Chapter 5

Summary

Grape is believed to be originated in Armenia near the Black and Caspian seas in Russia. An independent and recent origin of grapes is also traced to North America. Grape was introduced in India in 1300 AD by the Moghul invaders. Grape is a non-climacteric fruit that grows on the perennial, generally occurring in clusters and deciduous woody vines, of the genus *Vitis*.

Grapevines can be vegetatively propagated by cuttings. While almost all table grape cultivars belonging to the same species, *Vitis vinifera* but wine grapes have significant differences, through selective breeding.

In India the grape is developed under a different climatic conditions and soil types in three discrete regions, such as sub-tropical, mild tropical and hot tropical climatic areas in India. In India grapevines are developed on their own roots. There are two types of grape of grape cultivation Own Roots and Rootstocks.

The different types of varieties are grown namely Thompson Seedless and its clones (Tas-A-Ganesh, Sonaka, 2A clone), Anab-e-Shahi, Sharad Seedless and Flame Seedless, Manik Chaman these are the table grape varieties. The wine varieties are Chenin blanc, Chardonnay, Muscat, Sauvignon blanc, Cabernet sauvignon, Shiraz, Viognier, Merlot, and Zinfandel.

Grapes can work as a body coolants and sweet in taste. Also the grape is soft, not easy to digest and increase the humidity of body tissues. The grapes are rich in polyphenolic phytochemical compound like resveratrol, catechin and anthocyanin they play an important role in human health, these compounds have anti-oxidant activity which possess health-protective functions.

The grapes are also the rich source of micronutrient minerals like copper, potassium, calcium, iron, selenium and manganese and also of vitamins like vitamin C, vitamin A, vitamin K, carotenes, folate and B-complex vitamins. The grapes are having the capability to treat constipation, kidney disorders, fatigue, indigestion and prevention of cataracts and the macular degeneration on human health.

The grapes made up of antioxidant flavonoids, which can slow down aging and reduce the damage caused by free radicals. The grapevines sap ointment use to recover the skin and eye diseases was made by European folk healers. The grape leaves were used to stop inflammation, pain and bleeding. The unripe grapes were
used to recover the sore throats and dried grapes (raisins) can be used to lower the constipation and thirst.

The round, ripe, sweet grapes was used to recover a many health problems like cancer, cholera, smallpox, nausea, eye infections, skin, kidney, and liver diseases. Extracts of grape seed used to treat free radical damage, including heart disease, diabetes, and cancer. Grape seed extract has been effective against the bacterial infections caused by bacteria such as *Staphylococcus aureus*.

Wine is a fermented drink manufactured by the incomplete or complete grapes fermentation. The wine is making from the different types of the fruits like ripe berries of grapes, Cherrie, appeal etc. using fermentation technology these fruits are fermented. The mostly the wine is making from the grape which is belonging to the family vitaceae. The different species of grape was used for producing the wine.

For making a wine the mostly used *vitis* spices are *Vitis vinifera* and *Vitis labrusca*. The wine making is the natural process the grape juice is fermented by the yeast and bacteria which is present in the air and on the grape skin. The grape juice is mainly contains the sugars, acids, vitamins and different types of the minerals etc.

The wine grape varieties are mostly coloured but some wine varieties are also colourless. The wine production is very easy method because the wine is naturally producing from the grape juice this method is also carried out inside the house also. The wine is categorized through colorant like inflamed wine bleached wine and rosy wine with alcohol percentage ten to fourteen.

There are four main types of wines like bench, twinkling, heartened and fragrant. The age of the wine is playing a very important role on human body. The one year protection wine is known as the "outmoded ages." The red wine is well-preserved from the phase of more than nine years but white wine preservation time is only one year not more than the one year.

The wine manufacturing procedure is very old may be a very long years ago. The wine manufacturing is not a skill but it’s a discipline. The wine manufacturing it’s a natural procedure but for this technique human involvement is necessary. The wine manufacturer gives the directions to utilizing a different method for making a good quality wine.

The some important steps involved in the manufacturing the wine like harvesting, crushing and pressing, fermentation, clarification, preservation, aging and
bottling and labeling. The all wine manufacturers are strictly following this all steps with some addition and deletion.

The wine is mostly used for drinking purpose in all over the world. The wine drinking is happened on the any occasion or party. The red wine is more beneficial than the other wine because this wine is made up of a one type of the compound is known as the resveratrol which is playing a very beneficial role in human health.

The some researcher work on the benefits of wine on human health, some researchers are find out the improve the life of human, protect the human body from the some type of the cancers diseases, developed the brain and provide the good health to heart.

The grape is developed under a different climatic conditions and soil types in three discrete regions, such as sub -hot, mild hot and hot environmental areas in country. In India grapevines are developed on their own roots. There are two types of methods are used for the grape cultivation, Own Roots and Rootstocks.

The importance of soil is development of the healthy grapevine. The main important compounds for the development of the grapevine are the soil. The good quality soil menace the water holding capacity is good, nutrient rich and good texture etc.

The texture of soil is stony or grimy but not the waterless and soaked type of the soil. The soil is not the main part for the grapevine development but the fertilizers and manures are playing a very significant role in grapevine development. The grapevine growing plot is always want the circulation of air.

Another important part is the water irrigation. The proper irrigation is important for good grapevine development. The age of grapevine is long menace the grape vines are live very long time. The grapevines are having very long roots they are emerged in the soil. The very long roots of grapevines are spread in all over the soil. The soil is made up of the number of the microorganisms and these all microorganisms are having a different role in plant development.

The soil in grape vineyard is normally heavy clays or sandy loams. For improving the soil quality use the organic compost in India. The Weeding is very important practice in the grape vineyard because the weeds are very susceptible to the diseases. The weeds present within the rows remain detached automatically using the tractor haggard apparatuses. The grapes grown in hot areas where the water evaporation is fast there is need to give additional water or irrigation to the grapevine.
The physiological drawbacks are mostly connected with the less humidity and high temperature. These conditions are mostly observed in the hot area, in this climate the grapevine arms are dead and stem cracking is observed. Per shoot one or two bunches are kept, this ratio depends on the thickness of the arm.

In flat roof gable grapevine developing system the single length of shoot is expectant somewhat than the size of total canopy there use for inhibiting the berries from sunburn. For increasing the size of grapes physical resources are used like for grape bunch thinning and grape bunches dipping in gibberellic acid solutions using this practices the size of grapes is increased.

The weather data play a very important role in the important three disease management on grapevine like downy mildew, powdery mildew and anthracnose. The weather data provide major information about the disease incidence. The disease management schedule is depending on the weather forecasting data.

For disease prediction weather data is recorded on Metos automatic weather station was monitored and the first fungicide application was made when the weather was found favorable for initiation of disease. For using the weather data farmers able to plan the disease spray program. Farmers take a preventive spray before disease occurring this is an important benefit of the weather forecasting.

In prediction of high rain means the chance of occurring high disease in such case the farmers take sprays for disease prevention. The weather data also help in minimizing the cost of spray. The forecasting models help in disease management program and minimizing the economical losses of the farmer.

The commercial grape varieties belonging to Vitis vinifera are susceptible to several diseases. Due to the warm and wet climate during the south-west and north-east monsoon periods in the viticultural areas, the evergreen grapevines are attacked by a number of pathogens. The downy mildew, powdery mildew and anthracnose are the three important diseases in grapes. The virous is also the one of the important disease which causes the saviour disease problems in grapevines.

The virus is the obligate parasite it is not culture able microorganism. The virus is able to develop on living wage cells of a host. The virus in the infectious material which is the mainly made up of the genetic material is present in the protein coat. The virus is not very small in size which is not seen by the necked eyes this small molecule is only observed through the microscope.
The grapevines are very susceptible to the different types of the virus disease are occur in the all types of grape varieties. The mostly wine varieties are more susceptible than the table grape varieties. The virus infected grapevines are totally collapse and there is no such prevention methods are developed for controlling the viral diseases. There are different types of the viral diseases are occur on the healthy grapevine. The most important virus disease is Leafroll virus and fanleaf virus.

Downy mildew is one of the important diseases in grapes caused by *Plasmopara viticola* is an obligate parasite. The causative agent of powdery mildew *Erysiphe or Uncinula necator* this fungus is also obligate parasite.

The anthracnose is another most important disease of the commercial grape varieties in almost all the grape growing regions of the country in wet and warm weather. Thus during the monsoon season the young leaves and the growing shoot can be completely burnt out hampering required canopy development, unless protected by regular fungicide applications.

If it rains during fruiting season, bunches can get infected at any stage from pre-flowering to veraison, affecting their development and/or marketability. On stems, the bark may be completely killed and the infection may go down deep till the pith of the shoot.

The grapes harvesting is nothing but the removing of grape bunches from grapevine on the correct ripping stage in known as harvesting. In the time of harvesting more carefulness is very important. The harvesting is done by without damaging the grape. The postharvest pathogens are decaying and infect the grape berries when the grape bunches are not handled properly.

The improper handling of grape bunches resulted in loose berry and pedicel attachment, at this point the post-harvest pathogen grow very well. The commonly observed post-harvest pathogens namely are *Cladosporium, Alternaria, Aspergillus, Penicillium, Rhizopus, Botrodiplodia, Colletotrichum and Botrytis*.

For grape marketing the good quality grape production is the main task and another important thing is the sealing of grapes. For sealing the grapes we are having the very good local and international market because the farmer produces the grape for earning the many. The farmer always make good plan for developing the grape farming it means which variety develop, farmer developed those varieties which are having very demand in national and in international market.
Develop good quality grapes means there size, shape, in case of colour variety to delved the uniform colour and the most important thing is the ratio of sugar and acid. In now a days another big option in front of grape grower is the wine grape development because increase the day by day demand of the wine grapes for making the wine.

The growers also take care of the fungicide and pesticides spraying because the maximum sprays are creating the problems in residue analysis. For international marketing the pesticide residue analysis is the very important. When apply the maximum sprays for controlling the disease the pesticide residues are obtained in the grape fruit and reject the grapes from the international market.

Another option for sealing the grape is the local market. The local market also prefers the good quality including disease free table grapes which is used for the eating purpose. For grape marketing the important steps the grape harvesting carefully harvesting the grape without injury than packing, storage and transport these things are very important for national and international marketing.

The growers take very high dose spray for controlling the disease and these sprays are taken continually so there is big disadvantage in marketing the grapes. The more sprays are given the more residue in the grape so this kind of the grape are reject from the market.

The alternative source for controlling the disease is the biocontrol agents are apply in the farm for controlling the disease which caused by the pathogens or insect and pest. The demand of grape is very high in international market the India is one of the country which developed the good quality of grapes for eating and wine making purpose.

The previous researcher find out and described the anthracnose disease is caused by the pathogen *Elsinoe ampelina* but in some recent studies the worker indicated that the anthracnose disease is caused by the pathogen *Colletotrichum gloeosporioides*

These warming trends during the monsoon season could have had a significant impact on the shift in the pathogen populations on grapevines which occurred in the last few decades.

A shift in the etiology of grape anthracnose in India was noticed at the end of the last century and by 2010 it appears that *Elsinoe ampelina*, the known pathogen,
has gradually been replaced by *Colletotrichum* species mainly those belonging to *Colletotrichum gloeosporioides*.

Increase in the minimum (Tmin) temperatures which would have created conditions more favorable for *Colletotrichum gloeosporioides* than *Elsinoe ampelina* as the former can grow and infect at higher temperatures. Regression analysis of weather and disease data indicated that Tmin was significantly contributing to disease development. The different types of the *Colletotrichum* spices are find out which causing the serious disease in different crops.

The fungus, present deep in the lesions in the shoot doesn’t get killed by the fungicides and remains as the primary source of inoculum, becoming active under warm and humid conditions.

Shoots infected during monsoon in peninsular India, remain as the active source of inoculum in the vineyard during the fruiting period, and even light showers, increase the risk of development of the disease.

Moreover, as grape is a vegetatively propagated crop, the spread of the disease to new areas along with the infected planting material have frequently been observed.

For management of anthracnose disease only three fungicides were recommended form these two fungicides were non-systemic (copper oxychloride, propineb) and only one fungicide systemic in nature (carbendazim). Continuous use of fungicide carbendazim for anthracnose control has resulted in reduced sensitivity in the pathogen population.

To confirm the stability of fungicides are important for better disease control, the biology of fungicide resistance, how it develops, and how it can be succeeded in manage this fundamental. Fungal population consisting of the mycelium (the body of a fungus), sclerotia (large survival structures), spores (small reproductive structures) and the nucleus of single cells those are individually capable of reproduction and spread.

Single or multiple gene mutations are responsible for resistance mannerism. Resistance to site-specific fungicides by single-gene mutations are more likely to develop than the mutations in multiple genes responsible for resistance to multi-site inhibiting fungicides.

Mode of action (include alteration of the target site), reduced fungicide uptake, active export of the fungicide outside fungal cells, and detoxification or
breakdown of the fungicide are the different factors affecting the mechanisms of resistance.

Fungicide resistant management is the important strategy in agriculture. The pathogen developing resistance is greatly decreased by fungicide should not be used as alone but use as mixture or alternate sprays with another fungicide or bio-fungicide.

Disease management strategies only depend on well-timed practices of fungicides applications. These fungicides can prevent infection but they induce fungicide resistant and adverse effects on the environment and food safety, there is biocontrol agents is very important step in research to control fungicide resistance developed in plant pathogens.

In biocontrol agents mainly involved the promising microorganisms and there product it is a main alternative source to fungicide which is used to control disease which is caused by plant pathogen.

One of the best examples of biological control is the strains of the Bacillus genus are ubiquitous, “generally regarded as Safe” (GRAS).

The Bacillus is a vast and diverse genus belonging to the family Bacillaceae, which was initiated by Cohn. Fisher in 1895 was first articulated the family Bacillaceae. The genus Bacillus is able to produce endospores that are highly resistant to the heat and the cells are remaining live in the form of endospore in a starvation period for very long time.

The endospores are mainly round, oval, or cylindrical in shape and the endospores are formed inside the cell. Endospore formation is the very important characteristic of Bacillus.

Some of these Bacillus species showed effective control in field for controlling disease which is caused by pathogens and the bacillus species use as a biocontrol agent. The Bacillus species are mainly Gram-positive, rod-shaped and motile in nature. The some of the Bacillus species are obligate aerobes, facultative anaerobes.

Bacillus species are able to produce different types of metabolites and enzymes. The Bacillus species are also produce extracellular enzyme like chitinases, glucanases, proteases, nuclease, phosphatases, lipase, phospholipase C, thiaminase, and bacteriolytic enzymes.
These enzymes and metabolites play a vital role in plant disease management. This is one of the most powerful characteristics of *Bacillus* species to use as a biocontrol agents or as a bio-fungicide for controlling resistance in plant pathogens.

The different species of *Bacillus* showed good control in plant disease management via different applications like production of antibiotics which induce systemic resistance in plant. The secondary metabolites production is occurred in late logarithmic or early stationary growth stage of *Bacillus* species. The almost 169 secondary metabolites or antibiotics produce by the *Bacillus* species.

The most of the antibiotics or secondary metabolites are the peptide antibiotics. Antimicrobial peptides (AMPs) or non-volatile compounds produce by *Bacillus* species.

The antimicrobial peptides means lipopeptides like bacillomycin, fengycin, iturin, and surfactin it is present on the biosynthetic genes of antimicrobial peptides like bmyB, fenD, ituC, and srfAA these characteristics play key role in the biocontrol property for several plant pathogens management.

The *Bacillus* species also produce biosurfactins is one of the lipopeptide which have ability to induce motility of flagella and also solubilizing the protective lipid layers of cells.

The *Bacillus* species produce volatile metabolites of low-molecular weight lipophilic compounds in the form of complex mixture which is taken from different biosynthetic pathways. The volatile compounds freely cross the membranes and are easily released into the soil and atmosphere in the absence of a dissemination hurdle. The *Bacillus* species produce volatile metabolites which is very effective for controlling plant pathogens which cause disease.

The ‘biofilm’ is produce by *Bacillus* species in biofilm cells stick to each other on a surface of media. The biofilm producing organisms produce self-matrix of extracellular polymeric substance (EPS) in this matrix cells are attached to each other and are normally fixed in to matrix. The extracellular polymeric substance is conglomeration generally composed of extracellular DNA, proteins, and polysaccharides.

*Bacillus* produce biofilm and it colonize the plants, the *Bacillus* create association with plant and it association is found on the leaves, roots and giving that physical barring from plant pathogens. These all good characteristic shows *Bacillus* species is a promising biocontrol agent for management of plant disease.
To get a wide diversity in the pathogen which cause anthracnose for that infected grape samples were collected from 96 vines from 46 research or commercial vineyards in different grape growing regions of India i.e. Pune, Sangli, Solapur, Nasik in Maharashtra; Bijapur in Karnataka; Odiapatty in Tamil Nadu, Ludhiana in Punjab and Lucknow in Uttar Pradesh during July-September i.e. the period corresponding to the south west monsoon, in 2009-13.

The cultivars were Thompson Seedless, Tas-A-Ganesh, Sonaka, Clone 2A, Anab-e-Shahi, Manjri Naveen, Manik Chaman, Sharad Seedless, Sharad Jumbo, Krishna Seedless, Chardonnay and Merlot.

The geographical area covered was 16° 46.359' to 20° 28.557' N and 73° 59.052' to 75° 56.883' E in western India; 30° 54’ N and 75° 48' E in north India and in 09° 48.493’ N and 77° 18.120’ E in south India. The GPS data was recorded by Garmin’s GPS 76 marine navigator.

The pathogen was isolated from typical lesions on leaves, stem, petioles and berries. The tissues were surface sterilized in 4% sodium hypochlorite for 1 min, rinsed thrice in sterile distilled water, placed on Czapek Dox Agar plates and incubated at 28±0.1°C for up to 5 days. All isolates were purified by making single spore cultures.

For morphological diversity growth rate was studied on Czapek Dox Agar plates at 30±0.1°C in dark. The colony morphology and conidial characteristics were recorded after 7 days growth. The colony margin, appearance, type of mycelial growth, colour of surface and reverse of colony, sporulation pattern, and colour of conidial mass, conidia shape and size, and presence of setae in the acervuli were observed to form morphological groups.

Morphologically similar isolates were grouped together. The one isolate was used from each of the morphological groups for measuring length and width of 100 conidia by using Leica LAS Image Analysis software. The cultures were also examined for the formation of asci and ascospores after 15 and 30 days of incubation.

For pathological diversity the pathogenicity of all three hundred and seventy-nine isolates from each of the morphological groups was tested by Koch’s postulate on the 5th leaf from the top of a growing shoot of Thompson Seedless. After surface sterilization, the lamina was sprayed with a conidial suspension of *C. gloeosporioides* containing $1 \times 10^6$ conidia/ml and incubated in humid chambers (90% RH) at 22-28°C temperature under natural daylight.
For molecular identification DNA was extracted from 5 days old fungal mycelium grown in Czapek Dox broth at 28±0.1°C in dark from each of the three hundred and seventy-nine isolates belonging to the 14 morphological groups. Extraction was done by using the Plant DNeasy Mini Kits according to the manufacturer’s instructions and stored at -20°C.

Species specific primers were selected for molecular study of all three hundred and seventy-nine isolates. These primers were CgINT (5′-GGCCTCCCCGCTCCGGGCGG-3′) with ITS 4 (5′-TCCTCGGCTTATTGATATGC-3′) (Cai et al., 2009; Mills et al., 1992; White et al., 1990).

The 18s RNA of each of the three hundred and seventy-nine isolates was amplified using C. gloeosporioides specific primers. PCR reactions was done in a final volume of 25 μl, containing Taq buffer 2.5 μl, 1.2 μl of dNTP mix (10 mM), 1 μl of each primer (10 μM) (synthesized from IDT, USA), 1 unit of Taq polymerase enzyme (Bangalore GeNei, India) and 2.5 μl DNA (25 ng). PCRs were run on an ABI Gold Geneamp PCR System 9700 for 34 cycles with denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 1.5 min with an initial denaturation of 5 min at 94°C before cycling and final extension of 5 min at 72°C after cycling (Chowdappa et al., 2009).

PCR product was resolved in 1.2 % TAE Agarose electrophoresis gels stained with ethidium bromide and visualized using gel documentation system (Alpha Ease FCTM version 4.0.1, Alpha Innotech Corporation). The experiments were repeated with a single isolate from each of the 14 morphological groups with the above primer pair for ease of presentation.

Sequencing of ITS region of rDNA the ITS region of two isolates of C. gloeosporioides (23-P-1 and 17-P-1) was sequenced. The obtained sequence was compared with other sequences available in non-redundant nucleotide database (nr) in NCBI database and the sequence of first ten significant hits for both the isolates was downloaded and used for cluster analysis. The sequences of ITS regions of Elsinoe ampelina (accessions AY826762.1, AY826763.1 and AY826764.1) were also included for the cluster analysis.

Three hundred and seventy-nine isolates were isolated from forty-six vineyards and ninety seven vines representing wide geographical region, most of the popular cultivars and different susceptible plant parts were isolated and maintained. Maximum isolates (333) were from the popular table grape cultivar Thompson
Seedless and its clones viz. Tas-A-Ganesh, Sonaka and 2A Clone; rest were from other white and coloured table, wine and rootstock varieties.

Generally a single culture was maintained from all morphologically similar colonies obtained from any one plant part of an individual vine from each vineyard in each location, except from Solapur, Bijapur and Pune regions, where a larger number of the isolates were maintained.

The three hundred and seventy-nine isolates were classified into 16 groups based on the morphological character of the colony and the spore morphology produced hyaline, one celled both ends rounded, straight cylindrical conidia of 10.1–14.9 × 4.2–4.9 µ sizes. Setae were formed in the acervuli of the isolates belonging to morphological groups 11, 12 and 13, but were not observed in the acervuli of any of the isolates of morphological groups 1-10, 12-14.

All these three hundred and seventy-nine isolates were fast growing with growth rate of 5.1-6.9±0.05-2.0 mm per day. These fungi were identified as C. gloeosporioides. Thus there appears to be a shift in the etiology of the anthracnose pathogen from E. ampelina to Colletotrichum gloeosporioides.

The three hundred and seventy-nine isolates from each of the fourteen morphological groups produced symptoms on Thompson Seedless leaves within 3-6 days. The fungus was re-isolated from these lesions fulfilling Koch’s Postulates. Subsequently within 1-3 days, abundant acervuli of the inoculated fungi formed on the affected area. The isolates differed in their virulence. Isolated from a particular region were more virulent than those from all other regions.

The selected isolates belonging to group 1-14 gave single specific fragment of approximately 450 base pairs with CgINT-ITS4 primer pair confirming their identity as C. gloeosporioides. In the subsequent test, the all three hundred and seventy-nine isolates also gave a single amplicon of approximately 450 base pairs with CgINT-ITS4 primer pair, confirming that all the isolates belonged to C. gloeosporioides.

The DNA from two isolates (17-P-1 and 23-P-1) identified as C. gloeosporioides by morphological and molecular analysis were amplified with ITS1-ITS4 primer pair and PCR products were sequenced. The sequences from both the isolates showed 92-100% homology with other sequences from C. gloeosporioides available in NCBI database (nr).

Anthracnose is an important disease of grapes. Disease management mainly depends on fungicides. Regular use of fungicides developed resistance in pathogens.
This study was, therefore, undertaken to find the extent of resistance to carbendazim in natural field isolates of *C. gloeosporioides* taking a large population and to study the sensitivity of *C. gloeosporioides* to fungicides belonging to QoI and DMI which may provide insights for better disease management, in vineyards where carbendazim resistance is seen.

Study the sensitivity of three hundred and seventy-nine isolates to carbendazim on fungicide amended potato dextrose agar plates using the poisoned food technique. Minimum inhibitory concentration (MIC) was recorded as minimum concentration of the fungicide required to completely inhibit fungal growth.

*In vitro* screening of 53 *C. gloeosporioides* isolates to new generation fungicides for management of carbendazim resistant isolates in field. For this study 53 isolates was used those are representing different geographical regions, morphological groups and cultivars, to the commonly used fungicide carbendazim (Bavistin 50% WP) belonging to the benzimidazole group, copper oxychloride (Copper 50% WP), and propineb (Antracol 70% WP) these two fungicides belonging to dithiocarbamate.

Further triazole group fungicide viz. flusilazole (Nustar 40 % EC) and a strobilurin group fungicide viz. azoxystrobin (Amistar 23% SC) were studied. The effective concentration of each fungicide required for 50% mycelial growth inhibition (*EC*$_{50}$) was calculated for each isolates.

Sensitivity evaluation of three hundred and seventy-nine *C. gloeosporioides* isolates from grapevines showed that only five isolates had MIC less than 1 μg/ml (sensitive). Three hundred and fifty two isolates had MIC more than 10 μg/ml but less than 100 μg/ml (moderately resistant). Twenty two isolates were able to grow even at a high concentration of 1000 μg/ml (highly resistant).

The *EC*$_{50}$ values for carbendazim ranged from 0.09 to 75.39 μg/ml with the average very high at 28.88 μg/ml. The *EC*$_{50}$ values for copper oxychloride ranged from 243.79 to 399.46 μg/ml with the average at 302.98 μg/ml.

The *EC*$_{50}$ values for propineb ranged from 220.31 to 6664.50 μg/ml with the average at 3110.50 μg/ml. For azoxystrobin and flusilazole, all isolates gave *EC*$_{50}$ values 0.22 to 6.63 and 0.04 to 2.14 respectively indicating that all isolates are sensitive to azoxystrobin and flusilazole.

Development of sequence characterized amplified region (SCAR) marker for identification of carbendazim highly resistant *C. gloeosporioides* isolates. For this
study 12 RAPD primers were used and screen against 27 carbendazim moderately and highly resistant C. gloeosporioides isolates.

The unique fragment detected by RAPD primer OPA-13 in carbendazim highly resistant isolates was harvested than cloned and sequence.

The nucleotide sequence of the cloned RAPD fragment was used to design pairs of SCAR primers. The three primers are synthesized namely

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGPA13 F1</td>
<td>5’- CCACTCTGGGGAGTTCTTC - 3’</td>
</tr>
<tr>
<td>CGPA13 F2</td>
<td>5’- CAGCACCCACTCTGGGGAGTT - 3’</td>
</tr>
<tr>
<td>CGPA13 R1</td>
<td>5’- AGCACCCACATGGCTAAGAC - 3’</td>
</tr>
</tbody>
</table>

Specificity of SCAR primer pair was tested by PCR assays against forty-four isolates of carbendazim highly, moderately resistant and sensitive C. gloeosporioides isolates the validation and specificity of SCAR marker was tested by using different samples.

Out of these primers, the primer pair CGPA13 F1 (5’-CCACTCTGGGGAGTTCTTC-3’) and CGPA13 R1 (5’-AGCACCCACATGGCTAAGAC-3’) produced the specific band only in highly resistant tested isolates of carbendazim (1-YS-1, 38-P-2, 41-L-2, 49-S-5) and not in moderately resistant C. gloeosporioides (20-P-6).

The optimal PCR conditions standardized were as follows: one cycle of denaturation at 94°C for 4 min; followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min; and final extension at 72°C for 5 min.

To develop a diagnostic assay for carbendazim highly resistant C. gloeosporioides, the only selected 320 base pairs RAPD band generated by OPA13 was used to develop SCAR marker.

The primer pair displayed high specificity and distinguished carbendazim highly resistant isolates of C. gloeosporioides from, moderately resistant and sensitive isolates. The PCR product of 320 base pairs was present only in highly resistant isolates and absent in moderately resistant and sensitive isolates.

The SCAR developed was used for the detection of carbendazim resistant C. gloeosporioides from naturally and artificially infected leaf samples of grapes. Results can be obtained within 24 hrs, when compared with up to several days for conventional methods.
The specific assays reported in this work for identification of carbendazim resistant *C. gloeosporioides* provides a simple and efficient tool for early, rapid, sensitive and accurate detection.

The frequent use of systemic fungicide carbendazim for anthracnose control has resulted in developed resistance in the fungal pathogen population. This study was undertaken to identify a promising bio-control bacteria for management of resistance problem.

Fourteen *C. gloeosporioides* isolates, belonging to fourteen different morphological groups which were sensitive, moderately resistant and highly resistant for carbendazim were selected for study.

Eighty-seven bacteria were isolated from mature shoots of grapevines as single colony isolates. In dual culture assay, ten bacteria inhibited the radial growth of all the fourteen *C. gloeosporioides* isolates by 75% or more and these ten most antagonistic bacteria were selected for further studies.

The ten bacteria produced toxic volatile metabolites which inhibited the radial growth of fourteen *C. gloeosporioides* isolates by 29.78 to 81.15 %. Most toxic metabolites were produced by TS-45, TS-46, TS-31 which inhibited the radial growth of fourteen *C. gloeosporioides* isolates by 81.15%, 61.62% and 59.30% respectively.

The ten bacteria produced toxic non-volatile metabolites which inhibited the radial growth of fourteen *C. gloeosporioides* isolates by 44.67 to 82.80 %. Similar to volatile metabolites, the most toxic non-volatile metabolites were produced by TS-45, TS-46, TS-31 which inhibited the radial growth of fourteen *C. gloeosporioides* isolates by 82.80%, 76.54% and 73.25 respectively.

All ten bacteria showed dendritic growth and formed surface biofilm. The maximum biofilm formation was observed by the bacteria, TS-45, TS-46, TS-31 and TS-20.

The all ten bacteria were able to grow at temperatures ranging from 15°C to 37°C in nutrient broth. TS-45, TS-46, and TS-31 showed better growth at 15°C and also at 37°C. In pH TS-31 maximum growth in pH 9 and pH 5 but in TS-45 similar growth was obtained in all pH.

The ten most effective antagonistic bacteria were evaluated for their ability to inhibit *C. gloeosporioides* infection based on detached leaf bio-assay against *C. gloeosporioides* isolate 38-P-2. Four bacteria, TS-45, TS-20, TS-46, and TS-31 which recorded less than 1.00 disease ratings were taken for evaluation on field grown vines.
The four potential biocontrol bacteria, TS-20, TS-31, TS-45, and TS-46, were evaluated in a small scale field trial for control of anthracnose disease under natural epiphytotic conditions.

At the lowest concentrations of $10^4$ and $10^5$ CFU/ml, the AUDPC in vines treated with TS-45 were lower than that in carbendazim showing that it is the most effective bacteria for control of anthracnose. TS-20 was not as effective as the other bacteria at $10^6$ and $10^5$ CFU/ml.

The three bacteria TS-31, TS-45, and TS-46 which gave good control of anthracnose on leaves of field grown vines were also evaluated for biocontrol potential on detached grapes. TS-45 negligible infection (2.38 ± 4.12%) was observed showing good control of berry infection.

The morphological and biochemical characterization of biocontrol bacteria TS-31, TS-45 and TS-46. The colonies of TS-31 were wrinkled, white, lobate, opaque, rough, and raised; those of TS-45 were irregular, chreamy white, lobate, opaque, dry and flat; while those of TS-46 were irregular, white, undulate, opaque, rough and flat on nutrient agar medium after 48 hrs incubation at 28°C.

Microscopic examination revealed that all the three bacteria were Gram positive, endospore forming and the cells were rod shaped and in chains. The bacteria could tolerate sodium chloride concentration up to 10% and were able to grow at 15°C and 45°C.

The bacteria were positive for catalase, oxidase, Voges Proskauer, citrate utilization, starch hydrolysis and negative for methyl red tests. The carbohydrate utilization pattern showed utilization of manitol, manose and sorbitol resulting in acid production, while negative reaction was observed with utilization of xylose, maltose. The above features indicated that three promising biocontrol bacteria belonged to Bacillus and more specifically to B. amyloliquefaciens.

The identification of promising biocontrol bacteria based on 16S rDNA gene sequencing BLAST analysis of the partial 16S rDNA sequences of TS-31, TS-45, and TS-46 confirmed that all the three promising biocontrol bacteria belonged to the Bacillus spp.

In BLAST analysis 16S rDNA sequences of the three promising biocontrol bacteria showed the highest homology (99%) with B. amyloliquefaciens strain FZB42 (Gen-Bank accession no.: 075005) available in the NCBI database. After alignment of
the sequences of three promising biocontrol bacteria and different strains of Bacillus spp. the phylogenetic tree was constructed using MEGA6 program.

The tree showed that the bacteria TS-31, TS-45 and TS-46 formed a clade with reference B. amyloliquefaciens sequences at a bootstrap value of 95%.

The promising biocontrol bacteria TS-31, TS-45 and TS-46 was identified as B. amyloliquefaciens with 16s rDNA sequence analysis. The 16S rDNA sequences of these strains have been deposited in the NCBI nucleotide sequence database under the accession nos. KU500635 (TS-31), KU500636 (TS-45) and KU500637 (TS-46).

Biocontrol bacteria TS-31, TS-45, and TS-46 which showed good disease control on field grown vines, were studied for their sensitivity to fungicides commonly used in viticulture. There was no difference among the three strains in their sensitivity to fungicides and they can be used safely in combination with fungicides in an integrated disease control programme.

Check establishment of most promising biocontrol bacteria TS-45 on field grown vines. TS-45 showed good establishment on leaves and shoots at low concentration $10^4$ CFU/ml.

The three promising biocontrol bacteria TS-31, TS-45 and TS-46 identified as Bacillus amyloliquefaciens on the bases of morphological, biochemical and molecular characterization of the isolates. Promising biocontrol bacteria are not much inhibited by most of the fungicides and can be safely used in integrated disease management.

The microorganisms use as the biological controls is the very important strategy for controlling the plant pathogens which cause the various diseases on different types of the plants.

The different types of the microorganisms utilize as the biocontrol agents in agricultural practices but from all of the other microorganisms the bacteria is the very good example of the biocontrol agents. Why the bacteria is the good example of the biocontrol agents because the bacteria can able to develop in the high temperature and in high pH.

The bacteria very well developed in the high temperature because they form the endospores and they are resistant to the high temperature. The important point is the why bacteria is better than the fungus for controlling the plant diseases.

The bacteria is not inhibited by the all types of fungicides but the fungus not developed itself in the presence of fungicides. The different points are included for the bacteria is better option to control the plant diseases in different crops.
In other hand the biocontrol agents like fungus they are not able to grow in the high temperature and they are not surviving in high pH also. These fugal biocontrol agents are not well grow or they are inhibit there development in the presence of fungicides.

But the bacterial biocontrol agents are heat resistant because they produce endospores and they are easely grow in the high pH and salt. The fungicide action is only on the fungus but some fungicides like copper are act on both bactria and fungus.