Indexing of various viruses infecting Capsicum and their impact on its Phytochemical attributes

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Abstract: *Capsicum annuum* is a beneficial vegetable crop belongs to Solanaceae family, and is in demand for the abundance of vitamins and antioxidants which include phytochemical and Polyphenol. It is vulnerable to the attack of various pathogens like bacteria, virus and fungus. But incidence of plant viruses become prominent in Capsicum crop day by day in India and are one of the several factors responsible for loss of yield and quality of Capsicum crop. Losses caused by virus diseases are less perceivable than bacterial and fungal diseases. Viruses' infection causes metabolic and biochemical changes in plants. In order to detect the different virus that infect Capsicum crop and analyse their effect on its phytochemical constituents, a survey was conducted for sample collection on the basis of symptoms .All the collected samples were checked for the presence of virus infection through DAS-ELISA and RT-PCR. The tested samples showed positive result for CMV, PVX, PVY, TYLCV, GBNV and PSTVd. Thus, indexing showed that these were the common viruses in Himachal Pradesh, India that infect capsicum crop. All the samples showed positive result for virus were tested for phytochemical compounds. Phytochemical analysis showed that antioxidant activity, total carotenoids, total chlorophyll and ascorbic acid content were reduced significantly, whereas, total phenols and total flavonoids increased in virus infected samples. Thus, this analysis reflect the presence of virus and changes of nutritional status of capsicum under virus infection

Keywords: Capsicum, phytochemical, DAS-ELISA, RT-PCR, Antioxidant.

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1. Introduction

Vegetables crops grown worldwide play vital role in human nutrition are a good source of minerals, vitamins and phytochemical (Dias, 2011). It endows smallholder farmers with much higher income and helps in improving the socioeconomic status of the farmers (Wargovich,2000). India is endowed with varied agro-climatic conditions which are one of the important factor for the production of vegetable crops. *Capsicum annuum* a member of family *Solanaceae* is an important vegetable crop of Himachal Pradesh, India commonly known as Sweet pepper, Bell pepper or Shimla Mirch. There are 20-27 species of Caspsicum, out of which only five species are domesticated namely *Capsicum pubescens, Capsicum baccatum Capsicum annuum, Capsicum chinense* and *Capsicum frutescens* (Sharma *et al.*, 2017; AL-Snafi, 2015).

Capsicum annuum is an economically important cash crop and is globally known for bioactive compounds / antioxidants which include phytochemicals, carotenoids, polyphenols and exceptionally high amount of ascorbic acid (Duan *et al.,* 2007; Vanderlice *et al.*, 1990). These bioactive compounds inhibit oxidation of the essential fats in brain cells (Oboh & Rocha, 2008), play important role in prevention of the common degenerative diseases such as ulcers, cancer, cataracts, diabetes, obesity, cardiovascular diseases and certain neural disorders like Parkinson's and Alzheimer's disease (Gayathri *et al*.,2016; Materska and Perucka, 2005). Capsicum is raised in an area of 19 lakh hectares in the world (Bohra, 2015) and in India, it is cultivated in states like Himachal Pradesh, Karnataka, Jammu and Kashmir, Haryana, Uttarakhand, Madhya Pradesh, Orrisa and Maharashtra (Horticultural Statistics at a glance, 2018). In Himachal Pradesh, Capsicum is stands at third position after pea and tomato

and mainly grown by the farmers of Solan, Kullu, Mandi, Kangra, Sirmour, Chamba and Shimla (Horticultural Statistics at a glance, 2018).

Plants respond to environmental stress (Abiotic and Biotic stress) by producing defensive secondary metabolite (Dorantes-Acosta *et al.*, 2012). Biotic stress affects the productivity of Capsicum and are mainly due to biological units like bacteria, virus and fungi (Soleimani *et al.*, 2014). It is very difficult to identify the plant viruses as insects, herbicide and growth hormone causes similar symptoms in plants. Many bacterial, viral and fungal diseases cause economic losses to Capsicum cultivation. Among these, viral diseases are of great importance for farmers due to severe loss in quality and yield. Now, it becomes the main problem of farmers due to lack of screening techniques for virus identification (Nederhoff,1998).

Symptoms occur in host plant depend upon virus-host relationship may be different due to variety of cultivar, environmental factor and nature/strain of virus (Khan *et al.*,2007). There are several viruses that infect Capsicum and infection in mainly dependent on different types of vectors like Aphids, Whitefly, Thrips and non-invertebrate vectors. The genus *Potyviruses ,Cucumovirus, Poleroviruses* and *Alfamovirus* are the Aphid -transmitted viruses. Among aphids transmitted viruses, Capsicum annuum is more likely to be infected with subgroup-I of *Cucumber mosaic virus*, *Polerovirus* (Pepper vein yellow virus, Pepper yellow leaf curl virus, Pepper yellow virus, Beet western yellow virus and Capsicum yellow virus) and *Alfamovirus (Alfaalfa mosaic virus*) (Moury & Verdin, 2012). Among Whitefly transmitted viruses, *Crinivirus* and *Begomovirus* are the common one. *Tomato chlorosis virus* (ToCV) , a *Crinivirus and Tomato yellow leaf curl virus (TYLCV)* , a *Begomovirus* infects various Solanaceous host including pepper (Lozano *et al.*,2004; Lefeuvre *et al.*, 2010).

Tospoviruses (Family *Bunyaviridae*) are among the most damaging and economically important plant viruses also known as Thrips-transmitted viruses. *Tomato spotted wilt virus (TSWV)* and *Impatiens necrotic spot virus (INSV)* are the member of the Tospovirus. Both *TSWV* and *INSV* causes a similar disease in peppers (Haan *et al.*,1992). In addition to the above viral vectors, there are certain viruses that are not transmitted by invertebrate vectors: *Tobamoviruses* are the most important viruses of pepper that are not transmitted by an invertebrate vector. Among them, *Tobacco mosaic virus(TMV), Tomato mosaic virus (ToMV) and Pepper mild mottle virus (PMMoV)* are included. A total of 68 plant viruses are found to be infect Capsicum in world where only 19 different viruses (*Tobacco etch virus* (TEV), *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV), *Pepper mottle virus* (PeMV), *Potato virus Y (*PVY), *Tobacco mosaic virus* (TMV), *Alfalfa mosaic virus* (AMV), *Tomato spotted wilt virus* (TSWV) *and pepper mottle virus (*PeMV) are reported to be most destructive in nature) are known to infect Capsicum crop in India (Soleimani *et al.,*2014). It is very difficult to identify the physiological and biochemical changes occur in plants due to virus infection because insects, herbicide and growth hormone causes similar symptoms in plant. Plant viruses affects the metabolic pathways of plant and causes biochemical and physiological changes like growth retardation, loss of photosynthetic pigment and change in plant height/leaf area/biomass (Pazarlar *et al*., 2013; Siddique *et al.*, 2014). Plants showed structural deviation and formation of macroscopic (Chlorotic lesions, necrotic lesions and ring spots) and microscopic symptoms (histological changes like formation of inclusion bodies) on virus infection result in change in biochemical attributes (Hull, 2009; Muqit et *al.*, 2007; Meena *et al*., 2016). All these changes reduced the production/yield and quality/quantity of the crop. Deep knowledge related to effect of virus on biochemical attributes (Phytochemicals) is lacking. In the reference to above mention facts ,the present work was planned to identify various viruses infecting Capsicum crop and study their impact on phytochemical constituents of Capsicum.

2. Material and methods

2.1. Survey and sample collection: Surveys were conducted in three districts i.e Kangra, Kullu and Mandi of Himachal Pradesh and total 51 leaf samples from plants showing symptoms like curling, chlorosis, malformation of leaves, rosetting, severe stunting, mosaic, mottling and cupping were collected (Fig. 1). The leaf samples were collected and brought in the laboratory for further Analysis.

2.2. Screening for the presence of virus: All the collected leaf samples were tested for the presence of virus infection through DAS-ELISA and RT-PCR. Firstly, samples were screened for the presence of *Tomato mosaic virus* (ToMV), *Tomato Spotted wilt virus* (TSWV), *Tomato Bushy Stunt virus* (TBSV), *Tomato Yellow Leaf Curl virus* (TYLCV), *Potato leaf roll virus* (PLRV), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) and *Potato virus X* (PVX) by DAS-ELISA (Bioreba kit) using polyclonal antibodies (Clark and Adams, 1977). Further, these samples were tested (for confirmation) for *Alfalfa mosaic virus* (AMV), *Ground nut bud necrosis virus* (GBNV), *Impatiens necrosis virus* (INSV), *Potato mop-top virus* (PMTV), *Potato spindle tuber viroid* (PSTVd), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) and *Potato virus X* (PVX) by virus specific primers through RT-PCR.

2.3. Estimation of chlorophyll and carotenoids of virus infected plants:

Chlorophyll and carotenoids were determined spectrophotometrically following the method of Jayraman and Davies (Jayraman, 1981 ; Davies, 1976), respectively with two hundred mg of fresh Capsicum leaf samples using oxalic acid. Absorbance was measured at 645nm, 663nm and 480nm on Spectronic-20 using 80 per cent acetone as blank.

a)The total chlorophyll content (mg/g) was calculated by using the following equation

$$\textbf{Total chlorophyll (mg/g)} = \frac{(20.9 \times A_{645}) + (8.02 \times A_{663}) \times \text{Final volume}}{1000 \times \text{wt. of sample}}$$

b) The amount of total carotenoids (µg/g) was calculated with following equation:

$$\textbf{Total carotenoids} = \frac{A_{480} + (0.114 \times A_{663}) - (0.638 \times A_{645}) \text{ Volume made up}}{\text{Weight of sample}}$$

2.4. Estimation Of Ascorbic Acid

Ascorbic acid content was estimated by AOAC 2010 method (AOAC, 2010) with 2,6-dichloro-indophenol dye. Two gram of leaf sample was extracted with 2 ml of 2.0 per cent oxalic acid. The total weight of slurry was noticed and 0.8 g of this slurry was taken in a beaker. The volume of the slurry was made 2 ml with 1 per cent oxalic acid, filtered properly through Whatman Filter paper No.1 and 1.0 ml of this filtrate was titrated against 2, 6 dichlorophenol indophenol dye. Three concordant readings were observed for accurate result. The amount of ascorbic acid in sample expressed as mg/100g.

2.5. Estimation of Flavonoids

Total flavonoids in leaf samples were determined by Reis *et al.* method (Reis *et al.*, 2011). For extraction, one gram dried sample was mixed with 10 ml of methanol and shaked on water bath for 12 hrs at 37°C. The extraction solution were taken in replications and final volume was made 1.25 ml with distilled water followed by 0.075 ml of 5 per cent sodium nitrite was added to the mixture and mixed thoroughly. After 5 min, 0.15 ml of $AlCl_3$ (10%) was added and allowed to stand for 6 min. Then, 0.50 ml of NaOH and 0.275 ml of distilled water were added to the mixture. The solution was mixed properly and the intensity of pink colour was measured at 510 nm on spectrophotometer model Merck Spectroquant Pharo 100. The concentration of

total flavonoids in each sample was calculated from standard curve prepared from the working solution of Catechin (0.005 to 0.5 mg) .

2.6. Estimation of total Phenolic content

Dried leaf samples were tested for the variation in the total phenol content by the method of Makkar (Makkar, 2003). Finely ground dried leaf sample (0.2 g) in 10 ml of 70 per cent acetone was taken and extraction was carried out on water bath shaker for 2 hrs at 37°C. After extraction the contents were centrifuged at 10,000 rpm for 20 min and the supernatant was used for the estimation of total phenols. Supernatant (0.1 ml) was taken and final volume made to 1 ml with double distilled water followed by addition of 2.5 ml of 20 per cent sodium carbonate and 0.5 ml of FCR (1N). The content was incubated at ambient temperature for 40 min and absorbance was recorded at 725 nm on spectrophotometer model Merck Spectroquant Pharo 100

2.7. Determination of Antioxidant activity

The 2, 2-diphenyl-1- picrylhydrazyl free radical scavenging activity in leaf samples were estimated by Kordali *et al.*,2005 ; Sharma & Bhatt, 2009 methods. One gram dried leaf sample was ground in pestle and mortar with 10 ml of methanol. The content was transferred to a test tube and kept on shaking for 12 hrs at 37°C on shaking water bath. After 12 hrs, the content was dried on vacuum rotary evaporator (60°C) and recovered by adding 1-2 ml of methanol. Sample solution was taken in test tubes in the range of 20-100 µl and then methanol (AR Grade) was added to make the final volume of 3ml, followed by addition 1ml of DPPH solution (200 µM). The contents were vortexed properly, incubated at 30°C for half an hour in the dark and absorbance was recorded at 517 nm with spectrophotometer model Merck Spectroquant Pharo 100. IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH free radical concentration by 50 per cent) was calculated from the regression line obtained from the plot of per cent inhibition against concentration of each solution using the following equation:

$$\mathbf{IC_{50}\ value} = \frac{50 - \text{y intercept}}{\text{Slope}}$$

2.8. Statistical analysis: For each sample, estimations were carried out in triplicate to reduce the experimental error to a minimum and result was calculated with mean values and standard deviation The obtained data was analyzed statistically by using Analysis of Variance (ANOVA). A probability of ≤ 0.05 was selected as criterion for statistically significant difference.

3. Results

3.1. Screening for the presence of virus: The main objective of the present investigation was to identify the common viruses that effect Capsicum annuum in Himachal Pradesh and study their impact on its phytochemical constituents. Vegetables are the important source of phytochemical. Phytochemical present in vegetables play an important role in disease prevention as they have antioxidant activity, Thus, prevent many diseases (Chen *et al*., 2007; Zhang *et al.*, 2015). *Capsicum* is cultivated in tropical and subtropical areas of world and it has high economic value due to the presence of certain phytochemical like carotenoids, phenol, flavonoids (Olatunji & Afolayan, 2019). But viruses are major production constraints and resulting in reduction in yield and quality of crop. Thus, efforts were made to study the impact of viral infection on phytochemical compounds of Capsicum In order to achieve this goal, all of the leaf samples were serologically tested for the presence of the viral infections through DAS-ELISA and RT-PCR. In DAS-ELISA, out of 51 leaf samples, 30 samples were found to be infected with CMV, fifteen samples were found to be infected with PVX , 19 samples were found to be infected with PVY and four samples were found to be TYLCV positive. None of the 51 samples were found to be ToMV, TBSV, PLRV and TSWV positive in DAS-ELISA.

In RT-PCR, an amplified product of 831 bp corresponding to CP gene of GBNV was observed in one sample (Fig. 2a), product of 218 bp corresponding to CP gene of PVY was found in 19 samples (Fig. 2b), product of 562 bp corresponding to CP gene of PVX was observed in 15 samples (Fig. 2c), an amplified product of 200 bp corresponding to CP gene of PSTVd (viroid) was exhibited by one sample (Fig. 3a) and product of 540 bp corresponding to CP gene of CMV was observed in 30 samples (Fig. 3b). However, none of the 51 samples gave any amplified product corresponding to CP gene of AMV, TSWV, TBSV, INSV ,PLRV and PMTV. Number of leaf samples exhibit single ,double and multiple viral infections are given in Table 1. From this, it was concluded that the percent viral infection in samples from Kangra, Kullu and Mandi district was 90 percent, 31.2 percent and 60 percent, respectively (Fig. 4).

It was noticed the percent viral infection for CMV in Capsicum was 58.8 percent, PVY was 37.2 percent ,TYLCV was 7.8 percent, PVX was 29.4 percent, PSTVd (viroid) and GBNV was 1.96 percent.

Samples showed positive result for viruses (Table 2) by DAS-ELISA and RT-PCR were analyzed for variation in phytochemical compounds.

3.2.Antioxidant activity:

The antioxidant activity of CMV and TYLCV infected (Single infection) leaf samples varied from 0.324-0.585 µg/ml and 0.451-0.585 µg/ml, respectively. In case of leaf samples infected with CMV+PVY, CMV+PVX and CMV+TYLCV (Double infection) antioxidant activity ranged from 0.317-0.585 µg/ml, 0.341-0.585 µg/ml and 0.368- 0.585 µg/ml, respectively. Samples with single and double viral infection showed significantly lower level of antioxidant activity over control (0.585 µg/ml). Whereas, antioxidant activity of multiple viral infected i.e. CMV+PVX+PVY+PSTVd, CMV+PVX+PVY+GBNV and CMV+PVX+PVY leaf samples varied from 0.468-0.585 µg/ml, 0.492-0.585 µg/ml and 0.347-0.585 µg/ml , respectively. A significant variation was observed in antioxidant activity of all the multiple infected leaf samples. Overall, the antioxidant activity was found to be decreased in all virus infected leaf samples as compared to control. The percent range of decrease in antioxidant activity in Capsicum leaf samples with CMV, CMV+PVY, CMV+PVX+PVY+PSTVd, CMV+PVX+PVY+GBNV, TYLCV, CMV+PVX, CMV+PVX+PVY and CMV+TYLCV infection were 7.3-44.6 percent, 19.1-45.8 percent, 20 percent, 15.8 percent, 14-22.9 percent, 3.4-41.7 percent, 16-40.6 percent and 33.8-37.0 percent, respectively. Plant - virus interaction result in activation of plant defense system which result in production of Polyphenol oxidase, peroxidase, catalase etc. Studies showed that total antioxidant level get decrease in PMMoV susceptible pepper leaf samples as compared to resistant (Dikilitas *et al*., 2011). These findings are in direct conformity with the results obtained in the present investigation.

3.3. Total Phenol:

Plant phenols are secondary plant products /metabolites derived from the shikimate, pentose phosphate and phenylpropanoid pathways produced by the plant for protection against biotic and abiotic stress (Randhir *et al*., 2004; Bhattacharya *et al.*, 2010). These phenolic compounds can act as antioxidant, defence molecule against ultraviolet radiations and plant pathogen. It acts as a defense compound against ultraviolet radiation and plant pathogens (Dai & Mumper, 2010; Lin *et al*., 2016). The phenolic content on single virus infection (ranged from 10.1- 29.4 mg TAE/100g), double virus infection (varied from 10.1 -28.3 mg/100g) and multiple viral infection (ranged from 10.1-19.6 mg TAE/100g) increased significantly over control. In nutshell, all the virus infected samples showed significantly high phenol content over control. The results obtained in the present study are in conformity with findings of others researchers.

3.4. Total flavonoids content: Flavonoids are secondary plant product/polyphenolic

compounds produced by the polypropanoid pathway (Ghasemzadeh & Ghasemzadeh, 2011; Vicente & Boscaiu, 2018).Flavonids are the defensive molecule /phytoalexin with anti-inflammatory and anti-oxidative activity protect plants from biotic stress and behave like a UV filter (Samanta *et al.,* 2011; Panche *et al.*, 2016). Studies revealed that flavanoid content enhanced in virus infected plants, weaken the interaction between the coat protein subunits and thus inhibit plant virus action (French & Towers, 1992; Dikilitas *et al.*, 2011). Result of this experiment revealed that Flavonoid content ranged from 14.1-21.7 mg CE/100g on single infection,14.1- 25.3 mg CE/100g on double infection and 14.1- 29.6 mg CE/100g on multiple infection. In the present study, on single, double and multiple viral infection, flavonoid content increased in all the infected sample as compared to the healthy one (in order to inhibit the virus infection).

3.5. Total chlorophyll content: Green colour of the plant leaves are due to photosynthetic pigment known as chlorophyll. Chlorophyll is basic element for detection of plant stress and productivity (Palta, 1990). Chlorophyll content of any plant denotes its health and stress level. Hence, chlorophyll content was estimated in virus infected samples to analyse the effect of virus infection on primary pigment system. Chlorophyll studies demonstrated that on CMV and TYLCV infection (Single infection), total chlorophyll content varied from 3.23-3.94 mg/100g and 0.52-3.94 mg/100g, respectively; on CMV+PVY, CMV+PVX and CMV+TYLCV infection (Double infection) varied from 0.40-4.1 mg/100g, 0.57-3.94 mg/100g and 0.83-3.94 mg/100g, respectively and on CMV+PVX+PVY+PSTVd, CMV+PVX+PVY+GBNV and CMV+PVX+PVY infection (Multiple infection) varied from 0.80-3.94 mg/100g, 1.83-3.94 mg/100g and 0.72-5.82 mg/100g, respectively In this study, Chlorophyll content decrease on virus infection (Single, Double and Multiple infection) in infected leaves in comparison to the healthy plant.

3.6. Carotenoids content: Carotenoids are terpenoid and the second most abundant/ubiquitous pigments on earth after chlorophyll (Nisar *et al*.,2015). They play an important role in protection of photosynthetic apparatus from excessive light (Cazzonelli, 2011; Frank & Brudig, 2004) . They act as a precursor reservoir for the biosynthesis of bioactive compounds in plants, bacteria, fungi and even animals and have antioxidant potential due to their ability to quench singlet oxygen. The present study illustrate that carotenoids content reduced by 42.2-49.4% on CMV infection, 45.3-64.7% on TYLCV infection (Single infection). In case of Double infection i.e CMV+PVY,CMV+PVX and CMV+TYLCV carotenoid content reduced by 26.5-37.9%, 22.5-48.4% and 20.6-37.8% over control, respectively. Whereas, on multiple infection i.e. CMV+PVX+PVY, CMV+PVY+PVX+GBNV and CMV+PVY+PVX+PSTvd carotenoids content reduced by 14-33.3%, 13.7-15.8% and 17.2% respectively. A significant decrease in total carotenoids content was observed in all the virus infected leaf samples as compared to control. Carotenoids are highly sensitive to virus infection for example on CMV infection, carotenoids levels decreased drastically (Mali *et al.,* 2000). The main reason for decrease in carotenoid level was mainly due to obstruction in the expression of gene involved in synthesis of enzyme responsible for carotenoid synthesis like synthase, phytoene desaturase, z-carotene desaturase and carotene isomerase (Ibdah *et al.*, 2014). Similar trend was observed in sweet potato and grapevine (Kapinga *et al*., 2009; Sampol *et al*., 2003).

3.7. Ascorbic acid content: Ascorbic acid are present in milli molar concentration and act as a plant antioxidant (Zechmann, 2011). Plants respond to environmental stress by making certain physiological changes like increase in ascorbic acid level in plants. There is a communication between ascorbic acid and plant hormones during pathogen

attack (Khan *et al.*, 2011). In the present study, Ascorbic acid content was significantly low in infected leaf as compared to healthy .Several studies revealed that on virus infection (Tobacco mosaic virus and Pepper mild mottle virus) ascorbic acid content get reduced (Dikilitas *et al.*, 2011) . These findings support that during virus infection ascorbic acid content was decreased as observed in the present investigation.

The mean variation in Antioxidant activity, Total phenol, Flavanoid, Carotenoid content, Total Chlorophyll and Ascorbic acid content on single, double and multiple virus infection are given in Fig. 5, 6 and 7.

Correlation studies (Table 3) of these parameters showed that total phenol exhibit positive significant correlation with ascorbic acid and negative significant correlation with total carotenoids and total flavonoids content. whereas antioxidant activity, total carotenoids, total chlorophyll and ascorbic acid content showed no correlation with any phytochemical constituent

4. Discussion

Himachal Pradesh is an important state contribute toward the economy of the country by export of many vegetable crops. Export and import of seeds of many crops results in transmission of viruses as many viruses are seed-borne. Climate change and disturbance of nature by human have drastic impact on plant-virus interaction. Plant-virus interaction is responsible for cell to cell movement and virus replication (Kang *et al*., 2005). Increase in virus infection day by day and lack of early detection technique lead to the conduction of present study. In today's era plant virus disease become the major problem of farmers. It results into the loss of quality as well as quantity of the crop. There is no more effective pesticide which kill the virus. so identification and preventive measure is the primary step to protect the vegetable crops from virus infection. Virus infection become predominant due to lack of the proper identification and screening methods. So, the detection of the virus is the most important way to decrease the viral infection in the vegetable crops.

Therefore , in the present study an effort was made to identify the various viruses in Capsicum leaf samples collected on the basis of the symptoms from three districts of Himachal Pradesh. The study showed that percent viral infection in leaf samples from Kangra, Kullu and Mandi district was 90 percent, 31.2 percent and 60 percent, respectively. This study showed that CMV, PVY, PVX, GBNV, PSTVd (viroid) and TYLCV were the common virus that infect the Capsicum crop in Himachal Pradesh. In future, it will help the farmers of Himachal Pradesh to identify and control the viral disease by taking certain effective methods.

In addition to this, virus infection affect the nutritional status of Capsicum. Hence, effect of different virus were analysed on various phytochemical constituents i.e. antioxidant activity, total phenol, total flavonoids, total carotenoids, total chlorophyll and ascorbic acid content in virus infected Capsicum leaves. A significant decrease was observed in virus infected leaf samples of Capsicum for antioxidant activity, total carotenoids, total chlorophyll and ascorbic acid content, whereas, total phenols and total flavonoids increased in infected samples.

Depletion in antioxidant activity may be due to loss of non-enzymatic and enzymatic antioxidants on viral infection. The level of phenol content increased under stress condition in plants, high phenol content depict the plant defence response and it was 1.20 times higher than the normal condition (Dikilitas et al., 2011; Meena et al., 2008). Plant -virus interaction result in activation of phenol synthesis pathways and acceleration of synthesis of enzymes involved in this process. Evidence for increase in plant phenol on virus infection also come from the studies conducted in *Capsicum annuum L.* infected with Gemini virus in which level of total phenol was increased as compared to the healthy one (Meena *et al*., 2008). Plant viruses used biochemical and physiological attributes of plants at optimal level, causes

economic impact by leading change in concentration of different biochemical parameters. On virus infection, breakdown of chlorophyll pigment occur that leads to the origin of mosaic symptoms in leaf and finally low rate of photosynthesis (Shakeel *et al*., 2016; Bhattacharya & Chakraborty , 2018; Bhat *et al.,* 2013). In general with the progression of the disease in plants, a large number of metabolic and biochemical changes occurs that leads to yellowing (Chlorosis) and necrosis of leaf and finally may lead to decrease in chlorophyll content. The studies also revealed that interaction between virus and plants produced changes in photosynthetic parameters and level of chlorophyll pigments get reduced in single and multiple virus infection (Huseynova *et al*., 2018). Similar trend was observed in ginger and Capsicum plant as plant virus alter the chloroplast (Ananthu & Umamheswaran,2019; Meena *et al*., 2008). Reduction in chlorophyll and carotenoids due to viral infection may be due to reduction in number of chloroplasts in mesophyll cells, apart from a frequent involvement of colour change in most of the plants showing that chlorophyll content is either not synthesised at the same rate as in healthy plants or some amount of chlorophyll is destroyed as a consequence of viral infection. The reason for decrease in vitamin C content may be due to increased oxidation of ascorbic acid and deactivation of ascorbic acid recycling. The variation in phytochemical compounds may be the outcome of plant defence mechanism. Thus, the present study indicates that nutritional quality as well as yield of the Capsicum becomes very low on virus infection. This study suggest that potential methods can be promoted for bio-fortification of nutrients and antioxidants in virus infected crops for improving its quality in future.

5. Conclusions

The findings of present investigation showed that CMV, PVY, PVX, GBNV, PSTVd (viroid) and TYLCV were the common viruses which infect Capsicum crop in Himachal Pradesh. The percent viral infection studies confirmed that CMV, PVY, TYLCV, PVX, PSTVd resulted in 58.8 percent, 37.2 percent, 7.8 percent, 29.4 percent and 1.96 percent infection in Capsicum. Our findings related to impact of virus infection on Capsicum confirmed that certain markers of Biotic stress like total phenol and total flavonoids were increased whereas a significant decrease was observed in virus infected leaf samples of capsicum for antioxidant activity, total carotenoids, total chlorophyll and ascorbic acid content. This indicates that Phenols and Flavonoids act as a defence molecule against virus infection in plants so there level was increased (Dai & Mumper, 2010; Lin *et al*., 2016).

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Table 1: Number of samples showing positive results (single, double and multiple infection) for different viruses

Type of infection	Type of virus	No. of Sample
Single infection	CMV	5
	TYLCV	2
Double infection	CMV+PVY	8
	CMV+PVX	4
	CMV+TYLCV	2
Multiple infection	CMV+PVX+PVY	9
	CMV+PVX+PVY+PSTVd	1
	CMV+PVX+PVY+GBNV	1
Total		32

Table 2 : List of Samples code along with district and village names showing positive result for viruses

S.No.	District	Village	Sample Code	CMV	PVX	PVY	PMTV	PLRV	TYLCV	INSV	TBSV	GBNV	TSWV	TOCV	AMV	PSTV
1.	KANGRA	University Polyhouse	PLP-1	-	-	-	-	-	-	-	-	-	-	-	-	-
2.		University Polyhouse	PLP-2	-	-	-	-	-	-	-	-	-	-	-	-	-
3.		Tanda-1	PLP-3	+	-	-	-	-	-	-	-	-	-	-	-	-
4.		Tanda-2	PLP-4	+	-	-	-	-	-	-	-	-	-	-	-	-
5.		Baijnath-1	PLP-5	+	-	+	-	-	-	-	-	-	-	-	-	-
6.		Baijnath-2	PLP-6	+	-	+	-	-	-	-	-	-	-	-	-	-
7.		Bhattu 1	PLP-7	+	-	-	-	-	-	-	-	-	-	-	-	-
8.		Bhattu2	PLP-8	+	-	+	-	-	-	-	-	-	-	-	-	-
9.		Bhattu3	PLP-9	+	+	+	-	-	-	-	-	-	-	-	-	-
10		Jogipur	PLP-10	+	-	+	-	-	-	-	-	-	-	-	-	-
11		Barai-1	PLP-11	+	+	-	-	-	-	-	-	-	-	-	-	-
12		Lower Kohala	PLP-12	+	-	-	-	-	+	-	-	-	-	-	-	-
13		Barai-2	PLP-13	+	+	-	-	-	-	-	-	-	-	-	-	-
14		Tappa	PLP-14	+	+	+	-	-	-	-	-	-	-	-	-	-
15		Upper Kohala	PLP-15	+	+	+	-	-	-	-	-	-	-	-	-	-
16		KVK- Malah	PLP-16	+	+	+	-	-	-	-	-	-	-	-	-	-
17		Barai-3	PLP-17	+	-	-	-	-	-	-	-	-	-	-	-	-

18		KVK-kangra	PLP-18	+	+	+	-	-	-	-	-	-	-	-	-	-
19		Chadhiyar road	PLP-19	-	-	-	-	-	+	-	-	-	-	-	-	-
20		CSIR-IHBT	PLP-20	+	+	+	-	-	-	-	-	-	-	-	-	-
21	KULLU	Wadah	KL-1	-	-	-	-	-	-	-	-	-	-	-	-	-
22		Nagawag	KL-2	-	-	-	-	-	-	-	-	-	-	-	-	-
23		Katair	KL-3	-	-	-	-	-	-	-	-	-	-	-	-	-
24		Banjar	KL-4	+	-	-	-	-	-	-	-	-	-	-	-	-
25		Rotbah	KL-5	+	-	-	-	-	+	-	-	-	-	-	-	-
26		Binni-1	KL-6	-	-	-	-	-	-	-	-	-	-	-	-	-
27		Grammang	KL-7	-	-	-	-	-	-	-	-	-	-	-	-	-
28		Koladhar	KL-8	-	-	-	-	-	-	-	-	-	-	-	-	-
29		Kalehli	KL-9	-	-	-	-	-	-	-	-	-	-	-	-	-
30		Bramhambehr-1	KL-10	-	-	-	-	-	-	-	-	-	-	-	-	-
31		Bramhambehr-2	KL-11	-	-	-	-	-	-	-	-	-	-	-	-	-
32		Sar	KL-12	-	-	-	-	-	-	-	-	-	-	-	-	-
33		Binni-2	KL-13	-	-	-	-	-	-	-	-	-	-	-	-	-
34		Nagwain	KL-14	-	-	-	-	-	+	-	-	-	-	-	-	-
35		Bajoura	KL-15	+	+	-	-	-	-	-	-	-	-	-	-	-
36		KVK-B	KL-16	+	+	-	-	-	-	-	-	-	-	-	-	-
37	MANDI	Dodhwan	MD-1	+	+	+	-	-	-	-	-	-	-	-	-	-
38		Sundarnagar	MD-2	+	+	+	-	-	-	-	-	-	-	-	-	+
39		Ratti	MD-3	+	+	+	-	-	-	-	-	+	-	-	-	-
40		Jhiri-1	MD-4	-	-	-	-	-	-	-	-	-	-	-	-	-
41		Jhiri-2	MD-5	-	-	-	-	-	-	-	-	-	-	-	-	-
42		Jhiri-3	MD-6	+	-	+	-	-	-	-	-	-	-	-	-	-
43		Jhiri-4	MD-7	+	-	+	-	-	-	-	-	-	-	-	-	-
44	MANDI	Jhiri-5	MD-8	+	-	+	-	-	-	-	-	-	-	-	-	-
45		Jhiri-6	MD-9	+	-	+	-	-	-	-	-	-	-	-	-	-
46		Jhiri-7	MD-10	-	-	-	-	-	-	-	-	-	-	-	-	-
47		Jhiri-8	MD-11	+	+	+	-	-	-	-	-	-	-	-	-	-
48		Jhiri-9	MD-12	+	+	+	-	-	-	-	-	-	-	-	-	-
49		Mandi-1	MD-13	-	-	-	-	-	-	-	-	-	-	-	-	-
50		Mandi-2	MD-14	-	-	-	-	-	-	-	-	-	-	-	-	-
51		Mandi-3	MD-15	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Simple correlation coefficient between antioxidant activity, total phenol, total flavonoids, total carotenoids, total chlorophyll and ascorbic acid content in virus infected Capsicum samples

Parameter	Antioxidant activity	Total phenol	Total flavonoid	Total carotenoids	Total chlorophyll	Ascorbic acid
Antioxidant activity	1.0000					
Total phenol	-0.05902	1.00000				
Total flavonoid	-0.12672	-0.65080	1.00000			
Total carotenoids	0.11745	-0.66487	0.29432	1.00000		
Total chlorophyll	0.15542	-0.06957	-0.04429	0.06722	1.00000	
Ascorbic acid	0.23205	0.48710	-0.67501	-0.26685	0.03523	1.00000
Critical value at 5% (+/-) = 0.34338						

Figure 1: Capsicum leaf showing virus like symptoms

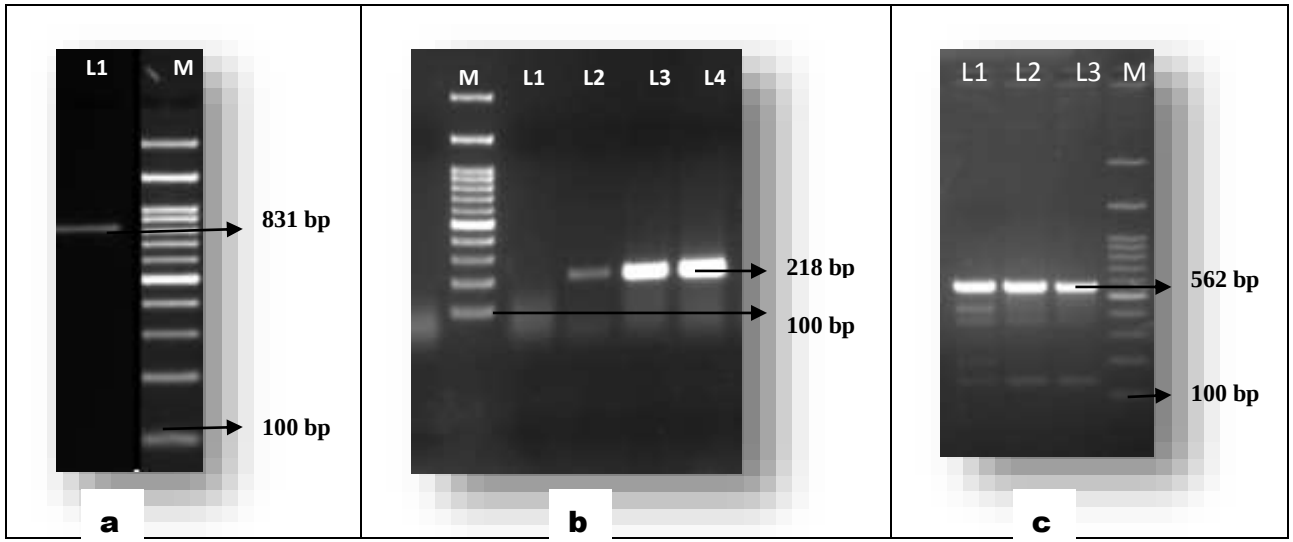

Figure 2: PCR amplification of (a) GBNV (~831 bp), (b) PVY (~218 bp) and (c) PVX (~562 bp) where M=100 bp DNA ladder and L=Lane

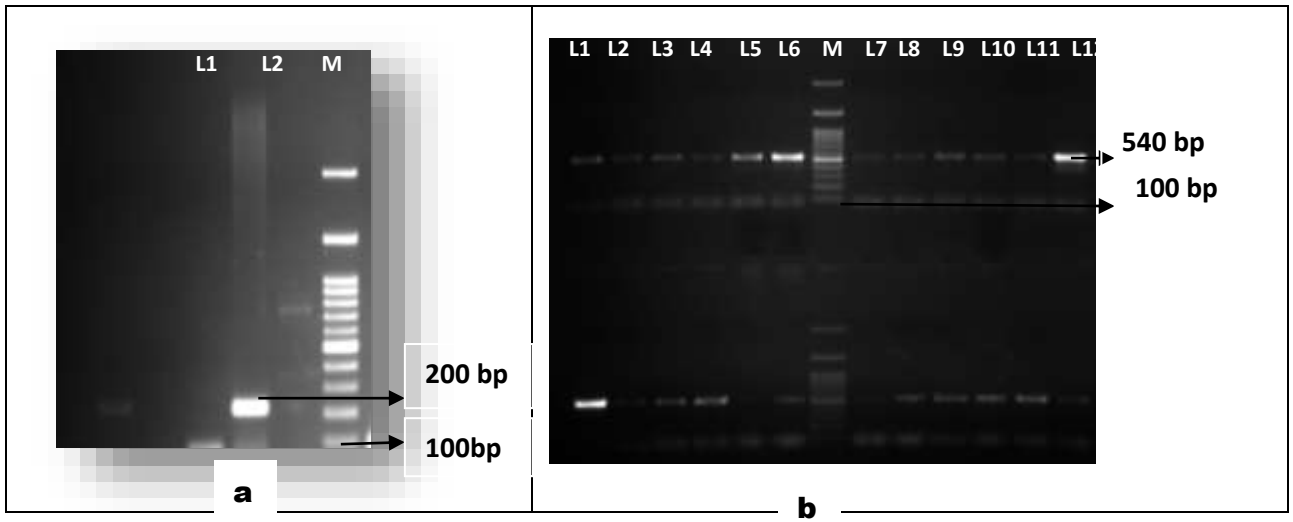

Figure 3: PCR amplification of (a) PSTVd (~200 bp) and (b) CMV (~540bp) where M=100 bp DNA ladder and L=Lane

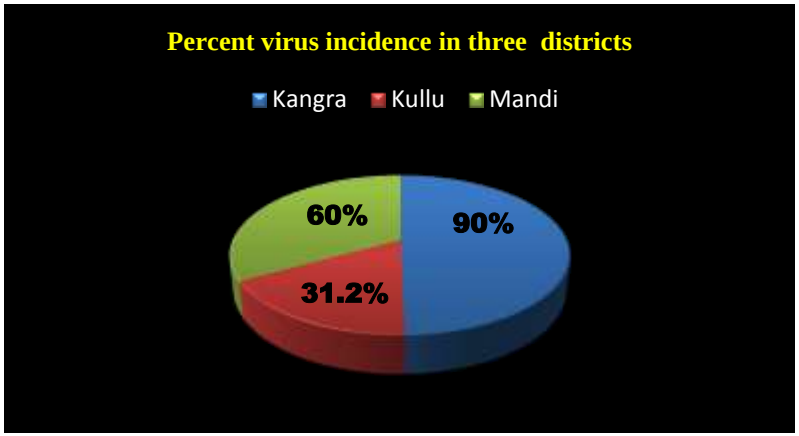

Figure 4: Percent virus infection in three district

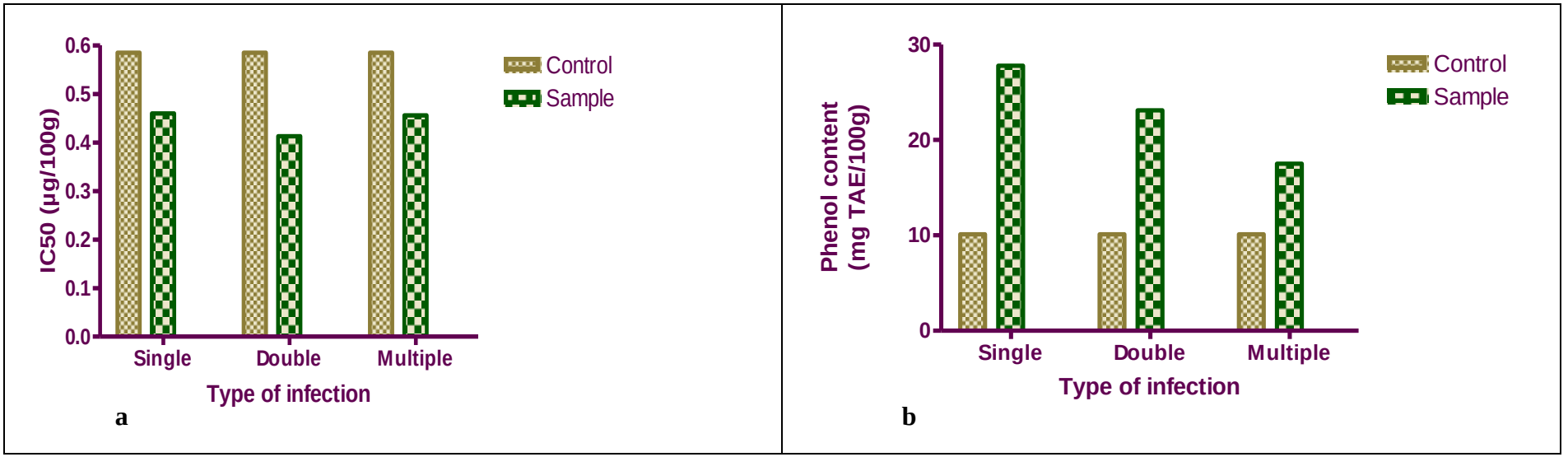

Figure 5: Mean variation in (a) Antioxidant activity and (b) Total phenol on single, double and multiple viral infections

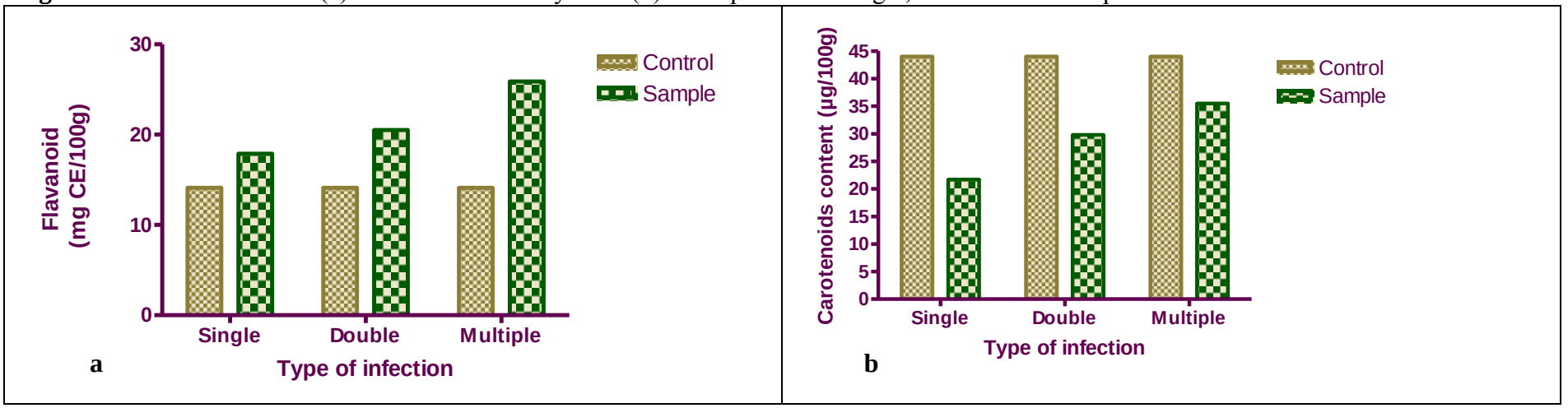

Figure 6: Mean variation in (a) Flavonoid and (b) Carotenoids content on single, double and multiple viral infections

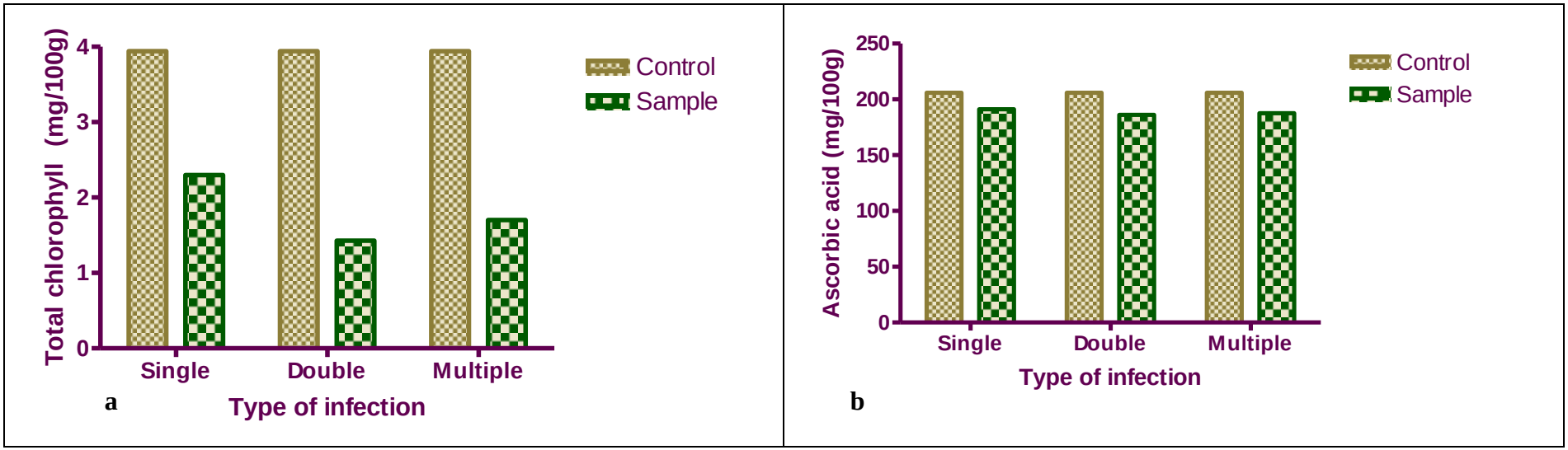

Figure 7: Mean variation in (a) Total Chlorophyll and (b) Ascorbic acid content on single, double and multiple viral infections