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FOLIAR APPLICATION OF *AGAVE CANTALA* ROXB. LEAF EXTRACT ENHANCES ANTIOXIDATIVE DEFENSE MECHANISM IN GRAPE (*VITIS VINIFERA* L.) LEAVES INFECTED WITH DOWNY MILDEW

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ABSTRACT

Field experiments were conducted to assess effect of aqueous leaf extract of *Agave cantala* on antioxidative mechanism in the leaves of *Vitis vinifera* infected with downy mildew. Enhancements of chlorophylls, carotenoids with total polyphenols were noticed in grape leaves infected downy mildew after application of *A. cantala* leaf extract. Further, it was also noticed that activities of enzymes peroxidase and polyphenol oxidase were increased and activity of enzyme catalase was decreased in sprayed grape leaves. From these results, it is concluded that *Agave cantala* leaf extract may be useful for controlling of downy mildew of grape.

KEYWORDS: *Agave cantala*, Antioxidants, Downy mildew, *Vitis vinifera*.

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most important sub-tropical fruit crop grown in the world. It is cultivated in more than 60 countries, with 9 million ha. area under cultivation.[1] It is very delicious refreshing and nourishing fruit grown mainly for making wine, raisin, juice, jelly and rest for table purpose. It is well known as nutrition source of natural sugars, vitamins and fibre.

In India, it occupies about 45,200 ha. of land under its cultivation. States like Karnataka, Andhra Pradesh, Tamilnadu, Maharashtra, Punjab, Haryana and Uttar Pradesh contribute major land under its cultivation. Maharasthra contributes 20,000 ha. with production more than 5 lakh tones / year and approximate turnover beyond 500 crores.[2] The major grape growing areas are situated in the districts of Nasik, Pune, Sangli, Solapur, Kolhapur, Satara, Jalgaon, Ahmednagar, Amravati, Nagpur, Parbhani and Aurangabad.

As grape is important commercial fruit crop it faces attack of several diseases and pests, which is a great problem in India. The fungal diseases such as downy mildew, powdery mildew, Anthracnose etc. are major, while, pests such as grapevine leaf hopper, thrips, mealybug, leaf roller, beetle etc.[3] To overcome these pest and disease, farmers use various chemical fungicides, pesticides which include mainly Bordeaux mixture, Sulfex, Wetasul, Bayleton, Saprol, Difolatan, Fenitrothion, Malathion, Endosulfan, Omethoate, Methomill, Monocroptophos, Acitate and Methadophos etc.[4]

India is one of the large scale grapes cultivating country of the world. The grapes cultivated in India are exported in countries like United Kingdom (UK), Netherlands, Belgium, Germany, France, Italy, Poland, Spain, Sweden, Greece etc. According to International Plant Protection Convension, 1951, Phytosanitary Certificate should be taken for export of fruits and other food products. The Phytosanitary Certificate is given to those products wich are free from pesticide residue and diseases. In European countries residue detection of 81 pesticides is made compulsory. If the residue is found in the grape berry above maximum residue limit (MRL) prescribed by FAO/WHO and Government of India, then it should not be exported in western countries. In India, due to climatic conditions and other factors, use of chemical pesticides is extensive. Over application of chemical control measures such as fungicides and pesticides on grape creates the residual effect on berries. Such grapes are not of export quality and high commercial market value (http://plantquarantineindia.nic.in/PQISPub/html/Exp-insp-cert.htm).

The residue of pesticide can be avoided by implementing Integrated Pest Management (IPM) programme. Pesticides which are less toxic to environment and easily biodegradable are preferred. Biological control of various pests is found more beneficial because it is target specific and easily biodegradable. The use of various biopesticides like *Trichoderma* spp., *Bacillus* spp., Neem (*Azadirachtra indica*), Karanj (*Pongamia pinnata*) and many are

reported. Number of active phytochemicals like polyphenols, steroidal glycosides, alkaloids and saponins were reported in *Agave cantala.*[5, 6]

Grape cultivation is on large scale in Maharashtra, especially in the districts like, Satara, Sangli, Kolhapur, Nasik, Ahamadnagar, etc. The farmers cultivating grapes in Sangli and Satara districts were using *Agave* leaf extract for controlling various diseases of grape. But the effect of *Agave* leaf extract on physiology of grape plants is still not investigated. In present investigation, an attempt has been made to study foliar application of *A. cantala* leaf extract on antioxidative defense mechanism in grape (*Vitis vinifera* L.) leaves infected with downy mildew.

MATERIALS AND METHODS

Preparation of *Agave* leaf extract

Mature, fully expanded, green leaves of *Agave cantala* Roxb. were used for preparation of extract. Selected leaves were first washed and cleaned with tap water and then by distilled water. The leaves were subsequently cut into small pieces. These pieces of leaves were kept in water. The proportion of *Agave* leaves to water was 1:5 i.e. 10 kg. *Agave* leaves in 50 litre water.

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

Foliar application of *Agave* leaf extract

Agave leaf extract was used only with water in the proportion of 1:3 (i.e. 1 litre *Agave* leaf extract in 3 litre water.). This extract was sprayed on grape plants with the help of hand pump (15 litre capacity).

Selection of Grape Plants

Grape plants (cv. Thompson seedless) were selected for study. 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. After 10 days of 1st treatment, leaves are collected randomly. Leaves of 5th row of twig containing flowers or fruits were collected. *Agave* leaf extract was sprayed for 1 year i.e. October cutting to the harvesting of grapes in March.

Methods

Chlorophylls

Chlorophylls were estimated following the method of Arnon.[7] 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. 0.5 g of fresh plant material was homogenized in cold mortar with pestle in dark. A pinch of $MgCO_3$ was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper using Buchner's funnel under suction. 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 a UV-VIS double beam spectrophotometer (Shimadzu UV-190) using 80% acetone as 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

$$Chl.a/Chl.b/Total\ chl.(mg/100g\ fresh\ weight) = \frac{X/Y/Z \times volume\ of\ extract \times 100}{1000 \times weight\ of\ plant\ material}$$

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.[8]

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:

$$Total\ carotenoids\ (mg/100g\ fresh\ weight) = \frac{Absorbance\ at\ 480 \times volume\ of\ extract \times 10 \times 100}{2500 \times weight\ of\ plant\ material}$$

Where, 2500 = extinction coefficient.

Total polyphenols

Polyphenols were estimated according to method of Folin and Denis.[9] From healthy, infected and sprayed grape leaves. Polyphenols were extracted from fresh leaf material in 80% acetone (25 ml) 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.. ml) of standard polyphenol solution (tannic acid, 0.1 mg/ml) 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 660 nm on Shimatzu double beam spectrophotometer. Polyphenols were calculated from the calibration curve of standard tannic acid.

Catalase (E.C.1.11.1.6)

Catalase activity was assayed by following the method of Luck[10] as described by Sadasivam and Manikam.[11] 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.[12]

Peroxidase (E.C. 1.11.1.7)

Activity of enzyme peroxidase from healthy infected and sprayed leaves was studied following the method of Maehly.[13] 1 g leaves of grape were homogenized in 15 ml ice-cold (1/15 M) 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, 2 ml 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.

Polyphenol oxidase (E.C.1.10.3.2)

To study polyphenol oxidase activity the methods of Mahadevan and Shridhar[14] 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.

RESULTS AND DISCUSSION

Chlorophylls

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. 1 A, B and C. 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. 1 C).

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.*,[15] and [16] 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.[17] In the view of Henningsen and Boynton,[18] the chlorophyll accumulation is controlled not only by the rates of process of chlorophyll biosynthesis or degradation but also by the formation of chloroplast ultra structure. The process such as shading also influences the chlorophyll content due to creation of irradiance gradient.

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.[19] Similar trend of decrease in chlorophyll content was observed by Dhumal[20] in sugarcane affected by GSD. Several workers observed the severe decline in chlorophyll contents in host infected by virus.[21]

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. 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 Tang *et al.*,[22] 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. Hence, inhibition of fungal growth due to *Agave* leaf extract spray decreases chlorophyll degradation in infected grape leaves.

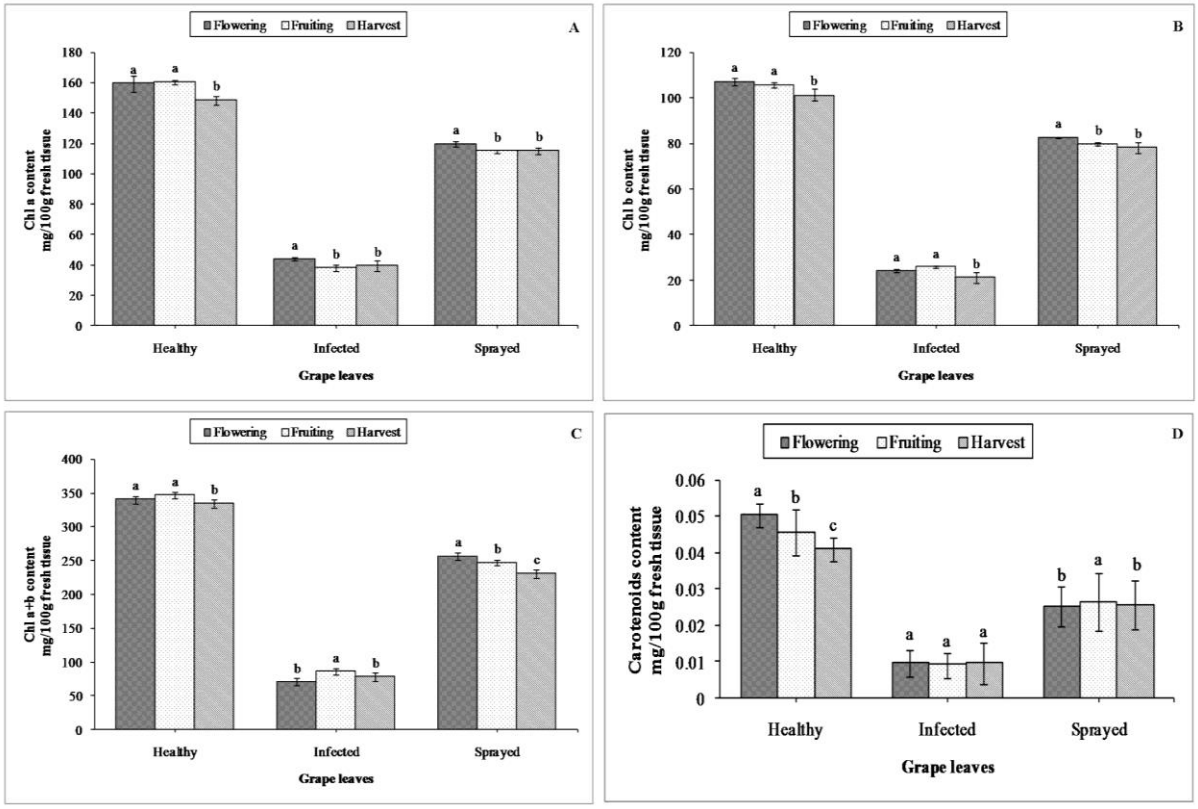

Figure 1. Effects of *Agave cantala* leaf extract on Chlorophylls (A, B, C) and carotenoids content (D) in healthy, infected and sprayed grapes leaves. Error bars indicating standard deviation (±SD). Means were compared by Duncan's Multiple Range Test (DMRT) at 0.05%, n=3.

Carotenoids

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. 1 D. From figure, 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 carotenoids content decreased from flowering to harvest stage.

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. 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.[17, 23]

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.[23] According to Razungles *et al*.,[24] carotenoid contents decreased progressively from the onset of grape berry development to the end of 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.[23]

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*.,[25] 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 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.[26] 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.

Total Polyphenols

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. 2 A. It is evident from 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 (1.37 g. 100^{-1} g fresh tissues) noticed at fruiting stage in *A. cantala* leaf extract sprayed grape leaves. While, lowest total polyphenol content (0.28 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.

Polyphenols play an important role in plant metabolism. According to Rice,[27] polyphenols influences fundamental plant processes such as photosynthesis, chlorophyll production and plant water relations. It also takes part in protein synthesis,[28] respiration[29] and membrane permeability.[30] According to Sharma *et al*.,[31] phenols are involved in stomatal movements. The involvement of phenols in plant defense resistance is based to a large extent on their cytotoxicity, which is associated with their oxidation products.[32] Phenolics consist of such compounds as condensed tannins, flavonoids, phenyl propyl etc. Flavonoids are fairly well distributed in the plant kingdom.[33] They are known to possess insecticidal and antimicrobial activity.[34]

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 phenols are regarded as preinfection inhibitors, providing plant with a certain degree of basic resistance against pathogenic microorganisms.[35] Plant infected with fungus show increase or decrease in phenol contents. The work of Scarpoari *et*

al.,[35] in cocoa affected by *Crinipellis*, Satisha *et al.*,[35] in grape infected by powdery mildew reported increase in phenol contents.

The grape cultivar Thompson seedless is susceptible to fungal diseases like downy mildew due low polyphenol content than wild, highly resistant variety like Mango.[36] 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[37] and Satisha *et al.*,[34] According to Kedge,[36] the grape cultivar containing high polyphenols show greater resistance against downy mildew. Satisha *et al.*,[34] 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.

Enzymes

Catalase

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. 2 B. 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.

Catalase is one of the main enzymes playing a role in the catabolism of hydrogen peroxide.[38] 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.

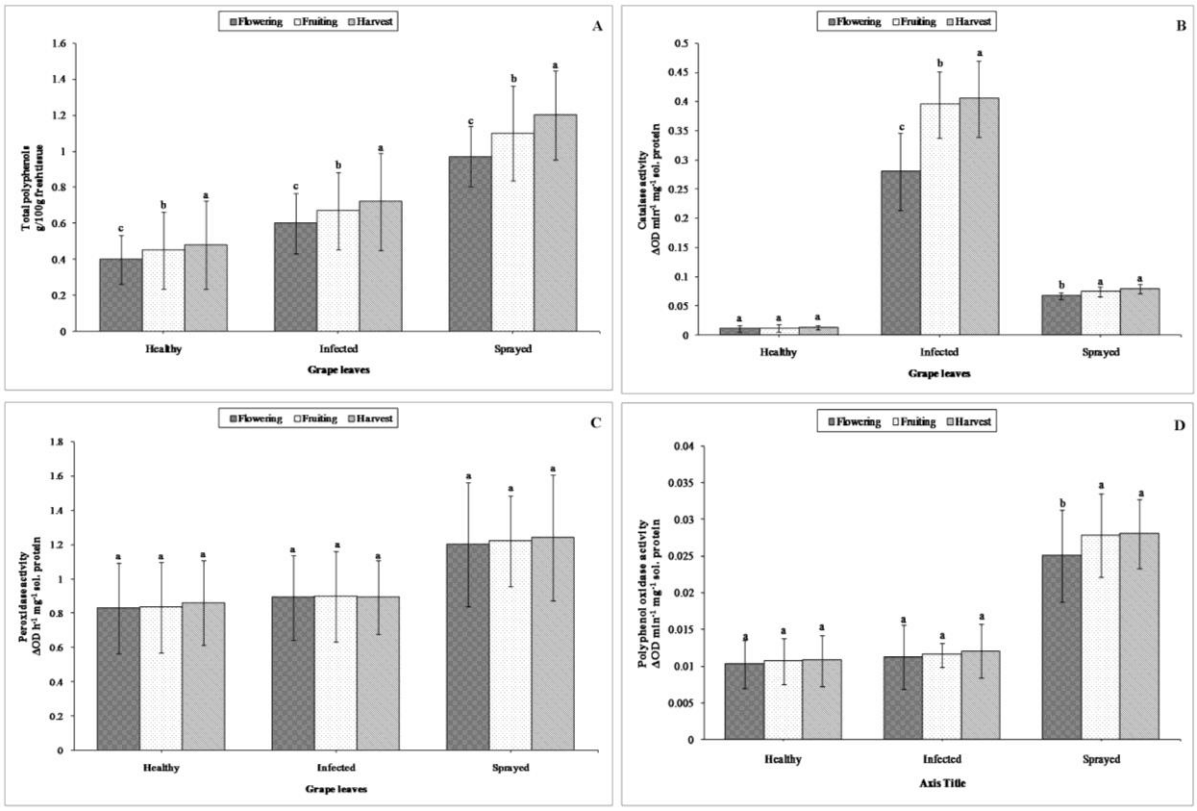

Figure 2. Effects of *Agave cantala* leaf extract on total polyphenols (A) and activity of antioxidative enzymes; catalase (B), peroxidase (C) and polyphenol oxidase (D) in healthy, infected and sprayed grape leaves. Error bars indicating standard deviation (±SD). Means were compared by Duncan's Multiple Range Test (DMRT) at 0.05%, n=3.

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.[39, 40]

The effect of infection on activity of catalase was reported by many researchers. Prasad[41] and Verma and Prasad[42] have reported greater activity of catalase in tobacco after viral infection. Low activity of enzyme catalase due to fungal infection was noticed by Montalbini and Marte[43] in beans infected by *Uromyces*.

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.[44] 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. 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.

Peroxidase

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. 2 C. 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.

Peroxidase plays an important role in growth and development of plants by controlling auxin catabolism,[45] H_2O_2 formation[46] and lignin and ethylene biosynthesis.[47] Peroxidase is involved in plant-pathogen interactions.[48] Moreover, role of peroxidase in disease resistance was noticed by Simmons and Ross.[49]

In case of fungal infection the activity of enzyme peroxidase is either increased or decreased. Vidhyasekaran[50] noticed low activity of peroxidase in finger-millet infected with *Helminthosporium*. Srivastava[51] in *Brassica* infected with *Marcrophomina*, Anjana *et al.*[52] in sunflower infected with *Alternaria* have reported high activity of peroxidase in host plant.

The high activity of enzyme peroxidase is noticed in *A. cantala* leaf extract sprayed grape leaves. Results also suggested that downy infection to 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.

Polyphenol oxidase

The importance of polyphenol oxidase (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. 2 D. From figure, 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.

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. Several reports indicated that polyphenol oxidase and other oxidases have a significant link to disease resistance in fruits and vegetables.[53, 54, 55] Moore and Stone[56] reported that the activity of these enzymes is usually increased in the cell surrounding the lesions where localization of the pathogen occurs. This enzyme is induced in response to mechanical wounding and signaling molecules such as methyl jasmonate and systemin. Hence, it play indispensable role in plant defense.[57, 58]

The slight increased activity of PPO in grape leaves infected with downy mildew suggests moderate resistance of the host. However, this increase in activity of enzyme PPO does not inhibit the growth and development of pathogen. Present studies are also on similar lines i.e. infected leaves show some higher rates than healthy leaves of grapes. The reason behind the susceptibility of cultivar Thompson seedless to fungal disease is confirmed with low activity of PPO after infection. This view is supported by the work of Gupta *et al*.,[59] and Srivastava.[51] According to them, susceptible cultivar exhibit low activity of PPO than resistant cultivar after infection.

The grape leaves infected with downy mildew and powdery mildew show high activity of enzyme PPO after *A.cantala* leaf extract spray. The enzyme PPO is involved in disease resistance. It oxidizes phenols to quinone, which play an important role in disease

resistance.[60] The high activity of PPO with peroxidase in *A. cantala* leaf extract sprayed grape leaves suggest high resistance induced in the host plant. Hence, high activity of PPO following with peroxidase and high concentration of copper and polyphenol content are the indicators of disease resistance shown by susceptible cultivar Thompson seedless after *A. cantala* leaf extract spray.

The studies on PPO and peroxidase as well as catalase in present investigation reveals that the *Agave* leaf extract may increases disease resistance in grape plant due to overall physiological activities related to the above enzyme. The confined effect may develop some resistance in grape plant. It may suggest the use of *Agave*s leaf extract spray may be beneficial to the farmers in controlling downy mildew in the Western Maharashtra.

CONCLUSION

It is evident from the results that after spraying the *Agave* leaf extract, the grape leaves infected with downy mildew shown enhancement in antioxidative defense mechanism. It is possible that active phytochemicals like polyphenols, steroidal glycosides, alkaloids and saponins present in *Agave cantala* leaf extract may be responsible for enhancement in antioxidative defense mechanism. From the results, it is concluded that *Agave cantala* leaf extract may be used to control downy mildew of *Vitis vinifera* L.

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