Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(9); 1195-1205
doi: 10.25258/phyto.v9i09.10306

ISSN: 0975-4873

Research Article

Impact of Virucide; Carrageenan in Suppression of Potato Virus Y in Potato Plants

Gomaa Hanaa H A*

Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Received: 12th Jul, 17; Revised 1st Sept, 17, Accepted: 14th Sept, 7; Available Online:25th Sept, 17

ABSTRACT

Background: Potato plants are usually subjected to numerous diseases caused by viruses. *Potato virus Y* belongs to a group of *potyvirus* that causes serious diseases to potato plants and is mainly responsible for the majority of losses caused in potato production. Objective: The present study aims to evaluate the potential effect of carrageenan (virucide) which was extracted from red algae (*Acanthophora specifira*) in an induction of systemic acquired immunity for bio-control of PVY infection in potato plants. Methods: Potato tubers (*Solanium tuberoisum* cv. spounta) were obtained. The virus isolate was obtained and maintained on *Datura metel* plant. Specimens of the red alga *Acanthophora specifira* were collected from El Shoaiba coast, the eastern part of Saudi Arabia; Red Sea. The tested material carrageenan was extracted from the selected seaweeds species as antiviral agents. The infectious sap was prepared and inoculated on the primary and free potato leaves. The tested plants were sub-classified into 5 groups; 2 healthy groups (control group and virucide-treated group), 3 PVY infected plants groups (one without virucide, PVY infected plants either before or after virucide). Reduction in the disease severity was recorded and inhibition in PVY infectivity as a result of periodical treatment of foliar potato plants with virucide (carrageenan) was assessed in pre and post-treatment groups. Results: PVY infection resulted in significant decrease in shoot length, leave an area and fresh and dry weight of shoots. Improvement of morphological characters of periodically-treated foliar with virucide (carrageenan) was observed. Chlorophyll and carotenoids contents were increased in PVY infected foliar potato plants treated with virucide (carrageenan). Biochemical markers as indicators for systemic acquired resistance were detected via significantly increased total proteins, phenol compounds, free proline (PO), polyphenol oxidase (PPO) and superoxide dismatase (SOD) enzyme activities. As well as expressed proteins of induced potato plants were determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The results indicated that newly expressed proteins were produced and a difference in number and density of bands in foliar treatment plants with virucide (carrageenan) were compared with healthy ones. Conclusion: It has been suggested that the induction of systemic acquired resistance (SAR) was successfully achieved; it also protected potato plants against PVY infection.

Keywords: Carrageenan, Polyphenol oxidase (PPO), Potato virus Y (PVY), Proline (PO), Sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Superoxide dismutase (SOD), Systemic acquired resistance (SAR).

INTRODUCTION

Viruses are responsive for considerable losses in crop productivity and quality. Several conventional strategies to control viral infection has been explored but without much success. In many approaches involving viral components, the induced resistance is very specific to a particular strain or group of viruses. The viruses basically differ from other crop pathogens and pests because they cannot be eradicated chemically. Management of plant viral diseases can be performed through the induction of natural plants' defenses e.g., systemic acquired resistance (SAR). SAR against viral infection has been confirmed using chemical and biological inducing agents[1].

Two types of induced resistance (IR), salicylic acid-dependent and jasmonic acid and ethylene dependent induced systemic resistance (lSR) are being characterized at the molecular level. Reports have shown that simultaneous expression of systemic acquired resistance (SAR) and ISR act synergistically; however, intricacies in the cross lack defense network have not been characterized well. The two types of resistance are effective against a wide range of pathogens[2].

Plants have developed a wide range of physical and chemical defense mechanisms to protect themselves against pathogens and pests through metabolic, biochemical and molecular defenses as well as physical/structural barriers such as thorns or waxy leaves. Plant defense mechanisms may be either preformed (passive or basal) or induced (active) resistance. Preformed resistance is constitutively present in plants, such as naturally occurring chemicals or physical barriers. In contrast, induced resistance occurs when a plant recognizes an invading pathogen through various elicitors[2]. The present study aims to evaluate the potential use of virucide (carrageenan) as a foliar treatment to

*Author for Correspondence: hgoumaa@hotmail.com

induce acquired systemic resistance for bio-control of PVY infection in potato plants[3].

The red algae family (Rhodophyta) contains species that are characterized by their content (non-fibrillar and sulphated polysaccharides) such as carrageenans. These polysaccharides are well known for their diverse biological effects[4,5]. Moreover, variant carrageenans and other sulphated polysaccharides from diverse red alga induce different defense response arsenals in several plant species[6].

Materials and Methods

Plant material and growing conditions: Potato tubers (*Solanium tuberoisum* cv. spounta) were obtained from mold brown structures project, Agriculture Research Center. This commercial cv. was chosen because it is commonly used in Egypt and for its sensitivity to *Potato virus Y* (PVY) infection. Tubers were transplanted in a mixture of sand and clay soil (1:3) w/w in pots (30 cm).

PVY isolate

The virus isolate was obtained from virology laboratory, microbiology department, faculty agriculture Ain Shams Univeristy, and maintained on *Datura metel* plant. The virus isolate was confirmed biologically by local lesion test on *Chenopodium amaranticolor* L. and kept under greenhouse conditions for the development of chlorotic local lesions as well as confirmed by DAS-ELISA using polyclonal antibodies specific PVY[7].

Algal material

The red alga *Acanthophora specifira* (Vahl) Børgesen Howe were collected from El Shoaiba coast, the eastern part of Saudi Arabia, Red Sea[8]. The selected species were identified by Papenfuss (1968)[9]; Cribb (1983)[10]; and Russell (1992)[11]. The specimens were washed with local sea water to get rid of attached epiphytes and other organisms. Then, they were gently brushed with tap water. The samples were air-dried at room temperature. The dried samples were grinded with an electrical blender into powdered form then kept in plastic bags at the dry and cool place until the time of extraction.

Extraction of carrageenan

carrageenan was extracted from the selected seaweeds species (red alga *Acanthophora specifira*) as antiviral agents. The dried seaweeds were extracted with hot distilled water, and then filtered on diatomaceous earth. The filtrate was poured in absolute ethanol with stirring. The precipitate was recovered and washed with 95°C ethanol, dehydrated with diethyl ether and then dried overnight at 50°C. Stock solutions (10 ml) were prepared in PBS (Phosphate buffer saline) buffer and were stored at - 4°C until use.

Virus inoculums

The infectious sap was prepared by grinding freshly infected leaves of *D. metel* plant in phosphate buffer solution (PBS) 0.1 M, pH 7.0. The extract was filtered through two layers of cheese- cloth. The primary and free potato leaves were inoculated with infectious sap (10^{-1} dilution). A few minutes later, the inoculated plants were washed gently with water.

Experimental design: Uniform potato tubers were transplanted in Botanical garden Botany Department, Faculty of Science, Suez Canal University Ismailia, Egypt in winter season 2013. The experiment was contained 16 ridges (8 for each plate). Treated tubers were completely randomly designed with 8 replicates. The irrigation and nutrition of potato plants were performed according to Ministry of Agriculture recommendations in Egypt. The potato plants were treated as shown in the table (1).

Leave Samples from the vegetative state (45 days) and yield stage (100 days) were taken for each treatment and the following parameters were estimated:

Pathogenic test

Disease severity induced by PVY isolate was recorded after 25 days of inoculation. The symptoms were recorded using the rating scale and calculated using formula according to Yang *et.al.* (1996)[12].

Virus infectivity

The infectious sap was extracted from each treatment in 0.1 Mm PBS PH 7.0. Plants of *D. metel* L. seedlings (10) were mechanically inoculated with the infectious sap of each treatment. The inoculated plants were kept under greenhouse condition and external symptoms were recorded. The virus infectivity was calculated as a number of infected plants per total inoculated plants. The results were confirmed using polyclonal antibody by DAS-ELlSA.

Detection of systemic acquired resistance

Photosynthetic pigments: Chlorophyll-A, Chlorophyll-B, and Carotenoids were estimated in the fresh foliage leaves. One gram of fresh leaf was extracted by grinding with 10 ml of 80% acetone. The mixture was then centrifuged for 5 min, at 3000 rpm. The supernatant was used for spectrophotometric determination according to method Lichtenlhaler (1987)[13].

Total soluble proteins were estimated according to the method of Lowery *et.al.* (1951)[14] using casein as a standard protein. Determination of total protein in leaves was performed by UV-spectrophotometer (UNICO 2000) at wavelength 750 nm.

Phenol compounds

One gm of fresh leaves were macerated in 50 ml 80% ethanol for at least 24 hr at 0°C, the alcohol was clarified the remained residue was re-extracted with 10 ml 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. Extraction and determination of phenolic compounds according to the method described by Daniel and George (1972)[15] using UV-spectrophotometer (UNICO 2000) at wavelength 725 nm.

Proline content: 0.2 gm of leaf samples were homogenized in 5 mL of 3% (w/v) sulfosalicylic acid and centrifuged at 3000 rpm at 4°C for 10 min. The supernatants were used for proline estimation according to the method described by Bates *et al*. (1973)[16] at 520 nm using UV-spectrophotometer (UNICO 2000).

Salicylic acid (SA)

Determination of salicylic acid in plant tissues by gas chromatography–mass spectrometry (GC–MS). SA from leaves extracted with 9:1 (*v*/*v*) methanol–chloroform was derived by use of

bis(trimethylsilyl)trifluoroacetamide (BSTFA) under the optimum reaction conditions (120 °C, 60 min). Quantitative analysis by GC–MS was performed in selected ion monitoring (SIM) mode using an internal standard. Procedures for sample preparation and reaction conditions were optimized. Analysis was completed within 2 hours. A sensitivity of 10 ng g^{-1} fresh weight and a relative standard deviation less than 5.0% for SA in leaves were achieved[17].

Enzymes activities

The plant materials used for estimation of enzymes were 2 gm of the terminal buds homogenized with 10 ml of phosphate buffer PH 6.8 (0.1M), then centrifuged at 2˚C for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source[18].

Peroxidase (POX) activity was assayed by measuring the inhibition of the auto- oxidation of pyrogallol using a method described by Bergmeyer (1974)[19] at 470 nm wave length using UV-spectrophotometer (Labomed, inc.23).

Polyphenol oxidase (PPO) activity was assayed by measuring the inhibition of the auto-oxidation catechol using a method described by Matta and Diamond (1963)[20]. The absorbance was measured at 495 nm wave length using UV-spectrophotometer (Labomed, inc.23).

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of auto-oxidation of pyrogallol using a method described by Marklund and Marklund (1974)[21] at 325 nm wave length using UV-spectrophotometer (Labomed, inc.23).

Determination of growth and yield

Plants were harvested 3 months after tuber planting. Shoot length, leave area, fresh and dry weight as well as yield parameter was recorded for all treatments.

Biochemical genetic detection

In the present study, leaves protein finger print were analyzed using SDS-PAGE based on the method of Laemmli, (1970)[22] modified by Studier, (1973)[23]. The revealed banding profile for different treatments was qualitatively and quantitatively compared.

RESULTS

PVY Isolate

The PVY isolate was confirmed biologically by appearance of chlorotic local lesions on *Ch. amaranticolor* and severe mosaic, leave narrowing and deformation on *D. metel*. Also, it gave positive precipitated serological reaction by DAS- ELISA with specific PVY polyclonal antibodies.

PVY Pathogenicity

Disease severity and PVY infection percentages in potato plants sprayed with virucide (carrageenan) are recorded in table (2). The obtained results revealed that the high level of disease severity (85 and 90 %) in PVY infected potato plants was observed at 45 and 75 days, respectively. The low level disease severity was obtained at 15, 0 % and 35, 10 % of foliar treatment, with virucide (carrageenan) pre and post PVY infection at 45 and 75 days, respectively (table, 2).

PVY infectivity

The foliar treatment with virucide (carrageenan) reduced PVY infectivity significantly. These results are recorded in table (2). It was observed that there is a high reduction of PVY infectivity especially at the second stage of plant growth (75 days) in pre and post foliar treatment with virucide. Concerning the effect of the foliar treatment with virucide (carrageenan) on the challenged potato plants with PVY, it was recorded in pre foliar treatment with virucide higher than PVY infection (table, 2 and figure 1).

Impact of foliar with virucide (carrageenan) on infected plants

Growth parameters: Foliar treatment with virucide (carrageenan) significantly increased shoot length, leave area, fresh and dry weight of healthy plants. These parameters were not significantly decreased in PVY infected plants compared with non-foliar treatment ones (table 2). Concerning the effect of the foliar treatment with virucide (carrageenan) on the challenged potato plants with PVY, it was noticed that, the foliar treatment pre PVY infection gave significant increase in growth characters of potato plants in comparison with the foliar treatment post PVY infection. These observed differences were found to be statistically significant especially at second stage (75 days) of plant growth.

Yield Character

Foliar treatment with virucide (carrageenan) increased the number and weight of potato tubers in plants infected with PVY and also in healthy plants not infected with PVY ones. The results recorded in table (3) revealed that the PVY infection caused significant decrease in number and weight of potato tuber per plant. On the other hand, application of virucide (carrageenan) foliar treatment resulted in highly increased potato tuber yield per infected plant.

Evaluation of virucide (carrageenan) for SAR induction: Systemic acquired resistance (SAR) was achieved using the foliar treatment with virucide (carrageenan) on potato plants pre and post PVY inoculation. Also, the effect of virucide (carrageenan) on virus pathogenic and infectivity were detected. The foliar treatment with virucide (carrageenan) was applied pre and post PVY inoculation intervals 15 days. One treatment pre PVY inoculation was firstly sprayed with virucide (carrageenan) then PVY inoculation was performed after 15 days. Leave samples were collected for each treatment and the following parameters were determined:-

Photosynthetic pigments

The results recorded in table (4) revealed that chlorophyll a and chlorophyll b was significantly decreased in PVY infected potato plants at two growth stages. The healthy foliar of treated plants showed a significant increase in chlorophyll a and b. Similarly, foliar of PVY infected plants treated with virucide (carrageenan) pre and post PVY infection showed significant increase in chlorophyll a and b compared with healthy control ones (Table 4). Concerning the effect of virucide (carrageenan) foliar treatment on carotenoid content of healthy and PVY infected potato plants, it was found that foliar treatment showed significant increase in healthy and both pre and

Table 1: Layout of experimental treatments.

Treatment Factors	Control (healthy)	Virucide (healthy)	PVY infected	Virucide then PVY infected	PVY infected then viruicide
Healthy	X				
PVY infected					x
Virucide		x			x

Table 2: Effect of foliar treatment with virucide (carrageenan) on potato plants and PVY infectivity.

Treatments Stage	Infected Potato plants			Foliar pre Inoculated PVY			Foliar Post Inoculated PVY		
	Symptoms	DS (%)	VI	Symptoms	DS (%)	VI	Symptoms	DS (%)	VI
Vegetative (45 days)	SM,VN L.N	85	8/10	mM	15	4/10	mM, LY	35	5/10
Yield 75 days	SM,VN LN	90	9/10	__	0	0/10	mM	10	3/10

LN=leave narrow, SM=severe mosaic DS = disease severity -- = no symptoms
LY= light yellow VN = venial necrosis VI= virus infectivity

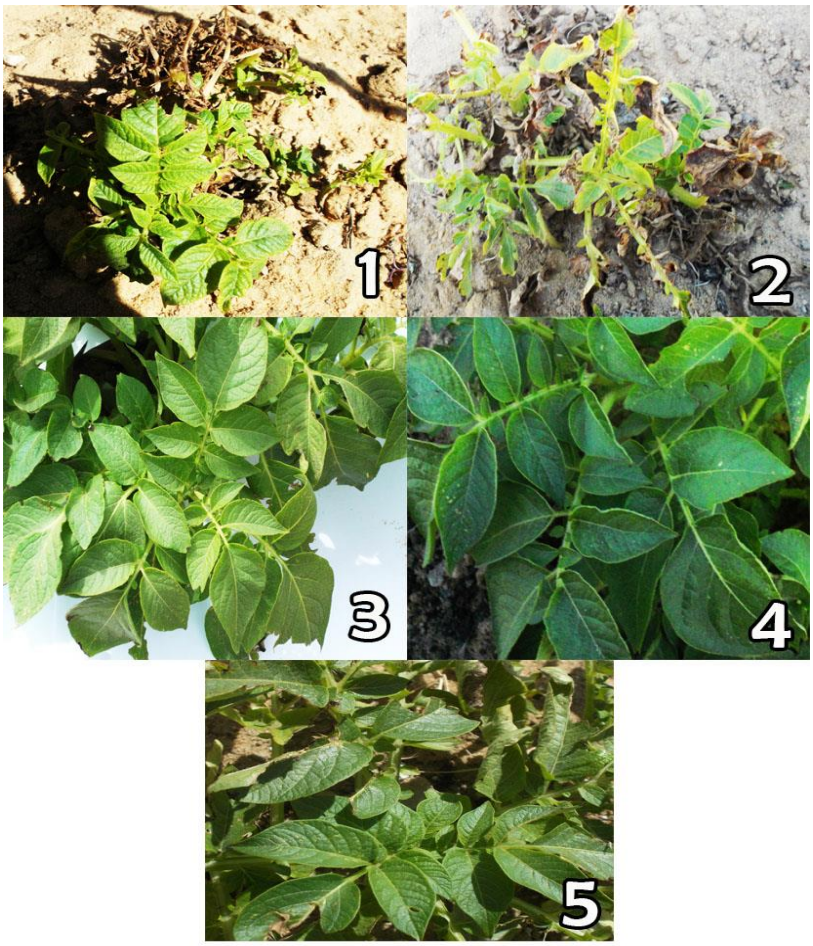

Figure 1: Potato leaves showing (1) PVY-infected plant sprayed with virucide post-infection, (2) PVY-infected plant, (3) Healthy plant, (4) Healthy plant sprayed with virucide and (5) PVY-infected plant sprayed with virucide pre-infection.

post PVY infection in comparison with PVY infected ones (table 4).

Total protein contents

The results in table (5) showed that, total soluble protein content in potato leaves was highly significantly increased due to virus infection during the two stages of plant growth. Regarding the effect of foliar treatment with virucide, it was found that both treatments significantly increased the total soluble protein content in potato leaves infected with PVY but not in potato plants treated with virucide.

Free proline content

Table 3: Impact of virucide (carrageenan) foliar treatment on growth and yield characters of infected potato plants with PVY.

Parameters	Shoot length (cm/Plant)		Leave area (cm/plant)		Fresh weight gm/plant		Dry weight gm/plant		Yield per plant	
Treatments	St- 1	St-2	St- 1	St-2	St- 1	St-2	St- 1	St-2	No.	Wt
Healthy	25.0	54.5	6.20	8.50	11.50	44.50	1.10	2.4	6.70	196.25
Virucide Foliar treatment	27.5	56.7	6.81	9.15	9.50	36.80	1.05	1.70	5.25	200.50
PVY infected Plant.	22.5	45.2	4.62	5.25	10.20	29.70	0.75	1.50	4.50	144.25
Virucide pre PVY infection	26.7	46.5	5.43	8.25	9.20	29.50	1.05	1.15	6.50	176.70
Virucide post PVY infection	24.5	44.7	4.24	7.50	11.50	37.70	1.07	1.46	6.00	175.50
L.S.D at 5%	2.5	3.9	2.2	3.2	2.31	3.32	0.21	0.22	3.3	3.5

No = Number of tubers Wt= Weight of tubers St-1= Vegetative stage (45 days) St-2= Yield (75 days)

Table 4: Photopigment contents in healthy and PVY infected potato and foliar treatment with virucide (carrageenan).

Photo pigments	Chlorophyll content				Carotenoid content	
	A		b			
treatments	St-1	St-2	St-1	St-2	St-1	St-2
Healthy plants	5.20	6.25	2.01	3.62	1.15	0.75
Virucide foliar treatment	5.75	6.50	2.25	3.75	1.25	0.91
PVY infected plants	4.15	3.45	1.35	2.52	0.95	0.65
Virucide pre PVY infection	5.25	6.12	2.15	3.10	1.20	0.90
Virucide post PVY infection	4.75	3.85	1.72	2.40	1.01	0.82
L.S.D.5%	0.38	0.05	0.21	0.12	0.08	0.06

St-1= Vegetative stage (45 days) St-2= Yield (75 days)

Table 5: Effect of PVY infection on protein, phenol, proline and salicylic acid content in potato plants.

Photo pigments	Protein (mg/g FW)		Total phenols		Proline		Salicylic acid	
Treatments	St-1	St-2	St-1	St-2	St-1	St-2	St-1	St-2
Healthy plants	45.56	60.70	0.84	0.82	0.40	0.42	0.10	0.12
Virucide foliar treatment	48.47	61.75	1.14	0.85	0.61	0.80	0.11	0.20
PVY infected plants	51.38	61.75	1.58	1.85	0.70	0.70	0.35	0.37
Virucide pre PVY infection	67.29	60.25	1.63	1.95	0.94	1.25	0.37	0.45
Virucide post PVY infection	64.10	60.50	1.83	1.86	0.82	0.87	0.42	0.47
L.S.D.5%	2.00	0.5	0.25	0.08	0.08	0.06	0.08	0.09

The results were calculated as mean of three replicates as ml /g FW
St-1= Vegetative state (45 days) St-2= Yield (75 days)

Concerning the effect of foliar treatment with viruicide (carrageenan) on healthy potato plants and PVY infected ones; it was found that, each of viruicide (carrageenan) and PVY infected plants had significant increase of proline content than healthy ones. As well as PVY infected potato plants, sprayed with virucide (carrageenan) showed highly significant increase at two growth stages compared with healthy ones (table 5).

Salicylic acid (SA)

The results revealed significant increase in SA content in PVY infected potato plants than healthy ones. On the other hand, it was found that SA contents in potato leaves were significantly increased in foliar response with virucide (carrageenan) and virus inoculation compared with potato plants non-foliar treated ones table (5).

Enzyme activities

Superoxide dismutase (SOD)

Data generated in table (6) showed the changes of SOD activity in PVY infected potato plants sprayed with virucide (carrageenan). During growth period the results revealed higher activity of SOD in PVY infected plants untreated with virucide. Foliar infected plants with virucide (carrageenan) pre and post PVY inoculation significantly had increased SOD activity compared with untreated plants (table 6).

Peroxidase activity (POX)

The results showed that POX activity was changed in infected potato plants sprayed or non–sprayed with virucide (carrageenan) than healthy ones as well as significantly increased in second stage of plant growth.

Table 6: Enzyme activities in foliar treatment with virucide (carrageenan) challenge infected potato plants with PVY.

Parameters	SOD activity (ug\g FW)		PO activity (ug\g FW)		PPO activity (ug\g FW)	
Treatments	St.1	St.2	St.1	St.2	St.1	St.2
Healthy	0.15	0.10	0.08	0.10	0.26	0.25
Virucide	0.20	0.21	0.16	0.18	0.27	0.26
Foliar treatment						
PVY infected plant	0.40	0.16	0.35	0.37	0.30	0.27
Virucide pre PVY inoculation	0.47	0.50	0.33	0.38	0.37	0.36
Virucide post PVY inoculation	0.35	0.37	0.30	0.39	0.31	0.29
LSD at 5%	0.06	0.06	0.08	0.06	0.06	0.06

St-1= Vegetative state (45 days) St-2= Yield (75 days)

All treatments were stimulated POX activity pre and post challenge with PVY during the two stages of plant growth compared with healthy virucide (carrageenan) treated and untreated table (6).

Polyphenoloxidase activity (PPO)

The results of present work (table 6) revealed that potato plant infected with PVY gave highly significant increase in PPO activity compared with healthy ones. It was found that healthy plants sprayed with virucide (carrageenan) mostly non-significant increased during the two growth stage. Concerning the effect of foliar treatment with virucide (carrageenan) on the challenged potato plants with PVY, it was found that pre or post PVY inoculation show significant increased of PPO activity during the two growth stages table (6).

Protein markers

The potato plants foliar treatment with virucide challenge with PVY showed variation in number and molecular weight and density of protein bands. The variability analysis among treated and untreated plants showed 20 bands. Regarding, the effect of foliar treatment with virucide on the challenged potato plants with PVY, it was found that, virucide treatment pre and post PVY inoculation revealed 22 and 21 bands respectively as well as, virucide treatment appeared 21 bands. The potato plants infected with PVY appeared 18 bands and healthy plants appeared 15 bands. Concerning, the virucide treated potato plants revealed unique polypeptide with molecular weight 120 KDa. As well as infected potato plants and foliar treated with virucide pre PVY inoculation appeared 45 KDa band (table, 7 and figure 2).

Discussion

The objective of this work was the induction of active acquired immunity (AAI) and systemic acquired resistance (SAR) in potato plants against PVY infection using carrageenan (a biotic elicitor).

The first criterion to judge the occurrence of AAI and SAR in potato plants periodically sprayed with the virucide (carrageenan) is the ability to reduce the percentage of PVY infectivity and to limits PVY multiplication. The obtained results showed that virucide inducer reduced the level of disease severity of PVY. The same results were obtained by Shahwan 2010 (CMV)[24], Gora, 2014[25] (APMV and APGV). The virucide may induce resistance or it may act as inhibitor of viral replication. Thus, biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development (EL-Dougdoug and Eskander, 2012)[26].

The obtained results showed a retarded PVY infected potato growth. Plant height, leave area, fresh, dry weights and tuber yield were statistically significantly decreased due to PVY infection. In this regards, the reduction in host plant growth may be correlated with the disturbances in the supply or distribution of the growth regulating hormones[27,28]. Viral infection induced reduction in amount of host plant growth of shoot and roots in potato plants which contributes to the diminished photosynthesis activity that is closely related with yield and quality products. Similar results were obtained by Elbadry and colleagues (2006)[29].

Considering foliar treatment periodically with virucide, it was found that PVY infected potato plants sprayed with virucide (carrageenan) were significantly improved in plant growth as shown by an increase of plant height, leave area, fresh and dry weighs, as well as yield tuberization. Our result are in accordance with those reported by Ksalami and Suprapta (2011)[30] and Gora (2014)[25].

Photosynthetic pigments content were positively markedly affected as result of foliar treatment with virucide (carrageenan). The obtained results clearly revealed a reduction in photopigments (a,b, and carotinoids) in potato leaves due to PVY infection. On the other hand, the results herein showed that foliar treatment with virucide (carrageenan) pre and post PVY infection improved plant health by increasing photopigments. The decrease in Chlorophyll and carotinoids might be due to the generation of reactive oxygen species (ROS) causing damage to chlorophyll and thalikoid membrane that means the plant failed to capture the light and so photosynthesis will decrease or stop. The effect of virucide as a regulatory role in plant physiology included inhibition of ethylene biosynthesis, interference with membrane depolarization, blocking wound responses and an increase in photosynthetic rate and chlorophyll content in potato leaves[31].

Thus virucide (carrageenan) may have an effect on plant defense mechanisms against harmful diseases. Therefore, it could be considered as effective bio-elicitor consists of essential components required to cell division, cell elongation and photosynthetic pigments formation because it is enriched in macro-and microelements, Phytohormones and vitamins. These results are in

Table 7: polypeptide pattern of protein content for PVY infected potato plants sprayed with viruicide.

MW peptide (KDa)	Healthy plants	Foliar treatment	PVY infected plants	Virucide pre PVY inoculation	Virucide post PVY inoculation	Polymorphism
200	_	+	_	+	+	Polymorphic
175	++	++	++	++	++	Monomorphic
152	_	+	++	++	++	Polymorphic
140	++	+++	+++	+++	+++	Monomorphic
125	++	++	++	++	++	Monomorphic
120	_	++	_	_	_	Unique
115	++	++	++	++	++	Monomorphic
105	++	+	+	+	+	Monomorphic
95	+	+++	++	+++	++	Monomorphic
90	+	+++	+	++	++	Monomorphic
85	_	+	++	+	+	Polymorphic
80		++	+	++	++	Monomorphic
78	+	+++	++	++	++	Monomorphism
75	++	++	+	++	++	Monomorphic
73	+	++	+	+	+	Monomorphic
70	++	++	+	+	+	Monomorphic
65	+++	+++	+++	+++	+++	Monomorphic
57	+++	+++	+++	+++	+++	Monomorphic
45	_	_	_	+++	_	Unique
40	_	_	_	+++	+++	Polymorphic
32	_	++	_	+	+	Polymorphic
30	++	++	++	++	++	Polymorphic
15	+	+	+	+++	+++	Polymorphic
Total polypeptide	15	21	18	22	21	

(-): Not detected, (+) weak protein expression density, (++) moderate protein expression density, (+++) strong protein expression density. Polymorphic = specific protein band, Monomorphic = common protein band, Unique = genetic marker band

harmony with the study carried by Ghobvial, *et. al.* (2009)[31] and Galal,(2006)[32].

Total protein was determined as response to induction treatment. The obtained results showed that the total soluble protein increased significantly in PVY infected leaves. The increase of the soluble protein due to viral infection has been reported by Mohamed, *et. al.* (2012)[33]. The increased protein in PVY infected leaves is possibly due to the synthesis of protein coat, protein related virus infection (PR); expressed protein and other virus associated non-structural proteins.

Moreover, treatment with virucide (carrageenan) significantly increased the soluble protein level in the healthy and PVY infected plants relative to their respective untreated plants. It has been reported that plants develop a complex variety of events involving synthesis and accumulation of new soluble proteins that have direct or indirect role in inducing plant resistance (Radwan *et. at.,* 2010)[34]. It has been suggested that the induced proteins may help to limit virus spread or multiplication (Chen *et. at.,* 2006)[35]. The continuous accumulations of newly-induced protein may help in the localization of viral infection; the reverse is not true, since the presence of a non-significant amount of induced proteins is a necessary condition to the observed systemic infection. Based on current knowledge of the biochemistry of resistance, it can be concluded that SAR results from the expression of several parameters, including changes in cell wall composition and *de novo* synthesis of phytoalexins and PR (pathogenesis related protein). Moreover, the *de novo* synthesis of phytoalexins is often related to the induced resistance stage (Walter, *et. at.,* 2007)[36].

In the present study, total phenol was significantly increased in the healthy and PVY infected shoots pre and post infection compared with healthy untreated control. On the other hand, it was found that foliar treatment with virucide (carrageenan) of potato plants infected with PVY in both pre and post infection showed significant increase in phenol contents. These phenolics mediate defense responses of potato plants against PVY infection that causes disease in potato plants. Phenolic components including flavonoids are structurally diverse group of plant secondary products that can play a variety of roles in plant defense against pathogens, such as phytoanticipins, phytoalexns, biosynthesis of lignin and structural barriers, which play a major role in resistance against pathogens[37]. Our results showed that the protein content increased significantly in PVY infected plants as compared with healthy plants.

Herein, the results revealed that protein content in leaves of virucide (carrageenan) treated plants were highly

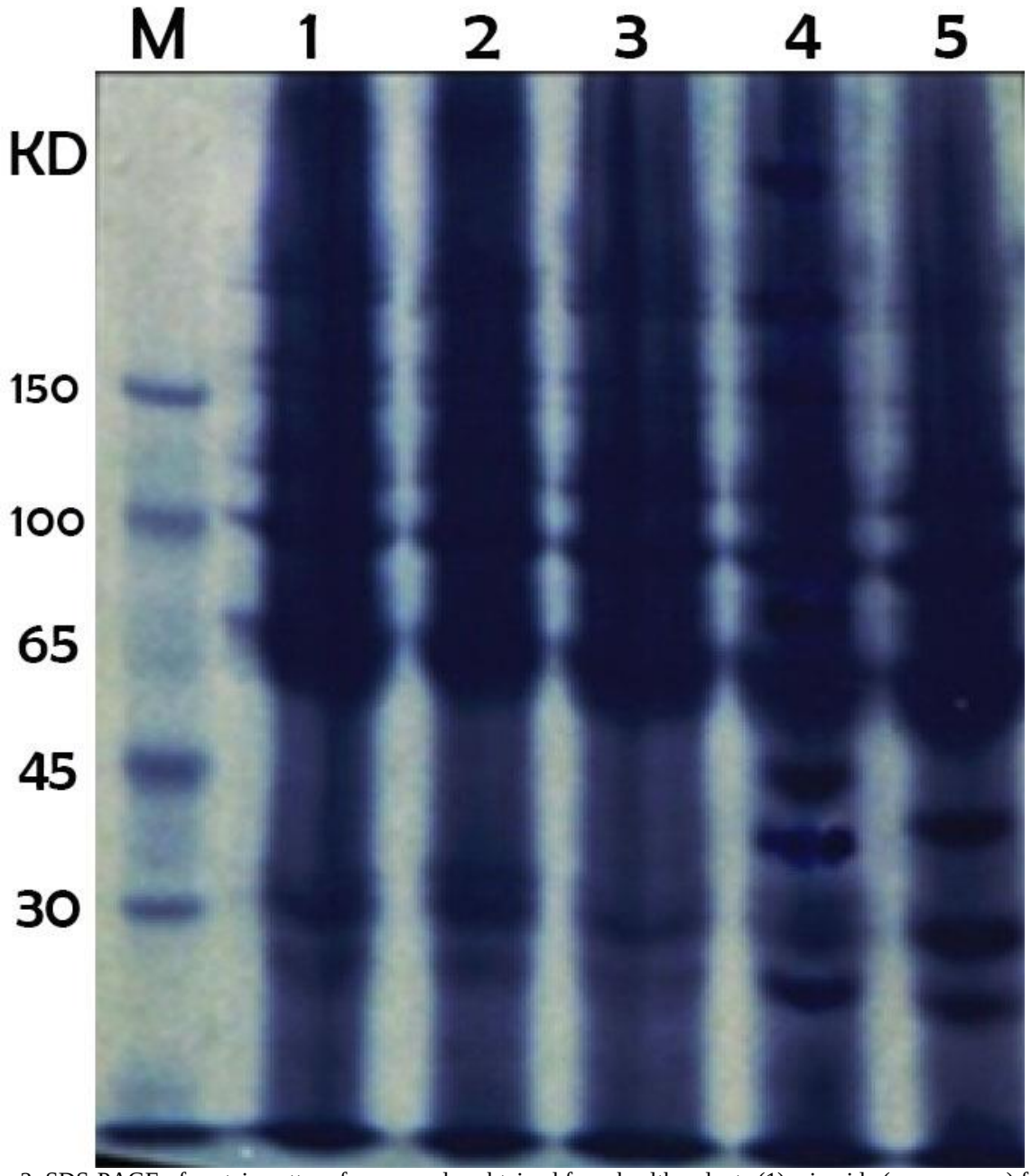

Figure 2: SDS-PAGE of protein pattern from samples obtained from healthy plants (1), virucide (carrageenan) foliar treatment (2), PVY infected Plants (3), virucide (carrageenan) per PVY inoculation (4) and virucide (carrageenan) post PVY inoculation (5).

significantly increased throughout the growth stages. Therefore, the results indicated that the defensive capacity of potato plants against PVY could be enhanced through virucide treatment. Proline accumulation is common metabolic response to a biotic stress[38].

The plants are exposed to microbial pathogens; they produce reactive oxygen species (ROS) that stimulate programmed cell death in the nearby plant cells in the infection site to effectively wall off the pathogen and terminate the disease process. The amino acid proline acts as a potent scavenger of ROS, thus prevents the induction of programmed cell death by ROS[39].

A large number of defense enzymes that have been associated with SAR include peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), lipoxgyenase (LOX), ascorate peroxidase (APX) and proteinase inhibitors. These enzymes also bring about liberation of molecules that elicit the initial steps in induction of resistance, phytoalexins and phenolic compounds[40]. Our results showed that antioxidant enzymes PO, PPO, SOD activities increased significantly in potato plants infected with PVY. The activity of PO, PPO and SOD enzymes were greater in the plants treated with virucide and challenged with PVY compared to healthy ones.

Induction of enzymes, that activate the phenylpropanoid pathway, have been reported to be among plant defense mechanisms by conferring mechanical and /or chemical

barriers in host tissues against pathogen as well as influences the levels of phenol compounds in the metabolic pool. A variety of plant defense-related processes have been implicated by POX activity including: hypersensitive response, cross-linking of phenolics with glycoproteins and cell wall lignifications[37,41,42].

The obtained result revealed that foliar treatment with virucide (carrageenan) on the challenged plants with PVY lead to increased contents of salicylic acid (SA), with significant increase in IAA (indol acetic acid), GA3 (gibberellins), ABA (abscisic acid) and jasmonic acid (JA) levels in plant leaves. It is reasonable to assume that several plant hormones either individually or in combinations modulate the complex processes involved in plant defense signaling pathways. The first hormones to be marked as central players in defense against pathogens was salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), with roles more recently attributed to abscisic acid (ABA) gibberellins (GA3) and auxin[43]. SA can trigger the SAR pathway in some plant species. SA induction is often linked with pathogenesis-related (PR) protein accumulation mainly PR-1. SA is considered to be a plant signal molecule and involved in induction of SAR, which activates many defense compounds including phenolic acids, coumarins, flavonoids and lignin[44].

PR-1 was detected to have enzymatic activity such as chitinase and gluconase, which increased the viral resistance by accumulating proteinase inhibitors. The term systemic acquired resistance referred to a change in the physiology of the plant. There were a number of biochemical and physiological changes that were established to be associated with SAR which included cell death and oxidative burst, deposition of lignin, accumulation of proline, phytoatexins, pathogenesis related protein PR -1 charges in pigment contents and Pox activity[45].

Biosynthesis of protein PR-1 was found as a response to virucide treatment. It has a distinguished role in the resistance to PVY either in healthy or challenged potato plants. The quantitative, qualitative and activity of antiviral proteins as protein content and patterns were determined by SDS-PAGE. The results indicated that a new pattern of proteins were produced as well as difference in number and density of bands among virucide treatments. It has been suggested that, the induced, patterns may help to limit virus spread or multiplication[24,25,35].

The continuous accumulations of newly-induced proteins may help in the localization of viral infection; the reverse is not true, since the presence of a non-significant amount of induced proteins is a necessary condition to the observed systemic infection. Based on current knowledge of the biochemistry of resistance, it can be concluded that SAR results from the expression of several parameters, included changes in cell wall composition and *de novo* synthesis of phytoalexins and PR. Moreover, the local *de novo* synthesis of phytoalexins is often related to the induced resistance stage[36].

REFERENCES

1. Gholizadeh, A., Kumar, M.; Balasubrahmanyam, A.; Sharma, S.; Norwal,. S.; loaha, M.L. and Kapoor, H.C. (2004). Antioxidant Activity of Antiviral proteins From Celosia Cristata. J. plant Biochemistry and Biotechnology B.13 -19.
2. Pieterse, C.M.J. and Van loon, L.C. (2004): NPRI: the spider in the web of induced resistance signaling pathways. Current Opinion in plant Biology 7: 456-464.
3. Hammerschmidt, R. (2009): Chapter 5 Systemic Acquired Resistance. Advances in Botanical Research, 51, 173-222.
4. Dietrich, C.P.; Farias, G.G.M.; De Abreu, L.R.D.; Leite, E.L.; Da Silva, L.F. and Nader, H.B. (1995): A new approach for the characterization of polysaccharides from algae:presence of four main acidic polysaccharides in three species of the class Phaeophycea. Plant Sci. 108:143-53 (11).
5. El Gamal, A.A. (2010): Biological importance of marine algae. Saudi Pharm J 18:1-25.
6. Vera, J.; Castro, J.; Gonzalez, A. and Moenne, A. (2011): Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. Marine Drugs 9:2514-2525.
7. Adams, A.N. and Clark, M.F.(1977): Characteristics of microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses,J. Gen.Virol., 34:475-483.
8. Gomaa H.H. and Elshoubaky G.A. (2016). Antiviral activity of sulphated polysaccharides Carrageenan from some marine seaweeds. IJCPR; 7(1): 34-42.
9. Papenfuss, G.F. (1968). A history, catalogue, and bibliography of Red Sea benthic algae. *Israel J Bot*; 17: 1-118.
10. Cribb, A. B., (1983): Marine algae of the southern Great Barrier Reef, Part I. Rhodophyta. Australia Coral Reef Society.
11. Russell, D.J. (1992). The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Bøerg. and *Hypnea musciformis* (Wulfen) J.Ag., and their association with two native species, *Laurencia nidifica* and *Hypnea cervicornis*. J.Ag. ICES Mar. Sci. Symp., 194: 110-125.
12. 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.
13. Lichtenthaler, H. K. (1987): Chlorophyll and cartenoids pigments of photosynthetic biomembranes. Methods Enzymols; 148:350-282.
14. Lowery,O.H., Rosebrough, N.J.,Farr,A.L. and Randall,R.J.(1951): Protein measurement with the folin reagent.J.Biol. Chem. 193:265-275.
15. Daniel, H.D. and George, C.M.(1972): Peach seed dormancy in relation to endogenous inhibitors and

applied growth substance. J. Amer. Soc. Hort. Sci.97:651-654.
16. Bates, L.S., Waldren, R.P. and Teare, I.D. (1973): Rapid determination of free proline for water stress studies plant and soil.39:205-207.
17. Chunhui Deng, Xiangmin Zhang, Jie Zhang Ji ,Qian Weimin Zhu(2003): Rapid Determination of Salicylic Acid in Plant Materials by Gas Chromatography–Mass Spectrometry. Chromatographia. Volume 58, Issue 3, pp 225–229.
18. Mukherjee, SP and Choudhuri,MA (1983):Implication of water stress-induced changes in level of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings Physiol. Plant.58:166-170.
19. Bergymeyer, H.U. (1974): Methods of enzymatic analysis.1- second edition Acadimic press. New York.
20. Matta, A. and Dimond, A.E. (1963): Symptoms of Fusarium wilt in relation to quality of fungus and enzyme activity in tomato stems. Phytopathol, 53:574-575.
21. Marklund, S and Marklund, G (1974): Involvement of superoxide Anion Radical in Autoxidation of Pyrolgallol and convenient Assay for Superoxide Dismatase. Eur. J. Biochem.47:469-474.
22. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T_4 Nature, 227: 680-685.
23. Studier, F.W. (1973): Analysis of bacteriophage T_7early Rana and protein of Slab gels. Molecular. Boil.79:237-248.
24. Shahwan, S.M. Eman. (2010): Inducing systemic resistance against some tomato virus diseases Ph. D. thesis: Fac. Agric Moshtohor Banha Univ. pp 175.
25. Gora, T.A. (2014). Studies cn some viruses affecting pome fruits in Egypt. Ph. D Thesis. Fac. Agric. Benda Univ . pp220.
26. EL Dougdoug K.H. and Eskander, A.L.(2012). Active acquired resistance (immunity) stimulated by localized viral infections in *N. glutinosa and D.metal*. 4th International Conference towards Strategy for controlling viral pathogens. November 14-16 Sharm El Sheikh Egypt (Abstract, No.50).
27. Guo, A.L., Salih, G. and KLessig. D.F. (2000). Activation of a diverse to TMV is independent of salicylic acid induction of a subset is also ethylene independent. Plant J.21: 409-418.
28. Ping,L. and Boland, M.(2004). Signals From the underground: bacterial volatiles promote growth in Arabidopsis. Trends in plant séance 9: 263-266.
29. Elbadry, M.; Taha, R.M.; EL Dougdoug, K.A and Gamal EL Dlin, H.(2006) .Induction of systemic resistance in Fabae bean (vicia faba L) to *Bean yellow mosaic poly virus (BYMV*) via seed bactorization with plant growth promoting rhizobacteria- journal of plant Disease and protection, 113 (6),247- 251.
30. Ksalami, K., and Suprapta, D.N. (2011). Induction of plant resistance against *Soybean stunt virus* using some formulations of *Pseudomonas aeruginosa*. Int. Soc. Southe.Asian Agric. Sci. 1,98-105.
31. Ghobvial, W. U. 1. ; Ahlam, A. ; Mehesen,1.; Jehan, M.Abass.; shalaby .M.E and Omar. A.F.(2009) : potential impacts of *Rhizobium* and compost tea enriched with Rhizobacteria for enhancing protection of fabae bean against *Broad bean mottle virus (BBMV*) . J. Agric. Res. Kafer EL-Sheikh. Univ.; 35.
32. Galal, A.M.M.(2006) : Induction of systemic acquired resistance in cumber plant ayainst *Cucumber mosaic Cucumovirus* by local Streptomyces strains. plant pathology journal Faisalabad, 5 (3) : 343- 349.
33. Mohamed, E.F, Azza, G. Farag, Osman, T.A.M. and Eman, A.A. (2012). Histo- pathological change in leaves cells of squash plants infected with *squash leaf curl begomovirus* (SqLCV). Rep Opinion 4(5): 65-75.
34. Radwan, D.E.M.; Faye2, A.K.; Mahmoud, Y.S. and Lu. G.(2010). Modifications in antioxidant activity and protein composition of bean leaf due to *Bean yellow mosaic virus* mention and SA treatments- Acta physiologde plantarum 32:315- 325.
35. Chen, C.; Qiu, D.W.; Lin, Z.; Yang , X.F. and Cao, K.Q. (2006) : inhibition of RNA replication and coat protein synthesis in *Tobacco mosaic virus* by plant activator protein- Chinese Journal of Biological Control, 22 (1): 63- 66.
36. Walter, D.; Newton, A. and Lyon, G.D. (2007): Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection. 1st. ed., Blackwell Publishing Ltd, Oxford, UK, 272pp.
37. Mondal, S. and Mitva, A. (2007): Reinforcement of cell wall in roots of *Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. physiology and Molecular plant pathology , 71: 201- 200.
38. Mazid, M.; Khan, T. A. and Mohammad, F. (2011) . Role of secondary metabolites in defense mechanisms of plants . Biology and Medicine, 3(2): 232-249.
39. Chen, C. and Dickman, M.B. (2005): proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii* . plant Pathology 102 (9) : 3459-3464.
40. Harish, S., Kavino, M.; Kumar, N.; Balosubramanian, P. and Samiyappan, R.(2009). Induction of defense related proteins by mixtures of plant growth promoting endophytic bacteria against *Banana bunchy top virus* .Biological control, 51:16-25.
41. Peraz-de- luque.A., Gonzalez- Verdejo,C-L.; Lozano, M.D., Dita. M.A.; Cubero, J.L., Gonzalez- Melinda P.; Risueno, M- C. and Rubiales, D. (2006). Protein cross- linking , peroxidase and B- 1, 3 – endoglucanase involved in resistance of pea against *Orobanche crenata.* Journal of Experimental Botany, 57:1461-1469.
42. Nafie, E. and Mazen, M.(2008). Chemical induced resistance against brown stem rot in soybean: The effect of benzothiadiazol . Journal of Applied Science Research. 4: 2046- 2064.
43. Kazan,K., and Manners, J.M. (2009). Linking development to defense auxin in plant- pathogen interactions. Trends Plant Sci. 14: 373-382.

44. Katoch, R., Mann, A.P. S. and Sohal, B.S. (2005). Enhanced enzyme activities and induction of acquired resistance in pea with elicitors. Journal of vegetation science, 11:67-83.
45. Radwan, D.E. M.; Fayez, A.K.; Mahmud, S.Y.; Harmed, A. and lu, G. (2007). Physiological and metabolic changes of *Cucunbita pepo* leaves in response to *zucchini yellow mosaic virus* (ZYMV) infection and salicylic acid treatments. Plant Physiol. Biochem.; 45:480- 489.