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دراسة لإعداد الذبابة البيضاء ومدى انتشار فيروس تجعد واصفرار اوراق البندورة في المناطق الشمالية من الضفة الغربية

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الملخص

أجريت دراسة لمعرفة مدى انتشار فيروس تجعد واصفرار أوراق البندورة على نبات البندورة في محافظتي طوباس وجنين وذلك خلال المواسم المختلفة لزراعة البندورة. وبشكل عام كانت الإصابة بهذا الفيروس عالية في محافظة طوباس مقارنة بمحافظة جنين، كذلك كان انتشار الفيروس أعلى ما يمكن خلال موسم زراعة البندورة الصيفي في جميع مناطق الدراسة مقارنة بمواسم الزراعة الأخرى حيث سجلت أعلى إصابة في منطقة الفارعة لتصل إلى ّ95% تلتها منطقة قشدة حيث وصلت الإصابة بها إلى 70%. احتلت الزراعة الخريفية للبندورة المرتبة الثانية من حيث مدى انتشار الفيروس فيها حيث تراوحت الإصابة من 40-70% في محافظة طوباس و 20-30% في محافظة جنين. بالنسبة للزراعة الربيعية والربيعية المبكرة كانت أكثر أمنا لدى المزارع كون الإصابة فيها قليلة مقارنة بالمواسم الأخرى حيث تراوحت الإصابة من 20-41% في محافظة طوباس ومن 21-29% في محافظة جنين. ومن ناحية أخرى بينت الدراسة التي أجريت على الذبابة البيضاء أن أعداد هذه الحشرة تتغير خلال السنة في محافظتي طوباس وجنين فقد سجل أعلى انتشار لهذه الحشرة في شهري تموز واب في محافظة طوباس في حين كان موعد الفوران لها هو شهر اب في محافظة جنين. إضافة لذلك كان ظهور هذه الحشرة مبكرا في محافظة طوباس حيث سجل أول ظهور لها خلال شهر شباط في حين تأخر ظهورها حوالي شهرين حتى ظهرت بمحافظة جنين.

ABSTRACT

Whitefly Population and Incidence of Tomato Yellow Leaf Curl Virus in Tomato Fields Grown in the northern regions of the West Bank

A survey was conducted in 2004/2005 to study the frequency of tomato yellow leaf curl virus's infection of tomato during different growing seasons in the Tobas and Jenin districts. The maximum viral incidence was recorded in Tobas, compared with Jenin district in all growing seasons. The viral incidence was much higher on summer grown tomato in both districts

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compared with the other growing seasons. The maximum viral incidence during the summer growing season was recorded in Al-Far'a (95%), followed by Kashda (70%). Autumn was considered the second most serious growing season of tomato as the viral incidence ranged from 40-70% and 20-30% in Tobas and Jenin districts, respectively. In spring and early spring growing seasons the viral infection was much lower in both districts compared with the other growing seasons. In this season the viral incidence was recorded to be 20-41% in Tobas and 21-29% in Jenin district. In addition, studying the population trend of the whitefly vector in Tobas and Jenin districts showed that the population had a seasonal change throughout the year. The maximum whitefly population was recorded between July and August to be in the Tobas district, and in August to be in the Jenin district. Also, in Tobas the whiteflies started to appear during February, which is two month prior to its appearance in the Jenin district.

Introduction:

Tomato (*Lycopersicon esculentum*) is the major vegetable crop in Palestine depending on the availability of irrigation. The annual production of tomato in Palestine is about 207,188 metric tons which occupies about 32% of the total vegetable production in the country (Palestinian Central Bureau of statistics (PCBS), 2006: 70-100). Tomato belongs to one of the most economically important families of angiosperms and contains many of the commonly cultivated plants. Solanaceae is the most variable of all crop species in terms of agricultural utility and the most valuable in terms of vegetable crops (van der Hoeven *et. al*. 2002). Worldwide, tomatoes are an important part of a diverse and balanced diet (Willcox *et. al.* 2003)

Tomato yellow leaf curl virus (TYLCV) is the generic name given to a complex of viral species occurring in the Middle East which causes severe disease in economically important crops, including tomato. The virus is the major disease infecting tomato in many tropical and subtropical regions, and yield losses can reach up to 100% (Czosnek *et. al.* 1990, Pico *et. al*. 1996, Moriones *et. al*. 2000, Czosnek *et al.* 2001). In Jordan, Anfokal *et. al*. (2005) reported that TYLCV was widely infecting tomatoes planted in several regions of the kingdom including Al-Mafraq, central and north Jordan Valley and north and south Amman. The highest rate of TYLCV infection was recorded in Al-Mafraq (76%), whereas, samples collected from the northern Jordan Valley showed the lowest disease incidence (13%).

The host range of the virus is quite broad, infecting plants belonging to several botanical families including solanaceae, malvaceae and

lequiminasae (Oetting and Yunis, 2004, Sawalha, 2000, Mansour and Al-Musa, 1992).

TYLCV is transmitted by a tropical whitefly, Aleurodid *Bemisia tabaci* Genn, and the virus failed to infect plants when inoculated mechanically (Brown and Czosnek, 2002; Polston and Sherwood, 2003). The virus, much like other Gemini viruses, is not seed-transmissible (Kashina *et al.,* 2003). The causal agent of the disease has been isolated and identified as a single-strand DNA containing geminivirus species of the genus *Begomovirus,* and most of which posses a monopartite genome (Czosnek *et. al.* 1988, Fauquet, et. al., 2003).

Tomato production in Palestine has not reached its full potential and tomato growers still take the risk and continue to grow the crop (PCBS), 2006: 70-100). The reasons for this kind of situation may be connected with climate and poor agricultural practices and, above all, the high level of pests and diseases. As TYLCV is expected to be the key disease of tomato responsible for the continuous crop failure, this research aims to study the incidence of the disease in tomato fields established in the major tomato growing sites in Palestine. Jenin and Tobas districts were selected for study because these regions are considered to be the major tomato growing sites in the country. The annual tomato production in these regions comprise 36% of the total tomato production in the West Bank ((PCBS), 2006: 70-100). Also, the research aims to study the population trend of the whitefly vector throughout the year to explain the variation in viral incidence in the studied regions.

Regions of study

Several regions of study were selected in the Jenin and Tobas districts since they are the major contributors for tomato production in Palestine. The fields were selected in Al-Zababdeih, Al-Jededeih, and Qabatyya regions to represent the Jenin district, whereas the fields of Al-Far'a and Kashda were selected to represent the Tobas district.

Growing seasons:

Tomato was planted in the studied regions as follows:

A. Open field culturing was mainly practiced by tomato farmers in the Tobas and Jenin districts in the following tomato growing seasons:
    1. Early spring: the seeds were planted in February under protected plastic tunnels and the seedlings were transplanted in the middle of March.

2. Spring season: the seeds were planted during the first week of March and the seedlings were transplanted in the middle of April. This is considered the main planting.
3. Summer season: the seeds were planted in April and May and the seedlings were transplanted starting from the middle of May until the middle of June.
4. Autumn season: the seeds were planted in June until August and the seedlings were transplanted starting from the middle of July until September.

In the summer and autumn growing seasons, the farmers used to cover tomato with muslin after transplanting for five to six weeks to avoid whitefly attack and other invasive insects.

B. Greenhouse culturing was practiced by few tomato farmers in Al-Jededeih and Qabatyya regions. Therefore, tomato was transplanted in the beginning of April and cultured for about eleven months under well-developed protective conditions and extensive pesticide spray.

Fields visitation and the duration of the study

The selected fields were visited weekly in a regular manner for sample collection starting from March 2004 until February 2005. Leaf samples were collected randomly from the top part of plant and prepared for lab testing.

Culturing practices

Tomato was planted in the studied area in wide rows of about 1 to 1.2 m wide with a distance between plants ranging from 30 to 50 cm, depending on the vegetative growth of tomato varieties. The land was first prepared by hoeing twice and then adding manure. Synthetic fertilizers were added in three equal parts, the first when preparing the land, the second after transplanting, and the third at fruit setting.

PCR testing

The polymerase chain reaction was employed as described by Navot *et. al.* (1992), Campbell and Reece (2005: 391-394) and Tortora *et. al.* (2002: 254-255) using TYLCV-specific oligonucleotide primers. Sub-genomic fragments of the virus genome were amplified. The primers were purchased from the Alltech Company, Paisley, UK. The primer sequences were from 5' to 3', P1V, ATACTTGGACACCTAATGGC, nucleotides (nt) 61-80, and P4C, TGGACATCTAGACCTAAG, nt. 2054-2071. The sequence of the P1V corresponds to the viron positive strand, whereas the P4C is complementary to the viron strand. Results were recorded as described by Sawalha (2000: 55-58).

Monitoring of whitefly population

The population trend of *B. tabaci* was monitored throughout the year in the Tobas and Jenin districts. Tomato growing sites were selected in Al-Far'a and Qabatyya regions to represent the Tobas and Jenin districts, respectively. Tomato leaves were inspected monthly and the number of adult whiteflies was counted and estimated per centimeter square of the leaf. The whitefly survey was carried out starting from January 2004 until December 2005.

Statistical analysis

Analysis of the data was conducted using the Two-Sample Tests of Proportions to compare virus occurrence in the studied regions. The data obtained from the last sample collection which revealed the maximum percentage of virus infection were used in the analysis (table 1). The results were analysed using a level of significance when ά = 0.05 (Lind *et. al.* 2005: 262-263).

Table 1: Sample collected during the last visit to the studied regions

	Early Spring		Spring		Summer		Autumn		Total	
Region	NSC	NIS	NSC	NIS	NSC	NIS	NSC	NIS	NSC	NIS
Far'a	100	30	100	41	100	95	100	70	Σ 400	Σ 236
Kashda	100	20	100	26	100	70	100	40	Σ 400	Σ 156
Al-Jededeih	100	22	100	22	100	30	100	20	Σ 400	Σ 94
Al-Zababdeih	100	21	100	21	100	32	100	22	Σ400	Σ 96
Qabatyya	100	24	100	29	100	50	100	30	Σ 400	Σ 133
Total	Σ 500	Σ 117	Σ 500	Σ 139	Σ 500	Σ 227	Σ 500	Σ 182	Σ 2000	Σ 715

NSC: Number of sample collected
NIS: Number of infected samples

Results

A subgenomic fragment of TYLCV with a fragment length of about 2000 base pair (bp) was amplified by a combination of P1V (20-mer primer from the intergenic region) with 18-mer primer (P4C). The PCR was able to detect TYLCV from infected tomato by developing obvious DNA bands when electrophoresed in agarose gel for 90 minutes (Plate 1).

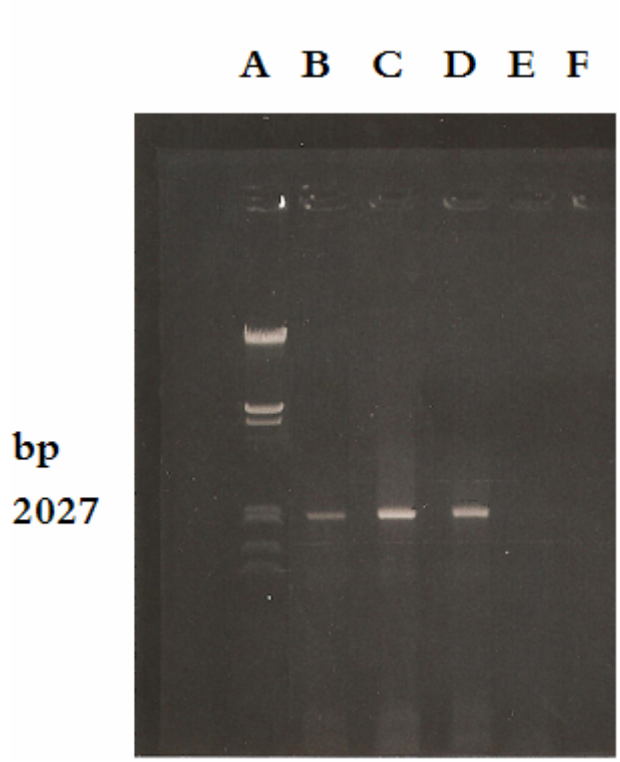
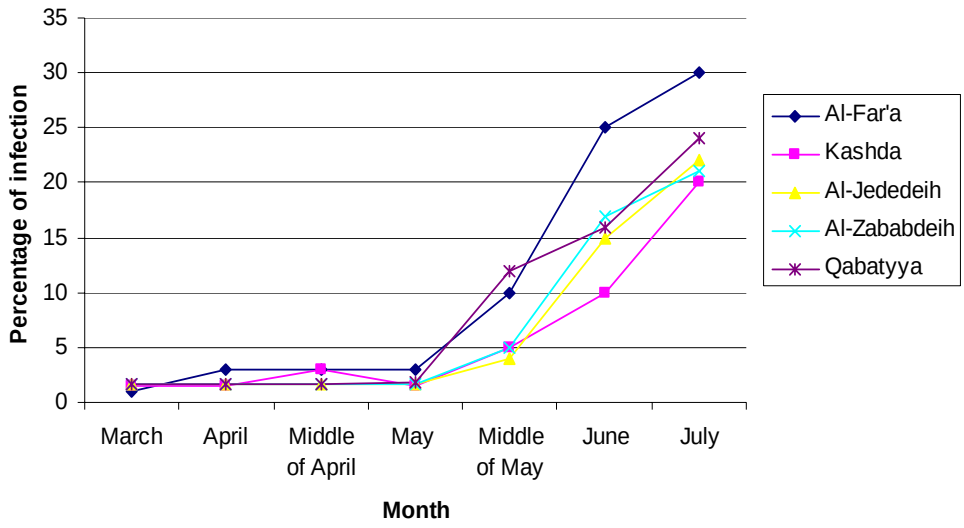


Plate 1: Agar gel electrophoreses of amplified PCR products of TYLCV DNA from infected tomato plants. The primers are P1V and P4C.
Lane A: DNA size marker (*Lambda Hind III Eco R1*, 123-21226 bp).
Lane B-D: Infected tomato samples
Lane E & F: Healthy tomato samples

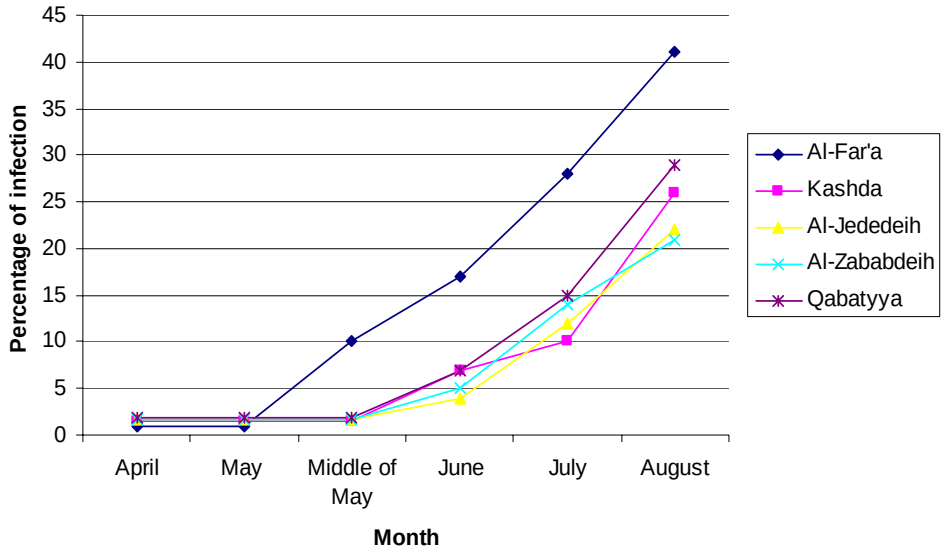
Tomato infection with TYLCV in early spring season began to develop about one month after crop transplanting in the studied regions except for Al-Far'a, which showed virus infection at least one week earlier than the other regions. Therefore, the viral incidence in Al-Far'a region started in the beginning of April and grew rapidly to reach 30% infection within 90 days. In Kashda region, the virus infection started 15 days after Al-Far'a, and the disease incidence grew less rapidly to reach the maximum infection of 20% within 3 months. As tomato transplanting in the Jenin district was delayed one month after the Tobas district, the first record of viral infection in this region occurred in the middle of May and grew gradually until 21-24% virus incidence (Fig. 1).

Figure 1: Incidence and disease development of TYLCV in tomato fields during the early spring season

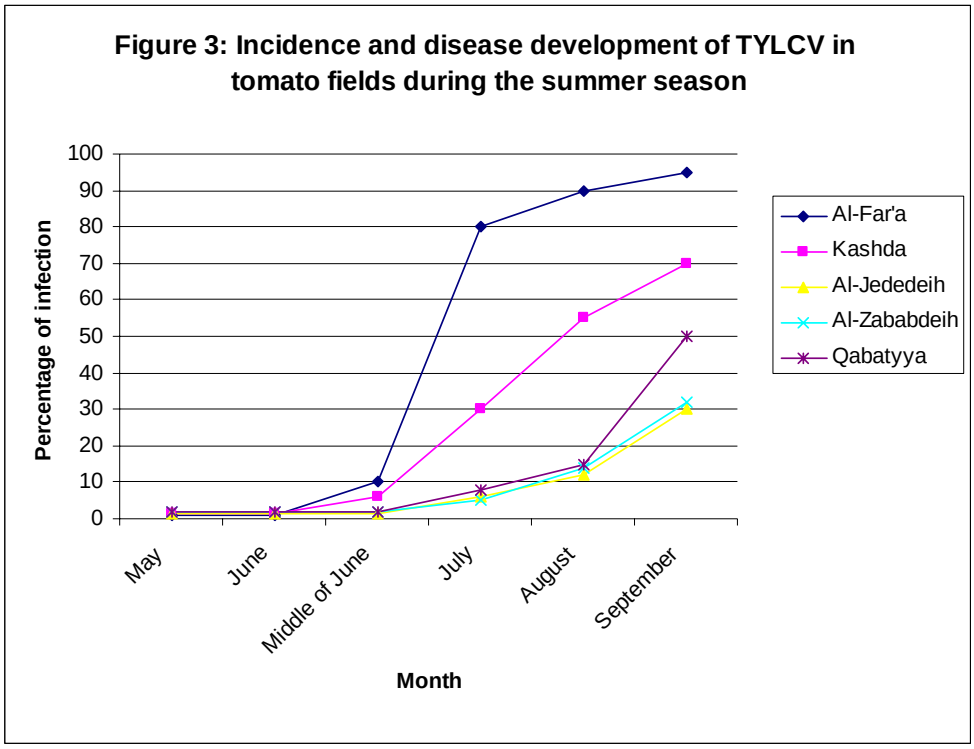


In spring season, the first record of viral infection in Al-Far'a region appeared in the middle of May and reached the maximum viral incidence of 41% in the middle of July and August whereas the virus infection in Kashda began about two weeks later to reach the maximum incidence of 26% in the beginning of August. In the Jenin district, the first virus infection started in June and the incidence increased in Qabatyya to reach 29% infection in August, while in case of Al-Zababdeih and Al-Jededeih region the maximum infection was recorded in the same month to be 21 and 22%, respectively (Fig. 2).

Figure 2: Incidence and disease development of TYLCV in tomato fields during the spring season

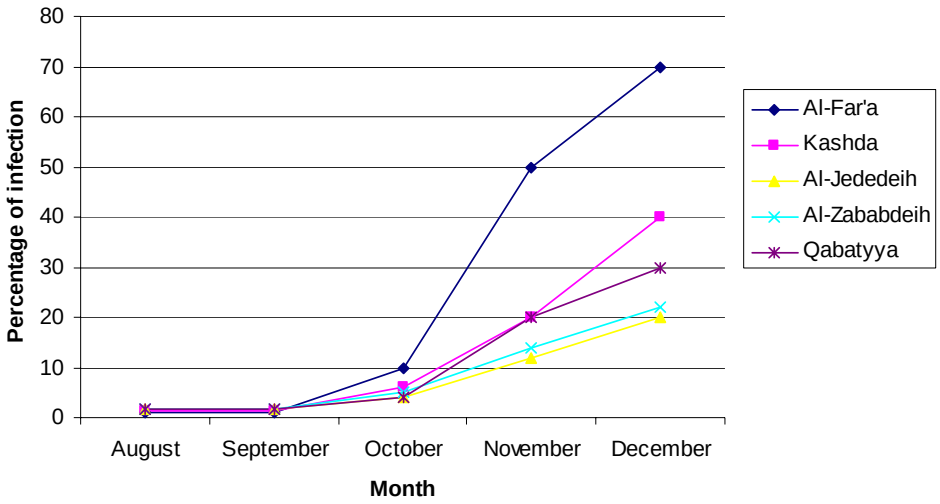


In summer season, the virus infection in Al-Far'a region started in the middle of June and grew rapidly to reach the maximum incidence of 95% in beginning of September. In Kashda, the same case occurred for the first virus record and the virus infection increased until it reached the maximum value of 70% in end of September. In Jenin district, the virus infection started in July and continued until the maximum infection of 30-50% occurred in September (Fig. 3).



In autumn season the first disease infection was recorded in beginning of October for all studied regions. In Al-Far'a the virus infection started from 10% in the beginning of October and continued until the maximum infection of 70% in December. In Kashda, the viral incidence started from 6% in October and increased until 40% as a maximum infection in December. In Jenin districts, the maximum viral infection of 30% was recorded in Qabatyya followed by Al-Zababdeih and Al-Jededeih in which the virus infection was recorded to be 22% and 20% respectively. The maximum viral infection for these regions was recorded in December (Fig. 4).

Figure 4: Incidence and disease development of TYLCV in tomato fields during the autumn season



For tomato planted under greenhouses in Qabatyya and Al-Jededeih regions the virus infection stayed zero from April until June then started to grow until it reached the maximum value during February of the next year. The maximum viral infection on tomato in these regions was recorded to be 14% and 11% in Qabatyya and Al-Jededeih respectively.

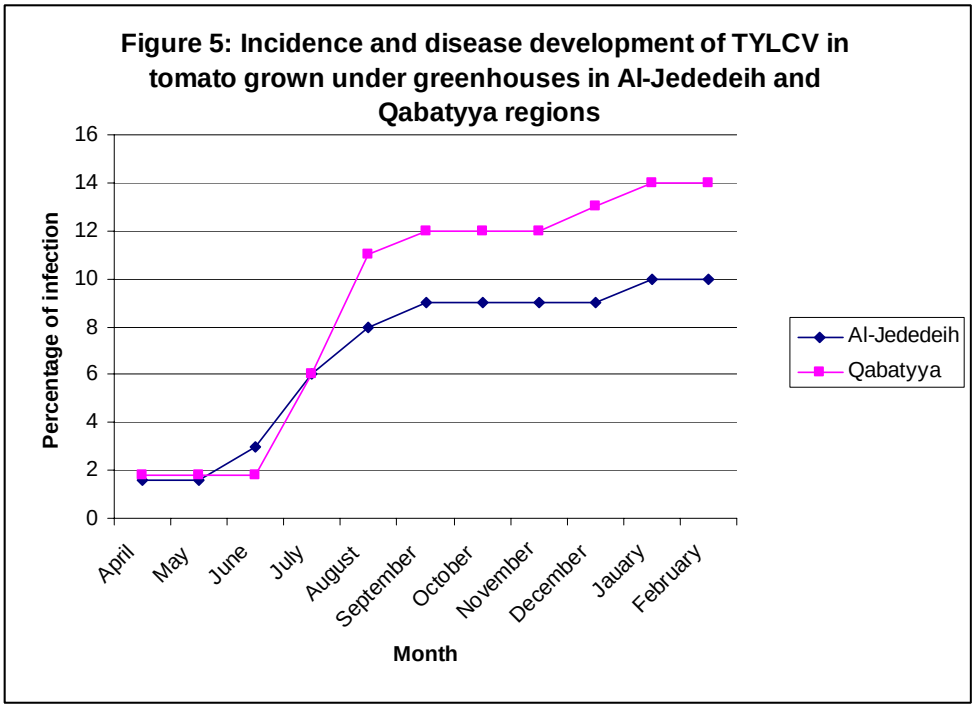


Figure 5: Incidence and disease development of TYLCV in tomato grown under greenhouses in Al-Jededeih and Qabatyya regions
Percentage of infection
16
14
12
10
8
6
4
2
0
April
May
June
July
August
September
October
November
December
Jauary
February
Month
Al-Jededeih
Qabatyya

Statistical analysis

The results of statistical analyses revealed that the virus infection of tomato plants during the summer growing season was more significant compared with the other growing seasons as the greatest Z-values were obtained mostly for all studied regions. Also, the results showed that the virus occurrence in Al-Far'a region was significant compared with most studied regions as the maximum Z-values of virus infection were obtained for this region (table 2).

Table 2: Statistical analyses and the Z-value of the Two-Sample Test of Proportion. The Z-table (critical value) = 1.645

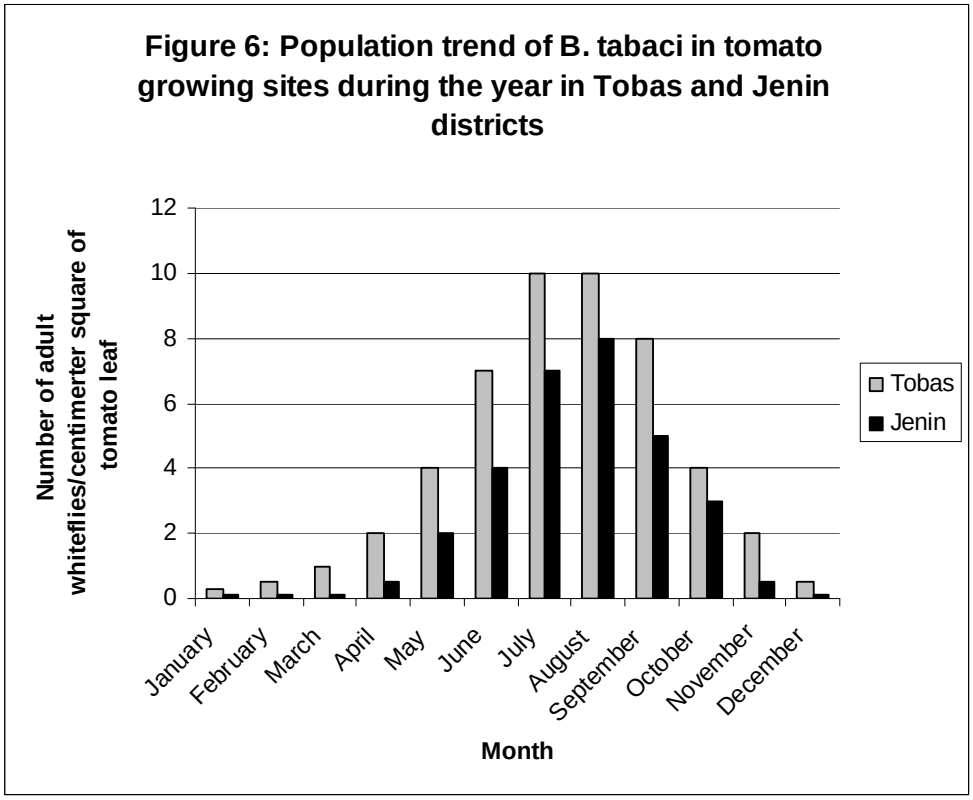
Area combination regarding tomato infection with TYLCV	Z-value of tomato growing seasons			
	Early Spring	Spring	Summer	Autumn
Al-Far'a/Kashda	1.63(NS)	2.26(S)	4.60(S)	4.26(S)
Al-Far'a/Al-Jededeih	1.29(NS)	2.90(S)	9.47(S)	7.11(S)
Al-Far'a/Al-Zababdeih	1.45(NS)	2.90(S)	9.23(S)	6.81(S)
Al-Far'a/Qabatyya	0.95(NS)	1.64(NS)	7.09(S)	5.66(S)
Kashda/Al-Jededeih	-0.35(NS)	0.66(NS)	5.66(S)	3.09(S)
Kashda/Al-Zababdeih	-0.18(NS)	0.84(NS)	5.36(S)	2.75(S)
Kashda/Qabatyya	-0.68(NS)	-0.48(NS)	2.89(S)	1.42(NS)
Al-Jededeih/Al-Zababdeih	0.17(NS)	0.17(NS)	-0.31(NS)	-0.35(NS)
Al-Jededeih/Qabatyya	-0.34(NS)	-1.14(NS)	-2.87(NS)	-1.63(NS)
Al-Zababdeih/ Qabatyya	-0.51(NS)	-1.31(NS)	-2.59(NS)	-1.29(NS)

S: Proportion of the 1st area in the combination is greater than the proportion of the 2nd area

NS: Proportion of the 1st area in the combination is less than the proportion of the 2nd area

Monitoring of whitefly population

The results showed that whitefly in Al-Far'a region started to appear during February and increased rapidly until it reached the maximum population during July and August. In Qabatyya the whiteflies started to appear in low population during April then increased to reach the peak during August. The results also showed that the maximum whitefly population in Al-Far'a was greater than its maximum population in Qabatyya region. Also, the whiteflies in Al-Far'a appeared on plants for a much longer period of time compared with Qabatyya region (Fig. 6).



Discussion

Studying the population trends of whiteflies proved that the population has changed throughout the year in the Tobas and Jenin districts. The greater population and longer persistence of the whiteflies in Al-Far'a compared with Qabatyya may be attributed to warm weather in this region, which prevails throughout most of the year. The warm conditions in this

region began during March and prevailed until nearly the end of November, whereas in Qabatyya the situation is a little bit different. The warm conditions in Qabatyya began after April and do not extend beyond October ((PCBS), 2005: 39-49). Jetter *et. al.* (2001: 1-60) and Gerling, (1990: 57-112) reported that the warm and dry conditions favored the whitefly, and therefore helped the spread of TYLCV. Also, the author reported that the whitefly populations decrease after heavy rain showers and this is explained in the drastic reduction in the number of whiteflies after November (Fig. 6).

With regard to the prevalence of TYLCV, the results revealed that the virus infection increased very rapidly and to a much higher level in the summer growing season in the studied regions (Fig. 3). Therefore, this season is considered the most critical season for tomato production in these regions because of the high rate of virus infection. In these seasons, tomato crops contaminated with TYLCV reach an infection rate of up to 95% in the Tobas district due to the suitability of weather for the spread of the vector (*B. tabaci*), and to the time of transplanting which occurs simultaneously with the period of whitefly activity. Similar results on the spread of the virus were recorded by international researchers in other Mediterranean countries. In this regard, Ioannuo (1985) and (1987) reported that TYLCV was an epidemic disease on tomato grown during the summer growing season in the southern coastal zone of Cyprus. The viral incidence was recorded to be ranged from 20-100%.

The second season most prevalent with the disease was recorded to be in autumn growing season in all fields selected in the studied regions. The spread of the virus in this season was also high for all regions (20-70%). Such a case may be attributed also to the suitability of the environmental conditions for the spread and activity of the whiteflies between June and September. Thus, because of the risk of TYLCV infection during these seasons, the farmers cover the transplants with muslin and spray insecticides extensively. The lower incidence of the disease during the autumn season compared with the summer one may be attributed to the cold weather, which prevails especially at night during this period in these regions ((PCBS), 2005: 39-49). Such conditions may reduce the whitefly numbers and suppress their activity (Gerling, (1990: 57-112)). In this regard, similar results were recorded in the neighbouring countries including Egypt and Lebanon. Moreover, in Egypt, TYLCV was considered the most serious disease of autumn-grown tomato in Fayoum, Giza, and Ismailia as the incidence ranged from about 80-99% (Nakhla *et. al.* 1993, Mazyad *et. al.* 1979). On the other hand, in Lebanon, Abou Jawdah *et. al*. (1995) reported that tomato grown in coastal plains during December were severely

infected with TYLCV, obliging farmers to abandon tomato production in this region. The viral incidence was recorded to be 85-90%. Such a difference in the frequency of the virus between Palestine, Egypt and Lebanon may be attributed to the climatic difference between these countries.

In addition the results revealed a low rate of viral occurrence on tomato plants grown during the early spring and spring seasons. The viral incidence varied between regions and ranged from 20-41%. This finding may be attributed to the time of transplanting which occurs between March and the middle April, during which the whiteflies have low activity to due the cold conditions which characterize these two months in the country ((PCBS), 2005: 39-49). This situation provides an opportunity for the tomato transplants to avoid viral infection during their early growth stages. Additionally, in the Jordan Valley, Al-Musa (1982) reported that the incidence of TYLCV increases more rapidly to reach a high occurrence rate of 93-100% in the fall grown tomato compared with spring-grown tomato, which reached the maximum viral incidence of 14% at the end of the growing season.

The results also demonstrated that the maximum occurrence rate for the virus was recorded in Al-Far'a for all tomato growing seasons. This finding may be attributed to the warm climatic conditions in this region which prevails throughout the year, stimulating the activity of the whiteflies and their reproduction (Jetter *et. al.* (2001: 1-60) and Gerling, (1990: 57-112). Therefore, in this region the whiteflies appear as early as one month compared with the other regions. Such a case increases the possibility of tomato infection during the early stages when the plants are still sensitive to viral infection.

The lower occurrence of the virus in Kashda compared with Al-Far'a especially during the autumn growing season may be attributed to the weather conditions which are cooler than Al-Far'a, and to the active west winds which prevailed most of the year ((PCBS), 2005: 39-49). These conditions have negative effects on the whiteflies as they can suppress their activities for long periods throughout the year.

In the Jenin districts, the lower occurrence rate of the virus in these regions may be attributed to the appearance of the whiteflies, which occurs one month after the whitefly appearance in Al-Far'a due to the cold environmental conditions in these regions((PCBS), 2005: 39-49). This type of environment may help the plants to avoid whitefly invasion and virus infection especially during the early growth stages.

For tomato planting in greenhouses in the Jenin district, the results illustrated that the viral incidence was low compared with the open fields. Also, under this planting the virus spread very slowly because it required about eleven months for it to reach its maximum frequency. This may be attributed to well-developed protection systems and the extensive pesticide spraying techniques practiced by the farmers in these regions. In addition, the results proved that the jump in the viral infection in these greenhouses occurred between July an August, which can be attributed to the whitefly population and its activity that prevails during these periods.

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