Chapter 1

Introduction

1.1. Grape information

Grape is a fruit of the genus *Vitis* that grows on the perennial, occurring in clusters and deciduous woody vines. Grapevines can be vegetatively cultivated by cuttings, so lack of seeds is not a problem for reproduction. The grapevine has long, woody stems rooted to ground with the stems that are covered with peeling bark. The grapevine can commonly grow up to 35 meters with alternative, broad, palmate leaves.

The unripe fruit is green and ripe fruit is dark purple in color (in case of coloured variety). Ripe fruits are covered with light grey wax. Grapes mainly contain 70 to 80% water and various organic and inorganic compounds like sugars, organic acids, phenolic compounds, nitrogenous compounds, aromatic compounds, minerals and pectic substances. The anthocyanin is the compounds which is present in the coloured grapes cultivars and which is play a very significant character on human health. The anthocyanin its work as an antioxidant and having abiomedicinal property its work against the human diseases and protect the human body from the different infections by improving the immunity power.

The grapes are good source of bioflavonoids. Grape originated in Armenia near the Black and Caspian seas in Russia, while recent origin of grapes is also found to North America. Moghul invaders introduced grape in India in 1300 AD. In India, grape cultivation declined after the fall of Moghul rulers but was reintroduced by Mohammed-Bin-Tughlak in South India (Aurangabad, Maharashtra). In India, grape is mainly cultivated in parts of peninsular India, especially Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh under tropical humid climate, covering an area of 116 thousand hectares occupying 1.70% of the total area, the country is also a major exporter of grapes.

1.2. Different grape varieties

1.2.1. Important table grape varieties

The grapes are cultivated commercially and mainly classified into table grapes and wine grapes. Nearly all table grape cultivars belonging to the species, *Vitis vinifera* but wine grapes belong to different species, by selective breeding. Table
grapes have large, seedless fruit with thick skin while wine grapes are smaller, seeded with thin skin. Green cultivars are Thompson seedless and its clones like Tas-A-Ganesh, Sonaka, Manik Chaman and 2-A Clone. These are popular and economically important table varieties of grapes. Sharad seedless, Fantasy seedless, Crimson seedless, flame seedless, are colored seedless varieties except Red Globe (seeded variety), while Chenin blanc, Chardonnay, Muscat, Sauvignon blanc, Cabernet sauvignon, Shiraz, Viognier, Merlot, and Zinfandel are some of the wine varieties. Jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed oil are prepared from the different cultivars of grapes and these products are used for commercial purposes.

1.2.2. Important varieties of wine

1.2.2.1. Cabernet Sauvignon

The Cabernet Sauvignon is one of the important wine varieties. The red wine is most popular and is best wine of the New World because of the Cabernet Sauvignon. The Cabernet Sauvignon is frequently mixed with Merlot and Cabernet Franc and its aroma is cedar wood or evocative of blackcurrants. The Cabernet Sauvignon wine is in demand all over the world.

1.2.2.2. Merlot

In many markets Merlot is one of the most popular red wine varieties. The Merlot variety gains second position to Cabernet Sauvignon in the best finest red wine blends. The Merlot wine is aromatic and generally softer than the Cabernet Sauvignon. The Merlot wine exhibit with best maturation of oak, but normally requires maturation with less bottle before which is prepared to drink. On the lunch and dinner table Merlot wines are perfect Merlot wines are naturally soft textures and rich flavors works well with a diverse array of foods.

1.2.2.3. Shiraz

The variety of Shiraz is also called Syrah. The Shiraz wine is soft and this wine is also distinguished by chocolaty and misty aromas. The Shiraz variety matures earlier than Cabernet Sauvignon.

1.2.2.4. Zinfandel

The zinfandel variety most likely produced in Southern Italy as the primitive grape. This variety is grown only in some wineries of India. The zinfandel wines quality is very good when the fermentation time is very appropriat.
1.2.2.5. Chenin Blanc

The Chenin Blanc is the most accepted white wine variety which is used for making a wine. The Chenin Blanc produces a broad range of wines from dry to sweet, plus luminous and immobile wines. Chenin Blanc dry wines are clean, fruity and Chenin Blancs sweet wines commonly used for drinking purpose.

1.2.2.6. Sauvignon Blanc

In recent days India can create wines of International excellence from Sauvignon Blanc and as a proof a small number of wineries are located in Pune and Nashik region. The environmental conditions of Maharashtra are appropriate for the growth of this variety.

1.3. Importance of grapes

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

The grapes are also a rich source of micronutrient minerals like Copper, Potassium, Calcium, Iron, Selenium and Manganese and vitamins like Vitamin C, Vitamin A, Vitamin K, carotenes, folate and B-complex vitamins. The different benefits of grapes on humans are they have the capability to treat constipation, kidney disorders, fatigue, indigestion, prevention of cataracts and the macular muscular degeneration.

Grapes are made up of antioxidant flavonoids, which can slow down aging and reduce the damage caused by free radicals. The grapevines sap ointment used 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 is used to treat free radical damage, including heart disease, diabetes, and cancer. Grape seed extract has been effective against the bacterial infections caused by Staphylococcus aureus.
1.4. Information about wine

- 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 which is 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.
- What kind of the wine making is depend on the manufacture and demand by the consumer because the in wine different types of the aromas, sugariness, percentage of alcohol and superiority of wines these are the important features of the wine.
- The roams, sugariness, percentage of alcohol and superiority these all things are organized through the fermentation procedure.
- The wine is categorized through pigment like red wine white 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 preservation wine is known as the "outmoded ages."
- The red wine is preserved from the age of more than nine years but white wine preservation time is only one year not more than the one year.
The old wines are the more exclusive than the new wines.

The some factors are involved in good quality wine is the age of wine, coloured, alcohol percentage, aroma, test and good observation during the fermentation process of grape juice etc. these are the some important points which make the good quality wine.

The traditional methods for making a wine from the grape juice is first important thing is the grape harvesting and then crushing the all methods involved in wine making is done by the manually.

But in earlier days the all methods involved in developing the wine is done by the machinery except the grape harvesting.

1.5. Wine manufacturing procedure

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.

1. Harvesting

The harvesting of grapes is main important steps in the wine manufacturing. The right time grape bunch harvest is the most important task because the grapes contain sugars, acids, vitamins etc. play a very important role and this content is developed on the basis of the berry development.

The harvesting of the grape bunches is the very important and preliminary step. The grape harvesting is mainly done by the using man power because for the wine making the ripe grapes are used those are containing the essential acids, esters, and tannins to without fail construct unusual and steady wine. The grape bunches are also harvested by the mechanically but the manufacturer prefer by manually harvesting because after harvesting sorting of bunches is important for making the good quality of wine.
2. Crushing and pressing

After the grape harvesting the next important step is the grape crushing in the past years the grapes are crushed by using the manually through the legs. But in now a day the grapes are crush by mechanically. The wine manufactures prefer the mechanically crushing because in machine the grapes are evenly crushed and that crushed material is known as the must.

In case of the white wine manufacturing the grape juice is immediately separated from the must it is containing the grape seed, skin etc. this west material reduce the white wine quality. In red wine the must is not separated immediately from the grape juice because the grape skin containing the colour, flavor so this things add slowly in juice and improve the red wine quality and texture.

3. Fermentation

The formation is another important step in the fermentation the grape juice containing the sugar so the abundant yeast present in the air so the natural fermentation procedure can occur within the 5 to 10 hours. But the most of the wine manufacturer add pure yeast culture for their confirmation of the end product.

For the production of sweet wine the grape juice is not fermented for the long time. In the fermentation procedure the sugar present in the grape juice it is utilized by the yeast and produces the alcohol is known as the fermentation procedure.

4. Clarification

The clarification process is start when the fermentation process is completed. After the completion of the fermentation procedure the wine is collected in the vessel or jar. After collection of wine the wine is clear from the dead yeast cells, proteins etc. by using different technique like filtration and fining method.

In the fining method the some adhering material put in the wine so all dead and settled particles adhered to the adhering material. After the clarification of wine the wine jars and vessels transferred in the racks for the next procedure or step.
5. **Preservation**

The wine preservation is also the very important step after clarification of the wine adds preservatives because the toxic producing microorganism can easily grow on the wine and get contaminate the wine.

When get other microorganism can grow on the clarifying wine the ultimately decrease the quality of wine. The sulfur dioxide or potassium sorbate are used as a preservative for wine preservation.

6. **Aging and bottling**

The aging and bottling of the wine is the final step of the wine manufacturing. The wine are transferred in to the bottles and packed for the sealing. The aging of wine menace the after bottling the wine is store for the limited time period after this time improve the wine quality in terms of the smoother and improve the wine colour.

For gaining of wine the wine is also preserve in the stainless steel tank also or in different types if the vessels. The wines are packed in bottles which are used for the marketing purpose.

7. **Labeling**

The labeling is the main and last step which is very important for identification. On the label add all details of manufacturing because in now days the different food product problems are arising about the human health.

The labeling is always very proper this is the safer side of the manufacturer. The bottles labels are always very attractive and easily seen by the eyes. The wine is good or not is only depend on the label. The after labeling the bottles are ready to seal in the market.

1.5.1. **Health benefits of wine**

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 play 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 health benefits of drinking wine is described in short such as-
1. **Decrease depression**
   
The some researcher work on the relationship between the drinking wine and depression so researcher find out that those gents and ladies drink the wine seven to eight glasses in a week they are reduce their depression.

2. **Controlling the cancer**
   
The some workers discovered that the benefits of the drinking a red wine. The red wine contain the resveratrol which play a very important role in the controlling the cancer disease.

3. **Anti-aging**
   
The red wine consumption is having different types of beneficial because it contains the resveratrol which is present in the skin of the red grapes. The resveratrol is play an important role in the reducing the age of human beings and they become always looks like a young. The resveratrol is also help in reducing the fat from the fatty person body.

   The red wine also contain the some different types of the compounds like the procyanidin which is help in to improve the health of blood vessels. When the blood vessels are the strong the ultimately increase the life of the human binges.

4. **Controlling the breast cancer**
   
The some researcher fined out that the red wine consummation help to control the breast cancer in woman body. The more alcohol consummation is the very dangerous to the human body but the red wine consummation is the beneficial to the human body.

   The red wine contains the different types of the compound which is present in the skin, seed and juice. The breast cancer disease can occur when increase the level of estrogen but the red wine components help to decrease the level of estrogen and alimentally minimizing the occurrence level of breast cancer.

5. **Controlling dementia**
   
The some researchers are finding out that the red wine contains the resveratrol which is help in minimzing the dementia disease. The resveratrol help to reduce the gumminess of the platelet cells which is present in the blood.
And also help in to opening the blood vessels and increase their flexibility. This thing is very helpful to maintain the blood flow to the brain. The resveratrol contain is more in the wine than the grape because in the time of making the wine the grape juice are not immediately separated from the grape skin, seed etc.

1.6. Development of grape 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.

1.6.1. Sub-tropical area

In the sub-tropical areas the region included are Uttar Pradesh, Haryana and Punjab. In these districts the only one variety grown subsequently is ‘Perlette’ is only variety which ripes very early. Because of rain damage in this area difficulty arise with Thompson Seedless multiplication. In this region Single cutting and single yield practice is accepted in this area.

1.6.2. Mild tropical area

This area encloses Karnataka, Andhra Pradesh and Tamil Nadu. In these regions Maximum temperatures is 36°C and minimum is 12°C. The mainly grown varieties are Bangalore Blue, Anab-e-Shahi and Gulabi. The Thompson Seedless is not grown usually, but only for Thompson Seedless two crops are harvested in a year.

Due to the non-prevalence of Phylloxera or nematodes, rootstocks are not employed, but in recent years, the ‘Dogridge’ rootstock is being employed to combat soil and water salinity problems.

1.6.3. Hot tropical area

In this district grape growing states involved are Maharashtra, Andhra Pradesh and Karnataka. These all areas are the important viticulture districts. In this region double cutting and a single yield is the universal training program was used from this region 70% discrete involved in grape growing. The main problems in this areas are salty water, soil and deficiency. In this areas different types of varieties are grown namely Thompson Seedless and its clones (Tas-A-Ganesh, Sonaka, 2A clone), Anabe-Shahi, Sharad Seedless and Flame Seedless, Manik Chaman.

1.7. Types of grape cultivation

There are two types of grape of grape cultivation, Own Roots and Rootstocks
1.7.1. Own Roots cultivation

The mostly grapes are grown by using own root system means for plantation use mature shoots cuttings with 3 to 4 buds and with a thickness 8 to 10 mm these shoots found in month of September or October. These new cuttings are dips in water for 24 hours. Before planting the cutting part soaked in solution of IBA for 5 minutes. The Management organization is not involved in the reproduction and supply of the planting material. The farmers themselves take the mature shoots cuttings from their selected and healthy grape vineyards and grow on their personal garden center.

1.7.2. Rootstocks cultivation

In the rootstock cultivation method mostly Dogridge rootstock are used. These Dogridge cuttings are grown in the polybags, further this Dogridge rooted cuttings are transferred in to the new field and then the shoots of desired variety are grafted on the Dogridge rootstock.

1.8. Development of vineyards

1.8.1. The plot creation and grapevine formation

The plot is cultivated and arranged into 120 m x 180 m detached by 3 m extensive ways. The level of plot is kept uniform because water flow and absorbance of the water in the soil must be uniform given by the drip system. The dugouts are 75cm in width, depth and length is 118m 3m cavity is kept in between the dugouts. These dugouts are closed with mud, up to a height of 45cm after 15 days contact to or exposed the sun. The residual cavity is occupied with a mixture of soil, manure, superphosphate, sulphate, potash and micro-nutrients.

1.8.2. The season for Planting and spacing

The top most seasons for cultivated cuttings planting in the main field is the September-October and for rootstocks plantation is the February-March. The spacing between the vine is different depends on the soil fruitfulness and variety. Almost for all variety the spacing is 4m x 3m or 6m x 3m but some varieties have a spacing of 3m x 2m or 3m x 3m.

At the time of the plantation utilize the expert advice because the exact distance between the two grapevine and the two rows are ver important. This distance for all the cultivars are the same for one of the cultivar may be different.
1.9. Maintenance and controlling of vineyards

1.9.1 Planting systems

There are 3 types of planting system practiced in India namely bower system, telephone or Y shaped and flat roof gable systems.

1.9.1.1 Bower System

In last days bower system was a most common system used by growers. The bower system is greatly suitable for various varieties like Bangalore blue, Anab-e-Shahi and Gulabi. The bower system is not very suitable with Thompson Seedless and its cloned Tas-A-Ganesh.

1.9.1.2 Telephone System or ‘Y’ systems

This system is mostly used for all type of varieties like Thompson Seedless and its clones and also for other seedless varieties in Maharashtra. In this system the crop yield is less than the bower system. In ‘Y’ system sun burn problem for berries is common because arms of the grapevine are exposed to the sun. In this system ‘Y’ shaped angle support is given to the grapevine.

1.9.1.3 Flat roof gable system

The flat roof gable system is mainly used for the different varieties grafted on the rootstocks. The advantage of this system is the grape bunches are not directly exposed to the sun rays and it is possible to prevent the sunburn problem of the grape berries and the grapevine arms are easily covered by the fungicides and pesticides sprays. In bower system worker face more problems during the time of grape thinning but in this system this problem is minimized so this system is normally used by growers for grape development in Maharashtra, Karnataka and Andhra Pradesh. The flat roof gable system is nothing but the combination of the benefit of bower system and Y systems minimize their drawbacks.

1.10. The grapevines pruning program

There are three types of pruning system is use in the main grape growing areas of India namely in sub-tropical, hot tropical and mild tropical

1.10.1.1 Sub-tropical area

In these areas the grapevines are pruned only one time in whole year in the month of December and the crop is also harvested once in a year. The grapevine half canes are pruned for regenerating the bud sprout and remaining are the fruiting canes.
1.10.1.2 Hot tropical

In hot tropical areas the grapevines are prune two times in a one year but crop is harvested once in a year. The all canes of grapevines are pruned only single bud is kept in one cane and this single bud sprouts in a month of March and May. This type of pruning is known as backward pruning. The canes developed in March and May are again pruned in the month of October-November. This type of pruning is called forward or fruitful pruning, in this pruning fruit is developed on grapevine.

1.10.1.3 Mild tropical

In the mild tropical areas the grapevines are pruned two times in a year and crop is also harvested two times in a year. This pruning system is applicable to some varieties like Bangalore Blue and Gulabi because these types of varieties are resistant to the main diseases of grapes.

1.11. Importance of soil in grape development

Importance of soil in the development of the healthy grapevine such points are explain in short likewise-

- 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 different organic and inorganic compounds. But the additional sources of manure and fertilizers are important for the better growth of the grapevine.
• The additional nitrogen, potash, magnesium etc. these all sources are playing an important role in the vigorous growth of the grapevine.

• The inconsumable nitrogen sources present in the soil these nitrogen source is converted in to the consumable through the some nitrogen fixing bacteria which is present in the fertilizers.

• When we are not giving the some additional manure and fertilizers to the grapevine its showed deficiency of these compounds.

• And when these additional manure and fertilizers doses are high that time it’s also showed some symptoms.

• The random use of the fungicides and pesticides to control the disease it’s may effect on the texture of soil and soil content.

• The soil is made up of the number of the microorganisms and these all microorganisms are having a different role in plant development.

• The maximum use of pesticides are become polluted the soil this is the big problem in front of the farmers.

1.12. The use of fertilizers and manure

The soil in grape vineyard is normally heavy clays or sandy loams. For improving the soil quality use the organic compost in India. The regular dose of organic compost is 500:500:1000 kg of N, P₂O₅ and K₂O per hectare this dose is normally use for light sandy soils and for heavy clay soils the dose is 660:880:660 kg per hectare.

The yearly compost dose is set on the bases of petiole analysis done after the forty-five days of the backward pruning. The forty percent compost dose given in the form of organic sources and the sixty percent fertilizers given in the form of inorganic sources. For heavy soils sulphate use as a source of potash instead of muriate. Now day’s soluble fertilizers are applied by using drip irrigation in grape vineyard. The fifty percent of P₂O₅, forty percent of N and thirty three percent of K₂O these use as a one year dose and apply in the grape vineyard in the time of backward and forward pruning.

1.13. Weeding

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 are removed mechanically using the tractor drawn implements. The weeds which are present
within the vine are removed manually. If the tractor drawn implements are absent then the weeds are removed by using weedicides sprays which are done very carefully because this weedicide may affect the healthy vines.

1.14. Additional irrigation

The grapes grown in tropical areas where the water evaporation is fast there is need to give additional water or irrigation to the grapevine. The requirement of water is calculated on the basis of pan evaporation using 0.8 as the crop factor. The application of water is dependent on the different growth stages of the grapevine and development of berry size. In the all grape growing areas less than ten percent areas are surface irrigated to the grape vineyards and the remaining areas are irrigation through the drip system.

1.15. Physiological draw backs

The physiological drawbacks are mostly connected with the less humidity and high temperature. These conditions are mostly observed in the hot tropical area, in this climate the grapevine arms are dead and stem cracking is observed. The high salt concentration present in water and soil cause damage to grapevine in areas like Maharashtra and north Karnataka. Other physiological drawbacks like shoots are not mature, berries contain water, the berry ripening is not uniform, pedicel attachment with berry is loose, excess water contain berry cracking and rotting and pink berry disorder.

1.16. Improve quality of grapes

1.16.1. Bunch and Shoot tapering

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.

1.16.2. Grape bunches production

The 10 ppm and 15 ppm Gibberellic acid (GA) is a growth regulator hormone which is used for increasing the size of grape. Sprays given in the prebloom stage of the grapes correspondingly when the bud breaks for bunch elongation this stage occurred on the 11th to 14th day of bud break. The grape bunches are cut to remain 8-10, berries per bunch is depend on the number of leaves present per bunches. The
grape bunches are put in the 30-40 ppm of GA solution and the for flower of bunch 10-20 percent GA solution is used

1.16.3. Increase berry size

For increasing the size of grapes physical resources are used like for grape bunch thinning and grape bunches dipping in GA solutions using this practices the size of grapes is increased. These all practices are depending on the man power for this practice mechanical resources are absent. In one grape bunch around 90-120 berries are kept this number depend on the number of leaves present for cultivate bunches and the each leaf depending on its size is nourishing the 8-10 berries.

When the berry size is 3-4mm then the grape bunches are dipped in 40-50 ppm GA solution, this same GA solution again used when berry size is 7-8mm. The grape bunch is dipped in 10 ppm BA + 25 ppm GA or 2 ppm CPPU + 25 ppm GA or 1 ppm brassinosteroid + 25 ppm GA when berry size is 16 mm or more than 16 mm in this stage instead of GA alone or its use in mixture.

1.16.4. Shoot tipping

The shoot tipping is mostly used for controlling the growth and it also gives good aeration and light transformation into the vine canopy. In peninsular India shoot tipping is carried out in both seasons backward and forward seasons but in North India shoot tipping is carried out in only forward pruning.

1.17. Grape diseases and Managements

The commercial grape varieties belonging to *Vitis vinifera* and these are susceptible for 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 which cause diseases like bacterial diseases, fungal diseases, virus diseases, miscellaneous diseases and disorders, Nematodes and insect, pests which reduce yields and quality of fruits.

Among the diseases, downy mildew, powdery mildew, anthracnose, rust and bacterial leaf spot are the important diseases of grapevine, which affect the green tender parts viz. young shoots, leaves, tendrils, flowers and berries; and can cause up to 100% crop loss. Downy mildew in grapes caused by *Plasmopara viticola* an obligate parasite. The causative agent of powdery mildew *Erysiphe or Uncinula necator* this fungus is also obligate parasite. Anthracnose disease in grapes is caused
by the pathogen *Colletotrichum gloeosporioides*. *C. gloeosporioides* pathogen is not obligate parasite.

The downy mildew, powdery mildew, anthracnose, rust and bacterial leaf spot are the very common grapevine diseases in India. But in current years another leaf spot pathogen *Alternaria* has emerged as a severe pathogen in grapes.

**1.17.1. Downy mildew**

The grapes are grown in the tropical areas of the nation face one of the most important disease i.e, downy mildew. The downy mildew disease is mainly observed on the leaves and also affects on the group of the flower and on young berries. When the downy mildew attacks the flower bunch, berry setting is hindered so there is a very high loss in the grape yield because berries are not developed very properly and the bunch appearance is disturbed.

For controlling downy mildew disease different types of fungicides are used like strobilin group which controls the downy mildew easily. Recently fungicides are recommended for controlling downy mildew such as azoxystrobin 494 mL/ha, the mixture of dimethomorph 50 WP + mancozeb 75WP 0.5 to 0.75 g/L + 2.0 g/L, this fungicide also given in mixture ametoctradin 27 + dimethomorph 20.27SC 800-1000ml/ha, kresoxim methyl 44.3 SC 600-700 ml/ha, mandipropamid 23.4% SC 0.8 ml/L and these two fungicides are given in a mixture fluopicolide 4.44% + fosetyl-Al 66.67% WG 2.25 to 2.5 kg/ha, pyraclostrobin 5% + metiram 55% 60WG 1.5-1.75 kg/ha. Some fungicides are used in the combination because of these practices it helps in minimizing the rate of resistance and increase the disease control.

![Fig. 1.1. Downy mildew symptoms on grapevine leaf](image-url)
1.17.1.1. Life cycle of downy mildew

The pathogen of downy mildew is *Plasmopara viticola*. The *Plasmopara viticola* produce spores by using two types of reproduction, the one is sexual reproduction and other is asexual reproduction. The sexual reproduction can occur at the end of the grape season. In asexual reproduction it produces biflagellated zoospores and in sexual reproduction it produces oospores. The mycelium is a septate. This pathogen belongs to the oomycete family and the order is Peronosporales. The oomycetes fungus distributes more ecological, biological and epidemiological characteristics with the plant pathogens.

The *Plasmopara viticola* is one of the obligate parasite, this parasite derives the food from the live host tissue by using the globose haustoria. The mycelium of this fungus penetrates in the host tissue. The sexual reproduction is carried out by forming the combination between the oogonia and antheridia within the host tissue. In the sexual reproduction the spores are produced is known as oospore.

These spores survive the dormancy period this period is also called starvation period. The oospores work as primary inoculum and these oospores are mainly present in the soil, decayed leaves, stem of the grapevine when the favorable condition is absent. The oospores are free in the rainy season and spread through the air and rain on the young leaves and immature berries.

The oospores are released in the green host tissue at the end of the season. The walls of the released oospores are thick and behave as live spores during the off session period, but these oospores also work as resource of genetic deviation. The oospores germination starts at the time of bud break of grapevine but in some grape growing areas the oospores are continuously germinate.

The oospores germinate and form a germ tube called sporangium and form sporangia. The essential conditions for germination of oospore is wet soil with temperatures more than 10ºC and in some grape growing area rainfall of at least 10 mm for 24 hours for soil wetness is important. The sporangia is the source of secondary inoculum for the spread of the pathogens The pathogen penetrates the hypae through the stomata into the host green tissue and form the tree like structure is called sporangiophores these are white in appearance and produce lemon-shaped sporangia on the tip of the sporangiophores. The sporangia spread by the air and by rain splatter. The zoospores are released from the sporangia and these zoospores
germinate and form the germ tube and inject in to the plant through functioning stomata (host tissue always green).

The life span of zoospores is very less; they easily dry out and die within 2 to 3 hours when exposed to sunlight and less humidity. The maximum downy mildew infection can occur after immediate releasing of zoospores. The zoospores live on leaf surface for 24 or more than 24 hours under the low temperature and high humidity. In good situation the infection can spread and they sporulate and this infectious spot appear like oilspots which is present on the lower surface of the leaf.

1.17.2. Powdery mildew

The Powdery mildew is another most important and very common disease in all grape growing areas. The powdery mildew importance is next to the downy mildew. The powdery mildew is identified on the bases of white powder like growth on the both sides of the leaves, immature shoots and young berries. The wettable Sulphur formulations easily control the powdery mildew disease.

The powdery mildew disease is mostly controlled by the broad range of fungicides like from zole group. now a days for controlling  powdery mildew the different type of fungicides are used penconazole 10 EC 0.50 ml/L, Triadimefon 25 WP 0.50-1.0 g/L, hexaconazole 5 EC 1.0 ml/L Myclobutanil 10 WP 0.40 g/L, flusilazole 40 EC 25 ml/200 L, fenarimol 10 EC 0.40 ml/L, difenoconazole 25 EC 0.50 ml/L, azoxystrobin 23 SC 494 ml/ha, kresoxim methyl 44.3 SC 600-700 mL/ha, dinocap 48 EC 0.30 - 0.35 ml/L and tetraconazole 3.8 EW 0.75 ml/L. These different groups of fungicides are used for controlling the powdery mildew.

1.17.2.1. Life cycle of powdery mildew

The powdery mildew disease caused by the pathogen *Erysiphe or Uncinula necator*. The powdery mildew pathogen is obligate parasite and takes nutrition from the live host tissue by entering through the epidermis. The powdery mildew disease developed by using infected buds is known as asexual reproduction. The Powdery mildew pathogen is live in the bud in the dormancy period these bud is called infected bud.

These infected buds sporulate and produce small shoot and spread the disease in whole grape vineyard. These infected small shots create spores known as conidium and its spreads through the air and these spores are set on the other
grapevine shoots. The conidia germinate within 24 hours in the lack of free water and they need more than 40% humidity for germination.

The initial inoculum is pennant shoots or chasmothecia or chlstrothecia are play an important role in spreading the disease in allover the grape vineyard in the presence of favorable condition the disease also spread very rapidly. The pathogen of Powdery mildew multiply within 5 to 12 days on the new infection place and this pathogen make a colony by germination of other conidia. The buds are bust for formation of infection but the after the 40 days the number of conidia is increased and spread sever infection in grape vineyard.

The all essentially wine grape and hybrid grape varieties are highly or moderately susceptible to powdery mildew. In wine grapes the Chardonnay variety is more susceptible to powdery mildew in table grapes varieties almost all varieties are susceptible to powdery mildew like Tas-A-Ganesh, Sonaka, 2A clone (clones of Thompson seedless), Shared seedless, manikchaman, Flame seedless etc.

The powdery mildew disease infects the all grapevine parts like leaf, petiole, stem, tendrils and berry. The initial symptoms appear in the form of light yellowesh spots and this spots turn to appear in the powder form. When the powdery mildew disease appear on the bunch in the time of harvesting its big problem for the farmer because the ultimately reduce the crop yield and crop price so farmer face big economical losses.

![Powdery mildew symptoms on grapevine leaf](image)

**Fig. 1.2.** Powdery mildew symptoms on grapevine leaf

### 1.17.2.2. Sexual reproduction

The powdery mildew pathogen produced by sexual fruiting bodies is known as chlstrothecia or chasmothecia. The chlstrothecia take 90 days for full maturation and
these chlstothecia are present on the surface of sever growth of powdery mildew on grapevine tissues. The young chlstothecia are initially yellow and slowly turn in to brown and then black when these chlstothecia are mature than they are seen by necked eye these chlstothecia are look like minute black pinpoint spots on the surface of the sever growth of powdery mildew on grapevine tissues.

The chlstothecia are normally produced in the mid-summer to autumn and they are alive through the winter session on the bark of the grapevine, grapevine top or barrier and on the soil. The chlstothecia are mature in the favorable condition and it produces ascospores. The ascospores are spread in grape vineyard by air and water and to infect the healthy tissues of grapevine. The powdery mildew infection can occur on the all parts of the grape vine like leaves the infection present on the both side of leaves, stem, petiole and grape berries (flowering to harvesting stage).

When the leaf is infected with powdery mildew infection the first symptom is the oily spot which is seen on immature leaf and then it turns to whitish patches is called colony growth and the green leaf turns to yellow. These colonies produce conidia by sexual reproduction and these conidia spread infection on grapevine. The early stage identification of powdery mildew infection is a difficult job because the infection starting stage is not easily seen by naked eye which is caused by conidia.

The powdery mildew disease incidence is only dependent on the favorable environment i.e. optimum temperature and relative humidity. The disease severity also depends on the last year inoculums present in the vineyard and the quantity of infective buds and chlstothecia which are carried on to the next session. The favorable temperature is 20-30°C and cloudy atmosphere; these two parameters are very important for developing the disease and increase the severity of disease

In the high temperature more than 35°C the decrease the growth of powdery mildew pathogen and ultimately disease incidence is less. When the rain is low approx. 2.5mm or greater and temperature is more than the 10°C the spores are not released from the fruiting bodies. The spores very well germinate in the presence of more than 40% humidity. The protected parts of the grape vineyard and dappled parts of the canopy give the suitable condition for the disease development

1.17.3. Anthracnose

In the 1970, dogwood anthracnose, a new form of the disease was identified in North America. Anthracnose is one of the important leaf spot diseases that lower the
quality of grapevine. Almost all the commercial grape varieties are vulnerable to anthracnose disease. Anthracnose causing fungi dormant in overwinter on infected leaves and twigs in the soil or in cankered twigs that remain on the tree in form of sclerotia.

The spores are dispersed in the form of sclerotia and they are driven and they are spread on the buds and young leaves the sclerotia germinate and enter the infection with favorable moisture and temperature conditions. The infection moves rapidly by due to the long rainy periods and its help the fungus to developed and spread. Anthracnose symptoms are present on all plant parts including leave, petioles, stems, tendrils, young shoots, and berries.

In initial symptoms of anthracnose, a circular brown spot is observed and this infection turns into light brown centers and dark brown to black margins with round or angular edges on leaf, petiole, shoot and berries. After long period of infection, the necrotic center of the lesion falls down and produces a shot-hole appearance. Young leaves are more susceptible for anthracnose infection than the older leaves. The veins of young leaves are mostly affected by anthracnose disease, resulting in complete burning. The lesions of anthracnose on the pedicels and rachis are similar in appearance with shoot lesions of anthracnose.

In the beginning the anthracnose lesions are small, reddish circular spots develop on berries and this symptom then look like a bird’s eye spot, and the anthracnose disease has been also known as bird’s eye spot. The anthracnose lesions usually developed from acervuli (fungal fruiting structures) of anthracnose pathogen. Due to the continued wet weather the pinkish mass of fungal spores called as conidia oozes from anthracnose lesion, this is the secondary source of inoculum which causes nonstop spread of the fungus throughout the growing season.

Anthracnose in grapes caused by the fungus *Elsinoe ampelina* but some of the earlier workers have reported caused by the fungus *Colletotrichum gloeosporioides* there has been a shift in the population. Morphologically, both *E. ampelina* and *C. gloeosporioides* produce one celled hyaline conidia, but the conidia of *E. ampelina* are 3-6×2-8 in size and the conidia of *C. gloeosporioides* which are 12-21×3.5-6 . *C. gloeosporioides* belonging to the -

Kingdom- Fungi  
Division - Ascomycota 
Class – Sordariomycetes
In grapes the most universal disease is anthracnose in all grape growing areas of this state. The anthracnose disease is identified on the basis of their appearance on the grape part. The anthracnose symptoms are greyish black or light brown on young shoots, immature leaves, petiole tendrils and immature berries. For controlling of anthracnose mostly carbendazim at 0.1 percent, copper oxychloride at 0.25 percent, propineb at 0.25 percent and Bordeaux mixture at 0.8 percent are used for controlling anthracnose.

![Anthracnose symptoms on grapevine](image)

**Fig. 1.3.** Anthracnose symptoms on grapevine

### 1.17.3.1. Disease cycle of anthracnose

The causative organism of anthracnose disease is the fungus *Colletotrichum gloeosporioides*. The dormant stage of the fungus is known as the sclerotia. The sclerotia present on the infected leaf, stem, petiole, young shoots and it produce abundant conidia or spores in the rainy season when condition is a wet for 24 hours and the temperature is more than 2°C.

The spores is formed in the asci is called as ascospores the ascospores present in the infected plant parts. The spores which are produced by the fungus are easily blown out to the different plant parts in the presence of rain water with the minimum 2mm or more than 2mm. The spores of pathogen is germinated in the presence of
favorable condition and cause the primary infection in the presence of rain water
maximum for 12 hours and the maximum temperature is in between 2-32°C.

The disease symptoms develop within 13 days when temperature is 2°C or in 4
days when temperature is 32°C. The acervuli produce by the asexual reproduction
these acervuli produce on infected area when disease is stable on plant parts. The
spores are produce in acervuli under humid condition and these spores play a role as
a secondary source of inoculum which causes infection throughout the growing
season. The humidity and temperature play a very important role for inducing the
disease development. The anthracnose sever infection can occur during the heavy
rainfall and hail.

1.17.3.2. Importance of weather forecasting for disease manegments

1. The weather data play a very important role in the important three disease
management on grapevine like Downy mildew, Powdery mildew and Anthracnose.

2. The weather data provide major information about the disease incidence. The
disease management schedule is depending on the weather forecasting data.

3. 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.

4. 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.

5. 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.

6. The forecasting models help in disease management program and minimizing
the economical losses of the farmer.

For identification of the shift the anthracnose disease causing pathogen use
following methodology explain in short-

- For example collect the monthly weather data of Solapur (1975 to 2009) and
  Ludhiana (1977 to 2008) was obtained from India Meteorological Department
  (IMD), Pune. From the monthly temperature data, maximum (Tmax) and
  minimum (Tmin) values were computed for all the years and also for the
monsoon season (June to September) as defined by IMD in each corresponding year.

- The rainfall and relative humidity (RH) data were similarly computed and linear regression equations were generated. The significance of the trend line was tested by using ‘t’ test and Mann-Kendall rank statistics (ô) on SPSS software. The magnitude of the trend was derived from the slope (value of ‘m’) of the regression line.

- The absolute change over the years under consideration was calculated by multiplying the slope value of the trend line by the number of years (Dhorde et al., 2009).

- Further, the highest maximum temperature per year for both the locations and the number of occasions when the temperatures remained above 35°C and above 40°C were also computed.

- The weather data on maximum (Tmax) and minimum (Tmin) temperature, relative humidity (RH), rainfall and leaf-wetness was recorded on Metos automatic weather station at the farm.

- Disease incidence was recorded on 10 leaves per plant following a 0-5 rating scale where, 0=no infection; 1=1-10% leaf area infected; 2=11-25% leaf area infected; 3=26-50% leaf area infected; 4=51-75% leaf area infected; 5=76-100% leaf area infected.

- The percent disease index (PDI) was calculated. Three replications of one plant each were maintained. The data was run on SAS system (‘Local’, W32_7PRO) using stepwise linear regression model.

1.17.3.3. Shift the antracnose pathogen

The previous researcher find out and described the anthracnose disease is caused by the pathogen *Elsinoe ampelina* but in recent study 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* sensu lato.

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.

1.17.3.3.1. The different *Colletotrichum* species

The different types of the *Colletotrichum* spices are find out which causing the serious disease in different crops the species of the *Colletotrichum* describe the differences between the *Colletotrichum* species such as-

1. *Colletotrichum gloeosporioides*

   The *Colletotrichum gloeosporioides* pathogen is having a very vast host range for the infection. The optimum temperature for their growth is the 22 to 25°C and pH range for the spore germination is the 5 to 8.

   The *Colletotrichum gloeosporioides* is the more virulent than the other species of the *Colletotrichum*.

   ![Fig. 1.4. Microscopic observation of *Colletotrichum gloeosporioides*](image)

2. *Colletotrichum falcatum*

   The *Colletotrichum falcatum* is one of the serious species of the *Colletotrichum* which cause the disease in different crops. The optimum temperature for their development is the 25 to 30°C. The optimum temperature for the conidia germination is the 24 to 27°C.

   When the production of approsorium is high than the conidia germination rate is low. The *C. falcatum* cause the disease in the different types of the crops like sugar cane, chilli etc.
3. *Colletotrichum capsici*

The *Colletotrichum capsici* is another important species which cause the disease in different crops this is the major pathogen in chilli crop. The optimum temperature for the growth is the 28 to 32°C.

The spore shape may create the confusions with the other species of *Colletotrichum* like *falcatus, truncatum*. The spore of the *Colletotrichum capsici* is slightly curved one end is the tapering end. The morphological identification get create the confusion in identification but in the molecular identification there is no confusion.

4. *Colletotrichum truncatum*

The *Colletotrichum truncatum* is another important species which is able to cause the disease in the different crops. The required temperature for the growth is 6 to 35°C and pH is 4 to 10. The best temperature and pH for the growth of *Colletotrichum truncatum* is 25 to 30°C and 6 to 7.

The *Colletotrichum truncatum* spores are very similar to the *Colletotrichum capsici*. In such cases the identification of the pathogen is not only based on the morphological but use molecular techniques for identification. In the molecular method used species specific primers so there is no confusion for identification of the pathogen.
5. *Colletotrichum acutatum*

The *Colletotrichum acutatum* causing the disease in different crops the because of this disease the loss in yield, damage the fruit quality these different types of the effects can occur due to this disease. The optimum temperature for the development of the conidia is the 21 to 31°C but the best temperature for the excellent growth is the 28°C.

There is chance to the mismatch identification of *Colletotrichum acutatum* with the *Colletotrichum gloeosporioides* because the spore shape makes the confusion in accurate identification. The best method used for the identification is the species specific marker used.

1.17.3.3.2. Parasitism of fungicide resistant pathogen by *Trichoderma* species explain in short

- PDA plates were seeded with a 5mm disc of the pathogen and the antagonist, cut from the margin of a 5 day old culture, on opposite ends of the plate.
- The plates were incubated at 28 ± 0.1°C in BOD incubator. Three replicates were maintained.
- Plates were observed for overgrowth of *Trichoderma* isolates over the fungicide resistant pathogen colony 6 days after contact.
• Slides were prepared from the zone of interaction, stained with cotton blue-lacto phenol and observed for parasitism at 400x using microscope.

• Observations were recorded on approximate percentage of mycelia of fungicide resistant pathogen exhibiting lysis in reach field.

• Four microscopic fields were observed for each treatment-replicate and the values averaged. Images were captured using microscope.

1.17.3.3.3. Biological control of resistant pathogen by *Trichoderma* species explain in short

• *Trichoderma* species which had shown a wide host range was reported by the number of researchers.

• Fungicide resistant isolates of pathogen were selected in the form of highly resistant isolate, moderately resistant isolate and sensitive isolate to the fungicides.

• Healthy leaves of field grown grape cultivar Thompson Seedless were harvested and wash with tap water than surface sterilized in sodium hypochlorite solution containing approximately 4% available chlorine for 1 min and then rinsed thrice with sterile distilled water and allowed to air dry.

• Conidia of fungicide resistant isolates of pathogen and *Trichoderma* species were harvested in sterile distilled water from a 10 day old culture are grown on Czapek Dox agar and potato dextrose agar respectively and the count was adjusted to $1 \times 10^6$ spores/ml by using a haemocytometer.

• The treatments were imposed as spray applications using an automiser.

• Leaves were first treated with the *Trichoderma* species, allowed to air dry and then sprayed with the isolates of pathogen (preinoculation) or first with the isolates of pathogen followed by the *Trichoderma* species (post-inoculation).

• Appropriate fungicide (carbendazim 50% WP at 5000 and 10,000 g/ml) and water controls were maintained.

• And recorded the disease incidence compared with the water control and fungicide control.
1.17.3.3.4. Fungicide evaluation of the efficacy in field explain in short

- Fungicide applications were made using an inter knapsack sprayer and the application was directed at both the adaxial and abaxial leaf surfaces.
- The experiment was laid out in randomized block design or complete randomize block design for example with four replications per treatment and nine grapevines per replicate.
- Border vines were considered as guard plants and observations were recorded on the central grapevine.
- Untreated control plants were included which did not receive any fungicide application.
- Disease severity was recorded before first spray application and subsequently at 4th day after each fungicide application.

1.17.3.3.5. Bio-assay to confirm the effectiveness of the fungicide

1. Acropetal movement

- To study acropetal movement, young shoots with seven leaves collected from unsprayed field grown vines.
- The leaves were washed under running tap water, placed with their stems dipped in sterile distilled water containing 2000 μg/m cytokinin and allowed to air dry.
- The adaxial and the abaxial surfaces of the 5th and 6th leaf were treated with any one of the fungicides
- The taking a care to not allow the fungicide to come in contact with the other leaves, allowed to air dry and kept in the humid chamber.
- After 24 hours all the seven leaves were inoculated with pathogen suspension and then incubated for five days and observation on infection was recorded.

2. Translaminar movement

- To study translaminar movement, sixth leaf from the apex of growing shoots was harvested.
- The growing shoots were washed underrunning tap water and arranged in humid chambers with their petioles dipped in sterile distilled water containing 2000 g/ml cytokine.
• The adaxial or the abaxial surface of each leaf was treated with any one of the fungicides, allowed to air dry and kept in the humid chamber.
• After 24 hours, a period considered sufficient to allow fungicide movement, the leaf surface opposite to the fungicide treated surface was inoculated with pathogen suspension.
• The leaves were incubated for five days and observation on infection was recorded.

3. Antisporulant effects
• To study antisporulent effects, the sixth leaf from the apex of growing shoots were harvested.
• The leaf were washed and arranged in humid chambers with their petioles dipped in sterile distilled water containing 2000 μg/ml cytokinin.
• The adaxial surface was inoculated with pathogen suspension and incubated for four days.
• Then it was treated with fungicide suspension and further incubated for 3 days.
• The lesions were harvested, measured and then suspended in distilled water containing 0.05% Tween 80, vortexed for 1 min and the spore count was taken using a haemocytometer. Dilutions were made wherever necessary before counting.

1.17.3.3.6. Biochemical and enzyme estimations are important for resistant mechanisms in grapevine
• The following defense related biochemical parameters and enzymes were analyzed separately in the leaves before and after challenge with the pathogen.
• The percent increase in contents after challenge with the pathogen was calculated.

1.17.3.3.7. Biochemical analysis explain in short
• Fifth leaf from apex of growing shoot was harvested from each of the genotypes which are used for the checking the resistance to the diseases.
• One gram of each sample was extracted in 80% methanol by overnight shaking at room temperature in the dark.
• The mixture was centrifuged at 12000 rpm for 15 min at 4°C.
The supernatant was separated and the residue was re-extracted for three times.
The complete extraction was ensured by qualitative Folin-Ciocalteu test on Whatman No. 1 filter paper till it comes negative.
The filtrates were pooled and filtered through 0.45 μm filters and stored at -20°C until used for analysis.
These extracted supernatant is used for the estimation of the different biochemical like total phenols, total sugars, flavonoids, flavon-3-ols and flavonols. These all biochemical play an important role in the defense mechanism.
In the resistant verity the total phenol, flavonoids and flavonols contents were high and total sugar contain is low.
But in the case of the susceptible genotypes total sugars were high and total phenol, flavonoids and flavonols contents were low.
In defense mechanism the important biochemical factors which inhibit the disease occurrence in the plant is the total phenol, flavonoids and flavonols.

**1.17.3.3.8. Assays of defense related enzymes explain in short**

- Peroxidase (E.C.1.11.1.7) and polyphenol oxidase (E.C.1.14.18.1) activities were measured which is play a very important role in the defense mechanism against the diseases.
- For Peroxidase (POD) activity, 1g of leaf sample was homogenized using pre-cooled mortar and pestle in 3 ml of 0.1Mphosphate buffer (pH 7.0).
- The homogenate was centrifuged at 12,000 rpm for 5 min at 4°C and the supernatant was collected and used for enzyme assay.
- For polyphenol oxidase (PPO) activity, 1g of leaf sample was homogenized using pre-cooled mortar and pestle in 4 ml buffer containing 50 mMTris HCL (pH 7.2), 0.4 M sorbitol and 10mMNaCl.
- The homogenate was centrifuged at 12,000 rpm for 10 min at 4°C and the supernatant was collected and used for enzyme assay.
- Peroxidase and polyphenol oxidase is very important enzymes to make the resistant verity.
- In the resistant genotypes the high contents of the Peroxidase and polyphenol oxidase are observed.
- But in the susceptible genotypes the low contents of the Peroxidase and polyphenol oxidase are observed.
- These two enzymes Peroxidase and polyphenol oxidase are play a very important role in the resistant mechanism of the genotypes.
- The peroxidase is the main defense related enzyme whose production is enhanced in response to anthracnose infection and thus, it might be a suitable biochemical marker for predicting resistance to anthracnose.

### 1.17.3.2. Control strategies for anthracnose disease management

Some greatest effective methods are use as of good cultural practices for managing the anthracnose disease. Cleanness is a serious factor in managing the anthracnose disease. For anthracnose disease management the control strategies like

1. The main part of disease management is the cut out and destroy the all diseased plant parts from the grapevines and from soil throughout inactive season. This main strategy will help to decrease the overwintering inoculum which spreads in the vineyard.
2. Another important strategy for minimizing the anthracnose disease incidence in vineyard is to escape the sensitive varieties like *Vitis vinifera* and some French hybrids.
3. Develop a new cropping and training systems to increase the air movement which helps in quick leaf drying and also help in full vine covering in the time of spraying and canopy diffusion.
4. For disease management another important control strategy is to remove anthracnose infected wild grapes and any plants close to the grape vineyard. The wild grape performs as an exceptional source of inoculum for developing the anthracnose disease on healthy grapevine.
5. If unable to remove the wild grapes from adjacent wooded plant parts, confirm that these wild grapes destroyed from the adjoining rows because spores are simply dispersed by the wind.
6. When anthracnose disease is present in the grape vineyard the well-timed spray schedule is required for controlling anthracnose disease.
7. In the undeveloped period of the fugues take lime sulfur sprays for managing anthracnose diseases.
1.17.4. Rust

In grapes the rust is one of the important diseases. The pathogen of rust is developed and live in the spots which are present on leaves or on the stems and in the dropped plant parts. The pathogen producing the symptoms on leaves in the presence of more than 20°C temperature and moisture which is important to develop a disease. The rust symptoms are normally present on the lower surface of leaf in the form of orange coloured granules. In severe infection these orange granules cover the whole lower surface area. The rust can controlled by using the protective fungicides like bordeaux mixture, captafol, difolatan, propiconazole, tebuconazole and azoxystrobin providing the greatest controller for the rust.

1.17.4.1. Life cycle of rust

The rust is the one type of grape disease is caused by the pathogen *Phakopsora euvitis*. The host range of rust disease is very less. The aeciospores are transferred on grape leaves through the wind. The aeciospores are generated on the different host from some of the basidiospore infections and their pathogenicity is not specific and these spores infecting the natural and cultured species of grape varieties. The urediniospores are collected from the diverse species of grapes varieties but these collected urediniospores have not showed infection on the same species of grapes varieties.

For teliospores the other host is available so these teliospores continually fall on the grapevine leaves these spores are germinated in rainy season and spread thin-walled basidiospores known as air borne. The teliospores are normally germinated in the dark condition at 18-20°C temperature. The infected grape vine tissues not produce spermogonia in 7-15 days but expect leaves create subcuticular spermogonia within 7-15 days. The asexual spores produce in aecia on the back side of the leaves within 7-14 days.

The infected leaves accept the uredinia and spread urediniospores, initially through the air, and the disease can occur by the repetitive cycles of the disease. The disease incidence and severity of the infection depends on the environmental conditions. With the help of this factor the infection spreads throughout the growing season in grape vineyard. For spore germination the optimum temperature is maximum 32°C and a minimum of 8°C.
Fig. 1.9. Rust symptoms on grapevine leaf

The leaf infected with urediniospores these spores produced young uredinia within 25 days on the grape vineyard. The rust pathogen enter in to the leaf though the stomata but in immature leaves the mature stomata is absent so the infection can occur in only mature leaves on the lower surface of leaves. But in some countries the both leaves immature and mature leaves are infected by rust.

On the back surface of the leaves near the urediniatelia is developing in autumn. The telia is the dormant part of the rust disease but this telia move exclusively in the uredinial condition in the absence of any other host this all procedure is occur in tropical and subtropical condition. The mycelium of uredinial might be alive in adverse conditions in inactive buds.

1.17.5. Bacterial leaf spot

The bacterial leaf spot is another important disease in all grape growing areas. This bacteria is able to cause the disease on the grape leaves, shoots and berries. The bacterial leaf spot is caused by the pathogen Xanthomonas. The very typical and unique symptoms of bacteria on leaf like a pinpoint water soaked spots specially present on the main and lateral veins on the lower side of the leaves.

Fig. 1.10. Bacterial leafspot symptoms on leaf
Generally these small spots join together and appear as larger patches. In severe infection these leaves looks like in wrecked form. For controlling the bacterial leaf spot different control majors are available such as in prophylactic spray streptocyclin at 500 ppm is used for preventing the disease. The Bordeaux mixture at 0.8 percent or copper oxychloride at 0.15 percent is used for controlling the bacterial disease.

1.17.6. Virus diseases of grapes

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 viral diseases are not the main problem in India but this is the most serious problem in other countries. But in the now days the viral disease are also the main problem in our country because the almost all types of the wine varieties are the imported from our country so this disease is transferred through the cutting of plants.

The grapevine affected viruses are directly causing the effect on the development of the vine, yield loss and decrease the quality and quantity of the grape bunches. The grapes value in the market is decrease because of the disease, the infected grapes are also not use in the wine preparation or making the resin. 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 in our country are the namely-

1. Leafroll virus
2. Fanleaf virus

These two viruses are explain in short as follows-

1. Leafroll virus

The leafroll virus is the most important virus which causes the disease on the different grape verities. This disease can occur in the summer session and infect the grape leaves. The leafroll virus is affected on the yield, quality
and quantity of the grape production. This virus causes the disease but they are not produce the symptoms on the grapevine.

This virus showed different symptoms on different grape varieties. The mostly cloned varieties are more susceptible than the hybrid varieties or the rootstock varieties of the grapevine. This virus spread the disease in optimum environmental conditions like temperature is the most important factor for spreading the disease in all over the vineyard. The symptoms of the leaf roll virus showed by the grapevine up to twelve months.

![Grapevine Leafroll virus infection on grape leaf](image)

**Fig. 1.11.** Grapevine Leafroll virus infection on grape leaf

This virus is only transmitted through the grafting or it may be transmitted by the different types of the insect or pest. The detection of virus disease is not easy because the spreading of virus disease is not very fast it take the twelve month for moving from the infection point to the another point.

There are different types of the grapevine leafroll virus (GLRaV) is present like 1, 2, 3, up to 9. But mostly found grapevine leafroll virus in our country re the GLRaV 1 and 3. The symptoms of the grapevine leafroll virus such as-

- The GLRaV 1 and 3 infected leaves are roll on the down side of the leaves.
- The yellowish colouer is developed on the leaf mostly main veins are not turn green to yellow.
- Some of the grape varieties leaves are turn green to colorless.
- In the some white grape verities the colouer change is not happen widely.
In this disease the grapevine shoots are not developed properly. The shoot length is very short.

When the shoots are not developed properly this may directly affect on the bunch development of the grapevine.

The grapevine leafroll virus disease reduces the age of the grapevine.

The grapevine leafroll virus disease also create the effect on the berry size, sugar and acid content and also effect on the colour development in case of the colour varieties.

This disease also effect on the berry ripening when the berries are not ripped timely so there is yield loss and no market value.

1.17.6.1. Disease management for leafroll virus

There are different practices are used for the controlling the leafroll virus disease in grapevine yard such as-

- The grafting material is showed be virus free.
- Take routine observation for presence of the grapevine leafroll virus infection in vine yard.
- For planting used disease free material and before planting take observation for disease.
- During the field observation there is the any chance of the grapevine leafroll virus present so confirmation is done by using the diagnostic test.
- The grapevines are infected with the grapevine leafroll virus are removed from the field this is the major control strategy to prevent the spreading the disease from the one vine to another vine.
- The field’s observations are taken for the mealy bug and insect because these are transmitted the disease from one to another and give proper spray for control.
- The only field observations are not sufficient for the presence or absence of the grapevine leafroll virus disease because this disease confuse with the deficiency of minerals like magnesium and phosphorus.
- For the confirmation of the grapevine leafroll virus disease the diagnostic tests are play a most important role.
1.17.6.1.1. Diagnostic methods

The diagnostic methods are used for the confirmation on the presence or absence of the disease in the grapevine yards. In this diagnostic method there are mostly two methods are used 1) enzyme linked immunosorbent assay (ELISA) 2) molecular detection by using Polymer chain reaction (PCR)

1. **Enzyme linked immunosorbent assay (ELISA)**

The enzyme linked immunosorbent assay (ELISA) is mostly used for the detection of the protein, hormone and antigen etc. for the detection of the GLRaV used the sandwich ELISA technique which is explain in the short as follows-

- The sandwich ELISA technique is carried out in the polystyrene plate which is contains with the 96 well.
- In this technique wells of the plates are firstly made up of the capture antibody.
- Then add our testing product is nothing but the antigen.
- For the detection of the antigen add the secondary antibody.
- The final step is for detection of the antigen antibody reaction adds enzyme conjugated antibody.
- Add substrate for the reaction of the enzyme with substrate and produce the colour.
- The colour intensity is measured by the ELISA reader on different wave length.
- The GLRaV disease presence or absences confirmation is based on the readings of the positive and negative control.
- The test readings are compared with the positive and negative control readings.

2. **Polymer chain reaction (PCR)**

The Polymer chain reaction is used for the confirmation of the GLRaV disease presence or absences the is the one type of the molecular method which is explain in short as follows-

- The PCR mixture is made up of the Taq buffer, dNTPs, for war and reveres primers, Taq polymerase and testing genetic material.
- The PCR condition is play an important role in the developing our interest of product.
The PCR conditions made up of the different temperatures and times like initial denaturation with the appropriate cycles denaturation, annealing, extension, final extension and cooling.

After the completion of the PCR program the PCR product load on the gel and the expected band size seen under the ultraviolet light.

When the expected band size is present in the both samples positive and negative so there is confirmation of the presence of the GLRaV disease in grapevine.

2. Fanleaf virus

The grapevine fanleaf virus (GFLV) is another most serious pathogen which causes the disease on the grapevine.

- The fanleaf virus is transfer from the one grapevine to another grapevine through the insect nematode.
- The source of fanleaf virus in the grape vineyard is coming from the nursery plant.
- The sources of fanleaf virus are remaining alive in the root of the dead grapevine.
- The grafting is the main source of fan leaf virus, so check the presence of fanleaf virus in grafting material.

There are the different types of the symptoms can occur on the grapevine such as-

- The fanleaf infected grapevines showed the irregularity in the development of the grapevine.
- The symptoms occurrence is dependent on the grapevine susceptibility.
- The fanleaf infected grapevines lifespan are reduce and they are collapse.
- The leaves are fall down, the shots are not developed properly and they are unable to developed the good quality grape bunches.
- The fanleaf virus developed the leaf colour from green to yellow.
- Mostly yellow colour developed on the vein of the leaf.
- The leaves are become in the cup shaped and the leaves are become looks like a fan that why this virus give a name fanleaf.
The fanleaf infected leaves are drop out and the internodes distance of the shoot is also not proper.

The uneven growth of shoot is occurring during the fanleaf infection on the grapevine.

The fanleaf infected grapevines not produce the normal size of the grape bunches so automatically losses in the grape yield.

The fanleaf infected grape bunches are not accepted in the market so grower face economic problem.

The fanleaf infected grapevines are removed from the soil and destroy them because this virus can live on the grapevine.

On the time of the new grapevine plantation one thing is remember the planting material is the free from the fanleaf virus infection.

The shoots are not developed properly so availability of shoots for the Grafting purpose is very less.

![Fanleaf virus symptoms on grape leaf](image)

**Fig. 1.12. Fanleaf virus symptoms on grape leaf**

### 1.17.6.2. Disease management for fanleaf virus

The different types of the parameters are used for the disease management program which is caused by the fanleaf virus-

- The planting material is the free from the fanleaf virus infection.
- The rootstock used for the grafting is also free from the fanleaf virus infection.
- The all grafted materials and the rootstock clones are free from the viral diseases.
- The planting material is resistant for the viral diseases and insect or pest infection.
There is no such treatment is available for the curing the fanleaf infection from the grapevine.

The fanleaf infected grapevine is remove and destroy there is only the option of curing.

In the time of the pruning used clean equipments and cleaning is necessary after pruning the each grapevine.

The pruning equipment is the source of spreading the contamination from one grape vine to another grapevine.

At the present day no such chemical control is available for the management of the grapevine fanleaf virus.

1.17.6.2.1. Detection of fanleaf virus

For the detection and identification of the grapevine fanleaf virus the different types of the techniques are used like-

- For the detection and confirmation of the grapevine fanleaf virus take the susceptible grape variety and inoculate the testing growth of the fanleaf virus on the leaves of susceptible grape variety.

- The inoculated leaves incubated on the controlled conditions and observe the symptoms and compare with the positive and negative controls.

- The sap inoculation method is also used for the identification in this method the sap of infected plant is inoculated in any other herbaceous plant.

- The sap inoculated herbaceous plant can grow in the controlled environmental conditions, the leaf, root and stem of this inoculated plants are infected with the virus check the symptoms and compare with the control plants.

- Another technique is the use enzyme linked immunosorbent assay (ELISA) for the detection and identification.

- In the ELISA methods use the specific antibodies for the detection of the antigen (testing material).

- The immune electron microscope is also used for the detection of the fanleaf virus this method is very easy than the other.
The other important method is the molecular method which is used for the detection of the fanleaf virus.

In the molecular method developed the species specific primes for the identification of the fanleaf virus.

The using the polymerase reaction chain (PCR) technique the developed primers are produced the specific amplicon with the genetic material.

The developed amplicon is load in agarose gel and seen under the ultraviolet light.

1.17.7. Minor diseases of grapes

The grapevine is the nutrient rich variety because of this parameter grapevine is very susceptible to the different diseases. The major diseases are seen above now explain the minor diseases very shortly-

1. **Phomopsis**

The *Phomopsis* is the one type of the microorganism which cause the disease on the different types of the crop like grape, soya been etc. This disease can occur in the rainy season. The different species of the *Phomopsis* which can able to cause disease on the different parts of grapevines like leaf, shoot, grape bunch and cane extra. The *Phomopsis* causing in disease on leaf is known as the leaf spot disease.

The *Phomopsis* produce different types of the symptoms on the leaf and shoot. The *Phomopsis* can produce the small dark brown spots on leaf after the few days of incubation these small spots may developed and produce the holes on the leaf and the leaf becoming yellow and fall down as well as the petiole also turn green to yellow.

![Fig. 1.13. Phomopsis infection on different parts of grapevine](image-url)
The similar types of the symptoms can occur on the shoot, mostly the brown colour spots with the black colour centers were developed in the rainy session and after few days of incubation this spots are spared all over the shoot and in seviour infection the shoot cracking is observed.

The dark colour brown and black patches are occurs on the cane and fungal growth is observed on the infected prat of the grapevine. The symptoms on the grape berry is similar to the leaf and shoot but the infected bunches are drop out and the resultant is the heavy yield loss.

2. *Agrobacterium*

The different species of *Agrobacterium* can cause the disease on grapevine is known as the crown galdisease. The fresh growth of the *Agrobacterium* is appears pale yellow colour in the bark or near the bark. The *Agrobacterium* species produce the crown gall is near to the root, soil and above the soil.

When the grapevine is injured it’s producing the some chemicals and *Agrobacterium* species attracted towards the compound. Than *Agrobacterium* enter in to the grapevine and cause the disease and produce the symptoms of crown gall.

![Fig. 1.14. Crown gall disease symptoms on grapevine](image)

3. *Alternaria*

The *Alternaria* can cause the leaf spot disease in the grapevine is known as the blight disease which is caused by the *Alternaria* species. The *Alternaria* cause the disease on grape leaf and on grape berries the yellowish colour small spots are developed on the margin of the leaf.

The small yellow spots are turn into the patches in the optimum weather conditions like humidity70% and atmospheric temperature range are 13 to 26°C. In the seviour infection the leaf and grape bunches are the turn
yellow and fall down. The similar type of the symptoms are appear on the grape bunches. The *Alternaria alternate* is the most common species is found out to cause the infection on grapevine.

**Fig. 1.15.** *Alternaria* infection on grape leaf

4. *Greenaria*

   The *Greenaria* able to cause the infection on different types of the crop but mostly caused the disease on the grapevine. This fungus can cause the disease on different plant parts of grapevines like leaf, shoot, berries and tendrils. The small red spots can occur on the green leaves and these red spots can turn in to the dark brown margin with light brown center.

   In the seviour conditions the pyknidia can appear on the center of the infection at the most appropriate temperature 21 to 26ºC. The *Greenaria* is mostly active on the grape berries the same type of the infection can occur on the grape berries. In the seviour infections the grape leaves and the grape bunches are the fallen down.

**Fig. 1.16.** Symptoms of *Greenaria* on the leaf and grape bunch

5. *Phoma*

   The *Phoma* is the one type of microorganism which is able to cause the serious disease in the grapevine is known as the stem canker. The species of *Phoma* are capable to cause the infection on leaf, shoot and stem. The *Phoma*
develop the spots on the leaf and in the seviour infection the fungus produce the pyknedia on the lesions.

This fungus also able to cause the disease on the stem this disease can occur in the summer session. The cracking on the stem is observed on the grapevine the initial symptoms on the stem is the light brown center with the dark brown margin.

![Image](image1.png)

**Fig. 1.17.** *Phoma* produce the symptoms on grape leaf

6. *Phytophthora*

In now a day the most serious disease is the root rot which is caused by the pathogen *Phytophthora*. The *Phytophthora* is the one type of the fungus which is able to cause the root rot disease in the number of crop. The root rot disease is caused by the additional water, nutrient shortage and deficiency of the water. The *Phytophthora* fungus is belonging to the oomycetes family.

The *Phytophthora* is the one type of the soil pathogen fungus which is present in the soil and cause the disease in unhealthy plant. The plant roots are the main important part of the plant because the all nutrient supply, water supply etc. is given through the root system only.

![Image](image2.png)

**Fig. 1.18.** *Phytophthora* cause infection on grapevines roots
7. *Pythium*

The *Pythium* is the one type of the fungus which is caused the root rot in the grape and on different crops. The root rot causing fungus is belonging to the oomycetes family and produce the zoospores which are actual the infectious material and they are causing the root rot infection in the root.

Fig. 1.19. Symptoms of *Pythium* on grape leaves

The different types of the symptoms produced by the *Pythium* fungus like the crops are not developed properly, the tips of roots are brown in coloured and in seviour infection the root tips are dead, the whole plant become turn green to yellow and they may be expire and the roots of the plants are becoming an attached with the spores of the pathogen.

1.18. Fungicide resistant development

To kill or inhibit fungi or fungal spores and to increase productivity of a crop biocidal chemical compounds or biological organisms used known as Fungicides. A large number of powerful fungicides are used for controlling the disease. There are two types of fungicides, systemic and non-systemic (contact). Non-systemic fungicides are not absorbed in plant tissue and are able to protect the only plant parts where they are deposited. Systemic fungicides are capable to transfer from the upper sprayed leaf surface to the lower unsprayed leaf surface and are taken up and spread through the xylem vessels of the plant. Few of the systemic fungicides are capable to transfer to all parts of a plant.

The fungicide resistance is a hereditary alteration in fungi, this finding is reduced fungicide sensitivity. The sensitivity reduced towards fungicide the consideration is hereditary change in sequences of nucleotides which is happen in less number or obviously forming sub-populations of resistant singles. The singles in a
population fungus it may be made up of spores, sclerotia, mycelium and the single cell’s nucleus which is able to reproduce and multiply. The fungicide resistant can occur in the fungus by single gene or multiple gene mutations.

The single gene mutation means the resistance is confirm for site-specific fungicides in the fungus. The mutation can occur in multiple genes of the fungus is confirmed that resistance to multi-site inhibiting fungicides. The resistant mechanisms are different which is mainly depending on the fungicide mode of action but involving the variation of the aim place, minimize the use of fungicide. The fungicide resistance level examined in the laboratory by observing feedback of field population members to the fungicide toxicity.

The fungicide toxicity reply is normally considered as fungal growth inhibition, inhibition of spore germination and when the fungus is obligate parasite the real plant infection. For find out the resistance in fungus for that calculate the effective concentration which inhibits growth, spore germination by 50% (EC$_{50}$) and then calculate the EC$_{50}$ values for all samples and find out the resistance.

The different types of mechanism are involved in fungicide resistance are such as:

- The one of the most important factor is continues used of fungicide for controlling one or different type of pathogen it is induce resistance against fungicide.
- The proper spraying technique and coverage of canopy of grape vineyard.
- The sensitive of fungicide is very short because of change in biochemical target site.
- Target proteins are increased their production.
- Alternative metabolic pathways are developed on that target site bypasses.
- The breakdown of the metabolic fungicide.
- Prohibiting or injecting of the fungicide from ATP-ase dependent transporter proteins.

The resistance of fungicide is a concept in that genetic mechanism involved and it decrease the sensitivity against fungicides. This resistance capability is gained from the evolutionary procedure. The resistance trait is might be present in the fungal inhabitants before the fungicide use. The fungicide resistant strains management is critical problem in front of growers once the resistance is developed in fungus the disease management is not easy.
The single site mode of action fungicides are usually resistance development at medium to high risk. The fungicide act on exact position in a biosynthetic pathway of the pathogen is known as single site mutation. The fungicide is less effective or unsuccessful for controlling the disease because the point mutation can occur in pathogen and these fungicides are listed in resistance development.

For resistance development the alteration in one base nucleotide is sufficient for guide to complete resistance (fungicide totally unsuccessful) this can form through strobilurin group fungicides. In older fungicides like chlorothalonil and copper has a small possible for devolve resistance in fungus because these fungicides have a multisite mode of action. In fungicide resistant pathogen genetically change in single major gene and the subpopulations pathogen are also completely resistant or sensitive to the fungicide. In the case of resistance the total loss of disease is observed and the control cannot be possible the resistance can occur by using high rates or regular applications fungicide.

1.19. **Fungicide resistance development in anthracnose pathogen**

There are two Nonsystemic fungicides copper oxychloride 50% WP, propineb 70% WP and only one systemic fungicide carbendazim 50% WP recommended for controlling anthracnose, of grapes. Carbendazim is a widely used broad-spectrum Benzimidazole fungicide. Benzimidazole fungicide acts as an antimitotic agent and binding to the β-tubulins. Carbendazim inhibits fungal mitotic microtubule formation.

Most frequent use of fungicides results in development of resistance to that fungicides. Reduced sensitivity due to the genetic modification in fungus that results in resistance to fungicide. Resistance can be a result of genetic mutations which occur at low rate (one in a million or less) or of naturally occurring sub-populations of resistant individuals. The development of fungicide resistance is induced by reasons likes the mode of action of the fungicide (how the active ingredient inhibits the fungus), use of fungicides, the biology of the pathogen, and the cropping system.

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.

1.20. Fungicide pathogen resistant management

The fungicide resistance management is a very important strategy for disease control. The different type of practices can be used for managing the fungicide resistant in grape vineyard namely

- The practices for controlling the fungicide resistance is mainly depend on the use of fungicides in grape vineyard.
- The resistance controlling practices should be incorporated earlier than the resistance trouble is raised.
- The fungicide resistant can be control by avoiding the use of high risk fungicide.
- But this not a useful solution for managing resistance because the number of new fungicides that are high risk for resistance problems and it supply more effective, broad-spectrum disease control.
- The resistance management practices are differs for the dissimilar fungicide groups and for aim of the pathogen and crop.
- In resistance management the use of fungicide should be minimum and timely application of fungicide.
- For controlling the resistance the pathogen exposure time to high risk fungicide should be minimized.
- For resistance management most important thing is to take alternative sprays with different group of fungicides or fungicides use in mixture or use bio-fungicides.
The numbers of fungicides are now available in market in the mixture form in this mixture 2 or more group of fungicides are involved.

For disease control use guide lines created by ICAR institute and take advice of experts and mimeses the same group of fungicides applications.

1.21. Biocontrol agents used for controlling pathogens, insects and pests

1. **Trichoderma harzianum**

   The *Trichoderma harzianum* is one of the microorganism which is used for the controlling the diseases caused by the fungal pathogens and diseases caused by the pest and insects. The *Trichoderma harzianum* is belonging to the phylum Ascomycetes and produce the abundant spores.

   This fungus grows very well at the room temperature about the 25 to 28°C. The *Trichoderma harziaum* routinely used by the farmers in their field for controlling the different types of the disease. The *Trichoderma harzianum* is available in the market in the form of liquid and powder.

   The different species of *Trichoderma* which is used for the controlling of the number of diseases and in now days this organism is consume for the drenching and find out there activity in ISR program.

   ![Microscopic observation of Trichoderma harzianum](image)

   **Fig. 1.20.** Microscopic observation of *Trichoderma harzianum*

2. **Verticillium lecanii**

   The *Verticillium lecanii* is also the one type of the microorganism and belonging to the fungus class. The *Verticillium lecanii* normally used in controlling or minimizing the diseases caused by the fungal pathogen and through the insect and pest. The *Verticillium lecanii* is belonging to the class Ascomycetes and this fungus also produce the abended spores.
The *Verticillium lecanii* use to control the powdery mildew disease which is very important disease in grapes and as well as used for controlling the mely bug from different crops including the grapes. The *Verticillium lecanii* developed by the number of company as a biocontrol product and used by the farmer for controlling the diseases in field.

![Image of Verticillium lecanii](image)

**Fig. 1.21.** Microscopic observation of *Verticillium lecanii*

3. *Beauveria bassiana*

The *Beauveria bassiana* is one of the entemopathogenic fungi which is used for the controlling the insect and pest which caused the different types of the infections on the different types of the crops. The *Beauveria bassiana* is belonging to the phylum Ascomycetes and produce more conidia.

When the insect larvae infected with the *Beauveria bassiana* than insect larvae die emidiatlly and the growth of *Beauveria bassiana* occurred all over the body of larvae. This product is also available in market as biocontrol agent and this product used by the farmer.

![Image of Beauveria bassiana](image)

**Fig. 1.22.** Microscopic observation of *Beauveria bassiana*

4. *Metarhizium anisopliae*

The *Metarhizium anisopliae* is also a one type of the entemopathogenic fungi which is used for the management of the insect and pest which cause the different kinds of the diseases in the different crops. The mode of action is
smellier with the *Beauveria bassiana* this entemopathogenic fungus also kills the insect and pest and easily devolved on the all over body of the insect and pest.

**Fig. 1.23.** Microscopic observation of *Metarhizium anisopliae*

This fungus used as a biocontrol agent for controlling the insect and pest development in farm. The *Metarhizium anisopliae* product is available in market in both type’s powder and liquid form. The *Metarhizium anisopliae* produce abundant spores the bullet shaped conidia is produced by the *Metarhizium anisopliae*.

5. **Paecilomyces fumosoroseus**

The *Paecilomyces fumosoroseus* is another type of the entemopathogenic fungi which is used for controlling the development of disease which is caused by the insect and pest. The conidia of the *Paecilomyces fumosoroseus* are circular in shape. This fungus utilized by the farmer for managing the disease which is caused by the entemopathogens.

**Fig. 1.24.** Microscopic observation of *Paecilomyces fumosoroseus*

1.22. **Management of pests**

The different types of pests can cause the infection and loss the quality of gapes the different pests like thrips, mealy bugs, leaf hoppers, flea beetles and stem borer these are the important pests of grapes in India.
1.22.1. Thrips

The thrips absorb the juice from the ovaries of flowers and newly set berries of grapevine. The grape berries affected by the thrips showed a corky layer and browning on grape berries. The thrips also from a scab on berry surface because these berries are affected in the time of ovaries or young berries these type of affected berries are not use for the selling purpose.

The thrips are successfully prohibited by spraying. The insecticide sprays are given controlling thrips one spray of insecticide for every five days from the beginning of the bloom to berry set. The recommended insecticides are emamectin benzoate 0.22 g/L, fipronil 0.05-0.06 g/L and lambda-cyhalothrin 0.50 ml/L is used for controlling thrips in grape vineyards.

![image of thrips on leaf]

**Fig. 1.25. Thrips on leaf**

1.22.2. Mealy Bugs

The one of the most important, severe and problematic pest is the mealy bugs of grapes in India. The nymphs and adults of the mealy bugs absorb the juice from the young tender shoots of the grapevine and resultant in crinkling and stunting of the new healthy shoots. The mealy bugs ooze honey and developed sooty mold on the honey on the leaves and berries. The mealy bug affected grape bunches are not accepted for selling purpose and losses in yield may be up to fifty percent. The mealy bugs are not easily control for their controlling different practices is used in India such as-

A) The broad-spectrum insecticides sprays are not taken because devolved resistance in the pest.

B) The spray of dichlorvas at 0.1 percent alone or mix with tridemorph at 0.1 percent or mix with neem oil 0.2 percent.

C) In recent days Buprofezin 1.00-1.50 ml/L and methomyl 1.25 g/L are recommended for controlling mealy bugs in grape vineyards.
D) There is one strong biological control option is *cryptolaemus montrozieri* liberate this beetles at 8,000-10,000 per hectare this beetles are release when the grape berries are started to ripening. For good results these beetles are always released in the mixed population of grubs and adults instead of only adults.

E) The main important practice is the removing the bark because in the bark mealy bugs laying the eggs and these eggs than developed and spread the infection in the entire farm yard.

1.22.3. Flea beetles

The scratch the sprouting buds and are eaten by the adult flea beetles after the both pruning backward and forward. When the buds are injured they dont sprout very well or they not succeed in sprouting. For the controlling the flea beetle different insecticides are used such as imidacloprid 0.30-0.40 ml/L andlambda-cyhalothrin 0.25-0.50 ml/L is used for controlling flea beetles in grape vineyards. These insecticides are sprayed from the fourth day till the leaves are appearing from the bud. Now a day another type of insecticides is used like thiophanate methyl 0.71-0.95g/L.
1.22.4. Leaf hoppers

In recent years these all pests have raised as a very severe problem in all over grape growing regions of India. These leaf hoppers adults and young nymphs absorb the whole juice from the lower side of the leaves. For controlling this pest different types of insecticide are used like mixture of quinalphos at 0.05 percent and phosalone at 0.05 percent is more effective on the nymphs while tridemorph at 0.1 percent only is effective on the adults.

![Fig. 1.29. Leaf hopper on leaf](image)

1.22.5. Stem borer

The stem borer is one of the important pest in grapes. The stem borer attacks on the stem and collapses the whole grapevine. The stem borer enters stem by creating a hole on the stem of the grapevine. The stem borers multiply within the stem and destroy the grapevine very rapidly. For controlling stem borer different practices are used viz, 2 ml of dichlorvos 76% injecting in to the vines, take spray of difenthiuron 0. 8 g/L and azadirachtin 1% 2 ml/L or 5% 1 ml/L.

![Fig. 1.30. Stem borer larvae on stem](image)  ![Fig. 1.31. Stem borer adult on grapevine leaf](image)
1.23. Information about the pesticides

- The pesticides is the made up of the one or more than one compound combination.
- This compound are mainly chemically developed or produced by the microorganisms for controlling the disease causing microorganisms, pest and insects etc. which cause diseases on the different crops.
- The pesticide compound is generated by the human being for there convinious.
- The roles of pesticide are to control the growth or stop the development of pest by interfering on their inert bio process and kill the pest.
- The pesticides are used form the thousands of year ago the first time sulfur is used for fumigation of the house and the arsenic is the chemical compound which is used for the managing the pest in garden.
- After the second world war different types of the inorganic and biological compounds incorporated in the pesticides world.
- There are different types of the fungicides are presently used namely- fungicides, nematocides, herbicides, molluscicides, algaecides, biopesticides, insecticides, ovicides, miticides and antimicrobials etc. these all pesticides action are explain in short such as :-

1. **Fungicides :-**

   The fungicides are used to kill the fungus which is cause the disease in the different types of the crops.

2. **Nematocides :-**

   The nematocides are used for the killing the nematodes which also cause in disease in crops.

3. **Herbicides :-**

   The herbicides are used for the controlling and killing the weed which can occur in farm.

4. **Molluscicides :-**

   The molluscicides are used for manage the molluscs, slugs and snails. Which cause the problems in field.
5. **Algaecides** :-
   The algaecides are playing an important role in controlling the algal development.

6. **Biopesticides** :-
   The biopesticides are the products which are developed from the live microorganisms and these products are used for the managing the growth of the microorganisms which cause the diseases in the crops.

7. **Insecticides** :-
   The insecticides are developed for controlling the development of the insect.

8. **Ovicides** :-
   The ovicides are used for killing the eggs of the insect or mites.

9. **Miticides** :-
   The miticides are used for controlling the mites which is present on the plant and on the animal surfaces. These mites are also present in the culture petri plate of microorganism.

10. **Antimicrobials** :-
    The antimicrobials are used for controlling the microorganisms which are dangerous to the crop.

### 1.23.1. Pesticides effects on environment

The effects of pesticides are explained in short such as-

- The pesticides are work as biocides for controlling the living things which are harmful to the plant health.
- The pesticides are mainly made up of the one chemical compound or the mixture of chemical compound.
- When these pesticides are applied to control the target pest, insect and fungus that time these pesticides are spread all over the environment by air.
- These pesticides are very specific to that target site this is known as site specific action.
- There are mainly two types of the pesticides which is based on their site specific action 1) single site 2) multiple site.
- The pesticides are mainly contaminated the air, water and soil regularly using by farmers.
The regular application of the different pesticides on crops, they are deposited in the crop products and they are very harmful to humans.

The pesticides effects on the human health like genetically disorders, effect on fetus body, brain damage etc.

The pesticides contaminate the air this air utilized by the all living things for surviving.

The human beings are mostly affected with the respiratory tract problems and this problem mostly occurs in the labor those taking a spray in the farm for controlling the diseases.

The water body is also contaminated through the pesticides and this contaminated water is used for the plant and for drinking purpose in the animal and human.

The pesticides are deposited in the soil and soil loses their natural sources like important microorganisms, organic, inorganic compounds etc.

In soil deposited pesticides are enter in to the plants and they remain intact in plant and then enter in to the fruit which is consumed by the humans.

The pesticide residues present on the surface or enter into the fruit very well. Before eating the fruit is washed by the water but the water dose not removes the pesticide residue.

The pesticides are enter in to the water body and they destroy the water life mainly fish are die and these died fishes are eaten by the humans.

The continuous uses of pesticides are creating resistant problems in the fungus population.

1.23.2. Pesticides effects on human health

- In the time of the new pesticides development the researcher first find out this molecule not harmful to the human health but only dangers to the pest.
- The pesticides are mainly used in the agricultural practices for controlling the pathogens which are harmful to the agricultural field.
- The pesticides application are given by the humans in the time of the handling the handling person are create more contact with the pesticides and which is very danger for that person.
- For the time for spraying using the gloves, apron and mask so mining’s the chances of person connection with pesticides.
• The time of the pesticide application the spraying person not taking a mask so his suffering from the respiratory tract problem.

• After handling the pesticides the hand properly washed with the soap or detergents.

• The maximum applications of the pesticides sprays are producing the pesticide deposition problem in edible crops.

• The pesticide contaminated food are eaten by the humans are developing the number of problems like stomach pain, brain disorders, hart problem and genetical disorders.

• The fruit contain pesticide residue these residue is not washable by the water.

• After eating this pesticide residue enter in our body and causes a different problems.

• The water body is also contaminated with the pesticides because all types of the waste materials are discarded in the water body.

• The aquatic life is also in the danger zone because the all living things are affected by the waste material and the some life are dead by the west materials.

• The numbers of people consume the dead fish as a food material and those peoples are also dying.

• In our country the number of people dies because of the food poisoning.

• Those peoples are work in pesticide factory do work very carefully.

• The some peoples are work in agriculture as labor for spraying carefully handling the pesticides because the pesticides are very harmful to the human health.

1.23.3. Pesticides effects on food

❖ The pesticides are utilized for controlling the disease on food plant and animals.

❖ The farmers are not following the spray schedule which is recommended by the experts.

❖ The farmers take high dose sprays for controlling the disease, the disease is control by the high dose but the residue is deposited in the food.

❖ Another adverse effect of the frequent use of pesticide is the developed the resistance in the community.
The pesticides applying food material is consume by the humans and they are suffering from the different diseases and disorders.

Develop another option for the pesticides for controlling the diseases which occurred on the different plants.

Develop some bio-control products which are not harmful to the environment, human health and food.

The some pesticides are used for controlling the animals and these animal meats eaten by the human.

Minimizes the spray schedule in the fruiting season and take non systemic pesticides spray for controlling disease.

Does not mix the west water in the fresh, marine water body so we are take care of aquatic life.

The fishes are eaten by the different animals and humans when the infected fish are eaten by the human they are also suffering from the infection.

In the time of spraying use hood for preventing the spreading of the spray in to the environment.

Prevent the food posing death of human beings by utilizing the experts spray schedule.

1.24. Insecticide resistance in pest

The gauge insects develop a resistance against inorganic insecticide was initially recognized by the A. L. Melander in 1914. The resistance was developed to organic insecticides like DDT that gave the intimation of development of resistance in pest. The development of resistance in many insects and mites is the because of continuous use and severe application of insecticides.

The random use of insecticide induces or develops the resistant gene in some insect and this resistance gene is transferred to their offspring. The ratio of development of insecticide resistance depends on different aspects containing the rate of insect’s production, the level of resistance of insects, and the insecticides determination and there specific action and the important point is amount, schedule and number of insecticides sprays these all things are very important for development of resistance in pest. The resistance rate is increased in the greenhouses because the mites or insects multiply very fast in the greenhouse and their no small chance of
sensitive insects is available in greenhouse and the farmers take regular spray with the same insecticide.

When the insects are resistant to the insecticide the ultimately increase the sprays for controlling the pest and these extra practices increase the insecticide bill cost and growers lost the many and yield. The growers can improve the yield and save a farm by taking an advice of crop experts and the grower can also minimize the rate of expenditure. The normally all types of insects are showed the resistance to the older insecticides but the manufacturing companies are not able to formulate or synthesize the new insecticide and they are not able to supply them in to the market for controlling the resistance development in the insect. The farmers don’t have patience to wait for new insecticide they are using these resistance fungicide for controlling insects. The resistance management is only depend on the farmers by using a well time practices of insecticides and control the resistance developed in the insect by using crop expert advice.

The insecticides are characterized in to the different classes like neonicotinoids, organophosphates, carbamates and pyrethroids. The MOA (common chemical structure and mode of action) is a defined procedure in this process the insecticides destroy the insect and prevent the insect growth. The target site of insecticide means the inhibition of particular place like disturbing the enzyme activity and then automatically disturbs the metabolic pathway. The resistance development in insect is cause due to the exhaustive use of insecticide for controlling insect. In all over the world greater than five hundred species of insects which are associated with the arthropods are showed resistance to the insecticides. The resistance can occurring insect is a serious issue in now days so there is need to work on integrated pest management (IPM).

1.25. Management of insecticides resistance

The insects are resistant to insecticide is can be explain as a ‘the genetically alteration in the susceptible insect and develop the resistant population. The excessive use of insecticide, recommended dose is not use and when the proper guide line is not follow which is given by the IRAC (label recommendation for that pest species) that time resistance is developed in the insect. The some insects are cross resistance means the one insect is resistant to the two different insecticides. The multiple resistances
means the one insect is resistant to the more than two insecticides. Common approach use for management of insecticide resistance such as-

- The most powerful approach is to avoid the insecticide which is cause the resistance in insect.
- The different groups of insecticides are used for controlling the insect.
- Avoid frequent use of insecticide and take alternate sprays of insecticide.
- Use entomopathogenic fungi like Beveria, Metarizium, Verticillium and Trichoderma.
- And follow the guidelines of the IRAC.

1.26. Harvesting of grape bunches

- The 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 correct stage of harvesting the grape bunches is decided on the colour changes of variety and also depend on touch the grape commencing apical portion of the grape bunch. The three type of colours are found in grapes namely green, black and red.
- The different principals are used for the evaluating the maturity of grapes which is depends on the purpose of grapes use for example for resin production the grape bunches are favored to harvest at late stage so develop more sugar in grapes when sugar is increased the ultimately increase the dried product weight.
- The all type of determinations the grape bunches ripening is tested on the basis of sugar and acid contain this combination provide correct information for harvesting table grape and wine grape varieties. The table grape varieties are used for eating purpose and wine grape varieties are used for making a wine. The best sugar and acid contain ratio is the in between 25-30.

The grapes are export in the national and international market so the following parameter are play key role in minimize the damages of grapes during harvesting like-

- The size of berry is must be greater than the 16mm in diameter.
- The TSS (Total sugar solid) is must be greater than 17° Brix.
- The weight of Bunch must be within the 300-750 g.
- The grape bunch colour must be milky green in the case of green variety.
- The grape bunches selected for marketing must be compact not loose.
- In the grape bunches all berries are equal in their appearance like size and colour.
- The rachis and pedicel must be green and fresh.

1.27. Management of post-harvest decay

1.27.1. Sorting of grape bunches

The grape bunches are mostly sorted on the basis of the weight, size and unique appearance of grapes and this same type of the grapes are used for the packing. For packing the berry size plays a very important role instead of the shape of the grape bunches. In the time of grouping the grape bunches are handled very carefully because the damaged bunches are infected by the post-harvest pathogens.

1.27.2. Cooling of grape bunches

After harvesting the grape bunches the grape bunches are kept in cold room for minimizing the heat, reduce the moisture loss and increase the storage ability of grape bunches. The grape bunches are required the cooling step before packing the grape bunches are cooled below 4.4°C temperature for minimum six hours after harvesting from the field. The normally grape bunches are cooled in a special rooms like room attached to the cold storage compartment. During the grapes transportation the grapes are transport in the cold storage compartment when the production site is far away.

1.27.3. Storing of grape bunches

The shelf life of grape bunches are checked at room temperature for seven days only. The storing life of grape bunches are increased when the grape bunches are stored at favorable temperature that minimize the rotting and post-harvest decay of grape bunches. The postharvest pathogens are Cladosporium, Alternaria, Aspergillus, Penicillium and Botrytis and the biochemical of grapes are disturb because of the presence of pathogenic fungi.

In the hot days grape bunches are harvested that time the grape bunches are not handled properly the grape berries are damage and injured in the time of harvesting and packaging the ultimately reduce the self-life of grape bunches. The shelf life of Thompson Seedless 30-60 days, Anab-e-Shahi is 40 days and Muscat 45
days, under the specific conditions of the storage. The best conditions for storage the
grape bunches is 0°C (low temperature) with humidity 92-96% (high humidity).

1.27.4. Postharvest pathogens of grapes

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.

**Cladosporium**-This is a one type of fungus, it can cause a post-harvest disease. This
fungus is present in the environment the high sugar present in the harvested grape
they are easily grown on the berries. Fevrebal temperatures for conidia formation and
infection are 5 to 25°C.

![Microscopic observation of Cladosporium](image1)

**Fig. 1.32.** Microscopic observation of *Cladosporium*

**Alternaria**- This is another type of fungus, which can cause a post-harvest disease.
This fungus is present in the environment the high sugar present in the harvested
grape they are easily grown on the berries. Fevrebal temperatures for conidia
formation and infection are 18 to 25°C.

![Microscopic observation of Alternaria](image2)

**Fig. 1.33.** Microscopic observation of *Alternaria*
Aspergillus-This is also a very important fungus can cause a post-harvest disease. This fungus is present in the environment the high sugar present in the harvested grape they are easily grown on the berries. Feverbal temperatures for conidia formation and infection are 17 to 42°C.

Fig. 1.34. Microscopic observation of Aspergillus

Penicillium-This is a one of the important post-harvest fungus can cause post-harvest disease. This fungus is present in the atmosphere the high sugar present in the harvested grape they are easily grown on the berries. Feverbal temperatures for conidia formation and infection are above 5 to 32°C.

Fig. 1.35. Microscopic observation of Penicillium

Rhizopus-This is another important fungus can cause post-harvest disease. This fungus is present in the environment and act as saprophyte. The harvested grapes contain high sugar and this fungus is easily grown on the berries in the form of brownish type of growth. Feverbal temperatures for conidia formation and infection are 4 to 2°C.

Fig. 1.36. Microscopic observation of Rhizopus
**Botrodiplodia** - This is a most important post-harvest fungus can cause post-harvest disease. This fungus grown on berries in the form of whitish growth after few days this growth turns into black. Fevrebal temperatures for conidia formation and for infection are 15 to 27°C.

![Botrodiplodia](image)

**Fig. 1.37. Microscopic observation of Botrodiplodia**

**Colletotrichum** - This is a type of fungus it can cause a post-harvest disease. This fungus is present in the environment the high sugar present in the harvested grape they are easily grown on the berries. Fevrebal temperatures for conidia formation and infection are 20 to 30°C.

![Colletotrichum](image)

**Fig. 1.38. Microscopic observation of Colletotrichum**

**Botrytis** - This is a one type of fungus it can cause a post-harvest disease. This fungus is present in the environment the high sugar present in the harvested grape they are easily grown on the berries. Fevrebal temperatures for conidia formation and infection are 59 to 77°F with moisture in the type of fog.

![Botrytis](image)

**Fig. 1.39. Microscopic observation of Botrytis**
1.28. Marketing of grapes

The some important points are playing a very significant role in grape marketing such as-

- 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 which 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.
- For wine grapes development expenditure is very low than the table grapes variety development. Because the disease controlling is not big issue and the size development is also not the very big issue.
- In the development of the table grape varieties the growers also make a schedule for the disease and pest, insect controlling sprays and sprays for gibberellic acids.
- 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 are grape harvesting carefully harvesting the grape without injury than packing, storage and transport these things are very important for national and international marketing.

In the current days the best option is the developed the wine grapes for marketing the wine makers are want to be the quality of grapes menace the diseased free the size of the grape berry is not issue.

For development of wine grapes the grower minimizing the cost of different practices which is very important in table grapes cultivation.

The important practices in table grapes but are not important in wine grape development like gibberellic acids treatment for grapes size development, thing of grape bunch like this so ultimately the cost is not so very high.

Recently increase the demand of wine and the importance of wine to the human health. So ultimately increase the wine production.

Increase the demand of wine grapes for making the wine and for wine production also a low cost biasness.

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.

1.29. Importance of molecular techniques

For classification of plants and microorganisms, several types of DNA markers (molecular markers) represent specific regions on chromosomes are used for characterization, identification and differentiation. DNA markers are genes or loci within coding regions.

Restriction fragment length polymorphism (RFLP), inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD) and simple sequence
repeats (SSR) and amplified length fragment polymorphism (AFLP) are commonly used types of DNA markers. Reliability, quantity and quality of DNA required, technical procedure for marker assay, level of polymorphism, and cost must be considered for the use of DNA markers.

Standard RAPD technique includes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nano gram (ng) amounts of total genomic DNA under low annealing temperatures by PCR. Changes in the priming sites will result in the presence or absence of bands due to nucleotide variation between different sets of template DNAs.

RAPD markers are detected as present or absent, thus are dominant as compared to co-dominant RFLP and isozyme markers. RAPD technique has found a wide range of applications in many areas of biology because it is a simple and cost effective. RAPD amplification is directed with a single, arbitrary and short oligonucleotides primer.

**1.30. Use of sequence characterized amplified region (SCAR) marker**

The sequence characterized amplified region (SCAR) marker are polymerase chain reaction (PCR) based marker that represent genomic DNA fragment at genetically defined loci. SCAR was developed and used for identification and differentiation of objective. The SCAR primer are made up of 15-20 bp of nucleotide sequences which is collected from cloned fragment sequences (RAPD marker) of RAPD primer.

The DNA fragments of test samples are amplified by using PCR technique. Problems regarding low reproducibility in RAPDs by use of longer PCR primers, but SCARs do not face such problem. Conversion of RAPDs into SCARs to obtain a co-dominant marker can be an additional advantage. Rapid and easy to use is the main advantage of SCARs. Also, SCARs have a better reproducibility and are locus specific. Less quantities of template DNA are required for the use of PCR.

**1.31. Biological control**

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.

The word “biological control” and its shortened similar word is “biocontrol” these words have been used in various fields of biology but mostly in plant pathology and entomology. In plant pathology this is used for antagonistic microorganisms these organisms inhibit the disease and also used for plant growth promotion. 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* is mainly Gram-positive, rod-shaped and motile in nature. The some of the Bacillus species are obligate aerobes, facultative anaerobes. The bacteria was described by Ehrenberg in 1835 as “*Vibrio subtilis*”. This bacteria renamed by Cohn in 1872 as the bacteria *Bacillus subtilis*.

*Bacillus* species are able to produce different types of metabolites and enzymes. The *Bacillus* species are also produce extracellular enzyme like chitinases, glucanases, proteases, nucleases, 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
\textit{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 \textit{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 \textit{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 \textit{Bacillus} species produce volatile metabolites which is very effective for controlling plant pathogens which cause disease.

The ‘biofilm’ is produce by \textit{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. \textit{Bacillus} produce biofilm and it colonize the plants, the \textit{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 \textit{Bacillus} species is a promising biocontrol agent for management of plant disease.

The plant disease maintain is the big challenge in front of the growers because in recent days fungicide resistant development is big issue and diseases of plants reduce the yield and quality of fruit and also disturb the health of plant. For this problem one solution is biological control this strategy is very use full for growers to minimize the disease problems and as well as resistant problem also. The biocontrol agent is one of the good alternatives for the fungicide and pesticide. These biocontrol agents are naturally present in the environment like in air, soil, and on the plant roots.

In entomology entemopathogenic fungi or nematodes are used as biocontrol agents to inhibit the insect and pest populations. In both fields, the microorganisms that inhibit the growth of pathogen and pest are known as the biological or bio control agent (BCA). In biocontrol mechanism the different types of the products involved which is produced by the antagonistic microorganisms such as fermented products, extra or intra cellular metabolites, enzymes etc.
The formulation of the antagonistic microorganisms is the composition of different inhibitory compounds which is act on the plant pathogens and pest. These formulations are known as bio-fertilizers or bio-pesticides this product is made up of live organisms or there metabolites.

The biocontrol agents are not easily commercialize and the grower acceptance is also very slow because of the organisms are very depend on the temperature and pH so there is problem is use in the field for controlling plant pathogen and pest. The environmental conditions are the only problem for the commercialization of the biological product so there is need to work on environmental factors. The biocontrol agents are formulated in the form of wet table powders, dusts, and granules and aqueous or oil based liquid products, with various additives to attain all the desirable attributes.

The biocontrol agents are having capacity to completely change the fungicide and chemical pesticides or insecticides. The biocontrol agents are having good option for replacing the fungicides and minimize the resistance development in pathogen. The antagonistic microorganisms having different approaches for controlling the plant pathogens and pest and best alternatives for minimize the use of fungicides and pesticides. The biological control agents may be used in integrated management program to attain the most excellent promising results.

1.32. Research objectives
1.32.1. To isolate anthracnose causing fungal isolates infecting grapevines in major grape areas
1.32.2. To characterize of anthracnose causing fungal isolates
1.32.3. To study fungicide resistance in fungal isolates infecting grapevines in major grape areas
1.32.4. To develop SCAR marker for identifying fungicide resistant isolates
1.32.5. To isolate potentially antagonistic bacteria from grapevines and study their antagonism
1.32.6. To characterize selected efficient bacterial isolates
1.32.7. To study the biocontrol efficiency of selected isolates in vitro
1.32.8. To study the compatibility of selected isolates with fungicides
1.32.9. To study the efficacy of selected efficient bacterial isolates in management of fungicide resistant fungal isolates
Describe above all the revealed points of the research topic in the following way -

1.32.1. To isolate anthracnose causing fungal isolates infecting grapevines in major grape areas

Grape is very important type of fruit which is used for the eating purpose. The grape is made up of different types of nutrients which is play a very important role on human health. The grape cultivates in all over India but main regions are involved in grape cultivation like Maharashtra, Karnataka, Andrapradesh Tamilnadu and Punjab, Uttar pradesh. The grape is susceptible for the different types of diseases like fungal, bacetial and insects. There are three important fungal diseases like downy mildew, powdery mildew and anthracnose these diseases cause economical loss of grapes. The on of the important disaes is anthracnose which appears on the leaf, stem, petiole, tendrils and berry and this disease reduce the grape quality. So very important step is to isolate the antracnose causing fungal pathogen and than identified and characterized by using different methods.

1.32.2. To characterize of anthracnose causing fungal isolates

The anthracnose causing fungal pathogen isolated from different grape growing region of India like Maharashtra, Karnataka, Andrapradesh Tamilnadu and Punjab, Uttar pradesh. The anthracnose infected grape samples collected from the grape field. For isolation Koch Postulate method have used. The isolated fungal pathogen characterized by using different types of methods like morphological, pathological and molecular. In morphological method study growth pattern and microscopic observation of spores (size and shape), in pathological test prove the pathogenicity of pathogen on detached grape leaf and molecular study universal or species specific primers have used for the identification of fungal pathogen.

1.32.3. To study fungicide resistance in fungal isolates infecting grapevines in major grape areas

The isolated fungal pathogen used for fungicide sensitivity. This study is very important because now a days increase the resistant population of pathogen in field by applying a frequent sprays of fungicides for controlling the disease. The mostly pathogen is resistant to the one group of fungicide which is use randamlly in field for controlling the disease. For minimizing the resistance the different group of fungicides is used in the field for controlling disease. For this fungicide sensitivity study different groups of fungicides are used by applying this option reduces the resistance to one group of fungicide.
1.32.4. To develop SCAR marker for identifying fungicide resistant isolates

In fungicide sensitivity one of the isolated pathogen showed resistance to the commonly used fungicide. For easy identification of the fungicide resistance pathogen one molecular method is developed like SCAR sequence characterised amplified region marker. The SCAR marker development is mostly depend on the some polymerase chain reaction (PCR) based molecular methods like RAPD, AFLP, SSR etc. these technique help in SCAR marker development. The advantages of this molecular method are very easy, cheap and time consuming.

1.32.5. To isolate potentially antagonistic bacteria from grapevines and study their antagonism

For controlling fungicide resistance plant pathogen use biological control these biological controls are present in the environment like soil, water and plant roots. The biocontrol agents is present in the plants is known as the endophytes which is increase induce systemic resistance in plants. The main advantages of biological control are minimizing the resistance, environmental pollution and reduce the fungicide and pesticide recedue. The biological controls are the microorganisms like bacteria or fungus. In bacteria different types of genus are play vital role in plant disease management one of the genera is *Bacillus* which is madeup of the different type of the biocontrol mechanism like enzymes, secondary metabolites and biofilm etc.

1.32.6. To characterize selected efficient bacterial isolates

The using Koch postulate bacteria isolate and the isolated bacteria identified and characterized using the different types of methods like morphological, biochemical and molecular. In morphological method examine colony characteristics and using Grams staining and motility confirm the genus of bacteria and using biochemical test confirmed bacterial species. In molecular technique 16sDNA gene is sequenced and compare our bacteria with known sequence of bacteria which is present in National Center for Biotechnology Information (NCBI) site.

1.32.7. To study the biocontrol efficiency of selected isolates in vitro

The isolated endophytes is used for there antagonistic activity those isolate showed good activity which is used for further study. The selected bacterial isolates is use for the check there efficacy in the form of antagonistic activity, production of secondary metabolites and biofilm formation which is carried out in the lab (*in vitro*).
1.32.8. To study the compatibility of selected isolates with fungicides

To check the compatibility of selected antagonistic isolates with commonly used fungicide. This study is very important because the biological controls are not showed good control in field because of the different environmental conditions. So in this case we are trying to apply biocontrol agents is mixed with fungicide for this trial we must know the biocontrol agent is compatible with fungicide or not. This test is carried out in the lab by using the different concentration of fungicides from different groups.

1.32.9. To study the efficacy of selected efficient bacterial isolates in management of fungicide resistant fungal isolates

The selected antagonistic bacteria is used for the check there activity on fungicide resistant pathogen by using detached grape leaf bioassay. The selected bacterial activity is checked at different concentration which bacteria showed good activity at lower concentration against anthracnose disease those isolate uses for further study.

1.33. Hypothesis

The objectives of the research work are able to build up under the hypothesis like -

1. \(H_0\): The anthracnose disease causing pathogen will be isolated
   \(H_1\): The anthracnose disease causing pathogen will not be isolated

2. \(H_0\): The anthracnose disease causing pathogen will be characterized and identified
   \(H_1\): The anthracnose disease causing pathogen will not be characterized and identified

3. \(H_0\): The fungicide resistance in pathogen which will be isolated
   \(H_1\): The fungicide resistance in pathogen which will not be isolated

4. \(H_0\): SCAR marker will be not developed for fungicide resistant pathogen
   \(H_1\): SCAR marker will be developed for fungicide resistant pathogen

5. \(H_0\): The antagonistic bacteria will be not isolate to control fungicide resistant pathogen
   \(H_1\): Will be isolate antagonistic bacteria to control fungicide resistant pathogen

6. \(H_0\): will be characterized and identified antagonistic bacteria
   \(H_1\): will not characterized and identified antagonistic bacteria
7. **H₀**: antagonistic bacteria will show efficacy against fungicide resistant pathogen  
   **H₁**: Antagonistic bacteria will not show efficacy against fungicide resistant pathogen

8. **H₀**: Antagonistic bacteria will show compatibility with fungicide  
   **H₁**: Antagonistic bacteria will not show compatibility with fungicide

9. **H₀**: Antagonistic bacteria will show good control against fungicide resistant pathogen *in vitro* and *in vivo*  
   **H₁**: Antagonistic bacteria will not show good control against fungicide resistant pathogen *in vitro and in vivo*

### 1.3.4. Methodology

The research worker will be used following techniques to complete her research work in explain in short –

- **Isolation of anthracnose causing fungal pathogens:**  
  Causative agent of anthracnose will be isolated from major grape growing regions all over India. For isolation Czapekdox and Potato dextrose agar will be used. Pure cultures maintain on Czapekdox agar at 4°C for further study (Koch’s postulate, 1890).

- **Characterization of anthracnose causing fungal pathogens:**  
  All the fungal isolates will be identified by morphological, pathological and molecular techniques (Ayodele 2006; Munaut 2001).

- **Fungicide sensitivity of different fungal pathogens:**  
  The sensitivity of the different isolates will be evaluated *in vitro* against recommended fungicides irrespective of the chemical groups. The sensitivity will be estimated based on the EC₅₀ values (effective concentration which causes 50% inhibition of growth) on liquid medium using the poisoned food technique (Nene and Thapliyal, 1979).

- **Development of Molecular Marker (SCAR):**  
  Fungicide resistant isolates will be differentiated by using RAPD primers. Bands specific to resistant isolates will be used for cloning, sequencing and designing primer. The validity of the primers will be confirmed using different types of the samples like pure isolated samples and natural field isolates etc (Rugienius R, 2006).
• **Isolation of antagonistic bacteria from grapevines:**
  Bacteria will be isolated from healthy shoots of grapevines growing in the research vineyards of NRC for grapes on nutrient agar (NA). Pure cultures will be developed by using dilution plate method and stored on nutrient agar at 4°C till required (Koch’s postulate, 1890).

• **Check antagonistic activity of purified endophytes:**
  The antagonistic potential of the isolated endophytes will be tested on nutrient agar by dual culture method. Bacteria will show strong zone of inhibition will be selected for further studies (Cubeta, 1985).

• **Characterization of selected endophytes:**
  The selected efficient isolates will be characterized based on morphological, biochemical and molecular analysis (Harley Prescott, fifth edition; Holt, 1994).

• **Studies of secondary metabolites of antagonistic bacteria against fungal isolates:**
  *In vitro* study sensitivity of fungal isolates to crude extract and volatile compounds of antagonistic bacteria for determines the percent inhibition of the pathogen which causes anthracnose disease (Mousivand et al., 2012).

• **Fungicide sensitivity of different endophytes:**
  The sensitivity of the different endophytes will be evaluated *in vitro* against recommended fungicides irrespective of the chemical groups. The sensitivity will be estimated on liquid medium (Basha et al., 2010).

• **Efficacy of selected efficient endophytes against fungicide resistant pathogens:**
  The selected endophytes will be tested *in vitro* for their antagonism against fungicide resistant isolates. The efficient ones will be evaluated on detached leaf bioassay under controlled conditions (Sawant IS 2012b).

• **Biocontrol efficiency of selected endophytes *in vivo***:
  The control of diseases will be studied on field grown vines after foliar applications of the antagonistic bacteria. Disease will be evaluated by following standard rating scales. (Sawant IS 2012b).