

OCCURRENCE AND PREVALENCE OF VIRUSES OF LEAFY VEGETABLES IN LAGOS, NIGERIA

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ABSTRACT

Four commercial leafy vegetable farms in Lagos State were surveyed from October 1989 to September 1990 for the occurrence and prevalence of leafy vegetable viruses. Mechanically transmissible viruses were isolated from *Amaranthus hybridus*, *Celosia argentea*, *Cucurbita moschata*, *Telfairia occidentalis* and *Brassica oleracea*, while virus-like symptoms (causal agents not sap-transmissible), were observed on *Solanum macrocarpon*. Two previously uncharacterized viruses, one each from *C. argentea* and *C. moschata* were isolated. The identification of viruses such as telfairia mosaic virus (TeMV) and amaranthus mosaic virus (AMV), that had been reported in the literature, were confirmed on the basis of host range, symptomatology, insect transmission and particle morphology. Virus-like symptoms were not observed on *Corchorus olitorus* and five other exotic vegetables. Amaranthus mosaic virus and celosia mosaic virus (CIMV), the latter isolated for the first time in Nigeria in the course of this study, were recorded from all the farms and were therefore the most prevalent. The incidence of AMV was 19.7% at Amuwo Odofin while it was 0.7% and 0.6% at Tejuoso and Abule Ado farms respectively. For CIMV, an incidence of 27.5% was recorded at Amuwo Odofin while Tejuoso and Abule Ado had 0.6% and 1.4% respectively. Telfairia mosaic virus and viruses on *C. moschata* and *B. oleracea* were limited to the Tejuoso farm. Generally, virus incidence was higher during the wet season for most of the viruses. Elimination of plant remains and volunteer crops may be exploited in the control of the viruses.

KEY WORDS: Survey, Leafy vegetables, viruses.

INTRODUCTION

Vegetables are edible plant parts that may be consumed raw or in cooked form (Okigbo, 1975). Their importance lies in their nutritive value as a source of vitamins, proteins and energy (Omueti, 1980; Oyenuga and Fetuga, 1975).

In Nigeria, vegetables such *Amaranthus* spp. *Celosia argentea* L., *Corchorus olitorus* L., *Cucurbita* spp (pumpkins) and *Telfairia occidentalis* Hoof. and grown mainly for their leaves. Recently, exotic vegetables such *Apium graveolens* L. (celery), *Brassica oleracea* L. (cabbage), *Cichorium intybus* L. (chicory), *Lactuca sativa* L. (lettuce) *Petroselinum crispum* (Mill) Nym. et. Hill (parsley) and *Raphanus sativus* L. (radish) were introduced into the Nigeria horticultural industry.

Viruses constitute one of the major factors limiting vegetable production world-wide (Grogan, 1980). In Africa, a number of viruses have been reported to infect fruit vegetables and they sometimes result in significant yield losses. They include potyviruses such as pepper veinal mottle virus (PVMV), papaya ringspot virus (PRV) (formerly watermelon mosaic virus-1 (WMV-1), a strain of WMV-2 and potato virus Y (PVY) as well

as tobacco mosaic virus (TMV), okra mosaic virus (OMV) okra leaf mosaic virus (OLMV), eggplant mosaic virus (EGMV) and eggplant severe mottle virus (ESMV) among others (Fauquet and Thouvenel, 1987; Ladipo, 1988; Brunt *et al.*, 1990).

In Nigeria, the first report of a leafy vegetable virus was by Nwauzo and Brown (1975) who described a mosaic disease of *T. occidentalis*. The aetiological agent provisionally designated as telfairia mosaic virus (TeMV) has been established to be a potyvirus (Shoyinka *et al.*, 1987). Cucumber mosaic virus (CMV) has been reported on the crop (Atiri, 1985). Ladipo (1976) reported a green mosaic disease of eggplant, *Solanum melongena* L while Taiwo *et al.* (1988) reported another potyvirus inciting a mosaic disease of *A. hybridus* L. Recently, potyviruses have been implicated as the causative agents of the dark green vein-banding of *Vernonia amygdalina* Del. and leaf curl and mosaic disease of *C. argentea* (Shoyinka *et al.*, 1994; Taiwo and Dijkstra, 1996; Owolabi *et al.*, 1998).

In a review of viruses of vegetables crops in Africa, Ladipo (1988) remarked that a few of the reported viruses were yet to be fully characterised and some, yet to be isolated. This study was

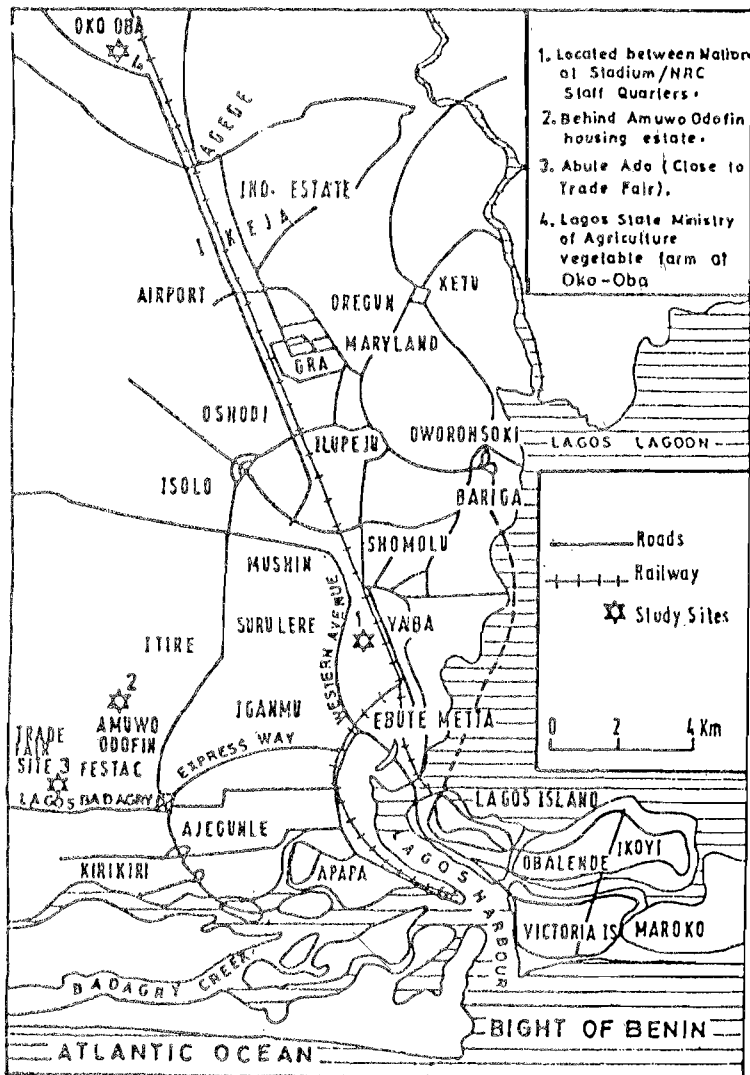


Fig. 1 Map of Lagos area showing the study sites.

therefore initiated to investigate the occurrence and prevalence of viruses on leafy vegetables in Lagos State with a view to isolating previously unreported viruses.

MATERIALS AND METHODS

Study Sites and Survey Procedures

The study sites were the Tejuoso Vegetable Farm located in the swamp between the National Stadium and its staff quarters in the Surulere area of Lagos Mainland, Amuwo Odofin Housing Estate and Abule Ado Farm situated some metres before the International Trade Fair Complex, Badagry expressway and a vegetable farm owned by the Lagos State Ministry of Agriculture at Oko Oba in Agege area of Lagos (Fig. 1).

In most of the farms, the method of cultivation was by crop rotation. In these farms,

seeds were sown in nurseries and the seedlings were transplanted at close spacing in well defined rows and columns on beds. This made sampling and assessment of the extent of virus infection easy. In addition, earth-dug wells, from where water was drawn for manual irrigation, ensured uninterrupted cultivation of vegetables all the year round. At Oko Oba farm however, seeds were sown by broadcasting and cultivation was rain-fed.

Surveys were conducted during the fourth week of each month from October 1989 to September 1990. Each site was divided into sections of about five (5) hectares. Beds to be surveyed were randomly selected from all sections. Sampling was by visual examination of the different vegetable types. The total of plants, as well as the number of plants showing symptoms on each bed were noted. Also, the characteristic symptoms of infected plants were recorded.

Virus Isolation and Identification

Selected representatives of virus infected plants were transferred from the fields into pots in the greenhouse (28 -33°C). Inocula were prepared from infected leaf tissues of *A. hybridus*, *C. argentea*, *C. moschata*, *B. oleracea* and *S. macrocarpon* by triturating leaf samples in 0.03M sodium phosphate buffer, pH 8.0 or 0.5M potassium phosphate pH 7.5 (1g/ml). Mechanical transmission was carried out on carborundum (600 mesh)-dusted test plants in the greenhouse.

Identification of the viruses isolated from *A. hybridus* and *T. occidentalis* was based on host range, symptomatology, mode of insect transmission, particle morphology, and compared with those of other viruses previously reported from these plants.

Identification of isolated viruses

For host range and symptomatology studies, some test plants were raised and inoculated mechanically with the virus isolates. The inoculated plants were observed for four weeks for symptom development.

For insect transmission test, *Aphis craccivora* obtained from *Glyricidia* sp. from the wild was used. Individual insect was starved for 2 hr and allowed acquisition access feeding for 2 - 5 min on detached symptomatic leaves of *A. hybridus* floated on water in Petri dishes. Ten insects were transferred to seedlings of the same plant and left overnight, after which they were sprayed with an aphicide. For TeMV, *T. occidentalis* was used as both source and test plants.

Leaf dip preparations of viruliferous sap

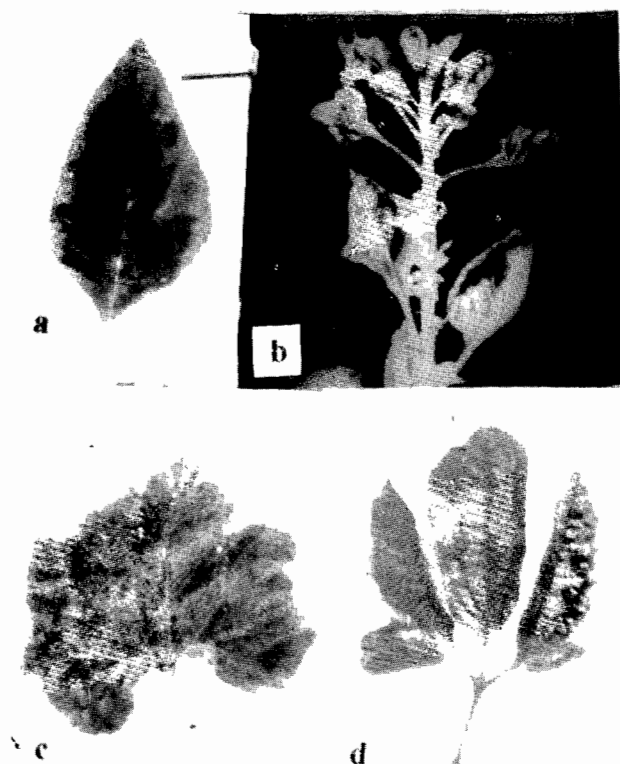


Fig. 2: Symptoms of infection observed on some leafy vegetables on the field. Mosaic on leaves of *Amaranthus hybridus* caused by amaranthus mosaic virus (AMV) (a); Severe leaf curl, mosaic, leaf puckering, moderate to severe stunting of *C. argentea* caused by the celosia virus isolate (b), green vein-banding, mosaic and blistering of the leaves of *C. moschata* caused by the cucurbita virus isolate (c) mosaic and leaf malformation induced by telfairia mosaic virus (TeMV) on *T. occidentalis* (d).

Table 1. Viruses on leafy vegetables in four farms in Lagos

Leafy vegetable	FARM LOCATION			
	Amuwo Odofin	Tejuoso	Abule Ado	Oko Oba
<i>Amaranthus hybridus</i>	+ ^a	+	+	+
<i>Celosia argentea</i>	+	+	+	+
<i>Cucurbita moschata</i>	- ^b	+	-	-
<i>Telfairia occidentalis</i>	-	+	-	-
<i>Brassica oleracea</i>	-	+	-	* ^c
<i>Solanum macrocarpon</i>	+	-	-	-
<i>Corchorus olitorus</i>	-	-	-	-
<i>Cusbara (Malvaceae)</i>	-	-	-	*
<i>Apium graveolens</i>	-	-	-	*
<i>Lactuca sativa</i>	-	-	-	*
<i>Petroselinium crispum</i>	-	-	-	*
<i>Raphanus sativus</i>	-	-	-	*

+^a = Virus present, -^b = Virus absent, *^c = Vegetable not cultivated

obtained from *C. moschata*, *C. argentea* were negatively stained with 2% phosphotungstic acid pH 6.0 and observed in Zeiss-902 electron microscope.

The viruses isolated from *C. argentea*, *C. moschata*, *B. oleracea* and probably *S. macrocarpon* had not been previously reported in Nigeria. They were tentatively referred to as celosia, cucurbita, brassica and solanum visuses respectively. Attempts were therefore made at preparing single lesion cultures of each isolate on *Chenopodium quinoa* and *C. amaranticolor*. Samples of young symptomatic leaves from plants infected by each virus were also cut into pieces, dried over calcium chloride and stored at 4°C for future use.

RESULTS

Disease symptoms in Field infected plants

Virus-like symptoms were observed on *A. hybridus*, *B. oleracea*, *C. argentea*, *C. moschata*, *S. macrocarpon* and *T. occidentalis* during the period of survey. Amaranthus mosaic virus (AMV) caused green vein-banding, mottle and mosaic of infected leaves (Fig. 2a) as well as stunting of plants. The major virus-induced symptom in *B. oleracea* was mosaic while the symptoms on *C. argentea* were characterized by severe leaf curl, mosaic, leaf puckering, moderate to severe stunting and apparent reduction in leaf size (Fig. 2b). Symptoms in *C. moschata* included green vein-banding, mosaic and blistering of the leaves (Fig. 2c). On *S. macrocarpon* the observed symptoms included mosaic, blistering and leaf

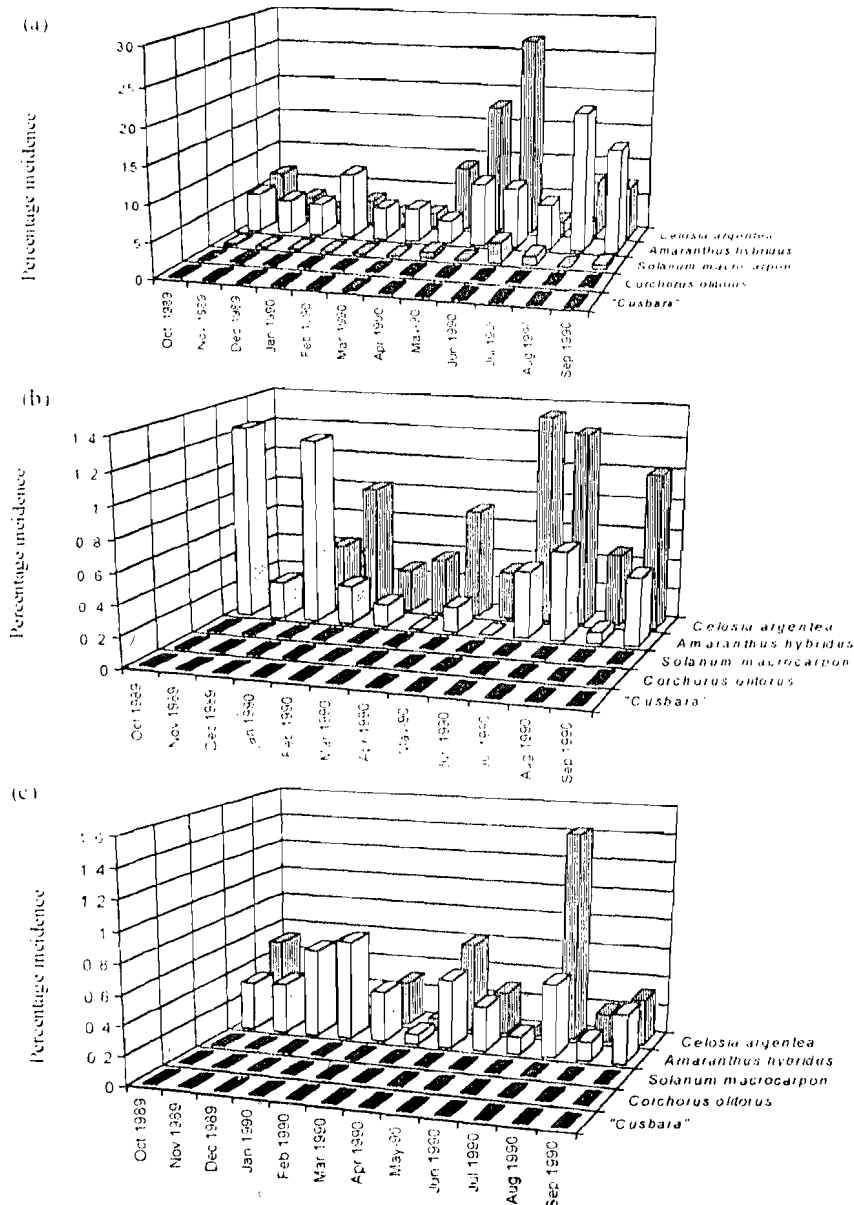


Fig. 3 Percentage incidence of viruses of leafy vegetables in Amuwo Okofin (a) Abule Ado (b) and Tejuoso (c) Farms

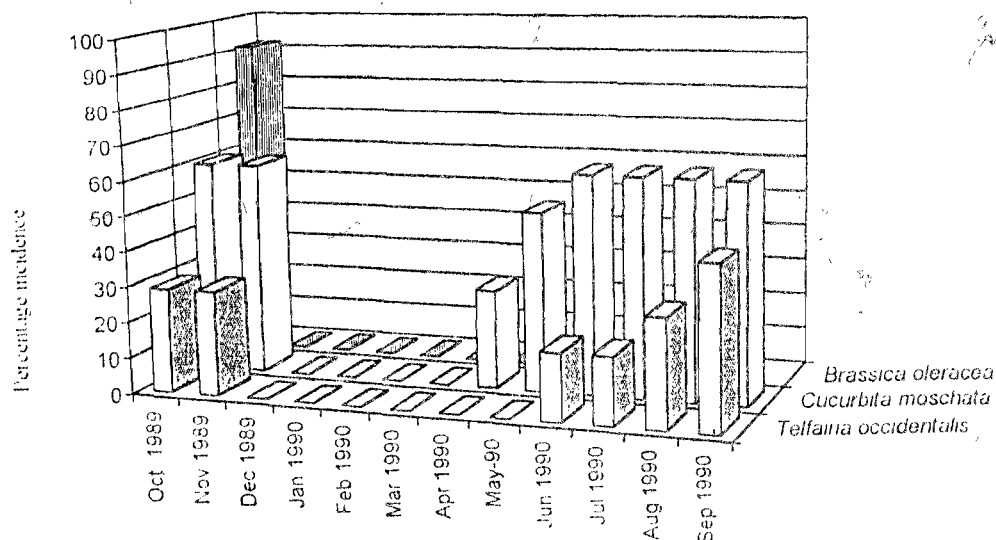


Fig. 4. Percentage incidence of virus infection of *Telfairia occidentalis*, *Cucurbita moschata* and *Brassica oleracea* in Tejuoso farm

glutinosa, *N. tabacum*, *Vigna unguiculata* var. "Ife Brown" were not infected by the virus. *Telfairia* mosaic virus induced leaf malformation in *T. occidentalis* but did not infect non-cucurbitaceous plants tested.

Electron microscopy of the leaf dip preparations from *A. hybridus* and *T. occidentalis* showed flexuous rod-shaped particles (Fig. 5).

Attempts to isolate the agent that induced the virus like symptoms in *S. macrocarpon* were not successful. The causal agent of the mosaic disease of *B. oleracea* could not be established because no suitable host could be identified immediately to ensure its propagation in the greenhouse and all attempts to re-isolate it from infected tissues dried over calcium chloride were unsuccessful.

DISCUSSION

The incidence of viruses in the surveyed farms was generally higher during the wet months of the year. Amaranthus mosaic virus (AMV) and CIMV were the most encountered in the course of this investigation. They were more prevalent at Amuwo Odofin than in Tejuoso and Abule Ado farms. Field observations at Amuwo Odofin farm showed that remnants of previous season crops, some of which were infected were commonly left unharvested in the farm. These remnants or volunteer plants could serve as a source of virus. This opinion is further strengthened when it was observed that *A. spiraecola*, which transmitted the two viruses in the greenhouse (Lobe 1987, unpublished data) was regularly found on both infected and healthy plants on the farm. The role of volunteer crops in the ecology and epidemiology of plant viruses

have widely reported (Bos, 1981). The practice of continuous cultivation of both *A. hybridus* and *C. argentea* in monoculture probably contributed to the high incidence of AMV and CIMV at Amuwo Odofin. Monoculture has been reported to increase inoculum build-up and disease severity (Lockhart, 1974).

The absence of virus-like symptoms on majority of the exotic vegetables might be due to absence of viruses in the seeds planted by the growers. This should prevent the accidental introduction of seed-borne viruses into new areas (Grogan, 1980). It is also probable that the exotic vegetables are fairly resistant or immune to the viruses existing in Nigeria or that the viruses associated with them are not present here.

The virus isolate obtained from *Telfairia occidentalis* was similar to that reported by Shoyinka *et al* (1987) with respect to host range, symptomatology and particle morphology. Also the virus isolated from *A. hybridus* in the course of this study was similar to that previously puckering while mosaic and leaf malformation were the symptoms observed on *T. occidentalis* (Fig. 2d).

Virus incidence and Prevalence

Amaranthus mosaic virus (AMV) and celosia mosaic virus (CeMV) were observed in all the farms surveyed and therefore the most prevalent of all the viruses (Table 1). Virus-like symptoms were observed on *C. moschata*, *T. occidentalis* and *B. oleracea* at Tejuoso farm only and on *S. macrocarpon* at Amuwo Odofin only. No discernible virus-induced symptoms were observed on *C. oltorus*, *A. graveolens*, *L. lactuca*, *R. sativa*, "Cusbara" (Malvaceae) in any of the farms throughout the period of the survey.

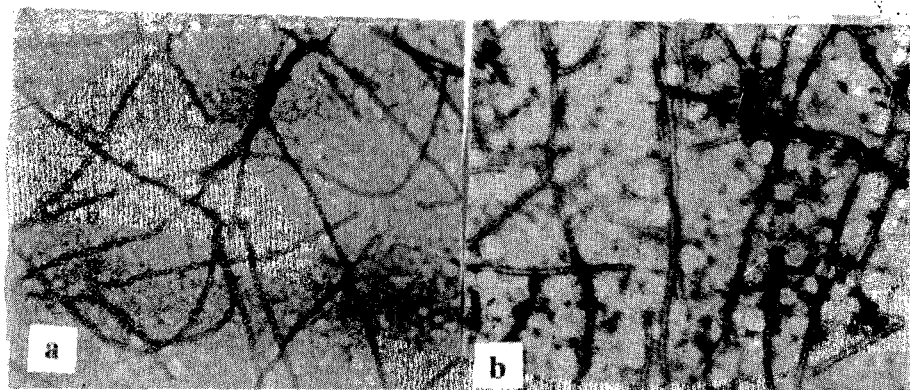


Fig. 5: Electron micrographs of amaranthus mosaic virus (AMV) (a) and telfairia mosaic virus (b) showing flexuous rod-shaped particles.

Generally, the results indicated that the incidence of virus-like symptoms was highest during the first and second growing seasons (March - July, and September - October) except for AMV which had the highest incidence of 19.7% at Amuwo Odofin (Fig. 3a) during the short dry spell at August 1990 and the lowest of 3.2% during the onset of rains in April. Similar trends were observed at Abule Ado and Tejuoso farms (Figs. 3b & 3c). The celosia virus had an incidence ranging from 1.7% to 27.1% in Amuwo Odofin with the highest in June 1990 and the lowest in February of the same year (Fig. 3a). The values recorded at Abule Ado and Tejuoso Farms were comparatively lower than those of Amuwo Adofin, but generally the incidence was highest around June and July and lowest during the dry season (Fig. 3b, c).

The incidence of the cucurbita virus in Tejuoso was 60% in October 1989 while by September the incidence was 66.1%. For TeMV a 29% incidence was recorded in October 1989 and this increased to 44.4% in September 1999 (Fig. 4). Symptoms of virus infection were observed on *S. macrocarpon* at Amuwo Odofin farm only. The incidence ranged from 0 - 2.9% with the lowest in October 1989 to March 1990. Virus infection of *B. oleracea* was recorded at Tejuoso farm in October 1989 to March 1990 when the vegetable was cultivated. Out of 500 plants examined in October 1989 459 were infected, representing an incidence of 91.6%. The survey conducted in April gave an incidence of 0.234% as only 2 out of 848 plants were infected (Fig. 4).

Identification of isolated viruses

The virus isolated from *A. hybridus* induced mosaic, green vein-banding and leaf malformation in *A. hybridus*, mosaic on *N. benthamiana* and chlorotic local lesions on

Chenopodium amaranticolor. Other test plants such as *Abelmoschus esculentus*, *C. argentea*, *Capsicum annum*, *Corchorus olitorus*, *N.* reported by Taiwo *et al* (1988). The virus obtained from *C. argentea*, one of the new but previously unreported leafy vegetables viruses in Nigeria, has since been characterized and designated as celosia mosaic virus (CIMV) (Owolabi *et al.*, 1998). Studies are in progress to confirm the identity of the mosaic-inducing agent in *C. moschata*.

Ladipo (1988) observed that some of the viruses of vegetable crops in Nigeria are yet to be isolated and characterised. The viruses inciting the symptoms observed on *C. moschata* and probably *S. macrocarpon* have not been characterised in Nigeria. The characterisation, identification and determination of the economic importance of these viruses form the basis of further research.

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