Chapter III

MATERIAL AND METHODS

Material and methods:-

A) Plant material:-

i) Cultivation of *Agave* plants:-

The plant of *Agave cantala* Roxb. was raised in the college garden soil by using bulbils for studies. However, the growth of *Agave* being very slow i.e. for well developed mature status of *Agave* requires about 4-5 years of period.

To overcome this difficulty during short research tenure, the *Agave* plants raised by farmers as hedge plants from Islampur and Shivnagar localities were used. About 10-15 plants of same age (5 years maturity) were selected for the study. These plants were provided with adequate amount of water in specific intervals. The required plant material i.e. leaves were taken from these plants and often used during experimental studies.

ii) Sampling method:

Mature, fully expanded, green leaves present at the middle of healthy and insect free *Agave cantala* Roxb. plants were selected from identical positions and used for preparation of extract.

B) Preparation of *Agave* leaf extract:-

The selected leaves were first washed and cleaned with tap water and then by distilled water. The leaves were subsequently cut into small pieces (2 inches) with the help of khurpi. Then the pieces of leaves were kept in water i.e. in 100 litre capacity container.

The proportion of *Agave* leaves to water is 1:5 i.e. 10 kg. leaves in 50 litter water.

After 10 days leaves of *Agave* were completely soaked in to water the colour of water become greenish black. This is an indication of well prepared leaf extract. Then the extract is filtered through double layered muslin cloth and used for spray treatment.

1) Application of Agave leaf extract :-

Agave leaf extract prepared in water can be used along with water or buttermilk or cow urine. In present investigation, extract used only with water in the proportion of 1:3 (i.e. 1 litre *Agave* leaf extract in 3 litre water.). This extract is sprayed on grape plants with the help of hand sprayer of 15 litre capacity (Paras Brand).

2) Selection of Grape Plants:-

Grape plants (cv. Thompson seedless) selected for study are cultivated by Mr. Jagannath Gavade (Junekhed). He has developed a vine farm of 2.5 acres and taking production of grapes from last six years. Hence the studies on infection of powdery mildew on grape plants were made in this field during research tenure.

10 vine plants infected with downy mildew are selected for study. The solution of *Agave cantala* leaf extract was sprayed with hand pump on infected vine plant for 4 days in sequence. According to Pady and Subaya (1970), spore release of fungus is high in early morning to midday. Hence, the treatment of Agave leaf extract was given at 8 to 9 am. After 10 days of 1st treatment, leaves are collected randomly. Leaves of 5th row of twig containing flowers or fruits were collected. Because, leaves of fruiting zone have positive effect on fruit yield and sugar content of grape berries (Petri *et al.*, 2002).

Use of *Agave* leaf extract spray to control the above diseases was made in the above field for 1 year i.e. October cutting to the harvesting of grapes in March.

Quantitative analysis of inorganic and organic constituents of healthy, infected and infected but *Agave* leaf extract sprayed leaves of grapes at different stages: flowering fruiting and harvesting has been made by following standard methods.

C) Methods:-

1) Inorganic Constituents:-

a. Preparation of acid digestion

Acid digestion method of Toth *et al.,* (1948) has been employed for the analysis of inorganic constituents. Plant material was carefully washed in water and blotted to dryness. The healthy, infected and *A. cantala* leaves extract sprayed leaves of *Vitis vinifera* were separated and subjected to drying at 60°C for 10 days till dried plant material had constant weight. The oven dried plant material was powdered. Five hundred mg of oven dried powdered leaf material was transferred to 150 ml capacity beaker to which 20 ml concentrated HNO3 was added. The beakers were covered with watch glass and kept till the primary reactions were completed. Then these beakers were heated slowly to dissolve solid particles. After cooling to room temperature, 10ml of perchloric acid (60%) were added to it and mixed thoroughly. Then these beakers were heated strongly until a clear and colourless solution (about 2-3 ml) was obtained. It was then cooled and transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day these extracts were filtered through dry Whatmann No.44 (Ash less) filter paper. Filtrates so obtained were used for estimation of different inorganic constituents.

b) Estimation of Phosphorus:-

The method of Sekine *et al.,* (1965) was employed for estimation of Phosphorus.

Phosphorus reacts with 'molybdate vanadate' reagent to give yellow coloured complex. By estimating calorimetrically the intensity of colour developed and by comparing it with the colour intensity of known standards, Phosphorus contentwas estimated. 4 ml of acid digest were taken in test tube and to two ml of 2 N HNO3 and 1 ml of 'molybdate vanadate' reagent (A-25 g ammonium molybdate in 500 ml of distilled water, B- 1.25 g ammonium vanadate in 500 ml 1 N HNO3, A and B were mixed at the time of experiment)

were added. Then final volume of each test tube was adjusted to 10 ml with distilled water. After 20 minutes, color intensity was measured at 420 nm using a reaction blank without acid digest or standard phosphorus. Calibration curve of standard phosphorus was prepared from standard phosphorus solution containing mg per ml (0.110 g KH2PO4 per liter = 0.025 mg P5+ ml -1) with the help of standard curve the amount of phosphorus in the plant material was calculated and it was expressed on dry weight basis.

2) Organic constituents:-

a. Moisture percentage of leaf:-

The healthy, infected and *Agave* leaf extract sprayed leaves of grape collected randomly. The leaves were washed, blotted dry and cut into small pieces. These pieces were thoroughly mixed and 10g leaf material was kept in oven at 70^0 C for drying till a constants weight was obtained the loss in weight per 100 g was expressed as moisture percentage.

$$M\ (\text{Moisture content})\ \% = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

$$\text{Dry matter}\ \% = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

b. Photosynthetic Pigments

i) Chlorophylls

Chlorophylls were estimated following the method of Arnon (1949). Randomly sampled fresh leaves from healthy, infected and *A. cantala* leaf extract sprayed grape plants were brought to laboratory, washed with distilled water and blotted to dry. Chlorophylls were extracted in 80% chilled acetone. 0.5g of fresh plant material was homogenized in cold mortar with pestle in dark. A pinch of MgCO3 was added to neutralize the acids released during

extraction. The extractwas filtered through Whatman No.1 filter paper using Buchner's funnel undersuction. Final volume of the filtrate was made to 100ml with 80% acetone. The filtrate was transferred into a conical flask wrapped with black paper to prevent photo-oxidation of the pigments. Absorbance was read at 663 nm and 645 nm on aUV-VIS double beam spectrophotometer (Shimadzu UV-190) using 80% acetoneas a blank.

Chlorophylls ($mg100^{-1}g$ fresh weight) were calculated using the following formulae:

Chlorophyll 'a' = 12.7 x A663 – 2.69 x A645 ------- X

Chlorophyll 'b' = 22.9 x A645 – 4.68 x A663 --------Y

Total chlorophylls (a + b) = (8.02 x A663) + (20.20 x A645) -------- Z

$$\text{Chl. a / Chl.b / total Chls. (mg 100 g}^{-1}\text{ fresh weight)} = \frac{\text{X / Y / Z x vol. of extract x 100}}{\text{1000 x weight of plant material (g)}}$$

ii) Carotenoids

Carotenoids were extracted from the weighed amount of leaf material as per the procedure described for chlorophylls earlier. Carotenoids were estimated following the method described by Kirk and Allen (1965).

The absorbance was recorded at 480 nm on a UV-VIS double beam spectrophotometer (Shimadzu UV-190). The total carotenoids were calculated using the following formula –

$$\text{Total carotenoids (mg 100 g}^{-1}\text{ fresh weight.)} = \frac{\text{A480 X vol. of extract X 10 X 100}}{\text{2500 X weight of plant material (g)}}$$

Where, 2500 = average extinction.

b. Carbohydrates:-

Carbohydrates were estimated according to the method described by Nelson (1944).

These were estimated from healthy, infected and sprayed leaves of grape. Five hundred mgs dry leaf material was homogenized in mortar with pestle and extracted with 80% alcohol. It was filtered through Buchner's funnel using Whatman No.1 filter paper. The filtrated was used for the estimation of soluble sugars while the residue was used for starch determination. The filtrate thus obtained was condensed on water bath till the volume was about 3 ml and treated with lead acetate and potassium oxalate (1:1) to decolourize it. To this distilled water was added and filtered. It was again washed with distilled water for 2-3 times collecting the washing in the same filtrate. This filtrate was used for estimation of reducing sugars (A). A known volume of this extract was hydrolyzed with concentrated HCl in pressure cooker at 15 lbs pressures for half an hour. The contents were cooled, neutralized with Na_2CO_3 and filtrate. The filtrate was used for the estimation of total sugars (B).

The residue obtained in the first filtration (alcohol extract) was transferred to a conical flask with 50 ml water and 2.5 ml of concentrated HCl. This was hydrolyzed, neutralized and filtered as stated earlier. This filtrate contains reducing sugars produced as result of hydrolysis of starch. The sugars so available were estimated to determine the starch present in the tissue (C).

The requisite quantity (preferably 0.1ml) of the above filtrates A, B and C was taken separately in 10 ml marked test tubes. In other such test tubes different concentrations (0.1, 0.2, 0.3, 0.4 ml) of standard glucose solution (0.1 mg ml^{-1}) were taken. One ml of Samogyi's alkaline copper tartarate solution [4 g $CuSO_4$, $5H_2O$; 24 g unhydrous Na_2CO_3; 16 g Na-K. tartarate (Rochelle salt and 180 g unhydraous Na_2SO_4 dissolved in 1000 ml distilled water] was added to each test tube. All the reaction mixtures were subjected to boiling water bath for about 10 minutes. After cooling to room temperature 1 ml of Arsenomolybdate reagent [25 g ammonium molybdate in 450 ml distilled water to which were added 21 ml concentrated H_2SO_4. To this was then added 3g

sodium arsenate, $NaHASO_4$ $.7H_2O$; dissolved in 25ml distilled water. All ingredients were mixed well and the solution was placed in an incubator at 37^0 C for 48 hours before use] was added to each reaction mixture the contents of each test tube were then diluted with distilled water to a volume (10ml). A blank was prepared by the same way but without sugar solution. After 10 minutes the absorbance of reaction mixture was read at 560 nm.

With the help of standard glucose curve, the amounts of these carbohydrate fractions were determined.

c. Total polyphenols:-

Polyphenols were estimated according to method of Folin and Denis (1915): from healthy, infected and sprayed grape leaves. Polyphenols were extracted from fresh leaf material in 80% acetone (25ml) Extract was filtered through Whatman No.1 filter paper using Buchner's funnel under suction. The phenolics were extracted repeatedly from the residue with acetone. The volume of the filtrated was made to 50 ml with acetone. One ml filtrated was taken in a 50 ml marked nesselor's tube. In other such tubes different concentrations (0.5, 1,2,3,4 ml) of standard polyphenol solution (tannic acid, 0.1 mg ml-1) were taken. Ten ml of 20% Na_2CO_3 was then added to each tube to make the medium alkaline. Two ml of Folin-Denis reagent (100 g of sodim tungstate and 20 g of phosphomolybdic acid dissolved in 500 ml distilled water were mixed with phosphoric acid (25%). This was refluxed for 2 ½ hrs, cooled to room temperature and diluted to one liter with distilled water] were then added to each test tube and finally the volume was made to 50 ml with distilled water. A blank was prepared without polyphenols. The ingredients were allowed to mix thoroughly. After some time the optical density of each mixture was read at 660nm on Shimatzu double beam spectrophotometer. Polyphenols were calculated from the calibration curve of standard tannic acid

3) Enzymes:-

a. Enzyme catalase (E.C.1.11.1.6) :-

Catalase activity was assayed by following the method of Luck (1974) as described by Sadasivan and Manikan (1992).

Healthy, infected and sprayed leaves were washed, blotted to dry. One gram leaves of grape was homogenized in 15 ml (1/15M) phosphate buffer (pH-6.8) and filtered through 4 layers of muscline cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes and supernatant was used as source of enzyme. The reaction mixture contained 3 ml of 0.05 H2O2 in 100 ml phosphate buffer (pH-7) and 0.1 ml enzyme extract, mixed well and change in OD was recorded at 240 nm. The enzyme activity is expressed as unit $min^{-1}.mg^{-1}$ protein as described by Bergmeyer (1974).

b. Enzyme peroxidase (E.C. 1.11.1.7):-

Activity of enzyme peroxidase from healthy infected and sprayed leaves was studied following the method of Maehly (1951).

1 g leaves of grape were homogenized in 15 ml ice-cold (1/15m) phosphate buffer (pH-6.8) and filtered through 4 layers of musline cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes and supernatant was used as source of enzyme. The reaction mixture contained 5 ml of 1/15m Acetate buffer (pH-5) 0.5 ml of 0.1% guaiacol, 1 ml enzyme extract, 2ml distilled water , 0.5 ml 0.08% H2O2. The reaction mixture was incubated at 30^0 C. Absorbance measured at 470nm. The enzyme activity was expressed as unit $h^{-1}.mg^{-1}$ protein.

c. Enzyme polyphenol oxidase (E.C.1.10.3.2):-

To study polyphenol oxidase activity the methods of Mahadevan and Shridhar (1983) was followed.

1g leaves of grape were crushed in 15 ml 0.1 M phosphate buffer (pH-6.1). The resultant homogenated was filtered through 4 layers of musline cloth. The filtrate was centrifuged at 10,000 rpm for 20minutes. The supernatant

served as enzyme source. The assay mixture contained 4 ml 0.1m phosphate buffer (pH-6.1), 1ml 0.01 M catechol prepared in 0.1 M phosphate buffer (pH-6.1), 0.5 ml enzyme and mixed well. The increase in OD at 30 seconds interval up to 180 seconds at 495 nm recorded. The enzyme activity was expressed as ΔOD min^{-1} $.mg^{-1}$ protein.

d. Enzyme invertase (E.C. 3.2.1.26):-

A method of Uppal and Kanwar (1992) with slight modifications was followed for the study of activity invertase enzyme.

1 g leaves of grape were homogenized in 10 ml 0.1 m phosphate bufter (pH-7.5) Homogenate was filtered through 4 layers of muslin cloth. Filtrate was centrifuged at 10,000 rpm for 20 minutes. Supernatant served as enzyme source. Assay mixture contained 4 ml 1 % sucrose solution, 1ml phosphate buffer (pH-7.5), 1 ml enzyme source. The reaction was terminated by boiling the mixture immediately and after 60 min. one ml reaction mixture was mixed with 2 ml Dinitrosalicylic acid reagent (DNS) and then boiled in water bath for 5 min. and cooled in ice bath. The volume was adjusted to 10ml with distilled water and absorbance was measured at 530nm. The enzyme activity was expressed as ΔOD $h^{-1}.mg^{-1}$ protein with the help of standard curve of glucose, the amount of glucose released corresponding to change in ΔOD hr^{-1} $.mg^{-1}$ protein was estimated.

Total Nitrogen:

The spectrophotometric method described by Hawk *et al.,* (1948) was used to estimate total nitrogen. 0.5 g oven dried plant material was digested with 10 ml dilute sulphuric acid (1:1) in Kjeldahl flask with a pinch of micro salt (anhydrous $CuSO_4$ and K_2SO_4 mixed in the proportion of 1:400). When the digest became colorless, it was cooled to room temperature and transferred quantitatively to 100 ml volumetric flask and volume (100 ml) was made with distilled water. It was then filtered through dry Whatman No. 1 filter paper and filtrate was used to estimate total nitrogen. 2 ml of filtrate was taken in a Nesslor's tube (35 and 50 ml marked) and to it a drop of 8% $KHSO_4$ and 1 ml dilute H_2SO_4 (1:1) were added and the volume was made to 35 ml with distilled water. For standards, different concentrations of ammonium sulphate (0.05 mg per ml) were taken in different Nesslor's tubes and after adding $KHSO_4$ and H_2SO_4 the volume was made to 35 ml with distilled water. To all these tubes 15 ml Nesslor's reagent was added (Nesslor's reagent: A-7 g KI and 10 g HgI_2 dissolved in 40 ml distilled water. B-20 % NaOH. A and B were mixed in the proportion 4:5). The reaction between the sample and the reagent produced orange-brown coloration. The intensity of the color produced was measured at 520 nm on UV-VIS Spectrophotometer. The absorbance reading for plant extract was compared with those of standard ammonium sulphate and total nitrogen concentration was calculated.

Result and Discussion

A) Inorganic constituents:-

a. Nitrogen: (N):-

Nitrogen is one of the important macro element required for plant metabolism. It is well known fact that nitrogen being important constituent of nucleotides, proteins, chlorophyll and enzymes involved in various metabolic processes which have direct impact on vegetative and reproductive phases of plants (Mengel and Kirkby, 1996). It is also found in the plant hormones like IAA and cytokinin.

Among the various forms of nitrogen available to the plant nitrate (NO_3) is the most preferred source for most plants. It is taken up by active transport through roots distributed through the xylem and assimilated by sequential action of the enzymes nitrate reductase (NR) and nitrite reductase (NiR). Optimum uptake of nitrate is the first step to enhance nitrogen use in any plant.

It has been established from a number of physiological studies that plant acquire their nitrate from the soil through the combined activities of a set of high and low affinity transport systems (Chopin *et al.*, 2007) with the influx of NO_3 being driven by the H^+ gradient across the plasma membrane. Some of these transporters are constitutively expressed, while others are nitrate-inducible and subject to negative feed back regulation by the products of nitrate assimilation. The identification of two gene families NRT_1 and NRT_2 on the basis of their deduced amino acid sequences (Chopin et al loc.cit.) has contributed towards unraveling the mechanisms of nitrate uptake in higher plants

A portion of nitrate taken up is utilized or stored in the root cells, while the rest is transported to the other parts of plants. Due to abundant of availability of photosynthetic reductants, leaf mesophyll cell are the main sites of nitrate reduction. This is initiated by the NAD or NADP-dependent NiR. Being the first irreversible and often rate determining step of the N assimilatory pathway nitrate reduction has been a favorite step for physiological and biochemical approaches to optimize fertilizer N use (Pathak *et al.*, 2008) NR

activity in leaf blades, expressed either as seasonal average or converted into seasonal input of reduced N, has been related to total reduced N, grain N and grain yield of cereals (Abrol and Nair, 1977).

The pattern of nitrate assimilation from different plant parts viz. the main shoot of wheat (Abrol *et al.,* 1976) developing ear of wheat plant grown at different soil N levels (Abrol *et al.,* 1978) and in the leaf blades at different stages of growth (Grover *et al.,* 1978) has revealed a direct positive correlation between increasing NR activity and increasing rates of nitrogenous fertilization. Most plant tissues have the capacity to assimilate nitrate, through their NR activity varies widely (Grover *et al.,* 1978; Pokhriyal *et al.,* 1980).

Nitrite accumulation is toxic to the plant and is also inhibiting to nitrate induction, whereas the effect of ammonium and glutamine vary depending on the tissue and plant type as well as condition of the study (Raghuram and Sopory 1999; Ali *et al.,* 2007) the addition of ammonium or nitrate to N-limited whole plants or cells induces enzymes of glycolysis and Kreb"s cycle, which are required for the synthesis of 2-OG (oxoglutarate) (Scheible *et al.,* 1997)

Glutamate and 2 oxoglutarate stimulate nitrate induction of NR and NiR in rice seedling (All *et al.,* 2007), the role of glutamate as a signaling molecule in plant nitrogen metabolism has been reviewed by Forde and Lea (2007), indicating that manipulation of nitrogen nutrition leads to dynamic alteration in plant response to changes in cellular energetic demands.

The tight regulation of C/N metabolism has been revealed through numerous studies which have indicated that net photosynthesis rate and amount of photosynthetic components are correlated with the leaf N content. The relative abundance of N pools in the plant plays a significant rote in regulating the C/N metabolism (Rolland *et al.,* 2006). Nitrate supply has been shown to result in the decrease of starch synthesis and diversion of carbon towards the conversion of organic acids into amino acids (Scheible *et al.,* 1997). On the other hand, nitrate deficiency results in the decrease of many amino and organic acids, along with an increase in the level of several carbohydrate,

phosphoesters and a handful of secondary metabolites (Fernie *et al.*, 2004). Total nitrogen reflects the total plant growth thus the studies of nitrogen uptake and its partitioning give a very good idea about essential measure to improve the overall crop productivity (Sprant, 1978).

According to Tisdale and Nelson (1984) an adequate supply of nitrogen to crop plants during their early growth period is very important for the initiation of leaves and florets primordial. Rajput *et al.*, (1988) reported more availability of nitrogen played a vital role in cell division. The amount of nitrogen absorbed by the crop increases, there is an increase in the number of tillers per square meter (Yoshida *et al.*, 1972). The application nitrogen increased the protein percentage in plant tissue (Kausar *et al.*, 1993). According to Evans (1989), most of N is used in the synthesis of photosynthetic apparatus components.

Effect of grape nitrogen status on vine vigour was briefly studied by Chone *et al.*, (2006). They found that nitrogen fertilizers increases secondary leaf area enhances secondary shoot growth and fresh berry mass. According to Bell and Henschke (2005), nitrogen plays a major role in many of the biological functions and processes of both grapevine and fermentative microorganism. Manipulation of grapevine nitrogen nutrition has the potential to influence quality component in the grape and the wine. The fermentation kinetics and formation of flavour-active metabolites are also affected by the nitrogen status of the must. The consistent nitrogen application in the vineyard increases grape berry quality and major nitrogenous compounds such as total nitrogen total amino acids, arginine proline and yeast assimilable nitrogen (YAN). Low must YAN adversely affect fermentation process and increase in undesirable quality of wine (Vrscaj *et al.*, 2005).

Delas *et al.*, (1991) and Chone *et al.*, (2001) found that higher vine nitrogen status has the depressive effect on the phenolic compound levels in white and red varieties of grape. Therefore, excessive nitrogen supply to grape vine increases the grape sensitivity to *Botrytis cinerea*. According to Chone (2006), the concentration of nitrogen is inversely proportional to phenolic

compound in grape variety Sauvignon Blanc. According to Sharma (2006), deficiency of nitrogen in grape vine causes yellowing of leaves and checks the growth. However, excess application of nitrogen fertilizers increases unwanted vegetative growth, leaf size and decrease in berry formation. The increased leaf size inhibits photosynthesis of leaves present in canopy. The increase in leaf size decreases the cell wall rigidity and hence susceptibility to fungal diseases.

The optimum concentration of nitrogen in glycophytes is 1.5% on dry weight basis (Epstein, 1972). According to Bhargava and Chadha (1993), the optimum concentration of nitrogen in the leaves of grape cv. Thompson seedless is 1.75%. The nitrogen content recorded in healthy, infected and sprayed leaves at flowering fruiting and harvest stage is exhibited in Fig. No.3.1. From Fig., it is clear that the healthy leaves have high concentration of nitrogen as compared to infected and sprayed. The highest N content is recorded at fruiting stage in healthy leaves the concentration of nitrogen is considerable low in leaves infected by downy mildew and powdery mildew. The increase in nitrogen content is observed in infected *A. cantala* leaf extract sprayed leaves.

The changes in concentration of nitrogen in host plant infected by various pathogens were observed by many workers. The increased concentration of nitrogen in sandal affected by sandal pike disease was observed by Parthasarathi *et al.,* (1962). According to Reghupathy and Jayraj (1973), and Prasad and Sahambi (1980), the nitrogen content increased in *Sesamum* leaves when infected by sesamum phyllody disease. While, the decrease in areca nut palm infected by yellow leaf disease (MLO) was observed by Yadava et al., (1973). Sivaprakasam *et al.,* (1976) reported low concentration of N in brinjal affected by little leaf disease. Similarly, low concentration of nitrogen in citrus affected by citrus greening disease (Verma and Singh, 1977) and sandal infected by spike disease (Raychoudhari and Verma, 1980) was reported.

The viral infection also changes nitrogen content in host plant. High nitrogen content was observed in cowpea due to viral infection (Khatri and

Table No. 3.1 Concentrations of nitrogen in grape leaves.

	Healthy	Infected	Sprayed
Flowering	1.3	0.6	0.9
Fruiting	1.5	0.8	1.0
Harvest	1.4	0.5	1.1

Values are expressed as g / 100g

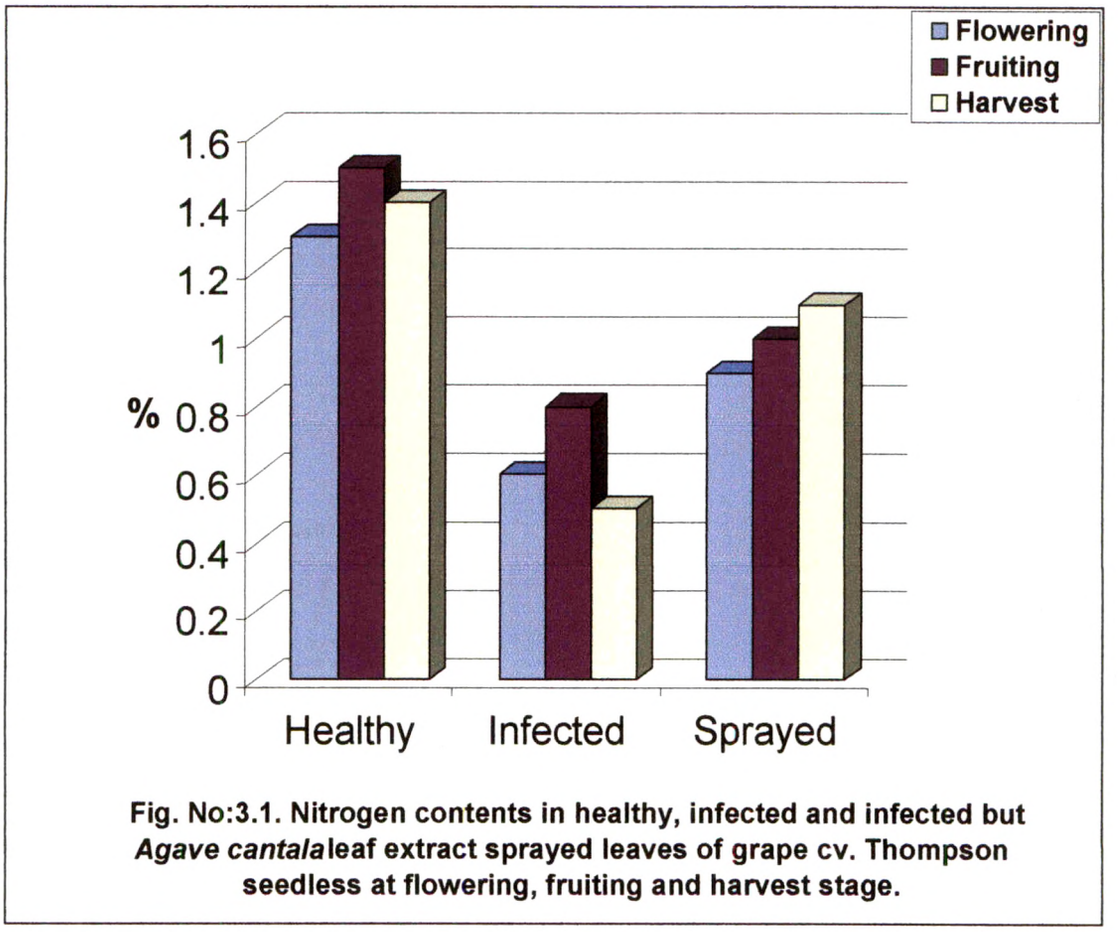


Fig. No:3.1. Nitrogen contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

Chemulu 1973). Similar increase in nitrogen concentration due to viral infection was observed in soybean (Demaski and Kuhn, 1975; Mali *et al.,* 1977), mung (Godse *et al.,* 1976), tomato (Sasikumaran *et al.,* 1979), chilli (Duggal *et al.,* 1981) and mungbean (Singh and Singh, 1983). On the other hand, decreased nitrogen content due to viral infection was noticed in tomato by Lodh *et al.,* (1971). Singh and Mall (1974) reported low N concentration in cowpea, urd and mung affected by virus.

Similarly low concentration of N was reported in papaya (Johri, 1975) black gram (Sivaprakasam *et al.,* 1976b), sugarcane (Ghorpade and Joshi, 1981) maize (Bose *et al.,* 1981) due to viral infections.

Nitrogen content in plant affected by fungal diseases shows an increase or decrease in concentration. The high concentration of nitrogen in sunflower infected by rust was reported by Sindh Mathar and Vidhyasekaran (1978). Sankpal and Nimbalkar (1980) observed an increase in nitrogen content in sugarcane infected by smut. On the contrary, decrease in nitrogen content was observed in beans infected by *Colletotrichum* (Hegde and Munjal, 1971), Majumdar and Rajchoudhari (1978) reported low concentration of nitrogen in pea infected by *Fusarium*, similar reports of decrease in nitrogen concentration in date palm infected by smut (Kapur *et al.,* 1978), and wheat infected by *Alternaria* (Vijayakumar and Rao, 1980).

The work of Vidhyasekaran and Kandaswamy (1971) and Rao *et al.,* (1978) reported protein decreases the phenol concentration in plant. Hence, plant becomes more susceptible to pathogen attack.

The high concentration of nitrogen in grape cv. Thompson seedless exhibits its susceptibility to pathogen attack. However, in present study concentration of N is decreased in infected plant, the slight increase in infected but *A. cantala* leaf extract sprayed grape leaves suggest the recovery of nitrogen assimilation in leaf tissue with increases in photosynthetic pigments (Fig. No. 3.15) and increase in berry sugar content.

The role of nitrogen in the present vine plant studies thus indicate its influence on disease recovery, polyphenol contents and possible for enzyme

system. The Agave leaf extract spray may be beneficial to increase N_2 covalent which is decreased due to infection in vine plants.

b. Phosphorus: - (P^{5+})

Phosphorus is an important macronutrient for plant growth and development. It remains as oxidized form in plant. It is present in plant cells as inorganic phosphorus, phosphate ester or energy rich phosphate bonds. It is a major component of biological energy i.e. ATP. The major photosynthetic product of light reaction NADPH, contain phosphorus. It is also constituent of DNA, RNA and phospholipids. Phosphorus in the form sugar phosphates is a main intermediate of Calvin cycle, glycolysis and pentose phosphate pathway. Vincent *et al.,* (1992) reported assimilation, storage and metabolism of Pi are of critical importance to plant growth and development.

Inorganic phosphorus is readily absorbed by the roots and accumulated in the leaf tissue. It is taken by plants mainly as $H_2PO_4^-$ and it remains as inorganic phosphate (Pi) or it is esterified through hydroxyl group to a carbon chain (C-O-P) as a simple phosphate ester (e.g. sugar phosphate) or adhered to another phosphate bond.

Phosphorus participates in various key reactions of biosynthesis and energy transfer reactions. It plays a key role in some enzyme reactions. It is either substrate or end product of many enzyme reactions Hence, compartmentation of inorganic phosphorus in cytoplasm and chloroplast is important for the regulation of metabolic pathway plant synthesize polyphosphatase which are linear polymer of inorganic phosphate have pyrophosphate linkages energetically homologous to ATP.

According to Marschner (1986), the optimum requirement of phosphorus to plant for growth is in the range of 0.3 to 0.5 % of dry weight basis. In the view of White (1973), the demand for phosphorus is associated with the rate of plant growth and level of metabolic activities. The uptake translocation accumulation and use of the essential mineral elements like phosphorus varied with genotype of crop species (Clark, 1983).

Phosphorus influences processes like photosynthesis and carbohydrate metabolism. Hence, its concentration in tissue decides yield of crops. At optimum level, increasing phosphorus content enhances plant growth but heavy accumulation of it results in stunted growth (Singh and Singh, 1977). The phosphorus content in plant is greatly influenced by environmental stress such as salinity, water logging, and water stress and pathogen attack.

The deficiency of phosphorus in Indian soil is rarely observed because of high application of phosphate fertilizers. However, high concentration of phosphorus affects plant growth (Singh and Singh, loc. cit)

Sharma (2005), phosphorus is responsible for bud differentiation and root development. It is accumulated in fertile flowers of grape. Its optimum concentration increases the bunch weight of grape berries. It's deficiency in vine plants causes immature inflorescence and decrease in fruit formation. Phosphorus deficient vine leaves appears to be bluish green. Severe deficiency causes violet colour of veins of leaves and petiole. Sharma (2005) reported that application of proper phosphorus fertilizers further reduces the incidence of downy mildew and powdery mildew. The high application of phosphorus fertilizers reduces the uptake of iron and zinc. Sahastrabudhe *et al.,* (1969), reported that the low moisture and high phosphorus containing soil of Sangli District decreases the fruit quantity.

The changes in phosphorus content during flowering, fruiting, and harvest stage in the healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape, cv. Thompson seedless are shown in Fig. No.3.2. It is evident from the figure that the healthy leaves of grape contain highest level of phosphorus as compared to infected and sprayed leaves. Among the three stages, at flowering stage the concentration of phosphorus is high in healthy leaves (0.25%). The leaves infected with downy mildew and powdery mildew contain least phosphorus content, at every stage, viz; 0.12%, 0.10% and 0.12% at flowering fruiting and harvest stage respectively. However, the increase in concentration of phosphorus (0.20%, 0.21% and

0.23% at flowering, fruiting and harvest stage respectively) in *Agave cantala* leaf extract sprayed grape leaves is observed compared to infected leaves.

The decrease in concentration of phosphorus in infected grape leaves is supported by the work of several research workers. The low content of phosphorus noticed in brinjal infected by little leaf disease (Sarkar and Joshi, 1977). On the contrary, accumulation of phosphorus in infected leaves of brinjal infected by little leaf disease (Sivaprakasam *et al.*, 1976; Srinivasan and Chelliah, 1979; Mitra and Mujumdar, 1980).

The changes in phosphorus content in plants infected by virus were observed by many authors. Ramiah *et al.*, (1973) reported increase in phosphorus content in bhendi affected by yellow vein mosaic virus. Similarly, increase in phosphorus concentration in groundnut affected by rosette of ground nut (Singh and Srivastava, 1974), hollyhock affected by yellow mosaic virus (Singh and Verma, 1974), mungbean infected by severe mosaic virus (Singh and Singh, 1983) was observed. While, Sasikumaran *et al.*, (1979) in tomato affected by tomato leaf curl virus and Ghorpade and Joshi (1981) in sugarcane affected by SCMV reported low phosphorus concentration in host.

Phosphorus content in plant, infected by fungal discuses also shows increase or decrease. According to Weste *et al.*, (1980), the accumulation of phosphorus was observed in *Isopogeon ceratophyllus* infected by *Phytophthora.* An increase in phosphorus content in sorghum infected by downy mildew was reported by Balasubramanian (1981). Ahmed *et al.*, (1982) also reported increase in phosphorus content in barley infected by brown rust. On the contrary, low concentration of phosphorus in infected plants was observed by many researchers. Sivaprakasam *et al.*, (1974) reported low phosphorus content in brinjal leaf infected by *Verticillium*. Prasad *et al.*, (1976) observed decrease in phosphorus concentration in safflower affected by rust. Similar reports of low concentration of phosphorus in sunflower infected by *Puccinia* (Patil and Kulkarni, 1977b) date palm affected by smut (Kapur *et al.*, 1978) and sugarcane affected by smut (Sankpal and Nimbalkar, 1980).

Table No. 3.2 Concentrations of Phosphorus in grape leaves.

	Healthy	Infected	Sprayed
Flowering	0.62	0.35	0.5
Fruiting	0.72	0.4	0.57
Harvest	0.66	0.38	0.53

Values are expressed as g / 100g

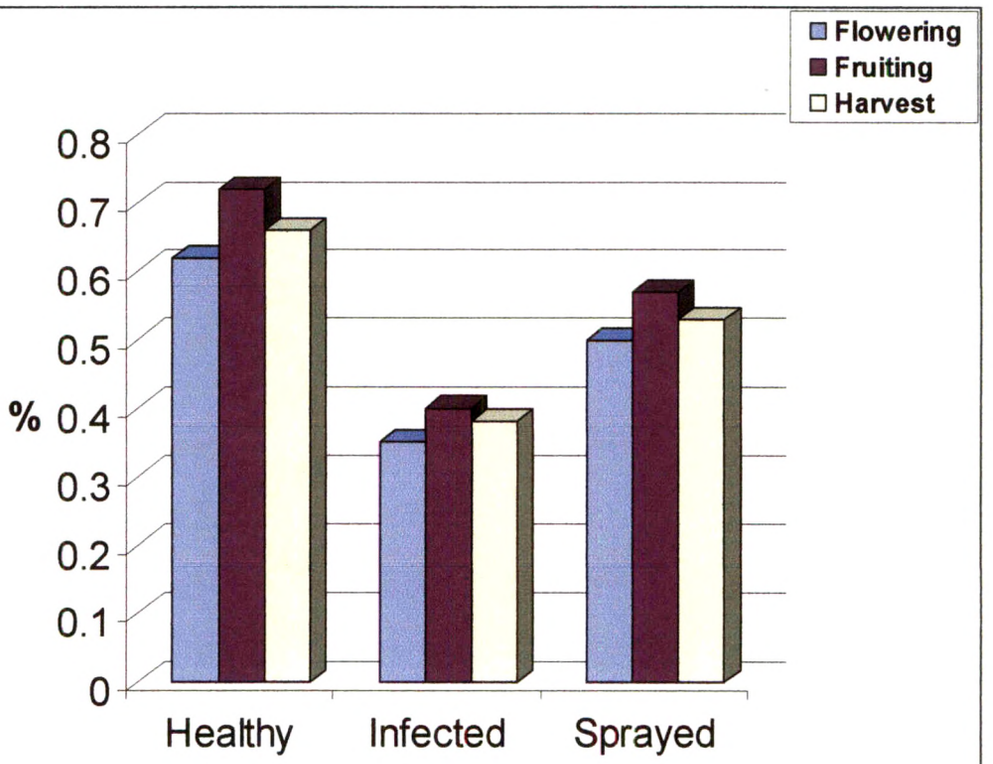


Fig. No:3.2. Phosphorus contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

An important aspect of phosphorus in plant is role in disease resistance. High concentration of phosphorus increase disease susceptibility of the host. According to Dhumal (1983), the sugarcane variety Co_{419} is more susceptible to GSD due to high phosphorus concentration. Similarly, the work of Kannaiyan *et al.,* (1973), Balasubramanian (1973), Massaux *et al.,* (1977), Kapur *et al.,* (1982), Bhaskaran and Ramanathan (1983) reports increase in phosphorus content in host increases the possibility of infection by pathogens.

However, in present study the phosphorus content in healthy leaves of grape cv. Thompson seedless is high. The concentration of phosphorus in *Agave cantala* leaf extract sprayed infected leaves of grapes is more as compared to infected leaves. The inhibition of development of diseases is observed in sprayed leaves. It appears that increase in phosphorus content in infected leaves after spray show disease resistance or disease inhibition. Due to high concentration of phosphorus in healthy and infected but *A. cantala* leaf extract sprayed leaves exhibit resistance against downy mildew and powdery mildew.

According to Sastry and Nariani (1962), Bains and Jhooty (1978), Kauraw (1979), increased phosphorus content in plant reduces the disease incidence by inducing the internal resistance to the various types of pathogens. Shrama (2006) also stated that application of phosphorus fertilizers decreases incidence of downy mildew. Hence, the spray of *A. cantala* leaf extract increases the phosphorus content with decrease in incidence of downy mildew and powdery mildew in grape cv. Thompson seedless.

c. Potassium :-(K^+)

Potassium plays an important role in plant growth and metabolism. It is monovalent cation involved in number of metabolic processes such as protein synthesis (Msio and Lauchli, 1986), photoreduction and phosphorylation (Pfluger and Mengel, 1972), starch formation (Hawker *et al.*, 1974) and nitrogen uptake (Melal *et al.*, 1975). According to Wakwoo (1965), potassium is involved in IAA synthesis and auxin metabolism.

Plant take potassium in an active and a passive way, as seen in cation K^+, depending on it's concentration in the nutritive medium. In low concentration, transport by carriers (active uptake) takes place. In high concentration, plasma membrane is more permeable and its passive movement with transpiration flow prevails (White, 1993). The electrochemical gradient decreases on the inner part the plasmatic membrane and because of this there is a non- specific inhibition o the uptake of other cations (Engels and Marschner, 1993).

Potassium is easily mobile in plant- through the xylem and phloem, it could be redistributed from older leaves to younger tissues, Potassium occurs during the whole period of fulfillment of its physiological function in the form of cation K^+. It means that potassium is not build into organic matter (Maschner 1997; Natr 2002). The significance of potassium it in plants is multilateral and its physiological function has not been yet fully clarified.

Potassium affects both phases of photosynthesis in the thylakoid membranes, it flows with cation Mg^{2+} in a different direction to H^+ flux and by this way it obtains electroneutrality. It influences the intensity of CO_2 fixation in the Calvin cycle (Peoples and Koch, 1979). Potassium impacts on the metabolism of sugars, their polymerization and the synthesis of starch (Preusser *et al.*, 1981). The potassium deficit results for example in thinner cell walls in a decrease of mechanical plant resistance and in a lower production of reserve polysaccharides. It influences tRNA band to ribosome and the next step to translation and subsequently the synthesis of proteins and their conformation (Wyn Jones *et al.*, 1979).

Potassium is a very important osmotic active matter. It activates the osmotic potential in sieve tubes and thereby the speed of transport of assimilates source sink (Deeken *et al.*, 2002). It activates the osmotic potential in cells of the stele of the root and the transport of matters through the xylem (Hsiao and Lauchli, 1986). According to Brag (1972), Potassium reduces water losses by transpiration. Hence, more organic matter can be produces per unit water consumed by a crop well supplied with potassium (Blanchett *et al.*, 1962;

Linser and Herwig, 1968). Potassium plays a key role in the stomatal movement and it impacts the water balance of plants (Hugouvlex *et al.*, 2002). According to Humble and Raschke (1971), the increase in turgor in guard cells associated with stomatal opening resulted from an increase in potassium concentration in the cells. Potassium influences the growth and the elongation of cells as well as the nastic movement of plants. Potassium increases the hydration of protoplast, it positively affects the synthesis of vitamins and it is an activator of many more than 50 enzymes like pyruvate kinase, phorphofructokinase, glutathione synthetase, fructors biophosphate, aldolase (Suelter, 1970), nitrate reductase (Gutierraz *et al.*, 1972) and RUBP carboxylate (Peoples and Koch, 1979).

Potassium is known to be very mobile in an upward and downward direction in the entire plant. Potassium is significant for the upward translocation of nitrate in the entire plant (Ben-Zioni *et al.*, 1971). Mengel and Kirkby (1982) reported that potassium is easily moved in plants and greater part of potassium is taken up during vegetative growth stage. The concentration of potassium at which 90% maximum yield is obtained is the critical concentration of potassium (Ulrich and hills, 1967). The growth reduced rapidly at lower concentration of potassium and above critical concentration growth shows little response (Leigh and Wyn Jones, 1984).

Several metabolic disorders are reported due to potassium deficiency. The potassium deficiency in sugarcane leads to yellowing and spotting of older leaves (Humbart and Martin, 1955) According to Ulrich and Onki (1966), potassium deficiency reduces growth rate and then chlorosis and necrosis in older leaves because older leaves supply it to younger leaves. Potassium deficiency leads to chlorosis and necrosis in the margins (Karadage, 1986) and tips of leaves (Morart, 1973).

Potassium is essential for vine growth. Grape berries are a strong sink for potassium, particularly during ripening; concentration of potassium in grape berry at harvest time determines the pH and quality of wine (Boulton, 1980). According to Schachtman *et al.*, (2003), excess potassium levels in grape

berries causes negative impact on wine quality, mainly because it decreases free tartaric acid resulting in an increase in the pH of grape juice must and wine. The transfer of high amounts of potassium from vine leaves to the ripening fruit has been related to the reduction of photosynthetic activity in mature leaves (Boulton 1960; Iland, 1988). Smart (1991), reported that high canopy densities causing shaded vine microclimates have also been implicated in higher grape juice potassium levels. The mechanism for such transfer of potassium is related to the senescence. Process when it is hastened by any cultural activity that reduces photosynthesis such as shading, lack of water and / or nutrients, pest and diseases. The concentration of potassium in grape varies with root stock (Ruhl 2002). Ruhl (loc. cit) reported that the mechanism responsible for the different potassium accumulation rates by shoots parts is located in the roots.

The optimum concentration of potassium in glycophytes is 1 % or 250 umole /g of dry tissue (Epstein, 1972). According to Bhargava and Chadha (1993), the optimum concentration of potassium in grape cv. Thompson seedless ranges from 2 to 3 % of dry weight at flowering fruiting and harvest stage.

The estimated values of potassium contents in healthy, infected and Agave leaf extract sprayed infected leaves of grape are depicted in the Fig. No: 3.3. It is observed that the potassium content in healthy leaves of grape at three stages i.e. flowering, fruiting and harvest time is 2.4 %, 2.4 % and 2.6 % respectively however. Due to infection of downy mildew, powdery mildew, the potassium content in infected leaves of grape are decreased considerably. The concentration of potassium in infected leaves of grape is below optimum level i.e. 1.25%, 1.0% and 1.4% at flowering fruiting and harvest stage respectively the potassium contents in infected but *Agave* leaf extract sprayed leaves are 2.1%, 2.3% and 2.2% of dry weight at flowing, fruiting and harvest stage.

It is observed by many authors that potassium translocation from senescent to healthy leaves (Ambike and Nimbalkar, 1975). In the present investigation, it is appears that due to Agave leaf extract spray the pathogen

Table No. 3.3 Concentrations of Potassium in grape leaves.

	Healthy	Infected	Sprayed
Flowering	2.4	1.2	2.1
Fruiting	2.4	1	2.3
Harvest	2.6	1.4	2.2

Values are expressed as g / 100g

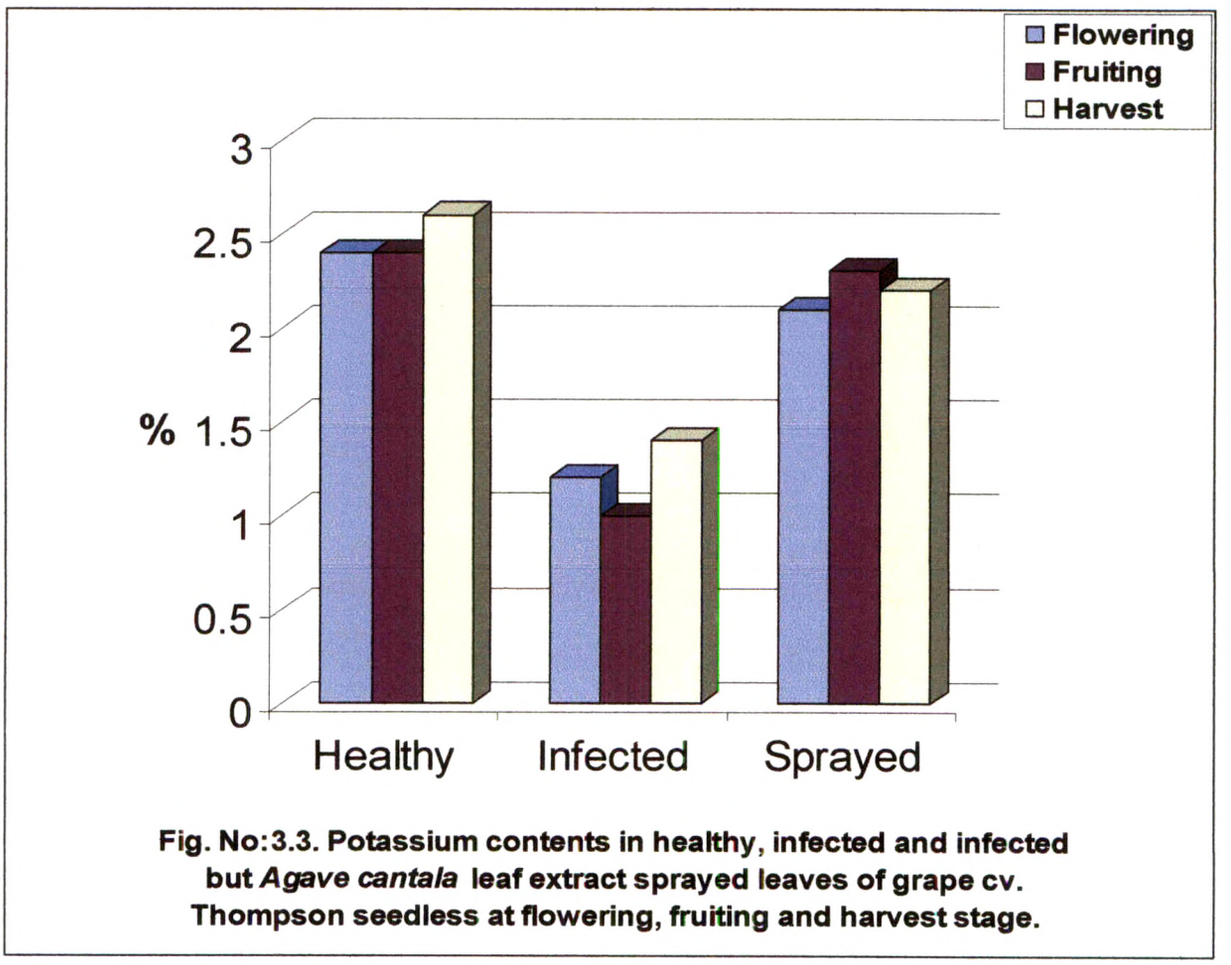


Fig. No:3.3. Potassium contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

growth inhibited in vine plant which ultimately reduces the chances and senescence process hence increase in potassium content in sprayed leaves of grape is in support and K-translocation.

Our results are on similar lines of the workers who studied the K-metabolism in grape and other plants, when pathogen is inducing the disease, as well as it is changing the host metabolism.

The concentration of potassium in plants is greatly influenced by pathogen attack. Several authors reported the change in potassium content in plant due to pathogen attack. Sivaprakasam *et al.,* (1976) and Srinivasan and Chelliah (1979) have also reported increased potassium content in of *Sesamum melongena* L. affected by little leaf disease. However, decrease in potassium content reported in areca nut palm affected by yellow leaf disease (Yadawa *et al.,* 1973), Sesamun affected by phyllody (Reghupathy and Jayraj, 1973), brinjal affected by little leaf disease (Sarkar and Joshi, 1977) and citrus affected by greening disease (Verma and Singh, 1977).

Potassium content in plant is greatly influenced by fungal pathogen. The concentration potassium in the leaves of sorghum was increased when infected by Sclerospora (Balasubaramanian, 1975). Similar reports of increase in concentration of potassium were reported in mango affected by *Capnodium* (Kulkarni and Kulkarni 1978), mung infected by *Drechslera* (Dwivedi and Shukla, 1981) and barley infected by brown rust (Ahmad *et al.,* 1982). On the other hand, Hegde and Mungal (1971), reported that the decrease in potassium content in bean infected by *Colletotrichum*. Similar reports of decrease in potassium content in brinjal infected by *Verticillium* (Sivaprakasan *et al.,* 1974), in sunflower infected by *Puccinia* (Patil and Kulkarni, 1977b). Similar trend of decrease in potassium content in infected leaves of grapes is reported in the present investigation. The potassium content in infected grape leaves is decreased considerably as compared to healthy leaves. According to Sharma (2006), the deficiency in potassium content is responsible for easy fungal pathogen attack like downy mildew on grape leaves. After *Agave* leaf extract spray the potassium content in infected leaves is increased because after spray

the growth of fungal pathogen is inhibited which ultimately lead to retranslocation of potassium from healthy leaves to sprayed leaves. It is possible that the early senescence developed due to pathogen attack is stopped due to the leaf+ extract spray.

The increase in potassium content in leaves of grapes infected by downy mildew after Agave leaf extract spray may afford greater resistance against the invading pathogen by reducing its rate of multiplication. Similar reports are reported by Sastry and Nariani (1962) and Mohan *et al.,* (1978). According to them, potassium may increase the disease resistance through secondary metabolites like phenols. The increase in potassium content increases the activity of polyphenol oxidase which influences the synthesis of phenol (Alagiangalingam *et al.,* 1977). Humbert (1968) reported that potassium increases resistance. The concentration of potassium in infective site directly influences the life span and receptiveness for pathogen and play important role to make the host susceptible or resistance to the disease (Allengton and Laird, 1954). According to Vaithilingam and Ragunathan (1977), the incidence of stem rot disease of rice decreased with increasing the application of potassium they reported that the higher levels of potassium may be inhibiting the establishment of pathogen inside the host tissue by increasing the mechanical or physiological resistance. Our reports are supported by Dastur and Bhatt (1964), Kannaiyan *et al.,* (1973), Kannaiyan and Prasad (1978), Ghorpade and Joshi (1981), Reis *et al.,* (1982) and Trolldenier (1982). It suggests that increase in potassium content in infected grape leaves after *Agave* leaf extract spray may increase disease resistance and lower the disease severity. Present investigation is on the similar lines indicating the application of *Agave* leaf extract spray as biofungicide and beneficial for disease control. The enzymes studies in the present investigation also support the K-metabolism in vine plants.

d. Calcium :-(Ca^{++})

Calcium is one of the major elements found in plant tissue. The chemical properties of calcium are relatively complex and calcium controls biochemical and physiological functions that are very different from those of other elements. The ionic radius of calcium is 0.09 mm which is relatively high compared with other divalent ions. It has a coordination number of six or higher (often seven or eight) to form octahedral complexes.

Calcium creates hardly soluble compounds with many organic substances especially organic acids and phosphates. Calcium with pectin forms a component of middle lamella of the cell wall and hence plays very important role in structural organization of plants. According to Claskeson and Hanson (1960), a major role of calcium appears to be affect cell adhesion, membrane and chromatic organization and enzyme confirmation. Calcium is essentially important for proper work of membrane and keeping the cell wall integrity. The fundamental role of calcium is reflected in the cell division it influences the structure of the mitotic spindle and middle lamella. Calcium included in the formation of ionic bridges increase in cell wall rigidity increase in hydrophobicity and reduction in water permeability and cell elongation (Clark, 1984). Calcium involved in controlling of membrane permeability to various ions, particularly to inorganic cation (Ven Stevenick, 1965).

According to Renzel (1992), operation of the plasma membrane Ca^{2+} transport systems strongly dependent on Ca^{2+} activity in their immediate vicinity. Calcium increases net absorption of potassium (Ortis *et al.,* 1994). Calcium plays a key role as the second messenger in the signal transduction of hormonal and environmental signals in plant cells (Roberts and Harmson, 1992). Protein phosphorylation and dephosphorylation is now regarded as one of the important mechanisms of regulation of enzyme activity changes in cytosolic Ca activity triggers the chain of events that results in turning a phosphorylation on and of thus ultimately affecting a large number of biochemical reactions (Swamy 1991).

According to Clark (1984) calcium uptake in plants is found to be influenced by number of factors such as soil pH, soil temperature, light, other cations and soil water potential. Calcium is very partially mobile through xylem and phloem. That is the reason why calcium does not any possibility of renutralization. Plants demand its regular uptake for whole growing season (Hocking, 1980; Mohr and Schopfer, 1995). Swamy (1991) reported that calcium influences the donor side of oxygen evolving complex in light reaction of photosynthesis in some studies.

Many enzymes have been reported to stimulated or inhibited by calcium some of which are or amylase esterase, pectinesterase, lipoxygenase, nucleases, proteins kinase, pyruvate kinase, poygalactyronic transeleminase, glucose-6-phosphate, dehydrogenase and adenosine triphosphatars (ATPase) (Clark 1984). Enzyme studies in the present investigation also suggest typical role of calcium in the grape vines if infected.

Calcium deficiency in plants leads to break down of cell walls, increase in permeability and leakiness of cell membrane. Its deficiency causes over 35 disorders in plant tissues (Swamy, 1997). Microscopic studies of plant tissues containing low calcium show considerably disorganization of cells (Fuller, 1980). Atkinson *et al.*, (1980) reported many calcium deficiency related disorders, e.g. fruit and vegetable mealiness. water core, internal breakdown, bitter pH, corking, tip burn and browning with breakdown and disorganization of cell wall of plants.

Calcium influences the yield and quality of grape berry. Calcium prevents rupture of grape berry. The deficiency in grape berry increases the susceptibility to various fungus pathogens (Sharma, 2006). Chardonnet *et al.*, (1997) studied effect of calcium treatment prior to *Botrytis cinerea* infection on the changes in pectic composition of grape berry. They found that calcium treatment inhibit fungal pathogen. According to Sharma (2006), optimum concentration of calcium in grape increases resistance against fungal disease, calcium increases cell wall rigidity of grape berry. The optimum concentration of calcium along with potassium and magnesium neutralizes toxicity of acids

produced in plant metabolism. The optimum concentration of calcium reduces the probability of leaf senescence and also fruit drop in grape (Sharma, 2006). Recently, Pang *et al.,* (2007) reported involvement of calcium signaling in dormancy release of grape buds.

According to Epstain (1972), the optimum value of calcium in terrestrial plants is 0.5 % or 125 umole/g of dry tissue. The critical concentration of calcium for dicots is 0.2 % dry weight which is higher than for monocots (less that 1% Salisburry and Ross, 1995). Bhargava and Chadha (1993) reported optimum concentration of calcium in the leaves of grapes cv. Thompson seedless at flowering fruiting and harvest stages ranges from 1 to 2 % of dry weight basis. Similar concentrations of calcium in the leaves of grapes are reported in our investigation (Fig. No.3.4). The value of calcium in healthy leaves of grapes is 1.2 %, 1.5 % and 1.7 % at flowering, fruiting and harvest stage respectively. But the concentration of calcium in the leaves of grape infected with downy mildew and powdery mildew increased considerably. The value of calcium in infected leaves is 2.0 %, 2.6 %, and 2.8 % at flowering, fruiting and harvest stages receptivity. Where as, the concentration of calcium in infected but *Agave* leaf extract sprayed leaves is 1.3 %, 1.5 % and 2.2 % at flowering, fruiting and harvest stages respectively. Our results suggest that the values of calcium in infected grape leaves increased and after spray the concentration of calcium in infected leaves is decreased.

Many researchers reported an increases or decreases in the concentration of calcium in infected leaves of different plant. Areca nut palm infected with yellow disease (MLO) of leaves shows increased calcium content (Yadawa *et al.,* 1973). However, Raghupathy and Jayraj (1973) and Sivaprakasam *et al.,* (1976), Sarkar and Joshi (1977) have reported considerable reduction of calcium in sesamum and brinjal leaves infected by sesamum phyllody and little leaf disease respectively.

An increase in calcium content in leaves of tomato and sugarcane infected by curly top, leaf curl and sugarcane mosaic virus respectively was reported by Panopulos *et al.,* (1972), Sasikumaran *et al.,* 1979) and Ghorpade

and Joshi(1981) However, decrease in calcium content was reported in leaves of pigeon pea infected by sterility mosaic (Nambiar and Ramakrishnan, 1969), bhendi infected by yellow vein mosaic (Ramiah *et al.,* 1977), cassava infected with cassava mosaic virus (Rosen *et al.,*1980) and sorghum infected by SCMC (Shukla and Joshi, 1981). The present results indicates role of calcium in development of disease tolerance due to *Agave* leaf extract spray to infected plants. However, amount of calcium may be controlled and slight increase than healthy plants.

The concentration of calcium is either decreased or increased in leaves infected by fungal discuses. Various author reported the significant increase in calcium content in infected leaves. Higher concentration of calcium in infected leaves then normal healthy leaves was reported by Sivaprakasam et al., (1974) in brinjal infected by *Verticillium.* Similarly, in sorghum infected by downy mildew (Balsubramanian, 1975), in sunflower affected by rust (Patil and Kulkarni, 1977b) and smutted sugarcane, the high calcium content was observed. On the contrary, low counteraction of calcium content was observed in bean infected by *Colletotrichum* (Hegde and Munjal, 1972), grape infected by leaf rot (Tanne and Spigel-Roy, 1973), coffee infected by grey rust (Massaux *et al.,* 1977), mango infected by *Capnodium* (Kulkarni and Kulkarni, 1978), *Eucalyptus* infected by *Phytophthora* (Halsall, 1980), *Ipomea* affected by *Albugo* (Misra and Padhi, 1981) and Pearl-millet infected by downy mildew (Mogle and Mayee, 1981).

Calcium increases disease resistance by governing the membrane permeability which regulates the nutrient supply available to invading pathogen (Bateman, 1969). Walter (1967) reported that gray mould disease of tomato may be controlled by with sufficient calcium. Similar role of calcium in disease resistance against fungal infection was reported by Kiraly and Gilly (1976). The work of Ghorpade and Joshi supported the role of calcium in disease mechanism. The balance between calcium and phosphorus increases the disease resistance (Stall *et al.,* 1965).

Table No. 3.4 Concentrations of Calcium in grape leaves.

	Healthy	Infected	Sprayed
Flowering	1.2	2	1.3
Fruiting	1.5	2.6	1.9
Harvest	1.7	2.8	2.2

Values are expressed as g / 100g

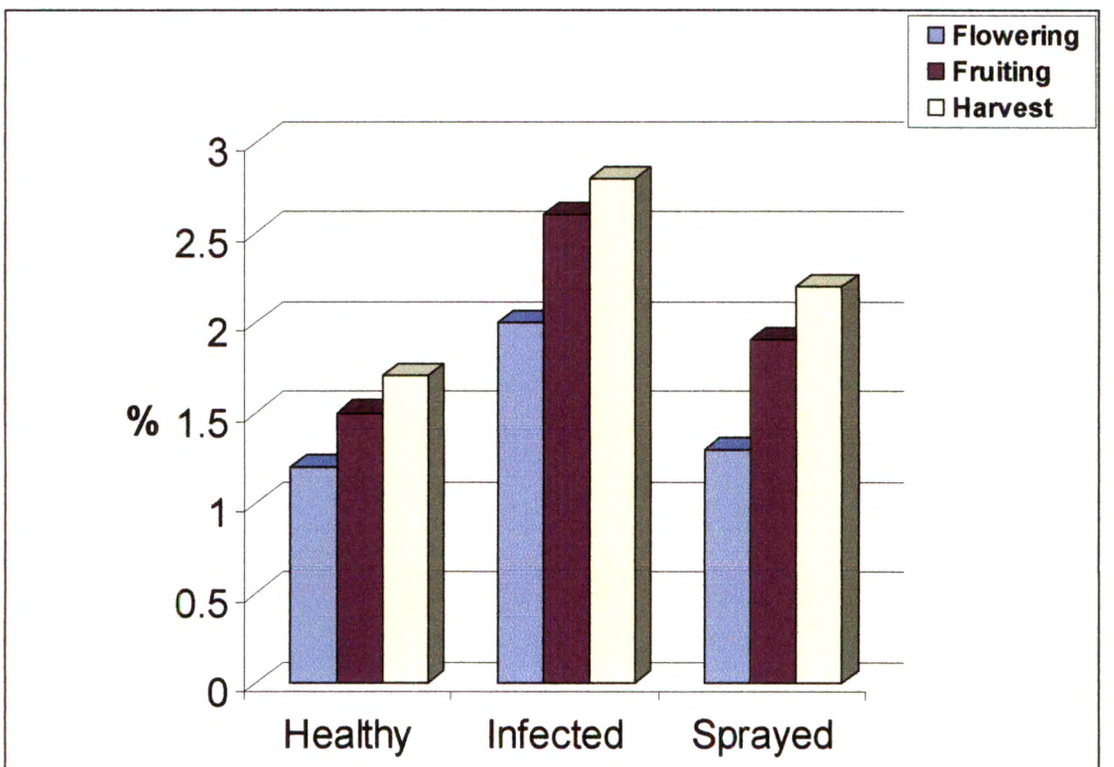


Fig. No:3.4. Calcium contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

In our investigation, the calcium content in *Agave* leaf extract sprayed leaves of grapes is increased with phosphorus concentration. Hence, the disease resistance may be induced in tissue. It is possible that due to high calcium content in *Agave cantala* leaf (Landge, 1988) may be the reason behind the increase in calcium in infected grape leaves which cause disease resistance. The increase in calcium concentration helps in protection of berry cell wall.

The concentration of calcium is increased in infected leaves. Several authors reported that at senescence, the high accumulation of calcium in leaves occur. The work of Waughman and Bellamy (1981) with 21 tree species reported the increase in calcium concentration in dead senescent leaves than live leaves. The relatively immobile nature of calcium is considered as one of the main reason for calcium concentration in senescent leaves. Mastmoto *et al.,* 1998) indicated that calcium concentration in the leaves of the oil palm increased with leaf senescence and abscission. The present investigation suggest that the grape leaves infected with downy mildew and powdery mildew undergoes senescence and therefore may be high accumulation of calcium takes place as compared to healthy leaves.

The role of calcium in the grape leaves is not its individual activity but possible along with other element like potassium, phosphorus and iron. It may induce the disease tolerance in infected grape leaves sprayed with by *Agave* leaf extract.

e. Magnesium :-(Mg^{++})

Magnesium is divalent cation which plays a crucial role in variety of physiological processes in plant. The most well known role of magnesium is its occurrence at the centre of chlorophyll molecule. Besides its function in the chlorophyll molecule magnesium is required in other physiological processes.

According to Mengel and Kirkby (1982), magnesium in plants is generally absorbed at lower quantities than either Ca^{2+} or K^{+}. Magnesium absorbed primarily from regions near the root apex, which supports the concept of passive uptake (Russell and Clarkson, 1976). According to Mulder (1950),

magnesium and potassium are inversely proportional to each other. High levels of potassium in the soil resulted in magnesium deficiency in apple leaves and high magnesium contents in plants cause a low level of potassium nutrition. Hall (1971) reported that calcium deficient tomato tissues have very high magnesium level. The level of magnesium in the nutrient medium is also of importance in relation to manganese uptake. According to Patil and Joshi (1972), the combination of magnesium and manganese was more effective in sucrose synthesis in sugarcane.

Magnesium is very mobile in the phloem and can be translocated from one tissue to another (Clark, 1984). Magnesium readily moves from roots, stems, cotyledons, primary leaves and secondary leaves to young newly developing leaves if plants are placed in solutions without magnesium. According to Mengel and Kirkby (1982), fruits and storage tissues are highly dependant on the phloem for their mineral supply. Thus, they are higher in potassium and magnesium than in calcium.

Magnesium is associated with organic anions such as malate, citrate, pectate and oxalate as well as inorganic ions (Kirkby and Mengel, 1967). This element also contributes to the electrical neutrality of organic compounds such as sugar phosphates, sugar nucleotides, organic acids and amino acids (Clark, 1984) one major role of magnesium is as co-factor in almost all enzymes activating phosphorylation process. Magnesium forms a bridge between the pyrophorphate structure of ATP or ADP and enzyme molecule. The activation of ATPase by Mg^{2+} is brought about by this bridging function (Balke and Hodges, 1975) phosphokinases, dehydrogenases, as well as anolases are activated by Mg^{2+}. In these enzymes however, the magnesium reaction is not specific and Mn^{2+}, is often a more efficient activator. A key reaction of Mg^{2+} is the activation of ribulose bisphosphate carboxylase.

According to Bandurski (1955), PEP-.case in spinach requires mg ($1x10^{-3}$m) for optimum activity, the enzyme reaction that requires Mg include Po_4 or nucleotide transfer (i.e. phosphatases, kinases, ATpases, synthass, nucleotide transferases,) carboxyl transfer (carboxylases, mutases and lyases) (Clarkson

and Hanson, 1980). According to Malkin and Niyogi (2000), the Calvin cycle is regulated by light via changes in pH and Mg^{2+} concentration since stromal pH and Mg^{2+} concentration are important regulators of enzymes such as Rubisco-b- biphosphate and phosphoribulokinase. It is also highly essential for the activity of sucrose phosphate synthase, one of the enzymes responsible for biosynthesis of sucrose which is a major form in which assimilated are transported in higher plants. The transfer of amino acyls from amino acyl transfer ribonucleic acid (tRNA) to a polypeptide chain appears to be activated by Mg^{2+} (Clark, 1984).

PEP-carboxylase the enzyme of Co_2 assimilation during night requires magnesium as co-factors in CAM plants (Sutton, 1975; Bartakke, 1977). According to Bartakke (1977), magnesium activates the enzymes PEP-case and RUBP-case in *Aloe barbadensis*. According to Jacob (1958), magnesium also promote the formation of vitamins especially carotene.

Magnesium is a key component of chlorophylls. One of the most familiar symptoms of Mg deficiency in plant is chlorosis. According to Bogorad (1966) and Ananthanarayana and Rao (1980), the chlorophyll contents increases with increased magnesium supply. According to Watson (1965), magnesium deficiency probably causes the dissociation of ribosomes into their subunits and destroys the ribosomal configuration that is necessary for protein synthesis.

According to Humbert (1968), under deficiency conditions growth was retarded, plant become weak and rind becomes soft. Khurana et al., (2005) reported that deficiency of magnesium reduces biomass, chlorophyll a and b levels and specific activates of catalase, acid phosphatase and ATPase in maize leaves. However, the deficiency also causes the increase in activities of peroxidase and RNase.

According to Sharma (2006), Magnesium is most ignored element in Indian viticulture. The deficiency of Magnesium is found more in Dogridge root stocks because it accumulate high amount of potassium. Magnesium deficiency in grape causes yellowing of leaves from margins. Early litter fall

was observed in magnesium deficient leaves. The most adverse effect of magnesium deficiency is mummification of grape berry. It causes great loss in grape yield (Sharma, 2006). Another reason for the deficiency of Magnesium in grape is high application of potassium fertilizers.

The optimum concentration of magnesium in glycophytes is 0.2 % or 80 umole/g of dry tissue (Epstein, 1972). In normal healthy leaves of grape cv. Thompson seedless, the optimum concentration of magnesium was found in the range of 0.40 % to .048 % (Bhargava and Chadha, 1993). In the present study healthy leaves of cv. Thompson seedless also shows magnesium content 0.45 % in flowering, 0.46 % in fruiting and 45 % in harvest stage (Fig No.3.5). The concentration of magnesium in infected leaves of grapes is 0.18 %, 0.15 % and 0.21 % at flowering, fruiting and harvest stage.

The considerable decrease in magnesium content observed because of fungal disease downy mildew. After spray of *A. cantala* leaf extract, the magnesium content in infected leaves of grape are observed as follows, 0.40 % at flowering stage, 0.41 % at fruiting stage and 0.42 % at harvest stage. The increase in magnesium content in infected leaves may be due to resistance devoted by *A. cantala* leaf extract.

Several investigators reported an increase or decrease in magnesium concentration. The leaves of sesamum plant infected by MLO shows higher magnesium concentration (Reghupathy and Jayaraj, 1973) and low concentration of magnesium was observed in brinjal plant infected by MLO (Sivaprakasam *et al.,* 1976).

Similar reports of increase and decrease in magnesium content was observed in plants infected by virus. Increased magnesium content in ragi infected with ragi mosaic virus and Phaseolus affected by bean yellow mosaic virus was reported by Ramachandran *et al.,* (1980 and Rosen *et al.,* (1980) respectively.

The changes in concentration of magnesium in infected leaves due to fungal disease were reported in controversial fashion. Hegde and Karande (1978) reported increased magnesium content in leaves of bajara infected by

Table No. 3.5 Concentrations of Magnesium in grape leaves.

	Healthy	Infected	Sprayed
Flowering	0.73	0.49	0.61
Fruiting	0.64	0.4	0.55
Harvest	0.69	0.48	0.57

Values are expressed as g / 100g

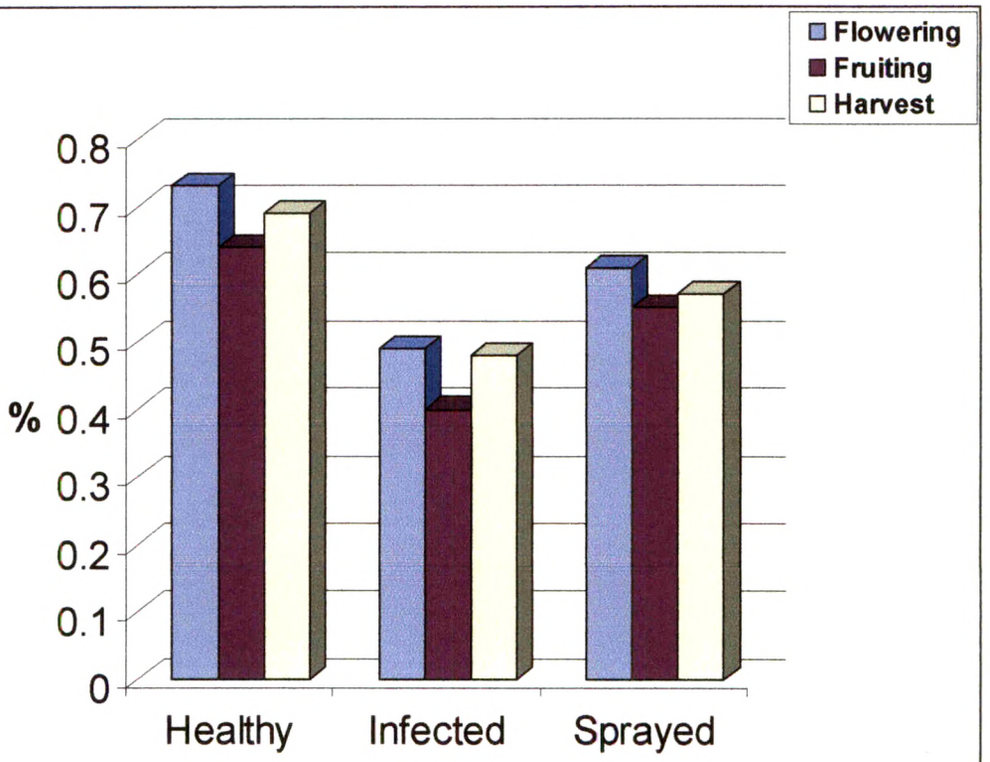


Fig. No:3.5. Magnesium contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

downy mildew. Similar trend of increase in magnesium content in Jowar and sugarcane leaves infected by downy mildew and smut was reported by Balasubramanian (1975) and Sankpal and Nimbalkar (1980) respectively. On the other hand, low magnesium was noted in *Ipomea* infected by white rust (Mishra and Padhi, 1981).

The magnesium deficiency in infected leaves is responsible for chlorophyll degradation. The mildewed grape leaves show symptoms like chlorosis. Magnesium is an activator of enzyme RuBPcase (Lorimer *et al.*, 1976) and as a co-factor in the reaction of sucrose synthetase and sucrose-P synthetase. Magnesium catalyzes the sucrose synthesis in higher plants. Reduction in magnesium in infected leaves of grape ultimately affects the berry sugar concentration. Under Mg deficiency, the mummification of berry was observed (Sharma, 2006).

Magnesium plays an important role in disease resistance (Seaker, *et al.*, 1982). The magnesium deficient plant is more susceptible to pathogen attack. The mycelium of powdery and downy mildew may inhibit the uptake and translocation of magnesium in leaves (Goswami *et al.*, 1976). The increased concentration of magnesium in sprayed leaves of grape suggests the inhibition of fungal growth and therefore, increase in magnesium content in infected but *A. cantala* leaf extract sprayed leaves. Data reveals the fact that the magnesium concentration increased after spray.

In case of sprayed plant recovery of magnesium is observed than infected plants. It may be one of the reasons of to induction of disease resistance due to *Agave* leaf extract spray in grape plants.

f. Iron :-(Fe^{+++})

Iron is a trace element required for number of metabolic processes in the plant. Fe is absorbed by plant roots as Fe^{2+} or as Fe chelate. Fe chelates are soluble and therefore available to roots. Romheld and Marschner (1981) observed that the uptakes and translocation of Fe is hormonally controlled, probably from shoot apex. They further suggested that Fe nutritional status of

the plant is transformed into a signal which induces distinct biochemical and morphological changes within the roots causing a fine regulation of Fe supply to the plant. This process is regulated by auxins. The Poaceae members are characterized by release of a non-protein amino acid phytosiderophores (an iron carrier) and by induction of high affinity uptake system for Fe III. The phytoseridophores transports the Fe III chelates an intact molecules and this strategy is more efficient and less pH dependent. In the grasses uptake of Fe III is more important.

Fe uptake in dicot roots requires a reduction step and subsequent translocation of Fe^{+2} across the cytoplasmic membrane (Prasad and Prasad, 1987) via ferric reductase FRO_2 to transport protein (Kochian, 2000) The uptake of iron is considerably influenced by other cations like Mn^{2+}, Cu^{2+}, Mg^{2+} K^{+}, and Zn^{2+} (Lingle *et al.,* 1963). Cu and Zn are known to displace Fe from chelate complexes, forming a corresponding heavy metal chelates. Iron is not readily mobile between different plant parts. According to Mengel and Kirkby (1982), iron is translocated in the xylem appears to be a ferric citrates as a major form.

Iron has a role in the synthesis of the common precursor of chlorophyll. It is required for the formation of photochlorpophyllide from Mg-protoporphyrin (Machold and Stephan, 1969). Price *et al.,* (1972) reported role of Fe in nucleic acid metabolism. Iron is an indispensable constituent of hemeprotein like cytochromes, thus playing a key role in the electron transport chains in respiration as well as photosynthesis. It is also an important constituent of ferrodoxin, a sulphur protein which is very necessary part of photosynthetic light reaction.

Numbers of iron suplhur proteins also participate in respiratory electron transport. According to Hsu and Miller (1968), Fe (II) is a component of the TCA cycle enzyme aconitase, where it is required for both stability and activity of the enzyme. Fe is also needed in the process of suphate reduction and nitrate reduction .this element is a constituent of oxidative enzymes like peroxidase,

catalase, cytochrome oxidase etc. and required for activation of number of other enzymes.

Guerinot and Ying (1994) reported Fe play a role in ribonucleotide dinitrogen reduction and energy yielding electron transfer chain. Fe is stored in stroma of chloroplast as phytoferritin, which can store about 5000 atoms can be converted into ferric ion, but its chemical properties place limitations on its accumulations (Guerinot and Ying, loc. Cit.).

High accumulation of iron in plant tissue cause severe cellular damage. Iron toxicity is often associated with deficiency of Zn and Mn and with a marked imbalance of nutrients due to the presence of H_2S (Perssarakli, 2005). High concentration of Fe in leaves lead to an increased uptake of Fe in chloroplast and thus, a dramatic impairment of total photosynthetic electron transport capacity. According to Vajpayee *et al.,* (2000) leaves under iron toxicity exhibit bronze spots. Beale (1999) reported that precipitation of iron in oxidation reduction reactions within the cells creates oxidative stress in plants when taken in excess and activates antioxidative enzymes.

Fe deficiency induces several morphological and physiological responses in many plants. According to Chapman (1975) and Shankar (1977), deficiency of Fe in leaves show complete chlorosis. Singh (1997) reported that deficiency of Mn and iron resulted in metabolic disturbances leading to deteriorated leaf. Fe deficiency decreases the protein fraction simultaneously with an increase in the level of soluble organic nitrogen compounds, (Bennett, 1945; Perur *et al.,* 1961).

In the view of Sharam (2005), the vineyard soil with high concentration of calcium carbonate (lime stone ($Ca\ CO_3$)) and high pH exhibit iron deficiency in grape plants. According to him, the iron deficient vine leaves show interveinal yellowing. The chlorosis along with retarded growth of primordia was observed due to iron deficiency. The vine plants which are irrigated with high bicarbonate water show iron deficiency. Sharma (2006) reported that the high clayed soil, low temperature and improper irrigation can reduce the uptake of iron by roots of vine plants.

In the present studies the Fe contents were studied in healthy, infected and *Agave* leaf extract sprayed vine plants during flowering, fruiting and harvest stages. It is observed that the Fe contents were decreased in infected plants than the control or healthy but due to leaf extract spray the Fe contents may recover considerable though plants are infected. It is possible that leaf extracts spray acceleration the Fe decrease as well as the disease tolerance in grape plants.

The changes in values of iron in healthy, infected and sprayed leaves are shown in Fig. No.3.6. From Fig., it is clear that healthy leaves of grape c.v. Thompson seedless show the concentration of iron in the range of 30 ppm to 40 ppm. The maximum iron contents observed at harvest stage.

The optimum concentration of iron in glycophyles is 2.0 u mole/ g of dry tissue or 100 ppm (Epstein, 1972). According to Stout (1961), 0.01% (of dry matter) is the requisite concentration of iron for optimal growth of plant. While, optimum concentration of iron in the leaves of grape cv. Thompson seedless is 30 to 80 ppm (Bhargava and Chadha, 1993). From Fig. the infected leaves of grape show decrease in iron content. The lowest concentration of Fe observed at flowering stage. The infected but *Agave* leaf extract sprayed leaves exhibit increased concentration of iron as compared to infected leaves.

An increase or decrease in iron values was observed by many authors in plants infected by MLO, virus and fungal infection. Accumulation of iron in sesamum leaves infected by MLO was reported by Reghupathy and Jayaraj (1973). Parthasarathi and Rao (1962) noted increased concentration of iron in leaves of sandal infected by MLO. While, low concentration of iron was reported by Sarkar and Joshi (1977) in brinjal leaves infected by MLO.

Similar controversial iron content in viral infected plant was observed by various investigators. High concentration of iron in bhendi (Ramiah *et al.* ,1973) infected by yellow vein mosaic and ragi (Ramachandran *et al.*, 1980) infected by ragi mosaic virus was observed. On the other hand, Ghorpade and Joshi (1981) reported low Fe content in sugarcane leaves infected by SCMV. Similar reports in sorghum infected by SCMV. (Shukla and Joshi, 1981),

Table No. 3.6 Concentrations of Iron in grape leaves.

	Healthy	Infected	Sprayed
Flowering	66	47	59
Fruiting	75	54	67
Harvest	80	48	72

Values are expressed as ppm

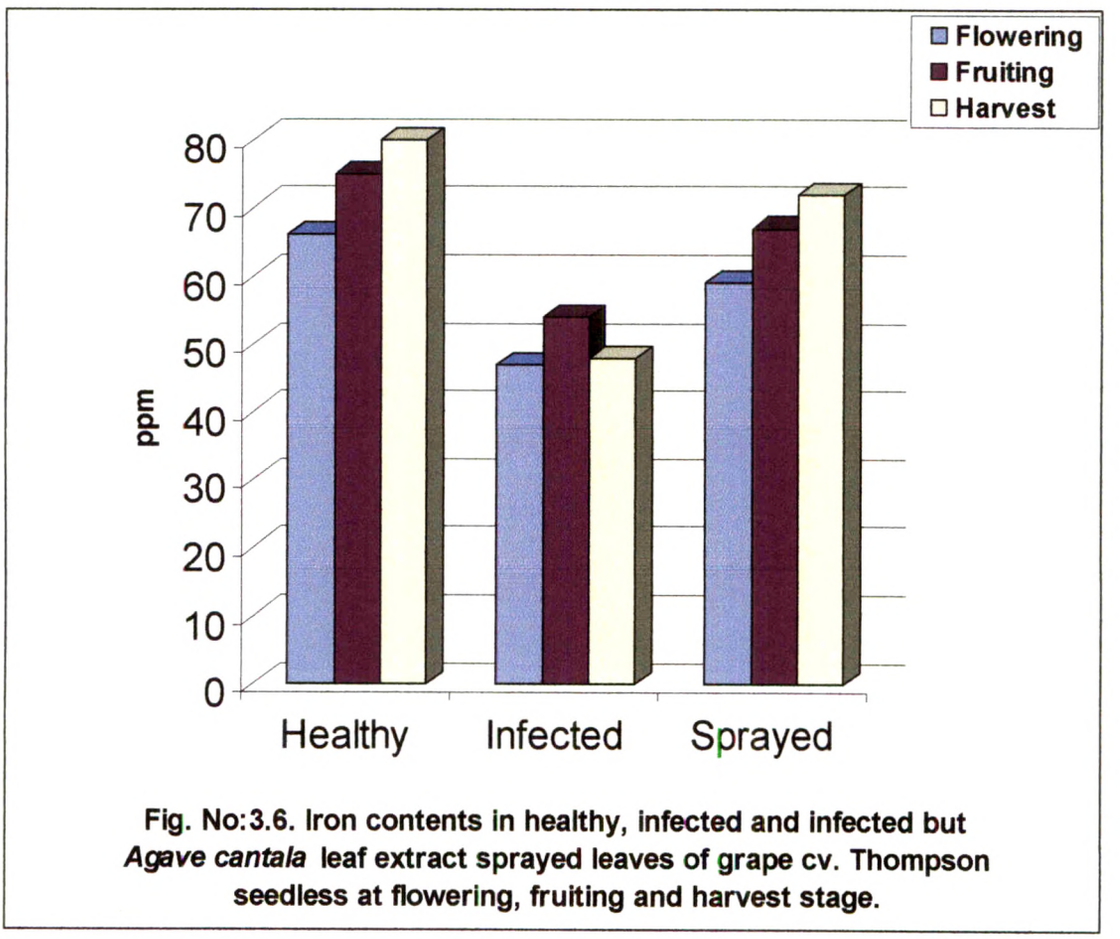


Fig. No:3.6. Iron contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

tomato infected by leaf curl virus (Sasikumaran *et al.,* 1979) and potato infected by potato virus X (Chandra and Mondy, 1981).

Fungal diseases influence the iron content in plants. Sunflower infected by rust exhibit high concentrations of iron (Patil and Kulkarni, 1977a), high concentration of iron in mango affected by *Capnodium* (Kulkarni and Kulkarni 1978), bean infected by *Colletotrichum* (Hegde and Munjal, 1971) and date palm infected by smut (Kapur *et al.,* 1978) was observed.

While, the work of Balasubramanian (1981) in sugarcane infected by smut and Sankpal and Nimbalkar (1980) in Jowar infected by downy mildew indicated that fungal pathogen caused decrease in the iron content. The role of iron in disease resistance was described by Sastry (1964), Hegde (1967) and Hegde and Munjal (1971). They noted that the concentration of active iron plays an important role in determining the growth and concentration of pathogen in host tissue, which governs the degree of disease resistance. The disease resistance decrease with increasing iron content (Hegde and Karande, 1978).

The results obtained in the present study are very interesting because the iron content in infected grape leaves is low as compared to healthy and sprayed. It is possible that due to high iron content in cv. Thompson seedless make susceptible to fungal pathogen attack. It is proved that Thompson seedless is highly susceptible diseases like powdery and downy mildew (Kedge, 2008) due to low phenolic content as compared to non-susceptible verities. The high concentrations of iron in healthy leaves of Thompson seedless suggest the susceptibility towards fungal attacks. In other view, it is possible that phenol concentration is influenced by iron content. However, in our studies Fe contents at three stages in grape plant may be due to resistance created by leaf extract spray, indicating *Agave* leaf extract play role in controlling the grape diseases.

g. Manganese :- (Mn^{++}):-

Manganese is one of the important micronutrient in plant. Plant requires very small quantity of manganese. It is highly essential for plant metabolism. It is quite abundant in the most of the soils.

Tifiin (1972) reported that plant manganese occurs as a free ion and partially moves through phloem. Like magnesium, manganese acts as a co-factor in the enzymatic synthesis of sucrose. Pandav *et al.,* (1981) described the importance of manganese in the synthesis of chlorophylls and 14 Co_2 fixation rate in pepper. According to Horiguchi (1987), in case of some species manganese is accumulated in large amount in roots while, in other in the shoot when they are grown in same culture solutions. Manganese is involved in photo-oxidation of H_2O in photosystem II of photosynthesis. Takahashi and Asada (1977) observed that in chloroplast large proportion of manganese is held in less tightly combined state and is most closely involed in oxygen evolution, while small fraction of manganese is entangled directly in stability of thylakoid.

Manganese is directly involved as a component of biotin enzyme in the biosynthesis of fatty acids (Marschner, 1986). According to Marschner (1986), manganese not only competes much more effectively but also in some way blocks the binding sites for magnesium. Both ion species bridge ATP with enzyme phosphokinase and phosphotransferases. Manganese activates malic dehydrogenase which oxidizes malic acid to give rise to oxaloacetic acid (Maumford *et al.,* 1962) reports manganese activates IAA oxidase and play important role in oxidation of IAA. In some cases manganese activates decarboxylases and dehydrogenase of TCA cycle (Mengel and Kirkby, 1982).

The function of manganese and magnesium are resembles to each other. Manganese is required for the conversion of isocitric acid and ά-ketoglutaric acid to succinic acid. The photo induced carboxylation of pyruvate, glyoxylate and glycolate occurred in isolated spinach chloroplast is increased considerably by manganese ions (Elstner and Heupes, 1973). Behra and Behra (1994) reported that increase in manganese concentration beyond critical leaves

Table No. 3.7 Concentrations of Manganese in grape leaves.

	Healthy	Infected	Sprayed
Flowering	70	56	67
Fruiting	74	53	65
Harvest	72	55	69

Values are expressed as ppm

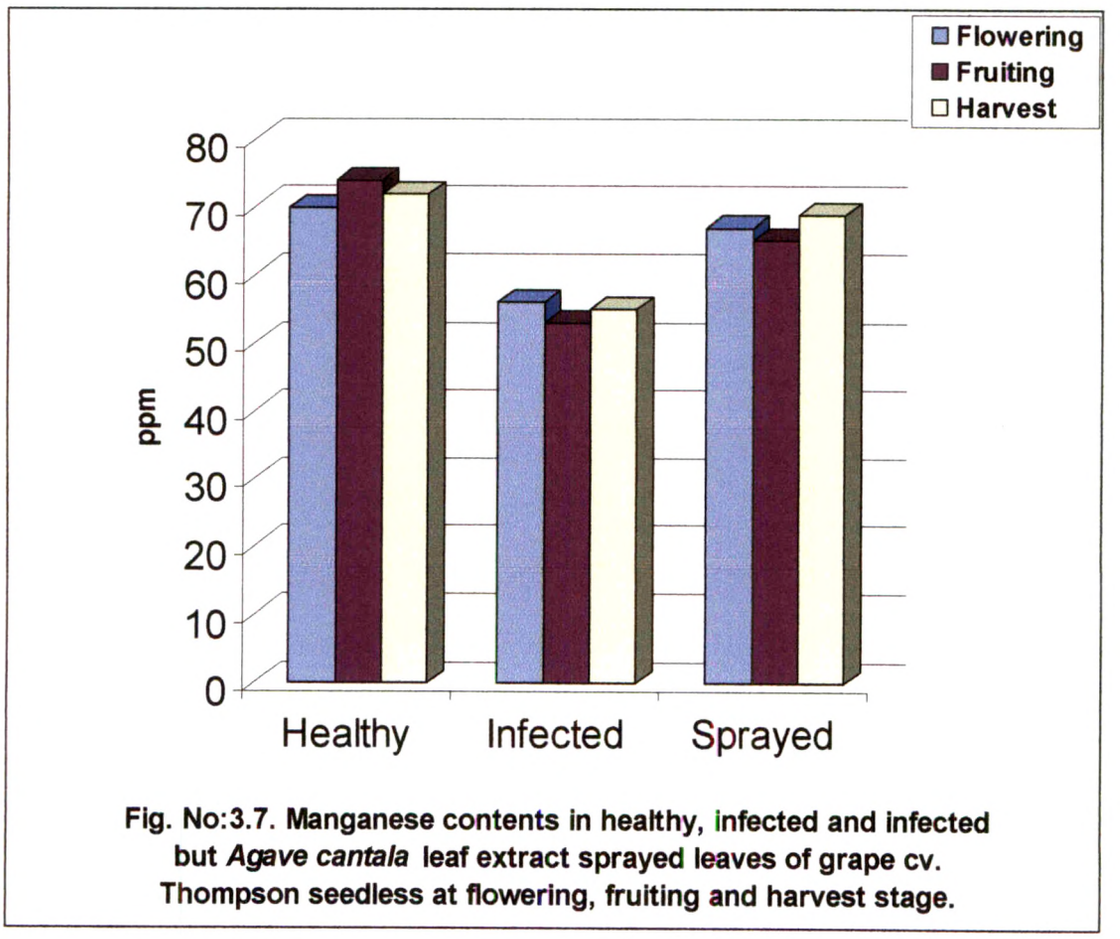


Fig. No:3.7. Manganese contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

decrease chlorophyll contents and activity of catalase in *Amaranthus* spp. Super oxide dismutase incorporating manganese in mitochondria plays an important role in scavenging of free radicals (Jimenez *et al.,* 1998 and Kukavica and Velijovic-Jovanovic, 2004). The optimum concentration of manganese for the growth of multicellular plant is 50 ppm (Stout, 1961). Manganese deficiency symptoms are very much similar to magnesium deficiency. Manganese deficient plants show the interveinal chlorosis in younger leaves, while magnesium deficient show marginal chlorosis.

The optimum concentration of manganese in higher plants is 1umole/g of dry tissue or 50 ppm (Esptein, 1972). The changes in manganese content in healthy, infected and *Agave* leaf extract sprayed and grape leaves is shown in Fig. No. 3.8. The healthy leaves of grapes exhibit optimum concentration of manganese as prescribed by Bhargava and Chadha (1993). The concentration of manganese in healthy leaves of grapes cv. Thompson seedless ranges between 70 to 72 ppm at three stages viz., flowering, fruiting and harvest. The low manganese content is shown in grape leaves infected by powdery and downy mildew. The lowest concentration of manganese is observed in flowering stage i.e. 53ppm. The *Agave* leaves extract sprayed infected leaves show increase in manganese content. The maximum manganese concentration is observed in sprayed leaves at harvest stage i.e. 69 %.

The studies on Mn contents in diseased plants were made by several workers. Low manganese content in the leaves of sugarcane infected by GSD (Grassy Shoot Disease) was reported by Singh *et al.,* (1967). Sasiksumaran *et al.,* (1979) reported an increase in manganese content in the leaves of tomato infected by leaf curl virus. On the contrary, Ghorpade and Joshi (1981) have reported low manganese content in sugarcane infected with sugarcane mosaic virus.

Fungal diseases also influence the manganese content in plants. Sunflower infected by rust exhibit low manganese content (Patil and Kulkarni, 1977a). Similar report of decrease in manganese content in sugarcane infected by smut was observed by Sankpal and Nimbalkar (1980). On the other hand,

Balasubramanian (1981) reported low manganese content in sorghum infected by downy mildew. Similarly, decrease in manganese content was observed in mango (Kulkarni and Kulkarni, 1978) and bean (Hegde and Munjal, 1971) infected by *Capnodium* and *Colletotrichum* respectively.

The work of Hassid and Bean (1955) reported increase in manganese and magnesium content enhances the activity of sucrose synthetase and sucrose-P synthetase. The increased manganese and magnesium content in *Agave* leaf extract sprayed leaves may enhance the activity of sucrose synthetase and sucrose-P synthetase in grape leaves. Hence, the increase in manganese content in infected but *A. cantala* leaf extract sprayed grapes exhibit the positive effect on grape berry sugar content. Thus it appears that there is specific role of manganese in grape plant like magnesium. in developing disease resistant. The mechanism may be accelerated due to *Agave* leaf extract spray.

h. Zinc :- (Zn^{+})

Zinc is micronutrient. Among various trace elements, the Zinc nutrition of plants has attracted a considerable attention of plant physiologists in recent years. Zinc is involved as a metal component of enzyme as a functional, structural or regulatory co-factor of a large number of enzymes like dehydrogenate, peptidases, carbonic anhydrate, Cu-Zn super oxide dismutase, proteinases, lactic acid dehydrogenate and peptidases. According to Marschner (1986), Zn is essential for activity of enzymes aldolase isomerase, transphosphorylase and DNA polymerase.

Zinc plays a role in membrane stability by regulating the level of oxidizing O_2 species (Pinton *et al.,* 1994). Reddy and Rao (1979) reported a key role of zinc in nitrogen metabolism. The work of Triveleka *et al.,* (1999) indicated that zinc (II) does not undergo reduction under any conditions compatible with life, its role as metallo enzyme is inherently different from that of other metals like Cu and Fe, which are capable of redox reactions.

Skoog (1970) and Bonner (1950), observed that the concentration of zinc in plants have positive correlation with auxin level. Nason *et al.*, (1951) and Tsui (1948) indicated participation of Zn in tryptophan and IAA metabolism respectively. Treatment of zinc induces noticeable increase in IAA by increasing the synthesis of IAA from its precursor tryptophan in seed potatoes (Puzina and Sorokina, 1981).

The characteristic visible symptoms of zinc deficiency in dicotyledons are the stunted growth due to shortening of internodes (resetting) and a drastic decrease in leaf size (little leaf.) Rangaswami *et al.*, (1978) reported zinc deficiency in roots alters the uptake of other nutrients (Cakmak and Marschner, 1990). Zinc is playing a prominent role in nitrogen metabolism. The deficiency of zinc in linseed verities causes a decrease in protein, nitrogen content and increase in free amino acid content (Ghildiyal *et al.*, 1986). Low zinc content in plant lowers the concentration of sulphydryl groups at the surface of plasma membrane (Welch and Norveal, 1993) and proton pumping activity in isolated plasma membrane vesicle is depressed (Pinton *et al.*, 1993). Recent studies have indicated close involvement of zinc in RNA transeription machinery. An increase in ribonuclease activity in French bean leaves may be due to zinc deficiency (Affab Hussain *et al.*, 1993).

The deficiency of zinc was observed in vine plants cultivated in sandy soil with high concentration of carbonates. Vine yards in Junnar and Narayangaon in Pune district (Maharashtra, India) exhibit high zinc deficiency (Sahastrabudhe *et al.*, 1969). According to Sharma (2003), zinc is required for the development of grape leaves and pollen grain, zinc deficiency in grape causes retarded petiole length and leaf size as compared to normal healthy leaves. The zinc deficiency reduces grape berry size with hard skin. The zinc deficient berry remains greenish at maturity. However, high concentration of Zn also affects grape leaf morphology. According to Sharma (2005), the pesticide containing high concentration of zinc can affect vine plant. High concentration of zinc in pesticide causes fan-shaped leaves of grapes. Rogiers

et al., (2006) studied mineral sinks within ripening grape berries. They found that seeds were the strongest sink for zinc.

The optimum concentration of zinc for terrestrial plant is 0-30 umole/g or 20 ppm (dry tissue basis) (Epstein, 1972). The critical deficiency levels of zinc are below 15-20 mg kg-1 dry weight of leaves and critical toxicity levels of zinc in leaves of crop plants are more than 400-500 mg Kg^{-1} dry weight basis (Marschner, 1986).

According to Bhargava and Chadha (1993), the optimum concentration of zinc for grape cv. Thompson seedless is 90 ppm dry weight of leaves. In the present studies the zinc contents in the healthy, infected and Agave leaf extract sprayed leaves of grape is shown in Fig. No. 3.9. It is clear from the data the zinc content in untreated leaves infected by downy mildew show considerably low as compared to healthy leaves at all three stage. While, leaves infected with downy mildew show considerable increase after *A. cantala* leaf extract spray.

The concentration of zinc observed by various workers in infected plants is either increased or decreased. Wutsher and Hardesty (1979) and Albrigo and Young (1979) observed increased zinc content in citrus plant infected by citrus blight disease and citrus decline. Increased zinc content in tomato and potato infected by virus, reported by Vijaya Rao *et al.,* (1967) and Chandra and Mondy (1981). The fungal diseases also show increase in zinc content. Sorghum infected by downy mildew (Weste *et al.,* 1980) and *Isopogon* infected by *Phytophthora* (Balasubramanian, 1981) show increase in zinc content.

In present investigation zinc concretion in grape leaves infected by downy mildew and powdery mildew is low as compared to healthy and sprayed leaves. It is possible that due to translocation of zinc from infected to young healthy leaves. Show low concentration in infected leaves.

The work of Till *et al.,* (1979), in wheat, Himelblau and Amasino (2001) and Kochian (2000) in *Arabidopsis thaliana* L. suggest that the level of zinc dropped significantly indicating that this nutrient is mobilized from senescing

Table No. 3.8 Concentrations of Zinc in grape leaves.

	Healthy	Infected	Sprayed
Flowering	64	40	59
Fruiting	66	43	56
Harvest	61	38	58

Values are expressed as ppm

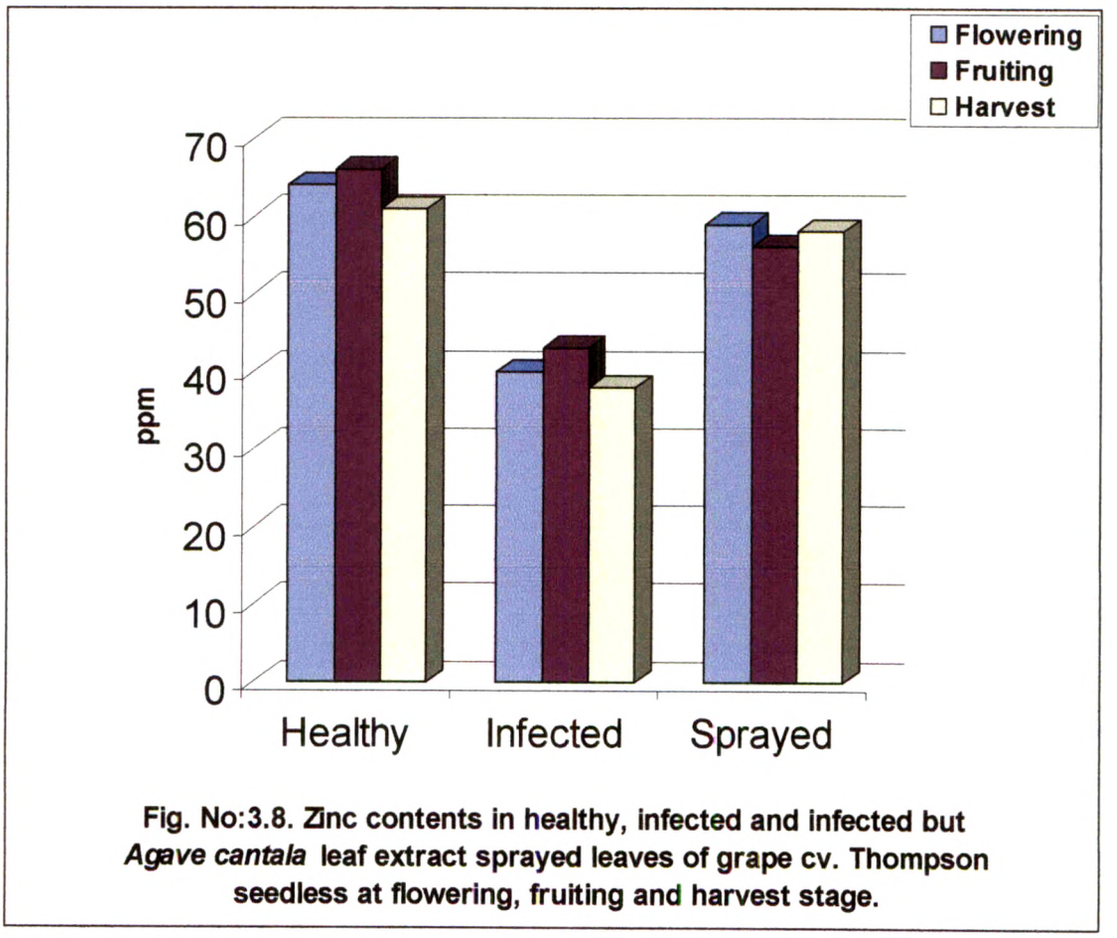


Fig. No:3.8. Zinc contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

leaves. In present work early senescence developed due to severe fungal infection can move zinc from infected to healthy leaves of grapes.

Zinc plays an important in disease resistance. The increased zinc concentration decreases the attack of viral and fungal pathogens. Zinc increases disease resistance in host tissue (Vijaya Rao *et al.,* 1967; Singh *et al.,* 1970; Deshmukh and Mayee, 1978 and Misra *et al.,* 1981). Due to this property all chemical pesticides contain high concentration of zinc. In other view, zinc increases the disease resistance by increasing the phenolic content (Shevchenko *et al.,* 1980). An increased concentration of zinc alters the host metabolism and checks the symptoms produced by *Fusarium* (Prasad, 1979). Moreover, according to Kedge (2007), the grape variety which contains high phenolic concentration shows more disease resistance.

Thompson seedless is susceptible variety of grape to fungal attack. The phenolic content present in Thompson seedless is very low as compared to wild varieties like mango (Kedge, 2008).

The phenolic contents in infected but *A. cantala* leaf extract sprayed grape leaves are more as compared to healthy leaves. The role of zinc in disease resistance was reported by various researchers. In present study, increase in phenol concentration (Fig. No.3.20) is observed in sprayed leaves of grape. The inhibition and growth of fungal disease viz., downy mildew and powdery mildew due to increase in zinc content with phenols by *A. cantala* leaf extract spray in infected leaves proves its role in disease control. Thus, our observations are showing interaction within zinc and phenolic contents in grape in overcoming the disease development.

i. Coppper (Cu):-

Copper is a redox-active metal. It is involved in protein and carbohydrate metabolism. It plays a vital role in reproductive growth. It is important constituent of enzymes like polyphenol oxidase, cytochrome oxidase, ascorbic acid oxidase and tyrosinase etc. It also plays an important role in

nitrogen metabolism. Copper involved in cell wall formation and strength. It is important constituent in electron transport chain.

Copper is a constituent of water soluble, blue colourd 10.5 Kda protein plastocyanin that transfers electrons between the cytochrome bf complex and P_{700} and serves as putative electron carrier between PSII and PSI (Kaim and Schwederski, 1995). The role of copper in the regulation of PS II mediated electron transport is either as a part of polypeptide involved in electron transport or as a stabilizer of the lipid environment close to electron carriers of PSII complex has been suggested by some investigators (Baron *et al.,* 1995; Makymiec, 1997). It is a component of several metalloenzymes an intermediate electron acceptor in the direct oxidation of substrate by molecular oxygen (Gupta, 1979). Copper also plays an important role in maintaining membrane structure of thylakoids (Henziques, 1989). It reacts with amino acids, proteins and other biopolymers producing stable complexes. According to Frieden (1968), copper provides metabolic control over auxin synthesis and is also involved in protein and carbohydrate metabolism. Copper ions have catalytic properties, which are enhanced upon binding of the ions to a protein molecule. Copper as a cupric ion is an essential trace element for algae and higher plants (Someer, 1945; Walker, 1953). The role of copper in photosynthesizing organisms depends greatly on its concentration. In concentration higher than 1.0μm, it is increasingly toxic to algae and higher plants (Gross *et al.,* 1970).

The critical deficiency level of copper in vegetative parts is generally in the range of 3 to 5 μg. g^{-1} dry weight. Its concentration depends upon plant species, plant organ, development stage and nitrogen supply (Rabson and Reuter, 1981).

The copper deficient leaves exhibit low soluble carbohydrate than normal leaves during vegetative stage (Mizumo *et al.,* 1982). The enzyme involved in Fe uptake, Ferric reductase show increased activity under copper deficiency. Copper deficiency may cause wilting of the upper tender leaves and drying tips, often without pronounced bleaching or change of colour.

Table No. 3.9 Concentrations of Copper in grape leaves.

	Healthy	Infected	Sprayed
Flowering	15	10	18
Fruiting	13	8	20
Harvest	12	9	15

Values are expressed as ppm

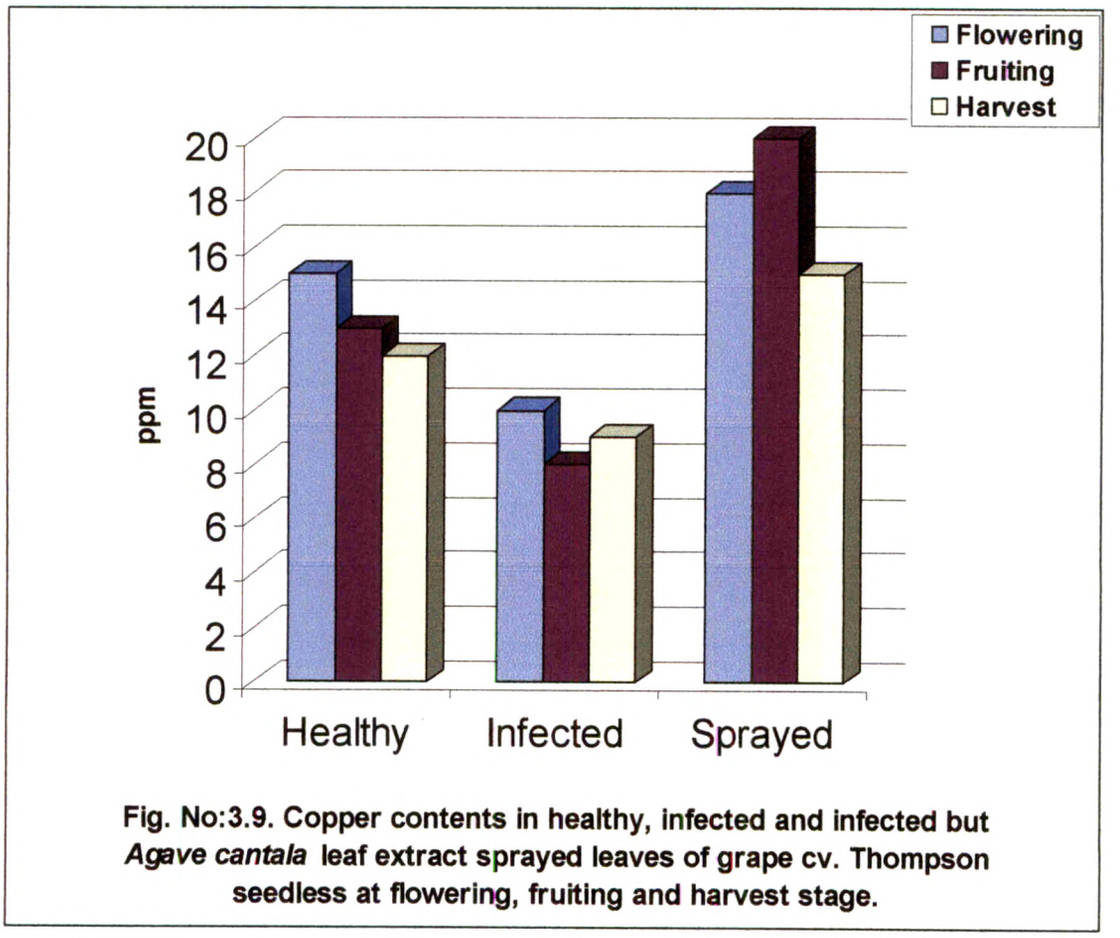


Fig. No:3.9. Copper contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

The deficiency of copper in vine plants is rarely observed because application of fungicide containing high concentration of it. Sharma (2006) reported that the fungicide containing high concentration of copper caused inhibition of pollen development due to deposition on stamens. Moreover, it also causes mummification of berries.

The optimum concentration of Cu in glycophytes is 0.10 µmole / g of dry tissue or 6 ppm (Epstein, 1972). According to Bhargava and Chadha (1993), the optimum value in grape cv. Thompson seedless is 7.5ppm.

The changes in copper concentration in healthy, infected and infected but *A.cantala* leaf extract sprayed leaves of grape at flowering, fruiting and harvest statge depicted in Fig. No.3.9. From Fig. it is confirm that the concentration of Cu in leaves infected with downy mildew and powdery mildew at all three stages are low as compared to healthy and sprayed leaves. i.e. 10 ppm, 8 ppm and 10 ppm at flowering, fruiting and harvest stage respectively. However, interesting value of copper in sprayed leaves is observed in all three stages. Thé leaves infected with downy mildew and powdery mildew shown increase copper content after A.cantala leaf extract spray. Copper concentration in sprayed leaves is observed more as compared to healthy leaves. The value of copper in healthy grape leaves ranges between 12 ppm to 15 ppm whereas in sprayed leaves it is 15 ppm to 20 ppm. The concentration of copper noticed in all three leaf samples at every stage is high as compared to concentration prescribed by Bhargava and Chadha (loc. cit.)

The change in concentration of copper in plants due to infection was reported by many authors. An increase in copper content in sunhemp affected by sunhemp mosaic virus was reported by Sastry (1963, 1964). According to Sasikumaran *et al.,* (1979), copper accumulated in tomato leaves when affected by virus. Similar increase in copper in ragi leaves affected by virus was noticed by Ramachandran *et al.,* (1980).

The variation in copper content in plants due to fungal infection was reported by many authors. Betz *et al.,* (1980) noticed increase in copper concentration in cabbage leaves infected by *Plasmodiophora.* Balasubramanian

(1981) reported accumulation of copper in leaves of sorghum infected by *Scelerospora* (downy mildew).

Many researchers reported conflicting role of copper regarding to disease resistance in plants. Sastry (1963, 1964) suggest that increase in copper content in diseased plant may promote the growth and development of the pathogen. Dhumal (1983) reported the positive relation of copper content and susceptibility in sugarcane cv. Co_{419}. According to him, the chances of GSD infection in sugarcane increases with increase in copper content.

On the other hand, increased copper content in leaves of wheat reduces the susceptibility to powdery mildew (Graham, 1979). According to him, copper deficient wheat plants were severely infected by powdery mildew. Griffioen *et al.,* (1994) reported that accumulation of copper in *Agrostis capillaris* reduces the chances of infection by VA-mycorrhizal fungi. Shevchenko *et al.,* (2004) reported that increased copper concentration in the leaves of *Lycopersicon esculentum.* L.cv Miliana showed signification reduction in amount of systematic tobacco mosaic virus (TMV) infection.

The present study with healthy, infected and infected but *Agave cantala* leaf extract sprayed grape leaves indicated that copper concentration in sprayed leaves is more as compared to healthy and infected leaves. The disease resistance induced in infected grape leaves against downy mildew and powdery mildew, due to *A.cantala* leaf extract spray, show positive correlation with increase in copper concentration in it. An increase in copper content in infected leaves after spray may inhibit the development of downy mildew and powdery mildew. These observations suggest some role of Agave leaf extract spray in controlling the mildews of vine to some extent by controlling the synthesis of trace element also.

j. Molybdenum:-

Molybdenum is trace element found in the soil and is required for growth of most biological organisms including plants and animals. Molybdenum is a transition element, which can exist in several oxidative states ranging from zero to VI in most agricultural soils.

The requirement of molybdenum for plant growth was first demonstrated by Arnon and Stout (1939) using hydrophonically grown tomato. They found that molybdenum is only trace element that could eliminate a phenotype commonly referred to as "whiptail". The role of molybdenum as an essential trace element for algae and higher plants in the process of nitrate reduction has been firmly established (Beevan and Hageman, 1969; Hewitt and Nicholas, 1964; Nicholas 1963; Vega *et al.,* 1971). It was early identified by Nicholas and Nason (1955) as the metal prosthetic group of nitrate reductase from soybean leaves. The work of Afridi and Hewitt (1964, 1966) and Hewitt and Nicholas (1964) have reported that in molybdenum deficient plant grown in the present of nitrate, molybdenum is required for the synthesis of nitrate reductase and have suggested that it is involved in the induction process and acts not merely as the constituent metal.

Molybdenum uptake in plant is often sequestered by Fe- or Al-oxihydroxides, especially in acidic soils, the concentration of the water soluble molybdate anion available for uptake by plants may be limiting for the plant, even when the total molybdenum content of the soil is sufficient. In contrast to bacteria, no specific molybdenum uptake system is known for plants, but since molybdeate and sulphate behave similarly and have similar structure, uptake of molybdate could be mediated unspecifically by one of the sulfate transporters. Transport of molybdenum in to the different plant organs proceeds via xylem and phloem (Zimmer and Mendel, 2008).

Molybdenum deficiency affects plant metabolism at different levels. The responses are strongly linked to the requirement of molybdenum for the various types of molybdoenzymes present in plant. Plant molybdoenzymes can be broken down to those involved in nitrogen reduction and assimilation i.e.

nitrate reduction (nitrate reductase; NR), Nitrogen fixation (nitrogenase), purine catabolism (xanthin dehydrogenase / oxidase; XDH), abscisic acid (ABA) and indole-3 acetic acid (IAA) sysnthesis (aldehyde oxidase; AO) and sulfur metabolism (Kaiser *et al.,* 2005). Since, molybdenum is involved in number of different enzymatic processes, defined plant response to molybdenum deficiency can be complex and thus difficult to assign causally to specific enzyme systems. This is particularly evident in molybdoenzymes involved in nitrogen metabolism where overall reduction in plant growth and health can alter plant development, susceptibitity to pest damage and fruit or grain development (Graham and Stangoulis, 2005).

In grapevine, molybdenum deficiency has recently been suggested as the primary cause of a bunch development disorder called Millerandage or 'hen and chicken' (Williams *et al.,* 2004). Millerandage is characterized by grapevine bunches that develop unevenly where fully matured berries are present in a bunch alongside a large number of fertilized undeveloped berries as well as unfertilized swollen green ovaries (Mullins *et al.,* 2000).

Millerandage has been reported primarily in *Vitis vinifera* Merlot. but it is also occurs in Cabernet Sauvignon and Chardonny cultivars. In Merlot vines displaying Millerandage also appear including shortened zig-zag shaped internodes, pale-green leaves, increased cupped and flaccid leaves and marginal leaf necrosis.

The changes in molybdenum content in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage are depicted in Fig. No.3.10. It is evident from the fig that the healthy leaves of grape contain highest leaves of molybdenum as compared to infected and sprayed. The decrease in molybdenum content in grape leaves infected by downy mildew and powdery mildew is observed. While, increase in molybdenum content is noticed in infected but *A. cantala* leaf extract sprayed grape leaves.

Molybdenum content in plant is related to diseases resistance. However, there is little direct evidence to conclude that improvement in plant

Table No. 3.10 Concentrations of Molybdenum in grape leaves.

	Healthy	Infected	Sprayed
Flowering	0.32	0.11	0.24
Fruiting	0.31	0.09	0.25
Harvest	0.3	0.1	0.22

Values are expressed as ppm

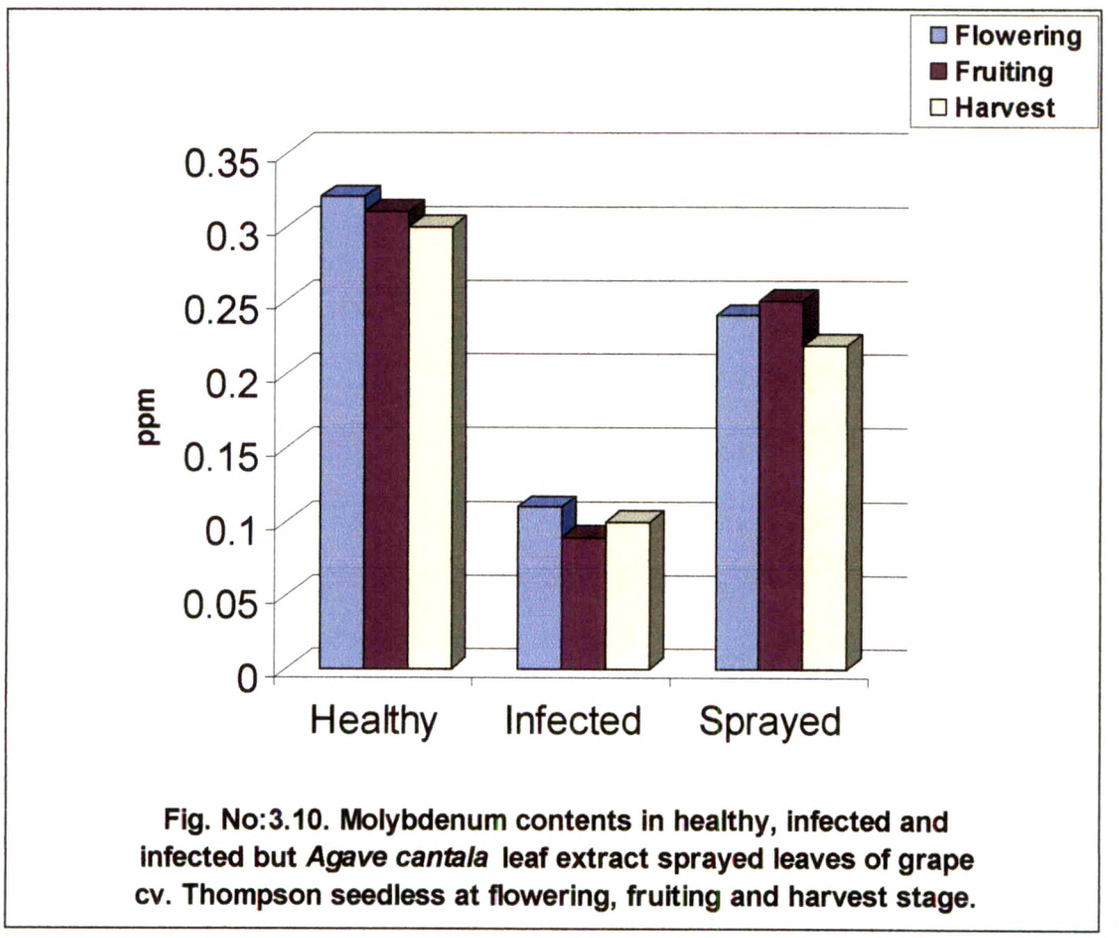


Fig. No:3.10. Molybdenum contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

molybdenum levels results in a decrease of disease. Bhargava and Khare (1986) observed high molybdenum concentration in resistant variety of chickpea than susceptible, regarding to *Alternaria blight*. They further conclude that high molybdenum content in chickpea is responsible for resistance against *Alternaria*. According to Sharma and Khanna (1969), higher concentration of molybdenum inhibited the spore germination and growth of *Alternaria tenuis* causing core rot of Mandarian oranges. Recently, Graham and Strangoulis (2005) reported that molybdenum can improve resistance in tomato against *Verticillium*.

In present study, the molybdenum content in infected grape leaves increased after *A. cantala* leaf extract spray. It is possible that increased molybdenum content in infected leaves exhibit resistance by inhibiting spore germination of *Plasmopara viticola* causing downy mildew. Hence, infected leaves show improved contents of other such as chlorophyll, nitrogen, magnesium etc.

The inorganic element synthesis and degradation in infected leaves may possibly controlled by Agave leaf extract and disease resistance may be induced in the vine plant successive stages of development up to fruiting stage.

k. Cobalt :- (Co^{++})

Cobalt is a transition element and essential component of several enzymes and co-enzymes. Depending on the concentration and status of cobalt in rhizosphere and soil, it affects growth and metabolism of plants, in different degrees.

The distribution of cobalt in plants is entirely species dependent. The uptake controlled by different mechanism in different species. Physical conditions like salinity, temperature, pH of the medium and presence of other metals influence the process of uptake and accumulation in algae, fungi and mosses. In higher plants, absorption of Co^{2+} by roots involves active transport. Cobalt is mainly transported in the xylem by the transpirational flow. Due to

low mobility of cobalt in plants, restricts its transport to leaves from stems (Palit *et al.,* 1994).

Several authors noticed involvement of cobalt in plant metabolism such as Yamada (1960) in fertilization, Niebroj and Kozubska (1964) in glycolysis and Lipskaya (1980) in hill reaction. Cobalt in higher plants participates in chlorophyll b formation. It is found in the plant as cyanocobalamin (coenzyme cobalamin) i.e. vitamin B_{12}. Cobalt helps in the fixation of molecules nitrogen in root nodules of leguminous plants. It is essential N_2 fixing microorganisms like *Rhizobium.* Hence, it is important for leguminous plant for symbiotic association with microorganisms.

It is also required for synthesis of leghaemoglobin. According to Shkolnik (1984), it play important role in synthesis of haem, a prosthetic group of iron porphyrine enzymes like cytochromes, catalase and peroxidase. Cobalt plays an important role in oxidative phosphorylation and structural and functional organization of leaves (Loecher and Liverman, 1964). According to Atta-Aly *et al.,* (1991), low concentration of cobalt in tomato leaves decreases activity of enzyme catalase and peroxidase. Also, low level of cobalt in soil increases the anthocyanin and flavons content in roselle (*Hibiscus sabdariffa* L.) (Eman *et al.,* 2007). They also found that the concentration of macroelements (N, P and K) and microelements (Ni, Mn, Zn and Cu) increased in leaves and calyses of roselle plant when treated with low level of Co i.e. 20 mg / kg soil. Bisht (1991) reported antagonistic relationship in cobalt and iron. Moreover, low level of cobalt in peanut leaves increases dry matter (Abo El-Seoud *et al.,* 1994)

According to Helmy and Gad (2002), plant growth of parsley i.e. plant height, number of leaves per plant as well as fresh and dry weight of leaves and root were significantly increased with low levels of cobalt in soil i.e. 25 mg/ kg soil.

The beneficial effects of cobalt include retardation of senescence of leaf, increase in drought resistance in seeds, regulation of alkaloid accumulation in medicinal plants and inhibition of ethylene biosynthesis (Palit *et al.,* 2008).

Table No. 3.11 Concentrations of Cobalt in grape leaves.

	Healthy	Infected	Sprayed
Flowering	0.03	0.04	0.03
Fruiting	0.03	0.04	0.03
Harvest	0.02	0.03	0.01

Values are expressed as ppm

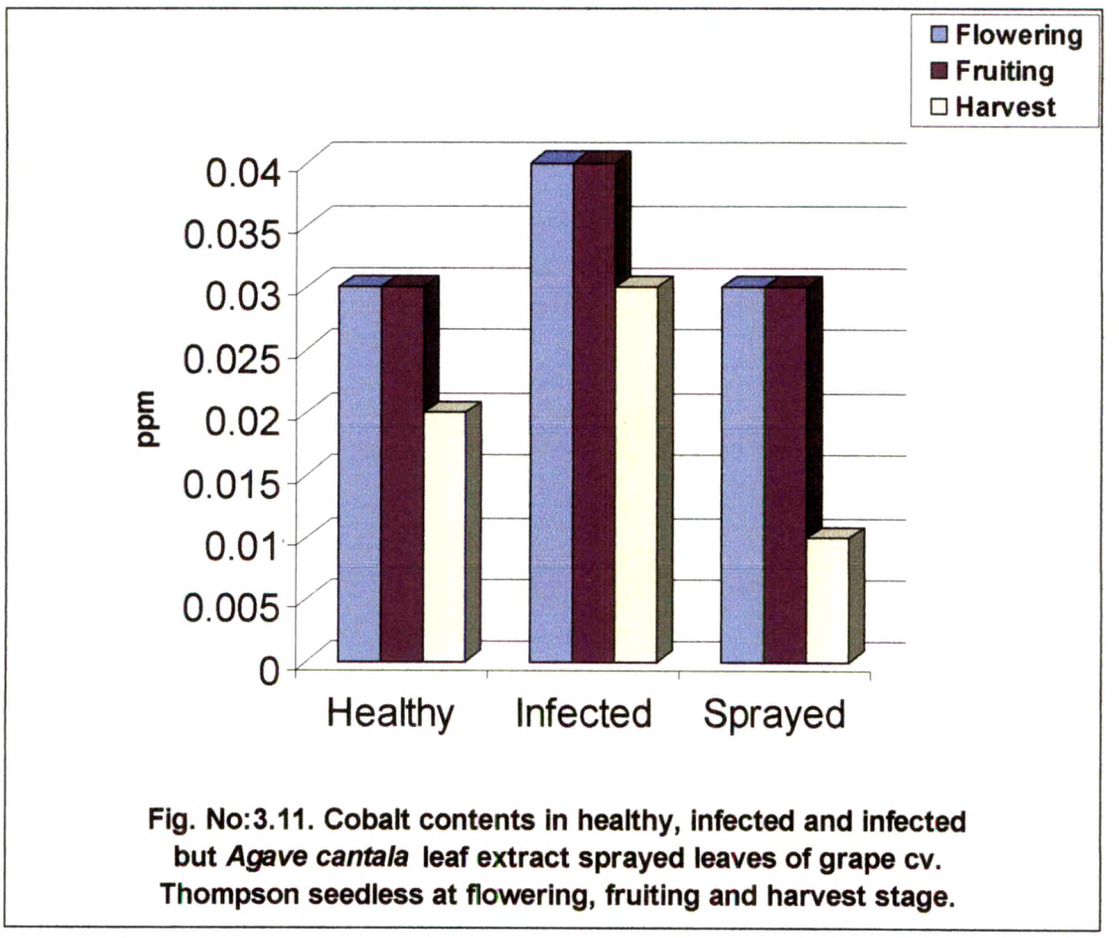


Fig. No:3.11. Cobalt contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

Table No. 3.11 Concentrations of Cobalt in grape leaves.

	Healthy	Infected	Sprayed
Flowering	0.03	0.04	0.03
Fruiting	0.03	0.04	0.03
Harvest	0.02	0.03	0.01

Values are expressed as ppm

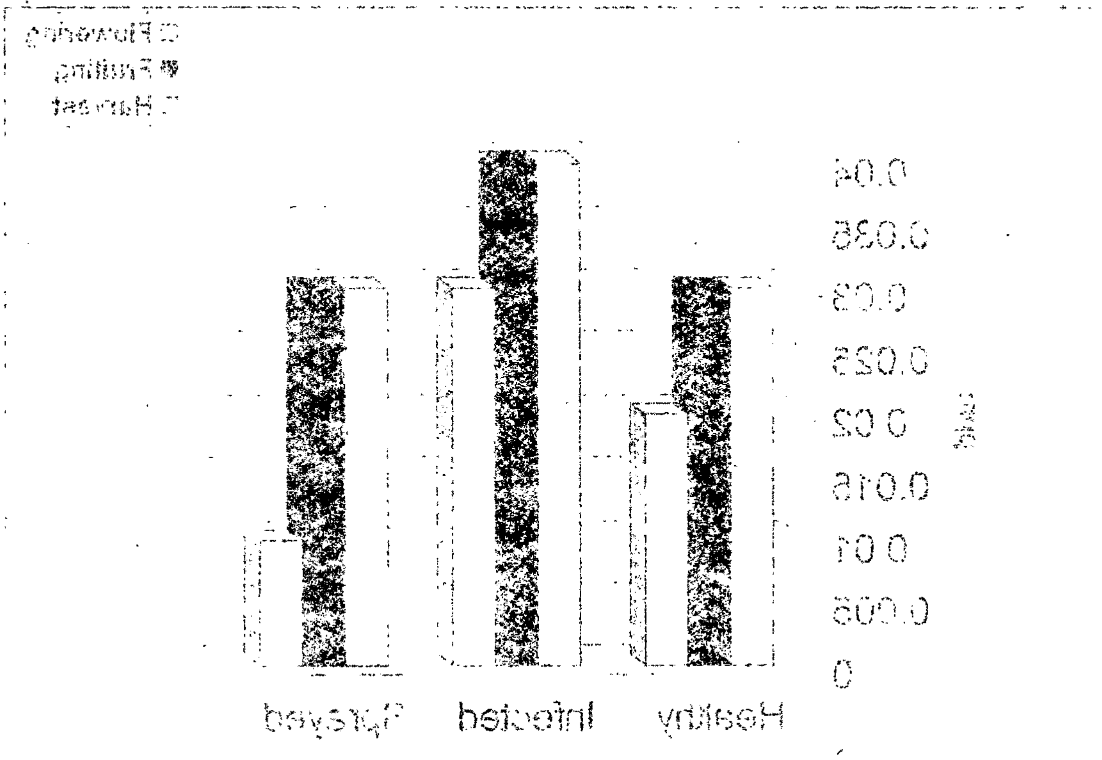


Fig. No. 3.11. Cobalt contents in healthy, infected and infected but Agave cantala leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

However, at high concentration, cobalt shows toxic effect on plants such as leaf fall, inhibition of greening, discolored veins, premature leaf closure and reduced shoot weight. Gopal *et al.,* (2003) observed cobalt toxicity effects on growth and metabolism of tomato. They found that excess cobalt causes chlorosis of young leaves from base, necrotic spots on chlorotic areas, loss of lamina and marginal scorching. The affected leaves were distorted and appeared hook like with rudimentary leaf lets at the top. Excess cobalt restricted the biomass, concentration of phosphorus, sulfur and iron, chlorophyll a and b DNA and RNA, reducing and non- reducing sugars, starch total proteins, protein and non- protein nitrogen and increased phenol concentration. In excess cobalt treated tomato, leaves, the activity of catalase decreased and peroxidase, ribonuclease and acid phosphatase increased.

The values of cobalt concentration in healthy, infected and infected but *A.cantala* leaf extract sprayed leaves of grape c.v. Thompson seedless at flowering fruiting and harvest stage are exhibited in fig. No.3.11. From fig., it is clear that there is no significant difference in cobalt concentration in healthy, infected and sprayed leaves of grapes. The lowest cobalt content was noticed in sprayed leaves at harvest stage i.e. 0.1 ppm. But the concentration of cobalt in infected leaves is slight greater than healthy and sprayed leaves.

From present data, it can be said that fungal infection like downy mildew and powdery mildew may increase cobalt concentration in grape leaves.

However, low concentration of cobalt in sprayed leaves at harvest stage may be responsible for better yield than infected. It is due to controlled organic metabolism of chlorophyll, sugars, proteins, etc. which increased the qualitative characters in the vine fruits. Thus, it is possible that cobalt has specific role not major but minor in controlling the physiological mechanism and may be due to Agave leaf extract spray on diseased vine plants.

B) Organic Constituents

a. Moisture percentage:-

Water is fundamental constituent of all living organism and it contributes about 90% of total protoplasm, it is essential for various enzyme reactions and play a key role in biochemistry and plants.

In plants water plays an important role in the process of light reaction which is characterized by photolysis of water is solvent and medium of translocation for metabolites and mineral within plants. It serves as a substrate for large number of hydrolytic reactions in the plant metabolism. Water is an essential constituent of membrane structure and function.

The water status of leaves merits a special consideration since it is the major site of metabolism in the plats. According to Ganga (2003), the water content of leaves is a balance between water uptake by the roots and water evaporated from the leaves and shoots by transpiration. The photosynthesis process which determines dry matter production has directly influenced by the leaf water content because it bearing various biochemical processes. The leaf water status is directly influenced by another important physiological process called transpiration which is associated with specialized leaf structure- stomata as well as leaf cuticle.

A reduction in moisture percentage in plants is first evidence caused by water stress. According to Lewitt (1956), water deficiency causes dehydration of protoplasm and loss of turgor. Water deficiency in plants causes production of reactive oxygen species (ROS) that damages plant tissue (Dat *et al.,* 2000).

The values of moisture percentage in healthy infected and infected but *A. cantala* leaf extract sprayed grape leaves (cv. Thompson seedless) at flowering, fruiting and harvest stage are depicted in Fig. No.3.12. From fig. it is evident that maximum moisture percentage observed in healthy grape leaves followed by sprayed leaves. Low moisture percentage observed in grape leaves infected with downy mildew and lowest moisture percentage i.e. 40.12% observed in infected leaves at fruiting stage. While, highest moisture percentage observed in healthy leaves at flowering stage (62.15%). Fig. NO.----

Table No. 3.12 Moisture percentage in grape leaves

	Healthy	Infected	Sprayed
Flowering	62.15	44.12	57.99
Fruiting	65.23	40.12	52.69
Harvest	64.89	38.56	55.12

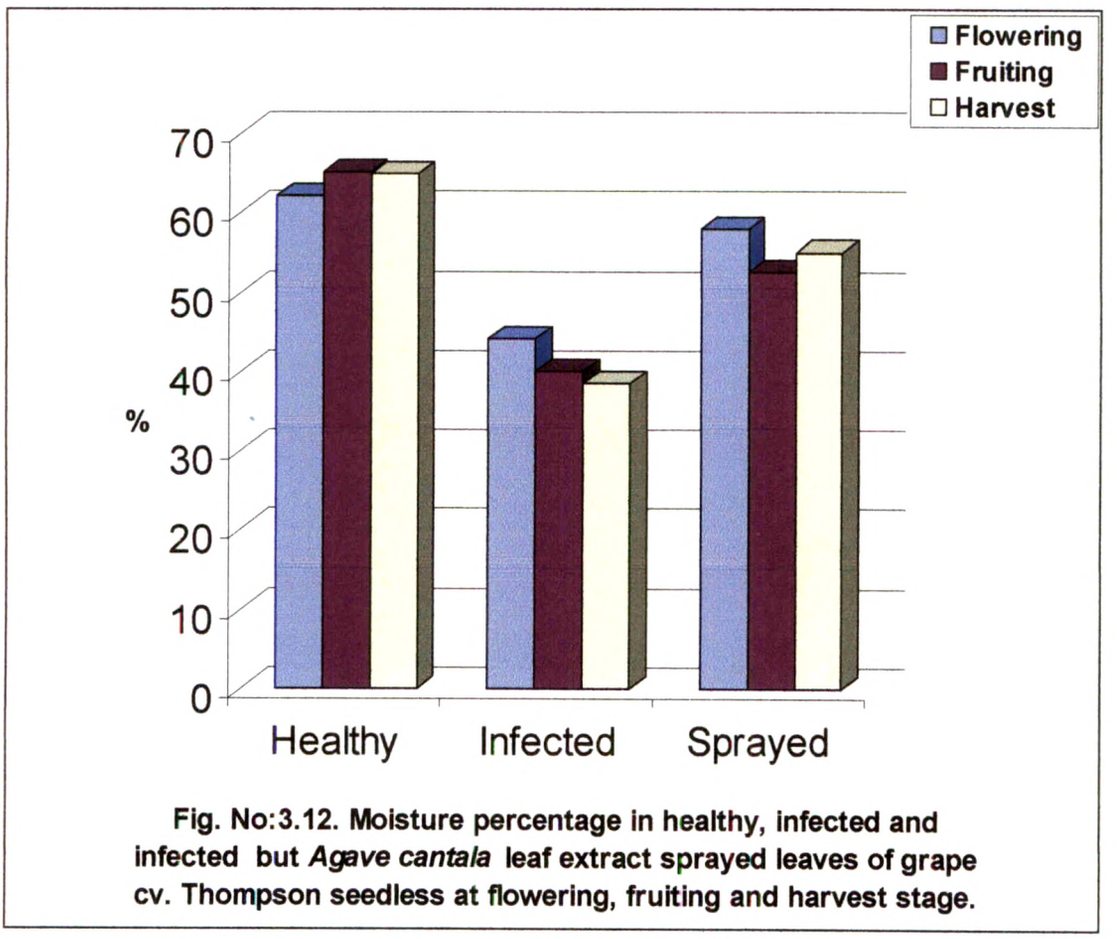
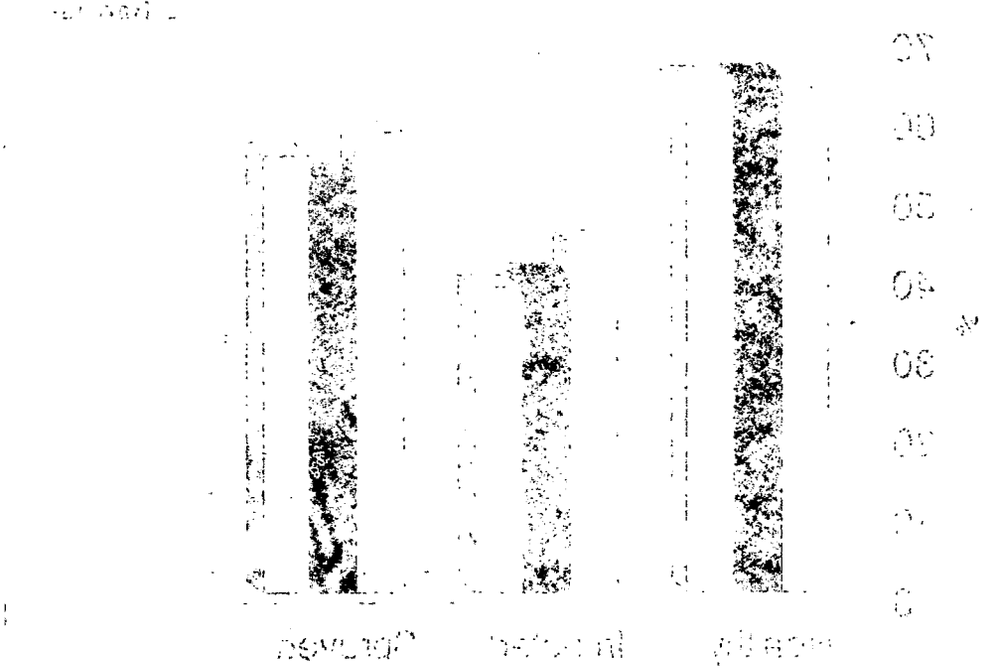


Fig. No:3.12. Moisture percentage in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.



exhibited the dry matter percentage in healthy, infected and sprayed leaves. It is clear from fig. that dry matter percentage is high in infected leaves as compared to healthy and sprayed leaves.

Various researchers reported increase or decrease in moisture content in plants infected with various pathogens. Dhumal (1983) reported increased water content in sugarcane infected with hollyhock yellow mosaic virus reported increase in moisture content. While, Ghorpade (1982) reported decreased moisture percentage in sugarcane infected with SCMV (sugarcane mosaic virus).

Fungal infection also causes increase or decrease in moisture percentage of plants. Dhingra *et al.,* (1982) observed increased moisture percentage in *Brassica* infected with *Albugo*. On the other hand, Tang *et al.,* (2005) have reported decrease in moisture percentage in mulberry leaves infected with *Cercospora moricola.*

The low moisture percentage followed by low potassium concentration in grape leaves infected with downy mildew and powdery mildew suggest utilization of photosynthates and minerals by fungus for it's own development. While, increase in moisture percentage at considerable level in infected leaves after *A. cantala* leaf extract spray with increase in potassium content (Fig. No.3.3) proves the inhibition of fungal growth development. Thus, the *Agave* leaf extract spray suggests the control of disease development by controlling the water status in infected grape leaf.

b. Photosynthetic pigments:-

i. Chlorophylls:-

The every existence of a life on this plant depends mainly on the process of photosynthesis means literally the assembly of a product from raw material using light. The "light reaction" of photosynthesis encompasses light harvesting i.e. the primary photochemical acts, electron transport and photophospharylation. They occur in or on the thylakoid membranes of the chloroplast.

Among various plant pigments, chlorophylls occupy a unique place in the life of green plants because these are primarily involved in harvesting the solar energy and converting it into chemical energy and as such act as an 'electron gun' of green plants. These pigments include chlorophyll a which is ubiquitous in all photosynthetic organisms capable of splitting water in photosynthesis. Chlorophylls are basically the magnesium chelates of closed tetrapyrrole rings derived from phytoporphyrin. The chlorophyll a assumes a number of forms within the thylakoid membrane. These are expressed as changes in absorption fluorescence spectra that presumably depend on the protein molecules with which the chlorophylls molecules are associated and their solvent environment (Seely, 1977; Thornber and Alberte, 1977).

The chlorophylls absorb in the blue (450nm) and red (650 to 700 nm) regions of the visible spectrum. Chlorophyll b is almost as wide spread as chlorophyll a but is absent from all the algae except the Chlorophyceae. In higher plants, chlorophyll a is a major pigment involved in harvesting solar energy while, chlorophyll b plays rather secondary role. The pigment-protein complexes are organized into two photosystems I and II (PS I and PS II) and a light harvesting complex (LHCP), which perform a central role in light reaction. PS- I act as a light driven plastocyanin ferredoxin oxidoreductase while, PS II acts as light dependant water-plastoquinone oxidoreducatse.

Chlorophyll pigments plays a main role in light reaction of photosynthesis, the rate of the pigment and content of the pigments have direct influence on the photosynthetic efficiency of the plant. According to Rosenow *et al.,* (1983) and Reddy and Prasad (1999), photosynthetic efficiency depends on chlorophyll content higher the chlorophyll content, higher the photosynthesis leading to enhanced leaf area index and yield. The content of chlorophyll in a leaf is the result of balance of steady chlorophyll synthesis and chlorophyll degradation (Sestak, 1985). In the view of Henningson and Boynton (1974), the chlorophyll accumulation is controlled not only by the rates of process of chlorophyll biosynthesis or degradation but also by the

formation of chloroplast ultrastructure. The process such as shading also influences the chlorophyll content due to creation of irradiance gradient.

The environmental factors such as drought (Virgin, 1965), salinity (Strogonov *et al.,* 1970), mineral deficiency (Natre, 1975), air pollution (Tanaka and Sugahara, 1980), and diseases (Carol and Kosuge, 1969) also causes decline in chlorophyll contents.

The changes in chlorophyll contents in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape cv. Thompson seedless are exhibited in Fig. No.3.13 and 3.14. It is evident from the figures that the healthy leaves of grape contain higher level of both chlorophyll a and b whereas, in infected leaves, chlorophyll contents are very low. The amount of chlorophyll a and b in infected but *A. cantala* leaf extract sprayed leaves is more as compared to infected leaves. Similar trend is observed for total chlorophyll content in healthy, infected and sprayed grape leaves (Fig.No.3.15).

The decline in chlorophyll content due to infection was observed by many researchers. Low chlorophyll content in brinjal leaf infected by little leaf disease was observed by Mitra and Sengupta (1980). Sandal affected by spike diseases (Parthasarathi *et al.,* 1976), *Vinca rosea* infected by MLO (Carling and Milliken, 1977) show low chlorophyll contents. Similar trend of decrease in chlorophyll content was observed by Dhumal (1983) in sugarcane affected by GSD.

Several workers observed the severe decline in chlorophyll contents in host infected by virus (El-Faham *et al.,* 1990; Milavec *et al.,* 2001).)

Fungal diseases also cause severe decline in chlorophyll content. The work of Heath (1974) in cowpea affected by rust reports low chlorophyll content. Sugar beet infected by powdery mildew (Magyarosy *et al.,* 1976), ground nut infected by rust (Siddaramiah *et al.,* 1979) show severe loss of chlorophyll contents. Similar reports of reduced chlorophyll content in plants affected by fungal diseases was reported in wheat infected by *Alternaria* (Vijaykumar and Rao, 1980), cowpea infected *Erysiphe* (Kaur and Deshpande,

1980), sugarcane infected by smut (Sankpal and Nimabalkar, 1980), ground nut infected by *Cercospora* (Johri and Padhi, 1981), wheat affected by *Puccinia* (Singh *et al.,* 1982), cocoa infected by withce's broom (*Crinipellis perniciosa*) (Orchard and Hardwick 1988), betelvine infected by *Colletotrichum* (Naik *et al.,* 1988), grape infected by *Sphaceloma ampalina* (Dhillon *et al.,* 1989), coriander infected with *Protomyces macrosporus* (Prasad *et al.,* 1989).

Grapevines infected by root feeding grape phylloxerra (*Daktuloshaira vitifolia* Fitch), *Fusarium solani* and *P. ultimum* were show significant reduction in chlorophyll contents (Omar *et al.,* (1995). Similar reduction in chlorophyll contents have reported by Tofazzal *et al.,* (1999) in mango infected by *Colletotrichum*, Khan *et al.,* (2001) in sorghum infected by leaf spot pathogen (*Drechslera sorghicola*), Srobarova *et al.,* (2004) in maize infected by *Fusarium*, Mahamud *et al.,* (2004) in faba bean infected with *Botrytis fabae*, Scarpari *et al.,* (2005) in cocoa infected by witches' broom, *Tang et al.,* (2005) in mulberry leaves infected by *Cercospora moricola.*

In the present studies on vines, it is observed that the chlorophyll content decreased in grape leaves infected by downy mildew and powdery mildew. However, the decrease in chlorophyll a was more pronounced than chlorophyll b in all three stages.

According to Farkas (1978), when a foliar pathogen establishes inside the host tissue, the chlorophyll contents are usually decreased, this is accompanied by yellowing of infected leaves. Various plant pathogens are known to produce toxic metabolites, which many destroy the chloroplast resulting into decrease of chlorophyll pigments (Fulton *et al.,* 1965; Pero and Main, 1970). Many authors noticed the low concentration of chlorophyll pigments in infected plant; however increased activity of enzyme chlorophyllase in infected leaves is directly proportional to the lowered chlorophyll contents (Bailiss, 1970; Chinnadurai and Nair, 1971; Parthasarathi *et al.,* 1976; Dhumal, 1983). Furthermore, the change in chlorophyll concentration was mirrored by the variation in N, Mg and Mn concentrations (Fig. no. 3.1 (N), 3.5 (Mg), 3.7 (Mn)) (Agarwala *et al.,* 1964; Kumar and

Table No. 3.13 Concentration of Chl. a in grape leaves

	Healthy	Infected	Sprayed
Flowering	156.6	43.16	118.04
Fruiting	159.84	36.75	112.89
Harvest	147.58	40.11	114.08

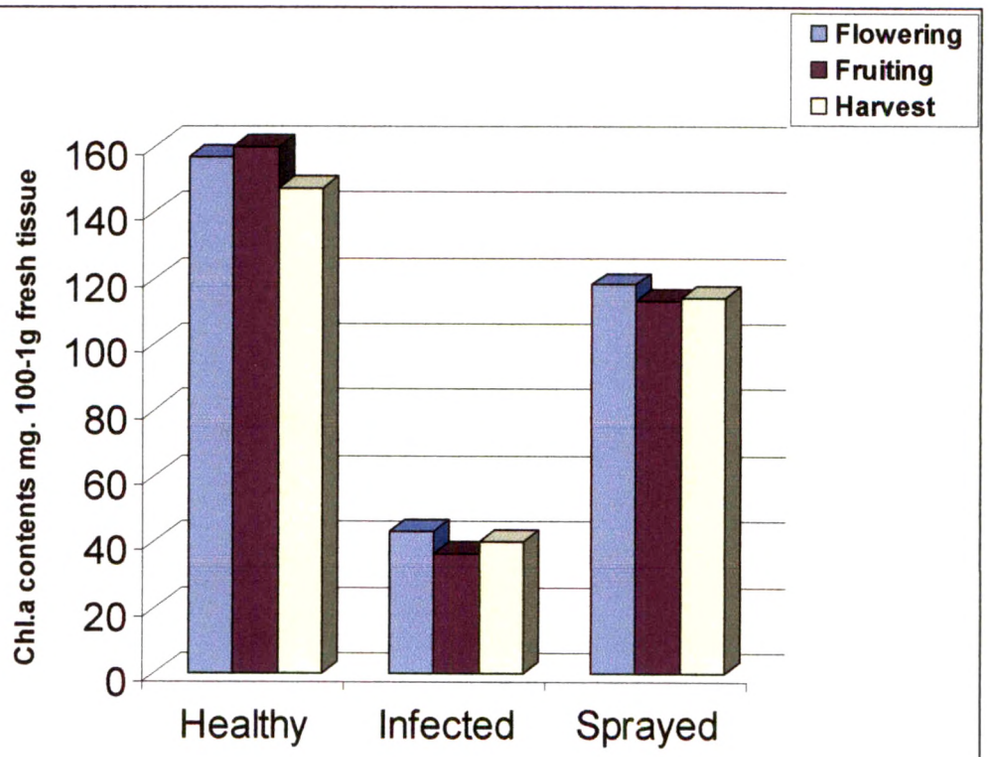


Fig. No:3.13. Chl.a contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

Table No. 3.14 Concentration of Chl. b in grape leaves

	Healthy	Infected	Sprayed
Flowering	106.26	23.62	81.1
Fruiting	105.38	25.36	78.45
Harvest	101.22	20.78	79.55

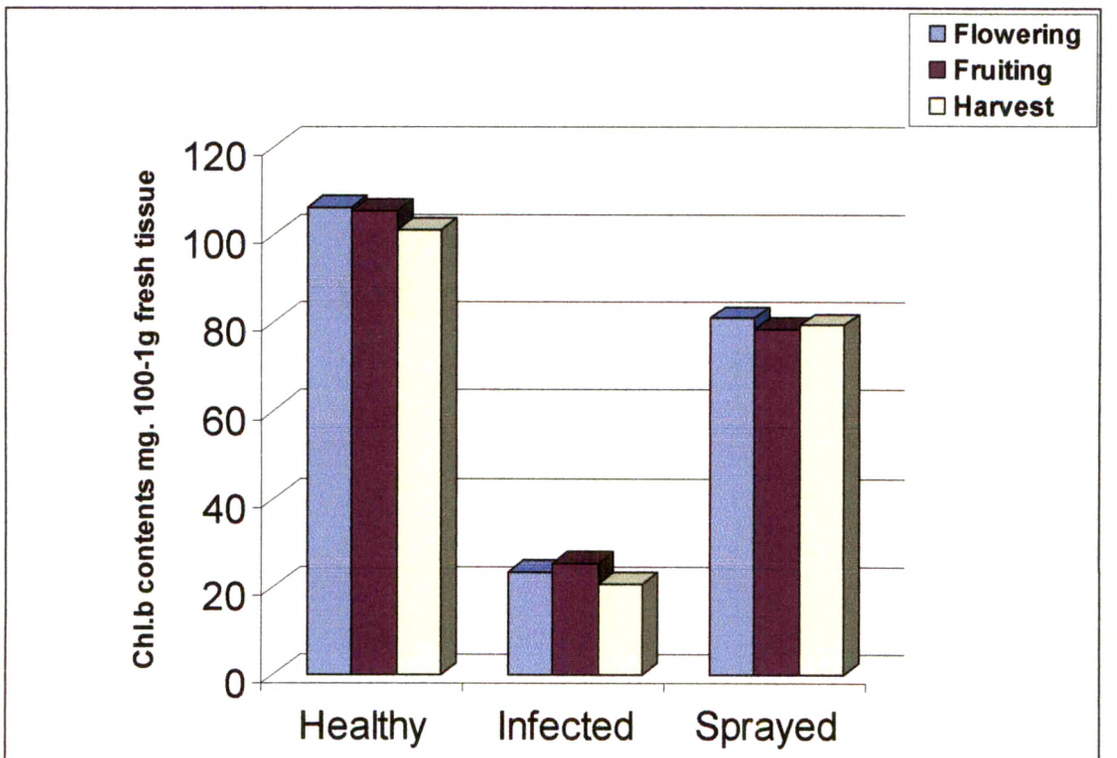


Fig. No:3.14. Chl.b contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

Table No. 3.15 Concentration of Total Chlorophyll in grape leaves

	Healthy	Infected	Sprayed
Flowering	340.32	70.64	256.86
Fruiting	346.56	86.25	246.95
Harvest	334.2	78.48	230.8

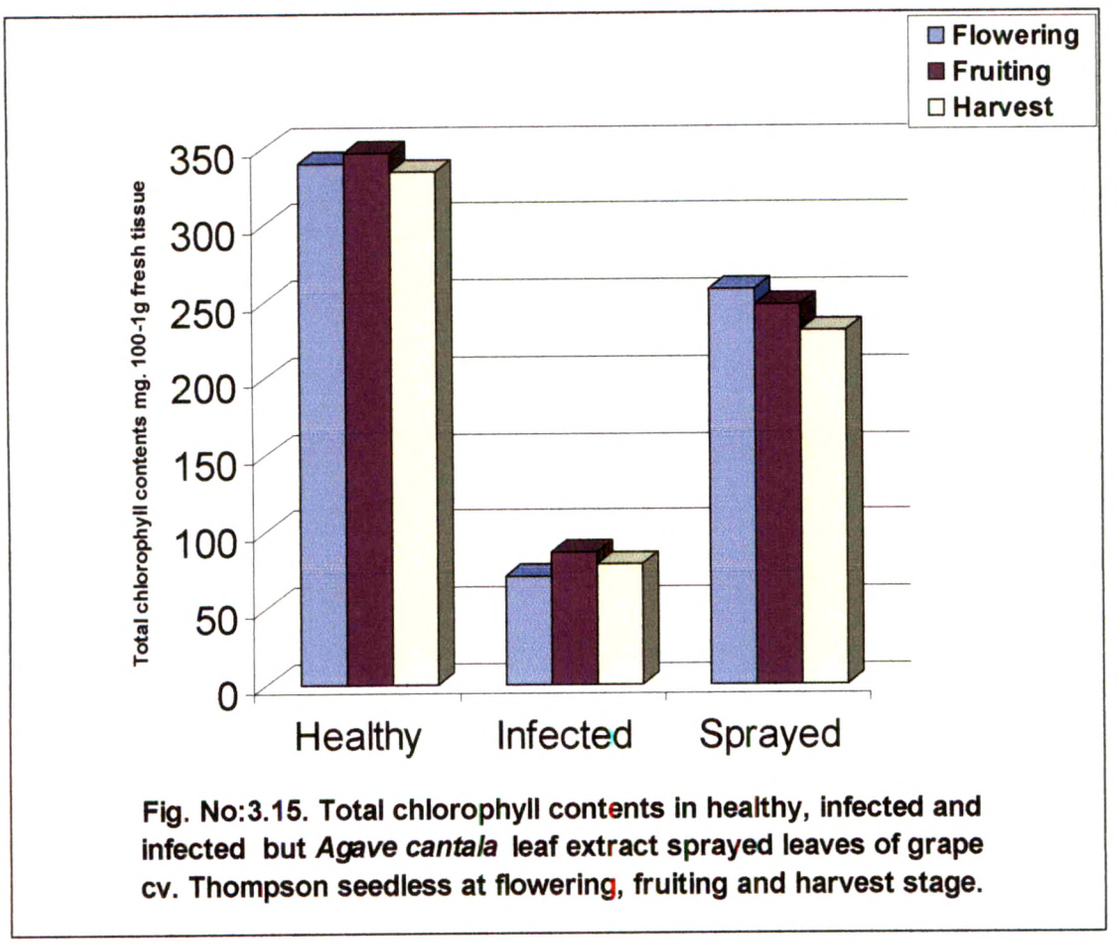
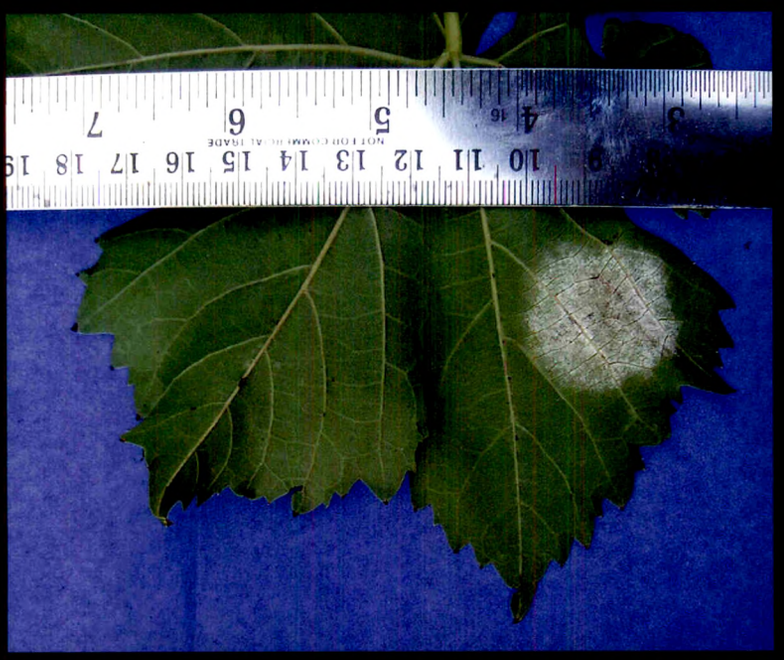


Fig. No:3.15. Total chlorophyll contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.



A : Before treatment of *Agave cantala* leaf extract

B : After treatment of *Agave cantala* leaf extract

Fig. 3 Effect of *Agave cantala* leaf extract spray on downy mildew infection

Alexander, 1972; Ananthanaryana and Venkata Rao, 1980; Ghorpade and Joshi, 1981; Dhumal, 1983). Nitrogen and Magnesium are major component of chlorophyll molecules (Abu-Grab and Ebrahim, 2000) and important involvement of Mn in photosystems (Krause and Santarius, 1975). Hence, low concentration of these elements suggests poor concentration of chlorophylls and photosynthesis and increased respiration.

The ratio of chlorophyll a / b noticed in grape leaves infected with downy mildew and powdery mildew is higher than healthy and *A. cantala* leaf extract sprayed leaves. This result is supported by the work of Mahadevan and Sridhar (1982) and Tang *et al.,* (2005) in mulberry leaves infected by *Cercospora.*

The high concentration of chlorophyll pigments and low chl. a / b ratio noticed in *A. cantala* leaf extract sprayed grape leaves than infected leaves. This result is supported by the increased content of Nitrogen, Magnesium and Manganese in sprayed leaves (Fig.No. 3.1(N), 3.5 (Mg), 3.7 (Mn)). Hence, inhibition of fungal growth due to *Agave* leaf extract spray decreases chlorophyll degradation in infected grape leaves. It is also indicate the interrelation with N, Mg and Mn contents and low and high chlorophyll contents in the healthy, infected and sprayed leaves.

ii. Carotenoids:-

Carotenoids are accessory pigments. These pigments play secondary role in the process of photosynthesis. Carotenoids are synthesized and accumulated in the chloroplast contribute to the organization of two photosystems in the thylakoid membrane associated proteins along with chlorophylls.

Role of carotenoids is rather secondary in comparison to a major role played by chlorophylls in the process of light reaction. According to Demming-Adams (1990), the role of carotenoids is two fold in photosynthetic systems (i) carotenoids functions as accessory light-harvesting pigments which trap light energy and pass it onto chlorophyll molecules. Carotenoids absorb light energy

in the 400-500 nm regions, which is not accessible to the chlorophyll molecules, and act as an accessory light harvesting pigment in the photosynthetic apparatus. (ii) Carotenoids play protective role in light mediated stress. Many workers have attributed a protective role of carotenoids offering photoprotection to chlorophylls against bleaching (Sestak, 1985; Ong and Tee, 1992)

Xanthophylls directly participate in the mechanism of photosynthesis, the violaxanthin cycle participating in oxygen transport, the function of β-carotene in the C-550 absorbance change. Young (1991) reported that production of carotenoid zeaxanthin is very important in setting up stress tolerance in plants. Carotenoids may also be performing some other functions because the carotenoids have been detected in non-photosynthetic plant species e.g. fungi, and in non-photosynthetic organelles like petals, anthers, pollen of some flowers, seeds and eyespot of *Euglena* species (Sestak, 1985).

The higher plants have carotences, α-carotene, β-carotenes and the xanthophylls, lutein, vioalaxanthin and neoxanthin. The ratio of chlorophylls/ carotenoids increases during the growth of leaf area and formation of chloroplast because carotenoids are synthesized more slowly than chlorophylls (Sestak, loc. cit).

Carotenoid contents in plants are influenced by number of endogenous and environmental factors. A decline in carotenoids during leaf senescence has been noticed by some workers (Biswas and Mohanty 1976; Cardini, 1983; Sestak, 1985; Cabello *et al.,* 2006). According to Matile and Martinola (1982), peroxidase is involved in the catabolism of carotenoids during leaf senescence. Mineral deficiency (Murumkar, 1986) and salt stress (Gururaj Rao and Rajeshwar Rao, 1981) are also causes decline in carotenoid contents.

Many researchers have studied carotenoid content in grape because carotenoids are precursors of secondary metabolites which are determinants of wine quality in grape berry tissue (Shultz *et al.,* 1998 Steel and Keller, 2000). According to Razungles *et al.,* (1988) carotenoid contents decreased progressively from the onset of grape berry development to the end of

Table No. 3.16 Concentration of Carotenoids in grape leaves

	Healthy	Infected	Sprayed
Flowering	0.0505	0.0096	0.0253
Fruiting	0.0456	0.0092	0.0266
Harvest	0.041	0.0098	0.0257

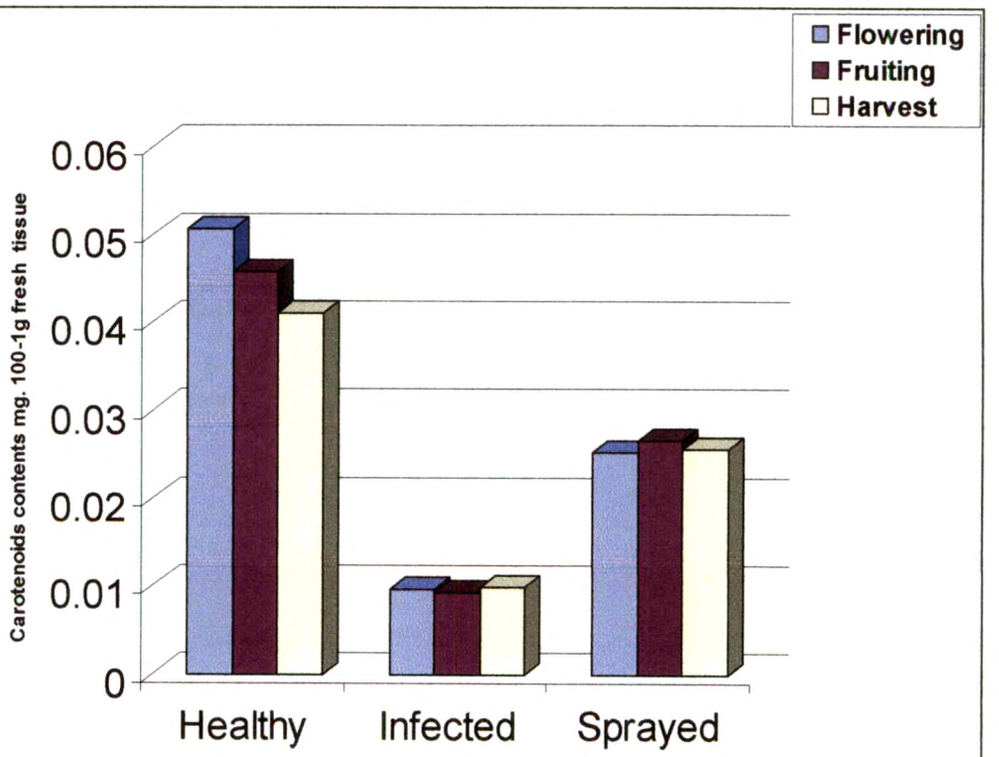


Fig. No:3.16. Carotenoids contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

	Healthy	Infected	Sprayed
Flowering	0.0255	0.0046	0.0287
Fruiting	0.0486	0.0092	0.0235
Harvest	0.041	0.0092	0.0207

maturation, with a sharp decrease during veraison. They observed higher carotenoid content in skin of berry than pulp. UV light causes severe loss of carotenoids in grape leaves (Steel and Keller, 2000).

The effect of some viticultural parameters such as grape cultivar, ripeness stage, sunlight and shade exposure, altitude, and vegetative height on the carotenoid profile was investigated by Oliveira *et al.,* (2004). According to them, carotenoid contents decreases during ripening carotenoid contents were consistently higher in grape exposed to shade than those exposed to direct sunlight in verities like Maria Gomes and Laureira. Low temperatures and high humidity during maturation period appeared to produce grapes with higher carotenoid values.

Grapes gown with higher vegetative height seem to have higher carotenoid levels. Furthermore, grape grown with lower vegetative height had higher weight and sugar concentrations.

The carotenoid content in healthy infected and infected but *A. cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage are recorded in Fig No.3.16. From fig., it is evident that the grape leaves infected with downy mildew and powdery mildew contain lowest carotenoid content as compared to healthy and *A. cantala* leaf extract sprayed leaves It is clear from data that carotenoid content decreased from flowering to harvest stage.

The work of Adelusi and Lawanson (1987), reported decreased carotenoid contents in yams (*Dioscorea* spp.) infected by *Botryodiplodia thiobromae* and *Aspergillus niger*. Naik *et al.,* (1988) have reported decreased carotenoid in betelvine leaves infected with *Colletotrichum.* Similarly, Prasad *et al.,* (1989) reported low concentration of carotenoids in coriander infected by *Protomyces macrosporus*. Fleischmann *et al.,* (2004) in beech (*Fagus sylvatica*) infected by *Phytophthora*, Scarperi *et al.,* (2005) in cocoa infected with Witches' broom, Pfeifhofer (2007) in Norway spruce needles affected by *Chrysomyxsa rhododendri* have reported decrease in carotenoid contents. Baranski *et al.,* (2005) observed changes in carotenoid content and distribution

in living plant tissue using NIR-FT-Raman spectroscopy. They found decrease in carotenoid content at infection site in sugar beet while, the carotenoid accumulation in parsley (*Petroselinum crispum* Mill. Nym.) infected with *Sesptoria petroselini*.

The high concentration of carotenoids in healthy leaves of grapes suggests its susceptibility towards fungal diseases. However, in *A.cantala* leaf extract sprayed grape leaves exhibit low carotenoid contents as compared to healthy leaves. Low carotenoid contents in grape leave show resistance against fungal diseases (Gangopadhay and Chattopadhyay, 1976). From this data, it can be said that disease resistance induced by *A. cantala* leaf extract may minimize carotenoid contents. Hence, the sprayed leaves show reduced development of downy mildew and powdery mildew as well as the fruit formation process may be enhanced.

c. Carbohydrates:-

Carbohydrates are compounds composed of carbon, hydrogen and oxygen. The carbohydrate group includes sugars, starches and cellulose. Sugars and starches provide energy to organisms for cell functions and cellulose for making up plant cell walls.

Carbohydrates are produced in the process of photosynthesis in plants. The carbohydrates produced by plants are an important source of energy for animals. In plants atmospheric CO_2 is converted into triose phosphates from which a whole range of monosaccharides and monosaccharide derivatives are synthesized and these are the precursors from which oligo and polysaccharides are formed. The most important monosaccharides are glucose, fructose and galactose having vital role in life processes. Disaccharides include sucrose, maltose and lactose. Polysoacharides are highly complex carbohydrates include starch, cellulose and glycogen. Starch is usual form in which carbohydrates are stored as energy by plants.

Carbohydrates also play protective role in plants. Sucrose is known to play an osmoregulatory role in many cases. According to Maximov (1929), the

accumulation of sugars protects the protoplasm from coagulation and desiccation and high concentration may prevent visible wilting for a long time inspite of an increasing water deficit.

In both cold and drought hardening, carbohydrates are effective as protective agent (Parker, 1972). In the view of Parker (loc. cit), the sugars bind with protein to form protein sugar complexes and have been found to harden the plant under drought. Vora *et al.,* (1974) have reported the accumulation of sugars in sorghum under water stress indicates a protective role of carbohydrates. The leaves of CAM plant show chloroplasts containing high starch contents Klug and Ting. 1978), suggest its adaptive feature to stress conditions. The work of Karmarkar (1965) in *Bryophyllum pinnatum*, Bartakke (1977) in *Aloe barbadensis* and Karadge (1981) in *Portulaca oleracea* have reported the high starch content. The starch content in *Agave cantala* is also very high in summer conditions (Landge, 1988).

Carbohydrate content leaves are influenced by various endogenous and exogenous factors. The process like senescence causes decrease in photosynthesis rate and consequently carbohydrate assimilation (Karadge, 1981). Plant infected with various pathogens also show increase or decrease in carbohydrate content (Singh, 2005).

The present studies reveal possible role and carbohydrate in controlling the infection status in vine plant when sprayed with Agave leaf extract.

i. Reducing sugars:-

The reducing sugars in healthy, infected and infected but *A. cantala* leaf extract grape leaves at flowering fruiting and harvest stage (cv.Thompson seedless) are recorded in Fig. No.3.17. It is evident from the fig. that healthy leaves of grape contain higher levels of reducing sugars at flowering, fruiting and harvest stage as compared to infected and sprayed leaves. The leaves infected with downy mildew and powdery mildew but *A. cantala* leaf extract sprayed, contain higher levels of reducing sugars as compared to infected

leaves at all three stages. The highest reducing sugars content is noticed in healthy leaves at fruiting stage while lowest at flowering stage.

Various researchers have been reported changes in reducing sugars content in host plant due to infection of MLO, virus and fungus.

The work of Bhargava *et al.,* (1980) has reported increased reducing sugar content in the leaves of brinjal infected with MLO. Similarly, Dhumal (1983) reported increased reducing sugar content in leaves and stalk of sugarcane infected by GSD. While, Prasad and Sahambi (1980) have noticed decreased reducing sugar content in *Sesamum* leaves affected by Sesamum phyllody disease.

The plants affected by virus also show increase or decrease in reducing sugars content. Singh and Singh (1983) have reported increase in reducing sugar content in mungbean infected by mung bean severe mosaic virus. On the contrary, Abu-El-Naser *et al.,* (1980), have reported decreased reducing sugar content in sugarcane and maize due to infection of sugarcane mosaic virus.

Similar trend have been reported in the case of fungal infection. The work of Sankpal and Nimbalkar (1980) in sugarcane infected with smut, Mohapatra (1982) in maize infected by *Sclerospora* and Goel et *al.,* (1983) in *Coriandrum* infected with *Protomyces* have reported increase in reducing sugar content in host. Similar accumulation of reducing sugar in host leaves was observed by Khan *et al., (*2001) in sorghum leaves infected with leaf spot and Scarpari *et al.,* (2005) in cocoa infected by witches' broom.

On the other hand, Garg and Mandahar (1977) in barley infected with *Pyrenophora.* Mandokhot *et al.;* (1979) in maize infected by *Drechslera,* Chahal and Kang (1979) in brown sarson infected by *Alternaria,* Kabsch (1982) in chicory infected by *Alternaria* and Singh and Singh (1983) in guava infected with *Aspergillus* have reported decreased reducing sugar content. Similar reports in lucerne leaves infected with downy mildew (Luthra *et al.,* 1988), mango infected by powdery mildew (Prakash *et al.,* 1989), coriander infected with *Protomyces macrosporus* (Prasad *et al.,* 1989), faba bean infected

Table No. 3.17 Concentration of Reducing sugars in grape leaves

	Healthy	Infected	Sprayed
Flowering	5.58	3.1	4.1
Fruiting	6.78	2.83	3.6
Harvest	6.2	2.7	3.21

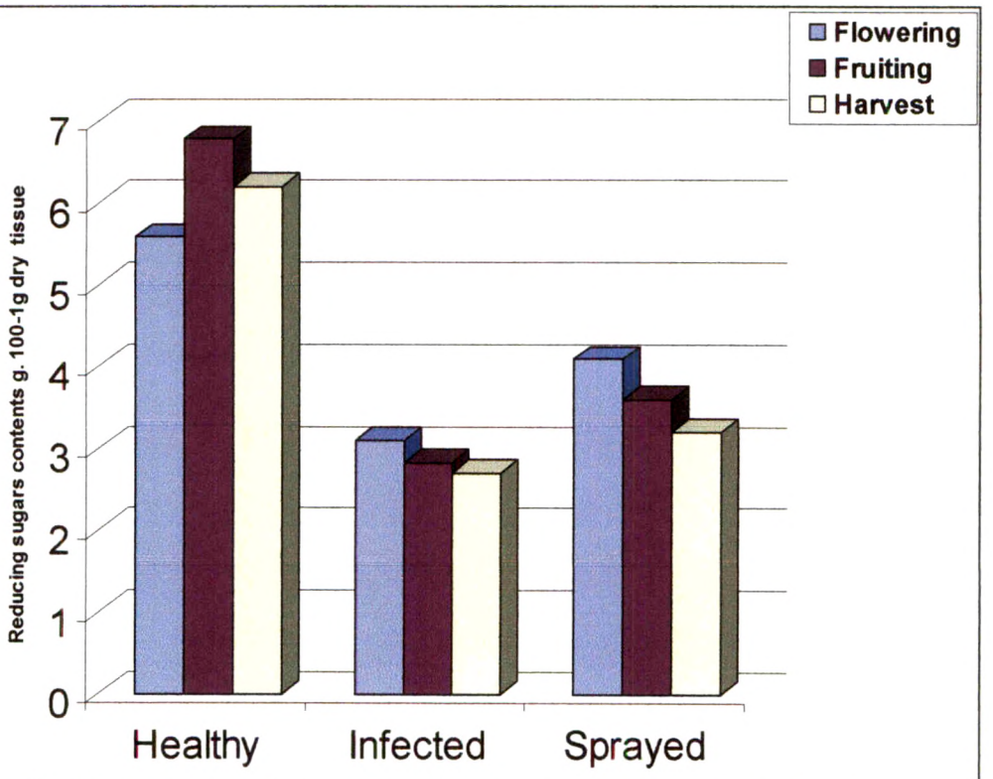


Fig. No:3.17. Reducing sugars contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

with *Botrytis fabae* (Mahmud *et al.,* 2004) and mulberry leaves infected with *Cercospora* (Tang *et al.,* 2005).

The depletion of reducing sugar may be due to increased respiration or utilization of sugars by the host fungi (Lilly and Bernett, 1951) According to Singh and Chohan (1977) fungi secrete some carbohydrate degrading enzymes which cause decline in sugar content in host tissue. The increased respiration and decreased photosynthesis in infected leaves, decrease carbohydrate content (Garg and Mandahar, 1976).

The high reducing sugars content in host suggest it's susceptibility towards fungal infection (Bhandari and Singh, 1976). According to Horsefall and Dimond (1957), Sohi and Rawal (1977), Gangopadhyay and Chattopadhyay (1977), Gupta and Chatarath (1979), Marimuthu and Kandaswamy (1981), Dhumal (1983), the total sugar content and phenol concentration in leaves are inversely proportional. The concentration of polyphenols in healthy grape leaves is low as compared to sprayed leaves. So, it can be said that *Agave cantala* leaf extract minimize activity of invertase, synthesis of reducing sugars and accelerates synthesis of polyphenols in infected leaves. In this way, Agave leaf extract inhibits the fungal growth and development after spray. Thus, present observations support the role sugar in infected leaves of vine plant

ii. Total sugars:-

The total sugars contents in healthy infected and infected but *A. cantala* leaf extract sprayed grape leaves of cultivar Thompson seedless at flowering fruiting and harvest stage are depicted in Fig. No.3.18. The total sugar contents are maximum in the healthy leaves in all three stages. While, lowest total sugar contents is noticed in grape leaves infected with downy mildew and powdary mildew. The level of total sugar content in *A. cantala* leaf extract sprayed grape leaves is higher than infected leaves. The highest total sugars contents are noticed at fruiting stage in healthy leaves while, lowest at harvest stage.

The changes in total sugar content in infected plant was observed by many workers, Srinivasan and Chelliah (1979) noticed accumulation of total

sugars in brinjal leaves affected by MLO. On the other hand, Prasad and Sahambi (1980) and Dhumal (1983) have reported decreased total sugar content in *Sesamum* infected with phyllody disease and sugarcane infected with GSD respectively.

Similar conflicting findings have been reported on the effects of viral infection on total sugars content of the host. Singh and Singh (1983) have reported high total sugars content in beans affected by yellow mosaic virus. On the other hand, Thind *et al.,* (1989) have reported decrease in total sugars content in summer moong infected with mung bean yellow mosaic virus and leaf crinkle virus.

The effect of fungal infection on total sugars content was observed by many researchers. The increase in total sugars content in barley infected with *Erysiphe* (Hwang *et al.,* 1983), lucerne leaves infected with *Alternaria* (Bhargava and Khare, 1988). While, decrease in total sugars content was noticed in sugarcane infected by *Cercospora* (Sankpal and Nimbalkar, 1980), guava infected by *Aspergillus* (Singh and Singh 1983), *Coriandrum* infected by Protomys (Goel *et al.,* 1983), sugarcane infected by *Ustilago* (Padmanaban *et al.,* 1988), betelvine infected with *Colletotrichum* (Naik *et al.,* 1988), mango infected with powdery mildew (Prakash *et al.,* 1989). Grape infected by *Sphacelona ampalina* (Dhillon *et al.,* 1989), coriander infected with *Protomyces* macrosporus (Prasad et al., 1989), faba bean infected with *Botrytis fabae* (Mahmud *et al.,* 2004) and mulberry infected with *Cercospora moricola* (Tang *et al.,* 2005)

The low level of total sugars content in grape leaves infected with downy mildew and powdery mildew might be due to increased activity of invertase. The work of Ghorpade and Joshi (1980), Sankpal and Nimbalkar (1979), Chen and Hou (1981), Mitchell (1982), Dhumal (1983), Prasad et al., (1989) supported above view that increased activity of invertase decreases total sugar content in infected leaves. Moreover, fungal infection also causes higher transpiration rate accompanied with low photosynthesis, resulted into low total sugars content in host tissue (Allen, 1942; Hopkins and Hampton, 1969;

Table No. 3.18 Concentration of Total sugars in grape leaves

	Healthy	Infected	Sprayed
Flowering	7.4	5.15	6.09
Fruiting	8.49	4.8	5.67
Harvest	8.4	4.5	5.4

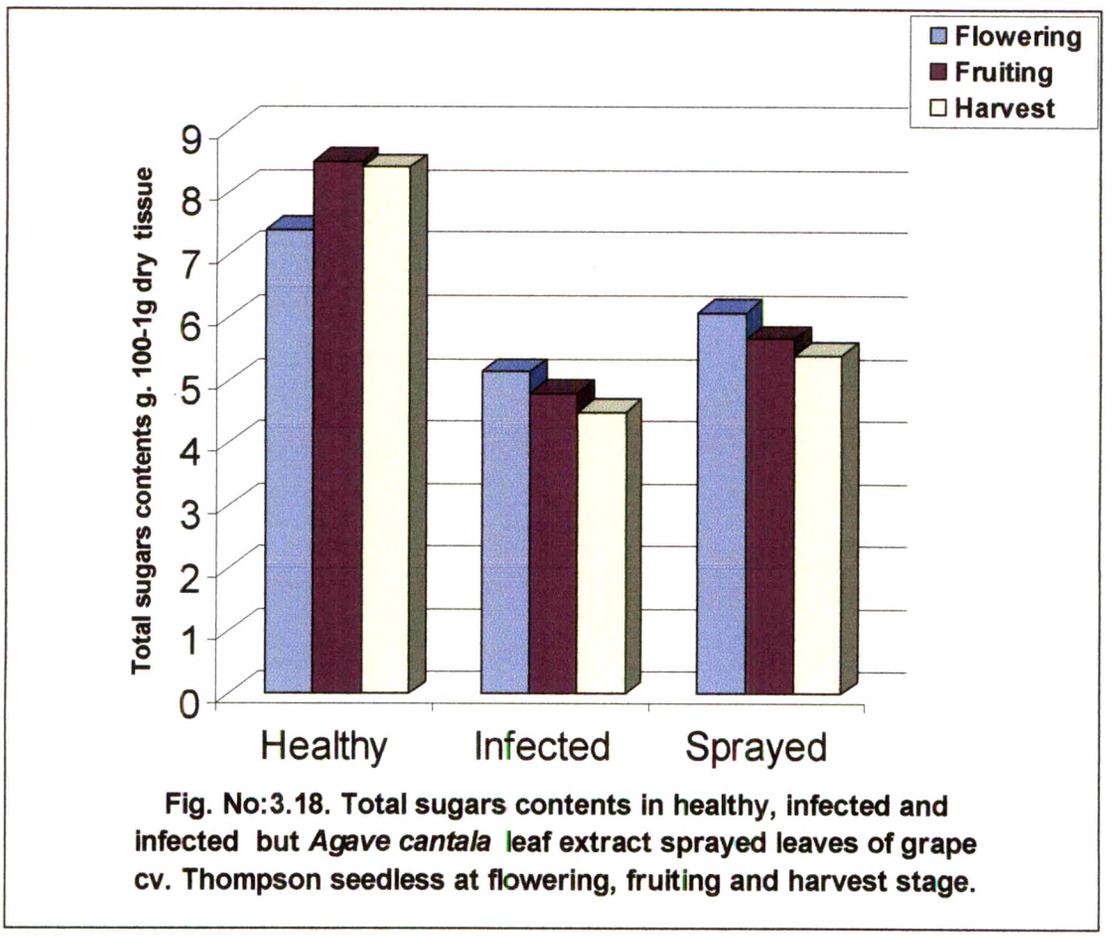


Fig. No:3.18. Total sugars contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

Jayarajan and Ramakrishnan, 1968; Mandahar and Garg, 1973; Dhumal, 1983). Low photosynthetic pigments noticed in infected grape leaves which suggest reduced synthesis of sugars due to low photosynthesis.

The higher level of total sugars content is noticed in infected but *A. cantala* leaves extract sprayed grape leaves as compared to untreated infected leaves. The higher chlorophyll content and low invertase activity supports the high total sugars content in sprayed leaves and exhibit disease inhibition due to *A. cantala* leaf extract spray.

The overall carbohydrate status in the infected leaves and sprayed leaves as compared to normal grape plant suggest that the after incidence of disease the level of carbohydrate changes i.e. reduced due to pathogens activity where its utilization may occur, however in Agave leaf extract sprayed plant sugar status is maintained due to inhibition of pathogens activity in the plants.

iii. Starch:-

The role of carbohydrates in plant disease resistance is an important aspect. The starch content in healthy, infected and infected but *A. cantala* leaf extract sprayed leaves of grape cultivar Thompson seedless at flowering, fruiting and harvest stage are recorded in Fig. No.3.19. The starch contents are maximum in sprayed leaves at all three stages as compared to healthy and infected leaves. In the infected leaves a marked decline in starch contents is noticed at all three stages. The highest level of starch content is noticed in sprayed leaves at flowering stage. While, lowest starch content is noticed in infected leaves at harvest stage.

The change in starch contents due to infection of MLO was observed by many researchers. Parthasarathi *et al.,* (1977) and Parthasarathi (1977) have reported increase in starch contents in sandal leaves infected by spike disease. While, Dhumal (1983) reported severe decline in starch content in sugarcane due to grassy shoot disease (GSD).

Similarly, viral diseases also cause variation in starch of host. Sugarcane affected by SCMV and chlorotic leaf spot virus show accumulation of starch

(Sreenivasulu and Nayudu, 1980). While, mung bean (*Vigna radiata*) infected by MBMV (Singh and Singh 1979), mung bean infected by yellow mosaic and leaf crinckle virus (Thind *et al.,* 1989) show markedly decline in starch contents.

Like MLO and viral diseases, fungal diseases also causes increase as well as decrease in starch contents of host. Sugarcane infected by smut (Sankpal and Nimbalkar, 1979), barley infected by *Erysiphe* (Hwang *et al.,* 1983), potato infected by *Synchytrium* (Santra, 1983), cocoa infected by witches' broom (Scarpari *et al.,* 2005) show increase in starch contents. On the other hand, bean infected with powdery mildew (Vidhysekaran and Kandaswamy 1972), barley infected by netblotch disease (Garg and Mandahar, 1977), maize leaves infected by *Sclerospora* (Chen and Hou, 1981) pear-millet infected with downy mildew (Mogle and Mayee, 1981b), *Coriandrum* infected by *Protomyces* (Goel *et al.,* 1983; Prasad *et al.,*1989), faba bean infected with *Botrytis fabae* (Mahmud *et al.,* 2004), mulberry leaves infected with *Cercospora* (Tang *et al.,* 2005) show decrease in starch contents.

In present investigation, starch content in grape leaves infected with downy mildew and powdary mildew decreased with disease development. This decrease in starch content has been related with the activation of starch hydrolyzing enzyme-β-amylase (Schipper and Mirocha, 1968; Vidhysekaran and Kandaswamy, 1972) which converts starch into sugar. However, in infected leaves of grape, activity of enzyme invertase is accelerated as compared to healthy and sprayed leaves. The increase in activity of enzyme invertase in infected host leaves suggest rapid cleavage of starch into glucose and fructose (Gibeaut *et al.,* 1990). Hence, the growth and development of pathogen accelerated by utilizing available reducing sugars. The low reducing sugar contents and increased activity of enzyme invertase supports the decreased starch contents in infected grape leaves. It is observed by many authors that high reducing sugars containing host has been susceptible to pathogens (Luthra *et al.,* 1988; Bhargava and Khare, 1988; Sugha and Singh, 1990; Sharma *et al.,* 1992). The grape cultivar Thompson seedless is

Table No. 3.19 Concentration of Starch in grape leaves

	Healthy	Infected	Sprayed
Flowering	4.8	4.19	7.12
Fruiting	5.13	4.09	6.78
Harvest	5.09	4.05	6.6

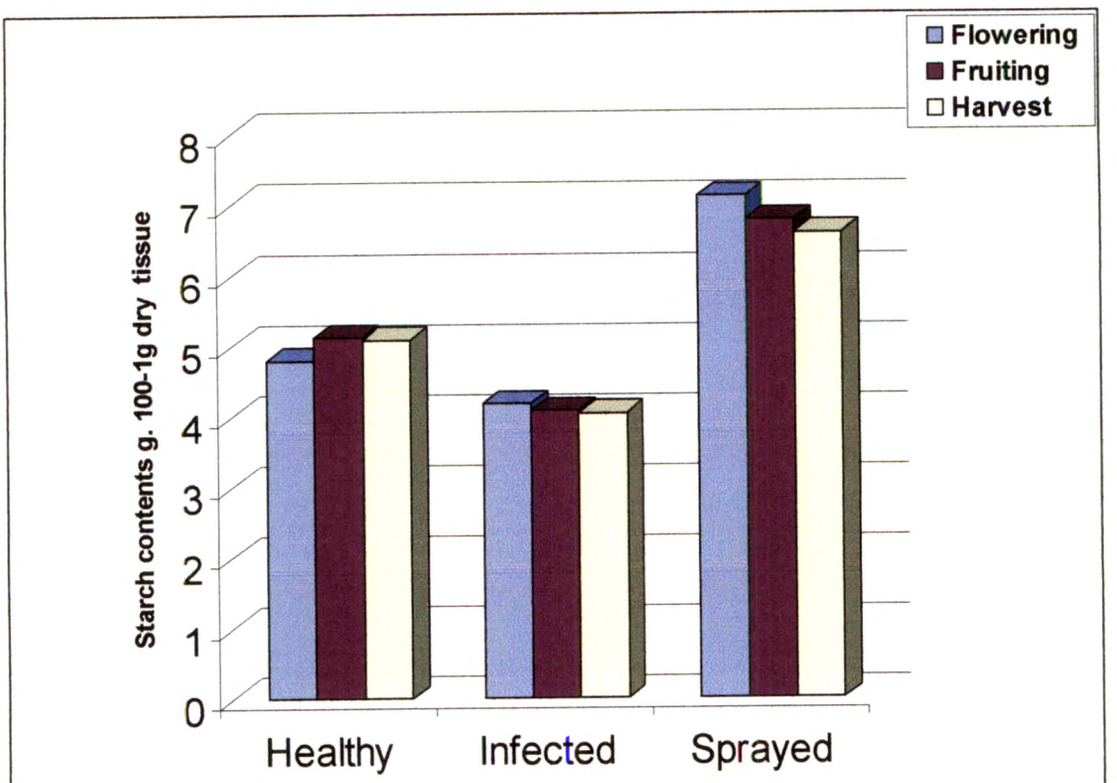
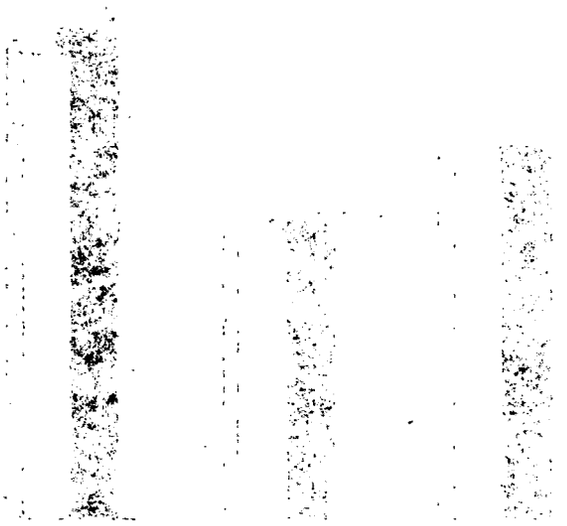


Fig. No:3.19. Starch contents in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.



susceptible to fungal diseases, may be due to high reducing sugar content in normal healthy leaves.

The starch contents increased in infected grape leaves after *A.cantala* leaf extract treatment. The increase in starch contents may be due to decreased activity of enzyme invertase (Fig No.3.23). The decreased invertase activity ultimately reduces production of reducing sugars, result into inhibition of utilization of reducing sugars by pathogens. Hence, the growth and development of pathogen inhibited by *A .cantala* leaf extract spray. The increased status of starch at three stages of presents studies suggest that in diseased plant, reduction in starch contents than healthy plants appears. However, the Agave leaf extract spray may control this decrease and maintained the starch level in increased status.

d. Total Polyphenols:-

Polyphenols are products of secondary metabolism in plants. These compounds have aromatic ring in their structures. Polyphenols is a group, having large variety of aromatic substances like tannins and betalins, anthocyanins, leucoantocyanins and anthoxanthins, hydroxyl benzoid acids, glycosides, flovonoids, sugar esters of quinine, shikkimic acid esters and coumarine derivatives. Although these compounds exhibit a wide array of structures, basically all compounds except flavonoids arise from a common biosynthetic intermediate, phenylalanine or its close precursor shikkimic acids. Flavonoids have one aromatic ring and its C_3 side chain arises from phenylalanine while the other arises from acetyl-CoA via the polyketide pathway.

Phenols are divided into following classes: simple phenols, phenolic acids, phenyl acetic acid, hydroxycinnamic acid, Cumarins and Isocumarins Naphthoquinones, Xanthones, Stilbenes and Anthraquinones, Ligans and Neoligans, Biflavonoid Ligins, Melanins, condensed tannins (Flavolons) (Goodwin and Mereer, 1983)

Flovonon, in particular hesperotince and naringenin are found in orange juice and grape fruit juice respectively. Citrus fruits present a broad range of flavonoids (4-oxo-flavonoids sometimes called citrus-flavonoids) which content is very high in juices. Anthocyanin are found especially in red fruit berries such as black berries, black currants, blue berries) and black grape, strawberries and raspberries. Polyphenols present in these fruits are found in processed fruits and vegetables and especially in juices (Lecerf,2006).

Polyphenols play an important role in plant metabolism. According to Rice (1979), polyphenols influences fundamental plant processes such as photosynthesis, chlorophyll production and plant water relations. It also takes part in protein synthesis (Dank *et al.,* 1975), respiration (Demos *et al.,* 1975) and membrane permeability (Glass and Dunlap, 1974). Kefeli reported the regulatory property of some polyphenols and its ability to cause leaf abscission. Some phenolics like lignin have a definite structural role in plants. According to Sharma *et al.,* (1988), phenols are involved in stomatal movements.

Polyphenols are a part of the complex immune system, which can be acquired in tissues under stress (Feucht, 1994). Contrary to animals, plants cannot synthesize antibodies for defense but can produce numberous phenolic substances - phytoalexins which can inhibit and kill pathogenic organisms (Bennett and Wallsgrove, 1994). Phenolics are known to inhibit the feeding of many insects and have demonstrated toxicity (Grayer *et al.,* 1992).

The involvement of phenols in plant defense resistance is based to a large extent on their cytotoxicity, which is associated with their oxidation products (Aver' yonav and Lapikova, 1994). Phenolics consist of such compounds as condensed tannins, flavonoids, phenyl propyl etc. Flavonoids are fairly well distributed in the plant kingdom (Hermann, 1988). They are known to possess insecticidal and antimicrobial activity (Barberan *et al.,* 1988).

It has been proposed that the first stage of the defense mechanism of plants involves a rapid accumulation of phenols at the infection, which function to slow down the growth of pathogens. Polyphenols play a vital role in the growth and propagation of plants and protect plant form damage. A number of

Table No. 3.20 Concentration of Total Polyphenols in grape leaves

	Healthy	Infected	Sprayed
Flowering	0.4	0.6	0.97
Fruiting	0.45	0.67	1.1
Harvest	0.48	0.72	1.2

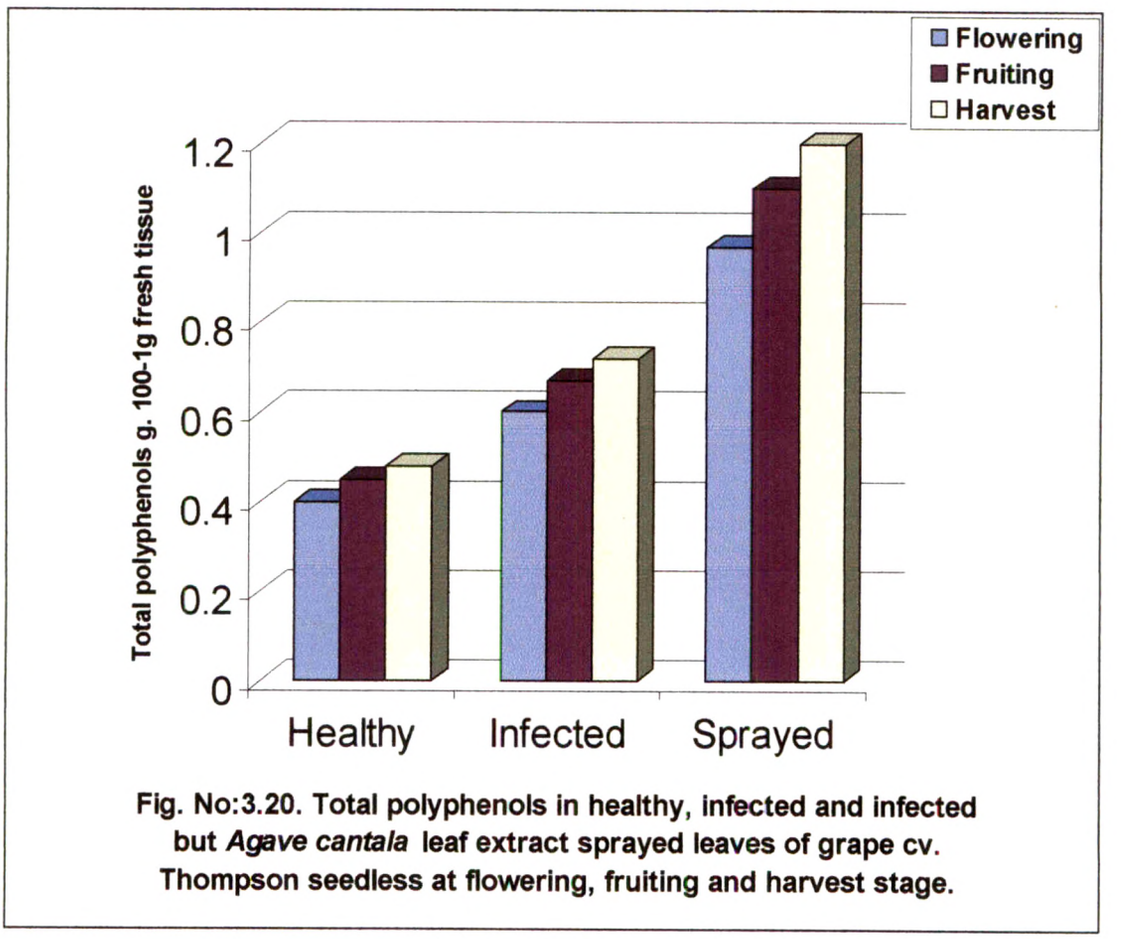


Fig. No:3.20. Total polyphenols in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

phenols are regarded as preinfection inhibitors, providing plant with a certain degree of basic resistance against pathogenic microorganisms (Satisha *et al.*, 2008).

The grapevine infected due to downy mildew disease also show variation on in polyphenol content depending up on disease incidence and development pathogen in the host plant. Present studies on effect of Agave leaf extract treatment in controlling downy mildew's development in infected plant above shows interesting observations in polyphenol content as follows. The total polyphenol content in healthy, infected and infected but *A. cantala* leaf extract sprayed grape leaves of cultivar Thompson seedless at flowering, fruiting and harvest stage are recorded in Fig. No.3.20. It is evident form the figure that the sprayed leaves of grape contain higher level of total polyphenols as compared to healthy and infected leaves. The highest total polyphenol content (1·2 g. 100^{-1} g fresh tissues) noticed at fruiting stage in *A. cantala* leaf extract sprayed grape leaves. While, lowest total polyphenol content (0·4 g. 100^{-1}g fresh tissues) is noticed in healthy leaves at flowering stage. The total polyphenol contents in leaves infected with downy mildew and powdery mildew are higher than healthy leaves at all three stages. Thus higher polyphenol contents in different stages of host development than the normal healthy plants suggest some possible controlling role in disease incidence.

Our results can also tally the observation of different workers in connection with disease development and variation in polyphenol contents. Such as plants affected by MLO show changes in polyphenol contents (Arya *et al,.* 1981). Similarly, viral infections to host plant also cause variation in total polyphenols (Thind *et al.,* 1989).

Plant infected with fungus show increase or decrease in phenol contents. The work of Arya *et al.,* (1981) in pearl-millet infected by *Sclerospora*, Vijayakumar and Rao (1980) in wheat infected with *Alternaria*, Sankpal and Nimbalkar (1980) in areca nut palm infected by *Ganoderma*, Tayal *et al.,* (1981) in phoenix affected by false smut, Agarwal *et al.,* (1982) in turmeric infected with *Taphrina*, Sharma *et al.,* (1983) in maize infected with leaf blight,

Singh and Singh (1983) in guava infected by *Aspergillus*, Padmanaban *et al.*, (1988) in sugarcane infected by *Ustilago*, Bhargava and Khare (1988) in chickpea infected with *Alternaria*, Gupta *et al.*, (1990) in mustard infected by *Alternaria*, Sharma *et al.*, (1992) in maize affected by *Turcicum*, Gupta *et al.*, (1992) in ground nut infected by leaf spot, Sugha *et al.*, (1992) in onion leaves infected by *Perenospora*, Dai *et al.*, (1995) in grape infected with downy mildew Khan *et al.*, (2001) in sorghum infected with *Drechslera*, Tang *et al.*, (2005) in mulberry infected by *Cercospora*, Scarpari *et al.*, (2005) in cocoa affected by *Crinipellis*, Satisha *et al.*, (2008) in grape infected by powdary mildew reported increase in phenol contents. On the other hand, the observations made by Santhakumari and Nair (1981) in hydrangea infected by *Colletotrichum*, Dhingra *et al.*, (1982) in Brassica infected by *Albugo*, Naik *et al.*, (1988) in betel vine infected with *Colletotrichum*, Prakash *et al.*, (1989) mango infected by powdery mildew, Dhillon *et al.*, (1989) in grape infected by *Sphaceloma* have reported decrease in phenol contents.

Phenols play a key role in disease resistance. Alteration of phenol metabolism following infection has been observed in many diseases (Farkas and Kiraly 1962; Jaypal and Mahadevan, 1968; Biswas and Purkayastha, 1988). The accumulation of oxidized phenolic compounds in plants, which are toxic to certain pathogens are believed to take part in plant defense reactions (Cardoso and Garraway, 1991; Matta *et al.*, 1969; Dimond, 1970; Satisha *et al.*, 2008) Peroxidase and polyphenol oxidase are capable of oxidizing phenols to quinones (Chattopadhyay and Bera 1980; Iwata *et al.*, 1982; Mansifield; 1983) and phenylalanine ammonia lyase is an intermediate enzyme (Burell and Rees, 1974) involved in the production of phenolic phytoalexines, namely pisatin and phaseolin. All these are associated with disease resistance of plants (Hardwiger and Hess, 1970; Rathmall, 1973; Dai *et al.*, 1995).

The grape cultivar Thompson seedless is susceptible to fungal diseases like downy mildew due low polyphenol content than wild, highly resistant variety like Mango (Kedge, 2008). Hence, in humid condition and at low temperature the grape cultivar Thompson seedless is easily attacked by fungal

diseases like downy mildew and powdery mildew. The polyphenol content in *A. cantala* leaf extract sprayed grape leaves after infection of downy mildew powdery mildew are very important with respect to disease resistance. The high polyphenol contents in infected leaves after Agave leaf extract spray may be due to increased activity of enzyme polyphenol oxidase, peroxidase and high concentration of copper.

The negative correlation between grape leaves infected with downy mildew powdary mildew and phenolic compounds was noticed by Kedge (2008) and Satisha *et al.,* (2008). According to Kedge (2008), the grape cultivar containing high polyphenols show greater resistance against downy mildew. Satisha *et al.,* (loc. cit.), also reported high resistance of grape against powdary mildew due to high phenol contents.

The increase in total polyphenol content in infected leaves after A. cantala leaf extract spray will enhances disease resistance and reduces disease development in different stages of host and pathogens. The enzyme studies above supports these observations of present work.

C) Enzymes

a. Catalase :- **(E.C.1.11.1.6**; Hydrogen Peroxide: hydrogen peroxide oxidoreductase).

Catalase is one of the main enzymes playing a role in the catabolism of hydrogen peroxide (Chance *et al.,* 1979). The catalase is a tetrameric heme protein occurring in almost all aerobic organisms. This enzyme is one of the few enzymes that exhibit dual enzyme activity. It has hyperoxide activity (Catalytic activity) when catalyzes the dismutation of hydrogen peroxide into water and oxygen:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O+O_2$$

The other catalase activity is peroxidase activity (Peroxidative acivity) when the substrates are one molecule of hydrogen peroxide and one molecule of hydrogen donor.

$$\text{donor} + H_2O_2 \xrightarrow{\text{Catalase}} \text{oxidized donor} + 2H_2O$$

The enzyme with catalase activity is present as multiple isoforms in plants and recent research on catalase cDNA clones showed that catalases exist as small gene families (Scandalios, 1994). Plant catalases are predominantly peroxysmal enzymes and most of them contain a carboxy terminal consensus sequence for peroxisomal import (Gould *et al.,* 1988). Catalalases play a role of specific peroxidase and their function is to protect cells from toxic effects of hydrogen peroxide.

The hydrogen peroxide (H_2O_2) generation is promoted due to biotic and abiotic factors such as drought, wounding, pathogenesis, phytochrome such as ABA, high temperature, excess excitation energy, ozone exposure and UV-radiations (Lebeda *et al.,* 1999, 2001; Neill *et al.,* 2002).

The changes in the activity of enzyme catalase in healthy, infected and infected but *A.cantala* leaf extract sprayed grape leaves of cultivar Thompson seedless at flowering, fruiting and harvest stage is shown in Fig. No...3.21.. It is evident from the figure that the infected leaves have the highest enzyme activity as compared to healthy and sprayed leaves. While, the grape leaves infected with downy mildew and powdery mildew show decrease in enzyme activity after *A.cantala* leaf extract spray.

The effect of infection on activity of catalase was reported by many researchers. Dhumal (1983) reported decrease in activity of enzyme catalase in sugarcane leaves affected by GSD. Prasad (1986) and Verma and Prasad., (1988) have reported greater activity of catalase in tobacco after viral infection. While, Singh (1983) reported low catalase activity in pumpkin infected with water melon mosaic virus. Low activity of enzyme catalase due to fungal infection was noticed by Serovova (1961) in *Cirsium* infected by *Puccinia* and Montalbini and Marte (1972) in beans infected by *Uromyces.*

High activity of enzyme catalase due to fungal infection was reported by several authors. Rubin and Chetverikova (1955) in cabbage infected with *Botrytis*, Uritani and Akazawa (1959) in sweet potato infected with *Ceratostomella*, Sathiyanathan and Vidhyasekaran (1981) in coriander infected with *Protomyces*, Gupta *et al.,* (1990) in mustard infected with *Alternaria*, Rao

Table No. 3.21 Activity of enzyme catalase in grape leaves

	Healthy	Infected	Sprayed
Flowering	0.0105	0.28	0.0668
Fruiting	0.0112	0.395	0.0737
Harvest	0.0131	0.405	0.0789

Enzyme activity expressed as Δ OD min^{-1} mg^{-1} sol. protein

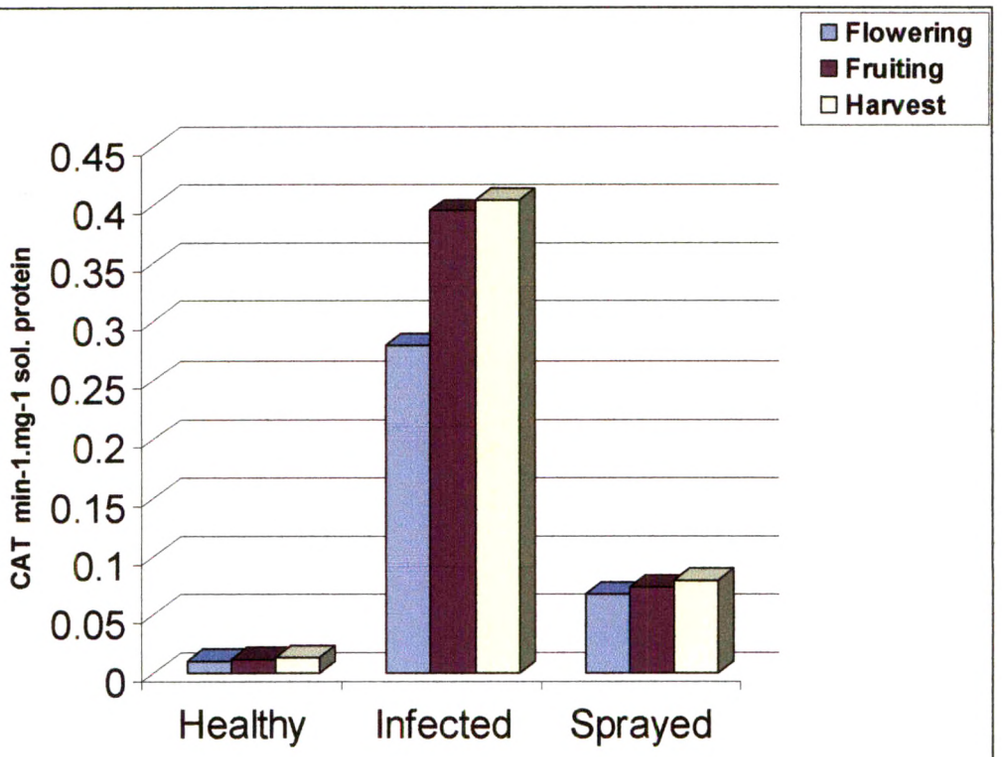


Fig. No:3.21.Catalase activity in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

et al., (1990) in raddish infected with *Aspergillus*. Thordal-Cristense *et al.,* (1997) in barley infected with powdary mildew and Mlickova *et al.,* (2004) in *Lycopersicon* infected with *Oidium*, have reported greater activity of enzyme catalase in host tissue.

The increase in catalase activity in grape leaves infected with powdary mildew and downy mildew is due to the increased concentration of H_2O_2 in tissues. Catalase plays a protective role by scavenging this hydrogen peroxide radical and protects cells from toxic effect of it (Luhova *et al.,* 2003). According to Bonner (1950) and Sasikumaran *et al.,* (1979), a change in catalase activity after infection was due to increased respiration at infection site. This enhancement is due to the respiration of fungus itself, which is more intense than that of the plant. Low chlorophyll contents in infected leaves are also reason behind the increased activity of catalase (Dekock *et al.,* 1960; Joshi and Dubey, 1977; Dhumal, 1983).

The activity of enzyme catalase in infected but *A.cantala* leaf sprayed grape leaves is low than infected leaves. The reason behind the decrease in catalase activity is reduced respiration and H_2O_2 production. However, according to Cohen and Schiffmann-Nadel (1976), decrease in catalase activity is related to the increased H_2O_2. They further suggest that increased H_2O_2 concentration at the infection site may be responsible for the death of fungus *Phytophthora* of lemon fruit. Hence, it is possible that low activity of catalase in grape leaves infected with downy mildew is due to increased concentration of H_2O_2 after *A. cantala* leaf extract spray. The fungal activities may affect over all mechanism of hest plant for their nutritional purposes. This is one of the reasons of increased activity of catalase during flowering, fruiting and harvest stages in infected grape leaves. However, the Agave leaf extract may control fungal activity due to which in sprayed plants again decreased in enzyme activities is observed.

Thus, the present work suggest catalase activity status may be controlled due to some fungicidal properties present in Agave leaf extract which manifest the disease development in grape at above three stages of the growth.

2) Peroxidase: - (E.C.1.11.1.7) [donor: hydrogen peroxide oxidoreductase]

Peroxidase is an oxidative enzyme. Plant peroxidases are monomeric heme-containing enzymes that are usually glycosylated and that catalyze a large variety of reactions (Siegel, 1993). According to Putter (1974), peroxidases from plant sources exhibit a very broad range of substrate specificity and catalyze the oxidation of cellular components such as $NADH_2$, ascorbic acid, ferrocytochrome c, aromatic amines and phenolic substances etc.

$$\text{donor} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{oxidized donor} + 2H_2O$$

The oxidative decarboxylation of amino acids like serine, alanine, methionine, phenylanine and tryptophan take place through the action of peroxidase (Mazelis and Ingraham, 1962). Peroxidases have been studied for their important role in lignification and suberization, for their active participation in the formation of diphenyl bridges, cross-linking of hydroxyproline-rich proteins (extensin) in the cell wall matrix and for their control function of redox state in the apoplast. The involvement of peroxidases in stress related physiological processes is well demonstrated (Low and Merida, 1996).

Peroxidase also play an important role in growth and development of plants by controlling auxin catalism (Ray, 1962), H_2O_2 formation (Gross *et al.*, 1977) and lignin and ethylene biosynthesis (Lieberman, 1979). Peroxidase is involved in plant-pathogen interactions (Montalbini *et al.*, 1995; Wojtaszek, 1997). The role of peroxidase in disease resistance was noted by Simmons and Ross (1970).

The changes in the activity of enzyme peroxidase in healthy, infected and infected but *A. cantala* leaf extract sprayed grape leaves at flowering, fruiting and harvest stage is shown in fig. No.3.22. From fig., it is clear that the *A. cantala* leaves extract sprayed grape leaves have the highest enzyme activity as compared to healthy and infected leaves. The leaves infected with downy mildew and powdery mildew has moderately high enzyme activity as compared to healthy leaves.

Table No. 3.22 Activity of enzyme Peroxidase in grape leaves

	Healthy	Infected	Sprayed
Flowering	0.83	0.89	1.201
Fruiting	0.835	0.898	1.219
Harvest	0.86	0.892	1.24

Enzyme activity expressed as Δ OD hr^{-1} mg^{-1} sol. protein

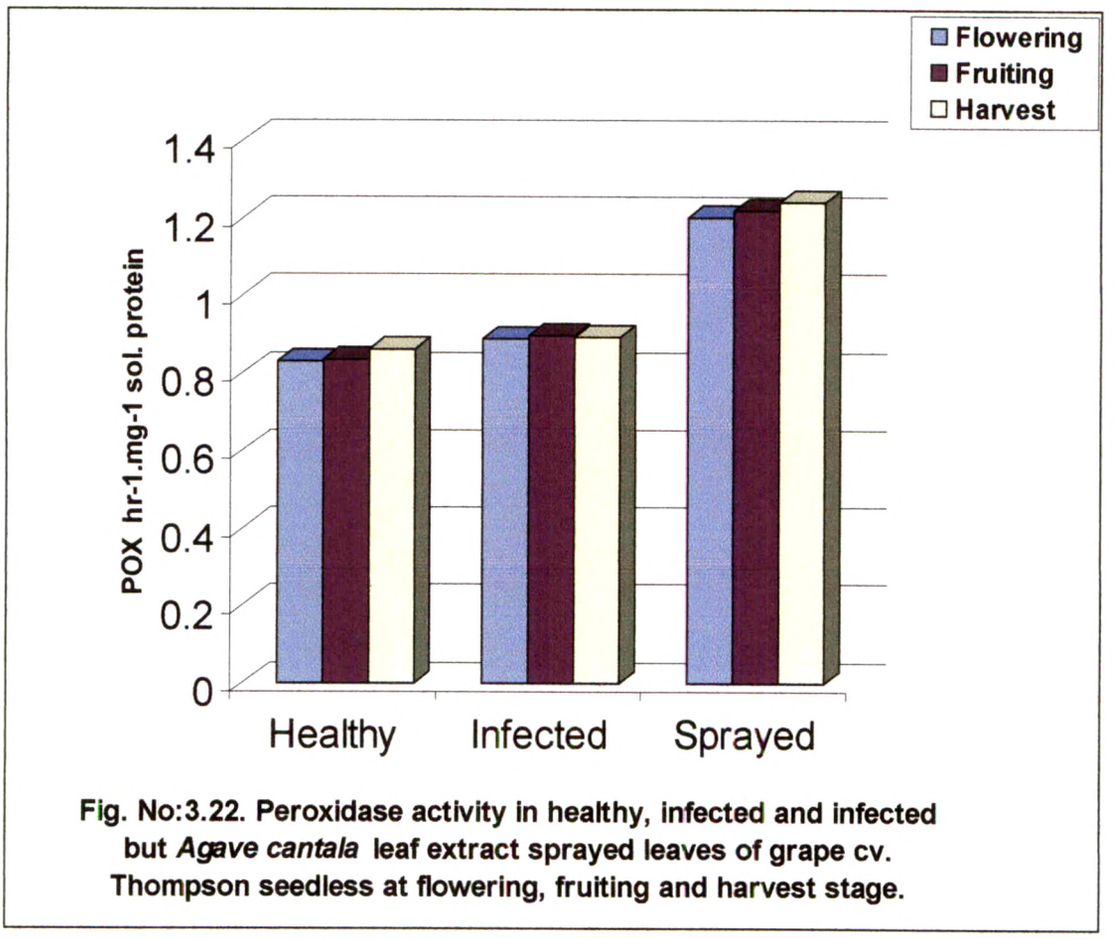


Fig. No:3.22. Peroxidase activity in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

The variation in activity of enzyme peroxidase is noticed due to infection of different pathogens. Sugarcane leaves infected with GSD show increase in acitivity of enzyme peroxidase as compared to healthy leaves (Dhumal, 1983). While, brinjal infected by little leaf disease (MLO) show low activity of peroxidase (Sarkar and Joshi, 1977). Similar trend of activity of peroxidase is noticed in case of viral infection. Simmons and Ross (1970), Prasad (1986) and Verma and Prasad (1988) in tobacco infected with lettuce mosaic virus have reported increase in activity of enzyme peroxidase. On the contrary, Narayanswamy and Ramakrishna (1966) reported low activity of enzyme peroxides in pigeon pea infected with sterility mosaic virus.

In case of fungal infection the activity of enzyme pervoxidase is either increased of decreased. Vidhyasekaran (1979) noticed low activity of peroxidase in finger-millet infected with *Helminthosporium*. On the contrary, Purohit *et al.,* (1980) in *Achyanthus* infected with while rust, Leson *et al.,* (1981) in wheat infected with brown rust, Kalyuzhnii and Bogdan (1981) in apple infected with powdary mildew, Arinze and smith (1982) in sweet potato root infected by *Colletotrichum*, Hammer-Schmidt *et al.,* (1982) in cucumber infected by *Colletotrichum,* Agarwal *et al.,* (1982) in turmeric infected with *Taphrina* observed low activity of peroxidase.

Goel *et al.,* (1983) in *Coriandrum* infected by *Protomyces,* Arora and Wagle (1985) in wheat infected with loose smut, Reddy and Reddy (1988) in Coccinia infected with *Rhizoctonia*, Dohroo (1989) in coriander infected with *Protomyces*, Gupta *et al.,* (1990) in mustard infected with *Alternaria*, Rao *et al.,* (1990) in radish infected with leaf spot, Kortekamp and Wind (1998) in grape infected with downy mildew, Khan *et al.,* (2001) in sorghum infected with *Drechslera*, Chakraborty *et al.,* (2002) in tea infected with *Exobasidium*, Mlickova *et al.,* (2004) in *Lycoperiscon* infected with Oidium, Nagy *et al.,* (2004) in Norway spruce infected with *Rhizoctonia*, Honty *et al.,* (2005) in pear fruit infected with *Eriwinia*, Parashar and Lodha (2007), in *Foeniculum* infected with *Ramularia* blight, Srivastava (2008) in *Brassica* infected with

Marcrophomina, Anjana *et al.*,(2008) in sunflower infected with *Alternaria* have reported high activity of peroxidase in host plant.

The activity of enzyme peroxidase is increased in grape leaves infected with downy mildew. According to Sasikumaran *et al.*, (1971) and Rao *et al.*, (1990) increase in activity of peroxidase is due to enhanced respiration. Low chlorophyll contents are also one of the reasons behind the increase in activity of peroxidase (Schwarze, 1954; Lele and Mukherji, 1979; Suseno and Hampton, 1966).

The high activity of enzyme peroxidase is noticed in *A. cantala* leaf extract sprayed grape leaves.Results also suggested that downy infectionto grape leaves increases peroxidase activity than healthy plant to some extent but spraying with Agave leaf extract show considerable increase in enzyme activity. It is possible that it may be a protective asset offered by Agave leaf extract to grape plant in controlling the disease development. Thus, peroxidase is involved in disease resistance. It is involved in oxidization of phenols to quinines along with polyphenol oxidase (Mansifield, 1983). It is highly active in resistant variety of crops (Rathi *et al.*, 1986; Verma and Prasad, 1988; Honty *et al.*, 2005). This view is supported by Martinez *et al.*, (1996). Srivastava (2008) reported involvement of peroxidase and polyphenol oxidase in disease resistance. According to him, resistant cultivars of *Brassica juncea* show greater activity of peroxidase than susceptible after infections of *Macrophomina.* Anjana *et al.*, (2008) reported higher peroxidase activity in resistant cultivars of sunflower against *Alternaria* infection. Similarly, Kortekamp and Wind, (1998) have reported high peroxidase activity in resistant cultivar of grape against downy mildew infection.

Hence, the slight increase in peroxidase activity in grape leaves after infection of downy mildew suggest moderate resistance exhibited by host and confirms the susceptibility of cultivar Thompson seedless.

The increase in peroxidase activity in infected leaves after *A. cantala* leaf extract spray suggests induction of more effective defense system by production of quinines, which play active role in disease resistance. Higher

latent form on the thylakoid membrane and is not involved in synthesis of phenolic compounds in leucoplasts, protoplastids or amyloplasts. PPO is often present in a latent form in rudimentary thylakoid. iii) It is normally function as a phenol oxidase in vivo only in senescent or damaged cells. iv)In the functional chloroplast, PPO may be involved in some aspect of oxygen chemistry - perhaps mediation of pseudocyclic photophosphorylation (Vaughn and Duke, 2006).

The importance of PPO in disease resistance in plants is considered during present investigation. The enzyme is studied for its activities in healthy, infected and Agave leaf extract sprayed plant leaves. The changes in the activity of enzyme PPO in healthy, infected and infected but *A.cantala* leaf extract sprayed grape leaves of cultivar Thompson seedless at flowering, fruiting and harvest stage is recorded in Fig. No.3.23. From fig. it is clear that the sprayed leaves have highest enzyme activity than healthy and infected leaves. The leaves infected with downy mildew exhibit greater activity of PPO than healthy leaves. Highest activity of enzymes PPO is noticed at fruiting stage in *A.cantala* leaf extract sprayed grape leaves.

The changes in activity of enzymes PPO due to infection was noticed by many researchers. High activity of enzyme PPO in brinjal affected by little leaf disease and sugarcane infected with GSD was reported by Mitra and Majumdar (1977) and Dhumal (1983) respectively. While, low activity of enzyme PPO noticed by Purohit *et al.,* (1979) in *Sesamum* affected by sesamum phyllody. Viral infections also cause variation in activity of PPO. Wagih and Coutts (1982) in cowpea and cucumber infected with TMV, Prasad et al., (1989) in tomato infected with virus have reported high activity of PPO. While, Ramiah *et al.,* (1973b) has reported low activity of PPO in bhendi infected with yellow vein mosaic virus. El-Fahaam *et al.,* (1990) reported no change in activity of PPO in lettuce infected with lettuce mosaic virus.

The activity of enzyme PPO is also influenced by fungal infection. Higher activity of PPO was noticed in pearl-millet infected by green year disease (Shekhawat *et al.,* 1980), *Achyranthus* infected with white rust (Purohit

activity of peroxidase in sprayed leaves is likely related with a systematic acquired resistance (SAR). Hence, the sprayed leaves exhibit high chlorophyll contents, carbohydrates and minerals essential for better yield. Thus, our results are also on similar lines but the use of Agave leaf extract as a biofungicide material is important. It alters the overall enzyme system in controlling the disease development in grape plant.

3) Polyphenol oxidase :- (E.C.1.14.18.1)

Polyphenol oxidase (PPO) is a copper containing enzyme. It is also known as catechol-oxidase, phenolase or diphenol oxygen oxidoreductase. It is widely distributed in the plant kingdom.

Polyphenol oxidase catalyzes the oxidation of monophenol and ortho-diphenols to insoluble polyphenols. This reaction is very important in maturation and ripening process of fruits and vegetables (Mayer and Harel, 1959). Since, it removes astringency by converting soluble phenolics into insoluble ones through oxidation and polymerization process. It has been suggested that the enzymes might be associated with many important physiological functions of plants such as defense, growth and differentiation (Maratte, 1973; Retig, 1974; Hyodo and Uritani, 1966; Ryan *et al.,* 1982; Gordan and Paleg, 1961).

Several reports indicated that polyphenol oxidase and other oxidases have a significant link to disease resistance in fruits and vegetables (Dong and Guo 1990; Okey *et al.,* 1997; Nema 1999). Moore and Stone (1972) reported that the activity of these enzymes is usually increased in the cell surrounding the lesions where localization of the pathogen occurs. This enzymes is induced in response to mechanical wounding and signaling molecules such as methyl jasmonate and systemin. Hence, it play indispensable role in plant defense (Boss *et al.,* 1995; Thipyapong and Stiffens, 1977).

Recent research in function of PPO in higher plants supported the following views: i) It is a plastidic enzyme that is unclear-coded but is inactive in until incorporated into the plastid. ii) In healthy green tissues PPO exists in a

Table No. 3.23 Activity of enzyme polyphenol oxidase in grape leaves

	Healthy	Infected	Sprayed
Flowering	0.0102	0.0112	0.025
Fruiting	0.0106	0.0115	0.0278
Harvest	0.0107	0.012	0.028

Enzyme activity expressed as Δ OD min $^{-1}$ mg^{-1} sol. protein

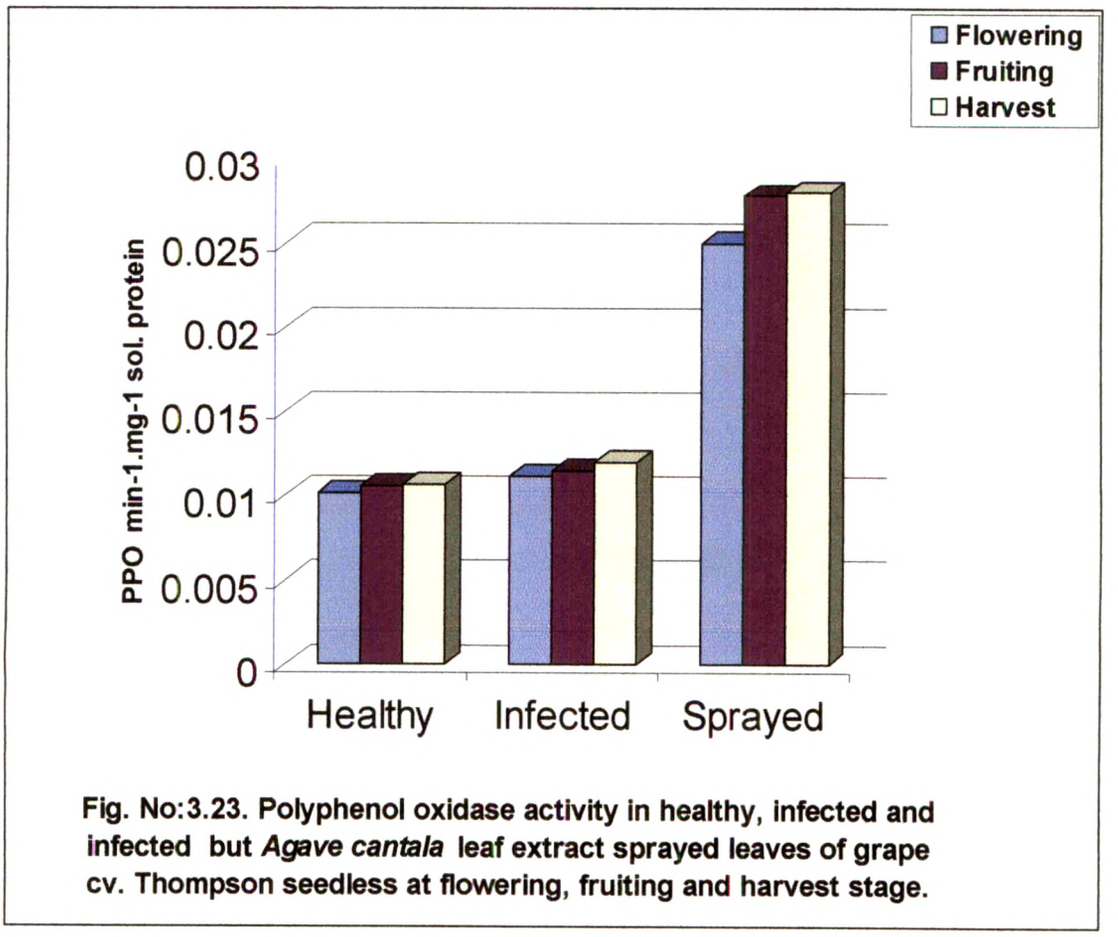


Fig. No:3.23. Polyphenol oxidase activity in healthy, infected and infected but *Agave cantala* leaf extract sprayed leaves of grape cv. Thompson seedless at flowering, fruiting and harvest stage.

production of glucose and fructose causes limited supply of carbon nutrition to pathogen and inhibits it growth and development.

The invertase study is on similar lines but possibly in addition supports the relation with pathogen activity during three stages of the studies. The enzyme concentration and activity may show constancy however increase status in infected plant may be found controlled in the sprayed vine plant and this is important observation of present studies.