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EFFECT OF TWO BIOTIC INDUCERS ON SALICYLIC ACID INDUCTION IN TOMATO INFECTED WITH *CUCUMBER MOSAIC CUCUMOVIRUS*

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ABSTRACT

To explore the inhibitory effect of two biotic inducers on tomato plant against *Cucumber mosaic cucumovirus* (CMV), salicylic acid (SA) activities were determined in the inoculated and non-inoculated tomato plants. Identify of CMV was confirmed by some differential hosts and dot blot immunoassay (DBIA) using polyclonal antibody specific CMV. Water extracts of *Mirabilis jalapa*, *Clerodendrum inerme* and Kombucha as antiviral to control the virus infection and detection systemic acquired resistance (SAR) were studied. SAR was detected by determination of salicylic acid (SA) resulted induction of protein related to biotic inducers, virus concentration and disease severity (DS). The obtained results from quantification of total SA in induced tomato plants (after 7 days of spraying inducers) showed high level of SA with kombucha treatment followed by *C. inerme* and *M. jalapa*, while mixed (*M. jalapa*+*C. inerme*) gave the lowest level of SA compared with healthy and infected controls. On the other hand, after virus inoculation tomato plants showed reduction of virus and disease severity. *M. jalapa*, *C. inerme* and kombucha showed lowest percentage of DS, while mixed *M. jalapa*+*C. inerme* showed high percentage of DS. Both of two biotics induced SA and inhibited CMV infection in the tomato plants.

Keywords: Plant defense, Antivirus, SA, *Mirabilis jalapa*, *Clerodendrum inerme* and Kombucha.

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populations were much lower on treated than control plants **(Verma and Kumar, 1982)**.

Antiviral protein designated as MAP [*Mirabilis* antiviral protein, 30 kDa (ribosome inactivating proteins, RIPs)] extracted from roots, leaves of *M. jalapa* was highly active against mechanical transmission and almost complete inhibition of cucumber mosaic cucumovirus **(Kubo *et al.*, 1990)**. **Hersanti (2005)** reported that leaf extract of *Mirabilis jalapa* is one agent induced systemic resistance against the attack of red pepper by CMV.

Praveen *et al.* (2001) isolated and purified a novel single resistance inducing protein (Crip-31) from the leaves of *Clerodendrum inerme*, which is a very potent, highly stable, basic in nature, 31 kDa in molecular mass having hydrophobic residues and induces a high degree of localized as well as systemic resistance against three different groups of plant viruses (*i.e.* CMV, PVY and ToMV), which differ at their genomic organization and having different replication strategies, infection in susceptible host *Nicotiana tabacum*. Minimum amount of purified preparation sufficient for systemic resistance induction was ~ 25 μgml^{-1}. The systemic inhibitory activity of the Crip-31 provides protection to whole plants within 40–60 min of its application. The systemic resistance inducing properties of this protein can be of immense biological importance, as it is similar to ribosome inactivating proteins (RIPs).

Shrivastava and Patel (2007) isolated, identified and characterized the chemical constituents and biotechnological prospects of the *Clerodendrum* genus. The major chemical constituents present in this genus were identified as phenolics, flavonoids, terpenes, steroids and oils.

Kombucha (the tea fungus) is a symbiotic colony of acetic and lactic acids bacteria with yeasts which can be divided for multiplication. Kombucha is mainly cultivated in sugared green tea to produce a slightly acidulous beverage including *Acetobacter xylinum* as a characteristic species and various yeasts **(Sreeramulu *et***

***al.*, 2000).** Acetic acid concentration may rise to levels as high as 20 g/L if the tea is allowed to ferment for up to 30 days **(Greenwalt *et al.*, 1998).**

Antioxidant and antimicrobial activities were achieved after fermenting sugared black tea, green tea or tea manufacture waste with tea fungus (Kombucha) for 12 to 15 days **(Jayabalan *et al.*, 2007).**

Therefore, this study was concerned with induction of systemic acquired resistance in tomato plants using water extracts of *M. jalapa*, *C. inerme* and Kombucha as antiviral to control the virus infection and detection SAR by SA level in treated plants as signaling in plant defense.

MATERIALS & METHODS

Virus isolate:

Cucumber mosaic virus (CMV.EG) was obtained from Virology Lab., Microbiology dept., Fac. of Agric., Ain Shams Univ. **(Megahed 2008)** and maintained in *Nicotiana glutinosa*. The identity of the virus isolate was confirmed biologically by characteristic reaction on some indicator hosts, namely *Chenopodium amaranticolor, C. murale*, *C. quinoa* and *Datura metel* and serologically by dot blot immunoassay (DBIA) **(Taha, 2010).**

Tomato plants:

The sterilized seeds of *L. esculentum cv.* Supermarmand VFN (Egyptian Company for Seeds, Oils and Chemicals, 2008) were sown in nursery. After one month, the seedlings were transplanted in other pots.

Biotic inducers:

Stock aqueous crude extraction (botanical) for each individual tested plant was made by blended 1 kg leaves tissue in 1 liter heated distilled water (65°C), Then filtered through 8 layers of muslin cloth. The supernatant was collected and stored in the refrigerator until use **(Vivanco *et al.*, 1999).**

Kombucha:

Crude kumbosha extract was prepared by fermenting sweetened green tea (100g sucrose, 10g Chinese green tea per liter of water) preparations with a symbiotic colony of yeasts and bacteria

(starter) for 12 days at 28°C, the extract was collected and centrifuged for 10 min. at 1000 rpm to separate any debris, then sterilized using sintered glass (G4) funnel **(Betsy and Sonford, 1996)**.

From each stock extract, one dilution was made (50%) using distilled water.

Greenhouse experiment:

The pots (five plants/pot) were divided into five groups and four of them were sprayed with inducers [(*Mirabilis jalapa, Clerodendrum inerme*, mixed (*M. jalapa* + *C. inerme*) and kombucha (30 ml per plant)] at the fourth leaves stage by the potential inducers at wet film, as well as control plants (the fifth group) sprayed with water according **(Vivanco *et al.* 1999).**

Samples:

Seven days after spraying tomato plants, samples from each previous treatments (for detection acquired resistance), were taken and other plants were rub-inoculated with CMV inoculum (CMV infectious sap 10^{-1} diluted in phosphate buffer 0.1 M and pH 0.7) by spatula to all treatments. Two separated groups of tomato plants served as control treatments were inoculated either by phosphate buffer or CMV, both not treated with tested inducers.

Quantification of total Salicylic Acid (SA):

Free and endogenous of SA were measured at once in the treatments by a method according to **Raskin (1992)**, with one modification by **Salem (2004)**. One gram of frozen tissue was ground in 3 ml of 90% methanol and centrifuged at 6000 rpm for 15 min. The pellet was back extracted with 3 ml of 99.5% methanol and centrifuged as above. Methanol extracts were combined and then centrifuged at 1500 to 2000 rpm for 10 min. The supernatant was dried at 40°C under vacuum using rotary evaporator (Heidolph.). The dried extracts were then resuspended in 3 ml of distilled water at 80°C and an equal volume of 0.2 M sodium acetate buffer, pH 4.5, containing 0.1 mg/ml β-glucosidase (22 unit/ mg, Sigma) was added, then the mixtures were incubated at 37°C overnight. After digestion, mixtures were acidified to pH 1 to 1.5 with

HCl. SA was extracted by adding (1:2, v: v) of sample: cyclopentan/ ethylacetate / isopropanol (50:50:1).

The organic extract was dried under nitrogen and analyzed by HPLC [SHIMADZO RF-10 AXL Fluorescence, HPLC Lab., National Research Center (NRC)]. One hundred microliters of each sample were injected into Dynamax 60A8 µm guord column (46mm x 1.5cm) linked to 40°C.

SA was separated with 23% v/v methanol in 20 mM sodium acetate buffer, pH 5.0 at a flow rate of 1.5 ml min -1. SA level was determined using standard curve.

Disease severity:

All tomato plants in each treatment were examined weekly for appearing of virus symptoms and determine disease severity, percentage of infection, reduction of virus and virus concentration after 25 days from CMV inoculation according to **Yang *et al.* (1996)**.

$$\text{Disease severity (DS)} = \frac{\Sigma\ (\text{disease grade} \times \text{number of plants in each grade})}{\text{Total number of plants} \times \text{highest disease grade}} \times 100$$

Reduction of virus infection:

The reduction of virus infection (R.I.) was calculated to all treatments as follow:

$$\text{Reduction Infection (R.I.)} = \frac{\text{Control - treated}}{\text{Control}} \times 100$$

RESULTS

The identity of CMV isolate was confirmed biologically where gave local lesion with morphological CMV character (chlorotic local lesion, **Figure 1-a)** as well as serologically using plyclonal antiserum specific for CMV by DBIA which gave purple color precipitation serological reaction **(Figure 1-b)**.

1- Quantification of total SA:

The obtained results from quantification of total SA in induced tomato plants before CMV inoculation were tabulated in **Table (1)** and **Figure (2-a:g),** it was observed that, the level of total SA has been increased in treated plants compared with untreated tomato plants with biotic inducers (H control) as well as with virus inoculated plants. However, the level of total SA in CMV infected

plants was higher than that of control but lower than that of biotic inducers.

The results in **Figure (2-a:g)** indicate that the healthy tomato plant (non treated) and tomato induced with *M. jalapa*, *C. inerme*, mixed *Mj+Ci* and kombucha extracts refers to the peaks obtained using HPLC, desired peak must be resulted in the retention time similar to the retention time of the standard. These peaks were used to calculate total SA based on the area under peak.

Kombucha gave the highest level of SA (9346.61 µg/g FW) followed by *C. inerme* (8652.78 µg/g FW), *M. jalapa* (7451.63 µg/g FW), while mixed *Mj+Ci* gave the lowest level of SA (3124.18 µg/g FW), compared with healthy and inoculated controls (293.79 and 1615.43 µg/g FW), respectively **(Table, 2).**

2- Virus infectivity:

The antiviral activity was assessed based on the number of local lesions formed on control and treated *C. amaranticolor* leaves.

All treatments in **Table (2)** decreased the percentage of infection and the decrease varied according to the treatment, being 24% with *M. jalapa* treatment, 40% with *C. inerme*, 50% with *Mj + Ci* and 40.7% with Kombucha. Disease severity parameter (DS) also showed different values due to inducers, *M. jalapa* has a low percentage of DS (11.5%), while mixed (*Mj+Ci*) has a high percentage of DS (24.1%). On the other hand, *C. inerme* and Kombucha were found to have moderate DS percentage, (13.7%) and (18.0%) respectively, compared with control (96.0%).

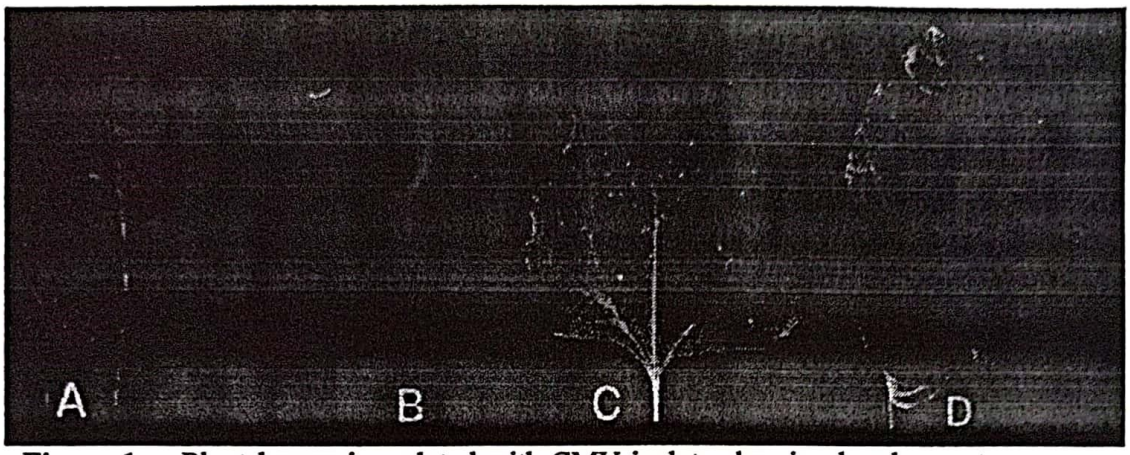


Figure 1-a. Plant leaves inoculated with CMV isolate showing local symptoms on *Chenopodium murale* (A), *C. quinoa* (B), *C. amaranticolor* (C) and *Datura metel* (D).

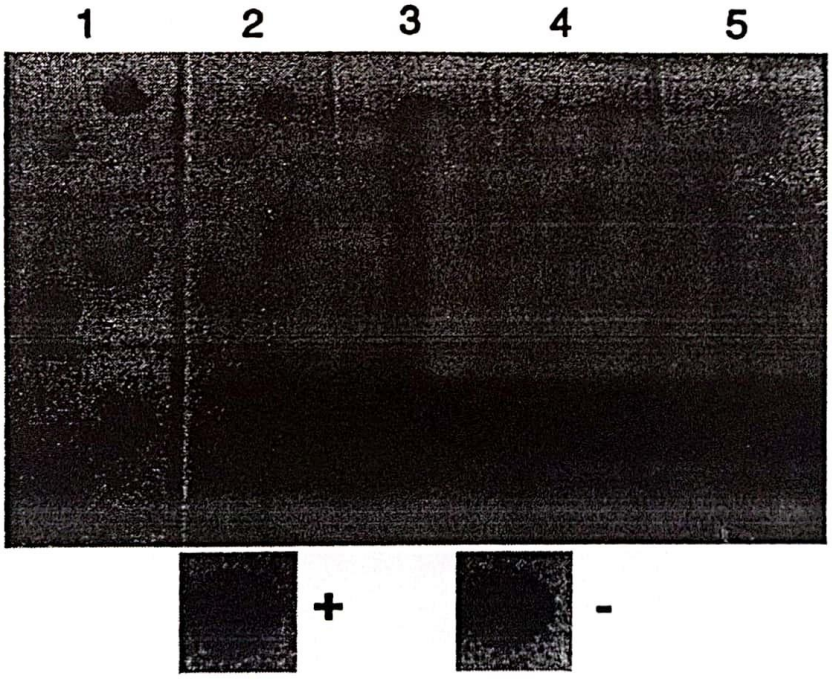


Figure 1-b. Dot Blot Immunoassay for CMV precipitation against specific IgG-CMV polyclonal.
+ : Positive - : Negative
Infected samples (Row,1) *N. glutinosa*, (row 2,3) Tomato, (row 4) *Datura* and (row 5) Cucumber.

Table 1. Quantification of total SA in tomato plants induced with biotic inducers compared with healthy and infected plants:

Treatments	No. peak	Area	Ret. time	Area %	Total SA (µg/g FW)
Standard SA	1	1195.321	4.301	100	-
Inoculated control	2	3861.915	4.951	57.5735	1615.43
M. jalapa (M)	1	17814.2	4.892	70.9815	7451.63
C. inerme (Y)	4	20685.7	4.050	37.1380	8652.78
Mixed (M+Y)	2	7468.814	4.944	57.4512	3124.18
Kombucha	6	22344.2	4.188	38.9318	9346.61
Healthy	4	702.349	4.626	92.5020	293.79

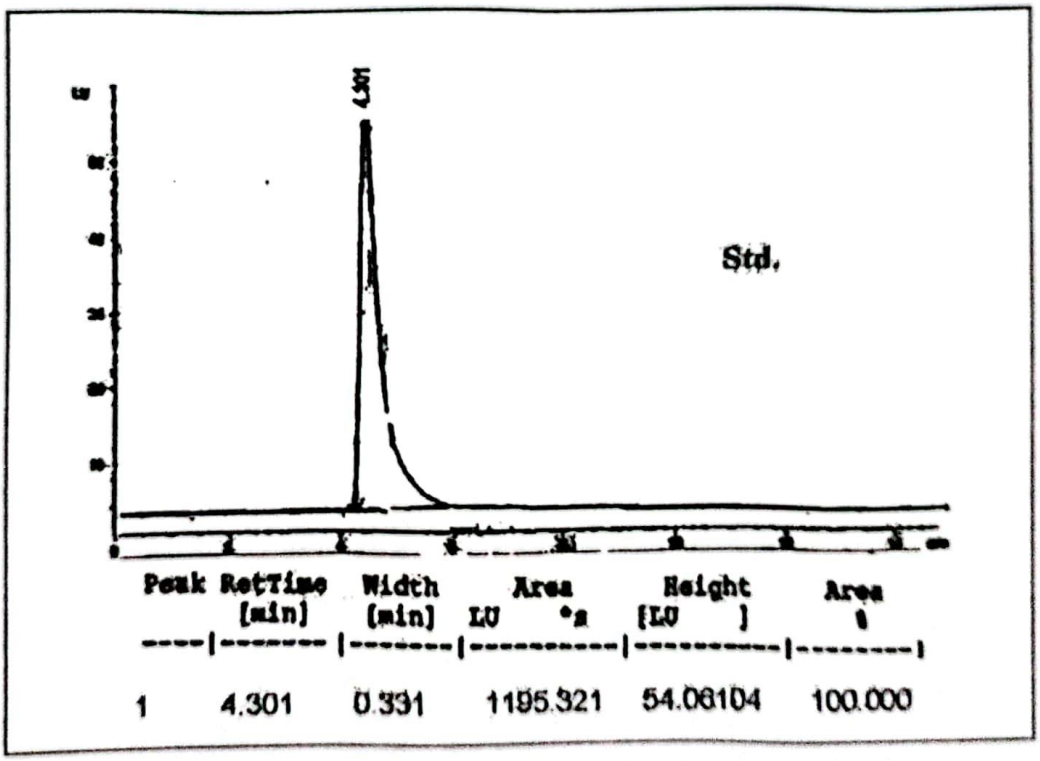
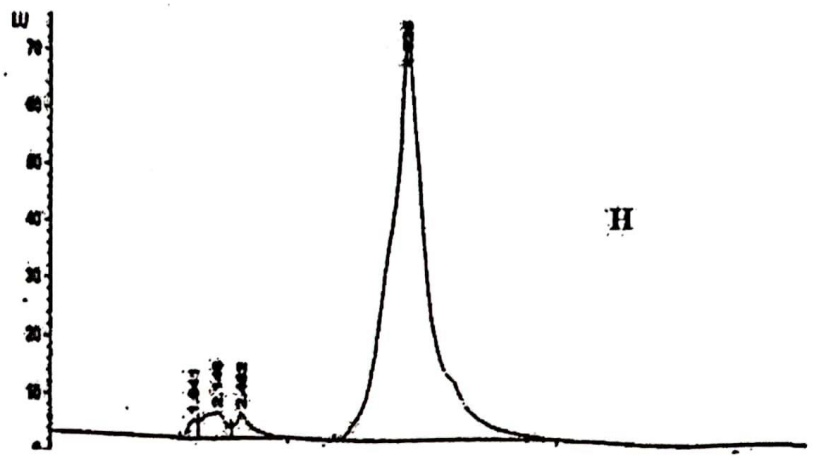
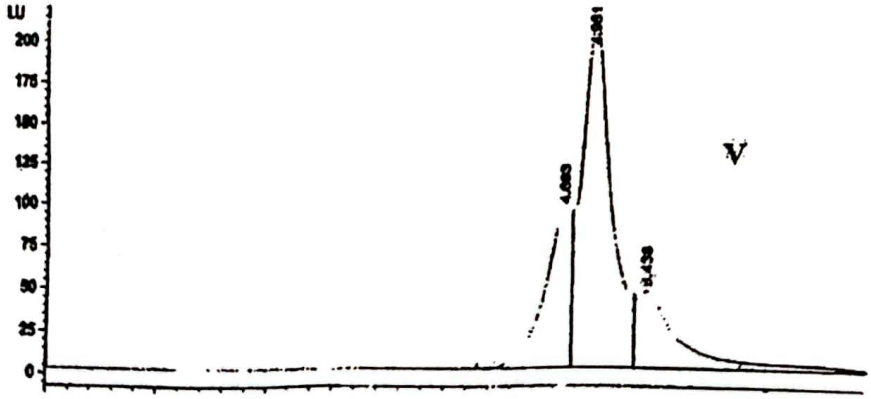


Figure 2-a. HPLC quantification of free and endogenous SA in induced tomato plants.



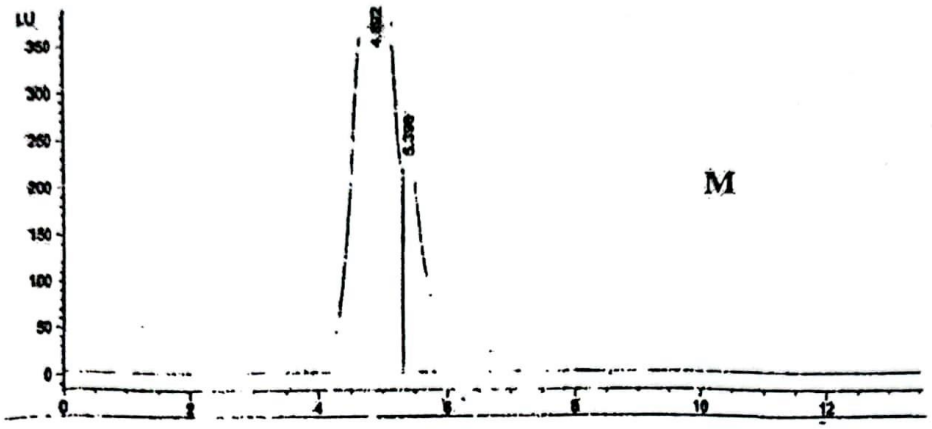
Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
4	4.626	0.4268	702.349	68.99808	92.5020

Continued Figure 2-b. (H) healthy plant.



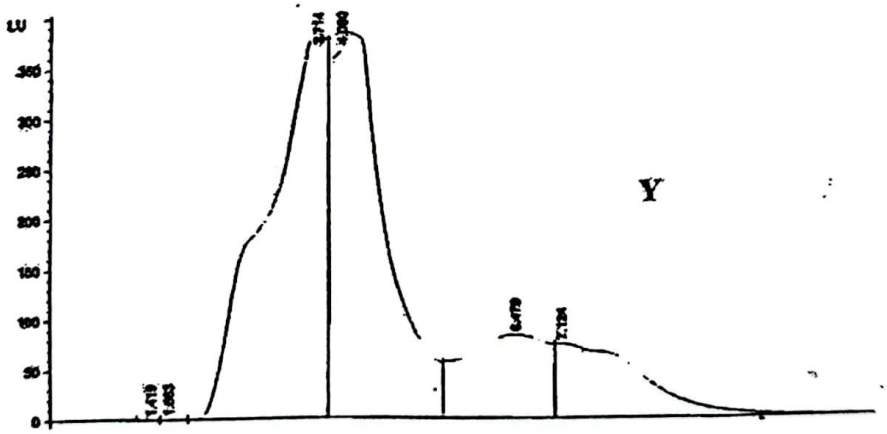
Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
2	4.951	0.2664	3861.915	207.9285	57.5735

Continued Figure 2-c. (V) Inoculated control.



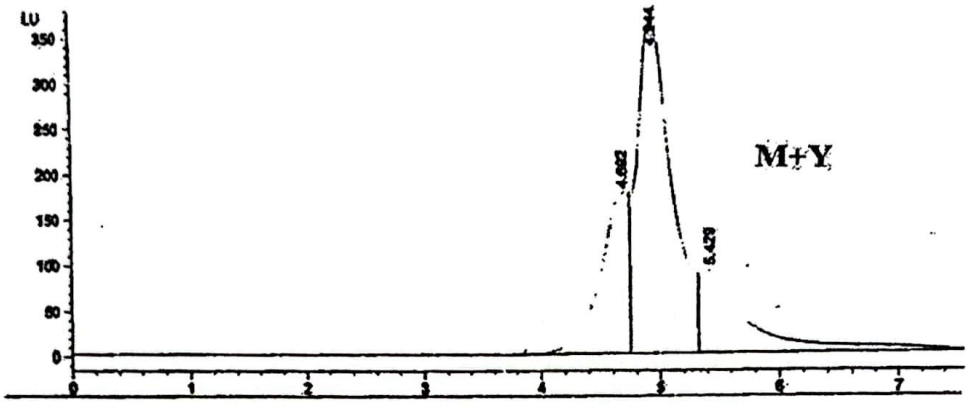
Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
1	4.892	0.5970	17814.2	368.1009	70.9815

Continued Figure 2-d. (M) Tomato plants treated with *M. jalapa*



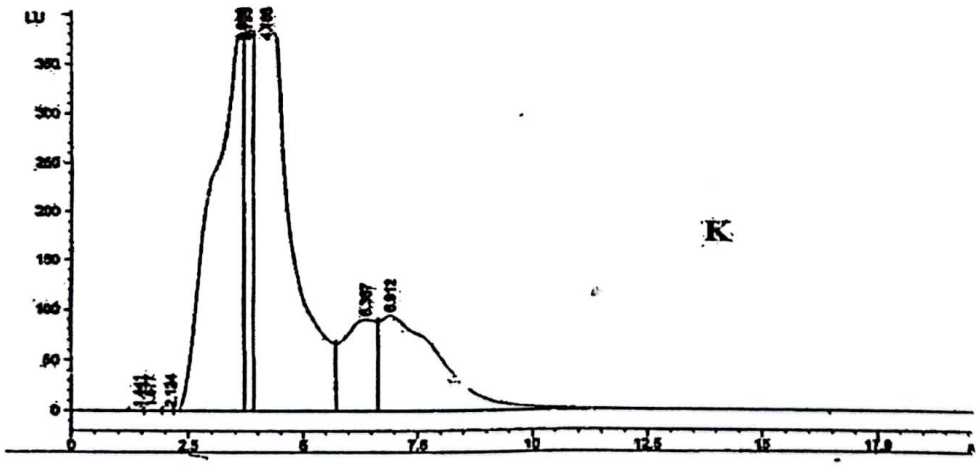
Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
4	4.050	0.6620	20685.7	383.3376	37.1380

Continued Figure 2-e. (Y) Tomato plants treated with *C. inerme*.



Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
2	4.944	0.2982	7468.814	359.9571	57.4512

Continued Figure 2-f. (M+Y) Tomato plants treated with (*Mj+Ci)*.



Peak	RetTime [min]	Width [min]	Area LU *s	Height [LU]	Area %
6	4.188	0.7147	22344.2	384.4832	38.9319

Continued Figure 2-g. (K) Tomato plants treated with Kombucha.

Table 2. Effect of biotic inducers on CMV infectivity in tomato plants.

Treatments	%of infection	%of R.I.	D.S. (%)	Conc.(Mean no. of L.L.)
Inoculated control	100.0	0.0	96.2	85.5
M. jalapa	24.0	76.0	11.5	10.2
C. inerme	40.0	60.0	13.7	12.2
Mixed (*Mj+Ci*)	50.0	50.0	24.1	21.4
Kombucha	40.7	59.2	18.0	16.0

R.I. = reduction of virus infection. D.S. = Disease severity.
Conc. = Virus concentration (Mean number of local lesions).

DISCUSSION

The induction of SAR by biotic inducers in tomato plants was biologically detected by percentage of infection, reduction of virus infection and disease severity and virus concentration, as well as by biochemical analyses determination: the level of salicylic acid.

Tomato plants induced with biotic inducers (M, Y, M+Y, K) as well as with CMV inoculated showed increase in the total SA than uninduced tomato plants ones.

The obtained results from quantification of free and endogenous SA using HPLC in induced cucumber plants were in harmony with percentage of infection, disease severity and CMV concentration.

The level of SA had been increased in treated plants. Kombucha and *C. inerme* treatments have the highest levels of SA being 9346.61 and 8652.78 µg/g FW, respectively, followed by *M. jalapa* (7451.63 µg/g FW), While mixed (*Mj+Ci*) gave the lowest level of SA (3124.18 µg/g FW).

The same results were obtained by many authors **(Mahmoud, 2003; Megahed, 2008; Taha, 2010 and Park *et al.*, 2010).**

Many evidences suggest that SA is a SAR signal **(Raskin, 1992 and Vernooij *et al.*, 1994).** In tobacco, SA levels increase as much as 180-fold after local lesion infection **(Malamy *et al.*, 1992 and Gaffney *et al.*, 1993)** and it was found that free and bound SA are produced around the infection site, whereas only free SA is detected in distal region **(Malamy *et al.*, 1992 and Yalpani *et al.*, 1994).**

Haung *et al.* (2006) measured SA quantitatively *in situ* in *Nicotiana tabacum* L. cv Xanthi-nc leaves inoculated with *Tobacco mosaic virus* (TMV). The biosensor revealed accumulation of apoplastic SA before the visible appearance of hypersensitive response (HR) lesions.

It has been found that SA acts as the internal general resistance in plants and induces the expression of messenger RNA, which presumably directs the synthesis of the PR proteins **(Moffat, 1992). Pieterse and Van Loon (1999)** indicated that, salicylic acid is an important signalling molecule involved in both locally and systemically induced disease resistance responses.

The second criterion used to judge the occurrence of SAR in tomato plants treated with biotic inducers is the reduction of percentage of infection. In our study, four inducers were able to reduce number of CMV infected tomato plants.

The reduction varied according to the inducer from 24 to 50%. The same results were obtained by many authors **(Raupach *et al.*, 1996; Zehnder *et al.*, 2000 and Megahed, 2008).**

All treatments have different percentage of DS, *M. jalapa* treatment has a low percentage of DS (11.5%) while *Mj+Ci* treatment has a high percentage of DS (24.1%).

Virus concentration was determined as mean number of local lesion on *C. amaranticolor* as indicator plant for CMV.

The obtained results show that *M. jalapa* and *C. inerme* treatments gave the lowest mean number of local lesions being 10.2 and 12.2 L.L, respectively. The highest concentrations were recorded for mixed (*Mj+Ci*) and kombucha.

Our results are in agree with **Mucharromah and Kuc (1991)** who mentioned that, oxalate and phosphates induced systemic resistance against CMV in cucumber plants by reducing the number of CMV chlorotic lesions, as well as with **Abo El-Nasr *et al.*(2004)** who found different reduction in ZYMV and PVY lesions produced on cucumber and pepper plants, respectively by induction of SAR with salicylic acid, di-potassium phosphate, chelated iron, acid and oxalic acid, potassium sulfate, magnesium sulfate, ammonium sulfate, calcium super phosphate and calcium nitrates treatments.

REFERENCES

Abo EI-Nasr, M.A.; El-Dougdoug, Kh.A.; El-Kattan, M.H. and Salem, E.A. (2004). Induction of salicylic acid in cucumber against ZYMV potyvirus by some nutrient chemicals. Egyptian J. Virol. 1:301-312.

Betsy, P. and Sonford, H. (1996). Kombucha Phenomenon. The Miracle Health tea how to safely make and use kombucha. 2nd ed Sierra Sunrise Publishing Inc.

Gaffney, T.; Friedrich, L.; Vernooij, B.; Negrotto, D.; Nye, G.; Uknes, S.; Ward, E.; Kessmann, H. and Ryals, J. (1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261: 754-756.

Greenwalt, C.J.; Ledford, R.A. and Steinkraus, K.H. (1998). Determination and characterization of the antimicrobial activity of the fermented tea Kombucha. Lebensmittel Wissenschaft und Technologie 31(3):291-296.

Hersanti, C. (2005). The Analysis of peroxidase activity and salicylic acid content of resistant red chilli plant to Cucumber mosaic virus (CMV) induced by crude extract of leaf *Mirabilis jalapa*. Indonesian Journal of Plant Protection, XI:1

Huang, W.E.; Huang, L.F.; Preston, G.M.; Naylor, M.; Carr, J.P.; Li, Y.H.; Singer, A.C.; Whiteley, A.S. and Wang, H. (2006). Quantitative in situ assay of salicylic acid in tobacco leaves using a genetically modified biosensor strain of *Acinetobacter* sp. ADP1. Plant J. 46(6): 1073-1083.

Jayabalan, R.; Marimuthu, S. and Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chemistry 102(1): 392-398.

Kubo, S.; Ikeda, T.; Imaizumi, S.; Takanami, Y. and Mikami, Y. (1990). A potent plant virus inhibitor found in *Mirabilis jalapa* L. Annals of the Phytopathological Society of Japan 56(4):481-487.

Levine, A.; Tenhaken, R.; Dixon, R. and Lamb, C. (1994).

H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79: 583–593.

Mahmoud, S.Y.M. (2003). Systemic acquired resistance induced in viral infected tobacco plants via viral necrotizing and plant decapitation. Assiut Journal of Agricultural Science 34(5): 334-351.

Malamy, J.; Hennig, J. and Klessig, D.F. (1992). Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to *tobacco mosaic virus* infection. Plant Cell, 4: 359-366.

Megahed, A.A. (2008). Effect of antiviral proteins produced by bacterial and fungal isolates on some viruses infecting vegetable crops. M.Sc. Thesis, Fac. Agric., Ain Shams University, Cairo, Egypt. 193p.

Metraux, J.P. and Raskin, I. (1993). In: Ihan, C. (Ed.), Biotechnology in Plant Disease Control. Wiley-Liss Inc., London, pp. 191–209.

Moffat, A.S. (1992). Improving plant disease resistance. Science. 257: 282-283.

Mucharromah, E. and Kuc, J. (1991). Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and virus in cucumber. Crop protection 10:230-265.

Park, S.W.; Liu, P.P.; Forouhar, F.; Vlot, A.C.; Tong, L.; Tietjen, K. and Klessig, D.F. (2010). Use of a synthetic salicylic acid analog to investigate the roles of methyl salicylate and its esterases in plant disease resistance. The Journal of Biological Chemistry 248(11): 7307-7317.

Pieterse, C.M. and Van Loon, L.C. (1999). Salicylic acid-independent plant defence pathways. Trends Plant Sci. 4(2):52-58.

Praveen, S.; Tripathi, S. and 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: 453–459.

Raskin, I. (1992). The role of salicylic acid in plant. Annu. Rev. plant Physiol. Mol. Boil. 43: 439-463.

Raupach, G.S.; Liu, L.; Murphy, J.; Tuzun, S. and Kloepper, J.W. (1996). Induced systemic resistance in cucumber and tomato against *cucumber mosaic cucumovirus* using plant growth-promoting rhizobacteria (PGPR). Plant Disease 80: 891-894.

Roossinck, M.J. (1999). Cucumoviruses (Bromoviridae)-general features. In: Granoof, L., Webster, R.G. (Eds.), Cucumoviruses (Bromoviridae)-general features. Encyclopedia of Virology, second ed. Academic Press, San Diego, pp. 315–320.

Salem, E.A. (2004). Induction of salicylic acid by some chemical substance used to control virus infection under protected Agriculture. M.Sc. Thesis. Fac. of Agric., Ain Shams. Univ. Cairo, Egypt, 118 P.

Shrivastava, N. and Patel, T. (2007). *Clerodendrum* and Healthcare: An Overview-Part II: Phytochemistry and Biotechnology. Medicinal and Aromatic Plant Science and Biotechnology 1(2): 209-223.

Sreeramulu, G.S.Y.; Zhu, Y. and Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. J. Agric. Food Chem. 48:2589-2594.

Taha, M.A.T. (2010). Biological control of *cucumber mosaic virus (cucumovirus)* by certain local streptomycetal isolates. M.Sc. Thesis, Fac. Agri., Ain Shams University, Cairo, Egypt. 217p.

Verma, H.N. and Kumar, V. (1982). Prevention of plant virus diseases by *Mirabilis jalapa* leaf extract. New Botanist 7:87-91.

Vernooij, B.; Friedrich, L.; Morse, A.; Reist, R.; Kolditz-Jawhar, V.; Ward, E.; Uknes, S.; Kessmann, H. and Ryals, J. (1994). Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant cell 6: 959-965.

Vivanco, J.M.; Querci, M. and Salazar, L.F. (1999). Antiviral and antiviroid activity of MAP containing extracts from *Mirabilis jalapa* roots. Plant Dis. 83: 1116-1121.

Yalpani, N.; Enyedi, A.J.; Leon, J. and Raskin, I. (1994). Ultra violet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. Planta 193: 373–376.

Yang, X.; Liangyi, K. and Tien, P. (1996). Resistance of tomato infected with *cucumber mosaic virus* satellite RNA to potato spindle tuber viroid. Ann. Appl. Biol. 129: 543-551.

Zehnder, G.W.; Yao, C.B.; Murphy, J.F.; Sikora, E.R. and Kloepper, J.W. (2000). Induction of resistance in tomato against *cucumber mosaic cucumovirus* by plant growth-promoting rhizobacteria. Biocontrol 45 (1): 127-137.