J. Basic. Appl. Sci. Res., 5(1) 18-26, 2015

ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Aphid-Transmission Efficiency of *Cucumber mosaic cucumovirus* and *Zucchini yellow mosaic potyvirus* on Squash and Their Control Using Essential Plant Oils

Ali. M. El-Borollosy

Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt

Received: November 14, 2014
Accepted: December 26, 2014

ABSTRACT

Cucumber mosaic cucumovirus (CMV) and *Zucchini yellow mosaic potyvirus* (ZYMV) were isolated from naturally infected squash plants serologically depending on indirect enzyme-linked immunosorbant assay (I-ELISA) and biologically by mechanical inoculation on *Chenopodium amaranticolor* as a local lesions host and finally maintained on *Nicotiana tabacum* cv. White Burley. Survey of different aphid species associated with squash plants in the field was carried out proving that *Myzus persicae* and *Aphis gossypii* were the most abundant species recording 65.3 and 57.5 insect per plant, respectively. Aphid virus transmission efficiency was studied and found that the winged forms of aphids were less efficient than the wingless ones in all tested species. The most efficient vector for CMV was *A. gossypii* and for ZYMV was *M. persicae* achieving 95 % and 100 %, respectively. After feeding aphids on virus infected squash plants for 10 min, insects were print-captured individually and virus was successfully detected by immunocapture reverse transcriptase polymerase chain reaction PC/IC-RT-PCR, as the 657 bp (CMV coat protein gene (*cp*))& 1.3 kbp (ZYMV helper component proteinase gene (*Hc-pro*)) bands were detected within agarose gel. Using *Lavendula officinalis* oil preparations for spraying squash plants revealed excellent results, as all plants sprayed with this soap emulsified oil confirmed to be CMV-free by I-ELISA and IC-RT-PCR, while only one plant confirmed to be infected with ZYMV by IC-RT-PCR. Results confirmed the high repellent effect of Lavender oil, as all plants sprayed with such oil preparations did not show any insects population. Concerning killing effect all preparations containing soap gave the highest killing effect on aphids, revealing that diluted soap preparations can be used as a safe vector insects controlling method in small fields and greenhouses.

KEYWORDS: *Cucumber mosaic cucumovirus*, *Zucchini yellow mosaic potyvirus*, Isolation, Aphid species, Essential oils, PC/IC-RT-PCR, Transmission control.

INTRODUCTION

Major viruses that infecting squash crop worldwide and affect its production are *Cucumber mosaic cucumovirus* (CMV) **(Brunt et al., 1996; Mauck et al., 2010)** and *Zucchini yellow mosaic potyvirus* (ZYMV) **(Provvidenti et al., 1984; Shehata and El-Borollosy, 2008)**.

CMV, genus: *Cucumovirus*, family: Bromoviridae, is one of the most widespread plant viruses with extensive host range infecting about 1000 species including cereals, fruits, vegetables and ornamentals **(Roossinck, 1999)**. The virus is readily transmitted in a non-persistent manner by more than 75 species of aphids **(Palukaitis et al., 1992)**.Virus particles are isometric of about 28-30 nm in diameter. CMV is a multicomponent virus with a single stranded positive sense RNA. RNAs 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein. Numerous strains of CMV have been classified into two major subgroups (subgroups I and II) on the basis of serological properties and nucleotide sequence homology **(Palukaitis et al., 1992; Madhubala et al., 2005)**. The subgroup I has been further divided into two groups (IA and IB) by phylogenetic analysis **(Roossinck et al., 1999)**.

ZYMV is one of the most economically important viruses of cucurbit crops. It is efficiently aphid-transmitted in a nonpersistent manner and it is also seed-borne in zucchini squash, which could have contributed to its rapid spread worldwide **(Lecoq et al., 2009)**.The virus has flexuous filamentous particles of 750 nm long, consist of a single-stranded RNA about 9600 nucleotides long **(Lisa et al., 1981)**. Two

***Corresponding Author:** Ali. M. El-Borollosy, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt

viral proteins are required for aphid transmission of ZYMV: the coat protein (CP) and a nonstructural protein, the helper component (HC) **(Pirone, 1991)**.

Transmission experiments of different isolates of Cucumber mosaic virus (CMV), Watermelon mosaic virus (WMV) and Zucchini yellow mosaic virus (ZYMV) were conducted using established colonies of *Aphis gossypii* Glover, *Myzus persicae* (Sulzer), *A. fabae* Scopoli and *A. craccivora* Koch as vectors. The transmission procedure used provided an estimate of the virus transmission efficiency for each aphid species. *A. gossypii* was found to be the most efficient vector of CMV (100 ± 0 %), while *M. persicae* showed the highest efficiency of transmiting WMV (67.9 ± 28.5 %) and ZYMV (96.4 ± 3.6 %) **(Garzo et al., 2004)**.

Aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Most of their properties are due to essential oils produced by their secondary metabolism **(Bell and Charlwood, 1980)**.

Today Lavender (*Lavendula officinalis*) essential oil is a component of many aromatherapy blends. Lavender essential oil contains up to 40 % linalyl acetate and 30 % linalool. Linalool is a terpene alcohol that is non-toxic to the human and naturally germicidal **(Bouzouita et al., 2005)**. It was found that linalool have the highest inhibitory effect over other aromatic plants' essential oils components, which representing the main component of *Ocimum basilicum*, and *Thymus vulgar is* essential oils **(Bassolé and Juliani, 2012; Oraby and El-Borollosy, 2013)**.

Natural oils have an advantage of less phytotoxic effect when applied to seedling in their early growth stages, compared with chemical insecticides **(Iovieno et al., 2002)**. Mineral oil is known from a long time such as an effective mean to control aphids and to reduce non-persistent viruses spread **(Brachet et al., 2001)**. Therefore, natural oils were recommended to be a harmless replacement of chemical insecticides for human biosafety.

Controlling stylet-borne plant viruses transmitted by aphids is one of the greatest challenges facing vegetable growers today. Attempts to control these diseases by killing winged aphids with potent chemical aphicides have proven futile and human health risky. Aphids transmit viruses in periods of time much less than required for an insecticide to work **(Oraby and El-Borollosy, 2013)**.

Plant essential oils that are both repellent, toxic and insect suffocating effect can be more effective for control in practice. Repellence prevents settlement of arriving aphids and the toxic properties kill the aphids that are already present on the crop before application of the oils. Oils may also interfere with insect feeding as it may cover mouse parts preventing feeding or virus transmission **(Munneke et al., 2004)**.

Therefore, the aim of this investigation is to survey different aphid species associated with squash plants in the field, studying aphids virus transmission efficiency, extract *Lavendula officinalis* and *Ocimum basilicum* essential oils and study its action as a natural, safe, and reliable bio-controlling agent against prohibiting virus transmission and observing the repellent and killing effect of oils spraying on vector insects.

MATERIALS AND METHODS

Isolation

Viruses used during this investigation were isolated from squash plants showing virus like symptoms (collected from a small squash field at Faculty of Agriculture, Ain Shams University, Kalubia Governorate, Egypt).

Isolation was performed depending on indirect enzyme linked immunosorbant assay (I-ELISA) according to **Koenig (1981)** using specific polyclonal antibodies against *Cucumber mosaic cucumovirus* (CMV) and *Zucchini yellow mosaic potyvirus* (ZYMV) (Agdia Inc., USA), and *Chenopodium amaranticolor* as local lesion hosts. Virus was maintained on *Nicotiana tabacum* cv. White Burley under greenhouse conditions (28ºC ±2).

Aphid survey

Survey of different aphid species associated with squash plants in the field (squash field at Faculty of Agriculture, Ain Shams University, Kalubia Governorate, Egypt) was made by direct counting method in which the total number of each aphid species (on the sample of 20 random plants) was counted. Collected aphid species were mounted, examined and identified according to the description of **Claude and Guy (2002)**.

Aphid transmission efficiency

Aphid species were collected and identified according to **Blackman and Eastop (1984)** from the same squash field. Aphid's samples were reared on turnip plants as a virus-free culture (biologically & serologically confirmed) in separate colonies of the species.

Aphids colonies of each species used for transmission experiments were started from a non-viruliferous single virginiparous female and reared on turnip plants in an environmental growth chamber under controlled conditions [23:16 ºC (day: night) and a photoperiod of 16:8 h (light: dark)].

Groups of 25-30 individuals of each aphid species were collected, starved for one hour and placed, for 30min acquisition period, on CMV or ZYMV infected squash plants, which had been inoculated 3 weeks previously. Aphids of different species and forms (winged or wingless) which were fed on infected plants transferred to feed on healthy squash seedlings (carrying 5 leaves) (10 aphids per plant & 20 plant per each insect species and form) for 1 h inoculation feeding period, then sprayed with insecticide, and kept in an insect proof cage. After 15 days, plants were assayed for CMV or ZYMV infection using I-ELISA.

Effect of essential oils on virus transmission by aphids

a) Preparation of vector insects

As the most predominant and virus transmission efficient, *M. persicae* and *Aphis gossypii* non-viruliferous wingless form colonies were used for ZYMV and CMV, respectively. Groups of 25-30 individuals of both aphids were collected, starved for one hour and placed, for 10 min acquisition period, on CMV or ZYMV infected squash plants, which had been inoculated 3 weeks previously. Negative controls were managed similarly using healthy plants.

The print capture immunocapture reverse transcriptase polymerase chain reaction (PC/IC-RT-PCR) was used to confirm the presence of CMV and ZYMV within individual aphid. A sample of 20 aphids for each virus were squashed individually on Whatman 3MM paper using the round bottom of an Eppendorf tube. Insect was extracted from paper with 100 µl of 0.5 % Triton X-100, the extract was then added to a 2 µg/ml of anti-virus IgG precoated PCR tubes and PCR protocol was performed according to **Olmos et al. (1997)**.

To detect the CMV coat protein gene (*cp*) and ZYMV helper component gene (*Hc-pro*), the following used primers (Invitrogen Corp., USA) were designed using Primer Premier software (PREMIER Biosoft International, USA) depending on the nucleotide sequence of ZYMV strain KR-PA (AY278998) **(Kown et al., 2005)**:

5'ATGTCGTCGCAACCGGAAGTTCAGTTCTTC3' (Sense)

5'TTACCAACTCTGTAATGCTTCATCT CGC3' (Antisense).

And designed depending on CMV *cp* gene sequences collected from PubMed GeneBank web site (http://www.ncbi.nlm.nih.gov) and according to **El-Afifi et al. (2007)**:

5'ATGGACAAATCTGAATCAAC3' (Sense)

5'TCAAACTGGGAGCACCCCAG3' (Antisense).

PCR products (10 µl) were analyzed by 1.5 % agarose gel electrophoresis. Bands size was determined from the gel photograph using Gel-Pro Analyzer software (Media Cybernetics, USA).

b) Essential oils source and preparation

The oil extracts were obtained from 50 g of *Lavendula officinalis* and *Ocimum basilicum* dried plants aerial parts by steam distillation according to **Barazandeh (2002)**.

Oils emulsion was prepared depending on commercial liquid dish wash soap (Peril®) as emulsifying agents. Oil dilution was prepared by adding 5 ml of oil to 100 ml of distilled water with 3 ml from soap. Controls were mixtures of corn oil with soap, water with soap and water only.

c) Aphid transmission

Healthy squash plants (carrying 5 leaves) were treated by spraying with different preparations mentioned before (using 5 plants for each treatment and 10 aphids per plant). Aphids (*M. persicae* and *A. gossypii* for ZYMV and CMV, respectively) which were fed on infected plants transferred to feed on treated healthy plants for 10 min inoculation feeding period, then sprayed with insecticide and kept in an insect-proof cage. After 15 days plants were assayed for CMV and ZYMV infection using I-ELISA. For confirming virus absence, plants which gave negative I-ELISA values were further tested with IC-RT-PCR as described by **Varveri (2000)**, using the same pairs of primers mentioned before.

Repellent and killing effect of oil spraying on vector insects

For such experiment squash plants (carrying 5 leaves) were sprayed with previously mentioned preparations in pots (5 plants for each treatment). Plants were then left under open field conditions near to plants with high insect's intensity (i.e., *M. persicae* and *Bemisia tabaci* (Whitefly)). Spraying was performed every 5 days till 20 days, in which plants were evaluated for insect's populations.

Another experiment was performed (in insects-proof cages) to study the killing effect of oil preparations on *M. persicae* aphids as the most abundant in squash fields. Treatments were applied by spraying on squash plants (5 plants for each treatment) each carrying 50 wingless aphids. After 24 h killing effect was studied by determination of surviving insect's approximate number per plant.

RESULTS

Virus isolation

Cucumber mosaic cucumovirus (CMV) and *Zucchini yellow mosaic potyvirus* (ZYMV) were isolated from naturally infected squash plants (showing mainly mosaic and malformation) **(Figure 1, A)**. Samples which gave positive I-ELISA results with either CMV or ZYMV specific antiserum produced chlorotic local lesions on *Chenopodium amaranticolor* **(Figure 1, B)**. Three cycles of local lesion isolation were performed and the last produced lesions were inoculated and maintained separately on *Nicotiana tabacum* cv. White Burley plants.

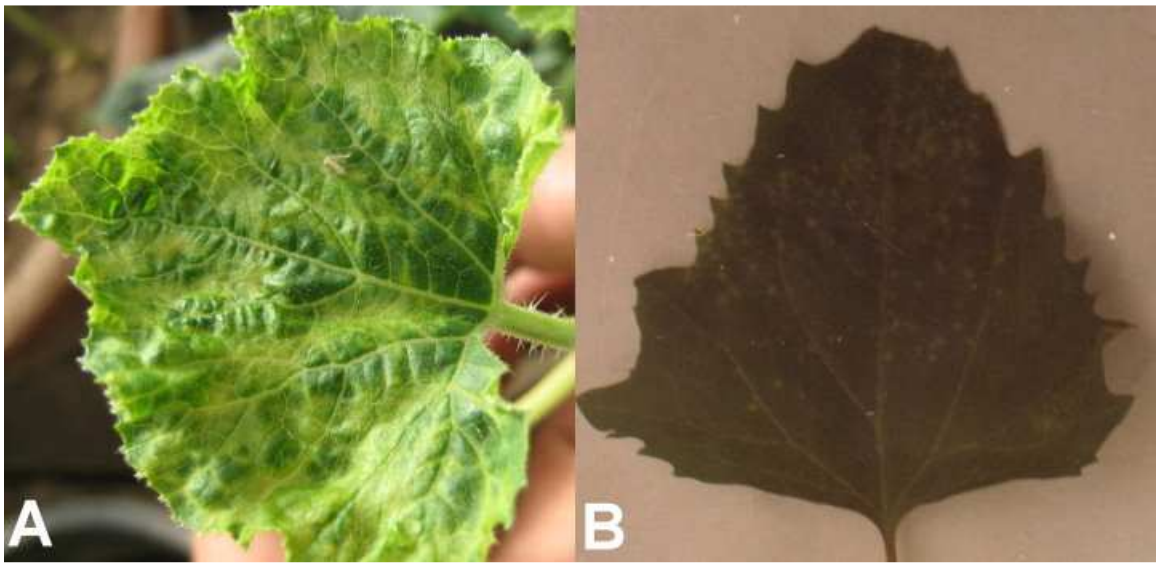

Figure (1): Isolation of viruses from squash plants with virus like symptoms (A), depending on chlorotic local lesions produced on *Ch. amaranticolor* (B).

Aphid survey

Five different aphid species were associated with squash plants in the field which were: *Myzus persicae*, *Aphis craccivora*, *A. gossypii*, *Acyrthosiphon pisum* and *A. nerii*. *M. persicae* and *A. gossypii* were the most abundant species recording 65.3 and 57.5 insect per plant, respectively, followed by *A. craccivora* and *A. nerii*, 30.1 & 21.8 insect per plant, respectively, whereas, *A. pisum* was the least abundant recording 10.7 insect per plant.

Aphid transmission efficiency

The five tested aphid species were able to acquire and transmit both viruses after 30 min from infected to healthy squash plants **(Table 1)**. The most efficient vectors for CMV and ZYMV were the wingless form of *A. gossypii* and *M. persicae*, achieving 95 % &100 % transmission rate, respectively. The lowest transmission rates were obtained with the wingless form of *A. pisum* which were15 % &10 % for CMV and ZYMV, respectively.

Obtained results proved that the winged forms of aphids were less efficient than the wingless ones in all tested species **(Table 1)**, as *A. gossypii* and *M. persicae* winged form gave 40 % & 45 % for CMV and ZYMV, respectively.

Table (1): Rate of transmission of both CMV and ZYMV using winged and wingless forms of 4 different aphid species

Aphid species & forms*	Number of infected squash seedlings per 20 tested			
	CMV		ZYMV	
	Number	%	Number	%
Myzus persicae				
Wingless	15	75	20	100
Winged	7	35	9	45
Aphis gossypii				
Wingless	19	95	17	85
Winged	8	40	5	25
Aphis craccivora				
Wingless	13	65	14	70
Winged	5	25	6	30
Aphis nerii				
Wingless	9	45	7	35
Winged	3	15	2	10
Acyrthosiphon pisum				
Wingless	3	15	2	10
Winged	1	5	0	0

* Ten insects were used per plant (30 min acquisition and 1 h inoculation periods)

Effect of essential oils on virus transmission by *Myzus persicae*

Detection of CMV and ZYMV within *M. persicae* using PC/IC-RT-PCR

Print capture PCR was used successfully for detecting CMV and ZYMV within individual aphid (*A. gossypii* for CMV and *M. persicae* for ZYMV). All of the 20 aphids for each virus studied gave positive PCR results in the form of *cp* and *Hc-pro* genes bands with the expected size of 657 bp & 1.3 kbp for CMV and ZYMV, respectively **(Figure 2)**. This result assumed that approximately all the insects which will be used to study virus transmission were carrying virus particles.

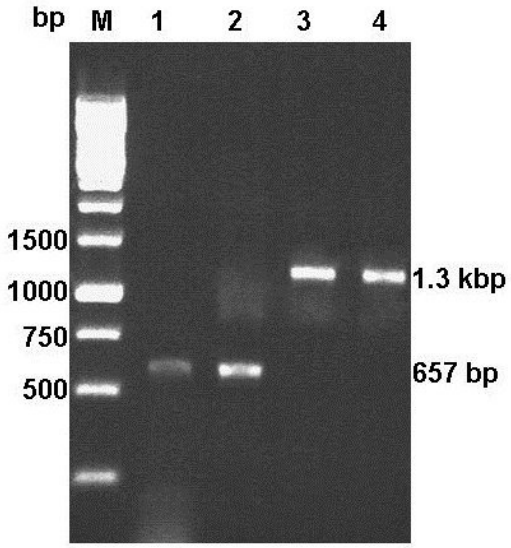

Figure (2): PC/IC-RT-PCR for detection of CMV *cp* (Lanes: 1-2) and ZYMV *Hc-pro* (Lanes 3-4) genes within individual aphid. M: 1kbp DNA marker (Promega, USA).

Aphid transmission

Using essential oils preparations for spraying squash plants revealed excellent results, as all plants sprayed with Lavender oil with soap confirmed to be virus-free by I-ELISA and IC-RT-PCR, while only

one plant confirmed to be infected with ZYMV by IC-RT-PCR. On the other hand corn oil with soap gave one of virus-free plants either with CMV or ZYMV. It was also found that treatment with *Ocimum basilicum* oil with soap gave 3 and 2 CMV and ZYMV free plants, respectively. The obtained results demonstrated in **Table (2)** favored the use of Lavender oil with soap, for oil can suppress insect infestation, while soap has a killing effect on delicate insects with sucking mouthparts like aphids.

Table (2): Effect of oil treatments on CMV and ZYMV transmission by aphids

Treatment* & Virus / Plant		I-ELISA and IC-RT-PCR results**									
		Lavendula officinalis		*Ocimum basilicum*		Corn		Water & soap		Water only	
		CMV	ZYMV	CMV	ZYMV	CMV	ZYMV	CMV	ZYMV	CMV	ZYMV
1	Ev	0.211	0.303	0.210	0.095	0.199	0.300	0.811	0.201	0.914	0.799
	PCR	-	+	-	-	-	-	ND	-	ND	ND
2	Ev	0.222	0.300	0.099	0.211	0.931	0.785	1.110	0.761	1.100	0.841
	PCR	-	-	-	-	ND	ND	ND	ND	ND	ND
3	Ev	0.105	0.149	0.214	0.914	0.955	0.681	0.970	1.001	1.091	0.911
	PCR	-	-	-	ND	ND	ND	ND	ND	ND	ND
4	Ev	0.094	0.231	0.814	0.799	0.873	0.869	1.104	0.916	0.911	0.899
	PCR	-	-	ND	ND	ND	ND	ND	ND	ND	ND
5	Ev	0.199	0.184	0.981	0.888	1.001	0.954	0.904	0.884	1.041	1.011
	PCR	-	-	ND	ND	ND	ND	ND	ND	ND	ND
Healthy* **		0.200	0.213	0.099							
Infected	CMV	1.051	0.911	0.798							
	ZYMV	0.854	0.771	0.914							

*: Essential oils with soap
**: Each I-ELISA value (Ev) (at 405 nm) was the average of 3 readings; IC-RT-PCR was performed on plants which gave negative I-ELISA results.
ND: not detected
***: Controls I-ELISA values for healthy and infected (15 days post inoculation) squash plants, without any treatment.

Repellent and killing effect of oil spraying on vector insects

Results in **Table (3)** confirmed the high repellent effect of Lavender oil, as all plants sprayed with such oil preparations did not show any insects population.

Concerning killing effect **Table (3)** data revealed that all preparations containing soap gave high killing effect on aphids especially *L. officinalis* followed by *O. basilicum* oils. Obtained data assumed that diluted soap oil preparations can be used as a safe vector insects controlling method in small fields and greenhouses.

Table (3): Repellent and killing effect of oil spraying on aphids

Treatment* / Plant		Insect population and surviving aphids number				
		Lavendula officinalis	*Ocimum basilicum*	Corn	Water & soap	Water only
1	P	+	-	+	+	+++
	S	6	11	15	20	50
2	P	-	-	+	+	+++
	S	3	10	20	23	45
3	P	-	+	+	++	+++
	S	0	12	13	19	50
4	P	-	+	-	++	+++
	S	5	9	16	11	48
5	P	-	-	+	++	+++
	S	5	7	15	25	43

Note: table gather the results of two separate experiments.
*: Essential oils with soap
P: Insects population, -: no, +: low, ++: moderate and +++: high population.
S: Surviving aphids number out of 50.

DISCUSSION

Cucumber mosaic cucumovirus was extensively isolated and studied on many Cucurbit crops mainly squash and cucumber **(El-Shamy, 2010; Vučurović et al., 2011)**, *Zucchini yellow mosaic potyvirus* was

isolated from squash plants showing virus like symptoms by depending on serology and symptoms **Shehata and El-Borollosy (2008)**.

Data achieved proved that the most efficient vectors for CMV and ZYMV were the wingless form of *A. gossypii* and *M. persicae*, achieving 95 % & 100 % transmission rate, respectively. **Napier (2009)** reported that there are a number of aphid species that attack cucurbits including melon aphid *Aphis gossypii*, cowpea aphid *Aphis craccivora*, potato aphid *Macrosiphum euphorbiae* and green peach aphid *Myzus persicae*.

The obtained results and transmission efficiency were also in harmony with what performed by **Varveri (2000)**, who detected potato Y *potyvirus* in single *M. persicae*, and for ZYMV studied by **Abdel-Reheem et al. (2006)**.

Obtained results proved that the winged forms of aphids were less efficient than the wingless ones in all tested aphid species. **Abd El-Wahab (2012)** studied the efficiency of sixteen aphid species as vectors of Lettuce mosaic virus (LMV) in Egypt, the obtained results proved that the winged forms of aphids were less efficient than the wingless ones in all tested species.

All of the 20 aphids for each virus studied gave positive immunocapture reverse transcriptase polymerase chain reaction (PC/IC-RT-PCR) results in the form of *cp* and *Hc-pro* genes bands with the expected size of 657 bp & 1.3 kbp for CMV and ZYMV, respectively. The results obtained were in harmony with that found by **Abdel-Reheem et al. (2006)** who successfully detected ZYMV by PC/IC-RT-PCR, as a 1.3 kbp helper component proteinase gene (*Hc-pro*) bands were detected within agarose gel.

The obtained results favored the use of Lavender oil with soap, for oil can suppress insect infestation, while soap has a killing effect on delicate insects with sucking mouthparts like aphids. Also data of insect killing and repellent effect assumed that diluted soap oil preparations can be used as a safe vector insects controlling method in small fields and greenhouses.

Concerning such point of view, in harmony results were also found by **(Asjes, 2000)** as he studied the efficiency of combining oil with an insecticide or soap for controlling virus vector insects. Also Lavender or soap will be harmless to human health when compared with the risk of chemical insecticide usage **(Iovieno et al., 2002)**.

Using oil in soap preparations can increase the killing effect on insects with sucking mouthparts by suffocation **(Asjes and Blom-Barnhoorn, 2002)**. This can manage the insects damaging effects on crops and also reducing their ability of pathogens transmission especially viruses, resulting an increase in crops quality and quantity.

Gorski and Tomczak (2010) studied the repellent and killing effect of some natural oils on Foxglove aphid (*Aulacorthum solani* Kalt.), i.e., sandalwood, basil, and grapefruit oil, he found promising results which were in harmony with the previous illustrated findings. In Egypt many gardeners have long been familiar with the toxic effect of organic substances like diluted dish wash liquid soap, which is harmless to human health and cheaper compared with chemical insecticides.

Studies show that prolonged contact and/or periods of exposure to high concentrations in the oil vapor phase (fumigation) are needed to kill the insects. Repelling insects from their host plants by spraying plant essential oils is even more difficult since they have to mask the host-plant odor for prolonged periods. Product formulation of these essential oils is critical for efficacy in time, phytotoxicity of the treated plants as well as prevention of washing off **(Masatoshi, 1999)**.

Several plant essential oils show potential to control aphids. Little is however understood about whether and how these oils disturb the plant-insect interaction. Despite the many unsolved problems related to using plant essential oils to control pest insects, it is expected that they will play an important role in future crop protection **(Munneke et al., 2004)**.

ACKNOWLEDGMENT

Thanks and grateful appreciation to Emeritus Chief Researcher Dr. Fatma M. Hatab and all my colleagues and stuff members in the Plant Protection Research Institute, ARC, Dokki, Giza, Egypt for their helpful guidance and providing facilities during this investigation.

REFERENCES

Abdel-Reheem, M.A.T.; Mona M. Oraby; A.M. El-Borollosy and M.F. Abd Elhamied (2006). Lavender Oil Constituents as a Natural Bio-Controler and an Aphid's Virus Transmission Suppressor. J. Biol. Chem. Environ. Sci., 1: 1219-1238.

Abd El-Wahab, A.S. (2012). Transmission Efficiency of Lettuce Mosaic Virus (LMV) by Different Aphid Species and New Aphid Vectors in Egypt. Aca. J. of Ento. 5: 158-163.

Asjes, C.J. (2000). Control of aphid-borne Lily symptomless virus and Lily mottle virus in Lilium in the Netherlands. Virus Res., 71: 23-32.

Asjes, C.J. and G.J. Blom-Barnhoorn (2002). Control of aphid vector spread of Lily symptomless virus and Lily mottle virus by mineral oil/insecticide sprays in Lilium. Acta Hort., 570: 277-281.

Barazandeh, M.M. (2002). Essential oil composition of *Lavandula latifolia* Medik from Iran. J. Essent. Oil Res., 14: 103-104.

Bassolé, I.H.N. and H.R. Juliani (2012). Essential Oils in Combination and Their Antimicrobial Properties. Molecules, 17: 3989-4006.

Bell, E.A. and B.V. Charlwood (1980). Secondary plant products. Encyclopedia of Plant Physiology. Vol. 8, Springer Verlag, Berlin.

Blackman, R.L. and V.F. Eastop (1984). Aphids on the Worlds Crops: An Identification and Information Guide, 1st Edition, Wiley, Chichester, pp: 466.

Bouzouita, N.; F. Kachouri; M. Hamdi and M.M. Chaabouni (2005). Volatile constituents and antimicrobial activity of *Lavandula stoechas* L. oil from Tunisia. Journal of Essential Oil Research, 17: 584-586.

Brachet, P.; F. Pinasseau; B. Cailleteau and J.L. Verrier (2001). Preventative control of non-persistent aphid transmitted tobacco virus in France. CORESTA Congress Proceedings, Cape Town (South Africa), p. 58.

Brunt A., K. Crabtree; M. Dallwitz; A. Gibbs and L. Watson (1996). Viruses of Plants: Description and Lists from the VIDE Database. C.A.B. International, Wallingford, U.K., pp.1484.

Claude, G. and B. Guy (2002). Field Identification Guide to Aphids in Quebec Vegetable Crops. Agriculture and Agri-Food Canada.

El-Afifi, Sohair I.; A.M. El-Borollosy and S.Y.M. Mahmoud (2007). Tobacco callus culture as a propagating medium for *Cucumber mosaic cucumovirus*. Inter. J. Virology, 3: 73-79.

El-Shamy, M.M.M. (2010). Molecular Analysis of *Cucumber Mosaic Cucumovirus* Symptoms Development on Squash Plants. J. of American Sci., 6: 94-103.

Garzo, E.I.; M. Duque and A. Fereres (2004). Transmission efficiency of different non-persistent viruses infecting melon by four aphid species. Spanish J. of Agric. Res., 2: 369-376.

Gorski, R. and M. Tomczak (2010). Usefulness of natural essential oils in the control of Foxglove aphid (*Aulacorthum solani* Kalt.) occurring on eggplant (*Solanum melongena* L.). Ecological Chemistry and Engineering, 17: 345-349.

Iovieno, P.; E. Lahoz; F. Porrone; Cristina Biondani; Angela Carella and R. Contillo (2002). Use of light mineral oil to reduce the severity of PVY and CMV infections on tobacco in Italy. Il Tabacco, 10: 57-61.

Koenig, R.C. (1981). Indirect ELISA methods for broad specificity detection of plant viruses. J. Gen. Virol., 55: 53-62.

Kown, S.W.; M.S. Kim; H.S. Choi and K.H. Kim (2005). Biological characteristics and nucleotide sequence of three Korean isolates of Zucchini yellow mosaic virus. J. Gen Plant Pathol., 71:80-85.

Lecoq, H.; C. Wipf-Scheibel; C. Chandeysson; A. Lê Van; F. Fabre and C. Desbiez (2009). Molecular epidemiology of Zucchini yellow mosaic virus in France: An historical overview. Virus Res., 141:190-200.

Lisa, V.; G. Boccardo; G. D'Agostino; G. Dellavalle and M. D'Aquilio (1981). Characterization of a potyvirus that causes zucchini yellow mosaic. Phytopathology, 71:667-72.

Madhubala, R.; V. Bhadramurthy; A.I. Bhat; P.S. Hareesh; S.T. Retheesh and R.S. Bhai (2005). Occurrence of Cucumber mosaic virus on vanilla (*Vanilla planifolia* Andrews) in India. J. Biosci. 30: 339-350.

Masatoshi, H. (1999). Antifeeding, settling inhibitory and toxic activities of labiate essential oils against the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Appl. Entomol. Zool., 34: 113-118.

Mauck, K.E.; C.M. De Moraes and M.C. Mescher (2010). Effects of Cucumber mosaic virus infection on vector and non-vector herbivores of squash. Commun. Integr. Biol., 3: 579-582.

Munneke, M.E.; A. Schuurman-de Bruin; J.R. Moskal and R.W.H.M. van Tol (2004). Repellence and toxicity of plant essential oils to the potatoaphid, *Macrosiphum euphorbiae*. Proc. Neth. Entomol. Soc., 15: 81-85.

Napier, T. (2009). Insect pests of cucurbit vegetables. Prime fact sheet No. 833, NSW Department of Primary Industries, Australia.

Olmos, A.; M. Cambra; M.A. Dasi; T. Candresse; O. Esteban; M.T. Gorris and M. Asensio (1997). Simultaneous detection and typing of plum pox potyvirus (PPV) isolates by heminested PCR and PCR-ELISA. J. Virol. Methods, 68:127-137.

Oraby, Mona M. and A.M. El-Borollosy (2013). Essential oils from some Egyptian aromatic plants as an antimicrobial agent and for prevention of potato virus Y transmission by aphids. Ann. of Agric. Sci., 58: 97-103.

Palukaitis, P.; M.J. Roossinck; R.G. Dietzgen and R.I.B. Francki (1992). Cucumber mosaic virus. Adv. Virus Res., 41: 281-348.

Pirone, T.P. (1991). Viral genes and gene products that determine insect transmissibility. Seminars in Virology, 2:81.

Provvidenti, R.; D. Gonsalves and H.S. Humayden (1984). Occurrence of Zucchini yellow mosaic virus in cucurbits from Connecticut. New York, Florida, and California Plant Dis., 68:443-446.

Roossinck, M.J. (1999). Cucumoviruses (Bromoviridae)-general features, In: "Encyclopedia of Virology, 2nd edition", pp. 315-320, (Granoof L. & Webster R.G. eds). Academic Press, SanDiego, USA.

Shehata, Sawsan F. and A.M. El-Borollosy (2008). Induction of resistance against *Zucchini Yellow Mosaic Potyvirus* and growth enhancement of squash plants using some plant growth–promoting rhizobacteria. Australian J. of Basic and App. Sci. 2: 174-182.

Varveri, Christina (2000). *Potato Y potyvirus* detection by immunological and molecular techniques in plants and aphids. Phytoparasitica, 28: 2.

Vučurović, A.; A. Bulajić; I. Stanković; D. Ristić; J. Berenji, and B. Krstić (2011). Karakterizacija virusa mozaika krastavca poreklom iz tikava u Srbiji. Pestic. fitomed. (Beograd), 26: 325-336.