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RESEARCH PAPER

Biophysical properties of *Sugarcane Mosaic Virus* (SCMV) and its management

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Key Message: This study reveals biophysical properties of sugarcane, and provides a baseline information for the sustainable management of sugarcane mosaic virus disease.

Abstract: *Sugarcane mosaic virus* (SCMV) is the main viral disease of sugarcane crops in Pakistan. The pathogen produces the specific symptoms on sugarcane plants are the irregular, light, dark-green mosaic pattern with upto 70% losses. The present study was subjected to confirm the mechanical inoculation of crude sap of SCMV with addition to check the longevity of SCMV extracted sap *in vitro***,** dilution endpoint (DEP), thermal inactivation point (TIP), effect of reducing agent on virus infectivity and management of SCMV through induced resistance in host plant by the application of different four chemical (citric acid, salicylic acid, potassium sulphate and, NPK solution) in vivo. The results of the present study revealed that the mechanical inoculation produced the symptoms of mosaic after 5 weeks of post inoculation. The longevity of SCMV reduced with the passage of time and dilution endpoint affected the infectivity of SCMV. The TIP of SCMV between 40-50 ºC was recorded. The reducing agent carbon tetrachloride (CCl_4) reduced the virus infectivity up to the maximum level. The maximum disease incidence inhibition was recorded in treatment one (T_1) citric acid 84.77% followed by salicylic acid 84%, NPK 67.27% and potassium sulphate (K_2 SO_4) 12% as compared to positive control 100% and negative control 0%. The proper management of SCMV is possible through virus free and modified virus resistance cultivars. Based upon current study, eco-friendly management strategies of SCMV could be devised in future. © 2021 Department of Agricultural Sciences, AIOU

Keywords: Dilution endpoint, Inoculation, Longevity, Reducing agents, Salicylic acid, Thermal inactivation point

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Introduction

Sugarcane (*Saccharum officinarum* L.) plants are known as a "heavy feeder crop" which is grown in tropical and subtropical regions of the world on both sides of equators with 35° S and 35° N approximately (Cheavegatti-Gianotto et al., 2011). To date, it has been grown in 107 countries of the world. The total cultivation area under sugarcane is 20.42-million-hectare with 1,333 million tonnes of production worldwide (Chaibandit et al., 2017; Ruan et al., 2018). Sugarcane is the main cash crop of Pakistan and has 5th position in the world for its production. In Pakistan, it is grown in an area of 1.1 million hectares with 63.75 million tonnes of production. In provinces wise cultivation of cane crop, Punjab has the first position with 705,000 hectares followed by others (Farooq & Gheewala, 2019). The main two sugarcane cultivation domains in province Punjab is Rahim Yar Khan (28.38 °N latitude and 70.38 °E longitude) and, Faisalabad (31.43 °N latitude and 71.10 °E longitude), these two area of Punjab produced the 30% (Rahim Yar Khan) and 29% (Faisalabad) of total production of the province (Farooq et al., 2001; Cheema et al., 2006; Azam & Khan, 2010).

The production and the cultivation area of Pakistan are decreasing gradually day by day. A number of reasons are present such as lack of resistant cultivars, old cultural practices of soil cultivation and management, biotic factors and water stressed conditions. Among these mentioned factors, the biotic factor has played a vital role in decreasing the production of sugarcane crops. Sugarcane crops are prone to a number of viral diseases. Sugarcane mosaic virus (SCMV) is the main viral disease of sugarcane crop in Pakistan. More than 13 strains of this viral pathogen had been known and among these 13 strains, two strains (SCMV A, F) were found in Pakistan (Yasmin et al., 2011). SCMV belongs to the family *potyviridae* and genus potyvirus (Anandakumar et al., 2020). This virus is of prime importance as it is used as a vector for screening the resistance potential of many transgenes in different crops (Chung et al., 2021). The detection of SCMV is accomplished by ELISA, PCR, RT-PCR and RT-loop mediated isothermal

amplification assay (Feng et al., 2020). The symptoms produced on sugarcane plants are the irregular, light, dark-green mosaic pattern develop and yellowish streaks along the veins of the leaves. Early stage of disease infection at fourth to fifth leaves may causes severe stunting such as "bushiness" (Perera et al., 2009; Li et al., 2019). It is considered as the most devastating pathogen of sugarcane crop as it incites significant economic losses and social impact in terms of destruction of sugarcane industry due to low production and quality deterioration (Chen et al., 2020). A vector Aphid (*Schizaphis graminis* or *Rhopalosiphum maidis*) mainly transmits this disease in a non-persistent manner and mechanically this disease is transferred by sap inoculation methods (Wu et al., 2012; Tran et al., 2020). Furthermore, the SCMV was reported from 25 countries of the world and has a broad host range which included sugarcane, corn, maize, sorghum, and Johnson grass (Shukla et al., 1994; Moradi et al., 2017).

The loss in yield due to this viral disease in Pakistan was recorded 10 to 32% annually (Aslam et al., 2018). The yield losses in sugarcane are attributed due to severe effects on photosynthetic, cytopathological, biological, transcriptome and proteome of the infected plants (Akbar et al., 2020a). The early sowing and avoidance from the host crops in surrounding areas results in low incidence of SCMV (Clemente-Orta et al., 2020). Rather than using the insecticides for indirect management of SCMV; the cross protection phenomena is the most reliable and eco-friendly management strategy for sustainable agricultural production (Cheng et al., 2020). The mutants of SCMV were identified by using high throughput sequencing techniques for the management through cross protection (Tuo et al., 2020). The aim of the present study is to check the affectivity and virulence of SCMV with the passage of time and the effect of reducing agents on virus infectivity by mechanically inoculation methods. The application of citric acid, salicylic acid, potassium sulphate and nitrogen, phosphorus and potassium (NPK) solution was also done for the management of SCMV disease.

Material and Methods

Incidence assessment of SCMV and sample collections

A field survey was conducted during 2017 of different five locations (Chak 38, University College of Agriculture, 49 Tail, Sillanawali and Kot Momin) of district Sargodha for the assessment of SCMV in the field. 20 plants of each location were tagged and the data of disease incidence of SCMV was collected on every 3rdday. Finally, the mean of the data revealed the incidence percentage of SCMV of each location. The infected samples of SCMV were collected based on their specific mosaic pattern symptoms in polythene ice bags and brought to the laboratory for the preparation of sap for inoculations purposes. Recorded the disease incidence (D.I.) percentage of SCMV by using the formula of Seem (1984):

$$\text{D.I. (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Sap extraction and inoculation on healthy plants leaves

Sap extraction procedure was followed by Addy et al. (2017). The small pieces of infected collected leaves of SCMV were homogenized in chilled mortar with 0.1 M of phosphate buffer having pH 8.0 in the presence of liquid nitrogen. Additionally 2% of polyvinyl pyrrolidone (PVP) v/v was added in the buffer and filtered the solution by muslin cloth. Before the sap application, the healthy plant leaves were tested through RT-PCR. The carborundum powder was used as abrasive on the leaf surface and inoculated the sap. The extracted sap applied directly on the healthy leaves of 7-week-old common sugarcane plant and inoculated leaves were then rinsed with distilled water (DW) to remove excess sap from the leaf surface and the plants were incubated in dark for an overnight. The leaves were covered with a polythene sheet to prevent the insect attack or other pathogens. The symptoms were observed 6 to 8 weeks later after the inoculation.

Longevity of SCMV extracted sap *in vitro*

The prepared sap of SCMV was taken and applied on the plants by different dates with different temperatures. The remaining sap was preserved in the same temperature to check the longevity of the virus *in vitro* condition.

Dilution end point (DEP)

For dilution endpoint (DEP), Teakle and Grylls method (Teakle & Grylls, 1973) was followed and prepared the fresh sap of infected SCMV leaves. 1 ml was taken from the prepared sap and mixed with the 9 ml water to make the volume 10 ml for DEP 1. Again, 1 ml was taken from the dilution end point 1 and mixed with the 9 ml water and made the volume of 10 ml DEP 2. Similarly, DEP 5 was made up to check the infectivity of SCMV on the healthy leaves (Fig.1).

Thermal inactivation point

Take the prepared SCMV sap in three DNA/RNA free 15ml small falcon tubes. The tubes were heated at different temperatures. First tube was heated at 40 ºC, second tube was heated at 50 ºC, third tube was heated at 60 ºC, each tube was heated for 10 min in a water bath. The application of heated sap was tested by mechanically inoculation method on healthy plant leaves to check the infectivity of the virus.

Mixing of reducing agents in extracted SCMV crude sap

A 2.5 ml of chloroform is dissolved in 100 ml distilled water in a beaker, making the 2.5% chloroform solution. Take the 5ml from the 2.5% chloroform solution, and mix them with extracted sap. Same as chloroform 5ml of carbon tetrachloride (CCl_4) was mixed with 100ml of distilled water in a beaker. Then, 5 ml from the 5% CCL4 solution was taken and mixed them with the sap. The effect of reducing agent in disease severity of SCMV was observed. The emerging symptoms and disease severity data was recorded.

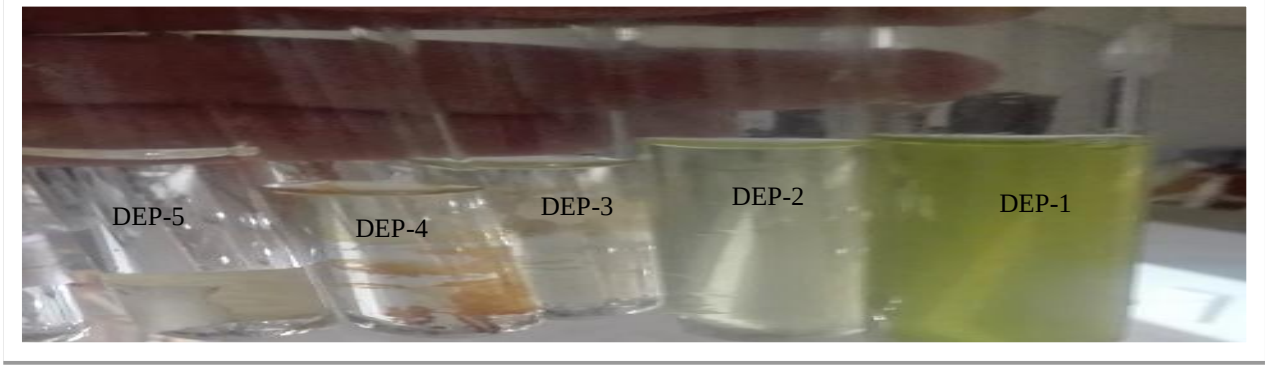

Fig. 1 Preparation of DEP 1 to DEP 5

Managements of SCMV by using different chemicals

The application of 0.1 % citric acid (T1), 0.1 % salicylic acid (T2), 1.5, 3 and 4.5 % potassium sulphate and 0.1 % NPK solution was applied to control the SCMV in vivo condition. A 1g of citric acid, salicylic acid and NPK were dissolved in 1 L water in a beaker and mixed thoroughly. These treatments were applied on mature and immature plants to check their effect on SCMV disease severity.

Results

Incidence assessment of SCMV

Disease incidence data of SCMV of selected locations (Chak 38, UCA, 49 Tail, Sillanawali and Kot Momin) were collected on every 3rd day regularly and noticed in the research folder. SCMV data was collected 10 weeks on selected dates, on the final date compared the mean value of each data, and inserted a graph for their comparisons. The maximum disease incidence percentage 82% SCMV was recorded in Chak 38 followed by 49-Tail 73%, Kot Momin 70%, Sillanawali 66% and UCA 49%, respectively (Fig. 2).

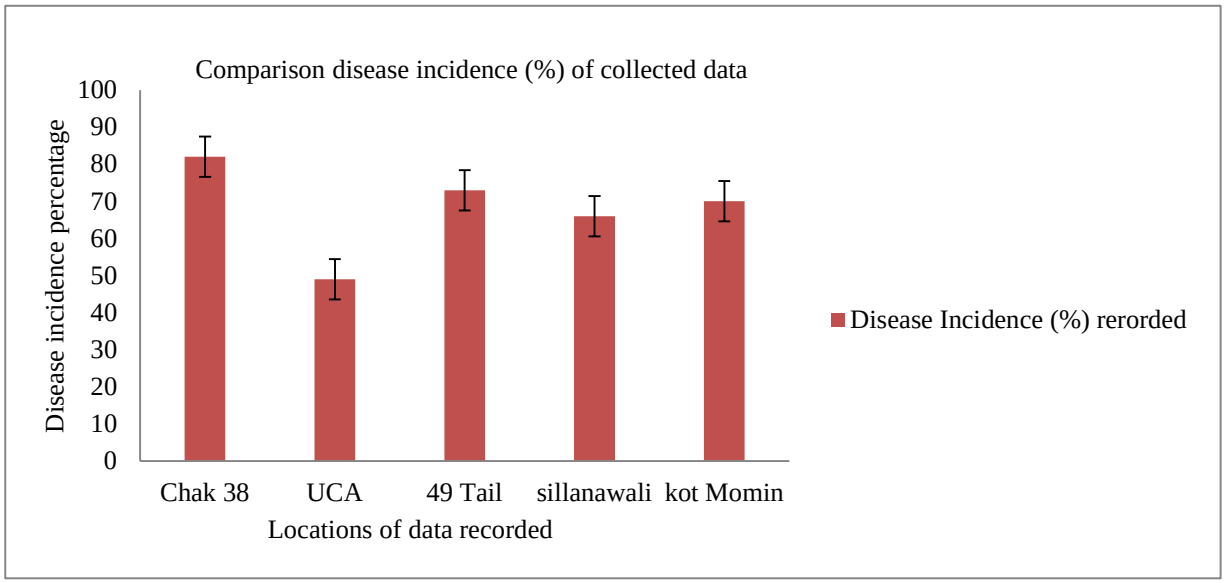

Fig. 2 Graphical representation of SCMV disease incidence (%) of all five locations of Sargodha

Mechanical inoculation of SCMV

Mechanically inoculation of SCMV on healthy plants produces the symptoms of moderate to severe mosaic patterns. The inoculated plant given in Fig. 3 showed the specific symptoms of SCMV after 4-5 weeks in controlled condition. After the 6 week of inoculation, the inoculated plant showed the severe yellowing pattern such in figure portion (d) and produced the mosaic. The yellow appearance due to SCMV is similar to nitrogen deficiency in plants but if we look carefully it is totally the mosaic pattern development due to inoculation results after the 6th week.

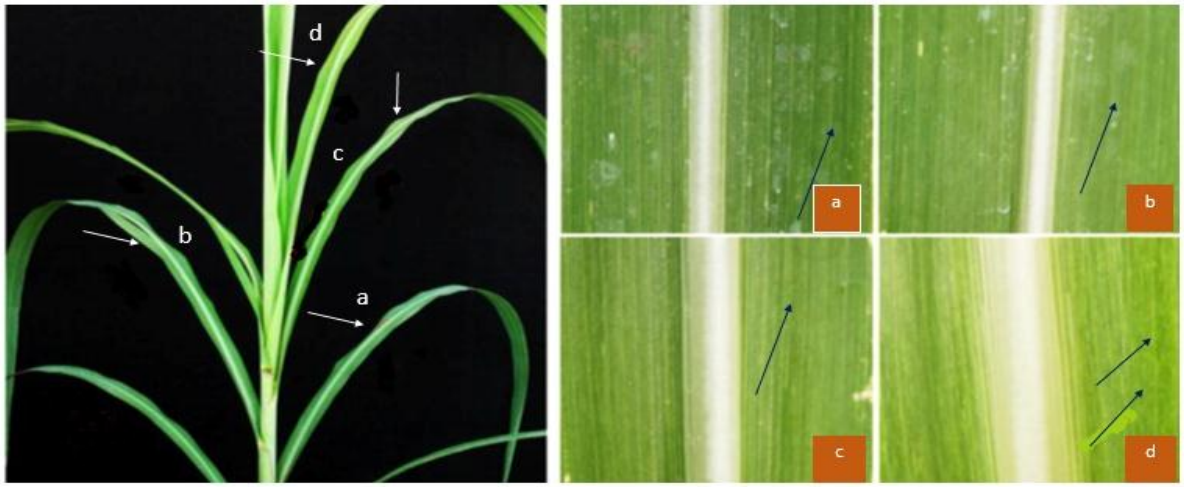

Fig. 3 Mechanical inoculation of SCMV through extracted sap

Longevity of SCMV extracted sap *in vitro*

The application of extracted sap on healthy plants at different time intervals decreased the longevity of the virus. At the initial application, the disease incidence was recorded 100% of inoculated plants at lab condition. However, with the passage of time the longevity of SCMV decreased. The second, third and fourth application of sap produced the 60, 30, 10 percent disease incidence of inoculated plants. Fifth application after the 10 days later of fourth application showed 0% disease incidences of inoculated plants (Fig. 4).

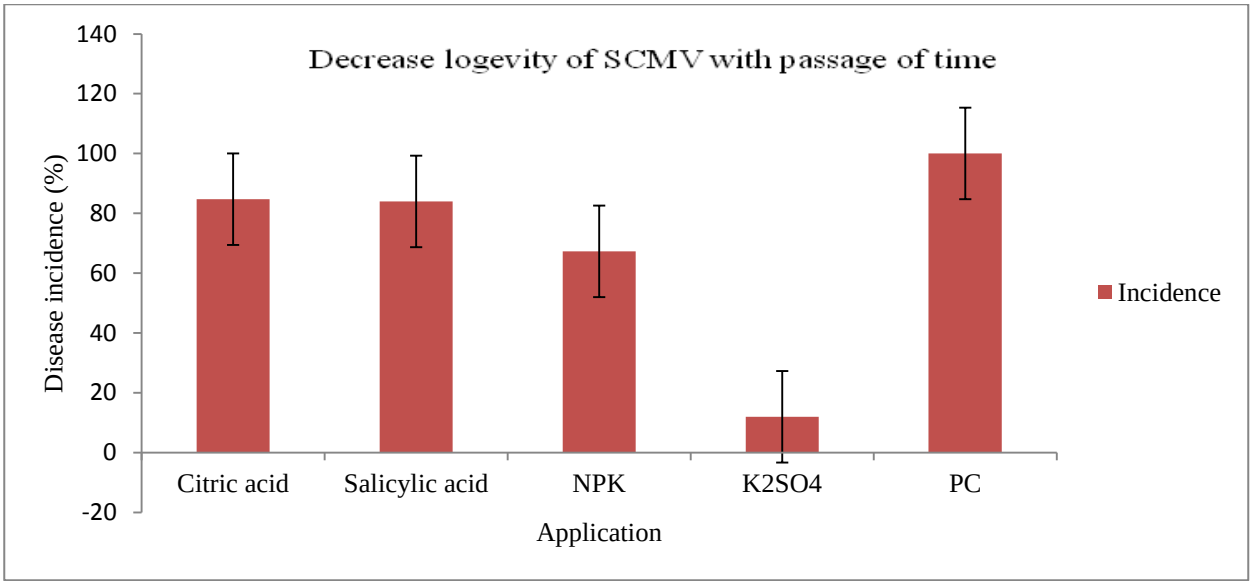

Fig. 4 Graphical representation showed the decreasing rate of SCMV with passage of time

Effect of dilution end point (DEP) on virus infectivity

According to the Teakle and Grylls (1973) if we dilute the virus sample five times, the infectivity of the virus will be reduced significantly. During study we observed that if we inoculated the plant with one time diluted sample of SCMV the disease incidence was recorded 40%. Furthermore, if we diluted the SCMV sample two, three, four and five times, then at 5^{th} DEP the infectivity of diluted sample became 0% and 0% disease incidence was recorded at 5^{th} dilution (Fig. 5).

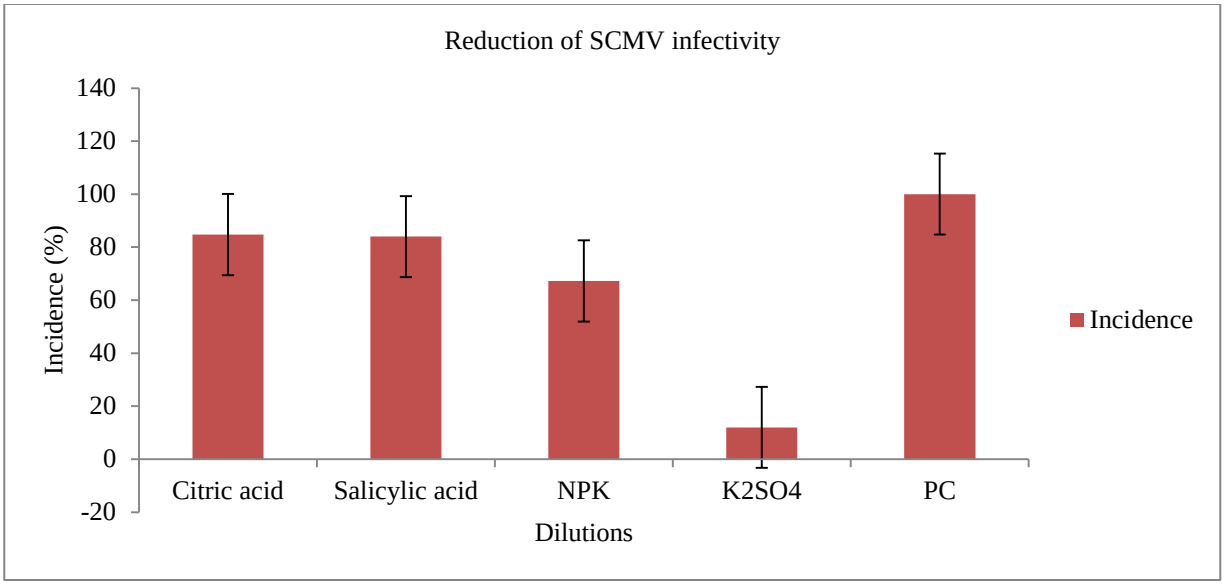

Fig. 5 Graphical representation of dilution end point (DEP) and reduction of SCMV infectivity.

Effect of heating on SCMV infectivity

On heating, the infectivity of the virus has been reduced according to Dijkstra & de Jager (1998); Ahamedemujtaba et al. (2019). During study, the crude sap of SCMV was heated at 40, 50 and 60^{o} C for 10 min in sonicator shaking heating water bath. After the incubation of 10 min in a water bath, the crude sap was applied on healthy plants by mechanically inoculation method to check the infectivity of SCMV samples after heating. The results revealed that on heating, the infectivity of virus has been reduced and disease incidence also decreased. The high temperature more affects the virus infectivity. At 60^{o}C the disease incidence was recorded minimum 18%, at 50 ^{o}C recorded 29% and at 40 ^{o}C was recorded 36%, respectively (Fig. 6).

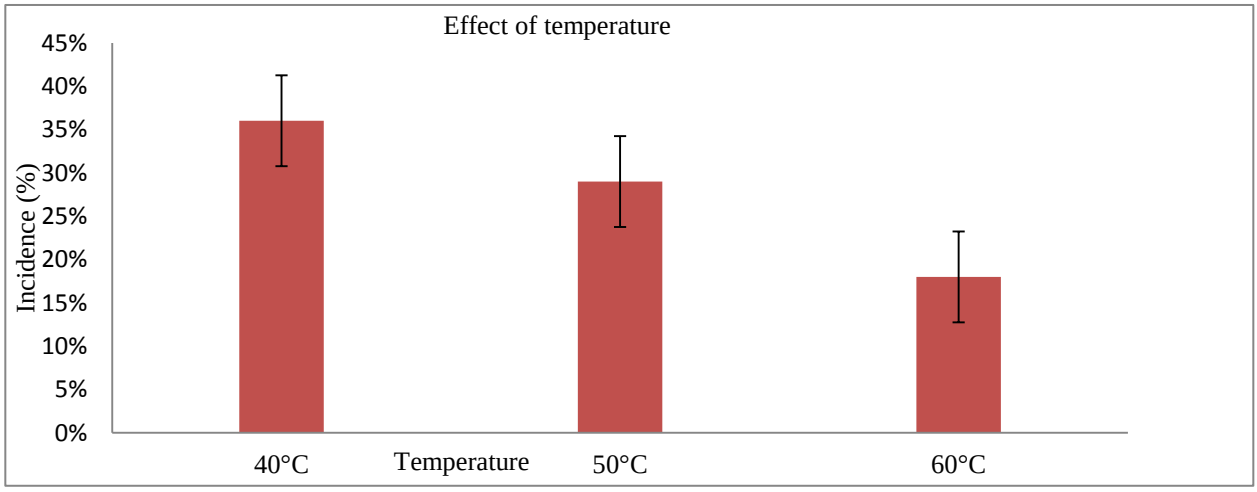

Fig. 6 Graphical representation in reduction of virus infectivity of SCMV crude sap on heating

Effect of reducing agents on virus infectivity

The reducing agent chloroform and carbon tetrachloride (CCl4) were mixed in the crude sap with ratio 2.5: 5 percent (%) and applied on the sugarcane healthy plant to check the infectivity of SCMV in control condition. Both the reducing agents decrease the infectivity rate of SCMV after the third and fourth application as compared to control plants where pure SCMV crude sap applied. Initially, the maximum disease incidence of chloroform 60% and Carbon tetrachloride (CCl4) 40% was recorded as compared to positive control 100% and negative control (DW) 0%, respectively (Fig. 7). After the third and fourth application of reducing agents, mixing crude sap produces normal disease incidence and CCl_4 considered as a best reducing agent as compared to chloroform.

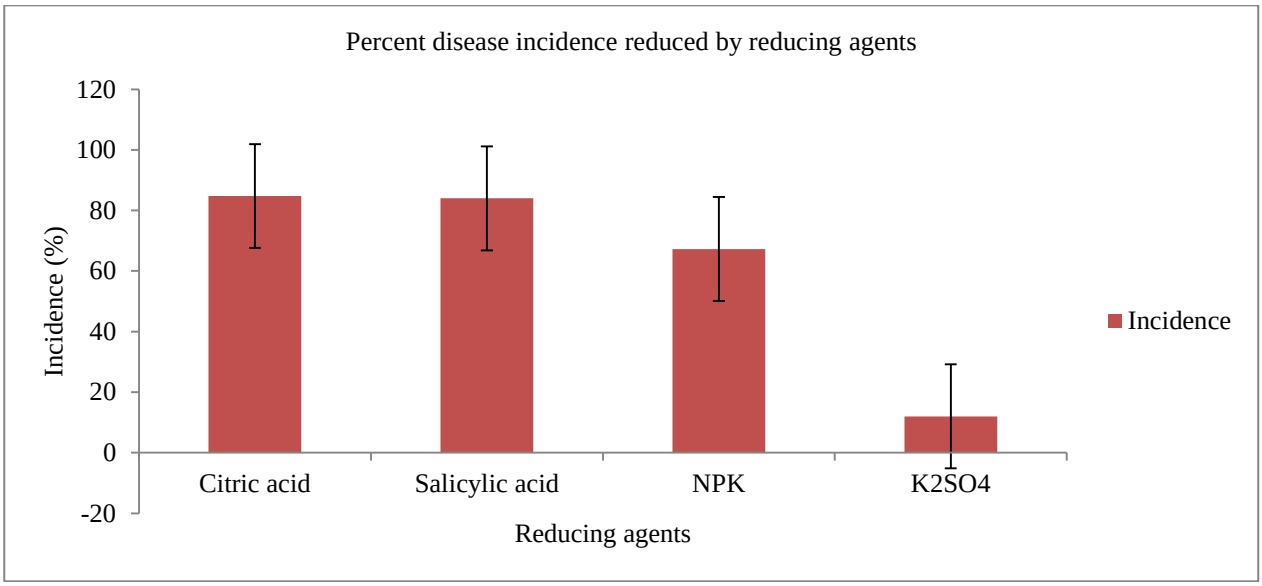

Fig. 7 Graphical representation showed the effect of reducing agents of virus infectivity, which are mixing in crude sap of SCMV

Managements of SCMV

In vivo the application of citric acid, salicylic acid, potassium sulphate and, NPK solution has shown significant results against the SCMV. Each treatment has four concentrations and five times applied on healthy sugarcane selected plants during study. At the final date, the recorded data was summarized and the mean value of each treatment compared with the positive control plant (only SCMV crude sap) and negative control (DW). The maximum disease incidence inhibition was recorded in treatment one (T_1) citric acid 84.77% followed by salicylic acid 84%, NPK 67.27% and potassium sulphate (K_2 SO_4) 12% as compared to positive control 100% and negative control 0% as recorded respectively (Fig. 8).

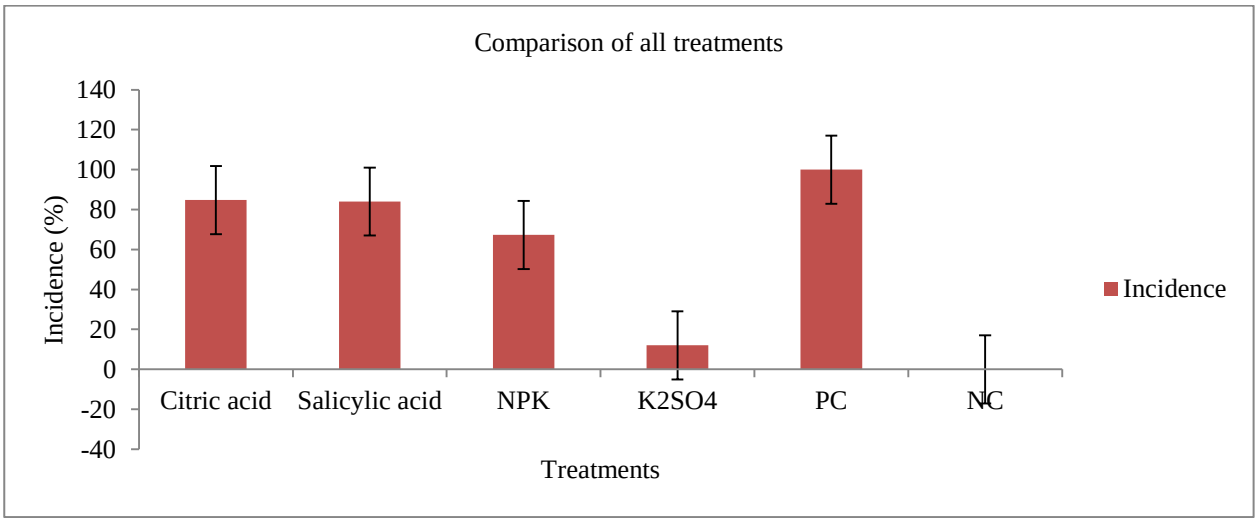

Fig. 8 Graphical representation of all the applied treatments and their inhibition of disease incidence as compared to positive and negative control

Discussion

SCMV is one of the most threatening viral diseases all around the world. It covers more than 107 countries worldwide and no resistant variety of sugarcane is present to control this viral disease (Braidwood et al., 2019). The losses due to this viral disease have been found between 40-70% worldwide (Espejel et al., 2006; Thorat et al., 2015). The vector of this disease spreads the infected sap from one plant to another through a non-persistent manner. The mechanical

transmission of SCMV through sap has been studied previously by many researchers (Adams et al., 2013; Liu et al., 2017; Akbar et al., 2020 a &b). SCMV has a broad host range and spread mostly in the member of Poaceae family (Shukla et al., 1989). Pakistan is included in the top sugarcane production countries. Annual loss due to SCMV disease has found 10-32 percent in Pakistan (Aslam et al., 2018; Ali et al., 2019).

The present study was done to confirm the SCMV infectivity on healthy plants *in vitro* and in vivo. A number of parameters by different ways such as sap inoculation on healthy plants at different intervals, Longevity *in vitro* (LIV), dilution end points (DEP), thermal inactivation point (TIP) and mixing of reducing agents were applied to check the SCMV infectivity *in vitro* conditions. The management of associated viral pathogens was also done through different four chemicals (citric acid, salicylic acid, potassium sulphate and, NPK solution) in vivo. The mechanical inoculation results revealed that the infected crude sap produces the mosaic pattern symptoms after the 5th month of inoculation in control condition. Singh et al. (2005) conducted an experiment to check the mechanical transmission of SCMV and applied the method of Bain's and Matz's. They concluded that the SCMV is mechanically transmitted by crude sap and four different Aphid vector species. The more severe infection of SCMV was found when aphid acquired the virus in just 30 second and transferred within 2 min in the host plant. (Putra et al., 2014) also described the mechanical inoculation and symptoms development of SCMV in cane plants and their management through different aspects.

The longevity of SCMV crude sap with the passage of time becomes decreased. During the present study, we preserve the crude sap at 30 °C for 10 days and observed that after the first application of sap the infectivity of the virus becomes low. In the beginning, the disease incidence rate is high and at fourth and fifth application, the incidence rate reached on zero. Finally, it was concluded that the sap longevity becomes slow and decreases with the passage of time. The longevity of virus became zero after five weeks of regular application in the field (Francki, 1980). The longevity of the virus becomes zero if we preserve it at 25 ºC for 20 days (Parvin et al., 2007). During this study, we also observed that the DEP decreased the infectivity rate of SCMV and resulted in a minimum incidence rate achieved. If we diluted the sap more and more than a time comes when the infectivity of the virus becomes zero and the host plant shows resistance and tolerance and no significant yield loss occurs. The infectivity of the virus through the DEP method became zero at a more diluted concentration point (Bhat & Rao, 2020). The virus infectivity became zero at 7th dilution after 7days of incubation at refrigerator (Sharma et al., 2018). Thermal inactivation point (TIP) of SCMV is between 40-50 ºC.

The present study confirmed the TIP of SCMV and concluded that at the highest temperature (60 ºC), the infectivity of the virus is low, and the maximum incidence rate has been found at 40 ºC and minimum at 60 ºC. The infectivity of the virus maintains at 45-53 ºC but if we crossed the temperature at 53 ºC then the infectivity would become low and decrease the incidence rate of viral disease (Damayanti & Putra, 2010). Most members of Potyvirus have the 50-58 ºC thermal inactivation point and become zero at high temperature from the thermal inactivation point. Reducing agents mixed in crude sap decrease the infectivity rate of SCMV. The reducing agent chloroform and CCl_4 were mixed in the crude sap with ratio 2.5: 5 percent (%). Both the reducing agents decrease the infectivity rate of SCMV after third and fourth application as compared to control plants where pure SCMV crude sap applied. Initially, the maximum disease incidence of chloroform 60% and CCl_4 40% was recorded as compared to positive control 100% and negative control (DW) 0%, respectively. After the 3rd and 4th application of reducing agents, mixing crude sap produces normal disease incidence and CCl_4 considered as a best reducing agent as compared to chloroform.

Elsharkawy and El-Sawy (2015) conducted an experiment and concluded that the mixing of plant extracted as biocontrol agent and chemicals in infected virus crude sap decrease the virus infection rate. Many other studies have shown the positive results when mixed the infectious crude sap with other reducing agents (Esseili et al., 2012; Beris et al., 2018; Al-Snai, 2019; Radwan & Ismail, 2020). In vivo the application of citric acid, salicylic acid, potassium sulphate and, NPK solution has showed a significant results against the SCMV. Each treatment has different four concentrations and five times applied on healthy sugarcane selected plants during study. At the final date, the recorded data was summarized and the mean value of each treatment compared with the positive control plant (only SCMV crude sap) and negative control (DW). The maximum disease incidence inhibition was recorded in treatment one (T1) citric acid 84.77% followed by salicylic acid 84%, NPK 67.27% and potassium sulphate (K_2 SO_4) 12% as compared to positive control 100% and negative control 0% as recorded respectively. Salicylic acid induced resistance in maize and sugarcane plant against SCMV in vivo during study (Yuan et al., 2019). The treatment of seeds with salicylic acid reduced the infection rate against the virus diseases (Mardani-Mehrabad et al., 2020).

Conclusion

The present research study concludes that the longevity of SCMV reduced with passage of time and dilution endpoint affected the infectivity of SCMV. During this study, the maximum reduction of virus infectivity was recorded by the reducing agent carbon tetrachloride (CCl_4). The maximum disease incidence inhibition was recorded in treatment one (T_1) citric acid 84.77% followed by salicylic acid 84%, NPK 67.27% and potassium sulphate (K_2 SO_4) 12% as compared to positive control 100% and negative control 0%.

Authors Contribution: A.A. conducted the research experiments. M.A.Z. supervised the research work. Y.I. contributed in laying out research work. M.U.G. helped to arrange the data for statistical

analyses. M.A.A. contributed in the collection of references, description and arrangement of the manuscript. M.A. contributed in editing the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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