"SEASONAL VARIABILITY IN GRAPEVINE PHYLLOPLANE MICROFLORA ASSOCIATED WITH DIFFERENT GRAPEVINE DISEASES AND ITS PROBABLE USE IN DISEASE MANAGEMENT"

By

Mr. Patil Digambar Jalindar

(Reg. No. R/13/137)

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
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APPROVED BY

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2015

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part there of has not been submitted by me or other person to any other University or Institute for a Degree or Diploma

Place : MPKV., Rahuri (Digambar J. Patil)

Dated : / /2015

Dr. S. V. Kolase
Jr. Plant Pathologist,
All India Coordinated Research
Project on Fruits, Department of
Horticulture, MPKV., Rahuri – 413 722,
Dist. Ahmednagar, Maharashtra

C E R T I F I C A T E

This is to certify that the thesis entitled, **"SEASONAL VARIABILITY IN GRAPEVINE PHYLLOPLANE MICROFLORA ASSOCIATED WITH DIFFERENT GRAPEVINE DISEASES AND ITS PROBABLE USE IN DISEASE MANAGEMENT"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, is a record of *bona fide* research work carried out by **Mr. PATIL DIGAMBAR JALINDAR**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and sources of literature referred to have been duly acknowledged.

Place : MPKV., Rahuri

(S. V. Kolase)
Research Guide

Dated: / /2015

Dr. B. R. Ulmek
Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra State (India)

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Place: MPKV., Rahuri (B. R. Ulmek)

Dated: / /2015

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Place : MPKV., Rahuri. (D. J. Patil)

Date :

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LIST OF ABBREVIATIONS

%	:	Per cent
µm	:	Micrometer
/	:	Per
@	:	At the rate of
^{0}C	:	Degree celcius
a.i.	:	Active ingradient
Abst.	:	Abstract
BOD	:	Biological oxygen demand
C.D.	:	Critical difference
cm	:	Centimeter (s)
CRD	:	Completely Randomized Design
CV	:	Coefficient of variation
et al.	:	And others (et alli)
etc.	:	And so forth (*et cetera*)
f.sp.	:	Formae specialis
Fig.	:	Figure
g	:	Grams
i.e.	:	That is
mg	:	milligram
ml	:	Millilitre (s)
mm	:	Millimeter
PGI	:	Per cent growth inhibition
PP	:	Page (s)
RBD	:	Randomized Block Design
S.E.	:	Standard error
spp.	:	Species
UV	:	Ultra violet

ABSTRACT

SEASONAL VARIABILITY IN GRAPEVINE PHYLLOPLANE MICROFLORA ASSOCIATED WITH DIFFERENT GRAPEVINE DISEASES AND ITS PROBABLE USE IN DISEASE MANAGEMENT

By

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A candidate for the degree
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Grape belonging to family *Vitaceae* is one of the consumers preferable and favourite dollar earning table fruit of tropical regions. It has high nutritional as well as medicinal values. It suffers from various diseases, amongst them powdery mildew and downy mildew caused by *Uncinula necator and Plasmopara viticola,* respectively, is an economically important diseases of grapes in Maharashtra. The powdery mildew disease affects all above ground plant parts including leaves, twigs, stem buds and fruit berries but it is more destructive when fruit berries are infected. For controlling these fungus tremendous numbers of pesticides are used all over the world and due to this, pesticide residues are detected in grape berries above MRL level and

therefore, a study was carried out to reduce the pesticide residue and also find out eco-friendly solution to mitigate the disease with the help of biological control agents found by collecting phylloplane microflora during the period of respective diseases.

The nineteen yeast and *Trichoderma* isolates were obtained from leaf samples collected from healthy vineyards of AICRP on furits, MPKV., Rahuri and farmers field from Pune, Nashik, Solapur, Ahmednagar, Satara and Sangli districts. Out of these eleven yeast isolates were evaluated against *Uncinula necator* under field condition. Out of eleven isolates, eight isolates were found effective against *Uncinula necator.* All eight isolates *viz.*, MPKVYI-9, MPKVYI-10, ANYI-1, ANYI-2, ANYI-3, SATYI-1, SOLYI-1 and NYI-1 were found to control the *U. necator* fungus upto 50 per cent. Out of these eight isolates, three isolates (ANYI-2, SATYI-1 and NYI-1) were found more effective and able to control powdery mildew fungi upto 75 per cent and out of these three isolates one (NYI-1) was found most effective and minimize the incidence of *Uncinula* upto 80 per cent under field condition.

Remaining eight isolates of *Trichoderma* were found effective against *Plasmopara viticola.* All eight isolates *viz.*, PTI-1, SATI-1, ANTI-1, ANTI-2, ANTI-3, SATTI-1, SOLTI-1 and NTI-1 were found to control the *Plasmopara viticola* fungus upto 55 per cent. Out of these eight isolates, three isolates (ANTI-2, SATTI-1 and NTI-1) were found more effective and able to control downy mildew fungi upto 80 per cent and out of these three isolates

one (NTI-1) was found most effective and minimize the incidence of *Plasmopara viticola* upto 80 per cent under field condition.

Phytoparasitism was the biocontrol mechanism of yeast was observed against *Uncinula necator* under microscopical studies. It was observed under microscope that spores of yeast attached to the surface of powdery mildew fungi and further epidermis of fungal spore get disturb and shrink.

The effect of yeast and *Trichoderma* isolates on leaf microflora was evaluated by leaf inprint method (Dorsal and Ventral).It revealed that the colonies of fungi, bacteria and actinomycetes were decreased and the colonies of yeast and *Trichoderma* were increased due to spray application of yeast and *Trichoderma* isolates.

1. INTRODUCTION

Grape (*Vitis vinifera* L.) belongs to family *vitaceae* is 90-95 million years old plant on the earth. It is believed that grape cultivation originated near Caspian Sea in Russia that spread westward to Europe and American continents and eastward towards Iran and Afghanistan. In India, grapes were introduced into the northern parts from Iran and Afghanistan by Muslim invaders in 12th century. Later it spread to south India in 1338.

Grape is the most important temperate fruit crop that has acclimatized to the subtropical and tropical agro-climatic conditions. It is a fairly good source of minerals like calcium, phosphorus, iron and vitamins such as B_1 and B_2. Grape is known for its cultural dualism between subsistence-oriented growers and export oriented large corporate growers in India. It has become the most remunerative commercial farming enterprise and as such, India exports a large quantity of fresh grapes

Grapes generally require a hot and dry climate during its growth and fruiting periods. It is successfully grown in areas where the temperature range is from 15-40 ^{0}C. Area with annual rainfall not exceeding 900 mm well distributed throughout the year is ideal. Grapes can be cultivated in variety of soils including sandy loams, sandy clay loams, red sandy soils, shallow to medium black soils and red loams. The soil should be

well drained, having good water holding capacity however, soils having pH range of 6.5-8.0 are considered ideal.

According to NHB data, In India Grapes are cultivated in an area of 111.4 thousand ha with a total production 1,234.9 thousand tons and productivity of 11.1tons/ha. Because of special arbour training systems provided for grape cultivation in India, productivity is highest among the grape growing countries of the world. Maharashtra is a leading state in production of grapes in the whole country. With regard to agricultural land under grape cultivation and grapes production, Nasik and Sangli districts are at forefront in the state. Apart from these, grapes are also grown in the district of Ahmednagar, Pune, Satara, Solapur and Osmanabad. Nowadays, grapes are produced in Latur district of Marathwada also. However, Nasik and Sangli districts are ahead in the production of grapes in a scientific manner. Area under grapes in Maharashtra is 86 thousand ha and production is around 774 thousand tons of grapes annually. Total export of grapes from India is 108.58 thousand tons during 2013-14 valuing of Rs. 602.88 crores, out of which, nearly 80% is exported from Maharashtra.

The grape production in India suffers from inherent constraints such as long dry spells, inadequate availability of rootstocks resistant to drought, salinity, pests and diseases, post harvest losses apart from the cultural aspects like improper nutrition, water management, improper training systems and physiological disorders that contribute towards overall reduction in the grape productivity. The losses due to insect pests and

diseases are high and for their management many sprays of several pesticides are required which accounts to 30 per cent of the total cost of production. Among the diseases occurring on grapes, fungal diseases are the most destructive followed by a few bacterial, viral and nematode infections of minor importance.

Major diseases of grapevine are anthracnose caused by the fungus *Elsinoe ampelina,* black rot is caused by *Guignardia bidwellii*, bacterial disease crown gall is caused by *Agrobacterium tumefaciens*, dead arm caused by the fungus *Phomopsis viticola*, downy mildew by fungus *Plasmopora viticola,* powdery mildew by *Uncinula necator*, while gray mold or bunch rot caused by the fungus *Botrytis cinerea.*

Although dry weather can slow the development of many grape diseases where as this is not the case with powdery mildew. This disease can be a serious problem even during periods of drought also. Losses can be severe if fruits are infected with powdery mildew disease, sometimes resulting in complete loss of the crop. The powdery mildew fungus not only affects fruit yield and quality, but it also reduces vine growth and winter hardiness.

Powdery mildew can infect all green parts of the grapevine. This disease is most easily recognized by the dusty appearance or white powdery growth occurring in patches on fruit, leaves and vines. When young expanding leaves are infected, they may become distorted and stunted. Severely affected leaves may curl upward during hot dry weather. Dark

brown to black blotchy lesions form on diseased vines. Infected berries often are misshapen or have rusty spots on the surface.

With increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world, the regulatory agencies have reacted to public pressure and introduced comprehensive to reduce pesticide use. There is also non-availability of effective chemicals for control of many diseases and some pest or disease pathogens have developed resistance to the certain pesticides. Biological control therefore appears to be one of the effective module in integrated pest or disease management schedule and viewed as alternative approach in 21st century. Several yeast e.g. *Picnia gulliermondii*, also parasities and inhibit the growth of plant pathogenic fungi such as *Botrytis* and *Penicillium*. Also *Trichoderma spp* e.g. *Trichoderma harzianum* inhibit the growth of downy mildew fungi Therefore antagonistic property of yeast and *Trichoderma spp* isolates were studied in present investigationwas planned for the study as entiteled "Seasonal variabiliy in grapevine phylloplane microflora associated with different grapevine diseases and its probable use in disease management" was undertaken with following objectives.

1. To isolate the phylloplane microflora associated with major diseases of grapes in their respective season of incidence.
2. To identify the microflora isolated during various seasons of incidence.
3. To find out the percentage frequency of fungal species isolated from the collected samples.

4. To find the effect of associated microflora for their probable use in control of major diseases.
5. Effect of fungicides on phylloplane microflora and pathogens of the diseases of grapes

2. REVIEW OF LITERATURE

The literature available and reviewed in support of present investigation, experimentation and results are summarised and categorized as under.

2.1 Occurrence of downy mildew and powdery mildew disease of grapevine

Arens (1929) reported for *Plasmopara viticola* that exomosis of material from external surface of plant which is parallel to root exdutes in the soil which affect germination of micro-organism.

Brown (1922 and 1936) showed the moisture film on plant surface inevitably carrier of both organic and inorganic substances solution released from underlying plant tissue which influence germination of micro-organism.

Mirdul Goel and Sinha (1961) isolated number of micro-organisms from surface of vegetable seeds and some of these are well known to produce antibiotics substances.

Ruinen (1961) examined the micro-organism of fresh leaves of various plants at Bogar Botanical Garden, Java and determined the water and nutrient relationship of phyllosphere.

Nikitina (1962) concluded a four year study at leningard Institute of Soviet Trade and found accumulation pytonicides in leaves of apple and pear varieties with different maturation time and their action against bacterial pathgens.

Preece and Dickinson,(1971); and Blackman, (1981) reported the habitat adjacent to the leaf surface is known as the phyllosphere and the surface of the leaf is called Phylloplane.

Sharma and Sinha (1972) studied on micro-organism found on surface of leaf i.e. phyllosphere play important role in associative or antagonistic manner on germination of pathogenic fungi.

Fry (1982) reported the rapid growth of foliage provides better opportunities for pathogen growth rather than organism in phyllosphere.

Doster and Schnathorst (1985) stated that *Uncinula necator* develops best on young grape leaves and will not usually infect leaves more than 2 months old.

Andrew (1985) reported that potential of exploiting antagonistic micro-organism of phylloplane in biological control of plant foliar diseases have been documented.

Puttoo and Razdan (1987) reported that epidemic of powdery mildew of grape occurred in June-August, 1985 in vineyards of Jammu and Kashmir on all important cultivars. In November, 1986 abundant Cleistothecia were produced on mature wood. The causal fungus was identified as *Uncinula necator*.

Singh and Munshi (1993) studied that Leaf maturity does not greatly influence the germination of conidia and formation of appressoria. However, growth and sporulation are adversely affected by advancing leaf maturity.

Olsen (1998) recognized the white to gray, powdery spots or large blotches on the surface of leaves, stems and fruits of host plants. The white powdery growth consists of the fungal mycelium and asexual reproductive spores.

Halleen and Holz (2000) studied the characteristics of the first symptoms developed on leaves of grapevine infected with powdery mildew. Symptoms observed on all the green parts of the vine: leaves, branches, inflorescences, rachises, pedicels and berries. Mildew colonies were found on either the lower surface of exposed leaves or on both sides of well-shaded leaves. The fungus forms a white to ash-grey, weblike mat of mycelial strands over the infected tissue.

Falconi *et al.* (2002) observed the biological control of airborne foliar diseases is the use of the antagonistic bacteria *Pseudomonas cepacia*and *Bacillus subtilis* to control Monilia pod rot in Cacoa

. Wilcox (2003) reported that powdery mildew fungus can infect all green tissues of the grapevine. On leaves, it appears as a white or grayish-white powdery covering of the upper and lower surfaces. Heavily infected leaves may turn dull, dry out and drop prematurely, whereas very young leaves that become infected may become distorted and stunted as they expand. Fruit infections may appear white and powdery or dark and dusty, and sometimes result in shriveling or cracking of the berries.

Jean *et al.* (2005) analyze the genetic variation and the population structure of grape powdery mildew fungus in Southern France. The sample comprised 101 isolates and was

mainly of flag shoot origin. RAPD analysis identified different haplotypes that clustered in two genetic groups i.e. A and B.

Kortekamp (2006) studied that This disease is present in many parts of the world. *P. viticola* reduces fruit quality and yield, either by direct infection of berries or as a result of the reductions in photosynthesis and plant vigor caused by leaf infections

Vinale *et al.* (2008) reported that the most studied biocontrol agents are the fungi of the *Trichoderma* genus, whose biocontrol mechanisms are largely investigated.

Trouvelot *et al.* (2008) studied the direct inhibitory effect of these products on the germination of *P. viticola* sporangia (zoospore release) was analyzed by mixing 500 µL of the sporangia suspension with 500 µL of a *T. harzianum*T39 conidia suspension (105 cfu mL−1 final) or 500 µL BTH (0.5 g L−1 final). After 15 min, changes in sporangial morphology were assessed and the percentages of germinated sporangia in three replicates of 50 sporangia per treatment were counted under light microscope.

Ellis (2008) stated that the powdery mildew fungus can infect all green tissues of the vine. Small, white or grayish-white patches of fungal growth appear on the upper or lower leaf surface. Affected berries may have patches of fungal growth on the surface similar to those on the leaves, or the entire berry may be covered with the white, powdery growth. Infected berries often misshapen or have rusty spots on the surface.

Hartman and Beale (2008) revealed that powdery mildew can infect all green parts of the grapevine. This disease was most easily recognized by the dusty appearance or white powdery growth occurring in patches on fruit, leaves and vines. When young expanding leaves were infected, they may become distorted and stunted. Severely diseased fruits may split open.

Bendezu-Euribe and Alvarez (2012) reported the perfect stage of powdery mildew of grapevine caused by *Erysiphe necator* found in Peru. Abundant, mature (black) and immature (brown), globose ascocarps were visible on the abaxial leaf surfaces covered by the anamorphic state of the fungus. The chasmothecia were epiphyllous and ranged from 93.3 to 157.5 µm in diameter.

Riaz *et al.* (2013) reported a new morphotype of *Erysiphe necator*, the fungal pathogen cause of grapevine powdery mildew. Compared to normal isolates, the new morphotype develops the first conidium on the tip of conidiophore by day five after inoculation and stays in arrested growth phase until day 9 or 10. On day 10 or 11, a branch appears at the base of first conidium that independently starts making conidia. Both main and side branches of conidiophores develop chains that were short with 2–4 conidia, their conidia take a longer time to mature, and they have stronger adhesion to sister conidia on the chain.

2.2. Isolation of phylloplane microflora

De Becze (1955) reported that yeasts are well distributed over warm and temperate zones of the surface of the earth and carry on life, utilizing the remenants and excretions of

plants and animals. They live principally on sugar-containing liquids such as juices of damaged fruits, leaves, stalks, roots and in the nectars of flowers. From the soil, where they carry on seasonal vegetation in dilute sugar solutions washed from the trees and where they mate, sporulate, or hibernate.

Breeze and Dix (1981) observed the population of fungi was different in different seasons. The highest population was observed during the month of July to October and sudden decrease started from November up to May noticed that in summer, the biomass of yeasts on leaves of Acerplatanoids was 50 times greater than of hyphal fungi.

Haridy (2002) isolated 87 yeast strains from rhizosphere and 115 strains from nonrhizosphere areas of potato, maize and vegetable marrow and cabbage plants. On the basis of 26 morphological and physiological properties, the isolated yeast strains were assigned to 9 genera and 15 species.

Aneja (2003) studied thatthe isolation of fungi was done by leaf impression method and serial dilution method.

Slavikova (2007) reported that the yeasts were isolated from the leaf surfaces of ten species of trees. One hundred and thirty seven yeast strains belonging to 13 genera were isolated from 320 samples of leaves and needles. Seventeen yeast species were isolated, but only seven occurred regularly *Aureobasidium pullulans*, *Cryptococcus laurentii*, *Pichia anomala*, *Metschnikowia pulcherrima*, *Saccharomyces sp.*, *Lachancea thermotolerans* and *Rhodotorula glutinis*. The remaining species were isolated from the leaves and needles of three or less tree species.

Aureobasidium pullulans, *Cryptococcus laurentii* and *P. anomala* were the most frequently found species and they occurred on leaves and needles of all ten tree species. *Saccharomyces sp.* occurred in leaf samples collected from eight kinds of trees.

Slavikova *et al.* (2009) isolated the yeast from leaf surfaces of five species of fruit trees located in southwest Slovakia. 155 yeast strains belonging to 11 genera were isolated from 300 samples of leaves. 17 yeast species were identified, but only three occurred regularly: *Aureobasidium pullulans, Cryptococcus laurentii* and *Metschnikowia pulcherrima*. Species such as *Hanseniaspora uvarum, Pichia anomala, Rhodotorula glutinis* and *Saccharomyces cerevisiae* were isolated less frequently. They found only few differences in the yeast community isolated from leaves of different tree species although dominant species occurred regularly on the majority of leaves.

2.3 Biocontrol mechanism of yeast and *Trichoderma*

Wisniewski *et al.* (1988) made several electron microscopic observations which showed that the antagonistic yeast cells may injure the pathogen directly. Co-culturing *Penicillium guilliermondii* with the pathogenic fungi *B. cinerae*or *P. expansum* demonstrated the ability of the yeast to attach fungal hyphae closely. Apart from the effects of direct contact of the antagonistic yeast with the pathogen hyphae. Filtrates of the antagonistic cells can produce higher level of gluconase than filtrates of the non-antagonistic yeast and the gluconase activity is the cause of cell wall degradation at the site of attachment. These findings raised the suggestion that the firm attachment to

the fungus, in conjuction with the enhanced activity of cell-wall degrading enzymes, may have an important role in the biological activity of yeast *Penicillium guilliermondii* and that it's efficacy is not dependent only on it's competition with the pathogen for nutrient.

Jarvis *et al.* (1989) were the first to report the efficacy of *Pseudozyma flocculosa* and *Pseudozy marugulosa* against cucumber, rose and wheat powdery mildew. The mode of action of this species was associated with the production of unusual fatty acids that naturally insert into powdery mildew cells and cause disorganization of cellular membranes and cell disintegration.

Dik *et al.* (1991) demonstrated that honeydew utilization by yeasts *Sporobolomyces spp.* and *Cryptococcus spp.* on wheat flag leaves dramatically reduced the level of carbon source available for nerotrophic pathogens.

Inge *et al.* (1993) reported that *Tilletiopsis albescens* grows well on powdery mildew fungi inoculated on barley or cucumber leaves and causes collapse of the colonies. Application of ballistospores or cut mycelium was equally effective for biocontrol and the effectiveness tended to increase exponentially with the concentration of germinating units (conidia and cut mycelium) applied. Seventy percent relative humidity or more is required for effective biocontrol. Two applications of *T. albescens* in the period from 3 days before to 3 days after inoculation with powdery mildew were more effective than one.

Wilson and Wisniewski (1994) revealed that competition for nutrients between yeasts and molds and parasitism were likely to be the main mechanisms of action. Many beneficial types of yeast can effectively deplete the sugar occurring in fruit wounds (place of infection) and inhibit germination of mold propagules.

Avis and Belanger (2002) reported that *Pseudozyma flocculosa*, a natural inhabitant of the phyllosphere, possesses unique means of defending its ecological niche by producing unusual extracellular fatty acids that are detrimental to powdery mildew fungi, an important group of plant pathogens. Results from these studies have shown that the fatty acids naturally insert themselves into powdery mildew fungi and cause disorganization of cellular membranes and cell disintegration.

2.4 Occurrence of phylloplane microflora

Schmid *et al.* (2011) reported significant difference in structure and function of above-ground grapevine associated microorganisms from organically and conventionally managed vineyard. The black fungus was strongly enriched in the communities of organically managed plants and yielded higher indigenous antiphytopathogenic potential.

Evan *et al.* (2012) studied the effect of aerated compost tea on grapevine microbial abundance on leaves. The numbers of culturable bacteria, fungi and yeasts on treated leaves were higher after application of aerated compost tea.

Martin *et al.* (2013) studied diversity of epiphytic bacteria on grape berries and other plant parts like leaves and

bark. Highest bacterial population counts found in soil samples followed by bark, berries and leaves.

2.5 Survival of powdery mildew pathogen

Pearson and Gadoury (1987) reported that *Uncinula necator* (powdery mildew of grape) survived in winter as mycelium in dormant infected buds. Cleisthothecia were found in spring on all plant parts infected during the previous growing season and also in leaf scars and in crevices of exfoliating bark. Most of cleistothecia borne on leaves, canes and cluster stems died during winter.

Cortesi and Bisiach(1997) studied that density and viability of populations of cleistothecia of *Uncinula necator* from bark, leaves and soil were determined in three vineyards. A higher density of cleistothecia was found on fallen leaves than on bark. However, the percentage of viable cleistothecia was higher on bark. No viable cleistothecia were recovered from soil. *U. necator* overwintered as mycelium in dormant infected buds, which gave rise to flag shoots. Cleistothecia were formed in autumn and their dispersal started in late September to mid-October, with the maximum number of cleistothecia trapped in funnels during the second half of October. Cleistothecia appear to function as the sole source of primary inoculums for grape powdery mildew. Some vineyards serve as additional sources of inoculums where the pathogen also overwinters in infected buds.

Halleen and Holz (2000) reported that the flag shoots were found shortly after budbreak in September 1997 in a Carignane vineyard. Cleistothecia were first observed during

April to May 1996 on severely infected leaves from three vineyards in the main grape growing areas of Stellenbosch. Cleistothecia occurred in small numbers on leaves (1-10 per leaf) and all were immature. Cleistothecia were dispersed by late summer and autumn rains from leaves to bark of grapevines, where they overwinter.

Grove (2004) studied the mode of perpetuation of *Uncinula necator* in Eastern Washington. Cleistothecia retrieved from bark fissures and senesced leaves contained viable ascospores at bud burst and later. Ascospore release in lab studies occurred from the late-dormant stage through the prebloom and (in some cases) the bloom stages. In vineyard studies, ascospores were trapped as late as 70 days after bud burst during rain events of 3.9 to 9.6 mm. Detection of ascospores in vineyard air preceded the initial occurrence of powdery mildew symptoms and signs and the occurrence of conidia in volumetric spore traps by several days. Cleistothecia are the only known source of primary inoculum in the grape-production regions of Eastern Washington.

Hajjeh *et al.* (2008) concluded that *Erysiphe necator* overwinters as mycelium or conidia in dormant buds as cleistothecia, but the importance of the two forms as sources of primary inoculums varies in different viticultural areas. The present study summarizes the observations made over 2001-2003, in 29 vineyards of southern Italy, on the occurrence and frequency of the two overwintering forms. Flag shoots were found in one-third of the vineyards, with a frequency highly variable

between year and vineyard, being more frequent in vineyards for wine-grape than for table-grape production.

Fathi and Khiavi (2012) reported that *U. necator* survived as mycelium in the dormant buds of the grapes during winter season. Also the effect of environmental factors on fungus biology showed that the pathogenic activity of the fungus began when the temperature was between 16-19 °C with a relative humidity more than 50 %. It was also found that optimum temperature and relative humidity for the sporulation of*U. necator* was 20-25 °C and 50-100 %, respectively.

Khiavi *et al.* (2012) studied the biology and epidemiology of *Uncinula necator* the causal agent of grape powdery mildew disease. Results indicated that the *Uncinula necator* survived as mycelium in dormant buds of the grape during winter season.

3. MATERIAL AND METHODS

The material used and methods followed during the course of present investigation are as follows

3.1 Material

3.1.1 Collection of leaf samples

The leaf samples of grapevine were collected from vineyard of AICRP on Fruits, Department of Horticulture, MPKV., Rahuri.

3.1.2 Isolation of Phylloplane microflora

The microflora were isolated from the leaves of grapevines collected from different varieties of grapes, MPKV., Rahuri.

3.1.3 Glasswares

Different types of Corning and Borosil brand glasswares used were conical flasks, petriplates, test tubes, glass rods, slides, coverslip, funnel, beaker, measuring cylinder, pipette, etc. the material available in the Department of Plant Pathology and Agril. Microbiology, MPKV., Rahuri.

3.1.4 Culture media

Glucose Yeast Extract Peptone Agar media (GYEPA media), was used for isolation of yeast. The media used during the course of studies are given here under.

Medium	Constituents	Purpose
1.GYEPA Medium		Isolation of yeast from leaves
Glucose	20 gm	
Bacto peptone	10 gm	
Bacto yeast peptone	5 gm	
Agar agar	20 gm	
Distilled water	1000 ml	
2.Nutrient Agar Medium		Study of phylloplane microbial count of grapevine leaf
Beef extract	3 gm	
Peptone	5 gm	
Sucrose	5 gm	
Dextrose	5 gm	
Agar agar	18 gm	
Distilled water	1000 ml	
3.Potato Dextrose Agar medium		Study of phylloplane microbial count of grapevine leaf
Potatoes	200 gm	
Dextrose	20 gm	
Agar agar	20 gm	
Distilled water	1000 ml	

3.1.5 Chemicals

The chemical used for different studies were of analytical grade and of standard firms *viz.,* M/s Merck (India) Pvt. Ltd. Mumbai, Glaxo Laboratories (India) Pvt. Ltd., Mumbai,

Bolts/Qualigens laboratories Mumbai, Hi-media Laboratories (India) Pvt. Ltd., Mumbai etc.

3.1.6 Experimental site

The present experiments were carried in laboratory, at Department of Plant Pathology and grapevine field at All India Coordinated Research Project on Fruits, Department of Horticulture, MPKV., Rahuri.

3.1.7 Laboratory instruments and equipments

Various laboratory instruments used during study were autoclave, laminar air flow, biological oxygen demand (BOD), hot air oven, incubator, refrigerator, electronic and physical balance, shaker, microscope etc.

3.1.8 Miscellaneous

The various other essential materials used were inoculating needles, forceps, sterilized water, blotting paper, sterilized cotton, muslin cloth, sprayers, scissor, syringe, rubbers, polythene bags, glass marking pens, sticky labels etc.

3.2 Method

1. The leaf and plant samples will be collected from vineyards at periodic interval during occurrence of major diseases.
2. Isolation of different microflora from phylloplane will be carried out on different media such as sucrose peptone agar or beef extract peptone agar or NA for bacteria and potato dextrose agar (PDA) and vegetable agar medium for fungi and yeasts will be used and will be inoculated with test sample using serial dilution technique (Aneja,

2003).

3. The percentage frequency of fungal species will be estimated as per the formula :

$$\text{Frequency (\%)} = \frac{\text{No. of samples showing occurrence of fungus}}{\text{Total no. of samples having all the fungi}}$$

4. After ascerting the purity, the cultures will be maintained.
5. Isolated microflora will be identified in laboratory of Department of Plant Pathology and Agril. Microbiology, MPKV., Rahuri.
6. From the isolated microflora, beneficial microflora will be tested against major diseases of grapevine by dual culture, food poison, spore germination, detached leaf test and whole plant method.

3.2.1 Isolation of phylloplane microflora

3.2.1.1 Isolation of phylloplane microflora after backword pruning

For isolation of phylloplane microflora leaf samples were collected from healthy as well as powdery mildew infected vineyards of AICRP on Fruits, MPKV., Rahuri and farmers field from Pune, Nashik, Solapur , Ahmednagar, Satara and Sangli districts. The isolation was done by leaf inprint method. For leaf samples, leaf prints were taken from abaxil and adaxil sides of leaves on NA as well as PDA media. The plates were incubated at 28 ± 1 ^{0}C for 48 hours. The following phylloplane microflora are observed in the backword pruning e.g. *Colletotrichum spp, Geotrichum spp,*

Cladosporium spp. Streptomyces spp. Curvularia affinis. Microbial colonies were identified and purified colonies of yeast was transferred on GYEPA slants and incubated at 28 ± 1 ^{0}C for 48 hours and stored in refrigerator as a pure culture for further studies. These yeast cultures were designated separately as per their location and allotted a numerical number for every yeast isolate.

3.2.1.2 Isolation of phylloplane microflora after forword pruning

For isolation of phylloplane microflora leaf samples were collected from healthy as well as powdery mildew infected vineyards of AICRP on Fruits, MPKV., Rahuri and farmers field from Pune, Nashik, Solapur , Ahmednagar, Satara and Sangli districts. The isolation was done by leaf inprint method. For leaf samples, leaf prints were taken from abaxil and adaxil sides of leaves on NA as well as PDA media. The plates were incubated at 28 ± 1 ^{0}C for 48 hours. the following phylloplane microflora are observed in the forword pruning e.g. *Aspergillus niger, A. flavus, A. fumigates, Penicillium spp, Alternaria alternate, Trichoderma spp.* microbial colonies were identified and purified colonies of yeast was transferred on GYEPA slants and incubated at 28 ± 1 ^{0}C for 48 hours and stored in refrigerator as a pure culture for further studies. These yeast cultures were designated separately as per their location and allotted a numerical number for every yeast isolate.

Details of leaf samples collected from vineyards

Sr. No.	Variety	Location
1	Thomson Seedless	MPKV., Rahuri
2	Flame Seedless	MPKV., Rahuri
3	Pusa Navrang	MPKV., Rahuri
4	Cabbernet Sauvignon	MPKV., Rahuri
5	Souvignon Blanc	MPKV., Rahuri
6	Red Globe	MPKV., Rahuri
7	Punjab Purple	MPKV., Rahuri
8	Tas-A-Ganesh	MPKV., Rahuri
9	Sharad Seedless	MPKV., Rahuri
10	Dogridge	MPKV., Rahuri
11	Teleki-5A	MPKV., Rahuri
12	Tas-A -Ganesh	Tal. Baramati, Dist. Pune
13	Sharad Seedless	Tal. Tasgaon, Dist. Sangli
14	Tas-A - Ganesh	Tal. Shrirampur, Dist. Ahmednagar
15	Thomson Seedless	Tal. Kopergaon, Dist. Ahmednagar
16	Thomson Seedless	Tal. Rahata, Dist. Ahmednagar
17	Thomson Seedless	Tal. Karad, Dist. Satara
18	Sharad Seedless	Tal. Sangola, Dist. Solapur
19	Tas-A- Ganesh	Pimpalgaon Baswant, Dist. Nashik

3.2.3 Testing of yeast isolates against powdery mildew on leaves of grapevine

The powdery mildew infected vine were selected from eight years old grape vineyards of AICRP on furits, MPKV., Rahuri. to test the efficacy of yeast isolates obtained from different leaf samples. The isolated yeast culture were sprayed as per treatment at 10^6 L^{-1} concentration on powdery mildew infected grapevine. The spraying was done three times at ten days interval and observations on PDI were recorded ten days after each spray. Ten leaves were observed from each direction of

vine and likewise fourty leaves were observed from four directions. Per cent disease intensity were recorded from all four directions.

3.2.4 Further testing of eleven effective yeast isolates against powdery mildew on leaves on grapevine

Efficacy of selected effective eleven yeast isolates out of twenty two isolates were further studied by spraying on powdery mildew infected vine three times as stated earlier. Per cent disease incidence and per cent disease control were recorded before every spraying of yeast.

3.2.5 Further more testing of three most effective *Trichoderma* isolates against downy mildew on leaves of grapevine

Efficacy of most effective three *Trichoderma* isolates out of eight effective isolates were further studied by spraying on downy mildew infected vine three times as stated earlier. Per cent disease incidence and per cent disease control were recorded before every spraying of *Trichoderma.*

Design and layout of experiment

1.	Crop	:	Grape (*Vitis vinifera* L.)
2.	Variety	:	Sharad Seedless
3.	Design	:	Randomized Block Design (RBD)
4.	Treatments	:	27 [one vine per treatment]
5.	Replication	:	Three
6.	Spacing	:	3 x 1.5 m
7.	Season	:	2014-15
8.	Age of the vines	:	8 years

N

R I		R II		R III	
T_1	T_{14}	T_4	T_{16}	T_6	T_{25}
T_2	T_{15}	T_7	T_{18}	T_{23}	T_{12}
T_3	T_{16}	T_2	T_{20}	T_{10}	T_{21}
T_4	T_{17}	T_5	T_{22}	T_{19}	T_{13}
T_5	T_{18}	T_3	T_{24}	T_9	T_{15}
T_6	T_{19}	T_1	T_{26}	T_{11}	T_{17}
T_7	T_{20}	T_8	T_{14}	T_{14}	T_{26}
T_8	T_{21}	T_{11}	T_{17}	T_8	T_1
T_9	T_{22}	T_9	T_{15}	T_{24}	T_3
T_{10}	T_{23}	T_{12}	T_{19}	T_5	T_{22}
T_{11}	T_{24}	T_{10}	T_{21}	T_2	T_{20}
T_{12}	T_{25}	T_{13}	T_{23}	T_{18}	T_7
T_{13}	T_{26}	T_6	T_{25}	T_4	T_{16}
T_{27}	T_{27}	T_{27}	T_{27}	T_{27}	T_{27}

Fig. 1. Plan of experimental layout

Treatment Details

Sr. No.	Treatment No.	Yeast isolate No.	Details of treatments
1.	T_1	MPKVYI-1	Mahatma Phule Agriculture University Yeast Isolate No. 1
2.	T_2	MPKVYI-2	Mahatma Phule Agriculture University Yeast Isolate No. 2
3.	T_3	MPKVYI-3	Mahatma Phule Agriculture University Yeast Isolate No. 3
4.	T_4	MPKVYI-4	Mahatma Phule Agriculture University Yeast Isolate No. 4
5.	T_5	MPKVYI-5	Mahatma Phule Agriculture University Yeast Isolate No. 5
6.	T_6	MPKVYI-6	Mahatma Phule Agriculture University Yeast Isolate No. 6
7.	T_7	MPKVYI-7	Mahatma Phule Agriculture University Yeast Isolate No. 7
8.	T_8	MPKVYI-8	Mahatma Phule Agriculture University Yeast Isolate No. 8
9.	T_9	MPKVYI-9	Mahatma Phule Agriculture University Yeast Isolate No. 9
10.	T_{10}	MPKVYI-10	Mahatma Phule Agriculture University Yeast Isolate No. 10
11.	T_{11}	MPKVYI-11	Mahatma Phule Agriculture University Yeast Isolate No. 11
12.	T_{12}	MPKVYI-12	Mahatma Phule Agriculture University Yeast Isolate No. 12
13.	T_{13}	MPKVYI-13	Mahatma Phule Agriculture University Yeast Isolate No. 13
14.	T_{14}	MPKVYI-14	Mahatma Phule Agriculture University Yeast Isolate No. 14
15.	T_{15}	PTI-1	Pune *Trichoderma* Isolate
16.	T_{16}	SATI-1	Sangli *Trichoderma* Isolate
17.	T_{17}	ANTI-1	Ahmednagar *Trichoderma* Isolate (Shrirampur)
18.	T_{18}	ANTI-2	Ahmednagar *Trichoderma* Isolate (Kopergaon)
19.	T_{19}	ANTI-3	Ahmednagar *Trichoderma* Isolate (Rahata)
20.	T_{20}	SATTI-1	Satara *Trichoderma* Isolate
21.	T_{21}	SOLTI-1	Solapur *Trichoderma* Isolate
22.	T_{22}	NTI-1	Nashik *Trichoderma* Isolate
23.	T_{23}	Fungicide	Beleton (1 g/lit.)
24.	T_{24}	Fungicide	Systhane (0.40 g/lit.)
25.	T_{25}	Tap water	
26.	T_{26}	Distilled water	
27.	T_{27}	Control (No spray)	

3.3 Biocontrol mechanism of yeast (Mycoparasitism)

For observing biocontrol mechanism by yeast the fresh conidial suspension of powdery mildew was prepared with 50 ml distilled water in 100 ml conical flask. The yeast suspension of each isolate containing 1 x 10^9 cells/ml was prepared in test tubes with 2 ml distilled water. Then 2 ml powdery mildew conidial suspension containing 1 x 10^8 cells/ml was poured in each test tube of yeast suspension and the tubes were kept at room temperature. After 7 hours the combined suspension of powdery mildew conidia and yeast from each tube taken on clear sterilized cavity slide, then kept cover slip over cavity. The parasitism of powdery mildew conidia with yeast cells was observed under microscope.

3.4 Biocontrol mechanism of *Trichoderma*

Biological control of plant disease is defined as the involvement of the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause. Different biological control agents (BCAs) can be used for the control of diseases. These include bacteria, fungi and actinomycetes. There are four different mechanisms by which BCAs control other microorganisms. The genus *Trichoderma* comprises a large number of species some of which act as biological control agents through one or more mechanisms. *Trichoderma* strains exert control against fungal phytopathogens either indirectly by competing for nutrients and space, modifying the environmental condition, promoting plant growth, plant defensive mechanisms

and antibiosis, or directly by mechanisms such as mycoparasitism. Activation of each mechanism implies the production of specific metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and permeases. Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which subsequently leads to induced systemic resistance. Mycoparasitism, the direct attack of one fungus on another, is a very complex process that involves sequential events, including recognition, attack, subsequent penetration, and killing of the host. The cell wall degrading enzymes (CWDEs) of Trichoderma such as different chitinolytic enzymes, glucanases and proteases are considered important in mycoparasitism. Chitin and -1, 3 glucan are the main structural components of the fungal cell wall and chitinases, and -1, 3 glucanases have been proposed as the key enzymes in the degradation of cell wall during mycoparasitism against phytopathogenic fungi. Genes encoding these enzymes are being used to impart resistance to various fungal plant pathogens. In addition to the direct action of hydrolytic enzymes on the cell wall of an invading pathogen.

3.4.1 Mycoparasitism

The mode of hyphal interaction and parasitism of *Trichoderma* spp. with several soil borne pathogenic fungi has been documented. *Trichoderma* grows tropically toward hyphae of other fungi, coil around them in a lectin mediated reaction,

and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. *Trichoderma* attaches to the host hyphae via coiling, hooks and appressorium like bodies, and penetrate the host cell wall by secreting lytic enzymes. The interaction is specific and not merely a contact response. *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetration. The cellwall degrading enzymes of *Trichoderma* such as *ß*-1, 3-glucanases and different chitinolytic enzymes have been suggested as the key enzymes in mycoparasitism. Endochitinase (42-kDa), chitobiosidase (40-kDa) and N-acetyl-b-D-glucosaminidase (73-kDa) from *T. atroviride* strain P1 and *T. virens* strain 41 were reported to have a substantial inhibitory effect on the germination of spores and hyphal elongation of several fungal pathogens, viz., *Botrytis cinerea, Fusarium* spp., *Alternaria* spp., *Ustilago avenae*, *Uncinula necator* and virtually on all fungi containing chitin in their cell-wall. Several enzymes have been purified and characterised, and their ability to inhibit the spore germination and hyphael elongation of pathogenic fungi has been shown *in vitro*. Scanning electron microscopy and fluorescence microscopy showed that *T. harzianum* and *T. hamatum* were mycoparasites of both *Sclerotium rolfsii* and *Rhizoctonia solani*. The antagonist attached to the pathogen and secreted glucanase and chitinase enzymes that act through the cell wall.

4. EXPERIMENTAL RESULTS

Grape (*Vitis vinifera* L.) is an important tropical and sub-tropical fruit crop of India. Substantial increase in area under grape and intensive cultivation of grape in last few years resulted in considerable increase in incidence of diseases. Now a days downy mildew and powdery mildew disease caused by Plasmopara viticola and *Uncinula necator* respectively. being an air borne disease is causing very heavy losses in grape production. The fungus developed resistance to different commonly sprayed fungicides and antibiotics and also the problem of pesticide residues associated with the grape berries. Therefore, an attempt was made to find out the ecofriendly solution to mitigate the disease with the help of naturally occurring biological agents like yeast and *Trichoderma.* The results obtained during the course of study on different aspects are given here under.

In the entire sampling period a total 18 fungal species were observed. Among this 9 isolates were identified as saprophytic, 7 as parasitic in nature and three were unidentified sterile mycelia forms. The most dominant fungal species were *Aspergillus niger* (23.28%), *A. flavus* (22.80%) *and Penicillium sp* (25.00%). The most prevalent species were *Alternaria alternata* (21.90%), *Curvularia lunata (*16.96%), *Trichoderma sp* (12.62%), *A. fumigatus* (06.12%) *and Fusarium sp* (04.83%) *which were observed in rainy season.* The less frequent species were *Colletotrichum sp* (03.10%), *Geotrichum sp* (02.87%), *Cladosporium cladosporoids* (02.63%), *Pestalotiopsis sp* (02.60%) and *Epicoccum sp* (1.10%) and *Streptomyces sp* (0.95%).

Tabale 1 Isolated phylloplane microflora

Sr No	Phylloplane microflora	Rainy season	Post monsoon	Summer season
1.	*Trichoderma spp*	+++	++	+
2.	*Alternaria alternata*	++	+	-
3.	*Aspergillus niger*	+++	++	-
4.	*Aspergillus flavipes*	++	-	--
5.	*A.terreus*	-	-	--
6.	*Penicillium spp*	++	+	--
7.	*yeast*	+++	++	++
8.	*Curvularia lunata*	+	++	-
9.	*Azospirillum spp*	-	+	--

+++ (Prominent), ++ (Moderate), + (Less), - (No growth).

4.1 Collection of leaf sample

Leaf samples were collected from healthy vineyards of MPKV., Rahuri as well as from farmers field from Pune, Nashik, Solapur, Ahmednagar, Satara and Sangli districts.

Eleven leaf samples were collected from the vineyards of MPKV., Rahuri, three samples from Ahmednagar district and one each sample were collected from Pune, Satara, Solapur, Nashik and Sangli districts.

Yeast and *Trichoderma* isolated from the leaf samples collected from vineyards of MPKV., Rahuri. the *Trichoderma* isolated from the leaf samples collected from Pune district was designated as PTI 1. Similarly, *Trichoderma* isolated from the leaf sample collected from Sangli district was designated as SATI-1, *Trichoderma* isolated from the samples collected from Ahmednagar district were designated as ANTI-1 to 3, *Trichoderma* isolated from the samples collected from Satara district was designated as SATTI-1, *Trichoderma* isolated from Solapur district designated as SOLTI-1 and *Trichoderma* isolate of Nashik district was designated as NTI-1 (Table 2).

Table 2. Details of leaf samples collected from healthy as well as powdery mildew and downy mildew disease infected vineyards

Sr. No.	Sample Designation	Variety	Location
1	MPKVYI-1	Thomson Seedless	MPKV., Rahuri
2	MPKVYI-2	Flame Seedless	MPKV., Rahuri
3	MPKVYI-3	Pusa Navrang	MPKV., Rahuri
4	MPKVYI-4	Cabbernet Sauvignon	MPKV., Rahuri
5	MPKVYI-5	Souvignon Blanc	MPKV., Rahuri
6	MPKVYI-6	Red Globe	MPKV., Rahuri
7	MPKVYI-7	Tas-A-Ganesh	MPKV., Rahuri
8	MPKVYI-8	Sharad Seedless	MPKV., Rahuri
9	MPKVYI-9	Fantacy Seedless	MPKV., Rahuri
10	MPKVYI-10	Dogridge	MPKV., Rahuri
11	MPKVYI-11	Teleki-5A	MPKV., Rahuri
12	PTI-1	Tas-A-Ganesh	Tal-Baramati, Dist-Pune
13	SATI-1	Sharad Seedless	Tal-Tasgaon, Dist-Sangali
14	ANTI-1	Tas-A- Ganesh	Tal-Shrirampur, Dist-Ahmednagar
15	ANTI-2	Thomson Seedless	Tal-Kopergaon, Dist-Ahmednagar
16	ANYI-3	Thomson Seedless	Tal-Rahata, Dist-Ahmednagar
17	SATTI-1	Thomson Seedless	Tal-Karad, Dist-Satara
18	SOLTI-1	Sharad Seedless	Tal-Sangola, ist-Solapur
19	NTI-1	Tas-A- Ganesh	Pimpalgaon Baswant, Dist- Nashik

4.2 Isolation of yeast and *Trichoderma*

Data presented in Table 2 revealed that yeasts and *Trichoderma* were isolated from all 19 samples collected from different vineyards. The population of yeast and *Trichoderma* were varied in leaf from sample to sample. It indicated that the

yeasts and *Trichoderma* were present in all leaf samples collected from six different districts.

4.3 Seasonal variation in microbial population

In the present study, it was observed that all the phylloplane samples collected from the six district harboured a variety of microorganisms. Yeast and fungi were abundant in all the samples where as the number of actinomycetes and bacteria were very low or absent in many cases. Bacterial population was very low in the phylloplane of both mature and immature grapes plants during the summer season and higher during the rainy season. It was most abundant during the post monsoon season. The phylloplane fungal population was observed to be higher during the post-monsoon season. Although there is a general trend towards increase of fungal population from summer to rainy and finally post-monsoon.

4.4 Effects of *Trichoderma harzianum* on *Plasmopara viticola* sporangia

Observations of sporangia following treatments with *T. harzianum* in water suspension demonstrated that none of these treatments had any effects on sporangia morphology or size until the release of the zoospores. The sporangia germination percentage following treatments with *T. harzianum* (86.7%), or water (89.3%) were not significantly different from one another (Kruskal–Wallis test; $P = 0.29$). Sporangia that had been treated with copper solution were normal in appearance, but did not germinate. The sporangia germination after treatments with *T. harzianum* or water was similar on grapevine leaves (data not

shown), confirming that both resistance inducers did not have significant direct toxic effect against *P. viticola*.

4.5 Effects of the number and timing of *Trichoderma harzianum* treatments.

Downy mildew severity was significantly reduced on *T. harzianum* treated grapevine leaves. In particular, repeated foliar applications of *T. harzianum* strongly reduced downy mildew symptoms, leading to a disease reduction of 48% and 63% with two and three treatments, respectively. The individual applications of the three treatments at one-day interval before inoculation resulted in disease control statistically comparable to that provided by copper hydroxide. However, the lowest level of downy mildew severity was observed following a single copper treatment (80% disease reduction). Observations with the light microscope did not reveal any penetration of *T. harzianum* into the leaf tissues or direct parasitism of *P. viticola*.

Repeated applications of *T. harzianum* did not increase disease control , which reached its maximum level with applications at 1 day before inoculation (83% disease reduction). The treatments at 1 day before inoculation were associated with levels of plant protection comparable with that of the copper treatment (84% disease reduction). However, *T. harzianum* applied 6 h before inoculation (63% disease reduction) was not as effective as the copper hydroxide treatment, suggesting that a minimal time interval sufficient for the activation of the plant systemic resistance system is required. When single treatments of *T. harzianum* was applied at different times, 14, 7 and 1 days

before inoculation, time-dependent grapevine resistance to downy mildew was observed . *T. harzianum* partially reduced the disease only when applied 1 day before inoculation (38% disease reduction), although not significantly when all experiment treatments are considered, and to a lesser extent than the copper. *T. harzianum* was ineffective when applied 7 and 14 days before inoculation (12% and 4% disease reduction, respectively), suggesting that the induced plant resistance was progressively reduced after elicitation and completely switched off within 7 days. In addition, in previous preliminary experiments we demonstrated that a single application 2 or 3 days before inoculation did not increase the level of resistance induction. Therefore the time the biocontrol agent needs to be on a leaf to express the elicitor seems to be less than 1 day, and the repeated applications of *T. harzianum* was important to significantly induce plant resistance.

Effect of single preventive *T. harzianum* treatments on downy mildew severity on leaves of grapevines grown under controlled greenhouse conditions. Treatments were applied to plants at 14, 7 and 1 days before inoculation with *P. viticola* sporangia. Copper hydroxide and water (Untreated) controls were applied 6 hr before inoculation (0). The mean severity and relative standard error of ten replicates (two experiments with experimental factor not significant, $P = 0.67$) for each treatment are presented.

4.6 Systemic effects of *Trichoderma harzianum* treatments

For the analysis of systemic resistance induction, grapevine roots or marked leaves were treated with *T. harzianum* and then inoculated with the pathogen. Downy mildew severity was then measured on untreated (systemic effect) and treated (local effect) leaves. These results demonstrated that root treatments with *T. harzianum* was ineffective in controlling downy mildew on leaves, but leaf treatments were able to induce local and systemic resistance both laterally and acropetally grapevine has alternated phyllotaxis, and if leaves on one side of a shoot were treated with *T. harzianum* disease severity on the untreated, opposite leaves of the same shoot was significantly lower than on untreated plants (60% and 56% systemic disease reduction, respectively). The activation of acropetal systemic resistance was also demonstrated by the reduced severity of symptoms on untreated leaves of plants whose basal leaves were sprayed with *T. harzianum* (49% systemic disease reduction)

Table- 3 Seasonal Variation of phylloplane microflora on grape leaf surface:

Microbial isolates	**Jan-Feb**	**Mar-Apr**	**May-Jun**	**Jul-Aug**	**Sep-Oct**	**Nov-Dec**
Aspergillus niger	++	++	+	+++	+++	++
A. flavus	+	++	+	++	+++	++
A. fumigatus	+	-	+	-	-	+
Penicillium spp	+	+	+	++	+++	++
Alternaria alternata	-	+	+	++	+	+
Curvularia lunata	-	-	-	++	++	+
Trichoderma spp	-	-	+	+++	++	+
Fusarium sp	-	-	-	+	+	+
Colletotrichum spp	-	-	+	+	+	-
Geotrichum spp	-	-	-	-	+	+
Cladosporium spp	-	-	+	+	-	-
Pestalotiopsis spp	-	-	+	+	+	-
Epicoccum spp	-	-	+	-	-	-
Streptomyces spp	-	-	-	+	-	-
Botryodiplodia theobromae	-	**+**	**-**	**++**	**++**	**+**
Alternaria spp	**-**	**-**	**-**	**++**	**+++**	**+**
Curvularia affinis	**-**	**+**	**-**	**+**	**++**	**+**
Aspergillus flavipes	**-**	**+**	**-**	**+**	**++**	**+**

+++ (Prominent), ++ (Moderate), + (Less), - (No growth).

Table 4. Comparative per cent change in powdery mildew disease intensity due to spray of yeast isolates

Sr. No.	Treatment No.	Before spray	After I spray	After II spray	After III spray
1.	T_1	51.50	52.50	44.50	58.00
2.	T_2	49.50	39.50	24.50	64.00
3.	T_3	75.50	50.50	34.50	55.00
4.	T_4	62.50	53.00	36.50	76.00
5.	T_5	36.00	44.00	35.50	67.00
6.	T_6	39.50	75.50	73.50	79.00
7.	T_7	38.50	63.50	41.00	38.00
8.	T_8	46.50	34.50	21.00	41.00
9.	T_9	58.50	64.00	52.50	77.50
10.	T_{10}	34.00	39.50	24.50	64.00
11.	T_{11}	50.00	40.00	37.00	55.50
12.	T_{12}	66.00	40.50	25.50	30.00
13.	T_{13}	59.50	40.00	33.50	45.50
14.	T_{14}	60.50	28.50	34.00	32.50
15.	T_{15}	98.50	63.00	42.50	51.50
16.	T_{16}	95.00	43.00	29.00	47.00
17.	T_{17}	54.50	21.00	15.00	27.00

Table 4 contd....

Sr. No.	Treatment No.	Before spray	After I spray	After II spray	After III spray
18.	T_{18}	44.50	31.50	36.50	38.50
19.	T_{19}	90.00	34.00	17.00	34.00
20.	T_{20}	97.00	55.50	30.50	21.50
21.	T_{21}	97.50	59.00	31.00	19.00
22.	T_{22}	99.00	46.00	21.50	11.50
23.	T_{23}	53.50	19.50	11.00	10.50
24.	T_{24}	56.00	28.50	20.50	10.50
25.	T_{25}	23.50	23.00	19.00	33.50
26.	T_{26}	23.50	27.00	20.50	68.00
27.	T_{27}	66.50	52.00	65.50	65.00
	S.E ±	1.720	3.205	3.349	2.759
	C.D. at 5%	4.80	9.094	9.505	8.647
	C.V (%)	4.95	12.83	17.87	13.94

Table 5. Per cent powdery mildew disease control after every spray of yeast isolates

Sr. No.	Treatment no.	Spray- I	Spray - II	Spray- III
1.	T_1	1.00	34.55	50.43
2.	T_2	22.50	61.56	0.56
3.	T_3	4.94	50.06	14.50
4.	T_4	0.96	42.96	15.18
5.	T_5	19.64	49.51	5.12
6.	T_6	44.07	10.40	19.87
7.	T_7	24.54	39.98	44.18
8.	T_8	32.31	66.99	35.50
9.	T_9	25.52	22.15	21.53
10.	T_{10}	23.49	62.56	0.56
11.	T_{11}	25.52	46.18	16.84
12.	T_{12}	20.54	60.01	52.68
13.	T_{13}	26.52	51.61	33.46
14.	T_{14}	47.07	49.83	51.78
15.	T_{15}	23.56	36.65	23.09
16.	T_{16}	15.64	54.58	26.12
17.	T_{17}	62.78	79.29	60.37
18.	T_{18}	41.19	44.96	42.4
19.	T_{19}	34.29	74.19	47.43
20.	T_{20}	5.86	53.26	66.96
21.	T_{21}	15.72	56.48	74.87
22.	T_{22}	10.76	67.21	82.59
23.	T_{23}	65.72	86.49	87.10
24.	T_{24}	44.07	67.76	83.15
25.	T_{25}	57.86	74.09	51.21
26.	T_{26}	48.01	68.76	5.12
27.	T_{27}	49.00	62.50	62.00

Table 6. Comparative per cent change in Downy mildew disease intensity due to spray of *Trichoderma* isolates

Sr. No.	Treatment No.	Before spray	After I spray	After II spray	After III spray
1.	T_1	57.50	50.50	45.50	57.00
2.	T_2	50.50	40.50	24.50	62.00
3.	T_3	70.50	45.50	30.50	50.00
4.	T_4	60.50	53.00	40.50	78.00
5.	T_5	37.00	48.00	39.50	69.00
6.	T_6	40.50	77.50	76.50	79.00
7.	T_7	44.50	66.50	42.00	39.00
8.	T_8	44.50	36.50	23.00	43.00
9.	T_9	60.50	63.00	55.50	68.50
10.	T_{10}	36.00	40.50	26.50	67.00
11.	T_{11}	52.00	42.00	39.00	57.50
12.	T_{12}	65.00	41.50	24.50	29.00
13.	T_{13}	62.50	42.00	35.50	47.50
14.	T_{14}	58.50	26.50	33.00	34.50
15.	T_{15}	74.50	64.00	43.50	52.50
16.	T_{16}	67.00	41.00	27.00	45.00
17.	T_{17}	56.50	22.00	16.00	28.00

Table 6 contd....

Sr. No.	Treatment No.	Before spray	After I spray	After II spray	After III spray
18.	T_{18}	46.50	33.50	38.50	40.50
19.	T_{19}	89.00	32.00	15.00	32.00
20.	T_{20}	95.00	52.50	20.50	18.50
21.	T_{21}	99.50	60.00	33.00	21.00
22.	T_{22}	98.00	47.00	20.50	10.50
23.	T_{23}	55.50	21.50	15.00	12.50
24.	T_{24}	55.00	29.50	21.50	12.50
25.	T_{25}	24.50	25.00	20.00	35.50
26.	T_{26}	22.50	23.00	25.50	70.00
27.	T_{27}	65.50	50.00	62.50	62.00
	S.E ±	2.100	2.890	1.901	1.626
	C.D. at 5%	5.959	8.202	5.395	4.615
	C.V (%)	6.00	11.52	9.96	5.97

Fig2: Seasonal variation in microbial population phylloplane of grapevine

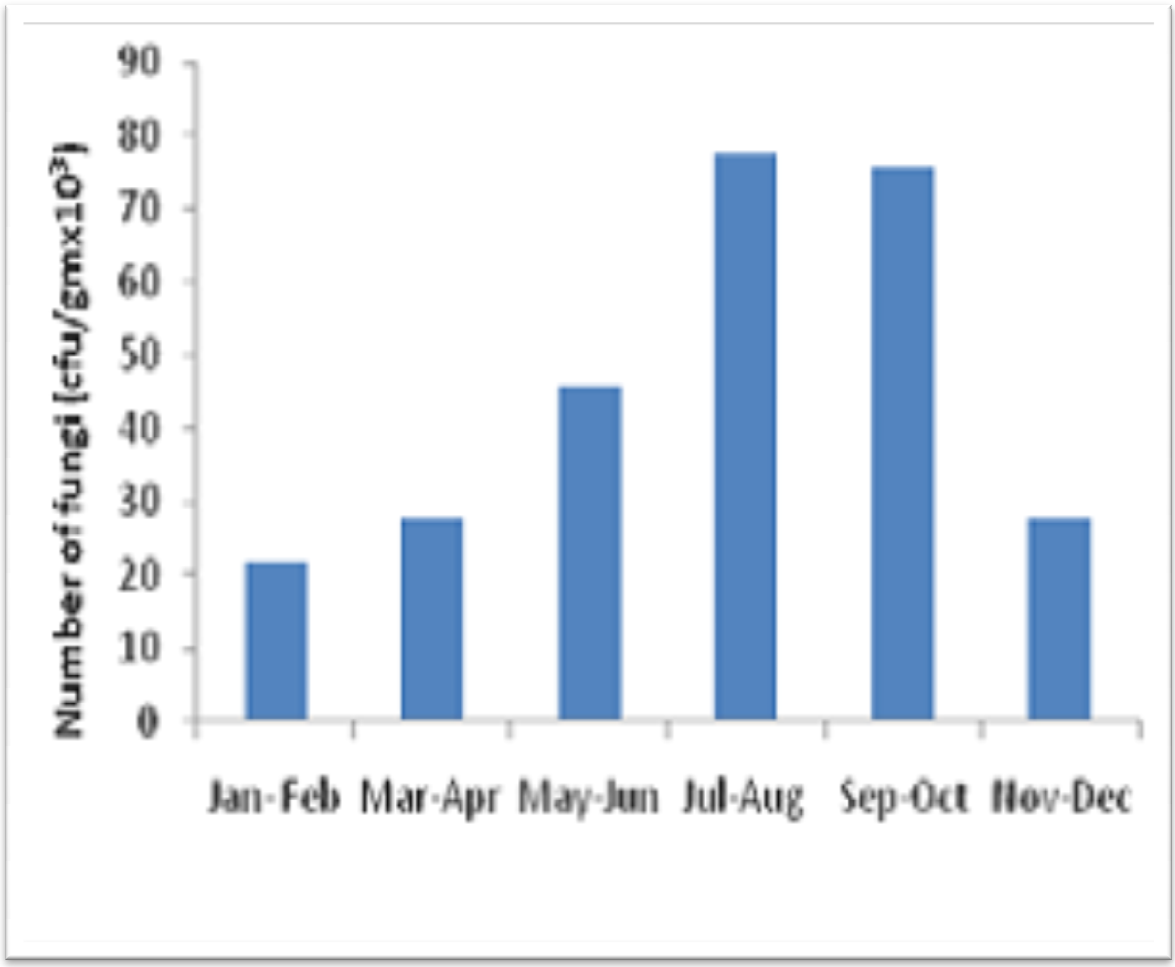
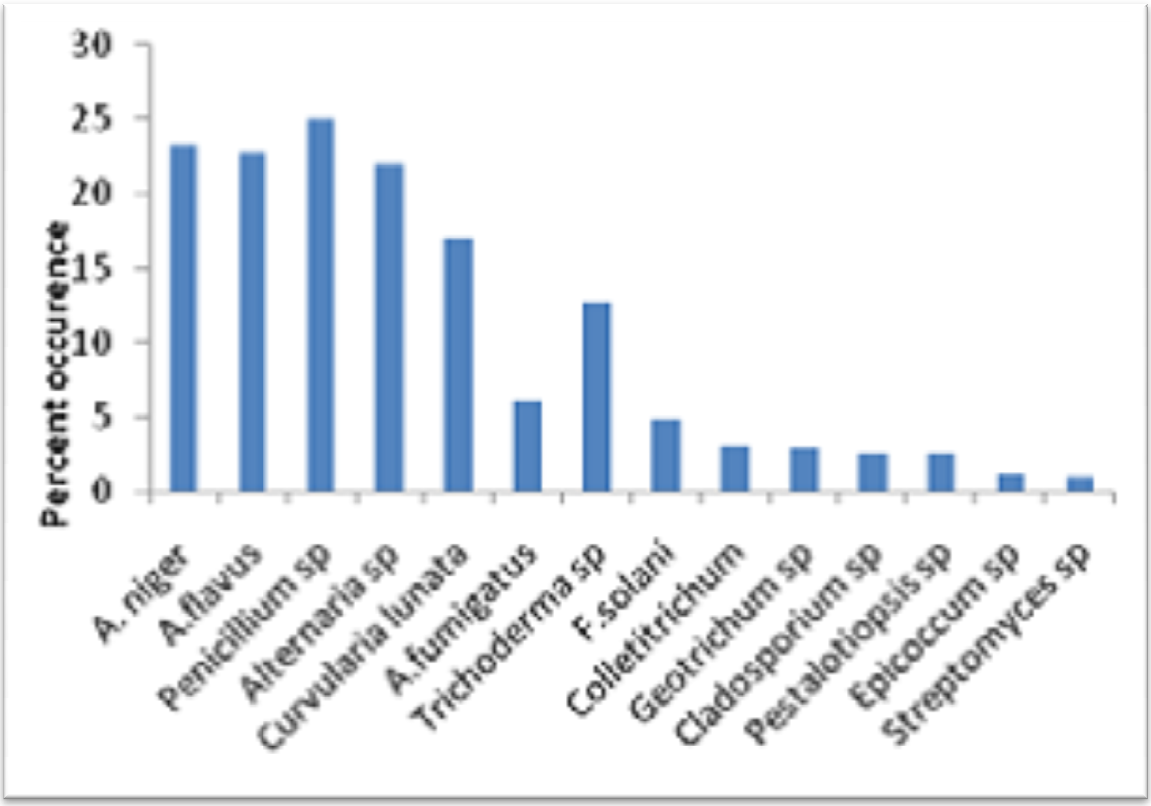


Fig 3: Fungal population in phylloplane of grapes



5. DISCUSSION

Major diseases of grapevine are anthracnose caused by the fungus *Elsinoe ampelina,* black rot is caused by *Guignardia bidwellii*, bacterial disease crown gall is caused by *Agrobacterium tumefaciens*, dead arm caused by the fungus *Phomopsis viticola*, downy mildew by fungus *Plasmopora viticola,* powdery mildewby *Uncinula necator*, while gray mold or bunch rot caused by the fungus *Botrytis cinerea*.

5.1 Collection of leaf sample

Leaf samples were collected from healthy as well as powdery mildew disease infected vineyards of MPKV., Rahuri as well as from farmers field from Pune, Nashik, Solapur, Ahmednagar, Satara and Sangli districts.

Eleven leaf samples were collected from the vineyards of MPKV., Rahuri, three samples from Ahmednagar district and one each sample were collected from Pune, Satara, Solapur, Nashik and Sangli districts.

Yeast and *Trichoderma* isolated from the leaf samples collected from vineyards of MPKV., Rahuri. The *Trichoderma* isolated from the leaf samples collected from Pune district was designated as PTI 1. Similarly, *Trichoderma* isolated from the leaf sample collected from Sangli district was designated as SATI-1, *Trichoderma* isolated from the samples collected from Ahmednagar district were designated as ANTI-1 to 3, *Trichoderma* isolated from the samples collected from Satara district was designated as SATTI-1,*Trichoderma* isolated from Solapur district designated as SOLTI-1 and *Trichoderma* isolate of Nashik district was designated as NTI-1.

5.2 Seasonal variation in microbial population

In the present study, it was observed that all the phylloplane samples collected from the six district harboured a variety of microorganisms. Yeast and fungi were abundant in all the samples whereas the number of actinomycetes and bacteria were very low or absent in many cases. Bacterial population was very low in the phylloplane of both mature and immature grapes plants during the summer season and higher during the rainy season. It was most abundant during the post monsoon season. The phylloplane fungal population was observed to be higher during the post-monsoon season. Although there is a general trend towards increase off ungal population from summer to rainy and finally post-monsoon.

5.3 Field testing of nineteen yeast and *Trichoderma* isolates against powdery mildew and downy mildew of grapevine

Nineteen yeast and *Trichoderma* isolates were tested against the powdery mildew and downy mildew disease of grape under field conditions. Among nineteen isolates of yeast eleven isolate were found effective against *Uncinula necator* under field condition.Out of eleven eight isolates were found effective against *Uncinula necator.* All eight isolates *viz.*, MPKVYI-9, MPKVYI-10, ANYI-1,ANYI-2, ANYI-3, SATYI-1, SOLYI-1 and NYI-1 were found to control the *U. necator* fungus upto 50 per cent. Out of these eight isolates, three isolates (ANYI-2, SATYI-1 and NYI-1) were found more effective and able to control powdery mildew fungi upto 75 per cent and out of these three isolates one (NYI-1) was found

most effective and minimize the incidence of *Uncinula* upto 80 per cent under field condition. and reaming eight isolates of *Trichoderma* were able to control the *Plasmopora viticola* upto 55 per cent after three sprays at ten days interval.

Further in continuation these eight effective *Trichoderma* isolates were tested against the downy mildew pathogen in field. Out of eight, three *Trichoderma* isolates significantly suppressed the growth of downy mildew causing pathogen up to 80 per cent. Mycelial growth of the downy mildew causing fungus was tremendously reduced due to antagonistic activity of effective *Trichoderma*. These three most effective *Trichoderma* isolates were again further tested in the same condition and even in same vineyard against the downy mildew pathogen.

Out of three *Trichoderma* isolates, isolate no. T_{19} (NTI-1) was observed as the most effective antagonist against pathogen. This isolate was obtained from the Tas-A-Ganesh variety of grape of Pimpalgaon Baswant of Nashik district. It indicated that this isolate carrying potential as a biocontrol agent of *Plasmopora viticola* which can be further evaluated in near future.

These results are in conformity with the report of Jarvis *et al.* (1989) who reported efficacy of *Pseudozyma flocculossa* and *Pseudozyma rugulosa* against cucumber, rose and wheat powdery mildew. Hijwegen (1992) reported the efficacy of *Tilletiopsis minor* in controlling cucumber powdery mildew. Ken K. Ng *et al.* (1997) reported *Tilletiopsis pallescens,* a

naturally occurring ballistospores forming yeast isolated from mildew infected leaves found as a biological agent of rose powdery mildew (*Spaerotheca pannosa*). Witting *et al.* (1997) examined the antagonistic effect of yeast *Aureobasidium pullulans* on brown rot blossom blight in green cherries under field condition. Paulitz and Belanger (2001) and Avis and Belanger (2002) reported the yeast *Pseudozyma flocculosa* reduce the powdery mildew diseases especially of greenhouse crops like rose, cucumber and tomato. Kiss *et al.* (2004) reported the effect of *Ampelomyces* in control of powdery mildew infection in various crops. El-Mehalawy *et al.* (2006) reported that two species of rhizosphere yeast fungi; *Saccharomyces unispora* and *Candida steatolytica* have antagonistic effects against fungal pathogen *Fusarium oxysporum*. Shalaby and El-Nady (2008) reported *Saccharomyces cerevisiae* as a biocontrol agent of *Fusarium oxysporum* and as plant growth promoter.

5.4 Biocontrol mechanism of yeast against *Uncinula necator*

The present study was undertaken to know the biocontrol mechanism of yeast isolates. Thus, the result obtained shows that all eight effective yeast isolates were able to parasitise the conidia of powdery mildew pathogen. Yeast cells were first attached to the conidia of powdery mildew then it ruptures the cell wall of conidia and entered into the conidial cell. Further, it disturbs cell materials of the conidia completely by killing them. The disruption of cellular membrane of powdery mildew conidia may be due to mycoparasitism or enzymatic activities.

These result are in conformity with the report of Wisniewski *et al.* (1988) was reported the ability of *Penicillium expansum* and *Penicillium guilliermondii* to attach fungal hyphae of *B. cinerae*. Further, it cause cell wall degradation at site of attachment by producing higher levels of gluconase which are cell wall degrading enzymes. Jarvis *et al.* (1989) who reported *Pseudozyma flocculossa* and *Pseudozyma rugulosa*produce the unusual fatty acids that insert into powdery mildew cells and cause disorganization of cellular membranes and cell disintegration. Dik *et al.* (1991) demonstrated the yeasts *Sporobolomyces* spp. and *Cryptococcus* spp. on wheat flag leaves reduced the level of carbon source available for nerotrophic pathogens.Inge (1993) reported that *Tilletiopsis albescens*grows well on powdery mildew fungi inoculated on barley or cucumber leaves and causes collapse of the colonies of powdery mildew.Wilson and Wisniewski (1994) revealed that competition for nutrients between yeasts and molds and parasitism are likely to be the main mechanisms of action and many beneficial types of yeast can effectively deplete the sugar occurring in fruit wounds (place of infection) and inhibit germination of mold propagules.

5.5 Biocontrol mechanism of *Trichoderma* against *Plasmopora viticola*

Biological control of plant disease is defined as the involvement of the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause. Different biological control agents (BCAs) can be used for the control of diseases.

These include bacteria, fungi and actinomycetes. There are four different mechanisms by which BCAs control other microorganisms.The genus *Trichoderma* comprises a large number of species some of which act as biological control agents through one or more mechanisms. *Trichoderma* strains exert control against fungal phytopathogens either indirectly by competing for nutrients and space, modifying the environmental condition, promoting plant growth, plant defensive mechanisms and antibiosis, or directly by mechanisms such asmycoparasitism. Activation of each mechanism implies the production of specific metabolites,such as plant growth factors, hydrolyticenzymes, siderophores, antibiotics, andpermeases. Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which subsequently leads to induced systemic resistance. Antibiosis occurs during interactions with other microorganisms involving low molecular weight diffusible volatile and nonvolatile toxic metabolite compounds or antibiotics like harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others. Mycoparasitism, the direct attack of one fungus on another, is a very complex process that involves sequential events, including recognition, attack, subsequent penetration, and killing of the host. The cell wall degrading enzymes (CWDEs) of *Trichoderma* such as different chitinolytic enzymes,glucanases and proteases are considered

important in mycoparasitism. Chitin and -1, 3glucan are the main structural components of thefungal cell wall and chitinases, and 1- 3glucanases have been proposed as the keyenzymes in the degradation of cell wall during mycoparasitism against phytopathogenic fungi. Genes encoding these enzymes are being used to impart resistance to various fungal plant pathogens In addition to the direct action of hydrolytic enzymes on the cell wall of an invading pathogen.

6. SUMMARY AND CONCLUSION

Phylloplane microflora at collected samples had highly significant seasonal variation in mature grapes trees while no significance was observed in immature trees. Highly significant variation was present in phylloplane microflora at sangli and satara district in the case mature grapes trees while it was lower in immature trees. Seasonal variation in phylloplane microflora on mature trees showed only low significance while in immature trees more significant variation was present. In general, the phylloplane microbial population was significantly high during post monsoon season and low during the summer season. The phylloplane fungi at collected samples showed significant seasonal variation in Tas-A-Ganesh mature trees at Satara there was significant seasonal variation in Thomson Seedless mature and immature leaves. Seasonal variation at Sangli was significant in Sharad Seedless mature and immature and Tas-A-Ganesh immature leaves while no significance was observed in mature Tas-A-Ganesh leaves. Significant seasonal variation was observed in samples from Pimpalgaon Baswant, Dist. Nashik. Slightly significant seasonal variation was observed in mature and immature Tas-A-Ganesh leaves at Pune while no significant variation was observed in mature Sharad Seedless leaves. All the phylloplane fungal samples at MPKV., Rahuri namely Tas-A-Ganesh mature and immature as well as Thomson Seedless mature and immature had significant seasonal variation.

In general, the phylloplane microbial population was high during the post monsoon season and less during summer season. The mature leaves showed higher population than immature leaves.

Powdery mildew is a major constraint in grapevine cultivation in Maharashtra, India and in world. The disease is caused by *Uncinula necator*. Locally it is also known as *Pandari bhoori*. The fungus attack all above ground parts including leaves, twigs, stems and berries. It was more destructive when berries are attacked. All commercially grown cultivars are susceptible to the disease. Also there is problem of pesticide residues in grape berries and due to this the Indian grape stock was rejected many times in foreign market. Therefore, to minimize the MRL level of pesticide in grape berries and environmental as well as soil pollution besides cost of cultivation, it was thought to undertake the study of yeast as biological agent against *Uncinula necator*. Powdery mildew disease of grape caused by *Uncinula necator* is one of the main hurdle in grape cultivation in Maharashtra and also in India. The fungus of powdery mildew disease is found to attack all above ground parts in general and leaves and berries in particular. Therefore, it causes tremendous losses in grape production and reduces the productivity of vine and increase the cost of production. On the other side there is main problem of pesticide residue in grape berries due to tremendous use of fungicides and pesticides against the various diseases therefore, it was thought to undertake study of yeast as biocontrol agent against *Uncinula*

necator. The results thus obtained from the studies are summarised and concluded here under.

Yeast and *Trichoderma* were isolated from the leaf samples collected from vineyards of MPKV., Rahuri as well as from farmers grape vine field from Pune, Nashik, Solapur, Ahmednagar, Satara and Sangli districts. Total ninteen isolates were obtained from different leaf samples. Out of eleven, yeast isolates were obtained from the vineyards of MPKV., Rahuri, three isolates from Ahmednagar district and one each isolate of yeast was obtained from the leaf samples of Pune, Satara, Solapur, Nashik and Sangli districts. It indicated that yeast and *Trichoderma* were present on leaves of all varieties collected from six districts.

In the study of field evaluation of yeast isolates against *Uncinula necator,* out of 11 isolates, 8 isolates *viz.,* MPKVYI-10, MPKVYI-9, ANYI-1, ANYI-2, ANYI-3, SATYI-1, SOLYI-1 and NYI-1 were found effective against the pathogen. All of eight yeast isolates inhibited the growth of powdery mildew pathogen upto 50 per cent under field condition. This indicated the preferred parasitism on *Uncinula necator.* Out of these eight *Trichoderma* isolates, three *Trichoderma* isolates were found more effective (ANTI-2, SATTI-1 and NTI-1) and from these three, one *Trichoderma* isolate i.e. NTI-1 found most effective against *Plasmopara viticola* under field condition.

In biological control mechanism study, the yeast cells were attached to the conidia from outside, then ruptured the conidial cell wall and entered into the conidia. After entering into

conidia, yeast disorganized the cellular material of conidia by parasitism.

In Phyllosphere study, it revealed that the colonies of fungi and bacteria were decreased while the colonies of yeast and *Trichoderma* were increased due to spraying of yeast and *Trichoderma* isolates.

Conclusion –

1. Studies on seasonal variation in phylloplane microorganisms of grapes trees showed that the microbial number in summer was much less than that in the other seasons.
2. The highest number was recorded during the post monsoon season. due to high humidity and atmospheric condition.
3. Actinomycete population was very low or absent in phylloplane samples across all the six locations.
4. Several microorganisms which were isolated from the phylloplane proved as antagonistic to the two known major pathogens of grapes trees. i.e *Uncinula necator, Plasmopara viticola.*
5. Yeast was isolated from all eleven leaf samples collected from different grape growing districts of Maharashtra indicated that the yeast is a commonly occurring fungi on phylloplane of grapevine.
6. Under field evaluation eight out of eleven isolates were effective *viz.*, MPKVYI-9, MPKVYI-10, ANYI-1, ANYI-2, ANYI-3, SATYI-1, SOLYI-1 and NYI-1 against the

Uncinula necator. Out of these eight isolates, NYI-1 was the most effective yeast isolate.

7. Out of nine, three *Trichoderma* isolates were effective (ANTI-2, SATTI-1 and NTI-1) and off these three, one *Trichoderma* isolate i.e. NTI-1 was most effective against *Plasmopara viticola* under field condition.
8. Spraying of yeast isolates over the grape vine helped to increase yeast population on leaf surface of powdery mildew disease. which will help to reduce the inoculums on leaves surface of grapevine.

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