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BIOLOGY OF *Bemisia tabaci* (GENNADIUS) ON HEALTHY AND SQUASH LEAF CURL CHINA VIRUS INFECTED PUMPKIN PLANTS UNDER DIFFERENT SEASONAL CONDITIONS

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ABSTRACT

Pumpkins are often referred to as the "king of cucurbits" due to their large fruit size. In 2021, we conducted studies on the biology of the whitefly, *Bemisia tabaci*, under insect-proof screen house conditions, focusing on both healthy and Squash Leaf Curl China Virus (SLCCNV)-infected pumpkin plants. Before reaching adulthood, the whiteflies went through three nymphal instars and a pupal stage. In terms of fecundity, female whiteflies laid between 42 and 57 eggs on healthy pumpkin plants, and between 35 and 49 eggs on SLCCNV-infected plants during the spring season. During the rainy season, fecundity ranged from 45 to 60 eggs per female on healthy plants and 31 to 53 eggs per female on SLCCNV-infected plants. The total life cycle of *B. tabaci*, from egg to adult, varied between 20 and 26 days on healthy plants and 19 to 27 days on SLCCNV-infected plants during the spring season. In contrast, during the rainy season, the life cycle duration ranged from 18 to 24 days on healthy plants and 18 to 23 days on SLCCNV-infected plants. These findings offer valuable insights into the reproductive and developmental dynamics of *B. tabaci* in relation to pumpkin plant health and viral infection status.

Keywords: SLCCNV, vector, virus, whitefly

Bemisia tabaci (Gennadius) is a minute, delicate sucking insect belonging to the order Hemiptera and family Aleyrodidae, commonly known as the whitefly due to the white, wax-like powder that dusts its wings and body (Rani *et al.*, 2020). Whiteflies are widely distributed across tropical and subtropical regions and extend into many temperate areas. They deposit their eggs on the underside of the upper canopy of host plants, where the immature stages subsequently develop (Sadhana *et al.*, 2024). *B. tabaci* serves as a vector for numerous plant viruses, with begomoviruses being among the most prevalent and impactful (Haq *et al.*, 2011). Temperature and host plant species are critical factors influencing its biology. Development of *B. tabaci* occurs within a temperature range of 14°C to 35°C, with optimal egg-laying occurring at an ambient temperature of 28°C (Martin, 1999). Pumpkin (*Cucurbita pepo*) is a major vegetable crop cultivated year-round in India. It is susceptible to various insect pests, including the fruit fly, red pumpkin beetle, aphids, and notably, the whitefly (Kesh and Yadav, 2023). Among these, *B. tabaci* is a particularly significant and destructive pest. It inflicts direct damage through sap-feeding and indirect damage by transmitting plant viruses, thereby acting as a vector for several diseases (Swathi *et al.*, 2023).

The genus *Begomovirus* belongs to the family Geminiviridae and includes viruses that infect a wide range of dicotyledonous plants. These viruses cause substantial economic losses to major crops such as tomatoes, beans, squash, cassava, and cotton. On pumpkin plants, *B. tabaci* not only damages the crop directly through sap extraction but also indirectly through the transmission of viral diseases, including Squash Leaf Curl China Virus (SLCCV) (Castillo *et al.*, 2011). Given its role as a vector for multiple viral infections affecting both cultivated and wild plants, the significance of *B. tabaci* has increased. However, there is limited information regarding the biology of *B. tabaci* on healthy versus SLCCV-infected pumpkin plants within the agroclimatic conditions of Punjab. This study aims to address this gap by investigating the biology of *B. tabaci* on both healthy and SLCCV-infected pumpkin plants under varying seasonal conditions. The findings are expected to enhance agricultural practices by identifying optimal periods and strategies for pest control. By understanding the seasonal variations in *B. tabaci* and SLCCV interactions, farmers and scientists can improve management strategies, thereby increasing agricultural sustainability and reducing crop losses.

MATERIALS AND METHODS

The study on the biology of *Bemisia tabaci* was conducted on both healthy and Squash Leaf Curl China Virus (SLCCNV)-infected pumpkin plants under insect-

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proof screen house conditions in May (spring season) and August (rainy season) of 2021. The research was carried out at the Entomological Research Farm, Punjab Agricultural University, Ludhiana, Punjab, India (30°54'3.4740" N and 75°51'26.1972" E). In May 2021, the mean temperature was 30°C with a mean relative humidity of 64%. In August 2021, the mean temperature remained at 30°C, but the relative humidity increased to 87%. Pumpkin seeds of cultivar Punjab Samrat were sourced from the Department of Vegetable Science, PAU, Ludhiana. The SLCCNV culture was maintained and propagated under insect-proof net houses by continuously transferring the virus from diseased to healthy Punjab Samrat plants (Plate 1).

Biology of *B. tabaci* on healthy and SLCCNV-infected pumpkin plants

Male and female whiteflies were distinguished based on their taxonomic characteristics. Males are smaller and have blunt abdominal tips, while females are larger with pointed abdominal tips. A pair of newly emerged male and female adults was introduced into each clip cage, with twenty cages placed on both healthy and SLCCNV-infected pumpkin plants (30 days old), resulting in five replications, each containing four plants. Each clip cage was attached to the lower surface of a fully expanded top leaf on both healthy and infected plants. Observations on the duration of various developmental stages were recorded daily. The developmental stages examined included:

i. Egg period

Twenty-four hours after releasing the pairs of *B. tabaci* adults, the adults were removed, and the number of eggs was counted using a microscope. Five randomly selected eggs were retained for further observation, while the remaining eggs were carefully removed using a fine brush and needle. To prevent additional egg-laying by outside whiteflies and to restrict the movement of crawlers outside the clip cage, the eggs were re-covered with a clip cage. The leaves containing the retained eggs on their abaxial surfaces were tagged and monitored daily until hatching occurred. This allowed for the recording of hatching percentages and the calculation of the incubation period.

ii. Duration of nymphal instars

In order to ascertain the number and duration of various nymphal instars, each nymph was identified individually and monitored under a microscope from the time the eggs hatched until they pupated. The duration and total period of the nymphal instar observation were noted.

iii. Pupal stage

Information on different nymphal and pupal stages was recorded at 24 hours interval. The puparium were kept under observation until the emergence of adults.

iv. Longevity of male and female & fecundity

Observations were records on adult fecundity, lifespan, and oviposition period. Duration of the newly emerged male and female adults was studied by pairing them in a leaf cage on pumpkin seedlings. In order to determine the lifetime of 20 adults and the fecundity of 20 females, newly emerged whiteflies were paired and placed in a leaf clip cage with pumpkin seedlings. The mortality of adults was recorded daily and longevity of both sexes was recorded as per the methods given by Ghelani *et al*. (2020).

Plate 1. Maintenance of virus culture under insect-proof net house

Efficiency of *B. tabaci* in transmission of SLCCNV during different seasons

The studies were conducted to evaluate the efficiency of *B. tabaci* in transmitting SLCCNV during two distinct periods: mid-April to June in the spring season and July to mid-September in the rainy season of 2021. Pumpkin test plants were sown in an insect-proof screen house during both seasons. Viruliferous whitefly adults were introduced to the healthy test plants during these periods. An acquisition access period and an inoculation access period, each lasting 24 hours, were provided on the two to three true leaf stage of the healthy pumpkin test plants. Following inoculation, the test plants were maintained in the insect-proof screen house for six weeks to observe the development of symptoms. For statistical analysis, the mean ± standard error was calculated for the durations of various developmental stages and periods.

RESULTS AND DISCUSSION

Study on biology of *B. tabaci* on healthy and SLCCNV infected pumpkin plants

The present investigation on the biology of *Bemisia tabaci* on pumpkin plants, both healthy and infected with Sweet Potato Leaf Curl Virus (SLCCNV), was conducted during the spring and rainy seasons of 2021. Data regarding the various life stages of *B. tabaci* are summarized in Tables 1 and 2.

Egg Stage: The pedicellate eggs were laid singly on the undersides of the leaves, with the pedicel anchored to the leaf tissue. Initially, the eggs exhibited a yellowish-white colouration, which subsequently darkened to brown prior to hatching. The incubation period for eggs laid on healthy plants ranged from 4 to 6 days in the spring, while on SLCCNV-infected plants, it was slightly shorter, ranging from 4 to 5.5 days. In the rainy season, the incubation periods were reduced to 3 to 5 days for healthy plants and 3.5 to 5 days for infected ones. The observed variations in incubation durations between the two seasons may be attributed to ecological differences. These findings align with the observations of Hussian and Tehran (1933), who reported an incubation period of 3 to 5 days from March to October. Similarly, Kedar *et al.* (2014) documented an incubation period of 2 to 5 days during July. Our results are consistent with the study by Mohanty and Basu (1987), which noted an incubation period of 2 to 4 days from March to October.

First instar nymph: After hatching, the first instar nymphs of *Bemisia tabaci* crawled on the lower surface of the leaf for 1 to 2 hours. The duration of the first instar stage varied, lasting 3 to 5 days, with an average of 4.10 ± 0.22 days on healthy plants and 3.70 ± 0.22 days on SLCCNV-infected plants during the spring season. In the rainy season, the duration was shorter, ranging from 3 to 4.5 days (mean: 3.45 ± 0.17 days) for healthy plants, and from 2.5 to 4 days (mean: 3.00 ± 0.21 days) for SLCCNV-infected plants. These findings are consistent with observations reported by Mohanty and Basu (1987) and Kedar *et al.* (2014) regarding nymphal periods in cotton cultivars.

Second instar nymph: The second instar nymphs of *Bemisia tabaci* exhibited a whitish-yellow colouration. During the spring season, the mean duration of the second instar nymphal period was 3.15 ± 0.16 days

Table 1. Biology of *Bemisia tabaci* on healthy and SLCCNV infected pumpkin plants in spring season

Stage/Unit	Healthy plants		Infected plants	
	Mean ± S.E (days)	Range (days)	Mean ± S.E (days)	Range (days)
Egg	4.80 ± 0.20	4-6	4.75 ± 0.18	4-5.5
Nymph				
1st instar	4.10 ± 0.22	3-5	3.70 ± 0.22	3-5
2nd instar	3.15 ± 0.16	2.5-4	3.05 ± 0.25	2.5-4.5
3rd instar	2.90 ± 0.24	2-4	2.75 ± 0.21	2-4
4th instar	4.10 ± 0.24	3-5.5	3.80 ± 0.28	3-5
Female				
Fecundity	49.70 ± 1.73	42-57	41.90 ± 1.42	35-49
Adult longevity	5.20 ± 0.16	4.5-7.5	5.15 ± 0.22	5-7
Life cycle	23.90 ± 0.78	21-26	23.45 ± 0.46	23-27
Male				
Adult longevity	4.47 ± 0.20	3.5-5.5	4.06 ± 0.19	2-4
Life cycle	22.05 ± 0.69	20-26	21.10 ± 0.56	19-25.5

*values are mean ± S.E

Table 2. Biology of *Bemisia tabaci* on healthy and SLCCNV infected pumpkin plants in rainy season

Stage/Unit	Healthy plants		Infected plants	
	Mean ± S.E (days)	Range (days)	Mean ± S.E (days)	Range (days)
Egg	4.20 ± 0.21	3-5	4.15 ± 0.22	3.5-5
Nymph				
1st instar	3.45 ± 0.17	3-4.5	3.00 ± 0.21	2.5-4
2nd instar	2.60 ±0.18	2-3	2.50 ± 0.16	2-3.5
3rd instar	2.50 ± 0.18	2-3.5	2.42 ± 0.14	1.75-3
4th instar	3.35 ± 0.15	3-4	3.17 ± 0.13	2.5-4
Female				
Fecundity	53.00 ± 3.61	45-60	40.80 ± 1.63	31-53
Adult longevity	5.75 ± 0.26	4-7	5.25 ± 0.32	4-7
Life cycle	22.15 ± 0.48	19.5-24	21.00 ± 0.36	19.5-23
Male				
Adult longevity	3.40 ± 0.22	2.5-5	2.85 ± 0.22	2-4
Life cycle	20.70 ± 0.77	18.5-23	19.62 ± 0.35	18-21

*values are mean ± S.E

on healthy plants and 3.05 ± 0.25 days on SLCCNV-infected plants. In the rainy season, the mean duration decreased to 2.60 ± 0.18 days for healthy plants and 2.50 ± 0.16 days for those infected with SLCCNV.

Third instar nymph: The mean duration of the third instar nymphs of *Bemisia tabaci* was 2.90 ± 0.24 days on healthy plants and 2.75 ± 0.21 days on SLCCNV-infected plants during the spring season. In the rainy season, the duration of the third instar nymphs decreased, ranging from 2.50 ± 0.18 days on healthy plants to 2.42 ± 0.14 days on SLCCNV-infected plants.

Fourth instar (puparium): The freshly formed puparium of *Bemisia tabaci* exhibited well-developed, red-coloured compound eyes that were distinctly visible. The duration of this instar was 4.10 ± 0.24 days on healthy plants and 3.80 ± 0.28 days on SLCCNV-infected plants during the spring season. In the rainy season, the duration decreased to 3.35 ± 0.15 days for healthy plants and 3.17 ± 0.13 days for those infected with SLCCNV. These findings are consistent with the observations reported by Kedar *et al.* (2014) and Aneja (2000) on cotton varieties at PAU, Ludhiana.

Adult: The fully developed adult *Bemisia tabaci* emerged from the pupal case through an inverted T-shaped slit. During the spring season, the longevity of female adults varied from 4.5 to 7.5 days on healthy plants and from 5 to 7 days on SLCCNV-infected plants. In the rainy season, the longevity of females was consistent, lasting 4 to 7 days on both plant types. The mean total life cycle duration was 23.90 ± 0.78 days on healthy plants and 23.45 ± 0.46 days on SLCCNV-infected plants in the spring. In the rainy season, the mean total life cycle was shorter, at 22.15 ± 0.48 days for healthy plants and 21.00 ± 0.36 days for infected plants. For male adults, the mean longevity during the spring season was 4.47 ± 0.20 days on healthy plants and 4.06 ± 0.19 days on SLCCNV-infected plants. In the rainy season, the mean adult longevity for males was 3.40 ± 0.22 days, with a range of 2.5 to 5 days on healthy pumpkin plants, and 2.85 ± 0.22 days, ranging from 2 to 4 days on SLCCNV-infected plants. These results align with those of Kedar *et al.* (2014), who reported that male longevity ranged from 3 to 5 days, while female longevity ranged from 5 to 7.5 days. Additionally, the mean life cycle duration was observed to be between 20 and 26 days on healthy plants and 19 to 25.5 days on SLCCNV-infected plants during the spring season. In the rainy season, the mean life cycle was found to be 20.70 ± 0.77 days (ranging from 18.5 to 23 days) for healthy plants and 19.62 ± 0.35 days (ranging from 18 to 21 days) for infected plants.

Fecundity: The fecundity of female *Bemisia tabaci* ranged from 42 to 57 eggs, with a mean of 49.70 ± 1.73 eggs on healthy plants. In contrast, on SLCCNV-infected plants, fecundity varied from 35 to 49 eggs, yielding a mean of 41.90 ± 1.42 eggs during the spring season. During the rainy season, fecundity increased, ranging from 45 to 60 eggs on healthy plants and from 31 to 53 eggs on SLCCNV-infected plants. The observed variations in fecundity may be attributed to ecological factors and differences among cultivars. Kedar *et al.* (2014) reported a fecundity range of 45 to 66 eggs, while Butter and Vir (1991) recorded fecundity between 22.5 and 86 eggs on various cotton varieties.

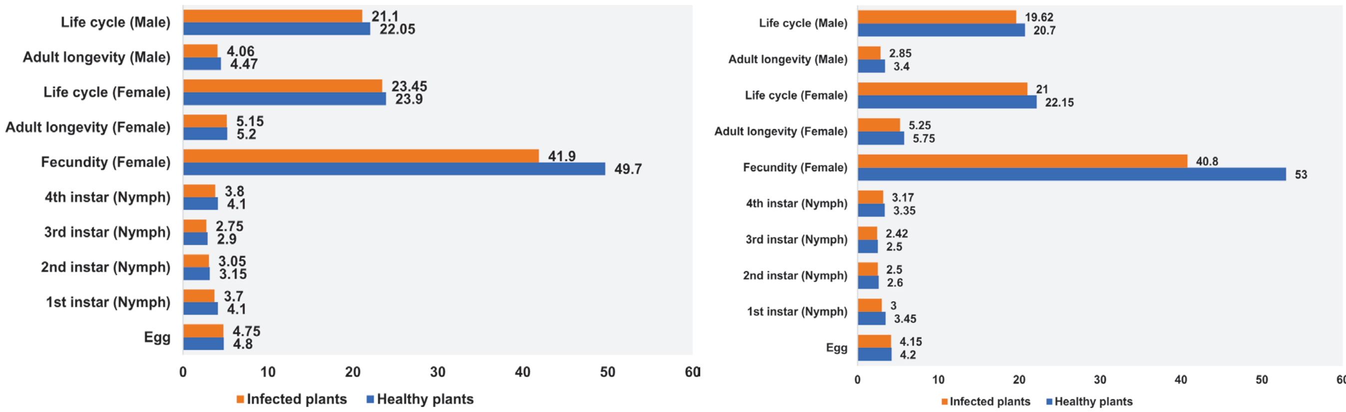

Fig. 1. Biology of *Bemisia tabaci* on healthy and SLCCNV infected pumpkin plants in rainy season

Efficiency of *B. tabaci* in transmission of SLCCNV during different seasons

Non-viruliferous adults of *Bemisia tabaci* were given a 24-hour acquisition access period on pumpkin plants infected with the yellow vein mosaic virus during both the spring and rainy seasons. Following this, the viruliferous whiteflies were provided a 24-hour inoculation access period on the test pumpkin plants. A total of fifteen whiteflies per plant were introduced, and twenty test plants were inoculated during each access period. As shown in Table 3, the transmission rate of pumpkin yellow vein mosaic virus was 95% during the spring season, while it was slightly lower at 91.66% during the rainy season. This minor variation in transmission rates may be attributed to differences in the titer values of SLCCNV in the virus source plants. These findings are consistent with those of Jayashree *et al.* (1999), who reported a 100% transmission rate of pumpkin yellow vein mosaic virus with an acquisition access period of 20 hours and an inoculation access period of 18 hours in Coimbatore, Tamil Nadu. Similarly, Maruthi *et al.* (2007) found that a 6-hour acquisition and inoculation access period each was sufficient to achieve 100% transmission of SLCCNV in pumpkin in the same region.

Table 3. Efficiency of *Bemisia tabaci* in transmission of SLCCNV during different seasons

Season	Transmission (%)	Incubation period range (days)
Spring	95.00	9-16
Rainy	91.66	10-17

The Squash Leaf Curl China Virus (SLCCNV) is obligately transmitted by insect vectors, primarily the whitefly species *Bemisia tabaci*, among others. Due to their indiscriminate feeding habits, whiteflies facilitate rapid and efficient viral transmission. The transmission occurs in a non-propagative, persistent, and circulative manner. This study examines the life cycle of *B. tabaci* on both healthy and SLCCNV-infected pumpkin plants, revealing slight seasonal variations. Specifically, the incubation period of the eggs was observed to be shorter during the rainy season compared to the spring season. This variation can be attributed to the whitefly's preference for high temperature and humidity, which promote faster reproduction rates. Additionally, the nymphal period was also shorter in the rainy season than in the spring. Furthermore, the fecundity of *B. tabaci* was found to be lower on SLCCNV-infected plants relative to healthy ones. The total life cycle duration for both male and female whiteflies was reduced on SLCCNV-infected plants compared to healthy pumpkin plants (see Fig. 1), and the life cycle was completed more rapidly during the rainy season than in the spring. This study elucidates how SLCCNV infection impacts the life cycle of *B. tabaci* on pumpkin plants and highlights seasonal variations in lifecycle parameters, demonstrating the adaptability of whiteflies to changing environmental conditions. The findings highlight the importance of timely interventions to mitigate the spread of viruses, contributing valuable insights to pest control strategies. Understanding the life cycle of *B. tabaci* under varying conditions is essential for developing sustainable pest management practices. Future research should focus on optimizing crop protection measures against virus transmission by whitefly vectors.

CONCLUSION

In conclusion, this study provides a comprehensive understanding of the biology of *Bemisia tabaci* on both healthy and Squash Leaf Curl China Virus (SLCCNV)-infected pumpkin plants across two seasonal conditions. The results reveal distinct variations in the developmental stages, fecundity, and overall life cycle of *B. tabaci* depending on the health of the pumpkin plant and the environmental conditions. Notably, the whitefly exhibited a shorter life cycle and reduced fecundity on SLCCNV-infected plants compared to healthy ones, with the rainy season promoting a faster development rate overall. These findings highlight the critical role of seasonal conditions and viral infections in influencing the population dynamics of *B. tabaci*. This information can guide the development of more targeted and effective pest management strategies, particularly in managing viral transmission by whitefly vectors. Future research should focus on optimizing control measures based on these insights to reduce crop losses and improve agricultural sustainability.

Authors' contribution

Conceptualization and designing of the research work (AK); Execution of field/lab experiments and data collection (BS and AK); Analysis of data and interpretation (BS); Preparation of manuscript (AK and BS)

Conflicts of interest: The authors declare that they have no conflicts of interest.

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