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Isolation, Identification and Inducing Systemic Resistance to Beet Mosaic Virus (BTMV) Infecting Sugar Beet Plants in Egypt

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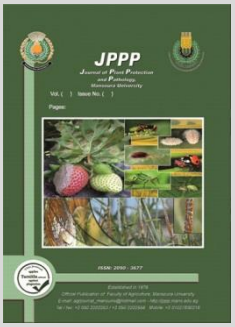

ABSTRACT

The objective of the study described in this paper was to isolate and determine a range of a *potyvirus* in sugar beet crop. The virus inoculum was taken from obviously diseased sugar beet plants (*Beta vulgaris* L.cv. Top), screening mild to severe mosaic complemented with leaves malformation and distortion, suspected to be caused by virus infection. Leaf samples were togethered from different areas in Menoufia Governorate, Egypt. The determination of the isolate was based on symptomatology, host range, modes of transmission (mechanical-insect), serological tests (Direct ELISA, DBIA and TBIA), light microscopy. BtMV infection distributed some of photosynthetic pigments in infected beet leaves, i.e. chlorophyll, carotenoids and also reduced the concentration of total carbohydrates and total sugar in roots. Currently, different inducers stemmed from several materials were used to decrease the virus concentration. Preliminary showing were actual in reducing the number of local lesions formed by challenge inoculation of BtMV. Both inducers (plant extracts and Salicylic acid (SA) showed reducing in the total numbers of local lesions and concentration of the virus. Among the inducers used for introduction of resistance against BtMV, *Dianthus caryophyllus* extract was found to be most effective. Also, Our results revealed that Salicylic acid (SA) used for screening was effective in reducing the concentration of virus and the number of local lesions formed by the challenge inoculation of BtMV .

Keywords: Beet mosaic virus (BtMV), sugar beet, serological tests, light microscopy, photosynthesis. Systemic acquired resistance (SAR)

INTRODUCTION

Sugar beet plants (*Beta vulgaris* L.) develops an very essential sugar crop cultivated in wide area in all over the world. It links the second essential sugar crop after sugar cane, producing annually about 40 % of sugar production all over the world (Gobarah Mirvat and Mekki, 2005). Lately, in Egypt Sugar beet plants have been presented , to meet with developing demand on sugar production. The cultivated area is increasing steadily and the ultimate goal is the gradual replacement of sugar cane by sugar beet crop as a highest source of sugar. The cultivated area with sugar beet during 2015/2016 on republic level touched was 903586 feddans (feddan = 4200 m^2), which yielded about 19509820 tons with an average of 21.591 tons/faddan*.

*According to Ministry of agriculture and land reclamation economic affairs sector. Agriculture Directorates of Governorate, 2015/2016.

Sugar beet is one of the *Chenopodiaceae* family, considered as the main source of sugar in the world. It is sensitive to infection by fungi, bacteria, phytoplasma and viruses (Mohammadi, 2016). Beet yellow virus(BYV), Beet mosaic virus (BtMV), Beet western yellow virus(BWYV), Beet soil-borne mosaic virus (BSBMV) and Beet curly top virus (BCTV) , are very important viral diseases to sugar beet all over the worldwide. Infection by assured viruses causes obvious yield reduction and economic losses. These viral diseases have an important rank because they not only cause direct destruction to the host but also present the plant to secondary attackers(Smith, 1972).

BtMV belongs to the genus *Potyvirus* in *Potyviridae* family of plant viruses, it is world-wide distributed virus in all major sugar beet plants and in varied infections with assured other viruses causes mosaic , stunting and major yield losses on susceptible sugar beet varieties .However, some sever strains of BtMV don't cause a significant economic damage to sugar beet (Abdel-Ghaffar, *et al*., 2003) .

Many conventional approaches to control virus infection, have been discovered but without much success. In several of new approaches involving viral components, the induced resistance is very specific to a particular strain or group of viruses (Gholizadeh *et al*., 2004). Systemic acquired resistance is considered one induced resistance which caused by pathogens that cause localized necrotic disease lesions or a hypersensitive reaction ((Hammerschmidt, 2009).

Plant extracts becomes very important actual against different pathogens because of it is a sources of antimicrobial compounds (Chakraborty and Chakraborty, 2010). Several efforts have been done to control the plant viruses infecting different plants.

Salicylic acid inducer is achieved to be an important for localization of the virus to the locality of the necrotic lesions and for the founding of systemic acquired resistance (SAR, intensified localization of the virus) in response to TMV control (Mure, *et al*., 1997).

This work was done to investigate the isolation and identification of BtMV on the basis of host range, modes of transmission, serological tests and light microscopy. Also,

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we showed the changes in the chemical constituents, especially photosynthetic pigments, Total carbohydrate and total sugar of beet plants due to BtMV. In this paper, induced systemic resistance (SAR) against BtMV in sugar beet using plant extracts and Salicylic acid.

MATERIALS AND METHODS

Isolation and identification:

Virus isolate:

Randomly samples of naturally diseased sugar beet, were placid from different location in Menoufia governorate, Egypt. Leaf samples showing severe mosaic, distortion and malformation, were observed attendant with scattered spots (suspected to be because of virus infection) were together and scanned serologically by ELISA (Clark and Adams, 1977). The positive samples were homogenized in a mortar and pestle, after adding phosphate buffer (1:5 w/v, 0.1 M, pH 7.2, Omar *et al.*, 2006), then the extracted sap was passed through a double layer of cheesecloth. Finally, the sap was back inoculated onto sugar beet plants. Inoculated sugar beet plants were kept in the greenhouse and used as a source of infection in the following experiments.

Symptomatology and host range.

Seedling of twenty plants cultivars and species belong to sex different families, were inoculated with the isolated virus. The inoculated plants were grown below greenhouse conditions (25 ± 5ºC) and perceived daily for 30 days for any virus symptoms. Inoculated plants showing visible or no visible symptoms were checked by a serological test.

Modes of transmission:

Mechanical transmission:

Infected sugar beet leaves were homogenized in a sterilized mortar after addition phosphate buffer (1: 5 w/v, 0.1 M, pH 7.1, Omar *et al.*, 2006), then the extract was expressed within cheesecloth and used to inoculate healthy the tested plants. The leaves of seedling were dusted with carborandum (600 mesh) before mechanical infection (Rawlins and Tompkins, 1936).

Insect transmissions:

In the course of studying insect transmission of the isolated virus, *Myzus persicae* L Sulz, and *Aphis fabae* Scorp, were used to study their ability to transmit the virus from infected (*Beta vulgaris* L. cvs Top) to healthy ones, as described by (Omar *et al*., 2006). The aphids were hungry to one hour. The virus free insects were permitted to feed on diseased sugar beet leaves for acquisition the virus for of 5 min. Then aphids (five aphids/plant) were transferred to healthy seedling of sugar beet and were allowed to eat for inoculation feeding period of 30 min, then the insects were killed with pesticide (Telfast, 20%). The inoculated plants were saved in insect resistant greenhouse and symptoms were recorded for 4 weeks .

Serological tests:

The isolate was identified by DAS-ELISA as described by (Clark and Adams 1977). ELISA kits (completely ready to use) were recommend by SANOFI, Sante Animale, Paris, France. Tissue blot immunoassay and Dot blot immunobinding assay) were used to detect the isolated virus as described by (Lin *et al*., 1990) .

Light microscopy:

Plant tissues of infected and healthy were slayed and fixed in formalin acetic acid(FAA) solution for 2 days(10 ml Formalin + 5 ml glacial acetic acid +35 ml distilled water + 50 ml HCl95%). Samples were dehydrated and cleared in n-butyl alcohol series (Willey, 1971) and embedded in paraffin wax of 56-58C. Cross and longitudinal slices 15 µ thick were cut using a rotary microtome, adhesive with Haupt's adhesive and stained with crystal violet erythrosine combination (Sass, 1961),cleaned in carbol xylene then fixed in Canada balsam, and then examined using light microscopy.

Viral Effect on chemical analysis of sugar beet plants:

Photosynthetic pigments:

For measuring the photosynthetic pigments, i.e. chlorophyll a, b and carotenoids, a vegetative sample from the fifth leaf was taken and the pigments were extracted by 85% aqueous acetone according to (Fadeel's method,1962). The absorbance was measured by using Spectrocolourimeter (Carl- Zeis) at 440, 644, 662 n.m wave lengths of. The data of obtained results was statically analyzed according to (Larcher and Cernsca, 1985).

Total carbohydrate contents (%):

Total carbohydrate were determined in tubers according to the method described by (Dogras and Psomakelis ,1991).

Sugar contents (%) was determined using the method of (Somogy, 1952).

Induced systemic acquired resistance against BtMV.

Efficacy of SA on indicator plant (*Ch. quinoa)* against BtMV.

Preparation of Aqueous plant extracts and Salicylic acid (inducers) for treatment :

Dry leaves and flower buds of three plants [belongins to 3 families] were placid from the market and farm of Faculty of Agriculture, Menoufia University (Table 1). According to (Mbata *et al.*, 2006) aqueous extraction method, about of (15 g) of each ground ingredients, were extracted by soaking for two days using distilled water (150 ml) in a flask (250 ml) on room temperature at shaker. The extracts were clarified by filter sheet (Whatman No. 1). Finally, we used vacuum freezer – dryer to concentrate the water extracts. The concentrates were kept in flasks (50 ml) and kept at 4 °C in refrigerated prior to use. Three concentrations of each stock aqueous extract were made (i.e., 5, 10, 15 %) using distilled water.

Table 1. Plant materials tested for their inhibitory effect against BtMV.

Plant extracts			
Family	**English name**	**Scientific name**	**Used part**
1- Caryophyllaceae	Carnation	*Dianthus caryophyllus*	Flower buds
2- Meliaceae	Neem	*Azadirachta indica*	Leaves
3- Solanaceae	Solanum	*Solanum nigrum*	Leaves

Salicylic acid (25 Mm , 50 Mm and 100 Mm) were sprayed using (500 ml) hand sprayer on ten leaves of *Ch. quinoa*, for every treatment 24 hrs pre-inoculation with virus isolate (every plant sprayed about 20 ml SA solution). The control plants were sprayed with distilled water. All Leaves of *Ch. quinoa* dusted with carborandum(600-mesh) and

mechanically inoculated with virus isolate. Total number of local lesions, from ten leaves for each treatment was counted (5-10) days after virus inoculation (Mahdy *et al*., 2007). The inhibition percentage of local lesions formation by every treatment as well as the control was calculated according to the number of local lesions produced using the following formula defined by (Devi *et al*., 2004):

$$\text{Inhibition \%} = \frac{A-B}{A} \times 100$$

Where:
A= Control
B= Treatment

Quantification of virus concentration by using DAS ELISA.

The leaves (100 mg) harvested from infected (untreated) and treated plants, were collected and studied serologically by ELISA (Clark and Adams, 1977) as described before.

RESULTS AND DISCUSSION

Isolation and identification:

The isolate under study, was identified as beet mosaic virus on symptomatology, host range, mechanical and insect transmission, serological tests and light microscope .

Symptomatology and host .

Twenty plants species belong to sex families were inoculated mechanically with virus isolate. The reaction of different plants to BtMV was classified to two categories depended on their responses on the host plants ; susceptible and non-susceptible hosts (Table2).

I. Susceptible hosts to BtMV: Results established in Table (2) show that the isolate of BtMV was able to infect 20 plant species and variety belonging to 6 families i.e. Amaranthaceae, Chenopodiaceae Compositae, Curcubitaceae and Leguminosae, these hosts were divided into two groups: local lesion and systemic hosts:

a. Host plants by systemic symptoms: *Beta vulgaris* cv. (Top (Fig 1,A) , Gazela (Fig 1,B) , Raspoly (Fig 1, C), *Beta patellaris, Beta vulgaris* var. rapa, *Beta vulgaris, Pisum sativum* L. (Fig 1, D) , *Vicia faba* L. (Fig 1, E), *Glycin max.* and *Cucurbita pepo* L, have introduced to systemically infected with BtMV isolate under study.

b. Host plants by local lesion symptoms: *Chenopodium album r* , *Ch. amaranthicolor* and *Ch. quinoa* L. (Fig 1, F). Our results have in agreement with that reported by Howell and Mink (1971), Lewellen (1973), Halliwell and Johnson (1988), Katis *et al*. (1997), Dusi and Peters (1999) and Omer *et al*., (2006)..

II. Unsusceptible hosts: Eight host plants were introduced to be unsusceptible to the studied BtMV isolate as presented in Table (2). The obtained results were in agreement with results obtained by **Bennett (1965) and Lewellen (1973).**

Modes of transmission:

.Mechanical transmission:

Results showed that BtMV was readily mechanically transmitted by sap extracted from infected sugar beet leaves using fore finger with (Phosphate buffer pH7.2 and carborandum 600 mesh) to healthy plants as described before. These results were in accordance with that reported by Bennett, (1965); Howell and Mink, (1971); Lewellen, (1973); Polak, (1981); Katis *et al*., (1997); Dusi and Peters, (1999) and Mali, (2000), Omer, *et al*., (2006) and Haggag Wafaa *et al*., (2010).

Table 2. The reaction of different hosts to BtMV isolate.

Host plant tested				Symptoms induced	ELISA test
Family	**Scientific name**	**English name**	**Variety**		
1-Amaranthaceae	*Gomphrena globosa* L.	Globeamaranth	—	No	-
2.Chenopodiaceae	*Beta vulgaris* L.	Sugar beet	Top	VC--Mal- M	+
			Gazela	M – SM-Ma- CLL l	+
	Beta patellaris L	Red beet	Raspoly	M - BL	+
	Beta vulgaris L	Fodder beet	-	Vb-M	+
	Chenopodium album	Ouares	Rapa	Vb - M	+
	Ch. amaranticolor.	Lamb'S	-	CLL	+
	Ch. quinoa.	Gooses Foot	-	CLL	+
			-	CLL	+
3- Compositae	*Lactucea sativa* L.	Lettuce	-	No	-
4- Curcubitaceae	*Cucurbita pepo*.	Squash	-	M	+
	Cucumis sativus L	Cucumber	-	No	-
5- Leguminosae	*Pisum sativum L.*	Garden pea	Mister B	M- VC-LL	+
	Vicia fabae L.	Broad bean	Balady	M- LR	+
	Glycine max L.	Soya bean	Lee	mM	+
	Phaseolus vulgaris L.	Common bean	Giza 6	No	-
	Vigna unguiculata L.	Cowpea	Black eye	No	-
6- Solanaceae	*Datura stramonium* L.	Jimson-weed	-	No	-
	Nicotiana tabacum L.	Tobacco	White-Burly	No	-
	Nicotiana glutinosa L	Wirginia plant	-	No	-

Abbreviation of symptoms:

CLL = chlorotic local lesion	**M = mosaic**	**mM: Mild mosaic**
MO = mottling	**No = No reaction**	**SM =severe mosaic**
LR= Leaf roll	**BL = Blisters**	**VC = vein clearing**
- = Negative reaction	**+ = Positive reaction**	**VC = Vein clearing**

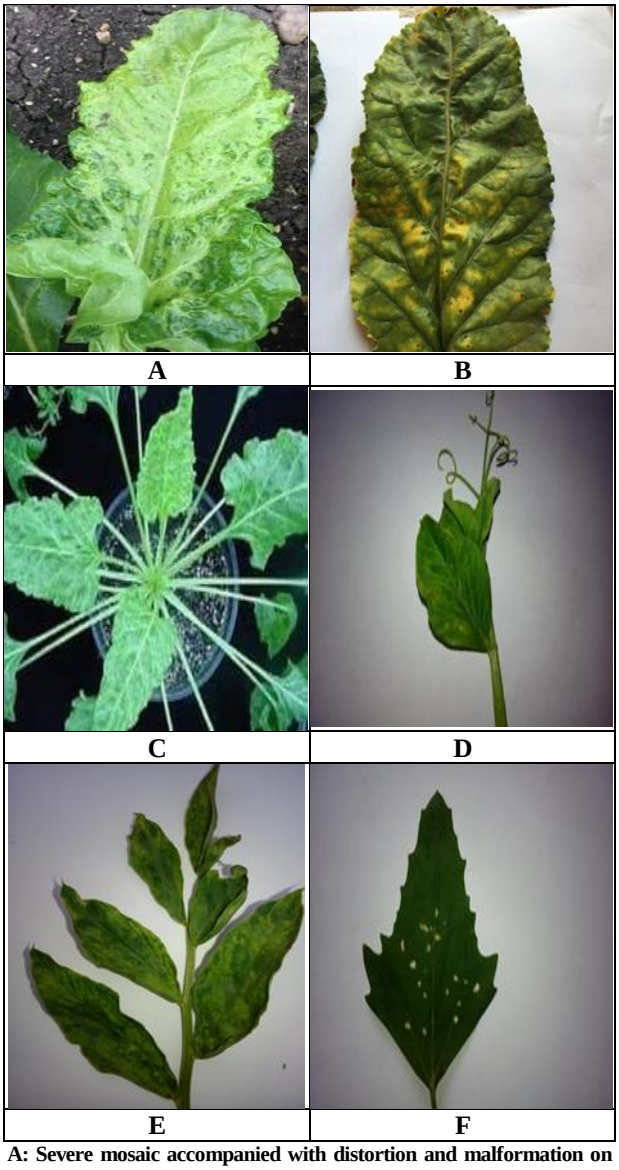
Insect transmission:

Two aphid insects were checked for capacity to transfer the virus isolate. Results revealed in (Table 3) indicated that *Myzus persicae* transmitted the virus by 70% only of the inoculated plants. However, *Aphis fabae* transmitted the virus to 40% only of the inoculated plants .

BtMV is spread by *Myzus persicae* Sulz in non-persistent method (Mali, 2000 and Omer *et al*., 2006). BtMV was also transferred by *Aphis fabae* Scop (Polak, 1981; Dusi and Peters, 1999).

Table 3. Insect transmission of BtMV from infected sugar beet leaves to healthy ones (every treatment repeated twice).

Aphid species	No. of inoculated plants	No. of infected Plants	Infection (%)
Myzus persicae (Sulz)	10	7	70
Aphis fabae (Scop)	10	4	40

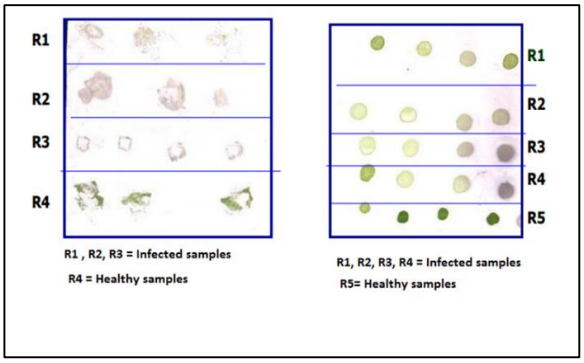


A: Severe mosaic accompanied with distortion and malformation on *Beta vulgaris* L.cv. Top. B: Mosaic and leaf malformation on *Beta vulgaris* L.cv. Gazela . C: Mosaic and blisters on *Beta vulgaris* L.cv. Raspoly D: Mosaic , vein clearing and local lesion on *Pisum sativum* L.cv. Mister B. E: Mosaic and leaf roll on *Vicia fabae* L.cv. Balady . F: Chlorotic local lesion on *Chenopodium quinoa.*

Fig .1. Reaction of several hosts to mechanical inoculation with the isolated virus .

Serological tests:

The virus isolate was well-known by DAS-ELISA, positive reaction obtained with the specific antiserum. BtMV readily detect immunologically using (DBIA)and Tissue blot technique (TBIA) on nitrocellulose membrane that using(1/1000) polyclonal antisera diluted in TPS buffer plus (1/8000) goat antibody rabbit alkaline phosphates conjugate , was used as secondary antibody (Fig2). DAS-ELISA test was used to approve the identification of BtMV. However, this technique was mentioned by Omer *et al*., (2006); Mohammadi, (2018) and Mohammadi, (2020) to identify BtMV. The specific antigen was immunologically limited with enzyme labeled antibody on nitrocellose membranes In TBIA test . Although this method is similar in principal to dot blot immunoassay of antigen on various membrane supports, the tissue blotting technique does not require mechanical disruption of tissue for antigen. Both techniques have been a higher compassion for the detection of BtMV. My results in agreement with those found by Hsu and Lawson (1991) reported that *Tomato spotted wilt virus* was identified using tissue blots from infected leaves and steams. The occurrence of TSWV in blot of diseased tissues was showed by the change of intense purple color. When the healthy stem and leaf blots did not advance purple color, but leaf blots retained main green color .

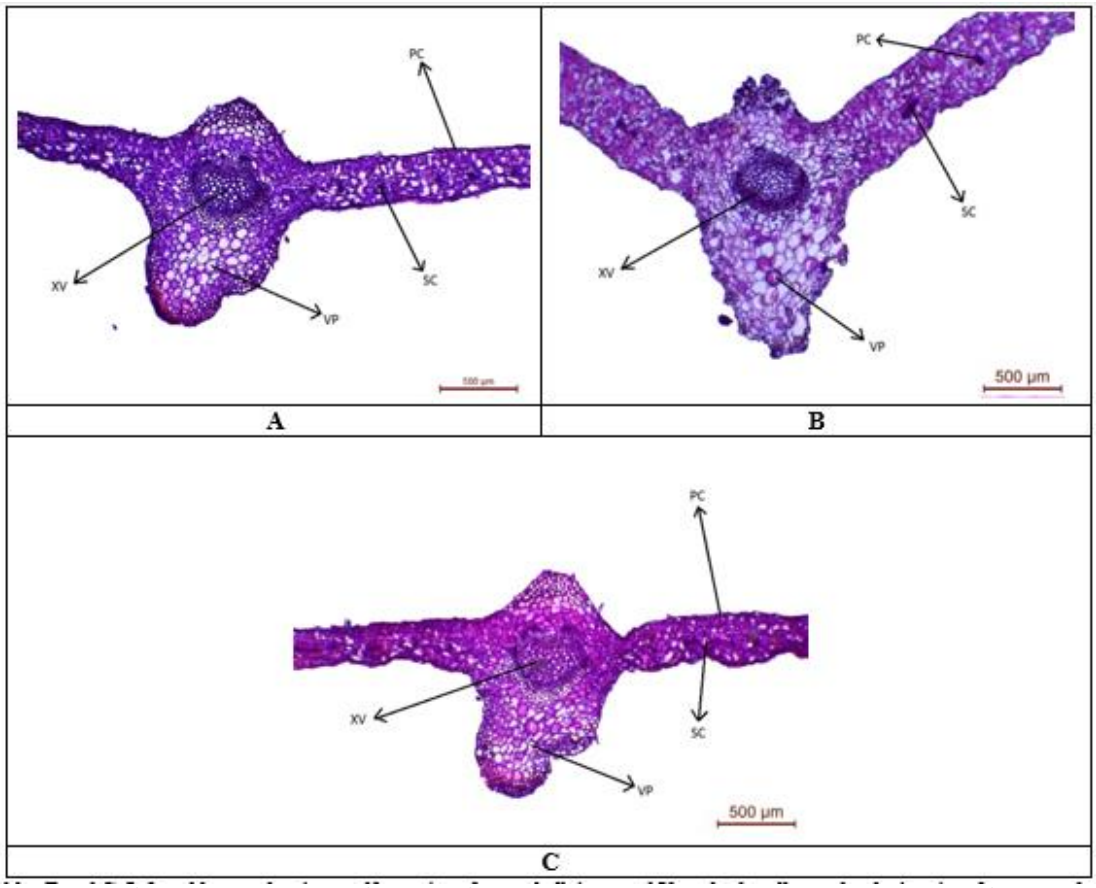


Light microscopy:

Semi thin sections of healthy and infected sugar beet leaves, were examined by light microscope after contrast staining. The examination revealed large difference between healthy and infected tissue . From Table (4) and Fig. (3), it could be observed that, BtMV reduce the measurements of Average (Av) of (Medvein thickness, Blade thickness, Palisade tissue thickness, Spongy tissue thickness, Vascular bundles length, Vascular bundles width, Number of xylem vessels and Xylem vessel diameter). Like results were noticed by Singh and Rathi (1996) and Mali (2000) . Semi thin sections of healthy and infected leaves were scanned after staining with fast green by light microscopy. The investigations shown great differences between healthy and infected; mesophyll layer was reduced and compact in the infected tissue and reduced size of spongy and palisade cell, obvious visually the number of xylem arms and phloem layers is reduced compared with the healthy, similar results were reported by Mohamed (2011).

Table 4. Anatomical (histological) measurements of healthy and infected sugar beet leaf tissues.

Measurements (µ)	Healthy	Infected
Average of Medvein thickness	1315	1030
Average of Blade thickness	188	160
Average of Palisade tissue thickness	88	60
Average of Spongy tissue thickness	75	55
Average of Vascular bundles length	271	235
Average of Vascular bundles width	198	158
Average of Number of xylem vessels/bundle	74	55
Average of Xylem vessel diameter	36	21



A: Healthy. B and C: Infected leaves, showing malformation of mesophyll tissue and Vascular bundles and reducing size of spongy and palisade cell, obvious visually the number of phloem layers and xylem arms.

Fig 3. Light micrograph of infected and healthy sugar beet leaves with BtMV, showing different changes in cells and tissues (500µm).

Viral Effect on chemical analysis of sugar beet plants:

Photosynthetic pigments:

In sugar beet plants infected with BYMV, there was a highly gradual decline in photosynthetic pigments. The obtained data in Table (5) showed that, Virus infection caused marked reduction in Chl a , Chl b and carotenoid contents (1.29 , 0.74 and 0.96 mg/gd.w) , compared with those (2.52 , 1.36 and 1.87 mg/gd.w) in healthy control for Chl a , Chl b and carotenoids respectively. Photosynthesis is considered one of the chief and important physiological processes for plant growth and it is extremely affected by viral infection (Arfan *et al.*, 2007). BtMV infection caused main reductions in both photosynthesis rates and pigment contents, resulting in general growth inhibition. Good to mention that, the content of photosynthetic pigments were positive markedly affected as result to application of the inducers and became one of visible react of sufficient of treatments. Clover *et al.*, (1999) reported that plant viruses reduce the level of photosynthesis in infected tissues over inhibition of photosystem activity and reduce in chlorophyll content. In this report, the reduction in Photosynthetic pigment contents could be clarified on a wide production of excitation energy that occurred under virus stress , leading to photo inhibitory damage in the reaction Centre within chloroplasts. However, the decrease in total pigment content in response to BtMV infection was observed.

Total carbohydrate and total sugar contents :

BtMV infection had significant effect on total carbohydrates and total sugar in sugar beet roots (Table 5). Our results showed that viral infection reduced the concentration of total carbohydrate and total sugar in roots (10.12 , 75.50 mg/g) compared with those (16.38 and 91.44 mg/g) in healthy control. This decrease was due to viral infection caused destruction of chlorophyll pigments in infected leaves and consequently in the process of photosynthesis and the result was the lack of the content of total carbohydrates in infected plants. The obtained results are similar to (Baker and Horton 1989 ; Clover *et al.*, 1999 ; Piszczek, 2000 and Abass, 2004). Infection had large effect on the storage root sugar concentration but sugar extraction efficiency would be down because of the increase in root layers (Clover *et al*., 1999).

Table 5. Effect of Beet mosaic virus on photosynthetic pigments, total carbohydrates and total sugars in infected sugar beet plants. They are means of three replications.

Measurements Treatments	Chlorophyll A (mg/g Dw)	Chlorophyll B (mg/g Dw)	Carotenoids (mg/g Dw)	Total carbohydrates (mg/100g)	Total sugar (mg/100g)
Healthy plants	2.52	1.36	1.87	16.38	91.44
Infected plants	1.28	0.74	0.96	10.12	75.50

Efficacy of SA on indicator plant (*Ch. quinoa)* against BtMV.

The results of preliminary trial recorded in Table (6) indicated that all three concentrations (*i.e*., 5 , 10, 15 %) of all tested plant extracts, inhibited BtMV infection with different degrees. The efficiency of inhibition was increased with increasing the concentration (15 %) of all extracts were the most effective one, where induced the highest systemic resistance against BtMV (as inhibitory percentage of local virus infection). Flower buds extract of *Dianthus caryophyllus* gave the highest inhibition rate at all concentrations, followed by *Solanium nigrum* extract. While, The least inhibitory rate was obtained using leaves extract of *Azadirachta indica* at all concentrations. These results were contract with the results achieved by Gupta and Naqvi (1991), aqueous extracts from leaves roots, green stems, bark, seeds, green fruits and rhizomes of (*Dianthus caryophyllus, Azadirachta indica, Capsicum annum, Chenopodium amaranticolar, Datura metel and Glycyrriza glabra*) obviously decreased the number of local lesions and systemic infection produced by *Brinjal necrotic mosaic virus.*

The results of using SA inducer in Table (6) showed reduction of number of local lesions on infected leaves with the virus. The efficiency of inhibition was increased with increasing the concentrations (75.8, 81.05 and 91.5 %) respectively. My results were greed with results of Madhusudhan *et al*., (2011), when he used SA inducer in contradiction of *Tobamoviruses* infection in Tomato and pepper plants. The results showed that the inducer used for screening was active in reducing the number of local lesions designed by the challenge inoculation of *Tobaco mosaic viruses*. According to the results reported by Carl *et al*., (2005) SA application induced resistance to *Cucumber mosaic virus* in Tobacco plants *(Nicotiana tabacum)* .

Table 6. The inhibitory effect * of SA on BtMV inhibition using local plant (*Ch. quinoa)*.

Inducer used		*Conc*	N. of local lesions		Inhibition
Source of plant extract	**Part used**	***(%)***	**Untreated (Control)**	**Treated with inducer**	**(%)**
1- *Azadirachta indica*	Leaves	5	95	79	16.6
		10	95	70	26.3
		15	95	50	42.1
1- *Dianthus caryophyllus*	Flower buds	5	95	25	73.7
		10	95	15	84.2
		15	95	4	95.8
3- *Solanium nigrum*	Leaves & Fruits	5	95	33	75.8
		10	95	20	81.05
		15	95	8	90.5
7- Salicylic acid (SA)		SA,25mM	95	23	65.3
		SA, 50mM	95	18	78.9
		SA, 100 mM	95	9	91.

*** Total number of local lesions on ten leaves used in every trial.**

Quantification of virus concentration by using Enzyme Linked Immunosorbent Assay (ELISA).

The ELISA reads showed lower concentration of virus in both inducers associated to control (Infected). In table (7) showed plant treatment with of *Dianthus caryophyllus* extract reduced the BtMV concentration (0.214) followed by *Solanium nigrum* extract (0.299) and *Azadirachta indica* extract (0.302).

While SA application reduced the BtMV concentration about (0.247). These results were agreement with that obtained by Madusudhan *et al*., (2011) observed the ELISA results showed lower concentration of tobamoviruses in both tomato and pepper seedling with inducers compared to control seedling. Also , Our results were consistent with Arslan, and Erkan (2012) they examined the effect of inhibitors in fourteen plant extracts and 15 chemical substances on *Tobacco mosaic virus* infection.

Table 7. The inhibitory effect * of SA treatment on the concentration of BtMV using systemic plant (*Beta vulgaris)*.

Inducer used	**ELISA read**
Healthy plants (Negative control)	0.103
Infected plants (Positive control)	0.417
Aza dirachta indica (15 %)	0.302
Dianthus caryophyllus (15 %)	0.214
Solanium nigrum (15%)	0.299
Salicylic acid (SA) 100 mM	0.247

According to their results on test plants, the highest inhibition in leaves were found with three plant extracts (*Capsicum annum, Dianthus caryophyllus* and *Yucca elephantipes and* Salicylic acid*).*

REFERENCES

Abass, M. (2004). Biological and serological studies on beet mosaic virus (BtMV) in Egypt. Ph.D.

Abdel-Ghaffar, M.H.; Salama, M.I.; Mahmoud, S.Y.M.(2003). Electron microscopy, serological and molecular studies on Egyptian isolate of beet mosaic *potyvirus* . Arab University J. of Agric. Sci., 11(2) : 469-484.

Arfan, M.; Athar, H. R. and Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. Journal of Plant Physiology, 164: 684-694.

Arslan, N. and Erkan. S. (2012). Investigation of the effects of some plant extracts and chemical substances on the infection. Bitki Koruma Bulteni, 52(2): 189-205.

Baker,N. R. and Horton, P. (1988). Chlorophyll fluorescence quenching during photo inhibition .In : Kyle DJ.Osmond CB, Arnt-zen CJ,eds. Photoinhibition .Amsterdam, Netherlands: Elsevier, 145-168.

Bannett, C.W. (1965). Partial or complete restriction of two mosaic viruses to parenchyma tissue. Plant reporter. 49 : 375-377.

Carl, N. M.; Kian-Chung, L.; Catherine A. M.; Sek-Man, W. and John, P. C. (2005). Salicylic Acid –Induced Resistance to *Cucumber mosaic virus* in Squash and Arabidopsis thaliana : Contrasting Mechanisms of induction and Antiviral action. Molecular Plant-Microbe Interactions 18 (5):428-434.

Chakraborty, D. and S. Chakraborty (2010). Bioassay guided isolation and identification of antibacterial and antifungal components from methanolic extract of green tea leaves *(Camellia sinensis)*. Res. J. Phytochem 4: 778-86.

Clark, M. F. and Adams, A. N. (1977). Characteristics of microplate method of enzyme - linked immunosorbent assay for detection of plant viruses. Journal. General.Virology.34 : 475- 483.

Clover, G.R. ; Azam, S.N. ; Jaggard, K.W. and Smith, H.G. (1999). The effects of beet yellows virus on the growth and physiology of sugar beet (*Beta vulgaris).*Plant pathology 48:129-139.

Devi, P. R. ; Doraiswamy, S.; Akkeeran, S. ; Rabindran, R.; Ganapathy, T.; Ramiah, M. and Mathiyazhagan, S. (2004).Antiviral action of *Harpulia cupanioides* and *Mirabilis jalapa* against *Tomato spotted wilt virus* (TSWV) infecting tomato. Archives of Phytopathology and Plant Protection, 37 (4): 245-259.

Dogras, C. A. S. and Psomakelis,C. (1991). Sugar and dry matter changes in potato stored in a clamp in a mountainous region of North Greec. Potato Research, 34: 211 –214.

Dusi, A.N. and Peters, D. (1999). Beet mosaic virus: its vector and host relationship. Journal of Phytopathology, 147:293-298.

Fadeel, A.A. (1962). Location and properties of chloroplasts and pigment determined in shoots. Plant physiology. 15: 130 – 137.

Gholizadeh, A. ; Kumar, M. ; Balasubrahmanyam, A. ; Sharma, S. ; Arwal, S.; Lodha, M. L. ang Kapoor, H.C. (2004). Antioxidant activity of antiviral proteins from Celosia crista. J. Plant Biochemistry & Biotechnology, 13 : 13-18.

Gobarah Mirvat, E. and Mekki, B.B., (2005). Influence of Boron application on yield and Juice quality of some sugar beet cultivars grown under saline soil condition . Journal of Applied Science Research 1(5) : 373 : 379.

Gupta, V. P. and Naqvi, Q. A. (1991). Inhibitory activities of plant extracts on virus infection, Acta Botany, Indica, 19: 18.

Haggag Wafaa , M. ; Younis , S.M. ; Farag Eman , M. (2010). Signaling Necessities and Function of Polyamines/ Jasmonate - Dependent Induced Resistance in Sugar Beet Against Beet Mosaic Virus (BtMV) Infection. New York Science Journal 3(8): 95-102.

Halliwell, R.S. and Johnson, J. (1988). Virus diseases of spinach in the Texas winter garden. Miscell aneous-Publication-Texas-Agricultural- Experiment-Station, MP-1656.4pp.

Hammerschmidt, R. (2009). Chapter 5 Systemic Acqured Resistance. Advances in Botanical Research, 51: 173-222.

Howell, W.E. and Mink, G.I. (1971). The relationship between volunteer sugar beets and occurrence of Beet mosaic virus and Beet westem yellows viruses in Washinton beet fields. Plant disease Report 55: 667-678.

Hsu, H. T. and Lawson, R. H. (1991). Direct tissue blotting for detection of *tomato spotted wilt virus* in impatiens. Plant Disease, 75: 292 – 295.

Katis, N.G. ; KoKinis, I.E. and Jones, P. (1997). Arable weeds identified as new sources of Beet mosaic poty virus in Greece. Annals of Applied Biology 130: 255-260.

Larcher, W.; Cernusca, A. (1985). Micro computer gesteuerte, mobile Anlage zum fluorimetrischen Nachweis von Photo-synthesestoerungen . Berchit der Oesterreichische Akadamie der Wissenschaften (Win) 194: 45-64.

Lewellen, R.T. (1973). Inheritance of Beet mosaic virus resistance in sugar in sugar beet. Phytopathology, 63: 877-881.

Lin, N. S.; Hsu, Y. H. and Hsu, H. T. (1990). Immunological detection of plant viruses and a mycoplasma like organism by direct tissue blotting on nitrocellulose membrans. Phtopathology, 80: 824 – 828.

Madhusudhan, K. N.; G. Vinayarani; S. A. Deepak; S. R. Niranjana; H. S. Prakash; G. P. Singh; A. K. Singa and Prasad, B.C. (2011). Antiviral Activity of plant extracts and other inducers against *Tobamoviruses* infection in Bell Pepper and Tomato Plants. International Journal of Plant Pathology, 2 (1): 35-42.

Mahdy, A. M. M.; Fawzy, R. N.; Hafez, M. A.; Mohamed Hanan .A.N. and Shahwan Eman. S. M.(2007). Inducing Systemic Resistance against *Bean yellow mosaic virus Potyvirus* Using Botanical Extracts. Egyptian Journal of Virology, 4: 223-240.

Mali, V.R.(2000). Natural infection of beet by Beet mosaic poty virus(BtMV) in west Slovakian. Zeitschnift fuer Pflanzenkran kheiten und Pflanzenschutz 107 : 539-547.

Mansfield, H.R.; Stern, M.D. and Oherby, D.E. (1994). Effect of beet pulp and animal by product on milk yield and *in vitro* fermentation by rumen microorganisms. J.Dairy Sci 77: 205-216.

Mbata, T. I.; Debiao, L. U. and Saikia, A. (2006). Antibacterial activity of the crude extract of Chinese green Tea (*Camellia sinensis*) on Listeria monocytogenes. The Internet Journal of Microbiology, 2 (2) : 20.

Mohamed, E.F.(2011). Changes in protein, amino acids composition and leaf cells of beet plants (*Beta vulgaris* L) due to Beet mosaic virus (BtMV) infection . Journal of American science 7(12): 845-853.

Mohammadi, M., ; Gibbs, A. ; Hosseini, A., and Hosseini, S. (2018). An Iranian genomic sequence of Beet mosaic virus provides insights into diversity and evolution of the world populayion. Virus Genes 54-272-279.

Mohammadi, M., ; Hosseini, A. (2020). Full genome sequencing of Iranian isolate of Beet mosaic virus. Iranian journal of plant pathology 55.4 : 339-351.

Mohammadi, M., ; Hosseini, A., and Hosseini, S. (2016). Investigation for recombination in P1 region of Iranian isolate of Beet mosaic virus (BtMV). Proceedings of 22nd Iranian Plant Protection Congress.

Mure, L. A. J.; Bi, Y. M.; Darby, R. M.; Firek, S. and Draper, J.(1997). Compromising early salicylic acid accumulation delays the hypersensitive response and increase viral dispersal during lesion establishment in TMV-infected tobacco. Plant Journal, 12:113-1126.

Omar, R.A. ; S. A. Sidaros ; S. A. El- Kewey ; Hayam, S. Abd El-Kader and Jehan, M. Abas (2006). Detection and identification of beet mosaic Poty virus (BTMV) infecting sugar beet plants in Egypt. Journal of Biotechno. 3(1-2) : 45-45.

Piszczek, J. (2000). Sugar beet diseases occurring in Poland-Gazeta- Cukrownicza,108(4):75-77.

Polak, I. (1981). Studies of virus diseases of sugar beet and its resistance to these diseases in Czechoslovakia. Principles of plant resistance to diseases and pests. 9:12.

Rawlins, T.E. and Tompkins, C.M. (1936). Studies on the effect of carborandum as abrasive in plant virus inoculation. Phytopathology, 26 : 578.

Sass, J.F. (1961). Botanical Microtechnique. 3rd ed. The lowa State Univ.Press, Ames, Lowa,USA.

Singh, A.K. and Rathi, Y.P.(1996). Histopathological studies in sterility mosaic affected sugar beet using fluorescent and light microscopy. Indian journal of virology 12(2) : 151-153.

Smith, K. M. A. (1972) . A Text Books of plant virus disease. 3rd Ed. Longman Group Ltd. London, 684 pp.

Somogy, N. (1952). Notes on suger determination. Journal-Biologychem., 145: 19 -23.

Specimen (Chapters 10) Plenum Press, New York, pp 155-173.

thesis, Faculty of Agriculture, Tanta University Pp.120.

Verma, H. N.; Baranwal, V. K. and Srivastva, S. (1998). Antiviral substances of plant origin. In plant disease control. APS Press. Paul, Minnesota, 154-162 pp.

Willey, R.I. (1971**).** Microtechniques: Alaboratory Guide. McMillan Publishing Co. INC., New

عزل وتعريف وإنتاج مقاومه مستحثة ضد فيروس موزيك بنجر السكر من نباتات بنجر السكر المصابة طبيعيا في مصر

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تم عزل فيروس موزيك بنجر السكر من نباتات بنجر السكر صنف توب المصابه طبيعيا فى الحقل من أماكن مختلفة من محافظة المنوفيه ولوحظت أعراض متفاوتة من موزيك وتشوهات علي اللأوراق. ثم عرفت هذه العزلة باستخدام الطرق البيولوجيه (الأعراض -المدى العوائلى والعوائل المشخصه) وطرق النقل (ميكانيكي وحشري) وأيضا تم التعريف بإستخدام الطرق السيرولوجيه مثل (طريقة الإليزا المباشرةDirect ELISA و طريقة البصمة النسيجية Tissue blot immunoassay وكذلك طريقة الإرتباط المناعى النقطى Dot blot immuno binding assay في تأكيد التعرف على الفيروس وأيضا تم استخدام الميكروسكوب الضوئى في التعريف . أدت الإصابة بفيروس موزيك بنجر السكر الي تحطيم في بعض صبغات في أوراق البنجر ومنها (كلوروفيل أ و ب والكاروتينات) كذلك أدت الإصابة إلي تقليل تركيز الكربوهيدرات الكلية والسكر الكلي في الجذور . تم دراسة تأثير بعض المستحثات للإصابة الفيروسية لهذا الفيروس وقد تم استخدام نوعين من المستحثات وهما المستخلصات النباتية وكذلك حمض السالسيلك وكانت النتائج الأوليه تشير إلي أن المستحثات المستخدمه في البحث أدت الي تقليل أعداد البقع الموضعيه الناتجة من الإصابة بالفيروس . أيضا أدي استخدام المستحثات إلي تقليل تركيز الفيروس في الأوراق المصابة . وقد أعطى مستخلص القرنفل أعلى نسبة تثبيط مقارنة ببقية المستخلصات النباتية الأخري – كذلك أوضحت النتائج أن استخدام حمض الساليسلك إلي تقليل عدد البقع الموضعية المتكونة وكذلك تركيز الفيروس باستخدام جهاز الإليزا المباشرة