Salix alba Extract Induces Systemic Resistance in *Cucumis sativus* Infected by *Cucumber mosaic virus*

Mahmoud R. Sofy*, Abd El-Monem M.A. Sharaf, Mohamed E. El-Nosary and Ahmed R. Sofy

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt
*mahmoud_sofy@yahoo.com

Abstract: In a greenhouse experiment, white willow (*Salix alba*) extract as soaked seeds was tested for induction systemic acquired resistance (SAR) in cucumber plants against *Cucumber mosaic virus* (CMV). The results demonstrated that CMV challenged plants were reductions in plant height, growth parameters, and soluble carbohydrates, while a significant increase in glutathione reductase and ascorbate peroxidase as a result of the viral infection compared with absolute control plants. On the other hand, challenged treatment (soaked seeds with *Salix* extract + CMV) showed an increase in all tested parameters when being compared with and challenged control plants. It can conclude that *Salix* treatment increases the *Cucumis sativus* L. alfa-beta plants resistance against CMV.
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Keywords: *Cucumber mosaic virus*, *Cucumis sativus*, *Salix alba* extract, Morphology and Biochemical measurements

1. Introduction

Viruses are among the most important kinds of plant pathogens causing severe economic losses in many crops. Viruses do not have self-movement or entry mechanisms like many fungi and bacteria do. They require a wound and direct delivery into that wound to infect a plant. Only once inside a plant cell, can the virus begin to multiply, high jacking the host machinery for its uses, thus causing a variety of disruptions to normal plant physiology [1].

Cucumber mosaic virus (CMV) is the most destroyer and economically important plant viruses having approximately 365 genera, 85 families, infecting 1200 dicotyledonous and monocotyledonous species in its host range [2].

The CMV is +ve sense tripartite virus having III genomic single-stranded RNA, which are encapsidated in a 28 nm icosahedral particle separately [3]. Proteins 1a and 2a are codded by RNA1 and RNA2, on a row those results in the consist of a replicas complex [4].

Family *Cucurbitaceae* contains approximately about 90 genera, but three genera are widely cultivated in Egypt, i.e., *Cucurbita spp*. (Squash and Pumpkin). *Citrullus spp*. (watermelon) and *Cucumis spp*. (melon and cucumber), cucumber (*Cucumis sativus* L.) is the main field and greenhouse vegetable crop of the coastal areas of the Middle East and Mediterranean Basin [5].

In Egypt, cucumber is among the very important *Cucurbitaceous* crops and the leading export vegetable. Cucumber is infected with several pathogens, where *Cucumber mosaic virus* is considered as the most virus infecting such plants in Egypt.

Replication of virus particles induces some changes in the biochemical profile of the infected plant cells, for example, chlorophyll, phenolic compounds, carbohydrates, protein and nucleic acids, etc. which is mainly related to the measure of crop losses [6].

Management of viral disease can also be accomplished through the induction of plants natural defenses, *e.g*., systemic acquired resistance (SAR), where SAR in plants is analogous to the innate immunity in animals called acquired physiological immunity [7].

The botanicals may induce resistance, or they may act as inhibitors of viral replication, biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in a reduction of disease development [8].

Salicylic acid is the main axis of the systemic resistance acquired is that it controls some physiological processes in plant [9].

Salicylic acid is a chemical carboxylic acid chemical formula $C_2H_4(OH)$ (COOH), It is found naturally in vegetables and fruits and was first extracted naturally from the *Salix sp* plant, and a plant hormone plays an important role in plant growth and development [10].

Sofy *et al.* [11] showed that *Salix* extract both foliar and seed treatment reduce the level of bean yellow mosaic disease severity with 52.6 and 42.8%, respectively related to infected faba bean plants with challenged control (82%). Also, Sofy *et al.* [12] reported that *Salix alba* extract increases the *Cucurbita pepo* cv. Eskandarani plants resistance to ZYMV.

The growing cost of pesticides and consumer demand for pesticide-free food have led to a search for substitutes for these chemical products. There are also some fastidious diseases, i.e., virus and viroid diseases, for which chemical solutions are few, ineffective, or non-existent [13].

So, this study aims to investigate the effect of CMV infection on growth, morphological change and metabolism of cucumber plants and also the use of some *Salix* extract hopping to abolishing the detrimental effects of the virus on these plants.

2. Material and Methods

2.1. Plant Material

For the present investigation, seeds of *Cucumis sativus* L. alfa-beta were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt, and germinated in seed trays containing perlite. Seedlings were cultivated under natural lighting, day/night temperature of approx. 22/20°C and 60% mean relative humidity. One-week-old seedlings were potted in soil and grown under the same conditions.

2.2. Preparation of the Plant Extract

Dried leaves and bark of the *Salix* plant (*Salix alba*) was obtained from the Botanical garden, Agriculture Research Centre (ARC) Giza Egypt. The dry leaves and bark of the *Salix* plant were crushed into powder. 0.5 gm of the powder was put in 50°C boiled water and left for one hr then filtered into a conical flask. The aqueous infusion was serialized by the bacterial filter. An equivalent of 10 mg dried material per ml of aqueous infusion was obtained [11, 12].

2.3. *Cucumber mosaic virus* (CMV) inoculation

A CMV isolate was isolated from naturally infected cucumber plants (*Cucumis sativus*) showing distinct viral symptoms were collected from Sharkia and Ismailia Governorates, Egypt. The virus isolate was detected using polyclonal antibody specific CMV kits provided by DAS-ELISA according to Clark and Adams [14]. The virus was checked on *Chenopodium amaranticolor* L. and reisolated from a single lesion. The extraction of resulted local lesions was inoculated on the healthy *Nicotiana glutinosa* plants as a CMV propagative host (unpublished data).

2.4. Methods of Planting, Treatments, and Collection of Samples

A pot experiment was carried out in the greenhouse of Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt to evaluate *Salix alba* water extract for inducing systemic resistance in cucumber plants against CMV. Surface sterilized seeds were soaked in the following treatments for 2 hrs before planting. A randomized complete block design was used with two treatments and two controls, where each consisted of eight replicate pots and two plants per pot. Treatments included sterilized seeds were soaked in *Salix alba* water extract (T1), T1 + CMV (T2), in addition to a virus challenged control (ChC, water-soaked seeds) and absolute control (AC, water-soaked seeds, and no virus). The pots were maintained in a greenhouse under natural lighting, day/night temperature of approx. 22/20°C and 60% mean relative humidity. After two weeks of growth, the two true-leaf seedlings of the plants with treatment T2 in addition to the challenged control (ChC) were challenged inoculated mechanically with CMV inoculum. The plant samples were collected for analysis when the plants were 45 days old.

2.5. Morphological, Physiological and Metabolic Changes

2.5.1. Morphological Measurements

The shoot height (cm), the root height (cm), leaves number, fruits number, fruits weight (g), fruits hold (%), as well as the fresh and dry weight of both shoot and root (g), were measured.

2.5.2. Determination of Soluble Carbohydrates

Total soluble carbohydrates were estimated according to the method of Umbriet *et al.* [15].

2.5.3. Antioxidant Enzymes Activities

Antioxidant enzymes were extracted according to the method of Mukherjee and Choudhuri [16]. Ascorbate peroxidase (ASC) and glutathione reductase (GR) activities were measured according to the methods of Chen *et al.* [17] and Karni *et al.* [18], respectively.

2.6. Statistical Analysis

All statistical calculations were done using SPSS (statistical package for the social science version 20.00) statistical program at 0.05 level of probability [19]. Quantitative data with parametric distribution were done using analysis of variance the two-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level of > 0.05, significant at the level of ≤ 0.05, 0.01 and highly significant at the level of ≤ 0.001.

3. Results

3.1. Virus isolation

Cucumber mosaic virus was detected in the naturally infected cucumber plants showing viral-like symptoms, where gave positive results by DAS-ELISA using specific CMV-IgG. It was biologically isolated by single local lesion from *Chenopodium amaranticolor* which gave chlorotic local lesions. It was propagated in healthy *Nicotiana glutinosa*, systemic host and confirmed by DAS-ELISA (unpublished data).

3.2. Growth Parameters

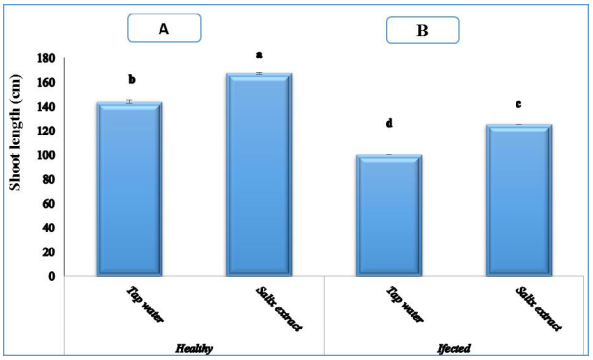

Figure 1. Effect of tap water and *Salix* extract on shoot length of healthy and infected cucumber plants

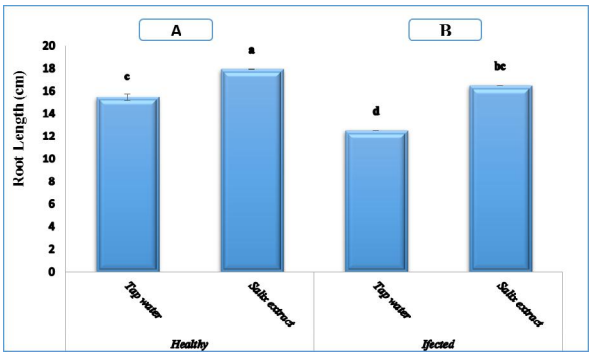

Figure 2. Effect of tap water and *Salix* extract on root length of healthy and infected cucumber plants

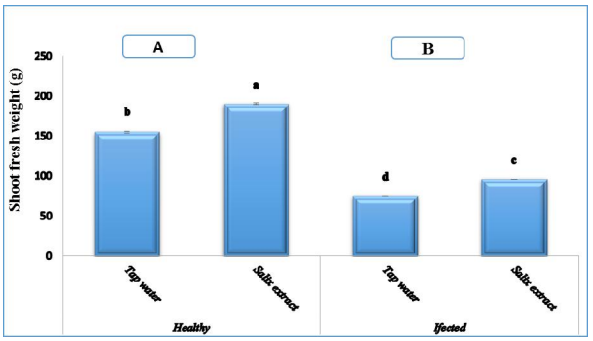

Figure 3. Effect of tap water and *Salix* extract on shoot fresh weight of healthy and infected cucumber plants

The results of the present work (Figs. 1, 2, 3, 4, 5 & 6) revealed that, cucumber plants infected with CMV, resulted in significant decreases in shoot length, root length, fresh and dry weight of both shoots and roots by 30.56%, 19.9%, 51.57%, 49.95%, 31.43% and 74.84%, respectively, when being compared with absolute control. On the other hand, cucumber plants infected with CMV and treated with *Salix* extract resulted in significant decreases in shoot length and fresh weight of both shoots and roots by 12.96%, 37.96%, and 39.73%, respectively, and significant increase in shoot dry weight by 39.62% and insignificant increase in root length, while insignificant decrease in root dry weight when being compared with absolute control.

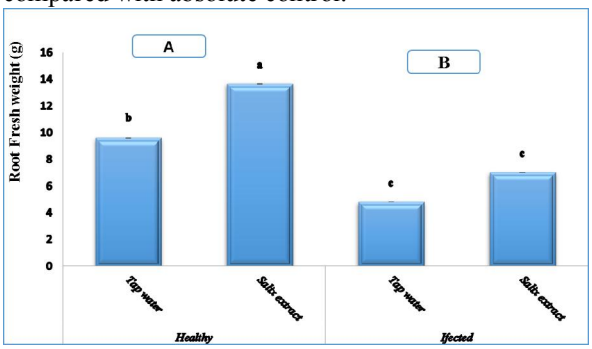

Figure 4. Effect of tap water and *Salix* extract on root fresh weight of healthy and infected cucumber plants

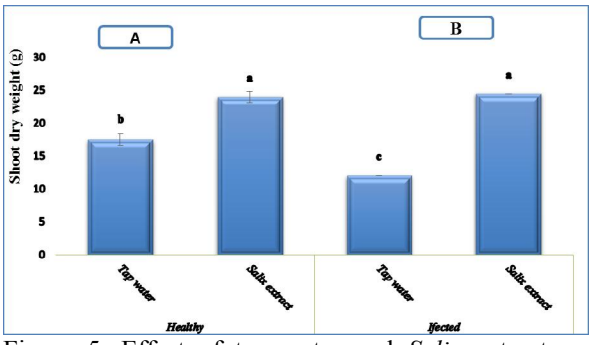

Figure 5. Effect of tap water and *Salix* extract on shoot dry weight of healthy and infected cucumber plants

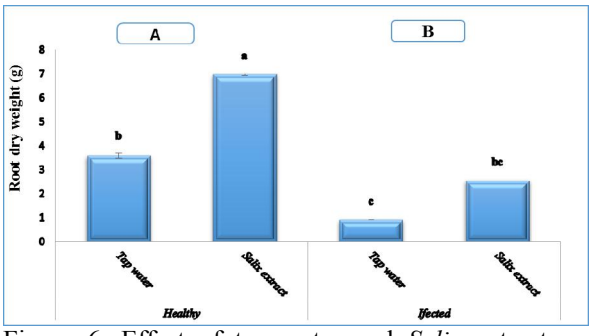

Figure 6. Effect of tap water and *Salix* extract on shoot dry weight of healthy and infected cucumber plants

The results of the present work (Figs. 7, 8, 9 & 10) revealed that, cucumber plants infected with CMV, resulted in significant decreases in leaves number, fruits number, and fruits weight by 43.84%, 48.38% and 32.49%, respectively, while insignificant in fruits hold when being compared with absolute control. On the other hand, cucumber plants infected with CMV and treated with *Salix* extract resulted in, significant decreases in fruits number and fruits weight by 6.38% and 11.27%, respectively, but

significant increase in fruits hold by 31.8% and insignificant in leaves number when being compared with absolute control.

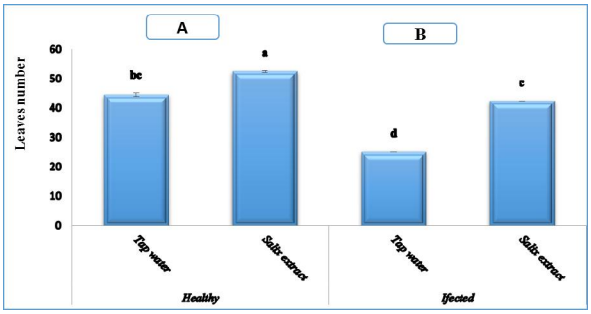

Figure 7. Effect of tap water and *Salix* extract on leaves number of healthy and infected cucumber plants

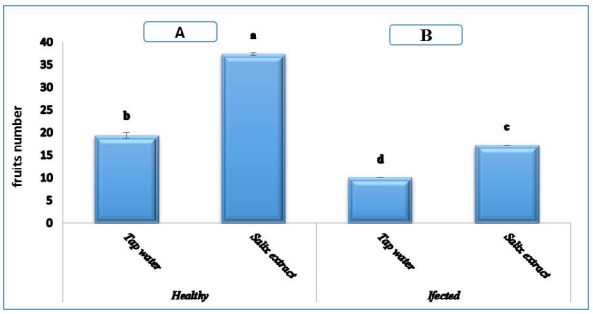

Figure 8. Effect of tap water and *Salix* extract on fruits number of healthy and infected cucumber plants

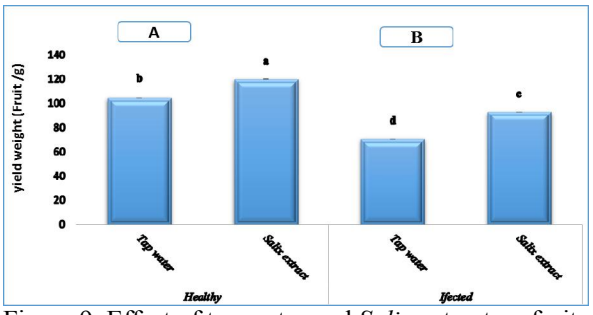

Figure 9. Effect of tap water and *Salix* extract on fruits weight of healthy and infected cucumber plants

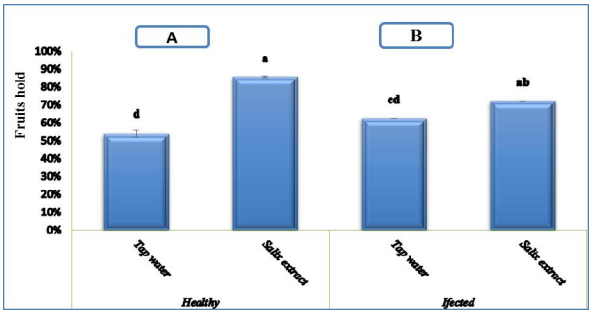

Figure 10 Effect of tap water and *Salix* extract on fruits hold of healthy and infected cucumber plants

3.3. Soluble Carbohydrates

Figures (11 & 12) illustrated that cucumber plants infected with CMV, resulted in significant decreases in carbohydrates shoot and fruits when being compared with absolute control. The reduction in carbohydrates shoot and fruits of infected plants was reduced by 27.21% and 44.27%, respectively in comparison to corresponding absolute control. Also, cucumber plants infected with CMV and treated with *Salix* extract resulted in, significantly decreasing in carbohydrates shoot and fruits was observed in cucumber plants by 6.32% and 12.69%, respectively when compared with absolute control.

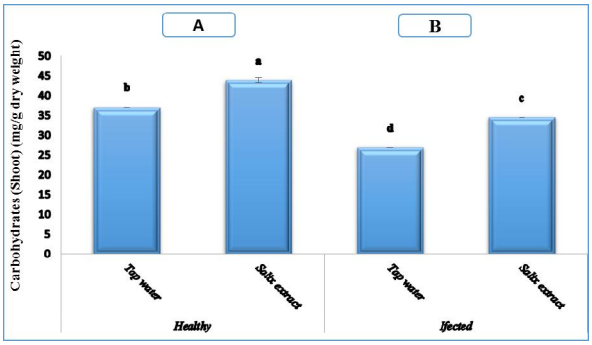

Figure 11. Effect of tap water and *Salix* extract on dry weight carbohydrates of healthy and infected cucumber plants

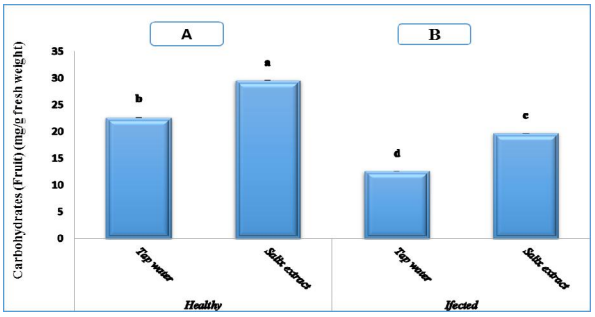

Figure 12. Effect of tap water and *Salix* extract on fresh weight carbohydrates of healthy and infected cucumber plants

3.4. Antioxidant Enzymes Activities

The effect of viral infection and treatment with *Salix* on the activities of ascorbate peroxidase (ASC) and glutathione reductase (GR) activities are shown in Figs. (13 & 14). In virus inoculated leaves, the antioxidant enzymes activities (ASC and GR) increase significantly by 107.53% and 88.2%, respectively, when compared to that of the absolute control. Also, the increase reached challenged treatment [*Salix* + CMV] with 79.86% and 66.21%, respectively, compared to that of the absolute control.

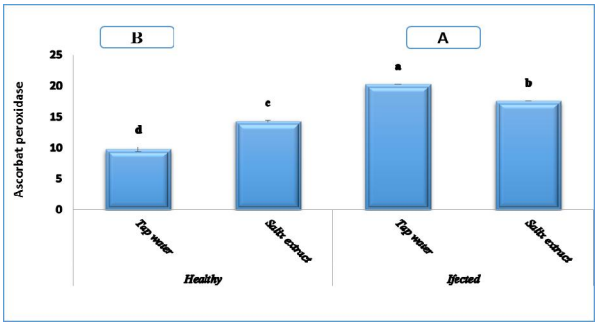

Figure 13. Effect of tap water and *Salix* extract on ASC of healthy and infected cucumber plant

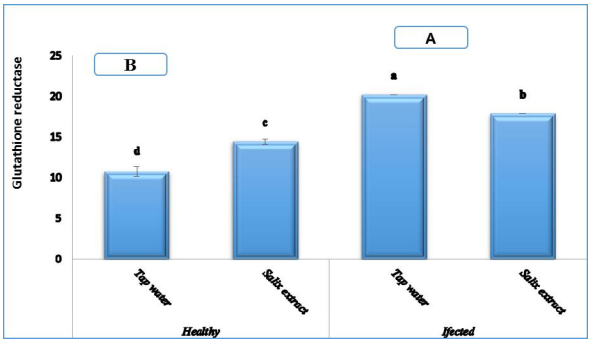

Figure 14. Effect of tap water and *Salix* extract on GR of healthy and infected cucumber plants

4. Discussion

Virus infection leads to many alterations of physiological and biochemical processes within the plant where the appearance of severe symptoms on virus-infected leaves in the form of severe mosaic and, the new leaves showed deformation of leaf morphology (filiform) occurs due to changes in metabolism, alteration of some enzyme activities. The objectives of this study were induction systemic acquired resistance (SAR) in *Cucumis sativus* L. alfa-beta plants against *Cucumber mosaic virus* infection upon priming with *Salix alba* extract treatment and subsequent challenge inoculation of the plant with the virus.

Our results indicated that cucumber plants infected with CMV, resulted in significant decreases in shoot length, root length, the fresh and dry weight of both shoots and roots when being compared with healthy plants. On the other hand, further enhancements of these data were obtained due to treatment seeds with *Salix* extract when being compared with challenged control. Our results in agreement with Sofy *et al.* [11], Deya Eldeen *et al.* [20] and Sercan *et al.* [21].

The results of present work indicated that cucumber plants infected with CMV, resulted in significant decreases in leaves number, fruits number, and fruits weight but insignificant in fruits hold when being compared with healthy plants. On the other hand, cucumber plants infected with CMV and treated with *Salix* extract resulted in significant decreases in fruits number and fruits weight, but significant increase in fruits hold and insignificant in leaves number when being compared with healthy plants. Thus, result similar to Sikora *et al.* [22] and Gianessi *et al.* [23].

The result in this present work illustrated that cucumber plants infected with CMV, resulted in significant decreases in carbohydrates shoot and fruits when being compared with healthy plants. The reduction in carbohydrates shoot of infected plants was reduced by 27.21% and 44.27%, respectively, in comparison to corresponding absolute control. Also, in infected treatment, significantly decreasing in carbohydrates shoot and fruits was observed in cucumber plants, where infected *Salix* extract, when compared with healthy plants. It has been known that the virus infection leads to decreased sugar levels in plant tissues, where the plants can modulate their sugar pools to act either as a source of carbon and energy or to use as signals and perhaps as putative priming agents to intensify immune reactions [24, 25]. Similar results were recorded where the cucumber, leaves infected with CMV were having a low concentration of carbohydrates, which increases the chances of the establishment of the virus.

A large number of defense enzymes that have been associated with SAR include, ascorbate and glutathione reductase (GR) [26, 27], where these enzymes bring about the liberation of molecules that elicit the initial steps in the induction of resistance, phytoalexins and phenolic compounds [28]. Our results showed that antioxidant enzymes activities indicated that the effect of viral infection and treatment with *Salix* on the activities of ascorbate and GR. In virus inoculated leaves, the antioxidant enzymes activities (ASC and GR) increase significantly, when compared to that of the healthy control. Also, the increase reached at challenged treatment [*Salix*] with 79.86% and 66.21%, respectively, compared to that of the absolute control when compared to that of the healthy control. Our results are in agreement with Vitti *et al.* [29], they reported that tomato plants infected with CMV had significantly increased ASC and GR compared with control, which may lead to the induction of defense responses according to several studies [30, 31].

The alterations in biochemical components in mesta plant infected with yellow vein mosaic disease were studied by Arpita and Subrata [32]. They reported that the enzyme assays revealed the lower activity of CAT and POD enzymes in diseased plants in comparison with healthy ones. In contrast, a marked increase in activities of PPO and ASC was found in diseased plants as compared with the

respective healthy plants. The compatible and incompatible plant-virus interactions between *Vigna mungo* and *Mungbean yellow mosaic india virus* were studied by Subrata *et al*. [27]. They reported that the activity of GR sharply increased upon MYMIV infection at three dpi (day post infection) during incompatible interaction and gradually increased up to 2.3-fold at 14 dpi compared to that of the control. While there was a steady increase in the ASC activity during compatible interaction from 7 dpi (day post infection) onwards and significant level was attained at 14 dpi, CAT activity declined significantly at 3 and 7, dpi as compared to that of the control during incompatible interaction. Whereas CAT activity increased significantly from 7 dpi during compatible interaction and 1.8-fold increment was noted at 14 dpi. The activity of APX was induced significantly from 3 dpi during the incompatible interaction, whereas a significant increase in APX activity was recorded from 7 dpi onwards during incompatible interaction.

5. Conclusion

The objective of this study has investigated the effect of CMV infection on growth, morphological change and metabolism of cucumber plants and also the use of some *Salix* extract hopping to abolishing the detrimental effects of the virus on these plants. It can conclude that *Salix* treatment increases the *Cucumis sativus* L. alfa-beta plants resistance against CMV.

Corresponding Author
Dr. Mahmoud R. Sofy
Botany and Microbiology Department
Faculty of Science
Al-Azhar University, 11884 Nasr City, Cairo, Egypt
E-mail: mahmoud_sofy@yahoo.com

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