.00	6.45±0.05	6.65±0.15
7th	0.50±0.00	0.00±0.00	0.00±0.00	7.35±0.65	7.40±0.60	7.70±0.30
9th	0.50±0.00	0.00±0.00	0.00±0.00	7.55±0.75	7.65±0.85	8.00±0.00
11th	0.50±0.00	0.00±0.00	0.00±0.00	7.65±0.85	7.65±0.85	8.10±0.10
13th	0.50±0.00	0.25±0.25	0.00±0.00	7.65±0.85	7.85±0.65	8.50±0.00
15th	0.50±0.00	1.00±1.00	0.00±0.00	7.65±0.85	7.85±0.65	8.50±0.00
18th	0.50±0.00	1.00±1.00	0.00±0.00	7.65±0.85	7.85±0.65	8.50±0.00
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.40±0.40	2.65±0.05	0.00±0.00	4.85±0.05	5.45±0.05	4.60±0.60
7th	5.05±0.25	3.55±0.05	2.00±0.00	5.80±0.20	6.25±0.25	5.15±1.15
9th	6.20±0.90	4.45±0.85	3.05±0.05	6.40±0.70	6.75±0.75	5.70±1.60
11th	6.65±1.35	5.25±1.65	3.60±0.00	6.85±1.15	6.90±0.90	6.05±1.95
13th	6.65±1.35	5.55±1.95	3.60±0.00	6.85±1.15	6.90±0.90	6.05±1.95
15th	6.65±1.35	5.55±1.95	3.60±0.00	6.85±1.15	6.90±0.90	6.05±1.95
18th	6.65±1.35	5.55±1.95	3.60±0.00	6.85±1.15	6.90±0.90	6.05±1.95

4.7.6 Elaeocarpus sphaericus

Plant extract of *E. sphaericus* showed variable results. Plant extract prepared using absolute alcohol and methanol was recorded almost 100% inhibitory for the growth of *B. cinerea*. Plant extract of ethyl acetate was also having 90% inhibitory

effect on *B. cinerea.* But extracts in ether, chloroform and benzene were not found restricting remarkably to the growth of *B. cinerea.* Extract prepared in ether, chloroform and benzene were also not having much effect on the growth of *F. oxysporum* and *A. alternata.* Plant extract of ethyl acetate, methanol and absolute alcohol were not having remarkable inhibitory effect on other pathogens also accept in case of *P. expansum* methanol extract was reported to have almost 74% inhibition on the growth.

4.7.7. Eupatorium odoratum

Plant extract of *E. odoratum* in general inhibited growth of all four fungi. In extract of absolute alcohol during initial days growth was not restricted but subsequently, decline in colony diameter of *F. oxysporum* was observed. Maximum inhibitory effect of plant extract was observed on *P.expansum, F. oxysporum* and *B. cinerea* under all the solvent treatment. Comparatively growth of *A. alternata* was not effected much. However, sometimes 50% inhibition in growth was occurred during the study period in different solvent extracts. Plant extract of ethyl acetate was found more effective on *P. expansum* as well as *F. oxysporum* whereas in case of *B. cinerea* extract in ethyl acetate, methanol and ether was found effective. Plant extracts in different solvent did not show any remarkable variation in their effect on *A. alternata.* All solvent extract have drastic effect on the growth of pathogenic fungi quite in similar manner.

4.7.8. Lantana camara

Plant extracts of *L. camara* found to inhibit the growth of four phytopathogenic fungi. In case of P. *expansum* and *A. alternata* growth of fungus colony was inhibited partially. *L. camara* extract does not inhibit cent percent growth of pathogen under any solvent. However, growth of pathogen treated with extracts always remains lesser than the control. The colony growth of *F. oxysporum* also inhibited at initial stage of incubation. But on 9th and 11th day increase in colony diameter was recorded and in case of absolute alcohol and chloroform extract colony growth was found almost similar as control. However, during subsequent period of incubation growth of pathogen was lesser than the control. In case of *B. cinerea* also inhibition in growth of fungus was not significant. Maximum reduction (27%) in growth was found under ether extract

treatment. Extracts in benzene and absolute alcohol did not show any inhibitory effect. But fungi treated with chloroform, methanol and ethyl acetate showed partial inhibition in the colony growth.

Table 4.23: Effect of *Eupatorium odoratum* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.90±0.00	1.00±0.10	1.45±0.25	1.00±0.00	1.05±0.05	1.20±0.10
7th	1.40±0.10	1.10±0.05	1.90±0.00	1.45±0.05	1.50±0.10	2.15±0.35
9th	1.50±0.00	1.60±0.20	1.95±0.05	1.90±0.10	1.85±0.35	2.15±0.35
11th	1.50±0.00	1.80±0.00	1.95±0.05	1.95±0.15	1.85±0.35	2.25±0.35
13th	1.50±0.00	1.80±0.00	1.95±0.05	1.95±0.15	1.85±0.35	2.25±0.35
15th	1.50±0.00	1.80±0.00	1.95±0.05	1.95±0.15	1.85±0.35	2.30±0.30
18th	1.50±0.00	1.80±0.00	1.95±0.05	1.95±0.15	1.85±0.35	2.30±0.30
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.55±0.05	1.70±0.30	3.35±0.25	2.25±0.05	2.1±0.00	3.25±0.15
7th	1.65±0.05	1.70±0.30	4.25±0.25	2.25±0.30	3.00±0.60	3.90±0.40
9th	1.70±0.00	1.75±0.35	4.40±0.20	2.65±0.35	3.30±0.30	4.00±0.40
11th	1.85±0.15	1.75±0.35	4.45±0.25	2.65±0.35	3.30±0.30	4.00±0.40
13th	1.85±0.15	2.20±0.80	4.45±0.25	2.65±0.35	3.35±0.35	4.00±0.40
15th	1.85±0.15	2.20±0.80	4.45±0.25	2.65±0.35	3.35±0.35	4.00±0.40
18th	1.85±0.15	2.20±0.80	4.45±0.25	2.65±0.35	3.35±0.35	4.00±0.40
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.15±0.05	0.85±0.15	2.40±0.50	1.15±0.05	1.30±0.10	2.00±0.00
7th	1.15±0.10	2.05±0.45	3.40±0.70	1.45±0.05	1.85±0.15	2.70±0.30
9th	1.85±0.05	3.00±0.20	3.50±0.70	1.90±0.10	2.30±0.10	3.15±0.35
11th	1.90±0.10	3.05±0.25	3.55±0.75	1.95±0.15	2.35±0.05	3.20±0.40
13th	2.00±0.20	3.05±0.25	3.65±0.65	1.95±0.15	2.35±0.05	3.20±0.40
15th	2.00±0.20	3.05±0.25	5.00±1.00	1.95±0.15	2.35±0.05	3.20±0.40
18th	2.00±0.20	3.05±0.25	5.00±1.00	1.95±0.15	2.35±0.05	3.20±0.40
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.80±0.00	1.30±0.10	1.90±0.10	1.55±0.05	1.20±0.20	1.40±0.00
7th	2.20±0.00	2.10±0.10	2.25±0.05	2.3.±0.00	2.10±0.20	2.35±0.05
9th	2.85±0.05	2.6±0.10	2.75±0.05	2.85±0.05	2.65±0.15	2.85±0.05
11th	3.15±0.05	2.95±0.05	2.95±0.05	3.10±0.10	3.10±0.10	3.15±0.05
13th	3.35±0.05	3.35±0.15	3.40±0.00	3.40±0.10	3.60±0.10	3.40±0.00
15th	3.65±0.05	3.70±0.00	3.85±0.05	3.95±0.15	4.00±0.20	3.70±0.10
18th	4.55±0.05	3.90±0.20	4.50±0.00	4.65±0.15	4.45±0.15	3.90±0.10

Table 4.24: Effect of *Lantana camara* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.30±0.02	2.00±0.00	2.10±0.10	2.20±0.10	2.15±0.05	2.60±0.20
7th	3.00±0.20	3.20±0.40	2.65±0.05	2.55±0.05	2.55±0.05	3.30±0.10
9th	3.30±0.20	4.80±1.10	2.80±0.10	4.75±0.95	2.85±0.05	3.80±0.10
11th	4.35±0.35	4.95±0.95	3.20±0.20	5.00±0.70	3.50±0.20	4.90±0.40
13th	4.40±0.30	5.35±0.65	4.40±0.40	5.25±0.45	3.85±0.15	5.20±0.20
15th	5.00±0.10	5.35±0.65	5.80±0.30	5.25±0.45	4.90±0.50	5.35±0.05
18th	5.60±0.20	5.35±0.65	5.80±0.30	5.25±0.45	4.90±0.50	5.35±0.05
Botrystis cinerea						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.80±0.10	3.25±0.15	3.20±0.00	3.50±0.10	2.85±0.25	3.80±0.20
7th	4.35±0.05	4.80±0.70	4.40±0.20	4.20±0.00	3.85±0.05	5.00±0.50
9th	4.60±0.20	4.80±0.20	6.00±0.00	4.60±0.30	5.10±0.30	5.65±0.65
11th	4.70±0.30	4.80±0.20	6.85±0.15	5.00±0.70	6.25±0.25	7.00±0.00
13th	5.25±0.45	4.95±0.15	7.35±0.45	5.25±0.65	6.35±0.15	7.10±0.10
15th	5.25±0.45	4.95±0.15	7.35±0.45	5.25±0.65	6.35±0.15	7.10±0.10
18th	5.25±0.45	4.95±0.15	7.35±0.45	5.25±0.65	6.35±0.15	7.10±0.10
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.40±0.00	2.05±0.05	2.45±0.05	2.30±0.20	2.95±0.05	3.30±0.10
7th	2.60±1.20	3.15±0.05	3.60±1.00	3.55±0.05	4.90±0.10	4.90±0.10
9th	4.95±0.10	5.35±0.15	5.80±0.10	4.75±0.25	5.95±0.05	6.10±0.20
11th	5.65±0.50	6.05±0.55	5.95±0.05	5.85±0.65	7.05±0.05	6.55±0.65
13th	5.90±0.10	6.05±0.55	60±0.10	6.15±0.45	7.50±0.30	6.75±0.55
15th	5.90±0.10	6.05±0.55	60±0.10	6.15±0.45	7.50±0.30	6.75±0.55
18th	5.90±0.10	6.05±0.55	60±0.10	6.15±0.45	7.50±0.30	6.75±0.55
Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.10±0.30	1.25±0.15	1.75±0.05	1.50±0.10	1.30±0.10	1.55±0.05
7th	3.10±0.30	2.15±0.15	2.35±0.35	2.20±0.00	2.15±0.05	2.65±0.05
9th	3.20±0.40	2.15±0.15	2.65±0.35	2.55±0.05	2.55±0.05	3.10±0.30
11th	4.25±0.45	2.25±0.25	2.65±0.35	2.75±0.15	2.55±0.05	3.20±0.20
13th	4.30±0.50	2.30±0.30	2.80±0.50	2.85±0.05	2.85±0.15	3.25±0.15
15th	4.30±0.50	2.30±0.30	2.80±0.50	2.85±0.05	2.85±0.15	3.25±0.15
18th	4.30±0.50	2.30±0.30	2.80±0.50	2.85±0.05	2.85±0.15	3.25±0.15

4.7.9. *Litsea cubeba*

Plant extract of *L. cubeba* was recorded inhibitory for the growth of all four fungi. Extract prepared in ethyl acetate, chloroform and benzene were noticed to restrict 100% growth of all four tested fungi. Plant extract using ether as solvent

was also severely inhibitory for the growth of all the four fungi. Plant extract of absolute alcohol and methanol did not show any remarkable effect on the growth of phytopathogenic fungi however growth always remain poor than the control one.

Table 4.25: Effect of *Litsea cubeba* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	2.60±0.20	2.70±0.10	0.50±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	3.05±0.05	3.00±0.30	0.50±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	3.05±0.05	3.00±0.30	0.50±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	3.05±0.05	3.05±0.35	0.50±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	3.05±0.05	3.05±0.35	0.75±0.25	0.00±0.00	0.00±0.00
15th	0.00±0.00	3.05±0.05	3.05±0.35	0.75±0.25	0.00±0.00	0.00±0.00
18th	0.00±0.00	3.05±0.05	3.05±0.35	0.90±0.10	0.00±0.00	0.00±0.00
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	4.60±0.10	5.10±0.20	0.50±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	5.00±0.20	5.80±0.20	0.50±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	5.00±0.20	5.80±0.20	0.50±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	5.10±0.10	5.90±0.20	0.50±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	5.15±0.15	5.90±0.20	0.50±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	5.15±0.15	5.95±0.25	0.50±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	5.15±0.15	6.00±0.30	0.50±0.00	0.00±0.00	0.00±0.00
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	1.80±0.10	1.80±0.10	0.50±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	3.50±0.10	2.30±0.60	0.50±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	3.50±0.10	3.00±0.30	0.50±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	3.65±0.15	3.10±0.40	0.50±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	3.70±0.10	3.10±0.40	0.50±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	3.80±0.20	3.15±0.35	0.50±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	3.90±0.30	3.15±0.35	0.50±0.00	0.00±0.00	0.00±0.00
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	2.85±0.25	2.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	2.85±0.25	3.10±0.00	1.00±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	3.65±1.05	3.30±0.00	1.40±0.00	0.00±0.00	0.00±0.00
11th	0.00±0.00	3.65±1.05	3.30±0.00	1.40±0.00	0.00±0.00	0.00±0.00
13th	0.00±0.00	3.65±1.05	3.30±0.00	1.40±0.00	0.00±0.00	0.00±0.00
15th	0.00±0.00	3.65±1.05	3.30±0.00	1.40±0.00	0.00±0.00	0.00±0.00
18th	0.00±0.00	3.65±1.05	3.30±0.00	1.40±0.00	0.00±0.00	0.00±0.00

Table 4.26: Effect of *Michelia champaca* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	2.05±0.05	1.50±0.50	1.85±0.15	2.00±0.00	2.05±0.05	2.05±0.05
7th	2.65±0.15	2.25±0.55	2.50±0.30	2.80±0.00	2.90±0.00	2.90±0.00
9th	2.75±0.25	2.45±0.75	3.35±0.35	2.95±0.15	3.20±0.20	3.70±0.00
11th	2.75±0.25	2.45±0.75	3.65±0.35	2.95±0.15	3.25±0.25	3.85±0.15
13th	2.75±0.25	2.45±0.75	4.15±0.35	2.95±0.15	3.30±0.30	3.95±0.25
15th	2.80±0.30	2.45±0.75	4.65±0.65	2.95±0.15	3.30±0.30	4.05±0.25
18th	2.80±0.30	2.45±0.75	5.15±1.15	2.95±0.15	3.50±0.50	4.20±0.40
Fusarium oxysporum						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	2.15±0.05	2.45±0.05	2.05±0.05	2.10±0.10	2.35±0.05	2.10±0.10
7th	3.80±0.00	4.20±0.00	3.65±0.05	3.85±0.25	4.20±0.10	3.85±0.25
9th	4.35±0.45	4.90±0.50	3.65±0.05	4.45±0.85	5.20±0.10	4.45±0.75
11th	4.40±0.50	5.20±0.80	3.65±0.05	4.80±1.20	5.55±0.45	4.60±0.90
13th	4.50±0.60	5.70±1.30	3.70±0.00	5.20±1.50	6.05±0.85	4.70±1.00
15th	4.60±0.60	6.20±1.80	3.75±0.05	5.60±1.90	6.60±1.40	4.80±1.10
18th	4.60±0.60	6.45±2.05	3.75±0.05	5.85±2.15	6.85±1.65	4.80±1.10
Botrytis cineria						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	1.25±0.25	1.45±0.15	1.05±0.05	1.05±0.05	1.00±0.00	0.95±0.05
7th	1.95±0.35	2.25±0.25	2.15±0.05	1.60±0.10	1.40±0.10	1.50±0.00
9th	2.55±0.55	2.80±0.20	2.50±0.00	1.85±0.35	1.45±0.15	2.00±0.00
11th	2.60±0.50	2.85±0.15	2.50±0.00	2.05±0.25	1.55±0.25	2.00±0.00
13th	2.75±0.55	2.95±0.15	2.50±0.00	2.20±0.20	1.65±0.25	2.00±0.00
15th	2.85±0.55	3.05±0.15	2.50±0.00	2.35±0.15	1.75±0.25	2.00±0.00
18th	2.95±0.45	3.10±0.10	2.50±0.00	2.35±0.15	1.90±0.40	2.35±0.05
Alternaria alternata						
Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
5th	2.05±0.05	1.90±0.10	1.75±0.15	1.55±0.05	1.90±0.10	1.35±0.25
7th	3.00±0.00	2.70±0.20	2.50±0.40	2.60±0.00	2.75±0.25	2.50±0.30
9th	3.35±0.15	3.05±0.45	2.85±0.15	3.05±0.05	2.80±0.20	2.70±0.50
11th	3.35±0.15	3.35±0.45	2.95±0.05	3.30±0.30	2.80±0.20	3.00±0.80
13th	3.35±0.15	3.60±0.20	3.00±0.00	3.60±0.60	2.85±0.15	3.25±1.05
15th	3.35±0.15	3.65±0.15	3.05±0.05	3.90±0.90	2.85±0.15	3.50±1.30
18th	3.35±0.15	4.05±0.25	3.10±0.10	4.20±1.20	2.85±0.15	3.85±1.65

4.7.10. *Michelia champaca*

Plant extract of *M. champaca* was not found to inhibit the growth of tested fungus almost 100% at any point of study. However, it was noticed that in extracts with some solvents growth reduced and inhibition percent was almost 75%. A remarkable observation was made in the growth of *P. expansum* with plant extract under all solvent that at initial stage inhibition was not severe but with passage of time reduction in growth was found in increasing order. Thus, inhibition effect was in ascending pattern whereas, growth of *B. cinerea* was reduced severely at initial stage but during subsequent period of growth effective was found decreasing and pattern of inhibition was in descending order. Growth of *A. alternata* and *F. oxysporum* was also inhibited and among the extracts prepared with solvents ethyl acetate, absolute alcohol and benzene were found more effective.

4.7.11. *Mikania cordata*

Plant extract of *M. cordata* was also found to restrict the colony growth of phytopathogenic fungi under all the solvents. The growth of *P. expansum* was severely affected by the plant extract prepared with methanol, absolute alcohol and chloroform. Plant extract prepared using methanol, ether, chloroform and benzene were recorded inhibitory for *F. oxysporum*. While in case of *B. cinerea* ether, chloroform and benzene extract restricted the growth of *B. cinerea* 56 to 63%. The growth of *A. alternata* was effected by all the solvent extracts under all the treatments and growth was effected more than 50%.

4.7.12. *Piper mullesua*

Results indicate that extract of *P. mullesua* remarkably restricted the colonial growth of tested fungi. Inhibitory effect was more significant in case of *B. cinerea* where chloroform extract inhibited 100% growth while extracts of ethyl acetate, methanol and ether do not allow any growth during initial period. After 9th day of incubation ethyl acetate extract show more than 90% inhibition in growth. In case of methanol and ether extract treated fungi inhibition ranges between 65 and 90 percent. In case of *P.expansum*, *F. oxysporum* and *A. alternata* growth of fungal colony was found inhibited and it ranges from 42 to 70% in *P. expansum*, 28 to 73 in *F. oxysporum* and 39 to 85% in *A. alternata*. Under all the solvents growth remain lesser than the control.

Table 4.27: Effect of *Mikania cordata* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Days	Ethyl acetate	Methanol	Alcohol	Ether	Chloroform	Benzene
Penicillium *expansum*						
5th	2.35±0.05	1.85±0.35	1.50±0.00	1.95±0.35	1.40±0.00	2.40±0.10
7th	3.20±0.00	2.45±0.05	2.50±0.10	3.05±0.25	2.55±0.05	3.15±0.15
9th	3.25±0.05	2.45±0.05	2.50±0.10	3.05±0.25	2.75±0.15	3.20±0.20
11th	3.25±0.05	2.45±0.05	2.50±0.10	3.15±0.15	2.75±0.15	3.20±0.20
13th	3.25±0.05	2.45±0.05	2.50±0.10	3.15±0.15	2.75±0.15	3.20±0.20
15th	3.25±0.05	2.45±0.05	2.50±0.10	3.15±0.15	2.75±0.15	3.20±0.20
18th	3.25±0.05	2.45±0.05	2.50±0.10	3.15±0.15	2.75±0.15	3.20±0.20
***Fusarium* oxysporum**						
5th	2.70±0.00	2.45±0.05	2.45±0.05	2.90±0.10	2.55±0.05	2.85±0.05
7th	3.05±0.05	3.25±0.45	3.55±0.35	3.60±0.50	3.75±0.25	3.70±0.20
9th	3.05±0.05	3.25±0.45	4.20±1.00	3.60±0.50	3.80±0.30	3.75±0.25
11th	3.05±0.05	3.25±0.45	4.70±1.50	3.60±0.50	3.80±0.30	3.75±0.25
13th	3.05±0.05	3.25±0.45	4.75±1.55	3.60±0.50	3.80±0.30	3.75±0.25
15th	3.05±0.05	3.25±0.45	4.85±1.65	3.60±0.50	3.80±0.30	3.75±0.25
18th	3.05±0.05	3.25±0.45	4.85±1.65	3.60±0.50	3.80±0.30	3.75±0.25
Botrytis cineria						
5th	2.10±0.20	1.70±0.00	1.20±0.00	1.60±0.10	1.75±0.05	1.90±0.10
7th	3.20±0.30	3.25±0.05	2.40±0.10	2.40±0.10	2.35±0.05	2.85±0.05
9th	3.60±0.70	3.95±0.15	3.30±0.20	2.60±0.20	2.65±0.35	3.10±0.30
11th	3.60±0.70	3.95±0.15	3.70±0.30	2.65±0.15	2.65±0.35	3.20±0.40
13th	3.60±0.70	3.95±0.15	3.70±0.30	2.65±0.15	2.65±0.35	3.20±0.40
15th	3.60±0.70	3.95±0.15	3.70±0.30	2.65±0.15	2.65±0.35	3.20±0.40
18th	3.60±0.70	3.95±0.15	3.70±0.30	2.65±0.15	2.65±0.35	3.20±0.40
Alternaria alternata						
5th	2.20±0.10	1.90±0.10	1.60±0.10	1.95±0.05	1.60±0.10	2.00±0.10
7th	2.70±0.20	2.40±0.10	2.00±0.10	2.40±0.10	2.00±0.10	2.85±0.05
9th	3.20±0.10	2.85±0.05	2.50±0.10	2.60±0.10	2.55±0.05	3.20±0.10
11th	3.25±0.05	2.85±0.05	2.75±0.05	2.95±0.05	2.55±0.05	3.20±0.10
13th	3.25±0.05	2.85±0.05	2.75±0.05	2.95±0.05	2.75±0.05	3.45±0.05
15th	3.25±0.05	2.85±0.05	2.75±0.05	3.05±0.05	3.05±0.05	3.45±0.05
18th	3.25±0.05	2.85±0.05	2.75±0.05	3.05±0.05	3.05±0.05	3.45±0.05

Table 4.28: Effect of *Piper mullesua* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.95±0.05	0.90±0.10	1.20±0.30	1.00±0.00	0.75±0.05	0.90±0.00
7th	1.45±0.15	1.25±0.15	1.55±0.05	1.50±0.50	1.15±0.05	1.40±0.10
9th	2.00±0.40	1.50±0.40	2.15±0.05	1.60±0.50	1.40±0.30	1.50±0.00
11th	2.50±0.80	2.10±0.90	2.40±0.00	1.60±0.50	1.90±0.80	2.00±0.10
13th	2.80±1.10	2.35±1.15	2.45±0.05	1.80±0.60	2.20±1.10	2.10±0.10
15th	2.80±1.10	2.35±1.15	2.55±0.05	1.80±0.60	2.25±1.05	2.50±0.10
18th	2.80±1.10	2.35±1.15	2.70±0.10	1.85±0.65	2.65±1.45	2.50±0.10
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.80±0.00	1.55±0.05	1.85±0.15	1.95±0.05	1.95±0.05	2.30±0.00
7th	3.35±0.15	3.15±0.05	3.75±0.05	3.45±0.35	2.35±0.45	3.60±0.10
9th	3.50±0.10	3.95±0.55	4.75±0.05	3.55±0.25	2.35±0.55	3.70±0.00
11th	3.55±0.15	4.10±0.50	4.95±0.05	4.55±1.25	2.35±0.55	4.00±0.00
13th	3.60±0.20	4.25±0.55	5.15±0.25	5.00±1.00	2.35±0.55	4.00±0.00
15th	3.65±0.15	4.25±0.55	5.20±0.30	5.00±1.00	2.35±0.55	4.00±0.00
18th	3.75±0.25	4.25±0.55	5.20±0.30	5.00±1.00	2.35±0.55	4.00±0.00
Botrytis cinerea						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.90±0.30	0.00±0.30	0.00±0.00	0.70±0.00
7th	0.00±0.00	0.00±0.00	1.50±0.70	0.30±0.30	0.00±0.00	1.20±0.10
9th	0.25±0.25	0.00±0.00	2.65±1.15	0.70±0.00	0.00±0.00	1.50±0.10
11th	0.35±0.35	0.50±0.00	3.80±1.10	1.45±0.35	0.00±0.00	1.60±0.10
13th	0.40±0.40	0.70±0.00	4.25±0.75	2.20±0.80	0.00±0.00	2.00±0.00
15th	0.50±0.50	0.85±0.15	4.25±0.75	2.40±0.90	0.00±0.00	2.00±0.00
18th	0.60±0.60	0.95±0.25	4.25±0.75	2.50±1.00	0.30±0.00	2.00±0.00
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.60±0.10	1.55±0.45	1.25±0.05	0.75±0.25	1.00±0.00	1.25±0.05
7th	0.60±0.10	1.85±0.15	1.35±0.05	0.75±0.25	1.00±0.00	1.25±0.05
9th	1.30±0.10	2.05±0.05	1.75±0.05	1.25±0.25	2.00±0.00	2.00±0.00
11th	2.00±0.00	2.35±0.35	2.00±0.00	1.75±0.25	3.00±0.00	2.80±0.10
13th	3.00±0.00	2.60±0.60	2.25±0.05	2.20±0.20	4.40±0.40	4.00±0.20
15th	3.35±0.05	2.75±0.75	2.30±0.10	2.20±0.20	4.40±0.40	4.60±0.20
18th	3.35±0.05	2.75±0.75	2.30±0.10	2.20±0.20	4.40±0.40	4.60±0.20

4.7.13. *Polygonum hydropiper*

Plant extract of *P. hydropiper* recorded to have significant inhibitory effect on colonial growth of all the tested fungus. Plant extract with chloroform was found to reduced 100% growth of *P. expansum* and *A. alternata*, the reduction of growth of *F. oxysporum* and *B. cinerea* was also 100% at initial stage but at the end of study it was found 87 and 92% respectively. Plant extract prepared with ethyl acetate was also noticed to inhibit growth of *P. expansum* and *A. alternata* 100% till 13th day of incubation but after that slight growth was occurred.

Table 4.29: Effect of *Polygonum hydropiper* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	1.10±0.00	0.90±0.10	0.00±0.00	0.00±0.00	0.60±0.10
7th	0.00±0.00	1.50±0.30	0.90±0.10	0.40±0.40	0.00±0.00	0.60±0.10
9th	0.00±0.00	2.05±0.75	0.90±0.10	0.50±0.50	0.00±0.00	0.60±0.10
11th	0.00±0.00	2.05±0.75	0.90±0.10	0.50±0.50	0.00±0.00	0.60±0.10
13th	0.50±0.00	2.10±0.80	0.90±0.10	0.50±0.50	0.00±0.00	0.60±0.10
15th	0.50±0.00	2.10±0.80	0.95±0.05	0.50±0.50	0.00±0.00	0.60±0.10
18th	0.50±0.00	2.20±0.70	1.10±0.10	0.50±0.50	0.00±0.00	0.65±0.15
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.25±0.25	0.60±0.10	1.10±0.00	0.75±0.05	0.00±0.00	1.00±0.00
7th	0.50±0.00	1.50±0.20	1.70±0.00	1.45±0.15	0.25±0.25	1.30±0.10
9th	0.95±0.05	2.40±0.10	2.80±0.00	3.00±0.00	0.70±0.20	1.80±0.20
11th	1.30±0.10	2.75±0.05	2.90±0.00	3.20±0.20	0.75±0.25	1.85±0.15
13th	1.75±0.05	3.00±0.10	3.00±0.00	3.45±0.45	0.75±0.25	1.90±0.10
15th	1.75±0.05	3.00±0.10	3.00±0.00	3.60±0.30	0.75±0.25	1.90±0.10
18th	1.75±0.05	3.00±0.10	3.00±0.00	3.60±0.30	1.10±0.60	1.90±0.10
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.90±0.00	1.15±0.25	0.75±0.05	0.00±0.00	0.00±0.00
7th	0.25±0.25	1.40±0.00	1.55±0.45	1.05±0.25	0.25±0.25	0.85±0.15
9th	0.75±0.25	1.95±0.05	1.65±0.35	1.35±0.25	0.25±0.25	1.40±0.40
11th	0.75±0.25	2.25±0.15	1.85±0.55	1.50±0.20	0.25±0.25	1.45±0.45
13th	0.75±0.25	2.55±0.25	2.05±0.75	1.65±0.15	0.25±0.25	1.50±0.50
15th	0.90±0.10	2.55±0.25	2.15±0.65	2.15±0.35	0.25±0.25	1.50±0.50
18th	0.90±0.10	2.65±0.35	2.15±0.65	2.15±0.35	0.50±0.00	1.50±0.50
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.75±0.05	0.85±0.15	0.80±0.00	0.00±0.00	0.00±0.00

7th	0.00±0.00	1.05±0.05	1.05±0.05	1.30±0.10	0.00±0.00	0.25±0.25
9th	0.00±0.00	1.30±0.00	1.50±0.00	1.85±0.05	0.00±0.00	0.60±0.60
11th	0.00±0.00	1.85±0.05	2.00±0.00	2.40±0.10	0.00±0.00	0.75±0.75
13th	0.00±0.00	2.55±0.05	2.50±0.00	2.90±0.20	0.00±0.00	0.95±0.95
15th	0.50±0.00	2.55±0.05	2.65±0.15	3.05±0.35	0.00±0.00	1.15±1.15
18th	0.50±0.00	2.60±0.10	3.40±0.90	3.45±0.75	0.00±0.00	1.15±1.15

The plant extract prepared with other solvent were also found effective in reducing the colony growth as compared to control. At any stage of study inhibition in growth of tested fungus was more than 50% in the extract prepared with methanol, absolute alcohol, ether and benzene.

Table 4.30: Effect of *samanea saman* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium *expansum*						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.25±0.05	0.00±0.00	0.00±0.00	1.25±0.05	1.35±0.15	1.45±0.15
7th	1.35±0.15	0.55±0.05	0.85±0.25	1.80±0.10	1.80±0.10	2.15±0.05
9th	1.40±0.20	0.95±0.05	1.05±0.45	1.85±0.05	1.80±0.10	2.15±0.05
11th	1.45±0.15	1.00±0.10	1.10±0.50	1.90±0.10	1.80±0.10	2.15±0.05
13th	1.55±0.05	1.00±0.10	1.10±0.50	1.95±0.05	1.85±0.15	2.20±0.10
15th	1.55±0.05	1.00±0.10	1.10±0.50	1.95±0.05	1.85±0.15	2.20±0.10
18th	1.55±0.05	1.00±0.10	1.10±0.50	1.95±0.05	1.85±0.15	2.20±0.10
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.15±0.05	0.00±0.00	0.00±0.00	1.60±0.30	2.40±0.10	2.65±0.15
7th	3.35±0.25	0.00±0.00	0.00±0.00	3.00±0.10	3.85±0.35	3.65±0.15
9th	3.35±0.25	0.00±0.00	0.00±0.00	3.10±0.20	4.00±0.30	3.70±0.10
11th	3.35±0.25	0.25±0.25	0.00±0.00	3.10±0.20	4.00±0.30	3.70±0.10
13th	3.35±0.25	0.50±0.50	0.00±0.00	3.10±0.20	4.10±0.40	3.70±0.10
15th	3.35±0.25	0.70±0.70	0.00±0.00	3.10±0.20	4.10±0.40	3.80±0.20
18th	3.35±0.25	0.75±0.75	0.00±0.00	3.10±0.20	4.10±0.40	3.80±0.20
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.50±0.10	0.00±0.00	0.00±0.00	2.95±0.05	2.85±0.05	3.75±0.05
7th	4.90±0.30	0.00±0.00	0.00±0.00	3.60±0.40	3.35±0.05	4.70±0.10
9th	5.50±0.50	0.00±0.00	0.00±0.00	3.75±0.55	3.35±0.05	4.70±0.10
11th	5.50±0.50	0.00±0.00	0.00±0.00	4.20±0.10	3.35±0.05	4.70±0.10
13th	5.65±0.35	0.00±0.00	0.00±0.00	4.20±0.10	3.40±0.00	4.70±0.05
15th	5.65±0.35	0.00±0.00	0.00±0.00	4.20±0.10	3.40±0.00	4.70±0.05
18th	5.65±0.35	0.00±0.00	0.00±0.00	4.20±0.10	3.40±0.00	4.70±0.05

Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.65±0.15	0.00±0.00	0.00±0.00	1.50±0.00	2.50±0.10	2.60±0.10
7th	2.90±0.10	0.00±0.00	0.00±0.00	2.00±0.10	3.55±0.05	3.65±0.05
9th	3.05±0.05	0.00±0.00	0.00±0.00	2.55±0.05	4.05±0.05	3.80±0.10
11th	3.15±0.15	0.00±0.00	1.00±0.00	3.00±0.10	4.05±0.05	4.25±0.05
13th	3.25±0.25	0.50±0.00	1.50±0.00	3.05±0.05	4.25±0.05	4.55±0.05
15th	3.25±0.25	0.95±0.05	1.90±0.10	3.05±0.05	4.25±0.05	4.60±0.00
18th	3.25±0.25	0.95±0.05	2.00±0.00	3.05±0.05	4.25±0.05	4.65±0.05

4.7.14. Samanea saman

In general plant extract of *S. saman* was found inhibitory for growth of all the fungi in all the solvent extracts. The solvent extract of absolute alcohol inhibited 100% growth of *F. oxysporum* and *B. cinerea* during the study period whereas in case of *A. alternata* 100% inhibition in growth was till 9th days but after that slight growth was observed. Plant extract in Methanol was also found to inhibit 100% growth of *B. cinerea* but in case of *F. oxysporum, A. alternata* and *P. expansum* 100% effect was observed for a shorter period. In subsequent period a slight growth of colony was observed. Plant extract in other solvent was also found effective against the growth of fungi.

4.7.15. *Solanum spirale*

S. spirale is one of the endemic plants, the extract of this plant was also not found to inhibit the growth of tested fungus significantly. The growth of *P. expansum* and *F. oxysporum* in extract prepared with solvent ethyl acetate, ether, chloroform and absolute alcohol was almost equal to control one. Thus, the inhibition in growth of fungi was negligible. The growth of *B. cinerea* and *A. alternata* was effected to some extent and inhibition in growth ranges from 15 to 86%. Among the different solvent used for extraction of plants chloroform was found more inhibitory for the growth of *B. cinerea* as well as *A. alternata*. The percent inhibition in growth of *B. cinerea* and *A. alternata* was more at initial stage which was found subsequently less in reducing the colonial growth.

4.7.16. Tagetes errecta

In general plant extract of *T. errecta* was recorded to restrict the colony growth of all the tested fungi. Among all the tested fungi *B. cinerea* was found to

inhibited significantly by extract of all solvents. During the initial stage of incubation up to 9th to 11th days there was 100% inhibition but in subsequent period colony diameter expanded. However, inhibitory effect was 66 to 93% in extract of different solvents. Growth of other tested fungus was also effected by plant extracts in different solvents and in most cases inhibition was 40 to 50%. In case of *P. expansum* and *F. oxysporum* at the end of study inhibition was recorded between 44 to 64%.

Table 4.31: Effect of *Solanum spirale* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.10±0.10	1.85±0.05	1.6±0.10	2.15±0.05	2.15±0.15	1.75±0.25
7th	3.30±0.10	2.40±0.10	2.45±0.15	3.30±0.10	2.80±0.10	2.55±0.55
9th	3.90±0.10	2.70±0.20	2.80±0.30	3.95±0.05	3.40±0.10	3.15±0.65
11th	4.65±0.15	2.95±0.45	3.35±0.45	4.60±0.30	4.05±0.15	3.65±0.85
13th	5.50±0.10	3.10±0.50	3.70±0.60	5.60±0.20	4.80±0.20	4.00±1.00
15th	5.90±0.20	3.40±0.40	4.05±0.95	6.35±0.15	5.65±0.35	4.65±0.65
18th	6.15±0.45	3.55±0.55	4.55±1.45	7.15±0.15	6.15±0.25	4.65±0.65
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.05±0.05	2.65±0.45	3.15±0.15	2.95±0.05	3.20±0.20	3.45±0.05
7th	4.55±0.05	3.90±0.40	4.20±0.10	4.45±0.05	4.40±0.30	4.55±0.05
9th	5.65±0.05	4.35±0.05	5.20±0.10	5.60±0.10	5.45±0.15	5.80±0.10
11th	6.95±0.05	4.85±0.05	6.40±0.40	6.90±0.10	6.95±0.05	6.95±0.05
13th	8.25±0.05	5.50±0.10	8.75±0.05	8.10±0.10	8.25±0.25	8.55±0.05
15th	8.65±0.05	6.50±0.50	9.00±0.00	8.60±0.10	8.80±0.20	9.00±0.00
18th	8.65±0.05	6.50±0.50	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.05±0.05	1.30±0.60	0.90±0.10	2.05±0.05	0.60±0.00	2.05±0.05
7th	3.00±0.00	1.65±0.95	0.90±0.10	3.50±0.10	0.60±0.00	3.10±0.10
9th	3.75±0.25	1.65±0.95	0.90±0.10	4.05±0.05	1.80±1.20	3.50±0.20
11th	4.25±0.25	1.95±0.85	1.15±0.05	4.65±0.15	2.15±1.35	3.80±0.10
13th	4.85±0.15	2.20±0.70	1.30±0.20	5.15±0.15	2.50±1.50	4.25±0.25
15th	5.20±0.30	3.00±0.50	1.95±0.45	5.60±0.40	2.90±1.70	4.50±0.50
18th	5.25±0.25	3.50±0.50	2.90±0.40	6.10±0.90	4.20±2.20	4.60±0.60
Alternaria alternate						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.05±0.05	1.10±0.10	0.85±0.05	2.05±0.05	0.70±0.00	2.15±0.05
7th	3.15±0.05	1.10±0.10	0.85±0.05	3.00±0.00	0.70±0.00	2.90±0.50

9th	3.65±0.35	1.35±0.15	1.45±0.05	3.55±0.05	1.35±0.65	3.60±0.40
11th	4.00±0.50	1.95±0.05	2.35±0.05	4.10±0.10	2.20±1.30	4.25±0.25
13th	4.45±0.85	2.75±0.25	3.05±0.25	4.85±0.05	3.00±2.00	4.75±0.25
15th	5.00±1.20	3.00±0.00	3.50±0.00	5.40±0.10	4.15±2.85	5.25±0.25
18th	5.10±1.30	4.10±0.10	4.40±0.30	5.95±0.15	4.85±2.85	5.25±0.25

Table 4.32: Effect of *Tagetes erectus* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.40±0.10	1.45±0.05	1.50±0.20	1.90±0.60	1.40±0.00	1.25±0.05
7th	2.30±0.00	2.35±0.25	2.25±0.25	2.45±0.35	2.60±0.20	2.45±0.15
9th	2.30±0.00	2.45±0.35	2.40±0.30	2.45±0.35	2.90±0.50	2.75±0.15
11th	2.30±0.00	2.65±0.45	2.55±0.35	2.45±0.35	3.25±0.85	3.15±0.15
13th	2.30±0.00	2.90±0.70	2.55±0.35	2.45±0.35	3.40±1.00	3.50±0.50
15th	2.30±0.00	2.95±0.75	2.55±0.35	2.45±0.35	3.80±0.80	3.70±0.70
18th	2.30±0.00	3.00±0.80	2.55±0.35	2.45±0.35	4.50±0.40	4.00±1.00
Fusarium oxysporum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.50±0.00	2.65±0.15	2.40±0.00	2.8±0.10	2.95±0.05	2.75±0.05
7th	3.45±0.15	3.90±0.60	3.25±0.05	3.75±0.25	3.55±0.05	3.75±0.05
9th	3.45±0.15	4.65±1.35	3.25±0.05	3.85±0.15	3.55±0.05	4.05±0.05
11th	3.45±0.15	5.70±2.40	3.25±0.05	3.95±0.05	3.55±0.05	4.30±0.10
13th	3.45±0.15	5.85±2.55	3.25±0.05	4.00±0.00	3.55±0.05	4.30±0.10
15th	3.45±0.15	5.85±2.55	3.25±0.05	4.35±0.05	3.55±0.05	4.30±0.10
18th	3.50±0.10	5.85±2.55	3.40±0.10	5.00±0.00	3.55±0.05	4.30±0.10
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7th	0.00±0.00	0.50±0.00	0.25±0.25	0.00±0.00	0.00±0.00	0.00±0.00
9th	0.00±0.00	0.50±0.25	0.50±0.00	0.00±0.00	0.00±0.00	0.25±0.25
11th	0.25±0.25	1.25±0.50	0.75±0.25	0.00±0.00	0.50±0.00	0.25±0.25
13th	0.75±0.75	1.60±0.50	1.40±0.60	0.50±0.00	0.80±0.00	1.40±0.60
15th	0.90±0.90	1.85±0.75	1.50±0.50	0.50±0.00	0.80±0.00	1.80±1.00
18th	2.20±0.20	2.20±1.10	1.60±0.50	0.50±0.00	0.80±0.00	2.15±1.35
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.05±0.15	1.85±0.15	1.95±0.15	1.55±0.05	1.65±0.15	1.50±0.00
7th	2.65±0.05	2.40±0.00	2.60±0.10	2.45±0.05	2.50±0.20	2.30±0.00
9th	2.65±0.05	2.55±0.05	3.05±0.05	2.75±0.25	2.55±0.25	2.65±0.35
11th	2.80±0.10	2.95±0.35	3.85±0.05	3.25±0.55	2.65±0.25	3.10±0.80
13th	2.85±0.05	2.95±0.35	4.40±0.10	3.60±0.80	2.65±0.25	3.35±1.05
15th	2.85±0.05	2.95±0.35	5.10±0.10	3.90±1.10	2.70±0.30	3.45±1.05
18th	2.85±0.05	3.00±0.40	6.00±0.10	4.30±1.50	2.80±0.40	3.70±1.10

4.7.17. *Taxus baccata*

Plant extract of *T. baccata* did not have remarkable inhibitory effect on the phytopathogenic fungi but growth of *P. expansum* to some extant was found restricted in extract of solvent like ethyl acetate, ether and methanol. In case of other fungi inhibition in their growth ranges from 4 to 60%. Plant extract of ethyl acetate and ether was found effective on *F. oxysporum* and *A. alternata*. Growth of *B. cinerea* was not remarkably effected by the plant extract of *T. baccata*.

Table 4.33: Effect of *Taxus baccata* plant extract on the growth of phytopathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	1.15±0.05	1.70±0.10	2.30±0.00	1.45±0.15	1.75±0.65	2.15±0.15
7th	1.25±0.05	1.90±0.10	2.55±0.05	1.45±0.15	3.30±0.90	2.65±0.25
9th	1.40±0.20	1.90±0.10	2.55±0.05	1.45±0.15	2.40±1.00	2.85±0.35
11th	1.60±0.40	1.90±0.10	2.70±0.10	1.55±0.25	2.40±1.00	2.90±0.40
13th	1.60±0.40	1.90±0.10	2.70±0.10	1.80±0.50	2.40±1.00	2.90±0.40
15th	1.60±0.40	1.90±0.10	2.70±0.10	1.80±0.50	2.40±1.00	2.90±0.40
18th	1.60±0.40	1.90±0.10	2.70±0.10	1.80±0.50	2.40±1.00	2.90±0.40
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.05±0.15	3.60±0.50	3.15±0.05	3.00±0.10	3.65±0.25	3.15±0.15
7th	3.20±0.10	4.15±1.05	3.15±0.05	3.05±0.05	3.75±0.25	3.15±0.15
9th	3.20±0.10	4.15±1.05	3.15±0.05	3.05±0.05	3.80±0.30	3.15±0.15
11th	3.20±0.10	4.20±1.10	3.15±0.05	3.10±0.00	4.05±0.15	3.15±0.15
13th	3.35±0.05	4.20±1.10	3.15±0.05	3.25±0.05	4.05±0.15	3.25±0.05
15th	3.35±0.05	4.30±1.20	3.15±0.05	3.25±0.05	4.05±0.15	3.25±0.05
18th	3.35±0.05	4.30±1.20	3.15±0.05	3.25±0.05	4.05±0.15	3.25±0.05
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.75±0.15	3.10±0.20	2.90±0.30	3.70±0.10	2.35±0.35	3.55±0.15
7th	4.00±0.00	3.95±0.05	4.05±0.05	4.70±0.10	4.05±0.05	4.00±0.00
9th	4.65±0.55	3.95±0.05	4.15±0.05	4.70±0.10	4.05±0.05	4.05±0.05
11th	4.75±0.65	3.95±0.05	4.20±0.10	4.70±0.10	4.05±0.05	4.05±0.05
13th	4.80±0.70	3.95±0.05	4.20±0.10	4.75±0.15	4.05±0.05	4.05±0.05
15th	5.65±1.55	3.95±0.05	4.20±0.10	4.80±0.10	4.05±0.05	4.05±0.05
18th	6.00±1.80	3.95±0.05	4.20±0.10	4.80±0.10	4.05±0.05	4.05±0.05
	Alternaria	***alternata***				
Days	**Ethyl acet**	**Methanol**	**Abs alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.00±0.00	2.20±0.10	2.50±0.10	2.10±0.10	2.05±0.05	2.40±0.10
7th	2.25±0.05	2.55±0.05	3.00±0.10	2.80±0.10	2.35±0.05	2.95±0.05

9th	2.25±0.05	2.75±0.05	3.35±0.05	2.80±0.10	2.75±0.05	3.55±0.05
11th	3.00±0.10	2.75±0.05	3.55±0.05	3.05±0.05	2.75±0.05	3.55±0.05
13th	3.45±0.05	2.95±0.05	4.05±0.05	3.05±0.05	3.55±0.05	4.05±0.05
15th	3.45±0.05	3.00±0.10	4.05±0.05	3.05±0.05	3.85±0.05	4.35±0.05
18th	3.45±0.05	3.00±0.10	4.05±0.05	3.05±0.05	3.85±0.05	4.35±0.05

Table 4.34: Effect of *Zanthoxylum oxyphyllum* plant extract on the growth of pathogenic fungi on different solvents. ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.50±0.00	0.50±0.00	0.50±0.00	0.50±0.00	0.50±0.00	0.50±0.00
7th	1.15±0.05	0.95±0.05	1.10±0.10	1.05±0.05	0.95±0.05	1.30±0.00
9th	1.60±0.00	2.15±0.05	1.80±0.10	1.35±0.05	1.30±0.00	1.45±0.05
11th	2.65±0.25	3.00±0.20	2.70±0.10	1.85±0.15	1.90±0.20	2.10±0.10
13th	3.35±0.25	3.65±0.35	3.40±0.10	2.90±0.10	2.50±0.40	3.25±0.05
15th	3.90±0.10	4.05±0.35	3.85±0.35	3.45±0.35	3.00±0.50	3.60±0.10
18th	3.95±0.15	4.35±0.15	4.45±0.65	3.75±0.65	3.50±0.60	3.60±0.10
***Fusarium* oxysporum**						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.75±0.25	1.00±0.10	1.00±0.00	1.55±0.05	1.35±0.15	1.80±0.20
7th	1.85±0.35	3.05±0.15	1.65±0.05	3.90±0.00	3.00±0.10	3.95±0.15
9th	3.15±0.15	4.35±0.15	3.35±0.15	5.55±0.05	4.55±0.05	5.75±0.05
11th	4.40±0.20	5.65±0.15	4.60±0.10	7.35±0.05	6.10±0.20	7.40±0.10
13th	5.85±0.25	7.20±0.20	6.10±0.00	8.00±0.00	7.30±0.20	8.10±0.10
15th	6.65±0.15	7.75±0.25	6.85±0.05	8.10±0.10	7.55±0.15	8.25±0.25
18th	7.45±0.55	8.25±0.25	7.65±0.65	8.75±0.25	7.70±0.30	9.00±0.00
Botrytis cineria						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.00±0.00	0.00±0.00	0.50±0.00	0.00±0.00	0.00±0.00	1.10±0.10
7th	0.25±0.25	0.25±0.25	0.90±0.00	0.00±0.00	0.00±0.00	3.15±0.15
9th	0.60±0.10	0.75±0.25	1.05±0.05	0.00±0.00	0.00±0.00	3.90±0.10
11th	1.10±0.20	1.05±0.15	1.40±0.10	0.25±0.25	0.25±0.25	4.65±0.15
13th	1.30±0.10	1.40±0.10	2.30±0.20	0.75±0.25	0.25±0.25	4.65±0.15
15th	1.50±0.10	1.55±0.05	2.80±0.70	1.10±0.20	0.35±0.35	4.65±0.15
18th	1.50±0.10	2.05±0.05	3.10±1.00	1.40±0.20	0.75±0.25	4.65±0.15
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	0.95±0.05	0.90±0.10	1.10±0.10	0.90±0.10	0.90±0.10	0.90±0.10
7th	1.70±0.20	1.65±0.15	2.15±0.25	1.45±0.05	1.35±0.15	1.45±0.05
9th	2.45±0.05	2.60±0.20	2.70±0.10	2.10±0.30	2.20±0.20	1.85±0.15
11th	2.95±0.15	2.90±0.10	3.35±0.25	3.10±0.30	2.75±0.05	2.40±0.40
13th	3.10±0.30	3.00±0.20	4.35±0.15	4.25±0.25	3.10±0.40	2.65±0.35
15th	3.15±0.35	3.30±0.20	5.00±0.10	5.40±0.20	3.50±0.70	3.25±0.25
18th	3.15±0.35	3.30±0.20	5.65±0.15	6.40±0.10	3.65±0.85	3.25±0.25

4.7.18. *Zanthoxylum oxyphyllum*

The inhibitory effect of *Z. oxyphyllum* plant extract was moderate on the tested fungus. The growth of *B. cinerea* was found severely affected by the *Z. oxyphyllum* plant extract under all the solvents. Plant extract prepared with ether, chloroform, methanol and ethyl acetate inhibit growth of *B. cinerea* 100% at initial stage of study period. After 11th days of incubation slight increase in colony growth was recorded as such growth of *B. cinerea* was affected by the plant extract prepared with different solvent. Growth of *P. expansum* was recorded more severely affected at initial stage but at the end of study a recovery was noticed. At initial stage inhibition in growth ranges from 75 to 81% whereas, at the end it declined and was between 33 to 43%. *F. oxysporum* was not effected much by the plant extract of *Z. oxyphyllum* but only extract prepared in ethyl acetate was found to restrict growth only on 5th day of incubation, at the end growth was almost similar to that of control one. At initial stage growth of *A. alternata* also inhibited by plant extract prepared with different solvents. During study period in most of the solvent extracts growth inhibition was almost 50% up to 13th day of study but only in extract prepared with ether and absolute alcohol inhibition percent declined and it was between 17 to 21% however, in other solvent extracts inhibition in growth was always remain more than 56%.

4.7.19. phytopathogenic fungi in media with solvent and without plant extracts (control)

Phytopathogenic fungi were also grown separately without plant extract in similar manner. However at the place of plant extract solvents were used. Different fungi grown in various solvents had variability in their growth but there was not any remarkable variation in growth of particular fungi under various solvents. *Penicillium expansum* grown in ethyl acetate, methanol, alcohol, ether, chloroform and benzene have almost equal colony diameter. Similarly *Fusarium oxysporum, Botrytis cinerea and Alternaria alternata* did not have remarkable difference in their colony diameter with different solvents. There was colony diameter difference between different fungi. The observations recorded for the growth of phytopathogenic fungi were compared with the growth of these fungi in different plant extracts. The percent inhibition in growth was calculated considering the growth of fungi under without extract treatment condition.

Table 4.35: Growth of phytopathogenic fungi in media with solvent and without plant extracts (**control**). ±S.E

Penicillium expansum						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	2.65±0.05	2.00±0.00	2.50±0.30	2.20±0.00	2.20±0.40	2.40±0.05
7th	3.40±0.10	2.90±0.10	2.75±0.35	2.65±0.35	2.40±0.60	2.90±0.10
9th	3.70±0.40	3.80±0.05	3.55±0.55	3.50±0.50	3.10±0.30	3.45±0.05
11th	4.35±0.15	4.50±0.10	4.30±0.60	4.35±0.55	3.90±0.60	4.00±0.00
13th	5.20±0.20	5.25±0.65	5.15±0.60	5.00±0.50	4.50±0.70	4.45±0.15
15th	6.30±0.10	6.50±1.20	6.45±1.35	6.20±0.60	5.40±0.70	5.05±0.25
18th	6.50±0.00	6.50±1.20	6.50±1.30	6.65±0.25	6.00±0.60	5.60±0.55
***Fusarium* oxysporum**						
Days	**Ethyl acet**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.90±0.10	3.50±0.10	3.00±0.00	4.00±0.20	4.20±0.20	4.30±0.10
7th	4.30±0.00	4.65±0.55	3.80±0.10	4.80±0.20	5.00±0.20	5.00±0.20
9th	5.05±0.15	5.60±0.60	4.35±0.15	5.65±0.25	5.70±0.20	5.85±0.15
11th	5.85±0.25	6.20±0.40	5.05±0.05	6.70±0.10	6.65±0.15	6.90±0.10
13th	6.55±0.05	7.00±0.50	6.25±0.25	7.80±0.00	7.85±0.25	7.55±0.05
15th	6.85±0.15	7.55±0.55	7.45±0.45	8.50±0.10	8.60±0.20	8.50±0.10
18th	7.00±0.00	8.50±0.50	8.65±0.05	9.00±0.00	9.00±0.00	9.00±0.00
Botrytis cineria						
Days	**Ethyl acet**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.80±0.20	4.25±0.05	4.25±0.05	4.05±0.05	4.30±0.20	4.05±0.05
7th	4.25±0.15	4.45±0.05	4.80±0.10	4.80±0.10	4.90±0.10	4.50±0.10
9th	4.85±0.15	5.00±0.20	5.25±0.05	5.35±0.15	5.40±0.10	5.05±0.05
11th	5.25±0.15	5.35±0.05	5.65±0.15	5.85±0.05	6.20±0.20	5.85±0.05
13th	5.85±0.25	5.95±0.25	6.00±0.10	6.35±0.05	6.35±0.15	6.25±0.05
15th	6.25±0.15	6.55±0.25	6.45± 0.15	6.95±0.25	6.75±0.05	6.95±0.15
18th	6.60±0.20	6.95±0.15	6.85±0.15	7.20±0.20	7.00±0.00	7.35±0.15
Alternaria alternata						
Days	**Ethyl acetate**	**Methanol**	**Alcohol**	**Ether**	**Chloroform**	**Benzene**
5th	3.40±0.15	3.00±0.00	2.80±0.00	3.90±0.10	4.00±0.10	3.60±0.10
7th	4.05±0.05	3.65±0.05	3.30±0.10	4.80±0.10	5.45±0.45	4.60±0.40
9th	5.10±0.10	4.75±0.25	4.05±0.05	5.75±0.25	6.25±0.05	5.15±0.35
11th	6.05±0.15	5.50±0.20	4.95±0.35	6.05±0.35	6.85±0.05	5.70±0.30
13th	6.85±0.15	6.05±0.15	5.75±0.35	6.85±0.15	7.35±0.05	6.55±0.45
15th	7.50±0.20	6.75±0.15	6.00±0.30	7.35±0.05	8.00±0.10	7.05±0.45
18th	8.00±0.10	7.50±0.10	6.85±0.25	8.05±0.05	8.55±0.05	7.65±0.55

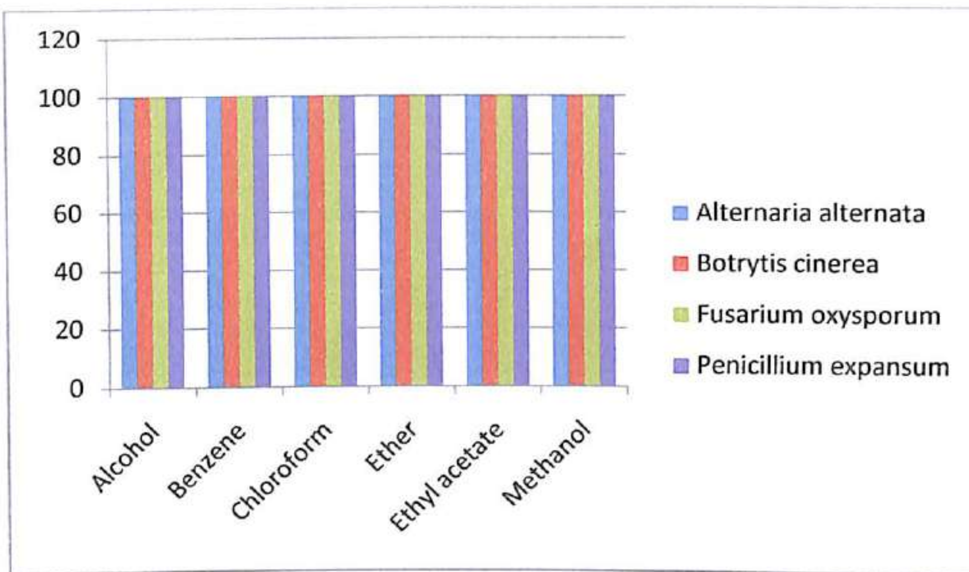

Figure 4.11: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Acorus calamus* Plant extract.

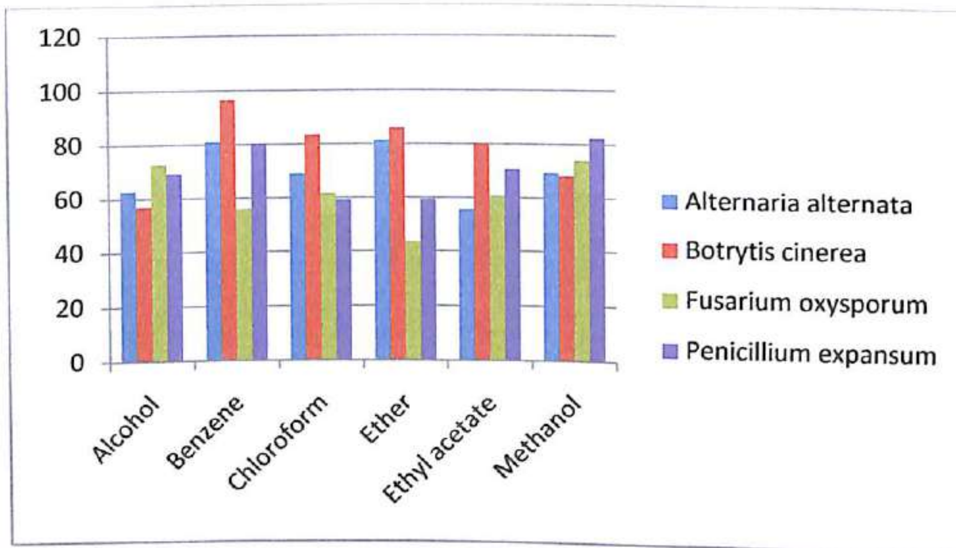

Figure 4.12: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Ageratum conyzoides* Plant extract.

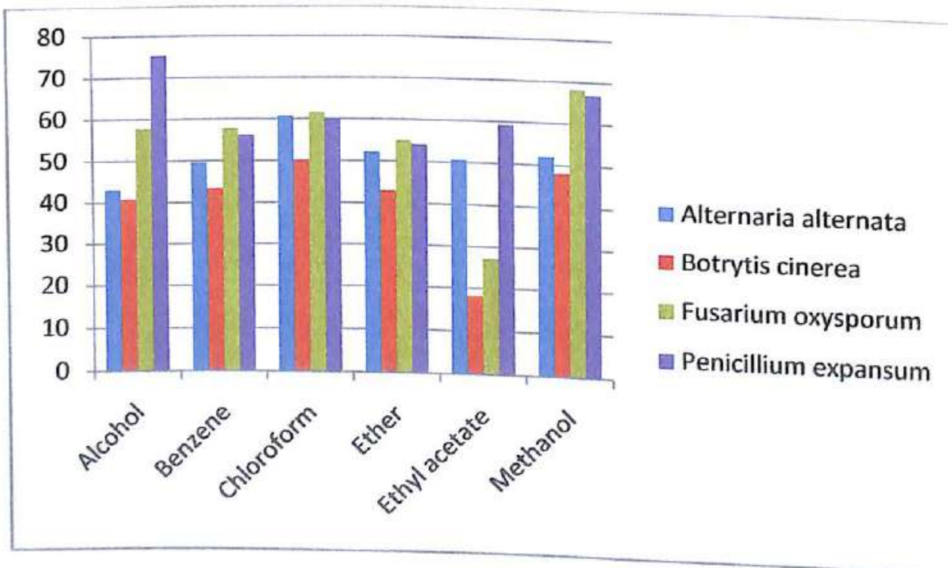

Figure 4.13: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Artimesia nilagerica* Plant extract.

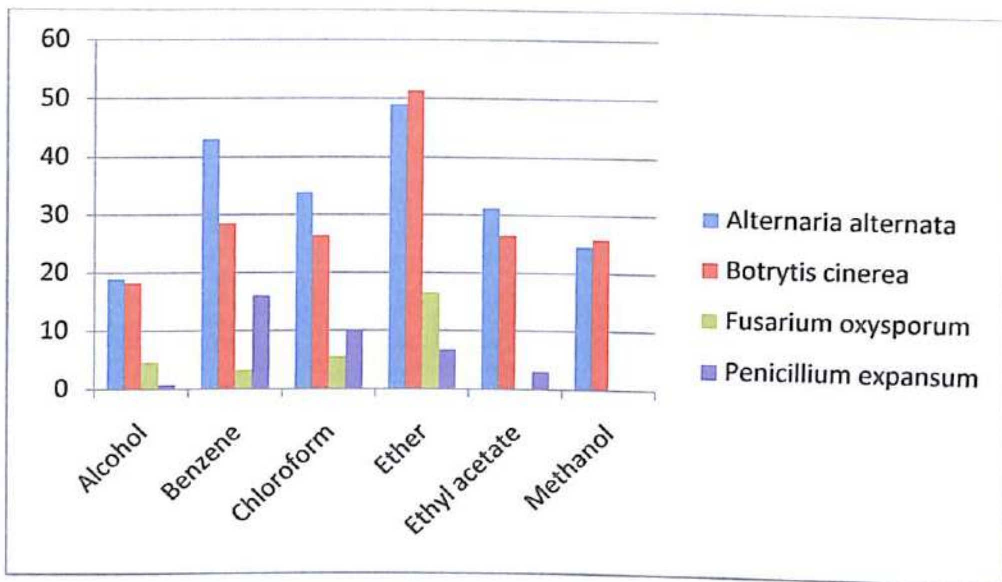

Figure 4.14: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Bauhinia purpurea* plant extract.

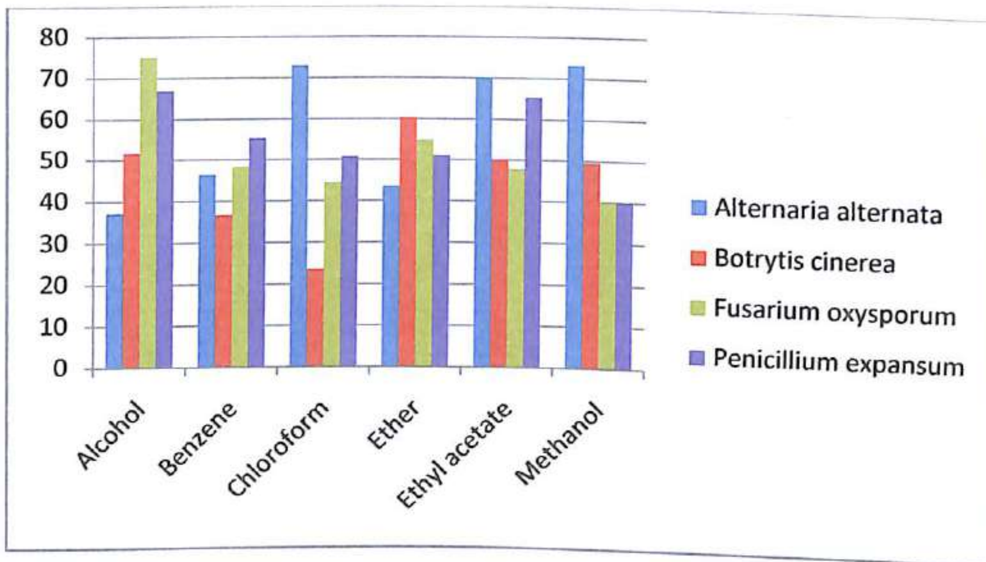

Figure 4.15: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Calicarpa arborea* plant extract.

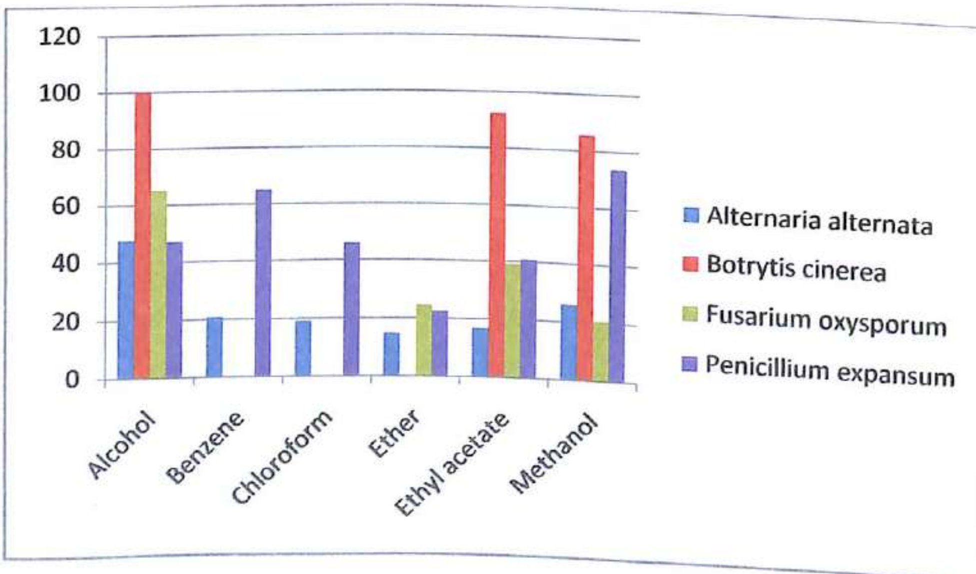

Figure 4.16: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Eleocarpus sphaericus* plant extract.

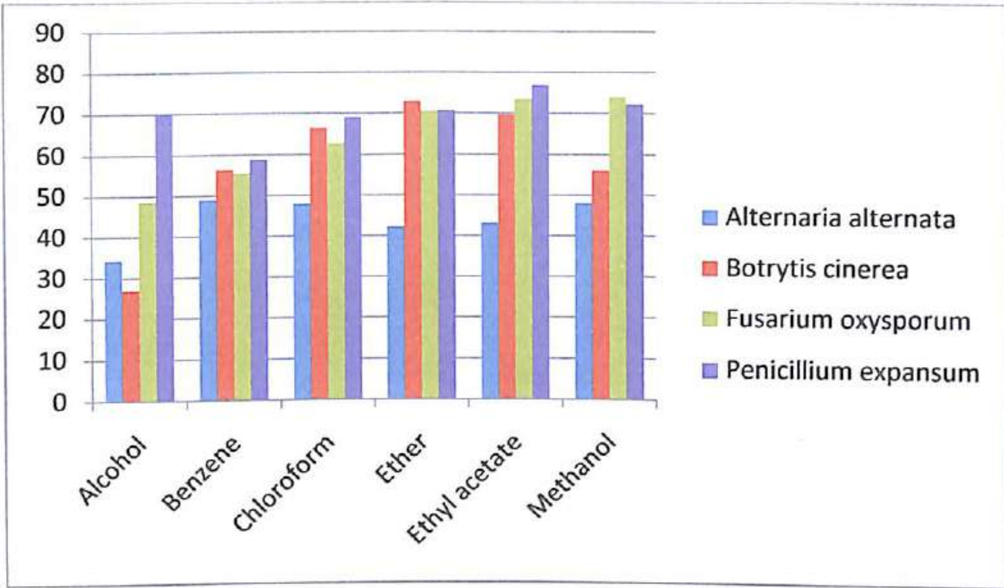

Figure 4.17: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Eupatorium odoratum* plant extract.

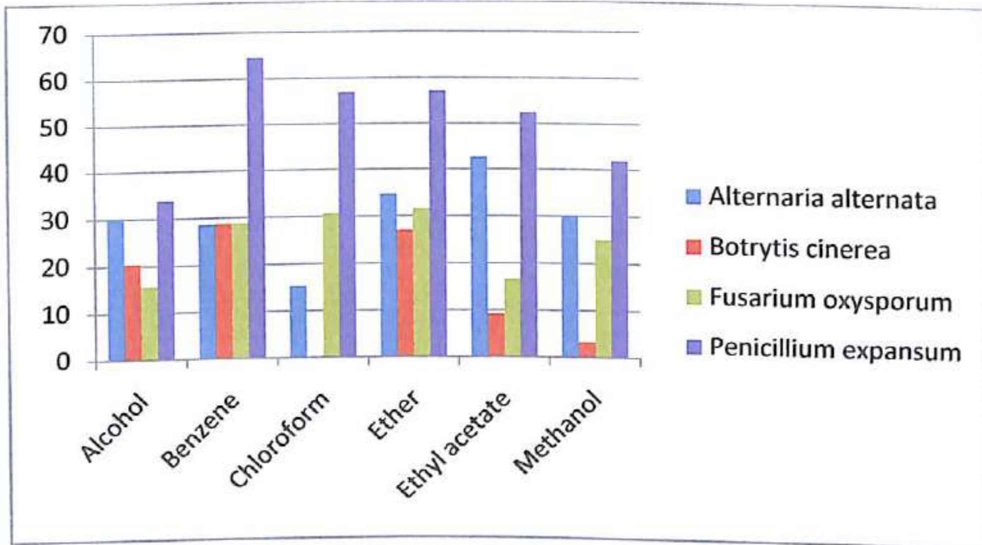

Figure 4.18: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Lantana camara* plant extract.

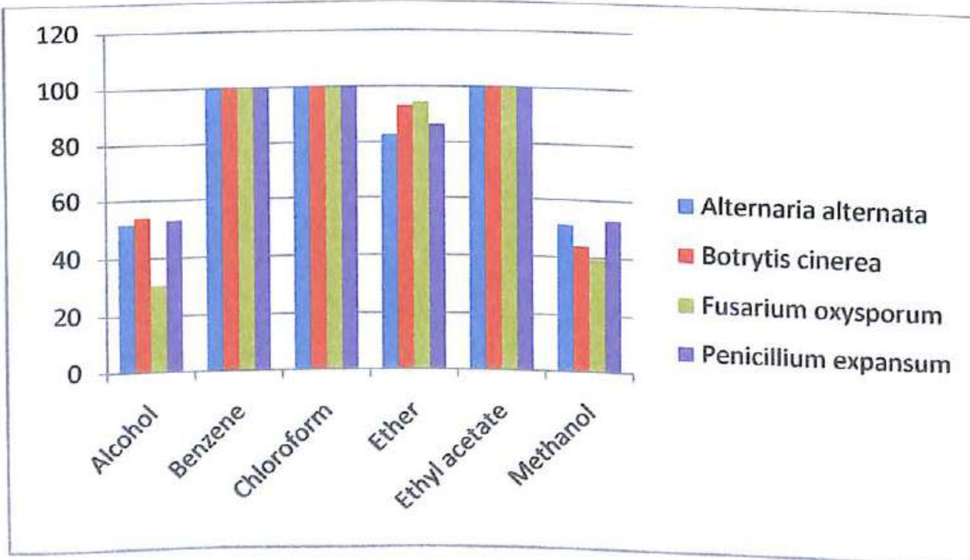

Figure 4.19: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Litsea cubeba* plant extract.

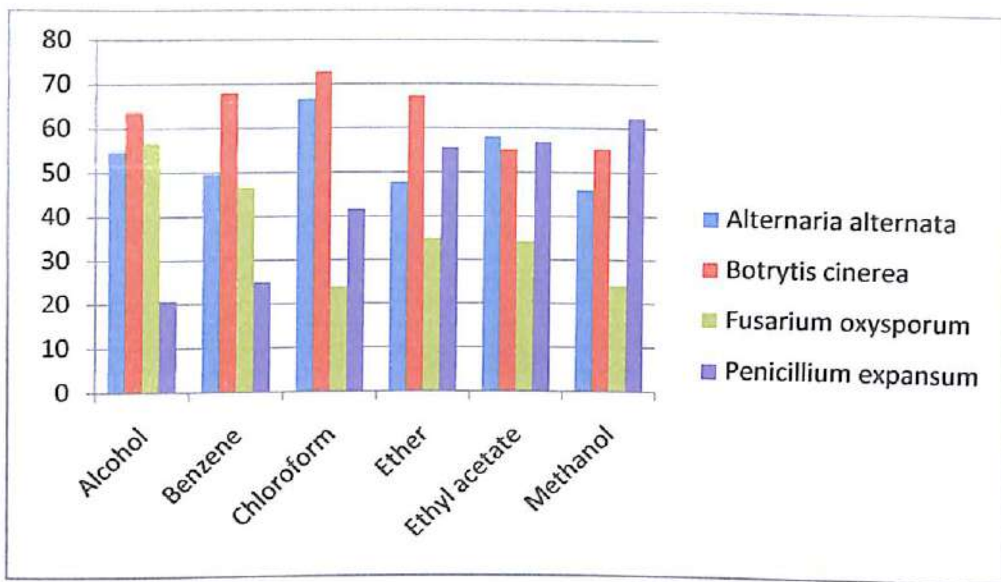

Figure 4.20: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Michelia champaca* plant extract.

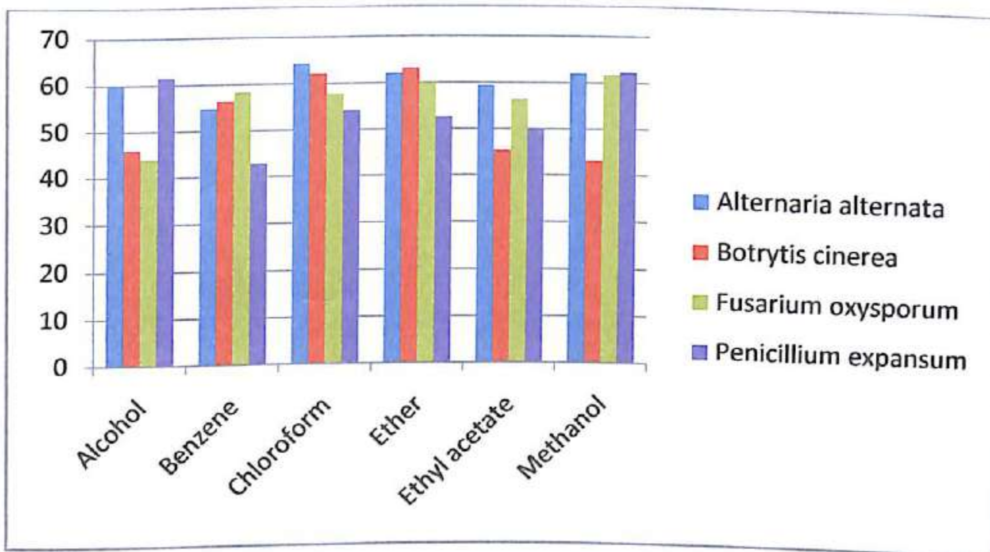

Figure 4.21: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Mikania cordata* plant extract.

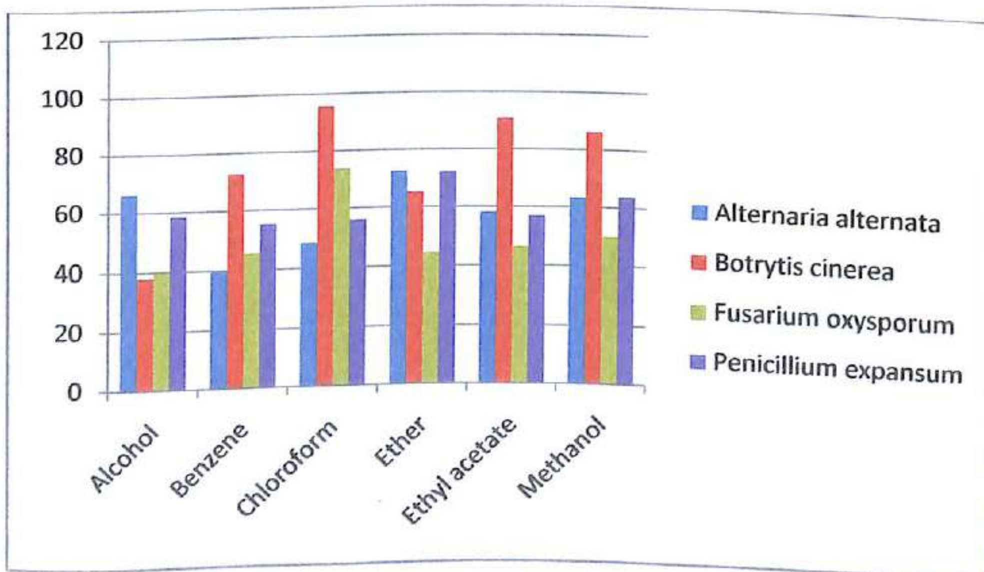

Figure 4.22: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Piper mullesua* plant extract.

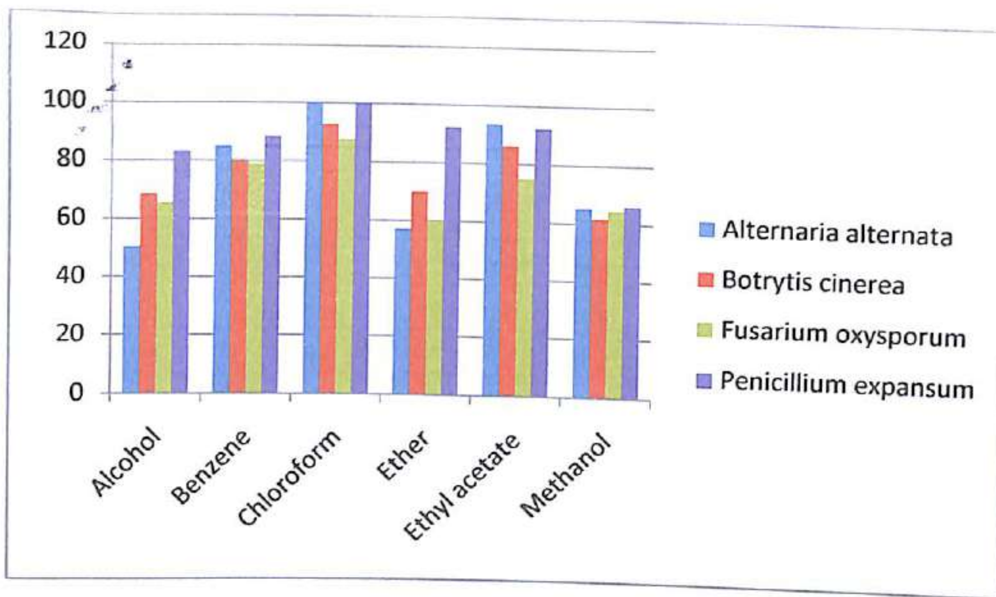

Figure 4.23: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Polygonum hydropiper* plant extract.

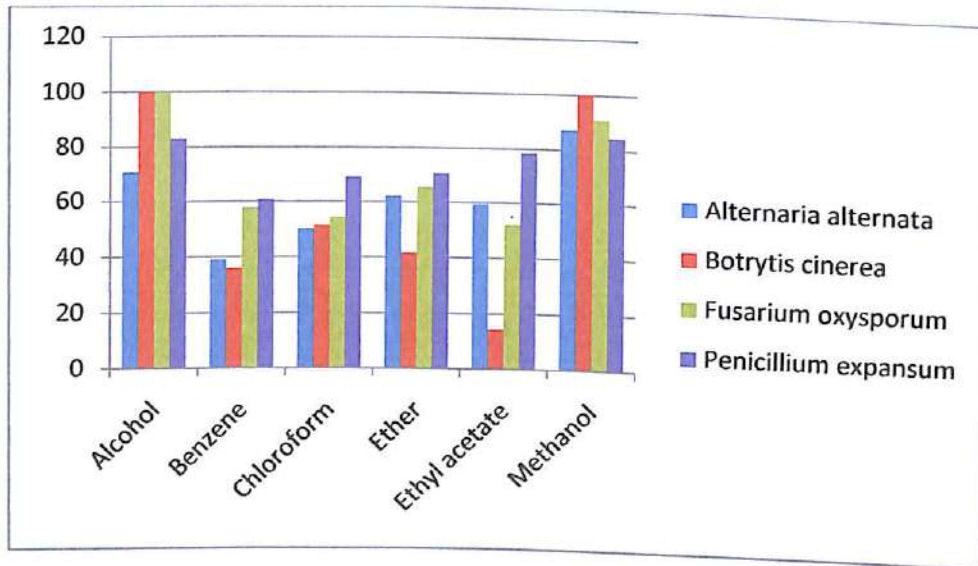

Figure 4.24: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Samanea saman* plant extract.

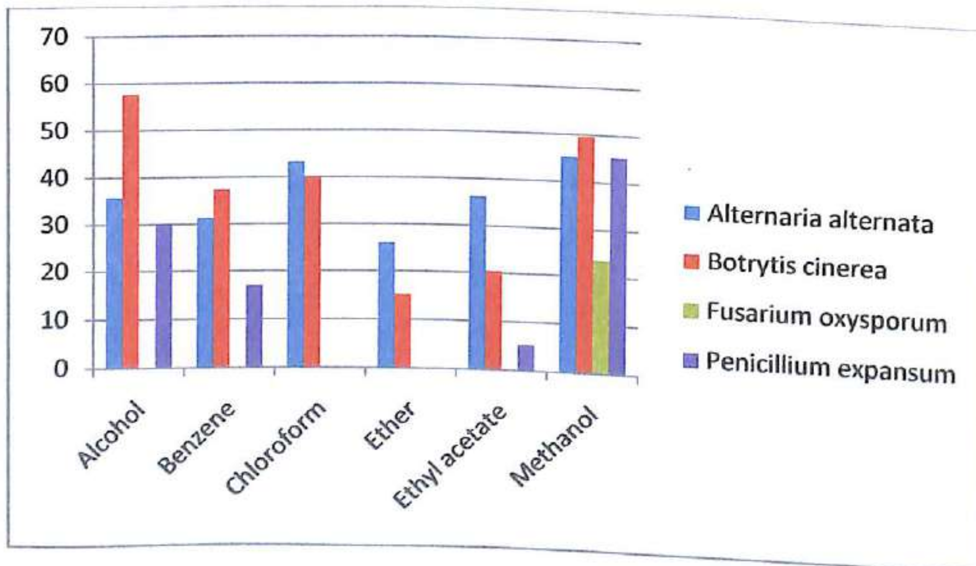

Figure 4.25: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Solanum spirale* plant extract.

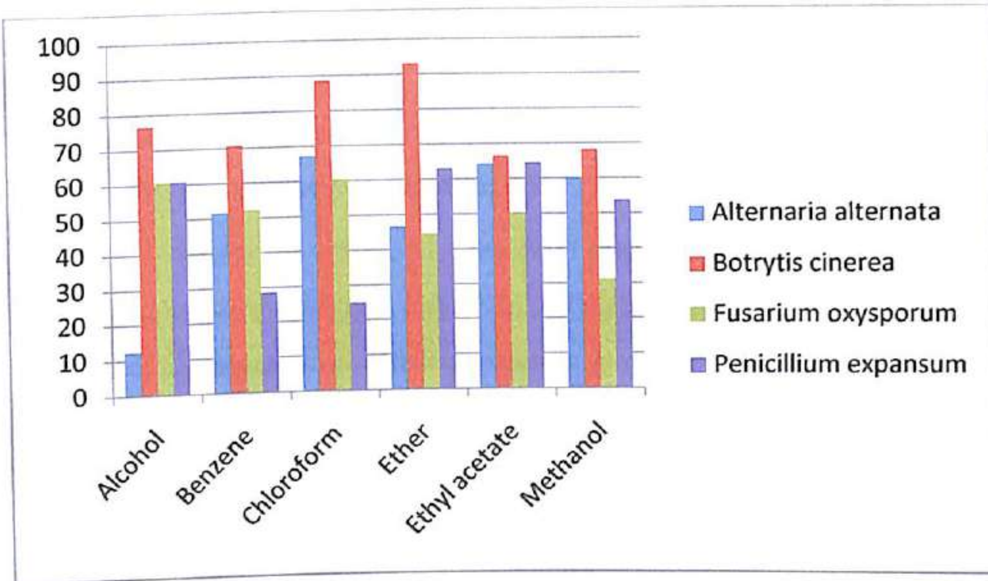

Figure 4.26: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Tagetes errecta* plant extract.

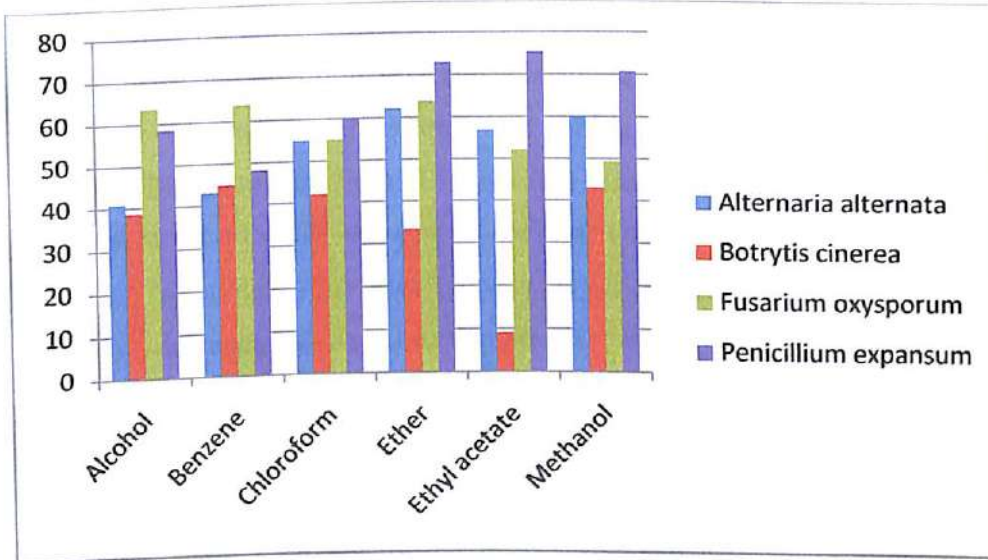

Figure 4.27: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Taxus baccata* plant extract.

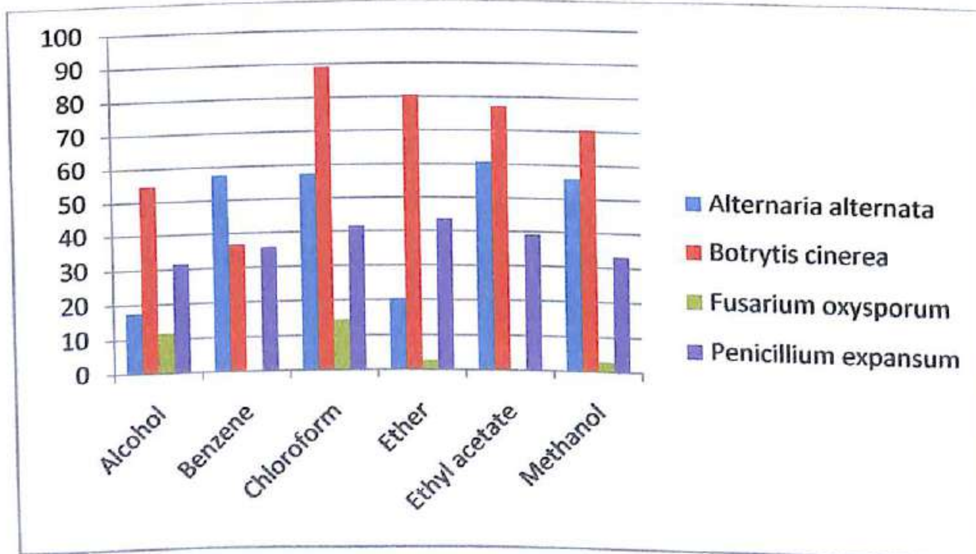

Figure 4.28: After 18 days of incubation percent inhibition in colony growth of pathogenic fungi treated with *Zanthoxylum oxyphyllum* plant extract.

4.8. *In Vivo* Efficacy of plants

4.8.1. *In vivo* efficacy of essential oils

To evaluate the efficacy of essential oils under *in vivo* condition only oils of four plants were selected on the basis of their performance in previous experiment relating to essential oil effect on colony growth of tested fungus. The four plant essential oils namely- *Acorus calamus, Ageratum conyzoides, Artemisia nilogirica* and *Litsea cubeba* were selected for further study. The concentration of oils for *in vivo* study was taken on the basis of their MIC.

4.8.1.1. Dip Method

The *in vivo* experiment carried out using dip method showed that fruits treated with *Litsea cubeba* oil were not having rotting even after 20 days of interval, also there was not infection at localized wound spot. Similarly, fruits treated with *Acorus calamus* oil did not have any rotting and localized wound infection. The kiwifruits inoculated with *P. expansum* and treated by *Artemisia nilogirica* oil found to rot after 12th days of incubation. The rotting initiated from the localized wound infection. *Ageratum conyzoides* oil treated fruits which were inoculated with *P. expansum* also start rotting after 12th days of inoculation.

Fungal pathogen *F. oxysporum* could not grow on kiwifruits till 12th day after inoculation on being treated with essential oil of different plants. Fruits treated with *L. cubeba* 1000ppm concentration of oil had localized wound spot after 9th days of inoculation but rotting started 12th day onward. In case of *A. calamus* treated fruits with 500ppm concentration of oils rotting begin after 12th days of inoculation. And similarly, *A. conyzoides* oil (1000ppm) treated fruits survived without any damaged till 12th day but after that only rotting was seen. In comparison to control fruit quality remain intact up to 12th days on being treated with essential oils.

Fruits artificially infected by *B. cinerea* and treated with different oils were also having more storage life as compared to control one. Fruits treated with *L. cubeba* oil did not have any infection spot or rotting even up to 16th days interval. Fruits treated with oil of *A. calamus* developed localized wounds after 20th day of inoculation by *B. cinerea* but there was no rotting till 30 day. In case of *A. nilogirica* oil treated fruits infection was occurred and rotting started after 12th day of inoculation. And similarly in *A. conyzoides* oil treated fruits rotting started quit

early and it was noticed in some fruits 7th day onward and most of fruits after 12th days of inoculation.

Table 4.36: Efficacy of essential oils by dip method under *in vivo* condition against pathogenic fungi

Essential oils	**Initiation of rotting of kiwifruit**	**Enhancement of storage life (in days)**
P. expansum		
Control	3	-
Acorus calamus oil (500ppm)	17	14
Ageratum conyzoides oil (1000ppm)	12	9
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	20	17
F. oxysporum		
Control	3	-
Acorus calamus oil (500ppm)	12	9
Ageratum conyzoides oil (1000ppm)	12	9
Litsea cubeba oil (1000ppm)	12	9
B. cinerea		
Control	3	-
Acorus calamus oil (500ppm)	20	17
Ageratum conyzoides oil (1000ppm)	12	9
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	16	13

4.8.1.2. Impregnated Method

The *in vivo* efficacy evaluation of essential oils carried out following impregnated method showed enhancement in storage life of the fruits. Fruit impregnated with essential oil of *L. cubeba* and were inoculated with fungi *P. expansum* were not having any rotting even after 24th days of inoculation except having only localized infection in wounds. *A. calamus* and *A. nilagirica* essential oil treated fruits had infection only after 14th and 12th days of inoculation. It was remarkable that rotting process in oil treated fruits was slower than control. *A. conyzoides* essential oil treated fruits were found to be infected and rotted after on 14th days of inoculation by *P. expansum.* The kiwifruits artificially infected *F.* oxysporum and treated with essential oil of *L. cubeba* could survive without infection and rotting till 20th day. Fruit treated with *A. calamus* survived till 26th days without infection and rotting started only after 26th day of inoculation. *A. conyzoides* essential oil was not effective as *A. calamus* and fruits treated with *A. conyzoides* oil start rotting after 12th day of inoculation. The kiwifruits treated with *A. conyzoides* and *A. nilagirica* oil start rotting after 12th days of inoculation

by *B. cinerea* and prior to that infection and localized wound was seen on 7th days after inoculation of fungi. *L. cubeba* oil treated fruit started rotting only after 12th day's interval whereas *A. calamus* treated fruit rotted after 24th days of inoculation. It shows that fruits treated with *A. calamus* have more storage life than essential oils of other plants.

Table 4.37: Efficacy of essential oils by impregnated method under *in vivo* condition against pathogenic fungi

Essential oils	**Initiation of rotting of kiwifruit**	**Enhancement of storage life (in days)**
	P. expansum	
Control	3	-
Acorus calamus oil (500ppm)	17	14
Ageratum conyzoides oil (1000ppm)	14	11
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	24	21
	F. oxysporum	
Control	3	-
Acorus calamus oil (500ppm)	26	23
Ageratum conyzoides oil (1000ppm)	15	12
Litsea cubeba oil (1000ppm)	20	17
	B. cinerea	
Control	3	-
Acorus calamus oil (500ppm)	24	21
Ageratum conyzoides oil (1000ppm)	12	9
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	12	9

4.8.1.3. Fumigation Method

To evaluate the efficacy of essential oils in *in vivo* condition fumigation technique was also followed. It was interesting to note that fruits treated with different essential oils through fumigation technique were not rotted except in case of fruits inoculated with *B. cinerea* and treated by *A. conyzoides*. Fruits inoculated by *P. expansum* and treated with *L. cubeba, A. nilagirica* and *A. conyzoides* essential oils developed only very small localized spots after inoculation of fungus but there was no rotting of fruits. Similarly fruits inoculated with *F. oxysporum* were not seen to have any infection or rotting till the end of study. Except in case of *L. cubeba* oil after 15th days of inoculation one or two localized spots were occurred at wound. A similar effect of essential oil in

enhancing the storage life of fruits was seen those were artificially infected with *B. cinerea*. Fruits treated with *A. calamus*, *A. nilagirica* and *L. cubeba* were not found rotting or having any infection. Fruits treated with oil of *A. conyzoides* had started rotting after 12th day of inoculation with *B. cinerea* and localized spots near wounds developed after 9th day of inoculation.

In general results indicate that essential oil treatment of fruits increased the storage life of fruits either of any method but fumigation technique was found more effective in increasing the storage life of kiwifruits.

Table 4.38: Efficacy of essential oils by fumigation method under *in vivo* condition against pathogenic fungi

Treatment	**Initiation of rotting of kiwifruit**	**Enhancement of storage life (in days)**
	P. expansum	
Control	3	-
Acorus calamus oil (500ppm)	17	14
Ageratum conyzoides oil (1000ppm)	14	11
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	17	14
	F. oxysporum	
Control	3	-
Acorus calamus oil (500ppm)	16	13
Ageratum conyzoides oil (1000ppm)	17	14
Litsea cubeba oil (1000ppm)	20	17
	B. cinerea	
Control	3	-
Acorus calamus oil (500ppm)	12	9
Ageratum conyzoides oil (1000ppm)	12	9
Artemisia nilagirica oil (5000ppm)	12	9
Litsea cubeba oil (1000ppm)	15	12

4.8.2. *In vivo* efficacy of plant extracts

In vivo efficacy of selected plant extracts was carried out on the basis of their effectiveness in previous experiment carried out using poisoned food technique. Selection of plant extract was based on their effect on a particular pathogenic fungus.

4.8.2.1. Dip Method

The kiwifruit treated with aqueous extract of *A. calamus* and *S. saman* and inoculated with *P. expansum* remain unaffected till 16th day after inoculation but rotting was recorded 17th day onward whereas in case of negative control storage life was lesser than treated one. Aqueous extract of *A. calamus* and *S. saman* was also able to restrict the growth of fungus *F. oxysporum* on kiwifruits. Fruits treated with *S. saman* were not having any rotting up to 20th days and after that also rotting was not recorded. Similarly *A. calamus* treated fruits were without any infection up to 17th day after that only two localized wounds were recorded. In case of *B. cinerea* plant extract of *A. calamus, S. saman, A. conyzoides* and *P. mullesua* were taken because of their positive inhibition effect on the tested fungus. *S. saman* and *A. calamus* treated fruits were without infection till 17th day and in subsequent period only two localized wounds were seen. *A. conyzoides* treated fruits and artificially inoculated with *B. cinerea* also survived for a longer time and one localized wound was seen after 11th day and another after 17th day and there was no rotting of fruits. Similarly to *A. conyzoides P. mullesua* treated fruits also survived for longer time and one localized wound was found on 11th day and another one on 17th day after inoculation of *B. cinerea.*

Table 4.39: Efficacy of plant extracts by dip method under *in vivo* condition against pathogenic fungi

Treatments	**Initiation of rotting of kiwifruit**	**Enhancement of storage life (in days)**
	P. expansum	
Control	3	-
Acorus calamus	17	14
Samanea saman	17	14
	F. oxysporum	
Control	3	-
Acorus calamus	22	19
Samanea saman	23	20
	B. cinerea	
Control	3	-
Acorus calamus	21	18
Ageratum conyzoides	20	17
Piper mullesua	20	17
Samanea saman	17	14

4.8.2.2. Impregnated Method

Plant extracts efficiency was also evaluated through impregnated method. Fruits artificially inoculated with *P. expansum* and treated by aqueous extract of *A. calamus* remain unaffected till 20th days and rotting started only in subsequent period whereas in case of *S. saman* treated fruits also there were no rotting for longer period but localized wound was seen after 5th day inoculation of *P. expansum*. The kiwifruits treated with *A. calamus* and *S. saman* and artificially inoculated by *F. oxysporum* were also remain unaffected for a period of 17th to 20th days and there was no rotting only in case of *S. saman* treated fruits three localized wounds were seen after 17th day. The kiwifruits those were artificially inoculated with *B. cinerea* and treated by extract of *A. calamus* and *P. mullesua* remain healthy up to 17th day but after that one or two localized wound were seen and rotting started after 20th day of inoculation. *A. conyzoides* and *S. saman* treated fruits had slightly more storage life and up to 20th days there was no infection or rotting. After 20th day only localized wound appeared and rotting started.

Table 4.40: Efficacy of plant extracts by impregnated method under *in vivo* condition against pathogenic fungi

Treatments	Initiation of rotting of kiwifruit	Enhancement of storage life (in days)
	P. expansum	
Control	3	-
Acorus calamus	20	17
Samanea saman	10	7
	F. oxysporum	
Control	3	-
Acorus calamus	23	20
Samanea saman	17	14
	B. cinerea	
Control	3	-
Acorus calamus	20	17
Ageratum conyzoides	20	17
Piper mullesua	20	17
Samanea saman	23	20

4.9. Organoleptic tests (sensory evaluation)

Results of organoleptic test are given in tables (4.41 to 4.44). Organoleptic tests were carried out for in *vivo* efficacy of essential oils as well as plant extracts.

4.9.1. Essential oils

4.9.1.1. Dip method

Fruits inoculated with *B. cinerea* and treated by *A. calamus* and *L. cubeba* were found equal to standard and without any changes in property, whereas, fruits treated with *A. nilagirica* and *A. conyzoides* essential oils and infected by *B. cinerea* after or at the end of study were below standard. *F.oxysporum* infected fruits and treated with *A. calamus* and *L.cubeba* essential oil were equal to standard whereas *A. conyzoides* essential oil treated were not to the mark and on numerical rating were found unacceptable. The fruits treated with *L. cubeba* and *A. calamus* essential oil and inoculated by *P. expansum* under dip method were found without deterioration of any property and were equal to standard. Whereas *A. nilagirica* and *A. conyzoides* treated fruits on the basis of taste were unacceptable.

Table 4.41: Sensory evaluation of essential oils treated Kiwi fruits by dip method

Fungi	**Name of plants and concentration of oil**	**Judges**						
		A	B	C	D	E	F	Average
Botrytis cinerea	*Acorus calamus* (500ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (1000ppm)	1	2	2	1	1	1	1.33
	Artemisia nilagirica (5000ppm)	3	3	2	2	3	1	2.33
	Litsea cubeba (5000ppm)	3	4	4	4	4	4	4
Fusarium oxysporum	*Acorus calamus* (500ppm)	4	3	4	4	4	4	4
	Ageratum conyzoides (1000ppm)	2	2	1	1	1	1	1.33
	Litsea cubeba (1000ppm)	4	4	4	4	4	4	4
Penicillium expansum	*Acorus calamus* (1000ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (5000ppm)	2	1	1	2	1	1	1.33
	Artemisia nilagirica (5000ppm)	1	1	1	1	1	1	1
	Litsea cubeba (1000ppm)	4	3	4	4	4	4	4

4.9.1.2. Impregnated method

Organoleptic test of kiwi fruits was carried out for *in vivo* efficacy of essential oils under impregnated method are given in table (4.42). Fruits those were inoculated with *B. cinerea* and treated by *A. calamus* and *L. cubeba* oil were

found almost equal to standard. Whereas *A. conyzoides* oil treated were below standard but without any off flavor. Fruits those treated with *A. nilagirica* were unacceptable. Similarly, fruits inoculated with F. oxysporum and treated with *A. calamus* and *L. cubeba* were equal to standard but *A.conyzoides* oil treated fruit were unacceptable in taste. Fruits inoculated with *P. expansum* and treated with *A.calamus* and *L. cubeba* essential oil found equal to standard. Whereas, *A. conyzoides* and *A. nilagirica* oil treated fruits were below standard. In general it was found that fruits treated with *A. calamus* and *L. cubeba* essential oil were in test equal to standard one in case of all phytopathogenic fungi inoculated fruits.

Table 4.42: Sensory evaluation of essential oils treated Kiwi fruits by impregnated method

Fungi	**Name of plants and concentration of oil**	**Judges**						
		A	B	C	D	E	F	Average
Botrytis cinerea	*Acorus calamus* (500ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (1000ppm)	4	4	3	2	3	2	3
	Artemisia nilagirica (5000ppm)	2	1	1	1	1	1	1
	Litsea cubeba (5000ppm)	4	4	3	3	4	4	4
Fusarium oxysporum	*Acorus calamus* (500ppm)	4	4	2	2	3	4	3
	Ageratum conyzoides (1000ppm)	1	1	1	1	1	1	1
	Litsea cubeba (1000ppm)	4	4	4	4	4	4	4
Penicillium expansum	*Acorus calamus* (1000ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (5000ppm)	1	1	2	2	2	1	1.5
	Artemisia nilagirica (5000ppm)	1	1	2	2	1	1	1
	Litsea cubeba (1000ppm)	3	4	4	4	4	4	4

4.9.1.3. Fumigation Method

The *in vivo* efficacy evaluation experiment under fumigation method for essential oil shows that *B. cinerea* inoculated fruits and treated with *A. calamus* and *L. cubeba* essential oil were found equal to standard whereas *A. conyzoides* oil

treated fruits were below standard but with no off flavor. Fruits artificially inoculated with *F. oxysporum* and treated by *A. calamus, A. conyzoides* and *L. cubeba* oil were having taste similar to normal fruits or were equal to standard one. Fruits inoculated with *P. expansum* and treated by *A. calamus* and *L. cubeba* oils were equal to standard. *A. conyzoides* and *A. nilagirica* treated fruits were below standard. The Kiwi fruits treated with essential oil of *A. calamus and L. cubeba* were found in good taste in case of all the pathogenic fungi treated one.

Table 4.43: sensory evaluation of essential oils treated Kiwi fruits by fumigation method

Fungi	Name of plants and concentration of oil	Judges						
		A	B	C	D	E	F	Average
Botrytis cinerea	*Acorus calamus* (500ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (1000ppm)	3	3	3	3	3	3	3
	Artemisia nilagirica (5000ppm)	1	1	2	2	1	1	1.5
	Litsea cubeba (5000ppm)	4	4	4	4	4	4	4
Fusarium oxysporum	*Acorus calamus* (500ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (1000ppm)	4	4	4	4	4	4	4
	Litsea cubeba (1000ppm)	4	4	4	4	4	4	4
Penicillium expansum	*Acorus calamus* (1000ppm)	4	4	4	4	4	4	4
	Ageratum conyzoides (5000ppm)	3	3	2	3	3	3	3
	Artemisia nilagirica (5000ppm)	4	4	4	4	4	4	4
	Litsea cubeba (1000ppm)	4	4	4	3	4	4	4

4.9.2. Plant extracts:

4.9.2.1. Dip Method

Organoleptic test of kiwi fruits was carried out for *in vivo* efficacy of plant extracts under dip method are given in table (4.44). Fruits inoculated with *Botrytis cinerea* and treated by *Acorus calamus* and *Samanea saman* plant extract were found equal to standard one. But *Botrytis cinerea* inoculated treated with *Ageratum conyzoides* and *Piper mullesua* plant extract were very poor in taste. Fruits inoculated with *Fusarium oxysporum* and treated by *Acorus calamus* was standard in taste whereas *Samanea saman* treated fruits were substandard. Similarly *Penicillium expansum* inoculated fruits and treated by *Acorus calamus* was standard in taste whereas *Samanea saman* treated fruits were poor in taste. It was recorded that *Acorus calamus* treated fruits were standard in taste for all the inoculated pathogenic fungi.

Table 4.44: Sensory evaluation of aqueous plant extracts treated Kiwi fruits by dip method

Fungi	Name of plant	Judges						
		A	B	C	D	E	F	Average
Botrytis cinerea	*Acorus calamus*	4	4	4	4	3	3	4
	Ageratum conyzoides	1	1	1	1	1	1	1
	Piper mullesua	1	1	1	1	1	1	1
	Samanea saman	4	4	3	3	4	4	4
Fusarium oxysporum	*Acorus calamus*	4	4	3	4	3	4	4
	Samanea saman	2	2	1	1	1	2	1.5
Penicillium expansum	*Acorus calamus*	4	4	4	3	4	4	4
	Samanea saman	3	3	2	2	1	1	2

Table 4.45: Sensory evaluation of plant extracts treated Kiwi fruits by impregnated method

Fungi	**Name of plant**	**Judges**						
		A	B	C	D	E	F	Average
Botrytis cinerea	*Acorus calamus*	4	4	4	4	4	4	4
	Ageratum conyzoides	3	3	3	3	3	3	3
	Piper mullesua	1	1	1	2	1	1	1
	Samanea saman	4	4	4	3	4	4	4
Fusarium oxysporum	*Acorus calamus*	4	4	3	3	4	3	3.5
	Samanea saman	1	1	1	1	1	1	1
Penicillium expansum	*Acorus calamus*	3	3	4	4	4	4	3.8
	Samanea saman	4	4	3	4	4	4	3.9

4.9.2.1. Impregnated Method

Organoleptic test of kiwi fruits was carried out for *in vivo* efficacy of plant extracts by impregnated method are given in table (4.45). Fruits inoculated with *Botrytis cinerea* and treated by *Acorus calamus* and *Samanea saman* plant extract were found equal to standard one. But *Botrytis cinerea* inoculated treated with *Ageratum conyzoides* and *Piper mullesua* plant extract were substandard in taste. Fruits inoculated with *Fusarium oxysporum* and treated by *Acorus calamus* were standard in taste whereas *Samanea saman* treated fruits were substandard. *Penicillium expansum* inoculated fruits treated by *Acorus calamus* and *Samanea saman* were standard. Results shows that *Acorus calamus* treated fruits were standard in taste for all the inoculated pathogenic fungi.

Kiwi fruit

Plate A. Fresh kiwi fruit

Plate B. Rotting Kiwi fruit

Phytopathogenic fungi isolated from Kiwi fruit

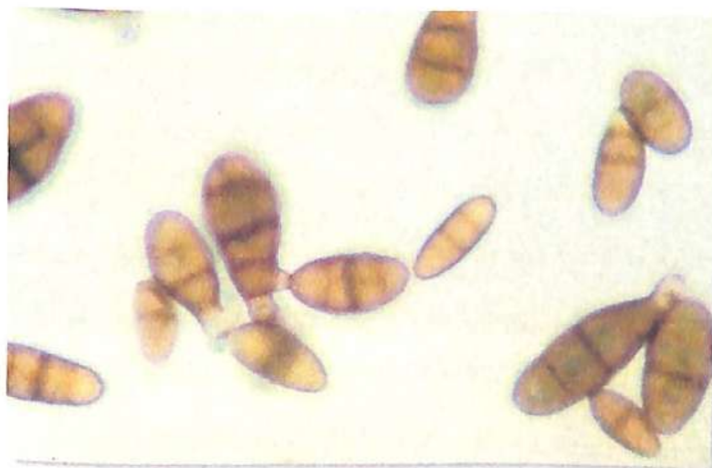

Plate 1. ***Alternaria alternata***

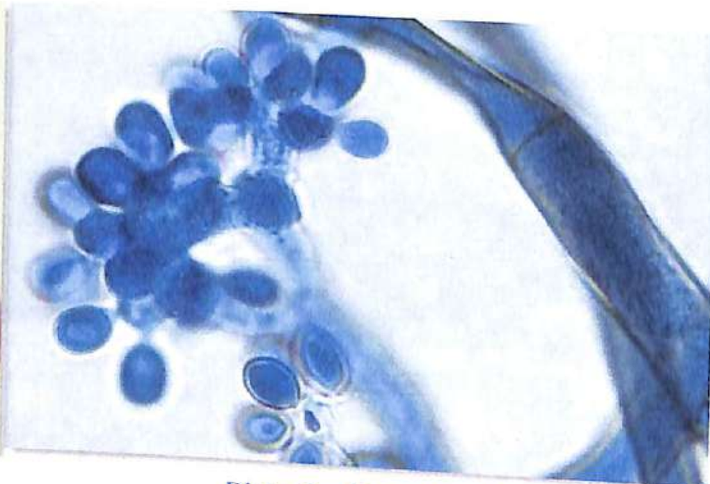

Plate 2. ***Botrytis cinerea***

Plate 3. ***Fusarium oxysporum***

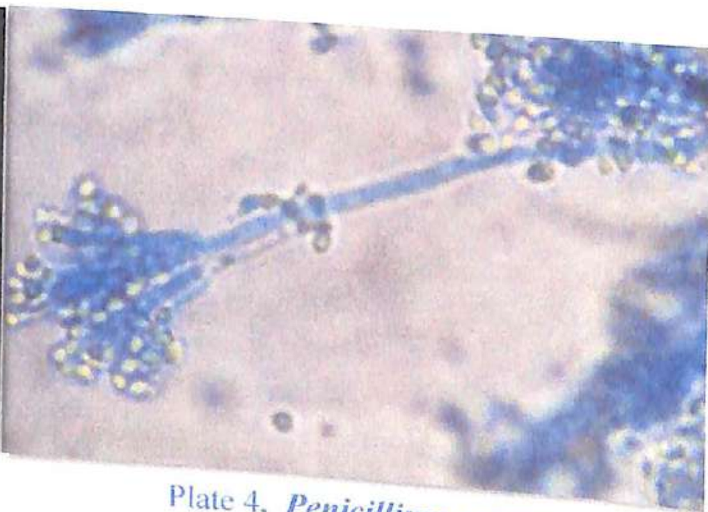

Plate 4. ***Penicillium expansum***

Fungi used for fungitoxic spectrum test

Plate 5. ***Aspergillus niger***

Plate 6. ***Cladosporium herborum***

Plate 7. ***Periconia microspinosa***

Plate 8. ***Rhizopus oryzae***

Effect of essential oils of different plants on the growth of phytopathogenic fungi

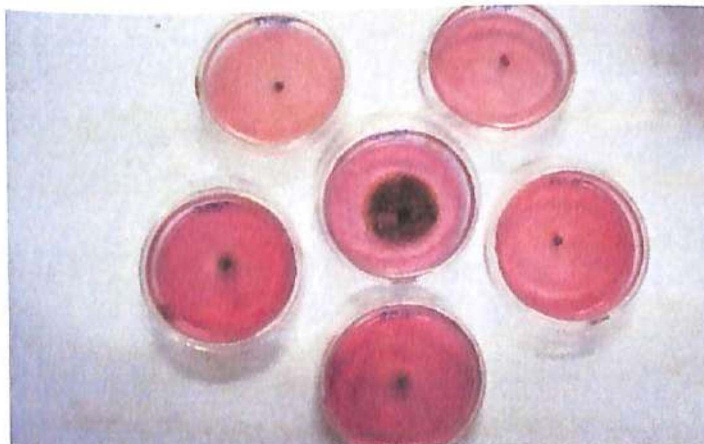

Plate 9. ***Acorus calamus*** **oil tested against** ***A. alternata***

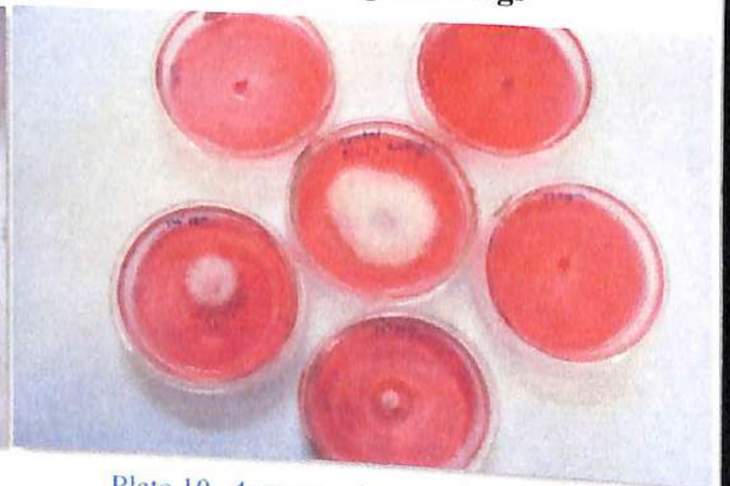

Plate 10. ***Acorus calamus*** **oil tested against** ***B. cinerea***

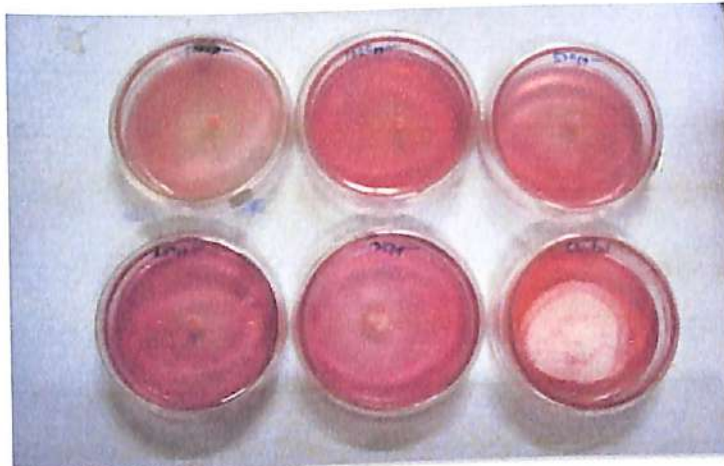

Plate 11. ***Acorus calamus*** **oil tested against**
F. oxysporum

Plate 12. ***Acorus calamus*** **oil tested against**
P. expansum

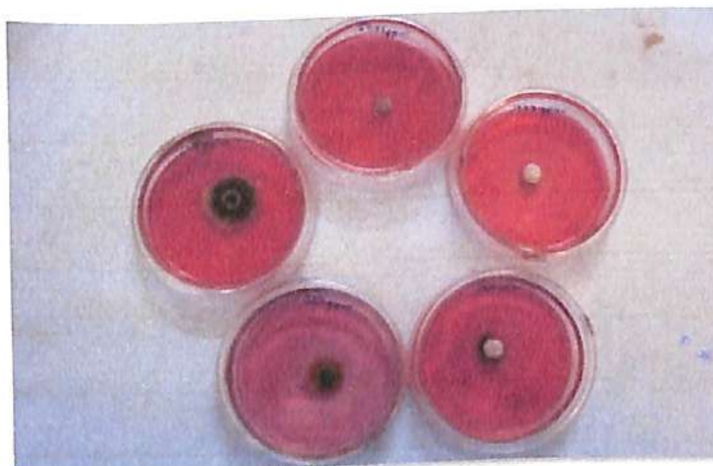

Plate 13. ***Ageratum conyzoides*** **oil tested against**
A. alternata

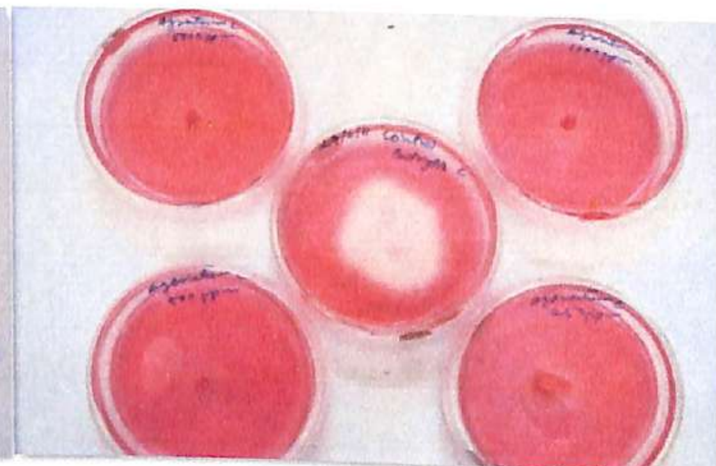

Plate 14. ***Ageratum conyzoides*** **oil tested against**
B. cinerea

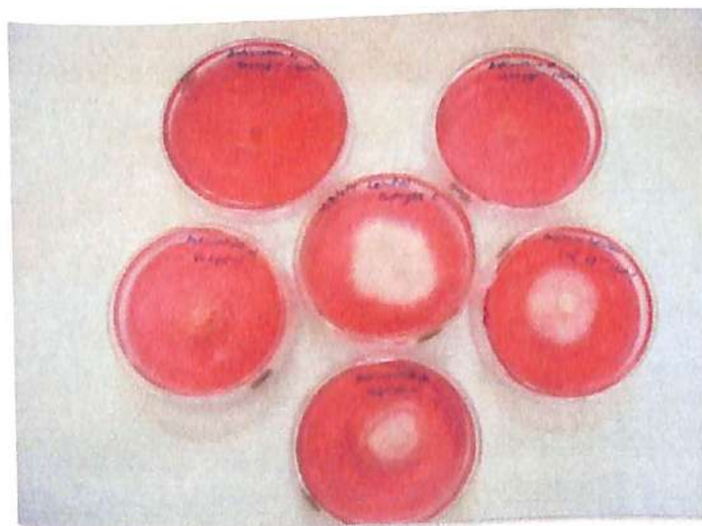

Plate 15. ***Artemisia nilagirica*** **oil tested against**
B. cinerea

Plate 16. ***Erigeron canadensis*** **oil tested against**
B. cinerea

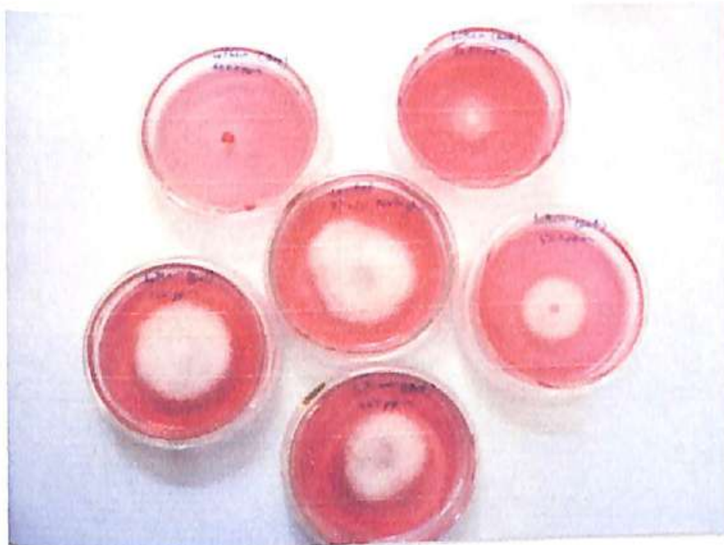

Plate 17. ***Litsea cubeba* oil tested against**
B. cinerea

Plate 18. ***Litsea cubeba* oil tested against**
F. oxysporum

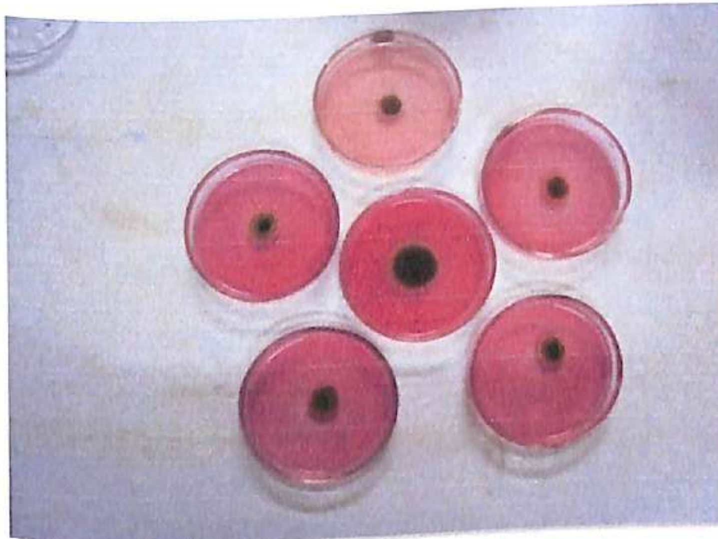

Plate 19. ***Mesua ferrae* oil tested against**
A. alternata

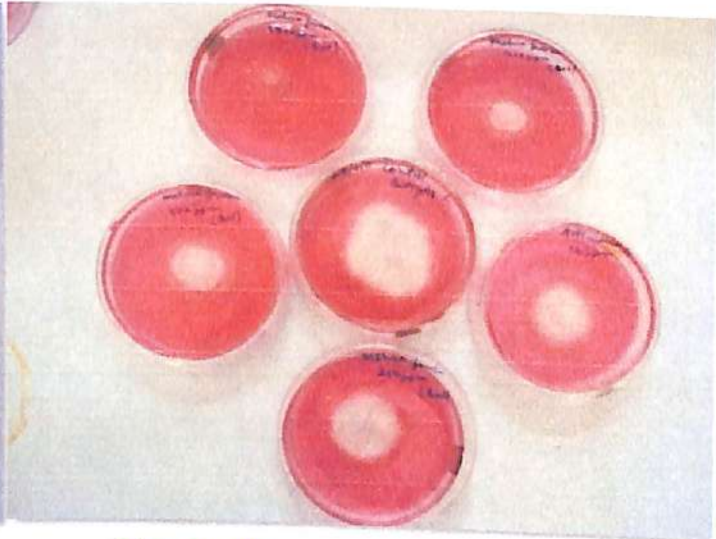

Plate 20. ***Mesua ferrae* oil tested against**
B. cinerea

Plate 21. ***Mikania cordata* oil tested against**
B. cinerea

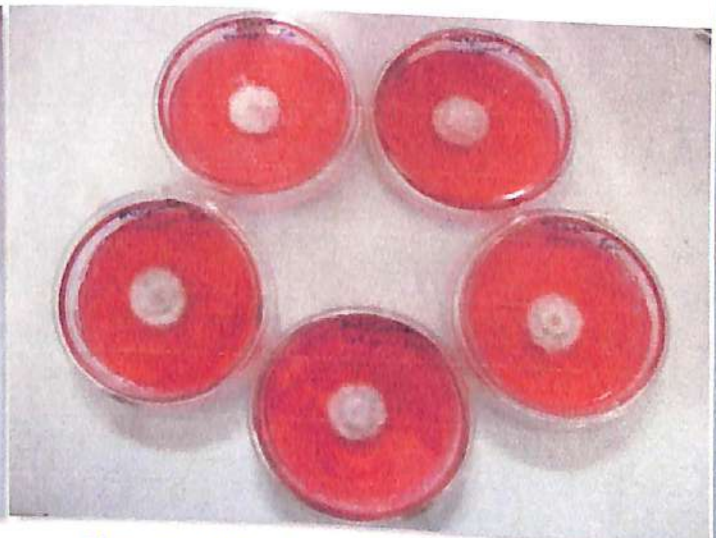

Plate 22. ***Mikania cordata* oil tested against**
F. oxysporum

Plate 23. ***Mikania cordata* oil tested against**
P. expansum

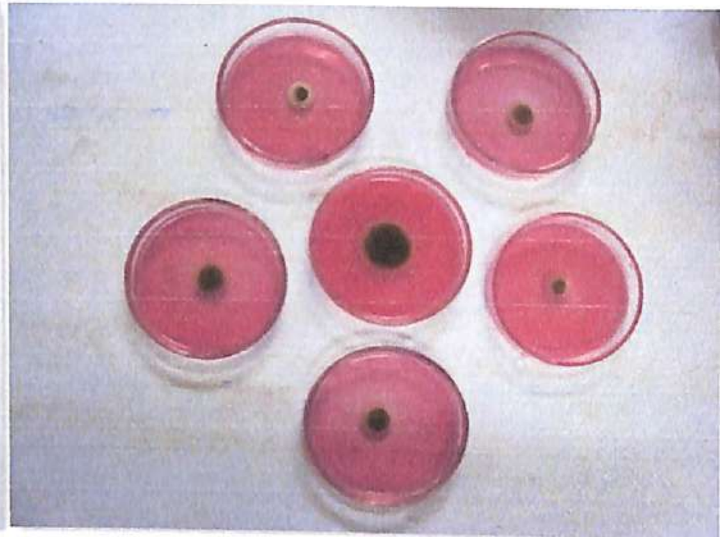

Plate 24. ***Piper mullesua* oil tested against**
A. alternata

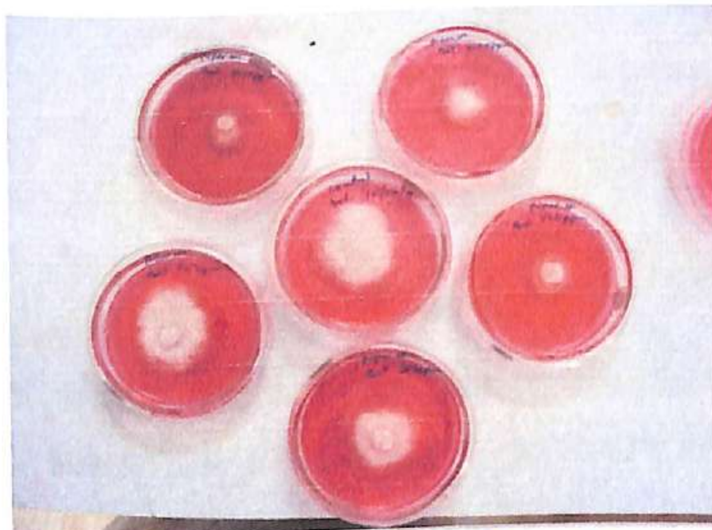

Plate 25. ***Piper mullesua* oil tested against**
B. cinerea

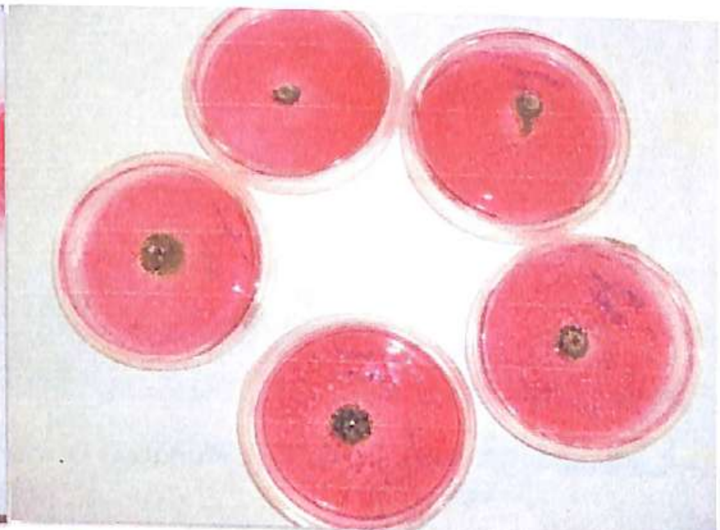

Plate 26. ***Pogostemon cablin* oil tested against**
A. alternata

Plate 27. ***Pogostemon cablin* oil tested against**
B. cinerea

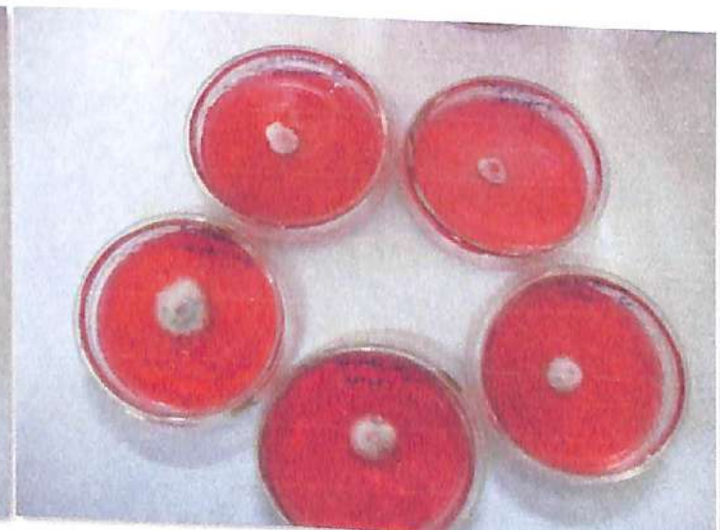

Plate 28. ***Pogostemon cablin* oil tested against**
F.oxysporum

Plate 29. ***Pogostemon cablin* oil tested against *P. expansum***

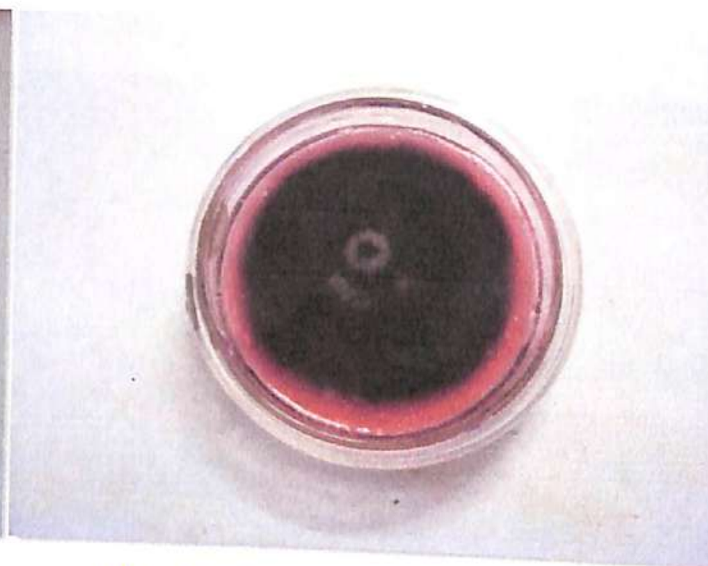

Plate 30. ***Alternaria alternate***(control)

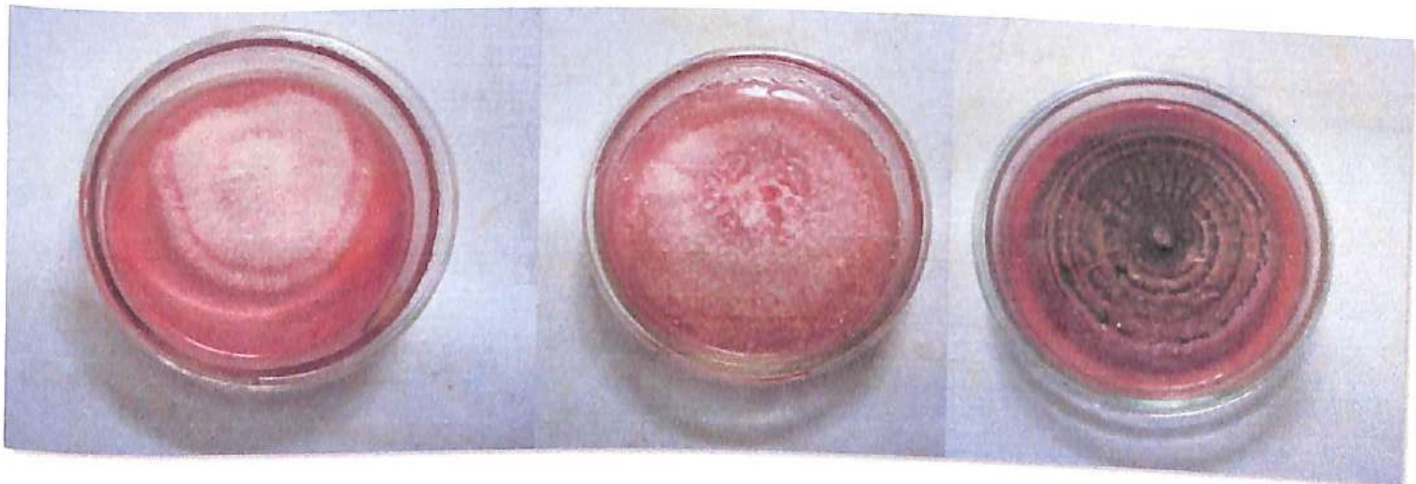

Plate 31. ***Botrytis cinerea***(control)

Plate 32. ***Fusarium oxysporum*** (control)

Plate 33. ***Penicillium expansum*** (control)

Effect of plant extract of different plants on the growth of phytopathogenic fungi (in solvent ethyl acetate, methanol, absolute alcohol, ether, chloroform, benzene respectively)

Plate 34. ***A. calamus* plant extract tested against *A. alternata***

Plate 35. ***A. calamus* plant extract tested against *B. cinerea***

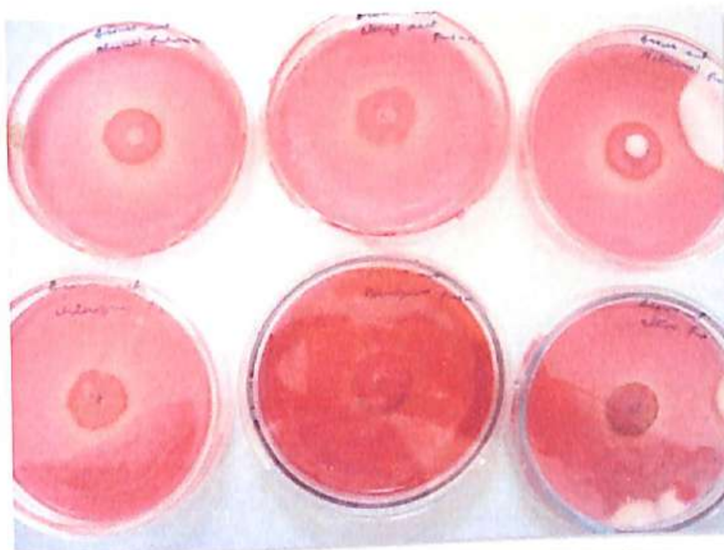

Plate 36. *A. calamus* **plant extract tested against** ***F. oxysporum***

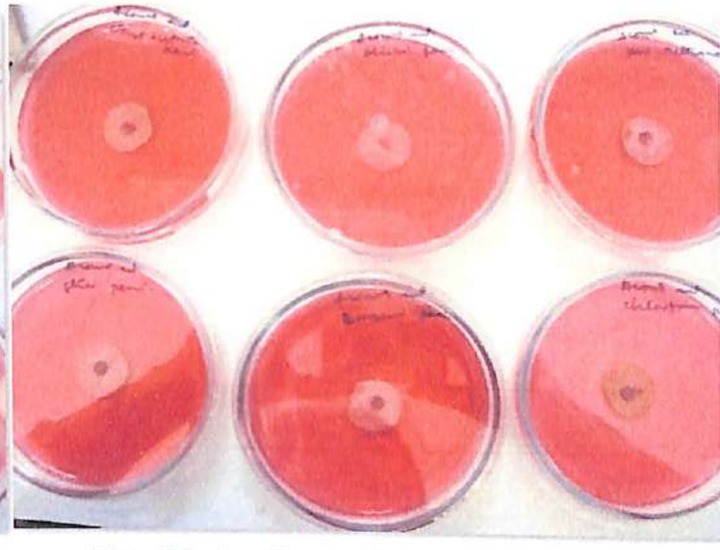

Plate 37. *A. calamus* **plant extract tested against** ***P. expansum***

Plate 38. *A . conyzoides* **plant extract tested against** ***B. cinerea***

Plate 39.*A . conyzoides* **plant extract tested against** ***F. oxysporum***

Plate 40. *A . conyzoides* **plant extract tested against** ***P. expansum***

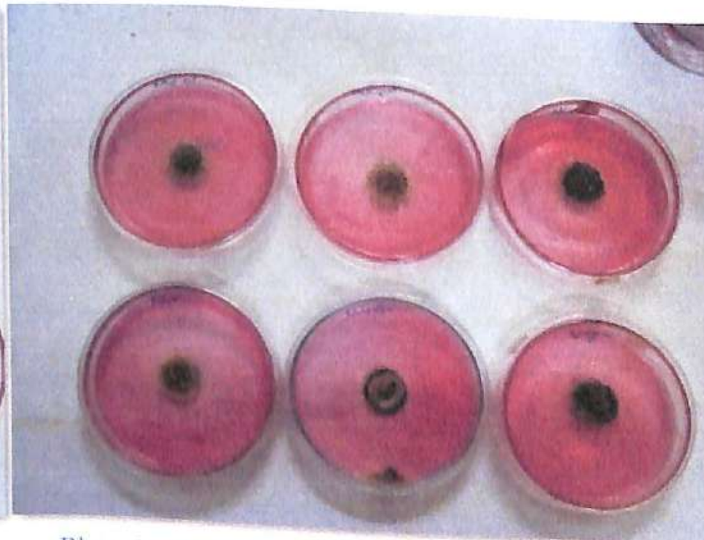

Plate 41. *E. odoratum* **plant extract tested against** ***A. alternata***

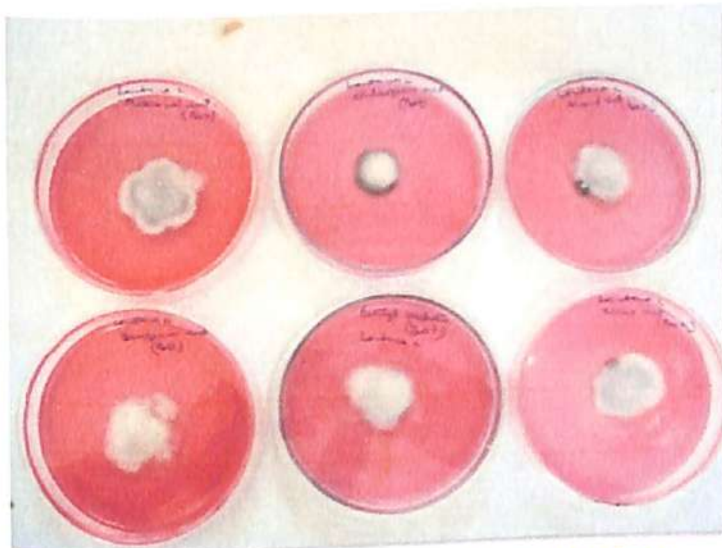

Plate 42. ***L. camara* plant extract tested against *B. cinerea***

Plate 43. ***L. camara* plant extract tested against *F. oxysporum***

Plate 44. ***M. cordata* plant extract tested against *B. cinerea***

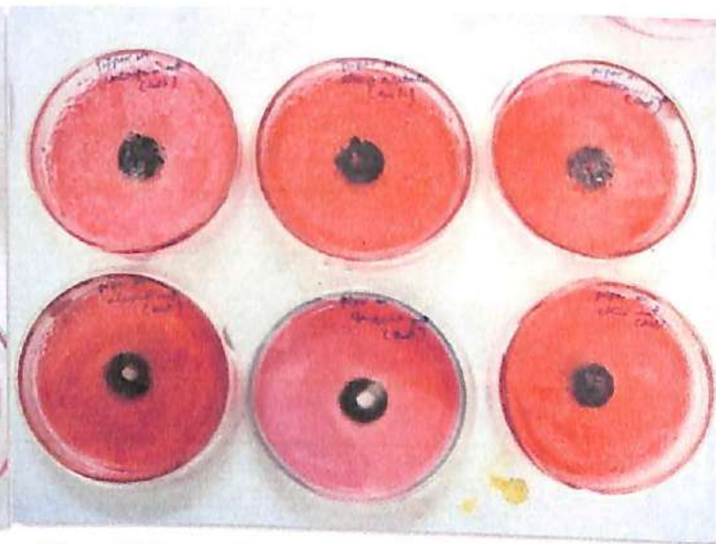

Plate 45.***P. mullesua* plant extract tested against *B. cinerea***

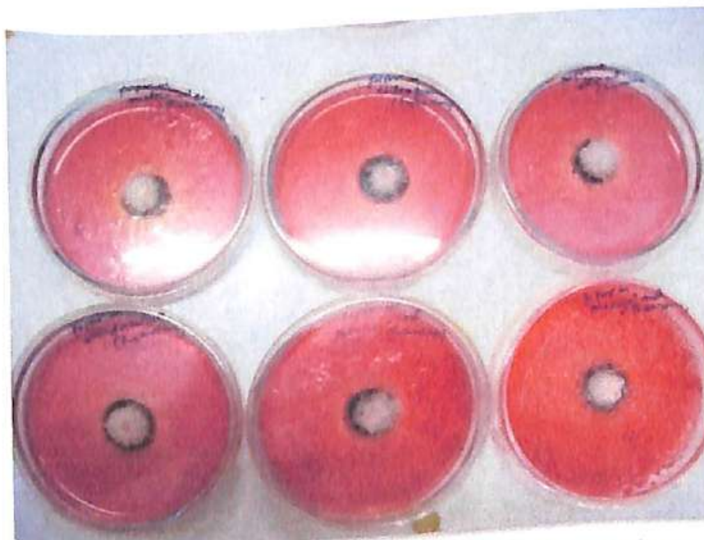

Plate 46. ***P. mullesua* plant extract tested against *F. oxysporum***

Plate 47. ***P. mullesua* plant extract tested against *P. expansum***

Plate 48. ***P. hydropiper* plant extract tested against** ***A. alternata***

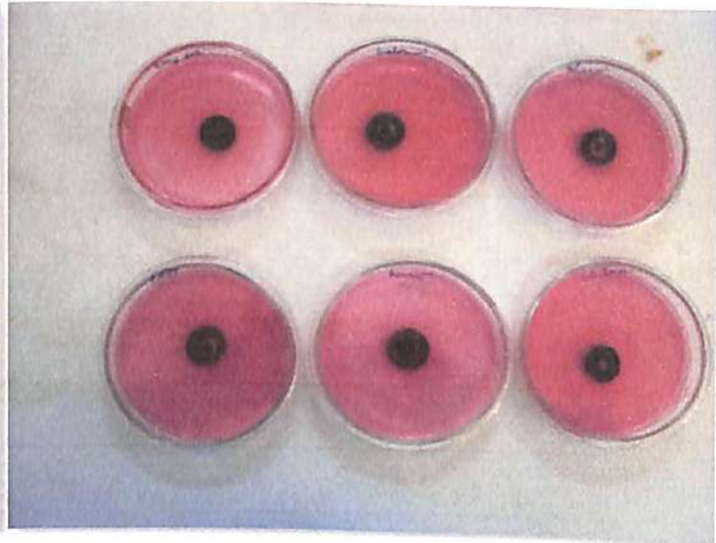
Plate 49. ***P. hydropiper* plant extract tested against** ***B. cinerea***

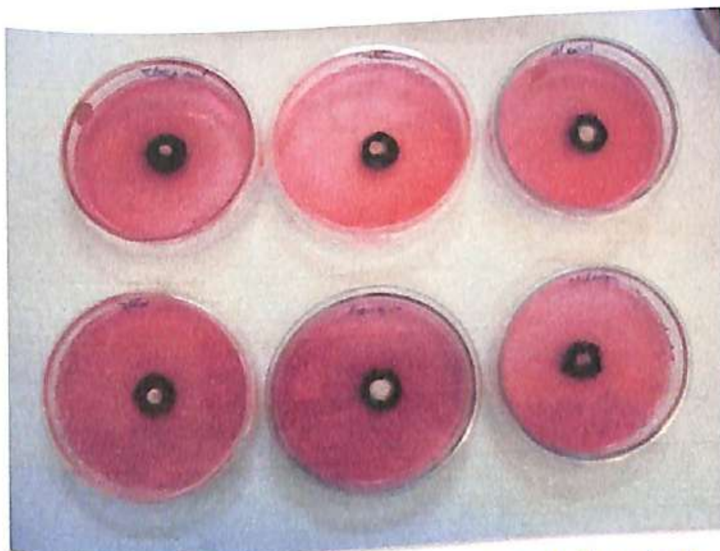
Plate 50. ***P. hydropiper* plant extract tested against** ***F. oxysporum***

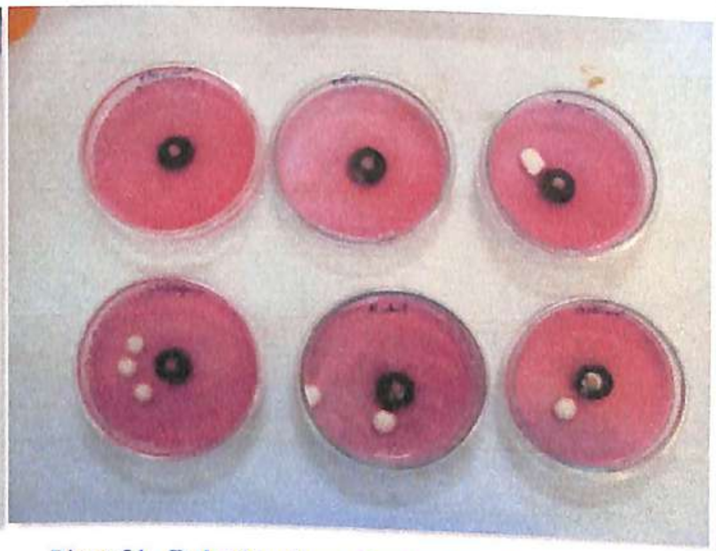
Plate 51. ***P. hydropiper* plant extract tested against** ***P. expansum***

Plate 52. ***S. saman* plant extract tested against** ***F. oxysporum***

Controls

Plate 53. *Alternaria alternata*

Plate 54. *Botrytis cinerea*

Plate 55. *Fusarium oxysporum*

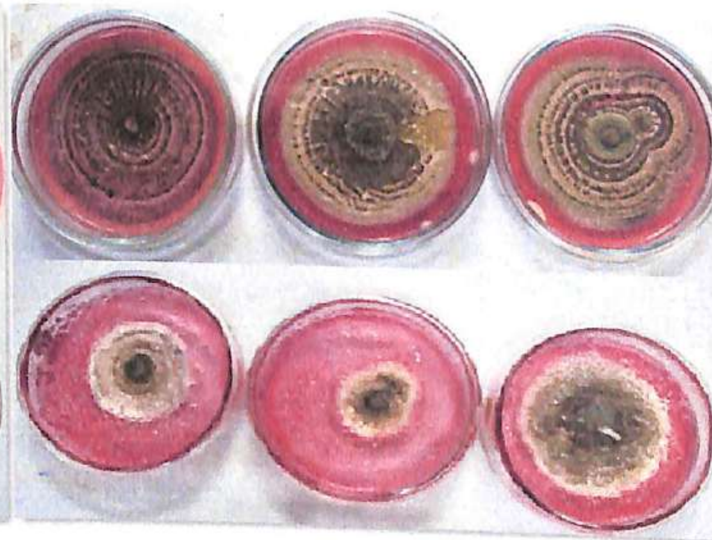

Plate 56. *Penicillium expansum*

In vivio efficacy of essential oils and plant extracts

Dip Method

Fumigation Method

DISCUSSION

Kiwifruits are climacteric and susceptible to fungal decays in postharvest stage and storage. After harvest, fruit rot diseases cause a severe loss of kiwifruit during cold storage, transportation, marketing, and in retail stores (Koh *et. al.*, 2003). Large area of land is under cultivation of Kiwi in Arunachal Pradesh. Fungal species *Alternaria alternata, Botrytis cinerea, Fusarium oxysporum* and *Penicillium expansum* were isolated from the rotting fruits of Kiwi which were collected from the local market. Many fungi are associated with post harvest fruit rots of kiwifruit (Pennycook 1985; Hawthorne *et. al.*, 1982). Botrytis gray mold rot caused by *Botrytis cinerea* is the most important and can directly invade the fruit or enter through wounds. Kiwifruit become much more susceptible to Botrytis (and other fungi) as they soften. Other fungal pathogens *Phomopsis* mali, *Botryosphaeria dothidea, Diaporthe actinidiae, Fusarium oxysporum* and *Penicillium expansum* have also been reported to cause post harvest fruit rots of kiwifruit (Lee *et. al.*, 2001; Koh *et. al.*, 2003). Enyiukwu *et. al.*, (2014) stated that most important losses in agricultural production which involve the greatest costs on the farm economy occur postharvest. It is estimated that worldwide between 10 and 40% losses of agricultural produce occur postharvest. Losses are more severe in developing than developed nations of the world. Several species of fungi and in some cases bacteria participate in postharvest deterioration and rots of tubers and agroproduce. These include species of *Aspergillus, Fusarium, Colletotrichum, Macrophomina, Penicillium* and *Rhizopus* amongst several others.

5.1. Effect of essential oils on phytopathogens

Essential oils were extracted from a number of locally grown plants and on the basis of easy oil extraction and yield only ten plants were selected for evaluation against different fungi. Essential oil of *A. calamus* was recorded inhibitory for the growth of all tested fungi. Colony growth of P. *expansum* and *F. oxysporum* restricted 100% at 5000 and 1000ppm concentration of *A. calamus* essential oil. At 500ppm concentration also 100% inhibition was found up to 9th day of growth in *P. expansum* and 7th day on *F. oxysporum*. Colony growth of A. *alternata* and *B. cinerea* was also inhibited by oil and at 5000, 1000 and 500ppm

concentration 100% inhibition was recorded. At 250ppm also in case of *A. alternata* it restricts 100% up to 7th day and after that slight growth was noticed during subsequent period of incubation. Results indicate that *A. calamus* essential oil have fungitoxic and fungistatic property against the phytopathogenic fungi. Mazza (1985) found, that Indian calamus oil contained high amount of β-asarone (77.7%) and 6.8% α-asarone, but in European calamus oil acorenone (8.1%), isoshyobunone (6.3%), β-gurjunene (6.7%), calamendiol (5.2%) and β-asarone (5.2%) were found to be major components. In the essential oil of the calamus leaves from Lithuania β-asarone (15.7 - 45.5%) was the most abundant compound, whereas acorenone (20.9%) and isocalamendiol (12.8%). The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (Wijesekara *et. al.*, 1997). Radusiene (2007) reported that essential oils of *A. calamus* were dominated by the presence of phenolic compounds: (*Z*)-asarone (15.7–25.5%) and (*Z*)-methyl isoeugenol (2.0–4.9%). Other identified major components were (*E*)-caryophyllene, α-humulene, germacrene, linalool, camphor and isoborneol. Due to presence of a number of compounds and high quantity of phenolics in essential oil of *A. calamus*, perhaps any one of that or in combination would have inhibited the colony growth of the tested phytopathogenic fungi. Satyal (2013) isolated a number of compounds from the essential oil of *A. calamus* and noted cytotoxicity and antifungal activity against *Aspergillus niger*. Sharma *et. al.*, (2007) reported antifungal activity of *Acorus calamus* oil against *Sclerotium rolfsii* and *Rhizoctonia bataticola*. Asha and Ganjewala (2009) found remarkable antifungal activity of *A. calamus* oil against *Aspergillus niger, A. flavus, Microsporum canis* and *Penicillium chrysogenum*. Lee *et. al.*, (2007) attributed antifungal activity of α-asarone and aldehydes present in the *A. calamus* oil.

Essential oil of *A. conyzoides* significantly inhibits the growth of all the three fungi (*P. expansum, F. oxysporum and B. cinerea*) at 5000 and 1000ppm concentration. In case of *A. alternata* impact was not remarkable. Increase in diameter of colony was always remained lesser than control one. Kamboj and Saluja (2008) found a wide range of chemical compounds including alkaloids, cumarins, flavonoids, chromenes, benzofurans, sterols and terpenoids from *A. conyzoides* essential oil. Inhibitory impact attributed to the presence of flavonoids, chromenes, benzofurans, sterols and terpenoids in the oil. Fungitoxic activity of

the oil is very well documented (Sharma *et. al.*, 1978; Kumar *et. al.*, 2010). Amadioha and Markson (2007) found that oil of *Ageratum conyzoides* and *A.melegueta* to significantly arrest the mycelial growth and biomass development of *Botrydiplodia acerina* causal agent of rot of cassava *in vivo*.

Essential oil of *A. nilagerica* inhibits the growth of all phytopathogenic fungi at higher concentration. In case of *P. expansum* at 5000ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* there was 100% inhibition up to 11th day at 5000ppm concentration of oil. However, slight growth was seen on subsequent period of incubation. At low concentration nature of oil was fungistatic. Sati *et. al.*, (2013) reported that essential oil contained approximately 79.91% monoterpenoids and 18.25% sesquiterpenoids. -Thujone (36.35%), -thujone (9.37%), germacrene D (6.32%), 4-terpineol (6.31%), -caryophyllene (5.43%), camphene (5.47%) and borneol (4.12%) as the major constituents. The essential oil exhibited significant antifungal activity against *Rhizoctonia solani* (ED50, 85.75 mg L1), Sclerotium rolfsii (ED50, 87.63 mg L1) and Macrophomina phaseolina (ED50, 93.23 mg L1). Padalia *et. al.*, (2014) found that essential oils were mainly composed of monoterpenoids (59.0%-77.3%) and sesquiterpenoids (15.7%-31.6%). The major constituents identified were artemisia ketone (38.3%-61.2%), chrysanthenone (1.5%-7.7%), germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%), germacra-4,5, 10-trien-1-α-ol (1.9%-4.9%) and artemisia alcohol (1.4%-3.6%). Stappen *et. al.*, (2014) reported that *A. nilagirica* essential oil have nonselective antifungal activity against plant pathogens *Colletotrichum acutatum, Colletotrichum fragariae* and *Colletotrichum gloeosporioides*. Presence of terpenoides in large quantity and other compounds in small quantity would have perhaps played antifungal property against the phytopathogenic fungi.

Essential oil of *Eupatorium odorata* was inhibitory against *P. expansum, F. oxysporum* and *A.alternata* at higher concentration of oil during their initial days of incubation, but during subsequent period of incubation growth of fungus colony was recorded. However, it always remains lesser than control. Joshi (2013) reported a total of twenty-nine compounds from essential oil of *Eupatorium odorata* have been identified, accounting 97.6% of the total oil. The main

constituents were himachalol (24.2%), 7-isopropyl-1,4-dimethyl-2-azulenol (17.6%), androencecalinol (14.1%), and 2-methoxy-6-(1-methoxy-2-propenyl) naphthalene (5.6%). The essential oil consists mainly of phenyl derivatives (41.6%), followed by oxygenated sesquiterpenes ((26.6%), long-chain hydrocarbons (18.9%), sesquiterpene hydrocarbons (6.8%), oxygenated monoterpenes (2.8%), and monoterpene hydrocarbons (0.9%). Owolabi *et al.*, (2010) also determined major Components in essential oil of *E. odoratum* as α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (E)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%). The oil was screened for antifungal activity against *Aspergillus niger* (MIC = 78 μg/mL). Presence of phenols and terpenoids found to be toxic and inhibitory for the growth of fungi.

Essential oil of *Erigeron canadensis* also inhibits the growth of phytopathogenic fungi *P. expansum* and *F. oxysporum* at higher concentration. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. While in case of *A. alternata* and *B. cinerea* at 5000ppm concentration of fungus colony was reduced initially but during subsequent period of incubation growth of colony increases but it always remain lesser than the control. Unnithan (2014) reported in the essential oil of *Erigeron canadensis* a total of 23 components and main constituents were monoterpenoids (limomene 57.2%), camphene (2.5%) α and β-pinenes (1.9 % & 2.1%) and sesquiterpenoids (caryophyllene (6.7%), germacrene D (4.9%) and α-curcumene (3.0%). A few non-terpenoid acetylenic compounds (4.8%) were also detected. Curini *et. al.*, (2003) found essential oils of *E. canadensis* under *in vitro* condition as growth inhibitors against phytopathogenic fungi *Rhizoctonia solani* Kuhn, *Fusarium solani* and *Colletotrichum lindemuthianum* but with weak fungicidal acitivity.

Growth of *P. expansum*, F. *oxysporum* and *A. alternata* was 100% inhibited at 5000ppm. While growth of *B. cinerea*, affected by *Litsea cubeba* essential oil even at 1000ppm concentration. Si *et. al.*, (2012) reported 59 compounds from *L. cubeba* oil out of which dominant components were monoterpenes (94.4–98.4%), represented mainly by neral and geranial (78.7–87.4%), and D-Limonene was in lesser constituent (0.7–5.3%). Several components were only detected in certain regions and compounds such as o-cymene and eremophilene have never before been reported in EOLC. Su *et. al.*,

(2012) identified main components in oil as β-caryophyllene (13.0%), τ-cadinol (11.1%), α-cadinol (8.6%), α-humulene (7.5%), α-pinene (7.0%), globulol (6.6%), and β-eudesmol (6.1%). The anti-wood-decay fungal activity of the oil showed that the oil was inhibitory for wood-decay-fungi species and compounds were determined to be τ-cadinol, α-cadinol, and β-eudesmol. Yang (2010) through preliminary bioassay study showed *L. cubeba* oil has good fungicidal activities against *Sclerotinia sclerotiorum, Thanatephorus cucumeris, Pseudocer-cospora musae* and *Colletotrichum gloeosporioides* at the concentration of 588 and 272 μM, and the essential oil has good fungicidal activities against *T. cucumeris* and *S. sclerotiorum*, with IC_{50} values of 115.58 and 151.25 μg/mL, repectively.

Essential oil of *Mesua ferrea* had fungistatic effect on all the fungi. At initial days there was inhibition but during their subsequent period of incubation, growth of fungus colony was recorded for all four fungi at all concentration of oil. However, the growth of fungus colony always remains lesser than the control. Chanda *et. al.,* (2013) reported that *M. ferrea* effect the growth of *A. niger*. The principal constituents of M. ferrea include mesuaferrone-A & B, mesuaferrol, mesuanic acid, amyrin and sitosterol present in the stamen (Subramanyam and Rao, 1977) while it is reported that seeds contain essential oils, xanthones and coumarins (Subramanyam and Raju, 1977). Sahu *et. al.*, (2014) analyzed that the different parts of the plant contain glycosides, coumarins, flavanoids, xanthones, triglycerides and resins. Specifically it contains α -copaene and germacrene D , ve β-amyrin, β-sitosterol, and a new cyclohexadione compound named as mesuaferrol, mesuanic acid, triterpenoids and resins, reducing sugars, and tannins , saponins, Mesuaferrone B, mesuol.

Essential oil of *Mikania cordata* inhibits the growth of all four phytopathogenic fungi. Hundred percent of inhibition in growth of tested fungus was not found at any concentration of oil. Comparatively *P. expansum, A. alternata* and *B. cinerea* growth was effected more than the *F. oxysporum*. At the end of study period growth of *F. oxysporum* at lower concentration of treatment was similar to control. *Mikania cordata* essential oil consist chemical groups like coumarins and derivatives, sesquiterpenes, sesquiterpenes lactones, diterpenes, phytosterols/terpenoids and flavonoids (Rufatto *et. al.,* 2012). Many authors attributed the pharmacological effect of guaco to coumarin (1, 2-benzopirone) (Santos *et. al.*, 2006). However, our results made clear that this is not the only

bioactive component present in the extracts we tested. Ethyl acetate is a solvent capable of extract coumarin and several terpenes and phenolic compounds, which have a wide range of biological activities. These include plant growth-regulators, secondary compounds against insects, and anti-bacterial properties (Huang *et. al.*, 2009). Some flavonoids and dicaffeoylquinic acid butyl esters have been recently described as bioactive for *M. micrantha* (Wei *et. a*l., 2004).

Essential oil of *Piper mullesua* also inhibits the growth of four phytopathogenic fungi. In case of all four fungi i.e. *P.expansum, F. oxysporum, B.cinerea* and *A. alternata* the effect of oil was quite similar. In case of *P. expansum* percent inhibition ranges between 11 and 60 while in case of *F.oxysporum* it was 28.5 to 71%. Growth of *A. alternata* was inhibited 38 to 62% at different concentration of oil. Myristicin, asarinin, sesamin and fargesin were found in *Piper mullesua* (Srivastava *et. al.*, 2000).

Essential oil of *Pogostemon cablin* was also found to inhibit the growth of all four phytopathogenic fungi. In case of *P. expansum* and *A. alternata* at 5000 and 1000ppm concentration inhibition in growth was almost 90%. At 500ppm also the growth was negligible. In case of *F. oxysporum* and *B. cinerea* during initial period of incubation growth was restricted but after 9th day of incubation enhancement in growth was recorded and difference was approximately between 19 to 37 percent. *Pogostemon cablin* essential oil consist about 70 compounds and out of those Patchoulol, pogostol, seychellene, nor-patchoulinol, patchoulipyridine, methylchavicol, limonene, pinine, p-methoxy cinnamaldehyde, Germacrene and Norpatchoulenol are the main chemical constituents (Chakrapani *et. al.*, 2013, Sallehhudin *et. al.*, 2013; Karimi, 2014). Kocevski *et. al.* (2013) reported antifungal activity of *Pogostemon cablin* essential oil against *A. flavus* at 1500ppm concentration. The Pogostemon cablin (Patchouli) is the most prominent member of family Lamiaceae which has been used against the common cold and as an antifungal agent in traditional medicine (Wu *et. al.*, 2004).

Essential oils are mainly conjugated to phenolic compounds which accumulate in some plant cells, and have positive effects on pathogen control (Plotto, *et. al.*, 2003). It is known that oxidation products of phlorsidzin (an *o*-dihydroxyphenolic compound) inhibit fungal growth, and are thought to inhibit growth of the apple scab fungus, *Venturia inaequalis* (Asghari Marjanlo, *et. al.*, 2009). Fungal pectinases hydrolyse pectin, a plant cell wall compound that is

abundant in the middle lamella and has a key role in cell adhesion. Thus, by inhibiting its pectinases, the ability of fungus to hydrolyse and invade plant cell walls would be compromised (Vermeriss and Nicholoson, 2006). A similar inhibitory role appears to be played by the phenolic compounds in essential oils. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven *et. al.*, 1994). Reports indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim *et. al.,* 1995). Thus, our findings revealed that exogenous essential oils may have a positive influence on the storage-life of kiwi fruit and reduce decay.

5.2. Effect of plant extracts on phytopathogens

On the basis of extracts effect on the tested fungi 18 plants were selected for further study. Plant extract of *Acorus calamus* was found to have significant effect on the growth of all the four phytopathogenic fungi. It was noticed that extract in ethyl acetate, methanol and alcohol was not effective against *Fusarium oxysporum*. But extract in other solvent was recorded inhibitory. Phytopathogenic fungi *Alternaria alternata, Botrytis cineria and Penicillium expensum* growth was significantly affected in all the solvent extracts. Overall restriction of phytopathogenic fungi was 100%. Begum *et, al.*, (2007) reported that ethanolic extracts of *Acorus calamus* and *piper betel* were the two most active plants showing potent antifungal activity against phytopathogenic fungi. The rhizome extract of *A. calamus* exhibited highest antifungal activity inhibiting the mycelial growth completely (100%) against all the 6 test pathogens. Devi *et. al.*, (2009) studied the antimicrobial and antifungal activity of *Acorus calamus* rhizome and leaf and reported that petroleum ether, chloroform, hexane, and ethyl acetate extract of rhizome and leaves and ethyl acetate was highly effective in antifungal and anti yeast activity. Singh *et. al.*, (2010) showed the antifungal activity using methanolic extract of *Acorus calamus.*

Ageratum conyzoides plant extract shows significant inhibitory effect on the growth of phytopathogenic fungi. In case of *P. expansum* and *B. cinerea* growth of fungal colony was found to be inhibited drastically. Methanol and benzene extracts of *A. conyzoides* inhibited 100% growth of *B. cinerea* up to 7th

day of incubation. Growth of *F. oxysporum* was inhibited 35 to 74% under different solvent plant extract treatment. Among the extracts methanol was found quite effective in reducing the growth. In case of *A. alternata* benzene extract inhibit 79 to 86% growth at different interval of incubation. Chandra and Dikshit (1981) screened leaf extracts of some plant species and found *Ageratum conyzoides* to inhibit the growth of *Colletotrichum capsici* and *Penicillium italicum*. Misra *et. al.*, (1988) found leaf extract of *Ageratum conyzoides* and *Chrysanthemum* sp. to exhibit strong activity against *Aspergillus sydoui*. Pal and Kumar (2013) found *Ageratum conyzoides* and *Argemone maxicana*, most effective against phytopathogenic fungi, *Fusarium oxysporum*. The MICs of the extracts were observed to be 6.25 X 10-4, 3.125 X 10-5 and 3.125 X 10-5 l/ml against the tested pathogen respectively. Pattnaik *et. al.*, (2012) reported that extract of *Ageratum.conyzoides* was efficient in inhibiting the growth of *Clavibacter Michigenesis*, mycelial growth of *Alternaria solani*, *Septoria lycopersici*, *Pythium debaryanum* and *Phytopthora capsici*. Hubert *et. al.*, (2013) reported that ethanol extracts of *A. conyzoides* totally inhibited the growth of pathogen *Phytophthora infestans* at 5000 ppm concentration.

In general plant extract of *Artemesia nilagerica* was found to have moderate effect on the growth of phytopathogenic fungi. Plant extract in ethyl acetate, methanol and absolute alcohol was found to inhibit growth of *P. expansum* from 60 to 75%. Extract in ether did not show much effect on the growth of *P. expansum*. In case of F. *oxysporum*, *B. cinerea* and *A. alternata* solvent extract of methanol, chloroform were found inhibitory for the growth. Extract prepared using other solvents were also noticed to restrict the growth of phytopathogenic fungi. Ahmeethunisa and Hopper (2010) reported leaf extract of the *Artemesia nilagerica* inhibitory against 15 bacterial strains. Zaker (2014) evaluated methanolic extracts (ME) and aqueous extracts (AE) of Artemisia for their antifungal effect against *Fusarium solani*, and found that methanolic extracts exhibited better antifungal activity compared to their corresponding aqueous extracts against *F. solani in vitro & in vivo*.

Plant extract of *B. purpurea* was also effective in reducing the colony growth of tested fungus. The growth of *P. expansum* and *F. oxysporum* was not affected by the extract prepared in ethyl acetate, methanol and absolute alcohol. However, growth of *B. cinerea* and *A. alternata* extract with above mentioned

solvent found to reduce the growth from 18 to 50%. Murugan and Mohan (2011) used leaves and stem bark of *Bauhinia purpurea* to prepare extracts in different solvents of various polarities such as petroleum ether, chloroform, acetone, methanol and water. They reported methanol extract most effective against the tested microorganisms and their phytochemical analysis revealed the presence of alkaloids, coumarin, flavonoids, phenols, tannins and terpenoids. Avinash *et. al.*, (2011) observed that aqueous extract of *Bauhinia purpurea* showed strong antifungal activity against *Candida albicans.* While methanolic extract showed moderate to strong antibacterial activity against *B. subtilis, E. coli* and *K. pneumonia.*

The plant extract of *Callicarpa arborea* was found to effect the growth of phytopathogenic fungi significantly. Plant extract in methanol, chloroform and ethyl acetate was recorded to restrict growth of *P. expansum, F. oxysporum,* and *B. cinerea* up to 50%. The colony growth of *A. alternata* was found affected remarkably at the end of study period and inhibition was between 70 to 73% in case of ethyl acetate, methanol and chloroform extract. Inhibitory impact on the colony growth was consistent. Results indicate that effect was till the end of study period and it could be fungitoxic in nature. The ethanolic and aqueous extracts of Calicarpa were antifungal against the fungi *G. fujikoroi, C. neoformans, C. albicans, M. verrucaria, A. niger, N. crassa* and *R. oligosporus* (Yadav *et. al.*, 2012). Yadav *et. al.*, (2012) reported that ethanolic and aqueous extracts of Calicarpa stem inhibit the bacterial growth. Leaves of Callicarpa contain ß-sitosterol, maslinic, oleanolic and ursolic acids and their methyl ester acetates, lupeol acetate and ß-amyrin acetate; heartwood contains ß-sitosterol and oleanolic acid. Bark contains methyl betulinate, baurenol and ß-sitosterol acetate (Ghani, 2003). Jones and Kinghorn (2008) reported that *Callicarpa* possesses antibacterial, antifungal, anti-insect growth, cytotoxic, and phytotoxic activities. In addition to that amino acids, benzenoids, simple carbohydrates, and lipids, numerous diterpenes, flavonoids, phenylpropanoids, phytosterols, sesquiterpenes, and triterpenes have been detected in or isolated from the genus *Callicarpa.* Presence of phenylpropanoids and ß-sitosterol acetate may be attributed to the antifungal activity of the *Callicarpa.*

Plant extract of *Eleocarpus sphaericus* showed variable results. Plant extract prepared using absolute alcohol and methanol was recorded almost 100%

inhibitory for the growth of *B. cinerea.* Plant extract of ethyl acetate was also having 90% inhibitory effect on *B. cinerea.* But extracts in ether, chloroform and benzene were not found restricting remarkably to the growth of *B. cinerea.* In case of *P. expansum* methanol extract was reported to have almost 74% inhibition on the growth. Phytochemicaly *Eleocarpus* consist phenols, flavonoids and tannins. Jayashree *et. al.*, (2014) reported *in vitro* antimicrobial activity of acetone, methanol and water extracts of leaf, stem bark and fruit of *Elaeocarpus serratus* L. (Elaeocarpaceae) against four bacterial species (*Shigella sonnei, Salmonella typhi, Staphylococcus aureus and Klebsiella pneumoniae*) and a fungal species (*Candida albicans*). The plant extracts displayed high antifungal activity against *Candida albicans* especially, the acetone extract was found to be more active antifungal. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen (Jayashree *et. al.*, 2014). Kumar *et. al.*, (2011) found plant extract exhibiting a broad spectrum of antimicrobial activity as it inhibited the growth of *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Penicillium* sp, *Aspergillus flavus, Candida albicans* and *C. tropicalis.*

Plant extract of *Eupatorium odoratum* in general inhibited growth of all four fungi. In extract of absolute alcohol during initial days growth was not restricted but subsequently, decline in colony diameter of *F. oxysporum* was observed. Maximum inhibitory effect of plant extract was observed on *P.expansum, F. oxysporum* and *B. cinerea* under all the solvent treatment. Plant extract of ethyl acetate was found more effective on *P. expansum* as well as *F. oxysporum* whereas in case of *B. cinerea* extract in ethyl acetate, methanol and ether was found effective. Umedum (2013) reported antifungal activity of ethanol extracts of *Eupatorium odoratum* leaf against *Xylohypha bantiana, Alternaria alternata* and *Wangiella dermatitidis.* Sontaya (2007) reported that ethanol extracts of *Eupatorium odoratum* inhibited mycelial growth of *Fusarium oxysporium* and *Collectotrium capsici* by 52.9% and 64.0% respectively. Owolabi *et. al.*, (2010) attributed antifungal activity of the *Eupatorium* due to presence of geijerene/pregeijerene (7.5%) and germacrene D (9.7%) which inhibited the growth of fungi.

Plant extracts of *Lantana camara* found to inhibit the growth of four phytopathogenic fungi. In case of P. *expansum* and *A. alternata* growth of fungus

colony was inhibited partially. In case of *B. cinerea* inhibition in growth of fungus was not significant. Maximum reduction (27%) in growth was found under ether extract treatment. Deena and Thopil (2000) found antifungal activity of Lantana against *Aspergillus niger, Aspergillus parasiticus, Rhizopus oryzae, Fusarium solani, Candida albicans, Colletotricum musae* and *Alternaria brassicola. Lantana camara* contain terpenoids, steroids and alkaloids as major constituents (Saleh, 1974). Perhaps presence of terpenoids effects the growth of phytopathogenic fungi. *Penicillium aeruginosa, Aspergillus niger, Fusarium solani, Candida albicans* appeared as the most sensitive ones due to presence of Umuhengerin, polymethoxylated flavone, isolated from the methanol extract of dried leaves exhibited the antifungal properties in vitro

Plant extract of *Litsea cubeba* was recorded inhibitory for the growth of all four fungi. Extracts prepared in ethyl acetate, chloroform and benzene were noticed to restrict 100% growth of all four tested fungi. Plant extract using ether and methanol as solvent was also severely inhibitory for the growth of all the four fungi. Plant extract of absolute alcohol did not show any remarkable effect on the growth of phytopathogenic fungi however growth always remain poor than the control one. Wong *et al.* (2014) reported strong antifungal activity of *Litsea elliptica* and *L. cubeba* methanol extract against *Fusarium oxysporum. L. cubeba* leaves consist a number of compounds and major constituents are terpenes, citral and phenolic compounds which possibly inhibited the growth of fungi. Gogoi *et. al.*, (1997) reported antifungal activity of L. *cubeba* at different concentrations against *Fusarium moniliforme, Fusarium solani, Alternaria alternata* and *Aspergillus niger.* Zhang *et. al.*, (2012) isolated five novel isoquinoline alkaloids (+)-N-(methoxylcarbonyl)-N-nordicentrin, (+)-N-(methoxylcarbonyl)-N-norpredicentrine, (+)-N-(methoxylcarbonyl)-N-norbulbodione, and (+)-N-(methoxylcarbonyl)-N-norisocorydione, and (+)-8-methoxyisolaurenine-N- oxide attributed to their synergistic effect on the fungi as antifungal activity.

Plant extract of *Michelia champaca* was not found to inhibit the growth of tested fungus almost 100% at any point of study. However, it was noticed that in extracts with some solvents growth reduced and inhibition percent was almost 75%. A remarkable observation was made in the growth of *P. expansum* with plant extract under all solvent that at initial stage inhibition was not severe but

with passage of time reduction in growth was found in increasing order. The previous study noted that the DCM extracts of *Michelia champaca* have the maximum number of growth inhibiting compounds against *Cladosporium cucumerinum* fungus (Oumadevi *et. al.*, 2007). Khan *et. al.*, (2002) used methanol extracts of leaves, seeds, stem and root barks, stem and root heart-woods of *Michelia champaca* and the obtained fractions (petrol, dichloromethane, ethyl acetate, butanol) exhibited a broad spectrum of antibacterial activity. Kumar *et. al.*, (2011) noticed strong antifungal activity of *M. champca* against *Candida albicans*.

Plant extract of *Mikania cordata* was also found to restrict the colony growth of phytopathogenic fungi under all the solvents. The growth of *P. expansum* was severely affected by the plant extract prepared with methanol, absolute alcohol and chloroform. Plant extract prepared using methanol, ether, chloroform and benzene were recorded inhibitory for *F. oxysporum*. While in case of *B. cinerea* ether, chloroform and benzene extract restricted the growth of *B. cinerea* 56 to 63%. The main chemical groups in Mikania leaves and stem are found coumarins and derivatives, sesquiterpenes, sesquiterpenes lactones, diterpenes, phytosterols/terpenoids and flavonoids (Rufatto *et. al.*, 2012). Amador *et. al.*, (2010) reported antibacterial activity in extracts against *Bacillus subtilis* and *Escherichia coli.* Ethyl acetate extracts of this plant exhibited significant antibacterial and anti-inflammatory properties.

Results indicate that extract of *Piper mullesua* remarkably restricted the colonial growth of tested fungi. Inhibitory effect was more significant in case of *B. cinerea* where chloroform extract inhibited 100% growth while extracts of ethyl acetate, methanol and ether do not allow any growth during initial period. After 9th day of incubation ethyl acetate extract show more than 90% inhibition in growth. In case of methanol and ether extract treated fungi inhibition ranges between 65 and 90 percent. In case of *P.expansum, F. oxysporum* and *A. alternata* growth of fungal colony was found inhibited and it ranges from 42 to 70% in *P. expansum,* 28 to 73 in *F. oxysporum* and 39 to 85% in *A. alternata*. Bioactive compounds present in leaves might be inhibitory for the growth of fungi. Srivastava (2001) reported Sesamin and Myristicin, major lignan of *Piper mullesua,* exhibited significant antifeedant activity and moderate growth inhibition towards 4th instar larvae of *Spilarctia obliqua.*

Plant extract of *Polygonum hydropiper* recorded to have significant inhibitory effect on colonial growth of all the tested fungus. Plant extract with chloroform was found to reduced 100% growth of *P. expansum* and *A. alternata,* the reduction of growth of *F. oxysporum* and *B. cinerea* was also 100% at initial stage but at the end of study it was found 87 and 92% respectively. Plant extract prepared with ethyl acetate was also noticed to inhibit growth of *P. expansum* and *A. alternata* 100% till 13th day of incubation but after that slight growth was occurred. Hasan *et. al*., (2009) reported anti fungal activity of *P. hydropiper* against *Aspergillus fumigates, A. niger, A. flavus, Candida albicans, Rhizopus oryzae* and *Trichophyton rubrum.* Shoots of *Polygonum hydropiper* L. (waterpepper), especially in the leaves and flower-heads, contain significant amounts of the sesquiterpenoid polygodial, a compound with a potential use as a natural pesticide. A survey of literature reveals that use of *P. hydropiper* as insecticidal (Roy *et. al.,* 2011) is very well established but antifungal activity of this plant is not yet explored. However, Hussain *et. al.,* (2010) reported antibacterial, antifungal and insecticidal activity in the plant species of under family polygonaceae.

In general plant extract of *Samanea saman* was found inhibitory for growth of all the fungi in all the solvent extracts. The solvent extract of absolute alcohol inhibited 100% growth of *F. oxysporum* and *B. cinerea* during the study period whereas in case of *A. alternata* 100% inhibition in growth was till 9th days but after that slight growth was observed. Plant extract in Methanol was also found to inhibit 100% growth of *B. cinerea* but in case of *F. oxysporum, A. alternata* and *P. expansum* 100% effect was observed for a shorter period. *Samanea saman* consist a number of phytochemicals like saponins, flavonoids, phytosterols and phenolic compounds which act as antibacterial (Raghvendra *et. al.,* 2008). Prasad *et. al.,* (2008) also reported antibacterial activity of *S. saman.* They also reported presence of tannins in the extract of plant. Tannins are water soluble plant polyphenols that precipitate proteins and due to this property microbial growth is stunted as it precipitate proteins in microorganism cells and making unavailable nutritional proteins to them. The growth of many fungi, bacteria and viruses was recorded to inhibit by tannins (Chung *et. al.,* 1998).

Solanum spirale is one of the endemic plants, the extract of this plant was also not found to inhibit the growth of tested fungus significantly. The growth of

P. expansum and *F. oxysporum* in extract prepared with solvent ethyl acetate, ether, chloroform and absolute alcohol was almost equal to control one. Thus, the inhibition in growth of fungi was negligible. The growth of *B. cinerea* and *A. alternata* was effected to some extent and inhibition in growth ranges from 15 to 86%. Prakash and Jain (2011) determined antifungal activity of aqueous and crude extracts from leaves of *S. nigrum* against *A. niger, A. flavus, C. albicans* by dry weight method. Extracts prepared using crude solvents exhibited higher antifungal activity as compared to their corresponding aqueous extracts. No good activity was observed in the aqueous extract. The preliminary phytochemical screening of the leaves revealed the presence of Alkaloids, Flavonols, Flavones, Flavanols, Saponins, Steroids (E)-Phytol (48.10%), n-hexadecanoic acid (7.34%), beta-selinene (3.67%), alpha-selinene (2.74%), octadecanoic acid (2.12%) and hexahydrofarnesyl acetone (Keawsa-Ard 2012).

In general plant extract of *Tagetes errecta* was recorded to restrict the colony growth of all the tested fungi. Among all the tested fungi *B. cinerea* was found to inhibited significantly by extract of all solvents. During the initial stage of incubation up to 9th to 11th days there was 100% inhibition, but in subsequent period colony diameter expanded. However, inhibitory effect was 66 to 93% in extract of different solvents. Shafique *et. al.*, (2011) studied the fungitoxic activity of T. erectus against *Ascochyta rabiei* the causal organism of chickpea blight disease. They found reduction in growth of fungi up to 55-73% *in vitro* condition. Plant leaves produce a substance called alpha-terthienyl which have antimicrobial activity and inhibit the growth of fungi, bacteria and also function as insecticide. Sharma *et. al.*, (2007) also reported antifungal activity of *Tagetes petula* against *Sclerotium rolfsii* and *Rhizoctonia baticola.*

Plant extract of *T. baccata* did not have remarkable inhibitory effect on the phytopathogenic fungi but growth of *P. expansum* to some extant was found restricted in extract of solvent like ethyl acetate, ether and methanol. In case of other fungi inhibition in their growth ranges from 4 to 60%. Plant extract of ethyl acetate and ether was found effective on *F. oxysporum* and *A. alternata.* Growth of *B. cinerea* was not remarkably effected by the plant extract of *T. baccata.* Khan *et. al.*, (2013) reported antifungal activity of *T. baccata* plant extracts in various solvents against different species of fungi *candida.* Bioassays of biflavones from *Taxus baccata,* were performed towards three fungi: *Alternaria alternata,*

Fusarium culmorum, Cladosporiumoxy sporum bilobetin exhibited the significant antifungal activity (Rinalda de Araújo G *et. al.*, 2007). The presence of bilobetin may be the reason for antifungal activity. Baranowskaa and Wiwart (2003) also found *T. baccata* plant extract inhibitory against the growth of fungi and reported presence of bilobetin and Amentoflavone compound in extract which were antifungal in nature

The inhibitory effect of *Z. oxyphyllum* plant extract was moderate on the tested fungus. The growth of *B. cinerea* was found severely affected by the *Z. oxyphyllum* plant extract under all the solvents. Growth of *P. expansum* was recorded more severely affected at initial stage but at the end of study a recovery was noticed. Krohn *et. al.*, (2011) isolated several biologically active alkaloids, including a new quinazoline-6-carboxylic acid from the plant *Z. rhetsa*, showing antibacterial and antifungal property. Chemically the plant contains a terpenoid, xanthyletin and sesamin, alkaloids and flavonoids and an essential oil, sabinene as its key constituents. Prabhash *et. al.*, (2014) determined phytochemical constituents as Flavanoids, glycosides, tannins, saponins, Terpenes, steroids and anthrocyanins. Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries. Volatile compounds from plants,especially essential oils have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematocidal activity (Amvam *et. al.*, 1998; Wilson *et. al.*, 1997). The efficacy of solvents for extracting volatile compounds from plants depends on to polarity of the solvent and the functional components.

Many plant extracts have potential as natural antimicrobial agents that can be applied to agricultural produces, foods and pharmaceutics (Horburg, 1998; Maoz and Neeman, 1998) because they contain a phytochemical that exhibits antimicrobial and cytotoxic effects on microorganisms (Feldberg *et. al.*,1988). Plant extracts contain a wide range of bioactive secondary metabolites which include alkaloids, flavonoids, tannins, saponins, phenols, phlobatannins quinones, lecitins, polyphenols, glycosides, terpenoids, polypeptides and steroids (Edeoga *et. al.*, 2005; Shukla *et. al.*, 2012; Enyiukwu and Awurum, 2013). These bioactive groups of natural products have been given as the reason for their inhibitive roles against pathogens in ethnobotany, drug

application and plant health management (Okwu and Njoku, 2009; Enyiukwu *et. al.*, 2014).

The inhibitory effect of garlic bulb extract on the mycelial growth of *A.tenuis* –causal organism of brinjal leaf spot was reported by Datar (1996). Two sprays of 10% leaf extract of Aegle marmelos combined with 0.01M nickel sulphate at the age of 100 and 115 days after sowing significantly reduced the disease intensity in chilli (Jharia *et. al.*, 1977). An antifungal peptide, Ay-AMP has been isolated, from *Amaranthus hypochondriacus* seeds and was found effective in controlling *A. alternata* at very low doses (Rivillas and Soriano 2007). Ay-AMP degrades chitin and is very resistant to proteases and heating. Cheng *et. al.*, (2008) showed that methanol extract from stems and leaves of *Myoporum bontioides* exhibited inhibitory activity against *A. alternata* with > 58% inhibition at 10gL-1after 12 hr. The active compound responsible for inhibitory activity was identified to be (–) epingaione. The ethanol extract of *Glycyrrhiza glabra* L. (commercial Licorice) and methanol extract of *Taverniera cunefolia* (a wild plant used as a substitute for commercial licorice) at 0.02% concentrations were proved very effective against *A. brassicicola* (Zore *et. al.*, 2004). Kumar *et. al.*, (2004) observed that amongst 4 neem products tested, achook and bioneem were quite effective as compared to furpume and nimbicidine against *A. brassicae*. The strong inhibitory action of ethanol or methanol extract of speed weed (*Polygonum perfoliatum*) against conidial germination of *A. brassicicola* causing leaf spot of spoon cabbage was reported by Ching (2007). Bhardwaj and Laura (2007) screened 20 plants for their antifungal activity against *A. brassicae* which causes leaf spot diseases of brassicaceae and brown rot of cauliflower and found that the maximum inhibitory effect was shown by leaf extracts of *Camellia sinensis* followed by root extracts of *Asparagus racemosus, Aloe vera, Acacia nilotica* and *Anthocephalus cadamba* whereas *Astercantha longifolia* showed moderate inhibition. Growth of *A. solani* on tomato was considerably inhibited by the extracts of garlic bulb and Prosopis leaf (Prasad and Naik, 2003).

5.3. *In Vivo* Efficacy of essential oils and plant extracts

To evaluate the efficacy of essential oils under *in vivo* condition only oils of four plants (*Acorus calamus, Ageratum conyzoides, Artimisia nilogerica* and *Litsea cubeba*) were selected on the basis of their performance for further study.

Kiwi fruits were treated through dip method, impregnated method and fumigation method. In general all the oils were found to enhance the storage life of fruits and increase in life was 9 to 24 days. It was noticed that rotting of fruits delayed for more days those were treated through impregnated method. Similarly four plants (*Acorus calamus, Ageratum conyzoides, Piper mullesua* and *Samnea saman*) were selected for *in vivo* efficacy against phytopathogenic fungi. In case of plant extract fruit rotting was found to delay 7 to 23 days. Likewise in impregnated method delay in rotting was more than dip method.

The compositions of the essential oils can vary greatly depending upon the geographical region, the variety, age of the plant, the method of drying and the method of extraction of the oil. Many essential oils and their constituents are found to exhibit antifungal properties, but the high cost of production of essential oils and the low concentration of active principles often prevent their direct use in the control of fungal diseases of plants and animals. In spite of this limitation, currently, there is much research performed for the development of safer antifungal agents such as plant-based essential oils and extracts to control phytopathogens in agriculture (Costa *et. al.*, 2000). Thus, essential oils and plant extracts are promising natural antifungal agents with potential applications in agro industries to control phytopathogenic fungi causing severe destruction to crops.

Solution contact is the most widespread technique of antimicrobial activity assessment (Zafar *et. al.*, 2002; Ezeifeka *et. al., 2004;* Somda *et. al.*, 2007; Ajibesin *et. al.*, 2008). The active compounds are able to directly inhibit the metabolism of microbial cells (Brogden, 2005). However, some volatile components that do not diffuse well may evaporate with the dispersing solvent during incubation which resulted in the presence of uninhibited activity in many extracts. The vapor contact method is suitable for estimation of the effect of volatile compounds. The vapor of volatile compounds not only circulates within the headspace above the agar medium but also adsorbed in the agar medium (Moleyar and Narasimham, 1986). The findings in this study are in agreement with those in other reports showing that essential oil components are efficient in preventing fungal growth by gaseous contact species (Inouye *et. al., 2000).* Inouye *et. al.*, (2001) reported that vapor of lavender oils and thyme show better antimicrobial activity than solution contact. Nonetheless, the bioactivity on hamster cells revealed that thyme oil

in the aqueous state had a higher activity than that in gaseous contact. This result is supported by Suhr and Nielsen (2003) reported that a large phenolic compound such as thymol in thyme in direct contact with the medium was very effective to control rye bread fungi. In this experiment, the inhibiting activity of vapor contact for *Lavendula angustifolia* and *Ginkgo biloba* was less than that of the solution contact method for these extracts. This would be due probably to insufficient vapor concentration which is related to the evaporation or decomposition in the vapor state of some of the components during long incubation periods. This was suggested by Kalemba and Kunicka (2003). Dorman and Deans (2000) supported this result, finding that some essential oil components such as limonene and ex-pinene were unstable in the vapor state, causing a rapid gas phase reaction with the atmospheric oxidants to yield oxygenated products, though these oil components were stable in an aqueous medium.

Chebli *et. al.*, (2003) indicated that essential oils of *Origanum compactum* and *Thymus glandulosus* inhibited the growth of the mycelium of *Botrytis cinerea.* Also, spore germination and germ tube elongation were also inhibited by the essential oils tested. The effect of essential oils on microbial growth has been reported by Fung *et. al.*, (1977). They thought it may be the result of phenolic compounds of essential oils that cause an altering of microbial cell permeability by interaction with membrane proteins. This would cause a deformation in cell structure and functionality, and permit the loss of macromolecules from their interior (Rattanapitigorn *et. al.*, 2006). Moreover, each of the essential oil components has its own contribution on biological activity of the oil. For example, carvacrol was found as the main compound in ammi oil, while anethole was found in anise as the main compound, and these compounds have more fungicidale effect (Takayuki *et. al.*, 2007).The results showed that used essential oils had the positive effect on storage life and reduce decay content. Previous reports indicated that reduced fruit decay during postharvest treatments with volatile compounds including raspberry and kiwifruit (Wang *et al.*, 2003, Williamson *et al.*, 2007). Essential oils mainly conjugated to phenolic compounds that accumulate in some plants cells and show useful effect for pathogen control (Plotto *et al.*, 2003).It is known those oxidations products of phlorsidzin (an orhto-dihidroxyphenolic compound) inhibit growth of the apple scab fungus *Venturia inaequalsi* (Asghari

et al., 2009). Fungal pectinases hydrolyze pectin, a cell wall compound that is abundant in the middle lamella and plays a role in cell adhesion. Thus, by inhibiting pectinases, the ability of the fungus to hydrolyze and invade the plant cell wall would be compromised (Vermerris *et al.*, 2006). It seems that similar role was done by phenolic compound of essential oils. Thus, these findings reveal that exogenous essential oils may have a positive influence on shelf life and reduce decay peach fruits. This study showed that essential oils were effective to maintain fruit quality. Treated fruits with essential oils had more total soluble solids, TA, anthocyanin and carbohydrate content comparison to control, which was in agreement with previous reports were shown that cinnamon and eucalyptus vapor had significant effect on TSS of strawberry infected to grey mould and increased TSS of fruits (Tian *et. al.*, 2011). Also, Asghari *et. al.*, (2009) reported that titrable acidity of strawberry infected to grey mould, increased with cumin essential oil application. These results indicate that essential oils application significantly decreased weight loss percentage. Previous experiments using natural antifungal compounds (eugenol, thymol and menthol vapors) revealed benefits due to reduced weight loss percentage in cherries and grapes (Serrano *et. al.*, 2005). Similar results were finding with eucalyptus and cinnamon oil in strawberry and tomato on reducing weight loss percentage (Tian *et. al.*, 2011). In fact, there was a linear correlation between ethylene and damage, and thus the fungus was responsible for the majority of ethylene production, a part of the basal level typical of non-climacteric fruits (Cristescu *et. al.*, 2002). Accordingly, it has been reported that grey mould produced greater amounts of ethylene as the concentration of conidia inoculated *in vitro* or in the climacteric tomato fruit increased. The respiration rate was clearly affected by these essential oils concentrations and dimension of infection (Cristescu *et. al.*, 2002).

Essential oils are natural mixtures of hydrocarbons and oxygen- (alcohols, aldehydes, ketones, carboxylic acids, esters, and lactones) containing organic substances of plants. Their constituents and derivatives have a long history of application as antimicrobial agents in the areas of food preservation and medicinal antimicrobial production (Voda *et. al.*, 2003). Biological activities of essential oils depends on the qualitative and quantitative characteristics of their components, which is affected by the plant genotype, plant chemotype, organ of plant, geographical origin, season, environmental, agronomic conditions, extraction

method and storage condition of plant and essential oils (Marotti *et. al.*, 1992; Suhr and Nielsen 2003).

The effect of essential oils on reduction of disease severity and possibility of the use their preservative property was studied to maintain postharvest quality of kiwifruits inoculated with *B. cinerea.* The results confirmed that the tested essential oils showed antifungal activity against *B. cinerea* in the highest concentration compared to lower concentrations. Generally, the results of our study were in accordance with previous study that showed origanum, sweet basil and thyme oils and had not significant effect in control of gray mold rot on inoculated kiwifruits with *B. cinerea* (Thanassoulopoulos and Yanna 1997).

Several reasons about the action of essential oils are presented: in some of reports on the antifungal activity of essential oils attributed to their most abundant components, especially phenolic compounds (Nychas 1995; Tripathi *et. al.*, 2009) and the major compounds detected in essential oils used in this study were thymol, carvacrol, trans-anethole and β-ocimene. The antifungal activity of some phenolic compounds such as eugenol, thymol and carvacrol on apricot, plum, sweet cherry and table grape was investigated previously and their potential in the reduction of fungal decay was confirmed (Liu *et. al.*, 2002; Serrano *et. al.*, 2005; Valverde *et. al.*, 2005). Phenolic components available in essential oils that have lipophilic character act in cell wall and interfere in action of membrane catalyzed enzymes and enzymes responsible for energy and protein production, as a result cause cell death (Kalemba and Kunicka 2003). Also, the antimicrobial activity of phenolic compounds could be related to the presence of an aromatic nucleus and OH group in their structure that caused cell wall degeneration (Farag *et. al.*, 1989). Additionally, the antimicrobial activity of plant extracts and essential oils may be related to total constituents available in their composition and existence of synergistic correlation between total components (Daferera *et. al.*, 2003). It is generally recognized that the antimicrobial action of essential oils depends on their lipophilic character. Rasooli and Owlia (2005) showed that cell wall and cell membrane are the main target of *T. eriocalyx* and *T. x-porlock* essential oils against *Aspergillus parasiticus* and treatment with essential oils caused a severe damage to cell wall, cell membrane, and cellular organelles such as mitochondria.

5.4. Organoleptic test

Fruits treated with *A. calamus* and *L. cubeba* oil were found equal to standard and without any changes in property, whereas, fruits treated with *A. nilagerica* and *A. conyzoides* essential oils and infected by *B. cinerea* after or at the end of study were below standard on being treated through dip and impregnated method while under fumigation method treatment all the four oil treated were found acceptable and almost equal to standard one. In general fruits inoculated with phytopathogenic fungi and treated by *Acorus calamus* and *Samanea saman* plant extract were found equal to standard one. The change in flavor and test of fruits treated with *A. nilagerica* and *A. conyzoides* essential oils might be due to presence some chemical substances which would have deteriorate the test of fruits.

Proper Organoleptic tests are also necessary before any recommendation. The product should be effective even for short duration treatments due to the limited postharvest life of fruit. The treatment should not have an effect on quality parameters such as acidity, flavour and aroma. The lowest suitable dose of the chemicals for practical application should also be determined. Keeping in view the merits of the botanicals as postharvest fungitoxicants, the products which are found efficacious during *in vitro* tastings, should be properly tested for their practical potency based on *in vivo* trials, Organoleptic tests and safety limit profil.

Until now several studies were planned to evaluate the antifungal property of plant essential oils, but a few was carried out to evaluate the effect of essential oils to maintain quality parameters of fruits (Ranasinghe *et. al.*, 2005; Serrano *et. al.*, 2005; Tzortzakis 2007). Serrano *et. al.*, (2005) reported that sweet cherry fruits treated with essential oil constituents such as eugenol, thymol and menthol showed benefits in terms of reduced weight loss and maintenance of fruit firmness in comparison to controls. However, sweet cherries treated with eucalyptol garnered off-flavor. Some of workers stated that cinnamon and clove essential oils did not change organoleptic and physicochemical properties of banana fruits (Ranasinghe *et. al.*, 2005).

Thus, the essential oils of *Acorus calamus, Litsea cubeba, Ageratum conyzoides, Artemesia nilagerica* and plant extracts of *Acorus calamus, Samnea saman, Ageratum conyzoides*, could be a possible new and effective biofungicides to control phytopathogenic fungi of kiwi fruits. Biofungicides are easily

biodegradable, selective and locally produced, especially for the farmers who cannot afford expensive synthetics fungicides. By using weed plant species as raw materials for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species.

In this study, *Acorus calamus, Litsea cubeba, Ageratum conyzoides, Artemesia nilagerica* essential oil and plant extracts of *Acorus calamus, Samnea saman, Ageratum conyzoides,* showed varying antifungal activities against phytopathogenic fungi. It would also be interesting to study the effect of essential oil and organic extracts of the mentioned plants against other important fungi for developing new antifungal agents to control serious fungal diseases in plant, animal and human beings.

Conclusion:

It can be concluded on the basis of present findings that the use of *Acorus calamus, Litsea cubeba, Ageratum conyzoides* essential oil and plant extracts from *Acorus calamus, Samnea saman* could be an alternative to synthetic fungicides for management of post harvest phytopathogenic fungal diseases of kiwifruits. Although in present investigation oil and plant extract are characterized by chemical composition and minimum inhibitory concentration however, some parameters like standardization of their doses during application are desirable before formulation as plant based biocides.

CHAPTER - 6

SUMMARY

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* Hayward] is a climacteric and susceptible to fungal decays in postharvest stage. After harvest, fruit rot diseases cause a severe loss of kiwifruit during cold storage, transportation, marketing, and in retail stores. Many fungi (*Alternaria, Penicillium, Fusarium, Aspergillus, Rhizopus, Phomopsis*) have been reported to create post harvest fruit rots of kiwifruit. Botrytis gray mold rot caused by *Botrytis cinerea* is the most important and can directly invade the fruit or enter through wounds. Kiwifruit become much more susceptible to Botrytis (and other fungi) as they soften. Other fungal pathogens *Phomopsis* mali, *Botryosphaeria dothidea* and *Diaporthe actinidiae* have also been reported to cause post harvest fruit rots of kiwifruit.

Postharvest losses of fruits and vegetables are a serious problem, because the values of fresh product significantly increase while passing from the farm to the consumers. It is estimated that worldwide between 10 to 40% losses of agricultural produce occur are postharvest. Losses are more severe in developing than developed nations of the world. Several species of fungi and in some cases bacteria participate in postharvest deterioration and rots of tubers and agroproduce. These include species of *Aspergillus, Botrytis, Fusarium, Colletotrichum, Macrophomina, Penicillium* and *Rhizopus* amongst several others.

Post harvest and storage pathogens of kiwifruit most often have been controlled by use of synthetic fungicides. In the recent years, pesticide residues on horticultural products, especially the fungicides used in postharvest stage, are of major concerns to the horticultural industry. Also, the increase in consumer's awareness about hazards of pesticide residues on fresh products and their demand to non-residue products, human health, and environmental pollution are considered. Additionally, the reduction in efficacy of fungicides and consequently development of resistant strains of fungi causes that agriculture researches assay new methods and substances to develop effective and safer alternatives to agrochemicals.

Biologically active plant extracts, including essential oils, represent rich potential sources of alternative and perhaps environmentally more acceptable

disease management compounds. Higher plants contain a wide spectrum of secondary substances viz. phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols. These chemicals bear a variety of properties viz. antibacterial, antifungal, antiviral, anthelmintic, anticancer, sedative, laxative, cardiotonic, diuretic and others. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. The preservative nature of some plant extracts has been known for centuries and there has been renewed interest in the antimicrobial properties of extracts from aromatic plants. Active constituents of the medicinal and aromatic plants have been found to be less phytotoxic, more systemic and easily biodegradable. Perusal of literature reveals that so far studies have not yet been carried out to control the post harvest diseases of kiwifruit by using essential oils and plant extracts. Based on the merits of exploitation of plant products for their biological activities, the work was framed to evaluate the potency of some essential oil and plant extracts of commonly occurring higher plants in the management of post harvest rotting of kiwi fruits.

A number of plant species were screened for oil extraction and their effect on phytopathogenic fungi and subsequently based on efficiency and extraction essential oils of ten plant species were evaluated to visualize their effect on the growth of four phytopathogenic fungi of kiwifruit viz. *Penicillium expansum, Fusarium oxysporum, Botrytis cinerea* and *Alternaria alternata* following Poisoned food technique method. Different concentration of oil such as 125ppm, 250ppm, 500ppm, 1000ppm and 5000ppm were taken to evaluate the effect. Simultaneously, a control was also maintained by inoculating culture disc on the medium without adding any oil.

Essential oil of *Acorus calamus* was found effective against the growth of all tested fungi. In case of *P. expansum* and *F. oxysporum* 100% inhibition of growth was recorded at 5000 and 1000ppm concentration. At 500ppm concentration 100% inhibition was found up to 9th day of incubation in *P. expansum* and 7th day for *F. oxysporum*. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* also the effect of oil was significant inhibitory. At 5000, 1000 and 500ppm concentration 100% inhibition was recorded. At 250ppm also in case of *A. alternata* it restricts

100% up to 7th day and after that slight growth was noticed during subsequent period of incubation. Essential oil of *A. calamus* inhibited the growth of all four phytopathogenic fungi, at 5000, 1000 and 500ppm concentration.

Essential oil of *Ageratum conyzoides* was also effective on the growth of all tested fungi at higher concentration 5000 and 1000ppm. In case of *P. expansum, F. oxysporum and B. cinerea* 100% or complete inhibition of colony growth was recorded at 5000 and 1000ppm concentration even after 15 days of inoculation. On *A. alternata* also the effect of essential oil on growth was observed. By and large in comparison to the control growth of fungus at all concentration of essential oil was lesser. Essential oil of *A. conyzoides* significantly inhibits the growth of all the three fungi (*P. expansum, F. oxysporum and B. cinerea*) at 5000 and 1000ppm concentration. In case of *A. alternata* impact was not remarkable.

Essential oil of *Artemesia nilagerica* inhibits the growth of all phytopathogenic fungi at higher concentration. In case of *P. expansum* at 5000ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* there was 100% inhibition up to 11th day at 5000ppm concentration thereafter slight growth observed. But in case of *F. oxysporum* even at 5000ppm concentration of oil slight growth was recorded. *Eupatorium odoratum* and *Erigeron Canadensis* essential oils were also inhibitory against the four phytopathogenic fungi. In case of *P. expansum, F. oxysporum* and *A.alternata* at higher concentration of oil during their initial days of incubation the inhibition was remarkable, but during subsequent period of incubation growth of fungus colony was recorded. However, it always remains lesser than control. Essential oil of *L. cubeba* shows drastic effect on the growth of fungus colony at higher concentration 5000 and 1000ppm. At lower level of concentration *i,e* 125, 250, and 500ppm of oil colony growth was recorded for all the four phytopathogenic fungi but it always remain lesser than the control. The effect of essential oil was corresponding to their concentration. Essential oil of *Mesua ferrea, Mikania cordata* and *Piper mullesua* also inhibits the growth of four phytopathogenic fungi during initial days of incubation there was sever inhibition but during their subsequent period of study growth of fungus colony was recorded for all four fungi at all concentration of oil. However, the growth of fungus colony always remains lesser than the control.

Essential oil of *Pogostemon cablin* was also found to inhibit the growth of all four phytopathogenic fungi. In case of *P. expansum* and *A. alternata* at 5000 and 1000ppm concentration inhibition in growth was almost 90%. At 500ppm also the growth was negligible. While in case of *F. oxysporum* and *B. cinerea* during initial period of incubation growth was restricted but after 9th day of incubation enhancement in growth was recorded and difference was approximately between 19 to 37 percent.

Inhibitory evaluation of essential oils against phytopathogenic fungi showed the effective results by only 4 plant species. Essential oil of *Acorus calamus* was found fungi toxic at 250ppm for *A. alternata* and 500ppm for rest of the three fungi. EO of *Ageratum conyzoides* was inhibitory at 1000ppm for *B. cinerea, F. oxysporum and P. expensum* but it was not found effective against *A. alternata. Artimesia nilagerica* EO was found fungitoxic at 5000ppm concentration against *A. alternata B. cinerea, and P. expensum. Litsea cubeba* EO inhibited growth of test fungus at 1000ppm and 5000ppm. Therefore considering fungi toxic property only four plant oils were selected out of 10 evaluated ones. Concentration for MIC was taken below 5000 ppm only.

Essential oils extracted from different plants were stored for a period of 730 days. Stored oils were tested for their fungi toxic effect at the interval of 30, 360 and 730 days respectively. It was noticed that oil of *Acorus calamus* remains effective even after 730 days on using 500ppm concentration. Similarly oils of *Ageratum conyzoides, Artemisia nilagerica* and *Litsea cubeba* were found inhibitory at 1000, 5000 and 1000ppm concentrations respectively. Results showed that quality of oils was deteriorated even after storage of such a long period. *Acorus calamus, Ageratum conyzoides* and *Litsea cubeba* oils was found fungicidal for all the phytopathogenic fungi. *Artimesia nilogerica* oil was fungistatic in nature only for *P. expansum* and for rest of fungi it was fungicidal in nature. Results related to effect of increased inoculums density of pathogenic fungi on the fungitoxicity of oils were also taken in consideration. In case of *Alternaria alternata* on increasing the inoculums density fungi toxicity of *Acorus calamus, Artimesia nilagerica* and *Litsea cubeba* oils was not affected. But *Ageratum conyzoides* Oil was found decreasing toxicity on increasing the inoculums density. Essential oils were found cent percent inhibitory on *Botrytis cineria* at all density of the fungus. Similar results were obtained in case of

Fusarium oxysporum except in case of *Artimesia nilagerica* oil which was not found affecting the growth of fungi at any concentration of fungal spores. In case of *Penicillium expansum* also fungi toxicity of oils was not altered due to variation in inoculums density of pathogens.

The fungotoxic spectrum of the plant based essential oils was studied by applying against a number of other fungi. Results showed that essential oil of *Acorus calamus* was fungicidal and cent-percent inhibitory for fungi like *Aspergillus niger, Cladosporium herbarum, Rhizopus oryzae.* Fungi *Periconia microspinosa* was found to grow at 500ppm concentration but at 1000ppm concentration colony growth was completely stopped. Essential oil of *Ageratum conyzoides* were recorded fungi static in nature. It stopped the growth of fungi during initial period of incubation up to 11th days but after that slight growth was occurred in petriplates. Essential oil of *Artimesia nilogerica* was also found in fungi static nature for the fungi and after initial inhibition growth started after nine days of incubation. *Litsea cubeba* oils were found fungicidal for all the phytopathogenic fungi and growth of fungi was completely stunted.

Total numbers of 57 plant species were collected to screen for their plant extract inhibitory effect on the fungi. Among the screened plant species eighteen were found inhibiting growth of fungi. On the basis of extracts effect on the tested fungi 18 plants were selected for further study. Plant extracts were evaluate for their effect on the growth of four phytopathogenic fungi of kiwifruit viz. *P. expansum, F. oxysporum, B. cinerea* and *A. alternata* following modified paper disc technique. Plant extracts were prepared using different organic solvents namely ether, benzene, chloroform, ethyl acetate, methanol and absolute alcohol. Simultaneously, a control was also maintained by similarly impregnating with the same amount of requisite respective solvent. Plant extract of *Acorus calamus* was found to have significant effect on the growth of all the four phytopathogenic fungi. It was noticed that extract in ethyl acetate, methanol and alcohol was not effective against *Fusarium oxysporum.* But extract in other solvent was recorded inhibitory. Phytopathogenic fungi *Alternaria alternata, Botrytis cineria and Penicillium expansum* growth was significantly affected in all the solvent extracts. Overall restriction of phytopathogenic fungi was 100%. *Ageratum conyzoides* plant extract shows significant inhibitory effect on the growth of phytopathogenic fungi. In case of *P. expansum* and *B. cinerea* growth of fungal colony was found

to be inhibited drastically. Methanol and benzene extracts of *A. conyzoides* inhibited 100% growth of *B. cinerea* up to 7th day of incubation. Growth of *F. oxysporum* was inhibited 35 to 74% under different solvent plant extract treatment. Among the extracts methanol was found quite effective in reducing the growth. *Artemesia nilagerica* Plant extract in ethyl acetate, methanol and absolute alcohol was found to inhibit growth of *P. expansum* from 60 to 75%. Extract in ether did not show much effect on the growth of *P. expansum*. In case of F. *oxysporum, B. cinerea* and *A. alternata* solvent extract of methanol, chloroform were found inhibitory for the growth. Plant extract of *Bauhinia purpurea* and *Callicarpa arborea* was also effective in reducing the colony growth of tested fungus. The growth of *P. expansum* and *F. oxysporum* was not affected by the extract prepared in ethyl acetate, methanol and absolute alcohol. However, growth of *B. cinerea* and *A. alternata* extract with above mentioned solvent found to reduce the growth effectively. Plant extract of *Eleocarpus sphaericus* showed variable results. Plant extract prepared using absolute alcohol and methanol was recorded almost 100% inhibitory for the growth of *B. cinerea.* Plant extract of ethyl acetate was also having 90% inhibitory effect on *B. cinerea*. But extracts in ether, chloroform and benzene were not found restricting remarkably to the growth of *B. cinerea*. In case of *P. expansum* methanol extract was reported to have almost 74% inhibition on the growth.

Plant extract of *Eupatorium odoratum* and *Lantana camara* in general inhibited growth of all four fungi. Maximum inhibitory effect of plant extract was observed on *P.expansum, F. oxysporum* and *B. cinerea* under all the solvent treatment. Plant extract of ethyl acetate was found more effective on *P. expansum* as well as *F. oxysporum* whereas in case of *B. cinerea* extract in ethyl acetate, methanol and ether was found effective. Plant extract of *Litsea cubeba* was recorded inhibitory for the growth of all four fungi. Extracts prepared in ethyl acetate, chloroform and benzene were noticed to restrict 100% growth of all four tested fungi. Plant extract of *Michelia champaca* was not found to inhibit the growth of tested fungus almost 100% at any point of study. However, it was noticed that in extracts with some solvents growth reduced and inhibition percent was almost 75%. Plant extract of *Mikania cordata* was also found to restrict the colony growth of phytopathogenic fungi under all the solvents and inhibitory effect ranges between 56 and 63%. Plant extract of *Piper mullesua* remarkably

restricted the colonial growth of tested fungi. Inhibitory effect was more significant in case of *B. cinerea* where chloroform extract inhibited 100% growth while extracts of ethyl acetate, methanol and ether do not allow any growth during initial period. Plant extract of *Polygonum hydropiper* recorded to have significant inhibitory effect on colonial growth of all the tested fungus. Plant extract with chloroform was found to reduced 100% growth of *P. expansum* and *A. alternata*, the reduction of growth of *F. oxysporum* and *B. cinerea* was also 100% at initial stage but at the end of study it was found 87 and 92% respectively. *Samnea saman* plant extract of absolute alcohol inhibited 100% growth of *F. oxysporum* and *B. cinerea* during the study period whereas in case of *A. alternata* 100% inhibition in growth was till 9th days but after that slight growth was observed. Plant extract in Methanol was also found to inhibit 100% growth of *B. cinerea* but in case of *F. oxysporum, A. alternata* and *P. expansum* 100% effect was observed for a shorter period. *Solanum spirale* plant extract in any solvent was not found fungitoxic for tested fungi. In general plant extract of *Tagetes errecta* was recorded to restrict the colony growth of all the tested fungi. Among all the tested fungi *B. cinerea* was found to inhibited significantly by extract of all solvents. During the initial stage of incubation up to 9th to 11th days there was 100% inhibition, but in subsequent period colony diameter expanded. However, inhibitory effect was 66 to 93% in extract of different solvents. The inhibitory effect of *Taxus baccata* and *Zanthoxylum oxyphyllum plant* extract was moderate on the tested fungus. The growth of *B. cinerea* was found severely affected by the *Z. oxyphyllum* plant extract under all the solvents. Growth of *P. expansum* was recorded more severely affected at initial stage but at the end of study a recovery was noticed.

To evaluate the efficacy of essential oils under *in vivo* condition only oils of four plants (*Acorus calamus, Ageratum conyzoides, Artimisia nilogerica* and *Litsea cubeba*) were selected on the basis of their performance for further study. Kiwi fruits were treated through dip method, impregnated method and fumigation method. In general all the oils were found to enhance the storage life of fruits and increase in life was 9 to 24 days. It was noticed that rotting of fruits delayed for more days those were treated through impregnated method. Similarly four plants (*Acorus calamus, Ageratum conyzoides, Piper mullesua* and *Samnea saman*) were selected for *in vivo* efficacy against phytopathogenic fungi. In case of plant extract

fruit rotting was found to delay 7 to 23 days. Likewise in impregnated method delay in rotting was more than dip method.

Fruits treated with *A. calamus* and *L. cubeba* oil were found equal to standard and without any changes in property, whereas, fruits treated with *A. nilagerica* and *A. conyzoides* essential oils and infected by *B. cinerea* after or at the end of study were below standard on being treated through dip and impregnated method while under fumigation method treatment all the four oil treated were found acceptable and almost equal to standard one. In general fruits inoculated with phytopathogenic fungi and treated by *Acorus calamus* and *Samanea saman* plant extract were found equal to standard one. The change in flavor and test of fruits treated with *A. nilagerica* and *A. conyzoides* essential oils might be due to presence some chemical substances which would have deteriorate the test of fruits.

Thus, the essential oils of *Acorus calamus, Litsea cubeba, Ageratum conyzoides, Artemesia nilagerica* and plant extracts of *Acorus calamus, Samnea saman, Ageratum conyzoides,* could be a possible new and effective biofungicides to control phytopathogenic fungi of kiwi fruits. Biofungicides are easily biodegradable, selective and locally produced, especially for the farmers who cannot afford expensive synthetics fungicides. By using weed plant species as raw materials for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species.

In this study, *Acorus calamus, Litsea cubeba, Ageratum conyzoides, Artemesia nilagerica* essential oil and plant extracts of *Acorus calamus, Samnea saman, Ageratum conyzoides,* were found having varying degree of antifungal activities against phytopathogenic fungi. It would also be interesting to study the effect of essential oil and organic extracts of the mentioned plants against other important fungi for developing new antifungal agents to control serious fungal diseases in plant, animal and human beings.

Conclusion:

It can be concluded on the basis of present findings that the use of *Acorus calamus, Litsea cubeba, Ageratum conyzoides* essential oil and plant extracts from *Acorus calamus, Samnea saman* could be an alternative to synthetic fungicides for management of post harvest phytopathogenic fungal diseases of kiwifruits.

Although in present investigation oil and plant extract are characterized by chemical composition and minimum inhibitory concentration however, some parameters like standardization of their doses during application are desirable before formulation as plant based biocides.

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