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Studies on Transmission Efficiency of *Bemisia tabaci* in *Vigna unguiculata (L)* for *Cowpea Golden Mosaic Virus*

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ABSTRACT

Whiteflies are very common in study areas and the experiments were conducted to study the feeding pattern of insect vector to develop control of virus transmission by understanding feeding pattern of vector. Optimum acquisition period was found to be 12 hour once the vector has acquired the virus it is able to transmit virus for as long as ten days and optimum infection feeding time is 24 hours, after which it transmit virus to other plants in intermittent manner. Even the post acquisition fasting has little effect on the efficiency of virus transmission although when post acquisition fasting was increased, decrease in efficiency of transmission is seen. Increasing the number of whiteflies per plant decrease the incubation period Virus persist in the vector for longer duration, which indicates it to be 'persistent type', virus acquisition and inoculation increases efficiency with increasing access time.

Introduction

The yellow mosaic diseases of a number of legumes across Southern Asia are caused by four species of whitefly-transmitted geminiviruses (genus Begomovirus, family Geminiviridae): Mungbean yellow mosaic virus, Mungbean yellow mosaic India virus, Dolichos yellow mosaic virus and Horsegram yellow mosaic virus. They cause losses to a number of important pulse crops, a major source of dietary protein in the region. The viruses have host ranges limited to plants of the family Fabaceae and efforts to limit losses are hampered by limited availability of conventional resistance sources and/or the lack of durability of the resistance that has been identified [1]

The cosmopolitan whitefly species, *Bemisia tabaci* (Gennadius) have always been regarded as pests to a large range of worldwide crops. This species is capable of transmitting plant viruses, with *B. tabaci* the vector of viruses in several groups. The largest group of viruses transmitted by *B. tabaci* is the geminiviruses and *B. tabaci* is known to transmit around 60 members [2].

Cowpea *Vigna unguiculata* (L) walp is one of the important pulse crop. Green pods of cowpea are used as vegetable in northern Indian states whereas in Bengal, Madras, Andhra Pradesh, Kerala and Maharashtra cowpea is cultivated as a pulse crop. In plains cowpea is grown in summer and rainy seasons, whereas on hills only in summers. Cowpea is an important legume crop and constitutes a vital food source of carbohydrate and proteins in many countries.

Whiteflies transmit viruses on cowpea [3] which causes major yield loss if infection occurs at early stage of crop [4]. These insects are very common in study areas and the experiments were conducted to study the feeding pattern of insect vector so effective control measures can be developed.

Materials & Method:

Young infected leaves of *Vigna unguiculata* cv. Pusa Komal with distinct virus symptoms were collected from glass house and used as food for the whiteflies. 2cm wide and 5cm. long straight glass tube whose one end in connected with rubber tubing with a cloth barrier between the glass and rubber was used as aspirator to collect whiteflies by sucking through rubber tubing . These white flies were allowed to feed on infected leaves for 12 hours. After feeding whiteflies were collected carefully and placed on test seedlings plants for 24 hours. About 10 whiteflies per plants were used for the transmission. Test plants were inoculated when first trifoliate emerged. 0.2% Imida-chloprid insecticide was used to kill the white flies. Test plants which were not inoculated with white flies served as control and kept under observations.

Effect of pre-acquisition fasting on transmission, Minimum time necessary for acquisition, Minimum infection feeding time for transmission, Effect of post acquisition fasting on transmission, Effect of number of insects on transmission and persistence of virus in the vector were studied using whiteflies as vector and cowpea as test plant. The experiments in the present study were carried out in an insect proof chamber, where usual precautions were taken to keep the plants free from insects and nematode infection.

Results:

For Pre-Acquisition Fasting white flies collected were not fed to know the effect of fasting on transmission before feeding on diseased plant. To ascertain the effect of pre-acquisition fasting on transmission through *Bemisia tabaci*, ,were kept on fast for 0, 0.5,2, 4 & 8 hours After fasting whiteflies were allowed to feed on infected plants for 12 hours Cowpea cv. Pusa Komal seedlings infected with the virus were used as the source of inoculums in the transmission and then the whiteflies were allowed to feed on healthy plants for 24hours with following experimental setup the observations are given in table 1.

Effect of Pre-Acquisition Fasting On Transmission:

Pre acquisition fasting time	- 0, 0.5, 2, 4, 8 hours
Acquisition feeding time	- 12 hours
Number of Whiteflies per plant	- 10
Number of plants treated with Whiteflies	- 10
Inoculation feeding time	- 24 hours

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Table 1. Effect of Pre-acquisition fasting on transmission of the virus by *Bemisia tabaci*

Sl. No	Period of Pre-acquisition fasting (h)	No. of infected plants out of total 10 plants exposed	Percent transmission	Incubation Period
1.	0	2.7	27%	18 days
2.	0.5	3.5	35%	15 days
3.	2	5	50%	14 days
4.	4	8	80%	13 days
5.	8	8	80%	13 days

Data presented in Table-1 showed that *Bemisia tabaci* could acquire virus, without any pre-acquisition fasting (i.e. pre-acquisition fasting is insignificant) but fasting has beneficial effect, after 4 hours of fasting transmission increases to 80% but increasing the time of fasting after 8 hours does not increase efficiency of transmission.

Minimum Time Necessary for Acquisition:

To know the optimum time required for acquiring virus from infected plants whiteflies were allowed to feed on infected cowpea cv. Pusa Komal seedlings for 12, 24, 36 & 48 hours then whiteflies were allowed to feed on healthy plants for 24 hours . To determine the minimum and optimum acquisition time (acquisition threshold) following treatments were given.

Pre acquisition fasting time - 4 hours
Acquisition feeding time - 5, 12, 24, 36, 48 hours
Number of Whiteflies per plant - 10
Number of plants treated with whiteflies - 10
Inoculation feeding time - 24 hours

Data from table-2 showed that *B. tabaci* may acquire virus in 5 hours, however optimum acquisition feeding was obtained after 12 hours. After that there is decrease in efficiency of virus transmission

Table 2. Minimum time necessary for Acquisition by *Bemisia tabaci*

Sl. No	Period of virus acquisition (Hours)	Number of plants infected	Percent infection	Incubation Period
1.	5	5	50%	13 days
2.	12	9	90%	12 days
3.	24	8	80%	12 days
4.	36	7	70%	14 days
5.	48	7	70%	13 days

Minimum Inoculation Feeding Time for Transmission :

To find out the minimum infection feeding period to infect healthy plants , whiteflies were fed on infected plants for 12 hours then allowed to feed on healthy plants for 5,12, 24, 36 & 48hours. To determine minimum inoculation time required for transmission, following treatments was given.

Pre acquisition fasting time - 4 hours
Acquisition feeding time - 12 hours
Inoculation feeding time -5,12,24,36,48 hours
Number of white flies per treatment - 10
Number of whiteflies per plant - 10

Data in table-3 shows that, the whitefly can acquire virus in 5 hours but optimum transmission is obtained after 24 hours. The percentage of infection is almost constant after 24 hours

Table 3. Minimum Inoculation feeding time for transmission of the virus by *Bemisia tabaci*

Sl. No	Time	No. of plants infected	Percent infection after inoculation feeding (P I Inoculation)	Incubation period
1.	5	7	70%	15
2.	12	8	80%	13
3.	24	9	90%	12
4.	36	9	90%	12
5.	48	8	80%	14

Effect of post-acquisition fasting on transmission:

Once virus has been acquired by white flies from infected plants and if they are not allowed to feed on healthy plant for how long they can carry the virus is important to know in controlling the spread of disease in nature. To find out, the starvation time after which white flies do not remain viruliferous, or they loss their ability to transmit the virus an experiment was conducted with the following treatments.

Pre acquisition fasting time - 4 hours
Acquisition feeding time - 12 hours
Post acquisition fasting time - 2, 4, 6, 8, 10h
Inoculation feeding time - 24 h
Number of whiteflies per treatment - 10
Number of whiteflies per plants - 10

The results in table-4 showed that there is no marked effect on transmission efficiency up to 6 hours. After 6 hours there is decrease in transmission efficiency but even after 10 hours of fasting vector is able to transmit the virus with considerable efficiency.

Table 4. Effect of Post-acquisition fasting on transmission of the virus by *Bemisia tabaci*

Sl. No	Post acquisition fasting period (hours)	Total No. Plant infected	Percent transmission	Incubation Period
1.	2	8	80%	13 days
2.	4	9	90%	12 days
3.	6	8	80%	12 days
4.	8	7	70%	14 days
5.	10	6	60%	15 days

Effect of the number of whiteflies on transmission:

To determine whether population of whiteflies effect severity of infection or high number of insects are responsible for early outbreak of disease, experiment was conducted to know the effect of number of insects per plants.2,4,8,10 & 15 whiteflies were allowed on single healthy plant with following treatments.

Pre acquisition feeding time - 4 hours
Acquisition feeding time - 12 hours
Inoculation feeding time - 24 hours
Number of insects per plant -2, 4,8,10 & 15

The Observation presented in table-5

Table 5. Effect of the number of whiteflies on Transmission

Sl. No	Number of White flies Per Plant	Total No. Plant infected	Percent transmission	Incubation Period
1.	2	2	20%	16 days
2.	4	4.3	43%	14 days
3.	8	7	70%	14 days
4.	10	8.2	82%	12 days
5.	15	7.9	79%	12 days

On increasing the number of whiteflies disease appeared early than with treatment with less number of whiteflies and transmission of disease to healthy plant was more with higher number of whiteflies.

Persistence of virus in the vector –

To know for how long virus can be transmitted by whiteflies after acquiring the virus the following experiment was conducted by feeding same insect to healthy plants after 2,4,6,8 & 10days by giving following treatment

Pre acquisition feeding time - 4 hours
Acquisition feeding time - 12 hours
Incubation feeding time - 24 hours
Same vectors fed on successive 5 test plant after and 10days -2,4,6,8

Table 6: Number of Successive Plants infected by *B. tabaci*

Days after acquisition	The number of plants exposed in a series					Total No. of Plants infected
	1	2	3	4	5	
2	+	-	+	-	-	2
4	+	-	+	-	+	3
6	-	+	-	+	-	2
8	-	-	+	+	+	3
10	+	-	+	-	+	3

Data presented in Table-6 showed that *B.tabaci* remains infective but it shows irregular behaviour in transmitting vector. It remains viruliferous up to 10 days or might be more and can infect plants in succession but it does not follow any regular pattern, it transmits virus intermittently [5].

Discussion

Whitefly *Bemisia tabaci* were found to be most common in the study region during month of August to September. These insects were tested for their efficiency as the vector for the virus under study. A pre-acquisition fasting has little effect on the efficiency of transmission, however there was some increase in percent transmission after 4 hour of pre acquisition fasting Pre-acquisition fasting might not be important for increasing the efficiency because when allowed to transmit without fasting, vector was able to transmit the virus with 70% efficiency (Table-1).

Optimum acquisition period was found to be 12 hour as the efficiency markedly increased at 12 hour when compared with 5 hours of acquisition period. The range of 12-24 hours is optimum because after 24 hours there is decrease in efficiency of transmission. The minimum acquisition feeding time resulting in successful transmission was 3 hours, and insect given a 48 hours acquisition feeding time were able to transmit virus to healthy plants. The 3 hours period was the shortest tested [5].

Once the vector has acquired the virus it is able to transmit virus for as long as ten days and optimum infection feeding time is 24 hours, after which it transmit virus to other plants in intermittent manner. Even the post acquisition fasting has little effect on the efficiency of virus transmission although when post acquisition fasting was increased, decrease in efficiency of transmission is seen (Table-4).

Increasing the number of whiteflies per plant decreases the incubation period (Table-5) [6] also found similar results with whiteflies in cotton leaf curl virus transmission.

Virus persists in the vector for longer duration, which indicates it to be 'persistent type', virus acquisition and inoculation increase in efficiency with increasing access time. *B. tabaci* is an efficient vector of the disease, a single whitefly is able to transmit the virus and the rate of transmission increased with increasing population intensity of the vector.

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