Iran Agricultural Research (2024) 43 (2) 87-93.

Iran Agricultural Research

Journal homepage: https://iar.shirazu.ac.ir

Effects of aqueous extracts and total protein content from different plant species on the inhibition of Tobacco mosaic virus infection

Fatemeh Kahani[a], Majid Siampour[a*], Masoud Ghasemi Ghehsareh[b]

[a] Department of Plant Protection, College of Agriculture, Shahrekord University, Shahrekord, I. R. Iran
[b] Department of Horticultural Sciences, College of Agriculture, Shahrekord University, Shahrekord, I. R. Iran

ARTICLE INFO

Keywords:
Antiviral activity
Glutinosa
Local lesion
Plant extract
Systemic

ABSTRACT- This study examined the inhibitory properties of aqueous extract and total protein of four plant species: carnation (*Dianthus caryophyllus*), Indian privet (*Clerodendrum inerme*), prickly pear cactus (*Opuntia stricta*), and periwinkle (*Catharanthus roseus*) against Tobacco mosaic virus (TMV). The aqueous extract and total protein from carnation, Indian privet, and cactus significantly reduced TMV infection in the local lesion host, *Nicotiana glutinosa*. Conversely, the periwinkle extract did not demonstrate a significant inhibitory effect on TMV. Results showed that the inhibitory effects of carnation and Indian privet were more effective than those of the cactus extract. The inhibitory effects of the plant extracts applied on the *N. glutinosa* leaves diminished after washing with water. Bioassay tests using total protein from carnation, Indian privet, and cactus indicated that the inhibitory effects of these proteins were comparable to those of their respective aqueous extracts. Thus, it can be inferred that the inhibitory agents present in these plants are proteins. The findings from both the aqueous extracts and the proteins extracted from carnation, Indian privet, and cactus suggest that the inhibitory effects of these substances are localized and not systemic, being confined to the leaves that were treated either simultaneously or prior to the virus inoculation. When *N. tabacum* (var. Turkish), a systemic host, was inoculated with TMV mixed with these plant extracts, there was no evidence of inhibition or a reduction in the incubation period. Therefore, using these extracts to manage TMV infections in its main systemic hosts may be ineffective.

INTRODUCTION

Given the limited availability of commercial antiviral pesticides, viral disease management primarily depends on developing resistant plant varieties and adopting disease avoidance strategies such as vector control using insecticides and planting virus-free seeds. These strategies include controlling vectors by insecticides and planting virus-free seeds. While developing resistant varieties is essential, it can be costly and time-consuming. Additionally, the use of insecticides against viral vectors poses significant environmental risks. Consequently, there has been sustained interest in the production and application of low-risk biologically-based compounds for the management of viral diseases (Verma and Baranwal, 2011; Hull, 2013). Using plant extracts or their protein derivatives with proven antiviral activity could be considered as an effective and economical approach for controlling viruses. These natural compounds may exert their effects against viral diseases through multiple mechanisms such as neutralizing the virus at the infection site or enhancing the plant's resistance (Verma and Baranwal, 2011). Most studies have focused on the impact of plant extracts on human and animal viruses, while less research has been conducted on the impact of these compounds on plant viruses (Verma and Baranwal, 2011). To date, a significant number of plant proteins with inhibitory effects against plant viruses have been identified. These include pathogenesis-related proteins, ribonucleases, and ribosome-inactivating proteins (Pushpa et al., 2013; Wong et al., 2014). In general, plant antiviral proteins can be categorized into two main groups based on their mode of action: 1) compounds that affect the virus in the extracellular environment or at the plant surface and 2) those that affect the virus intracellularly. The latter group can be further divided into two subgroups. The first includes proteins with ribosome-inactivating property that inactivate the ribosomes of infected host cells. The effect of these proteins induces local resistance and is most effective when applied simultaneously with or before virus inoculation on the plant. The second subgroup includes those that induce systemic antiviral resistance and must be applied several minutes to hours before virus inoculation (Verma and Baranwal, 2011; Duarte et al., 2021).

Previous studies have identified antiviral proteins in extracts from various plant species. Notable examples include the root extract of *Boerhavia diffusa* and leaf extracts from *Clerodendrum aculeatum*, *Bougainvillea spectabilis* (paper flower), *Cuscuta reflexa* (dodder), and *Chenopodium quinoa*. The protein extracts from these plants have shown activity against several plant viruses. The antiviral activity of these plant proteins has been mainly evaluated by quantifying local lesions induced by the virus in designated hosts, commonly referred to as local lesion hosts (Verma and Baranwal, 2011; Awasthi and Verma, 2006; Awasthi et al., 2009; Biniaz et al., 2023).

*Corresponding author: Associate Professor Department of Plant Protection, College of Agriculture, Shahrekord University, Shahrekord, I. R. Iran.
E-mail address: siampour@sku.ac.ir
https://doi.org/10.22099/iar.2025.51800.1652
Received 28 November 2024; Received in revised form 09 January 2025; Accepted 12 January 2025
Available online 29 March 2025

The present study investigates the effectiveness of aqueous extracts and proteins derived from prickly pear cactus (*Opuntia stricta*), carnation (*Dianthus caryophyllus*), periwinkle (*Catharanthus roseus*), and Indian privet (*Clerodendrum inerme*) against Tobacco mosaic virus (TMV), a model virus of significant economic importance, in both local lesion and systemic hosts. Previous studies have shown that some of these plant species, or closely related ones, possess antiviral properties. For example, extracts from *Opuntia streptacantha* have been effective against various human and animal viruses (Ahmad et al., 1996). Recent studies have highlighted the local inhibitory effects of extracts and proteins derived from *O. ficus-indica* on several plant viruses, including *Cucumber mosaic virus* (CMV) and TMV (Rasoulpour et al., 2017; Rasoulpoor et al., 2018). However, there are currently no confirmed reports regarding the effects of extracts from the prickly pear cactus species *O. stricta* or periwinkle on plant viruses. The systemic antiviral activity of extracts and proteins from *C. inerme* against several viruses, including TMV, has been reported. Moreover, the local antiviral properties of extracts and proteins from carnation (*D. caryophyllus*) against various plant viruses have also been established (Weintraub et al., 1952; Cho et al., 2000; Mahdy et al., 2007; Duarte et al., 2021). This study evaluates (or reevaluates) and compares the TMV inhibitory activities of extracts from various plant species in both local and systemic hosts, while also examining their modes of action as observed in this study and those reported in previous studies.

MATERIALS AND METHODS

Virus source

In this experiment, a Shiraz isolate of TMV maintained on *Nicotiana tabacum* var. Turkish (the systemic host) was used.

Plants used for antiviral testing

The anti-TMV activity of four plant species was examined: *Catharanthus roseus* (periwinkle), *Clerodendrum inerme* (Indian privet), *Dianthus caryophyllus* (carnation), and *Opuntia stricta* (prickly pear cactus). Periwinkle plants were obtained from seeds in the greenhouse at Shahrekord University. Indian privet was obtained from an urban green space in Ahvaz, where cuttings were rooted and subsequently maintained in the Shahrekord University greenhouse. Carnation was obtained from local greenhouses in Shahrekord. A sample of prickly pear cactus was collected from an urban green space in Isfahan and used after propagation. The effects of aqueous extracts and total proteins from these plants against TMV were investigated in *N. glutinosa* (a local lesion host) and *N. tabacum* var. Turkish (a systemic host) (Adams and Antoniw, 2006).

Determining antiviral activities in local lesion hosts

N. glutinosa plants were grown from seeds in pots. The inoculum for TMV was prepared by extracting fresh tissue from Turkish tobacco infected with TMV, using a 100 mM phosphate buffer at pH 7. Fresh leaves of periwinkle, carnation, Indian privet, and cladodes of prickly pear cactus were extracted in phosphate buffer to evaluate their antiviral properties (Rasoulpour et al., 2018).

Moreover, to evaluate the inhibitory effects of plant proteins, total proteins from the leaf tissue of Carnation, Indian privet, and prickly pear cactus were extracted using ammonium sulfate, following the modified method described by Park et al. (2015). Specifically, 10 grams of fresh plant tissue were extracted in 50 ml of extraction buffer (37.5 mM Tris pH 7.5, 50 mM sodium chloride, 15 mM EDTA, 75 mM sodium citrate and 0.2% sodium bisulfite). The extract was then centrifuged at 8000 rpm for 25 minutes. The pH of the supernatant was adjusted to 5.1 by adding acetic acid. The solution was subsequently centrifuged at 10,000 rpm for 30 minutes, and the pH of the supernatant was adjusted to 7 using 3 M Tris. Ammonium sulfate was added to the solution to a final concentration of 70%. After stirring overnight at 4 °C, the resulting solution was centrifuged at 10,000 rpm for 30 minutes. The obtained pellet was dissolved in 5 ml of extraction buffer and centrifuged at 10,000 rpm for 30 minutes. The supernatant was then dialyzed twice for one hour and a third time for eight hours against fresh phosphate buffer at 4 °C.

To evaluate the antiviral activity of aqueous and total protein extracts from treatment plants, as well as their mode of action against TMV in *N. glutinosa*, six different experiments were conducted (local lesion tests, sections 3-1 to 3-6). In these experiments, extracts or plant proteins and the TMV inoculum were mechanically rubbed onto the leaves of the *N. glutinosa* plants by hand. Additionally, for TMV inoculation, dusting with carborundum was performed. The TMV inoculum used in each of these six experiments was identical. Five days after inoculation with TMV, the number of local lesions for each treatment was counted and statistically analyzed. Local lesion tests were conducted using either half-leaf or whole-leaf assays. Each experiment was performed on a minimum of three *N. glutinosa* plants. The percentage of TMV inhibition (I) was calculated using the formula $I = (1 - T/C) \times 100$, where C and T represent the average number of local lesions in the control and treated leaves, respectively (Verma et al., 1996). Unless mentioned otherwise, the concentrations of the plant extracts and the TMV inoculum used in the experiments were 200 and 100 mg/ml, respectively.

Effect of extracts on the inhibition of TMV infection in vitro

The TMV inoculum (100 mg/ml), was mixed in a 1:1 ratio with extracts from each treatment plant (200 mg/ml). The mixture was then applied to half-leaves of several *N. glutinosa* plants (4 or 5 plants for each treatment). The opposing half-leaves were only inoculated with TMV (50 mg/ml). Each treatment was carried out with at least 12 replicates (half-leaves). Additionally, ten half-leaves from three *N. glutinosa* control plants were solely inoculated with TMV (50 mg/ml).

Application of extracts on leaf surface before TMV inoculation

In this experiment, we examined the inhibitory effects of the plant extracts (treatments) applied to the leaf surface before virus inoculation. Half-leaves from three or four *N. glutinosa* plants were treated with each plant extract. After

20 minutes, both the treated half-leaves and the untreated half-leaves (control half-leaves) were inoculated with TMV. Each treatment was replicated on ten half-leaves. Moreover, ten half-leaves from four *N. glutinosa* (control plants) were inoculated solely with treated TMV.

Systemic inhibitory activity of plant extracts

Twelve leaves from four *N. glutinosa* plants were treated with each of four different plant extracts. Careful randomization of the leaves was implemented to account for potential variances in the responses of different leaves from the same plant to the treatments. After 24 hours, these treated leaves (referred to as "on-sites") and several untreated leaves from the same plants (referred to as "remote sites") were inoculated with TMV. For comparison, leaves from four *N. glutinosa* plants were also inoculated solely with TMV, serving as control plants. The leaves used in this experiment were randomly selected from mature leaves with approximately similar sizes.

Inhibition durability of plant extracts after washing

Nine half-leaves from three *N. glutinosa* plants were treated with extracts from carnation, Indian privet, and prickly pear cactus. Two hours later, these treated half-leaves were thoroughly washed with water. The entire surface of both the treated and untreated half-leaves was then inoculated with TMV. The number of local lesions was compared between the treated and untreated half-leaves.

Antiviral activity of plant proteins in vitro

Total protein from each treatment plant was prepared at a concentration of 10 mg/ml and mixed in a 1:1 ratio with TMV inoculum (100 mg/ml). This mixture was then inoculated onto ten half-leaves from three *N. glutinosa* plants. The opposing half-leaves, along with ten half-leaves from three control plants, were inoculated solely with TMV (50 mg/ml).

Durability and systemic activity of antiviral plant proteins

In this experiment, the systemic antiviral activity of each plant's total protein was examined over a 24-hour period post-treatment. A total of ten randomly selected leaves (replicates) from four *N. glutinosa* plants were treated with each of the total proteins at a concentration of 5 mg/ml. After 24 hours, both the treated leaves and ten additional untreated leaves from the same plants (remote sites) were inoculated with TMV. Additionally, ten leaves from another four *N. glutinosa* plants were inoculated solely with TMV to serve as control samples.

Inhibitory effect of the extracts against TMV in a systemic host

In this experiment the inhibition of TMV infection in the systemic host, *N. tabacum* var. Turkish, by concurrently inoculating TMV with aqueous extracts from Indian privet, carnation, and prickly pear cactus. Individual young tobacco seedlings were transplanted into separate pots. Aqueous extracts from each treatment plant (200 mg/ml) were mixed in a 1:1 ratio with the TMV inoculum (100 mg/ml) and applied to two leaves of ten Turkish seedlings. In parallel, another set of ten Turkish plants was inoculated solely with TMV (100 mg/ml), serving as the infected control plants. Furthermore, another ten plants were treated with phosphate buffer and designated as the healthy control plants. The plants were spaced apart under greenhouse conditions for symptom development. The antiviral properties of the extracts were evaluated by monitoring the incubation period and the appearance of mosaic symptoms until two months after treatment. The presence of TMV infection in the plants was evaluated using an indirect ELISA test (Konig, 1981). Three leaves from each plant were randomly sampled and tested for TMV infection.

Statistical analyses

The t-test was used to compare the mean values of the two treatment groups. When evaluating the effects of more than three treatments, the data were subjected to ANOVA analysis, followed by Tukey's test for post-hoc comparisons (https://www.statskingdom.com/).

RESULTS AND DISCUSSION

Effect of aqueous extracts on the inhibition of TMV in N. glutinosa

TMV inhibitory effect in vitro

The comparative analysis of each extract treatment against its respective control treatment showed that all extracts, except for the periwinkle, significantly reduced the number of local lesions (Fig. 1 and Fig. 2A). ANOVA analysis indicated that the inhibitory effects of Indian privet and carnation were similar and significantly greater than those of the prickly pear cactus extract (Fig. 2B). The calculated inhibition percentages for the extracts were as follows: Indian privet 94.8%, carnation 96.4%, cactus 87.4%, and periwinkle 17.3%. In summary, the results of this experiment demonstrated that the extracts from Indian privet, carnation, and cactus were effective in neutralizing TMV when mixed with it prior to inoculation (in vitro), while the periwinkle extract did not exhibit such properties.

Inhibitory effect of extracts on leaf surface

In this experiment, extracts were applied to the half-leaves, followed by the inoculation of TMV on both the treated and untreated (control) half-leaves, as well as on control plants. Comparative analysis revealed that the mean number of lesions on half-leaves treated with Indian privet, carnation, and cactus extracts was significantly lower than that observed on the untreated half-leaves. However, the periwinkle extract did not show a significant effect in reducing the number of local lesions (Fig. 3). These results were consistent with those obtained in the previous experiment (3-1-1), demonstrating that the application of these extracts to the leaf surface prior to TMV inoculation had antiviral effects. According to our findings, the compounds in the aqueous extract of periwinkle did not exhibit anti-TMV effects. While some alkaloids found in periwinkle have shown antiviral activity against certain human viruses through mechanisms like protein synthesis inhibition (Pal and Lal, 2023), such properties were not

observed in the aqueous extract of periwinkle when tested against TMV.

Inducing systemic resistance 24 hours post-treatment

This experiment aimed to evaluate the potential for inducing systemic resistance 24 hours after treating *N. glutinosa* leaves with extracts from Indian privet, carnation, cactus, and periwinkle. In treatments involving all extracts, except for periwinkle, the mean number of local lesions on the treated leaves (on-sites) was significantly lower than that on the untreated leaves (remote sites). Furthermore, the mean number of local lesions on the untreated leaves (remote sites) did not show a significant difference compared to the leaves of the control plants (Fig. 4). These results indicate that the antiviral effects of Indian privet, carnation, and cactus extracts were localized in the treated leaves, and systemic resistance was not induced in the plants. The observed percentages of inhibition 24 hours post- treatment in the treated leaves (on-sites) were 92%, 87%, and 81% for Indian privet, carnation, and cactus extracts, respectively. No significant percentage of inhibition was observed with the periwinkle extract in the treated leaves.

Durability of inhibitory effects after washing

In this experiment, washing treatments were applied to evaluate the durability of the TMV inhibitory effects of Indian privet, carnation, and cactus, all of which exhibited anti-TMV effects in previous tests. Statistical comparisons indicated that none of the three extracts differed significantly in the number of local lesions from the control group (the untreated half-leaves). Conversely, the number of local lesions in the unwashed treatments was significantly lower than that of the control treatments (Fig. 5A). Additionally, the results indicated that for all three extracts, the percentage of inhibition in the unwashed treatments was higher than that in the washed treatments (Fig. 5B). Our findings indicate that the inhibitory effects of Indian privet, carnation, and cactus are limited to the leaf surfaces of *N. glutinosa*. The inhibitory compounds do not penetrate the plant cells; rather, they can be removed through washing. Therefore, it can be suggested that when applied at sufficient concentrations before or simultaneously with viral inoculation, the inhibitory components from these plants occupy specific sites on the plant's surface, which prevents the attachment of TMV and its subsequent entry into the cells through wounds. Similar conclusions have been reported in other studies concerning carnation (Duarte et al., 2021). Alternatively, the compounds from the plants may have effectively inactivated the TMV. Conversely, some other studies have indicated that the anti-TMV activity of carnation is due to the inactivation of host ribosomes (RIPs) (Taylor et al., 1994) or the induction of systemic resistance in the host plant (Ostermann et al., 1987; Duarte et al., 2021), which contradicts our findings. Regarding cactus, it can be proposed that its inhibitory compounds may function through ribonuclease activity, similar to that observed in another species of Opuntia, *O. ficus-indica* (Rasoulpour et al., 2017; Rasoulpour et al., 2018). In contrast, our results regarding Indian privet challenge earlier studies that propose a systemic antiviral effect of this plant extract against TMV and some other plant viruses (Verma and Prasad, 1992; Praveen et al., 2001).

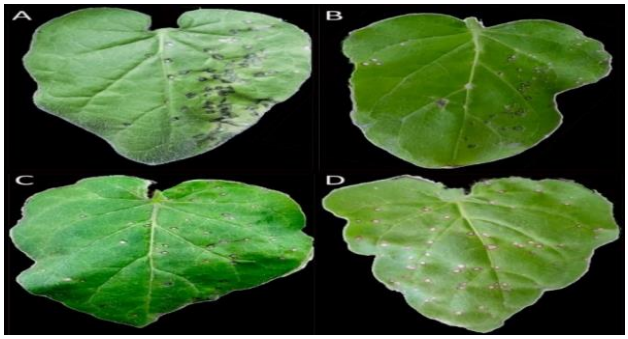

Fig. 1. Local lesions developed after inoculation of Tobacco mosaic virus (TMV) alone (right half-leaves) or mixed with aqueous extracts of (A) *Clerodendrum inerme*, (B) *Dianthus caryophyllus*, (C) *Opuntia stricta*, and (D) *Catharanthus roseus* (left half-leaves) in *Nicotiana glutinosa*

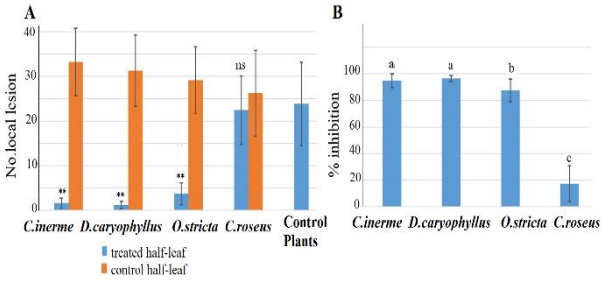

Fig. 2. (A) The mean number of local lesions per half leaves (± standard deviation bars) and (B) percent of virus inhibition resulting from the inoculation of half-leaves of *Nicotiana glutinosa* with Tobacco mosaic virus alone (control half leaf), in comparison to the corresponding half-leaves treated with TMV mixed with aqueous extracts from four plant species (treated half-leaf). Different letters on columns (B) represent significant differences at $P < 0.05$. **: significant difference at the 1% level between each treatment and its corresponding control. ns: not significant.

TMV inhibitory effect of total proteins

The total protein concentrations extracted from 10 grams of fresh tissue were 25 mg/ml for Indian privet, 19 mg/ml for carnation, and 12 mg/ml for cactus. Approximately 4 ml of total protein solution was extracted from each plant. Due to the ineffectiveness of the periwinkle extract in inhibiting TMV infection, the antiviral activity of its proteins was not evaluated.

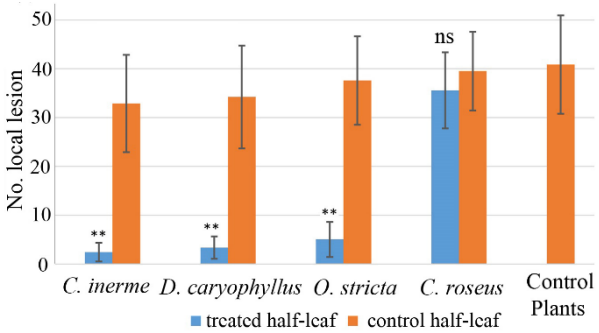

Fig. 3. The mean number of local lesions (± standard deviation bars) resulting from the Tobacco mosaic virus inoculation of half-leaves of *Nicotiana glutinosa* alone (control half leaf), in comparison with the corresponding half-leaves pre-treated with aqueous extracts of various plant species (treated half-leaf). Control plants were inoculated only with Tobacco mosaic virus

(TMV). **: significant difference at the 1% level between each treatment and its corresponding control. ns: not significant.

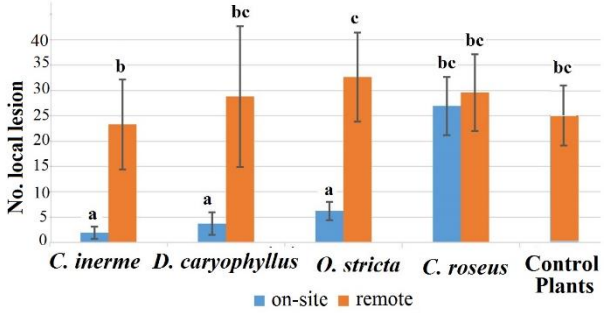

Fig. 4. Evaluation of different plant extracts for systemic resistance induction against Tobacco mosaic virus infection in *Nicotiana glutinosa*. The assay involved application of aqueous extracts onto whole leaves of *N. glutinosa*, followed by inoculation with TMV, 24 h later, on these treated (on-site) as well as non-treated (remote) leaves. Data are presented as mean number of local lesions per leaf (± standard deviation bars). Different letters on columns represent significant differences at $P < 0.05$.

In vitro TMV inhibitory effects of total proteins

This experiment aimed to evaluate the effects of plant proteins on TMV prior to inoculation. Results showed a significant reduction in the mean number of local lesions in the treated half-leaves compared to the control half-leaves for all three plant proteins (Fig. 6A). The percentage of inhibition observed was 92% for carnation, 91% for Indian privet, and 79% for cactus. This indicates that the total proteins of Indian privet and carnation were more effective than that of cactus in inhibiting TMV infection (Fig. 6A). Notably, the percentage of inhibition associated with the proteins from these three plants was comparable to that of their respective aqueous extracts (Fig. 1 and Fig. 6). Thus, it can be suggested that the antiviral effects of the aqueous extracts of these plants are mainly attributed to their protein content.

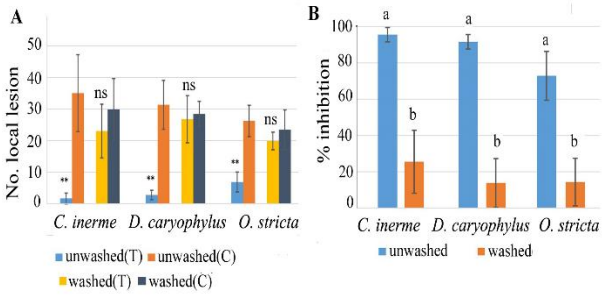

Fig. 5. (A) The mean number of local lesions per half leaves and (B) percentage of inhibition resulting from plant extracts applied to *Nicotiana glutinosa* in washed and unwashed treatments prior to Tobacco mosaic virus (TMV) inoculation. In washed treatments, extracts were applied to half of the leaves, washed after 2 hours, and inoculated with TMV (washed (T)) along with untreated halves (washed (C)). In unwashed treatments, extracts were applied to half of the leaves and then both halves (unwashed (T) and unwashed (C)) were inoculated with TMV after 2 hours. B compares inhibition percentages for washed and unwashed treatments. Different letters on columns denote significant differences at P < 0.05. **: 1% level significance between treatments and corresponding controls. ns: not significant.

Examining systemic resistance by plant proteins

Total proteins extracted from Indian privet, carnation, and cactus were applied to the leaves of *N. glutinosa*. Twenty-four hours later, TMV was inoculated on these treated leaves (on-sites), as well as on untreated leaves from the same plants (remote sites) and on control plants. The mean number of local lesions on the leaves treated with each of the three plant proteins (on-sites) was significantly lower than on the untreated leaves from the same plants (remote sites). Furthermore, no significant difference was observed between the number of local lesions at remote sites and those on the control plants (Fig. 7). These findings confirm that the anti-TMV properties of these protein treatments function locally and do not induce systemic resistance. These results are consistent with the non-systemic effects observed from the aqueous extracts of these plants. These results indicate that the anti-TMV properties of aqueous extracts from Indian privet, carnation, and cactus are likely related to their protein content. Plant proteins are known for their antiviral properties, effectively inhibiting viral infections (Verma and Baranwal, 2011; Duarte et al., 2021). Three hypotheses explain the antiviral mechanisms of the plant proteins. First, inhibitory proteins may function as ribosome inactivating proteins (RIPs), which terminate protein synthesis in infected cells and lead to cell death (Cho et al., 2000; Domashevskiy et al., 2017). However, our findings suggest that the inhibitory effects of Indian privet, carnation, and cactus are confined to the leaf surfaces of *N. glutinosa*. Second, these proteins could trigger the production of virus inhibitory agents (VIA) that alter cellular metabolism to promote systemic resistance (Awasthi et al., 2016). Nonetheless, the study found localized surface activity in Indian privet, carnation, and cactus without any systemic effects. Third, components in some plant species may exhibit ribonuclease activity, which can lead to virus inactivity (Rasoulpour et al 2017). Finally, antiviral proteins may bind to virus particles, competing for receptor sites on the plant's surface (Van Kammen et al., 1961; Yang et al., 2012). This aligns with our findings, suggesting that proteins from these plants, when applied in sufficient quantities, can prevent TMV attachment and entry.

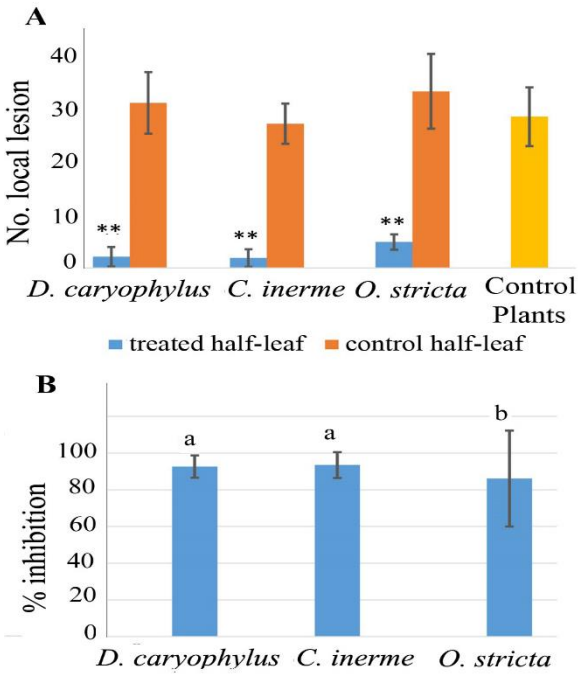

Fig. 6. (A) The mean number of local lesions (± standard deviation bars) and (B) percent of virus inhibition resulting from the inoculation of half-leaves of *Nicotiana glutinosa* with Tobacco mosaic virus alone (control half leaf), in comparison to the corresponding half-leaves treated with TMV mixed with total protein extracts of various plant species (treated half-leaf). (B) Different letters on treatment bars represent significant differences at $P < 0.05$. **: significant difference at the 1% level between each treatment and its corresponding control.

Inhibitory effect of the extracts against TMV in systemic host

In this experiment, all Turkish tobacco plants inoculated with TMV, including both treated and untreated controls, exhibited mosaic symptoms in 2-3 weeks. In contrast, none of the healthy control Turkish plants displayed any symptoms throughout the 2-month post-inoculation period. All plants showing mosaic symptoms tested positive for TMV in ELISA. Additionally, TMV infection was not detected in any asymptomatic plants by ELISA tests.

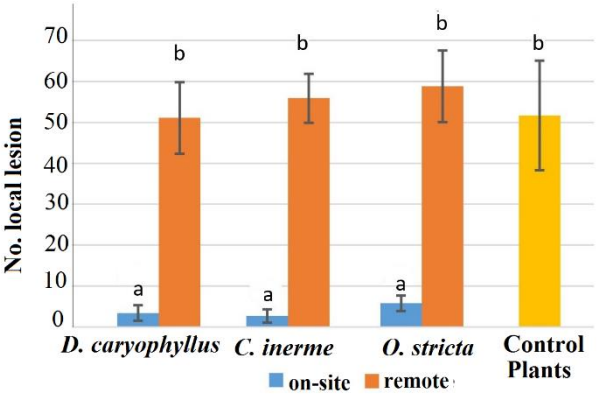

Fig. 7. Effects of total protein extracted from different plants species on systemic resistance induction against Tobacco mosaic virus infection in *Nicotiana glutinosa*. The assay involved application of total protein onto leaves of *N. glutinosa*, followed by inoculation with TMV, 24 h later, on these treated (on-site) as well as non-treated (remote) leaves. Data are presented as mean number of local lesions per leaf (± standard deviation bars). Different letters on treatment bars represent significant differences at $P < 0.05$.

These findings suggested that the inhibitory effect of extracts is not absolute, allowing a small amount of virus to enter the plant and cause systemic infection in Turkish tobacco. Furthermore, the plant extracts did not influence the latency period of TMV when comparing Turkish plants treated with the extracts to infected control plants (data not shown).

CONCLUSION

The findings of this study indicate that extracts and total proteins derived from Indian privet, carnation, and cactus can locally inhibit TMV infection on treated leaves of a local lesion host. The significant inhibitory effects of these plant extracts on TMV infection in local lesion hosts suggest their potential use in protecting natural host plants (systemic hosts) against TMV and other Tobamoviruses. In this regard, a previous study (Ostermann et al., 1987) has shown the successful use of carnation extract to manage TMV in the systemic host of tobacco. However, our study found that the plant extracts used in this study were ineffective against TMV infection in the systemic host, *N. tabacum* var. Turkish. Further investigations are needed to elucidate the factors contributing to the local anti-TMV action of the Indian privet used in this study, in contrast to the systemic activity of this plant species reported elsewhere (Verma and Prasad, 1992; Praveen et al., 2001).

FUNDING

This research was funded by Shahrekord University, Shahrekord, Iran.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Majid Siampour and Fatemeh Kahani; Methodology: Fatemeh Kahani and Majid Siampour; Validation: Majid Siampour and Masoud Ghasemi Ghehsareh; Formal analysis: Fatemeh Kahani, Majid Siampour, and Masoud Ghasemi Ghehsareh; Investigation: Fatemeh Kahani and Majid Siampour; Resources: Fatemeh Kahani; Data curation: Fatemeh Kahani, Majid Siampour, and Masoud Ghasemi Ghehsareh; Writing—original draft preparation: Fatemeh Kahani and Majid Siampour; Writing—review and editing: Fatemeh Kahani, Majid Siampour, and Masoud Ghasemi Ghehsareh; Visualization: Fatemeh Kahani and Majid Siampour; Supervision: Majid Siampour; Funding acquisition: Fatemeh Kahani.

ETHICAL STATEMENT

All authors are aware on content of the manuscript and consented to submit it to *Iran Agricultural Research* Journal. We did not send this article to another journal.

DATA AVAILABILITY

The raw data of this research are available at the request of the reviewers and editors

REFERENCES

Adams, M. J., & Antoniw, J. F. (2006). DPV web: A comprehensive database of plant and fungal virus genes and genomes. *Nucleic Acids Research*, *34*(suppl_1), D382-D385 https://doi.org/10.1093/nar/gkj023

Ahmad, A., Davies, J., Randall, S., & Skinner, G. R. B. (1996). Antiviral properties of extract of *Opuntia streptacantha*. *Antiviral Research*, *30*(2-3), 75-85. https://doi.org/10.1016/0166-3542(95)00839-x

Awasthi, L. P., & Verma, H. N. (2006). *Boerhaavia diffusa*– A wild herb with potent biological and antimicrobial properties. *Asian Agri-History*, *10*(1), 55-68.

Awasthi L. P., & Singh S. H. Y. A. M. (2009). Management of ringspot disease of papaya through plant products. *Indian Phytopathology*, 62, 369-375.

Awasthi, L. P., Verma, H. N., & Kluge, S. (2016). A possible mechanism of action for the inhibition of plant viruses by an antiviral glycoprotein isolated from *Boerhaavia diffusa* roots. *Journal of Virology and Antiviral Research*, *5*(3), 2. https://doi.org/10.4172/2324-8955.1000159

Biniaz, Y., Ahmadi, F., Niazi, A., & Afsharifar, A. (2023). Antiviral activity of three plant species, *Rhus coriaria*, *Chenopodium quinoa*, and *Ailanthus altissima* against tobacco mosaic Virus. *Journal of Agricultural Science and Technology*, *25*(1), 199-211. https://doi.org/10.1080/09670874.2021.1985653

Cho, H. J., Lee, S. J., Kim, S., & Kim, B. D. (2000). Isolation and characterization of cDNAs encoding ribosome inactivating protein from *Dianthus sinensis* L. *Molecules and Cells*, *10*(2), 135-141. https://doi.org/10.1007/s10059-000-0135-0

Domashevskiy, A. V., Williams, S., Kluge, C., & Cheng, S. Y. (2017). Plant translation initiation complex eIF iso 4F directs pokeweed antiviral protein to selectively depurinate uncapped Tobacco etch virus RNA. *Biochemistry*, *56*(45), 5980-5990. https://doi.org/10.1021/acs.biochem.7b00598

Duarte, L. M. L., Alexandre, M. A. V., Chaves, A. L. R., dos Santos, D. Y. A. C., de Souza, A. C. O., & Bernacci, L. C. (2021). Plant-virus infection inhibitors: The great potential of Caryophyllales species. *Physiological and Molecular Plant Pathology*, *113*, 101597. https://doi.org/10.1016/j.pmpp.2020.101597

Hull, R. (2013). *Plant virology*. New Yourk: Academic press.

Mahdy, A. M. M., Fawzy, R. N., Hafez, M. A., Mohamed, H. A., & Shahwan, E. S. (2007). Inducing systemic resistance against Bean yellow mosaic potyvirus using botanical extracts. *Egyptian Journal of. Virology*, *4*, 129-145. https://doi.org/10.21608/JPPP.2017.46340

Ostermann, W. D., Meyer, U., & Leiser, R. M. (1987). Induction of plant virus resistance: 2. leaf extract from carnation plants (*Dianthus caryophyllus* L.) as inducer of resistance. *Zentralblatt für Mikrobiologie*, *142*(3), 229-238. https://doi.org/10.1016/S0232-4393(87)80020-3

Pal, D., & Lal, P. (2023). Plants showing anti-viral activity with emphasis on secondary metabolites and biological screening. *Anti-Viral Metabolites from Medicinal Plants*, 29-95. https://doi.org/10.1007/978-3-031-12199-9_2

Park, S. R., Lim, C. Y., Kim, D. S., & Ko, K. (2015). Optimization of ammonium sulfate concentration for purification of colorectal cancer vaccine candidate recombinant protein GA733-FcK isolated from plants. *Frontiers in Plant Science*, *6*, 1040. https://doi.org/10.3389/fpls.2015.01040

Praveen, S., Tripathi, S., & Varma, A. (2001). Isolation and characterization of an inducer protein (Crip-31) from *Clerodendrum inerme* leaves responsible for induction of systemic resistance against viruses. *Plant Science*, *161*(3), 453-459. https://doi.org/10.1016/S0168-9452(01)00425-3

Pushpa, R., Nishant, R., Navin, K., & Pankaj, G. (2013). Antiviral potential of medicinal plants: An overview. *International Research Journal of Pharmacy*, *4*(6), 8-16. https://doi10.7897/2230-8407.04603

Ragetli, H. W. J., & Weintraub, M. (1962). Purification and characteristics of a virus inhibitor from *Dianthus caryophyllus* L.: II. Characterization and mode of action. *Virology*, *18*(2), 241-248. https://doi.org/10.1016/0042-6822(62)90010-7

Rasoulpour, R., Afsharifar, A., & Izadpanah, K. (2018). Antiviral activity of prickly pear (*Opuntia ficus-indica* (L.) Miller) extract: Opuntin B, a second antiviral protein. *Crop Protection*, *112*, 1-9. https://doi.org/10.1016/j.cropro.2018.04.017

Rasoulpour, R., Afsharifar, A., Izadpanah, K., & Aminlari, M. (2017). Purification and characterization of an antiviral protein from prickly pear (*Opuntia ficus-indica* (L.) Miller) cladode. *Crop Protection*, *93*, 33-42. https://doi.org/10.1016/j.cropro.2016.11.005

Taylor, S., Massiah, A., Lomonossoff, G., Roberts, L. M., Lord, J. M., & Hartley, M. (1994). Correlation between the activities of five ribosome-inactivating proteins in depurination of tobacco ribosomes and inhibition of tobacco mosaic virus infection. *The Plant Journal*, *5*(6), 827-835. https://doi.org/10.1046/j.1365-313x.1994.5060827.x

Van Kammen, A., Noordam, D., & Thung, T. H. (1961). The mechanism of inhibition of infection with tobacco mosaic virus by an inhibitor from carnation sap. *Virology*, *14*(1), 100-108. https://doi.org/10.1016/0042-6822(61)90137-4

Verma, H. N., & Baranwal, V. K. (2011). Potency of plant products in control of virus diseases of plants. In Dubey, N. K. (Ed.) *Natural products in plant pest management* (pp. 149-174). Wallingford UK: CABI.

Verma, H. N., & Prasad, V. (1992). Virus inhibitors and inducers of resistance: Potential avenues for biological control of viral diseases. In Mukerji, K. G., Tewari, J. P., Arora, D. K. & Saxena, G. (Eds) *Recent development in biocontrol of plant diseases* (pp. 81-110). Aditya Books Pvt. Ltd, New Delhi, India.

Verma, H. N., Shalini Srivastava, S. S., Varsha, V., & Dhirendra Kumar, D. K. (1996). Induction of systemic resistance in plants against viruses by a basic protein from *Clerodendrum aculeatum* leaves. *Phytopathology 5*, 485-492. https://doi.org/10.1094/Phyto-86-485

Wong, K. L., Wong, R. N. S., Zhang, L., Liu, W. K., Ng, T. B., Shaw, P. C., Kwok, P. Ch. L., Lai, Y. M., Zhang, Zh. J., Zhang, Y. T., Cheung, H. P., Lu, J., & Sze, S. C. W. (2014). Bioactive proteins and peptides isolated from Chinese medicines with pharmaceutical potential. *Chinese Medicine*, *9*, 1-14. https://doi.org/10.1186/1749-8546-9-19

Yang, J., Jin, G. H., Wang, R., Luo, Z. P., Yin, Q. S., Jin, L. F., & Lin, F. C. (2012). *Spinacia oleracea* proteins with antiviral activity against tobacco mosaic virus. *African Journal of Biotechnology*, *11*(26), 6802-6808. https://doi.org/10.5897/AJB11.2654