Characterization of *Strawberry latent ringspot virus* (SLRSV) on strawberry in Egypt

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ABSTRACT

Strawberry latent ringspot virus (SLRSV) was isolated from symptomless strawberry plants and identified with a specific antiserum (Loewe Biochemica GmbH) using Double Antibody Sandwich ELISA (DAS-ELISA). Virus survey was carried out during 2013 - 2014 in different locations on commercial strawberry fields. The percentages of infection were 3.7, 4.5, 15.7 and 20% in El-Behera, El-Kalubeia, El-Ismalia and El-Menofia respectively. SLRSV was transmitted either by *Xiphinema americanum*as nematode vector or mechanically from infected strawberry plants onto 16 host species belonging to seven families. Typical leaf curling, chlorosis, vein clearing and stunting were appeared on indicator host (cucumber) 3 weeks post inoculation. Stability experiments of SLRSV showed that the thermal inactivation point was 48-62°C; the dilution end point was 10^{-4} to 10^{-6} and the longevity in vitro 10 - 18 days at room temperature. The diagnosis of SLRSV in the infected tissues of strawberry plants was performed using both serological (DAS-ELISA) and molecular assays. Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify 497 bp fragment using PCR primers specific for the viral coat protein gene as a tool for molecular diagnosis. The PCR detection was confirmed with direct DNA sequencing and phylogenetic analysis for the coat protein gene. Further insurance of SLRSV infection was performed using light microscopy which showed presence of amorphous inclusion bodies, electron microscopy and chemical analysis.

Key words: Survey, antiserum, DAS-ELISA, RT-PCR, and microscopy.

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) has been widely cultivated in Egypt. It is one of the most important vegetable crops for local consumption and exportation. Strawberries contains high levels of antioxidant compounds, which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease in addition to a number of other health benefits (Ames *et al*, 1993: Wang *et al*, 1996). Strawberry production is increasing annually, the world production exceeding 4 million tons, virus diseases of strawberry are also increasing as the crop is planted in new regions and exposed to new viruses (Ioannis et al, 2013). The total world area (241109 Ha) and total world production of strawberries about (4516810) per ton. Egypt Occupies the fourth position of strawberries production after USA, Turkey and Spain. The total area of Strawberries orchards in Egypt are (13999.2) feddans with an approximate yearly production (242297) per ton according to the statistics of (FAO, 2012). *Strawberry*

latent ringspot virus (SLRSV), was first identified more than 40 years ago (Lister, 1964), has been listed as quarantine past in the United States prior to 2004, SLRSV has a host range that exceeds 125 plant species belonging to 27 families of both monocots and dicots (Schmelzer, 1969 and Murant, 1974). The virus occurs naturally in many species of wild and cultivated plants and infects, often symptomless, a wide range of commonly used herbaceous test plants;SLRSVwas isolated from leaves and flowers parts, can be transmitted with plant sap by mechanical inoculation, SLRSV are efficiently transmitted by members of the nematode genera *Xiphinema*and *Longidorus*, as well as via pollen and seed. Both adults and larvae transmit, and virus may be retained for up to 84 days in the vector kept without plants(Murant, 1974), (Lister, 1964; Lamberti *et al*., 1986). The strawberry nematode-transmitted viruses have wide host ranges and can cause significant losses in the crop especially when present in mixed infections with other viruses. (Stace-Smith, 1970; Milkus, 2001). There are about 30 virus species affecting Strawberry (Martin *et al*.2006). SLRSV was first record in Egypt (2001), Italy (1979), Portugal (1990), Spain (1998), USA (2001), Turkey (2004), Lebanon (2005), Syria (2005), Croatia (2007), Tunisia (2009), and Albania (2009) (Giovanni,2013). SLRSV is a single stranded RNA, isometric particles of ~ 30 nm in diameter (Faggioli *et al*., 2002), among molecular methods, RT-PCR has proved to be the most rapid, sensitive and reliable technique for detecting RNA viruses in infected plants (Hadidi and Candresse, 2001). Thus, the use of PCR technology is an important step to optimize and speed up strawberry virus diagnosis. The aims of this study were to isolate and identify the occurrence of *Strawberry latent ringspot virus* (SLRSV–Egyptian isolate) through biological, serological and molecular studies.

MATERIAL AND METHODS

Source of virus isolate and filed survey:

Filed survey for viral infection was carried out during 2013 – 2014 in spring and early summer on strawberry fields to determined SLRSV occurrence using Double-antibody sandwich enzyme-linked immune-sorbent assay (DAS – ELISA) using SLRSV specific polyclonal antibodies. Samples were collected randomly from several commercial strawberry fields in four governorates [El-Kalubia, El-Behera, El-Monofia and El-Ismailia governorates]. The samples which reacted positively with the SLRSV antiserum were used for virus isolation and identification by biological reaction on herbaceous hosts. Different serological and molecular techniques were also applied.

Isolation and propagation of virus isolate:

The naturally infected plants which gave positive results with DAS-ELISA were used as a SLRSV source. The virus isolate was inoculated on leaves of *C. quinoa* plants using mechanical inoculation. Single local lesions were then propagated on *Cucumissativus* by mechanical inoculation on three cycles of purification were repeated (Tang *et al.,* 2013). Isolated virus was confirmed by studying host range, symptomatology, mode of transmission and stability. Also, viruses were insured by serological, molecular and histological studies.

Host range and Mechanical transmission

Sixteen species and varieties belonging to families (*Rosaceae, Solanaeae, Cucurbitaceae, Chenopodiaceae, Fabaceae, Lamiaceae* and *Poaceae*), were mechanically inoculated with the virus isolate by grinding in[0.05M phosphate buffer (pH7.0), nicotinic acid 0.02%] and the sap was clarified through two layer of cheesecloth; an equal number of healthy plants of the same age and cultivar were rubbed with buffer and kept to serve as control. Indicator plants and control were maintained in darkness for 16 to 24 h before inoculation and dusted with number 600 carborundum as an abrasiveprior to inoculation. Four to six leaves were inoculated per plant, and the inoculated leaves were briefly rinsed with cold water after inoculation. Plants were examined daily up to 35 days for symptoms development under greenhouse conditions.Plants showed no symptoms were checked by back inoculation to the indicator hosts; results were confirmed by DAS–ELISA.

Nematode transmission

The procedures used were those described by (Brown and Trudgill, 1983). Experiments were done with 25 cm^3 plastic pots maintained in temperature controlled cabinets and with three-week-old seedlings of *C. quinoa* used as manually infected sources of SLRSV. Groups' virus-free nematodes, mainly adults and fourth-stage juveniles, were given access to these virus-source plants for four weeks. They were then extracted, counted and in groups of two or five placed in clean 25 cm^3 pots in which were planted three *C. quinoa* virus free bait plants. After four weeks the nematodes were extracted and counted. The roots of the virus-source and bait plants were washed to remove any nematodes and/or virus that may have become entangled with orbed adhering to the roots. Root galls, indicative of nematode feeding, were counted and the roots tested for virus by comminuting them and rubbing the resultant suspension onto the leaves of *C. quinoa* assay plants. The aerial parts of the bait plants were frozen (-20') and some of those in which virus had been detected in the roots were subsequently tested for systemically trans-located virus. Virus from some of the *C. quinoa* assay plants was used in serological tests to confirm its identity. *C. quinoa* was compared as virus source and as bait plants using the procedures described above with SLRSV and groups *X.americanum*. After four weeks they were extracted, counted and discarded; the root galls were counted and approximately half of each bait plant root system was excised and the plant transferred to a clean 25 cm^3 pot. The excised portion of root was tested for the presence of virus and those bait plants which had virus detected in the excised portion of their roots were used as virus-source plants in the subsequent test. This procedure was repeated until virus was not detected in any of the bait plants used with *X.americanum* (Gibbs and Gower, 1960).

Stability of the virus

Thermal end point (TEP), Dilution end point (DEP) and Longevity *in vitro* (LIV) of the virus isolate were determined according to the technique described by Noordam (1971). Using infectious crude sap obtained from infected strawberry plants and then performed on healthy *C.quinoa* as an indicator host plant for SLRSV infection according to (Schmelzer, 1969). Each experiment was repeated twice.

Enzyme-linked immune sorbent assay

Strawberry latent ringspot virus (SLRSV) were tested using (DAS – ELISA) technique demonstrated by Clark and Adams (1977), was performed on selected symptomatic herbaceous indicator plants using commercial polyclonal antiserum raised against SLRSV (Loewe Biochemical GmbH), according to the manufacturer's instructions.Healthy leaves of each species were used as negative controls. Absorbance was measured at 405 nm on a Bio-Rad M-680 micro-plate spectrophotometer (Bio-Rad Laboratories Inc.). A sample was interpreted as positive when the absorbance value was more than three times the value of the negative control.

Molecular Detection of SLRV

Nucleic acid extraction and RT-PCR

Total RNA was extracted from infected cucumber plants as a propagative host for the virus using gene jet ™ RNA purification kit (Fermentas, USA). The extracted RNA was used as a template for one tube RT-PCR amplification reaction using Verso TM one step RT- PCR kit (Thermo scientific) utilizing specific primers for the SLRSV **SLRSV-F: 5`**-CCT CTC CAA CCT GCT AGA CT-3` and **SLRSV-R: 5`**-AAG CGC ATG AAG GTG TAA CT-3`.RT-PCR was performed in 25 µl total volume containing 4.75 µl of nuclease - free water, 3ng/µl of total RNA, 12.5 µl of one step PCR master mix,3 µl of 10 µM of each primer,0.5 µl Verso enzyme mix and 1.25 µl RT-Enhancer. RT reaction started with incubation at 50 C for 15 min, followed by denaturation at 95C for 5min. The amplification reaction was performed through 35 cycles in T-Gradient thermal cycler (Biometra, Germany) starting with denaturation at 94ċ for 30 sec, primer annealing at 52ċ for 30 sec and extension at 72ċ for 2 min. Final extension at the end of the 35th cycle was performed at 72ċ for7 minutes. The PCR products were stained with gel star (Lonza, USA) and analyzed by electrophoresis in 1.0% agarose gel and visualized by UV illumination using Gel Documentation System (Gel Doc 2000, Bio-Rad, USA).

Nucleotide sequencing:

The PCR fragment of the partial coat protein gene for the SLRSV was gel extracted using QIAquick Gel Extraction Kit from QIAGEN. The purified PCR product was directly sequenced using Automated DNA sequencing. The forward and the reverse primers (SLRSV-F and SLRSV-R) those used for PCR were also used for DNA sequencing. The nucleotide sequence was analyzed using DNAMAN Sequence Analysis Software (Lynnon BioSoft. Quebec, Canada) and compared with the coat protein sequences of the SLRSV isolates available in GenBank.

Histo-pathological studies

Light microscopy of inclusion bodies

Strawberry latent ringspot virus isolate was transmitted by inoculation of sap to *visia faba* plants growing under greenhouse conditions, healthy and infected *visia faba* leaves were collected at the same age of two weeks from infection to examine amorphous inclusion bodies under light microscopy, the method described by (Christie and Edwardson, 1986), epidermal strips taken from the lower

surface of systematically infected *visia faba* leaves were treated with 5% triton X-100 for 10 minutes to disrupt the plastids and facilitate the observation of the inclusions. Then, stained for 15 min [immersing in 100mg bromophenol blue and 10g mercuric chloride in 100 ml distilled water], then placedin 0.5% acetic acid for 15 min and mounted in water. Finally, the strips were examined by light microscope to detect the protein contents of inclusion bodies.

Electron microscopy (Ultrathin section of virus-infected tissues)

Ultra-structure of SLRSV-infected *C. quinoa* leaf cells was investigated to detect the effect of viral infection. Small pieces of leaves of both healthy and infected plants were cut with razor blade to 1x1mm sections, then fixed in 3% cold 0.1M phosphate buffer glutaraldehyde pH7 for 6h, the pieces were further treated as described earlier (Roland, 1978; Verma *et al.,* 2003). The ultrathin sections were examined with SEO (Sumy Electron Optics) TEM-100 at the Electron microscopy unit, Military Veterinary Hospital.

Chemical constituents changes of strawberry fruits in response to viral infection:

Determination of total carbohydrates (mg/100gm dry weight). Total carbohydrates were determined according to Dubois *et al.,* (1956). The outcome data was expressed as mg/100gm dry weight.

Determination of fruit pigments (mg/100gm of fresh matter). The fruit anthocyanin was calorimetrically determined in fruit samples (mg/100gm of fresh matter) according to Hsia et al., (1965). Chlorophyll –a; Chlorophyll – b and total carotenoids were colourmetrically determined in fruit samples (mg/100gm of fresh matter) according to Saric *et al.,* (1976). The chlorophyll content and the carotenoids pigments were calculated using the following equations (as µg/ml):

Chl a = 9.784* E 662 − 0.99* E644 =X1

Chl b = 21.426*E 644 –4.65 * E662 =X2

Carotenoids = 4.695* E440– 0.268 (X1 + X2)

E: Reading on the device by nm

X1: value of Chla; X2:value of Chl b

The Content: (mg/100gm of fresh matter)

$$\text{The Content} = \frac{\text{X} * \text{volume of alcohol}}{\text{Sample WT.(mg)}}$$

Determination of vitamin C in fruit as gm/100gm. Vitamin C as mg ascorbic acid was determined and estimated per 100 ml fruit juice, according to the method cited in A.O.A.C. (1990).

Determination of the total indoles and total phenols in fruits as (mg/100gm of fresh matter). Extraction of indolic and phenolic compounds was conducted according to the method described by Daniel and George (1972).

Determination of fruit mineral content (gm/100gm Dry Wt.). Half gram of the dried samples was digested using the H_2SO_4 and H_2O_2 as described by Cottenie (1980), the extracted samples were used to determine the following minerals: Nitrogen (N^{+2}) content was determined in the digested solution by the modified micro-kjeldahl method as described by (Plummer, 1971). Potassium (K^{+}) content was determined by flame-photometer (Piper, 1950). Iron (Fe^{+3}) and calcium (Ca^{+2}) these elements were determined by using Atomic Absorption Spectrophotometer, Pyeunican SP1900, according to Brandifeld and Spincer (1965).

Determination of Acidity % (gm/gm fresh Wt.). Total acidity as of anhydrous citric acid was determined and estimated

per 100 ml fruit juice, according to A.O.A.C. (1990).

Determination of total soluble sugar (gm/100gm of fresh Wt.). Soluble sugars were determined in the fruit ethanolic extract. Colorimetric determinations of total soluble sugars expressed as glucose were determined calorimetrically according to the method of Dubois et al. (1956).

Estimation of total flavonoids (mg/100 mg of fresh Wt.). The total flavonoids (antioxidants), was determined in the fruit ethanolic extract by spectrophotometer using Djeridane et al. (2006).

RESULTS

Source of virus isolate and filed survey

Survey was carried out during 2013 –2014, in spite of the absence of visible symptoms; virus was detected using DAS- ELISA on random samples. Data presented in Table (1) indicated that the percent of infection were 3.7, 4.5, 15.7 and 20% in El-Behera, El-Kalubeia, El-Ismalia and El-Menofia respectively in strawberry fields. These results indicated that the dissemination of the virus was varied according to locations and distributed in the country in different levels.

Host range and Mechanical transmission

Results presented in Table (2) showed that the tested plant species varied in their response to viral infection. Some plant species didn't develop any symptoms (*Fragaria ananassa, Solanum lycopersicum, Solanummelongena, Petuniahybrid, Triticumaestivum and Hordeummurinum*), and other showed different symptoms on the different plant species when inoculated mechanically with SLRSV as shown in Table (2) and Fig. (1), to determine the host range and differential hosts. SLRSV infection produced systemic local lesion on the inculcated leave of *C. amranticolor* Cost & Reyng and *C.quinoa*, systemic leaf crinkles on the inculcated leave of *C.quinoa* wild, vein clearing on *Cucumissativus*L. *cv Beita alpha*, mosaic on the inculcated leave of *visiafaba*and *Nicotianatabacum* (white burley),mosaic and leaf malformation on the inculcated leave of *Phaseolusvulgaris*,mottling on the inoculated leave of *Capsicum annuum*and leaf blotches on *Nicotianatabacum* L. results were confirmed by ELISAtests. The symptoms were observed 10 – 20 days from infection. ELISA tests failed to detect SLRSV from these hosts (*Solanum lycopersicum, Solanummelongena, Petuniahybrid, Triticumaestivum and Hordeummurinum*). The obtained data reviled that SLRSV Egyptian isolate had limited host range, these results agreed with Lister (1964) he reported that SLRSV infects restricted species in and outside family *Rosaceae*.

Nematode transmission

Xiphinemaamericanum was able to transmit SLRSV from infected strawberry and *Ch. Amaranticolor* to Strawberry seedlings (100 %). These results also agree with those of (Hancock and James, 2008).

Stability of the virus

Thermal end point (TEP), Dilution end point (DEP) and Longevity *in vitro* (LIV) were determined separately for SLRSV. Results showed that (TIP) was between 48-62˚C, DEP was 10^{-4} to $10^{-6}$and LIV was 10 - 18 days at room temperature. Virus stability of SLRSV was studied using *C.quinoa.* These results are in harmony with Lister (1964)and Schmelzer (1969) on *C. quinoa*, Tomlinson and Walkey (1967) on rhubarb and Richter & Kegler (1967)

on peach but survived only up to 7 days at room temperature.

Enzyme-linked immune sorbent assay

Leaf samples of the symptomatic local lesion on *C. amaranticolor* Cos t& Reyng, *C. quinoa,*systemic leaf crinkles on *C.quinoa* wild, vein clearing on *Cucumissativus*L. *cv Beita alpha,* mosaic on *visiafaba*and *Nicotianatabacum* (white burley), mottling on *capsicum annuum*and leaf blotches on *Nicotianatabacum* L. plants were tested for the presence of SLRSV using ELISA. These herbaceous species inoculated from samples listed in Table (2) tested positive for SLRSV. No reactions were observed for healthy controls of each species.

Table (1): Survey of *Strawberry latent ringspot virus* (SLRSV) years 2013-2014.

Location	El-Behera			El-Qalubia			El-Ismalia			El- Monofia			Total		
Year	Tested	Infected	Percent of Infection %	Tested	Infected	Percent of Infection %	Tested	Infected	Percent of Infection %	Tested	Infected	Percent of Infection %	Tested	infected	Percent of Infection %
2013	250	15	6.0%	300	16	5.3%	235	35	14.9%	300	68	23%	1085	134	12.4
2014	290	5	1.7%	280	10	3.6%	244	40	16.4%	200	32	16%	1014	87	8.6
Total	**540**	**20**	**3.7%**	**580**	**26**	**4.5%**	**479**	**75**	**15.7%**	**500**	**100**	**20%**	**2099**	**221**	**10.5**

Table (2): Host range, symptoms, incubation period and ELISA tests of *Strawberry latent ring spot virus* (SLRSV) after mechanical inoculation.

Host range	Symptoms/ Infectivity	Incubation period(Days)	ELISA Test
Family Rosaceae			
Fragaria ananassa	**Ns (9/10)**	**20**	+
FamilyChenopodiaceae:			
C.quinoa	**SLL(7/10)**	**14**	+
C.amranticolor	**SLL(9/10)**	**14**	+
C. quinoa wild	**SLC(4/10)**	**10**	+
FamilyCucurbitaceae:			
Cucumis sativus	**VC(6/10)**	**17**	+
FamilyFabaceae:			
Phaseolus vulgaris	**M , LM(8/10)**	**15**	+
Vicia faba	**M(4/10)**	**14**	+
Family Lamiaceae			
Mentha spicata	**YVB(2/10)**	**20**	+
FamilySolanaceae:			
Capsicum annuum	**MO(6/10)**	**17**	+
Solanum lycopersicum	**Ns**	**20**	-
Solanum melongena	**Ns**	**20**	-
Nicotiana tabacum(white Burley)	**M(7/10)**	**20**	+
Nicotiana tabacum L	**LB(8/10)**	**19**	+
Petunia hybrida	**Ns**	**20**	-
FamilyPoaceae			
Triticum aestivum	**Ns**	**20**	-
Hordeum murinum	**Ns**	**20**	-

SLL= systemic local lesion, SLC= systemic local crinkle, VC=Vein clearing, M=Mosaic, LM=leaf malformation, MO=Mottling, LB = leaf blotch, YVB= Yellow vein banding, Ns=no symptoms. + = Positive ELISA and - = Negative ELISA

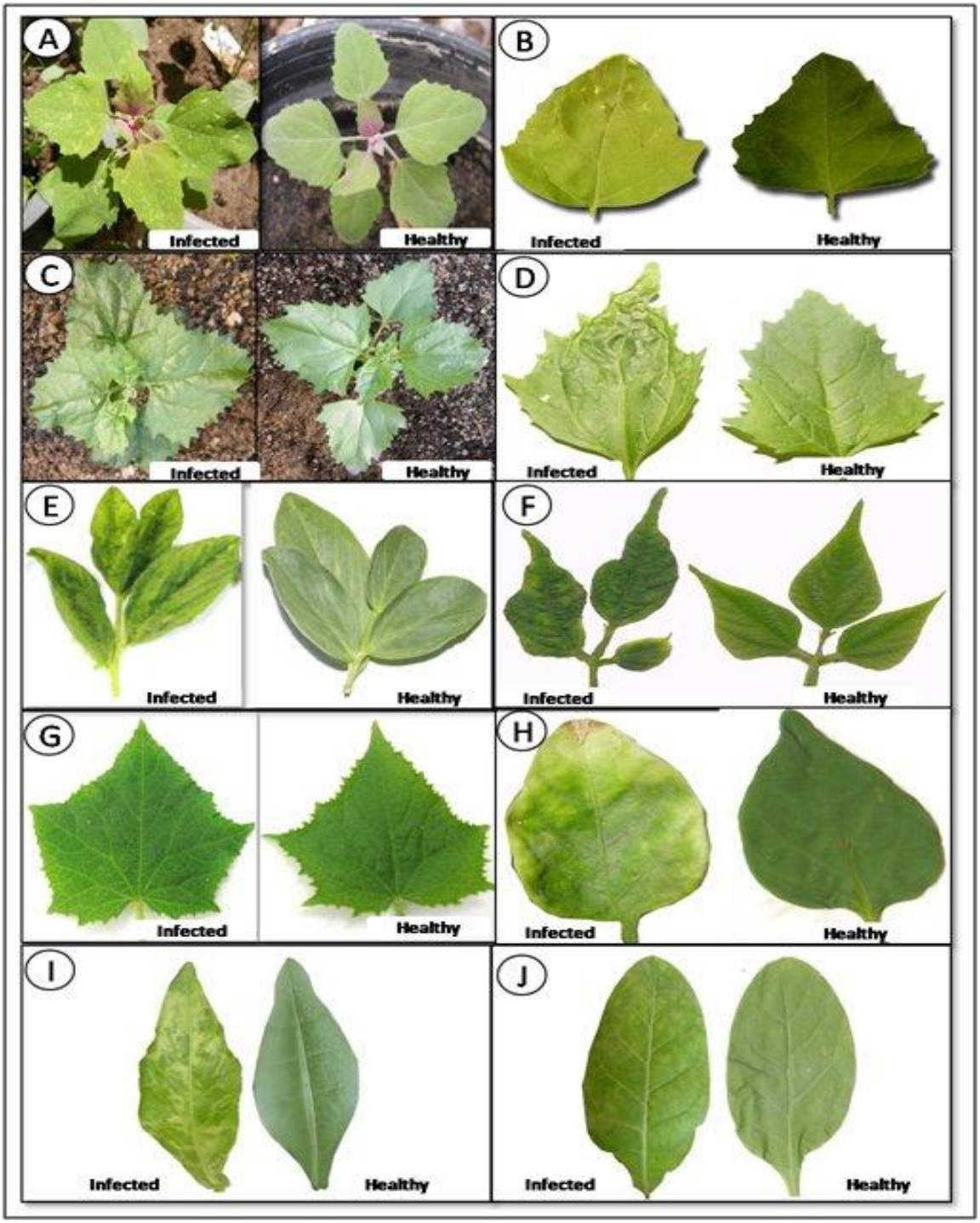


Fig. (1): Symptoms on some susceptible host to *Strawberry latent ringspot virus* (SLRSV). A and B: *Chenopodium amranticolor* Cost & Reyng and *C.quinoa* showing systemic local lesion. **C**: *C.quinoa* wild showing systemic leaf crinkles. **E** and **J**: *visiafaba*and *Nicotianatabacum* (white burley) showing mosaic. **F**: *Phaseolus vulgaris*showing mosaic and leaf malformation. **G**: *Cucumissativus* L. *cv Beita alpha* showing vein clearing. **H**: *Capsicum annuum*showing mottling. **I**: *Nicotianatabacum* L. showing leaf blotches.

RT-PCR

The total RNA isolated from each infected sample was used as a template for the one step RT-PCR amplification. Electrophoresis analysis of RT-PCR product showed a single amplified fragment at 473 bp Fig. (2).

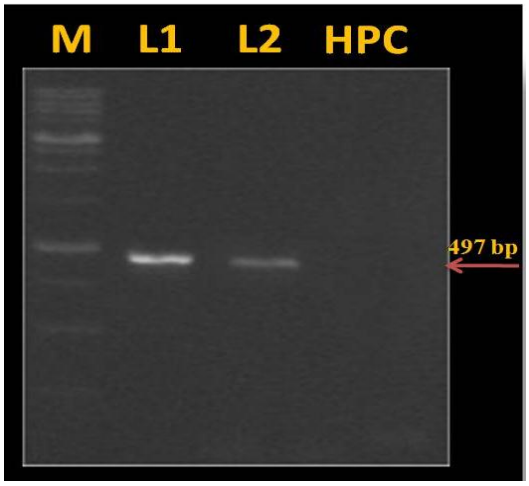


Fig. (2):Electrophoresis analysis for the RT-PCR detection of SLRV in infected cucumber samples. L1 and L2: different samples; L3 Healthy plant control and M: 100 bp DNA ladder.

Sequence analysis:

The purified RT-PCR fragment representing a partial coat protein gene of SLRV was sequenced and compared with corresponding sequences of other isolates of SLRV available in GenBank. The genetic code was translated into the equivalent amino acid using the standard universal code and shown in fig (5a). The obtained data were assembled using DNAMAN software. Multiple sequence alignment of the nucleotide sequence of the partial coat protein gene (Egyptian Isolate) was done with the corresponding sequences of the following SLRV isolates available in GenBank:

DQ324376- isolate Gorz 2, HM591195- New Zealand, AY438666, AY461735 Canada, HQ456535 - isolate Mint 454, DQ324375.1- isolate W and DQ324374 - isolate N 31. Sequence analysis and the phylogenetic tree among the DNA nucleotide sequences showed range of 80 to 85 % similarity with the different SLRV isolates in the GenBank Fig (5b).

Histo-pathological studies

Light microscopy of inclusion bodies

Amorphous cytoplasmic inclusion bodies have been seen near the nucleus induced by SLRSV as evidence of the viral infection which observed with light microscopy in infected epidermal strips of *visia faba* two weeks after inoculation; whereas these inclusions were not observed in healthy *visia faba* leaves as shown in (Fig.3).

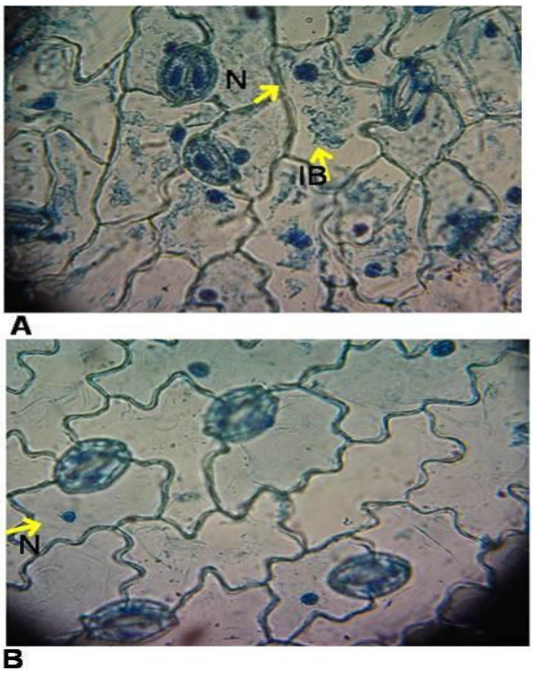


Fig. (3): A. Transverse sections in infected plant showing granular structures amorphous inclusion bodies near the nucleus **B**: Transverse sections in healthy plant showing Stomata and clear cell with Nucleus (N) only**.**

Inclusion bodies play an important role in diagnosis of plant viruses especially in preliminary studies(Christie and Edwardson, 1986).These results were in agreement with those reported by (Roberts and Harrison, 1970).

Ultrathin section of virus-infected tissues

Electron microscope examination of ultrathin section of healthy and infected *C.quinoa*leaf with SLRSV was done. Ultrathin section of healthy samples showed normal cell wall, nucleus, mitochondria and chloroplast. The chloroplasts were ellipsoidal, elongated with numerous thylakoids having a good granular organization .rare plastoglobuli, small starch little grains. However infected samples showed that infected cells contain virus particles in cytoplasmic tubules (Fig. 4). Where the plasmodesmata and vacuoles had no morphological change in the cellular organization of meristemic cells. On the other hand extensive changes have been observed in infected mesophyll cells including severe degeneration of chloroplast structure and development of cell wall protrusions and the mitochondria appeared to be round than normal and .These results in harmony with (Robert et al, 1970; Joneset al, 1973 and Kheder et al., 2004).They reported that nepovirus-infected cells often contained virus particles in cytoplasmic tubules and no morphological changes have been reported in the cellular organization of meristemic cells. Including severe modification of chloroplast and mitochondria structure.

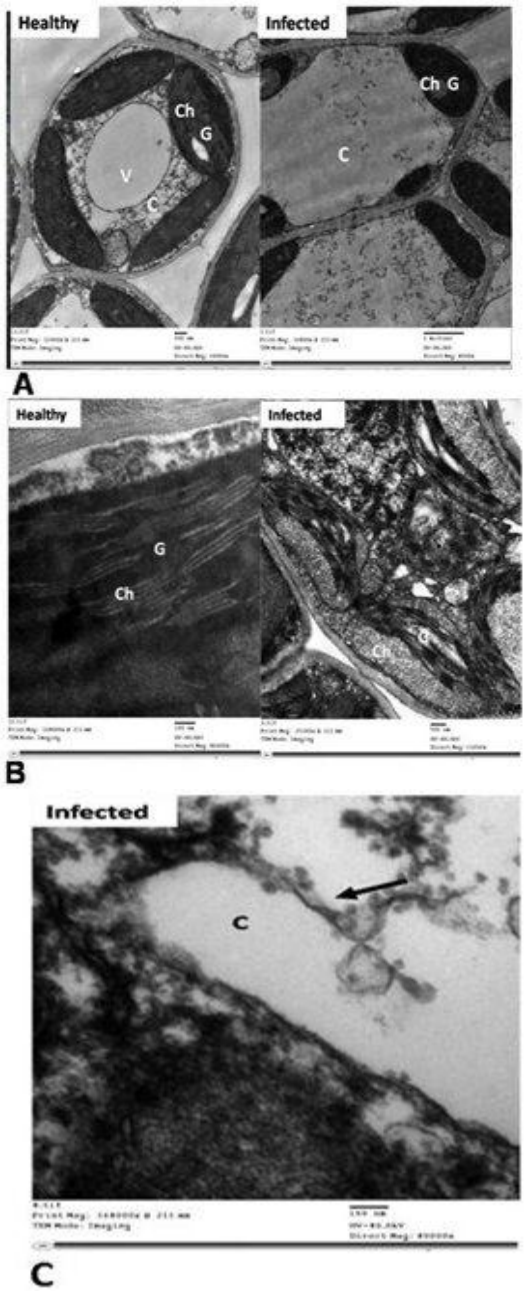


Fig. (4) **A**: Ultrathin sections of infected leaves showing sever deformation in cytoplasm structure Ch = Chloroplast, G = grana V = Vacuoles and C = Cytoplasm. **B**: sever modification of chloroplast structure. **C**: Infected cells contain virus particles in tubules scattered in the cytoplasm or associated with plasmodesmata which they often penetrate.

Effect of viral infection on fruit-organic contents:

The results showed that the changes of total carbohydrate in strawberry fruit infected with SLRSV was amounted 11.534 mg/mg fresh wt. this results indicate that a notable decrease in total carbohydrates contents (14.855 mg/mg fresh wt.) by 23%. Data represented in Table (1), reviled that, the virus infection decreased the chlorophyll a, chlorophyll b, compared with the control plant. While, carotenoids and total Anthocyanin contents remarkably reduced by infection with SLRSV as compared with control healthy plant.Table (3), also showing significant reduction in vitamin C contents, by SLRSV infection.

On the other hand the data represented in Table (3), indicated that total Indol contents were greatly increase by infection with SLRSV as compared with control plant. This increase was estimated by 98.3%, in fruit compared to control plants. The same trend was observed in phenols where the phenols of the infected fruits measures 0.34031mg/g fresh wt. compared to 0.17825 mg/mg fresh wt. in control plant. The results outlined in Table (3), showed that the soluble sugars decreased in the ethanolic extract of the infected fruits. On the other hand, the flavonoids increased nearly double of its value.

Changes in Mineral contents and acidity

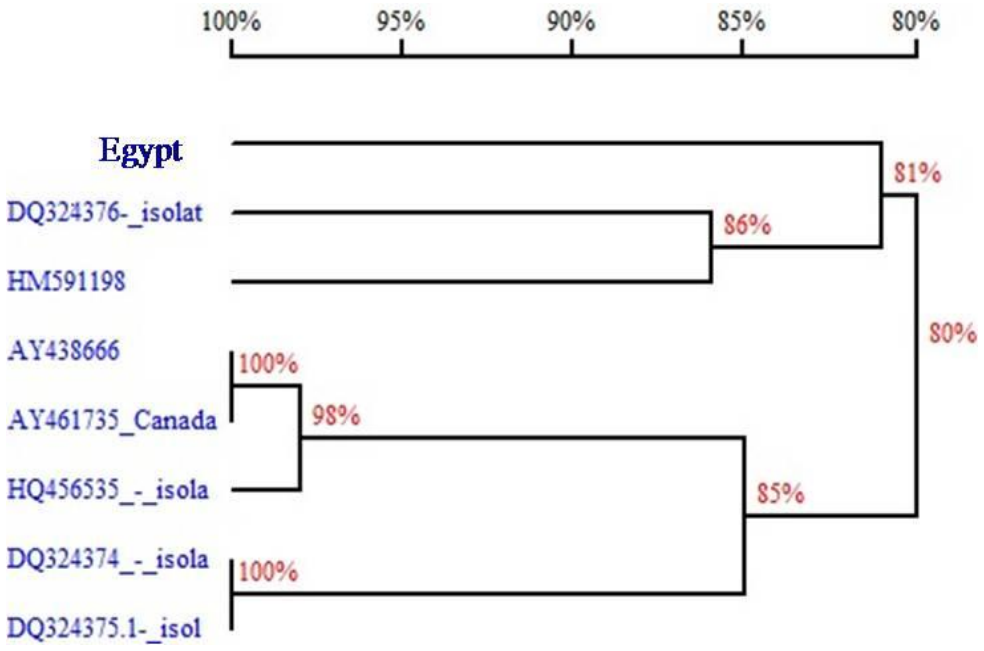
The results obtained (Table 3), showed decrease in the contents of inorganic cations (Ca^{++}, K^{+}, Fe^{+++} and N^{++}) in SLRSV infected strawberry fruit. The percentage of acidity in the fruit juice was measured as citric acid and showed a remarkable increase (Table 3). Finally, the phytochemistry of strawberry (Fragariaananassa Deuch.) received a great interest in the last decade among other fruits (Clark et al., 2002); due to its economic and commercial value. The present study was carried out to assess the effect of SLRSV infection on the strawberry fruit content especially the sugar, vitamens (Vit. C), indols and phenolics. The results showed a notable decrease in total carbohydrates contents in the fruits infected with SLRSV (Table 3). In addition to chlorophyll a, chlorophyll b, carotenoids and clear reduction in antioxidant namely, total anthocyanin and vitamin C contents, by SLRSV infection. This indicates that the SLRSV infection negatively affect the fruit postharvest quality preservation, this observation was recently reported by (Rekika et al., 2005), the clear reduction in anthocyanin content (Table 1), as the main antioxidant compound indicating the negative effect of this viral infection on the fruit quality as flavorants, colorants or antioxidant; these fruit uses were reported by (Maas et al., 1991).

The infected fruits showed remarkable increase in phenols, total flavonoids, indols and percentage of acidity in the fruit juice as citric acid (Table 3). This increase in the antioxidant compounds by the viral infection may be attributed to the role of these antioxidant molecules as defense mechanism to stress factors. Similar results were reported by Rogier et al., (1998), they claimed that the antioxidant compounds sustain the life and defense against external stresses such as fruit ripening and senescence. Also, Prusky (1996) reported that phenolic compounds play important role in the plant tissues against pathogen attack. The mineral composition (Table 3), showed decrease in SLRSV infected strawberry fruits; this indicates the

negative effect of the viral infection of the fruits as mineral source.

Table (3). Chemical composition of the fruits (control value between bracts): Values measured as mg/mg fresh wt. except minerals as mg/mg dry wt.

Sample constituent	Value (infected/ control)
Total Carbohydrate	11.534 (14.855)
Chl a	0.013 (0.035)
Chl b	0.007 (0.019)
Carotens	2.609 (2.686)
Anthocyanin	0.051 (0.092)
Vit. C	8.625 (12.246)
Phenols %	0.340 (0.178)
Indols	4.182 (2.407)
Calcium	0.748 (0.831)
Potassium	1.236 (1.409)
Ferric	102.150 (102.300)
Nitrogen	2.317 (2.703)
Acidity %	0.200 (0.010)
Total flavonoids	1.522 (0.803)
Total sugars	8.141 (10.559)



[A]

```
1          CCTCTCCAACCTGCTACACGTACGAGCAGAGCTTCTCATATGCCTATTCAGCGATCAGGA
1           P  L  Q  P  A  T  R  T  S  R  A  S  H  M  P  I  Q  R  S  G

61         GCGAAGACGCTTGTCACTTTTGAGGACAATCAGGCATCCCTGAAGGAGCAGCAACCTTTG
21          A  K  T  L  V  T  F  E  D  N  Q  A  S  L  K  E  Q  Q  P  L

121        CAGGCTCGTGCTTCTTTTTCTTTTTCTGGGGTTTCCTATGGGCAACAGGACCGTGCCGCT
41          Q  A  R  A  S  F  S  F  S  G  V  S  Y  G  Q  Q  D  R  A  A

181        CTTCCTTCAGCACCCTCCCCAAAATGTCCTTTTCATAATAGGGTGGGTTGTCCTTGTAAG
61          L  P  S  A  P  S  P  K  C  P  F  H  N  R  V  G  C  P  C  K

241        CAGCATACCGGTGAAGCGGAGCCGAGTGGGTTGCATGAAGACCTTGTCCCTGCGGCATCT
81          Q  H  T  G  E  A  E  P  S  G  L  H  E  D  L  V  P  A  A  S

301        GGTGGTACCGAAGCCATTTTCTTTTCACCCAAGAGCATTCCTGTTCCAGGTGGTGCTAAG
101         G  G  T  E  A  I  F  F  S  P  K  S  I  P  V  P  G  G  A  K

361        TTTGTTGGCTCTCACCCCTTTTCGTTTCCTATCAATAGCAATGTTGGTACCACGGTCTAC
121         F  V  G  S  H  P  F  S  F  P  I  N  S  N  V  G  T  T  V  Y

421        TGTCTACCTTTGATCAGTACCTCCTTGAAAGATACAGAGTGGGGAAGGTCTTGTACGACC
141         C  L  P  L  I  S  T  S  L  K  D  T  E  W  G  R  S  C  T  T

481        TATACCTTCATGCGCTT
161         Y  T  F  M  R
```

[B]

Fig. 5: [A] phylogenetic tree showing relationships among reported isolates of SLRV and the Egyptian isolate based on the nucleotide sequences of the partial CP gene. Horizontal distances indicate degree of relatedness. [B] The amino acid translations for the patial coat protein gene.

DISCUSSION

Strawberry latent ringspot virus (SLRSV) has been identified and characterized in the virus-diseased strawberry samples those were collected from different locations and governorates (see materials & Methods). This way can confirm, in future study, having Most of viral-genomic isolates to for further study to identify gene ontology (J. Dutkowski et al, 2013). Identification was done using different technologies i.e. a. Means of transmission (mechanical and nematode transmission), b. Serological analysis (ELISA) using PAbs specific for SLRSV, c. Molecular biology (RT-PCR) and both electron and optical microscope (Figs. 3, 4, 5 and 6). Moreover, host range (Fig. 2 and Table 1). Previous result data are confirmed by Tang J. et al, (2013), who has used the same technologies and got the same results. Moreover, confirmation of virus occurrence as external-symptom expression in correlation with serological analysis (Table 1) was also done. Tang J. et al, (2013) has made the same correlation between symptoms expression and RT-PCR analysis. More than virus-stability tests were carried out

for more confirmation on SLRSV identification (Materials & Methods part)

The RNA of the Egyptian isolate of SLRV was detected using RT-PCR according to the described procedure utilizing specific primers designed for a certain part of the coat protein gene. A PCR product of approximately 497 bp (Fig 2) was amplified from viral RNA extracted from infected leaves after converting it into cDNA through reverse transcription reaction that agreed with Tang J. et al, (2013). No bands were amplified from the healthy plant controls that reflected the sensitivity as well as the specificity of the PCR primers used in this study. To confirm the RT-PCR results as well as the incidence of the SLRV, molecular characterization of the amplified SLRV fragment was performed. The amplified coat protein gene fragment resulted from the RT-PCR was purified and prepared for DNA direct sequencing. The nucleotide sequencing of the SLRV partial CP gene was performed and the multiple sequence alignment was done to compare the sequence of 497 nucleotides of our findings with the corresponding sequences of different SLRV CP genes available in GenBank. The sequence of the partial coat protein gene was translated into 165 amino acids according to the standard universal code.

Sequence analysis and the phylogenetic tree among the DNA nucleotide sequence showed a very low identity that ranged between 80 to 85% when compared with the different SLRV isolates in the GenBank. This low identity may due to the use of the amplified PCR product directly for sequencing instead of cloning it. Also the sequence was performed in this study for a partial coat protein sequence only, instead of the complete gene sequence. Therefore, further studies, comprising cloning of the complete nucleotide sequence of the coat protein gene are recommended to find more accurate relationship between our isolate and the other available SLRV isolates.

SLRSV can infect naturally different hosts i.e. *Apium graveolens*, *Fragaria*, *Lilium*, *Prunus*, *Rosa*, *Rubus* and*Vitis vinifer*. Previous economical hosts, lead to study the negative effect of the virus as histopathological and internal deformation of plant-cell organelles (Figs 4, 5 and 6). Determination of chlorophyll A & B which indicate effect of virus on phtothynsis process (Table 3), all items shown in Table (3) were reduced except phenolic compounds. These results in harmony with (Robert *et al*, 1970; Jones *et al*, 1973 and Kheder *et al*., 2004).It also, indicates that nepovirus-infected cells often contained virus particles in cytoplasmic tubules and no morphological changes have been reported in the cellular organization of meristemic cells. Table (3) also shows SLRSV-infection negative effect on fruit postharvest quality preservation, this observation was recently reported by (Rekika et al., 2005). Deleterious effect of Identified SLRSV was shown in Table (3) and figures (2, 3,4, 5 and 6). The goal of this study has been able to get the work aim done and future study as well.

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