Mesta yellow vein mosaic virus: *application of loop-mediated isothermal amplification method to study efficiency of acquisition, retention and transmission by* Bemisia tabaci *(Hemiptera: Aleyrodidae) in Kenaf*

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ORIGINAL RESEARCH ARTICLE

Mesta yellow vein mosaic virus: application of loop-mediated isothermal amplification method to study efficiency of acquisition, retention and transmission by *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Kenaf

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Abstract
Whitefly is an important pest and vector of many plant *begomoviruses*. Investigations were carried out to understand the acquisition, retention, transmission, and gender basis transmission efficiency of *Mesta yellow vein mosaic virus* (MeYVMV) by whitefly. The minimum acquisition access feeding period (AAFP) was 0.10 h which resulted in 11.1% infected plants. Based on LAMP assay and yellowing symptoms, 100% virus acquisition was achieved by whitefly with an exposure of 18 to 48 h. Virus was transmitted in tested plants up to 88.8% after 1 to 10 days retention time of viruliferous whiteflies, whereas no symptoms were observed after 35 days. Fifteen minutes of exposure to viruliferous whiteflies on healthy plants resulted in 11.1% transmission. Cent percent transmission of virus was achieved in plants with 12 h exposure period. Highest virus transmission efficiency of 55.60% was observed in female whitefly compared to 33.3% in single male whitefly. Present study shall help to understand the interaction of insect-plant–virus relationship, epidemiological knowledge, and formulation of management strategies against virus and its vector.

Keywords *Mesta yellow vein mosaic virus* · *Bemisia tabaci* · *Hibiscus cannabinus* · LAMP method

Introduction

Kenaf (*Hibiscus cannabinus* L.) an annual, herbaceous, lignocellulosic crop belongs to *Malvaceae* is grown in several countries including India (Mahadevan et al., 2009). It is mainly grown for bast fibers which provide traditional packaging material and a good quality pulp for production of paper. It is also used as leafy vegetables and seeds are rich in oil with a low level of unsaturated fatty acids with potential medicinal values (Duke 1986; Pal and Jain 1998; Wong et al. 2014).

Whitefly *(B. tabaci* (Gennadius) is a pest of >600 host plants including fiber crops, ornamental plants, vegetables, weeds and spices (Secker et al. 1998; Martin et al. 2000; Oliveira et al. 2001; Jones 2003; Liu 2007; Zhang et al. 2007; Naranjo and Ellsworth 2009; Wan et al. 2009). *B. tabaci* is a highly adaptive insect that feeds on various plants (Greathead 1986; Oliveira et al. 2001) and continues to infest new host species (Simmons et al. 2008) vectoring >100 plant viruses (Jones 2003). New biotype of viruses exhibit difference in virus transmission, endosymbionts, developmental rate, transmission efficiency, and physiological host damage. Populations of whiteflies induce huge losses and occur worldwide on fiber crops. Direct feeding cause physical damage and secretion of honeydew and contamination with fungus also cause crop loss once virus transmitted by whiteflies (Gerling 2002). The polyphagous feeding habit also increases probability to acquire and transfer plant viruses from weeds and other host species. Kenaf and roselle are natural hosts for MeYVMV, while other species of Malvaceae and Fabaceae and two species of Vigna (*V. umbellata*, *V. unguiculata* sp. *unguiculata* cv. V-240) were also found alternate hosts for this virus (Chatterjee et al. 2008).

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Mesta yellow vein mosaic virus (MeYVMV) transmitted by whitefly in a persistent manner has a monopartite genome belongs to the genus *begomovirus*, (family: *Geminiviridae*) cause severe economic losses in kenaf in various parts of India. (Chatterjee et al. 2005a, b; 2006; Chatterjee and Ghosh 2007a, b; Das et al. 2008; Roy et al. 2009). First natural infection of kenaf with MeYVMV causing yellow vein mosaic was reported in West Bengal (Chatterjee et al. 2005a). When infected at an early growth stage, fail to bear flowers, while at the late growth stage, noticed malformed flowers and fruits causing low seed yield. In general, infected plant shows stunted growth with reduced leaf size. Due to increased population of infected whiteflies, MeYVMV reduced fiber and seed yield of kenaf by 18 to 24% respectively (Roy et al. 2008).

Both polymerase chain reaction (PCR) and non-PCR-based diagnostic methods have been successfully used for detection of MeYVMV in host plants and its vector (Chatterjee et al. 2005a, 2007; Ghosh et al. 2007). Although, the PCR- based method is widely used for the detection of several viruses in vector but have several intrinsic disadvantages, such as the requirement for rapid thermal cycling using specialized instruments, sometimes a low amplification efficiency, and more time-consuming. Also, virus expression in latent form could be very difficult to identify in the crop at early growth stages. Taking such disadvantages into account, a simple DNA amplification-based technique known as loop-mediated isothermal amplification (LAMP) was developed and used for the detection of MeYVMV in both infected plants and whitefly. LAMP was more efficient, robust, accurate, and rapid method for the detection of MeYVMV in kenaf and whitefly then the conventional PCR method (Meena et al. 2019). Information on acquisition, retention, and transmission of MeYVMV by *B. tabaci* is very limited. In vector transmitting diseases, disease epidemiology mainly depends on the efficiency of the vectoring population through acquisition, retention, and transmission of the virus. Understanding interactions of insect-plant-virus relationships is essential for epidemiological knowledge and for the formulation of management strategies (Mehta et al. 1994). In the present study, the LAMP method was used to assess acquisition, retention, and transmission efficiency of MeYVMV transmitted by *B. tabaci*.

Materials and methods

Source of MeYVMV MeYVMV detected by LAMP method (Meena et al. 2019) was maintained on kenaf plants (variety, JBM 81) through transmission of whitefly were kept in a growth chamber at 28-30 °C for 2-3 weeks. Yellow vein mosaic symptom appeared on young leaves were positive for MeYVMV by LAMP method.

Source of whitefly Whiteflies culture collected from Crop Protection Division, ICAR-CRIJAF, were maintained in glasshouse on a non-MeYVMV host brinjal plant under controlled conditions. For establishing viruliferous whitefly culture in glasshouse, non-viruliferous whiteflies were allowed to feed for 48 h on MeYVMV infected kenaf plants. Viruliferous adult whiteflies were then aspirated and released on 21 days old five healthy kenaf plants. Pot covered with a small plastic cylindrical cage (7.5 x 2.5 cm). Pots were kept at 30 °C in a controlled glasshouse with 18: 6 h (L: D) photoperiod and 60-70% RH. Three pots with kenaf plants similarly caged without whiteflies served as control. After 15 days exposure period of whiteflies at 30 °C , all kenaf plants expressed typical yellow vein mosaic symptoms were further used to establish viruliferous whiteflies in glasshouse. Non-viruliferous (MeYVMV free) whitefly colony were also reared and maintained on a non-MeYVMV brinjal host.

Primer for LAMP assay For detection of virus in infected whiteflies and kenaf plants, previously designed MeYVMV LAMP primer sets (Meena et al. 2019) were used in LAMP amplification. The specific LAMP primers of MeYVMV were amplified DNA obtained from infected whiteflies and plants. The primers, oligonucleotide sequences and expected size of amplified products for LAMP are depicted in Table 1.

DNA extraction from plants and whiteflies for LAMP detection Total DNA was isolated from virus-infected kenaf leaves by using CTAB method (Doyle and Doyle 1987). 150 mg of leaf tissue was ground in liquid nitrogen, resuspended in 500 µl preheated CTAB extraction buffer (2.5% CTAB, 100 mM Tris-HCl, pH 8, 1.4 M NaCl, 20 mM EDTA , 0.2% beta-mercaptoethanol, 1% polyvinylpyrrolidone) and incubated at 65 °C for 1 h. Following this 2 µl of RNase A was added to each sample and incubated at 37 °C for 10 min. One ml of dichloromethane was added and centrifuged at 12000 × g for 15 min. The supernatant was mixed with an equal volume of chloroform: isoamyl alcohol (24:1 v/v) and centrifuged at 12000 × g for 10 min. The aqueous phase was transferred to new tubes containing 1 ml of ice-cold isopropanol. Ten µl of sodium acetate was added to each sample and incubated at -20 °C for 1 h to precipitate the DNA. Samples were centrifuged at 7000 × g for 5 min and pellets obtained were washed twice with 500 µl of 70% ice-cold ethanol, followed by washing with absolute ethanol. Nucleic acid pellets were dried and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, PH 8). Similarly, DNA of whiteflies was extracted as described by Fukuta et al. 2003. Leaf tissue from healthy kenaf plants was used as negative control.

MeYVMV acquisition assay To assess the acquisition efficiency of whiteflies, about 1500 virus-free adult whiteflies were maintained on non-host brinjal plants and aspirated into glass

Table 1 Oligonucleotide primers used in loop mediated isothermal amplification (LAMP) assays for detection of *Mesta yellow vein mosaic virus*

Assay	Primer	Type	Length of primer	Sequence (5′ - 3′)[a]	Genome position[a]
LAMP	F3	Forward outer	21 nt	AGTCATTGTATTTTGTTGGCA	626–646
	B3	Backward outer	18 nt	AAAACGATCACGCTGGTC	802–819
	FIP	Forward inner	46 nt	ACGCCTATCTCTAACAATCCAAA -TGGATGGACGAGAACATCAAGAC	701–723, 654–674
	BIP	Backward inner	40 nt	CTTCAGGAACCCCAAACGATTT -TCTTCACAGTAGCCGTAG	725–746, 782–799
	LF	Loop Forward	16 nt	TTGGATGGACGAGAAC	651–666
	LB	Loop Backward	25 nt	GTTCAATGTTTATGATAACGAGCCC	756–780

[a] Nucleotide sequence of MeYVMV (Gen Bank accession number: EF428256) available in NCBI database was used for designing the primers

vials and released immediately on MeYVMV infected plants. For each exposure time interval, 10 adult infected whiteflies were aspirated into separate vials and immediately released on healthy kenaf plants maintained separately in cage house. Ten kenaf plants were tested at 0.16, 0.25, 0.50, 1, 3, 4, 6, 8, 12, 18, 24 and 48 h respectively. Except for the last interval, all whitefly samples were aspirated back into vials at the end of each time interval. Whiteflies present on the surface of cage were carefully removed. Assay plants in the cage were maintained at 30 °C under controlled glasshouse condition with a photoperiod of 18: 6 h (L: D) and 60-70% RH. Yellow vein mosaic symptoms were noticed 15 to 65 days later and presence of virus was confirmed by novel LAMP method. Experiment was repeated thrice.

MeYVMV retention assay A whitefly retention experiment was also conducted to ascertain how long the viruliferous adult whitefly could retain MeYVMV infectivity when transferred to non-MeYVMV plants. To achieve complete viruliferous whitefly population, about 1500 whiteflies were aspirated in glass vials and released immediately into a cage where MeYVMV-infected kenaf plants were maintained. Viruliferous adult whiteflies were transferred after giving of acquisition access period started elsewhere to (30 days old non-MeYVMV brinjal plants maintained in control cage chamber). Brinjal plants were replaced every 10-12 days to avoid egg deposition by newly emerging adult whiteflies. From first day and at 5 days interval, 10 adult viruliferous whiteflies were aspirated from non-host plants. Each sample of viruliferous whiteflies was immediately released into healthy kenaf plants kept in cylindrical cage. The tested retention days of kenaf plants to whiteflies were 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 days. All assay plants were maintained in a controlled cage as described earlier for 60 days. Experiment was repeated thrice and presence of MeYVMV in tested plants was confirmed by LAMP method.

MeYVMV transmission assay MeYVMV transmission assay was accomplished by employing infected adult whiteflies on kenaf plants kept in insect proof cage at 30 °C in a controlled glasshouse with an 18: 6 h (L: D) photoperiod and 60-70% RH . Ten MeYVMV infected adult viruliferous whiteflies were allowed for inoculation access feeding period (IAFP) on healthy kenaf plants for 0.25 to 48 h kept in cylindrical cage. After completion of each transmission time series, all whiteflies were carefully aspirated and removed from the tested plants. The tested plants were then placed in a clean insect-proof cage kept under control glasshouse conditions. The exposure time to kenaf plants with whiteflies was 0.25, 0.50, 1, 2, 3, 5, 7, 9, 12, 18, 24, and 48 h. Assay plants were regularly observed for symptoms and presence of MeYVMV was confirmed by the LAMP method. Experiment was repeated thrice.

Transmission efficiency (MeYVMV) of whitefly on gender basis An experiment was conducted to ascertain the transmission efficiency of MeYVMV by a single adult male and female whitefly. Non-viruliferous male and female whiteflies reared on a non-host plant were used to carry out this experiment. A single adult male whitefly aspirated from MeYVMV infected plants and released into a cage with virus-free kenaf plants. Cage was kept in a control glasshouse condition described elsewhere with same arrangement done for each single adult female whitefly. Ten adults of male and female each were set up for each experiment. In a similar way, additional kenaf plants were set up for each non-viruliferous male and female whitefly. After 15-35 days, assay plants were evaluated for the virus symptom expression, and the presence of the virus was confirmed by the LAMP method. Experiment was repeated thrice.

Statistical analysis Statistical analysis was performed on acquisition, retention, and transmission efficiency of MeYVMV by whitefly. Observations recorded on MeYVMV infected plants were transformed to arcsine values. Mean with a standard deviation of observations on MeYVMV infected plants was calculated. Duncan's multiple range test (DMRT) was used to determine a significant difference ($p<0.05$).

Results

Detection of MeYVMV in plants and whitefly by LAMP method The presence of the virus in plants and whiteflies was confirmed by LAMP method. Three primer pairs, (FIP, BIP, F3, B3, LF and LB) successfully detected MeYVMV both in infected plants and whiteflies (Fig. 1). These primer pairs produced amplicons with size ranging 0.1 kb to 1.0 kb in infected plants and whiteflies only. While, the same primer pairs did not produce any amplicon in DNA isolated from healthy kenaf plants.

MeYVMV acquisition The minimum acquisition access feeding period (AAFP) for adult whitefly to acquire MeYVMV was 0.10 h with 11.1% infection in assay plant. When time series of AAFP was increased to 0.15, 0.30, 1, 3, 4, 6, 8 and 12 h, the percent infection increased to 11.1, 22.2, 25.0, 37.5, 42.8, 50.0, 62.5 and 87.5% respectively (Table 2). Percent infection of MeYVMV at time series of 0.10, 0.15, 18, 24, and 48 h were found non-significant while 0.30, 1, 3, 4, 6, 8, and 12 h were found significant at $p<0.05$. Assay plants from time series 18 to 48 h showed cent percent infection with yellowing symptom expression of virus. Result clearly indicated that the acquisition period of whitefly was directly related to the infection of MeYMV in healthy kenaf plants.

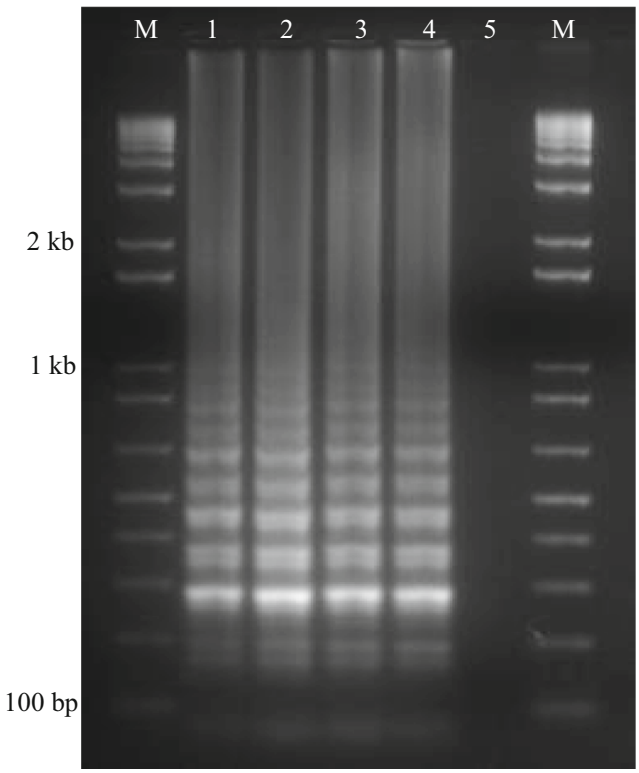

Fig. 1 LAMP detection of MeYVMV in infecting kenaf plants and whitefly (Lane 1 and 2; LAMP with DNA from infected plants), (Lane 3 and 4; LAMP with DNA from whitefly), Lane 5: LAMP with DNA from healthy plants (Negative control) and Lane M: 1 KB Plus DNA ladder

Table 2 Acquisition time for MeYVMV by whitefly based on its transmission and LAMP detection

Acquisition access feeding period (h)	Number of plants tested*	Infected plants with MeYVMV (%)*
0.16	9	1 (11.1)[a]
0.25	9	1 (11.1)[a]
0.50	9	2 (22.2)[b]
1	8	2 (25.0)[c]
3	8	3 (37.5)[d]
4	7	3 (42.8)[e]
6	8	4 (50.0)[f]
8	8	5 (62.5)[g]
12	8	7 (87.5)[h]
18	8	8 (100)[i]
24	5	5 (100)[i]
48	5	5 (100)[i]
mean (SD) = 54.14 ± 35.01	–	–

Acquisition access feeding period (0.16 to 48 h) given to whitefly to acquire MeYVMV and caged with kenaf plants (Ten whiteflies per plant). Percent infected plants with MeYVMV in each AAFP time series were calculated in parenthesis; *Mean of three replications; Means followed by the same superscripts in a column are not significantly different at ($p < 0.05$)

MeYVMV retention Viruliferous whiteflies retained and transmitted MeYVMV up to 35 days after removing from infected plants. Highest transmission rate (88.8%) was observed when plants were exposed to viruliferous whiteflies from 1 to 10 days of retention time. Virus retention capacity rate of whiteflies gradually decreased (after 15 to 25 days) and the transmission rate in infected plants was 66.6, 56.5 and 33.3% respectively (Table 3). Number of an infected plant in 30 and 35 days with MeYVMV was only one with the transmission rate of 11.1%. After 35 days, no symptom of virus transmission was observed in infected plants. Infected plants with MeYVMV at 1, 5, 10, 40, 45, 50, 55, and 60 days of retention time found statistically non-significant to each other. Similarly, 15 to 35 retention days were found significant at $p<0.05$. Presence of the virus in infected plants was confirmed by LAMP method. At the end of each trial, only a few adult whiteflies remained alive.

MeYVMV transmission Rate of IAFP and virus transmission in healthy kenaf plants had a similar trend. One plant with 11.1% virus infection was observed at 0.15 h IAFP which increased to 22.2, 33.3, 50.0, 66.7, 83.3, 83.3, and 85.7% when assay plants were exposed to whiteflies at 0.50, 1, 2, 3, 5, 7, and 9 h of IAFP respectively (Table 4). Similarly, tested plants with whiteflies at 12 to 48 h exposure time showed cent percent virus transmission. Besides this, assay plants used in control, and exposed with non-infected whiteflies did not show symptoms and negative with LAMP method. Percent infection of

Table 3 Retention time for MeYVMV by whitefly based on its transmission and LAMP detection

Retention time (days)	Number of Plants tested*	Infected plants with MeYVMV (%)*
1	9	8 (88.8)^{f}
5	9	8 (88.8)^{f}
10	9	8 (88.8)^{f}
15	9	6 (66.6)^{e}
20	9	5 (56.5)^{d}
25	9	3 (33.3)^{c}
30	9	1 (11.1)^{b}
35	9	1 (11.1)^{b}
40	8	0 (0.00)^{a}
45	8	0 (0.00)^{a}
50	8	0 (0.00)^{a}
55	8	0 (0.00)^{a}
60	9	0 (0.00)^{a}
mean (SD) = 34.23 ± 36.59	–	–

Whiteflies acquired MeYVMV during 24 h caged with kenaf plants (Ten whiteflies per plant) for 1 to 60 days. Percent infected plants with MeYVMV in each retention days series were calculated in parenthesis; *Mean of three replications; Means followed by the same superscripts in a column are not significantly different at ($p < 0.05$)

MeYVMV at time series of 0.15, 0.50, 1, 2, 3, 5, 7, and 9 h of IAFP found significant at $p<0.05$. However, IAFP at 12, 18, 24, and 48 h showed similar percent infection and were non-significant to each other.

Table 4 Transmission time for MeYVMV by whitefly based on its transmission and LAMP detection

Inoculation access feeding period (h)	Number of plants tested*	Infected plants with MeYVMV (%)*
0.25	9	1 (11.1)^{a}
0.50	9	2 (22.2)^{b}
1	9	3 (33.3)^{c}
2	6	3 (50.0)^{d}
3	6	4 (66.7)^{e}
5	6	5 (83.3)^{f}
7	6	5 (83.3)^{f}
9	7	6 (85.7)^{g}
12	7	7 (100)^{h}
18	5	5 (100)^{h}
24	5	5 (100)^{h}
48	5	5 (100)^{h}
mean (SD) = 69.63 ± 31.23	–	–

Whiteflies acquired MeYVMV during 24 h caged with kenaf plants (Ten whiteflies per plant) for 0.25 to 48 h IAFP. Percent infected plants with MeYVMV in each IAFP time series were calculated in parenthesis; *Mean of three replications; Means followed by the same superscripts in a column are not significantly different at ($p < 0.05$)

Transmission efficiency (MeYVMV) of whitefly on gender basis

Investigation based on transmission efficiency of whitefly on gender basis revealed that both male and female whitefly acquired MeYVMV from infected plants and transmitted the virus into healthy kenaf plants. The maximum transmission rate of the virus was observed by female whitefly (55.6, 43.3 and 50.0%) compared to male whitefly with 33.3, 13.3 and 25.0% (Table 5). Results revealed that female whitefly had more capacity to feed, retain and transmit the virus from infected plants to healthy plants compared to male whitefly. Control plants remained healthy and negative with LAMP method and expression of virus symptoms.

Discussion

The present study showed that with the availability of virus inoculums and efficient transmission of whitefly, MeYVMV could potentially cause devastating effect on kenaf fiber production. Average incidence of disease was more in rainy day due to lush growth of crops and weeds that act as an alternative host for whiteflies. Previous study highlighted environmental conditions prevailing during the crop period and favored of maximum buildup of the whitefly population (Chatterjee et al. 2007). Whitefly population build up very rapidly and serve more reservoirs for the virus and causes infection in the young crop as noted in *Okra yellow vein mosaic virus* (Dhanju et al. 1993) and *Cotton leaf curl virus* (Narula et al. 1999). Information on the ability of vectors to acquire, retain and transmit a virus has been very important for understanding vector relationship and managing the virus outbreaks (Polston et al. 1990). We determined 0.10 h minimum AAFP and 18 h maximum AAFP for whitefly. When acquisition time increased then infection rate of MeYVMV was also increased. According to Varma 1952; Nene 1973; Noor et al. 2002, adult whiteflies require a minimum acquisition period of about 10-30 min to acquire virus. Similarly,

Table 5 Transmission efficiency of MeYVMV by whitefly on gender basis

Trials	Gender	Infected plants/total plants	Transmission (%)
1	Female	15/27	55.6
	Male	9/27	33.3
2	Female	13/30	43.3
	Male	4/30	13.3
3	Female	14/28	50.0
	Male	7/28	25.0

Whiteflies acquired MeYVMV during 24 h caged with kenaf plants (one insect per plants) for a 12 h IAFP. Insects that survived for 12 h on the plants were taken into account for calculation of transmission efficiency

Pollard 1955 reported minimum time (10-30 min) required by whitefly to reach the mesophyll region and to acquire virus from phloem tissues that also supported to our study. The present study reveals that kenaf plants were more succulent and efficiency of virus acquisition and transmission by whitefly from infected plants to healthy plants was high. Rashid et al. 2008 reported minimum feeding period required by whitefly was 30 min for acquisition and successful transmission of virus. Similar results were also observed by Seetharama and Yaraguntaiah 1981; Jayashree et al. 1999; Jiang et al. 2000; Rashid et al. 2008 that also corroborated to our results.

Long retention period of 35 days after virus acquisition suggested that single viruliferous whitefly could have ample time to cause serious damage after feeding on MeYVMV plants. It was observed that initially whiteflies retained high quantity of virus titer, while whitefly virus titer retention efficiency was decreased later. Also observed that up to 10 days in tested plants, virus titer quantity was high and causes 88.9% infection. Virus retention efficiency mainly depends on the proportion of cells with high virus titer content in the feeding tissue and the virus titer content in the cells that the insect feeds from. When retention efficiency of whitefly increased, virus transmission rate gradually increased. In our findings we found duration of retention time and population density of vector that play a key role in virus retention efficiency. In a related study, more than 100 h retention of *Tomato leaf curl virus*-Ban 4 demonstrates a persistent type of virus-vector relationships (Gibbs and Harrison 1976). Similarly, *begomovirus* DNA acquired over 24 to 48 h remains associated with the insect for several weeks, much longer then infectivity (Caciagli and Bosco 1997; Rubinstein and Czosnek 1997; Muniyappa et al. 2000). However, flying pattern and feeding habit of whiteflies on different natural host plants could transmit MeYVMV efficiently. Due to such prevailing conditions, MeYVMV transmitted by whiteflies has a serious effect on kenaf fiber production. In a recent year, a new *Kenaf leaf curl virus* was detected on the same crop (Paul et al. 2006) thus, the findings obtained from this study will also help in understanding the factors influencing the epidemiology of this relatively new virus on kenaf crop and hopefully on finding solutions to manage this disease.

Our study suggested minimum IAFP required by whiteflies for transmission of MeYVMV in plants was 0.15 h. When IAFP increased, infection of MeYVMV was also increased. Transmission efficiency of whiteflies was mainly dependent on number of hours increasing for IAFP on assay plants. When IAFP time was up to 12 h then infection of MeYVMV was cent percent. In a similar study, 100% of whiteflies were infected with *Tomato leaf curl virus China* and *Tobacco curly shoot virus* after 12 h of exposure time (Jiu et al. 2006) and at 0.5 h, transmission rate of whitefly was 40% for each virus. Whereas, in the case of *Tomato yellow leaf curl China virus*, transmission rate with one adult whitefly was 55% after 48 h of exposure. While, transmission rate on tomato plants was cent percent when 10 adult whiteflies exposure with 48 h (Jiu et al. 2006). Earlier maximum infection rate 77.3% with 20 whiteflies was observed (Capoor and Ahmad 1975). Our findings clearly suggested that transmission efficiency of whitefly mainly depends on virus-host interaction.

Transmission efficiency of female whitefly was higher then male whitefly on gender basis experiments. Female whitefly transferred MeYVMV more efficiently in healthy plants compared to male whitefly after 18 h AAFP and 12 h IAFP period. It seems to assume that transmission rate of MeYVMV might be related to body size and content of virus particles acquire more by larger female whitefly compared to smaller male whitefly. Larger female whitefly may efficiently acquire and transmit virus in the plant due to more sucking power. In a similar report, female whiteflies transmitted bipartite *Tomato leaf curl Benglore virus* (Muniyappa et al. 2000) and monopartite *Tomato yellow leaf curl virus* (Cohen and Nitzany 1966) with higher efficiency then male whiteflies. However, in most cases, gender and age of whiteflies was ignored. Forty-eight hours of acquisition access period of TYLCV shows 50% infection in tomato plants with adult female whiteflies while same-age adult male whiteflies were able to infect only 20% of plants (Czosnek et al. 2001). The present study confirmed that infection capacity of whiteflies decreased with the age period. It has to be reported that adult male and female whiteflies transmitted *Squash leaf curl virus* with the same efficiency (Polston et al. 1990). The male whiteflies were less efficient in virus transmission then the female whiteflies (Boulehya et al. 1997). The reason for these differences was not cleared and need to further detailed study. Our findings further suggested that the female whitefly was more efficient in the transmission of MeYVMV then male whitefly.

The impact of the disease on yield and quality of agricultural produce have been reported to be influenced by globalization of trade, pathogen's evolution, cultural practices as well as climatic and several other factors (Garrett et al. 2006; Meena et al. 2014a, b). Therefore, development of simple but accurate diagnostic techniques for the identification of disease causing organisms is a need of the hour to boost the existing crop management practices. In present study, LAMP method used for successful detection of MeYVMV in leaves and whitefly could improve the reliability of viral transmission information. In field conditions, presence of latent infection of virus without showing any symptoms, population of various age groups of whitefly (adult and nymphs) is mixed and effect of age group and their virus vector relationship is not known. In such conditions, we concluded that plants are completely free from virus infection. Likewise, molecular detection techniques also given false negative results due to presence of low titer content of virus in vector as well as in

infected plants. Due to higher primer efficiency, LAMP easily detected latent infection of the virus in plants as well as in vector. LAMP based detection procedure could be used to detect MeYVMV from single whitefly and a group of nymphs, which makes it a potential tool for understanding epidemiological aspect of viral disease.

The present study indicated more precisely the ability of whitefly to acquire, retain, and transmit MeYVMV in kenaf plants. Whiteflies multiplied the virus in larger copy numbers in their body and transmit it to the healthy plants. When exposure time of whitefly increased the transmission rate of the virus was elevated. Hence, understanding the interaction of insect-plant–virus relationship with LAMP method will help for epidemiological knowledge and in formulation of management strategies.

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Compliance with ethical standards

Conflict of interest They have no conflict of interest.

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