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Transmission studies of tomato leaf curl New Delhi virus infecting cucurbits and tomato

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Abstract

Two sets of experiment were performed to study the transmission rate of *Tomato Leaf Curl New Delhi Virus* among different cucurbitaceous host. In first experiment six different varieties of different crop were taken as treatment. In first set of different crop maximum transmission per cent was seen in case of tomato to tomato with 68.52 per cent, followed by tomato to sponge gourd and tomato to bitter gourd with 53.81 and 45.83 per cent transmission respectively. Similarly, in second set of different variety of crop transmission per cent of tomato to tomato was maximum with 63.43 per cent followed by tomato to sponge gourd and bottle gourd with 54.89 and 44.32 per cent respectively. In first set of experiment minimum transmission percent was observed in case of tomato to pumpkin with 28.70 per cent followed by 38.82, 44.72 per cent in case of transmission from tomato to bottle gourd and cucumber. Whereas, in second set minimum percent of transmission of 33.77 in case of tomato to pumpkin followed by tomato to bitter gourd and cucumber with 42.08 and 43.58 per cent transmission respectively was observed. Correspondingly efficiency of white fly based on population were also checked with 1,3,5 and 10 number of whiteflies with 24 hrs. Acquisition feeding period on all the 6 crops and it was confirmed that a single whitefly is efficient to transmit begomovirus up to 37.2,38,43,37,33 and 41% in host crop tomato, bottle gourd, pumpkin, sponge gourd, Bitter gourd and cucumber respectively and it reaches maximum up to 100% if number of whiteflies increases more than 10.

Keywords: transmission, efficiency, begomovirus, tomato leaf curl New Delhi virus

1. Introduction

The members of the genus begomovirus are transmitted by the hemipteran vector whitefly, *Bemisia tabaci* (Gennadius; Hemiptera: Aleyrodoidea) in a circulative manner. The whitefly, being a phloem-feeder is considered as double whammy due to its ability to affect the plant as sucking pest and its role as a vector of dreaded Begomoviruses (De Barro *et al.*, 2011; Dinsdale *et al.*, 2010) [5, 7]. The transmission efficiency of the virus relies on virus strain indicating the co-evolution of the virus and vector biotype (Hogenhout *et al.*, 2008; Gottlieb *et al.*, 2010) [8, 9]. Varma (1955) [15] gave first ever evidence of *Pumpkin Yellow Vein Mosaic Virus* (PYVMV) being transmitted by whiteflies and also established minimum population of viruliferous vectors needed for transmission. These results were further established by Capoor and Ahmad (1975) [4]. Further, Jayashree *et al.* (1999) [10] proved the identity of PYVMD causing virus as a Begomovirus. Capoor and Ahmad (1975) [4] suggested, transmission efficiency of a single viruliferous whitefly is about 30 per cent which goes up to 60 percent when 3 viruliferous whiteflies are present. Whereas, Muniyappa *et al.* (2003) [11] recorded cent per cent transmission with five whiteflies. Capoor and Ahmad (1975) [4] reported that, about 30 seconds of minimum Acquisition Feeding Period (AFP) is required to acquire PYVMV by *B. tabaci*. A minimum of 5 min of Acquisition Access Period (AAP) and 30 minutes of Inoculation access Period (IAP) was suggested by Jayashree *et al.* (1999) [10]. Whereas Muniyappa *et al.* (2003) [11] recorded minimum IAP of 10 minutes and about 6 hours of IAP resulted in cent per cent infection. According to Butter *et al.* (2008) [3], the transmission of ToYLCV is 30% with single whitefly and it goes up to 100% when inoculated with 10 whiteflies. Similarly, in cucumber 5 insect were enough to transmit when the number of whiteflies were increased up to 10 the efficiency reached to 75% correspondingly (shad *et al.*, 2019) [14]. In a study on bitter gourd single white fly transmitted 19.5% Bitter gourd yellow mosaic virus and it increases to 100% with increase in number of whiteflies up to 15 (Naik *et al.*, 2019) [12]. the extent of yield loss caused by some Geminiviruses is as high as 100 per cent. Several Begomoviruses have been reported to infect in different cucurbits across the globe (Al-Musa *et al.* 2011) [1]. The increasing incidence of whitefly transmitted begomoviruses is alarming and there is a need to develop a comprehensive strategy for transmission of the

begomovirus and its host range, so by keeping this in mind, the host range and pattern of transmission were studied on different six crops including one Solanaceae and 5 cucurbits.

2. Methods and Materials

2.1 Transmission rates of Begomovirus between different host

The experiment was conducted under Completely Randomized Design (CRD). Transmission experiment was carried out with 2 varieties of 6 different crops (2×6 treatments) and 5 replications for each treatment. Plants were infected artificially with viruliferous White Fly and transmission percent was calculated after one week. One more parallel study was done on optimum number of whiteflies required for successful transmission by providing 24 hrs. Feeding Acquisition period. CRD was used for this experiment with 4 replications of 4 treatments of different number of white fly (1, 3, 5, 10) on all the sets of variety.

2.1.1 Collection of white-fly

To collect the whiteflies from the field, aspirator made of a glass tube and a rubber tube of 40 cm length was used. The leaves colonized with whiteflies on an apparently healthy crop were turned slightly upward and the whiteflies were sucked in using aspirator glass tube and later blown into the plastic tubes.

2.1.2 Establishment and maintenance of whitefly

The pure culture of whitefly (*Bemisia tabaci*) used for the current investigation was originally collected from pumpkin, cucumber and pointed gourd plants at Bihar agricultural university, Sabour Bhagalpur. The collected whitefly was maintained on bitter gourd, cucumber, and sponge gourd and kept under the insect-proof wooden cage or insect free net box covered with muslin clothes on the all the side and with transparent plastic on the front side to aid in supervision. These cages were having temperature of about 28- 30 °C in the insect free house or box. The healthy culture was obtained after continuous growing of whitefly for 2nd generation.

2.1.3 Maintenance of disease caused by the Begomoviruses

The cucurbits infected with the Begomoviruses showing symptom like chlorosis, mosaic, blistering, mottling, cupping, blistering with stunted growth were identified and collected

from naturally infected plant and the virus was transmitted from infected to one week healthy with the help of whitefly (*Bemisia tabaci*) by giving 24 hrs to each AAP and IAP. The culture was maintained under the insect free condition by regularly inoculating with viruliferous whitefly.

2.1.4 Preparation of cages for acquisition access feeding by *Bemisia tabaci*

Plastic or polyvinyl chloride bottles tapering towards the narrow mouth were taken, the base of the bottle were removed with the soldering and covered with the black muslin clothes, the narrow mouth of the bottle was cut up to 1/4th of the length below the screw cap and plugged in with the cotton to prevent whitefly escaping during the usage.

2.1.5 Acquisition Access period

A group of non-viruliferous whitefly was released on the infected sample of the tomato maintained under the insect-proof house, and whitefly is allowed to feed for the successful acquisition of the virus by the whitefly for at least 24 hours.

2.1.6 Inoculation Access period

The whiteflies collected after 24 hrs. of AAP were released on one-week old healthy cucurbits seedling to inoculate virus by using inoculation tube for 24 hrs. After the IAP of 24hrs, the plants were kept under insect free condition by keeping them under cage for symptom development and observation was recorded after one week of inoculation

2.1.7 Transmission of Begomovirus to healthy plants through vector white fly

Collected white fly were allowed to feed on the infected plant with AAP of 24 hours and the viruliferous whiteflies were carefully released into cages having healthy plants and allowed to feed on them for 24 hours of IFP. After inoculation the plants were again placed in insect free chamber to avoid further infection. Plants were observed after 7 -10 days after artificial inoculation with vector and observations were recorded. Number of plants artificially infected with the vector whitefly, Number of plants showing symptoms of apparent symptoms of Begomovirus and the Transmission percent was calculated. The details of the treatments in transmission is presented in (table 1)

Table 1: Details of the host employed in transmission study

SI No	Name of host	Variety 1	Variety 2
1	Tomato (<i>Solanum lycopersicum</i>)	Punjab Swara	Kashi Vishal
2	Bottle gourd (<i>Lagneriasiseraria</i>)	NDBGH-4	Raj Chamatkar
3	Pumpkin (<i>Cucurbita maxima</i>)	Pusa Vikas	Kashi Harit
4	Sponge gourd (<i>Luffa cylindrica</i>)	Rajendra Nenua 1	Raj Nenua 1
5	Bitter gourd (<i>Momordica charantia</i>)	Pusa Rasdar	Pusa do Mousami
6	Cucumber (<i>Cucumis sativa</i>)	Punjab Naveen	Swarna

2.1.8 Transmission of begomovirus based on whitefly population

Similarly, efficiency of the viruliferous white fly were calibrated by releasing it on one-week old seedling of different crop under controlled environment to know the infection capacity of whitefly even in the low population. For this CRD, was used with 4 treatments and 4 replications. The second-generation pure cultured whitefly was provided 24 hrs. Acquisition feeding period on maintained ideal plant for the Begomovirus. Afterwards different number of

Viruliferous Whitefly were used as 4 treatments (1, 3, 5 & 10) for infecting the different set of crops by providing 24hrs. Inoculation feeding period.

2.1.9. Molecular Confirmation of Transmission by whitefly

The symptomatic leaf of the infected field showing begomovirus like symptoms and each plant leaves showing the symptom of the Begomovirus phenotypically were further processed for the molecular confirmation. Total DNA was

extracted by using Promega DNA Extraction kit. PCR were performed with the specific degenerative primers of the begomovirus, Cp Deng 541 F/ Cp Deng 540 R (Deng *et al.* 1994) and ToLCNDV-CPF: 5' TACGATCTTGCCGAGATCTCA3'/ToLCNDV-CPR: 5' ACCCAGGTCCTTAAGTACCT 3' Primers were designed from the DNA-A of ToLCNDV (MF807949). Materials used for preparation of PCR reaction mixture of 25µL were 12.5µL Master mix, 8.5µL Nuclease free water, 1µL Forward primer and 1µL Reverse primer. Specific primer of begomovirus. PCR performed with the master cycler (Nexus Eppendorf, Germany) programmed 40 cycles of denaturation at 95 °C annealing for 1 min at temperature 49° C for Deng primer and 53 °C for ToLCNDV-CPF/ ToLCNDV-CPR followed by 1 min final extension with final 10min extension at 72 °C. DNA was assessed through gel electrophoresis system (GeNei biosystems) at 1 per cent agarose gel in TAE buffer (1%) containing 0.1% ethidium bromide and visualized in gel documentation system (UVITECH, UK).

3. Result

3.1 Transmission pattern of ToLCNDV from tomato to cucurbitaceous crops

Experiments were conducted in the department of Plant Pathology, BAU, Sabour with six different crops as treatments, having five replications and repeated the experiments with second type of variety of same crop. Experiment was conducted with the six varieties of different crops as treatment *viz.*, Punjab Swarna of tomato (*Solanum lycopersicon*), NDBGH-4 of bottle gourd (*Cucurbita moschata*), Pusa Vikash of pumpkin (*Cucurbita maxima*), Rajendra Nenua-1 of sponge gourd (*Luffa acutangula*), Pusa Rasdar of bitter gourd (*Momordica charantia*), Punjab Naveen of cucumber (*Cucumis sativus*). After inoculating the plant with the infected whitefly, the transmission per cent were calculated after symptom appearance. Maximum transmission per cent was seen in case of tomato to tomato with 68.52 per cent, followed by tomato to sponge gourd and

tomato to bitter gourd with 53.81 and 45.83 per cent transmission respectively. The minimum transmission percent was observed in case of tomato to pumpkin with 28.70 per cent followed by 38.82, 44.72 per cent in case of transmission from tomato to bottle gourd and cucumber (Table 2). In order to confirm the transmission rates, a second set of experiment was conducted with same crop but with different cultivars, *viz.*; Kashi Vishesh of tomato, Rajendra Chamatkar of bottle gourd, Kashi Harit of pumpkin, Nenua local of sponge gourd, Pusa Do Mausami of bitter gourd and Swarna Ageti variety of cucumber. The transmission results presented in table 2 suggested a similar trend of transmission in both set of experiments. Transmission per cent of tomato to tomato was maximum with 63.43 per cent followed by tomato to sponge gourd and bottle gourd with 54.89 and 44.32 per cent respectively. Whereas, minimum percent of transmission of 33.77 in case of tomato to pumpkin followed by tomato to bitter gourd and cucumber with 42.08 and 43.58 per cent transmission respectively was observed.

Table 2: Transmission rates of ToLCNDV from tomato to other cucurbitaceous hosts

Whitefly mediated transmission (Host)	transmission	
	Cucumber	Cucumber
Tomato to Tomato	68.52(55.87)*	63.43(52.79)
Tomato to Bitter gourd	38.82(38.45)	44.32(41.72)
Tomato to Pumpkin	28.70(32.34)	33.77(35.50)
Tomato to Sponge gourd	53.81(47.17)	54.89(47.79)
Tomato to Bitter gourd	45.83(42.57)	42.08(40.42)
Tomato to Cucumber	44.72(41.87)	43.58(41.29)
C.D.	7.85	3.58
SE (m)	2.521	1.15

In both the set of experiment show the similar pattern of Transmission. (No.) * is the angular transformed data. With SE(m)= 2.25 and 1.15 respectively, maximum % transmission is in Bold character.

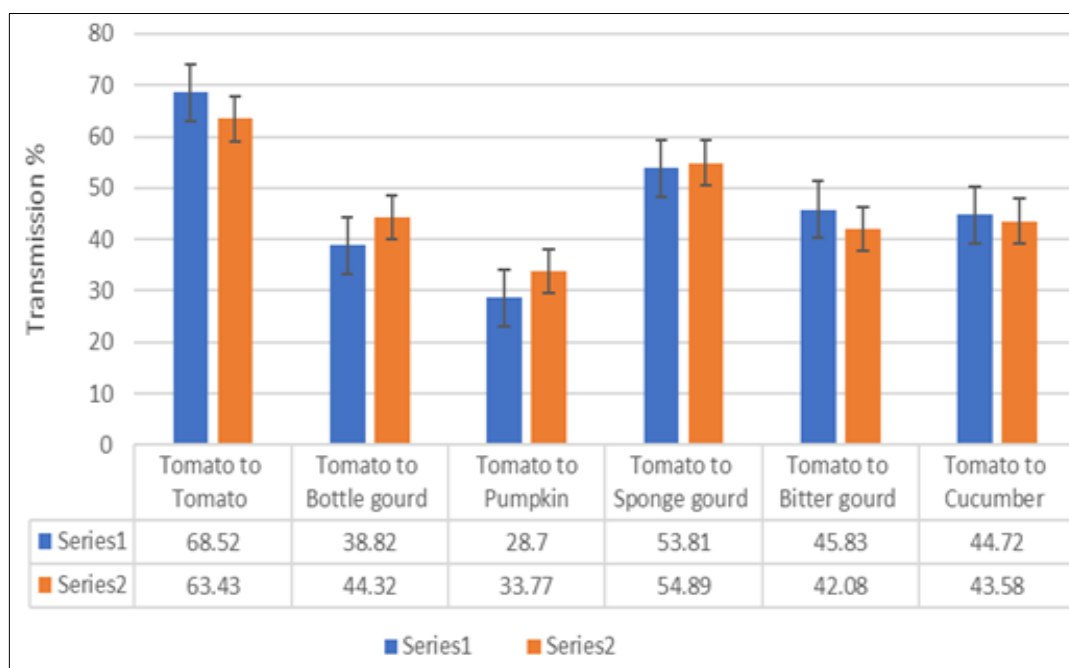


Fig 1: Graph showing the similar pattern of transmission of virus in both the set of Varieties of different Crops

3.2 Transmission of Begomovirus vs Number of whiteflies

This experiment was also done in the virology laboratory Department of Plant pathology. CRD was applied with 4 treatment and 4 replications. This experiment was done with the one set of the variety of all the crop which were used for host range determination. The treatment was 1, 3, 5, 10 number of white flies as inoculating agent or vector of virus. In case of tomato, the transmission was 37 percent when inoculated with single viruliferous whitefly, while when inoculated with 3 viruliferous white flies the percent transmission was successful with 52.75 per cent. Similarly, there was 65 and 91.25 percent when inoculated with 5 and 10 viruliferous whiteflies respectively. In case of Bottle gourd, the maximum efficiency was with the population of 10 viruliferous white flies with 87.2%. Even a single viruliferous whitefly was much efficient to transmit the virus with 38%

ascendingly followed by 3 and 5 whiteflies with transmission percent of 43.25 and 71.5 percent respectively. There was similar pattern of transmission in other cucurbits also like in pumpkin, there was 43, 41.75, 77.25 and 78.62% transmission when inoculated with 1, 3, 5 and 10 viruliferous whiteflies as treatments. In sponge gourd, Bitter gourd and Cucumber the transmission was maximum even in case of 5 viruliferous whiteflies inoculation with 77.25, 78.5 and 72.75 percent respectively. Similarly, in all the three crop the transmission percent with 10 viruliferous whiteflies are 67.32, 68.76 and 74.52% respectively. In case of sponge gourd and bitter gourd transmission efficiency was more in case of single whitefly inoculation than 3 whiteflies. In cucumber transmission was directly proportional to the increase in the number of white flies as it increases consequently.

Table 3: Transmission efficiency of whitefly with treatments of different number of Whiteflies (T1 =1, T2=3, T3=5 &T4=10). Transmission % is calculated by total number of symptomatic plant /numbers of plant inoculated with infected whiteflies. The maximum and minimum are marked with ** &*.

Crop	T ₁	T ₂	T ₃	T ₄
Tomato (<i>Lycopersicon esculentum</i>)	37.2*	52.75	65	91.25**
Bottle gourd (<i>Cucurbita lagenaria</i>)	38*	43.25	71.5	87.2**
Pumpkin (<i>Cucurbita maxima</i>)	43	41.75*	77.25	78.62**
Sponge gourd (<i>Luffa cylindrica</i>)	37.6	31.25*	78.5**	67.32
Bitter gourd (<i>Momordica charantia</i>)	33.6	33.5*	72.75**	68.76
Cucumber (<i>Cucumis sativa</i>)	41*	58.8	75.5	74.52

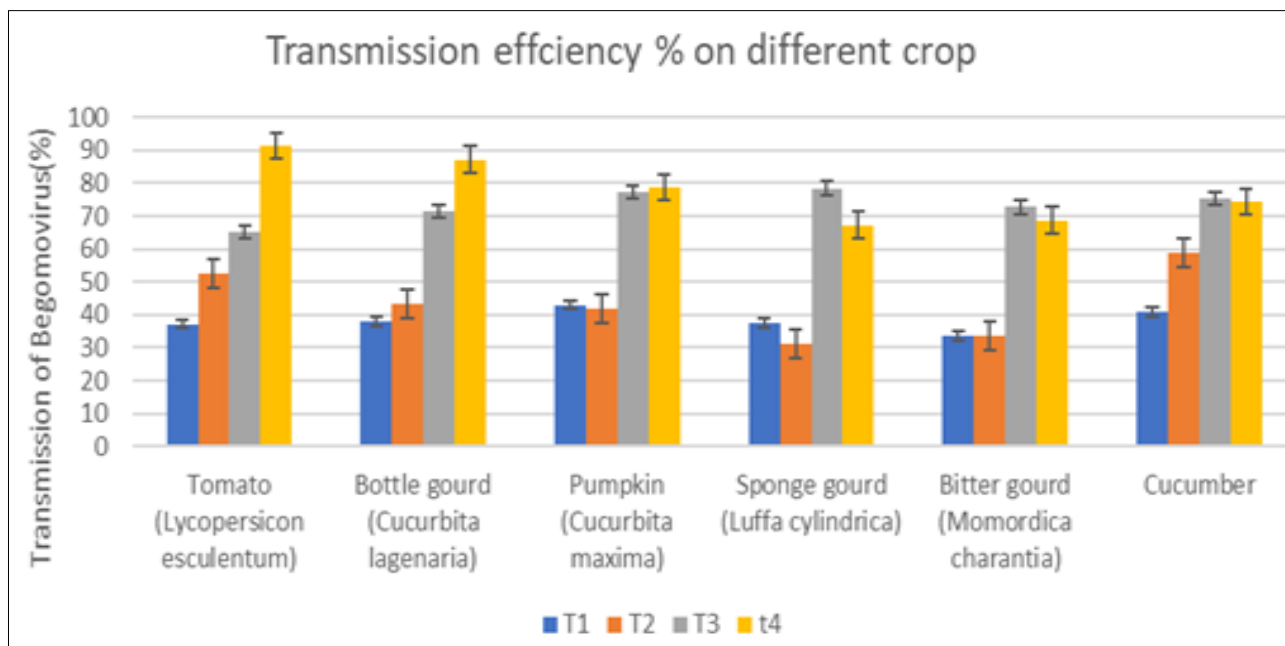


Fig 2: Graphical representation of Transmission efficiency of whitefly with treatments of different number of Whiteflies (T1 =1, T2=3, T3=5 &T4=10 whiteflies)

3.3 Molecular confirmation of the Begomovirus to confirm the transmission by whitefly

3.3.1 Molecular detection and confirmation of Begomovirus

Plant leaves showing symptom of begomovirus were selected and their DNA was isolated with the help of genomic DNA isolation Kit (“Wizard Genomic DNA Purification Kit”; Make: Promega Bioscience). The standard protocol for total DNA extraction was followed as per manufacturers instruction (Anon, 2019), the quality of the DNA was assessed through gel electrophoresis system (GeNei biosystems) at 1 per cent agarose gel in TAE buffer (1%).

Polymerase chain reaction was carried out for extracted DNA in PCR machine (Eppendorf Master Cycle) with Cp Deng 541 F/ Cp Deng 540 R and specific primers of ToLCNDV up to 40 cycle of denaturation, annealing and extension. PCR product were analyzed with the gel electrophoresis using 1 per cent agarose gel with the 1 kb ladder. The electrophoresis was run for 30 minutes at 50V. Gel were checked under the Gel documentation system (GeNei). Gave positive amplification. The positive amplification of approximate ~570bp band indicated the involvement of one or more begomoviruses. Positive amplification of approximate ~760bp confirmed the presence of *ToLCNDV*.

4. Discussion

4.1 Transmission pattern *ToLCNDV* from tomato to cucurbitaceous crops

Maximum Transmission percent was seen in case of Tomato to Tomato with 68.52 per cent, followed by Tomato to sponge gourd and tomato to Bitter gourd with 53.81 and 45.83 per cent transmission percent respectively. The Minimum transmission percent was in case of Tomato to Pumpkin with 28.70 per cent followed by 38.82, 44.72 percent in case of transmission from tomato to Bottle gourd and cucumber. The transmission followed a similar trend under two sets of experiments having different cultivars. The maximum transmission from tomato to tomato among different treatments under both experimental sets indicate a possible local adoption of whitefly population to particular crop and a possible role of virus-vector interaction. But, the fact that significant amount of transmission was also observed from tomato to cucurbitaceous host indicating its epidemiological significance as temporal continuation of the virus. The co-evolution of begomoviruses with whitefly and their efficiency in transmission has been well documented (De Barro *et al.*, 2011; Dinsdale *et al.*, 2010) [5, 7]. The transmission efficiency of the virus relies on virus strain indicating its co-evolution of the virus and vector biotype (Hogenhout *et al.*, 2008; Gottlieb *et al.*, 2010) [8, 9].

4.2 Transmission of Begomovirus based on Whitefly population

When Tomato was treated with different number of whiteflies maximum percent transmission was seen in case of 10 viruliferous whiteflies with 91.25% followed by 5 viruliferous whiteflies with 65 per cent. Even in case of single viruliferous whitefly inoculation, there was a successful transmission with 37.2 per cent. as according to Butter *et al.* (2008) [3], a single whitefly resulted 30% transmission of *TLCV* after providing 24hrs acquisition feeding period. While in Bottle gourd single whitefly shows transmission with 38% with increase in number of whiteflies to 3,5 and 10 the transmission increases correspondingly with up to 43,71.5,87.2% respectively as similar as Capoor and Ahmad described the transmission of *PYVMV* through whiteflies in the year 1975. In case of pumpkin also, similar pattern of transmission has been observed, the maximum transmission was with 10 viruliferous whiteflies with 82.5% of transmission. In sponge gourd single viruliferous whitefly was capable of transmitting virus up to 37.5% and the maximum transmission efficiency was with 5 number of whiteflies with 78.5% as it is described in the previous literature that a single whitefly efficiently transmits begomovirus in sponge gourd and with 10 number of whiteflies the efficiency reach maximum to the 100% (Patil *et al.*, 2017) [13]. Similarly, in bitter gourd also, with the single viruliferous white fly there was 33.6 5 of transmission and with increase in viruliferous whitefly number to 10 the transmission raised up to 72.75% the similar pattern of transmission is described in the literature of Naik *et al.* (2019) [12], in which it has been documented that 100% transmission of *Bitter gourd yellow mosaic virus* if inoculated with 15 viruliferous whiteflies. In case of cucumber also, when viruliferous whiteflies released in ascending order of 1,3,5 and 10 resulted 41,58.8,75.5 and 74.52% of begomovirus transmission as it has been well documented that 5 whitefly were enough to transmit the virus in cucumber and with increase in whiteflies to 10 the transmission reach to 75% (Shaad *et al.*, 2019) [14].

5. Conclusions

In first two sets of experiment when the whiteflies were released after giving 24hrs Acquisition feeding period on the infected tomato with begomovirus, the maximum transmission% was found on tomato to tomato followed by tomato to sponge gourd, bitter gourd, bottle gourd. High levels of transmission from tomato to cucurbitaceous host indicating its epidemiological significance in temporal continuation of the virus. In second experiment of virus vector relationship, it was found that even a single whitefly can transmit begomovirus efficiently and with increase in number of whiteflies, the transmission efficiency increases correspondingly and 10-15 viruliferous whiteflies are enough for 100% transmission efficiency. After transmission, the symptomatic leaves of the infected plant were found positive to the specific degenerative primers of Begomovirus and specific primers of *ToLCNDV*.

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