BIBLIOGRAPHY

PATRICIO, JOEYLITO G. APRIL 2011. Incidence and Severity of Squash Leaf Curl Virus Infection on Chayote in La Trinidad, Tublay and Atok, Benguet. Benguet State University, La Trinidad, Benguet.

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ABSTRACT

This study was conducted to assess the incidence and severity of Squash Leaf Curl Virus on Chayote in La Trinidad, Tublay and Atok, Benguet.

Results in the greenhouse experiment revealed that the municipality of Tublay had the lowest Squash Leaf Curl Virus local lesion and systemic severity. The Municipality of Atok and La Trinidad had the highest disease severity.

In the field survey, the chayote plantation in Tublay, Benguet had the lowest Squash Leaf Curl Virus disease severity and disease incidence. The Municipalities of Atok and La Trinidad, Benguet had the highest of disease severity and disease incidence of Squash Leaf Curl Virus.

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INTRODUCTION

Chayote (*Sechium edule)* is a perennial, training vine bearing edible fruits which belong to family Cucurbitaceae. Knot and Deanon (1980) stated that the chayote is a nature of Mexico, Central America and the West Indies.

Chayote and other crops belonging to the Cucurbit family are given on overhead trellis (Knot and Deanon, 1980). In addition, side trellis is used to withstand strong wind in wind prone areas. This practices combined with judicious pruning will enhance more yield for the crop. If they reach the desirable size, they are harvested manually (Velasco, 1994).

The symptoms exhibited by a virus infected plant may be due to other factors such as mineral deficiency, insects, nematodes, fungi and other environmental factors. But, oe of the many ways to determine virus infected plant is the shortening of internodes (Bawden, 1964) and curing of leaves.

Virus is one of the pathogen that is difficult to control because it is highly dependent on the disseminating agents such as insects. In addition, viruses could also be transmitted mechanically by the use of contaminated garden tools of manual handling of plant part by man.

The squash leaf curl virus affects most cucurbits, with cucumber showing the least symptoms. Leaves are cupped upward, new growth bends upward, and mid-vein portions of leaves are severely mottled. Flowers and fruit are small and abnormally developed. Control measures focus on lowering the potential virus inoculums in the area. These measures include that weeds around the fields do not harbor whiteflies and destroy

infected crops as soon as practical after harvest. Controlling the whiteflies directly has not proved successful (Horn *et al.*, 2004)

Squash leaf curl is transmitted persistently by both biotypes of the sweetpotato whitefly, *Bemesia tabaci*. It has been a problem in Arizona in fall watermelons in the central part of the state. Infection of young plants causes severe curling, and small leaves that are mottled and yellowing. Often an entire field may be affected, but young plants have been seen to grow out of the disease. It occurs primarily in squash and watermelon. No resistant varieties are known, but a host free period with sanitation and weed control may reduce subsequent infections.

Like in most municipalities of Benguet, vegetable farming is one of the sources of livelihood among the municipality population of La Trinidad, Tublay and Atok. Chayote is raised throughout the year and is preferred by farmers of Benguet because it is easy to sell at local and city markets.

At present, one of the problems faced by farmers in growing chayote is the incidence of disease exhibiting the upward cupping of leaves bending of new growth upward, and severe mottling of the mid-vein portions of leaves. No management strategies are being employed for these disease infections. Thus, information on the disease characteristics like incidence and severity would be of great help to the chayote farmers of La Trinidad, Tublay and Atok, Benguet.

The study aims to assess the incidence and severity of squash leaf curl virus infection on chayote La Trinidad, Tublay and Atok, Benguet.

The survey was conducted in selected existing chayote plantation at La Trinidad, Tublay and Atok, Benguet. The mechanical transmission activity was conducted in the Plant Pathology Greenhouse, College of Agriculture, Benguet State University, La Trinidad, Benguet.

REVIEW OF LITERATURE

About the disease

Squash leaf curl virus (SLCV) was detected for the first time in Jordan using degenerated oligonucleotide primers. Two isolates of the virus, SLCV-E and SLCV-R, were detected using specific oligonucleotide primers in symptomatic *Cucurbita pepo*. SLCV was also found to occur naturally in *Malva parviflora*, which showed severe leaf curling, yellowing and stunting of the whole plants. The full-length genomes of *Squash leaf curl virus*-Malva (SLCV-Malva) isolate were amplified using the bacteriophage Φ DNA polymerase enzyme. Nucleotide sequence analysis showed that SLCV-Malva shared high nucleotide identity (98% and 97%) with SLCV-EG and SLCV-E from Egypt and USA, respectively. A survey using dot-blot hybridization indicated that squash leaf curl disease occurred in all surveyed areas. The highest disease incidence (95%) was recorded in Dir Alla area, whereas disease incidence did not exceed 69% in squash samples collected from North Ghor (*Al_Muza*, 2007).

Virus Symptoms

Diener (1967) as stated by Singh (1978) cited that infected plants show histopathology changes as hyperthrophy, hyperplasia, and necrosis. The physiology of the plant is affected as shown in the decreased photosynthetic activity, increased activity of phenophenoloxides and accumulation of oxidized polyphenol derivatives and increased activity of growth regulating substances.

Janick (1972) as cited by Angadol (1983) grouped the virus diseased into two; the yellow viruses and mosaic viruses. Symptom of the yellow viruses include yellowing,

leaf curling, dwarfing, excessive branching, shortening of the mosaic viruses includes a mosaic appearance and mottling which is due to chlorosis in small areas of the plant following the death of the tissues.

Smith (1972) as cited Angadol (1983) mentioned that in the field, infected plants are readily recognized by their reduced size and different growth habits and by marked changed in leaf color and shape, flower buds are killed and reduced yields are obtained when infection occurs before motivation of plants or fruits.

Tarr (1972) as cited by Angadol (1983) stated that symptoms of virus diseases in plants are varied, often severe and sometimes so slight that it cannot be noticed immediately except on close examination. Symptoms may disappear under certain environmental conditions such as high temperature, or following the application of certain fertilizers. Some plants may show no outward symptoms even if they contain the virus.

Singh (1978) also mentioned that plant viruses are infections and sometimes highly contagious. However, Mathews (1981) mentioned that virus infections do not necessarily caused disease at all times in all parts of an infected plants. There are at least five (5) situations in which disease may be absent. They are, infection with a very mild strain of the virus; a tolerant host, nonsterile “recovery” from disease symptoms in newly formed leaves; leaves that escape infection because of their age and positioned in the plant; and dark green areas in a mosaic pattern.

Transmission of Viruses

One of the cardival characters of a virus is the fact that it has infections. However, it was recognized early, that viruses differ widely in the means and in the ease with which they may be transmitted. Some can be transmitted easily while others are difficult to transmit by artificial means. Some viruses have been transmitted by only one method while others are has been transmitted by two or more methods. This was brought about by the coincidence that the two viruses studied first, peach yellow and tobacco mosaic represents extremes. The common tobacco mosaic virus is transmitted readily by simple mechanical means, while peach yellow virus is transmitted by us of buds and graft.

As cited by Ilag (1987), viruses are transmitted by mechanical means, by nematodes, by certain soil-borne fungi, by mites, through infected seeds and through infective vegetative planting materials.

Moreover, Singh (1978) further stated other means through which virus can be transmitted by vegetatively reproductive parts, transmission by grafting and budding, transmission of viruses by pollen, transmission by seed, transmission of viruses through weeds and flowering plants parasites, transmission of viruses through insects and transmission by contact of diseased plant and healthy plants.

The same author further stated that the insects can transmit only one specific virus to only one species of the host plant while other transmit as single virus to more than one species of the host.

Walker (1969) reported that viruses which are readily more transmitted mechanically are aphids and those which do not yield to such transfer are leaf hopper-borne. Furthermore, there are differences among vectors and their relation to the virus

they transmit. Some viruses transmitted by aphids and whiteflies are carried in such a way that the vectors remain viruliferous for a period of only a few minutes to a few hours. These virus as cited by Ligat (1983) are called stylet-borne viruses. The same species of aphids and whiteflies may transmit the virus after several hours after it is possible that the virus infected by the insects is later transmitted through the body into the saliva, by which channel it eventually reaches the next host plant.

Ilag (1987) also reported that viruses are transmitted mechanically. Bawden (1964) stated that the many reported failures to infect plants in mechanical transmission by inoculation with extract from infected ones, reflect the intrinsic behavior of the infective virus is doubtful. He further stated that the failures most probably occurred because the properties of either the plant yielding the extract or the one inoculated prevented infection. Moreover, virus inoculum containing adequate concentration of virus, transmission and deposition of virus through abrasive (such as carborandum) causing injuries on the plant, and establishment of infection by placing the polycestronic viral genome first, are the factors essentially for mechanical transmission.

MATERIALS AND METHODS

Survey of Squash Leaf Curl Virus on Chayote

A survey and documentation of the incidence and severity of Squash Leaf Curl Virus infection on chayote was done at the selected municipality of La Trinidad, Tublay and Atok, Benguet. From the selected sites samples of infected leaves with SLRV, were gathered and was used for inoculation at the greenhouse of BSU.

Mechanical Transmission of Squash Leaf Curl Virus On Chayote

1. Source of samples. Leaves suspected of being infected with Squash Leaf Curl Virus were collected in existing chayote farms in the municipality of La Trinidad, Tublay and Atok, Benguet.

2. Preparation and inoculation of test plants. Previously germinated seedlings of *Vigna umbellate* were used as indicator plant in the study. This plant was used because; it showed prominent manifestation of Squash Leaf Curl Virus symptoms (Cohen *et al*. 1983). The seedlings were transplanted in black plastic bags. The seedlings at four to six leaf stages were used as test plants.

3. Mechanical Transmission experiment. Prior to bioassay, suspected Squash Leaf Curl Virus infected chayote leaf samples were weighed to determine the amount of phosphate buffer that was added. The sap was extracted with the use of mortar and pestle. Before inoculation, the upper left surface of test plants was dusted with carborundum powder # 320. Sap inoculation was done by dipping the forefinger into the pestle containing the mixture of sap and buffer and then rubbing it gently on the carborundum-dusted leaves. After inoculation, the inoculated leaf surfaces were washed with distilled

water to remove carborundum dust. Each test plants were labeled properly. Inoculated test plants were incubated inside the greenhouse and then observed for reactions daily for three weeks. Photographs of the plants showing symptoms were taken.

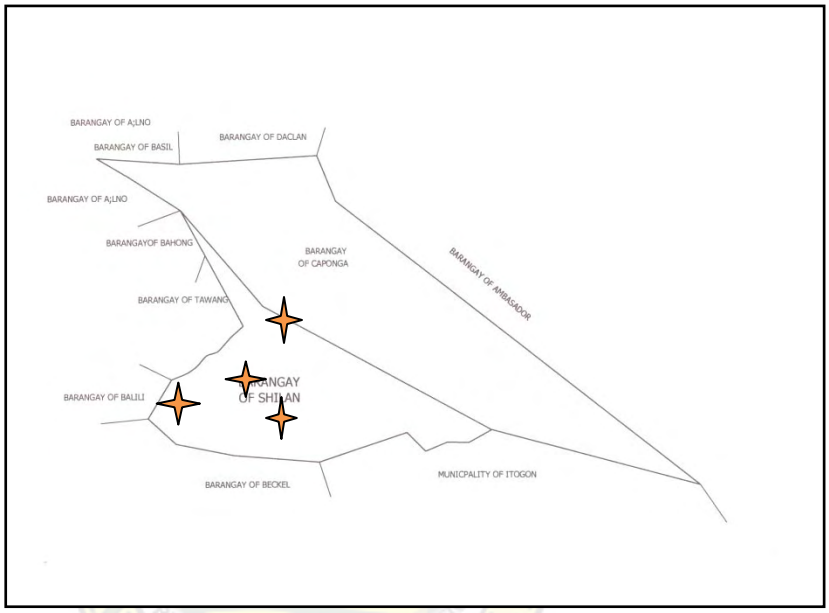

Figure 1. Map of the surveyed sitios in La Trinidad, Benguet

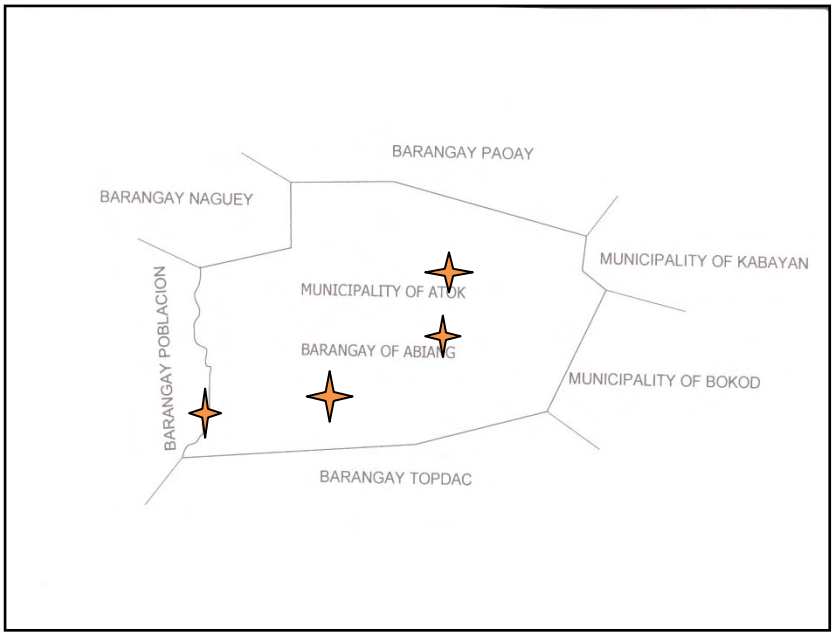

Figure 2. Map of the surveyed sitios in Atok, Benguet

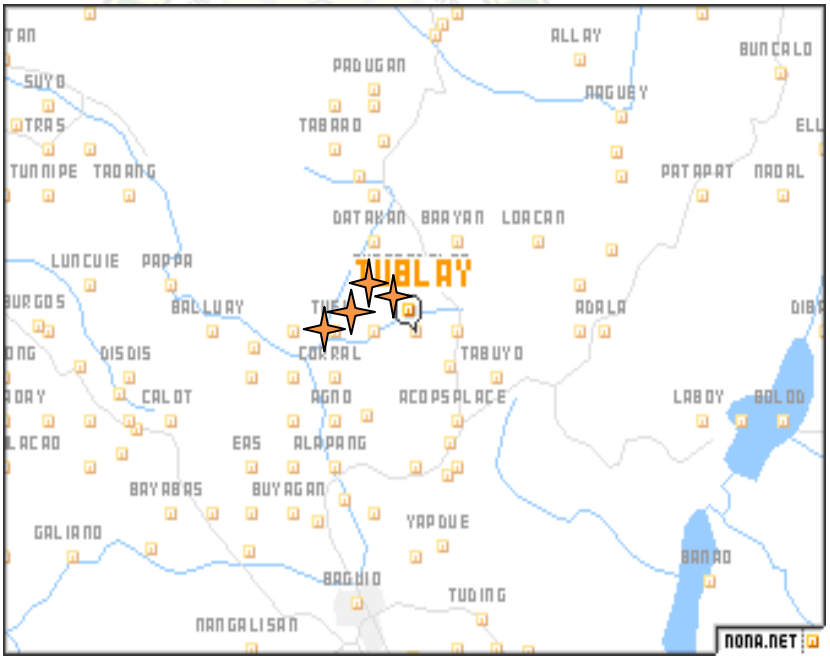

Figure 3. Map of surveyed sitios in Tublay, BengueT

Figure 4. Mechanical transmission activities in the greenhouse

Data Gathered

1. Severity of Squash Leaf Curl Virus on Chayote

A. Green House

The number of necrotic spots on the inoculated *Vigna umbellata* (as test plants), were assessed using the arbitrary rating scale below (Ligat and Lumiqued, 2000).

Scale	Description
0	No infection
1	Mild infection, 1-10 necrotic spots in inoculated leaf, local infection.
3	Moderate infection, 11-25 necrotic spots in inoculated and uninoculated leaf, systemic infection
5	Severe infection, 26-50 necrotic spots in inoculated and uninoculated leaf, systemic infection.

Disease severity will be expressed

$$DS = \frac{n(0) + n(1) + n(3) + n(5)}{N \times 5} \times 100$$

Where:

n- Number of necrotic spots classified by scale.

N- Total number of sample plants

B. Field Condition

Twenty plants were selected per surveyed area and rated for disease severity. The severity was assessed using the arbitrary rating scale below (Ligat and Lumiqued, 2000).

Scale	Description
0	No infection
1	Mild infection, 1-10 infected leaves per plant.
2	Moderate infection, 11-25 infected leaves per plant.
5	Severe infection, 26-50 infected leaves per plant.

Disease severity will be expressed as:

$$DS = \frac{n(0) + n(1) + n(3) + n(5)}{N \times 5} \times 100$$

Where:

n = number of infected leaves per plant classified by scale.

N = total number of plants

3. Incidence of Squash Leaf Curl Virus on chayote. The presence or absences of Squash Leaf Curl Virus - infected chayote plants were recorded. The datum was determined by dividing the number of infected plants over the total number of sample plants multiplied by 100.

4. Location map showing the different sites.

RESULTS AND DISCUSSION

Visual Symptoms Of Squash
Leaf Curl Virus on Chayote

Infected leaves are frequently puckered and mottled yellow or light green leaf symptoms are more evident on younger leaves (Figure 5). Plants can be stunted (Figure 6) especially when the infection occurs early in the development. Although infected fruit can be reduced in size and show variation in colors, the most obvious symptoms is distorted or lumpy appearance (Figure 7).

Figure 5. Leaf stunting on Chayote caused by SQLV

Figure 6. Stunting of young Chayote caused by SQLV

Figure 7. SQLV symptoms on Chayote

Severity of Squash Leaf Curl Virus
on Chayote in the Greenhouse

Squash Leaf Curl Virus infected chayote leaf samples from the three municipalities were transmitted mechanical to *Vigna umbellate* which served as indicator plant in the greenhouse. Six days after inoculation, local lesions were recorded in infected samples from Tublay, which had the lowest disease infection (Table 1). The Municipality of Atok and La Trinidad had the highest disease severity of 70.34 and 68.66 percent.

Twelve days after inoculation (Figure 10), the disease infection became systemic where infection exhibiting necrotic lesions after six days of inoculation. This was observed in the samples from Tublay municipality which had the lowest Squash Leaf Curl Virus severity and in Atok and La Trinidad which registered the highest of 68.33 and 58.66 percent.

It could be noted from the results that there was a virulent strain of Squash Leaf Curl virus in Tublay, Benguet. Another implication could be the phenomenon of Squash Leaf Curl Virus latent infection in Atok and La Trinidad, Benguet.

The Chayote plantation in Ambassador, Tublay, Benguet were intercropped with a tree (alnus), trumpet flower and flower of the night or “Dama de Noche” which are non-hosts of Squash Leaf Curl Virus.

The presence of alnus trees may have resulted to the low the Squash Leaf Curl Virus in Tublay which conforms with the findings of Ligat (2004) that alnus trees enhanced growth vigor of chayote and develop resistance against Squash Leaf Curl due to the nitrogen-fixing capacity of alnus species, saturated by light of one-tenth the higher intensities which increases photosynthetic rate it also releases low molecular weight substances from decayed debris which taken up by chayote resulting to an increased metabolism and influence development of resistance against the virus disease.

Table 1. Severity of squash leaf curl virus in the BSU (Greenhouse Experiment)

SOURCE OF INOCULUM	LOCAL LESION	SYSTEMIC INFECTION
La Trinidad	68.66	58.66[a]
Tublay	57.67	39.00[b]
Atok	70.34	68.33[a]
Coefficient of Variation	27.14%	27.35%

Means followed by common letters are not significantly different at 5% level DMRT

a b c

Figure 8. Samples of *Vigna umbellate* as indicator plant (Internet) (a. uninoculated b. necrotic lesion, c. systemic infection)

Figure 9. Samples of *Vigna umbellate* as indicator plant (Present study) a. uninoculated b. inoculated

Figure 10. Systemic infection of SQLV after 12 days of inoculation

Field Survey on the Severity and Incidence of Squash Leaf Curl Virus

The Chayote plantation in Tublay, Benguet had the lowest Squash Leaf Curl severity which had comparable results with the chayote plantation in Atok, Benguet (Table 2, Figure 11 and 12). The highest disease severity was observed in La Trinidad, Benguet (Figure, 13).

Tublay, Benguet (Figure 12) had the lowest incidence of Squash Leaf Curl Virus and La Trinidad municipality (Figure 13) had the highest disease incidence.

The Chayote plantation in La Trinidad, Benguet had abundant wild sunflowers around the vicinity of the farm which provided a haven for whiteflies which is a vector of the virus. The surrounding was also covered with lime stones which emit heat and enhance the dryness of the plantation making conditions favorable for whiteflies population increase.

Table 2. Severity and incidence of squash leaf curl virus in the field

SOURCE OF INOCULUM	SEVERITY	INCIDENCE
La Trinidad	54.25	50[a]
Tublay	47	46.25[a]
Atok	47.27	48.75[a]
Coefficient of Variation	8.36%	18.33%

Means followed by common letters are not significantly different at 5% DMRT

Figure 11. Chayote plantation in Atok, Benguet which had the lowest systemic Symptom of SQLV

Figure 12. Chayote plantation in Tublay, Benguet which had the lowest local lesion Smptom of Squash Leaf Curl Virus severity

Figure 13. Chayote plantation in La Trinidad, Benguet which had the highest Symptom of Squash Virus severity and incidence

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was conducted to assess the severity and incidence of Squash Leaf Curl Virus on chayote in La Trinidad, Tublay and Atok from August to March 2011.

In the greenhouse study it was noted that six days after inoculation, the representative test plants of Tublay had the lowest number of necrotic spots while the more necrotic spots were observed in plants inoculated with samples from Atok and La Trinidad. Twelve days after inoculation, systemic infection was noted where samples from Tublay had the lowest infection and in Atok and La Trinidad with the highest infection.

In the field survey, the chayote farm in Tublay had the lowest severity and incidence of SQLV and the highest was observed in La Trinidad. Incidence of the virus in La Trinidad and Atok was 100%.

Conclusions

Based on the results, the incidence and severity of SQLV infection on chayote in La Trinidad, Tublay and Atok, Benguet ranged from 47% to 100%. SQLV is transmitted by whiteflies but can also be mechanically transmit.

Recommendations

It is recommended that farmers should take precautions in obtaining chayote planting materials from La Trinidad, Tublay and Atok as there were high severity and incidence of Squash Leaf Curl Virus. Further, farmers should initiate proper care and control measures not to spread the virus as it could be mechanically transmitted. Field

sanitation should be observed by farmers in order to prevent the proliferation of alternative hosts and white flies as vector of the disease.

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APPENDICES

Appendix Table 1. Severity of squash leaf curl virus on the greenhouse 6 days after Inoculation

SOURCE OF INOCULUM	REPLICATION I	II	III	IV	TOTAL	MEAN
LA TRINIDAD	62.67	66.66	77.33	68	274.66	68.66[a]
TUBLAY	69.33	73.34	46.67	33.33	222.67	55.67[a]
ATOK	90.67	88	42.67	60	281.34	70.34[a]

ANALYSIS OF VARIANCE TABLE

SOURCE OF VARIANCE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SUM OF SQUARES	COMPUTED F	TABULATED F .05	.01
Replication	4					
Treatment	2	515.812615	257.9063075	0.83 [ns]	3.98	7.20
Error	9	2792.113475	310.2348306			
TOTAL	11	3307.92609				

[NS]= Not significant Coefficient of Variation=27.14%

Appendix Table 2. Severity of squash leaf curl virus on the greenhouse (systemic Infection)

SOURCE OF INOCULUM	REPLICATION I	II	III	IV	TOTAL	MEAN
LA TRINIDAD	53.33	53.33	65.33	62.67	234.66	58.66[a]
TUBLAY	64	66.66	72	70.67	273.33	68.33[a]
ATOK	35.33	10.67	56	64	156	39[b]

ANALYSIS OF VARIANCE TABLE

SOURCE OF VARIANCE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SUM OF SQUARES	COMPUTED F	TABULATED F .05	.01
Replication	4					
Treatment	2	1787.42445	893.712205	3.90[ns]	3.98	7.20
Error	9	2061.326775	229.0363083			
TOTAL	11	3848.75122				

[NS]= Not significant Coefficient of Variation=27.35%

Appendix Table 3. Severity of squash leaf curl virus (Field Survey)

SOURCE OF INOCULUM	REPLICATION I	II	III	IV	TOTAL	MEAN
LA TRINIDAD	55	57	51	54	217	54.25[a]
TUBLAY	47	46	54	41	188	47[b]
ATOK	44	47	49	49	189	47.25[a]

ANALYSIS OF VARIANCE TABLE

SOURCE OF VARIANCE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SUM OF SQUARES	COMPUTED F	TABULATED F .05	.01
Replication	4					
Treatment	2	135.5	67.71	3.94[ns]	3.98	7.20
Error	9	102.83	17.14			
TOTAL	11	352836				

[NS]= Not significant Coefficient of Variation= 8.36%

Appendix Table 4. Incidence of squash leaf curl virus (Field Survey)

SOURCE OF INOCULUM	REPLICATION I	II	III	IV	TOTAL	MEAN
LA TRINIDAD	55	65	40	40	200	50[a]
TUBLAY	45	45	40	55	185	46.25[a]
ATOK	50	45	50	50	195	48.75[a]

ANALYSIS OF VARIANCE TABLE

SOURCE OF VARIANCE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SUM OF SQUARES	COMPUTED F	TABULATED F .05	.01
Replication	4					
Treatment	2	29.2	14.6	0.19[ns]	3.98	7.20
Error	9	470.8	78.47			
TOTAL	11	336400				

[NS]= Not significant Coefficient of Variation= 18.33%