CHAPTER – IV

RESULTS AND DISCUSSION

A) Organic constituents:

a) The moisture percentage:

The moisture percentage is an important aspect in the study of healthy and disease plants. There is decrease in dry matter contents is reported due to higher moisture percentage developed by disease incidence. It may be because respiratory and other metabolic activities of pathogenic fungi in the host plants.

Sharma et al; (1990) reported higher moisture content of diseased leaves than the healthy leaves of peach leaves attacked by leaf – curl disease. However *Trichoderma* treatment on infected levees observed maximum increase in moisture percentage is 87.6% as compared to control leaves is 80.5%. Very low moisture percentage was observed Infected and very high moisture percentage was observed in *Trichoderma* treated leaves of *Piper betel* L.Var. kapoori. This changes are due to excessive hypertrophied and enlargement of leaves. Live (1976) reported *Taphrina deformance* infected peach leaves were 7 times heavier than healthy leaves and contain 2 to 3 times more water.

The infected plant leaves show disappearance of chlorophylls due to cause of pathogen and show senescent appearance. Root-Rot leaves show complete degradation of chlorophylls & leaves become pale-yellow to dark yellow in appearance. Joshi and Mishap (1970) have reported more moistures content in senescent leaves of *Cleroldendron inerme*. Similar results were found by Jamaal (1975), Desponded (1981) and Bizarre (1984)

Table.No. 1.

Moisure % Dry Matter % of Control, infected & *Trichoderma* treated leaves of *Piper betle* L. var. kapoori.

Sr. No.	Parameter	Control	Infected	*Trichoderma*
1.	Moisture percentage Dry matter percentage	80.5 1.95	81.5 1.24	87.6 1.85
2.	Soil pH	7.17	7.31	7.44
3.	Temperature		20-24^{0}C	29^{0}C

in mangroves, Sugarcane & *Alternantera* respectively. Rane (1989) also reported similar results in groundnut.

The fungal infection also causes increase or decrease in moisture percentage in plants. Increased moisture percentage is observed in Brassica infected with Albugo(Dhingra et,al1982). Tong et al (2005) have reported increase in moisture percentage in infected mulberry leaves by *Cercospora moricola.*

Our results in Table.No.(1)show the effect of infection & *Trichoderma* treatment after infection on moisture percentage of Betel

vine. The data show due to fungal, pathogenic infection the moisture percentage in Infected leaves increased 81.5% while the control leaves show 8o.5 % moisture percentage, while *Trichoderma* treated leaves show 87.6 % moisture percentage.

It may be stated that infected leaves show increased water content. This increase in moisture percentage may be due to more accumulation of ions in the infected leaves. So reduce the toxicity of ions by dilution indicated by Jennings (1968).

Dry matter :

The dry matter percentages decreased in Infected leaves of *Piper betle* L.Var.Kapoori infected by *Phytophthora* Foot-Rot disease. T he are depicted in Table. No.(1). The maximum dry matter was observed in Control leaves are 0.085 % ,in Infected leaves was observed 0.11 % while in *Trichoderma* treated leaves are 0.111 %.

According t Johair (1975) reported decrease in the dry matter percentage of mosaic virus infected *Carica papoya* L. The result represented lower dry matter content in the Infected leaves. Also in *Trichoderma* treated leaves moisture percentage increased while dry matter in control leaves was observed maximum than *Trichoderma* treated Infected leaves It might be due lower amount of chlorophyll along with an enhanced activity of enzymes.

The result represented increase in dry matter content in *Trichoderma* treated infected leaves than the Infected leaves

b) soil p^H:

Soil is one of the important ecological factor .Soil is the natural habitat of plants ,animals and microbes.It is the outermost layer of the earth's crust.However soil is olso defined as ,any part of earth crust in wich plant root penetrates.The soil environment is dynamic and complex. Soil is "weathered surface of the earth crust's swich is mixed with organic materiaks in wich micro organisims live and plants grow".

The study of oil science is Pedology .The soil is complex having solids ,liquids, and gaseous components with their own physiological properties and definite relationship with the otheir. The important facters are minerals, soil texture, soil water, siol air , soil temperature, soil humus and soil microorgasims.

Soil texature is associated with minerial nutrition of plants .Bacteria and fungi play an important role in the geo-chemical, biochemical and biophysical processes of pedogenesis. The soil acidity or pH is important facter in the control of type of vegetation which will grown in a particular area.

Phyptphthora leaf and foot rot varies among seasions and sites , the pathogen is highly favoured by excessives rainfall and poor soil drainage. Disease symptoms include soft, water- soaked leasions on shoots slightly above or below the soil line. S tandard soil conditions from rainfall or excessive irrigation have been implicated in increase of Phytophthora root rot disease , outbreake of many perennial and annul crops . However wet soil conditions stimulate the release of zoospores from sporangia increasing inoculum leavels.

The soil pH neutral to slightly acidic is suitable for growth of Actinomycetes group . The also found plenty in soils ,rich in organic matter , result in decomposing the resistance parts of organic matter like cellulose .Also they synthesize chemical subastances in the soil and produce the familier odour when the first rain showers are received after a hot summar .

The pH of soil is also important for growth of algal member. They are microscopic and very size plant s and are usually found on the surface of wet soils of paddy field .They help in adding organic matter to soil , improving the soil aeriation.

The soil acidity is associated with the presence of hydrogen and aluminium ions on the exchange complex and the existence of an equilibrium solution of hydrogen ions in the interstitial water of the soil. Redman and Patrick (1965) studied the pH behaviour of a large number of soils on submergence and noited those originally abhove pH 7.4 decreased in value while those below pH 7.4 increased. As an intencity factor it may be defined by the conenvitional physical chemical concept of hydrogen ion activity expressed as pH. Soil pH is now usually measured electrometrically using a glass electrode referred to a calomel half-cell. (J.R.etheritington First edition (1975).

Despite the difficulties of interpreting soil pH value they show strong correlation with soil type, vegetation type , profile horizon and agriculturally ,with crop groth , lime requirement and mineral nutrition. Natural soil usually have pH values between about pH 3.0 and 8.4. The upper value being the calcium carbonate equilibrium with atmospheric

mull is characteristic of the more cation –saturated soils of pH 4.8 -5.0 and above ,between 3.8 and 4.8 intermediate organic matter forms occur.

Many differences between soils of differing pH are due to the processes of 'soil metaboilsim' wich vary strongly wiyh soil pH. Between pH 5.0 and 8.0 ,both bacterial and fungal decomposition procssd rapidly, but below about pH 5.0 ,bacterial activity is reduced and fewer fungel species are found. There are also a change in fauna, the numerious and snails of higher pH soils being replaced with a less diverse population of arthropods ,mainly mites and springtails.

The PH of a soil influences the solubility of phosphate, trace elements (Fe, Mn, Zn, Cu, Co, Mo) and toxic ions (e.g. AL^{3+}) as well as controlling the activity of soil micro-orgasims . For example ,the fixitation and mineralision of nitrogen by free living bacteria cease af soil pH value below 4. Becaues of these effects, the pH values of agriculturial soils are norminaly maintained wirh the range 5.5 to 7.0 ,to insure adequate supplies of phosphates and trace elements and active microbial populations , coupled with low solubilities of toxic ions . However plant species can colonise soils whose pH values lie outside this range , if they evolved adaptations to overcome the adervese factors associated with extrems of pH (e.g. trace element deficiencics at pH 7.7).

The soil pH plays an important role during infection and after *Trichoderma* treatement of *Piper betle* L. Var. kapoori are shown in Table No.(1). However soil pH of Infected leaves infected by

carban dioxide concentration and the lower value, the soil solution equilibrium with a highly hydrogen- saturated soil . More sxtreme values do occur in unusual soil types. Soms alkali soils with high sodium carbonate content rich values of pH 10.0- 10.5 while drained gleys may produce sulphuric by oxidation of ferrous sulphide , their pH falling to 2.0 or below .Such low pH may also be found in soils, heaps soils derived frome sulphides ores or from coal spoils with a high ferrous sulphides content.

The pH range for some typicals soils of departement of Agriculture , Maharashtra is given and Ratnigari soil lateric and pH rang is 5.0 to 5.5 , pH of Red loam Igatpuri is – 6.0 to 6.5 , in Kargat pH of Clay loam is 6.2 to 6.8 , Jat (Sangli) has sandy loam (alkaline) is 8.5 to 9.0 soil pH, in Jalgaon clay soil pH is 7.5 yo 7.8 and Sindewani contain loam soil having soil pH is 5.8 to 6.2 (v.g. vidya and s.r. sahasrabuddhe first edition (1970)).

Pearsall (1952) delimited various boundry pH values wich are ecologically significant . Plants regarded as calcicoles usually occur above pH 6.5 and contrast with the extreme calcifugus of heate and moorland soil below pH 3.8 -4.0 . Soil` above pH 6.5 are generally cation – saturated and have a subclass of calcareous soils containing free calcium carbonate , while soils below pH 3.8 -4.0 are strongly desaturated and have a considerable content of exchangeable hydrogen.

These pH limits are also reflected in the nature of the soil organic matter, raw humus or mor is associated with soils of below pH 3.8 while

phytophthora Foot –Rot disease while soil pH of *Trichoderma* treated leaves are

From this results low level of pH of soil are observed during infection of *Phytophthora parasitica* Var.piperina which is slightly increased after treatement of *Trichiderm* .

In Gumosis or Gum disease of Citrus Tsao (1969) and Tsao and Bricker (1964 ,1968) have made extensive studies on the behaviour of *Phytophthora parasitica* in soil . It shows heavy soils , high soil moisture , soil pH 5.4 -7.5 and are conductive for disease development . pH is a crucial factor in many otheir aspects of agricultural Science . to give an other example , the pH of freshly prepared silage must fall rapidly sto 4.0, otheirwise undesirable microorganisims (e.g. *Clostridium* spp.) will invade the mixture giving a poor quality oa toxic silage.

Among with the otheir microorganisms . Fungi are also associated with microbiological activity remains confined to the surface of the roots ,called the rhizoplain . In many cases the roots of resistance varities of plants have been to produce hydrocynic acid which is however , not produced in the susceptible varities.It is presumed that this acid has an inverse effect on the pathogenic fungi by preventing them from flourishing near the roots (B. P. Pandey 2007) .

C) Soil Temperature :

Temperature is a physiochemical ecological factor. It is defined as the "intensity aspect of heat". It is a form of energy called thermal

energy. It penetrates into each and every region in the biosphere, and affects almost all forms of life. It influences various life activities such as growth, metabolism, reproduction, movement, distribution, death etc.

Also Anthracnose disease in betelvine reported that maximum temperature morning relative humidity and rainfall had significant negative correlation and minimum temperature, evening pH and number of rainy days had positive co-relation with the on the incidence and spread of the disease at AAU, Jorghat.

At OUAT, Bhubaneswar, the regression analysis of PDI with that of the weather parameters revealed that only the minimum temperature towards significant minor leaf spot disease incidence to the extent of 78.34%.

Two varieties, viz. Simurali Deshi and Halisahar Jhal inoculated with *Phytophthora spp.* during end of May,2003. The incidence of diseases was recorded from 1st week of June,2004 to November,2004 at 7 days interval, results that x-coefficients for min. temperature (1.79), max. RH (1.38) and rainfall (0.02) had positive significant effect on percent disease incidence while that of max. temp. (1.09) and min. RH (0.18) had negative significant effect non foot rot caused by *phytophthora* spp. While the results of the correlation studies of different weather parameters with the leaf rot caused by phytophthora spp. containing leaf

vine variety simurali Deshi with the disease incidence revealed that X-coefficients for min. temperature (2.45), max. RH (2.05) and rainfall. (0.031) had positive significant effect on percent disease incidence while that of the max. temp. (1.67) and min. RH (0.36) had negative significant effect.

In Jawaharal Nehru Krishi Vishwa Vidyalaya Jabalpur (2005-2006) worked on Phytophthora foot and leaf rot results indicated that the incidence of disease recorded in the second week of June with 1.5 and 1.61%. The disease index increases gradually and reached to 22.15 and 22.35 % during both the years respectively in second week of September when the difference between maximum and minimum temperature was less and humidity was ranging from 87 to 93 and 89 to 90%. In the month of October the incidence of stem rot increase and it was observed that it increased from 18.33% to 22.15% during (2004-05) and 17.86 to 22.2% during (2005-06).

In International Journal of the American phytopatholigical society volume 98, October,2008 observed the effect of temperature on mycelial growth of 46 *Phytophthora i*solates examined when inoculated on to AR-V8 juice agar, all isolates grew when incubated at temperature between 10 & 25 ^{0}C, with optimum growth of 25^{0}C. (P=0.0001). The average colony diameter after 3 days of mycelial growth at 25^{0}C, was

34mm. At 7 days after inoculation the diameter of the majority of the isolates measured approximately 75mm, Sporangia and oospores were produced at temperatures between 10 and 25^{0}C at 7 days after inoculation. From this work, temperature study indicated mycelial growth of *Phytophthora spp.* occured between 10 and 25^{0}C with the greatest growth of 25^{0}C and no growth about 25^{0}C. This is comparable to findings of Boesewinkel (6) who determined temperature optimum of 20 to 22^{0}C for mycelial growth of a phytophthora spp. infectingt asparagus in New Zealand Fallaan et al (19) noted that high losses occurred in the early part of the asparagus harvest season after periods of heavy rainfall when soil temperature were between 9 and 24^{0}C.

Generally plants can grow in any area where the temperature is between 26^{0}C and 60^{0}C. The normal life activities starts to decline from 40^{0}C onwards and stops almost at 90^{0}C. The rate of absorption of water increases with the increase in temperature from 27-35^{0}C. If the temperature exceeds 35^{0}C or lowers 27^{0}C, the water absorpotion in plants decreases due to the adverse effect of temperature on the permeability of plasmamembrane towards water. So hot soil is considered to be physicalloy dry soil, but cold soil is considered to be a physiologically dry soil.

In some temperature plants, the growth of root cambium increases if the soil temperature is high. Therefore, they show a marked decrease in root growth if they are exposed to low soil temperatures. In other waords temperature creates suitable conditions for the growth of the plants decreases when the soil temperature is low.

According to Tiwari (1973) optimum temperature for growth of the fungus in culture is 30^0C. The fungus is killed at 40^0C in 24 hours. Maximum sporangial development in culture is at 30^0C none at 5-10^0C, and 37-40^0C.

In Journal of Indian Phytopathological society (2nd Feb.,1969) gives effect of temperature on the development of Betel vine wilt and economic control. It was observed that in all the years a high percentage of the plants wilted during the month of January, February, November and December.

In vitro germination of sporangia by zoospores and germ tubes is maximum at 25^0C and 30^0C, respectively. The zoospories of *Phytophora* germinate best at 20-25^0C (Tiwar, 1973). However, selvaraj et al (1973) have stated that the maximum growth of the fungus occurs at 22-24^0C, so the soil temperature favouring maximum disease development.

Sporulation & zoospore liberation is best at 22^{0}C.Husain and Ahmad (1961) had found maximum liberation of zoospores at 21-23^{0}C.

The koleroga or rot disease of areca palms affected by *Phytophthora arecae* (coleman) pethybridge shows that the optimum temperature for the growth of the fungus in cultures is 30^{0}C.

The temperature effect on the Wilt disease of betel vine caused by *Phytophthora parasitica* Dast. showed that the minimum temperature, the number of days showing a temperature below 23^{0}C and the Wilt disease incidence will be observed that in all the years high percentage of the plants wilted during the month of January, February, November & December.

Effect of soil temperature of infected leaves and *Trichoderma* treated leaves of *Piper betle* L. var. Kapoori is recorded in Table No.(1) which shows that temperature of soil in infected leaves are observed minimum infectred by *phytophthora parasitica* var. Piperina. However, maximum increase in soil temperature are observed in *Trichoderma* treated infected leaves of betel vine, also infected by *Phytophthora parasitica* var. piperina.

(C) Organic constituents :

i) Chlorophylls –

Chlorophyll is pigment present in the chloroplast of green plant are capable of photosynthesis. Chlorophyll pigment alone is capale of absorbing the light energy and converting into light energy.

Seven types of chlorophyll pigments are known, of these chl.a & chl.b are the most important. Each chl. Molecule is made up of a tetrapyrrole skeleton formed into a ring with a central atom of magnesium. Each pyrrole molecule consists of a ring made up of four carbon and one nitrogen atom. Attached to the pyrrole rings is a tail called the 'Phytol' chain.

The main role of chlorophyll is to absorb the light energy and convert it into chemical energy. The light energy is available in the form of energy packet called 'photons' or 'quanta'. This is observed by chlorophyll and converted into chemical energy.

Fisher and Stern (1940) made an extensive study of chlorophylls which resulted in the elucidation of the structure of chlorophyll-a and chlorophyll-b.The proposed structure of chlorophyll-a has been confirmed by Wood-Ward (1960) who synthesizewd it in the laboratory.

Table No.2.

Chlorphylls of Control, infected and *Trichoderma* treated leaves of *Piper Betle* L. var. kapoori.

Sr.No.	Parameter	Control	Infected	*Trichoderma* treated
1.	Chlorophyll-a	5.711	1.519	4.603
2.	Chlorophyll-b	14.64	7.332	12.69
3.	Total Chlorophyll (a+b)	20.351	8.851	17.293
4.	Ratio a/b	0.3900	0.2071	0.3627

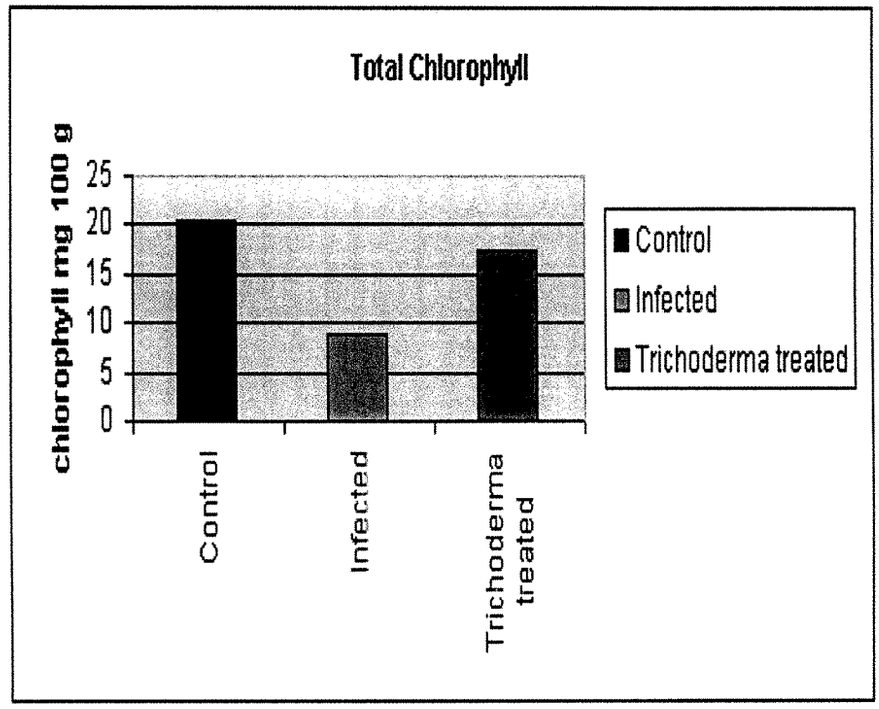


Fig. 1 *Value of chlorophylls are expressed in mg 100^{-1} gm. fresh leaves

chlorophyll-b differs from chlorophyll-a in having a formyl groupt instead of a methyl group at Carbon-3 of the tetrapyrrole ring.

The absorption bands of chlorophyll-a lie in the visible region of the light spectrum. Although the fact that chlorophyll is present in different forms (a,b,c,etc.) has been long established, the observation that these different kinds of chlorophyll occur in multiple molecular forms in vivo has not been widely appreciated. Well planned experiments in the laboratory of Dr. C.S. French of Stanford University have revealed the existence of at least four multiple molecular forms of chlorophyll-a alone. The same is true of other chlorophylls and also of various kinds of macromolecules found in the living cell in general.The chlorophyll content are usually, is accompanied by yellowing of infected leaves. (Farkas, 1978).

According to Rosenow et al ; (1983) and Reddy and Prasad (1999) , efficiency of photosynthesis depends upon presence of chlorophyll contents. The maximum photosynthesis results to enhanced leaf area index and leaf yield. The chlorophyll accumulation is controlled not only by the rates of process of chlorophyll biosynthesis or degradation but also due to the formation of chloroplast structure.

Photosynthetic pigments were analysed in the leaves of *Piper betle* plants before infection & after *Trichoderma* treatment. Results are represented in Table (2) & figure-1 about 1.519 % chlorophyll a, 7.332% chl.b observed in infected leaves while total chlorophylls (a+b) are 8.851 & Ratio of a/b are 0.2071 was observed in infected leaves. However *Trichoderma* treated leaves show increase in chlorophylls as chl.a are 4.603 %, chl.b are 12.69%, Total chlorophylls 17.293% & ratio of a/b are 0.3627%, while in control leaves Chl.a – 5.711% , Chl.b 14.64%, Total Chlorophylls (a+b) – 20.351 and Ratio of a/b are 0.3900%

There are many evidences in literature showing a decrease in chlorophyll content of plant after infection & increase in chlorophyll content after *Trichoderma* treatment of infected plant. Many reports on fungal infection have noted similar trends Allen (1942) & Scot & Simillie (1963) suggested the decrease in chlorophyll content in wheat and barley leaves infected by powdery mildew respectively. Kaur & Deshpande (1980) reported the decrease in chlorophyll in cowpea infected by *Erysiphae.*

Accoring to Abu - Grab and Ebrahim (2008) nitrogen and magnesium are major component of chlorophyll molecules and they so

important involvement in Mn in photosystems .(Krause and Santarius, 1975).

Several workers reported the Treatment of *Trichoderma Spp.* Increases yield and chlorophyll in several plant. According to Gomma et al, (2000) an increase in weight & size of Gladiolus Cv.of corms might be due to Treatment of *Trichoderma Spp.* Results in increased in chlorophyll-a and chlorophyll-b of leaves and leaf area.

Fungicides also plays an important role in disease treatment & increase in chlorophyll content. According to Y.K. Sharma' and B.B.L. Thakare (2004) in influence of systematic fungicides on physiological & biochemical constituents of Chill crop., observed spray treatment of some systemic fungicide4s on die-back of chill results in increased the chlorophyll content as compared to untreated. The effect of chlorophyll contents, photosynthesis and respiration of barley plant treated with systemic fungicides (Carlson,1970). These findings were also confirmed by Mathre (1972) and Singh and Kang (1983) in groundnut plant treated with fungicides like benzimidazole & oxithilns, were increased production and chlorophyll. The increase in chlorophyll content, due to effect of systemic fungicides has been reported in several crops viz. *capsicum annum, vigna radiata, solanum melongena aned pennisetum*

americanum (Ahmed and Siddiqui,1995; Siddiqui and Ahmed,1996; Siddiqui and Khan,2001; Siddiqui,1997 and Siddiqui et al;1999)

In the present investigation we observed the Estimation of chlorophyll content in control, Infected & *Trichoderma* treated leaves of Betel variety Kapoori. From the value of chlorophyll the results show decrease in chlorophyll contents in infected leaves of Betel vine. It was also found that the greater reduction of chlorophyll in Infected leaves as compared to *Trichoderma* treated leaves and Control leaves. However, the treatment of *Trichoderma* on infected leaves show increased chlorophyll content. Greater value of chlorophyll are observed in *Trichoderma* treated leaves of Betel vine variety Kapoori.

(ii) Carotenoids

Carotenoids in all higher plants are synthesized and located in chloroplast along with the chlorophylls. They are accessory light harvesting pigments which trap light energy and pass it on to chlorophyll molecule. They absorb light energy in 400-5—nm regions which is not accessible to chlorophyll molecule. Main role of carotenoids is protect the chloroplast from stress damage. According to Demming-Admas (1990) an additional protective mechanism is attributed to the Carotenoids present in higher plant chloroplasts, where they are involved in the interconversion of three xanthophylls viz. Violaxanthin, Antheraxanthin, Zea xanthin & related to the dissipiation of excess excitation energy under adverse condition.

The important role of carotenoids is light mediated etress.In the view of Sestak , (1985) , Ong and Tee, (1992)attributed r a protective role ofcarotenoides offering photoprotection to chlorophylls against bleaching. The important photosynthetic pigment is Xanthophylls also directively participate in the mechanisim of photosynthesis. The violoxanthin cycle participating in oxygen transport ,the function of ß – carotene in the C- 550 absorbance change. The production of caroteniod ,Zeaxinthin is very important in setting up stress tolerance in plants.

TABLE NO. 3.

Carotenoid, polyphenol & TAN in Control, infected & *Trichorderma* treated leaves of *Piper betle* L. var. kapoori.

Sr.No.	Parameter	Contr ol	Infected	*Trichoderma* treated
1.	Carotenoids	18.24	16.68	17.45
2.	Polyphenol	304.75	396.38	298.41
3.	TAN			
	a)Afternoon TAN(12pm)	178.125	2.8125	34.416
	b)Evening TAN(6pm)	95.83	41.66	68.75

*Values are expressed in mg100^{-1}gm.fresh weight for carotenoid & polyphenol.

*Values of TAN are expressed in ml. of decinormal alkali in TAN.

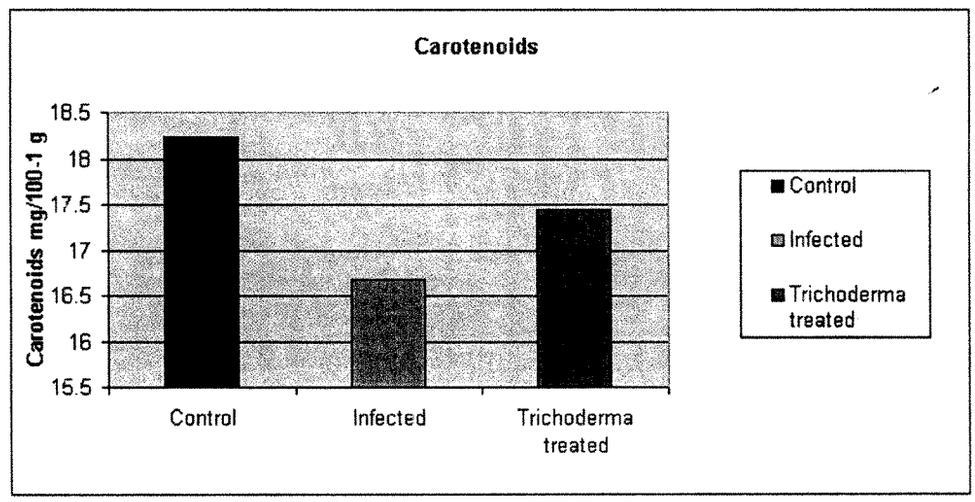


Fig.2.*Values are expressed in mg 100^{-1} gm. fresh weight for carotenoid

(Yong,1991). The ratio of chlorophylls / carotenoids increases during the growth of area of leaf and chloroplast formarion due to carotenoids are synthesized more slowly than chlorophylls.(Sestak , loc. Cit).

According to Sestak, (1985) the other same function of carotenoids are due to the presence of carotenoids habe detected in non-photosynthetic plant species of fungi and non – photosynthetic organellies like petals , pollens of some flowers , seeds and eyespot of Euglena .

In the present investigation there are decreased in carotenoids are observed in infected leaves of *Piper betle* L.- kapoori. Reduction in carotenoids of downy midew infected leaves of opium reported by Singh et at; (1986). Naik et al;(1988) also observed reduction in carotenoids due to infection in Betle leaves.

The number of factors influnced by carotenoid content in plants. Carorenoids are declined due to leaf senescence (Biswas and Mohanty 1976; Cardini, 1983; Sestak, 1985 ; Cabello et al, 2006). Murumkar,(1986) observed mineral deficiency and salt stress (Gururaj Rao and Rajeshwar Rao,1981).)causes carotenoid contents are declined.

According to (Shultz et al , 1998; Steel and Keller, 2000) carotenoides are precursors of secondary metabolites wich are

determinants of wine quality in grape berry tissue . Also activity of peroxidase is involved in the catabolisim of carotenoids during leaf senescence (Matile and Martinola 1982).The change in carotenoid content and distrubition in living plant tissue using NIR-FT- Raman spectroscopy observed by (Baransik et al , 205).

The carotenoid contents increased progressively after *Trichoderma* treatment or infected Betle leaves, infected by *phytophthara parasitica* var. pipernia. The results are depicted in Table (2) show carotenoids in control leaves are 18.24%, *Trichoderma* treated leaves contain 17.45% carotenoids, while infected leaves observed 17.45% carotenoid. The maximum corotenoid was observed in control leaves and *Trichoderma* treated leaves as compared to infected leaves of *Piper betle* L.var.kapoori.

From our results of the present investigation it is clear that there are increased carotenoid contents after treatment of *Trichoderma* on infected leaves of *Piper betle* L. var.kapoori.

The decreased carotenoid contents are observed in Yams (*Dioscores spp.)* infected by *Botryodiopladia thiobromae* and *Aspergillus niger.* are recorded by (Adelusi and Lawanson 1987). In

beech (*Fagus sylvatica*) infected by *Phytophthora* (Fleischmann et al ; 2004). The coca infected with Witche's broom (Scarperi et al , 2005). In Norway spruece needles affected by Chrysomyxsa rhododendri show decrease in carotenoid content. (Pfeithafer 2007).

The increase in corotenoid content after *Trichoderma* treatment may due to the disappearance of pathogen in host tissue and metabolic balances leading to the increase of synthetic activity of carotenoids.

ii) Polyphenols

The compounds with more than one hydroxyl groups are called polyphenols. Polyphenols are aromatic compounds formed during secondary metabolism, and function in oxidation and reduction reactions. Phenolics also interfere with growth and reduction reactions. Phenolic compounds affect fundamental process such as chlorophyll synthesis, photosynthesis, protein synthesis, Respiration, membrane permeability & water relations (Glass and Dunlop,1974; Dank et al;1975; Rice,1979).

Phenols are found in a wide variety of plants, from alga to angiosperms (Walker,1975). The aromatic ring can be substituted by other organic groups, thus giving rise to a vast range of phenolic compounds. Chemically the basic skeleton of phenols includes an

aromatic ring containing at least one hydroxyl group & its functional derivative. Phenols are found in plants in the form of their glycosides in cell vacuoles. A few phenolic derivatives seem to act as phytoalexins in defending host plants against Fungal or Pathogenic attacks (Cruicks hank,1963). Also phenolic compounds play a very important role of increase resistance of infected plant after bioagent treatment.

Several workers have reported either increased or decreased content of polyphenol in plant. viz. parthsarthi et.al;(1970) in Sandal spike disease, Prasad and sahambi (1980) in Sisamum affected of *sessamum phyllody.* Agarwal et.al; (1982), Singh and Singh (1983) in Turmeric and Guava affected by *Taphrina and Aspergillus* respectively have reported increase in polyphenols.

Influence of disease in treatment on polyphenols in Piper betle L. var.kapoori is depicted in Table (3) & Fig.3. A maximum increasesin polyphenol are observed in control leaves and *Trichoderma* treated leaves while very low polyphenols are observed in infected leaves infected by *phytophthora parasitica* var.piperina. the polyphenol contents in infected plant observed less than in control but it show increased in *Trichoderma* treated leaves. From this result it is clealr that *Trichoderma* treatment plays important role in polyphenol metabolism during control of *phytophthora* infection in Betel vines.

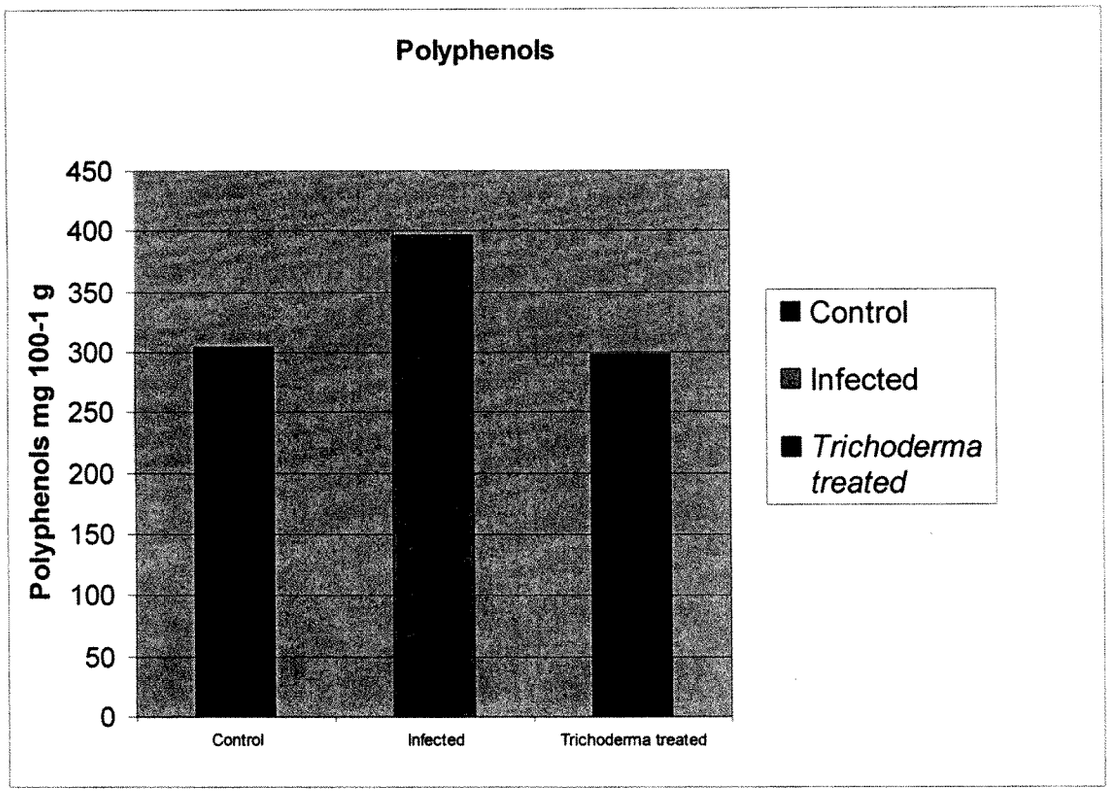


Fig.3.*Values are expressed in mg100^{-1}gm.fresh weight for polyphenol.

Fungicide treatment are also found to best in control infection of plant. Y.K. Sharma' and B.B.L. Thakare (2004) reported all systemic fungicides when sprayed for control of die-back of Chilli that, the phenols are found to show the similar increase is treated plant. the phenol content of Chilli Plant also found to increased with the application of systemic fungicides as compared to untreated plants.

Several workers viz. (Gautam et al; 1984,Marshall et al;1991, Mohmed et al; 1988, Singh and Kang,1983 and Siddiqui,1997) are

agreed & reported treatment of systemic fungicides increases phenol contents in crops like soybean, peanut, groundnut, onion and brinjal. Mhatre,(1972) and Singh and Kang (1983) confirmed in groundnut plant treated with systemic fungicides, were increased production was noticed in polyphenol. The systemic fungicides have positive effects are reported in crops like capsicum annum, solanum melanogena and vigna radiate (Ahmed and Siddiqui,1995; Siddiqui and Ahmed,1996; Siddiqui and Khan,2001; Siddiqui,1997 and Siddiqui et al;1999).

According to Minoka Sharma and Bishwanath Chakraborty (2004) Antifugal phenolics (AFP) were extracted from healthy and inoculated with (E.veans) tea leaves. They observed inherent components of polyphenols were greatly enhanced in the tea leaves, found hypersensitive reactions in comparasion to susceptible ones.

The significant negative correlation between the post harvest pathogens and ascorbic acid, total phenols are Orthro Dihydroxy (OD) phenols were observed in susceptibility of Tomato Genotype, relation to fruit qualitative characters. They observe that the ascorbic acid & total polyphenol were the main factors which increased resistance significantly, during regression of coefficient of variables on the prevalence of post harvest rot. The total polyphenols in correlation between fruit rot and qualitative characters are 25.20 to 52.80 mg /100

gms. Also they found there are simple correlation of storage rot fungi in total polyphenols and Orthro Dihydroxy (OD) phenols as one of the significant.

Our present investigation whowed that there is decreaser in polyphenol content in infected leaves of *Piper betle* L.var.kapoori. The decreased polyphenol content in infected leaves showed lower resistence to disease.

Cruickshank, I.A.M. and Perrin , D.R. (1964) pathological function of phenolic compounbds in plants show increase or decrease activity also Farkas et. Al.(1962) show role of phenolic compounds increased or decreased in physiology of diseases and disease resistance. Again it was reported that oxidation products of phenol also help in disease resistence.

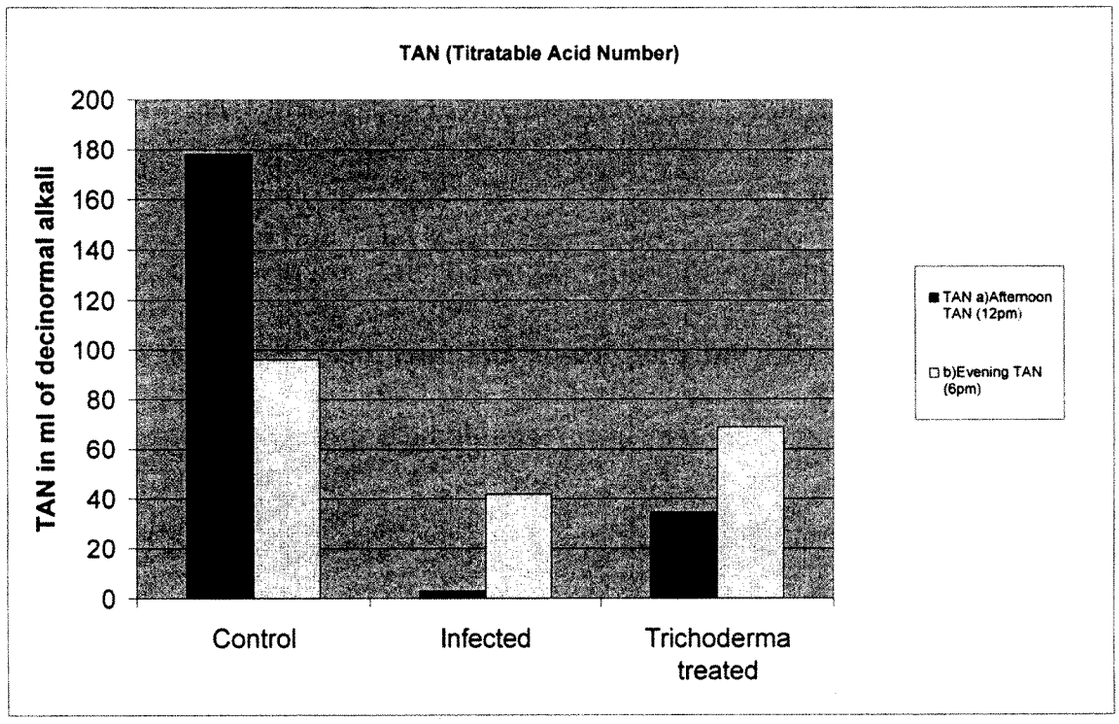


Fig. No. 4. *Values of TAN are expressed in ml. of decinormal alkali in TAN.

iv) TAN (Titratable Acid Number) :

The various acids were synthesized during the metabolic processes. Acidity status of the leaves can be determined by titrating the plant extract against standard alkali, TAN can also be defined as number of ml. of dicinormal NaOH required toneutralise the acid content from 100 g of fresh plant material.

In our present investigation TAN value decipted in Table No.(3) and Fig. No. (4) shows Afternoon (12 PM) TAN in control leaves was , in Infected leaves and in *Trichoderma* treated leaves was of Betle

vine Var. Kapoor. However enening TAN (6 PM) in control leaves was, in Infected leaves was and in *Trichoderma* treated leaves found value of evening TAN .

Organic acids areperhapes the most important comman metabolities present in all aerobic organisims since these compounds are intermediates of a central metabolic pathway, TCA cycle . During the metabolic processes various acids were synthesized . Malic acid is one of the comman organic is involved in CAM and C4 pathways. The two acids like Isocitric acid and Succinic acid are important intermediate in glyoxilate cycle. The carbon skeleton for the synthesis of number of important metabolities are provided by these compounds. It is well established that aculumilation of organic acids in plant in revelent to the afjustment of the cation –anion balance in plant (Poop and Kinzel ; Triplett et al ; 1980) . However oxalic acid is important to participate in osmoregulation oin halophytes such as Atriplex (Osmond , 1963). The organic acid level in plant yissue can be modified by the relative levels of available anions (Cl^- , SO_4^{2-}, NO_3-) or a certain cation ($NH4^+$) which can be metabolized by the plants.

When the rate of respiration increased ultimetly the level of TCA cycle increased , results of increased TNA value are observed in

plants. The TAN values are increasd due to the respiration after stimulation of TAN in free pod.

The value of TAN are increased after infection are reported by many workers .The highest TAN observed in teak leaves affected by powdery mildew.(Thite et al ; 1980). The increased TAN observed in mango leaves infected by *Capodium ramosum* cooks(Kulkarni and Kulkarni 1976).Also increased TAN values are reported by Jagtap et al ; (1981),Tutenol;(1981),Hardikar(1978) and Sasikumaran et al;(1979).

Our results show various TAN values in *Piper betle* var. kapoori after infection and *Trichoderma* treated treatment.The two times TAN values shows variation in reading.The data shows that afternoon TAN (12 PM) values are decreased in infected leaves than *Trichoderma* treated infected leaves. In control leaves TAN are very high as compared to other two sample.The very low TAN value are observed in infected leaves during infection of *phytophthora parasitica* var. piperina.

Also, our results Table No.(3)and Fig.No(4) shows TAN during evening (6 PM) of three sample of *Piper betle* L. var. kapoori. Maximum TAN value are observed in control leaves. The TAN values are decreased in two samples infected by *phytophthora parasitica* var. piperina. However, value of TAN in *Trichoderma t*reated leaves are

maximum as compared to infected leaves, but less than TAN of control leaves Enhanced chlorosis and breakdown of many metabolities leading to increased TAN in infected leaves during evening.

Table.No 4.

Carbohydrates & Total soluble Proteins of Control, infected & *Trichoderma* treated leaves of *Piper betle* L.var. kapoori.

Sr.No.	Parameter	Control	Infected	*Trichoderma* treated
1.	**Carbohydrates**			
	i)Reducing sugars	140	778	771
	ii)Non-reducing sugars	411	890	466
	iii)Total sugars	552	116	537
	iv)Starch	144	849	749
	v)Total carbohydrates	697	101	128
2.	**Total soluble proteins**	0.085	0.122	0.111

*** Values of carbohydrates are expressed in mg 100 $^{-1}$ g. dry weight**

***Values of proteins are expressed in 100 $^{-1}$ g fresh weight**

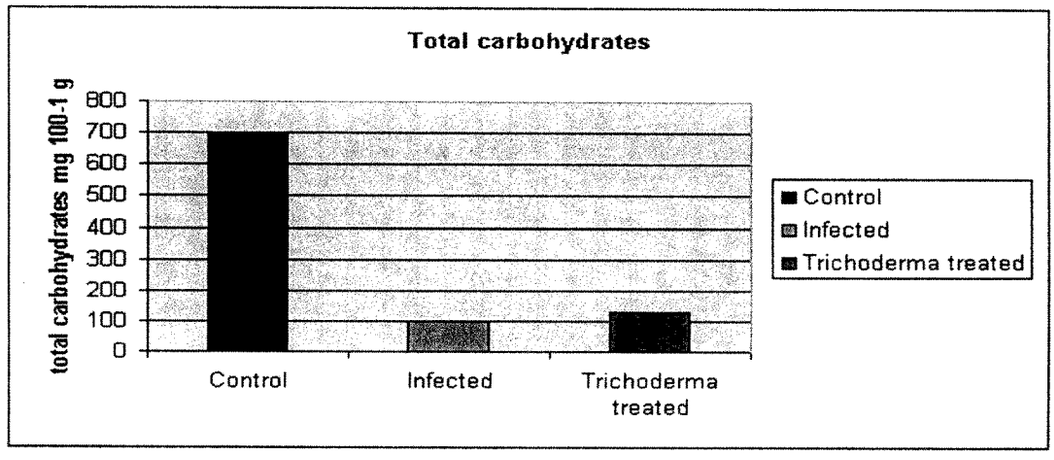


Fig.5. *Values of carbohydrates are expressed in mg 100 $^{-1}$ g. dry weight

V) Carbohydrates :

Among the various organic metabolites that are found in plant cells, carbohydrates present is the most important compound so far as dry matter production and energy relations of cells are concerned carbohydrates are usually available in the complex forms. They are among the most widely distributed compounds in plant kingdom plants can build up carbohydrates from carbondioxide by photo synthesis. It is also important structural components. Many plants contain large quantities of carbohydrates as reserved food material. Sugars also provide the building blocks for amino acrols and fatty acids. Besides the key role in carbon metabolism and energetics of a plant cell, carbohydrates perticularly soluble sugars, play an important role in asmoregulation (Morgon,1992).

The role of carbohydrates are very important in plants. Sucrose plays an important osmoregulatory role in many cases. The accumulation of sugars protects the protoplasm from coagulatiom and desiccation and high concentration may prevent visible wilting for a long time inspite of an increasing water deficit. (Maximov, 1929). The accumulation of sugars in Sorghum under water tress indicates a protective role of carbohydrates.

(Vara et al , 1974). Milyaeva and Kumarov ,(1996) , observed sugars also play a role in signaling pathway in plants.

According to Singh (2005) plant infected with various pathogens show increase or decrease in carbohydrate content . The many pathogenic fungi generally utilise ready carbohydrates of host plants for their life activities. The fungi have the ability to convert complex form of carbohydrates in to simple water soluble sugars and utilize. The fungi utilize monosaccharides directly while oligo, polysaccharides are firstly converted into simple compound and then utilized.

Many workers have reported increase in reducing sugar content while few have reported while few have reported decrease in Kaley and Nagaich (1976), Kaley et al., (1977), Nagoich (1978) have reported considerable increase in reducing content in different infection of potato leaves. Bhandari And Singh (1976) reported the high reducing content in host suggests its susceptibility towards fungal infection.The greated quantities of reducing sugar in bean infected with *collelatricum* has been reported has been reported by Hegde and Munjal (1971). Also, increase in sugars in have been reported by Sankpal and Nimbalkar (1989) in

GSD affected sugarcane; Mohaptra (1982) in maize infected with sclerospora Goel et al., (1983) in coriander infected by protomyces, karande & Hegde (1984) reported in Bajra affected by Downy Midew fungus. Nagaraja (1990) reported increase in sugars while Achor (1994) reported reduction in reducing sugar and starch in redish infected by *perinospora* . Accumulation of soluble carbohydrates in Arabidopsis infected by Albugo reported by Chou et al.,(1995). According to Khan et al, (2001) observed accumulation of reducing sugar in leaves of Sorghum infected with leaf spot and Scarpari et al, (2005) . *Witches' broom* infected by

Our results of present investigation are depcted in Table No.(4) and Fig.No.(5). In *Piper betle* leaves infected by *Phytophthora parasitica* var. piperina show increase in reducing sugars. However reducing sugars in *Trichoderma* treated infected leaves also increased than control leaves but show less than Infected leaveof Betle vine Var. Kapoori.The different opinions have been reported to increased or decreased reducing content in diseased plants. The accumulation of reducing sugar is due to the distribution of normal phalem transport or due to the release of Amylase in the host cell are reported by Okasha et al., (1968). while parthsarthi (1977) suggested increase in reducing

sugar is due to phloem necrosis. Joshi (1976) Billet et al.,(1977) Ghorpade and Joshi (1980), chen and HOU (1981), Mitchelli (1982) attributed due to manifold increased activity of invertase and synthelase might increase the reducing in diseased plants.

Thus, increase in content of reducing in *Piper betle* infected by *phytophthora parasitica* in Table No.(4) and Fig. No.(5).The value of reducing sugars in Control leaves are 140 % , in Infected leaves are 778 % and in *Trichoderma* treated contain 771 % value of total sugar of Betle vine Var. Kapoori . The maximum reducing sugar observed in Infected leaves as compaired to *Trichoderma* treated leaves while total suger in control leaves was observed very low as compaired to Infected leaves and *Trichoderma* treated leaves of Betle vine Kapoori. This due to more accumulation of sugars in infected leaves

On the contrary lower content of total sugars are reported by Nagvi (1977) Kapur et al., (1978). Sankpal and Nimbalkar (1980), Agarwal et al,(1982) Mahaptra (1982)Goel et al.,(1983) reported lowered total sugar content in groundnut affected by cercospora, date palm, sugarcane infected by Smut turmeric infected by *Taphrina* , coriander infected by protomyces.

The change in total sugar content in infected plant was observed by many workers . According to Srinivasan and Chellliah (1979) total

sugar accumulated in brinjal leaves affected by MLO. On the other hand decreased total sugar content in Sesamum infected with GSD reported by

(Prasad and Sahambi ; 1980 and Dhumal , 1983).

The change sugar contents are observed due to viral infection . The maximum sugar content in beans affected by yellow mosaic virus reported by (Singh and Singh ;1983). On the other hand decrease in total sugar contents are reported by many workers. Thin et ; al (1989) content of total sugars decreased in summae moong infected with mung bean yellow mosaic virus and leaf wrincle virus.

Our investigation shows more decrease in total sugar content in infected leaves than *Trichoderma* treated leaves of *Piper betle* L.var. kapoori However highest percentage of total sugars are observed in Control leaves than another three sample.

The effect of fungal infection on total sugar content was observed by many wprkers .According to Mahmud et al ,(2004) reported decrease in tital sugar content in faba bean infected with *Botrytis fabae* . Similar results are observed to Tang et al,(2005) in mulberry infected with *Cercospora manicola*

Change in starch following infection have been observed in many foliage diseases. The facultative parasites such as cercospara also causes

a depletion of starch. Kalandarsamy (1964) reported the reduction in starch in leaves of Banana and initial major content. Of ground nut leaves infected by cercospora personeta Nagaraja and Thite (1988) and Nagaraja (1990) reported increase in starch and total carbohydrates in Rubia and *phyllanthius emblica* after infection. Thus this increased content of starch may not be nomally utilized for the growth of pathogen hence it accumulates in the infected parts.

i) Reducing sugars :

The effect of *Trichoderma* treatment on inforced leaves of *piper betle* L. var. kapoori is shown in Table. No. (4) and depicted in Fig No.(5). It is evident from the result that the amount of reducing sugars in *Trichoderma* treated leaves increased as compared to control leaves. However reducing sugars maximally accumulated in infected leaves as compaired to *Trichoderma* treated and Control leaves .The concentration of reducing sugar in infected leaves are increased due to infection of pathagen. The amount of reducing sugar also increased in *Trichoderma* treated leaves however it is less than infected leaves of *Piper betle.*

ii) Non reducing sugar :

The amount of non reducing sugar was observed maximum in Infected leaves than *Trichoderma* treated leaves ,while contral leaves

shows less value of non reducing sugar than Infected and *Trichoderma* treated leaves.

iii) Total Sugars :

The total sugar content of Trichoderma treated leaves of *Piper betle* L. var. kapoori increased after treated conditions. However, it is not more than Root Rot leaves. The amount of total sugars in infected leaves is decreased as compaired to control, Tricha derma treated and Root Rot

(yellow) leaves. The total sugar can centration is very low in infected leaves and total sugar concent ration is very high in Trichoderma treated leaves and Root Rot leaves respectively.

iv) Starch :

The starch content of all sample of leaves in piper bettle L. var. kapoori is greatly influenced by infection and treatment.However, maximum starch are observed at infection level. Higher levele of starch observed in Infected leaves as compaired to *Trichoderma* treated leaves ,while the starch value in control leaves was observed very less than Infected and *Trichoderma* trated leaves.Which is more exceed that in the. The starch content of leaves also adversly affected by infection, increased due to infection of pathagen. However out of threer sample of *Piper betle*. Very high starch observed in infected leaves.

The different trend is observed for total carbohydrate content of *Piper betle* under both Infection & *Trichoderma* treatment.

v) Total Carbohydrates :

The amount of total carbohydrates decreased in infected leaves than *Trichoderma* treted leaves of *Piper betle*. However Maximum amount of total carbohydrotes are observed in control leaves.

vi) Total soluble protein :

Proteins are important cell constituents for plant growth. They are linear polymers of high molecular weight. Cells contain a very large number of proteins. They are tuiding blocks of many amino acids. Hence, their reduction in infected plant parts will ultimately hamper metabolic activities and affect the growth of plants.

Protein are generally correlated with nitrogen content. Therefore, high nitrogen content in the infected Root Rot leaves Ultimately show high protein content. Increase in protein is due to protein synthesis of the pathogen or host. Accumulation of proteins observed in the present study might be due to decreased translocation from leaves to other plant parts under roltining of root of betel vine. Increase protein in infected plant parts is due to protein synthesis of the pathogen or host are reported staples and ledbetter, (1958). On the other hand stimulated protein

synthesis in host tissue around the infection centre also infected potato by *Phytophthora* and infected sweet potato by ceratocystic (Goodman et al.,1967).

Effect of *Trichoderma* treatment on total soluble protein in infected leaves and foot- rot leaves of *Piper betle* L. var. kapoori is depicated in Table No.(4). The value of protein content in control leaves was observed 0.085 , In Infected leaves were 0.122 and *Trichoderma* treated leaves were 0.111. The value of protein are increased maximum in Infected leaves of *Piper betle* infected by *Phytophthora parisitica* var. piperina. However protein of *Trichoderma* treated leaves of infected vine are observed maximum than control leaves. It is observed that very high level of protein accumulated in Infected leaves than *Trichoderma* treated leaves while very low level of protein are observed in control leaves.

In infected beans (*uromyces phaseali*) there is a great incorporation of cysteine labeled with S35 in the protein fraction of the leaf at the infection centres than in the uninfected parts of the leaf. This indicates that there is greater protein synthesis in diseased tissue. The increase of protein in infected plant part is mainly due to the synthesis of proteins by the pathogen rather than the host as experiment done with

triticum labeled glycine is mainly incorporated into the fungal mycelium and uredospores (staples and led better,1950)

A parallel proteolysis and ptotein synthesis can occur in rust diseases. Rodolf (1963) compaired nitrogen metabolism of wheat infected with rust (puccinia graminis tritici) in intact (attached) and detached leaves, rusting in an accumulation of free amino acids and amide, while a relative increase in protein synthesis was suggested after infection in detached leaves accompanied by a decrease in free amino acids and amides.

Kiraly et al. (1966) reported in attached leaves of bean infected with rust *Uromyces phaseoli*, there is an increased protein synthesis in the tissues of the green island surrounding the infection centre, while the tissue zone between the green islands become senescent are by a low capacity for protein synthesis (shaw and Kolotel; (1961). The resistant varities display upon infection a decrease in total soluble and protein nitrogen which is in contrast to susceptible varities.

Protein increase in rust infected leaves of ground nut are reported by patel and vaishav (1986). Vidhyasekan et al., (1973) have shown that there is an increase in protein content of rice grain due to infection of *Helminthosparium.* Vidhyasekaran and Durairaj (1971)observed increase in protein content n citrus due to xanthomanas infection. Patel and

Vaishav (1986) also reported increase in protein in rust infected leaves of groundnut.

In the present study increased protein content in *Trichoderma* treated infected leaves as compaired to control leaves due to resistance nature of *Trichoderma* against *phytophthora parasitica* var. piperina is phythogenic fungi while increased protein content in root rot than infected leaves may be due to systhesis of proteins by pathogen in the host tissue, because planty amino acid are available in the break down of proteins in host cells synthesis of proteins seems to play a role in disease resistance. In fungus infected plants, the total protein content of the host pathogen complex increase. The influence of host parasite interactions on proteins and resistance has been discussed by stahmann (1967). Nucleic acid and protein changes in wheat leaf nuclei during rust infection were studied by Bhattacharya et al. (1965).

E) Inorganic Constituents :

(I) Sodium (Na+)

The values of sodium content are recorded in Table No.(5) and Fig .No. (6) which shows the sodium content, decreased in infected leaves while maximum sodium content is observed after *Trichoderma* treatment, in infected leaves of *Piper betle* L. var. kapoori, while low

sodium contents are observed in control leaves as compared to *Trichoderma* treated leaves.

Both favourable as well as adverse effects of infection on sodium content have been reported by Nambiar and Ram Krishnan (1969) in pigeon pea affected by mosaic virus.

Sodium is a functional element for all terrestrial plants (Nicholas 1961). Sodium is also one of the most dominant $CaHO_4$ in saline soils. It is activator of transport ATP-ase in animal and possibly in plants. This monovalent nutrient is required for photosynthesis to decrease the $C0_2$ assimilation. Brownwell and crossland (1972) reported that C4 plants require Na^+ as an essential nutrient. Sodium also can replace potassium partly. Kulkarni and Kulkarni (1978), Patil and Kulkarni (1977). in mango, sunflower affected by capnodium and *puccinia* respectively reported decrease of sodium content in the infected leaves of plants.

On the other hand increase of sodium content after biological treatment in infected leaves have been reported by many workers.

In our present investigation the sodium content is decreased in infected leaves. On the other hand sodium content increased in *Tichoderma* treated leaves of *Piper betle* L. var. kapoori. The decrease of sodium content may be due to either destructive changes in host tissue or infection have inhibited the absarbance of sodium. The increase of

sodium content may be due to either normal changes in host tissue or *Trichoderma* treatment have return absorbance of sodium.

Our result of the present investigation show the similar trends of decrease in accumulation of potassium in infected leaves of Betel leaves infected by *Phytophthora* foot - rot disease.However the because the ratio of K / Na also greatly increased because of increased K and decreased sodium.There are some correlation between leaf N and K to disease susceptibility tolerance of vines

(Balasubrahmanyam, Chourasia and Rawat ;1982.)

(II)Potassium (k+) :

Potassium is considered as a most essential, mocronutrients and its uptake is highly selective and closelycoupled with metabolic activity. Potassium lons are used as an activater by many enzymes. It plays an important role in maintenance of cellular organization, permeability of membrane, balance of water in protoplasm K plays an important role in activation of about 60 plant enzymes, protein symthesis, stomatal movement, photosynthesis and osmaregulation (Marschner 1986). At all level it is highly mobile element in plant. It may be directly promotes the synthesis of various organic compounds such as proteins, sugars and

polysaccharides. Potassium also plays an important role in photophosphorylation (pfluger and mengal,1972) and Starch formation (Hawker et al.,1974).

Potassium enhances the plant's ability to resist cold disease and other adverse conditions.It is essential for grain or seed development, improves the quality of fruits, seeds and vegetables (colour, flavour and size). The critical concentration of K is in the range of 0.5 to 2% in dry matter and K requirement for optimum growth of plant is 2-5% High K concentration in cytoplasm and chloroplast is necessary to neutralize the soluble and insoluble macromolecule onion and to stabilize the pH between 7-8 which is optimum for enzyme reaction.

It is essential for carbohydrate breakdown during respiration. Potassium lons are used to maintain electroneutrality in cells. Under potassium deficiency, phloem transport is affected, NR activity is declined, protein synthesis is disburbed, amino acids and soluble organic nitrogenous compounds are accumulated. Potassium deficiency when is very acute, the plant dies, when it is mild, growth in shoot system is affected. The essentiality and physiological role of potassium in fungal metabolism have been little investigated. Among all the metabolic minerals, potassium is present in the largest amount in both mycelium

and spares, concentration of 0.001 to 0.004 M of this metal are adequate for most of the fungi (Steinberg,1946, Jarvis and Johnson,1950).

Potassium deficiency is responsible for physiological diseases like i) Die-back disease and ii) Rosethe disease.High K. concentration in cytoplasm and chloroplast is necessary to neutralite the soluble and insoluble macromolecule onion and to stabilize the pH between 7-8 which is optimum for enzyme reaction.The muriate or sulphate of potash are generally used in potassium deficient soils, to remove potassium deficiency in plants.

(iii) Calcium (Ca^{++}) :

Calcium is an important and essential contain required by the plants. Most of the soils contain enough calcium for adequate plant growth. Its optimum value for terrestrial plants is about 0.5% or 125 mole per gram of dry tissue (Epstein 1972).Atomic weight of calcium is 40.08. It is available in Ca^{2}+ form to plants.Concentration in dry tissue is 5,000 ppm and 0.5%.Calcium is of fundamental importance for membrane permeability and maintenance of cell integrity common calcium ores are Apetite (calcium phosphate) calcite ($CaCO_3$) and Dalomite ($CaCO_3$, $MgCO_3$) calcium is absorbed from soil as calcium ion (Ca_2+).Uptake

and transport of calcium in plant is almost passive. It has also a role to play in ion uptake. It helps in formation of middle lamella by the formation of calcium pectate.Calcium in many organisms, including fungi, acts as a protection against the injurious effects of certain common monovalent cautions, especially H^+, Na^+ and K^+. It is required for the normal functioning membrane and has been implicated as a second messenger for various plant responses to both environmental and harmonal stimulus.In its function as second messenger calcium may bind to calmodulin, a protein found in the Cytosol of plant cell.The calmodulin–calcium complex regulates many metabolic processes calcium stabilize cell membrane by binding phosphate and carbonate group of phospholipids (cold well, and Haug 1981).Calcium plays important role in cell division and extension,Ca++ regulates the spindle activity.Calcium is also involved in pollen tube growth clarkson and Hanson (1980) also viewed that Ca ++ maintains calcium play important role in controlling permeability of membrane of various ions mainly inorganic cation. (Ven steveninck,1965).

The plasma membrane Ca_2+ transport stems strongly dependent on Ca_2+ activity in their immediate vicinity while reflecting of Ca_2+ transport Renzel (1992). According to (Roberts and Harmson,1992) phosphorylation and dephosphorylation protein is now regulated by one

important mechanism of regulation of enzyme activity turning a phosphorylation on results of changes in cystolic Ca++ activity triggers the chain of events, so it altemately affect a large number of biochemical reactions (Swamy,1991).

Calcium is partially mobile through xylem and pholem while uptake of calcium in plants is found to be influenced bynumber of environment edapic factors of soil pH light, soil temperature, other cautions and plant demand of calcium is its regular uptake for the growing season. It promotes root.

According to Rains (1972), Ca++ protects injurious effect of H^+ and other toxic ions. Both calmodulin and calcium dependent protein kinase contribute to the role of calcium as a second messenger.

Calcium in many organisms, including fungi, acts as a protection against the injurious effects of certain common monovalent cautions, especially H+, Na+ and K+. However calcium controls the translocation of Na & K plants. Calcium is essential to reduce metal toxicity in plant.

The calcium contents in the healthy Control leaves were 1920.% In *Trichoderma* treated leaves were observed 2560. % while in the infected leaves it is 1520 .%. The *Trichoderma* treated leaves also show increased calcium during control of disease.The maximum calcium content is observed in *Trichoderma* treated betel vine than

infected and control leaves. It appears from the data that calcium has some role to changes the metabolism in infected as well as *Trichoderma viride* treated vine.

Calcium acts as cofactor for activities of several enzymes such as - amylase, ATpase, succinic dehydrogenase and enzyme involved in nitrogen metabolism (Clark,1984) reported that many enzymes has been reported to be stimulated or inhibited by calcium are ATpase, protein kinase, pyruvate kinase, Nuclease - amylase, pectine esterase and lipoxigenase.

According to (Swamy,1991) deficiency of calcium in plants leads to break down cell walls, increase in permeability leaflessness of cell membrances chlorosis generally occurs along the margins of younger leaves, its deficiency also cowes down ward hooking and malfomation of younger leaves, symptams are occured in tissue may be due to its immobile nature in plant. According to Atksinson et al., (1980) there are many calcium deficiency related disorders are seen in plants are fruit and vegetable mealiness, internal breakdown corking, cracking bitter pH tip burn & browing results disorganisation of cell walls of plants calcium deficiency results in accumulation of starch in leaves.

The folior analysis of betelvine cultivated at Mohoba indivated the Ca content is 0.71 on dry basis under closed system of cultivation (

Balasubramanyan et al.,1984). According to (Gour et al.,1972) the incareased nutrient could be due to the conductive soil environment provided by the organic manures.However, they reported calcium varied widely but seemed to bear no relationship to leaf yield (V.R. Balasubramanyan,Ahmanyam, R.S. Chaurasia and K.K. Singh 1989) reported calcium content in two varities of Betel vine is Desawari & C.V. Bangla variety.According to them calcium content in Cv. Desawari under high performance are observed 0.018 – 1,17/ ha In average performance is 0.060 – 1.143 and in low performance calcium is 0.072 – 1.107 the values are given in percent dry wt. However, in Bangla Cultivated variety Ca is 0.102 – 0.819 in High performance, 0.180 – 1.296 in Average performance and calcium in low performance is 0.279 – 0.954 in percent dry weight. They showed the values of calcium & Mg in leaf ranged and was high in low performance plantation.

Nutrient element content at betel leaves gives in Desawari, 0.60 in Bangla the values are given in percent dry wt. (V.R. Balasubrahmanyam and K.S. Rawat 1988). The calcium content differ from variety to variety in betle vine.

The calcium content in healthy and infected leaves of Cv. Bangla (44)* & Cv.Desawari (205)* reported that the common diseases observed in these plantations are foot and leaf rot by *Phytophthara*

parasitica var. piperina, coller rot *Athracnose*, and bacterial leaf blight wilt. The leaves at healthy Desawari (205)* showed 0.057, Moderate in 0.46 and 0.29 in high disease incidence in percent dry weight. However, In Bangla (44)* Ca in Healthy is 0.58, 0.56 in Moderatw & in high disease incidence calcium value is 0.18 disease index in percent (V.R. B alasubrahmanyam,R.S. Chaurasia and K.K. Singh1989).

At present similar concentration of calcium in the leaves of *Piper betle* L.var.kapoori are reported. The concentration of calcium in the *Trichoderma* treated infected leaves of Kapoori increased considerably. The decreased calcium in infected leaves is observed as compaired to *Trichoderma* treated leaves.while in control leaves calcium value slightly increased but less than *Trichoderma* treated leaves. The maximum calcium was observed in *Trichoderma* treated leaves of Betle vine Var.Kapoori.

Higher concentration of calcium have been reported by Balasubramanyan (1975) in soghum affected by downy mildew, Sankp[al and Nimbalkar (1980), Ghorpade and is smutted sugarcane and sugarcane mosaic virus reported higher calcium concentration, Sivaprakasam et al.,(1974) reported higher concentration of calcium in brinjal infected by verticillium, panopoulos et al.,(1972), Sasikumaran et al., (1979) and Ghorpade and Joshi (1981) have been reported increased

calcium content in leaves of tomato and sugarcane infected curl top, leaf curl and sugarcane mosaic virus respectively. The intensity of conidation in *Trichoderma viride* is also affected by Ca concentration (Brian and Hemming 1950).

The present results indicates calcium role in development of disease tolerance due to *Trichoderma* treatment to the infected betel vine Var. Kapoori. However, amount of calcium may be slightly increased. It may be due to development of disease resistance in the Betel vine.

iv) Magnesium :

Magnesium is secondary nutrient. It is a divalent cation (Mg^{++}) which plays a important role in all physiological processes in plants.Magnesium is an important constituent of chlorophyll molecule and therefore, essential for chlorophyll synthesis, it is essential for all green plants.

Magnesium in plants is very moble in the pholem and can be translocated from one tissue to another (Clerk,1984).According to Mengel and Kirlsby (1982) fruit and storage tissues are highly dependant on the pholem for their mineral supply, so they are higher in potassium and magnesium than in calcium. It acts in the uptake of phosphorus and regulates uptake of other nutrients. The magnesium level in the nutrient

medium is also of important in relation to manganese uptake. The combination of magnesium and manganese was more effective in surose synthesis in sugarcane (Patil and Joshi 1972).

Magnesium primarly absorbed the root apex which act as passive uptake concept (Russell and Clarkson,1976).In plants Mg is generally absorbed at lower concentration either Ca2+ or K+. According to Hall (1971) the calcium deficient tomato tissues has very high level of magnesium. In the form of phosphate carrier, magnesium is essential for phosphate metabolism.

Magnesium behaves similarly to calcium in soils being derived from alumino.Silicate, silicate or sulphate minerals or non-carbonate parent materials or from dolomite (MaCa (C03)2) and magnesite (MgC03).The total soil content of Mg, like Ca, is widely varjable ranging from 0.003% to 0.6% Mg in normal soils (Bould 1963) to much higher values in dolomite soils ehen 1.2% Mg may be exceeded (Bear,1964).

According to Mulder (1950), Potassium Mg and Magnesium K are inversly proportional to each other. When potassium level high in soil resulted in Mg deficiency in apple leaves while high level of Mg in plant cause low level of potassium.

One most important role of Mg is as co-factor in almost all enzymes activator during phosphate transfer, ATPase synthesis respiration and synthesis of nucleic acids. It forms a bridge between the pyrophosphate structure of ATP or ADP and enzyme molecule. According to (Balke and Hodges,1975) activation of ATPase by Mg2+ is brought about by bridging function, phospho - kinases, dehydrogenases and analases are activited by magnesium.

Magnesium is assiciated with organic onions as malale citrate, pectate and axalate and inorganic ions (Kirkby and Mengel,1967).The presence of this elements contributes to the electrical neutrality of organic compound like sugar phosphates, sugar nuceoticles, organic and amino acids (Clark,1984).

Exchangeable Mg in soils is usually less the exchangable Ca despite the fact that the total Mg content of non-carbonate soils often exceeds tota Ca. Brar (1968) gives a mean value of 1.6 me/100 g for exchangeable Mg and 40.7 mg /100 g for total Mg in a nmumber of N.Amnerican soils. The concentration of Mg in soil solution is similar to that of Ca of about 10 me/I (C.Fried and Broeshart,1967). According to Sharma (2006), Mg++ is most ignored element in Indian tissue culture.

Magnesium value in folior analysis of Betelvine plantation in Mahoba suggest magnesium content varied widely but seemed to bear no relationship to yield of leaves.

Nutrient element concentration in Cv. Desawari & Bangla leaves of high average and low performance plantations observed that in Desawari the value for leaf magnesium ranged very narrowly and was high in low performance plantations.There was negative signification correlation of Mg is (- .158) between yield and leaf nutrient concentration.

Magnesium content in Cv. Desawari betel leaves resulted & Cv. Bangla betel leaves of apparently healthy and disease condition showed the result that in Desawari Mg value are high/maximum in disease plant (1.116), as compaired to Moderote (1.06) & Healthy (0.97).

In Bangla (44)* Mg. content maximum in disease affected vine is (1.09%), as compared to Healthy (1.02 %) and Moderate (0.96%). It is suggested that higher Mg in the diseased tissue may be due to concentration effect.

Nutrient element concentration of Bangla and Desawari of healthy leaves showed value of Magnesium in Desawar is 0.70% In Bangla varidfy magnesium value is 0.99% (% dry weight). (Sultan 1975; Bartakke 1977)

The PEP-corboxylase the enzyme of Ca_2 assimillation during night need magnesium as a Ca-factors in CAM plants. Bartakke (1977) Reported Magnesium activities the enzyme PEP-case and RUBP-case in Aloe borbadensis Jacob (1958) reported the magnesium also promote the formation of vitamins is carotene. Mummification of being was observed undedr magnesium deficiency (C. Sharma,2006).

Magnesium present in betelvine shows differences due to the type of different writers Magnesium value in Bangla is 0.63 (% dry wt.),Desawaris–0.75(% dry wt.) kapoori 0.55 (% dry wt.),

(Balasubramayamet al ; 1994).

Enzyme peroxidase and RNase also due Mg deficiency magnesium deficiency cause development of unthocynin pigments in aerial organs. Also, it is responsible for physiological diseases such as (1) Sand-drawn disease (ii) Matted chlorosis and necrosis in leaves.

Fungi also need about 7 elements to meet their nutritional requirements. Mg is also important element for fungi they utilize magnesium in the form of specific compounds as ions and free elements. Also, Mg are found in the ash obtained after burning the spares and the mycelium.Mg also known as funtional element, because they also have structural property.The most adverse effect of Mg eficiciency observed at the time of mummification of grape berry (Sharma, 2006) reported Mg

deficiency causes great loss in grape yield.High application of potassium fertilizers also important cause of Mg deficiency in groups.

The values of Magnesium depicted in Table No.(5) and Fig. No.(6), in show the value of Magnesium in Control leaves were found 3260. % , Infected leaves 2480. % and in *Trichoderma* treated leaves were 4230. %.The maximum increase in Magnesium content is observed in *Trichoderma* treated leaves of *Piper betle* infected Var. Kpoori than in Infected leaves infected by *phytophthora parasitic* var. piperina. While In control leaves value of Magnesium is less than *Trichoderma* treated leaves may be due to resistance developed by *Trichoderma* treatment. However, decrease Magnesium content may be due to the minimum level of potassium content in infected leaves of Betle vine Kapoori.

Many workers reported increase or decrease in Magnesium content in infected leaves. An increased Magnesium content has been reported by Hegde and Karande (1978) in Bajara affected by downy mildew, Sankpal and Nimbalkar (1980) in smutted sugarcane. Raghupathy and Jayaraj,1917 reported higher magnesium concentration in leaves of sesamum plant infected by MLO. While lower Magnesium concentration was observed in brinjal plant infected by MLO (Sivaprakasam et al. 1976).

The deficiency of Magnesium in infected leaves are due to the result of chlorophyll degradation. The leaf spot and root rot leaves shows symptoms like chlorosis, Magnesium catalyse the sucrose synthesis in higher plants. it is an activator of enzyme RUBP case

(Larimer et al;1976) and a co-factor in the reaction of sucrose synthetase and sucrose synthetase. The sucrose synthesis in higher plants catalyse the Magnesium.However accumulation or Magnesium in susceptible line during initial stage of growth. Reduction in Mg in Infected leaves of *Piper betle* ultimately affects the sugar concentration. Seaker et al.,(1982) also reported role of magnesium in disease resistance. Under Magnesium deficient the plant have greater concentration of pathogen. However due to infection fungal attack inhibit the uptake translacation of Magnesium in leaves.

The increased concentration of Magnesium in *Trichoderma* treated infected leaves of Betel vine suggests the inhibition of fungal growth due to the recovery of Mg in observed than infected vine. It may be one of the reason of induction of disease resistance due to treatment of *Trichoderma* in Betel vine.

The present observations indicate that Mg concentration has some role to play in development and disease resistance in connection with the

enzyme activities and other metabolic reactions in Betel vine treated by *Trichoderma viride.*

(V) Phosphorus :

Phosphorus is an important macronutrient essential for all living organisms. This non-metalic element is essential for all forms of life, including fungi. A Phosphorus forms the basic component of nucleic acids DNA, RNA the high energy Phosphorus, ATP, ADP & the phosphalipids. Phosphorus is Primary Plant nutrient it is obsorbed from soil in the form of phosphate ion (HPO_42) and (HPO_42).The H_2PO_4 is being favoured below pH 7 and H_2PO_4 above pH7. Automatic wt. is 30.98. The concentration of Phosphorus in dry tissue is 2,000 ppm and 0.2% Much of the Phosphate is converted into organic forms upon entry into the roots or after transport through the xylem into shoot either free or bound to organic forms of ester. It play major role is energy transfer during plant metabolism like respiration, photosynthesis in the form of ATP, NADP and also in cell division and cell expansion phosphorus is involved in the formation of cell membrance lipids, which play a vital role in ionic regulation (Bieleski and Ferguson,1983). Phosphorus concentration of the terrestrial plants is 0.2% of dry weight (Epstein 1972). Phosphorus is also a constituents of a variety of organic

compound which are essential for the structure and metabolism of plants. It is present in the plant cell as inorganic P or phosphate or energy rich phosphate bound. Hall and Baker, (1972) have shown that inorganic Phosphorus plays a vital role in phalem transport. There are two forms of phosphate Pool i.e.,metabolic pool and non-inetabolic pool. The cytoplasmic pool contains phosphate enters while vacuolor pool inorganic phosphate. The inorganic P (ip) is a substrate as well as product of number of enzymatic reaction and hence compartmentalized to regulate metabolic reaction of cytoplasm. According to Marschner, (1986), the Phosphorus influences processes like photo synthesis and carbohydrate metabolism so its concentration in tissue decides yield of crops. In plan, Phosphorus content is greatly influenced by several environmental stress such as salinity water logging, water stress, and pathogen attach. According to White, (1973) Phosphorus demand is associated with the rate of plant growth and level of metabolic activities.

TABLE . NO. 6

Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Copper of Control, infected & *Trichoderma* treated leaves *of Piper betle* L. var.kapoori.

Sr.No.	Parameter	Control	Infected	*Trichoderma* treated
1.	**Sodium**	170.	150.	180.
2	**Potasium**	440.	400.	620.
3.	**Calcium**	1920.	1520.	2560.
4.	**Magnesium**	3260.	2480.	4230.
5.	**Phosphorus**	310.	220.	360.
6.	**Iron**	10.4	8.3	10.5
7.	**Copper**	2.9	1.5	2.2

***Values are expressed in mg/100 gm dry weight.**

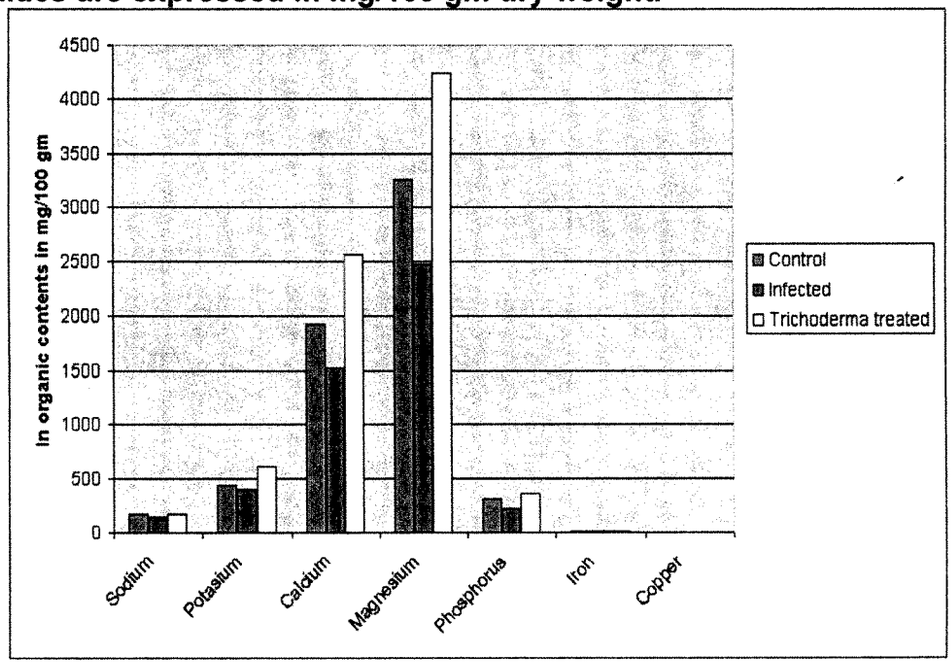


Fig.6. *Values are expressed in mg/100 gm dry weight.

The optimum level results increasing phosphorus content & it enhances plant growthbut due to heavy accumulation of phosphorus cause stunted growth (Sing and Sing,1977).

High concentration of Phosphorus affect plant growth plant recover from Phosphorus deficiency when phosphate fertilizers are added in the soil and phosphate is made available to the plant Sharma; (2005) observed that the phosphorus is responsible for bud differentiation and root development. The optimum concentration of phosphorus increase the weight of grape berries. The high application of Phosphorus fertilizers reduces the uptake of iron and zinc. Sharma,(2006) further reported application of proper phosphorus fertilizers reduces the incidence of downey mildew and powdery mildew.

Phosphorus requirement of plant species is the range of 0.3 to 0.5% dry weights during vegetative growth. The P uptake is pH dependent and at low pH enhances Phosphorus uptake. It is also controlled by the internal status of the root and age of the plant heavy concentration of Phosphorus are found in meristematic regions of actively growing plants. Therefore, it stimulates root development early growth and early maturity of crops. It stimulates flowering, helps in seed and fruit formation, that is reproductive growth of plant. It contributes to general hardiness of plant. There is close relationship between

phosphorus and nitrogen regarding maturity in plants, excess nitrogen delying maturity and abundant Phosphorus causes early maturity. Also, excess Phosphorus causes more root growth, relative to shoot growth while excess nitrogen cause low shoot to root ratio.

It aids legume in nodule formation. It is essential part of many sugar phosphates involved in photosynthesis, respiration and other metabolic pathways. Phosphorus is both a structural and functional element in the growth of fungi. The fungi apparently utilize P in the form of phosphates. Different phosphates support different amount of growth. Both inorganic and organic phosphates may be utilized by the fungi. Raulin (1869) recognized the essentiality of P in nutrition of *A.niger* Fungi exhibit varying requirement of P. According to Jacob and Lawior (1992) plant with low p had low photosynthesis rate and less carboxylation efficiency than plant with ample P.deficience of Phosphorus affects all aspects of plant growth and metabolism, resulting shunted growth, poor development of root rowth, premature leaf fall or early senescence. Deficiency also causes accumulation of carbohydrates in plant. Deficiency of Phosphorus leads to less total soluble protein and Rubisco/unit area. Phosphorus is major element which plays an important role in productivity and hence it is essential ingradient of N.P.K. fertilizer.

The values of Phosphorus content in control, Infected leaves and *Trichoderma* treated leaves of *Piper betle* L.Var.kapoori are given in Table.No.(5) and Fig.(6). The value in Control leaves are 310. % , in Infected leaves are 220. % and in *Trichoderma* treated leaves are observed 360. %.It shows increased Phosphorus content in *Trichoderma* treated, infected leaves of Betel vine.The maximum decrease in phosphorus content are observed in infected leaves, while Phosphorus in control leaves are less than *Trichoderma* treated leaves. Also maximum increased value of phosphorus are observed in *Trichoderma* treated leaves.

Decreased phosphorus in infected vine have been reported by some workers viz. Sivaprakasam et al.,(1974) reported low Phosphorus content in brinjal leaf infected by verticilium.Patil and Kulkarni (1979) reported decreased Phosphorus content in sunflower infected by rust,similar report of low concentration of Phosphorus in date palm offered by Smut (kapur et al.,1978).Sankpal and Nimbalkar (1980) reported decrease in Phosphorus content sugarcane affected by Smut.

On the other hand increased Phosphorus in infected plant have been reported by sarghum infected by downy mildew (Bata Subramanian (1981). Weste el al.,(1980) reported increased has phorus content in *Isopugan ceratophyllus* infected by *Phytophthora* Ahmed et al.,(1980) in

barley infected by brown rust, chattopadhyay and Bera (1981),Ayres (1981), Koenigil affected by *Helminthosporium erysiphae* and Colletatrictium respective has suggested increased Phosphorus in infected plant.

The present investigation reveals that the Phosphorus content is highly increased after treatment of *Trichoderma* on infected leaves of *Piper betle* L.Var. kapoori. While several reasons have been attributed to the increased level of Phosphorus after treatment.

According to kannaiyan et al.,(1978),Balasab,Bhaskaran and Ramanathan (1983) reported increase in Phosphorus content in host increases the possibility of infection by pathogen. It is reported that high concentration of phosphorus content increases disease susceptibility of the host. Dhumal (1983), reported that sugarcane variety CO419 is more susceptible to GSD due to Phosphorus concentration is high. The inhibitation of development of disease is observed in *Trichoderma* treated leaves, it shows that after *Trichoderma* treatment Phosphorus content increases in infected leaves it shows disease resistance or disease inhibition exhibit resistance against leaf spot and foot root disease Sharma, (2000). reported that application of Phosphorus fertilizers decreases incidance of dowing mildew and powdery mildew in grape vine, Shastry and Nariani (1962), Bains and Jhooty (1978), Kauraw

(1979) reported, the increased Phosphorus content in plant reduces the disease incidence by inducing the internal resistance to the various types at pathogens.

The nutrient element concentration of Bangla and Desawari of healthy leaves are shown that the Phosphorus in healthy leaves of Deswari is 0.23% while Phosphorus contents in Healthy Bangla leaves are 0.34%. However, value of minerals of five Betel leaf cultivors are given and it is showed that the mineral concentration varies from variety to variety in Betlel vine. Phosphorus content observed in Bangla variety is 0.23% In kapoori is 0.61%, in Desawari 0.35%, Meetha is 0.25% and in Sanchi it is 2.65 % while Phosphorus concentration ranges between 0.12 and 0.22 in the stem and 0.13 and 0.16 % in leaves.(Balasubranayam et al;1994).

The studies conducted by National Botanical Research, Lucknow have shown that betel vine removes appreciable quantities of nutrients from the 50%. It has been estimated that a crop of 10 tonnes of leaves per hectare removes about 80 kg. nitrate 14-15 kg Phosphorus and about 100 kg. potassium. These three elements need to be supplied to the soil in insufficient quantities.

Betel vine does not readily manifest sign of distress caused by inadequate supply of nutrients. Hence, the production may suffer long before the symptoms appear. It is therefore, necessary to know the needs

commom disseases observed in these plantations are foot and leaf rot caused by *Phytophthora parasitica* var. piperina, collor rot, anthracnos, bacterial leaf spot, wilt, blight. In Deswari (205)* phosphorus element in healthy is 0.46%, In moderate – 0.32 % and in High disease incidence p is 0.46%, while value of phosphorus in Bangla (44)* in healthy is 0.32%, In moderate is 0.33% and in high disease incidence P – is 0.57 % from it is shown that the higher nutrient (N.P. and Mg) in diseased tissues may be due to concentration effect similar trend was also observed in Cv. Bangla vine.

The high disease incidence in some of the vineyards surveyed may be due to unusually high rates of nitrogen fertilization practiced in the area. The present observations agree with that of Hursch (124) as quoted by Lundegarh and Ashby (1981) that excessive nitrogen seems to facilitate the attack of pathogens. The increased suscentibility is sord to be the formation of collenchyma instead of sclerenchyma. Application of high rates of nitrogen fertilizer increases disease incidence of Leaf spot and root rot Hence, the *Trichoderma* treatment and low concentration of Nitrogen (N 4.01%), minimis disease incidence and increases maximum leaf yield in *Piper betel* L. var. kapoori. The phosphorous contents in the present studies also on similar likes and it may be in connection with diverse resistance development in Betel vine.

(VI) Iron (Fe ++):

Iron is a micronutrient for plants it has a much wider pedogenetic and microbiological significance than its quantative uptake would suggest Iron makes up some 0.7 – 4.2% of temperate zone soils and between 14-56% of many tropical latosols. Small percentages of iron occur at lathe components of the clay alumina-silicates but the greater part is present either as hydrous ferric sesquioxide in particulate from may form organo ferrous complexes or precipitate as FeS, $FeC0_3$ or Fe $(OH)_2$. In normal neutral or alkaline soils the

Fe3- connection in the soil solution is extremely low, but falling redox potential may allow Fe2- to be produced sometimes rising to toxic concentrations of several hundred.

Iron forms a integral part of fungal protoplasm. It is an electron carrier in the oxidation reduction of respiration and is a constituent of certain enzyme and association of iron with various enzymes including cytochromes, cytochrome oxidase catalase and have lent so much support to the possible role of iron Iron seems to occupy a intermediate position between the macro and micro essential elements.

According to Guerinat and Ving (1994) Fe play a role in ribanucleotide dinitrogen reduction and energy yielding electron transfer chain. Iorn taxicity is often associated with Zn and Mn deficiency and with a marked

imbalance of nutrients due to the presence of H_2S (C .perssarakli,2005). Excess accumulation of iron in plant cause severe cellulase damage. According to vajpayee et al., (2000), leaves under iron toxicity exhibit branze spots, maximum concentration of iron in leaves show an increased uptake of Fe in chloroplast and thus, a dramatic imparement of total photosynthetic electron transport capacity According to Beale ;(1999) precipitation of iron in oxidation reduction reactions within the cells creates oxidation reduction oxidative stres in plants when it taken in excess and activates antioxidative enzymes.

Iron is a micronutrient for plants, it is a trace element required for number of metabolic processes in plant. Plant absorb iron as Fe2+ or as Fe chelate. Fe chelates are soluble so they are easily available to roots. The uptake and translation of Fe is hormanally controlled probably from shoot apex (Romheld and Marschner 1981).

Fe deficiency causes morphological and physiological responses in several plants. According to Sing; (1997) Mn and iron deficiency resulted in metabolic disturbances leading to deteriorated leaf. Intervenial 'white chlorosis', appearing first in young leaves, Fe deficiency causes simultaneous loss of chlorophyll and degeneration of chicroplast structure in young leaves.Chapman (1975) and Shankar (1997), reported the deficiency of iron in leaves causes complete

chlorosis.In some plants, iron deficiency results, young leaves become yellow, exhibiting chlorosis,it causes 'induced chlorasis'

Iron content in folior analysis of healthy Betel vine plantations in Mohoba suggested iron content varied widely but seemed to bear no relationship to yield of leaves.

Remarkable influence of Azosprillum was noticed in the Fe contents of the leaf. In un-inoculated vine the Fe content was 10.26 ppm which increased to 13.44 ppm in the combined vines. However, Triacontanal spray also found significantly increased the Fe to 12.57 ppm while the untreated control had only 10.96 ppm. Interaction had no significant effect on Fe content in leaf.

Its optimum concentration in glyclophytes is 2.0 mole per of dry tissue (Epstein 1972). Ramkrishnarao and Ramalingas Wamy (1981) noted a range from 127.20 to 270.6 ppm in crop plant.

Many workers have noted that increase or decreased content of iron under pathogenesis e.g. Hegde and Manjal (1971) reported in Bean Pods infected with *Colletotrichum*, Iron affects the production of penicilin (Koffled et at.,1947). Iron has been found to influence the pectic emzyme system of the pathogen *Fusarium oxysporum* var. vasinfectum (Subramanian,1956). Apparao (1959) showed that iron is an effective antagonistic against certain toxic ions, such as Cu and Zn,

during studies with *Pyricularia oryzae*. Kulkarni and Kulkarni (1978) noted that there is accumulation of iron in leaves of sunflower infected with puccinia and mango infected by *Capnodium* respectively philip and Devadath ; (1981) in rice infected with bacterial blight reported increase in iron content as compaired to healthy ones.

On the contrary Sankpal and Nimbalkar (1980), Dhumal and Nimbalkar (1982), Mogle and Mayes (1981) in sugarcane with smut G.S.D. with sugarcane, bajara infected by sclerospora graminicola reported decrease iron content in infected leaves respectively.

In our present investigation Table No.(5) and Fig.No (6) shows there is maximum decrease in iron content in infected leaves of *Piper betle* var. kapoori. But there is no change in iron content in healthy, control leaves of kapoori variety. The maximum increased iron content was observed *Trichoderma* treated leaves.Thus decrease in iron content after infection may be due to its translation to physiologically active site as reported by Brown (1976) same results are observed in control and *Trichoderma* treated leaves of *Piper betle* vine.. However, iron content are observed maximum in *Trichoderma* treated leaves as compared to Infected leaves while it is slight less than control leaves.

The decrease in iron content in infected leaves of turmeric was observed in varieties of salem and Rajapure (M.Phil.Thesis 1997). Iron deficiency

decrease in the process of growth due to slow respiration. Deficiency of iron in plant decreases the protein fraction simultaneously with an increase in the level of soluble organic nitrogen compounds (Bennett,1945; perur et al.,1961). Department of Botany Shivaji University,1997). From Fig.No.(6) the infected leaves of *Piper betle* show decrease in the content. The *Trichoderma* treatment on infected leaves exhibit increased concentration of iron as compared to infected leaves. The results obtained in the study are very intreresting because . It is possible that due to high iron content in Var. Kapoori make susceptible to fungal pathogen attacks. It is proved that it is highly susceptible to disease like foot rot caused by *Phytophthora parasitica* var. piperina. The high concentration of iron in control leaves and *Trichoderma* treated leaves of Kapoori suggest the suscentibility towards fungal attacks. In other hands it is due to the Phenol concentration in leaf tissue influenced by iron content.

However, in our studies Fe contents at infection level may be due to creation of resistance indicating Trichoderma treatment has important role in controlling the disease of *Piper betle.*

(Vii)Copper (Cu++) :

Copper is constituent of plastocyanin, a compound forming a part of photosynthetic electron transport chain and component of cytochrome – C – oxidase of respiratory chain. It activates a group of oxidizing enzymes and is a constituent of certain proteins, organic matter, soil micro-organism and soil pH all are important for its availability to the plant, copper is a essential tomaintain C/N ratio, for induction of flowering.

However, copper is known to act as an electron carrier in enzyme which bring about oxidation reduction and regulates respiratory activity in plants. Copper is needed in a very small quantity 0.01 -0.1 ppm – as compaired to their elements. It is very difficult to prepare a medium free of Cu ions. According Roberg ; (1931) made use of postels method of adding ammonium sulphide to convert heavy metals to sulphides and absorbing these impurities with charcoal. Saraswathi Devi (1958) could not establish the indispensibility of Cu for the growth of some soil *Fusarium* studied by her. It may also play a role in chlorine metabolism of fungi. However its deficiency causes reduction in rates at vegetative and reproductive growth. The work of Ramkrishnarao and Ramlingaswamy (1981) indicated that the copper concentration depends on the growth phase of sugarcane crop. copper deficiency causes many

morphological changes in plant parts. It causes wilting in terminal region and in young shoots, followed by death. However, leaf colour is often faded, due to reduction in amount at pigments. copper deficiency causes reduction in the rates of vegetative and reproductive growth, also young leaves show marginal chlorasis.

Deficiency of copper causes 'Summer die-back' disease of fruit trees (e.g. citrus) and Reclamation disease cereals and legumes. Also due to deficiency plants are weak and show symptoms like chlorosis and spotting.

Many workers have noticed that increased or decreased content of copper under pathogenesis. According to Ramlingaswamy (1981) indicated that the concentration of copper depends on the growth phase of sugarcane. The copper content increased in infected leaves of all turmeric varieties (M.Phil Thesis, 1997). Blank and Talley (1941) found that Cu was toxic to *Phymatotrichum amniverum*. The fungal disease couse an increase or decrease in copper content of host plant. An increased copper content in the cabbage leaves and sarghum infected by *Plasmodiophora* and *Sclerospora* reported by Betz et al. (1980) and (Balasubramanian; 1981).

The present studies undertaken gives the estimated values of copper contents in control, Infected and and *Trichoderma* treated leaves of Betel vine of Var. Kapoori dipicted in the Table No.(5) and Fig.(6). It is observed that copper content decreased in Infected leaves while Maximum increase was observed in control leaves. However, the value of copper content is increased in *Trichoderma* treated leaves as compaired to Infected leaves while it is less than in control leaves.

In research paper micronutrients content of betel leaf under open system of cultivation stimulated new investigation after conducting the preliminary studies, using urea, form yard manure Azospirillum and Triacontanol observed that at reduced N level the leaf Cu content was also get reduced with pre-plant vine dipof Azosprillium, the cu content was 0.04 ppm while it was 0.05 ppm in the combined inoculation. Also Tricacontanol sprayed vines registered 0.04 ppm while the untreated check had also registered the same. The interaction effects were not found to be significant. Cu content increased after inoculati on with Azosprillium could absorb more nutrients than uninoculated control (Lim et al,1983 and Sarig et al,1986).

The combined inoculation Azosprillum recorded, the cu 0.05 ppm, increased nutrient content in betel leaves could be due to the formation

of cytokinin, GA and INA activities in the roots leading to high absorption of nutrients.

The Triacontanal sprayed vines had registered higher micronutrients in betel leaves. The mean values of copper influenced by the iriacontanal spray is Cu - 0.04 ppm due to higher water uptake and cell enlargement which confirmed with the findings of Hangartar et al,1978

Journal of plantation crops 27 (30:221-224, December 1999 Integrated Nutrient Management on Micronutrients contents of Betel leaf under open system of cultivation

(received 20-7-98 revised; 1-3-99; accepted 22-7-99)

The nutrient element concentration of Bangla and Desawari leaves shows the copper content is 26 ppm, present in Desawari, while copper content is 30 ppm was observed in Bangla variety (Research paper - Morphology & chemisty of *Piper betle.*

The copper value in Bangla variety of *Piper betle* is 27 ppm, In Desawari is 20 ppm. (Balasubramanyam et. al. 1994).

According to subramanian (1956) revealed that low concentration at Cu (10-6M) stimulated pectine methyl esterase (PME). as well as polygalacturonase (PG) activity in pectic enzyme system of Fusarium Vasinfectum.However, steinberg (1936-1950) reported lack of Cu in the media reduced the growth of Number of fungi.

In our present investigation it is clear that increase in copper content after *Trichoderma* treatment on Infected leaves of *Piper betle* may be due to the accumulation of copper for its higher amount in healthy plants than treated plays. The infection decreases the Cu contents, however *Trichoderma* treatment returns the Cu contents upto creditable status. It is possible that Cu may plays some role in disease resistance in Betel vine.

(E) ENZYMES STUDIES

(I)Polyphenol Oxidase (E. C. 1. 10. 3 .2)

Polyphenol oxidase are copper proteins of wide occurance in nature which catalyse the aerobic oxidation of certain phenolic substrates to quinononones which are autooxidised to dark brown pigments generally known as melanins. This quinines formed are quite toxic to extracellular enzymes produced by pathogens, due to their activity. According to Sanchez–Amat and Solono (1977), the enzymatic browing is the main function of polyphenol oxidase (PPO) in fruits and vegetables. It is estimated that about half of words fruits and vegetable crops are lost due to the post harvest browning reactions involving the polyphenal oxidase. However this importants property of PPO makes it an important enzyme in food industry. It is also play an important role as efficient reagent for cleaning waste water containing polyphenols.

The polyphenol oxidase category refers to two enzymes in this category – laccase or p.diphenol oxygen oxidoreductase and catechol oxidase or O.diphenol oxygen oxidoreductase catechol oxidase which is also referred to as phenolase, polyphenol oxidasc, tyrosinase or catecholase. The presence of phenolic compounds in plants, their oxidation following injury, either mechanical or due to infection and the relatively high

TABLE NO. 6.

Enzyme activity in Control, Infected & *Trichoderma* treated leaves of *Piper betle* L. var. kapoori.

Sr.No.	Parameter	Control	Infected	*Trichoderma* treated
1	Polyphenol oxidase	0.20	1.15	5.48
2.	Peroxidase	0.68	8.37	3.40
3.	Cellulase	23.808	98.550	11.618

. *Values of polyphenol oxidase are expressed in ΔOD = min -1 mg -1 soluble protein

*Values of peroxidase are expressed in ΔOD = hr -1 mg 1 soluble protein.

*Values of cellulase are expressed in mg glucose released per minute per mg protein.

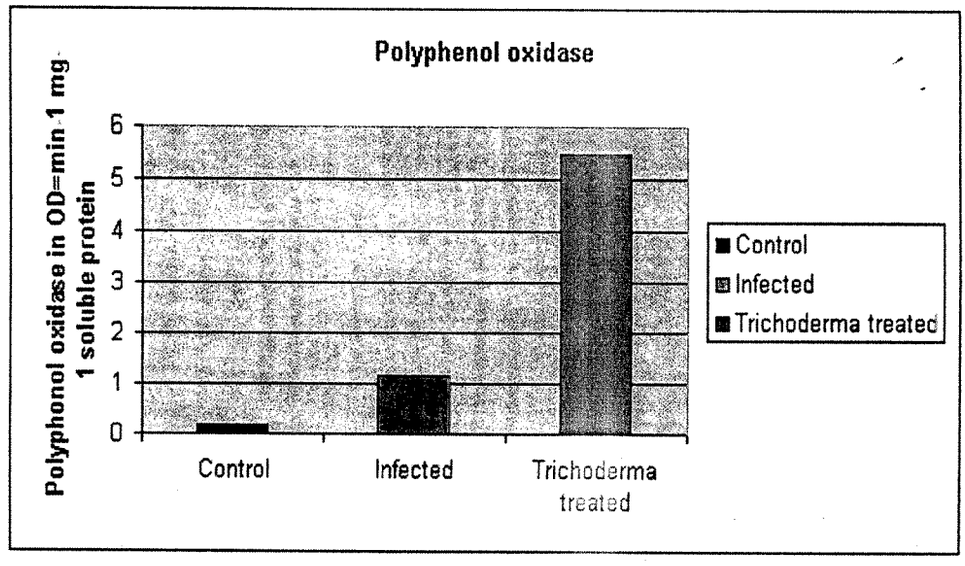


Fig.7. *Values of polyphenol oxidase are expressed in ΔOD = min -1 mg -1 soluble protein

toxicity of the oxidation products have along drawn attention. The possible relationship of these properties to plant resistance to disease has prompted many research workers to ascribe a role to catechol oxidase in disease resistance. Catechol oxidase has been found to increase following infection by virus, bacteria fungi or mechanical injury. Even nematode infection may induce increased catechol oxidase activity (Brueske & Drapkin,1973). The catechol oxidase may function by producing such quinines (Mayer and Hard,1979). Latency of catechol oxidase and the occurance of endogenous inhibitors may, on the other hand, explain the failure to detect the enzyme in some tissues. The enzyme has been reported from a variety of plant organs and tissues and particular cases or pollen grains, latex, crown gall tissue and relatively high leaves in guard cells.

Each individual enzyme tends to catalyze the oxidation of one particular phenol or phenolic compound more readily than others. The polyphenol oxidase (PPO) comprises of catechol oxidase and laccase. The activity of these enzymes are important with regard to plant defence metabolism against pests and diseases and appearance polatibility and use of plant products. Fresh, fruit vegetables, mushroom etc. contain these enzymes considerably.

The physiological function of Polyphenol oxidase (PPO) in higher plants have been unequivaocally determined, there is evidence, which suggests that the enzyme plays an important role in defense mechanism (Mayer, 1987). The plant polyphenol oxidase is induced in response to mechanical wounding and signaling molecules such as, Methyl Jasmonate and Systemine, suggests that polyphenol oxidasemay play a role in plant defense (Boss et al; 1995; Thipyopong and Steffens,1997). Several workers studied activity of polyphenol oxidase enzyme in infected plant groups. In general, phenol oxidase catalaze the oxidation of phenolic substances with molecular oxygen. Increase in polyphenol oxidase activity has been observed in the Stem tissues of *Fusarium* infected tomato plants (Sanwal,1956) Openions on the role of phenolase in host resistance have been summarized in several reviews (Forkas and Kiraly,1958; Rubin and Artsikhovskaya,1964 and 1967; Goodman et al; 1967; Hore, 1966; Wood,1967,Kosuge,1969). According to Kirlay (1959) observed and has found polyphenol oxidase activity in leaf tissues of wheat varities resistance to *Puccinia graminis* while no such activity was seen in the succeptible vartiety.

According to R.S.Mehrotra (1980) plant pathologist stated that the increased phenolase activity in diseased or wounded areas of plant tissues is generally accompanied by an increased concentration of

phenolic substances in diseased plants. Polyphenol oxidase activity is considered due to the hypersensitive reaction or resistant variety of any plant groups. While the oxidation products is quinines formed in Phenols are toxic to the host cell as well as to the pathogen.

The activity of Polyphenol oxidase is considered to be important in pathogenic infection.The higher concentrations of fungitoxic phenolic substance and oxidation products results in increased polyphenol oxidase activity which generally, ascribed from resistance to several plant pathogenic infection.The polyphenol oxidase enzyme role important for oxidation of phenolic compounds to quinines which may be more fungitoxic. The final product obtained after oxidation is polymerization in to dark – coloured melanins may then occure. The pathogenic organism produces polyphenol oxidase oxidize the host polyphenols to more highly Fungitoxic Substances. They thus prevent further development of the pathogens The melanin is apparently fungitoxic but in other cases these insoluble pigments are relatively nontoxic to fungi. However in blight of rice polyphenol inhibitor reduces the toxicity of catechol to *Cochliobolus miyabeanus* and it thus prevent melanin development, presumably by they preventing conversion of catechol into fungitoxic quinines (OKU, 1960). The phenols and

polyphenol oxidase released in germinated uredospores of *Puccinia graminis* (Farkas and Ledingham,1959), Also in resistance of barley plants to *Erysiphe graminis* appears partly with the collapse of the mesophyll cells with an accompanying release of a phenolic substance & they are accumulated around the houstaria but they inhibit further development of the fungus (Scott, Millerd and White, 1957)

In plant tissues phenolic compounds produced are primarily shikimic and acidic pathways. According to Hare (1966) polyphenols,phenolic glucosides, flavonoids, anthocyanins, aromatic amino acid and coumaria are aromatic substances tend to accumulate around infected plant tissue and also in the tissues adjacent to wounds where they might exert a effect of fungistatic. Also phenolics are produced by a head to tail condensation of acetate units and these arederived from the sugar breakdown after respiration. Shikimic acid pathway is very useful for phosphoenol pyruvate from glycolysis these reacts with erythrose which is produced during pentose pathway. The activity result is increased or decreased in diseased plants, and at final stage they form dehydroquinic and shikimic acid, some intermediate unknown compounds are produced. The most important phenolic compounbd in the plant defense are caffeic acid, ferulic acid, chlorogenic acid, phloretin and various phytoalexins.

Chakravarty and Srivastava (1967) have reposted the resistance the resistance of carrot roots to *pythium aphanidermatum* to an unidentified, accarently performed phenolic substance. The phenolic contents of rice are responsible for resistance in *pyricularia oxyzae*, Wakimoto and Yoshil (1958). According to Sporston (1957) reholds that balsam (*Impatiens balsamina*) leaves observed free from fungle diseases due to maximum phenolic or glycosides which are oxidized to fungitoxic quinines.

The phenotic substances produced in plant tissues in response to infection. Also induced proteins synthesis and enzyme synthesis seems to play a role in disease resistance. The marked changes in 9 to 13 enzymes examined by Webster and Stahmann (1966) in black rot of sweet potato caused by fungus *Ceratocystis fimbriata*, in sweet potato tissues inoculated with the pathogen and with a non-pathogen that induces immunity.,

The present work on enzyme activities in Infected and *Trichoderma* treated vine is made . The change in Polyphenol oxidase activity were analysed in the leaves of Piper betle L. Var. Kapoori , before infection and after Trichoderma treatement . The results are repreasented in Table No. (5) and Fig .(5) . The value of Polyphenol oxidase in Control leaves are 0.20, In Infected leaves – 1.15 and

Trichoderma treated leaves are 5.48 . The maximum value of polyphenol oxidase activity were observed in *Trichoderma* treated leaves as compaired to Infected leaves. However very low activity were observed in Control leaves than *Trichoderma* treated leavea and Infected leaves of Betle vine.

The The activity of Polyphenol oxidase (PPO) would seem to be important in that it can oxidize phenolics to quinines which may be more fungitoxic. According to Tomiyama et al. (1958), the resistance to *phytophthora* is reversed by the copper enzyme inhibitars that interfere with the formation of toxic quinines via polyphenol oxidase. The inoculation with *phytophthora infestans*, both polyphenol and polyphenol oxidase increased in resistance potato varieties but not in susceptible varieties which suggests a resistance mechanism to the enzyme (Rubin et al.,1974). However voras et. Atl; (1957) have reported that when streptomycin is obsorbed through the root, it gives resistance to potato against *Phytophthora*. This is explained the fact that antibiotic activity generally enhances polyphenol oxidase activity, and hence, the resistances in plants(C.R.S. Mehrotra 1980).

According to Arnon, D.I. (1949) copper enzymes in isolated chloroplasis and polyphenol oxidose in *Beta vulgaris* Plant Physiol, 24:1-15. also Cochrane M.P.(1994)studied and observe the germ aleurane of barley show change in polyphenol oxidase and peroxidase activity.KGC,M.and M:Shrg,D.(1976) studied catalase peroxidase and polyphenol oxidase activities during rice leaf senescence.

According to M.K.Mahatma * R, Bhatnagnigar[1] and P.Rawals[2] (2008) the Polyphenol oxidase activity increased , was found in all genotypes ot seven days after infection. Thet also observed higher activity of enzyme polyphenol oxidase in resistance genotypes wich had about 2-3 folds than susceptible genotypes. Due to higher activity of poltphenoil oxidase and ß -1,3- glucanase in resistance genotypes ,they confers resistant against downe mildew pathogen. A nother enzyme is Malate dehydrogenase show decrease in activity was found in all genotypes but it was higher in resistance genotypes at seven days after infecti

The present studies thus reveals the decrease in Polyphenol oxidase activity in Infected leaves .It was also found the greater reduction in activity in Infected leaves caused by *Phytophthora parasitica* Var.

piperina. The treatement of *Trichoderma* on Infected leaves shows maximum activity may be resistance development to the pathogenic infection.Due to *Trichoderma* treatment in Infected leaves results higher concentration of phenolic susbestances and oxidation product results an increased Polyphenol oxidase activity wich is generally ascribed for resistance to infection of Foot - Rot disease to Betle leaves . It is possible that phenolic compound contents and enzyme activity interrelated for the development of disease tolerance in the vine

(ii) Peroxidase (EC 1.11.1.7)

Peroxidase enzyme are widely distributed in plant tissues, and are of immense physiological interest because of their associationwith numerous catalytic function. Peroxidase is located in subcellular components like nucleus, ribosome, mitochandria, cell walls and membrane. Peroxidase (POD) includes in its widest sense of group of enzymes such as NAD-Peroxidase NADP-Peroxidase, fatty and peroxidase etc. as well as group of very non specific enzymes from different sources which are simply known as peroxidase (Donor : H_2O_2 – Oxidoreductase I.II.I.7). The Peroxidase (POD) catalyses the dehydrogenation of a large number of organic compounds such as phenols, aromatic amines hydroquinanes etc. organisms, The important functions has been proposed by many workers among these is the ability

to oxidize indole-3. acetic acid (Siegal & Galston,1955; Stonier et al;1979). However, other functions of peroxidase in which role of this enzyme has been implicated include ethylene biosynthesis (Mapson & Wardale,1972), hydroxylation of praline (Ridge & Osborne,1970), Lignification C.Fielding & Hall,1978), Wound – healing (Kawashima & Vritini,1963), and disease –resistance (Johenson & Cunninghiam,1972) and is of potentially considerable importance.

Peroxidase is an oxidative enzymes whose primary reaction is to oxidize molecule at the expense of H_2O_2. It is from the plant sources exhibit a very range of substrate specificity and catalyse the oxidation of cellular components such as pnenolic substances, aromatic amines, ascorbic acid, ferrocytochrome C, $NADH_2$, etc. by either H202 or organic hydroproxides (Putter,1974).

$$A\,H_2 + H_20_2 / (ROO4)\ \text{peroxidase}\ A + 2H_20 / ROH + H_20$$

Peroxidase enzyme are useful for assay stem of producing hydrogen peroxidase.

Peroxidase Enzyme also shows oxidative activity besides the peroxidation i.e. it catalyse the oxidation of different substances by atmospheric oxygen under arobic conditions without exogenous peroxide e.g. $NADH_2$ (petrochenko and Kolesoiko,1966), phenol pyruvate (Jaynes et al.,1972) and indole acetic acid (Platee et al;1964). In addition to this,

enzyme peroxidase also play an important role in growth and development of plants through their control in auxin catabolism (Ray,1962),H_20_2 formation (Lieberman,1979).

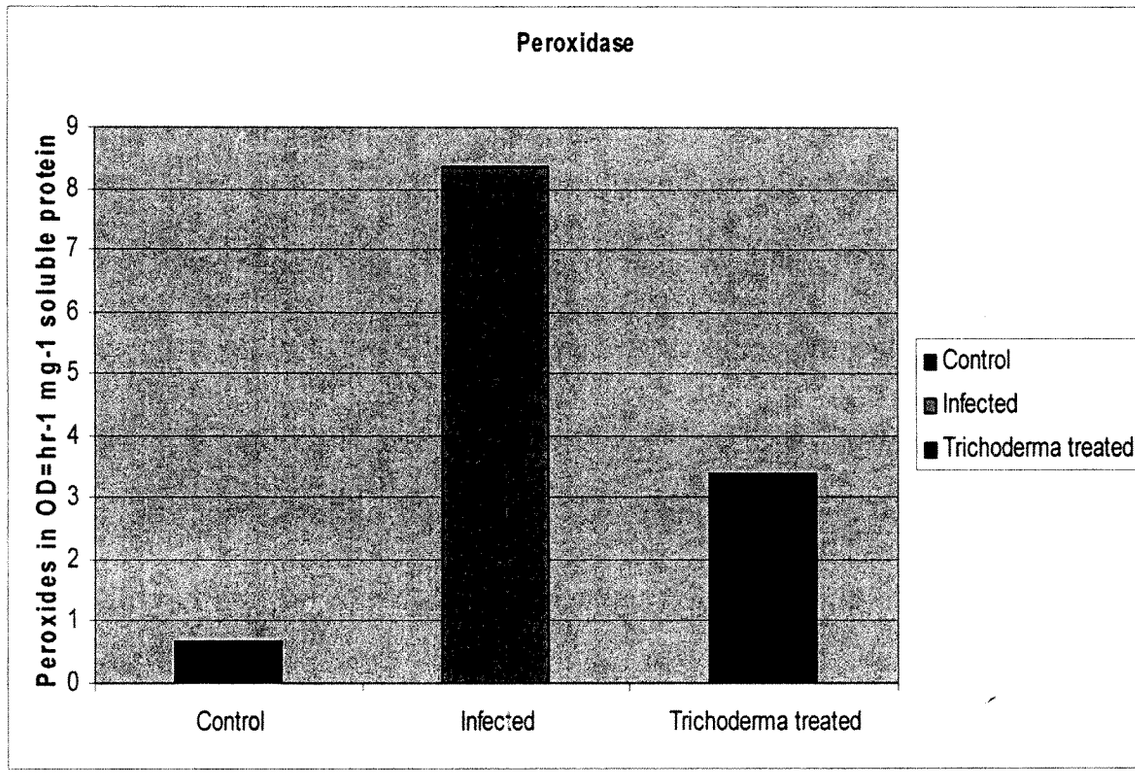


Fig.8. *Values of peroxidase are expressed in ΔOD = hr -1 mg 1 soluble protein.

According to Pilet and Gasper (1968), they may also have a function of IAA oxidase in plant cells. A number of investigation & showed that the peroxidase activity in the host causes to the plant resistance (Loverkonch et al.,1968) Seevers et al. (1971) reported that the higher peroxidase activity was associated with rust resistance in wheat. It is an indicator of respiration rate and may be involved in the catabolism of chlorophylls during senescence.

Peroxidase is haemprotein enzyme. The enzyme is two important characteristics of stability and ability to yield chromogenic products it make suitable for the preparation of enzyme conjugated antibodies. Peroxidase also used for a variety of applications are immunohistology probes for demonstration of tissue antigens. They also used in enzyme amplified immunoassay system for the quantitative demonstration of soluble and insoluble antigens. (A.G.Murugesan, C. Rajakumari 1960) .

The present study regarding of the the activity of enzyme peroxidase in four samples of *Piper betle* var. kapoori is recorded in Table No.(6) & depicted in Fig.(8) show activity of peroxidase in Control leaves is 0.68 , In Infected is 8.37 and *Trichoderma* treated leaves is 3.40. The maximum increased activity observed in Infected leaves than *Trichoderma* treated leaves while activity of peroxidase in

control leaves were observed minimum than Infected leaves and *Trichoderma* treated leaves of Betle vine.

The higer activities of Peroxidase may be due to increased activity of pathogen inside the host tissue i.e.catabolic type. However the *Trichoderma* treatement controls the pathogen's growth as well as reduces the enzyme activity. The antagonistic effects of *Trichoderma* in controlling disease resistance in *Piper betle* vine coincidence the reduced activity of peroxidase also.

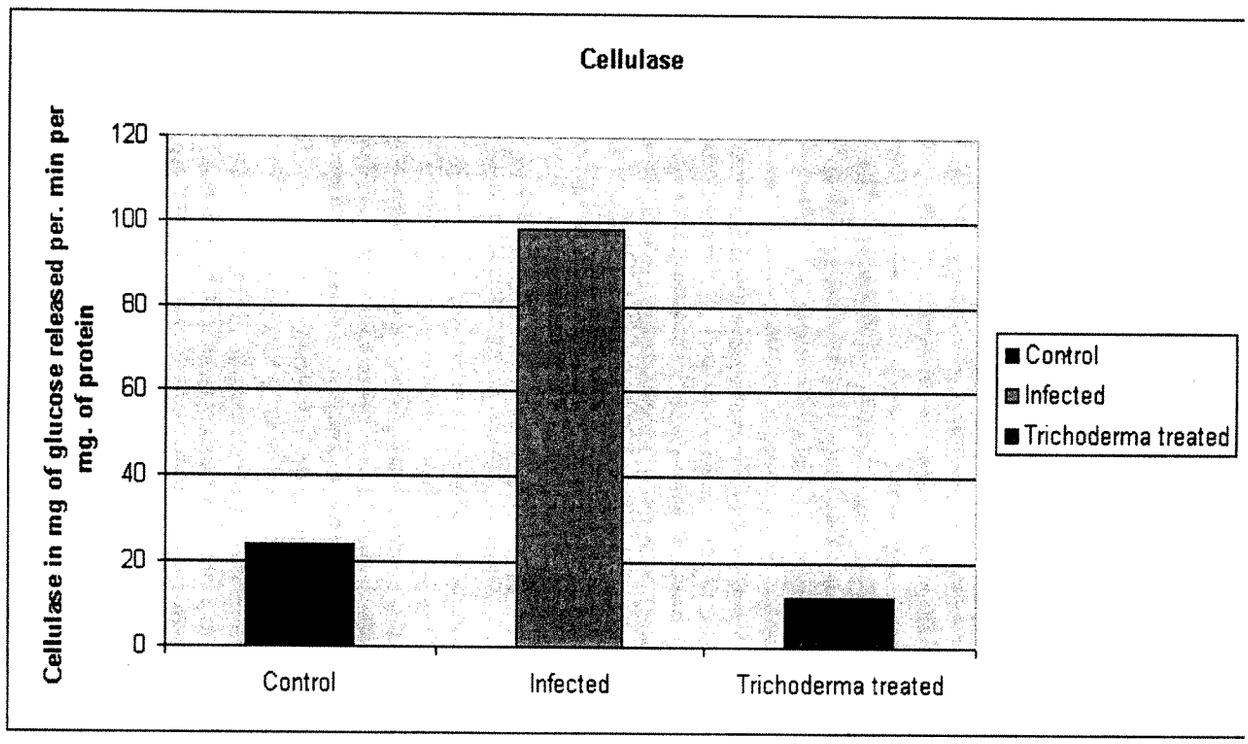


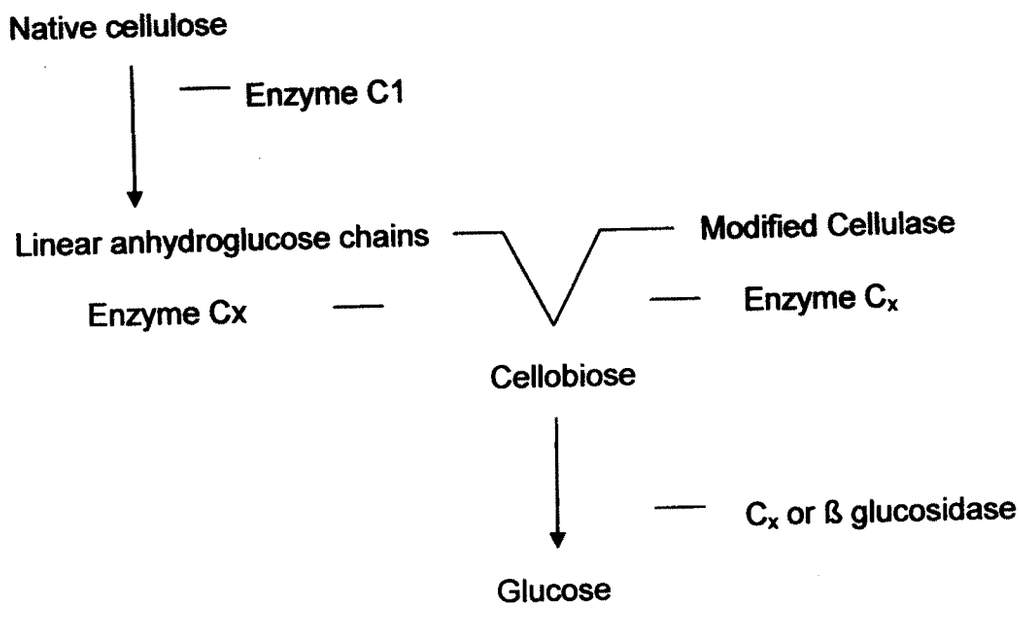
Fig.9. *Values of cellulase are expressed in mg glucose released per minute per mg protein.

iii) Cellulase (E.C. 3.2.1.4.)

The enzyme cellulases degrade cellulose and its derivatives, degrade destroying its crystalline structure are designated as C, enzymes and expose the glucon chains to B-1, 4 endoglucanases, termed Cx enzymes, which degrade the glucon chains to cellobiose (Reese,1956). The conversion of cellulose to glucose also requires a cellobiase or B. glucosidase.

The presence of cellulalytic enzymes and the degradation of cellulose by bacteria and fungi have been reviewed by Wood (1960) and Norkrans (1963). Gascoigne and Gascoigne (1960), siu and Reese; (1953). Greathouse and Wessel; (1954), & Reese (1957).

Wood, (1967). proposed a scheme of cellulalytic enzymes when native cellulose is degraded as fallows.



Cellulalytic enzymes have been implicated in wilt diseases (Husain and Kelman , 1957, Talboys, 1958, Husain and Diamond, 1960) caused by *Pseudemonas solanaceraum*, *Verticillium alboatrum* and *Fusarium oxysporium f.spp. Lycopersici.* Wood (1860) suggested that the conditions in the tracher favour the production of cellulases by vascular wilt pathogens and that local action of these enzymes might result in the formation of the compounds of high molecular weight wich could improve the flow of sap in the transpiration stream.

The production of cellulaytic enzymes in vitro is often reduced by sugars and by various in hibitors such as polyphenols (Mandels and Reese, 1963).The germiniting uredospores of *Puccinia graminis tritici* are known to produce a cellulase but its role is uncertain. .(Van Sumeae et; al 1957).

Also micro – organisims including cellulotic fungi such as *Chaetomium, Fusarium myrothecium* and *Trichoderma spp.* and oter species are *Penicillium* and *Aspergillus* species play important role in synthesis in cellulose. According to [1]M.A.Milala et.al; (2005). The cellulase enzyme production by Aspergillus niger in submerged culture with agricultural wastes like millet ,guinea, corn straw , rice husk and Maize straw as substrate was studied .

Fusorium Moniliforme are reported to produce both cellulolytic enzymes and has got

F.rolfsii produces cellulalytic enzymes and has got the capacity to cause soft rot as well as the capacity to cause soft rot as wel as degradation of hardened tissues (Hussain and Kelmon,1957). In the root - rot and foot - rot of many plants and in leaf spot diseases, cellulases seem to play an important part in pathogenesis.

Cellulase (a complex multienzyme syetem) which acts as collectively to hydrolise cellulose from agricultural wastes to produce simple glucose units . Also micro- organisms tor getting their nutrition from the surrounding substrate secreate extra cellular enzyme (s) which degrade different types of materials or substrate in to simpler forms. This material or substrste wich are usually utilized by them to survive and flourish in different types of habitates. (Sunanda Soni; 2008).

Cellulase enzyme were analysed in the the leaves of *Piper betle* after infection and after *Trichoderma* treatement. The results are repreasented in Table No. (5) and Fig. (9).The value of cellulose in Control leaves are 23.808, In Infected leaves are 98.550 and in *Trichoderma* treated leaves are 11.618.

Cellulase is a collective term for a group of enzymes which act on the substrate cellulose. They are abundant in fungi and bacteria. The reaction of this involves two step. (1) prehydrolysis In this sep the hydration of anhydrous glucose take place. The enzyme involved is Enzyme I usually they act on highly ordered cellulose substrate like cotton fibres. However they exhibit weak activity an soluble derivatives of cellulose. Second step is hydrolysis. In this step breakdown of polymers are occurs in the presence of the enzyme (Enzyme x). the enzyme with exo - and endo – B –1, - 4 glucanase target on soluble derivaties of cellulose (A.G Murugesan, C. Rajakumari ,1960).

Recently, pectolytic and cellulalytic enzymes are implicated almost routinely as a feature of host-parasite interactions and their involvement in the degradation of the pectine and cellulolytic constituents of cell walls and of the middle lamella in the plant tissues has been reported for such diverse hazards of diseases as soft rots, dry rots, charcoal rots, damping off, wilts, blights and leaf spots.

Cellulalytic enzymes have been investigated in those organisms which bring about the deterioration of textile and wood but not much a tension has been given to their significance in plant – disease.There are imperfect understood for the enzymic degradation of cellulose. The

The wild strain of *Trichoderma lignorium* was isolated from the Banana cultivated as raton crop, continuously for seven years. The *T. lignorium* was grown on Banana waste based medium for production of cellulotic enzyme also *T.lignorium* synthesized cellulases wereused for saccharification of agro- waste . According to Baig et, al ;(2003) the lignocellulasic waste of Banana plant left over for naturally degration in field was effectively used as component in the medium for the production of enzymes.

In the presnt investigation change in cellulase activity werw observed in Control , Infected and *Trichoderma* treated leaves of *Piper betle* L.Var. Kapoori. It is clear that the decreased activity of cellulase were observed in *Trichoderma* treated leaves than Infected leaves and Control leaves ,while maximum increased activity of cellulase were observed in Infected leaves than Control leaves of Betle vine.

There are increasing evidence that a number of fungi, particularly the wood rotting fungi, produce several cellulalytic enzymes (Petterson et al;1963). *Polyparus versicolor* produces a multiple component cellulase system the plants.