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RESEARCH

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Effect of the entomopathogenic fungus, *Beauveria bassiana* inhibiting whitefly transmission of squash leaf curl virus infecting squash

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Abstract

Background Squash leaf curl virus (SLCV) is efficiently transmitted and spread by the whitefly, *Bemisia tabaci* (Gennadius), which is the only vector that transmits *begomoviruses* naturally causing huge crop losses through feeding damage. The widespread use of chemical insecticides to control the whitefly *B. tabaci* has become extremely hazardous to the environment. Alternative methods such as biological control have been advocated. Entomopathogenic fungi (EPF) have been found as promising whitefly bio-pesticides.

Results Naturally infected squash plants that showed symptoms of squash leaf curl disease were collected from Giza Governorate, Egypt. SLCV was detected using a PCR assay using coat protein-specific primers and generated an amplicon of 419 bp. Multiple sequence alignment showed that the SLCV-Giza isolate has a significant identity of 99.2% with the SLCV-Mx:BCS: La Paz isolate from Mexico and 99% with the SLQV.Q2521 isolate from Egypt. Phylogenetic analysis showed that SLCV-Giza is closely related to the SLCV-Mx:BCS: La Paz isolate from Mexico. The whitefly transmission test revealed that the virus transmitted to an extent of 13.3% and reached 100% of transmission using 15–20 viruliferous whiteflies; while the efficiency of syringe injection was (60%). *B. tabaci* newly emerge adults were able to acquire and transmit SLCV after an Acquisition Access Period (AAP) of 15 and 30 min by low rates of 13.3 and 22.2%, respectively. The transmission rate was increased gradually to reach the maximum of 100% after 24, 48, and 72 h (AAP). *B. tabaci* was able to inoculate SLCV after an Inoculation Access Period (IAP) of 15 and 30 min with rates of 46.7 and 62.2%. The whitefly was allowed to acquire SLCV from a squash plant (virus source) treated previously with EPF (*Beauveria bassiana*) and allowed to transmit the virus to the test plants. The transmission effectiveness of viruliferous whitefly was lower (33.4%) than that of non-treated whitefly (100%). The transmission efficiency was decreased on the second day by 6.8% and by the third day by 2.2% of treatment with the EPF. The results were validated by PCR assay for SLCV from tested squash plants and the PCR did not reveal specific amplification.

Conclusions The use of EPF (*B. bassiana*) for *B. tabaci* control had a direct impact on SLCV accumulation and transmission.

Keywords Squash leaf curl virus (SLCV), *Bemisia tabaci*, PCR, Entomopathogenic fungi (EPF), *Beauveria bassiana*

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Background

Squash plant *Cucurbita pepo* L. subsp. *pepo* has great nutritional value and is grown all over the world. Viral infections including squash leaf curl virus (SLCV) are responsible for the majority of squash crop losses (Venkataravanappa et al. 2021). The virus (SLCV) was first detected in California in the 1970s and recently in the Greater Middle East. In Egypt, SLCV was first isolated from squash (*Cucurbita pepo* L. cv. Eskansarani) plants growing in Qaluobiya Governorate where leaf curl and severe stunting effects were seen on freshly produced leaves of squash plants infested with whiteflies (Amro et al. 2014).

Squash leaf curl virus is one of the members of the genus Begomovirus, Family: *Geminiviridae,* and is a New World begomovirus. The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is the only vector that transmits begomoviruses naturally causing massive crop losses through feeding damage (Castillo et al. 2010). SLCV is transmitted efficiently in a persistent manner by *B. tabaci* but is not transmitted by mechanical inoculation (Mc Creight and James 1991). SLCV is bipartite with a twinned-icosahedral (18–20 nm) structure. It contains a bipartite single-strand genomic DNA, DNA A, and DNA B. DNA A encodes five proteins and DNA B encodes two proteins (Lazarowitz and Lazdins 1991). The DNA-A component encodes five proteins, the capsid protein (AV1), the replication-associated protein Rep (AC1), the trans-activator protein Trap (AC2), the replication enhancer protein Ren (AC3) and AC4, a symptom specific protein. DNA-B encodes a movement protein MP (BC1) and a nuclear shuttle protein NSP (BV1)). Thus, the DNA-B component is responsible for cell-to-cell movement and long-distance movement within the affected plant (Fontenele et al. 2021). A squash begomovirus has also been reported from China, a squash leaf curl virus China called (SLCCNV), and appears to be of Old World origin based on DNA sequencing analysis; and is an important bipartite virus that causes severe economic losses in Cucurbitaceous crops, such as wax gourd, squash, and pumpkin. It differs from SLCV-like isolates from the USA and Mexico. The silver leaf whitefly transmits it, also informally referred to as the sweet potato whitefly, and infects cucurbit crops in various parts of the old World (Wu et al. 2020).

The widespread use of chemical insecticides to control the whitefly *B. tabaci* has become extremely hazardous to insect resistance, the environment, and human health. As a result, using alternative methods such as biological control was recommended as an environmentally friendly solution. EPF has been identified as a promising biopesticide for whitefly management (Malekan et al. 2013). *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) as an EPF, has a wide host range of insects. Several studies have reported the efficacy of *B. bassiana* against all life stages of whitefly eggs, all nymphal stages, and adults (Zafar et al 2016). The aim of this study was to investigate the relationship between adult whitefly, plant virus, host plant, and EPF. In addition, it was to study the potential use of EPF as a biological control agent to control *B. tabaci* whitefly and observe the SLCV transmission.

Methods

Source and maintenance of virus isolate

The virus isolate understudy was isolated from squash plants collected from the Experimental Station, Agriculture Research Center (ARC), Giza, Egypt showing disease symptoms like mottling, yellowing, leaf curling, and stunting and a high population of the whitefly. Virus isolate was maintained in a greenhouse for two months on squash seedling plants cv. Eskandrani by serial transmission using the whitefly vector, *B. tabaci*. Infected plants were kept in the insect-proof greenhouse. Samples were tested by PCR using Squash Leaf Curl Virus (SLCV) specific primers.

DNA extraction and detection of SLCV

Total DNA was isolated from naturally infected squash plant and from six squash plants inoculated with viruliferous whitefly using Dellaporta extraction procedure (Dellaporta et al. 1983). DNA from an asymptomatic squash plant was taken in the field as a negative control. Nucleic acid was precipitated by adding 500 µl absolute ethanol, centrifuged, washed twice in 70% ethanol, dried, and re-suspended in 50 µl of water. The primer pair A1/A2 was used in direct PCR tests. For PCR amplification of the SLCV coat protein gene core region (CP), the oligonucleotide-specific primers A1 (5'-cgagcagcggcatgatatct-3') and A2 (5'-ccggcttcctggtggttat-3) were used according to Azza et al. (2005). Five µl of extracted DNA were utilized in a total PCR reaction mixture of 25 µl, which included 10 pmol of each primer and 12.5 µl of Mangotaq DNA polymerase (Bioline GmbH, Luckenwalde). The DNA amplification process began with a 3-min denaturation stage at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s., annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final 10 min extension step at 72 °C. The PCR products were stained using Biomatik's EZ View nucleic acid dye, electrophoresed in a 1% agarose gel, and then viewed under UV light.

Nucleotide sequencing

The identification of the virus isolate was carried out based on DNA sequencing. The PCR product of the coat protein gene was purified using Gel/PCR DNA

Fragments Extraction Kit (Geneaid, Taiwan). The purified PCR product was partially sequenced in both directions by the Analysis Company using ABI 3730xl automated DNA sequencer. DNAMAN version 8.0 sequence analysis software (Lynnon Corporation, Canada) was used to analyze the nucleotide sequences and generate the maximum likelihood rooted (Ml) Phylogenetic tree. The nucleotide sequence was compared with the corresponding sequences of 11 sequences of other squash leaf curl virus isolates (SLCV) from different geographical regions that were available in GenBank (Table 1). The obtained sequence data were analyzed using DNAMAN sequence analysis software. The nucleotide sequence was submitted to GenBank as an SLCV-Giza isolate with accession number MT260406.

Maintenance of whitefly colony

Adults' whitefly were collected from squash plants and grown on healthy sweet potatoes in a controlled growth chamber at 28 °C, 30–50% humidity and 14:10 L:D hrs photoperiod. Sweet potato plants are chosen for a non-viruliferous colony because it is a non-host for SLCV to avoid contamination in the colony. The eggs were allowed to hatch and tagged to the newly grown sweet potato in a separate glass cages covered with a 40-mesh size nylon net and kept in a controlled glasshouse and maintained for transmission studies (Venkataravanappa et al. 2017).

Maintenance of entomopathogenic fungi *B. bassiana*

The EPF *B. bassiana* was isolated from soil collected from East Owainat at New Valley Governorate, Egypt. According to Zimmermann (1986), greater wax moth, *Galleria mellonella* L larvae was used as trap of the EPF. *B. bassiana* was grown on autoclaved Sabourad dextrose yeast agar media (SDAY), at 25 ± °C for 14 days (Nada 2015). Spores were collected from the culture medium by carefully rinsing with a sterilized aqueous solution of 0.02% Tween 80, and then filtered through cheesecloth to reduce mycelium clumping. Spores were counted using Haemocytometer (Neubauer improved HBG, Germany 0.100 mm × 0.0025 mm^2) and suspension were diluted with sterile distilled water to the concentration 5×10^7 spores/ml.

Table 1 The Accession numbers, description and the identity matrix with different SLCV isolates available in GenBank

Isolates' name	Accession No	Country	Homology matrix (%)
SLCV-Giza (the current isolate)	MT260406	Egypt	100
SLCUV-Mx:BCS:La Paz	MF187211	Mexico	99.2
JO3-126	KM595202	Jordan	98.7
SLQV.Q2521	MN422078	Egypt	99.0
Ismailia	KC895398	Egypt	98.7
Segment DNA-A	MH346454	Egypt	98.7
PA2-Q340	KM595229	Palestine	99.0
JO1-617	KM595172	Jordan	98.4
EG2-9	KM595148	Egypt	98.4
PA3-T6	KM595238	Palestine	98.7
IL3-98	KM595121	Israel	98.2
LB2-2-9	KM595139	Lebanon	99.0

Transmission tests

1-Syringe inoculation test

Twenty seedlings at the 2–3 leaf stage of healthy squash plants cv. Eskandrani were inoculated with sap from naturally infected squash leaves by syringe using fresh 0.2 M potassium phosphate buffer (pH 7) containing 0.02 M sodium sulfite. The virus-containing extract (1:2 wt/vol) was inoculated by syringe according to Allam et al. (1994). On the other hand, healthy squash plants cv. Eskandrani were used as a control plant and injected by a syringe using healthy squash leaf sap. The test and control plants were kept under observation in the insect-proof greenhouse for 30 days. Symptoms and the percentage of transmission were monitored and recorded for one month. The inoculated plants were used as a virus source for further testing of insect transmission.

2-Insect transmission test

The virus was transmitted by the whitefly as described by Venkataravanappa et al. (2017). Squash plants were sprayed with imidacloprid 0.05 after infestation with the viruliferous whiteflies and placed in insect-proof cages until symptoms appeared. The symptomatic plants were maintained in the green house as SLCV source plants.

1-Number of whiteflies required for SLCV transmission

To determine the required number of newly emerged adults for transmit SLCV, whiteflies were given an acquisition access period (AAP) of 24 h on infected squash plants as virus source. The viruliferous whiteflies were released on squash seedling plants at 0, 1, 5, 10, 15 and 20 insects per plant and in each treatment 15 plants were inoculated. After 24 h of the inoculation access period (IAP), the Imidacloprid was sprayed to kill the whiteflies. The plants were kept under insect-proof cages for the development of viral symptoms; the percent transmission was recorded based on symptoms expressed by the plants.

2-Acquisition access period (AAP) of SLCV

Adult whiteflies were administered for 15 and 30 min; 6, 12, 24, 48 and 72 h AAP, on virus source plants. Post AAP, 10 insects per each test plant were used, 15 test plants

and three replicates per each test were used. The viruliferous insect given 48 h IAP on susceptible squash plant, After IAP the test plants were sprayed with Imidacloprid (0.05%) 17.8 SC. The plants were kept under insect proof cage for development of viral symptoms appearance.

3-Inoculation access period (IAP) of SLCV

Inoculation test was done using viruliferous whiteflies, collected from the virus source after AAP 24 h and placed on the test plants inside the cylindrical glass cages to IAP 48 h (10 insects per plant) then removed using the insecticide imidacloprid and maintained under greenhouse condition for symptoms appearance.

4-Ability of viruliferous whiteflies to inoculate SLCV into B. bassiana—treated plant

Adult whiteflies were transferred to SLCV-infected squash plant to acquire virus (AAP). The test plants were sprayed with 15 ml of *B. bassiana* conidia suspension at concentration (5×10^7 spores/ml). Directly after drying, viruliferous adults were transmitted to treated test plant after 24, 48, 72 h, respectively, from virus acquisition (AAP) as 10 insects/test plant, Fifteen test plants for each treatment were used. After 48 h IAP, whitefly were killed using the imidacloprid and test plants were transferred to insect proof cages maintained under greenhouse condition. Symptoms development was recorded for next one month. The control test plants were sprayed with sterile aqueous solution of 0.02% Tween 80. Positive control (C+), where fungus free-test plants inoculated with viruliferous adult whitefly, while the negative control (C−) were fungus free-test plants inoculated with healthy adult whiteflies.

5-Ability of whiteflies to acquire the SLCV from infected plant treated with B. bassiana

B. bassiana suspension (15 ml) with concentration (5×10^7 spores/ml) was sprayed on the plant virus source (SLCV), after drying, Number of 500 virus free-whitefly adults (*B. tabaci*) was released to feed on this treated virus source for (AAP) 24, 48 and 72 h, respectively, as illustrated in Fig. 1. After AAP the viruliferous whitefly adults at the rate of 10 insects/plant were released on healthy squash plant. Fifteen test plants for each treatment was used. Each treatment was caged for IAP to 48 h. The control test plants were sprayed with sterile aqueous solution of 0.02% Tween 80. Positive control (C+), while fungus free test plants were inoculated with viruliferous adult whitefly, while negative control (C−), fungus free test plants were inoculated with healthy adult whitefly. The whitefly insects were killed using the insecticide imidacloprid and maintained under greenhouse conditions for symptoms appearance (Fig. 2). The tested plants from the two previous experiments were tested by PCR for detection of SLCV using the specific primers and procedure described above.

Results

Syringe inoculation and SLCV symptoms

The naturally infected squash plant collected from Giza governorate exhibited typical SLCV symptoms as shown in (Fig. 2). Infected plant with SLCV showed leaf curling, leaf crinkle, chlorosis and stunting symptoms. Virus inoculation using the syringe injection method showed 60% efficiency in transmission of SLCV from infected squash plants cv. Eskandrani to healthy plants. The symptoms appeared at 2–3 weeks post-inoculation. The inoculated plants showed yellow spots; vein chlorosis, leaf curling and leaf crinkle Fig. 3A–C.

Detection of SLCV

SLCV was detected in the collected sample of symptomatic squash by PCR analysis. Total DNA isolated from naturally infected and healthy squash plants was used as a template for endpoint PCR. All symptomatic squash samples generated amplicons of about 419 bp (Fig. 4). However, no amplicons were detected in healthy squash plant.

Sequence analysis

The sequence comparison showed that the SLCV-Giza isolate had 99.2% nucleotide identity to SLCV-Mx:BCS:La Paz (MF187211) from Mexico. The sequence comparison also showed that the nucleotide identity was about 99.0% to isolates SLCV.Q2521 from Egypt, PA2-Q340 from Palestine and LB2-2-9 from Lebanon with accession numbers MN422078, KM595229 and KM595139, respectively. The lowest nucleotide identity of 98.2% was with the isolate IL3-98 (KM595121) from Israel. The Maximum likelihood (Ml) phylogenetic tree (Fig. 5) showed three main clades of SLCV isolates. SLCV-Giza isolate formed a monophyletic group with SLCV.Q2521 isolate from Egypt and SLCV-Mx:BCS:La Paz isolate from Mexico and clustered with JO3-126 isolate from Jordan.

Insect transmission tests

Effect of viruliferous whitefly numbers on SLCV transmission rates

Whiteflies can transmit the virus to an extent of 13.3% and reach to 100% of transmission using 15–20 viruliferous whiteflies (Table 2).

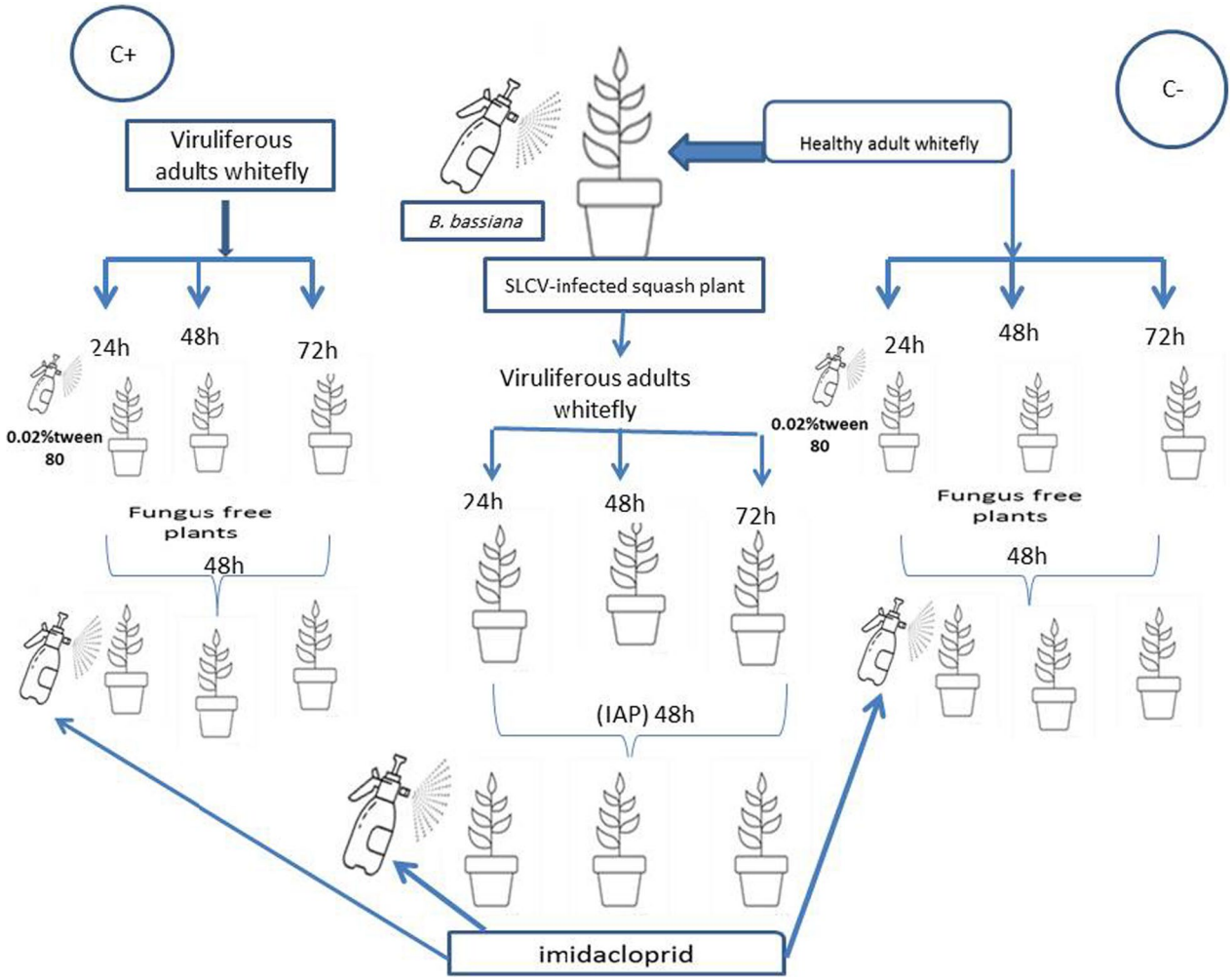

Fig. 1 The indicating diagram for experiment of effect of treatment plant virus source with *Beauveria bassiana* on ability non-viruliferous whitefly adults *Bemisia tabaci* to acquisition SLCV virus

Fig. 2 Naturally infected squash plants exhibited SLCV symptoms

Effect of acquisition access period (AAP) on the transmission % of SLCV

B. tabaci was able to acquire and transmit SLCV after an Acquisition Access Period (AAP) 15 and 30 min by low rates of 13.3 and 22.2%. The transmission rates were increased gradually to reach the maximum of 100% after 24, 48 and 72 h (AAP) Table 3. Obtained results recommended using AAP 24 h at the least to obtain 100% of virus transmission. *B. tabaci* were able to transmit SLCV after an Inoculation Access Period (IAP) 15 and 30 min at the rates of 46.7 and 62.2%, respectively (Table 4).

The result recommended using IAP 24–48 h at the least to obtain 100% of virus transmission.

Effect of B. bassiana ability on viruliferous B. tabaci adults to inoculate SLCV virus on treated test plants (Treatment 1)

When viruliferous whitefly was allowed to feed on tests plants that had fungus *B. bassiana* applied to them, transmission trials showed that viruliferous whitefly was able to transmit SLCV but with lower efficiency (73.3%) than non-treated (100%). The transmission efficiency was decreased on the second day at 44.5% and on the third day at 31.7% of treatment with the EPF, *B. bassiana*, respectively (Table 5).

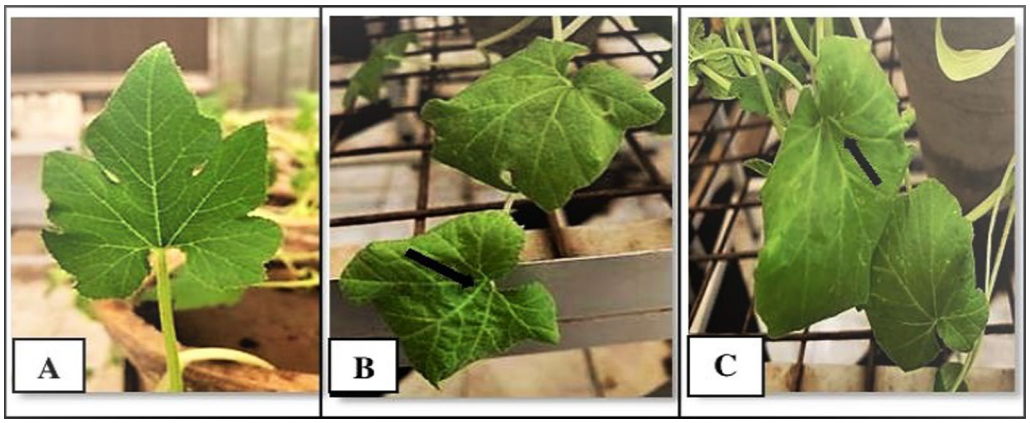

Fig. 3 SLCV symptoms upon syringe inoculation. **A** Healthy plant. **B** leaf crinkle and **C** yellow spotting and downward curling

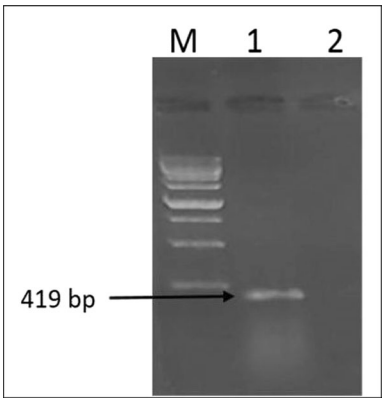

Fig. 4 1% Agarose gel electrophoresis for SLCV PCR amplification. M: 1 Kb ladder; Lane1: from naturally infected squash plant. Lane 2: from healthy squash plant as a negative control

Effect of treatment plant virus source with B. bassiana on the ability of non-viruliferous whitefly B. tabaci adults to acquire SLCV virus (Treatment 2)

Transmission experiments conducted using squash treated with fungus as an inoculation source revealed that non-viruliferous whiteflies were able to acquire the virus. However, the transmission rate was greatly reduced (33.4%). The transmission rate decreased to 6.8 and 2.2% after the second and third days of treatment with fungi, respectively (Table 6).

SLCV detection in squash plant inoculated using viruliferous whitefly

The SLCV DNA was detected in fungi-treated squash plants inoculated by viruliferous whitefly after they were given 24, 48, and 72 h AAP, and 48 h IAP on treated plants (Fig. 7 lanes 2, 3 and 4). On the other hand, no SLCV-specific amplicons were detected in squash plants subjected to feeding the fungus-treated viruliferous whiteflies (Figs. 6, 7 lanes 5, 6 and 7). The SLCV-specific band was detected in the positive control while no band was observed in the negative control (Fig. 7).

Discussion

Naturally infected squash plant collected from Giza Governorate, Egypt, exhibited typical symptoms of SLCV such as leaf curl, chlorosis, and stunting as described previously by (El-Dougdoug et al. 2009). PCR using specific primers A1/A2 for SLCV enables rapid and accurate detection of SLCV from naturally infected squash plants collected from Giza Governorate, Egypt. In this study, DNA sequencing analysis was performed to determine whether the current SLCV-Giza isolate is New-World or Old-World SLCV. Partial nucleotide sequence comparison of SLCV-Giza for DNA-A genome had high nucleotide similarity with all New World SLCV reference isolates retrieved from GenBank. The highest similarity was with the isolate SLCV-Mx:BCS:La Paz from Mexico. Based on DNA sequence analysis, SLCV-like isolates from the United States and Mexico (New World SLCV) differed from the reported SLCV from China, (SLCCNV) which appeared to be from the Old World SLCV (Wu et al. 2020). The phylogenetic analysis indicated that the isolates; SLCV-Giza, SLCV.Q2521 from Egypt, and SLCV-Mx:BCS: La Paz from Mexico were descendants of the same ancestor. The Phylogenetic analysis showed that the SLCV-Giza was more closely related to the SLCuV-Mx:BCS:La Paz isolates from Mexico and SLQV.Q2521 338 isolates from Egypt than the isolates from Jordan and Palestine (JO1-6, PA2-Q34, and PA3-T) along with another Egyptian isolate from Ismailia Governorate. Signifying current SLCV-Giza isolate might have been introduced independently from Mexico. Such a situation also explains why in the present study, no variations in the coat protein gene of SLCV-Giza were seen as earlier

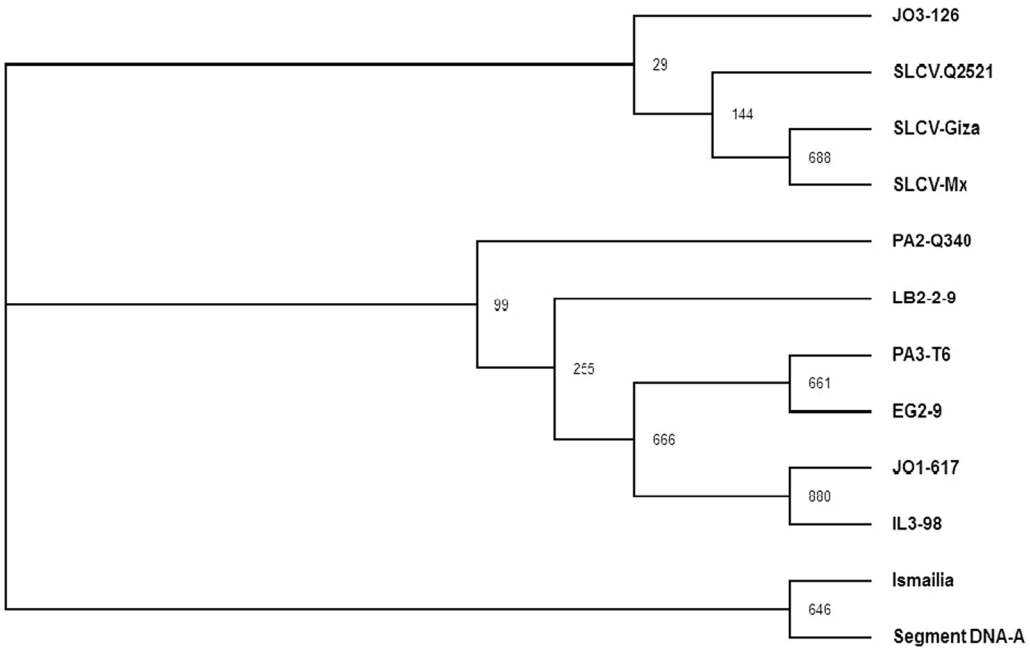

Fig. 5 Maximum likelihood rooted (Ml) phylogenetic tree for the SLCV-Giza isolate based on the partial nucleotide sequence alignment of DNA-A genome with SLCV isolates available in GenBank using DNAMAN software. Each node's numbers represent the bootstrap value based on 1000 replications

Table 2 Number of whiteflies' adults required for transmission of SLCV

No. of whiteflies/plant	No. of I/T*	Transmission (%)
1	2/15	13.3
5	8/15	53.3
10	14/15	93.3
15	15/15	100.0
20	15/15	100.0

*I Infected plants, *T* Tested plants

reported by Farrag et al. 2014 who used another SLCV isolate. These results revealed that a high genomic structural stability of the core region of the SLCV CP gene in the DNA-A genome was observed. Obtained results are inconsistent with Farrag et al. (2014) who reported that the coat protein gene examined for Egyptian SLCV from three different isolates infecting common bean plants showed relatively high diversity. On the other hand, the present data came in line with the conclusion of Lapidot et al. (2014) that SLCV contained small amounts of sequence variation.

The numbers of whitefly required for SLCV transmission revealed that one whitefly can transmit the virus by 13.3%, while the maximum percent of the transmission is 100% obtained when using 10–20 whitefly insects in the transmission test. This result came in line with that obtained by Onkara et al., (2019). A whitefly required at least 15 min. to become viruliferous resulting in 13.3% transmission and increased transmission efficiency with

Table 3 Tasting acquisition access period on transmission (AAP) of SLCV

Acquisition access period (AAP)							
Replicates	**15 min**	**30 min**	**6 h**	**12 h**	**24 h**	**48 h**	**72 h**
R1	2/15*	3/15	4/15	11/15	15/15	15/15	15/15
R2	3/15	3/15	6/15	13/15	15/15	15/15	15/15
R3	1/15	4/15	7/15	10/15	15/15	15/15	15/15
Total	6/45	10/45	17/45	34/45	45/45	45/45	45/45
%	13.3	22.2	37.8	73.3	100	100	100

*No. of infected plants/inoculated (tested)

Table 4 Testing of the inoculation access period (IAP) on the transmission of SLCV

Inoculation access period (IAP)							
Replicates	**15 min**	**30 min**	**6 h**	**12 h**	**24 h**	**48 h**	**72 h**
R1	7/15*	8/15	12/15	15/15	15/15	15/15	15/15
R2	9/15	9/15	14/15	14/15	15/15	15/15	15/15
R3	5/15	11/15	11/15	15/15	15/15	15/15	15/15
Total	21/45	28/45	37/45	44/45	45/45	45/45	45/45
%	46.7	62.2	82.2	97.8	100.0	100.0	100.0

*No. of infected plants/inoculated (tested)

Table 5 Transmission rates of SLCV using viruliferous *Bemisia tabaci* on fungi-treated squash plants

Replicates	**Transmission Rates of SLCV using *Bemisia tabaci***					
	D1		**D2**		**D3**	
	No.	**Rates %**	**No.**	**Rates %**	**No.**	**Rates %**
R1	9/15*	60**	8/15	53.3	6/15	40.0
R2	11/15	73	5/15	33.3	3/14	21.4
R3	13/15	87	7/15	47.0	5/15	33.3
Average	33/45	73.3%	20/45	44.5%	14/45	31.7
C+	100.0		100.0		100.0	
C−	00.0		00.0		00.0	

C+ Positive control, *C−* Negative control, *D1* Day 1 after treatment, *D2* Day 2 after treatment, *D3* Day 3 after treatment

*No. of infected plants/inoculated (tested)

**Percentage of plants infected

Table 6 Transmission rates of SLCV using fungi treated viruliferous whiteflies *Bemisia tabaci* adults

Replicates	**Transmission rates of SLCV using *Bemisia tabaci***					
	D1		**D2**		**D3**	
	No.	**Rates %**	**No.**	**Rates %**	**No.**	**Rates %**
R1	4/15*	27.0**	2/15	13.3	0/15	0.0
R2	6/15	40.0	0/15	0.0	0/15	0.0
R3	5/15	33.3	1/15	6.7	1/15	6.7
Average	5/15	33.4	1/15	6.8	0.3/15	2.2
C+	100		100		100	
C−	00.0		00.0		00.0	

C+ Positive control, *C* – Negative control, *D1* Day 1 after treatment, *D2* Day 2 after treatment, *D3* Day 3 after treatment

*No. of infected plants/inoculated (tested)

**Percentage of plants infected

increasing AAP. These results are consistent with those presented in Fiallo-Olive et al. (2020) study. An acquisition period of 12 h or more resulted in 100% transfer of SLCV. This result was parallel to the result obtained by Jayashree et al. (1999) who inoculated the pumpkin plants with PYVMV that was transmitted by viruliferous whiteflies. Viruliferous whitefly was able to inoculate SLCV after IAP of 15 min by 46.7%. This percentage increased gradually by increasing IAP to reach the maximum after 24 h IAP these results agree with (Ghanim et al. 2001) who reported that Begomovirus requires a 30–60 min inoculation access period (IAP) on the host plant. Once acquired, viruses are transmissible for the life of the whitefly. A gradual increase occurred in the number of

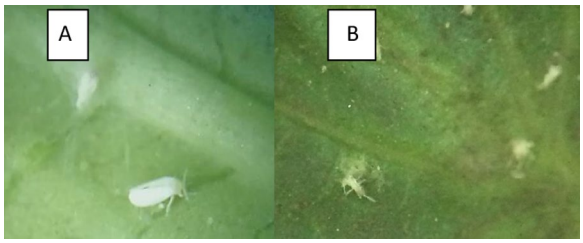

Fig. 6 **A** Healthy whitefly adults **B** Whitefly adults treated with fungi

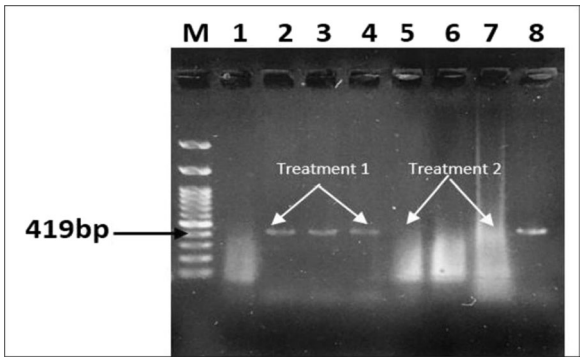

Fig. 7 1% agarose gel electrophoresis analysis of the PCR products from tested squash plants. M: 100 bp DNA Marker; Lane 1: healthy control plant; Lane 2, 3 and 4: (Treatment 1) fungi-treated squash plants inoculated by viruliferous whitefly after 24, 48 and 72 h AAP respectively. Lane 5, 6 and7: (Treatment 2) tested plants subjected to feeding the fungus-treated viruliferous whiteflies after 24, 48 and 72 h AAP. Lane 8: positive

infected plants as virus AAP and IAP increased, reaching 70 and 75% at an AAP and IAP of 24 h, respectively.

It is hypothesized that plant biochemistry changes could also influence plant virus transmission by insect vectors (Chang et al. 2021). In contrast to previous reports, in the current study, whiteflies were able to successfully inoculate the fungi-treated plants. This could be attributed to the very low inoculation time required for SLCV transmission, as shown in this study. When whiteflies were treated with fungi, SLCV transmission dropped significantly. Results in the present study on reduced virus transmission by EPF-treated whiteflies are in agreement with previous reports (Sune et al. 2021). On the other hand, Zhang et al (2016) found that EPF *Isaria fumosorosea* infection in B-biotype *B. tabaci* directly affected levels of TYLCV in whiteflies, decreasing TYLCV accumulation in the vector and reducing virus transmission. Similarly, Sune et al. (2021) reported that when viruliferous whitefly's nymphs and adults were infected with *Isaria javanica*, the amount of virus carried decreased significantly. This might be because fungi might have altered whitefly feeding habits or affected virus stability in whiteflies. Many studies have been conducted to investigate the relationship between EPF and insect feeding behavior and many studies have reported a significant reduction in insects upon infection with EPF (Mohammad and Port 2015). However, the exact mechanism of how fungi affect viral transmission by insect vectors is unknown and warrants further investigations.

Conclusions

In this study, SLCV was isolated from naturally infected squash plant showing leaf curl, chlorosis, and stunting symptoms. Comparison of sequences for the current SLCV-Giza isolate revealed high nucleotide identity with all New World SLCV reference isolates. Whereas, the SLCV-Giza isolate was closely related to the SLCuV-Mx:BCS: La Paz isolate from Mexico and SLQV.Q2521 338 isolate from Egypt. Phylogenetic analysis indicated that isolates SLCV-Giza and SLCV.Q2521 from Egypt and SLCV-Mx:BCS:La Paz from Mexico have a common ancestor. These results imply that the present SLCV-Giza isolates might have been introduced independently from Mexico. The present study investigated the relationship between SLCV transmission and adult whitefly *B. tabaci*. The results documented that the whitefly, *B. tabaci* is a very efficient transmission vector of SLCV-Giza isolate, that the viruliferous whitefly was able to inoculate SLCV 15 min after IAP. Additionally, the potential use of an EPF as a biological control agent to control *B. tabaci* whitefly and to control SLCV transmission was examined. Infection of *B. tabaci* whitefly by EPF (*B. bassiana*) had a direct impact in reducing SLCV transmission. Therefore, spraying *B. bassiana* into squash production system could help in the control of SLCV transmission by *B. tabaci*. Our findings provide fundamental information needed to develop an integrated for managing SLCV disease in Egypt.

Abbreviations

SLCV	Squash leaf curl virus
AAP	Acquisition access period
IAP	Inoculation access period
ARC	Agriculture research center

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