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ASSESSMENT OF BIOCHEMICAL CONTENTS IN OKRA PLANTS INFECTED WITH OKRA YELLOW VEIN MOSAIC VIRUS

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A B S T R A C T

Okra (*Abelmoschus esculentus* L.) belongs to family *Malvaceae* and it is a rich source of carbohydrates, minerals and vitamins. Okra yellow vein mosaic disease (OYVMD) is a devastating biotic stress that is caused by okra yellow vein mosaic virus (*OYVMV*). This virus is vectored by whitefly (*Bemisia tabaci*) in a persistent and circulative manner. The current study aims to evaluate the effect of *OYVMV* on biochemistry of okra plants. An extensive survey about the epidemiology of OYVMD in geographical location of Tehsil Sahiwal, District Sargodha (Punjab Province-Pakistan) on okra fields was conducted. Symptomatic samples subjected to *OYVMV* confirmation were collected. The healthy and infected samples were processed for determination of biochemical parameters such as total phenolic contents (TPC), total soluble sugars (TSS), and enzymatic antioxidants i.e. superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) to assess the effect of virus infection on okra plants. TPC were measured by using Folin-Ciocalteu reagent (FCR); TSS determined through anthrone sulfuric acid assay and enzymatic antioxidants by using nitroblue tetrazolium (NBT) solution. The quantity of TPC was decreased while TSS and enzymatic antioxidants increased in diseased plants. TPC in healthy bark and leaves was 0.12 and 0.14 mg/g as compared to 0.2 mg/g of diseased samples. TSS in diseased bark and leaves was 223 and 214 mg/g while enzymatic antioxidants SOD, POD and CAT were also higher. The present study would be helpful in developing environment friendly management approaches by using biochemical profiling of infected plants.

Keywords: *OYVMV*, Management, Evaluation, Biochemistry, Okra.

INTRODUCTION

Okra or lady finger is widely grown angiospermic pod vegetables from mallow family (Agbo *et al*., 2008). Okra primarily developed for young ovary, which are spent as plants uncooked, boiled. The shoot utilized in the rag industry, while the dry kernel used manufacture plants. Stews, soups, baked items, and meal preparation are all made using it (Akingbala *et al*., 2003). In Pakistan, it is grown on 14465 hectares, yielding around 109239 thousand tons of green pods (FAO, 2020). This is a very low amount of production in relation to other countries throughout the world. The pathogenic stresses i.e. damping off, Fusarium wilt, powdery mildew are to blame for its poor productivity (Prakasha *et al*., 2010). Okra yellow vein mosaic disease caused by okra yellow vein mosaic virus (*OYVMV*) is among the deteriorating factors resulting in reduced okra yields. *OYVMV* belongs to the genus *Begomovirus* and the *Geminiviridae* family (Zerbini *et al*., 2005). Whitefly (*Bemisia tabaci*) that belongs to order *Hemiptera* and *Aleyrodidae* family spread *OYVMV* in a persistent and circulative manner (Ghanem, 2003). In Pakistan, OYVMD incite 20-30%

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yield losses, but during epidemics, these losses increased up to 90% (Ali *et al*., 2005). The yield losses caused by OYVMD may reach up to 90% in severe cases in Pakistan.

OYVMV causes yellowing of veins; stunted growth, smaller and deformed fruit with yellow or creamy colour, fibrous, small, and harsh in texture (Kucharek, 2004). Chemical control is an important aspect of an integrated pest management (IPM) program to prevent agricultural losses caused by insect pests and viruses since it is a quick method of pest control. Many insecticides have been employed to reduce the population of whiteflies (Satpathy *et al*., 2004). The synthetic insecticides are toxic many to beneficial insects and microbes apart from whiteflies and also cause environmental contamination (Kavitharaghavan and Vijaya, 2005). Rashid *et al*. (2012) employed innovative insecticides to combat the yellow vein mosaic disease spread by *B. tabaci*. The whitefly population was reduced in the diasenthiuron 50 WP, imidacloprid 70 WG and thinmethoxmm 25 WG treatments and subsequent incidence of OYVMD. The repeated usage of chemical insecticides develop resistance in the insects and it could not be controlled (Avicot *et al*., 2014). The most effective method of combating OYVMD is to adopt resistant cultivars. On a natural hot environment, many okra cultivars were tested for OYVMD tolerance. In the field, Singh *et al*. (2003) examined 24 okra types for *OYVMV* susceptibility. There were no highly resistant cultivars, 3 resistant, 8 moderately susceptible, 3 susceptible, and 10 highly susceptible genotypes. The resistance of already developed genotypes would be broke down due to the extensive climate change conditions which force the virus mutation and vector population development (Jones and Naidu, 2019). Considering the above described background studies, the present experiment was planned to study the effect of *OYVMV* on the biochemical contents of infected okra plants because it would pave for accurate disease diagnosis, disease progression and plant microbe interaction. The biochemical analysis of healthy and diseased okra plant samples would be helpful to find out the possible sustainable management tactics for OYVMD.

MATERIALS AND METHODS

Survey and symptomology: A survey of different okra fields was conducted randomly based upon visual inspection from Tehsil Sahiwal, District Sargodha (Pakistan). Salient features of *OYVMV*D (mosaic patterns of leaves, vein clearing, stunted plant growth, and creamy yellow color of leaves) were used for symptomatic identification in the field survey (Bhagat and Kumar, 2022).

Collection of diseased samples: Based upon symptoms, plant leaves and fruit samples were collected from the fields for the bioassay of okra *yellow vein mosaic virus* (*OYVMV*). The foliage having salient symptoms was collected from disease hotspot areas and relevant crop information was recorded. Samples were then stored in plastic bags and stored in a cold box and then transferred to the laboratory for further studies (Bock *et al*., 2022).

Pathogenicity test: A pathogenicity test was carried out on 10 okra plants grown in plastic pots comprising potting mix (75% peat, 25% sand, v/v). Suspected viruliferous whiteflies were collected from the diseased field through aspirator and released onto the healthy okra plants grown under controlled conditions. The plants were covered with plastic sheet before whitefly inoculation so that the released whitefly may stay on the plants for standard inoculation feeding period. The plants were watered and fertilized as per requirement till the appearance of the characteristic symptoms of *OYVMV* (Kashina *et al*., 2007).

Effect of *OYVMV* on biochemical contents of the okra plants: Determination of total soluble sugar (TSS): TSS was measured by using anthrone sulfuric acid assay; the plant samples were ground in distilled water and filtered. The anthrone reagent was prepared by mixing anthrone and concentrated sulfuric acid followed by adding in plant extract in a test tube. The test tube was heated in water bath and then cooled at room temperature. The absorbance of sample was measured by using spectrophotometer at 620 nm and sugar contents were calculated by comparing with standard glucose curve (Grandy *et al*., 2000).

Determination of total phenolic contents (TPC): TPC was determined using the Folin-Ciocalteu reagent technique with minor modifications. Ethanol with gallic acid was used a standard and total phenols were expressed as (GAE) gallic acid equivalents / 100 g as a standard of fresh weight sample. All determinations were made 3 times, although tests were done twice.

Chemical Reagents: 20% Na_2CO_3 solution was made by dissolving 20 g sodium carbonate in to 80 ml distilled water.

- 10% Folin reagent was prepared by dissolving 10 mL of Folin in 90 ml distilled water.

Prepared sample in various test tubes (about 500 mL) and made 3 copies of each sample. Folin Solvent 10%, 500 mL of 20% Na_2CO_3 was added to test tubes and left to sit for 6 minutes, At 760 nm, the absorbance level was determined using a spectrophotometer. The spectrophotometer was calibrated with a blank before running the samples.

Determination of Antioxidant enzyme: Leaf samples from healthy and diseased okra plants were collected in zip lock bag and shifted to ice bucket before use. The samples were transferred to laboratory for further processing. All the samples were washed in 3 changings of tap and distilled water to ensure removal of inert material and dust particles. The samples were ground in extraction buffer with a ratio of 1:2 i.e. 100 g of samples were put in 200 ml of extraction buffer. The supernatant was removed through muslin cloth and remaining sample was used for determination of antioxidant enzymes. Superoxide dismutase (SOD) was determined by measuring the ability to inhibit photoreduction of nitroblue tetrazolium (NBT). The determination of catalase (CAT) and peroxidase (POX) were accomplished by using modified method.

RESULTS

Effect of *OYVMV* disease on total phenolic contents (TPC) in okra: The present experiment depicts that TPC contents in healthy leaves of okra were significantly higher than diseased samples (Figure 1). The amount of TPC recorded in healthy leaf and bark was 0.12 mg/g and 0.14 mg/g, respectively while in diseased plants these values were 0.2 mg/g, both in bar and leaves, respectively. These values are average of 3 replicates from each variety sample both in healthy and diseased leaves and bark of okra plants.

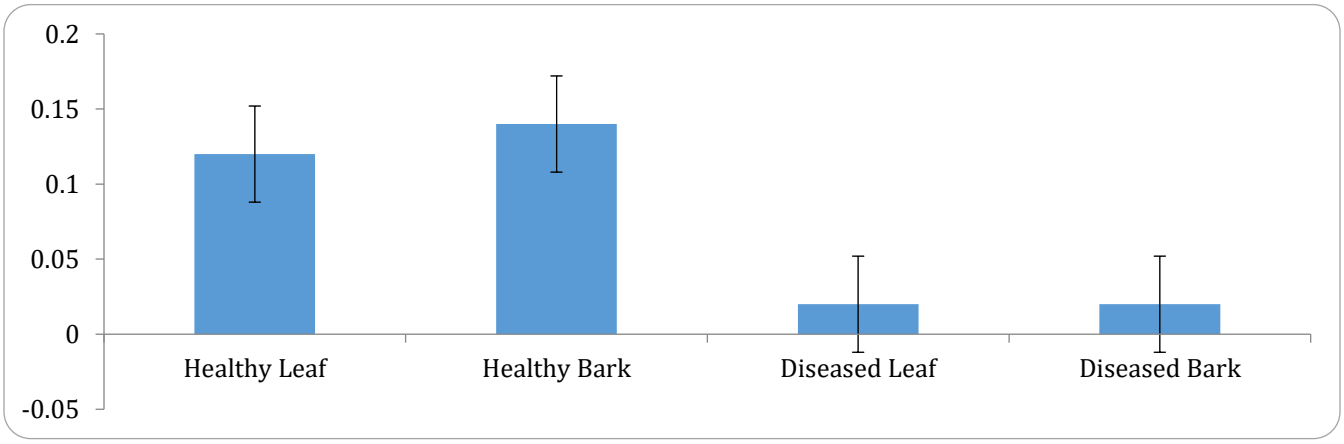

Figure 1. Effect of *OYVMV* disease on TPC on healthy and diseased leaves and bark of okra samples (Unit for TSP mg/g)

Effect of *OYVMV* disease on Flavonoid contents of okra plants: The results of flavonoid analysis in healthy leaves and symptomatic leaves and bark indicated the higher contents as compared to diseased parts of okra plants (Figure 2). The amount of flavonoid content recorded in healthy and diseased leaf was 0.75mg/g and 0.17 mg/g while, in healthy and diseased bark was 0.80 and 0.25 mg/g, respectively.

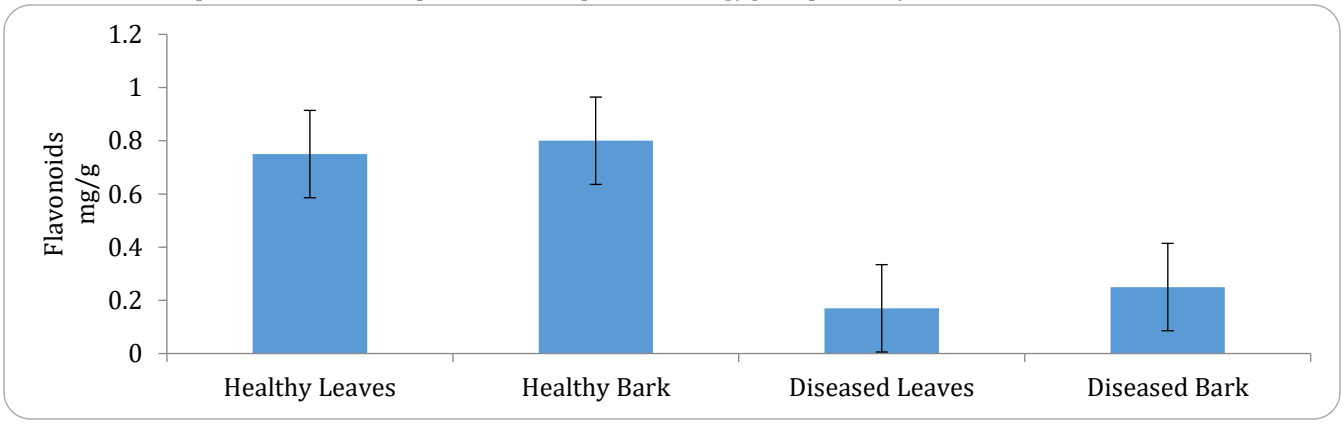

Figure 2. Effect of *OYVMV* disease on Flavonoids contents of bark and leaves of okra plants (Unit for Flavonoids mg/g).

Effect of *OYVMV* disease on antioxidant enzymes in leaves and bark of okra plants: The quantity of antioxidant enzymes i.e. SOD, POD and CAT was significantly higher in the relative samples of infected plants as compared to healthy plants. Among the 3 famous antioxidant enzymes; catalase concentration was higher as compared to superoxide dismutase and peroxidase both in healthy and diseased samples. As far as the SOD contents are concerned, "Sabz Pari" variety showed higher antioxidant content in both healthy and diseased plants with 320 to 410 U/ml, respectively followed by "Diamond 2525" having 325 to 410 U/ml, respectively. While SH 8225 and BS 782 have the least content having 320 and 350 U/ml, in healthy and diseased respectively (Figure 3).

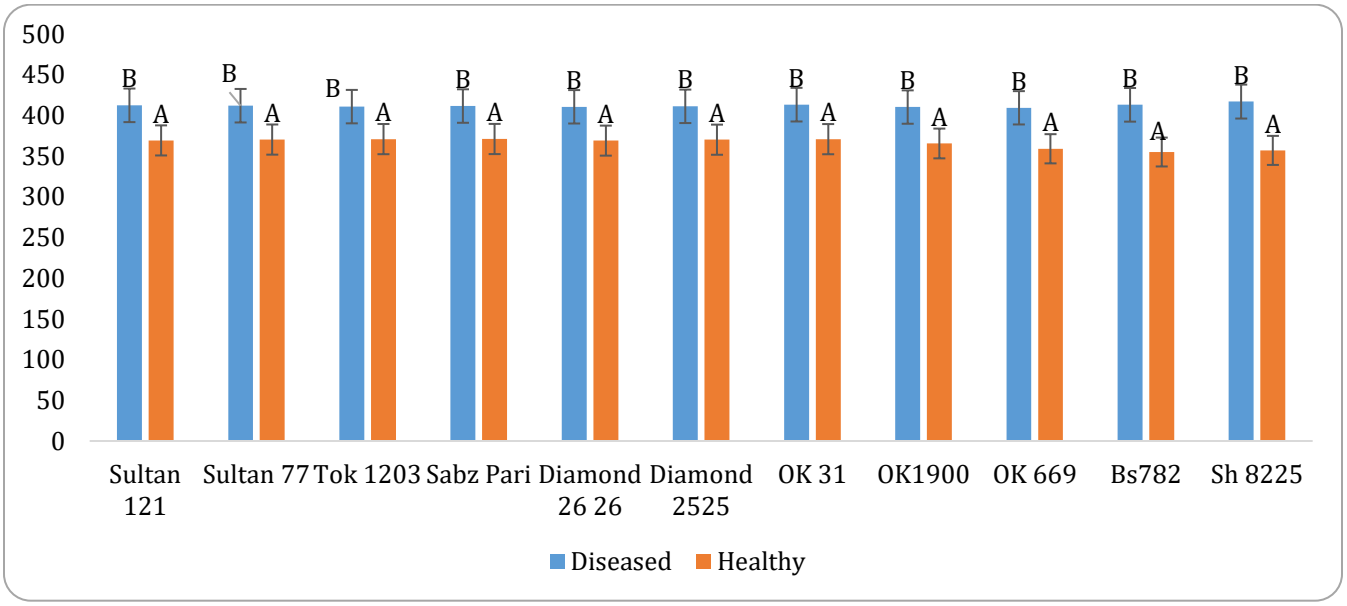

Figure 3. Effect of *OYVMV* disease on superoxide dismutase in different okra cultivars

The analysis of healthy and infected okra plants indicated that Sh-8225 variety shows higher antioxidant content in both healthy and diseased plants with 140 and 160 U/ml, respectively (Figure 4).

The CAT contents ranged from 104 to 112 U/ml in diseased plants while Sultan-121 showed only 05 U/ml in case of healthy plants (Figure 5).

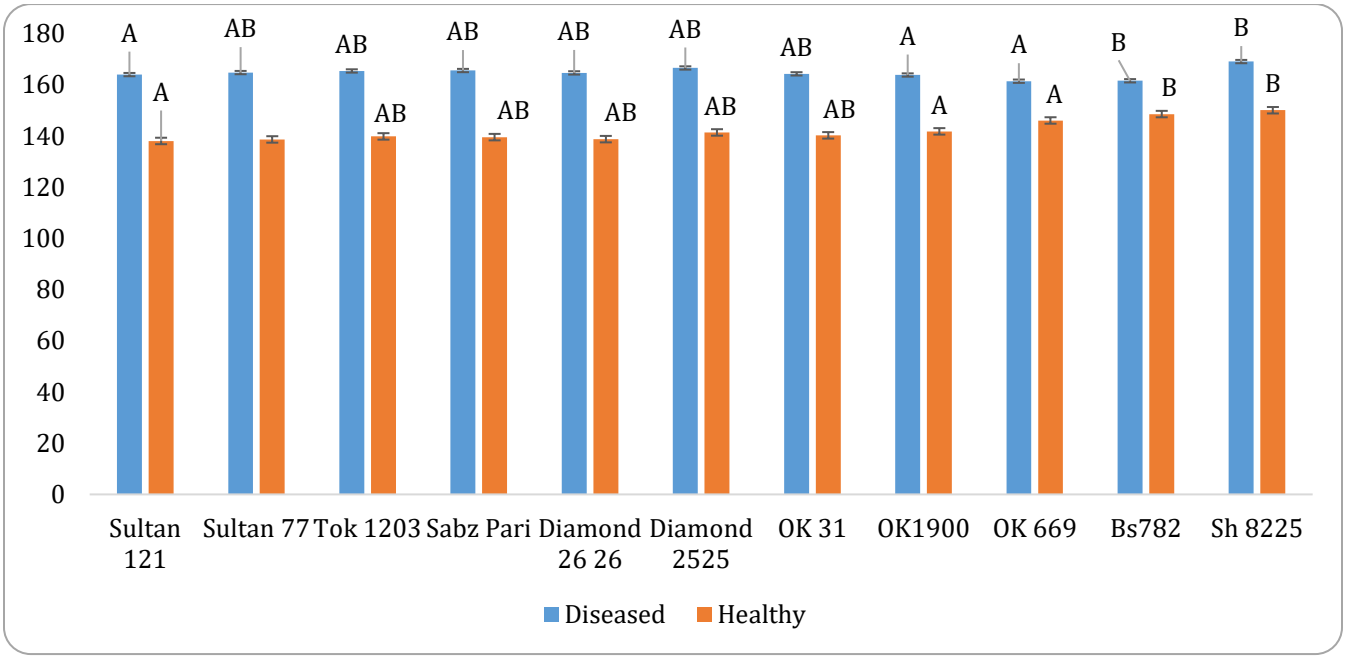

Figure 4. Effect of *OYVMV* disease on peroxidase in different okra cultivars

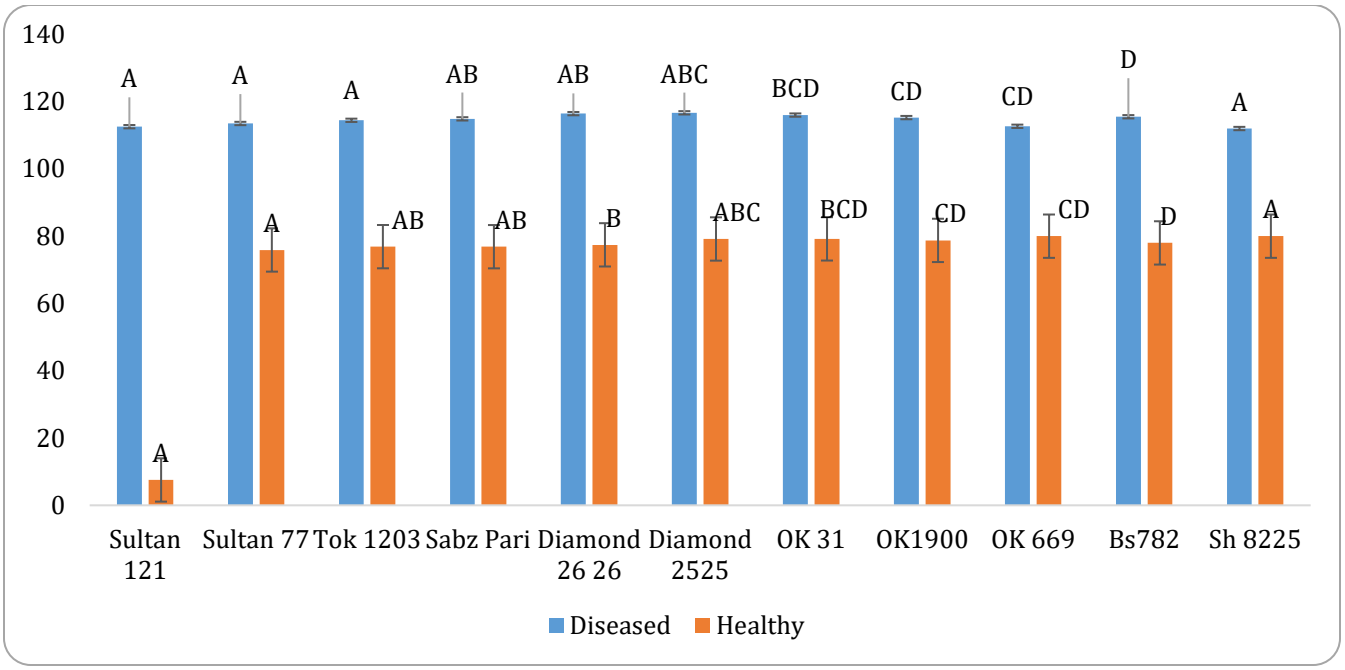

Figure 5. Effect of *OYVMV* disease on catalase in different okra cultivars

Effect of *OYVMV* disease on α-diphenyl-β-picrylhydrazyl (DPPH): The total amount of DPPH on healthy leaves & bark was lower than infected leaves. The amount of DPPH recorded in healthy leaf and bark was 0.16 and 0.21 U/ml, while in diseased bark it was 0.37 and 0.32 U/ml, respectively (Figure 6).

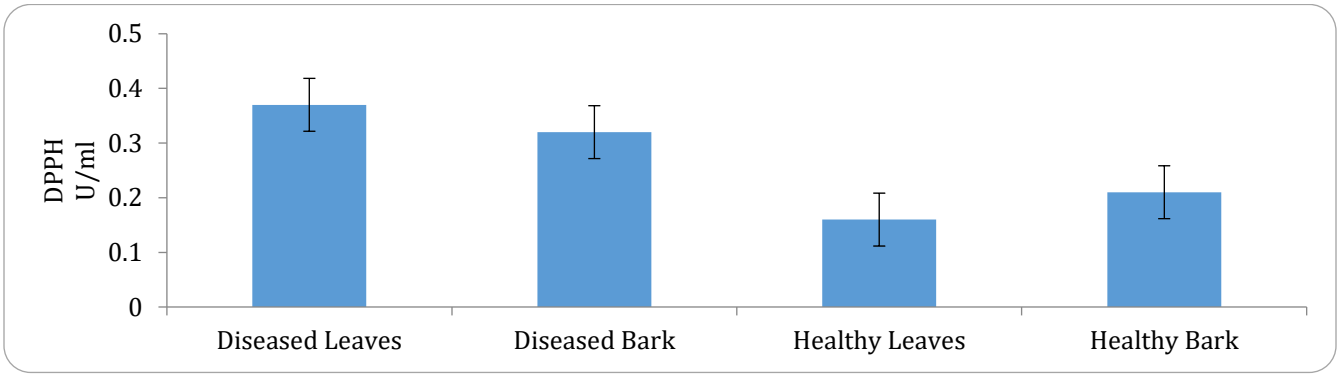

Figure 6. Effect of *OYVMV* disease on DPPH in different okra cultivars

Effect of *OYVMV* disease on TSS of okra plants: Total soluble sugars (TSS) were significantly in higher in diseased leaves and bark of okra plants as compared to healthy one. In diseased leaves and bark, the quantity of TSS was 223 and 214 mg/g, respectively (Figure 7).

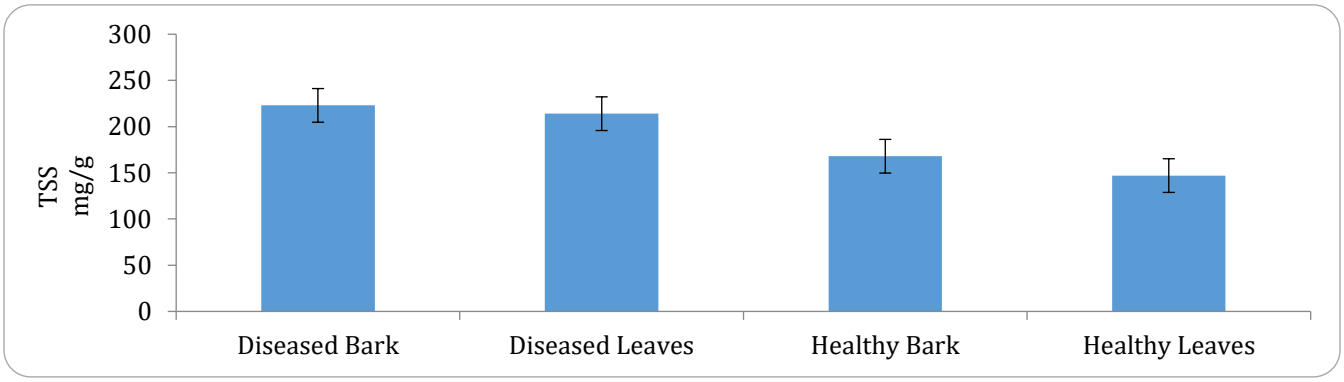

Figure 7. Effect of *OYVMV* disease on TSS in different okra cultivars

DISCUSSIONS

There were higher phenolic contents in healthy samples of leaves and bark of okra plants as compared to *OYVMV* infected samples. The increased phenolic contents helped the plant to be saved from virus infection as it has been proved from many previous studies that phenols play a significant role in plant protection from insect attack (Kumar *et al*., 2020). This finding seems sound in the present case, as *OYVMV* is vectored by a sucking insect whitefly; due to the presence of higher phenolic contents there were fewer possibilities of insect attack and subsequent virus transmission in healthy okra plants. The relationship between high phenols and plant health is proven in many experiments as it may activate plant defense mechanism against various biotic stresses like bacteria, fungi and viruses (Caliskan *et al*., 2017). The observations made by the researchers strengthen the outcomes of present study as the healthy plants resisted the viral attack due to ample quantity of phenols. Kulbat *et al*. (2016) conducted different experiments to assess the role of phenols in plant defense mechanisms and concluded that phenols can escape pathogen invasions and save the host from noxious effect of ROS (reactive oxygen species). *OYVMV* is entered into the plant phloem during feeding by the whitefly; phenolic contents exacerbate the production of different antibiotics that hinder the growth and development of the insect (Tayal *et al*., 2020). The increased concentration of phenols may also be attributed the plant safety from insect attack as the phenols reduce non-protein thiols in the midgut of the insect that ultimately leads impaired growth and efficiency (Singh *et al*., 2020).

In this present study, the results of phenol analysis in healthy leaves were significant ($P<0.05$). In the case of diseased leaves results were not significant ($P<0.05$). The total amount of phenols on healthy leaves was higher than infected leaves & bark. The amount of phenols recorded in healthy leaf and bark was 0.12 and 0.14, respectively while in diseased plants these values were 0.2, respectively. The amount of flavonoid recorded in healthy and diseased leaf was 0.75 and 0.17 while, in healthy and diseased bark was 0.80 and 0.25. The amount of anti-oxidant recorded in healthy leaf and bark was 0.2 and 0.4, respectively. While in diseased plants these values were 0.2 to 0.18, respectively. The amount of DPPH recorded in healthy leaf and bark was 0.16 to 0.21, respectively while in diseased plants these values were 0.37 to 0.32, respectively.

As the plant biosynthetic machinery is occupied by the viruses during replication, defense system is disturbed and subsequently the quantity of all the enzymatic and non-enzymatic antioxidants decrease significantly. The decreased amount of these antioxidants indicates that plant is unable to overcome the disturbances posed by the viruses (Basu *et al*., 2010). Although in current study, few enzymatic and non-enzymatic antioxidants were increased in diseased condition, the condition may due to the response of plant defense to fend off the viral infection. It is because of the increased production of reactive oxygen species (ROS) in diseased plants that may be toxic for the system when produced in higher quantities. The increased amount of enzymatic and non-enzymatic antioxidants is an attempt of the plant to save itself from the harmful effects of ROS (Miller *et al*., 2010). The attack of *OYVMV* disturbed the chloroplast cells which interrupted the formation of chlorophyll leading to the appearance of mosaic symptoms. In many experiments, it was noted that amount of SOD increased in chloroplast cells; that was an attempt to overcome the consequences of ROS production (Boguszewska *et al*., 2010). Catalase converts the hydrogen peroxide into water and oxygen and thus increases the ability of the plant to resist pathogen invasion (Chen and Gallie, 2006).

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Muhammad Umair	:	Biochemical Analysis
Rana Binyamin	:	Statistical Analysis
Misbah Ali	:	Collected Data
Salman Ghaffar	:	Reviewed Manuscript
Muhammad N. Sajid	:	Sample Collection and Pathogenicity