



Biological and Physico-chemical Properties of Causal Agent of Virus Disease of *Coccinia barteri* (Hook. f.) Keay in Calabar, Cross River State

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Authors' contributions

This work was carried out in collaboration among all authors. Author VEU designed the study. Authors VEU and OTU wrote the protocol and wrote the first draft of the manuscript. Authors ATT and OMU managed the pathological investigations of the study. Authors VEU, OTU, ATT and OMU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The physico-chemical properties of the causal agent of virus disease of *Coccinia barteri* (Hook. f.) Keay were studied. The virus causing the disease was characterized using diagnostic tools such as host range, longevity *in vitro*, thermal inactivation point, dilution endpoint and aphid transmission. The virus was mechanically transmitted from the natural host (*C. barteri*) to the healthy test plants in the green house. In the biological properties, the virus was successfully transmitted by *Aphis spiraecola* (obtained from *Chromolaena odorata* (L.) R. M. King & H. Rob.) from infected *Cucumeropsis mannii* Naudin to a healthy *C. mannii* in a non-persistent manner and had a narrow host range limited to the family Cucurbitaceae. In the physico-chemical properties based on crude sap with an unknown virus concentration, beyond which infectivity was lost. It was readily inactivated by heating to 35 – 65°C for 10 minutes in determination of thermal inactivation point. The virus had a longevity *in vitro* of between 4 – 5 days beyond which it was non-infectious. Symptoms induced by the virus were leaf cupping, mottle chlorosis, blisters, stunted growth, rugosity, leaf malformation and mosaic patterns.

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1. INTRODUCTION

Viruses are one of the important pathogens of plants. A virus particle (virion) is composed of a nucleic acid and a coat protein. A decade ago, plant viruses are ultramicroscopic and can only be seen with the aid of electron microscope. They do not reproduce sexually, rather they replicate autonomously. They are obligate parasites that cannot survive outside a living host. Interestingly, viruses can be 'crystallized' and can remain virulent for decades [1]. According to [2], viruses can be defined as "a set of one or more nucleic acid template molecules, normally encased in a protein coat or coats of protein or lipoprotein, which is able to organize its own replication only within suitable host cells. Within such cells virus production is dependent on the host's protein synthesizing machinery, organized from pools of required materials rather than by binary fission and located at sites which are not separated from the host cell contents by a lipoprotein bi-layer membrane". The interaction between viruses and host plants negatively affects host morphology and physiology, resulting in disease [3]. In a majority of cases, viruses are virulent and cause disease in crops during their mono-cultivation in open fields or greenhouses for food production. Not surprisingly, the current taxonomy of plant viruses is primarily based on viruses isolated from cultivated crops showing disease symptoms [4,5]. Wild plants are often latently infected with viruses in nature without any apparent disease symptoms [6,7]. a decade ago, Roossinck and colleagues discovered and emphasized the beneficial effects of viral infections for host plants [8,9], meanwhile, several virulent strains of plant viruses such as cucumber mosaic virus (CMV) Fny strain [CMV(Fny)], bromo mosaic virus (BMV) Russian strain, tobacco mosaic virus (TMV) U1 strain, and tobacco rattle virus (TRV) have been shown to confer drought or cold tolerance to their host plants [8,10,11]. Although the molecular mechanisms underlying this conferred drought and cold tolerance have not yet been elucidated, several metabolites, including osmoprotectants and antioxidants that are associated with improved drought and cold tolerance, were observed to increase in these virus-infected plants [8].

The Cucurbitaceae family is commonly known as gourd, melon and pumpkin family. *Coccinia* is a genus from this family. The genera *Coccinia* is

commonly called the scarlet gourds with 25 species. *Coccinia* species are perennial climbing or creeping herbs. Climbing is supported by simple unequal tendrils. The species are dioecious, meaning that individual plants produce flowers with only male or only female organs. All species occur in sub-saharan Africa, from semi-arid savannas to rain forests and rarely also in montane forests [12].

C. barteri occurs in evergreen forest [13]. It is herbaceous characterized by unbranched tendrils which arise from the axils of leaves by which it attaches itself to supports. The leaves are variable in shape, more or less deeply lobed 3-5 lobed, shining, glossy and dark green in colour often with white blotches. The male and female flowers are borne in a raceme and the fruits are streaked and ellipsoidal in shape [14].

C. barteri is a pot herb and of medicinal importance in Cross River State [14]. It is used for treatment of venereal disease [15].

1.1 Objective of Study

The objective of this study was to determine the biological and physicochemical properties of causal agent of virus disease of *C. barteri* (Hook. f.) key in Calabar, Cross River state. Properties investigated included host range, symptomatology, insect transmission, thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV).

2. MATERIALS AND METHODS

2.1 Source of Planting Materials

Seeds used for this study were gotten from Etinan market in Akwa-Ibom state. The seeds were dried to prevent rotting in the soil and stored in the refrigerator to maintain viability.

2.2 Preparation of Buffer

The buffer used for inoculation of virus was 0.03 M of sodium hydrogen phosphate buffer (Na₂HPO₄) pH 8.0. The buffer was prepared by dissolving 4.68 g of Na₂HPO₄ in one litre of distilled water. The pH was adjusted to 8.0 by adding a few drops of 0.5M of sodium dihydrogen phosphate (NaH₂PO₄). The buffer was kept in the refrigerator before use.

2.3 Virus Isolation, Propagation and Maintenance

Symptomatic leaves obtained from *C. barteri* in Calabar were brought from the field in sealed polyethylene bags. The infected leaf tissues were triturated in cold buffer pH 8.0 in sterilized pestle and mortar until a fine homogenate was obtained. The virus was mechanically inoculated and maintained in *C. manni* (Naudin) in the green house ($23 \pm 2^\circ\text{C}$). The nine-day old test seedlings were dusted with carborundum and the infective homogenate was rubbed onto the leaves with the pestle or fore-finger. The inoculated leaves were rinsed with water and left for four weeks for symptom development. *C. manni* was used as source of inoculum for subsequent inoculation.

2.4 Host Range and Symptomatology

Host range study was carried out using twelve plants from five different families. The seeds of the test plants were sown in perforated pots filled with sterilized soil. After germination, the seedlings were supplied with water until the inoculation stage was reached. The cucurbits and legumes were inoculated at two leaf stage while others were inoculated at four leaf stage and left for four weeks with continuous watering for symptoms development. Some plants from each species were inoculated without carborundum only to serve as a control check.

2.5 Properties in Crude Sap

Three tests were carried out on crude sap from infected *C. manni* in order to determine the virus stability, concentration and longevity. The tests were thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV).

2.6 Determination of Thermal Inactivation Point

A homogenate of infected leaves was prepared as described previously in. About 2 ml of the solution was apportioned into seven test tubes. The tubes were heated to temperature between 35°C and 65°C at intervals of 5°C in a water-bath beginning from the highest to lowest temperature. The content of each test tube was heated for ten minutes and rapidly cooled in a beaker containing cold water. The content of each test tube was used to inoculate at least 3 – 4 seedlings of the test plant (*C. manni*).

2.7 Determination of Dilution End-Point

Infectious homogenate from infected leaves was prepared as described previously in. Six clean test tubes were filled with 9 ml of distilled water. 1 millilitre of the homogenate was added to the first test tube (10^{-1}) and stirred. From the first test tube was added to the second test tube 1 milliliter and stirred to give 10^{-2} . This dilution continued to 10^{-6} . Then each of the dilutions were used to inoculate 3 – 4 seedlings of the test plants. The inoculated plants were kept for 4 weeks for symptoms development.

2.8 Determination of Longevity *in vitro*

The prepared infectious homogenate was dispensed into six test tubes. The test tubes were sealed with a cellophane and left under laboratory condition. The content of the first test tube was inoculated on 3 – 4 seedlings of the test plants. The process was repeated for six consecutive days. The inoculated plants were kept for 4 weeks for symptoms development.

2.9 Aphid Transmission

Wingless *Aphid spiraecola* was tested for its ability to transmit the virus. The aphid species was sourced from *Chromolaena odorata*. Aphid insects were singly removed of the aphids were removed after breathing heavily on the leaves and tapping lightly over a white sheet of paper. The aphids were transferred into a transparent container covered with a gauze for aeration. The aphids were starved for two hours and allowed acquisition period for 3 minutes to feed on the detached infected leaf. Ten aphids were transferred with a paint brush onto the cotyledonary leaves of the seedlings and left overnight. The test plants were covered to prevent the spread of aphids to other plants during the inoculation period.

3. RESULTS

3.1 Host Range and Symptomatology

The result for the host range of the virus isolate is summarized in Table 1. The virus had a narrow host range limited to the cucurbits.

3.2 Thermal Inactivation Point of the Virus

Table 2 shows the result for thermal inactivation point of the virus isolated from *C. bateri*.

Table 1. Host range determination of the virus isolated from *C. barteri*

Test plants	Symptoms
Cucurbitaceae	
<i>Cucumeropsis mannii</i> (Naudin)	Leaf cupping, mottling, chlorosis, stunted growth, reduced leaf size.
<i>Citrullus lanatus</i> (Thunb.)	Stunted growth, mottle chlorosis, reduced leaf size
<i>Cucumis melo</i> L.	No symptom
<i>Luffa cylindrica</i> M. Roem.	No symptom
<i>Cucumis sativa</i> L.	No symptom
Fabaceae	
<i>Glycine max</i> L.	No symptom
<i>Phaseolus vulgaris</i> L.	No symptom
<i>Arachis hypogaeae</i> L.	No symptom
<i>Crotalaria retusa</i> L.	No symptom
Poaceae	
<i>Zea mays</i> L.	No symptom
Solanaceae	
<i>Capsicum annum</i> L.	No symptom
<i>Lycopersicon esculentum</i> Mill	No symptom

Table 2. Thermal inactivation point determination of the virus isolated from *C. barteri*

Temperature 0°C	Number of inoculated plants	Number of infected plants	Percentage infection
35	4	0	0
40	4	0	0
45	3	0	0
50	4	0	0
55	3	0	0
60	4	0	0
65	4	0	0

Table 3. Dilution end-point determination of the virus isolated from *C. barteri*

Dilution	Number of inoculated plants	Number of infected plants	Percentage infection
10 ⁻¹	3	1	33.3
10 ⁻²	4	0	0
10 ⁻³	4	0	0
10 ⁻⁴	3	0	0
10 ⁻⁵	3	0	0
10 ⁻⁶	4	0	0

3.3 Dilution End-point of the Virus

Table 3 shows the result for dilution end point of the virus isolated from *C. bateri*.

3.4 Longevity *in vitro* of the Virus

Table 4 shows the result of the longevity *in vitro* of the virus isolated from *C. bateri*.

3.5 Insect Transmission

The virus was transmitted by *Aphis spiraecola* from infected *C. mannii* to the healthy test plants in a non-persistent manner. The insect

inoculated plants developed symptoms characteristic of infection by the virus isolate such as leaf cupping which was an acute infection.

4. DISCUSSION

Plant viruses are one of the major phytopathogens that infect plant leading to decrease yield in food crops. They can be transmitted by insects, through grafting, vegetative propagation, fungi, nematodes or mechanically. Different methods were used for the detection of the virus under study such as

Table 4. Longevity *in-vitro* determination of the virus isolated from *C. barteri*

Days	Number of inoculated plants	Number of infected plants	Percentage infection
0	4	3	75
1	5	3	75
2	6	3	50
3	5	3	60
4	3	1	33
5	5	1	20
6	3	0	0

host range and symptomatology, longevity *in vitro*, thermal inactivation point, dilution end-point and aphid transmission. These methods were used to determine the biological and physico-chemical properties of the virus. However, according to [1], for successful transmission, the young symptomatic leaf tissues, which contains higher virus titer than older leaf tissues were used and homogenized in a cold buffer in a pre-cooled sterilized pestle and mortar. The molar concentration and pH of the buffer was also important because too high or too low molar concentration and pH will abort the virus transmission.

Only two plant species tested were susceptible to the virus, which were *C. manni* (Naudin) and *Citrullus lanatus* (Thunb.) belonging to the family Cucurbitaceae. Symptoms exhibited included stunted growth, leaf cupping, mottling, mottle pods, blisters and rugosity while other test plants were not susceptible. This shows that the virus had a narrow host range limited to the cucurbits. This virus is similar to the virus isolated from *Lagenaria breviflorus* and *Coccinia barteri* reported in Calabar by [1] which was also limited to the cucurbits while non-cucurbits were not susceptible.

Thermal inactivation point (TEP) as described by [16] is the temperature required for the complete inactivation of a virus in crude plant sap when exposed to heat. The result in Table 2 shows that at temperature between 35°C – 65°C the virus was completely inactivated and the inoculated test plants did not show symptoms of infection by the virus. This result contrasted with [17]. Where the isolates were inactivated by heating between 60°C–65°C. However, the virus was not inactivated when kept in room temperature.

The dilution end-point (DEP) is the highest dilution of sap from a virus-infected plant which is still infectious, but is usually given as the range between the dilution and the next one at which the infectivity is lost [16]. Table 3 shows that the sap can only be diluted once and is still

infectious. From 10^{-2} to 10^{-6} , there were no symptoms of infection.

Longevity *in vitro* (LIV) is the length of time after which a crude sap from a virus-infected plant loses its infectivity when kept at room temperature (28 ± 2°C) [16]. Continuous keeping of the inoculum for 6 days, the infectivity of the virus reduced progressively and was completely non-infective in day 6 (Table 4). This shows that the buffer can maintain the infectivity of the virus for five days beyond which it is non-infective.

Virus transmitted in a non-persistent manner shows that the virus was retained in the foregut of the aphids for a short period, usually a few hours or less [1]. This virus under study was transmitted in a non-persistent manner by *Aphis spiraeicola* from infected *C. manni* to the test plants. Most members of the genus potyvirus are transmitted in a non-persistent manner [1]. The result is similar to that of [18] where two strains of Moroccan watermelon mosaic virus was transmitted by *Aphis spiraeicola* in a non-persistent and this was supportive of the genus *Potyvirus*.

5. CONCLUSION

Plant viruses cause major loss to several agricultural and horticultural crops in the world. Several kinds of plant viruses infect cucurbit and some of them are not related to the family Cucurbitaceae such as PRSV-W, TRSV etc. There are no direct methods to control plant virus, hence, methods for their detection play a critical role in disease management such as biological properties and physico-chemical properties. Biological properties were related to the interaction of the virus with the host and physico-chemical properties were longevity *in vitro*, thermal inactivation point and dilution end-point. The virus under study was relatively stable having a narrow host range that was limited to the cucurbits. These results emphasized the need to combine biological and physico-chemical

properties in characterization of the virus understudy. This is because other plant viruses may have similar biological properties to the one under study. It also provides preliminary information for further identification of the virus which may be carried out such as serological and molecular characterization, cytopathology, electron microscopy, Immunosorbent Electron Microscopy (ISEM) and coat protein gene sequencing.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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