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Age-related Induced Resistance Effect on Tomato Seedlings for Producing Tomato Yellow Leaf Curl Virus (TYLCV)-Free Plants and High-quality Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Egypt is facing a major problem in the field of tomato seed production, as infection with the yellow tomato leaf curl virus (TYLCV) is one of the most important factors in the success of this important production process, which has an impact on national food security, in addition to facing the steady increase in the costs of importing tomato seeds in particular vegetable crop seeds in general. Therefore, the main objective of the current study is to study plant age-related induced resistance (ARIR) against tomato yellow leaf curl virus (TYLCV) in tomato plants. Several research points were studied, respectively: first, the effect of plant age on resistance to TYLCV virus in tomato

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plants that is transmitted by whitefly. Second, the detection and identification of tomato yellow leaf curl virus (TYLCV) in seeds obtained from seedlings of different ages (35 and 90 days old). Third, study the behavior of the whitefly in terms of the number of eggs and larvae, the percentage of the number of infected plants that showed symptoms of infection with the virus, and its relationship to the age of the seedlings. The results of this study proved that the age of the plant is closely related to the ability of the plant to withstand infection with the tomato yellow leaf curl virus (TYLCV). The DNA of the tomato yellow leaf curl virus (TYLCV) was identified from a sample of seeds obtained from plants obtained from 35-day-old seedlings. On the contrary, the DNA of tomato yellow leaf curl virus (TYLCV) was not detected in the seed sample obtained from plants produced from 90-day-old seedlings that were cultivated and adapted inside the nursery. The results also showed that in both protocols, using or without insecticides did not prevent the white fly from laying eggs and producing larvae on the plants. The increase was also gradual in the numbers of eggs and larvae of the white fly, as this activity peaked in the third week of transferring the seedlings to the open field, then those numbers decreased after the third week. This study also demonstrated the effect of positive seedling age (90 days old) on morphological traits related to vegetative growth, fruit production, and seed yield. Among the important benefits obtained was the ability to obtain seeds free of TYLCV in tomato plants, as well as the ability to remove nursery plants that showed early symptoms of the virus, and thus reduce the economic losses caused by the whitefly through the spread of the virus in the open fields.

Keywords: Tomato; Lycopersicon esculentum L.; age-related resistance effect; ARIR; whitefly; seed production; tomato yellow leaf curl virus; TYLCV.

1. INTRODUCTION

One of the most important food crops in the world is the tomato, which is the second-most produced and consumed vegetable globally [1]. Together with its derivatives, tomatoes are one of the main food sources of carotenoids, contributing to the western diet's estimated 80% daily need for lycopene, ascorbic acid, flavonoids, a-tocopherol, and potassium [2,1]. The favorable role of tomato consumption in the prevention of chronic diseases including cancer and cardiovascular disease has been highlighted by several epidemiological studies [3,4]. Lycopene, a highly effective radical scavenger that can combat reactive oxygen species and prevent cell damage, has been given the most credit for this effect's antioxidant activity [5]; however, other carotenoids' beneficial effects on health may also be explained by other mechanisms [6]. Additionally, tomatoes contain a variety of other substances, including phenolics and vitamin C, which may have synergistic benefits in preventing human disease [1]. Egypt produces roughly 6.246 million tonnes of fresh fruit annually from 150109 hectares, compared to the global average of 189.134 million tonnes from 5.167 million hectares [7]. One of the most destructive diseases of grown tomatoes is the tomato yellow leaf curl geminivirus (TYLCV), which is spread by the whitefly *Bemisia tabaci* (Gennadius) (*Lycopersicon esculentum* Mill.). In many tropical and subtropical countries, TYLCV causes economic losses of up to 100% in tomato crops and is expanding into new areas. Accurate detection and identification processes are required as TYLCV's economic significance grows [8]. The Tomato Yellow Leaf Curl Virus (TYLCV), one of the most well-known begomoviruses that infect tomatoes, is spread by the bacterium *Bemisia tabaci*, according to [9]. Some RNA viruses have been known to spread through seeds in the past, but TYLCV has never been identified as a seed-borne virus. Without whitefly-mediated transmission, TYLCV was found in young tomato plants in 2013 and 2014 that had grown from fallen fruits from tomato plants that had been TYLCV-infected in the previous farming season. Additionally, TYLCV-Israel (TYLCV-IL) was found in seeds and seedlings of tomato plants infected with TYLCV through both agro-inoculation and viruliferous whitefly-mediated transmission. The average transmission rate to seedlings was also 84.62% and 80.77%, respectively, and the seed infectivity ranged from 20 to 100%. Although TYLCV-infected seeds were also produced by TYLCV-tolerant tomato plants, there was less viral genome present than in TYLCV-susceptible tomato plants. TYLCV was discovered in whiteflies and recipient tomato plants six weeks after TYLCV-infected tomato plants, non-viruliferous whiteflies, and healthy tomato plants were all housed together in an insect cage. TYLCV-IL can spread through seeds, and tomato plants that grew from TYLCV-infected

seeds may act as an inoculum source. This is the first account of tomato TYLCV seed transfer. All seed bundles were determined by [10] to be TYLCV-infected. Additionally, in three of the 14 bunches, virus transmission was confirmed. Additionally, it was discovered that seeds and seedlings replicate viruses. This is the first report to show that white soybean is TYLCV's host and that the virus may be transmitted from seeds to soybean. It has been demonstrated that age-related induced resistance (ARIR), also known as age-related resistance in plants, has a significant impact on how viruses interact with plants [11,12]. For instance, as a plant's age increased, it became less susceptible to diseases like bean pod mottle virus and tomato spotted wilt tospovirus [13-15]. Likewise, it has been demonstrated that plant age increases the expression of genetic resistance to TYLCV [16]. More and more studies are being done on the plant immunity system, and novel components and functions are being assigned to participate in the stress response. The new language is also used to distinguish various participants in the overall immunity scenario [17]. To defend themselves against pathogen attacks, plants have developed a highly developed immune system that includes both innate and induced processes. Inducing the defense response in plants artificially has been done so over the past 50 years using a variety of biological and chemical inducers, which has led to the development of "induced resistance" (IR) to a subsequent pathogen attack. In glasshouse and lab settings, IR has successfully controlled disease, but it has rarely provided the same level of field protection as synthetic pesticides. But, legislation to limit the use of synthetic chemicals in agriculture is what has sparked a resurgence in interest in IR for crop protection. When combined with fungicides, bactericides, and other biological control methods, inducers can support integrated crop management plans. By integrating inducers in this way, chemical inputs can be decreased without losing effectiveness. Furthermore, novel inducers are being developed, and fresh approaches to their application in sustainable crop protection are being guided by developments in our understanding of plant defense. The use of IR in specific cropping systems will be covered in this review, along with chances to maximize its potentials, such as the creation of more potent inducers and their integration with traditional and cultural management methods [18].

This research sought to understand how age-related induced resistance (ARIR) in tomatoes develops and how it may be used to the tolerant virus. This study looked at tomato plants' age-related induced resistance (ARIR) to the tomato yellow leaf curl virus (TYLCV). First, we'll look at how plant age affects whiteflies' ability to resist the TYLCV virus in tomato plants. Second, TYLCV (Tomato Yellow Leaf Curl Virus) detection and identification in seeds derived from seedlings and transplants of two different ages (35 and 90 days old). Third, research how whitefly behavior and plant age are related. The capacity to transplant plants from the nursery that exhibit virus symptoms early and thereby lessen the financial losses caused by the white fly by spreading the virus to open fields. The fourth step involved measuring the extent to which morphological characteristics related to vegetative growth, fruit yield, and seed yield were affected by viral infection.

2. MATERIALS AND METHODS

From 2019 to 2021, this study was conducted at the Kaha Vegetable Research Farm in Egypt's Qalyubia Governorate. The experimental site's soil is categorized as clay soil. The heirloom tomato cultivar Castle Rock cv., which is TYLCV-susceptible, was employed as the sole genotype. The Vegetable Seed Production Unit, Vegetable Research Departments, Dokki, Giza, Egypt, was where the seeds were obtained. The same field environment was used to compare yield performance. To use in the two procedures for seedlings and transplanting at ages 35 and 90 days; respectively, seeds from the same lot were separated into two groups and collected from the same lot. In the current study, we investigated the effects of age-related induced resistance on tomato seedlings to produce plants that are free of the tomato yellow leaf curl virus (TYLCV). Tomato seeds, which are typically grown from seedlings, grew on January 5th (2019) in nursery trays, one seedling growing on each hill under ideal conditions for fertilization, irrigation, and pest control. After 35 days, the seedlings were moved to the field (February 10th). Second, the following is the suggested method for growing seedlings, in which the seedlings spend 90 days inside the nursery.

On November 15th, 2019 in nursery trays, one tomato seedling was planted on each hill. After 35 days (December 20th), the seedlings were transplanted into one-liter plastic round pots with a 25 cm space between each one and a 25 cm space between plant rows. 24 transplants are placed in each square meter of the nursery. After

55 days, the seedlings were moved to the field (February 15th). To get hardened transplants, a dark purple hue, and the trichomes of the transplants, are developed under conventional irrigation and fertilization settings. Whereas the number of glandular trichomes was favorably connected with the number of caught insects and negatively correlated with the attractiveness of the whitefly and the number of oviposition per leaflet and leaf. The quantity of non-glandular trichomes linked favorably with whitefly oviposition per cm^2/leaflet and per cm^2/leaf and negatively with the number of caught insects.

Additionally, several cultivars exhibited strong antixenosis (ovipositional non-preference) levels for the B. tabaci B biotype, which is connected to the type IV glandular trichome [19].

For another season, the two approaches were compared against one another. Seedlings were planted in the field under field circumstances with 50 cm between each one and one meter between plant rows. In the production of commercial tomatoes, all approved cultural methods were used. For the standard technique of generating seedlings plants and the proposed method of producing seedlings plants, respectively, harvesting began 90 and 60 days after transplanting and continued twice weekly throughout the growing season, which ended on May 28. The vegetative growth parameters for the seedling and transplanting stage were then calculated using the average of three randomly chosen plants every two weeks (fresh weight (g), dry weight (g), height (cm), number of leaves, stem diameter (cm), number of branches, the weight of leaf (g), petiole diameter (cm), the length of the leaflet (cm), the width of the leaflet (cm), length of compound leaf (cm), fresh root weight(g), dry root weight(g), root length (cm^2), Branch number per plant and plant height (cm) were measured. Fruit and yield characteristics, including average fruit weight (g), fruit number per plant, yield per plant, firmness for fruits (kg), fruit diameter (cm), fruit length (cm), and total soluble solids (TSS)(g/100g) were noted. Additionally, seed quality, including seed yield per fruit (g), germination percentage (%), seedling emergence (%), speed emergence index, and third, the association between whitefly behavior and plant age, was investigated.

2.1 Data Parameters

Observations were made on several different traits. These are:

Germination percentage (%)

This was calculated as the percentage of seedlings that emerged 21 (DAP) relative to the number of seeds that were germinated with germination paper in the laboratory.

$$\mathrm{GP\%} = \frac{\text{Seeding emerged by 21 DAP}}{\text{Number of seeds planted}}\ X\ 100$$

Speed emergence index (EI)

Seedling emergence was recorded at 9, 11, 13, 15, 17, and 19 days after planting (DAP) and used to compute EI according to the modified formula of [20].

$$\mathrm{EI} = \Sigma \frac{(\text{Plants emerged in a day})\ (\text{Day after planting})}{\text{Plants emerged by 19 days after planting}}$$

Seedling emergence percentage (E %)

This was calculated as the percentage of seedlings that emerged 21 DAP relative to the number of seeds sown per plot.

$$\mathrm{E\%} = \frac{\text{Seeding emerged by 21 DAP}}{\text{Number of seeds planted}}\ X\ 100$$

Growth rate parameter

Relative growth rate (RGR) (g/g/day) was calculated by the formulae outlined by [21].

$$\text{Relative growth rate (RGR)} = \frac{(\log e\ W2 - \log e\ W1)}{(t2 - t1)}$$

Where, W2 and W1 are the total dry weight values at times t2 and t1, respectively.

2.2 Infestation of Insects

Numbers of insect infestations

After seven days of alternate transplanting, samples of the plant's leaves were taken every seven days until the beginning of tomato fruit coloring. Each replication had 20 leaves, which were chosen at random. Paper bags containing the gathered leaf samples were used to transport them to the lab for analysis. A binocular microscope was used to carefully inspect the upper and lower surfaces of each leaf, counting and recording the number of whiteflies (*Bemisia tabaci*) (eggs and larvae). The number of whitefly eggs and larvae without and with pesticides (on 35-day-old seedlings), and the number of

whitefly eggs and larvae without and with insecticides were all noted and tallied (90-day-old transplanting). From the start of the transplantation procedure in the field until the conclusion of the plant's life, insecticide control was done at intervals of four days, taking note that the chemical pesticides are controlled right away after taking leaf samples.

The percentage of the number of tomato plants exhibiting virus symptoms (TYLCV)

The virus symptoms expressed as cumulative numbers were estimated three times, every 15 days after 45 days from transplanting. Symptoms were evaluated morphologically. The percentage of the number of plants exhibiting virus symptoms was recorded and the percent plants showing virus symptoms were estimated visually and calculated according to the following equation:

$$\text{plants showing virus symptoms: } = (plants \text{ showing } virus\ symptoms\ as\ cumulative\ numbers)/(\text{Number of total plants})\ X\ 100$$

Molecular detection of tomato yellow leaf curl virus (TYLCV) in tomato seeds:

Seeds obtained from both procedures were germinated under controlled laboratory conditions to detect the presence of tomato yellow leaf curl virus (TYLCV), the following procedure was followed:

2.3 DNA Extraction

Total DNA was extracted from leaves of infected and healthy tomato plants collected for the detection of tomato yellow leaf curl virus using the CTAB method [22].

PCR Reaction Component

The reaction mixture for DNA amplification consi sted of 1X PCR buffer, primer AV1F (5'ATGGCG AAGCGACCAG3') and AV1R (5'TTAATTTGTG ACCGAATCAT3'), $MgCl_2$, dNTPs, Taq DNA polymerase and genomic DNA. The total reaction volume was 17 µl.10X PCR buffer 2.5 µl, 1.5 Mm $Mgcl_2$ 1.5 µl, dNTPs (0.2mM) 1.00 µl, Primer (forward) 1.00 µl, Primer (reverse) 1.00 µl, Taq DNA Polymerase 0.5 µl, Genomic DNA 1.00 µl, and Sterile distilled Water 8.50 µl. All the reactions were carried out under aseptic conditions to avoid contamination for false amplifications. The thermal cycler was switched on 5 minutes before the experiment. The reaction mixture of each 17 µl was dispensed in PCR tubes (0.2ml) using a micropipette and PCR amplification was performed with thermal profile as listed below: Cycle 1: 95ºC for 5 minutes (Initial denaturation) Cycle 2: 95ºC for 1 minute (Denaturation) 50ºC for 40 seconds (Primer annealing) 72ºC for 1 minute (Polymerization) Cycle 3 72ºC for 5 minutes (Final elongation) After completion the amplified PCR products were stored at 4ºC till gel electrophoresis.

Agarose gel electrophoresis of PCR product:

1.2 % agarose gel (100 ml) was prepared using 1X TAE buffer [23]. It was properly homogenized by heating it in a microwave oven. Ethidium bromide (5 microliters) was added as a stain. The agarose solution was then poured into the gel casting tray with the combs attached to form wells. After solidification, the combs were removed and the gel was transferred in an electrophoresis unit in such a way that the wells were at negative poles. The tank was filled with 1X TAE buffer till the surface of the gel was covered. 5µl of each PCR product mixed with 2 µl of gel loading dye was slowly loaded into the wells using disposable micropipette tips. A 1kb molecular ladder was also loaded to estimate product sizes in base pairs (bp). The electrophoresis was then carried out at 120 volts till the dye migrated to the end of the gel. After the completion of this process, the gel was visualized and photographed in the gel documentation system. The size of the PCR product was determined by comparing it with the marker used in this study [23].

2.4 Experimental Design and Statistical Analysis

The statistical analysis consisted of a two-treatment experiment (two procedures; the first procedure is the common method of producing seedlings and the second procedure; is the proposed method for producing transplanting). The acquired data were statistically evaluated using Fisher's analysis of variance (given as a pairwise comparison procedure called the least significant difference (LSD) test). This test should be employed only if the overall F test rejects the hypothesis that all means are equal. If the overall test is significant, any pair of means is tested using a process similar to a standard Student's t-test. No additional tests are run if the total F ratio is not significant. When it is used, the two procedures for seedlings and transplanting

at the age of 35 and 90 days; respectively, are deemed different if the absolute difference between the two sample means is more than 5% using combined ANOVA across years with one-way randomized blocks analysis (Multiple comparisons and trends among treatment means) [24]. Minitab software was used to do all computations [25].

Confidence interval formula:

$$Confidence\ interval = \overline{x} \pm z \frac{s}{\sqrt{n}}$$

Where:

X is the mean
Z is the chosen Z-value (1.96 for 95%)
S is the sample standard deviation
N is the sample size

Understanding Confidence Intervals, we will assume that you, as a researcher, wanted to know the average weight of students in a university and since you will not measure the weight of all students; you took a random sample of 50 students and measured their average weight. Let's assume that the number you got is 70 kilograms, which somehow expresses the weight of the students of this university... But if we assume that we have already measured the weight of every student in the university, can we get the same result? Maybe yes and often not... If we assume that we take another sample, we may get the same result, or we may not get it ... So if we take a sample, and calculate the arithmetic mean, we are not entirely sure that this number is the average of absolutely all students. What do you think if instead of this single number, we take a range of numbers that has an upper and lower limit and we say we are pretty sure that the arithmetic means of all the students located between these two numbers 65 and 75 kg and this is called the Confidence Intervals (CI). It must be accompanied by a percentage that expresses the degree of confidence. So, for example, 95% CI, and 99% CI. The sense is that if we repeated the experiment a large or infinite number of times, the average weight of university students would be between these two numbers 95% of the time. If the period contains the number zero - for example (-2; 8), this means that the difference between the two groups may be zero, and therefore there may not be a significant difference.

Confidence intervals are conducted using statistical methods, such as a t-test. A t-test is a type of inferential statistic used to determine if there is a significant difference between the means of two groups, which may be related to certain features.

3. RESULTS AND DISCUSSION

Analysis of variance (One-way ANOVA) - mean square and Fisher Pairwise Comparisons for morphological traits, growth rate parameter, and seed quality in two procedures for tomato Plants were produced from seedlings and transplanting at the age of 35 and 90 days, respectively.

Results in Tables (1 and 2) the ANOVA table show a significant mean difference among the 35-day-old seedling and 90-day-old transplant procedures for all various characters at 0.05 level of significance. The means and the periods of confidence intervals for all traits of the 90-day-old transplant procedure were significantly greater than the means and the periods of confidence intervals for all traits of the 35-day-old seedling procedure.

Examination of the numbers of whitefly eggs and larvae for both procedures during various weeks with and without pesticide application 90-day-old transplants and 35-day-old seedlings.

Two things may be inferred from the data in Fig. 1. First, using insecticides in either methodology did not prevent the whitefly from being present, laying eggs, and developing larvae on plants. The second point is that the whitefly's egg and larval populations gradually increased; they peaked during the third week of transferring seedlings and transplants to the open field and then started to decline.

Comparing the various treatments for both procedures for 35-day-old seedlings and 90-day-old transplants, it will be possible to determine the numbers of whitefly eggs and larvae as well as the proportion of tomato plants that are showing signs of the virus (TYLCV).

According to Fig. 2's findings, whether insecticides were used or not, the Interval plot for the number of whitefly eggs and larvae with the 90-day-old procedure was lower than the number of eggs and larvae with the 35-day-old procedure.

Table 1. Analysis of variance (One-way ANOVA)-mean square and Fisher Pairwise Comparisons for morphological traits in two procedures for tomato seedlings and transplanting at the age of 35 and 90 days, respectively

Traits[1]		Analysis of Variance[2]		Fisher Pairwise Comparisons[3]	
		Procedures (df=1)	Error df=10)	Mean	95% C.I
FW	S-35	170006*	44	3.658[b]	(2.362;9.678)
	T-90			241.71[a]	(235.69;247.73)
DW	S-35	2574.83*	0.03	1.003[b]	(0.849;1.156)
	T-90			30.299[a]	(30.145;30.453)
H	S-35	2852.08*	2.82	12.333[b]	(10.807;13.860)
	T-90			43.167[a]	(41.640;44.693)
NL	S-35	3675.00*	1.90	5.500[b]	(4.246;6.754)
	T-90			40.500[a]	(39.246;41.754)
SD	S-35	5.527*	0.038	0.261[b]	(0.0823;0.4397)
	T-90			1.618[a]	(1.440;1.797)
NB	S-35	14.083*	0.083	0.00[b]	0.262;0.262)
	T-90			2.167[a]	(1.904;2.429)
WL	S-35	12.507*	0.011	0.598[b]	(0.5018;0.6952)
	T-90			2.640[a]	(2.543; 2.737)
PDL	S-35	0.324*	0.001	0.123[b]	(0.102;0.144)
	T-90			0.452[a]	(0.430;0.473)
LL	S-35	147.554*	0.801	2.627[b]	(1.813;3.441)
	T-90			9.640[a]	(8.826;10.455)
WIL	S-35	47.1121*	0.1781	1.284[b]	(0.900;1.668)
	T-90			5.246[a]	(4.862;5.630)
LCL	S-35	1147.66*	1.38	7.274[b]	(6.208;8.341)
	T-90			26.833[a]	(25.767;27.900)
FRW	S-35	18548.2*	1.4	1.112[b]	(0.026;2.198)
	T-90			79.743[a]	(78.657;80.829)
RL	S-35	950.769*	0.566	5.277[b]	(4.593;5.961)
	T-90			23.079[a]	(22.395;23.763)
NF	S-35	6.750*	0.150	0.00[b]	(0.352;0.352)
	T-90			0.548[a]	(1.148;1.852)
WF	S-35	34.527*	0.326	0.00[b]	(0.520;0.520)
	T-90			3.392[a]	(2.872;3.913)
DF	S-35	11.0419*	0.072	0.00[b]	(0.244;0.244)
	T-90			1.919[a]	(1.674;2.163)
LF	S-35	6.661*	0.008	0.00[b]	(0.084;0.084)
	T-90			1.490[a]	(1.405;1.574)

*[1]: FW =The fresh weight; DW =The dry weight; H =The height; NL =The number of leaves; SD =The Stem diameter; NB = The number of branches; WL= The weight of leaf; PDL = The Petiole diameter; LL = The length of the leaflet; WIL = The width of the leaflet; LCL = The length of compound leaf; FRW = The fresh root weight; RL= The root length; NF = The number of fruits; WF = The weight of fruit; DF = The diameter of fruit; LF = The length of fruit; S-35 = 35-day-old seedlings; and T-90 = 90-day-old transplants. [2]: df = Degrees of freedom. [3]: 95% C. I = confidence intervals that contain 95% of expected observations. The means within columns followed by the same letter for one character are not statistically different at the 5% level (Fisher Pairwise Comparisons). * = Significance at 0.05 level of significance; and ns = non-significance*

The number zero, which corresponds to a difference that is not statistically significant, appears in the Fisher individual 95% confidence intervals for the number of whitefly eggs of 35-day-old seedlings treated with insecticides and the 35-day-old seedlings treated without insecticides. The same is true for the Fisher individual 95% confidence intervals for the number of whitefly eggs of 90-day-old transplants treated with insecticides and the 90-day-old transplants treated without insecticides. Fisher's individual 95% confidence intervals for the remaining treatment periods do not contain the number zero, indicating a significant difference.

Table 2. Analysis of variance (One-way ANOVA) - mean square and Fisher Pairwise Comparisons for morphological traits, growth rate parameter, and seed quality in two procedures for tomato Plants were produced from seedlings and transplanting at the age of 35 and 90 days, respectively

Traits[1]		Analysis of Variance[2]		Fisher Pairwise Comparisons[3]	
		Procedures (df=1)	Error (df=10)	Mean	95% CI
PH	PS-35	3018.06*	1.47	48.259[b]	(47.156;49.362)
	PT-90			79.977[a]	(78.874;81.079)
NB	PS-35	127.205*	0.131	8.251[b]	(7.922;8.580)
	PT-90			14.763[a]	(14.434;15.092)
NL	PS-35	3026.28*	1.95	45.930[b]	(44.659;47.201)
	PT-90			77.691[a]	(76.420;78.962)
LA	PS-35	34631.4	29.3	159.081[b]	(154.157;164.004)
	PT-90			266.52[a]	(261.60;271.45)
FW	PS-35	3278.56	4.04	55.516[b]	(53.686;57.345)
	PT-90			88.574[a]	(86.745;90.403)
NF	PS-35	630.069*	0.760	20.954[b]	(20.162;21.747)
	PT-90			35.447[a]	(34.654;36.239)
AY	PS-35	3713089*	5384	1882.0[b]	(1815.2;1948.7)
	PT-90			2994.5[a]	(2927.8;3061.2)
FF	PS-35	2.087*	0.006	0.886[b]	(0.8133;0.9577)
	PT-90			1.720[a]	(1.6473;1.7917)
DF	PS-35	12.471	0.078	4.490[b]	(4.2349;4.7441)
	PT-90			6.528[a]	(6.274;6.783)
LF	PS-35	15.568*	0.120	3.274[b]	(2.9588;3.5899)
	PT-90			5.552[a]	(5.237;5.868)
TSS	PS-35	8.796*	0.033	4.378[b]	(4.2110;4.5450)
	PT-90			6.090[a]	(5.9233; 6.2574)
RGR	PS-35	0.163*	0.003	0.633[b]	(0.5864;0.6803)
	PT-90			0.867[a]	(0.8197; 0.9136)
SY	PS-35	0.066	0.0002	0.417[b]	(0.40420;0.42980)
	PT-90			0.565[a]	(0.55236;0.57797)
GSP	PS-35	432.000*	0.567	83.833[b]	(83.149;84.518)
	PT-90			95.833[a]	(95.149;96.518)
SE	PS-35	225.333*	1.333	54.000[b]	(52.950;55.050)
	PT-90			62.667[a]	(61.616; 63.717)
SEI	PS-35	141.460*	0.681	27.988[b]	(27.238;28.739)
	PT-90			34.855[a]	(34.104;35.606)
SF	PS-35	3246429*	9587	1498.37[b]	(1409.30;1587.44)
	PT-90			2538.6[a]	(2449.6;2627.7)
USF	PS-35	290814*	338	317.77[b]	(301.05;334.49)
	PT-90			629.12[a]	(612.40;645.84)

*[1]: PH = The plant height; NB = The number of branches per plant; NL = The number of leaves per plant; LA =The leaf area per plant; FW = The fruits weight per plant; NF = The number of fruits per plant; AY = The average yield per plant; FF = The firmness of fruit; DF = The diameter of fruit; LF = The length of fruit; TSS = The Total soluble solids in fruit; RGR = The Relative growth rate; SY = The seed yield per fruit; GSP = The Germination seeds percentage; SE = seedling emergence; SEI = The speed emergence index; SF = The suitable fruit yield for extracting seeds; USF = The unsuitable fruit yield for extracting seeds; PS-35 = The plants were produced from seedlings at the age of 35 days; and PT-90 = The Plants were produced from transplants at the age of 90 days. [2]: df = Degrees of freedom. [3]: 95% C. I = confidence intervals that contain 95% of expected observations. The means within columns followed by the same letter for one character are not statistically different at the 5% level (Fisher Pairwise Comparisons). * = Significance at 0.05 level of significance and ns = non-significance*

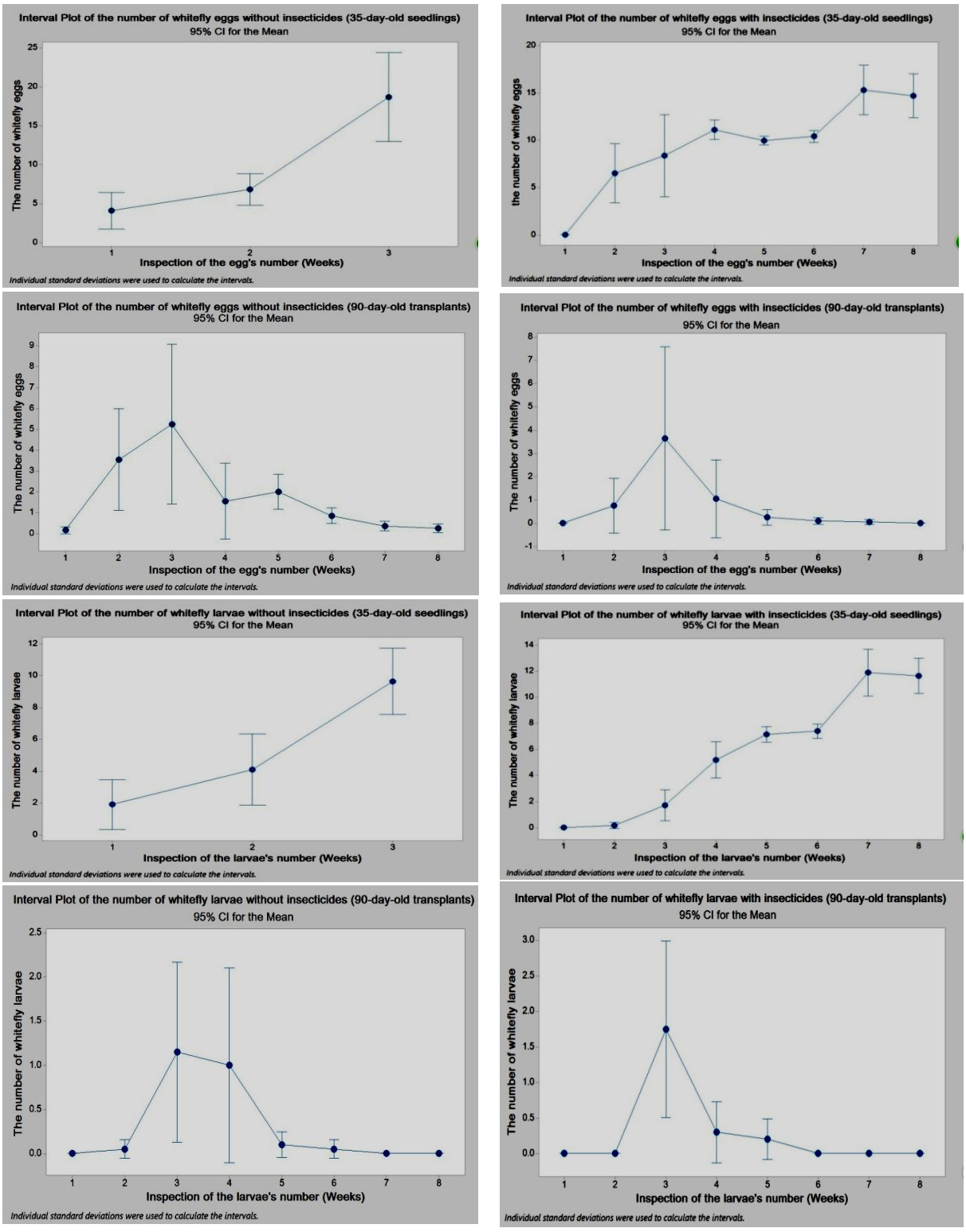

Fig. 1. Interval plot for inspection of the number of whitefly eggs and larvae during different weeks with and without insecticide application for both procedures 35-day-old seedlings and 90-day-old transplants

95% C. I = confidence intervals that contain 95% of expected observations

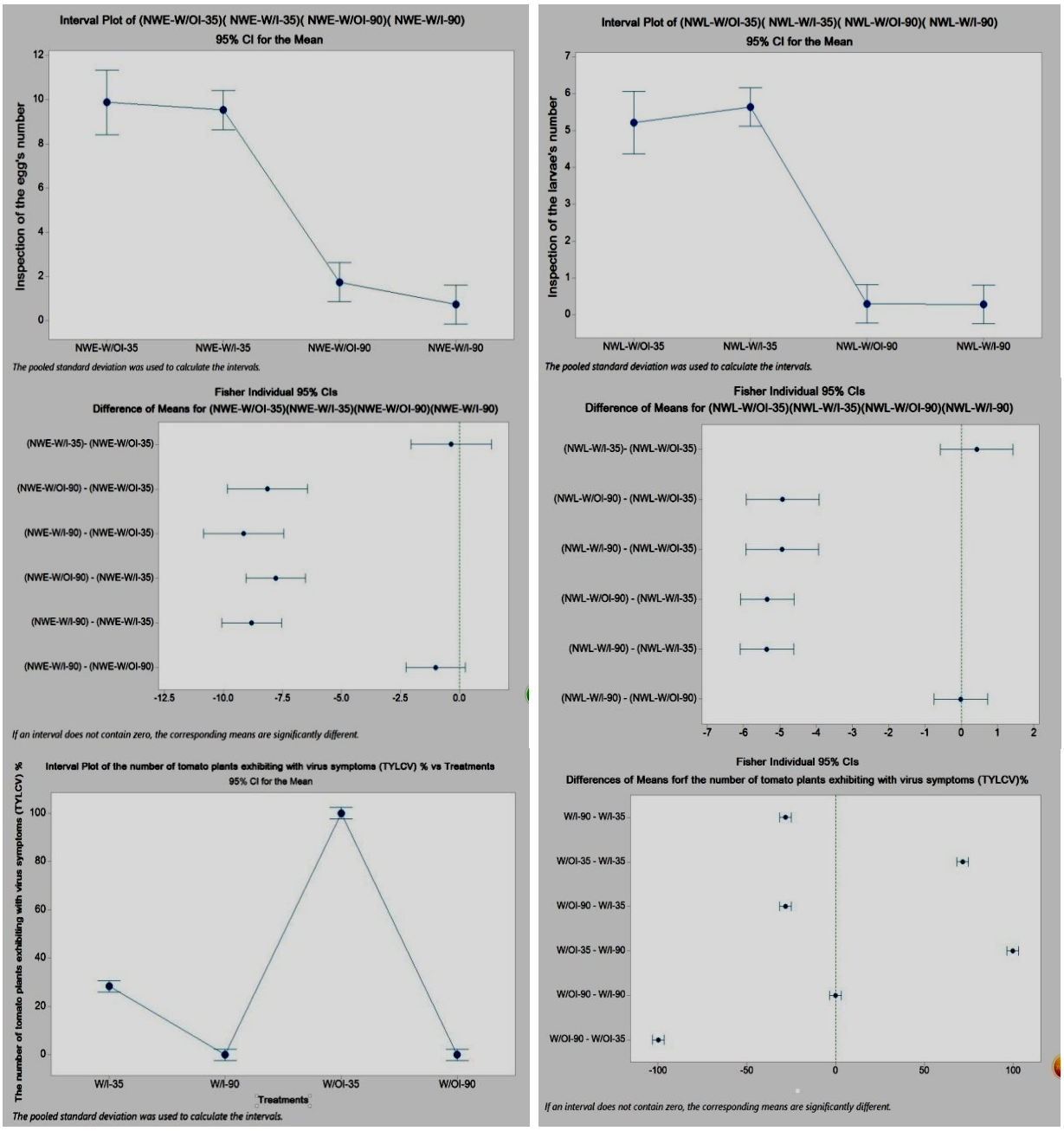

Fig. 2. Interval plot and Fisher individual 95% confidence intervals for inspection of the number of whitefly eggs and larvae and the percentage of the number of tomato plants exhibiting virus symptoms (TYLCV) by comparing the different treatments to each other for both procedures for 35-day-old seedlings and 90-day-old transplants

95% C. I = confidence intervals that contain 95% of expected observations. NWE-W/OI-35 = the number of whitefly eggs without insecticides (35-day-old seedlings); NWL-W/OI-35 = the number of whitefly larvae without insecticides (35-day-old seedlings); NWE -W/I-35 = the number of whitefly eggs with insecticides (35-day-old seedlings); NWL-W/I-35 = the number of whitefly larvae with insecticides (35-day-old seedlings); NWE -W/OI-90 = the number of whitefly eggs without insecticides (90-day-old transplants); NWL-W/OI-90 = the number of whitefly larvae without insecticides (90-day-old transplants); NWE -W/I-90 = the number of whitefly eggs with insecticides (90-day-old transplants); NWL-W/I-90 = the number of whitefly larvae with insecticides (90-day-old transplants); W/OI-35 = 35-day-old seedlings-without insecticides; W/I-35 = 35-day-old seedlings-with insecticides; W/OI-90 = 90-day-old transplants-without insecticides; and W/I-90 = 90-day-old transplants-with insecticides

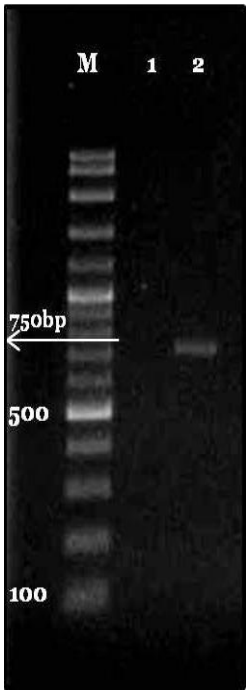

Fig. 3. TYLCV amplified product on agarose gel using primers AV1F (5'ATGGCGAAGCGACCAG3') and AV1R (5'TTAATTTGTGACCGAATCAT3'). Molecular detection of tomato yellow leaf curl virus (TYLCV) in tomato seeds; Seeds obtained from both procedures were germinated under a controlled laboratory. The aqueous phase was subjected to agarose gel electrophoresis and stained with ethidium bromide. DNA of sample (2), yielded one band of TYLCV (720 bp), and no PCR products were amp1ified from samples (1)

*WHERE, **M** = marker; **sample 1** = a sample of fresh leaves obtained from germinating seeds of the procedure (90-day-old transplants); and **sample 2** = a sample of fresh leaves obtained from germinating seeds of the procedure (35-day-old seedlings)*

The number zero, which corresponds to a difference that is not statistically significant, appears in the Fisher individual 95% confidence intervals for the number of whitefly larvae of 35-day-old seedlings treated with insecticides and the 35-day-old seedlings treated without insecticides. The same is true for the Fisher individual 95% confidence intervals for the number of whitefly larvae of 90-day-old transplants treated with insecticides and the 90-day-old transplants treated without insecticides. Fisher's individual 95% confidence intervals for the remaining treatment periods do not contain the number zero, indicating a significant difference.

By comparing the remaining treatments, the interval plot of 35-day-old seedlings without insecticide treatment showed the highest percentage of tomato plants exhibiting virus symptoms (TYLCV) (100%) and the interval plot of 90-day-old transplants with and without insecticide treatment showed the lowest percentage of tomato plants exhibiting virus symptoms (TYLCV) (0%).

The percentage of tomato plants showing virus symptoms (TYLCV) of 90-day-old transplants treated with insecticides and the 90-day-old transplants treated without insecticides have Fisher individual 95% confidence intervals that both contain zero, which indicates that the treatments are not statistically different from one another.

3.1 The Efficiency of the Plant Age on Tomato Yellow Leaf Curl Virus (TYLCV) Multiplication

Results in Fig. (1) showed that the DNA of sample 2 (=35-day-age seedling procedure) yielded one band of TYLCV (770 bp), and no PCR products were amp1ified from sample 1 (= 90-day-age transplants procedure) by multiplex PCR using two sets of primers AV1F (5'ATGGCGAAGCGACCAG3') and AV1R

(5'TTAATTTGTGACCGAATCAT3'). The age of the plant is closely related to the plant's tolerance to infection with the Tomato yellow leaf curl virus (TYLCV), and this was represented in the production of 90-day-old seedlings before transferring them to the open field.

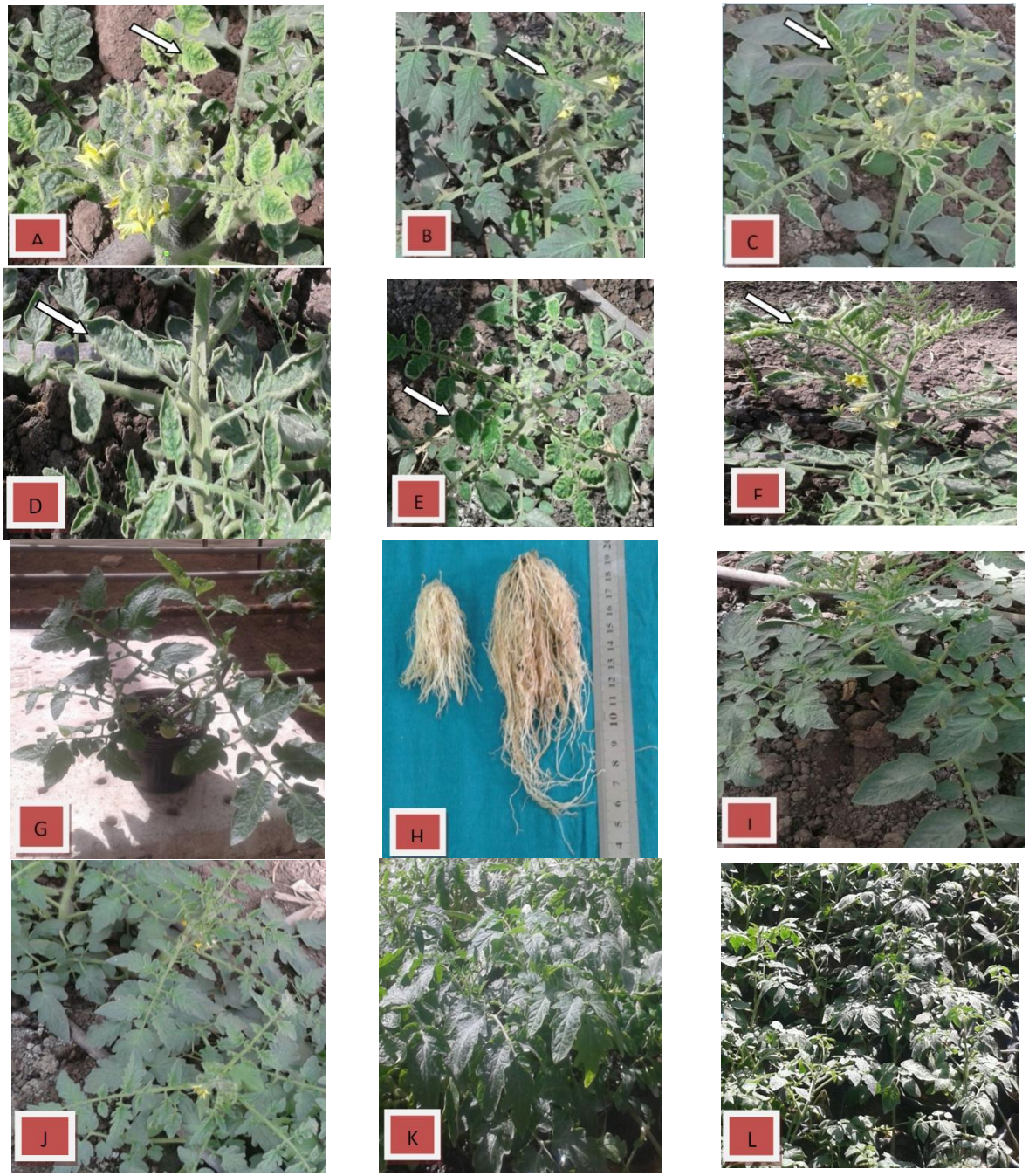

Fig. 4. Tylcv symptom severity scale. Severity scores were based on a 0 - 4 scale developed by avrdc where; 0- no symptoms (pictures for healthy plants g; i; j; k; and l) 1- slight yellowing (mild symptom - picture b); 2- leaf curling and yellowing (moderate symptom – picture a); 3- yellowing, curling and cupping (severe symptom pictures c;d; and e); 4- severe stunting, curling, and cupping; plant stops the growth (very severe symptom - picture f)

Source: [26]. Tylcv symptom severity scale that shows leaf reduction, leaf curl upward with yellowing of the new leaves on tomato was presented with the 35-day-old seedlings procedure, compared with healthy plant produced from the 90-day-old transplants procedure. Picture h = fresh root for the 90-day-old transplant procedure (on the right) and fresh root for the 35-day-old seedlings procedure (on the left)

All symptoms (leaf reduction, leaf curling, distortion, and general stunting with or without yellowing) of Tomato yellow leaf curl virus (TYLCV) infection appeared on the plants resulting from the 35-day-age seedling procedure, Contrary to the plants resulting from the other procedure, the 90-day-age transplants procedure which did not show any symptoms of the virus (Fig. 2).

The previous results related to many different crops have been found to have host plant resistance to insects. In certain instances, the resistance only manifests itself at particular plant developmental stages. For instance, the plant part, plant age, and environmental conditions can all affect the epicuticular lipids that contribute to resistance against herbivorous insects [27]. Another illustration is how, as plants get older, a cultivar of Brassica oleracea becomes more resistant to the cabbage whitefly [28]. Similar results have been reported for Solanum lycopersicum's resistance to tomato leaf miners [29]. Additionally, it has been demonstrated that resistance levels can range between various plant sections [30,31]. Depending on a variety of internal and external circumstances, the outcome of virus-plant interaction following virus penetration into plants might range from immunity to severe disease progression [32]. The interactions between plants and plant pathogens like viruses can be strongly impacted by environmental conditions like temperature and water availability, which can change how diseases develop [33]. The most significant parameters influencing virus-plant interactions are, fundamentally, plant resistance and virus infectiousness. On the plant side, a variety of elements, including plant cultivar and age, among others, have been proven to alter virus resistance [32]. It has been demonstrated that age-related induced resistance (ARIR), also known as age-related resistance in plants, has a significant impact on how viruses interact with plants [11,12]. For instance, as a plant's age increased, it became less susceptible to diseases like bean pod mottle virus and tomato spotted wilt tospovirus [13-15]. Likewise, it has been demonstrated that plant age increases the expression of genetic resistance to TYLCV [16]. On the other hand, little is known about tomato ARIR's defense against TYLCV.

It could be concluded that The DNA of the Seed sample obtained from plants of the 35-day-old seedling procedure yielded one band of TYLCV (770 bp), and by multiplex PCR using two sets of primers AV1F (5'ATGGCGAAGCGACCAG3' and AV1R (5'TTAATTTGTGACCGAATCAT3'), no PCR products were amplified from the Seed sample obtained from plants of the 90-day-old transplants procedure. The creation of 90-day-old seedlings before transferring them to the open field reflected the fact that the age of the plant is closely related to the plant's tolerance to infection with the Tomato Yellow Leaf Curl Virus (TYLCV). The development of tomato seeds free of the virus confirmed that the expression of TYLCV resistance increased with plant age. Regarding the behavior of the whitefly, the findings demonstrated that utilizing or not using pesticides in either procedure did not prevent the whitefly's presence, egg laying, or larval generation on plants. The number of whitefly eggs and larvae increased gradually as well; they peaked in the third week after the seedlings and plantings were moved to the open field, and then they started to decline.

4. CONCLUSION

The results of this study proved that the age of the plant is closely related to the ability of the plant to withstand infection with the tomato yellow leaf curl virus (TYLCV). The DNA of the tomato yellow leaf curl virus (TYLCV) was identified from a sample of seeds obtained from plants obtained from 35-day-old seedlings. On the contrary, the DNA of tomato yellow leaf curl virus (TYLCV) was not detected in the seed sample obtained from plants produced from 90-day-old seedlings that were incubated and acclimatized inside the nursery. The results also showed that in both protocols, using or without insecticides did not prevent the white fly from laying eggs and producing larvae on the plants. The increase was also gradual in the numbers of eggs and larvae of the white fly, as this activity peaked in the third week of transferring the seedlings to the open field, then those numbers decreased after the third week. This study also demonstrated the effect of positive seedling age (90 days old) on morphological traits related to vegetative growth, fruit production, and seed yield. Among the important benefits obtained was the ability to obtain seeds free of TYLCV in tomato plants, as well as the ability to transplant nursery plants that showed early symptoms of the virus, and thus reduce the economic losses caused by the whitefly through the spread of the virus in the open fields.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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