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CHEMOTHERAPY OF TOMATO MOSAIC DISEASE USING RIBAVIRIN AND 8-AZAGUANINE

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ABSTRACT

Tomato mosaic disease (ToMD) caused by Tomato mosaic virus (ToMV) is a major threat to tomato production globally. In Pakistan, tomato is one of the most popular vegetables, and ToMD causes significant losses in fruit production, quality, and size. Various management strategies are implemented to control the disease. In this study, the efficacy of chemotherapy using ribavirin and 8-azaguanine was investigated. Seedlings of tomato plants were inoculated with sap from symptomatic tomato plants using the sap transmission technique to test the pathogenicity of the virus. Disease incidence and severity were recorded at weekly intervals for one month after inoculation. Ribavirin and 8-azaguanine were applied to tomato plants at different concentrations, and disease incidence and severity were observed weekly for one month after treatment. The results showed that the mechanically inoculated plants exhibited characteristic symptoms of ToMD, such as a mosaic pattern of color on the leaves. The disease incidence did not reduce with the application of ribavirin and 8-azaguanine, but the severity of the disease was significantly reduced. The plants treated with 1000 µM of ribavirin and 8-azaguanine had significantly lower severity scores compared to the untreated plants. However, the treated plants did not recover from the disease. The study found that ribavirin and 8-azaguanine are effective chemotropic agents in reducing the severity of ToMD, although they cannot cure the disease completely. Further studies are required to investigate the underlying mechanism of how ribavirin and 8-azaguanine suppress the symptoms of ToMD.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetables in the world, including Pakistan. In Pakistan, the tomato crop was harvested on 57.838 thousand hectares in 2020, yielding an average of 10,273 kg/ha and 594.21 million tons of tomatoes (FAOSTAT, 2020). Tomatoes are known for their health advantages (Cammarano et al., 2020). However, tomatoes are known to contain numerous components that are crucial in the prevention of cancer, heart disease, cataracts, and many other common health issues. People have discovered the value of including tomatoes in a balanced diet (Olaleye et al., 2014). After potatoes, tomatoes are the second most popular vegetable crop cultivated worldwide (Agarwal et al., 2020). Tomatoes and tomato-based products account for a significant portion of global agricultural output. Over 80% of the tomatoes cultivated globally are processed into tomato juice, paste, puree, ketchup, sauce, and salsa. Vitamins and phytochemicals are abundant in tomato fruit (Viskelis et al., 2015).

It is commonly used as a source of vegetables for a daily diet, eaten both raw and cooked, and used to make soups, salads, preserves, pickles, sauces, and many other items. It contains essential macro- and micronutrients, such as carbohydrates, proteins, lipids, minerals, and vitamins (Talvas et al., 2010). In 2020, 5.052 million hectares of the tomato crop were harvested worldwide, with a yield of 186.821 million tons (FAOSTAT, 2020). Many biotic and abiotic factors can affect tomato crops. Viral infections continue to be a significant factor limiting its production among biotic stressors, with estimates for quantitative and qualitative yield losses in tomato harvests frequently reaching up to 100% (Oladokun et al., 2019).

One of the most devastating diseases, tomato mosaic disease (ToMD), which is induced by tomato mosaic virus (ToMV, Genus: Tobamovirus, Family: Virgaviridae), causes major losses to tomato fruit production, quality, and size globally (Aseel et al., 2019). ToMD in India reduced the weight and number of tomatoes by 59.77 percent and 34.30 percent, respectively (Giri and Mishra, 1990).

Initially, ToMD was reported in the Netherlands in 1910 and in the USA in 1916 (Broadbent, 1976). ToMD commonly occurs in Pakistan (Imran et al., 2012). ToMV contains a linear, single-helical RNA genome. Its particle structure is rod-shaped, and the virus is around 300 nm long and 18 nm wide. Protein makes up 95% of virus particles, with 5% nucleic acid. The genome is monopartite (Silva et al., 2011). ToMV has a broad host range and is mainly transmitted through mechanical contact with contaminated cultivating instruments, farmer activities, or diseased plants (Aseel et al., 2019). Additionally, biological properties, such as symptomatology, transmission assays, and amplification of coat protein and nucleic acid, are used to confirm viral infection in plants as an intrinsic property (Naidu and Hughes, 2001).

On susceptible tomato plants, ToMV can cause internal browning, mosaic, curling, and distortion of leaves, as well as deformation of fruits (Kumar et al., 2011). The severity of the disease can be conveniently determined based on its characteristic symptoms and is strongly correlated with crop yield losses (Cooke et al., 2006).

Although the major methods for managing ToMD are removing contaminated plants from the area, using seeds or seedlings that have been proven to be virus-free, and choosing cultivars of plants that are resistant to the virus (Aseel et al., 2019), certain chemical compounds are also employed to treat plant viruses (Yang et al., 2014; Fan et al., 2011; Hernández et al., 2017; Sekerz et al., 2015; Balamuralikrishnan et al., 2002).

Research supports the fact that ribavirin and 8-azaguanine are potential antiviral agents that can be used to prevent viral infection in tomato plants. Therefore, the following goals were set for this study: to evaluate the efficiency of ribavirin and 8-azaguanine against ToMD in tomato plants grown in greenhouses.

MATERIALS AND METHODS

The experiment was conducted at the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam to evaluate the effectiveness of different concentrations of ribavirin and 8-azaguanine on the development of tomato mosaic disease under greenhouse conditions.

Pathogenicity test for confirmation of viral infection at test plants using mechanical transmission technique

Tomato seedlings were raised in earthen pots and protected in insect-rearing cages to prevent cross-contamination and insect infestation. At 30 days of age, the seedlings were inoculated with tomato leaf sap containing tomato mosaic disease symptoms using the rubbing method. The sap was obtained by grinding five grams of ToMV-infected leaf tissue in a sterile pestle and mortar and adding 10 ml of phosphate buffer. Carborundum powder was used to provide minute injuries to facilitate the entry of virus particles into the test plants. Tap water was used to remove any extra traces from the wounds. The plants were then moved into the dark after leaf inoculation to allow for symptom development, as described by Akbar et al. (2015). In the control group, plants were inoculated with sterilized distilled water instead of sap. The experiment was repeated three times. The plants were observed until symptoms appeared. The incidence and severity of symptoms were recorded weekly for one month after sap inoculation. Disease incidence was calculated using the following formula:

$$\text{DI (\%)} = \frac{Xi}{Xn} \times 100$$

Where, *Xi*= Number of symptomatic tomato plants
Xn= Total number of tomato plant
The disease severity was also recorded using 0-4 disease rating scale (Matsuura et al*.,* 2014). Where,
0= No symptoms,
1= slight yellowing and a few necrotic spots,
2= distinct yellowing and necrotic spots,
3= more severe yellowing and necrotic spots with dwarfing,
4= seedling dead.

Preparation of phosphate buffer

1.781 g of Sodium phosphate monobasic (Na2HPO2.2H20) was dissolved in 1L of deionized water (dH_2O) to make the Na solution and 1.362 g of Potassium hydrophosphate ($KH_2PO_4.2H_20$) was dissolved in 1L of deionized water (dH_20) to constitute the K solution. Phosphate buffer was then made by adding 51 ml of the Na solution to 49 ml of the K solution, as reported by Rizk et al. (2020).

Effect of ribavirin and 8-azaguanine on the development of Tomato mosaic disease

Various doses of ribavirin and 8-azaguanine such as 100, 500 and 100 µM were sprayed on the tomato plants. About one month prior to the treatment of ribavirin and 8-azaguanine, the tomato plants were inoculated with sap obtained from symptomatic tomato as described above. There were two control panels: in one panel, plants were inoculated simultaneously with sap from symptomatic tomato plants, and in another panel, plants were treated with sterilized distilled water instead of sap. The experiment was repeated three times. There were three plants per repeat. On a weekly basis following the application of treatments, observations were taken on the incidence and severity of tomato mosaic disease as described above.

Data analysis

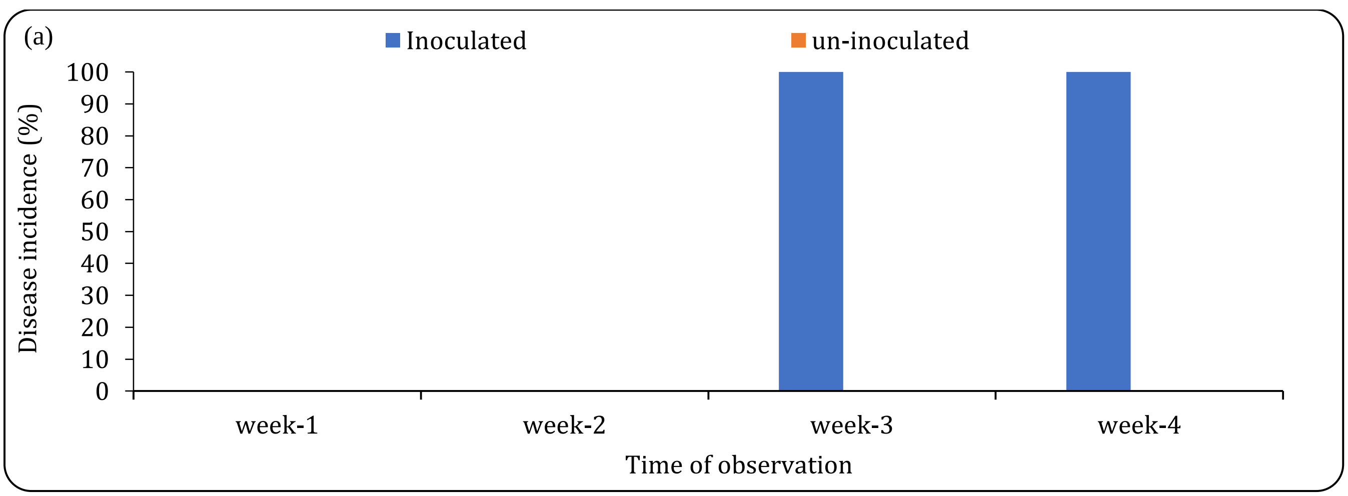
Mean values of the treatments were compared for significant differences using the LSD test (α = 0.05) of a completely randomized design, using STATISTIX v. 8.1 software (Analytical Software).

RESULTS

The development of tomato mosaic disease on mechanically inoculated plants and the effectiveness of different concentrations of ribavirin and 8-azaguanine were tested in greenhouse conditions. The obtained results are presented here.

Development of tomato mosaic disease on mechanically inoculated tomato plants

The plants that were inoculated with sap from symptomatic plants showed characteristic symptoms of the disease, such as a mosaic pattern of color on leaves, while un-inoculated plants did not exhibit any symptoms. No symptoms were observed in the inoculated plants in the 1st and 2nd week after inoculation. Mechanically inoculated plants showed 100% incidence and a severity score of 1 for tomato mosaic disease in the 3rd week of inoculation (Figure 1a and b). The incidence of tomato mosaic disease was the same (100%) in the 4th week of observation, but the severity score had increased up to 3 (Figure 1a and b). However, un-inoculated plants did not show any symptoms of tomato mosaic disease at any assessment time point (Figure 1a and b).



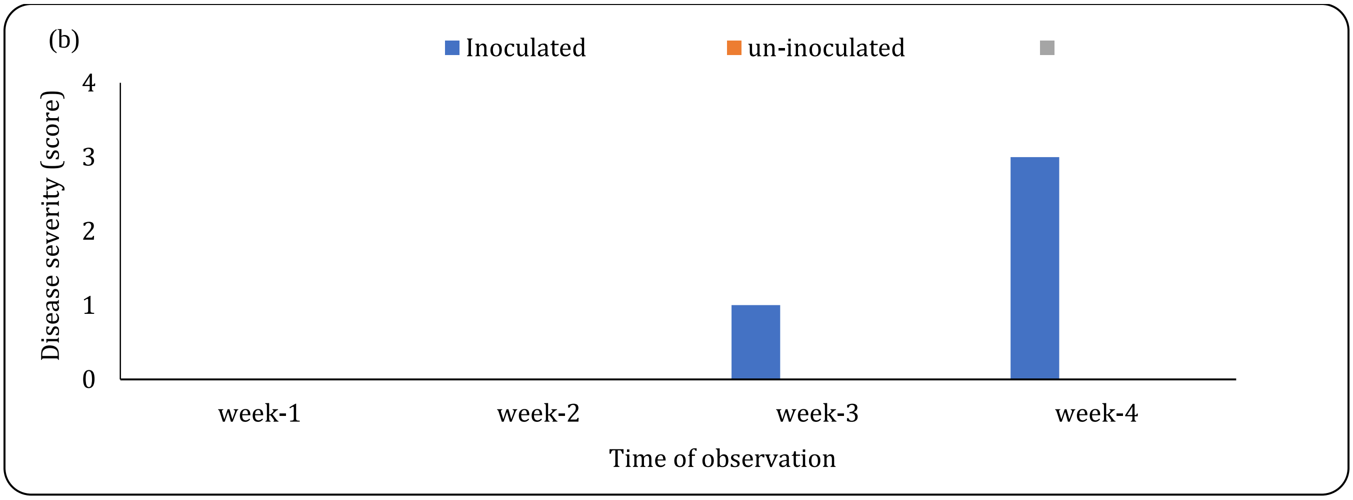


Figure 1: Incidence (%) (a) and severity score (b) of tomato mosaic disease in inoculated and un-inoculated tomato plants at different time points.

Incidence of tomato mosaic disease at different concentration of ribavirin and 8- azaguanine

Before applying different concentrations of ribavirin and 8-azaguanine, plants were inoculated with sap from diseased plants. The obtained results showed 100 ± 0% incidence of tomato mosaic disease from week 1 up to week 3 of observation (Figure 2 and Plate 1). Similarly, inoculated but untreated plants also showed 100 ± 0% incidence of the disease (Figure 2). Un-inoculated plants did not show any symptoms until the 3rd week of observation (Figure 2). The results showed that there was a significant difference in incidence among all treatments (DF=7, F=6.8, P=0.000).

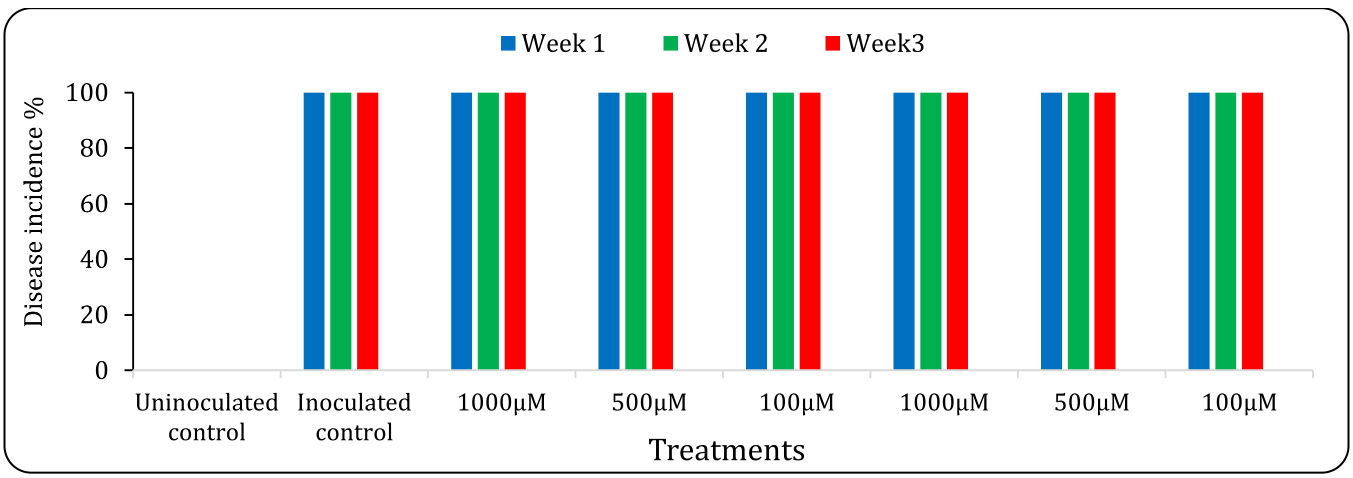


Figure 2: Effect of ribavirin and 8-azaguanine at different concentrations on the incidence (%) of tomato mosaic disease at different time points.

Severity of tomato mosaic disease at different concentrations of ribavirin and 8-azaguanine

While incidence was recorded after one month of mechanically inoculated plants to evaluate the effects of different concentrations of ribavirin and 8-azaguanine, the severity of tomato mosaic disease was also recorded. Statistical analysis showed that there was a significant difference between the treatments (DF = 7, F = 22.85, P = 0.0000) and in the interaction of treatment × week (DF = 14, F = 2.22, P = 0.0217). Significantly lower severity scores of 1±0 and 1±0.4 were recorded in 1000 µM of ribavirin and 8-azaguanine, respectively, at the 3rd week of observation (Figure 3). However, when the same plants were observed to record the severity score at the

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1st week of treatment, severity scores of 2±0.43 and 2±0 were recorded (Figure 3). In the remaining treatments, either 500 µM or 100 µM of ribavirin and 8-azaguanine showed an increasing trend of severity score from the 1st week of observation to the 3rd week, as in the inoculated plants (Figure 3).

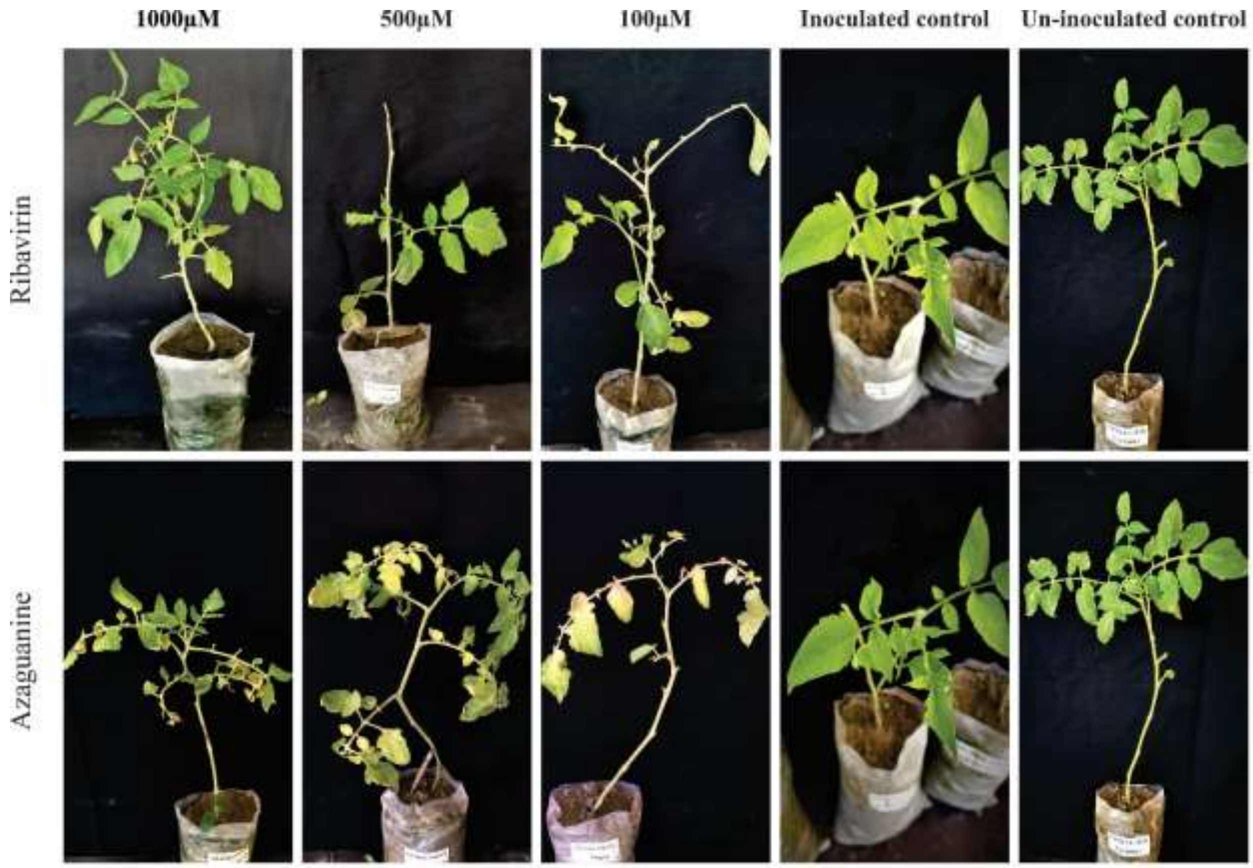

Plate 1: Symptoms of tomato mosaic disease under treatment of different concentrations of ribavirin and 8-azaguanine at different time points.

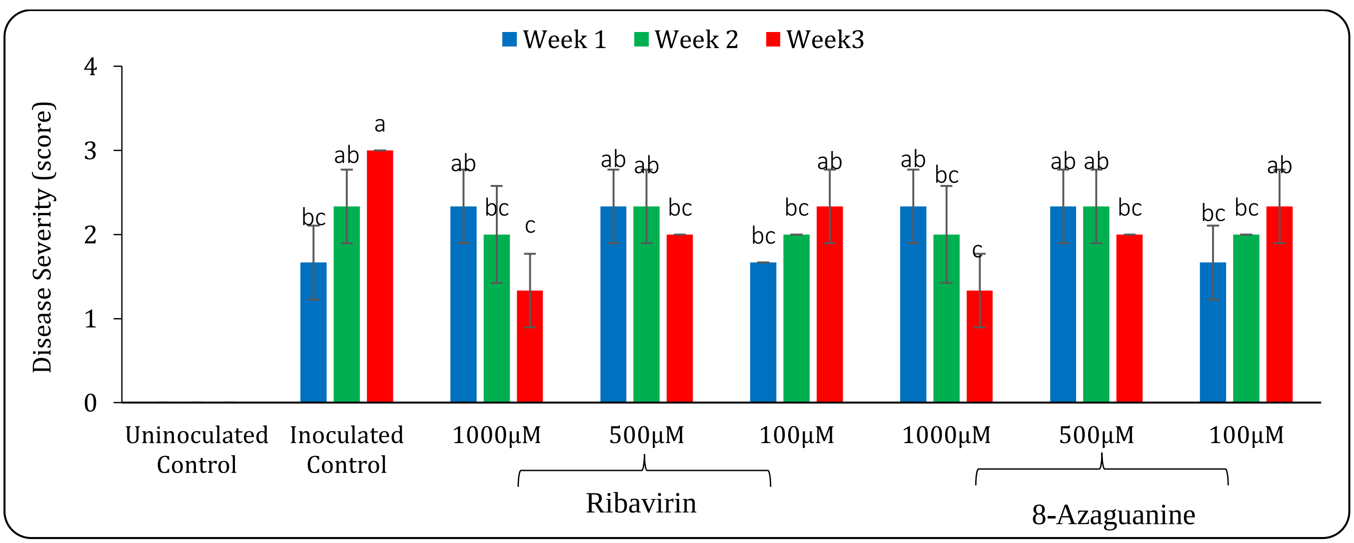

Figure 3: Effect of ribavirin and 8-azaguanine at different concentrations on the severity (score) of tomato mosaic disease at different time points.

DISCUSSION

Tomato mosaic disease (ToMD), caused by tomato mosaic virus (ToMV, Genus: Tobamovirus), can lead to significant losses in tomato production in terms of both quality and quantity (Aseel et al., 2019). The disease is commonly observed in Pakistan (Imran et al., 2012). Growers in the Sindh province are facing issues with tomatoes exhibiting symptoms such as internal browning, mosaic, curling, and leaf distortion, as well as fruit deformation and distortion, which are associated with ToMD. However, in the present study, we observed through pathogenicity tests that mechanically inoculated plants showed 100% incidence and a severity score of 1 of tomato mosaic disease at the 3rd week of inoculation. The severity of the disease showed progression until the 4th week of observation, up to a score of 3. In comparison, un-inoculated plants did not exhibit any symptoms of the disease. ToMV can cause mosaic, stunting, and leaf deformation in susceptible tomato plants (Broadbent, 1976). Chitra et al. (1999) and Hoon and Jin (2002) also reported that tomato plants infected by ToMV exhibit symptoms including leaf necrosis, mosaic mottling, deformation, and yellowing. On mechanical inoculation, tomato plants showed typical symptoms of ToMD, while such symptoms were not observed in the un-inoculated control. Therefore, it can be concluded that the expression of such symptoms was associated with ToMV infection.

Numerous management strategies are employed to manage ToMD, such as removing contaminated plants from the area, using seeds or seedlings that have been proven to be virus-free and choosing cultivars of plants that are resistant to the virus. These are the major methods for managing ToMD (Aseel et al., 2019). However, certain chemical compounds have been used to treat plant viruses (Yang et al., 2014; Fan et al., 2011; Hernández et al., 2017; Şekerz et al., 2017; Balamuralikrishnan et al., 2002). In the current study, we used ribavirin and 8-azaguanine to manage ToMD. The obtained results showed that neither of the tested chemicals was effective in curing the plants of the disease, but the severity of the disease was reduced with the application of these chemicals. Such findings have been reported for many pathosystems. For example, Spak et al. (2010) showed that ribavirin has the most powerful antiviral effect against TYMV. Similarly, Fan et al. (2011) studied the activity of O, O'-diisopropyl (3-(L-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl) thioureido) (phenyl) methyl phosphonate (2009104) against tobacco mosaic virus (TMV) using the half-leaf method. They reported it as an effective compound for TMV through inhibiting the polymerization process. Likewise, Yang et al. (2014) investigated the effect of MS medium amended with ribavirin on the growth of the plantlets and the efficiency of eliminating single infection of VA, PLRV, PVS, PVX, and PVY and mix-infection of PVM, PVS, and PVY.

Significant results were recorded for eliminating these viruses with the application of ribavirin. Furthermore, no change in the genetic makeup was observed in the potato plants due to the application of ribavirin (Singh, 2015). Perez et al. (2017) compared two different antiviral compounds viz., Inhibitovir and Q-2000VI, against PRSV before and after the development of symptoms under field conditions. They concluded that the application of Inhibitovir can prevent loss induced by PRSV and reduce symptom development. Şekerz et al. (2017) have reported that Plum pox potyvirus (PPV) is the most severe viral disease affecting stone fruits of the *Prunus* genus, and it may be completely eradicated utilizing *in vitro* applied procedures. Therefore, by treating infected apricot shoot tips *in vitro*, the possibility for using thermotherapy, chemotherapy, and cryotherapy procedures was evaluated. While chemotherapy and cryotherapy showed promising results, the medium containing quercetin produced the highest survival of the shoot tips (100%) while ribavirin produced a considerably lower survival (80%). Inclusion of 8-azaguanine was also discovered to be phytotoxic because no survival was attained following treatment. Cryopreserved apricot shoot tips showed a 10% survival rate when using the two-step freezing approach in the cryotherapy case. Chemotherapy and cryotherapy might be used to *in vitro* remove PPV from infected apricots and other stone fruits, according to an RT-PCR study that revealed PPV was abolished in the survivors after those treatments.

CONCLUSION

According to the results of our study, ribavirin and 8-azaguanine have been shown to be effective chemotropic agents in reducing the severity of Tomato Mottle Disease (ToMD). However, it is important to note that these chemical compounds cannot completely cure the disease. While the findings are promising, there is still a need for further research to fully understand the mechanisms through which ribavirin and 8-azaguanine work to suppress the symptoms of ToMD. Such research can help to shed light on the potential for developing even more effective management strategies for this disease.

AUTHORS' CONTRIBUTIONS

JDH, ZAN and AAG conceived and designed the experiments, MSK performed the experiments, JDH, MSK, AAG and GHJ analyzed the data, ASK contributed materials/analysis/tools, MSK, MAK and JDH wrote the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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