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V Summary

A. Ecological studies

Population density of the whitefly (adult and nymphal stage) and virus infection ratio on squash leaves

During the studied seasons 2004 and 2005, squash plant leaves were chosen to estimate the infestation of whitefly different stages and virus infection ratio in Dakahlia Governorate.

A-1.) Summer Plantation

This study was carried out to classify the natural infestation of the whitefly and associated virus infection ratio on squash leaves. The first appearance of the whitefly occurred during the late of April 2004 with low adult number and then gradually increased to reach the highest level of infestation during the mid of July of the same season and during early of August for nymphal stages .However, the same trend of population was recorded in 2005 summer season but with different numbers recording the peak of abundance during the last week of August for adult stage and during the early of the same month for nymphal stages population

A.2.-) Nili plantation

Inspection of squash leaves throughout the period of experiment revealed that the early infestation was determined during the early of September 2004 with high number (36 insects). The population was changed reaching the least population level during the first of December (15 insects). The population of whitefly nymphal stages was slightly lower than that of adult stage recording the peak of abundance during mid of November (36 individuals) during 2004.The virus infection ratio during Nili plantation 2004 season recorded the highest ratio during early of November (24 %).However, during 2005 Nili cultivation season, the adult and nymphal infestation of whitefly was obviously denoted that the

population was in a gradually increase reaching their highest level during early of November(68 adult stages) and mid of November(42 nymphal individuals). Moreover, the virus infection ratio in this study during Nili plantation recorded the highest ratio from mid of November till mid of December 2005 (40 %).

Effect of the different planting time on the mean number of white fly stages and virus ratio infection on squash

The regular inspection of squash plantations during the different sowing dates through the experiment revealed that the following seasons Summer 2004, summer 2005 and summer 2006 were the preferable time for growing the population of the whitefly nymphs but the Nili 2004 and Nili 2005 were the lowest period for harboring the nymph population. From the obtained results there was highly significant differences between the time of planting and the infestation of insect nymphal stages.

However, the population of the adult stages of whitefly on squash plants in the tested regions was obviously highly than that occurred in nymphal stages. The plants sowing in summer season of 2005 was the best for growing the adult stages of this insect in comparison with other planting times. Summer 2006 come in the second order followed by summer 2004 but the Nili planting time was the least preferable time in this respect.The virus ratio infection was clearly higher when the plants were sowing during summer 2004 but the least was obviously observed during the period of Nili 2004.It was noticed that there were highly significant difference between the plant sowing in different planting dates and virus ratio infection on squash.

The correlation between adults and nymphs of whitefly population

The obtained results shows a positive and lower significant relation in summer 2004 and positive and highly significant relation in Nili 2004 and Summer 2005. However the relation between the different stages nymphs and adult of whitefly was non-significantly positive relation.

The correlation between nymphs of whitefly population and virus ratio infection.

The correlation values show that for Summer 2004, Summer 2005 and Summer 2006, the relation expressed as highly significantly positive, while it was negatively insignificant during the season of Nili 2005

The relation between adult of whitefly population and virus ratio infection.

The change in the population of the whitefly adult stages in relation to the ratio of infection on squash was highly significant and positive in the Summer 2004, Summer season 2005**,** Nili 2005 and Summer 2006. On the other hand the period of Nili 2004 expressed highly and positive significant relation between the insect and virus ratio infestation. The activity of the virus ratio infection is mostly related o the single effect of whitefly nymphs and to combined action of the studied factors. It seems that the whitefly insect population has a preferred condition for the virus growth.

Effect of inoculation access period (IAP) on the transmission of squash leaf curl virus by whitefly *Bemisia argentifolii*

The inoculation access period on the transmission of squash leaf curl virus significantly affected with time proceeding, where the complete transmission was clearly observed after spending 72 hours, but the exposure time 15 minutes was not affected on the transmission process. The obtained data showed that the number of *Bemisia argentifolii* exposed to virus with acquisition period and the inoculation period was

24 hours significantly affected on the infection ratio. However, no infection with the virus when a single insect exposed to the virus. On the other hand, when the number of exposed *Bemisia argentifolii* to virus was 10 or more the infection ratio was reached to the maximum level 100 %

The ratio of infection with the squash curl leaf virus in the adult stage of whitefly *Bemisia argentifolii*

The infection symptoms did not observed in the first and the second day after adult emergence of *Bemisia argentifolii*. The obtained date also, denoted that the ratio of symptoms infection began to increase with the developments of the emerged adults where the ratio of infection reached to 20% after three days of emergence increased to reach to their maximum level (100 %) after the 8^{th} emergence day. However, no infection symptoms were appeared for the emergence of adults of *Bemisia argentifolii* after the 9^{th} and 10^{th} emergence day.

Effect of infection of whitefly *Bemisia argentifolii* with Squash Leaf Curl Virus on the components of the squash leaves.

In this study, performance of whitefly *B. argentifolii* was compared on SLCV infected and healthy squash plants of the same age. From obtained results, it was observed that the total sugar ratio in the diseased leaves of squash was considerably lower than in healthy leaves. The ratios were 1.909 and 4.859, respectively. Also a marked decreases in reducing sugar ratio in diseased leaves was recorded (1.070 % than healthy ones (2.858 %). From the study also, it was noticed that a very high decrease in the total carbohydrates % content of the diseased squash leaves (11.619 %) in comparison with those of the healthy leaves (24.880%).

III.Biological studies of the whitefly *Bemisia argentifolii*:-

This study was carried out to estimate the effect of virus infection on the silver leaf whitefly, *Bemisia argentifolii*. In this study the insect spent three different generations for this study.

The incubation period of insect which did not significantly affected in this study ranged from 2 – 5 days for the different insect state and generations. However, there were not clearly observed differences between the different insect life cycle of the two insect sexes in the different three generations. The lowest period in different tested replicates was recording 11 days in case of healthy insect state in the third generation and the highest value was recorded 18 days. The longevity period of the adult female was highly significant between the different insect three generation and this effect was not recorded between the different healthy insects and infected once. The least longevity of adult males was recorded 8.2 days in the case of healthy insects of the third generation. But the longest longevity duration period of *B. argentifolii* (19 days) was determined when the adult females were exposed the infection with the virus. Also, the fecundity of the adult female affected by the presence of virus where the number of laid eggs was about 108 eggs in case of the health insects in comparison with 189 eggs were laid when the insects were infected with the virus

III-) Nucleotide sequence of Egyptian isolate of Squash leaf curl virus.

Squash leaf curl virus (SLCV) is a virus with geminate particles, 22 x 38 nm. The circular ssDNA genome is bipartite and consists of two similar-sized species. Known hosts are in the Cucurbitaceae, Leguminosae, Solanaceae and Euphorbiaceae. The virus is transmitted by the whitefly, *Bemisia argentifolii*, and by inoculation with sap. Squash leaf curl virus (SLCV) Geminivirus was isolated for the first time in

Egypt from squash (*Cucurbita pepo)* plants growing in Qaluobia governorate. This virus is transmitted by whiteflies and is cause a problem in Egypt. Symptoms of the disease are crumpled leaves with yellowed, mottled areas. Leaves have shortened petioles that cluster around the vines. Squash is also susceptible to this virus. Severe yield losses are associated with infection of young seedlings, usually when whitefly populations are high. Infections of older plants do not affect yield. Polymerase chain reaction (PCR) assay was used for the detection and identification of the isolated virus from nucleic acid extracts of infected squash plants using universal oligonucleotide primer for detection of Geminiviruses group. The viral DNA amplified product was approximately 1.350 kb as estimated by agarose gel electrophoresis This primer was predicated to direct the amplification of fragment of SqLCV DNA-A consisting of approximately 460 bp. DNA-DNA hybridization assays of viral genome have been used for detection of the present virus isolate using specific DNA probe prepared for SqLCV. No PCR product, no hybridization reactions were observed with samples containing DNA extracted from healthy plants.

The bipartite geminiviruses such as tomato golden mosaic virus (TGMV) and squash leaf curl virus (SqLCV) have two single-stranded circular genomic DNAs. Through cloning and molecular analysis we have identified two highly homologous bipartite geminiviruses as were identified causing squash leaf curl disease. Nucleotide sequence analysis of the genome of this virus have the same bipartite component organization characteristic of other whitefly-transmitted geminiviruses. Sequence comparison with the genomic components of tomato golden mosaic virus and bean golden mosaic virus revealed a close evolutionary relationship with these two bipartite geminiviruses. Polymerase chain reaction (PCR) was used to amplify SQLCV isolates 1, 2 and 3 using

specific primers designed for each. The amplified DNA of the three fragments and isolates of the isolated SLCV were cloned into TOPO-TA vector™ (3.9 kb) and transformed into competent cells of Escherichia coli.

Nucleotide sequence for cloned DNA 1, 2 and 3 of isolated SLCV were obtained from each insert of recombinant plasmids (SqLCV1841v. 5 CACACCTTCT TGATTATACT AGTTGG 3 and SqLCV547c 5 CTGCTAATTA TACAGACTTA CACAAAAGCG 3) using DNA sequence. The nucleotide sequences were analyzed and compared with the sequences of SLCV of The comparison between Egyptian isolate (Kaha) and Giza (DQ364057), Jordan (EU057179.1), Arizona,USA(DQ285016.1),Qualifiers(M38183.1)(AF256203.2,Arizona, USA, Oman (EF532620), Costa Rica (AY064391.1), and Israel (AY206998.1) using DNAMAN program.

Isolates 1 and 2 have been completely sequenced, while only fragments of the other isolate (3) has been sequenced. The identity of the sequences of the isolated SLCV with isolates was between 93 % to 98 % but was less than 50 % with isolate. The sequenced DNA fragments 1, 2 and 3 were submitted in the Gen Bank. Dot blot hybridization methods were developed for detection of SQLCV. The DNA probe was labeled with 32P for radioactive detection, with biotin for a chemiluminesecent technique, and with digoxigenin for a colorimetric method. The radioactive method was slightly more sensitive than the chemiluminesecent method, whereas the digoxigenin technique was the least sensitive