CHAPTER - V

SUMMERY AND CONCLUSIONS

Betle vine (Piper *betle*) is a member of family piperaceae . Piper is larger genus with more than 1000 species. It is perennial , dioecious , climbing shrub with glossy hearte shaped leaves used for chewing as fresh along with some supplements. The leaves are aromatic, digestive, stimulant and carmanitive. It is also medicinal and nutritional value.

Betle vine is native of Malaysia. It is important commercial cash crop of India , Bangladesh, Shrilanka and to some extent of Singapur, Thialand , Phillipines and New guinea . In India it is grown over an area of 40,000 hecters. The vine is grown on commercial scale on cash crop sin the states such as Assam , West Bangal , Bhiar , Maharashtra , Utter Pradesh , Meghalaya, Orissa, Karnataka, Kerla , Andhra Pradesh and Madhya Pradesh. About 45,000 hectare area is preasently under Betle vine cultivation.

In Maharashtra 1100 hectare region is under the crop in Sangli,Satara,Kolhapur,Solapur, Pune, Nasik, Jalagaon,Buldhana ,Akola , Nagpur and Thane district The annual turnover of Betle vine is more than Rs. 67 crores engaging 15 lack families in Maharashtra.

The Betle vine crop salthough is a source of livehood for hundreds of people sengaged either directly for its cultivar or indirectively in its trade did not get its due importance in terms of research and development in the country until 1981.It is highly labrous intensive crop therefore, it gives livehood to many marginal , submarginal and landless and provide reasonable income.

The All India Coordinated Research Projcct On Betle Vine was sanctioned and started functioning during 1981on recommendation of a special team constituted by the ICAR. There are several varaties of Betle vine distinctly differing in respect of morphology,colour of leaf , taste and aroma. Some of the important varities of Betle vine are Desi , Kali, Ramtake pan of Maharashtra , Bangla, Mithu of Assam, Desi , Kapoori, Banarasi, Calcuttia , Bangla pan of Uttar Pradesh , Mar pan of Tamil Nadu , Lavangi in Andhra Pradesh. The two Betle vine varities viz. Kapoori and Bangla are commenly grown in India .Variety Kapoori is mostly grown in Maharashtra , Andhra Pradesh , Tamil Nadu, and Kerla while Bangla variety is grown mostly in Uttar Pradesh , Bihar, Madhya Pradesh and West Bengal and Assam. In the state of Maharashtra there are quite good acerage under betle vine and the Kapoori type is mostly grown.The familiar Betle vine commonily known as'' Banarasi pan''is grown in Uttar Pradesh and oter Eastern states of the country.

India is the largest producer of Betle leaves and the value of annual production of leaves has been estimated to be about Rs. 700 crores. The normal economic life of Betle vine is under closed conservatory is 3 – 4 years.

The Betle vine crop is highly laborious intensive and particularly suited to small holdings. Once established , it becomes a perennial source of employment and cash crop flow for day to day requirement to the farmer.

Piper betle is climber, it requires support system. It is cultivately primerly in 1) Under natural ecosystem 2) Under artificially created shade known as Boroj , Baraja. Also third system known as mixed cropping with arecanut, coconut, jackfruit, mango etc. is followed in a limited scale.

The growth of Betle vine is very fast in September - November and consequently the irrigation demand becomes rather heavy , that is fallowed by a light spray in December – January.

The leaves are used traditionally for chewing purpose with arecanut and lime. They are medicinally important used in catarrhal and pulmonary afflications. The low temperature (4- 8^0c) storage of Betle leaves reduced the senescence up 40 days. It is considered useful for many disease affecting human beings.

In Maharashtra mostly local types of *Piper betle* are grown . The vine is delicate, woody climber associated with inherent problems such as low yield potential (in terms of number of leaves per vine), smaller leaf size, more pungent taste and susceptibility to various diseases such as *Phytophthora* leaf spot and foot rot ,*Anthracnose* and *Sclerotium wilt.* The Betle vine dangerously affected by *Phytophthora* leaf rot and root rot a fungal disease appreciably reduces the market value of the produce resulting in low economic out – turn. The leaf rot and root - rot is the most comman fungal disease identifided as *Phytophthora parasitica* Var. piperina. The report of such disease are seen from other countries such as Malayasia , Srilanka , Bangladesh , Myanmar and Sarawak.

However resent reports indicate that the yield is reducing day by day due to all ill managed agricultural practices. More – over Betle vine diseases leaf spot and Root –Rot are also contributing to the decline in leaf yield.

In the present investigation four samples of *Piper betle* L. of variety Kapoori is Control , Infected and *Trichoderma* treated leaves hane been selected for change in enzymes of mycoparasitism by *Trichoderma* spp. in control of Fot –Rot disease. The Betle vine containing variety Kapoori have been selected.It is affected by Root – Rot disease caused by *Phtyophthora parasitica* Var. piperina.It was observed infection of leaf spot and Root – Rot disease.These diseases change the morphology and physiology olso change the activity of enzymes of vine, which ultimetly affect the vine metabolisim , growth and yield adversely.

The *Phytophthora* disease occurance on Betle vine is severe problem to the cultivers in Maharashtra.The importance of antagonic effects of the fungus *Trichoderma* in controlling vine pathogens was studied by several workers. The overall review of this work given by A.M. Deshmukh; (2001) The soil borne pathogenic suppression is due to antibiotics produced by *Trichoderma* spp. due to mycoparasitism.Hence in the present investigation pathophysiological studies in connection with *Phytophthora* disease occurance in Betle vine were undertaken.

Although overall retardation due to infectiom is well established.Chemical remedy causes health hazards to human being also

minimize leaf quality,leaf taste also chemical treatement are laborious and chief .It degread leaf quality .Therefore considering this view attempts have been made in the present work to gives treatement of *Trichoderma* treatement meet only cure disease of Betle vine but also increase yield and quality of leaves also increases economic conditions of farmers and cultivers.

The studies are made to analysed the Organic, Inorganic constituents and changes in the enzyme activities after treatement of *Trichoderma viride* on Betle vine i.e. Control, Infected and *Trichoderma* treated leaves of variety Kapoori.

The significant findings in Control ,Infected and *Trichoderma* treated leaves of Betle vine are summerised.The possible conclusions regardingdisease control mechanisims by *Trichoderma* mycoparasitism in Betle vine infected by foot-root are explained

A) Physicial parameters

a) Moisture percentage

The leaf moistuer percentage increased maximum in *Trichoderma* treated leaves than Infected leaves and Control leaves of *Piper betle* L.Var. Kapoori. This is due to excessive hyrertropied and enlargement of leaves. It is mainly influenced by pathogenesis,while maximum increased moistuer percentage are in *Trichoderma* treated leaves are due to activities for controlling disease development in the host tissue

Dry matter percentage

Dry matter percentage decreased in Infected leaves wich is due to loss of moisture , chlorophylls and changes in catabolic activities of enzymes in Infected leaves whereas it is higher in Control leaves than *Trichoderma t*reated leaves of Betel vine L.Var. kapoor.

b) Soil pH

The soil pH of selected pam – mala show variations in pH in the infection of *Phytophthora parasitica* Var. piperina . Infected leaves grown in soil contain alkaline pH , after *Trichoderma* treatemt alkaline soil shows slight increase in pH . The possible variation in pH contents may be due to pathogenic activities of fungus in root sphere zone .

c) Soil Temperature

Soil Temperature of selected pan mala of *Piper betle* were stuide wich show loss in soil temperature is observed during infection of *Phytophthora* disease.However increase in soil temperature in normal state were observed. After treatement of *Trichoderma* , soil temperature may be influnces the growth of pathogen. Pathogen grow vigerously at low temperature while treatement of *Trichoderma* were made soil free from pathogen .Variation in temperature has significant negative correlation with disease development in Betle vine.

B) Organc Constituents

i) Chlorophylls

The maximum decrease in the value of total chlorophylls were observed in Infected leaves during pathogenesis while Control leaves contain higher chlorophyll content as compared to *Trichoderma* treated leaves . The ratio of chlorphyll –a to chlorophyll-b were also decreased inInfected leaves of *Piper betle* during pathogenisis. This decrease in chlorophyll contents may be due to metabolic disturbances and loss of structural integrity of chloroplast in Infected leaves than in Control leaves . The catabolic activity enhances due to pathogens activity wich results in chlorophyll content variations.

ii) Carotenoids

The Carotenoide contents are also affected by the infection of Phytophthora *parasitica* in Betle vine . The vine is very sensitive to Foot-Rot disease. Control leaves contain maximum carotenoids while *Trichoderma* treated leaves show maximum carotenoid as compaired to Infected leaves.but it observed minimum than Control leaves .This decrease in carotenoide content may be due to pathogenic activities and the contents to *Trichoderma* treatement to Infected vine brings carotenoide contents to there normal status in Betle vine.

iii) Total polyphenols

The total polyphenol contents in Infected leaves are observed less than in Control leaves while it show increased after *Trichoderma* treated leaves may be due to the *Trichoderma* treatement thus plays an important role polyphenol metabolisim during control of *Phytophthora* infection in Betle vine.Major role of polyphenols in development of disease resistance may be appears in the present studies.

iv) TAN

AfternoonTAN

The value of TAN are increased in Control leaves than *Trichoderma* treated leaves and Infected leaves while decreased value of TAN are observed in Infected leaves than *Trichoderma t*reated leaves of *Piper betle* L.Var.Kapoori.

Evening TAN

The value of TAN are increased in Control leaves than *Trichoderma* treated leaves and Infected leaves of *Piper betle* L.Var. Kapoori. The value of TAN is increased in *Trichoderma* treated leaves as compaired to Infected leaves of Betle vine. Infection enhanced chlorosis and breakedown of many metabolities leading to This due to pathogenic increased TAN in Infected leaves of variety Kapoori.

The total amount of acids e.g. malic acids and others are synthesized in relation to infection to the leaves. The CAM is not dected in *Piper betle* varities.However acid contents may in flunce the disease development process to the pH of leaves may changes due to treatement and it is due to the reason in controlling disease.

i) Carbohydrates

i) Reducing sugar – Contents of Reducing sugar were enhanced during infection of pathogen. Maximum reducing sugars were observed in Infected leaves than Control leaves and *Trichoderma* treated leaves of *Piper betle* L.Var.Kapori.

ii) **Non reducing sugar-** Values of non reducing sugar content were remarkably affected by infection .decreased value of non reducing sugar were observed in Control and *Trichoderma* treated leaves while maximum content were observed in Infected leaves of Betle vine.

iii) Total sugar - the total sugar are maximum increased in *Trichoderma* treated leaves and Control leaves while very low value are observed in Infected leaves of *Piper betlel.*Var. Kapoori.

iv) **Starch** - Starch contents are observed maximum in Infected leaves than *Trichoderma* treated leaves and Control leaves leaves .Very low starch were observed in control leaves of *Piper betle L.*Var. Kapoori.

v) Total carbohydrates - the maximum increased value of total carbohydrate were observed in Control leaves . However *Trichoderma* treated leaves contain increased value of total carbohydrate than Infected leaves while it observed less than Control leaves of Betle vine Var. Kapoori.

The variations in total carbohydrate contents are due to metabolic activities of *Phytophthora* in diseased vine.The mycoparasitic activities of *Trichoderma* induces utilization of thease starch , sugars in control of disease or the development of disease resistance in treated vine.

v)Total soluble Protein

The total soluble protein content were positively influenced by pathogenic infection . The maximum value were recorded in Infected leaves than *Trichoderma* treated and Control laves of *Piper betle* L.Var.Kapoori. This increase in protein nay be due to synthesis of protein and enzyme by pathogen in host tissue because plenty amino acids are available in the breake down of proteins in host cell. However value of protein content were observed maximum in *Trichoderma* treated leaves as compaired to Control leaves.

C) Inorganic Constituents

i) Sodium :

Sodium content is slightly decreased in Infected leaves during pathogenic infection .The maximum increased value of sodium were observed in *Trichoderma* treated leaves than Control and Infected leaves of *Piper betle* L.Var.Kapoori. More decreased sodium were observed in Infected leaves.The decrease of sodium may be due to either catabolic changes in host tissue. It may possible that the infection might have inhibited the absorption of sodium. However the increased sodium content after treatement of *Trichoderma* may be due to either favourable activities and tolerance of host tissue of Infected leaves for disease resistance.

ii) Potassium

Accumulation of potassium were observed in the *Trichoderma* treated infected leaves than Control leaves .However due to pathogenic infection potassium content were decreased in Infected leaves of *Piper betle* of Variety Kapoori. Very low potassium were observed in Infected leaves infected by *Phytophthora* disease.

Thus potassium contents may affect the enzyme synthesis and activities in diseased vine. It may be due to important role of ' K' in

plants metabolisim , in both plants. It is possible that ' K' has important in development of disease tolerance in infected vines by *Trichoderma* treatement.

iii) Calcium

Calcium values are higher in *Trichoderma t*reated leaves. The maximum increase of calcium were observed in *Trichoderma t*reated leaves than control leaves.while more decreased value of calcium were observed in Infected leaves of *Piper betle* L. Var. Kapoori. The vital role of calcium in disease resistance against against infection in Betle vine may be possible . However present observations are not on the similar lines. Variations in the results may be awaiting .

iv) Magnesium

Magnesium content increased in *Trichoderma* treated than Control leaves while maximum decreased calcium were observed in Infected leaves of *Piper betle* L.Var. Kapoori.

The metabolic activities related to Mg concentration in plants mostly affect the enzyme synthesis and degreadation.Mg thus play important role in conservation of enzyme status , hence it is

possible that Mg status in Betle vine Control and Infected and *Trichoderma* treated is due to mycoparasitism of these members.

V) Phosphorus

Phosphorus content is considerabely increased in *Trichoderma* treated leaves than Infected treated leaves.The value of Phosphorus were observed less in Control leaves than *Trichoderma* treated leaves while it is observed maximum than Infectrd leaves. of *Piper betle* L.Var. Kapoori.The maximum content of phosphorus are due to positive effect of *Trichoderma* treatement on Infected leaves . It appears that phosphorus has some role to play in disease resistance in Betle vine .

Vi) Iron

It is observed that the decrease in Iron content in Infected leaves but there is considerable maximum Iron content is observed in *Trichoderna* treated leaves than the Control and Infected leaves of Betle vine Kapoori. It is due to internal changes of pathogen activities in imbalasing the overall metabolisim during disease development . The controlling mechanism by *Trichoderma* to the *Phytophthora* incidence may be correlated with Iron and other element status in the Betle vine.

Vii) Copper

Copper content are negatively influnced by pathogenic activities.In the present studies maximum Copper content are observed in Control leaves whereas least values of Copper content are observed in Infected leaves.The Copper content in *Trichoderma* treated leaves are observed maximum than Infeeted leaves but it observed less than Control leaves of *Piper betle* L.Var. Kapoori. This may be due to requirement of copper for the growth of *Trichoderma* in Infected host tissue as well as pathogenic activities created by *Phytophthora* fungus . The Cu is an trace element and certainly shows its preasence during disease controlling mechanism.

D) Enzyme studies

i) Polyphenol oxidase (E.C.1.10.3.2)

The activity of Polyphenol oxidase is positively influnced by pathogenesis in infected leaves of *Piper betle* L.Var. Kapoori.The maximum change in activity are observed in Infected leaves as compaired to *Trichoderma treated* and Control leaves. The maximum decrease in peroxidase activity are observed in Control leaves . The increased activity of peroxidase in Infected leaves may be due to change metabolic shifts in infected parts wich may be due to more accumulation

of hydrogen peroxidase metabolisim . The low activity of enzyme in Control is due to balanced metabolisim.Infection increases H_2O_2 concentration hence enzyme activity is recorded maximum .There are increase in activity is recorded in *Trichoderma* treated vines. It may be due to mycoparasitic properties of *Trichoderma* in disease resietance.

polyphenol oxidase is observed in control leaves. It is possible that Polyphenol oxidase is developing disease resistance in most of the vines. Thus its higher activity is good example for changed metabolism in the treated vines. Preasent studies also reveals the similar control mechanism interrelated with increase activities of Polyphenol oxidase in *Trichoderma* treated vines than diseased and Healthy, Control vines. The polyphenol contents thus inhibits the pathogens development in treated vines.

ii) Peroxidase (E.C. 1.11.1.7)

The activity of Peroxidase are positively influnced by infections. However peroxidase activity changes in *Trichoderma* treated leaves observed maximum as compaired to Infected and Control leaves.However maximum decreased activity are observed in Infected leaves of *Piper betle* L.Var. Kapoori .

iii) Cellulase (E.C.3.2.1.4.)

The enzyme Cellulase are influnced by pathogenic activity . The activity of cellulase is increased in Infected leaves than Control and *Trichoderma* treated leaves of *Piper betle* L. Var.Kapoori .The higher activity of enzyme are observed in Infected leaves while very low activity of cellulase are observed in *Trichoderma* treated leaves . The infection causes mazimum activity and it causes severe damage to enzyme system .However the *Trichoderma* may balances the enzyme mechanisim. In nature degreadtive function of cellulase is observed during pathogen's incidence in the vine. This phenomenon is also appears in the present work on Cellulase studies.

E) The significant findings of present work :

The present work is related tostudies on enzymes of mycoparasitism by *Trichoderma spp.(T.viride*) in control of foot – rot disease of *Piper betle* L. In Maharashtra . This disease causes severe economic tocultiver and changes agricultrial economics of the country . The chemical treatements are very chief and causes hazards harmful effects on human being, Hence use of *Trichoderma spp.(T. viride*) treatement in disease control is adapted the significant result are enlisted as fallows . The organic, inorganic constituents and enzyme study

suggest that pathogen activities changes all these constituents in infected of Betle vine. The treatment of *Trichoderma spp (T. viride)* control the pathogen development during leaf initiation and leaf maturation in Betle vine.Simultanesiouly , the above mineral status is maintened in relation to disease control mechanisim.

The organic contents like moisture percentage , pigments, carbohydrates and total polyohenols studies revels intresting results indicating interrelation between host *Piper betle* vine and the pathogen *(Phytophthora parasitica)* Var.piperina interactivity during leaf initiation and leaf maturation stag in Betle vine.The pathogen's incidences ,development as well as inhibitory of growth due application of Trichoderma treatement to the infected Betle vine. Naturally , the decrease in organic contents due to catabolic activities of pathogen in Infected vine are recorded whereas *Trichoderma* treatement balances the amount of individual constituents in better position . It suggests the metabolic processes showing dendency towards disease resistance status of infected Betle vine.

The important aspect of present research work is to study some enzymes related to pathogenesis mechanisim in infected vine. Simultaneously these studies are extended further to observe effect of *Trichoderma spp.(T.viride)* treatement to infected Betle vine

controlling *Phytophthora* foot –rot disease.The observation and result obtained are interested reveling the role of enzyme polyphenol oxidase, peroxidase and cellulase .

It is observed that the activities of these enzymes are considerable on control leaves of Betle vine.All enzymes are showing maximum activities in infected leaves than control leaves of vine .It suggests the prevent role against disease by treatement of *Trichoderma spp.(T. viride)* to infected Betle vine.Thus disease resistance may be developed due to antagonism and mycoparasitism property of *Trichoderma spp.(T. viride*). As a bio logical control agent nature.

It is possible that fungal growth requires specific pH, temperature, minerals etc. The *Trichoderma spp. (T. viride*) are producers of antibiotics which are involved in antagonisim and mycoparasitism nature.It produces cell wall degreading enzymes also due to production of hydrolytic enzymes including cellulose by *Trichoderma* posseses antagonisim against large number of soil borne plant pathogens. Hence , disease incidence is inhibited and vine becomes come to normal position due to sequential treatement of *Trichoderma* after infection. Finally the cultivers may obtain disease free ,residual free leaves with high quality tastes sugar percentage and more durability. It possibly increases the total yield of cultivar. Thus present investigation suggests

the use of *Trichoderma* spp. *(T.viride)* treatement to the Betle vines controls the foot – rot disease incidence and increases the disease resistance in in *Piper betle* vine. These results are confirmed by biochemical and enzymes studies in the present work.