STUDIES ON THE MANAGEMENT OF LEAF BLIGHT OF GRAPE CAUSED BY *Alternaria vitis* (Cav.) Sacc.

By
K. Praveena Deepthi
B.Sc.(Ag)

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ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY,
RAJENDRANAGAR, HYDERABAD-500030, ANDHRAPRADESH,
INDIA.

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CERTIFICATE

Miss. K. Praveena Deepthi has satisfactorily prosecuted the course of research and that the thesis entitled, "**STUDIES ON THE MANAGEMENT OF LEAF BLIGHT OF GRAPE CAUSED BY *Alternaria vitis* (Cav.) Sacc.**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

Date:

Place: **(Dr. T. VITHAL REDDY)**
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON THE MANAGEMENT OF LEAF BLIGHT OF GRAPE CAUSED BY *Alternaria vitis* (Cav.) Sacc.**" submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N G Ranga Agricultural University, Hyderabad, is a record of the bonafied research work carried out by Miss. K. Praveena Deepthi under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

(**Dr. T. VITHAL REDDY**)
Chairman of the Advisory Committee

Thesis approved by the Student Advisory Committee

Chairman : (Dr. T. Vithal Reddy) ____________________________
Professor and Head,
Dept. of Plant Pathology,
College of Agriculture,
Rajendranagar,
Hyderabad - 500030.

Member : (Dr. T. Narsi Reddy) ____________________________
Principal Scientist,
Grape Research Station
Rajendranagar,
Hyderabad – 500030.

Member : (Sri. V.V. Narendranath) ____________________________
Asst. Professor,
Dept. of Statistics and Mathematics,
College of Agriculture,
Rajendranagar,
Hyderabad – 500030.

DECLARATION

I, **K. Praveena Deepthi,** hereby declare that the thesis entitled "**STUDIES ON THE MANAGEMENT OF LEAF BLIGHT OF GRAPE CAUSED BY *Alternaria vitis* (Cav.) Sacc.**" submitted to Acharya N G Ranga Agricultural University for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is a result of original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Date:

Place: **(K. PRAVEENA DEEPTHI)**

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LIST OF SYMBOLS AND ABBREVIATIONS

%	**:**	**percent**
±	**:**	**plus or minus**
µm	**:**	**micrometer**
CD	**:**	**Critical Difference**
cfu	**:**	**Colony Forming Units**
CRD	**:**	**Completely Randomized Design**
cm	**:**	**centimeter**
et al.,	**:**	**and his co workers**
etc	**:**	**and so on**
Fig	**:**	**Figure**
FME	**:**	**FYM + Fish meal + Cellulose**
FYM	**:**	**Farm Yard Manure**
FYME	**:**	**FYM + Paddy straw**
g	**:**	**gram**
GBJ	**:**	**Grape berry fermented Juice**
ha	**:**	**hectare**
i.e.,	**:**	**that is**
in vivo	**:**	**on live host**
in vitro	**:**	**in lab conditions**
lt	**:**	**litre**
mg	**:**	**milligram**

min	**minute**
ml	**millilitre**
mm	**millimeter**
NSKE	**Neem seed kernel extract**
^{o}C	**degree centigrade**
PDA	**Potato dextrose agar**
ppm	**parts per million**
PPB-1Y	**Yellowish phylloplane bacterial isolate**
PPB-2R	**Reddish phylloplane bacterial isolate**
PPB-3Ro	**Rose phylloplane bacterial isolate**
PPB-4W	**White phylloplane bacterial isolate**
PPB-5Cr	**Cream coloured phylloplane bacterial isolate**
PDI	**Percent Disease Index**
SA	**Salicylic acid**
S.EM.	**Standard error of mean**
SEM	**Scanning Electron Microscopy**
SPME	**Sugarcane press mud extract**
VCE	**Vermicompost extract**
w/v	**weight / volume**

Author : K. Praveena Deepthi

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ABSTRACT

Studies were carried out to derive a suitable management strategy for Alternaria leaf blight of grapevine caused by *Alternaria vitis* (cav.) sacc. which is becoming serious in Andhra Pradesh.

The cold sterilised extracts of different fermented materials (Neem seed kernels, Vermicompost, Sugarcane press mud, FYM + Paddy straw, FYM + Fish meal + Cellulose and Grape berries), fungicides, salicylic acid and biocontrol agents were tested against the mycelial growth and spore germination of pathogen.

Grape berry fermented juice is superior over all other treatments in inhibiting the mycelial growth as well as spore germination of *Alternaria vitis*, at its higher concentration. 10 day fermented juice is more effective compared to 20 and 30 day fermented extracts. This is followed by neem seed kernel extract. Other water extracts of composts considerably inhibited the spore germination but not the mycelial growth.

Among the fungicides tested Iprodione 25% + Carbendazim 25% (Quintal) at 0.1% and Indofil M-45(Mancozeb) at 0.25% completely (100%) inhibited the mycelial growth and spore germination of pathogen. Iprodione 25% + carbendazim 25% (Quintal) at 0.05% given 100% inhibition of mycelial growth.

Salicylic acid is not consistent in its performance in inhibiting the mycelial growth. The maximum inhibition was 14.05% at its higher test concentration of 125 ppm. At the same concentrations it inhibited the spore germination upto 45.62%.

The biocontrol agents *Trichoderma viride* and *Pseudomonas fluorescens* showed 66.67% and 55.6% of inhibitions respectively.

The microflora, isolated from the grapevine phylloplane include ten fungal species like *Trichoderma harzianum, T. viride, Aspergillus niger,* *A. tumarii, A. fumigatus, Paecilomyces variotii, Thielavia terricola, Cladosporium cladosporoides, Oidiodendron sp.*, *Alternaria sp.* and five bacterial isolates PPB-1Y , PPB-2R, PPB-3Ro, PPB-4W, PPB-5Cr. were observed. Among the fungi *Trichoderma harzianum* recorded highest mycelial growth inhibition of 72.8% and *Pseudomonas fluorescens* was the best among the bacteria with 65.91% growth inhibition and 5.34mm of inhibition zone.

The treatments, which were proved effective in *in vitro* studies, were tested in the glass house on grapevine rooted cuttings of Thompson seedless variety for their efficacy against the Alternaria leaf blight. In all the methods of testing 10 day unsterilised grape berry fermented juice recorded minimum Percent Disease Index. Prophylactic application of 10 day unsterilised grape berry fermented juice at 25% and Iprodione25% + Carbendazim25% (Quintal) at 0.1% recorded minimum Percent Disease Index of 14.8% each followed by native *Trichoderma harzianum*, native *Pseudomonas fluorescens* with 17.29% each and *Pseudomonas fluorescens* (Bio control agent) with 22.23%. All the treatments were considerably effective in reducing the Percent Disease Index.

CHAPTER I

INTRODUCTION

Cultivated Grapevine (*Vitis vinifera* L.) belongs to the family Vitaceae. It is a perennial climber with woody shoots and leathery leaves which bears flowers in clusters in the leaf axils. It is best grown in red loamy soils with moderate rainfall.

The commercially grown grapes are used mainly as table grapes, raisins and for wine making. Grapes are a good source of minerals like calcium, phosphorous, iron, and vitamins like B_1 and B_2. Its juice is mild, laxative and acts as stimulants for kidneys (Chadha & Sikhamany, 1999.).

Grape is cultivated in Europe, North and Central America, India, China, Turkey, Israel, Argentina, Chile, South Africa and Algeria etc. In India, it is grown in the states of Utter Pradesh, Haryana, Punjab, Maharashtra, Andhra Pradesh, Karnataka and Tamilnadu (Bhosale *et al.,* 2004) in over 34000 ha with an annual production of 10 lakh tonnes (Ramarethinam *et al*., 2004).

In Andhra Pradesh grape is cultivated in an area of 3500 ha with an annual production of 80,000 tonnes, mainly in Rangareddy and partly in Mahaboobnagar, Medak, Nalgonda, Ananthapur and Chittor districts (CMIE-2004). Important varieties grown in this state are Anab-e- Shahi and Thompson seedless.

In recent times grape has emerged as promising export oriented fruit crop and is being exported to 45 countries all over the world, mainly to UK, UAE, Netherlands, Bangladesh, Srilanka, Saudi Arabia and Oman earning nearly Rs 100 crores annually (Bhosale *et al.,* 2004).

This high value crop is often subjected to the risk of yield loss to a considerable extent due to unprecedented biotic and abiotic stresses like pests, diseases and deficiencies.

Nearly 100 types of fungal, bacterial, viral and mycoplasmal diseases are occurring all over the world on grape (Pearson, 1993). Among them downy mildew, powdery mildew, anthracnose are of major importance. Due to usage of specific fungicides to manage the above major diseases, the diseases like Alternaria leaf blight etc. are becoming major diseases in grape growing districts of Andhra Pradesh causing considerable losses.

This disease was first reported by Vidhyasekaran *et al*., (1969) on Khandari variety in Tamilnadu and the symptoms were clearly defined. Later this disease was reported and studied by Suhag *et al.,*

(1980) in Haryana on Thompson seedless, Beautiseedless, Perlette, Delight, Kishmishcherni and Kishmish beli etc.

In AP the leaf blight caused by *Alternaria vitis* (Cav) Sacc. occurs through out the growing season under favourable conditions and cause huge losses at times. The symptoms of the disease vary from small minute chlorotic spots to complete blighting of the leaves.

In view of frequent occurrence of Alternaria leaf blight disease and its increase in severity season after season and non-availability of suitable management strategies, the present research programme is taken up with the following objectives.

1. *In vitro* testing of composts, organic amendments, bio control agents, Agro chemicals, and fungicides for their antipathogenic properties.
2. Evaluation of phylloplane microflora of grapevine for their antagonism to *Alternaria vitis*.
3. *In vivo* testing of the effective treatments for the management of the disease on grapevine in pot culture.

<u>Plate 1:</u> Grapevine orchard with Alternaria leaf blight infection.

<u>Plate 2:</u> Severely blighted grapevine foliage due to infection of *Alternaria vitis*.

CHAPTER II

REVIEW OF LITERATURE

The work done on the grapevine leaf blight disease is reviewed under this title. As the specific research is very less on the particular pathogen *Alternaria vitis*, the investigations done on the efficacy of fungicides and biocontrol agents against different *Alternaria spp* are also considered for review. The subject of compost teas is recently gaining importance in plant disease management. So their efficacy against diverse phytopathogens is summerised , rather specifically on *Alternaria spp*. Similarly the importance of salicylic acid in protecting the various crop diseases, influence of phylloplane microflora on different grape pathogens, reports on antagonistic activity of yeasts against phytopathogens are also reviewed briefly.

2.1 DISEASE - PATHOGEN

In India, first report on the occurrence of Alternaria leaf blight of grapevine was given by Vidhyasekaran *et al*., (1969) in which the conidial morphology of *Alternaria vitis* was described as slightly verrucose, brownish, swollen at the base and gradually tapering at the proximal end. They had a filiform apex, which may be lighter in shade than the rest of the spores and ending in a straight false beak. Spores were produced singly and not in chains. Spores measured 25.0 – 62.5µ m X 10-17.5 µm with a mean of 39.25µm x 14 µm.

Suhag *et al*., (1980) studied the effect of temperature, relative humidity and precipitation on the four pathogenic fungi of grapevine namely *Sphaceloma ampalina*, *Alternaria vitis*, *Helminthosporium rostratum*, *Pseudocercospora vitis*. They reported that the best temperature for *A. vitis* is 30-40^{O}C and the incidence of disease decreases with increase in relative humidity during monsoon. The four pathogenic fungi cause extensive damage to succulent vines. The suitable temperature and pH for spore germination of *A. vitis* are 30^{O}C and 4.5 respectively (Sushag and Kaushik, 1982).

Liu *et al*., (1996) discussed the integrated prevention steps to control *Alternaria viticola* on grape vine, which include strengthening cultivated management to increase disease resistance and application of shajunbao 300 or carbendazim which gave good control of the disease. The optimum temperature reported for conidial germination is 25-27^{O}C.

2.2 COMPOST WATER EXTRACTS AND COMPOST TEAS

2.2.1 AN INTRODUCTION

Compost water extract is made from compost suspended in a barrel of water for 7-14 days (Steve Diver, 2002). The key factors influencing the effectiveness of the compost tea were the age of the compost and nature of its source ingredients (Brinton *et al*., 1996).

The primary benefit of the compost teas will be a supply of soluble nutrients, which can be used as liquid fertilizer (Steve Diver, 2002). In addition to the nutrition they can be used potentially in agriculture for plant disease suppression (Riggle, 1996). Compost extracts can be sprayed on crop to coat leaf surfaces, and so that they provide resistance to infection from pathogens (Grobe, 1998).

Compost teas are very beneficial in plant disease management and they can be included in the integrated disease management strategies of field and horticultural crops (Block, 1997; Orlikowski and Wolski, 2000; Quarles, 2001). Even the addition of this organic extracts to growing media encourage the growth of benign organisms, which suppress the plant diseases (Dixon *et al*., 1998).

Compost teas show multiple modes of activity in suppressing plant diseases like, induced resistance, antibiosis and competetion. Regardless of the mode of action, preventive application before pathogen infection appears necessary for optimal control through all known mode of actions (Scheuerell and Mahaffee, 2002).

2.2.2 COMPOST TEAS AND COMPOST WATER EXTRACTS AGAINST PLANT DISEASES

Scheuerell and Mahaffee (2002) reported that a variety of plant foliar pathogens and diseases have been suppressed by application of non aerated compost tea while few organic extracts were with limited control options. Later (2004) they reported the effect of aerated and non aerated compost tea , produced with or with out additives for the suppression of damping off of cucumber caused by *Pythium ultimum*. Aerated compost tea produced without the molasses based additives, had a threshold of bacterial population density above 7 log 10 cfu/ml which makes compost teas suppressive.

Dittmer *et al*., (1990) reported that the treatment of extracts of grape marc and cow manure resulted in a reduction in the incidence of *Erysiphe graminis* fsp *hordei* and an increase in barley yield. Similar results were reported by Winterscheidt *et al*., (1990) while working with downy mildew of cucumber caused by *Pseudoperonospora cubensis*. The water extracts of cattle and horse manure and grape marc reduced mycelial growth and inhibited conidial germination of *Botrytis cinerea* and reduced the incidence of *B.cinerea* on detached grape leaves. The disease incidence was influenced by incubating time (Ketterer *et al*., 1992).

The efficacy of extracts of fermented mature compost prepared from cattle manure, chicken manure and grape marc was tested by Elad and Shtienberg (1994) against grey mold on tomato and pepper plants and on grape berries. All the extracts reduce the disease by 56-100 % after a fermentation lasting >10 days. Tsror *et al*., (2003) indicated that the water extracts from cattle manure compost, Kaligrin and Difol (Fish oil) grape marc compost AQ10 significantly reduced the incidence of powdery mildew caused by *Leveillula taurica* on pepper in comparison with the non treated control.

Wickramaarachchi *et al*., (2003) assessed the possibility of inducing resistance in tomato against *Alternaria solani* using foliar spraying of compost water extracts. Foliar spray of 100% cattle manure based compost extracts amended with *Trichoderma viride* was superior over the other treatments. Similarly, a significant reduction in the incidence of early blight caused by *Alternaria solani* was observed with 14 day old compost extract prepared in a ratio of 1:5 compost to water and the yield of tomato in treated plants was significantly higher than in the control (Tsror and Bieche, 1999).

Papegeorgiou *et al*., (2002) reported that the mode of extraction affects the efficacy of watery compost. The extract incubated at 20^{O}C reduced the incidence of *A. solani* by 43% compared to the control.

Tosi *et al*., (2004) reported that the efficacy of aqueous extracts of composts used for foliar spraying depends on the method of composting and stabilisation time which varies from compost to compost.

An experiment was carried out by El Marsy *et al*., (2002) *in vitro* and *in situ* to determine the effect of various composts and their water extract on different fungi. *In situ* result indicated that the concentration of compost water extract at 5, 10 and 15% suppressed *Sclerotium bataticola,* by 83% using 5% crop compost and by 94% using 5% leaf compost and 10% garden compost. Treatments with compost extracts obtained from 2-3 year old cattle manure and nettle extract had an inhibitory effect on leaf diseases like anthracnose and Septoria leaf spot and increased yields of white currant during the two year organic cultivation experiment (Parikka *et al*., 2002).

Yohalem *et al*., (1994) reported that of more than 30 compost materials tested, anaerobically fermented aqueous extracts of spent mushroom substrate was most effective in the inhibition of conidial germination of *Venturia inaequalis*, causal agent of apple scab. Smilarly, the aqueous extracts obtained from anaerobically fermented spent mushroom substrates, significantly reduced the leaf area affected by *V. inaequalis* (Yohalem *et al*.,1996). Cronin *et al*., (1996) also reported similar results, that the clarified water extracts of slurries of spent mushroom substrates inhibited *in vitro* germination of conidia of the apple scab pathogen *Venturia inaequalis* by upto 98% relative to germination in water controls.

Nakasone *et al*., (1999) reported that the anaerobic aqueous extracts obtained by mixing vermicompost and organic compost at a ratio of 1:1 with water were effective in suppressing the mycelial growth of *Colletotrichum sp, Alternaria solani, Botrytis cinerea, Sclerotinia sclerotiorum, Sclerotium rolfsii, Rhizoctonia solani and Fusarium oxysporum* fsp *lycopersici*. The vermicompost extract at a concentration of 40 µg/ml caused a 50% reduction of zoosporangia formation of *Phytophthora cryptogea* and completely inhibited the sporulation at 1000 µg/ml (Orlikowskii, 1999). But Szczech (1999) observed negative results while working with wilt of tomato. He reported that the sterilised extracts of vermicompost added to PDA stimulated the growth of *Fusarium oxysporum* f.sp *lycopersici* mycelium a wilt pathogen in tomato.

Ishida *et al*., (2001) observed that the sprays of the aqueous extracts of vermicompost with a concentration higher than 50% and application twice a week reduced the severity of the zuchhini squash powdery mildew caused by *Sphaerothaeca fuliginea*. Singh *et al*., (2003d), showed that aqueous extracts of vermicompost inhibited spore germination of several fungi at very low concentrations 0.001-0.5% and also effected the powdery mildew of balsam and pea caused by *Erysiphe cichoracearum* and *Erysiphe pisi* by inducing the synthesis of phenolic acids which develops resistance in plants against diseases.

Orlikowski and Skrzypezak (1997) reported that post plant drenching of *Pelargonium* with 0.1% of keratin bark urea extract protected 60% of plants against *Phytophthora nicotinae*. A preventing spraying with keratin bark urea extract at 0.1 and 0.2% significantly reduced the spread of Myrothecium leaf spot in *Pelargonium* (Orlikowski *et al.*, 1998). Wolski and Glinski (2001) reported that the extracts from bark urea, keratin bark urea granules, vermicompost, *Echinacea purpurea,* grape fruit and garlic preparation of furano coumarins and chitosan were appeared to be effective against certain plant diseases.

Ma Li Ping *et al*., (1996) observed that compost extracts of horse and cow manure gave good control compared to extracts obtained from goat and sheep manure against cucumber downy mildew caused by *Pseudoperonospora cubensis* under green house conditions with relative efficacies of 67.33% and 66.1% compared with untreated plants. The macerated extracts of horse, cow and pig manure significantly reduced the incidence of cucumber powdery mildew caused by *Sphaerotheca* *fuliginea* by 72.3 – 79.7% compared with control (Ma Li Ping *et al*., 1999b). They tested the efficacy of the above extracts against the cucumber wilt pathogen *Fusarium oxysporum* fsp *cucumerinum* also (Ma Li Ping *et al*.,1999 a), and recorded the relative efficacy of compostextracts from pig, horse and cow manure were 58.9 – 92.5%, 18.6%, 38.5% respectively against the pathogen in *in vitro*. They (Ma Li Ping et al., 2001) observed similar results on sweet pepper. The compost extracts obtained from pig, horse and cow manure could significantly control sweet pepper Fusarial wilt caused by *Fusarium oxysporum* fsp *vasinfectum* in a green house pot test.

Al Dahmani *et al*., (2003) tested the efficacy of compost water extracts prepared from composted cow manure, pine bark, organic farm compost against the tomato bacterial spot caused by *Xanthomonas vesicatoria* and observed that the population of *X. vesicatoria* in infected leaves was reduced significantly by the extracts prepared from composted cow manure.

Sackenheim *et al*., (1994) reported that fermented aqueous extracts of composted microbiologically active substrates significantly reduced infestation of grape leaves by *Plasmapara viticola* under growth chamber conditions. Briton *et al*., (1996) examined the compost teas in relation to their development and use, for controlling plant pathogenic fungi such as *Venturia inaequalis* (apple scab) *Uncinula necator* (grape powdery)and *Phytophthora infestans* (late blight of potato)and proved effective.

The compost extracts obtained from manure straw composts and water extracts made by incubating in water for 3-18 days, inhibited conidial germination of *Botrytis cinerea* on glass slides and reduced mycelial growth on agar (Mc Quilken *et al*., 1994).

2.2.3 COMPOST WATER EXTRACT AND COMPOST TEA - STERILISATION

Yohalem *et al*., (1994) reported that of anaerobically fermented aqueous extracts of spent mushroom compost maintained its inhibitive properties after filter sterilisation and autoclaving. Similar observations were made by Zhang *et al*., (1998a) who reported that the water extracts were heat stable and then reduced the bacterial speck disease of arabidopsis even after passing through a 0.2 μm membrane made up of cellulose acetate. They (1998 b) also reported that autoclaving destroyed the SAR (Systemic Acquired Resistance) inducing effect of the compost water extracts.

Non autoclaved organic compost extracts were more effective compared to autoclaved extracts in reducing the mycelial growth of *Colletotrichum sp, Alternaria solani, Botrytis cinerea, Sclerotinia* *sclerotiorum, Sclerotium rolfsii, Rhizoctonia solani* and *Fusarium oxysporum* fsp *lycopersici* (Nakasone *et al*., 1999). Cronin *et al*., (1996) confirmed that extracts filtered with 0.1 µm pore sized membranes were most effective in reducing the apple scab compared to autoclaved extracts.

Hardy and Sivasithamparam (1991) reported that the chlamydospore production by *Phytophthora cinnamoni* was suppressed by filter sterilised composted eucalyptus bark extracts. The filtrate of water extracts from spent mushroom compost could reduce production of zoospores by the *Pythium aphanidermatum* causing cucumber damping off by the water extracts from spent mushroom substrates(Wang and Huang, 2000).

Labrie *et al*., (2001) reported that the filter sterilised water extracts obtained from two mature biphasic composts, reduced the growth of two oomycete plant pathogens. Filter sterilised aqueous extracts of composts made form conifer bark and grape skin inhibited the growth of *Rhizoctonia solani* and the sporulation of *Gibberella fusicuroi*. (Weilguny and Macek , 1995)

Mc Quilken *et al*., (1994) reported that the compost extracts obtained from manure straw composts, lost their ability on filter sterilization and autoclaving. Szczech, (1999) reported that the sterilised extracts of vermicompost added to PDA stimulated the growth of *Fusarium oxysporum* f.sp *lycopersici* mycelium a wilt pathogen in tomato.

Otero *et al*., (1998) studied on the microfilteration and reported that 100% of yeasts and molds and > 90% of bacteria were retained on the 1.4 μm membrane filter.

2.3 AMENDMENTS

Zaman *et al*., (1997) showed that the spray application of garlic and neem leaf extracts in the field reduced the incidence of seed borne fungi of mustard in storage like *Alternaria, Fusarium, Aspergillus, Penicillium, Rhizopus*, *Chaetomium* and *Curvularia* etc. Spray of garlic and neem leaf extract in crude form at flowering stage suppressed the incidence of leaf blight of mustard caused by *Alternaria brassicae* and also increased yield (Ferdous *et al*., 2002).

Raj Purohit (2003) reported that the plant products AFF 3 and neem gold at 6.5 ml/l were effective against leaf spot of sesame caused by *Alternaria sesami* and phyllody. Nimin recorded highest growth inhibition of *Alternaria triticina* in *in vitro* followed by neem gold, nimbicidine, neem oil, neem bark extracts and neem leaf extracts (Singh *et al*., 2003e).

Patil *et al*., (2001) indicated that the plant products, namely neem seed extract and neem leaf extract at 5% and tobacco decoction at 2% were effective in reducing disease incidence of tomato early blight caused by *Alternaria solani* and increased the fruit yield. Ambhore *et al*., (2003) reported that Tagetes leaf, neem seed significantly reduced the intensity of leaf blight of irrigated wheat caused by *Alternaria triticina*. Leaf extracts of neem and bishkatali were effective in controlling the leaf blight of cauliflower caused by *Alternaria brassicae* and *Alternaria brassicicola* (Hosna Kohinoor *et al*., 2003).

Neem seed kernel extract caused greater inhibition of mycelial growth of *Alternaria helianthi* compared to other leaf extracts. But during the *in vivo* evaluation no treatment was effective in controlling the leaf blight pathogen of sunflower (Amaresh *et al*., 2000a). Mohan *et al*., (2001) reported that neem oil at 3% reduced the disease incidence of onion leaf blight caused by *Alternaria porri*.

Theodore and Toribio (1995) prepared composts from mixture of sugarcane bagasse + filter press mud, bagasse + vinasses + filter mud were able to suppress the *Pythium aphanidermatum* on cucumber in climate chamber experiments. Neem cake, sugarcane pressmud effective against damping off of FCV caused by *Pythium aphanidermatum* and observed improved seed germination and survival compared with the untreated control (Narayanswamy *et al*., 1998).

Dissanayake and Hoy (1999) reported that cotton gin trash compost, sugarcane filter press cake and biosolids suppressed the root rot disease caused by *Pythium arrhenomonas* and the plant growth was increased.

Karegowda *et al*., (2000) assessed the effect of FYM and sugarcane pressmud against the wilt pathogen *Fusarium oxysporum* fsp *lycopersici* and recorded the lower pathogen survival in pot mix treated with pressmud at 200 gr/pot + FYM at 200 gr / pot.

Gorodecki and Hadar (1990) reported that media containing composted grape marc or composted separated cattle manure reduced diseases caused by *Rhizoctonia solani* and *Sclerotium rolfsii* on radish. Wojdyle (2000) reported that all the treatments like Triforine, garlic juice, Amtify 25SC (20% vermicompost), Atpoplan 80EC (70% mineral oil), Biosept (grape fruit juice) and Atonik AL caused almost complete collapse of conidia and hyphae of *Sphaerotheca pannosa* var *rosae.*

Lee *et al*., (2001) found that a treatment, effective microorganisms + fermentation matter (80% bran, 15% Fish meal and 5% water) decreased the incidence of blossom end rot and bacterial wilt of tomato caused by *Ralstonia solanacearum*.

2.4 FUNGICIDES

2.4.1 AMISTAR (AZOXYSTROBIN)

Robak (1998) reported that applications of azoxystrobin 250 SC, propineb significantly reduced Alternaria leaf spot on chinese cabbages in Poland. The seed obtained from plants sprayed with Amistar 250SC at 0.1% were the least infected by pathogens while Amistar 250SC at 0.04% reasonably limited seed infection by seed borne pathogens like *Alternaria, Botrytis*, *Fusarium* etc. in onion crop (Janas and Robak 1999). Robak and Sobolewski (1997) observed that the most effective fungicide for control of cucumber powdery mildew, onion downy mildew and late blight of tomato was Amistar 250 SC at 0.8 l / ha.

Sidlauskiene *et al*., (2003 a) reported that the fungicide Amistar (Azoxystrobin) was most effective on *Alternaria alternata* and *Alternaria brassicicola* cultures *in vitro* inhibiting 57.9 – 69.6 and 87.3 - 91.2% at 0.05 and 0.1% solution respectively. They (2003 b) also tested several fungicides against various *Alternaria* diseases of tomato, cucumber and cabbage seed plants in field conditions and reported that Amistar 250 SC at 0.8 – 1 lt /ha was the most effective among the fungicides and reduced disease incidence by 88.3 – 93.3%.

Bertelsen *et al*., (2001) reported the control of saprophytes like *Alternaria alternata, Cladosporium macrocarpus* by azoxystrobin and epoxyconazole treatments caused delay in the accelerated senescence, but without significant increase in the above ground biomass and yield in wheat. Azoxystrobin inhibited spore germination, mycelial growth of the saprophytes and reduced *Alternaria alternata* induced papilla formation in wheat leaves under glass house conditions. Mazur *et al*., (2002) tested the efficacy of some fungicides against the pathogenic fungi of chickpea and reported that Amistar (Azoxystrobin 250 mg/lt) was the most effective fungicide against all the fungi tested *Fusarium oxysporum, F. avenaceaum, F culmorum, Alternaria tennussima A. alternata, Botrytis cinerea in vitro* and *in vivo*. The activity of azoxystrobin, difenoconazole, polyxin B and trifloxystrobin on one or more stages of the life cycle of *Alternaria alternata* and on decay development in fruits suggests that these compounds potentially could provide control of mouldy core disease in apple (Reuveni and Sheglov, 2002). Pasche *et al*., (2004) reported that severity of early blight on potato plants treated with azoxystrobin and pyraclostrobin was significantly greater with reduced sensitivity to *Alternaria solani* isolates compared with sensitive isolates.

The effect of Amistar (azoxystrobin) is considerably higher in reducing the severity of tan spot of wheat caused by *Drechslera tritici repentis* (Jorgensen and Jensen, 2003) where as Jorgensen *et al*., (2003) observed the efficacy against tan spot of wheat. Amistar was less effective in field trials compared to Opera & Acanto treatments.

Hellemen (1999) reported that Amistar (Azoxystrobin) and Folicur (Tebuconazole) were effective fungicides for the control of Septoria leaf spot in wheat. Triademenol tebuconazale, tetraconazole, cypraconazole, bromuconazole and Amistar (Azoxystrobin) were suitable fungicides for wheat diseases like powdery mildew, yellow rust, brown rust, black rust and various other leaf spots (Pancaldi and Alberti,2001).

The fungicide Amistar is highly effective against spring wheat diseases like mildews, Septoria leaf spot, brown rust etc. (Petrova, 2002). Armour *et al*., (2003) compared the effect of fungicides Opus (epoxyconazole) at 250 g ai/lt and Amistar, (azoxystrobin) at 250 gr / lt in a disease model of speckled leaf blotch, a foliar disease of winter wheat caused by *Septoria tritici* in New Zealand.

Difenoconazole was reported on the most effective fungicides in controlling rosette of boysenberry caused by *Cercosporella rubi* while azoxystrobin and benomyl also gave good control (Langford *et al*., 2003). Baltrami *et al*., (2002) has given the optimum rates of Amistar as 0.4 and 0.6 lt /he for the control of *Cercospora* in beet in Italy.

Wicks and Hitch (2002) evaluated the effect of strobilurin fungicides, Amistar (azoxystrobin) and Flint (trifloxystrobin) with various combinations of other fungicides against powdery and downey mildew diseases of grapevine and reported that Flint was more effective for powdery mildew while Amistar at 0.5 gr/lt provided excellent for downey mildew control.

Thind *et al*., (2004) assessed the efficacy of 3 strobilurins fungicides Azoxystrobin (Amistar 250 SC), Tryfloxystrobin (Flint 50W G) and kresoxyn methyl (Stroby 50WA) against 10 diverse fungi like *Fusarium moniliformi*, *Drechslera oryzae*, *Aspergillus niger*, *Penicillium digitatum*, *Rhizoctonia solani*, *Colletotrichum capsici,* *Uncinula necator*, *Sphaerotheca fuliginea*, *Phytophthora infestans* and *Pseudoperonospora* *cubensis* and proved that Amistar 5 mg/ml was effective against all but the percent disease control of each was different.

2.4.2 INDOFIL M-45 (MANCOZEB)

Maheswari and Singh (1997) reported that Indofil M-45 controlled the leaf spot of *Dolichos* caused by *Alternaria alternata*. In 1998 they concluded that Alternaria leaf spot of *Lablab purpureus* can be effectively controlled by 3 applications of either Indofil M-45 at 0.2% or carbendazim at 0.1% at an interval of 10 days.

Dubey *et al*., (2000) reported that 3 sprays of Topsin M 0.5% + Indofil M-45 0.1% controlled the leaf blight disease caused by *Alternaria alternata* in the field. And similar results were observed in controlling *Alternaria cucumerina*. The disease was effectively controlled by 3 sprayings of Indofil M – 45 at 14 day interval after 30 days of sowing. (Shahid Ahamed and Udit Narain, 1998).

Significant reduction in spore germination of *Alternaria alternata* was reported by (Rashmi *et al*., 1998) at all the tested concentration of 10 to 20 ppm of Indofil M-45 similar observation were made by Lal *et al*., (2000) who reported that Rovral and Indofil M-45 more effective in *in vitro* conditions against *Alternaria tennussima* the casual organism of leaf blight in pigeon peas.

Inhibition of mycelial growth and sporulation of Alternaria blight of marigold caused by *Alternaria tennussima* was reported with Indofil M-45 (Barnwal *et al.*, 2002). Singh *et al.*, 2002 also observed 100% inhibition of mycelial growth of *Alternaria cucumerina* the causal organism of cucumber leaf spot. In pot culture experiments also similar results obtained with Indofil M-45.

Mohit Singh *et al*., (1997) found that the combination of emisan 6 with Indofil M-45 was most effective in reducing the potato early blight caused by *Alternaria solani* and increased the seed yield. Spraying with 0.2% Indofil M-45 in the linseed plants exhibited maximum reduction of disease intensity of leaf blight caused by *Alternaria lini* and given the highest seed yield (Khan *et al.,* 2004). The two sprays of Indofil M-45 at 0.2% at 20 day interval significantly reduced the Alternaria blight caused by *Alternaria brassicae, Alternaria brassicicola*, *Alternaria raphani* and enhanced the yield over the untreated control (Yadav *et al*., 2002). While working with foliar disease of linseed like Alternaria blight and powdery mildew, Jyothi Singh and Singh (2002) reported that Indofil M-45 at 0.2% controlled the disease and given higher yields as compared to control. Similarly spraying of Indofil M-45 at 0.25% controlled the Alternaria leaf blight of Marigold caused by *Alternaria dianthi* and increased the flower yield over the check. (Mazumdar, 2000) and Ved Ratan (2003).

Ashok Kumar and kumar (1996) recorded minimum Alternaria blight caused by *Alternaria brassicae* infection in mustard with iprodione at 0.2% followed by Indofil M-45 but Indofil M-45 is recommended on the basis at benefit cost ratio.

Becerra *et al*., (1988) reported that citrus leaf spot disease caused by *Alternaria spp* is effectively controlled by Indofil M-45 at 2-3% treated after 7-15days of shoot emergence. Spraying of Indofil M-45 was not effective for controlling Alternaria leaf blight of sunflower caused by *Alternaria helianthi* (Smitha Ranjan *et al*., 1999).

Akbari *et al*., (1996) reported that mancozeb as Indofil M-45 was the most effective followed by propiconazole and hexaconazole for controlling the cumin blight caused by *Alternaria bursnii* through fungicides captaf 0.25%, COC 0.35%, Indofil M-45 0.25% when sprayed at 20 days intervals significantly reduced disease severity of leaf blight of aubergine caused by *Alternaria sp* in microplot trials and also increased the yield. (Yadav *et al*., 1998). Annapurna Kumari *et al*., (1999) observed reduction in the Alternaria leaf blight of cauliflower caused by *Alternaria brassicae* was effectively controlled by 3 sprays of

Indofil M-45. Rai *et al*., (2002) reported that seedling blight of sugarcane caused by *Alternaria alternata* was effectively controlled by Bavistin and Redomyl MZ followed by Indofil M-45 when applied at the time of emergence and 5 days after the emergence. Kuwar Singh and Rai (2003) showed that 0.2% Indofil M-45 was most effective in controlling leaf spot of brinjal caused by *Alternaria alternata* . In the same year Ambhore *et al*., reported that all the fungicidal treatments including Indofil M-45 at 0.25% reduced the intensity of leaf blight of wheat caused by *Alternaria triticina*.

2.4.3 QUINTAL (IPRODIONE25%+CARBENDAZIM25%)

Surinder Kumar *et al.,* (1990) reported that Calidan (iprodione + carbendazim) was the most effective against Alternaria blight of carrot caused by *Alternaria dauci*. Similar work was done by Vulsteke *et al*., (1995). They tested difenoconazole, bitertenol and iprodione + carbendazim against leaf pathogen of carrot like *Erysiphe herachei* and *Alternaria dauci* and reported that all the treatments are equally effective. They observed similar effect in controlling the *Erysiphe*, *Alternaria sp* with sprayings of iprodione + carbendazim (Vulsteke *et al*., 1996). They also reported that 3 applications of Quintal has controlled the mustard leaf blight caused by *Alternaria dauci*. (Vulstake *et al*., 1997).

Biswas (2004) studied the efficacy of different fungicides like Monceran 250 SC, Anvil 5 SC, Folicur 250 SC, Swing 250 EC, Quintal 50 WP, Asmur 30 EC and Sheathmar 3L against sheath blight of rice caused by *Rhizoctonia solani.* Monceren 250 SC at 0.15% was proved to be effective. Quintal 50 WP is less effective compared to other treatments. Abaso (1995) reported that Apron, Plus 50 DS, Rovral TS (Carbendazim + Iprodione), Fernasan D and Vitavax 200 were ineffective as seed dressers on anthracnose and charcoal rot.

2.4.3.1 CARBENDAZIM , IPRODIONE

Quintal 50 WP is a combination fungicide of carbendazim 25% + iprodione 25%. So, the efficacy of carbendazim and iprodione as individual fungicides against *Alternaria spp* is reviewed briefly.

Pieta and Pastucha (1993) found that thiram and carbendazim gave the best control of *Phoma exigua* and *Alternaria alternata* which were predominant seed borne pathogen of soybean. Dipping the chilli fruits in carbendazim solution (1000 μg/ml) for 10 min effectively controlled the seed borne pathogens including *Alternaria alternata* (Datar, 1996b). Shivankar *et al*., (2000) reported that the carbendazim treatment at 0.1% recorded the highest germination shoot length (9.37

cm) and shoot length (12.35 cm) in the *Alternaria alternata* infected wheat seeds.

Patil *et al*., (1988) reported that maximum growth inhibition of *Alternaria tennussima*, a casual agent of leaf blight of groundnut was given by Panolil, Rovral (Iprodione). Later (1990) they tested two systemic and seven non systemic fungicides against this pathogen and a maximum inhibition of mycelial growth was recorded with iprodione at all concentrations tested.

Spraying with 0.2% iprodione gave maximum disease control of sunflower blight caused by *Alternaria helianthi*. (Amaresh and Nargund, 2000b). Subsequently they (2002c) reported minimum intensity of Alternaria blight and maximum oil yield in chlorothalonyl treatment followed by cypraconazole and iprodione. Mondal *et al.,* (1999) also worked on leaf blight of sunflower and reported that rovral at 0.2% had the highest efficacy in controlling the blight caused by *Alternaria helianthi* and increasing the sunflower yield. Rao and Ranganadha (2003) reported that leaf blight incidence (7%) and plant disease index (2.1%) were lowest and crop yield and benefit cost ratio were highest with the applications of iprodione at 0.05% in sunflower.

But Srinivas *et al.,* (1997) reported that carbendazim was the most effective control followed by iprodione against blight of sunflower caused by *Alternaria alternata*.

Fitt *et al.,* (1991) reported that growth of all the three species of *Alternaria* like *Alternaria alternata*, *A. infectoria*, *A. linicola* on V-8 juice agar was decreased greatly by iprodione but little variation was observed between isolates. Similar observations were made by Giri and Peshney (1993) who reported that the mycelial growth and spore germination of leaf pathogens of mung bean *Alternaria alternata* was effectively inhibited by iprodione, carbendazim, foestyl AL, mancozeb etc. 91.31% growth inhibition of leaf spot pathogen of safflower i.e., *Alternaria carthami* was recorded by Krishna *et al*., (1998) with aureofungin followed by mancozeb and iprodione at 1500 ppm but was at par with 1000 ppm. Selmaoui *et al*., (1997) reported that iprodione was the most effective fungicide for controlling apple rot caused by *Alternaria tenuis* during storage.

Shivpuri *et al*., (1988) observed that iprodione at 0.2% was the best treatment against Alternaria blight of mustard. The defoliation was minimum in this treatment. Mondal *et al*., (1989) working on radish seedling blight caused by *Alternaria brassicae* reported that iprodione applied to 55 day old radish seedlings at 10 day interval was the best treatment to control the disease. Iprodione at 0.2% reduced the Alternaria blight severity of mustard and increased seed weight and yield (Ayub *et al*., 1996).

Annapurna Kumari *et al*., (1999) concluded that spraying with Rovral at 2 kg/ha reduced the intensity of Alternaria leaf blight and increased the yield of cauliflower. Similar observations were reported by Khoda *et al.,* (2003) with Rovral 50 WP at 0.2% which gave best control of Alternaria blight of cauliflower caused by *Alternaria brassicae* and increased the yield.

Application of iprodione and carbendazim reduced tomato early blight incidence like mancozeb, carbendazim etc. were also equally effective. (Choulwar and Datar, 1992). Prasad and Nayak (2003) reported highest yield and benefit cost ratio in tomato under iprodione treatment in addition to reducing the disease incidence of early blight caused by *Alternaria solani*.

Datar (1996a) studied the effect of fungicide against onion purple blotch caused by *Alternaria porri* and reported that all the fungicides including iprodione and carbendazim proved effective. Carbendazim increased the bulb yield also. But in the experiments conducted by Kolte *et al*., (1993) on the same disease Rovral (Iprodione) was least effective treatment among all.

Kucumierz *et al*., (1989) found Rovral (Iprodione) was effective against several fungal diseases of seed carrot caused by *Botrytis spp*, *Fusarium spp, Alternaria radicina*, *Sclerotium sclerotiorum*, *Rhizoctonia solani* etc. Sutherland *et al*., (1990) assessed the efficacy of four fungicides, carbendazim, prochloraz, iprodione, vinclozolin against late diseases of winter oil seed rape in Scotland caused by *Alternaria brassicae*, *A. brassicicola, Botrytis cinerea*, *Sclerotium sclerotiorum*. The disease control was variable. Russo (2001) reported that iprodione was the best fungicide to control *Bipolaris oryzae* of rice.

Mercer *et al.,* (1992) reported that weekly sprays of iprodione effectively controlled *Alternaria linicola* on linseed. Lal *et al*., (2000) reported that rovral 2.5 kg / ha was the best against Alternaria leaf blight, which recorded highest yield in pigeon pea.

Xu ling *et al*., (1999) reported that sprays of iprodione 5 gr/l on grapes at 65 days and 9 days before harvest markedly decreased berry shattering due to *Alternaria, Botrytis, Cladosporium* and other species during storage at $50^{O}C$ for 2 months.

Swart *et al*., (1998) reported that mancozeb at 0.01% applied at fortnightly interval was the most effective against brown spot on *Minneola tangelo* caused by *Alternaria alternata*.

2.5 SALICYLIC ACID

Kalaichelvan and Mahadevan (1987) reported that few of the phenolic treatments inhibited spore germination or linear growth of test fungi *Alternaria alternata*, *Curvularia spicata*. Anthranilic, Gentisic salicylic and vanillic acids were not very inhibitory to these fungi. All the tested phenols inhibited spore germination of *Curvularia spicata* and anthranilic, salicylic and vanillic acids were inhibitive to *Alternaria alternata*. Gentisic and salicylic acid had an effect on oxygen uptake in both fungi (Kalaichelvan and Mahadevan, 1988).

Salicylic acid and Bion (Acibenzolar) reduced spore germination and radial growth at the pathogen *Alternaria helianthi* of sunflower (Ratnam *et al*., 2004).

Kassemeyer *et al.,*(1998) studied the possibility of controlling *Plasmapara viticola* and *Uncinula necator* by inducing resistance with *Pseudomonas syringe* pv *syringe*, *Botrytis cinerea* and salicylic acid on grape cv pinot noir clone FR 54-86 and it is concluded that induced resistance may have potential for biological control of powdery and downy mildew of grapes. Feeding of 200 μm salicylic acid to tomato plants can significantly elevate the foliar SA levels, induce PR-1B gene expressions and activate systemic acquired resistance to the early blight pathogen *Alternaria solani* (Matthew and Alexander, 1999).

Deloire and Renault (1996) reported that *Botrytis cinerea* and to a lesser extent, salicylic acid were found to induce synthesis of pathogenesis related proteins in leaves of grape cv chardonnay. The most effective group of elicitors was represented by salicylic acid, chitosan, methyl jasmonate and elicitor released from all walls of *Botrytis cinerea*. These four representative elicitors highly stimulated accumulation of pathogenesis related proteins (Repka, 2001)

2.6 BIOCONTROL AGENTS

Ray *et al*., (1990) reported that the two fluorescent *Pseudomonas* isolates obtained from soil were shown to reduce growth of *Aspergillus niger*, *Fusarium spp*, *Alternaria solani*, *Drechslera oryzae* etc. Twenty one isolates among the several *Pseudomonas spp* isolated from soil inhibited the growth of a number of plant pathogenic fungi including *Fusarium*, *Alternaria*, *Cladosporium, Pyricularia, Pythium, Verticillium*, *Cylindrocarpon* and *Rhizoctonia* etc. (Hasegawa *et al*., 1990).

Hebber *et al*., (1991) reported that *Pseudomonas fluorescens*, *P. putida*, *P. maltophilia*, *P. cepacie* were associated with sunflower leaves and roots and inhibited *in vitro* growth of *Alternaria helianthi*, *Sclerotium rolfsii* and *Rhizoctonia solani*. Similarly from brinjal and tomato Atef (2000) isolated several bacteria from phylloplane and rhizoplane of the crops. Among all the isolates *Pseudomonas fluorescens* SM 8, *Erwinia herbicola* SM 81, 182 ,*Bacillus subtilis* IB 18 were proved to be the best antagonists against several pathogens like *Macrophomina phaseolina*, *Helminthosporium tetramera*, *Alternaria alternata* and *Fusarium spp*.

Trichoderma viride was the prominent antagonist among all the microflora of citrus against all the members of the sooty mold like *Alternaria alternata*, *Botryodiplodia theobromae*, *Cladosporium oxysporum*, *Colletotrichum gloeosporoides* and *Curvularia spp* etc.(Srivasthava and Thakre, 2000).

Khmel *et al*., (1997) reported that a novel strain of *Pseudomonas fluorescens* VKM CR 330 D at $10^8 - 10^9$ cells / ml showed broad spectrum of antipathogenic properties and Agricultural antibiotic activity against *Alternaria solani*, *Helminthosporium sativum* and *Rhizoctonia solani*. The preparation of a strain of *Pseudomonas fluorescens* VKM CR 328 D containing 0.1 – 1 gr cells / lt was effective against *Alternaria sp*, *Botrytis cinerea*, *Fusarium heterosporum*, *Fusarium moniliformi*, *Helminthosporium sativum* etc. (Sorokina *et al*., 1995)

Saspiotrowska and Dorszewski (1996) observed that *Trichoderma viride* and *Trichoderma harzianum* exhibited antagonistic activity against *Alternaria alternata, Colletotrichum coccoides*, *Phoma exigua*, *Phoma exipyrina* etc. Ghaouth *et al*., (2002) discussed the antagonistic activity of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and *Saccharomyces cereviceae* against the post harvest diseases of fruits and vegetables caused by *Alternaria citri*, *Penicillium digitatum*, *P italicum*, *Botrytis cinerea* etc.

Anju Puri *et al*., (1994) reported that *Trichoderma viride, Trichoderma hamatum, Trichoderma harzianum* inhibited *Alternaria zinniae.* The inhibiting principle is pectolytic and cellulolytic enzyme activity of these biocontrol agents. Hoffland *et al*., (1996) demonstrated that pretreatment with *Pseudomonas fluorescens* strain WCS 417 protects radish through induction of systemic resistance not only against *Fusarium oxysporum* fsp *raphani* but also against the avirulent bacterial leaf pathogen *Pseudomonas syringe* pv *tomato* and the fungal leaf pathogens *Alternaria brassicicola*.

Palazon *et al*., (1988) reported that *Trichoderma viride* was strongly antagonistic to fruit not pathogens *Alternaria tenuis* and *Botrytis cinerea in vitro*. Similarly Sesan (1990) showed that *Trichoderma viride* was a strong antagonist against *Sclerotinia sclerotiorum* and *Alternaria radicina* on stored carrot *in vitro.* Vamnacci (1991) reported that among all antagonists *Trichoderma harzianum* gave the best control against seed borne *Alternaria raphani* in Radish.

The new strain of *Trichoderma viride* VKMF 3269 D was useful as a biological control agent in grapevine plantations, mainly against the grey mold pathogen *Botrytis cinerea* and also against *Alternaria alternata* (Khirton *et al.,* 1994). *Trichoderma viride* showed highest antagonistic activity *in vitro* compared to other isolates against the purple blotch pathogen of gerbera *Alternaria porri*. Conidial sprayings at 10000 spores / ml inhibited growth of the pathogen in the field (Sasthrahidayat, 1995).

Babu *et al.,* (2000a) reported that *Trichoderma harzianum*, *Trichoderma viride* were significantly effective in inhibiting the mycelial growth of *Alternaria solani*, the casual agent of tomato early blight *in vitro*. But there was no significant difference between the effectiveness of *Trichoderma spp* in pot culture studies. *Trichoderma viride*, *Aspergillus awamori, Trichoderma hamatum* effectively inhibited the growth of gerbera leaf spot pathogen *Alternaria alternata in vitro* (Ghosh *et al* .,2002). Similar observations were made by Mazur *et al* .,(2003) who reported that *Trichoderma viride* inhibited the growth of test pathogen of chick pea *Fusarium oxysporum, Rhizoctonia solani*, *Sclerotinia sclerotiorum, Botrytis cinerea* and *Alternaria alternata* but did not showd any activity *in vivo*.

Chen *et al*., (1993) reported that fluorescent *Pseudomonas* strains B 526-7 and a mixture of these were highly antagonistic to phytopathogenic fungi of apple like *Physalospora piricola, Glomarella cingulata* and *Alternaria mali* in *in vitro* and *in vivo*. The sunflower leaf blight pathogen *Alternaria helianthi* was effectively suppressed by fluorescent and non fluorescent *Pseudomonas* in *in vitro* conditions (Prasad and Kulshreshta, 1999). Babu *et al* .,(2000b) tested the strains of *Pseudomonas fluorescens* in *in vitro* and *in vivo* for their biological control potential against *Alternaria solani* of tomato. All the six strains inhibited the growth by 98 to 100% *in vitro* and reduced leaf blight disease by 15-38% compared to control.

Abada *et al*., (2002) indicated that the biocontrol agents *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* caused different degrees of antagonistic action either by inhibition or hyperparasitism to the growth of *Botrytis cinerea* and *Phytophthora* *cactorum*. Experiment under field conditions by spraying of culture filtrate of the biological control agents resulted in significant reduction of fruit rots of straw berry and an increase in the marketable fruits. The antagonism of *Trichoderma viride, Pseudomonas fluorescens, Bacillus subtilis* against onion leaf blight pathogen *Alternaria porri* was less when compared to other treatments (Mohan *et al.,* 2001).

Mercer *et al*., (1992) reported that the weekly sprays of *Trichoderma viride* gave most effective control against *Alternaria linicola* on linseed capsules. Similar work was done by Pratella and Mari (1993) who reported that the spray treatments of *Trichoderma viride, T. harzianum, Gliocladium roseum, Paecilomyces variotii* to the fruits, partially controlled *Botrytis cinerea* in straw berry and kiwifruit, *Fusarium oxysporum* in potato and *Alternaria citri* in lemon.

Abada (1994) reported that *T. harzianum* caused a great reduction in the infection level of damping off and root rot diseases of straw berry caused by *Alternaria sp, Mucor sp, Fusarium sp* and also increased root weight both in pot and field experiments during two successive growing seasons.

Trichoderma viride was more effective than *Pseudomonas fluorescens* in controlling the leaf blight of sunflower caused by *Alternaria helianthi* (Mathivanan *et al*., 2000).

A combination of *Trichoderma viride* and carboxin effectively controlled the leaf blight of wheat caused by *Alternaria triticina* in the field (Kumar *et al*., 2002).

Lal *and* Upadhyay (2002) showed that 2 sprays of *Trichoderma viride* at 4 gr/lt gave 35.59 % control of leaf blight of pigeon pea caused by *Alternaria tennussima* followed by *Gliocladium virens* which showed 31.32% disease control.

Meena *et al*., (2003) assessed the antagonism of isolates of *Trichoderma viride* against white rust and leaf blight of mustard caused by *Alternaria brassicae* and powdery mildew, and determined *T. viride* is less effective compared to other chemical treatments.

Slusarski and Pietr (2003) showed that the combined application of dazomet and *Trichoderma viride* was an acceptable alternative to methyl bromide fumigation in controlling the leaf diseases of cabbage, celary and tomato caused by *Alternaria brassicae* and *Phytophthora infestans*. The better control of the pathogenic fungi of *Fusarium solani*, *Alternaria alternata, Fusarium oxysporum, Verticillium alboatrum* was achieved with *Trichoderma viride* was applied to the soil before planting (Stonkeviciene and Snieskiene, 2003).

Silva *et al.,* (1998) reported that among the antagonists tested, *Pseudomonas fluorescens* JA4 and BJ 22 were the most effective showing a reduction in disease severity of 20.9% and 17.8% respectively when applied simultaneously with *Alternaria ricini* or castor under green house conditions.

The efficacy of *Trichoderma viride* and *Pseudomonas fluorescens* against the sunflower blight caused by *Alternaria helianthi* was tested and the results revealed that *Trichoderma viride* was equally effective as that of fungicides but, *Pseudomonas fluorescens* was ineffective (Mathivanam *et al* .,2000)

Leifert *et al*., (1992) reported that *Serratia* and *Pseudomonas* strains showed *in vitro* antagonism against *Botrytis cinerea* and *Alternaria brassicicola*, they were also tested *in vivo* and *Pseudomonas fluorescens* isolates CL42, 66, 82 *Serratia plymuthica* isolate CL 43 and *S. liquifaciens* isolate CL 80 provided the best control of both fungi at 4^{O}C. Seed treatment or spraying with spore suspensions of *Trichoderma viride* on growing plants controlled *Alternaria linicola* on linseed (Mercer *et al* .,1993). Deshmukh *et al*., (1994) showed that

Trichoderma viride seed treatments were not as effective as the fungicides treatments and reduced seed borne fungi of jowar by only 25-30%. Sawant *et al*., (1999) reported that of all the *Trichoderma viride* treatments, seed treatment combined with soil applications was the most effective and recorded the lowest incidence of early blight of tomato caused by *Alternaria solani* seed treatment with *Trichoderma viride* completely eliminated the seed borne pathogens *Alternaria alternata*, *Phyllostricta cajani, Rhizoctonia bataticola, Curvularia lunata* etc of Red gram (Pradeep Kumar *et al* .,2000).

Prasad and Kulshreshta (2002) reported that seed treatment with *Pseudomonas fluorescens* at 8.7 to 9.4 x 10^{11} cfu /ml isolated IV gave the greatest seedling emergence 92-100% and lowest incidence of Alternaria blight infested seedlings in sunflowers caused by *Alternaria helianthi.*

Singh *et al*., (2003a) evaluated the fungicides and biocontrol agents against seed microflora of pearl millet includes *Alternaria alternata*, *Aspergillus flavus, Trichoderma harzianum* and *Pseudomonas fluorescens* were proved to be effective. They conducted another similar experiment and studied the effect of *Trichoderma harzianum, T. viride*, *Pseudomonas fluorescens* on different seed borne pathogens of bajra like *Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliformi, Rhizopus stolonifer* etc. and concluded that all the biocontrol agents significantly increased seed germination percentage and seedlings vigor (Singh *et al* .,2003b).

The seedlings from healthy seeds and seedlings from the seeds treated with *Pseudomonas fluorescens* and *Trichoderma viride* at 4 gr/kg showed good shoot and root lengths compared to control, eliminated the seed borne disease of sorghum caused by *Fusarium, Alternaria, Bipolaris, Curvularia*, *Exherohilum* etc. (Indira and Muthusubramanian, 2003 and 2004). The size and quality of seeds from plants treated with *Pseudomonas fluorescens, Trichoderma harzianum, Bacillus subtilis* were greater or equal to those treated with the control (Pieta and Pastucha, 2004).

Smouse *et al.,* (1999) reported that seven of the 55 *Trichoderma viride* isolates, were associated with complete inhibition of fungal discolouration caused by *Ophiostroma piceae* and *Alternaria alternata* in panderosa pine.

2.7 YEAST

Suzzi *et al.,* (1995) tested the biocontrol activity of 586 natural and wine yeasts against several plant pathogens including *Alternaria alternata* and some of them completely inhibited the pathogens growth and *Saccharomyces cereviceae* N 826 and N 831 showed a broad spectrum of activity. Native yeast isolates significantly reduced the diameter of leisons of *Alternaria alternata* on tomato fruits and exhibited the highest biological control activity (Fuentes *et al* .,2002).

Lima *et al*., (1997) reported that among all the isolated yeasts and yeast like fungi obtained from fruits and vegetables, *Candida vanderwatii* L 60 and *Candida oleophila* L66 were the most effective antagonists of *Botrytis cinerea* and *Rhizopus stolonifer* of grapevine.

Cirvilleri *et al*., (1999) reported that of the 12 test antagonists yeasts from healthy table grapes a strain identified as *Kloeckena spp* consistently reduced the infections and decay of grape gray mold caused by *Botrytis cinerea*.

2.8 PHYLLOPLANE MICROFLORA

Ferreira, (1990) isolated 8 species of epiphytic bacteria and fungi from leaves and bunches of wattham gross grapes and tested for their inhibition against *Botrytis cinerea*. One bacterial isolate completely inhibited and 13 other fungi includes *Trichoderma harzianum* were proved effective.

Over 100 isolates of *Trichoderma harzianum* were obtained from soil samples of kiwi fruits and from phylloplane of grapes, oranges, *Eucalyptus globulus* and apricot in Chile and *Trichoderma harzianum* I proved effective for grape bunch rot caused by *Botrytis cinerea* (Latorre *et al.,* 1997).

Krol (1998) studied the biotic interactions of 282 isolates of epiphytic bacteria of grapevine with *Botrytis cinerea in vitro* and 17 isolates were more or less inhibited the growth, 6 of these isolates formed, 10 mm inhibition zone which includes *Bacillus sp, Pseudomonas fluorescens.* Machowicz Stefanak (1998) isolated 9 saprophyte species of fungi from grapevine phylloplane and tested their antagonism against *Botrytis cinerea*. Among all, only *Trichoderma harzianum* protected individual fruits against infections.

Magnoli *et al*., (2003) isolated the phylloplane microflora from grape using surface disinfection method and identified. Among them *Alternaria, Aspergillus and Penicillium* were predominant. Rodolfi (2003) detected 49 genera of phylloplane fungi from randomly collected grapevine leaves from green house plants using an SAS air sampler. The species includes *Aspergillus, Penicillium spp, Conidiobolus spp, Gliocladium vermoesenii, Graphium sp, Zygosporium spp*, etc. Krol, (2004) isolated 23 species of fungi from healthy canes and grape shoots. Among them *Alternaria alternata* and *Fusarium spp* are predominant.

Baicu and Opera (1992) observed significant changes in leaf microflora of grape cv chasselas dore immediately after treatments with COC, Ridomil, Perozin. Their effect started to decrease after 16 days. But no changes were observed in colonies of *Alternaria*. Suppini *et al.,* (1995) observed that *Aureobasidium*, *Cladosporium* and Phomaceae members as predominant epiphytic species of grapevine carposphere and studied the interactions between *Uncinula necator* and the saprophytic fungi on the presence and absence of fungicidal treatments like tridemenol 2.5% + sulfer 50%. The results demonstrated a good selectivity of the fungicidal mixture and showed that the influence of spray volume and concentration was limited.

14 species of fungi, like *Aspergillus spp, Chaetomium spp*, *Cladosporium herbarum* were present on grapevine phylloplane. *Fusarium roseum, Mucor, Alternaria alternata* and the number of species varied with season, age, variety, maturity of the leaves (Fateh Singh, 1995). Rousseau and Doheche (2001) isolated 7 yeast strains and 10 bacteria strains from grapevine phylloplane and studied the influence of environmental conditions. Results confirmed that most bacteria are sensitive to water deficit and yeasts are less sensitive. Also studied their antagonism against *Botrytis cinerea*.

Sackenheim *et al*., (1994) found correlations between the total number of colony forming units (cfu) of yeast and filamentous fungi, enterobacteia, *Pseudomonas* and aerobic bacilli on leaves, to compost extracts and micro climatic conditions.

Bugaret *et al*., (1989) reported that *Botrytis cinerea, Sphaeropsis, malorum, Epicoccum sp, Alternaria, Cladosporium sp, Aureobasidium sp* were the most frequently isolated from grapevine canes in winter. Grape juice splashing on canes could increase the number of isolated mycelial colonies upto 25%.

CHAPTER III

MATERIALS AND METHODS

This chapter includes all the materials used for screening and methods adopted in the investigation. All the techniques detailed under respective headings and references quoted.

3.1 CONFIRMATION OF PATHOGENICITY

3.1.1 ISOLATION

Diseased leaves showing typical symptoms were collected from the vines of Thompson seedless variety from the Grape Research Station, Rajendranagar. These leaves were first washed with tap water followed by sterilised distilled water. Diseased portions with some healthy portion were cut into small bits of 3-5 mm size. These bits were surface sterilised by dipping them in sodium hypo chlorate (0.1%) solution for one minute and then 3-4 bits were transferred aseptically to the presterilised petriplates containing the PDA culture medium. These petriplates were incubated at $25 \pm 2^{O}C$ to encourage the mycelial growth from the leaf bits (Aneja,2003).

3.1.2 PURIFICATION

The culture was purified by single spore isolation (Ho and Ko,1997) (Plate4) 0.05 ml of spore suspension with a density of 100 spores/ml was placed on water agar above the circles marked on the bottom of the petriplate. After the incubation of 12 hours a single germinating spore in each circle was transferred separately to a petriplate containing PDA medium and incubated at 25 ± 2^{O}C to get pure culture.

3.1.3 PATHOGENICITY

The culture was identified as *Alternaria vitis* based on the spore characteristics i.e., length, width, colour, shape etc. and pathogenicity. To test pathogenicity the spore suspension with 100X10^{4} spores / ml was sprayed on both the leaf surfaces of the grapevine grown in polybags. The inoculated twigs were covered with polythene bags for 12 hours to maintain humidity for infection. The inoculated plants were observed for typical symptoms. The pathogen was reisolated from these diseased leaves and morphological & cultural studies (Plate 5&6) were carried out to compare with the original isolate.

3.*2* *IN VITRO* TESTING OF COMPOSTS, FUNGICIDES AMENDMENTS AND CHEMICAL

Different composts , amendments, plant sources as extracts, fungicides, chemical and biocontrol agents, which were tested against *Alternaria vitis in vitro* are listed in Tables 1-4.

3.2.1 PREPARATION OF EXTRACTS

3.2.1.1 MAKING COMPOST

Pits of 1 x 1.5 x 0.45 m^3 were dug at the glass house , filled with the compost materials separately and were covered with black polythene sheet. These composts were turned manually at every 3^{rd} day and sprinkled with water regularly to maintain moisture for better decomposition, for 45 days to get the ripe compost.

3.2.1.2 PREPARATION OF COMPOST WATER EXTRACTS

The water extracts of the composts were prepared using a procedure adapted by Steve Diver (2002) with few modifications. Ripe composts and amendments were soaked in water in the ratio of 1 : 1 (w/v) in plastic drums. They were aerated periodically by turning them with a wooden stick and fermented for 10, 20 and 30 days. The water extracts were obtained by squeezing and coarse filtering followed by filtering through whatman, No.4 (Particle retention, >20µm) blotters.

3.2.1.3 FERMENTATION OF GRAPE BERRIES

Grape berries of Dilkush variety were punctured slightly and fermented anaerobically for varied periods of 10, 20 and 30 days in separate air tight plastic containers. The clear fermented juice collected and filtered with whatman, No.4 (Particle retention, >20µm) blotter.

3.2.1.4 COLD STERILISATION

The extracts and grape berry juice were sterilised with bacterial proof membrane filters. The cellulose acetate membrane with 47 mm diameter and 0.45 µm pore size was fixed in polysulfone filter holder (plate 7) and wetted with distilled water then autoclaved at 120°C for 15 min. To facilitate the active filtration, the sterilised filter holder bearing the membrane was attached to the vaccume pump (Plate 8) (Dhingra and Sinclair, 1995). The extracts were filter sterilized and the standard extracts were collected into the presterilised conical flasks aseptically(Plate 9). Sterility of extracts was confirmed by spreading 0.1 ml of each extract on 0.1 strength Difo tryplicase soy agar medium. Plates were incubated at 28°C for 48 hours.

3.2.2 EFFICACY OF DIFFERENT EXTRACTS, FUNGICIDES AND CHEMICAL ON THE MYCELIAL GROWTH OF *A.vitis*

The efficacy of extracts, fungicides and chemical was tested using Poisoned food technique (Nene and Thaplial, 2002). The extracts, fungicides and chemical solutions of known concentrations were mixed in the PDA medium before pouring into the sterilized petriplates and labeled accordingly. On this poisoned medium 6mm diameter discs of the pathogen was inoculated at the center and incubated at 25 ± 2°C.

The mycelial growth was recorded at every 24 hrs. The effectiveness of the treatments was decided based on the Percent inhibition.

I =C-T/T X100

I = Percent inhibition

T = Radial growth in treatment

C = Radial growth in control

3.2.3 EFFICACY OF DIFFERENT EXTRACTS, FUNGICIDES AND CHEMICAL ON SPORE GERMINATION OF *A.vitis*

Effect of different extracts, fungicides and chemicals on spore germination was studied using Slide germination technique (Reddik and Wallace, 1910). The required concentration of extracts, fungicides and chemical solutions were prepared with sterilised distilled water. The spore suspension was prepared from 7 day old culture with a density of 20 spores / microscopic field (10X). A 0.05 ml of test solution was placed on a clean cavity slide with the help of 1 ml pipette and allowed to dry. Then 0.05ml of spore suspension was placed in each of the cavities and covered with cover slips. The slides were placed in petriplates lined with moistened blotter papers and incubated for 12 hours at $25 \pm 2^{O}C$. Control was also maintained using sterilised distilled water. The percent inhibition was recorded as follows.

I =C-T/T X100

I= Percent inhibition

C= Spores germinated is control

T= Spores germinated in treatment

3.3 ISOLATION OF PHYLLOPLANE MICROFLORA OF GRAPEVINE

The micro flora was isolated from the grapevine phylloplane using serial dilution technique (Aneja, 2003). Leaf washings from the 3rd, 5th and 7th leaves from the bottom of the cane of Thompson seedless variety were taken for the isolation of phylloplane microorganisms. One gram of 1 cm discs from the leaves were cut and transferred into conical flask with 10 ml of sterile distilled water. The leaf bits were thoroughly shaken for 20 min on vortex shaker to dislodge the microflora adhering to the leaf surface. Serial dilutions of the leaf washings were made and 1 ml each of these dilutions were plated on the plain agar. Fungal colonies from the individual spores were transferred onto PDA slant and bacterial colonies onto the nutrient agar slants and maintained as pure cultures.

3.4 *IN VITRO* TESTING OF BIOCONTROL AGENTS AND PHYLLOPLANE MICROFLORA

For testing the antagonistic activity of the biocontrol agents and phylloplane microflora against *Alternaria vitis*, Dual culture technique (Aneja,2003) with some modifications was employed.

3.4.1 EFFICACY OF FUNGAL ANTAGONISTS

To test the effectiveness of *Trichoderma viride* and phylloplane fungi equal sized 6mm diameter discs of test pathogen and biocontrol agent or phylloplane fungus were aseptically transferred and placed opposite towards the peripheries of the petriplate containing appropriate medium. The plates were incubated at 25 ± 2^{O}C and the Percent inhibition was calculated using the formula

I = C-T / T X 100

I= Percent inhibition

T= Growth in treatment

C= Growth in control

3.4.2 INTERACTIONS OF FUNGAL ANTAGONISTS – SEM STUDIES

Interactions of antagonistic fungi against *A. vitis* was studied using SEM. The processed samples (Bozzola and Russela, 1999) were examined under scanning electron microscope (model JOEL – JSM

5600) at various magnifications in Ruska Lab of ANGR Agricultural University, Hyderabad.

3.4.3 EFFICACY OF BACTERIAL ANTAGONISTS

The antagonistic properties of *Pseudomonas fluorescens* and phylloplane bacteria against the pathogen was tested. 6 mm discs of medium bearing the test pathogen was placed at the center of the petriplates with PDA medium, to create more favourable conditions to the pathogen than the bacterium. Bacteria were streaked on four sides near the periphery and labelled. The petriplates were incubated at 25 ± 2^{O}C. Radial growth of the test pathogen was recorded compared to the control and the inhibition zone was measured in millimeter.

3.5 GLASS HOUSE TESTING OF THE EFFECTIVE *IN VITRO* TREATMENTS

The treatments found effective in the *in vitro* studies were tested on the rooted cuttings of Thompson seedless variety of grapevine in glass house.

3.5.1 MASS MULTIPLICATION OF PATHOGEN

To produce adequate conidial inoculum of the test pathogen, *Alternaria vitis* was grown on the host leaf extract medium. For this purpose medium was prepared by dissolving 20 g of dextrose and 15 g of agar agar in the host leaf decoction, prepared from 200 g of grape leaves and made upto to 1 lt. The pathogen was inoculated on the petriplates containing the host leaf extract medium and incubated at 25 ± 2^{O}C. Conidia from the 7 day old culture were harvested by placing a few ml of sterilised distilled water in the culture plates and by a gentle scraping on the surface with camel hair brush. The spore suspension was diluted with sterile water to get a spore density of 100 x 10^4 spores / ml.

3.5.2 MASS MULTIPLICATION OF BIOCONTROL AGENTS AND PHYLLOPLANE ANTAGONISTS

The fungal antagonists were grown on PDA at 25 ± 2^{O}C and spore suspension of 50 x 10^3 spores / ml was prepared from the 5-6 day old cultures.

The bacterial antagonists were grown on nutrient agar at 25 ± 2^{O}C. Bacterial cell suspension of 10 x 10^3 to 10 x 10^4 cfu / ml was prepared from 2 day old cultures with the help of haemocytometer (Aneja, 2003).

3.5.3 TESTING OF DIFFERENT TREATMENTS AGAINST THE DISEASE IN GLASSHOUSE CONDITIONS

To develop a suitable management strategy for the leaf blight disease, the treatments were imposed as follows.

1. The treatment was imposed 7 days before the inoculation of the pathogen. (Prophylactic).

2. Treatment was imposed 24 hours before inoculation of the pathogen. (Simultaneous).
3. The pathogen was inoculated 7 days before the application of treatment. (Curative).

The inoculations and treatments were imposed by spraying on both the surfaces of leaves of Thompson seedless variety of grapevine grown in polybags. After inoculation with pathogen the inoculated twigs were covered with polythene covers for 12 hours to maintain the humidity for better infection. The effectiveness of different treatments was recorded using 0-9 scale (Mayee and Datar, 1986) and Percent Disease Index (PDI) was calculated using the formula.

$$\text{PDI} = \frac{\text{Sum of disease ratings}}{\text{Total ratings}} \text{ X } \frac{100}{\text{Maximum grade}}$$

3.6 STATISTICAL ANALYSIS

All the experiments were carried out with three replications and the results were statistically analysed by following Completely Randomized Design.

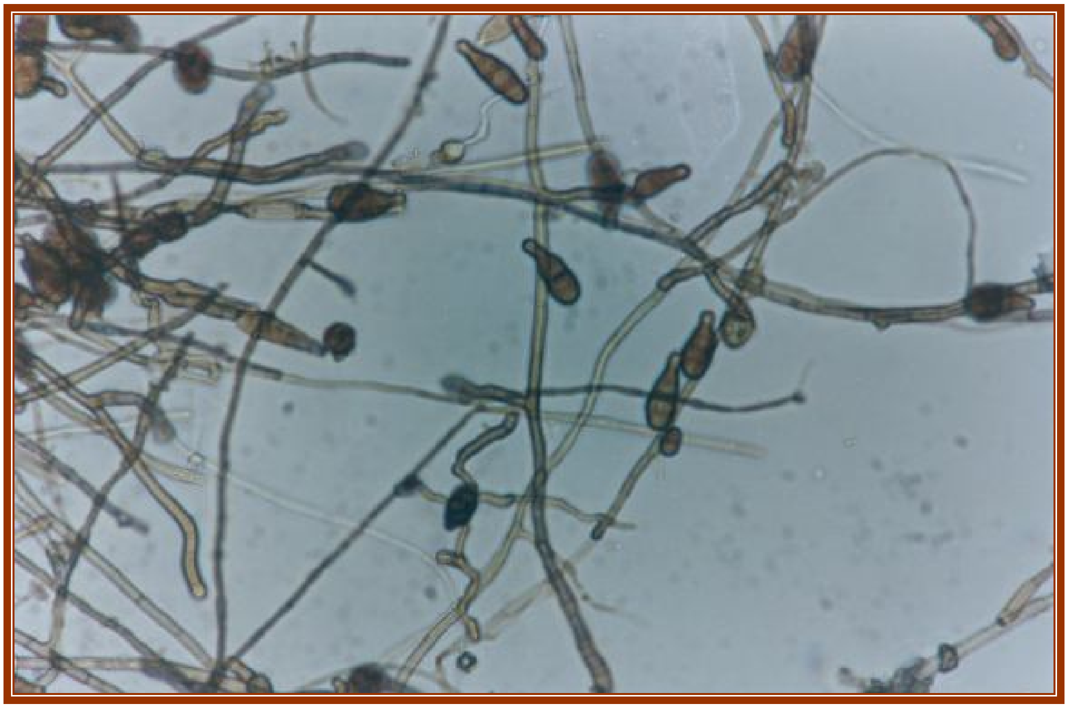


Plate 5: **Photomicrograph of *Alternaria vitis* showing mycelium and conidia (10X40X).**

Plate 6: **Photomicrograph showing conidia of *Alternaria vitis* (10X63).**

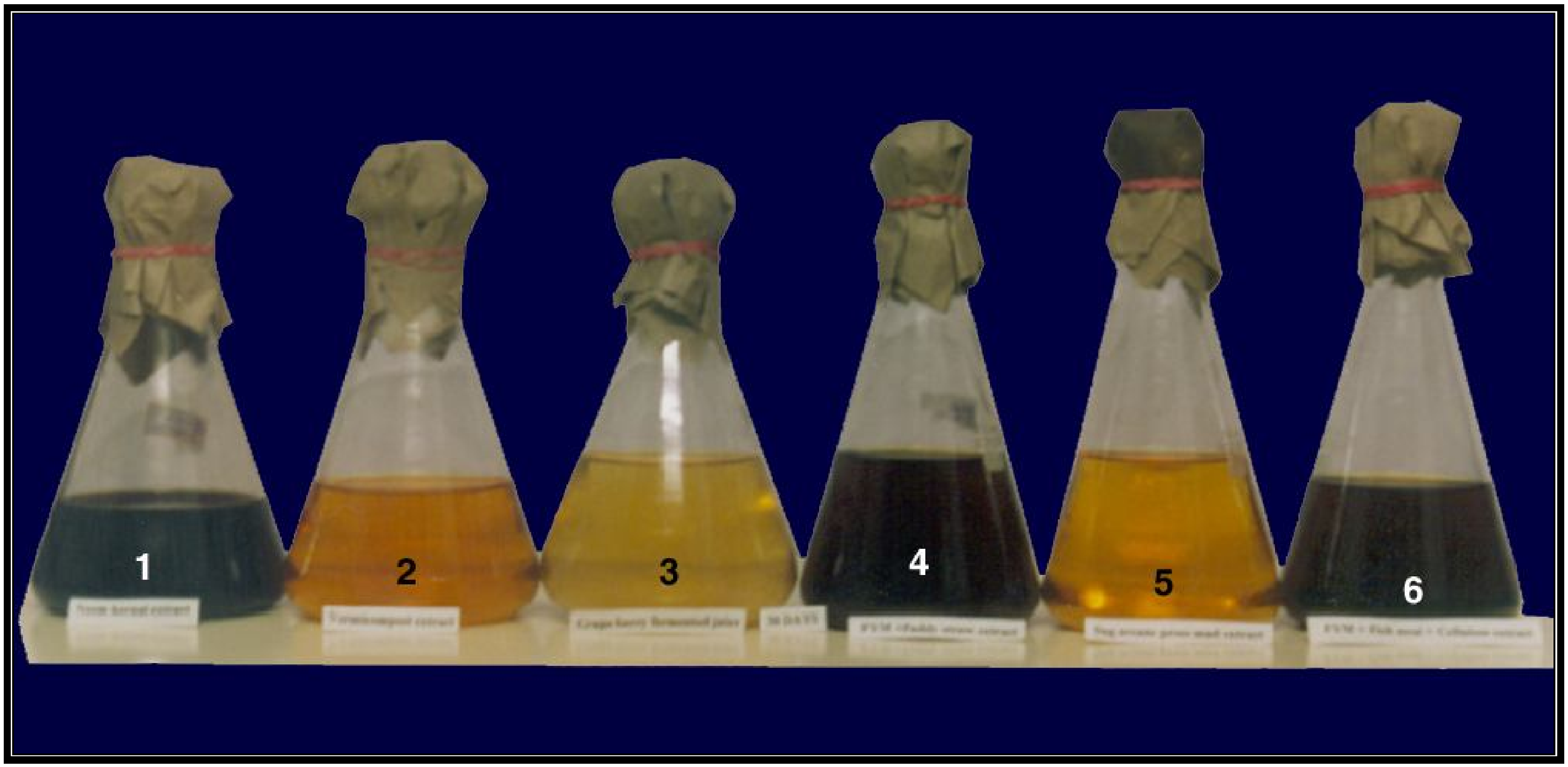


Plate 9: Cold sterilised extracts. (10 days) 1. Neem seed kernel extract 2. Sugarcane press mud extract 3. Grape berry fermented juice 4. FYM+Paddy straw extract 5. Vermicompost extract 6. FYM+Fish meal extract.

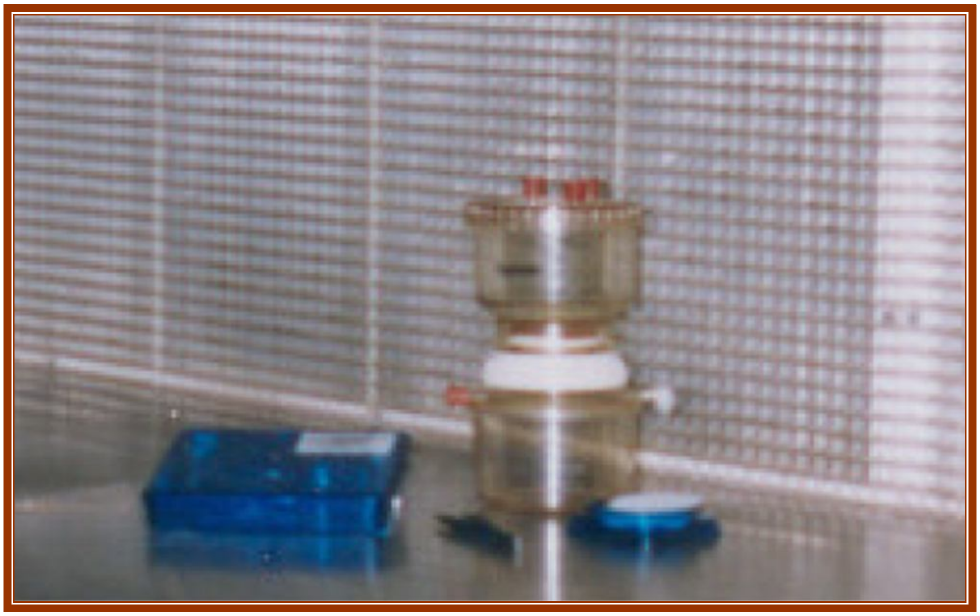


Plate 7: **Polysulfone filter holder and Cellulose acetate membranes.**

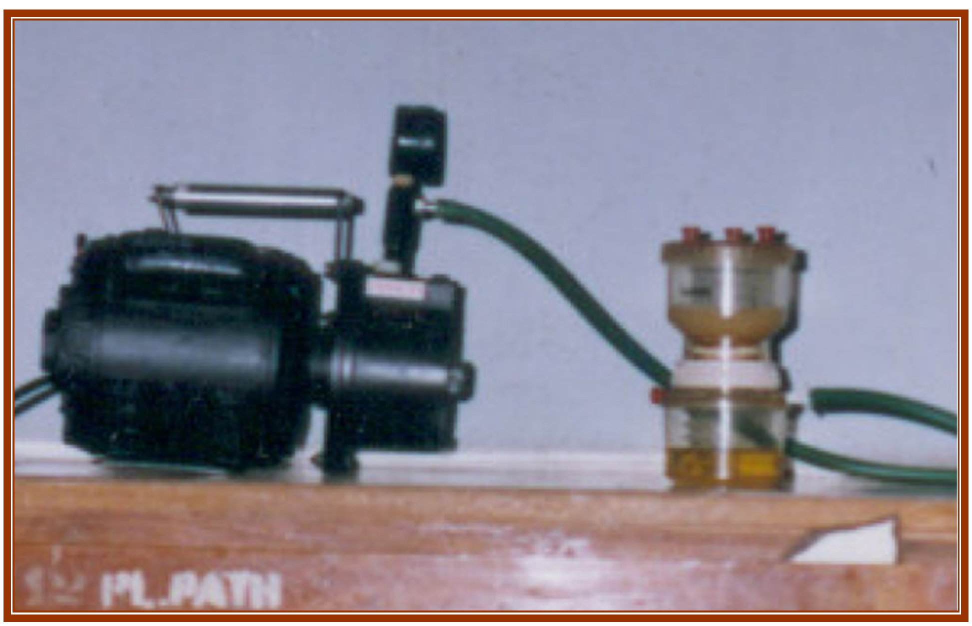
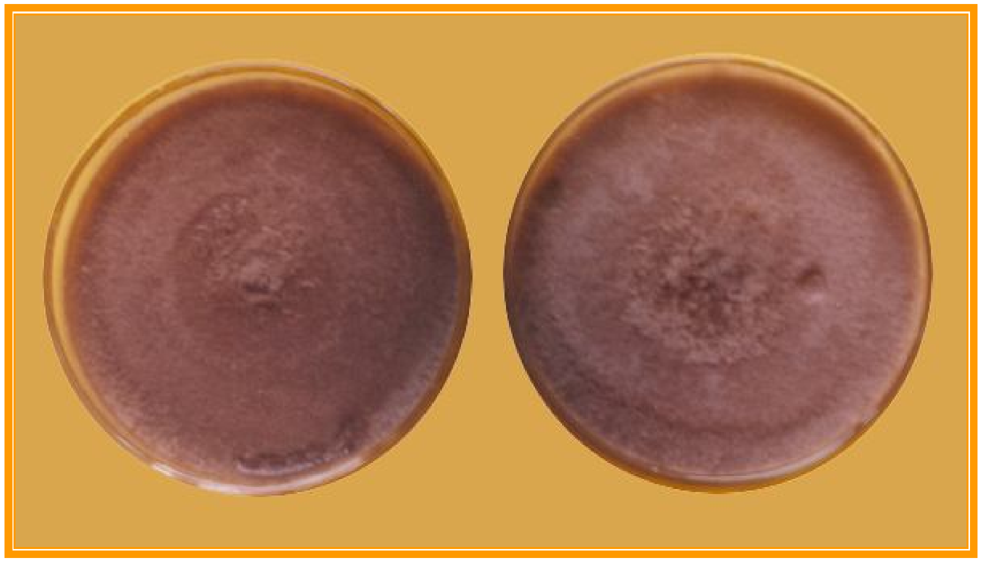
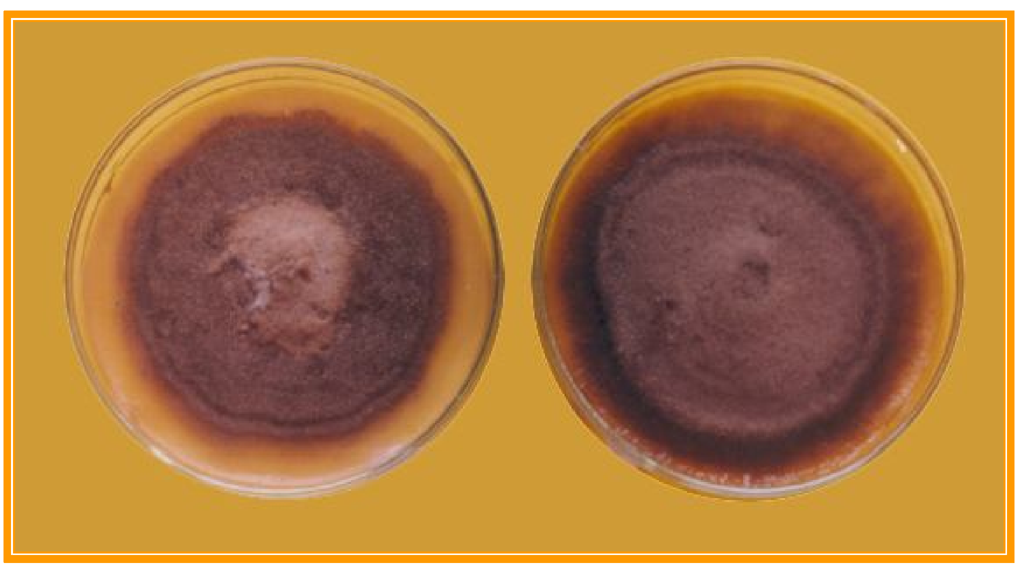


Plate 8: **Cold sterilisation – The micro filtration assembly.**



<u>Plate 3:</u> Pure culture of *Alternaria vitis* (Hyphal tip method).



<u>Plate 4:</u> Pure culture of *Alternaria vitis* (Single spore isolation method).

CHAPTER IV

RESULTS

The efficacy of extracts of composts, amendments and plant sources, fungicides, chemicals, bioagents, phylloplane microflora was tested individually on the grapevine leaf blight pathogen *Alternaria vitis* as well as on the disease in the glass house. Results of the experiments are presented here.

4.1.1 CONFIRMATION OF PATHOGENICITY

The pathogen isolated from the infected grape leaves was identified as *Alternaria vitis* based on the cultural characters, spore morphology (Vidhyasekaran *et al*., 1969). On inoculation the leaves of grapevine expressed the symptoms from 4^{th} or 5^{th} day on words. Initially light greenish yellow coloured, chlorotic areas were observed on the lamina which later turned into small specks that expanded into spots. Blighting observed mostly from leaf margins. Fungus reisolated from the inoculated leaves showing typical symptoms is compared and found quite similar to the original culture in morphological and colony characters, thus establishing the pathogenicity on grape.

4.1.2. CULTURAL AND MORPHOLOGICAL CHARACTERS OF *Alternaria vitis*

The pathogen could produce visible growth after 24-36 hours on PDA. After 72 hours whitish colonies were observed which were later turned greyish after 5-6 days. The fungus showed radiating growth. Some times sectoring is observed.

Microscopic studies revealed that, hyphae was light brown, profusely branched and septate. Conidiophores were simple or branched, branched and comparatively dark brown in colour. Conidia were brown in colour swollen at base gradually taper towards the proximal end. Beaks were comparatively lighter in colour and also varied in size. Conidia were produced singly. Very rarely chains were observed. Conidia measured 25.0- 65.25µm X 10.30-17.5µm in size. On the basis of these cultural and morphological characters and pathogenicity the fungus was identified as Alternaria vitis (Cav.) Sacc.(Vidhyasekaran *et al*., 1969)

4.2 EFFICACY OF DIFFERENT EXTRACTS

Six extracts i.e., Neem seed kernel extract (NSKE), Vermicompost extract (VCE), Sugarcane pressmud extract (SPME), FYM + Fish meal + Cellulose extract (FME), FYM + Paddystraw extract (FYME) and Grape berry fermented juice (GBJ) were used for studying the effect on *A.vitis*.

4.2.1 ON MYCELIAL GROWTH

4.2.1.1 10 DAY FERMENTED EXTRACTS

Alternaria vitis differed in its reaction to the ten day fermented extracts of composts and GBJ. (Table 5 & Plate 10). GBJ at 50% and 25% showed 100% growth inhibition and at 12.5% the inhibition was 58.76%. Except GBJ all other treatments were less effective in inhibiting the mycelial growth of the test pathogen.

4.2.1.2 20 DAY FERMENTED EXTRACTS

Among all the treatments GBJ at 50% was highly effective with 100% inhibition of growth (Table 6 & Plate 11) followed by NSKE at 10% with 50.75% inhibition. Except these two the remaining treatment were less effective against the mycelial growth of the pathogen.

4.2.1.3 30 DAY FERMENTED EXTRACTS

With an increased fermentation time of 30 days also GBJ showed 100% and 82.08% respectively at 50% and 25% concentration respectively. And at 12.5%, the growth inhibition of *A.vitis* was 43.58% (Table 7 & Plate 12). All the GBJ concentrations were significantly superior over rest of the treatments.

4.2.2 ON SPORE GERMINATION

4.2.2.1 10 DAY FERMENTED EXTRACTS

Spore germination differed with different organic extracts fermented for 10 days (Table 8). GBJ at 50% recorded 96.04% inhibition followed by FYME at 50% with 67.08%, which was at par with 10% SPME (65.87%) and 25% GBJ (64.67%). All the treatments at 10 days fermentation were considerably effective in inhibiting the spore germination.

4.2.2.2 20 DAY FERMENTED EXTRACTS

The efficacy of extracts fermented for 20 days are presented in Table 9. GBJ at 50 percent concentration showed highest inhibition of 82.92% followed by FME at 50% with 80.42% and SPME at 10% with 73.75% that were at par with GBJ 50%. All the extracts fermented for

20 days were considerably effective in the inhibition of spore germination.

4.2.2.3 30 DAY FERMENTED EXTRACTS

Results of 30 day fermented extracts on the spore germination of *A. vitis* are presented in Table 10. Among all the treatments GBJ at 50% recorded 85.42 % inhibition followed by the same extract at 25% with 67.5% inhibition.

4.2.3 INTERACTION OF TREATMENTS WITH FERMENTATION PERIODS

The mean percent inhibition values of growth were analysed through factorial CRD to compare the interactions of treatments and different fermentation periods. The results are presented in Tables 11 & 12. Most of the treatments showed an increased inhibition of mycelial growth at a higher fermentation period of 30 days. But difference in the means of % inhibition of spore germination at the three fermentation periods tested were not significant.

4.3 EFFICACY OF FUNGICIDES

The fungicides viz., azoxystrobin (Amistar 25SC), mancozeb (Indofil M-45 75WP) and iprodione25% + carbendazim25% (Quintal 50WP) were tested at different concentrations to study their effect on mycelial growth and spore germination of *A.vitis.* Results described in Table 13

4.3.1 ON MYCELIAL GROWTH

Results indicated that the sensitivity of *Alternaria vitis* varied with fungicides and its concentrations. Iprodione 25% + carbendazim 25% (Quintal) at 0.1% and 0.05%, mancozeb (Indofil M-45) at 0.25% showed 100% inhibition of mycelial growth, followed by mancozeb (Indofil M-45) at 0.125% with 81.6% inhibition. (Plate 13)

4.3.2 ON SPORE GERMINATION

The effect of different fungicides on spore germination test is given in Table 13. Iprodione25% + carbendazim25% (Quintal) at 0.1% and mancozeb (Indofil M-45) at 0.25% showed 100% inhibition of spore germination. Mancozeb (Indofil M-45) at 0.125% with 97.62 % inhibition is at par with above best treatments. Azoxystrobin (Amistar) at 0.025% was found to be least effective in inhibiting the spore germination of the fungus with 86.91% inhibition.

4.4 EFFICACY OF SALICYLIC ACID

Effect of Salicylic acid on the mycelial and spore germination of *A.vitis* has studied at five concentrations. The effect of SA on mycelial growth and spore germination is presented in Table 14.

4.4.1 ON MYCELIAL GROWTH

Salicylic acid was not effective in inhibiting the mycelial growth of *A.vitis* at all the concentrations tested (Plate 14). However at a higher concentration of 125 ppm showed 14.05% inhibition. While all other treatments were at par.

4.4.2 ON SPORE GERMINATION

Results indicated that SA showed a maximum inhibition of 45.62% at higher concentration of 125 ppm which was significantly superior over other concentrations tested. The remaining treatments were at par with an inhibition ranging between 36.60% and 32.44% .

4.5 EFFECT OF BIOCONTROL AGENTS ON MYCELIAL GROWTH OF *A.vitis*

The bioagents i.e., *Trichoderma viride* and *Pseudomonas fluorescens* were found to be effective in inhibiting the growth of *A.vitis* in culture (Table 15 & Plate 15) . The growth inhibition of *A.vitis* due to *Trichoderma viride* was 66.67%, while the inhibition was 55.56% with *Pseudomonas fluorescens.*

4.6 EVALUATION OF PHYLLOPLANE MICROFLORA

4.6.1 ISOLATION AND IDENTIFICATION

Phylloplane microflora of grapevine were isolated from washings of leaf surface. Ten fungal and five bacterial isolates were observed. The fungal species isolated were identified at ITCC, Division of Mycology and Plant Pathology, IARI, Pusa, as *Trichoderma harzianum, T. viride, Aspergillus niger, A. tumarii, A. fumigatus, Paecilomyces variotii, Thielavia terricola, Cladosporium cladosporoides, Oidiodendron sp.*, *Alternaria sp.* (Plates 16-19). And the five bacterial isolates (Plate 24) were named as PPB-1Y , PPB-2R, PPB-3Ro, PPB-4W, PPB-5Cr. Bacterial isolates were studied for their morphological and colony characters. Description of the characters of bacterial isolates is presented in Table 17 (Plates 38-42). Antagonistic properties and activity of these microflora on the growth of *A.vitis* is described.

4.6.2 EFFICACY OF PHYLLOPLANE FUNGI

Among all the phylloplane fungi tested against the *A.vitis*, *Trichoderma harzianum* showed highest inhibition of 72.8% against

A.vitis in culture followed by *T.viride* with 63.05 % inhibition of growth. The results are presented Table 16 (Plates 20-23).

4.6.3 EFFICACY OF PHYLLOPLANE BACTERIA

The results on the effect of phylloplane bacteria on *A.vitis* in culture are presented in Table 17(Plate 25). Among all the bacterial isolates PPB-1Y showed highest percent inhibition of 65.91% and 5.34 mm inhibition zone, followed by PPB-2R with 56.3% inhibition and 2.67mm inhibition zone.

4.7 INTERACTIONS OF FUNGAL ANTAGONISTS – SEM STUDIES

The antagonists *Trichoderma viride* (Bio control agent) and *Trichoderma harzianum* (phylloplane fungus) showed mycoparasitism and lytic activity against the *Alternaria vitis*. Various interactions were observed like Penetration of antagonists into pathogen hyphae, formation of holes , pits, groves and knots, coiling, disintegration of mycelium etc.

4.8 GLASS HOUSE TESTING OF THE EFFECTIVE *IN VITRO* TREATMENTS

The treatments proved best under *in vitro* conditions were evaluated for their performance against the leaf blight disease under glass house conditions. Along with these treatments unsterilised 10 day GBJ at 25%, 20 day NSKE at 10 % were also included for testing. The different treatments tested against the disease in glass house conditions are listed below.

1. 10 day sterilised GBJ at 25%
2. 10 day unsterilised GBJ at 25%
3. 20 day NSKE at 10%
4. Iprodione25% + carbendazim25% (Quintal 50WP) at 0.1%
5. Mancozeb (Indofil M – 45 75WP) at 0.25 %
6. *Trichoderma viride* at 5 X 10^4 conidia /ml
7. *Pseudomonas fluorescens* at 10^4-10^5cfu /ml
8. Native *Trichoderma harzianum* at 5 X 10^4conidia /ml
9. Native *Pseudomonas fluorescens* at 10^4 –10^5cfu /ml
10. Salicylic acid at 125ppm

The treatments were tested individually and were imposed in three different methods of applications viz. prophylactic, simultaneous and curative applications as described in chapter III, Materials and Methods. Results presented in Table 19.

4.8.1 PROPHYLACTIC APPLICATION

Among the different prophylactic treatments tested spraying of 10 day fermented 25% unsterilised GBJ and iprodione25% + carbendazim25% (Quintal) showed minimum PDI of 14.83% each indicating the superiority over other treatments in controlling the leaf blight disease. Among the bioagents tested it was observed that native isolates of *Trichoderma harzianum* and PPB –1Y were found to be superior over commercially available bioagents with 17.29 of PDI each.

4.8.2 SIMULTANEOUS APPLICATION

Among these treatments also, 10 day fermented unsterilised GBJ at 25% showed minimum PDI of 22.23 followed by iprodione25% + carbendazim25% (Quintal) at 0.1% and *Pseudomonas fluorescens* which were at par with the best treatment with 27.18 and 27.17 PDI indicating the superiority over treatments in controlling the leaf blight disease of grapevine is glass house conditions. However all these treatments are at par and superior over the other treatments.

4.8.3 CURATIVE APPLICATION

Different treatments applied one week after the inoculation of the pathogen were found to be not effective in controlling the leaf blight pathogen under glass house conditions. In curative applications 20 day NSKE at 10% recorded the least PDI of 41.97% followed by the 10 day unsterilised GBJ at 25% with PDI of 42.44%. However these two treatments are at par with each other. The antagonists like Native PPB – 1Y, *Trichoderma viride*, native *Trichoderma harzianum* with 45.59%, 46.29% and 47.14% of PDI have registered significantly less PDI compared to control with 61.11% of PDI.

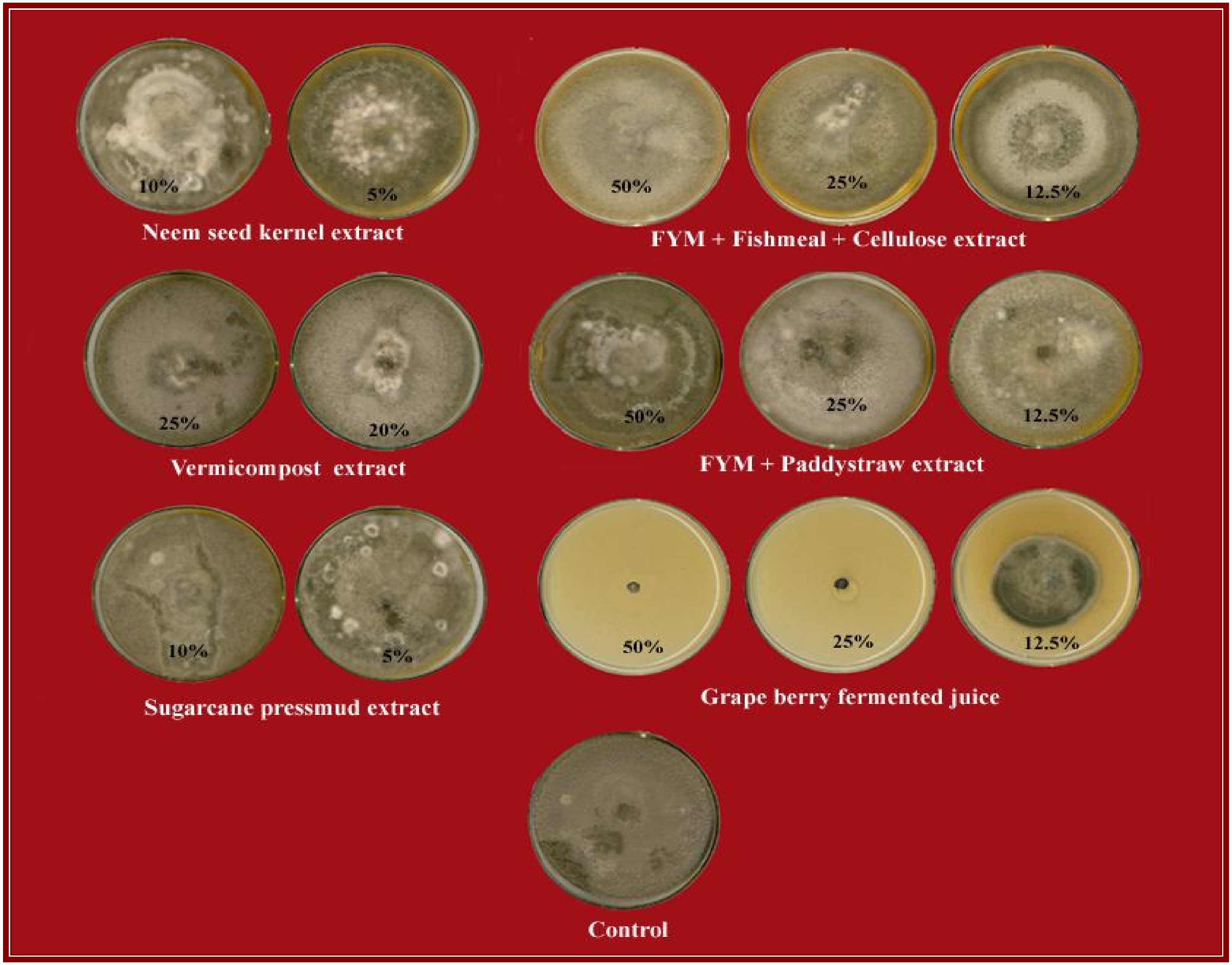


Plate 10: ***In vitro*** **efficacy of 10 day fermented extracts on mycelial growth of** ***Alternaria vitis*****.**

Plate 11: ***In vitro*** **efficacy of 20 day fermented extracts on mycelial growth of** ***Alternaria vitis.***

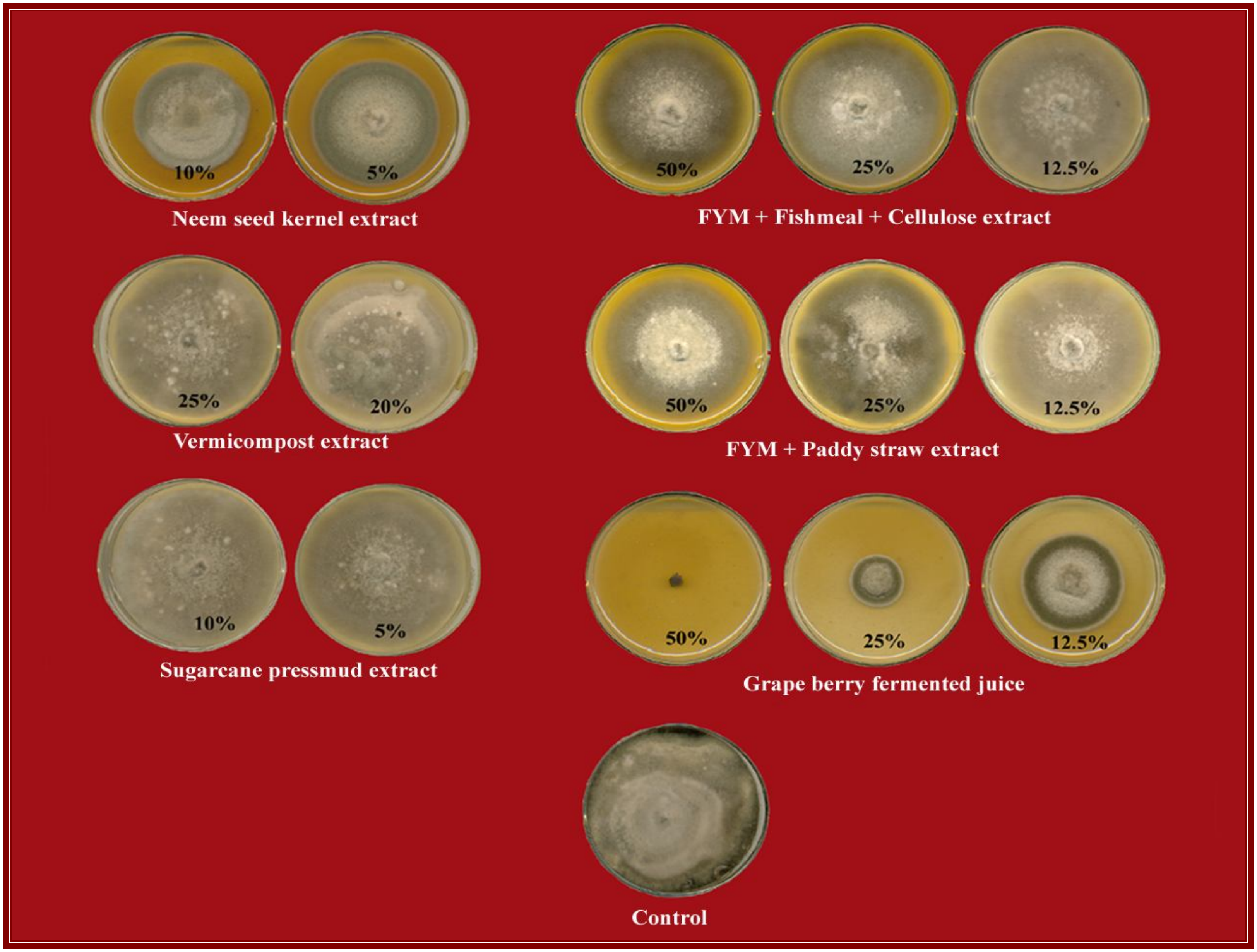
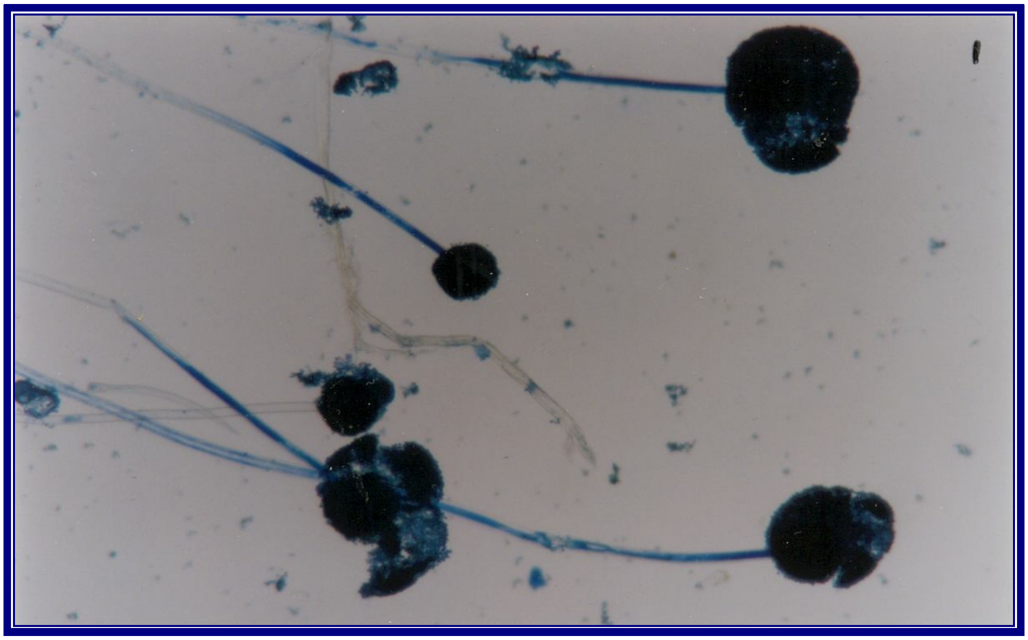
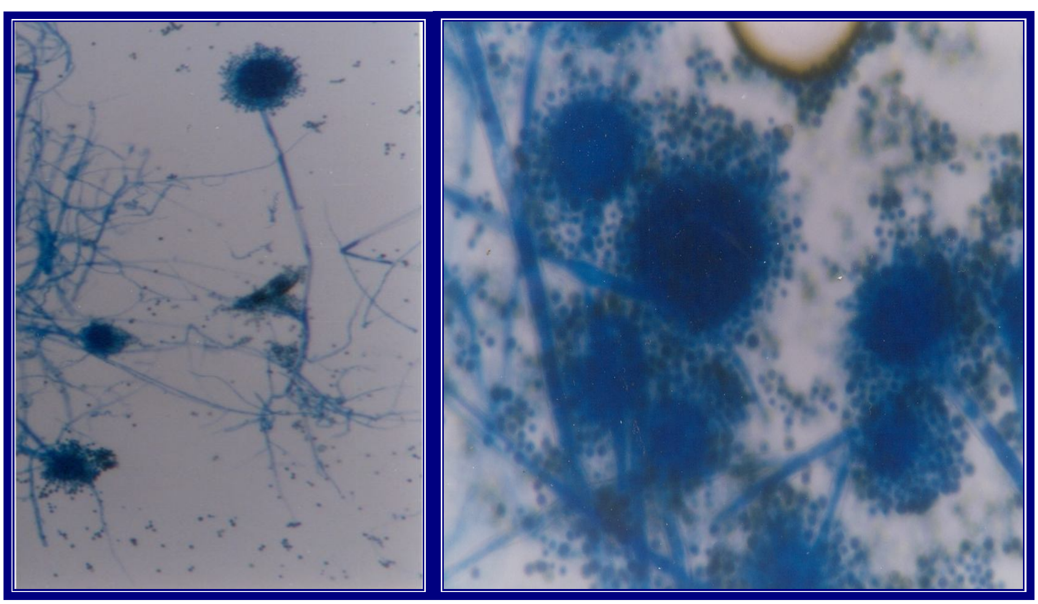


Plate 12: ***In vitro*** **efficacy of 30 day fermented extracts on mycelial growth of** ***Alternaria vitis.***

<u>Plates 28-42:</u> Photomicrographs of phylloplane microflora of grapevine

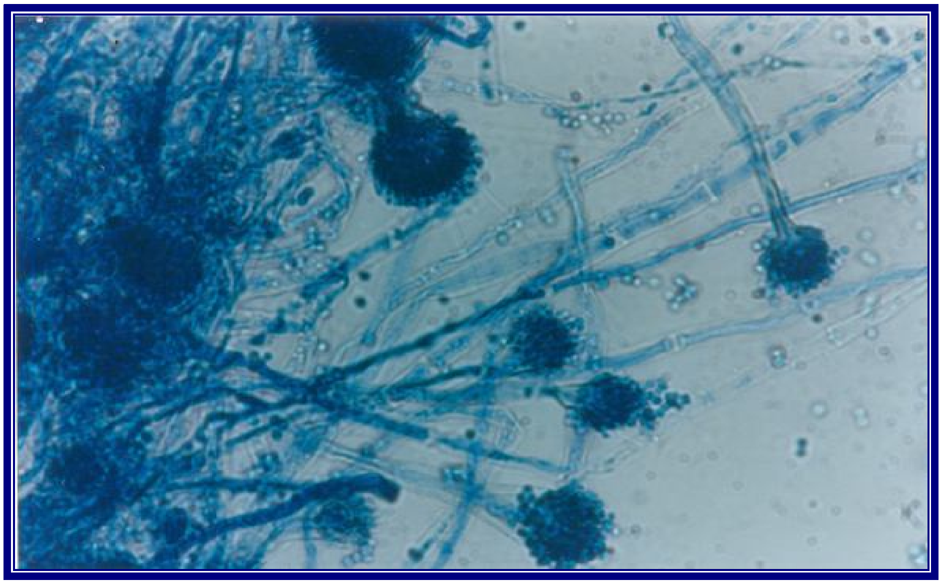


<u>Plate 30:</u> Conidiophores and conidial mass of native *Aspergillus niger* .

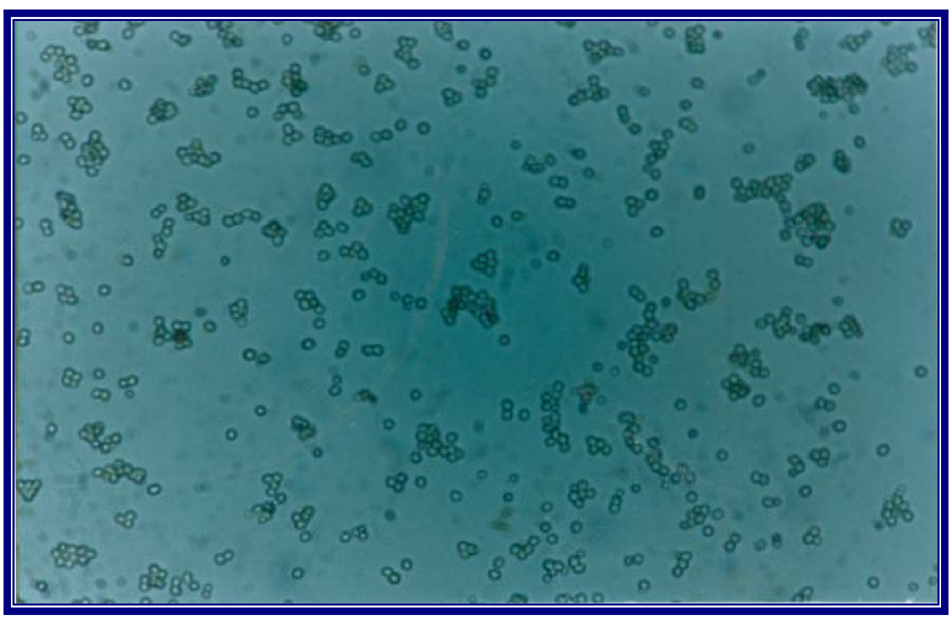


<u>Plate 31:</u> Mycelium, conidiophores and conidia of native *Aspergillus tumarii*.

<u>Plates 28-42:</u> Photomicrographs of phylloplane microflora of grapevine.



<u>Plate 32:</u> Conidiophores and conidia of native *Aspergillus fumigatus.*



<u>Plate 33:</u> Conidia of native *Paecilomyces variotii*.

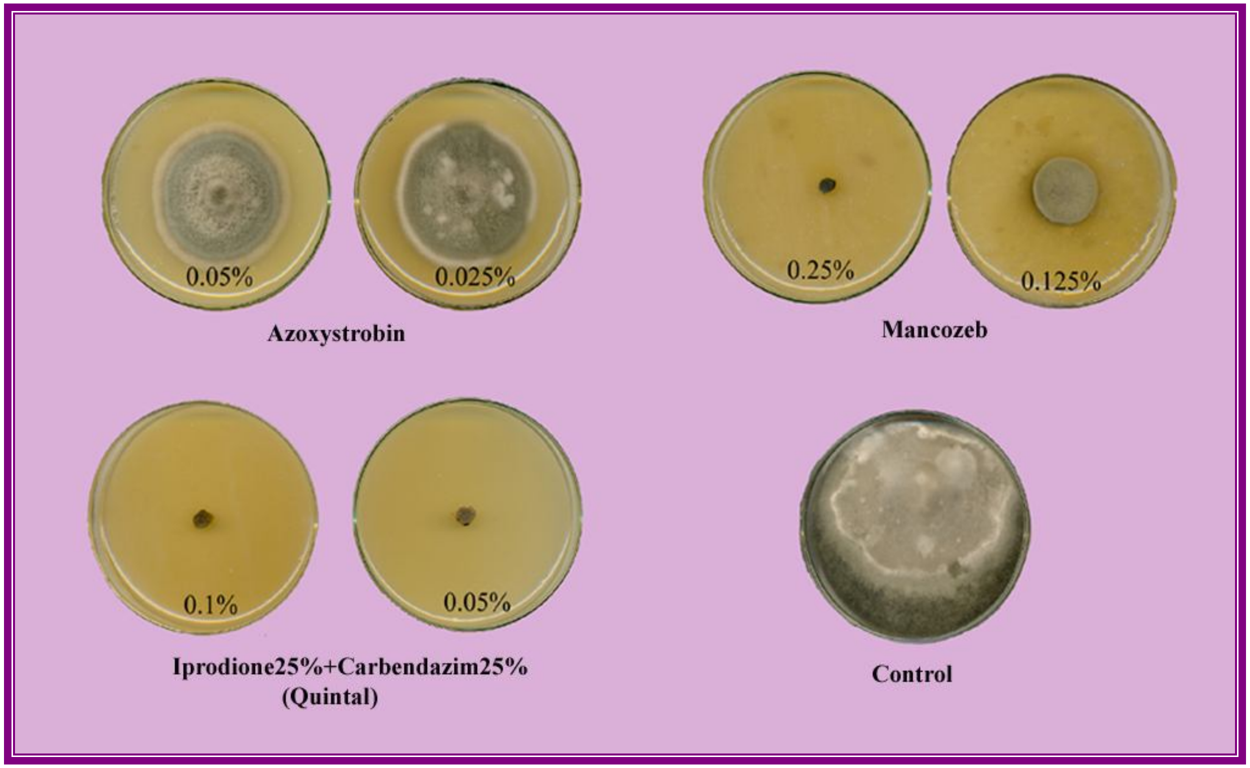
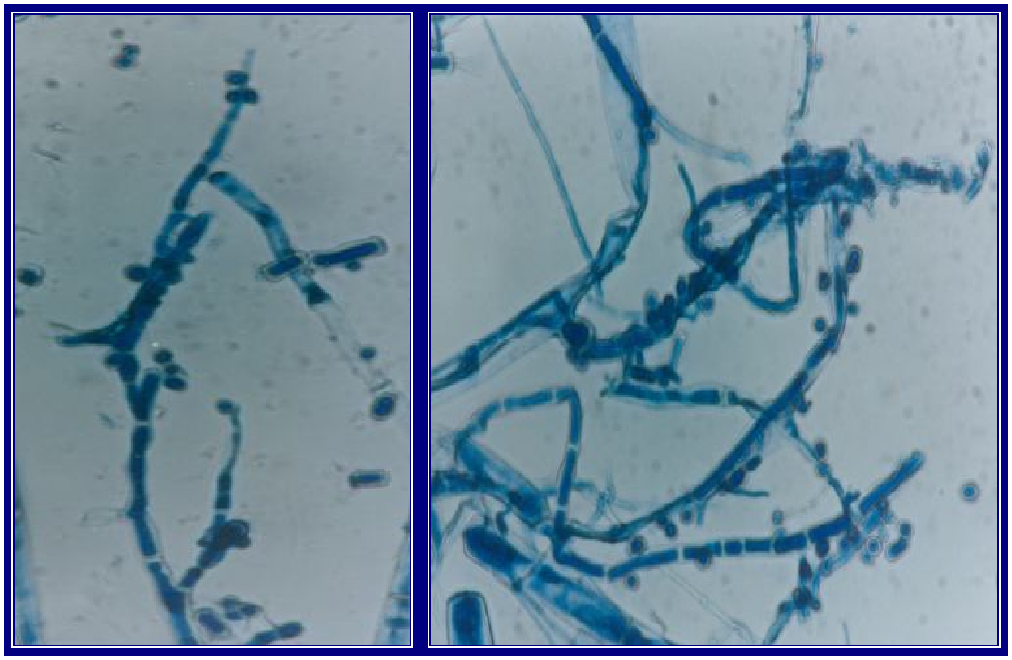
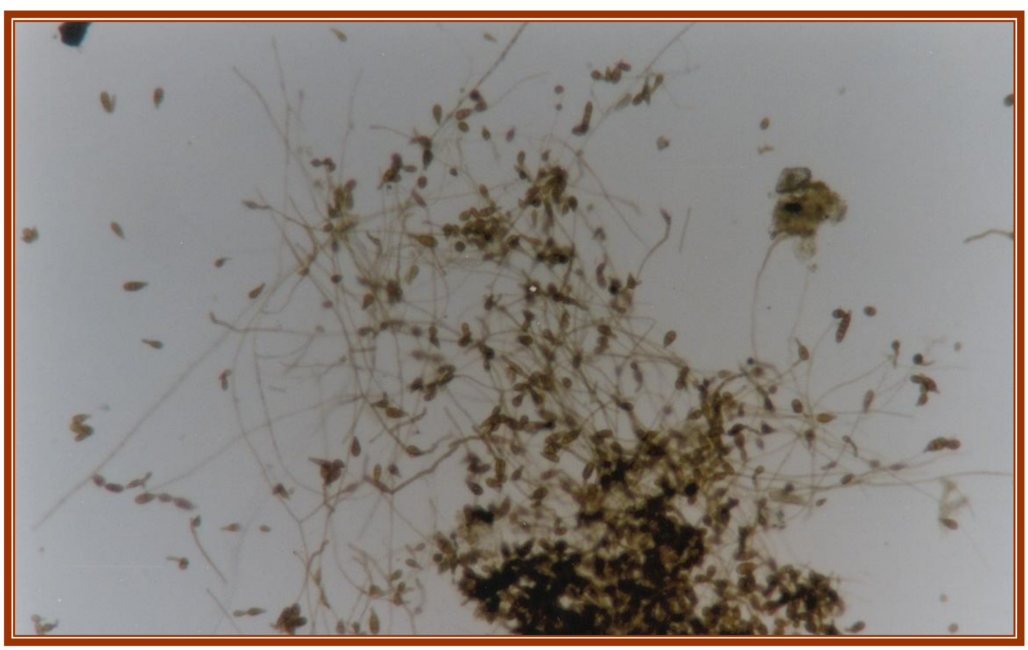


Plate 13: ***In vitro*** **efficacy of fungicides on mycelial growth of** ***Alternaria vitis.***

<u>Plates 28-42:</u> Photomicrographs of phylloplane microflora of grapevine



<u>Plate 36:</u> Mycelium, mycelium converting into barrel and spherical shaped arthrospores of native *Oidiodendron sp.*



<u>Plate 37:</u> Mycelium and conidia of native *Alternaria sp*.

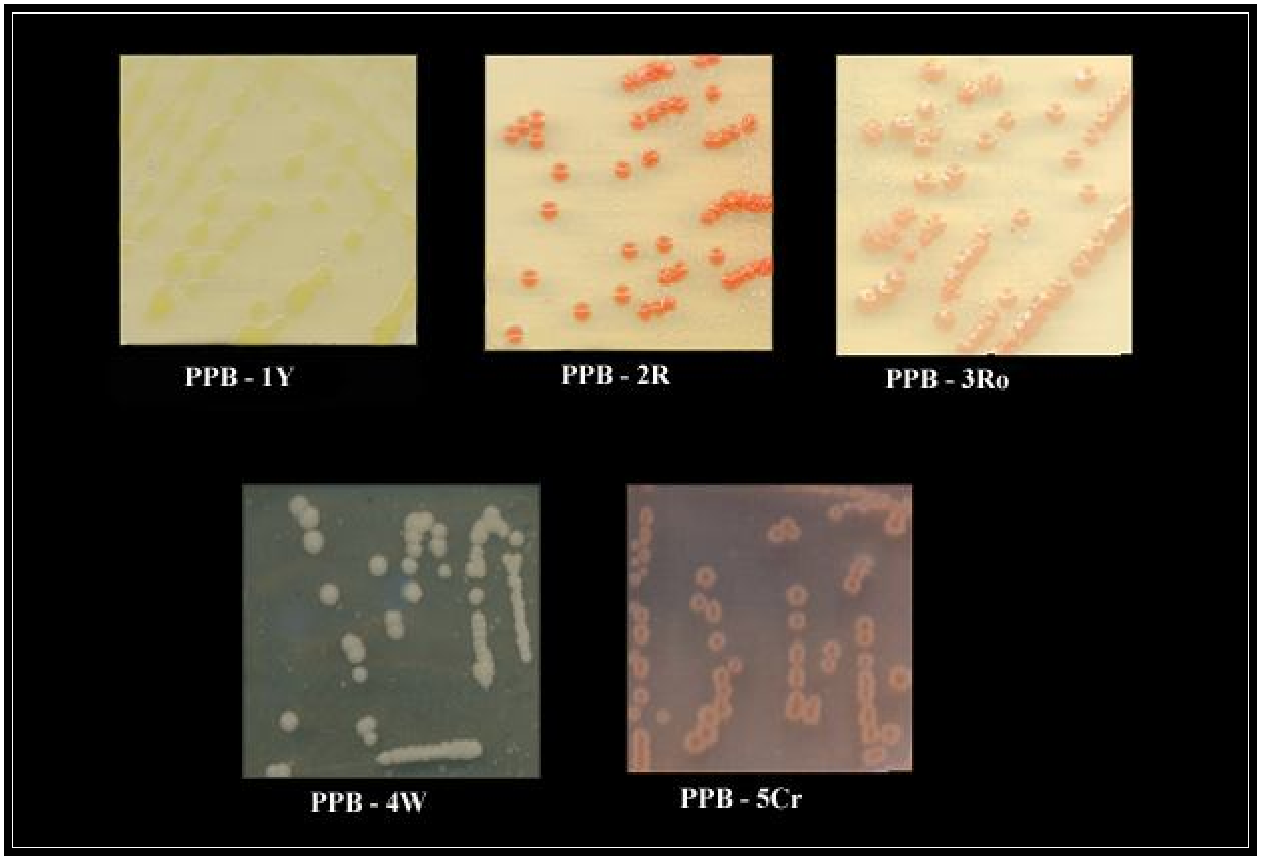


Plate 24: Phylloplane bacterial isolates of grapevine in pure culture.

Plate16 – 19: Phylloplane fungi of grapevine in pure cultures.

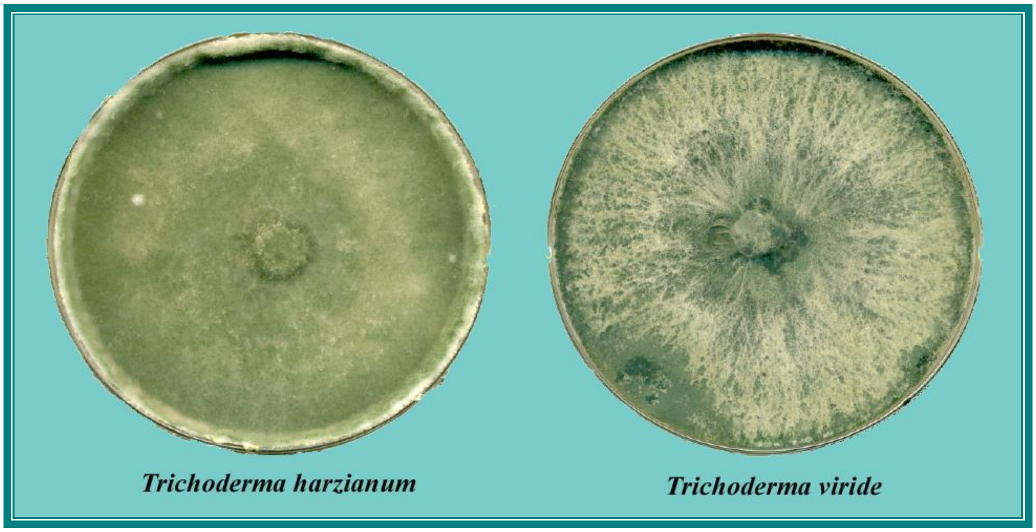


Plate 16: Pure cultures of *Trichoderma harzianum* and *T. viride* isolated from grapevine phylloplane.

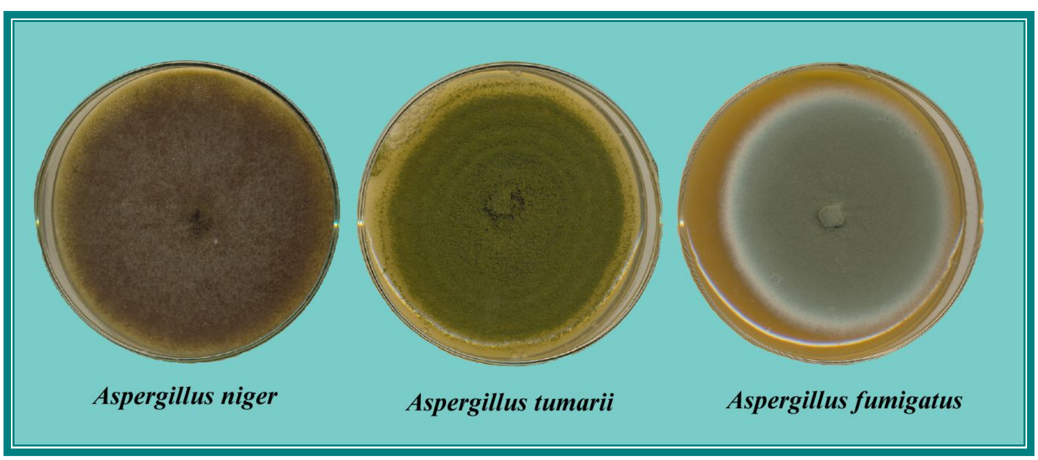


Plate 17: Pure cultures of *Aspergillus niger, A. tumarii* and *A. fumigatus* isolated from grapevine phylloplane.

Plate16 – 19: Phylloplane fungi of grapevine in pure cultures.

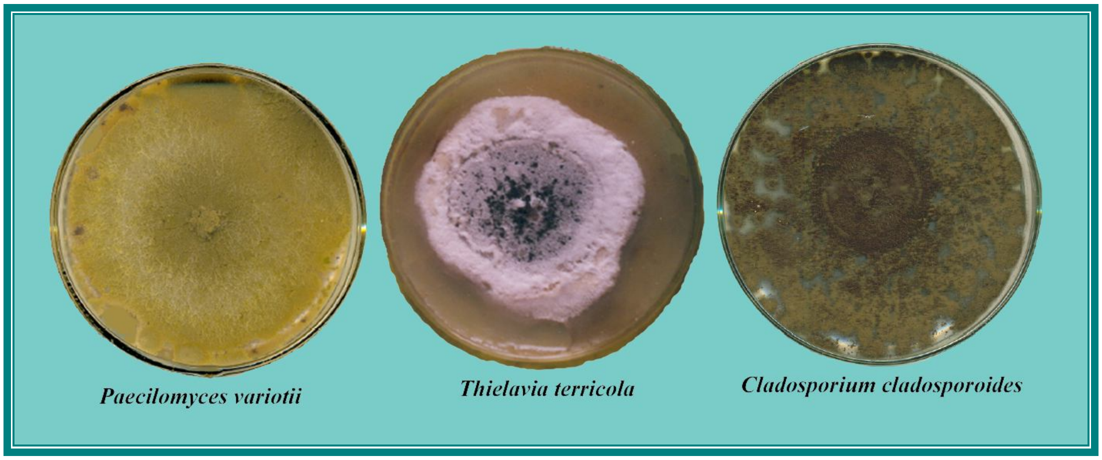
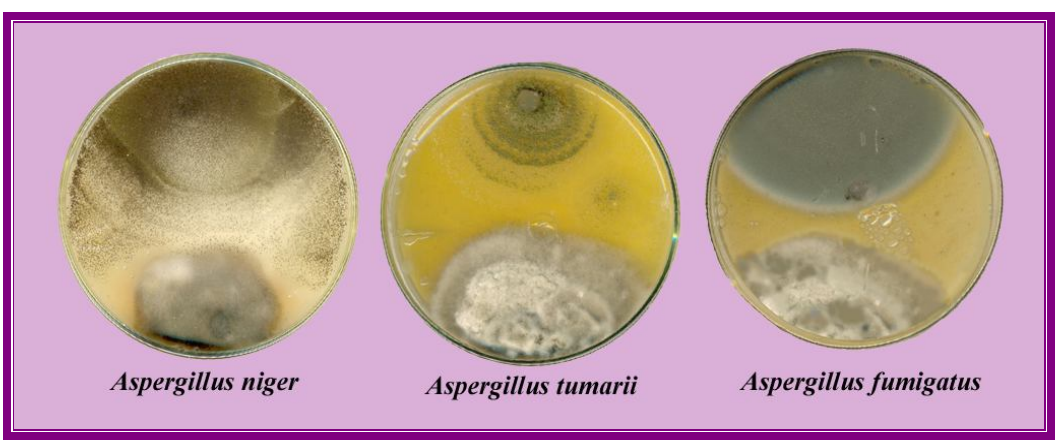


Plate 18: Pure cultures of *Paecilomyces variotii, Thielavia terricola* and *Cladosporium cladosporoides* isolated from grapevine phylloplane.

Plate 19: Pure cultures of *Oidiodendron sp* and *Alternaria sp* isolated from grapevine phylloplane.

<u>Plates 20 -23:</u> Efficacy of phylloplane fungi on *Alternaria vitis* in dual culture.

<u>Plate 20:</u> Efficacy of native isolates of *Trichoderma harzianum* and *T.viride* on *Alternaria vitis.*



<u>Plate 21:</u> Efficacy of native isolates *Aspergillus niger, A. tumarii* and *A. fumigatus* on *Alternaria vitis.*

Plates 20 -23: Efficacy of phylloplane fungi on *Alternaria vitis* in dual culture.

Plate 22: Efficacy of native isolates of *Paecilomyces variotii, Thielavia terricola* and *Cladosporium cladosporoides* on *Alternaria vitis*

Plate 23: Efficacy of *Oidiodendron sp* and *Alternaria sp* on *Alternaria vitis.*

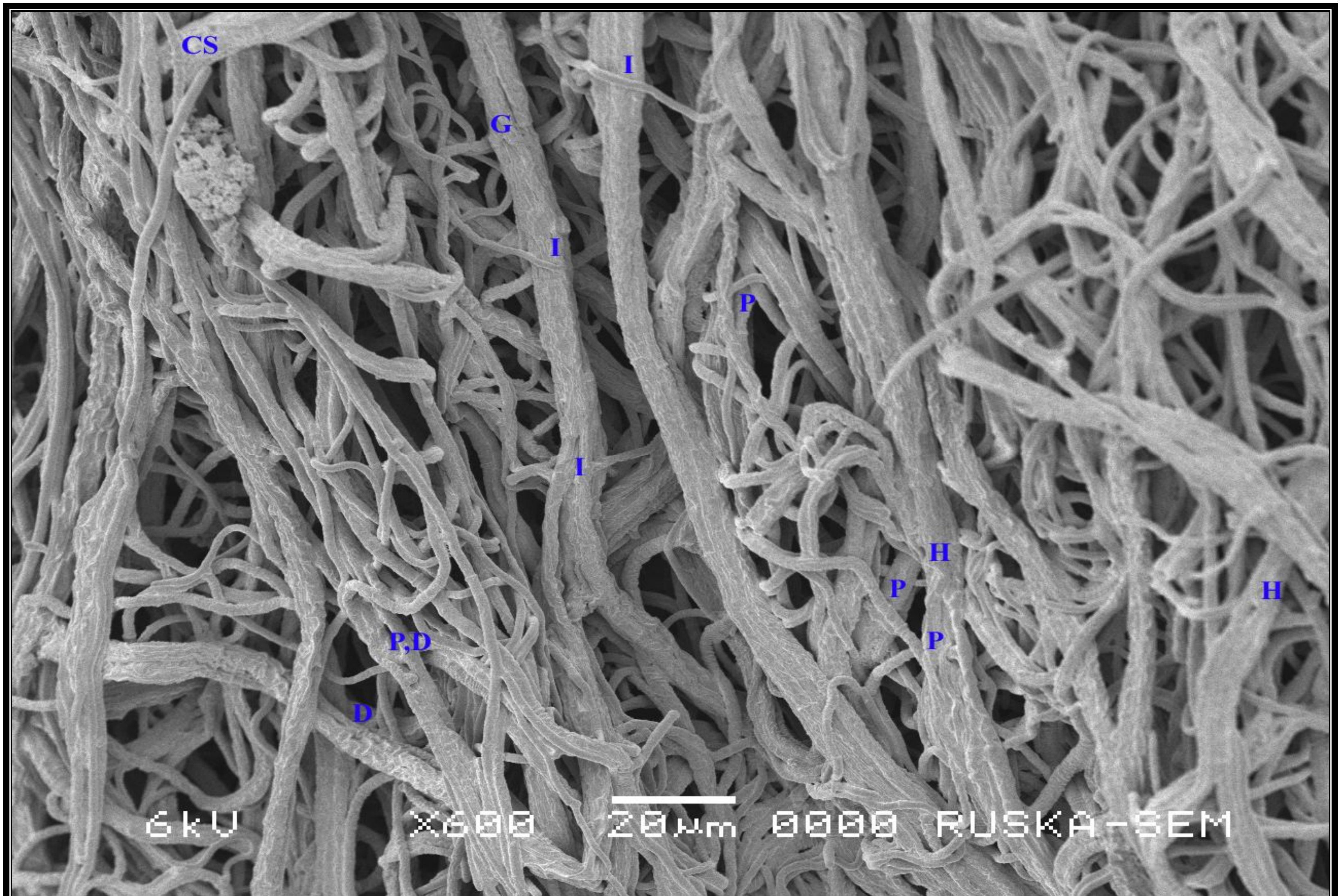
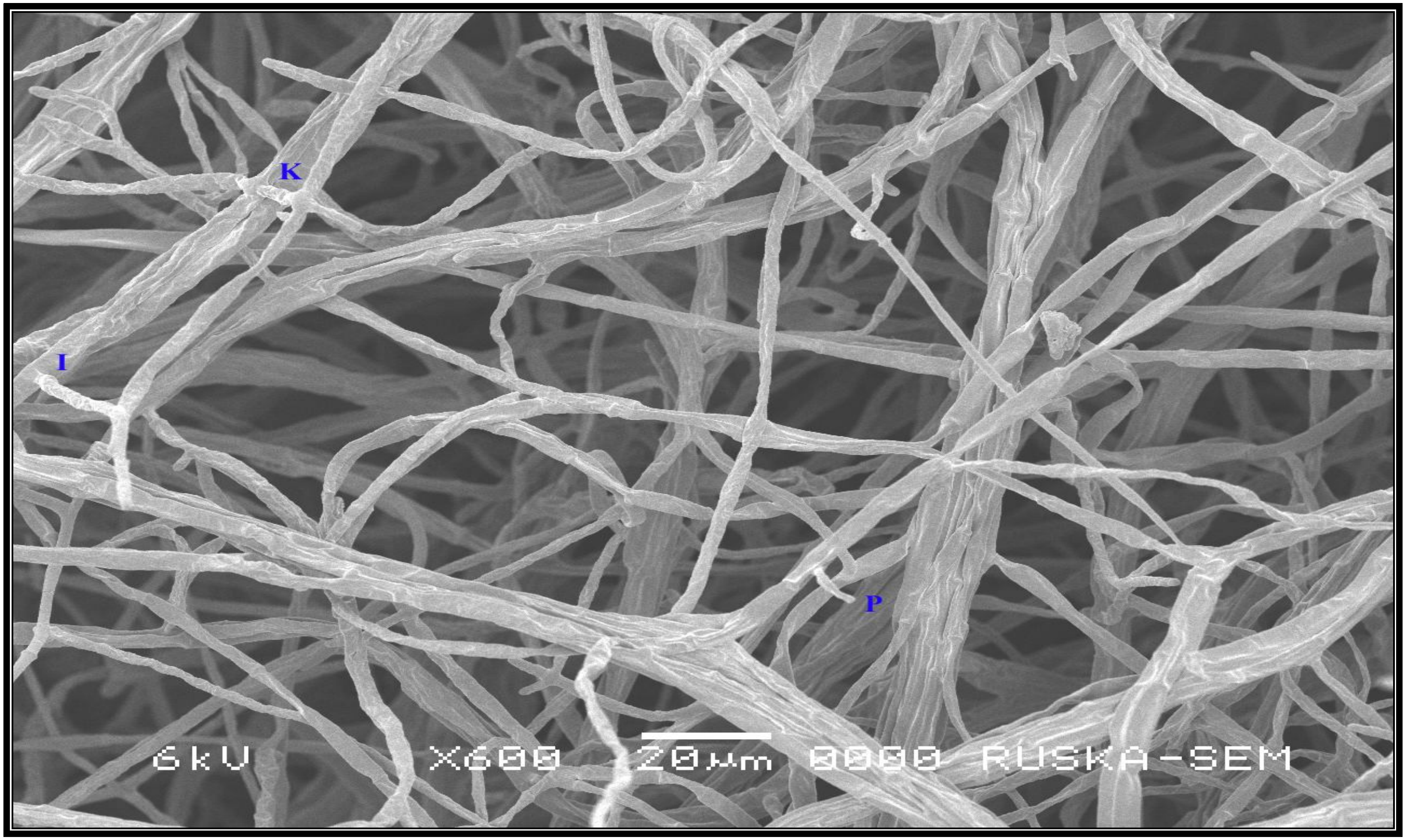
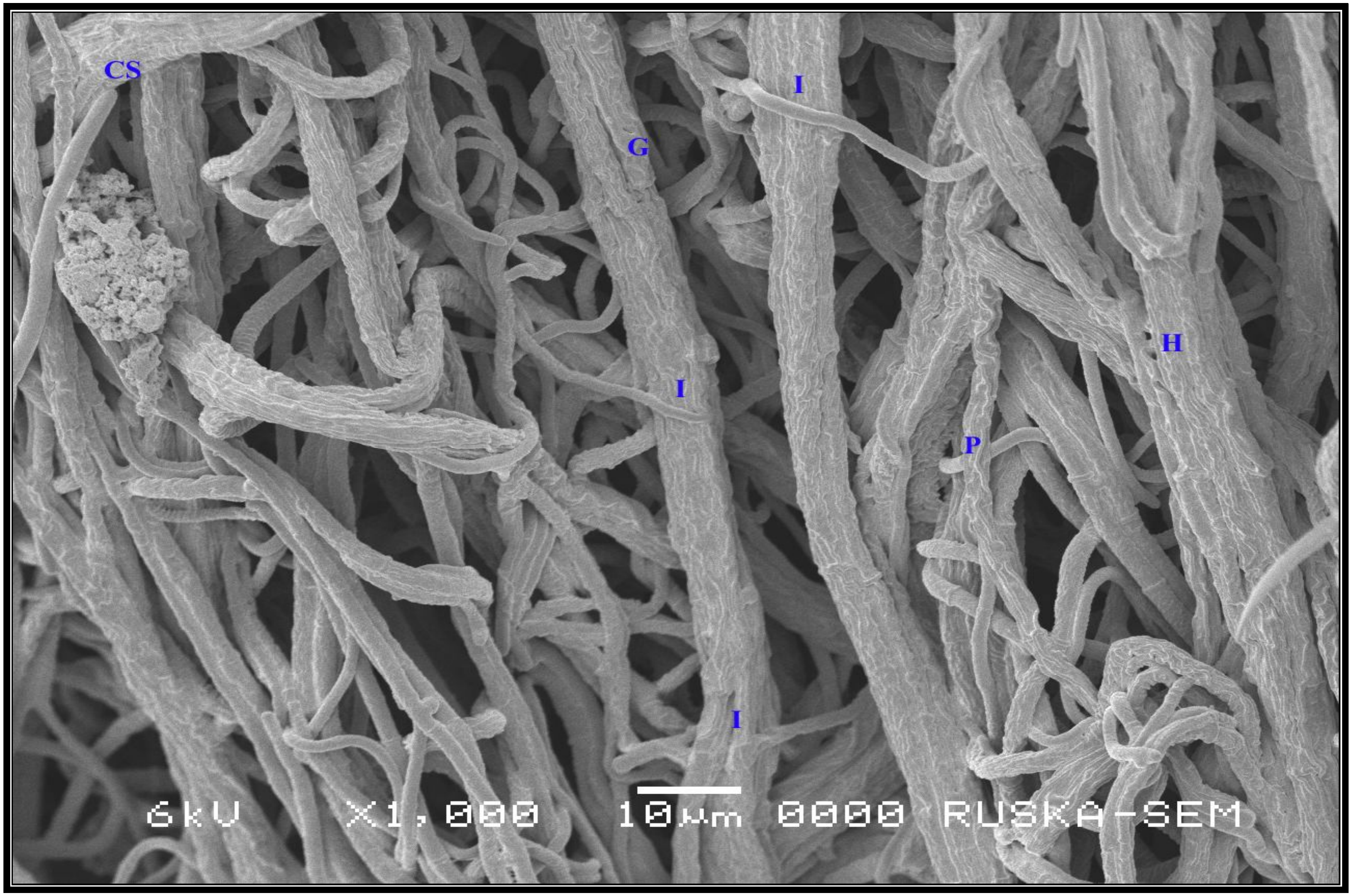


Plate 44: **Mycoparasitism of native *Trichoderma harzianum* on *Alternaria vitis*. CS – Chemotropic stimulus; D – Disintegration; P – Penetration; I – Interaction; G – Groves formation; H – Holes formation.**



<u>Plate 46:</u> Mycoparasitism of native *Trichoderma harzianum* on *Alternaria vitis*. K – Knots formation; I – Interaction; P – Penetration.



<u>Plate 45:</u> Mycoparasitism of native *Trichoderma harzianum* on *Alternaria vitis*. CS – Chemotropic stimulus; D – Disintegration; P – Penetration; I – Interaction; G – Groves formation; H – Holes formation.

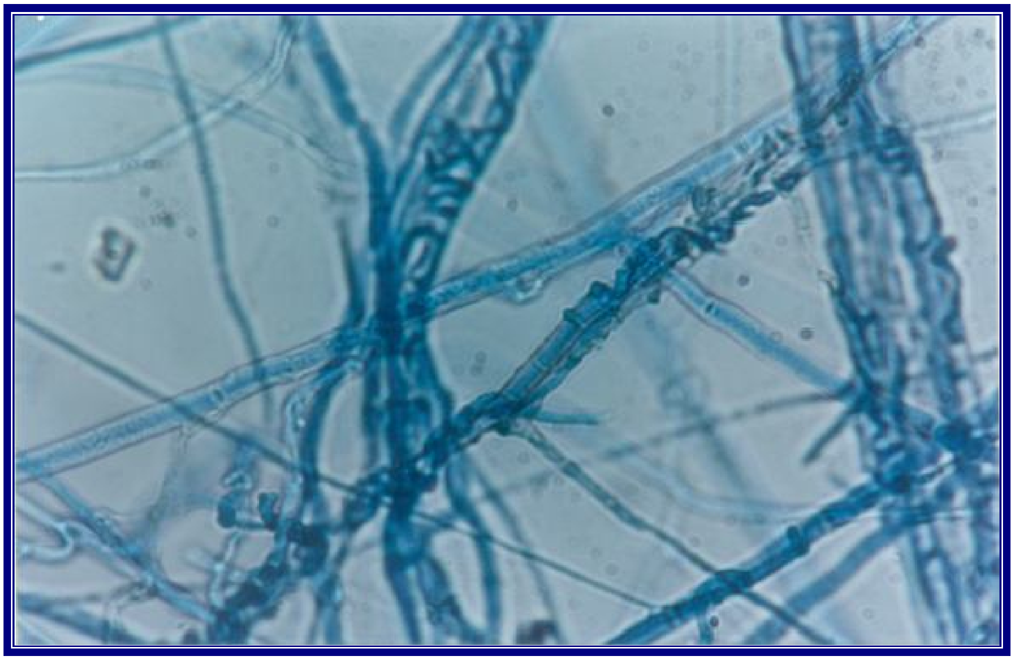


Plate 47: **Photomicrograph showing interactions of native *Trichoderma harzianum* and *Alternaria vitis.***

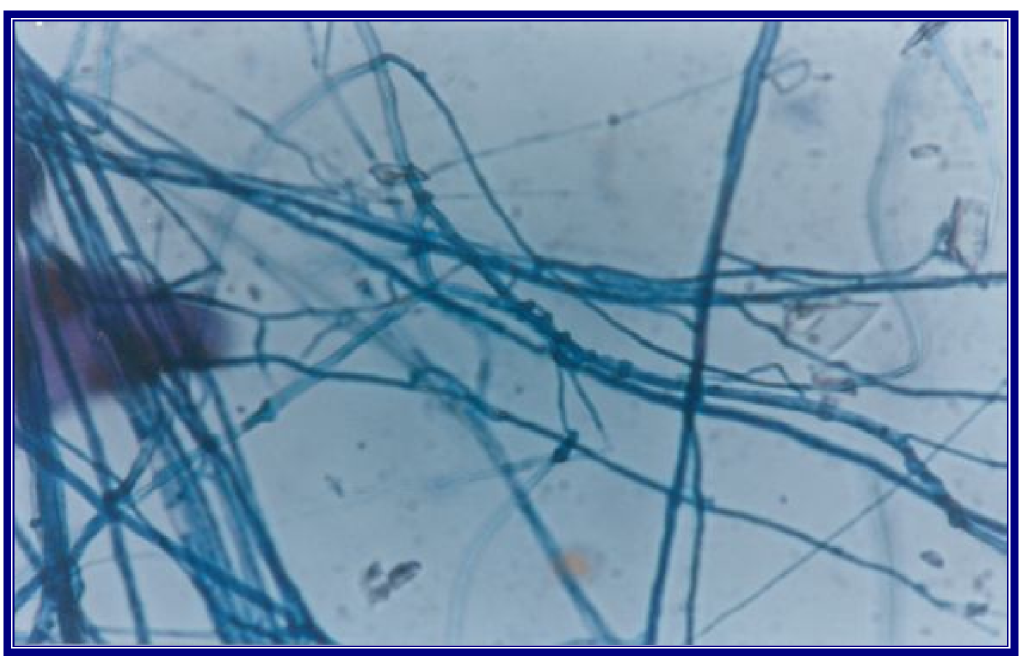


Plate 48: **Photomicrograph showing interaction of native *Trichoderma viride* and *Alternaria vitis.***

Plates 28-42: Photomicrographs of phylloplane microflora of grapevine

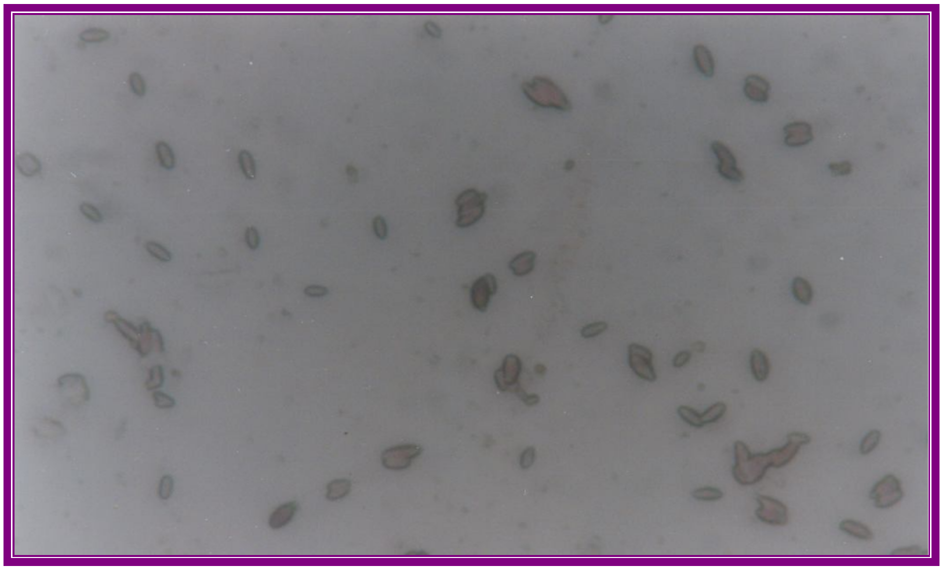


Plate 38: Individual cells of native bacterial isolate PPB-1Y

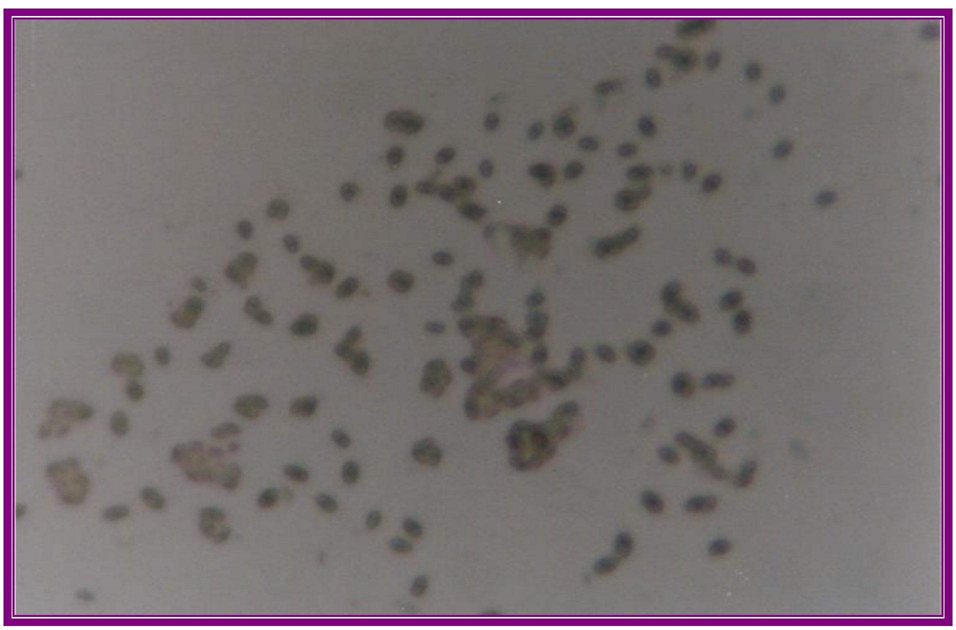


Plate 39: Individual cells of native bacterial isolate PPB-2R.

Plates 28-42: Photomicrographs of phylloplane microflora of grapevine

Plate 40: Individual cells of native bacterial isolate PPB-3Ro.

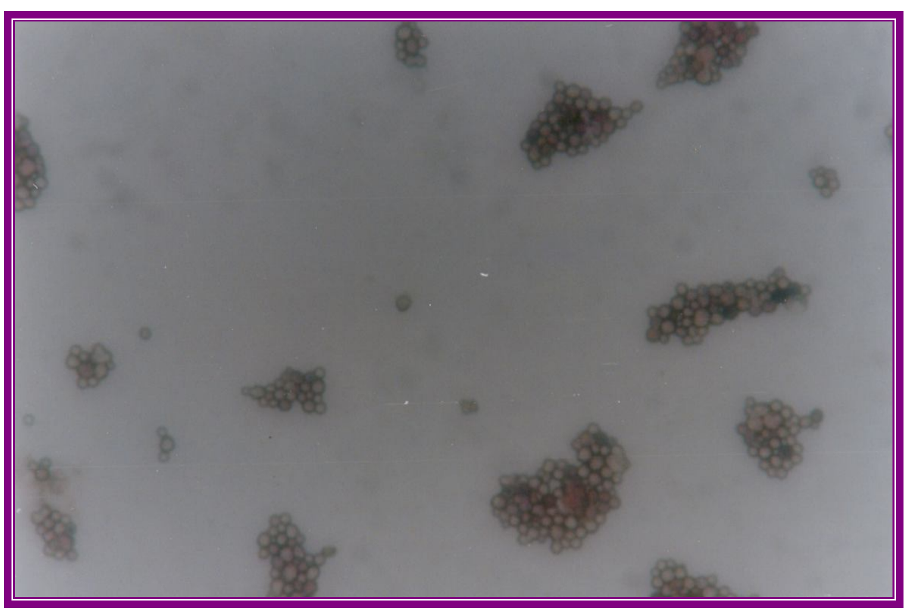


Plate 41: Cells of native bacterial isolate PPB-4W.

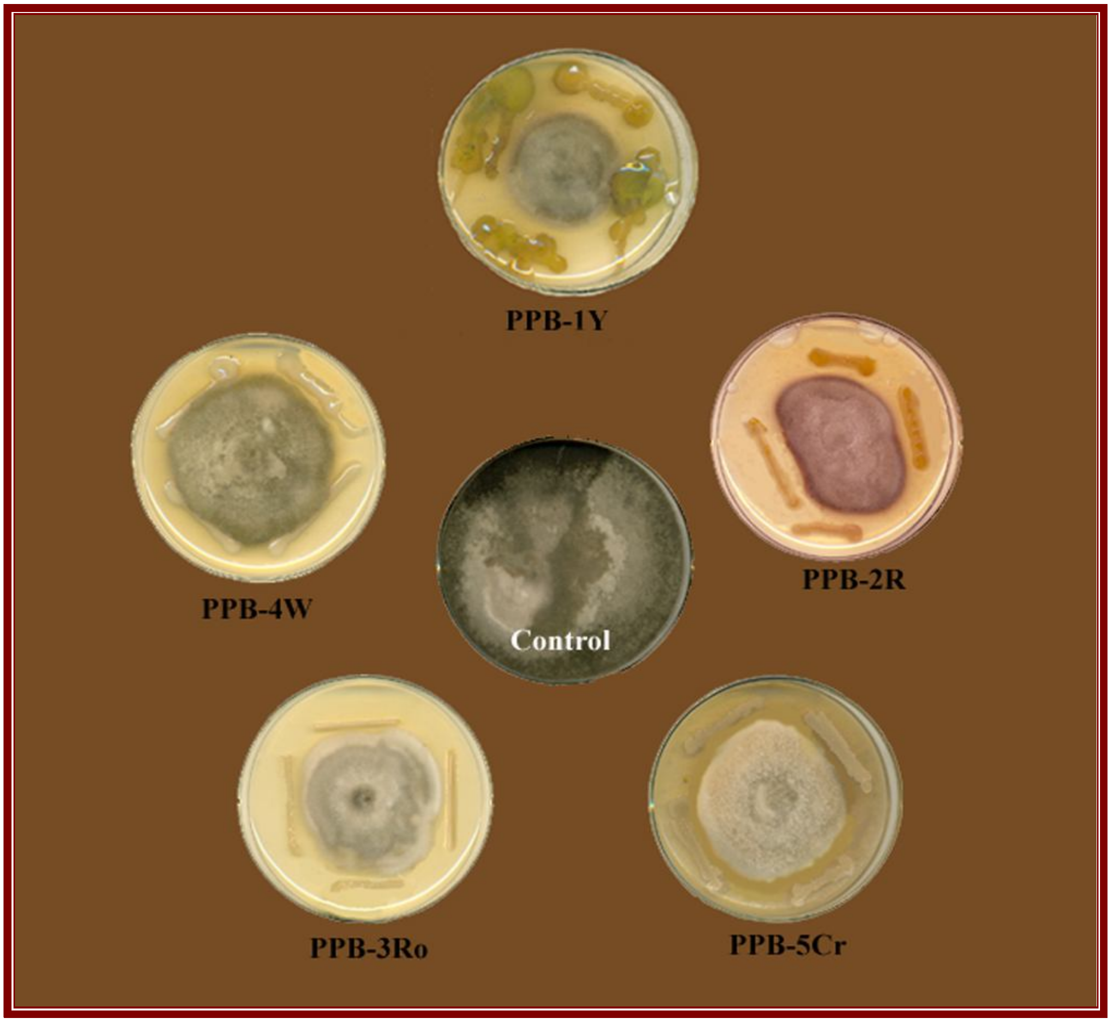


Plate 25: **Antagonism of grapevine phylloplane bacterial isolates against *Alternaria vitis.***

Plates 28-42: Photomicrographs of phylloplane microflora of grapevine

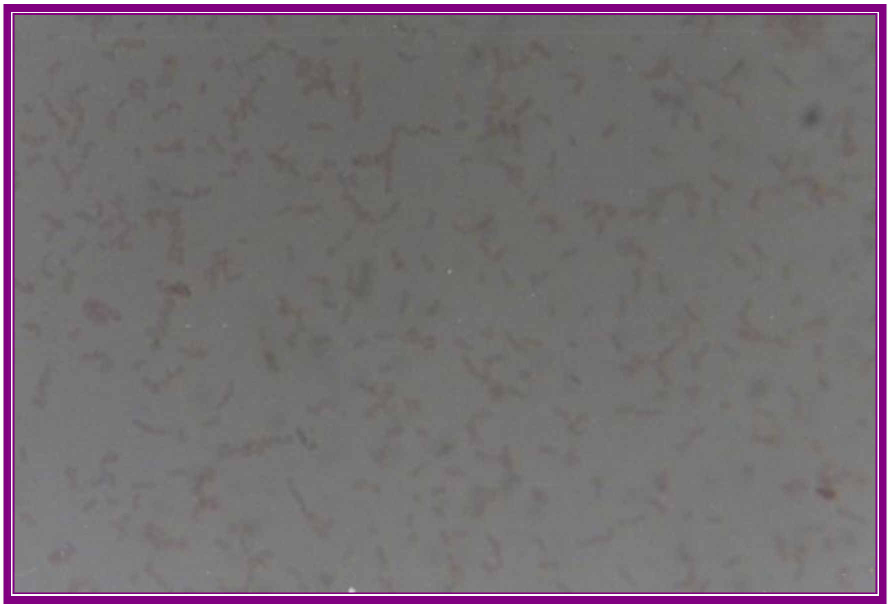


Plate 42: Cells of native bacterial isolate PPB- 5Cr.

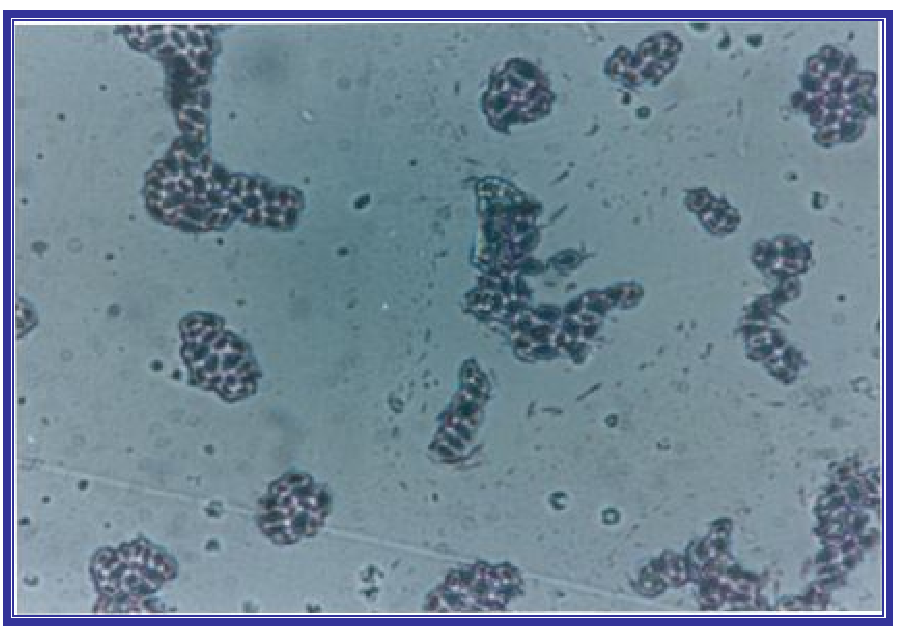
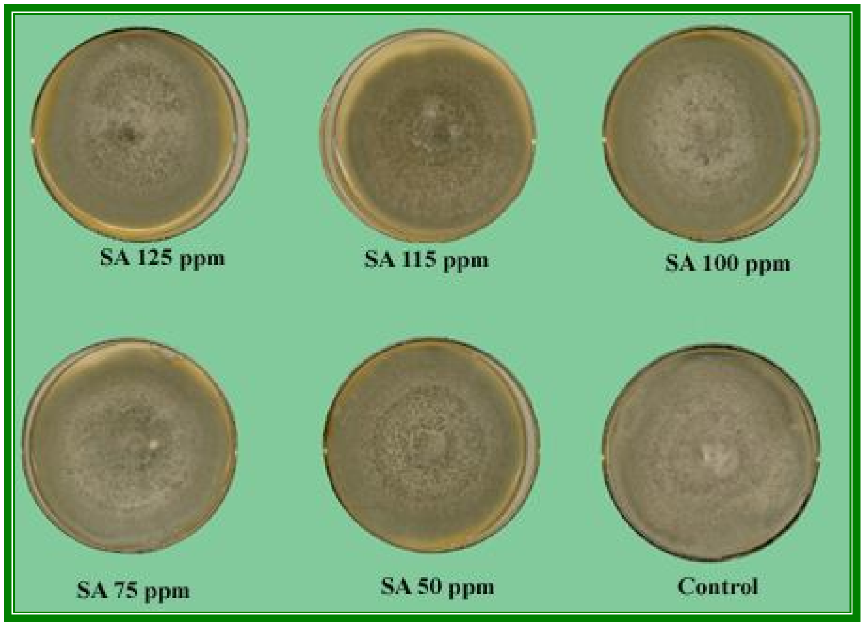
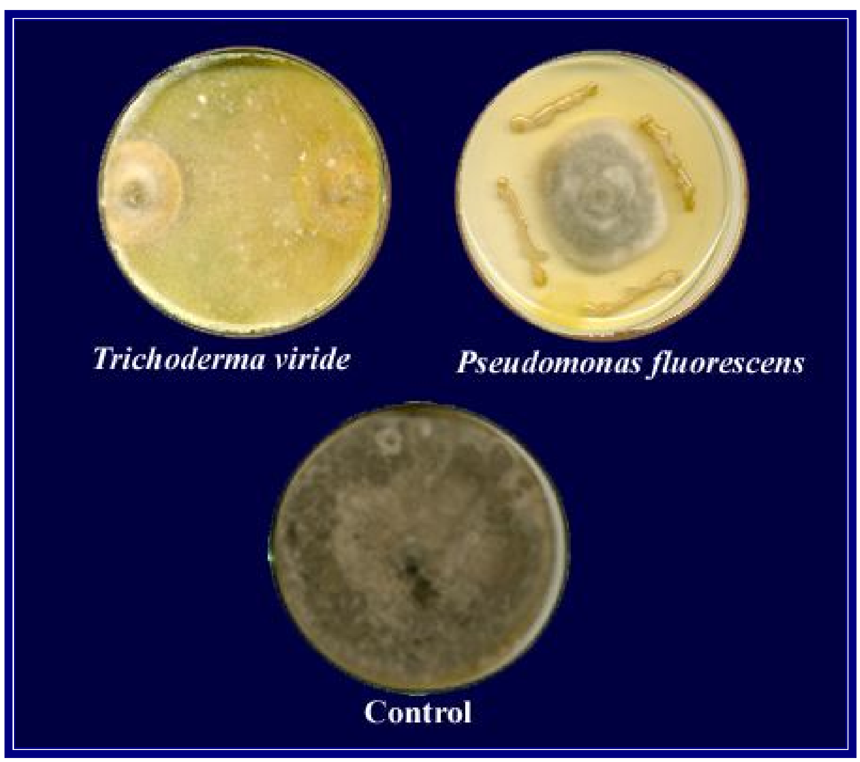


Plate 43: Yeast cells isolated from 10 day fermented grape berry juice.



<u>Plate 14:</u> *In vitro* efficacy of salicylic acid on mycelial growth of *Alternaria vitis.*



<u>Plate 15:</u> Effect of biocontrol agents on *Alternaria vitis*.

<u>Table 5:</u> Efficacy of 10 day fermented extracts on mycelial growth of *Alternaria vitis.*

S. No	Treatment	%Concentration	% Inhibition of mycelial growth
1	NSKE	5	10.32 (18.56)*
2	NSKE	10	18.56 (25.10)
3	VCE	20	9.52 (17.74)
4	VCE	25	7.96 (16.12)
5	SPME	5	5.55 (13.15)
6	SPME	10	7.93 (16.18)
7	FYME	12.5	8.75 (16.94)
8	FYME	25	6.36 (13.34)
9	FYME	50	7.17 (14.89)
10	FME	12.5	7.17 (14.88)
11	FME	25	3.18 (10.13)
12	FME	50	3.18 (10.13)
13	GBJ	12.5	58.76 (50.05)
14	GBJ	25	100.0 (90.00)
15	GBJ	50	100.0 (90.00)
SEM ±			2.44
CD (0.05)			7.05

*** Values in parentheses are angular transformed values.**

<u>Table 8:</u> Efficacy of 10 day fermented extracts on spore germination of *Alternaria vitis.*

S.No	Treatment	% Concentration	% Inhibition of spore germination
1	**NSKE**	**5**	47.77 (43.71)*
2	**NSKE**	**10**	40.50 (39.47)
3	**VCE**	**20**	44.3 (41.72)
4	**VCE**	**25**	55.49 (48.16)
5	**SPME**	**5**	45.65 (42.46)
6	**SPME**	**10**	65.87 (54.31)
7	**FYME**	**12.5**	51.34 (45.77)
8	**FYME**	**25**	50.77 (45.43)
9	**FYME**	**50**	67.08 (55.15)
10	**FME**	**12.5**	37.40 (37.61)
11	**FME**	**25**	41.14 (39.89)
12	**FME**	**50**	40.34 (39.31)
13	**GBJ**	**12.5**	55.14 (47.96)
14	**GBJ**	**25**	64.67 (53.7)
15	**GBJ**	**50**	96.04 (78.54)
SEM ±			**2.79**
CD (0.05)			**8.05**

*** Values in parentheses are angular transformed values.**

Table 6: **Efficacy of 20 day fermented extracts on mycelial growth of *Alternaria vitis.***

S. No	Treatment	%Concentration	% Inhibition of mycelial growth
1	**NSKE**	**5**	45.23 (42.26)*
2	**NSKE**	**10**	50.75 (45.43)
3	**VCE**	**20**	5.55 (13.57)
4	**VCE**	**25**	3.18 (10.13)
5	**SPME**	**5**	3.18 (10.13)
6	**SPME**	**10**	3.18 (10.13)
7	**FYME**	**12.5**	7.14 (15.36)
8	**FYME**	**25**	3.18 (10.13)
9	**FYME**	**50**	24.67 (29.77)
10	**FME**	**12.5**	7.14 (15.35)
11	**FME**	**25**	8.73 (17.15)
12	**FME**	**50**	8.75 (16.94)
13	**GBJ**	**12.5**	23.05 (28.64)
14	**GBJ**	**25**	39.25 (38.78)
15	**GBJ**	**50**	100.00(90.00)
SEM ±			**1.24**
CD (0.05)			**3.58**

*** Values in parentheses are angular transformed values.**

Table 9: **Efficacy of 20 day fermented extracts on spore germination of *Alternaria vitis.***

S. No	Treatment	%Concentration	% Inhibition of spore germination
1	**NSKE**	**5**	23.68 (16.17)*
2	**NSKE**	**10**	52.92 (46.67)
3	**VCE**	**20**	52.92 (46.67)
4	**VCE**	**25**	56.25 (48.70)
5	**SPME**	**5**	58.75 (50.10)
6	**SPME**	**10**	73.75 (59.18)
7	**FYME**	**12.5**	31.25 (33.95)
8	**FYME**	**25**	34.17 (35.63)
9	**FYME**	**50**	53.75 (47.15)
10	**FME**	**12.5**	52.92 (46.68)
11	**FME**	**25**	59.58 (50.57)
12	**FME**	**50**	80.42 (63.78)
13	**GBJ**	**12.5**	50.00 (45.00)
14	**GBJ**	**25**	65.00 (53.77)
15	**GBJ**	**50**	82.92 (65.67)
SEM ±			**2.95**
CD (0.05)			**8.52**

*** Values in parentheses are angular transformed values.**

Table 10: Efficacy of 30 day fermented extracts on spore germination of *Alternaria vitis.*

S. No	Treatment	%Concentration	% Inhibition of spore germination
1	NSKE	5	52.50 (46.46)*
2	NSKE	10	55.84 (48.36)
3	VCE	20	46.67 (43.06)
4	VCE	25	53.34 (46.92)
5	SPME	5	38.75 (38.45)
6	SPME	10	29.17 (32.61)
7	FYME	12.5	34.17 (35.59)
8	FYME	25	52.92 (46.68)
9	FYME	50	49.58 (44.76)
10	FME	12.5	48.34 (44.04)
11	FME	25	54.58 (47.63)
12	FME	50	55.67 (48.33)
13	GBJ	12.5	49.58 (44.76)
14	GBJ	25	67.50 (55.32)
15	GBJ	50	85.42 (67.58)
SEM ±			2.84
CD (0.05)			8.19

*** Values in parentheses are angular transformed values.**

<u>Table 7:</u> Efficacy of 30 day fermented extracts on mycelial growth of *Alternaria vitis.*

S. No	Treatment	%Concentration	% Inhibition of mycelial growth
1	**NSKE**	**5**	29.83 (33.07)*
2	**NSKE**	**10**	40.84 (39.72)
3	**VCE**	**20**	11.11 (19.44)
4	**VCE**	**25**	12.70 (20.86)
5	**SPME**	**5**	13.50 (21.54)
6	**SPME**	**10**	8.73 (17.06)
7	**FYME**	**12.5**	12.7 (20.86)
8	**FYME**	**25**	11.57 (19.75)
9	**FYME**	**50**	16.79 (24.17)
10	**FME**	**12.5**	7.14 (14.95)
11	**FME**	**25**	4.75 (12.59)
12	**FME**	**50**	127 (20.86)
13	**GBJ**	**12.5**	43.58 (47.52)
14	**GBJ**	**25**	82.08 (65.21)
15	**GBJ**	**50**	100.00 (90.00)
SEM±			**1.39**
CD (0.05)			**4.03**

*** Values in parentheses are angular transformed values.**

Table 15: Effect of biocontrol agents on the growth of *Alternaria vitis*.

S.no	Biocontrol agent	% Growth Inhibition
1	*Trichoderma viride*	66.67
2	*Pseudomonas fluorescens*	55.56

Table12: Effect of fermentation periods of extracts on the spore germination of *Alternaria vitis.*

S. No	Treatment	Concentration %	% Inhibition			
			Fermentation Period (days)			Mean percent inhibition
			10	20	30	
1	**NSKE**	**5**	47.77 (43.71)*	23.68 (16.17)	52.50 (46.46)	**38.18 (37.91)**
2	**NSKE**	**10**	40.50 (39.47)	52.92 (46.67)	55.84 (48.36)	**49.75 (44.84)**
3	**VCE**	**20**	44.3 (41.72)	52.92 (46.67)	46.67 (43.06)	**47.96 (43.81)**
4	**VCE**	**25**	55.49 (48.16)	56.25 (48.70)	53.34 (46.92)	**55.02 (47.93)**
5	**SPME**	**5**	45.65 (42.46)	58.75 (50.10)	38.75 (38.45)	**47.72 (43.67)**
6	**SPME**	**10**	65.87 (54.31)	73.75 (59.18)	29.17 (32.61)	**56.26 (48.70)**
7	**FYME**	**12.5**	67.08 (55.15)	31.25 (33.95)	34.17 (35.59)	**44.17 (41.56)**
8	**FYME**	**25**	50.77 (45.43)	34.17 (35.63)	52.92 (46.68)	**45.95 (42.98)**
9	**FYME**	**50**	51.34 (45.77)	53.75 (47.15)	49.58 (44.76)	**51.55 (45.90)**
10	**FME**	**12.5**	37.40 (37.61)	52.92 (46.68)	48.34 (44.04)	**46.22 (42.77)**
11	**FME**	**25**	41.14 (39.89)	59.58 (50.57)	54.58 (47.63)	**51.77 (46.03)**
12	**FME**	**50**	40.34 (39.31)	80.42 (63.78)	55.67 (48.33)	**58.81 (50.47)**
13	**GBJ**	**12.5**	55.14 (47.96)	50.00 (45.00)	49.58 (44.76)	**51.57 (45.91)**
14	**GBJ**	**25**	64.67 (53.7)	65.00 (53.77)	67.50 (55.32)	**65.72 (54.26)**
15	**GBJ**	**50**	96.04 (78.54)	82.92 (65.67)	85.42 (67.58)	**88.12 (70.60)**
Mean			**53.57 (47.55)**	**54.72 (47.81)**	**51.60 (46.04)**	**53.29 (47.13)**
				Fermentation period	**Treatment**	**Interaction**
			SEM ±	**0.74**	**1.65**	**2.86**
			C.D (0.05)	**-**	**4.64**	**8.03**

*** Values in parentheses are angular transformed values.**

Table 11: Effect of fermentation periods of extracts on the mycelial growth of *Alternaria vitis.*

S. No	Treatment	% Concen-tration	% Inhibition			
			Fermentation period (days)			Mean percent inhibition
			10	20	30	
1	NSKE	5	10.32 (18.56)*	45.23 (42.26)	29.83 (33.07)	**28.56 (31.30)**
2	NSKE	10	18.56 (25.10)	50.75 (45.43)	40.84 (39.72)	**36.72 (36.75)**
3	VCE	20	9.52 (17.74)	5.55 (13.57)	11.11 (19.44)	**8.73 (16.92)**
4	VCE	25	7.96 (16.12)	3.18 (10.13)	12.70 (20.86)	**7.95 (15.70)**
5	SPME	5	5.55 (13.15)	3.18 (10.13)	13.50 (21.54)	**7.41 (14.94)**
6	SPME	10	7.93 (16.18)	3.18 (10.13)	8.73 (17.06)	**6.61 (14.46)**
7	FYME	12.5	8.75 (16.94)	7.14 (15.36)	12.7 (20.86)	**9.53 (17.72)**
8	FYME	25	6.36 (13.34)	3.18 (10.13)	11.57 (19.75)	**7.03 (14.41)**
9	FYME	50	7.17 (14.89)	24.67 (29.77)	16.79 (24.17)	**16.21 (22.94)**
10	FME	12.5	7.17 (14.88)	7.14 (15.35)	7.14 (14.95)	**7.15 (15.06)**
11	FME	25	3.18 (10.13)	8.73 (17.15)	4.75 (12.59)	**5.55 (13.29)**
12	FME	50	3.18 (10.13)	8.75 (16.94)	12.7 (20.86)	**8.21 (15.97)**
13	GBJ	12.5	58.76 (50.05)	23.05 (28.64)	43.58 (47.52)	**43.11 (40.76)**
14	GBJ	25	100.0 (90.00)	39.25 (38.78)	82.08 (65.21)	**73.78 (64.66)**
15	GBJ	50	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	**100.0 (90.00)**
Mean			**23.63(27.81)**	**22.20(26.25)**	**27.46(30.91)**	**24.43(28.32)**
				Fermentation Period	**Treatment**	**Interaction**
			SEM ±	**0.46**	**1.02**	**1.77**
			C.D (0.05)	**1.29**	**2.88**	**4.98**

*** Values in parentheses are angular transformed values.**

Table 2: **Fungicides used for testing and their concentrations.**

Sl. No.	Fungicides (Trade name)	Common name	Formulation	Nature	Toxicity	Concentrations tested		Manufacturing company
1	Amistar	Azoxystrobin	25 SC	Systemic		0.025%	0.05%	Syngenta India LTD.
2	Quintal	Iprodione 25% + Carbendazim 25%	50 WP	Contact & Systemic	Danger	0.05%	0.1%	Bayer crop science
3	Indofil m-45	Mancozeb	75 WP	Contact	Caution	0.125%	0.25%	Indofil chemicals company

Replications: 3

<u>Table 13:</u> Efficacy of fungicides on mycelial growth and spore germination of *Alternaria vitis*.

S. No	Fungicide	Trade name	Concentration %	% Inhibition	
				Mycelial growth	Spore germination
1	Azoxystrobin	Amistar	0.05	43.90 (41.50)*	91.27 (72.85)*
2	Azoxystrobin	Amistar	0.025	42.50 (40.69)	86.91 (68.79)
3	Mancozeb	Indofil-M 45	0.25	100.00 (90.00)	100.00 (90.00)
4	Mancozeb	Indofil-M 45	0.125	81.60 (64.66)	97.62 (82.84)
5	Iprodione+ Carbendazim	Quintal	0.1	100.00 (90.00)	100.00 (90.00)
6	Iprodione+ Carbendazim	Quintal	0.05	100.00 (90.00)	92.07 (73.71)
SEM ±				0.52	1.63
CD (0.05)				1.55	5.02

*** Values in parenthesis are angular transformed values.**

<u>Table 13:</u> Efficacy of fungicides on mycelial growth and spore germination of *Alternaria vitis*.

S. No	Fungicide	Trade name	Concentration %	% Inhibition	
				Mycelial growth	Spore germination
1	Azoxystrobin	Amistar	0.05	43.90 (41.50)*	91.27 (72.85)*
2	Azoxystrobin	Amistar	0.025	42.50 (40.69)	86.91 (68.79)
3	Mancozeb	Indofil-M 45	0.25	100.00 (90.00)	100.00 (90.00)
4	Mancozeb	Indofil-M 45	0.125	81.60 (64.66)	97.62 (82.84)
5	Iprodione+ Carbendazim	Quintal	0.1	100.00 (90.00)	100.00 (90.00)
6	Iprodione+ Carbendazim	Quintal	0.05	100.00 (90.00)	92.07 (73.71)
SEM ±				0.52	1.63
CD (0.05)				1.55	5.02

*** Values in parenthesis are angular transformed values.**

<u>Table 19:</u> Efficacy of different treatments on the Alternaria leaf blight of grapevine in glass house conditions.

S.No	Treatment	Concentration	PDI		
			prophylactic	Simultaneous	Curative
1	**10 day sterilised GBJ**	**25%**	33.34 (35.22)*	43.21 (41.09)*	54.08 (47.34)*
2	**10 day unfiltered GBJ**	**25%**	14.83 (22.36)	22.23 (27.63)	42.44 (40.62)
3	**20 day NSKE**	**10%**	28.40 (32.00)	35.19 (36.04)	41.97 (40.20)
4	**Quintal**	**0.1%**	14.83 (22.36)	27.18 (31.34)	51.86 (46.07)
5	**Indofil M-45**	**0.25%**	29.64 (32.89)	37.04 (37.45)	48.15 (43.88)
6	***Trichoderma viride***	**5X10^4spores/ml**	38.76 (38.48)	37.04 (37.45)	46.29 (42.87)
7	***Pseudomonas fluorescens***	**10^4-10^5cfu/ml**	22.23 (28.13)	27.17 (31.34)	55.56 (48.19)
8	***Trichoderma harzianum*** **(Phylloplane)**	**5X10^4spores/ml**	17.29 (24.36)	33.34 (35.26)	47.14 (43.36)
9	**PPB-1Y (Phylloplane)**	**10^4-10^5cfu/ml**	17.29 (24.36)	40.74 (39.62)	45.59 (40.73)
10	**SA**	**125ppm**	34.57 (35.90)	35.19 (36.37)	48.15 (43.88)
11	**Control**		60.76 (51.22)	56.79 (48.92)	61.11 (51.44)
SEM ±			**2.40**		**2.74**
CD (0.05)			**7.05**		**8.03**

***Values in parentheses are angular transformed values.**

Table 16: **Effect of phylloplane fungi on mycelial growth of *Alternaria vitis*.**

S. No	Phylloplane Fungus	% Inhibition of mycelial growth
1	***Trichoderma harzianum***	72.8 (58.63)*
2	***Trichoderma viride***	63.05 (52.58)
3	***Aspergillus niger***	49.17 (44.53)
4	***Aspergillus fumigatus***	30.14 (33.29)
5	***Aspergillus tumarii***	29.32 (32.78)
6	***Paecilomyces variotii***	30.94 (33.78)
7	***Alternaria sp***	4.75 (12.59)
8	***Thielavia terricola***	6.14 (14.24)
9	***Oidiodendron sp***	41.34 (40.00)
10	***Cladosporium cladosporoides***	30.38 (33.43)
SEM ±		**0.95**
CD (0.05)		**2.81**

*** Values in parentheses are angular transformed values.**

<u>Table 17:</u> Efficacy of phylloplane bacteria on the mycelial growth of *Alternaria vitis* and Inhibition zones produced by them in culture.

S. No	Phylloplane bacteria	% Inhibition of mycelial growth	Inhibition zone (mm)
1	PPB-1Y	65.91 (54.28)*	5.34 (13.34)*
2	PPB-2R	56.30 (48.62)	2.67 (9.36)
3	PPB-3Ro	37.04 (37.48)	1.00 (5.74)
4	PPB-4W	34.82 (36.16)	0.34 (1.91)
5	PPB-5Cr	41.48 (40.09)	2.00 (8.13)
SEM ±		0.72	0.92
CD (0.05)		2.28	2.89

*** Values in parentheses are angular transformed values.**

Table 18: Colony and morphological characters of bacteria isolated from grapevine phylloplane.

Sl No.	Isolate	Colony character	Gram reaction	Cell shape and size
1	**PPB-1Y**	Yellowish, shiny, flat, irregular shaped, 5-7mm diameter, mats also observed, Fluorescent, Greenish granules on older colonies.	(-)	Rods, 10-11 µm
2	**PPB-2R**	Reddish orange, shiny, circular, raised, convex, 2-3 mm diameter	(+)	Shorter rods, 6.5µm
3	**PPB-3Ro**	Rose, convex, shiny, circular, 5-6 mm diameter	(+)	Short rods, 8.7µm
4	**PPB-4W**	Milky white, opaque, circular, convex, 3mm diameter	(-)	Cocci, 3.1µm
5	**PDB-5Cr**	Light brownish or dull white with pink coloured center convex, 2 – 3mm in diameter	(-)	Minute rods , 1µm

<u>Table 14:</u> Efficacy of Salicylic acid on mycelial growth and spore germination of *Alternaria vitis.*

S. No	Concentration (ppm)	% Inhibition	
		Mycelial growth	**Spore germination**
1	**50**	13.06 (21.10)*	33.75 (35.50)*
2	**75**	14.05 (21.96)	36.60 (37.22)
3	**100**	12.30 (20.5)	32.44 (34.71)
4	**115**	12.30 (20.5)	35.55 (36.56)
5	**125**	14.05 (21.96)	45.62 (42.47)
SEM ±		**1.17**	**1.6**
CD (0.05)		**3.68**	**5.05**

*** Values in parentheses are angular transformed values.**

<u>Table 3:</u> Chemical used for testing and its concentrations.

Sl.No	Chemical	Concentration (ppm)
1	Salicylic acid	50
2	Salicylic acid	75
3	Salicylic acid	100
4	Salicylic acid	115
5	Salicylic acid	125

Replications: 3

<u>Table 4:</u> Biocontrol agents used for testing.

Sl No	Biocontrol agent
1	***Trichoderma viride***
2	***Pseudomonas fluorescens***

Replications: 3

Table 1: Extracts used for testing and their concentrations.

Sl. No.	Composts /amendments / Plant sources	Concentrations		
		10 days*	20 days*	30 days*
1	Neem kernel extract	10%,5%	10%,5%	10%,5%
2	Vermicompost extract	25%,20%	25%,20%	25%,20%
3	Sugarcane press mud extract	10%,5%	10%,5%	10%,5%
4	FYM + fish meal + cellulose extract In ratio of 70:15:15	50%,25%,12.5%	50%,25%,12.5%	50%,25%,12.5%
5	FYM + paddy straw extract In ratio of 75:25	50%,25%,12.5%	50%,25%,12.5%	50%,25%,12.5%
6	Grape berry fermented juice	50%,25%,12.5%	50%,25%,12.5%	50%,25%,12.5%

* Fermentation period.
Replications:3

CHAPTER VI

SUMMARY

The results of the studies on the Alternaria leaf blight of grapevine are summerised under this heading.

As the Alternaria leaf blight of grapevine caused by *Alternaria vitis* (Cav) Sacc. is becoming serious problem in Andhra Pradesh, the present investigation was carried out to derive a suitable, cost effective and ecofriendly management strategy.

The pathogen was isolated from the infected leaves, purified and pathogenicity was established in the glass house.

Various water extracts prepared from composts and organic amendments, grape berry fermented juice, fungicides, salicylic acid, bio control agents were tested for their efficacy against the mycelial growth and spore germination of the pathogen.

The composts and amendments were soaked in water in 1:1 ratio w/v aerobically for different brewing periods like 10, 20 and 30 days. Grape berries were fermented anaerobically for the same periods. The extracts were coarse filtered followed by cold sterilisation by passing them through 47 mm diameter membrane filter made up of cellulose acetate with pore size 0.45 µm.

Among all the extracts grape berry fermented juice performed best at all concentrations tested at all fermentation periods in inhibiting the mycelial growth and spore germination compared to other treatments followed by Neem seed kernel extract. Other compost extracts are not effective for mycelial growth inhibition but effective for inhibition of spore germination.

Among the fungicides tested iprodione25% + carbendazim25% (Quintal) at 0.1% and Mancozeb (Indofil M-45) at 0.25% showed 100% inhibition of mycelial growth as well as spore germination of pathogen. Quintal at 0.05% also gave 100% inhibition of mycelial growth.

The biocontrol agents *Trichoderma viride* and *Pseudomonas fluorescens* showed their efficacy with 66.67% and 55.6% of inhibition of mycelial growth respectively.

Salicylic acid is not consistent in its performance in inhibiting the mycelial growth with 14.05% even at 125ppm, while the same concentration inhibited the spore germination upto 45.62%.

Phylloplane microflora were isolated from grapevine leaves of Thompson seedless variety. Among them 10 fungal isolates and 5 bacterial isolates were observed. The fungal isolates were identified as *Trichoderma harzianum, Trichoderma viride*, *Aspergillus niger, Aspergillus tumarii, Aspergillus fumigatus, Paecilomyces variotii, Theilavia terricola, Cladosporium cladosporoides, Oidiodendron sp, Alternaria sp*. Bacterial isolates were named as PPB-IY , PPB-2R, PPB-3Ro, PPB-4W, PPB-5Cr.

The efficacy of phylloplane microflora was tested. Among fungi, *Trichoderma harzianum* with 72.8% and among bacteria PPB-IY with 65.91 % inhibition of mycelial growth and 5.34 mm of inhibition zone were found effective against *A.vitis*.

The best treatments in *in vitro* were tested *in vivo* on Thompson seedless variety of grape in glass house. These treatments were imposed in three different modes i.e., prophylactic, curative, simultaneous applications.

In all the methods of testing 10 day unsterlised Grape berry juice recorded minimum PDI. Prophylactic applications of 10 day unsterilised Grape berry fermented juice at 25%, Quintal at 0.1% recorded minimum PDI of 14.83% each followed by prophylactic application of native *Trichoderma harzianum*, native PPB-1Y with 17.29% of PDI each and *Pseudomonas fluorescens* (Bio control agent) with 22.23% proved best.

All the *in vivo* tested treatments were considerably effective against the Alternaria leaf blight of grapevine in reducing the PDI.

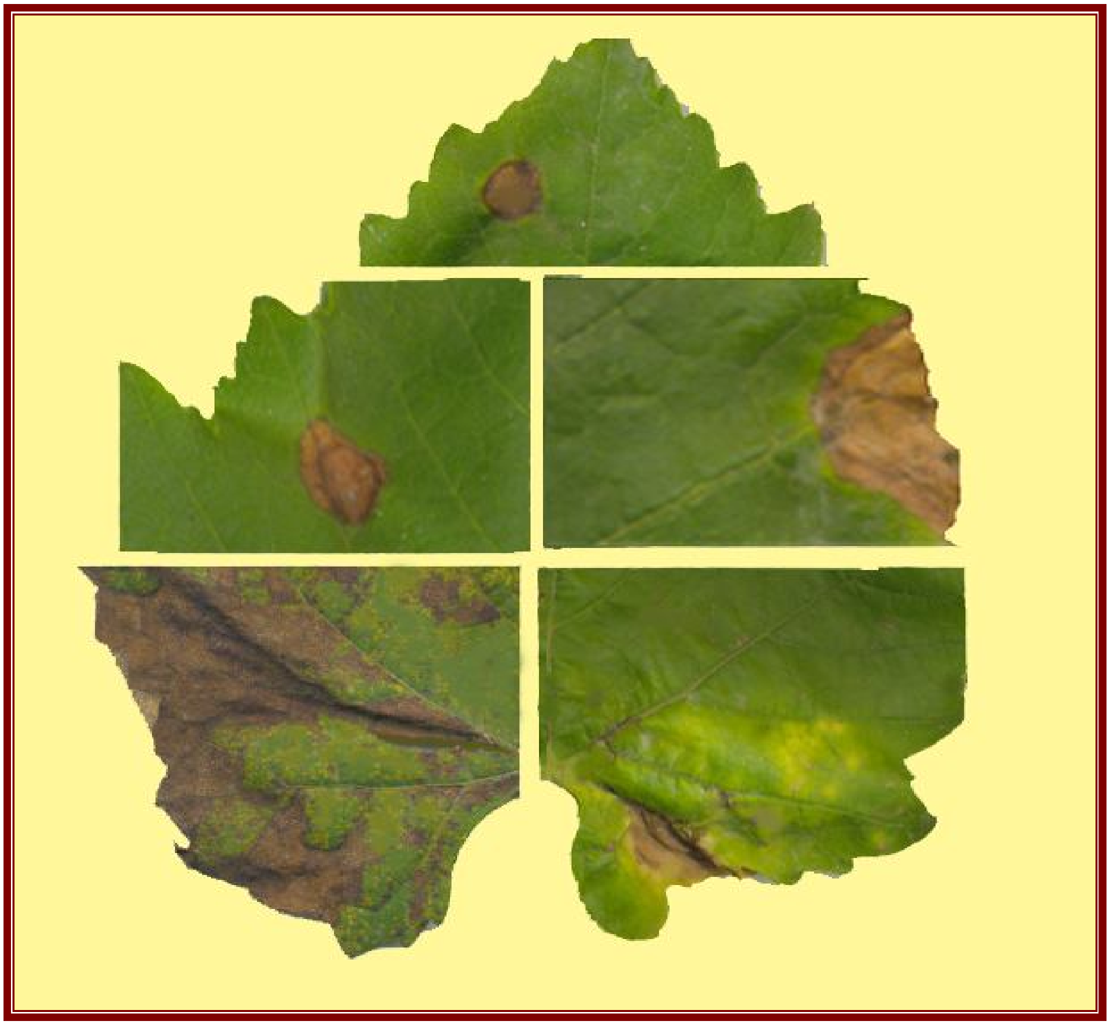
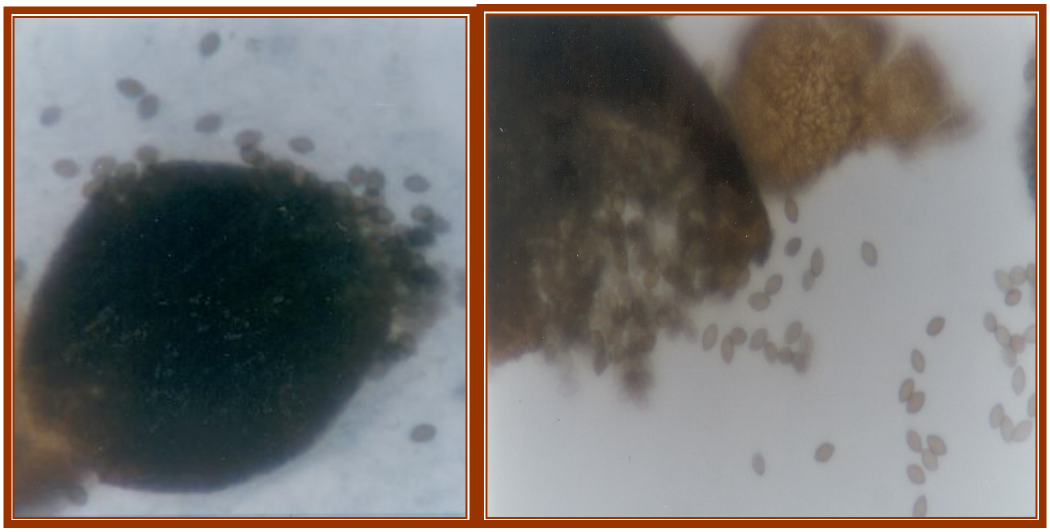
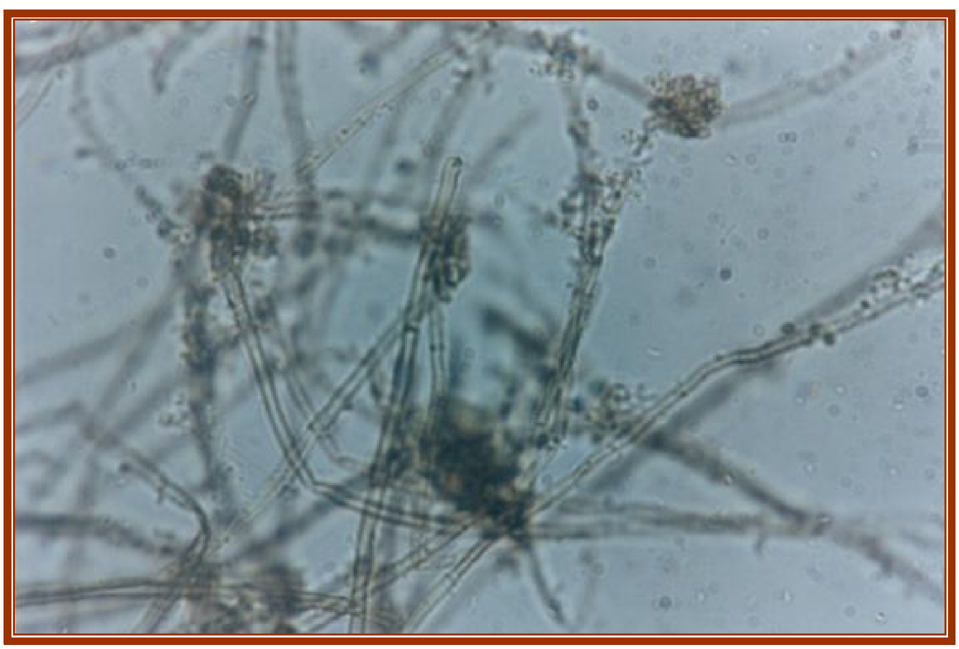


Plate 51: **Various symptoms produced on foliage of grapevine in glass house (clockwise from upside). i. Brown circular spot. (up) ii. Concentric brown lesion from margin. iii. Chloratic areas on the lamina. iv. Blighting from margin. v. Enlarging brown spot with concentric circles on the leaf lamina.**

<u>Plates 28-42:</u> Photomicrographs of phylloplane microflora of grapevine

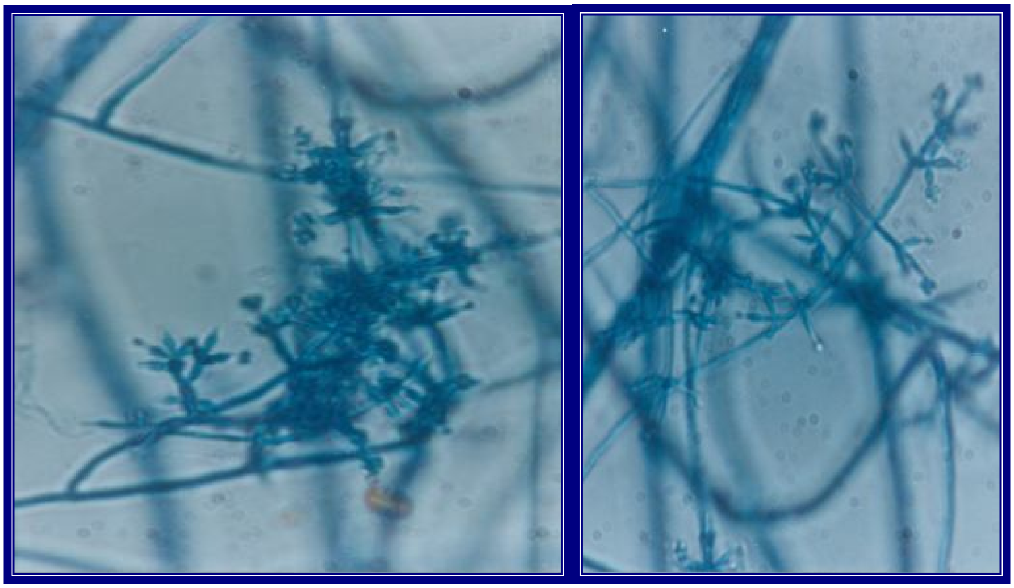


<u>Plate 34:</u> Perithecium and ascosopres of native *Thielavia terricola*.

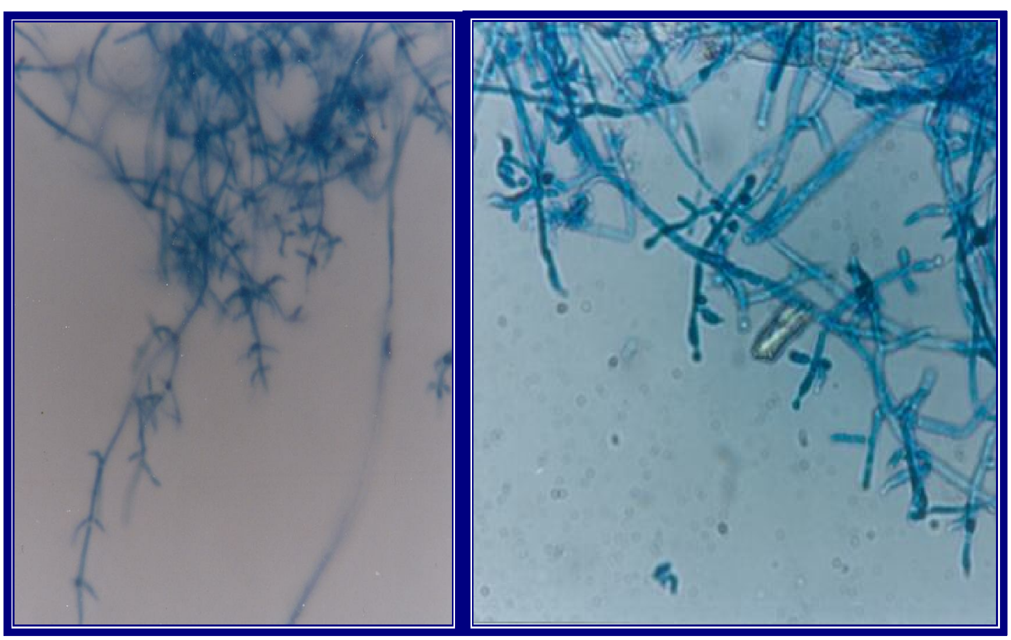


<u>Plate 35:</u> Mycelium, conidiophores and conidial bunch of native *Cladosporium cladosporoides*.

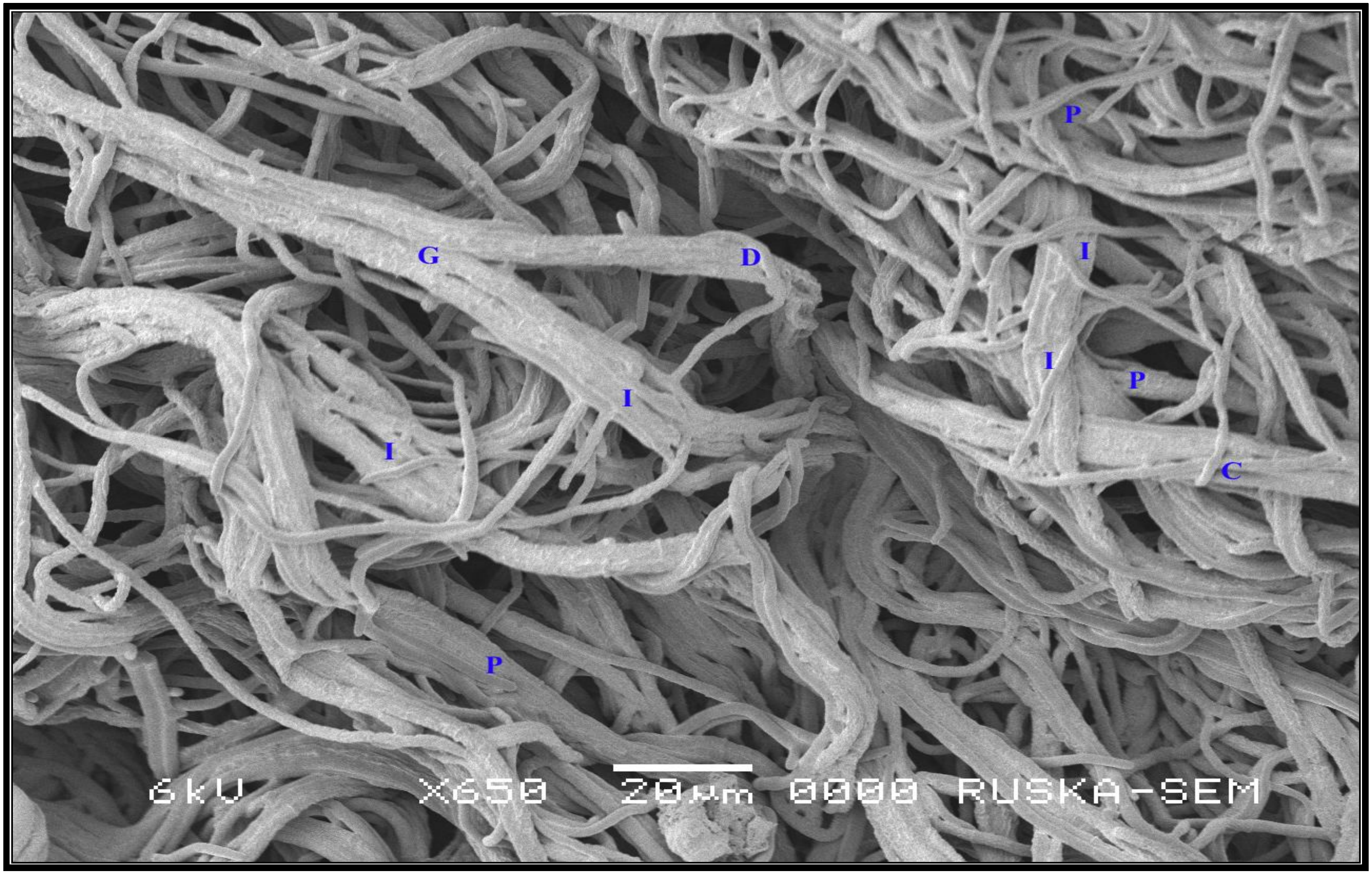
<u>Plates 28-42:</u> Photomicrographs of phylloplane microflora of grapevine



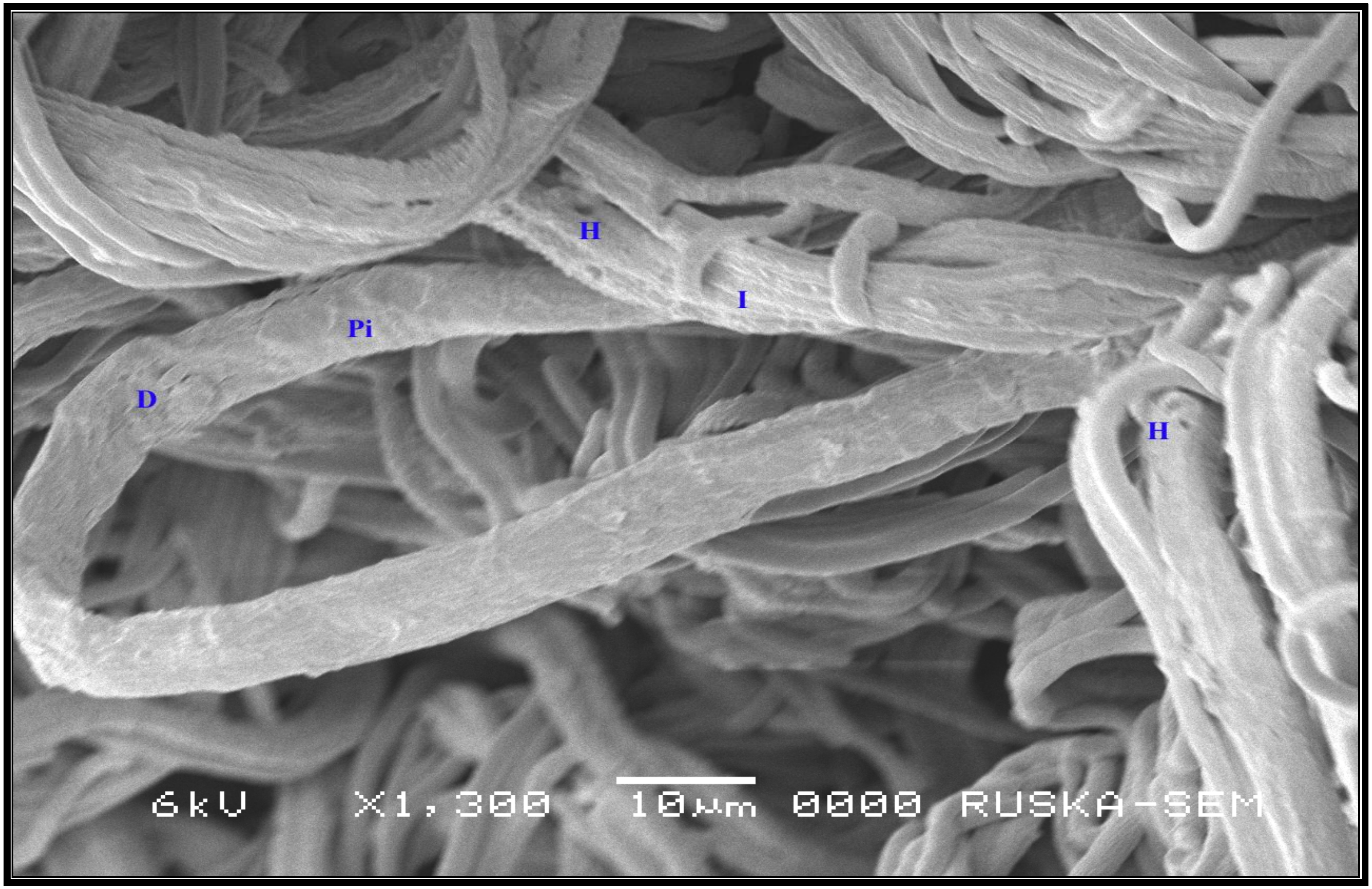
<u>Plate 28:</u> Mycelium, phialides and conidia of native *Trichoderma harzianum*.



<u>Plate 29:</u> Mycelium, phialides and conidia of native *Trichoderma viride*.



<u>**Plate 49:**</u> **Mycoparasitism of *Trichoderma viride* (Biocontrol agent) on *Alternaria vitis*. I – interaction; P – Penetration; D – Disintegration; G – Groves formation; C-- Coiling.**



<u>Plate 50:</u> Mycoparasitism of *Trichoderma viride* (Biocontrol agent) on *Alternaria vitis*. Pi – Pits formation; D – Disintegration; H – Holes formation; I – Interaction.

Plate 26: **Pure culture of yeast isolated from 10day fermented grape berry juice**

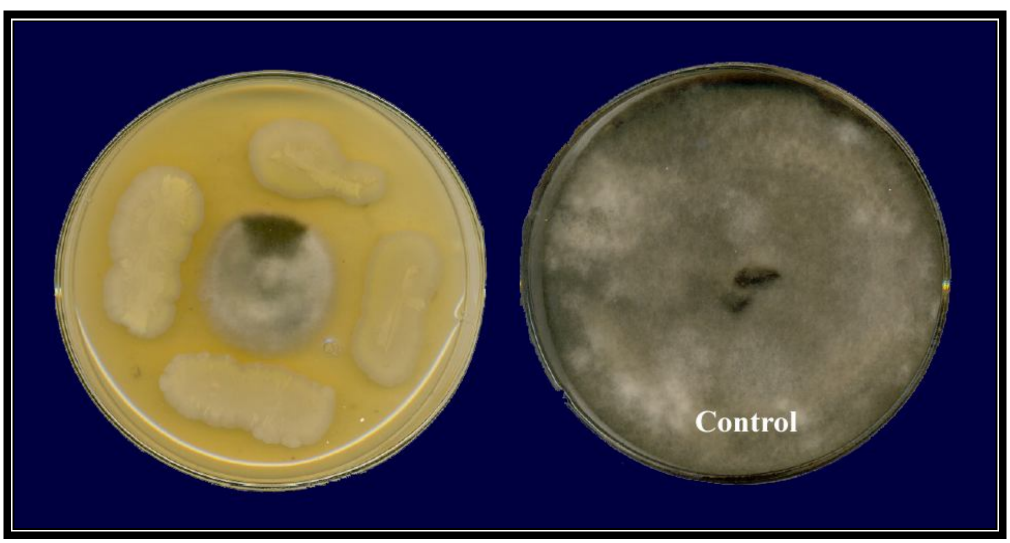


Plate 27: **Efficacy of yeast isolated from 10 day grape berry fermented juice against the *Alternaria vitis.***

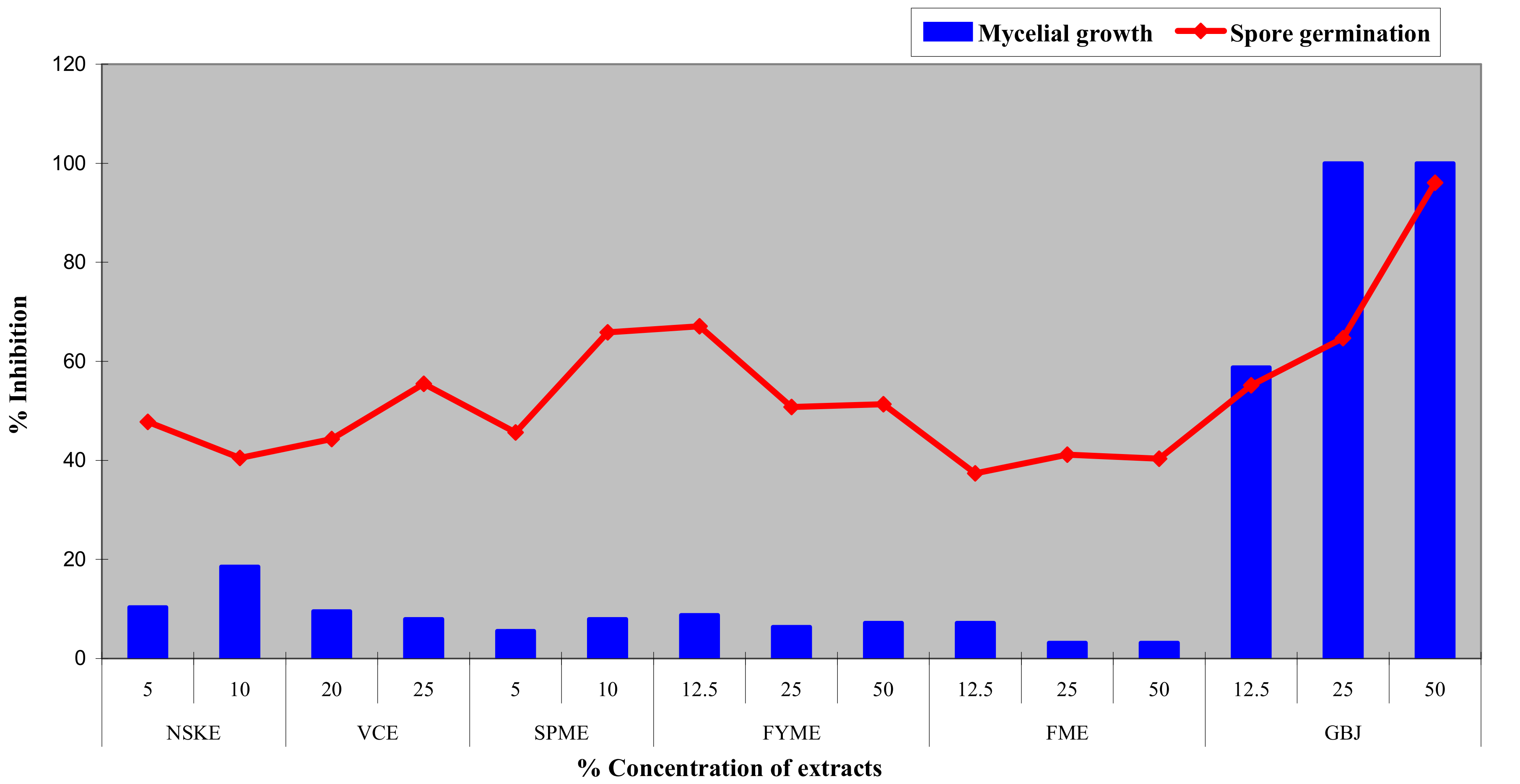


Fig 1: ***In vitro*** **efficacy of 10 day fermented extracts on mycelial growth and spore germination of** ***Alternaria vitis.***

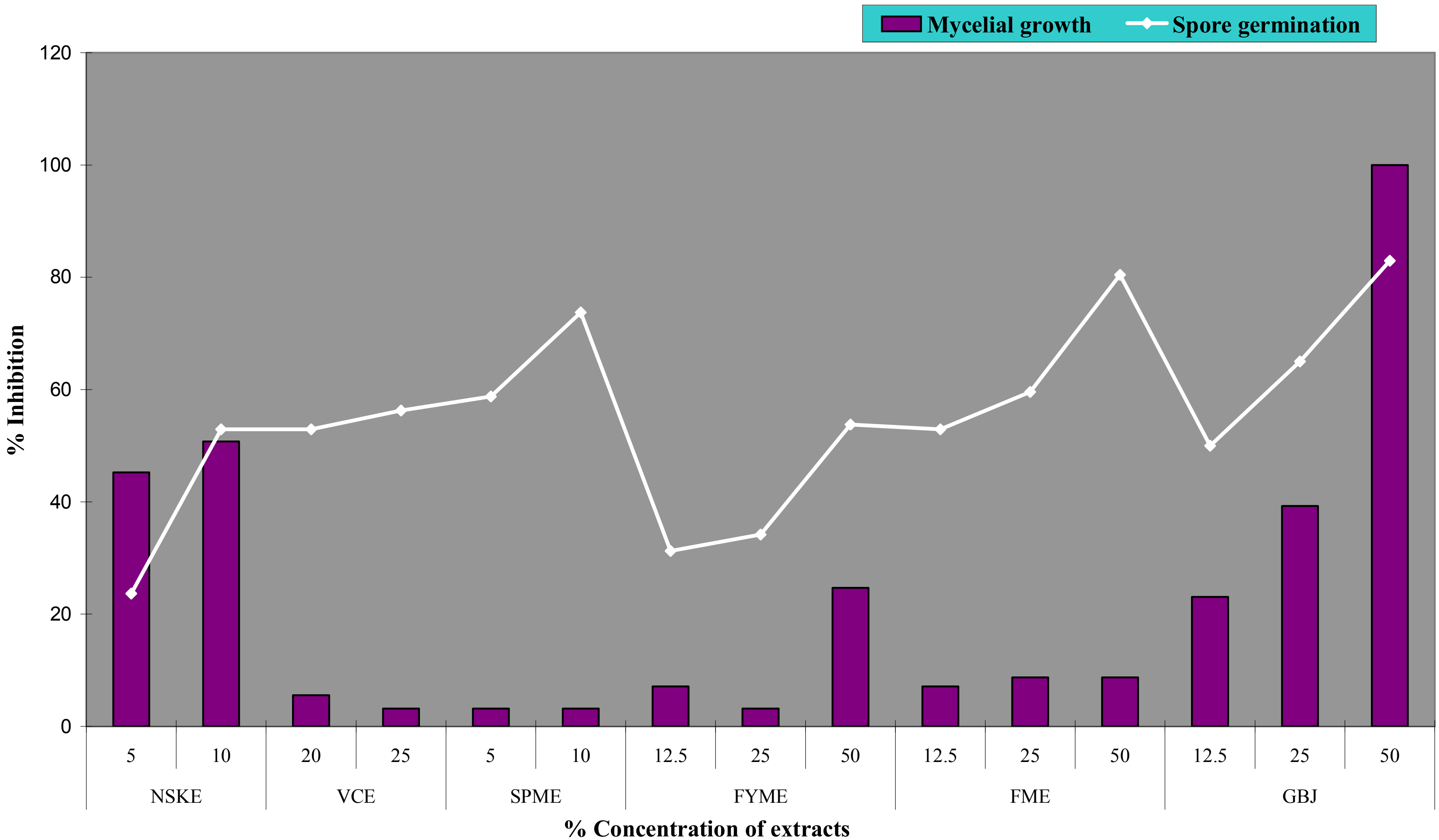


Fig 2: ***In vitro*** **efficacy of 20 day fermented extracts on mycelial growth and spore germination of** ***Alternaria vitis*****.**

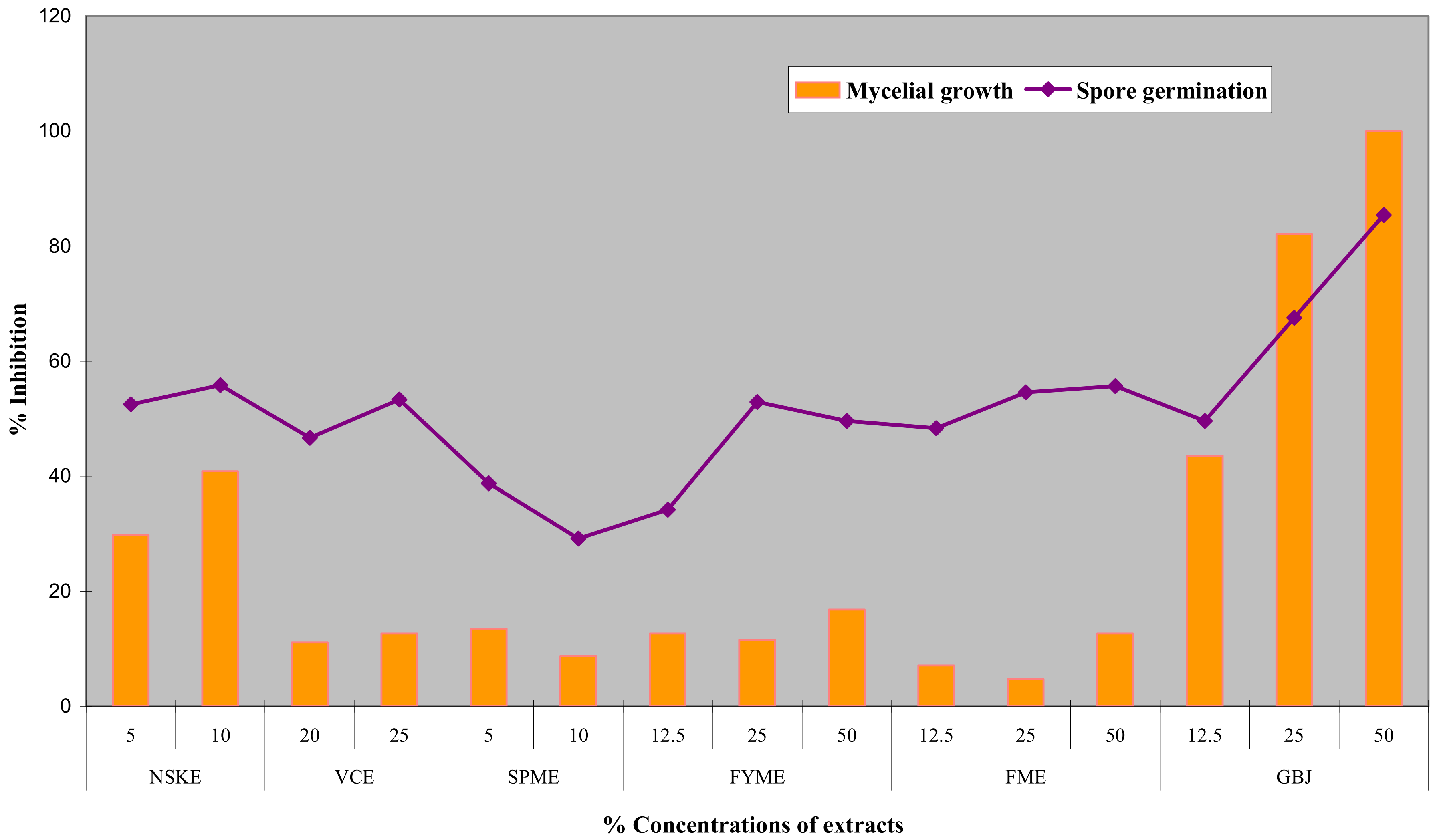


Fig 3: ***In vitro*** **efficacy of 30 day fermented extracts on mycelial growth and spore germination of** ***Alternaria vitis*** **.**

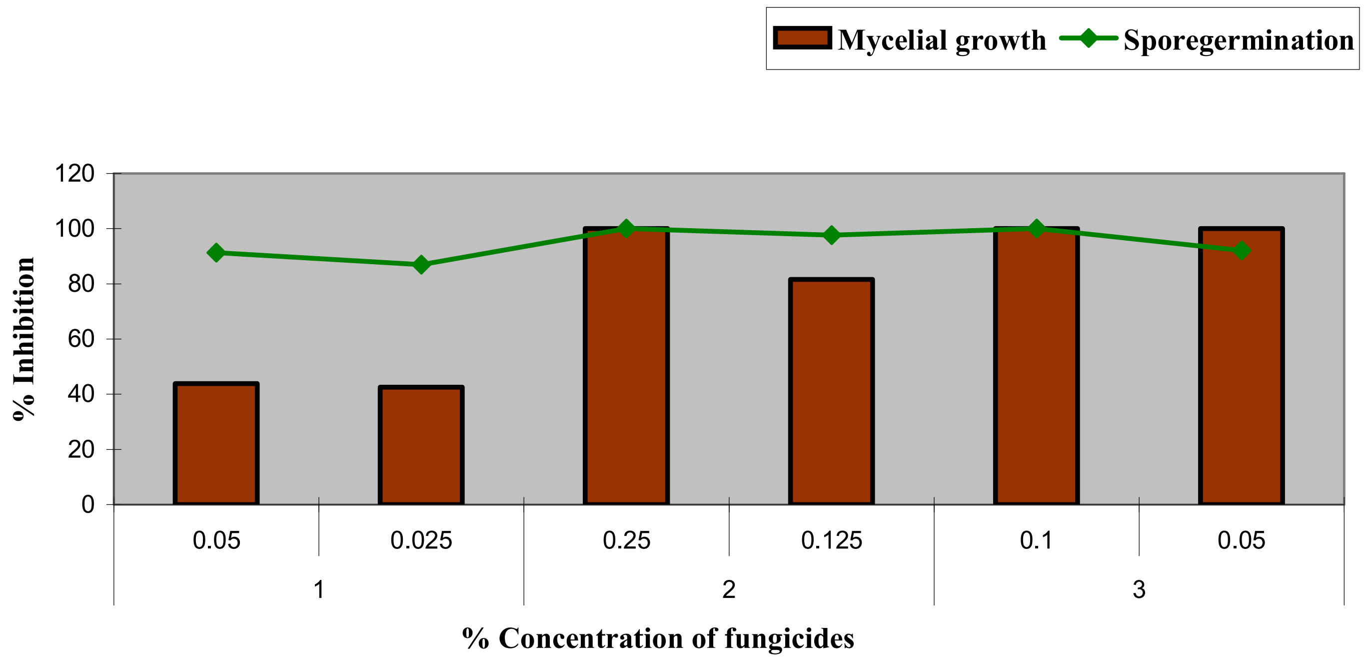


Fig 4: ***In vitro*** **efficacy of fungicides on mycelial growth and sporegermination of *Alternaria vitis***

Fungicides indicated on X-axis of Fig: 4 are

1. Azoxystrobin
2. Mancozeb
3. Iprodione25%+Carbendazim25% (Quintal)

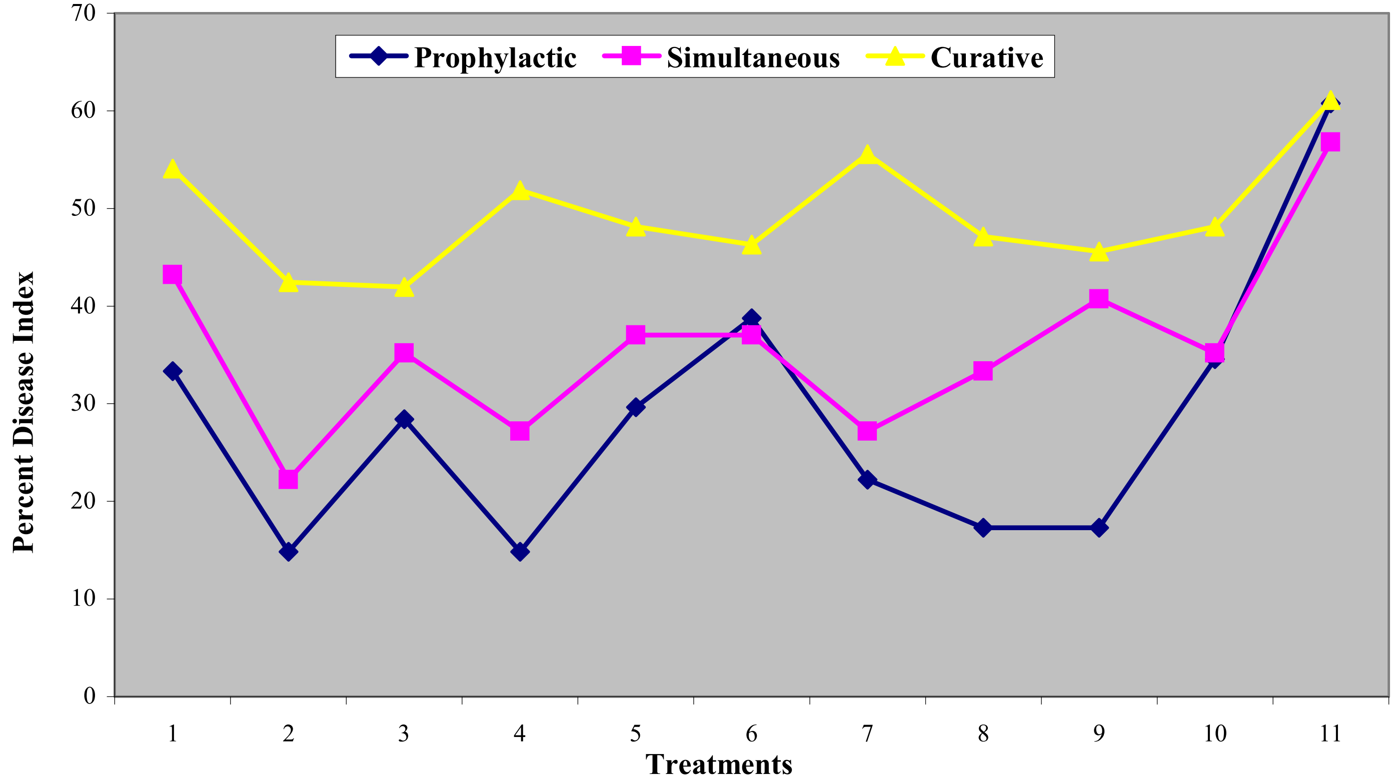


Fig 8: ***In vivo*** **efficacy of different treatments on Alternaria leaf blight of grapevine in glass house conditions.**

The different fungitoxicants indicated on X-axis in Fig: 8 are..

1. 10 day sterilised GBJ at 25%
2. 10 day un sterilised GBJ at 25%
3. 20 day NSKE at 10%
4. Iprodione25% + Carbendazim25% (Quintal) at 0.1%
5. Mancozeb (Indofil M – 45) at 0.25 %
6. *Trichoderma viride* at 5 X 10^4 conidia /ml
7. *Pseudomonas fluorescens* at 10^4-10^5cfu /ml
8. Native PPB-1Y at 10^4 –10^5cfu /ml
9. Native *Trichoderma harzianum* at 5 X 10^4conidia /ml
10. Salicylic acid at 125ppm
11. Control

Phylloplane bacteria indicated on X- axis of Fig: 7 are

1. PPB-1Y
2. PPB-2R
3. PPB-3Ro
4. PPB-4W
5. PPB-5Cr.

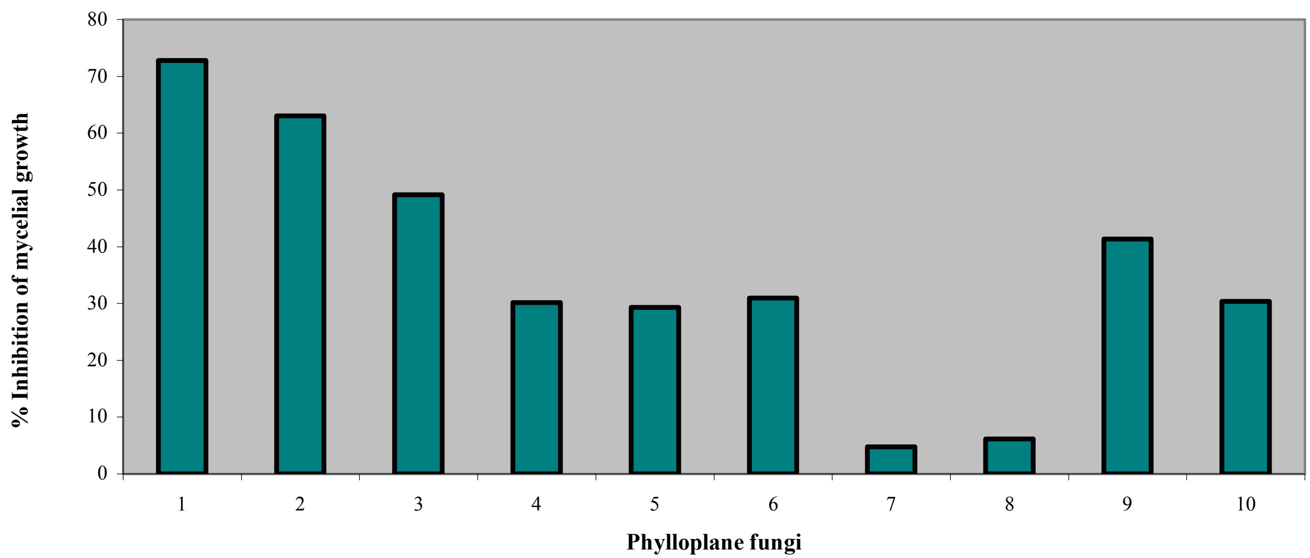


Fig 6: ***In vitro*** **efficacy of Phylloplane fungi on mycelial growth of** ***Alternaria vitis***

Phylloplane fungi indicated on X- axis of Fig:6 are are

1) *Trichoderma harzianum*

2) *Trichoderma viride*

3) *Aspergillus niger*

4) *Aspergillus tumarii*

5) *Aspergillus fumigatus*

6) *Paecilomyces variotii*

7) *Alternaria sp.*

8) *Thielavia terricola*

9) *Oidiodendron sp.*

10) *Cladosporium cladosporoides*

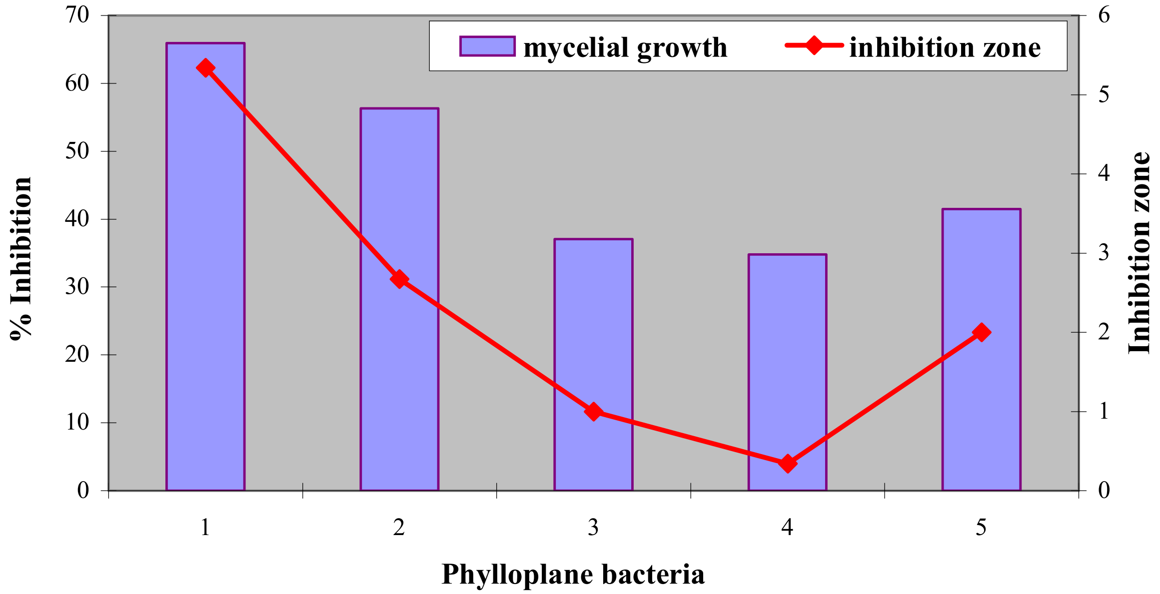


Fig 7: ***In vitro*** **efficacy of phylloplane bacteria on** ***Alternaria vitis*****.**

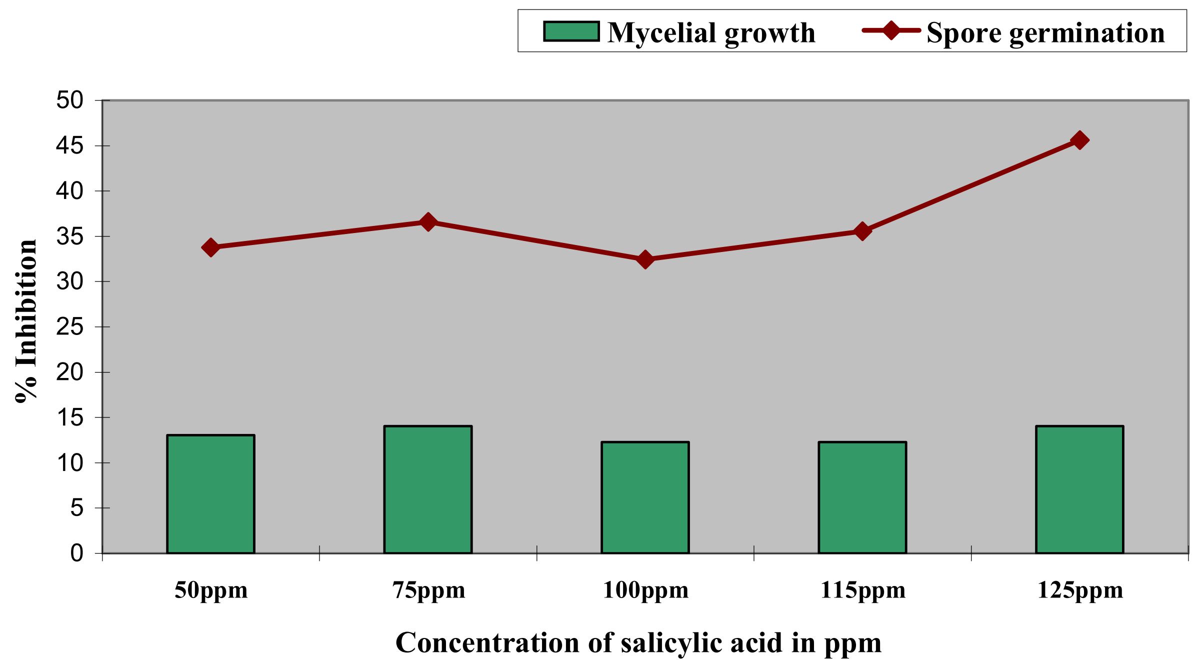


Fig 5: *In vitro* efficacy of salicylic acid on mycelial growth and spore germination of *Alternaria vitis*.

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