

**Chapter V**

***SUMMARY AND CONCLUSIONS***

## **Summery and Conclusions**

Grape (*Vitis vinifera* L.) is most important fruit crop grown in western Maharashtra. It is an important cash crop cultivated mainly for making wine, raisin, table purpose and other products like juice jelly etc.

The climatic conditions in Western Maharashtra are not suitable for healthy grape cultivation. The humid atmosphere and low temperature with highly clayed soil are not preferred for healthy grape cultivation. Hence, occurrence of various pests like downy mildew, powdery mildew and mealybugs is common in Western Maharashtra. The period of December to March months is very important for grape production during which severe attack of various pests have been reported which affect grape production up to 80 % to 90 %. To avoid this loss, extensive use of chemical pesticides for spray is made by grape cultivators. The frequently fluctuating climatic conditions like rains and cloudy atmosphere with the susceptible grape varieties suffers and severe loss is reported. The use of harmful chemical pesticides creates many problem like residual effect to fruits and consequently to the consumers and environment.

In recent years, grapes are cultivated on large scale for export to foreign countries. The food products with residue of chemical pesticides are completely banned in Western countries. Hence, according to International Plant Protection Convension, 1951, Phytosanitary Certificate should be taken for export of fruits and other food products. According to, plant Quarantine Order, 2003 (Government of India), the farmer who want to export the products will satisfy the conditions mentioned in the act. The products free from residue can be only exported to the foreign countries.

The extensive use of chemical pesticides adversely affects the export of grapes to foreign countries due to residue of banned chemicals. Hence, according to Plant Quarantine Order, 2003, the grapes are not sprayed with harmful chemicals before the period of harvest which develops residual effect in them.

Hence, various biopesticides prepared from medicinal plants are used under the Integrated Pest Management Programme. The use of biopesticides like Azadirachtin prepared from *Azadirachta indica* (Neem) is well pronounced and adopted by cultivator. Last 5 Years or so the extract prepared from *Agave cantala* leaves is widely used as biopesticides in Western Maharashtra. *Agave cantala* is a CAM succulent and commercially known as fibre crop. The plant parts possess some active phytochemicals like, saponins, steroidal glycosides and alkaloids. The present study deal with application of *A. cantala* leaf extract on grape leaves infected with downy mildew in comparison with healthy and infected leaves.

In the present work, the biochemical studies in grape leaves infected with downy mildew and infected but *A. cantala* leaf extract sprayed in comparison with normal healthy, disease free leaves of cultivar Thompson seedless are undertaken. The results are summarized and conclusions derived from the same are presented as follows:-

#### **A) Mineral nutrition studies:-**

##### **1) Nitrogen:-**

The analysis of nitrogen content in healthy infected and infected but *A. cantala* leaf extract sprayed leaves of grape cv. Thompson seedless indicated that healthy leaves contain highest level of nitrogen as compared to leaves infected with downy mildew and sprayed the highest nitrogen content recorded in healthy leaves was 1.5 % at fruiting stage. While, lowest nitrogen content recorded in infected leaves was 0.5% at harvest stage. The leaves infected with downy mildew showed considerable recovery of nitrogen after *A. cantala* leaf extract spray. The nitrogen contents in sprayed leaves were 0.9%, 1.0 % and 1.1% at flowering, fruiting, and harvest stage respectively. The high nitrogen contents suggested susceptibility of cultivar Thompson seedless to fungal diseases. However, *Agave* spray decreased the amount at considerable proportion

## **2) Phosphorous:-**

The analysis of phosphorous contents revealed that healthy leaves have highest concentration of phosphorous than infected and sprayed leaves. The highest concentration of phosphorous was recorded at fruiting stage i.e. 0.72 % in healthy leaves. The level of phosphorous in leaves infected with downy mildew was very low as compared to healthy and sprayed leaves. The lowest phosphorus content (0.35 %) recorded in infected leaves at flowering stage. After *A. cantala* leaf extract spray, the infected leaves shows consider warble high concentration of phosphorous. The values of phosphorous in sprayed leaves were 0.50 % 0.57 % and 0.53 % at flowering, fruiting and harvest stage respectively. The increased amount of P is may be in disease resistance response of grapes due to leaf extract spray.

## **3) Potassium:-**

The analysis of potassium contents in healthy infected and infected but *A. cantala* leaf extract sprayed leaves of grape cultivar Thompson seedless indicated that accumulation of potassium in infected leaves after Agaves leaf extract spray. The healthy grape leaves contain highest concentration of potassium than infected leaves. The leaves infected with downy mildew have lowest potassium contents i.e. 1.2 %, 1.0 % 1.4 % at flowering, fruiting, and harvest stage respectively. Potassium is an important macro element in relation to disease resistance (Sastry and Nariani, 1962). Hence, the greater concentration of potassium in infected leaves after *A. cantala* leaf extract treatment also suggested a possibility of induction of disease resistance.

## **4) Calcium:-**

In the mineral nutrition studies, it was recorded that calcium contents of grape leaves infected with downy mildew were high as compared to healthy and *A. cantala* leaf extract sprayed leaves. The values of calcium recorded in infected leaves were, 2.0% 2.4% and 2.8% at flowering fruiting, and harvest stage respectively. While, the sprayed leaves exhibited 1.3 %, 1.4 % and 1.6 %

of calcium at flowering, fruiting, and harvest stage respectively. The accumulation of calcium in infected leaves may be due to early senescence developed by fungal disease. The low level of calcium in infected leaves after the treatment of Agaves leaf extract may indicated that the process of senescence may stopped with inhibition of pathogen growth and development in the successive stage of grape life cycle.

### **5) Magnesium:-**

In present studies, high magnesium contents were recorded from infected leaves after *A. cantala* leaf extract spray. While, untreated infected leaves showed low magnesium contents. The concentrations of magnesium in sprayed leaves were 0.61%, 0.55%, 0.57 at flowering fruiting and harvest stage respectively. The leaves infected with downy mildew contain 0.49%, 0.40% and 0.48 % of magnesium at flowering, fruiting, and harvest stage respectively, this analysis was supported by the values of chlorophyll contents. The spray may increases disease resistance.

### **6) Iron:-**

The concentration of iron recorded in healthy leaves of grape c.v. Thompson seedless were higher than infected and Agave leaf extract sprayed. The increase in iron contents were recorded in infected leaves after *Agave cantala* leaf extracts spray. The values recorded in sprayed leaves were 59ppm, 67ppm and 72ppm at flowering fruiting and harvest stage respectively. Whereas, concentration of iron in untreated leaves were 66ppm, 75ppm and 80ppm. The increase in iron content in sprayed leaves suggested induction of disease resistance. The disease resistance increases with increase in iron concentration (Hegde and Karande, 1978). Thus, the role of iron may possibly on the similar lines toward development of disease resistance in grape.

### **7) Manganese:-**

An increase in concentration of manganese was noticed in Agaves leaf extract sprayed grape leaves. While, the leaves infected with downy mildew shown considerable low manganese contents. The manganese contents noticed in infected leaves were 56ppm 53ppm and 55ppm at flowering, fruiting and harvest stage respectively. Whereas, infected but *A. cantala* leaf extract sprayed leaves contains 67ppm, 65ppm and 69ppm manganese at similar stages. The highest concentration of manganese was observed in healthy leaves at fruiting stage i.e. 74ppm. The high manganese content due to Agave leaf extract spray possibly interrelated to disease resistance development.

### **8) Zinc:-**

In the present studies, concentration of zinc were more in *A. cantala* leaf extract sprayed grape leaves than non-treated leaves infected with downy mildew. The concentration of zinc in Agave leaf extract sprayed grape leaves were 59ppm, 56ppm and 58ppm at flowering, fruiting and harvest stage respectively. While, those in leaves without treatment infected with downy mildew were 40ppm, 43ppm and 38ppm. The highest zinc concentration noticed at fruiting stage, 66ppm in healthy leaves. The accumulation of zinc in sprayed leaves may enhances phenol contents and develop immune system in the grape plant.

### **9) Copper:-**

The analysis of copper content in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape cv. Thomson seedless indicated that the higher values of copper contents were recorded in sprayed leaves i.e. 18 ppm 20 ppm and 15 ppm at flowering, fruiting and harvest stage respectively. Whereas, in leaves infected with downy, low levels of copper were noticed at every stage. The values of copper recorded in infected leaves

were 10 ppm, 8 ppm and 10 ppm at flowering, fruiting and harvest stage respectively. An accumulation of copper in leaves infected with downy mildew after *A.cantala* leaf extract spray may enhances disease resistance of grape.

#### **10) Molybdenum:-**

The concentration of molybdenum in the healthy leaves recorded 0.32 ppm, 0.31 ppm and 0.30 ppm at flowering, fruiting and harvest stage respectively. While, in leaves infected with downy mildew, values of molybdenum were 0.11 ppm, 0.9 and 0.10 ppm. The level of molybdenum contents increased in *A.cantala* leaf extracts sprayed leaves. The values of molybdenum in sprayed leaves were 0.24 ppm, 0.25 and 0.22 ppm at different stages. Hence, accumulation of molybdenum in infected leaves after Agave leaf extract treatment would be important aspect in developing disease resistance in all 3 stages of growth in grape.

#### **11) Cobalt:-**

The analysis of cobalt in healthy, infected and infected but *A.cantala* leaf extract sprayed grape leaves of c.v. Thompson seedless indicated that the leaves infected with downy mildew shown greater concentration of cobalt (0.04 ppm, 0.04 ppm, and 0.03 ppm) than healthy and Agave leaf extract sprayed leaves. The values of cobalt noticed in healthy and sprayed leaves were same except, at harvest stage i.e. 0.03 ppm in healthy and 0.01 ppm in *A. cantala* leaf extract treated leaves.

The cobalt plays important role in some enzymes system operations in plants. It is possible that increased concentration of cobalt may play role in disease resistance development by controlling enzyme activities in grape plant.

## **B) Organic constituents:-**

### **1) Moisture percentage:-**

The status of moisture % in healthy infected and infected but *A. cantala* leaf extract sprayed leaves of vine plants (cv. Thompson seedless) was analyzed at different stage. The moisture percentage of healthy leaves recorded at flowering, fruiting and harvest stage were higher than infected and sprayed leaves. The moisture percentages noticed in sprayed leaves were considerable high than infected leaves. High moisture percentage in infected leaves after *A. cantala* leaf extract spray suggested involvement of Agave leaf extract in disease reduction mechanism by balancing the moisture contents which is necessary factor for growth of pathogen.

### **2) Pigments:-**

#### **a) Chlorophylls:-**

The chlorophyll contents recorded in healthy grape leaves were higher as compared to infected and *A. cantala* leaf extract sprayed leaves. The increase in chlorophyll contents were noticed in infected leaves after Agave leaf extract spray. The ratio and chl.a/b was higher in infected leaves than healthy and sprayed leaves. The concentration of chlorophyll pigments is related to the rate of photosynthesis. Hence, increase in chlorophyll contents after spray in infected leaves suggested decrease in chlorophyll degradation due to infection, increased biosynthesis of chlorophylls and high rate of photosynthesis. From these studies, the possible role of Agave leaf extract spray in disease resistance development in grape may be altered photosynthetic metabolism in infected plants.

#### **b) Carotenoids:-**

The healthy leaves of grape cv. Thompson seedless exhibited high carotenoids contents than infected and *A.cantala* leaf extract sprayed leaves however , sprayed leaves exhibited considerable carotenoids content as



compared to untreated infected leaves. It is important reasons of destruction of pigments due to pathogen's activities and utilization of substances for its growth.

### **3) Carbohydrates:-**

The carbohydrates were analyzed at flowering, fruiting and harvest stage from healthy infected and infected but *Agave cantala* leaf extract sprayed grape leaves in the form of reducing sugars, total sugars and starch.

#### **a) Reducing sugars:-**

The analysis of reducing sugars contents in healthy, infected and sprayed leaves of grape c.v. Thompson seedless indicated that leaves infected with downy mildew contains low level of reducing sugars than healthy and sprayed leaves. The infected leaves showed high level of reducing sugars after the treatment of *A. cantala* leaf extract. High reducing sugars content in healthy leaves suggests susceptibility of cultivar Thompson seedless. While, high reducing sugars in sprayed leaves as compared to infected leaves indicated disease inhibition after Agave leaf extract treatment.

#### **b) Total sugars:-**

The healthy leaves of grape cv. Thompson seedless contain higher level of total sugars as compared to infected and *A. cantala* leaf extract sprayed leaves. The lowest total sugars contents were recorded in leaves infected by downy mildew at every stage of grapevine development. The total sugars content were higher in infected but sprayed leaves, may be due to inhibition of pathogen growth and development.

### **c) Starch :-**

The starch contents recorded in infected but *A. cantala* leaf extract sprayed leaves were higher than healthy leaves. The leaves infected with downy mildew contained low level of starch at every stage of grapevine development. The highest value of starch was recorded in sprayed leaves at flowering stage. The increased contents of starch were found related to decreased activity of invertase in infected leaves after the treatment of Agave leaf extract.

Collectively the carbohydrate status in infected leaves was increased before *A. cantala* leaf extract sprayed of Mg indicate that for the nutrition process of pathogen, total carbohydrates are utilized on large scale. Hence, decrease in carbohydrate contents was recorded in infected plants than the control one. However, the Agave leaf extract sprayed plants showed higher values of the same. It is possible that the fungal activities for growth and development are controlled by the leaf extract spray and amount of carbohydrates spray and amount was restored similar to control plants.

### **4) Total polyphenols:**

The total polyphenol contents were recorded in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape cultivar Thompson seedless at flowering, fruiting and harvest stage. The levels of total polyphenols were increased in grape leaves after infection of downy mildew. However, the increase in total polyphenols in Agave leaf extract treated grape leaves was higher than infected leaves. Polyphenols are important factors in determination of disease resistance. High level of polyphenols in infected leaves after Agave leaf extract spray confirms the induction of disease resistance mechanism. The level of total polyphenols was higher in sprayed leaves at flowering, fruiting and harvest stage. It was noticed that healthy leaves contain low polyphenols. Hence, the present studies confirm that grape cultivar Thompson seedless is susceptible to downy mildew. However the Agaves leaf extract increases the

total polyphenol contents in the sprayed plant which may possible asset in development of disease resistance in grape plant.

### **C) Enzymes:-**

#### **1) Catalase:-**

The activities of enzyme catalase were recorded in healthy infected and *A. cantala* leaf extract sprayed grape leaves of cultivar Thompson seedless at flowering, fruiting and harvest stage. The leaves infected with downy mildew exhibited high activity of enzyme catalase than healthy and sprayed leaves at every stage. While, leaves infected with downy mildew showed low activity of catalase after *A. cantala* leaf extract treatment. Hence, low activity of catalase in treated leaves suggested partial relief to host from stress caused due to infection of downy mildew. The pathogen increases catabolic processes in the host which are related to enzyme synthesis and activity. The Agave treatments control this degardetive mechanism by controlling catalase activity in the infected grape leaves which finally leads to the disease resistance mechanism.

#### **2) Peroxidase:-**

The activites of enzyme peroxidase were recorded from healthy, infected and sprayed leaves of grape. The high activity of enzyme peroxidase was notice in infected leaves after *A. cantala* leaf extract treatment than healthy and infected leaves at every stage of grapevine development. High activity of peroxidase and high polyphenol contents in sprayed leaves suggested induction of disease resistance to inhibit pathogen growth due to Agave leaf extract contents. The induction of enzyme activity is one of process in disease resistance.

#### **3) Polyphenol oxidase:-**

The activity of enzyme polyphenol oxidase were recorded in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape (cv. Thompson seedless) at flowering, fruiting and harvest stage. The

high activities of polyphenol oxidase were noticed in leaves infected with downy mildew after the treatment of *A. cantala* leaf extract than infected and healthy leaves. The high polyphenol oxidase activity accompanied with high polyphenol content and copper suggested induction of disease resistance mechanism in infected leaves after treatment of Agave leaf extract possible in disease resistance by reducing enzyme activities.

#### **4) Invertase:-**

The activities of enzyme invertase were recorded in healthy, infected and infected but *A.cantala* leaf extract sprayed leaves of grape (c.v. Thompson seedless) at flowering fruiting, and harvest stage. The high activities of enzyme invertase were noticed in leaves infected with downy mildew. While, decrease in invertase activities were notice in infected leaves after *A. cantala* leaf extract spray. High activity of invertase in infected leaves suggested rapid degradation of sucrose into glucose and fructose for development of pathogen. The low activity of invertase in Agave leaf extract treated leaves indicated limitation of nutrition supply to pathogen and involvement of *Agave cantala* leaf extract in disease resistance. The possible degradation of carbohydrates is controlled due to control of invertase activity. It is an important and recordable mechanism of disease resistance development in the grape by a CAM succulent like Agave.

#### **D) The significant findings of present work:-**

The present work is related to pathophysiological studies in infected grape crop by downy mildew disease in Western Maharashtra. This disease causes severe economic losses to cultivar and changes agricultural economics of the country. Hence, use of Agave leaf extract treatment in disease control is adopted and the significant result are enlisted as follows. The mineral metabolism studies suggest that pathogen's activities changes N<sub>2</sub>, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Mo, and Cobalt contents in infected plants. The application of Agave leaf extract controls the pathogen development during

flowering, fruiting and harvest stage in grapevine. Simultaneously, the above mineral status is maintained in relation to disease control mechanism.

The organic contents like moisture percentage, pigments, carbohydrates and total polyphenols studies reveals interesting results indicating interrelation between host grape plant and the pathogen (downy fungus) interactivity, during flowering, fruiting and harvest stage. The pathogen's incidences, development as well inhibitory of growth is due to application of Agave leaf extract treatment to the infected plant. Naturally, the decreases in organic content due to catabolic activities of pathogen in infected plant are recorded whereas spray treatment balances the amount of individual constituent in better position. It suggests the metabolic processes showing tendency toward increasing disease resistant status of infected plants.

The important aspect of present research work is to study some enzymes related to pathogenesis mechanism in infected plant. Simultaneously these studies are extended further to observe effect of Agave leaf extract spray to grape in controlling the downy disease. The observation and result obtained are interested revealing the role of enzyme catalase, peroxidase, polyphenol oxidase and invertase at different stages of development in grape.

It is observed that the activities of these enzymes are considerable in healthy non infected grape plants. All enzymes are showing maximum activities in infected plant than the control in all 3 stages studied. Whereas, the enzyme activities in infected but Agave leaf extract treated plant become normal i.e. similar like healthy plant. It suggests the preventive role against disease by spraying of Agave leaf extract to grape plant. Thus disease resistance may be developed due to absorption of leaf contents of Agave as its biofungicidal nature.

It is possible that fungal growth requires specific pH, temperature, minerals etc. The Agave leaf extract contain the organic acids (Malic acid) in large amount, along with minerals, polyphenols, alkaloids and saponins etc. They create unfavorable environment for proper growth of fungus by changing

pH status. Hence, disease incidence is inhibited and plant becomes normal due to sequential spraying of extract during specific time interval. Finally the cultivars may obtain disease free, residual free fruits with high quality of tastes, sugar percentage, and more durability. It possibly increases the total yield of cultivar.

Thus, present investigation suggests the use of *Agave cantala* leaf extract spray treatment to the grape crops controls downy disease incidence and increases the disease resistance in grape plant. These results are confirmed by biochemical and enzymes studies in the present work.