Curative control of ACMV, viral disease of cassava (Manihot esculenta Crantz) "Yacé" by the use of a virucide and an organic fertilizer

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ABSTRACT
Cassava virus causes huge yield losses in cassava cultivation in West Africa and especially in Côte d'Ivoire. However, according to prior research, little work has been done for effective curative control against the ACMV. Our study aims to propose methods of fight against the responsible vector of the incidences. Test were conducted in both controlled area and in a peasant area with stark virucide and Herbagreen bio-fertilizer at different concentrations to evaluate their effects on the growth and development of plants affected by the disease. The results of this work showed that ACMV is a disease that affects the growth and development of affected plants. The Herbagreen bio-fertilizer favored the growth and development of the affected plants and the virucide STARK permanently canceled the degree of severity of the disease during the experimentation time. In view of these results, stark virucide and Herbagreen bio-fertilizer can be used to foster the growth and development of cassava plants with ACMV.

Keywords:
ACMV, Viricidal, Control, Biofertilizer.

1. Introduction:
Cassava, Manihot esculenta Crantz (Euphorbiaceae), is a plant that was introduced into Africa in the 16th century. It is cultivated for the richness of its leaves in iron, and for the abundance of starch in the tubers. In 2004, the world production of cassava was estimated at 184 million tons, 55% of which was produced in Africa [1]. It is one of the most dominant starchy foods in the daily diet in sub-Saharan Africa [2]. Located in humid tropical Africa, Ivory Coast is an agricultural country. With a production of about 2.8 million tons, and a yield between 5 and 8 tons per hectare, cassava is the second most important food crop in Ivory Coast after yam (FAO, 2009). Cassava is mainly cultivated in all agro-ecological zones (AEZ) with the exception of Agro-ecological Zone VI (AEZ VI), comprising the locality of Bouna [3]. Several varieties such as "Yacé", "Bonoua", IM84, are used. The processing of tuberous cassava roots is used for different dishes such as attié ké, foutou, placali,... However, many biological constraints contribute to limit its productivity, among which vascular bacterial disease, anthracnose, mealy bug, mites and African mosaic are the most important. African cassava mosaic disease (ACMV) is one of the most important factors responsible for the decline in cassava yields in Africa [4]. The FAO estimates that yield losses average between 14 and 24% per year, equivalent to 15 to 27 million tons of tuberous roots lost. In the case of ACMV infection on susceptible cultivars, losses are more important and approach 95% of the yield [5-6].

In view of the economic consequences that this disease could cause, anticipating appropriate control strategies will make it possible to guarantee sustained cassava production, which is a guarantee of food security and improved income for producers.
It is in this context that this study was conducted, the general objective of which is to contribute to the establishment of an effective control method against African cassava mosaic in Ivory Coast. Specifically, this will involve:

- To evaluate the effect of an organic fertilizer on the growth and development of plants affected by African cassava mosaic disease;
- To evaluate the effect of a virucide on virus-affected plants;
- To evaluate the cumulative effect of the virucide and the organic fertilizer.

2.Materials and methods:

2.1 Plant material:
The plant material consisted of healthy and infected cassava cuttings of the Yace variety taken from fields in Ivory Coast. This variety is much more used in the preparation of many dishes for its quality.

2.2. Biological control equipment:
The biologics used in this study are:
STARK is a natural herbal biostimulant, which has an anti-viral effect, and is used to control viral diseases of all plants, especially cassava and cocoa. It is an organic virucide that protects plants and also increases their resistance against viruses. The product is composed only of a rare plant mixture that immediately stops the viruses, and allows the plant to resume its development. It has been used for the treatment of plants affected by ACMV.
The HERBAGREEN, a natural bio fertilizer composed mainly of calcite and zeolite. It favours a healthier growth because the new leaves that appear are no longer virosed and the plant grows faster. It comes in two forms: a liquid form called Herbagreen Fluisan and a powder form called Herbagreen Z20 has been used for plant treatment [7].

2.3. Technical equipment:
The technical material used in this study is [8]:
- A machete that was used for cleaning the plot,
- A data for ploughing,
- A LAVAL Roberval scale (CAP.44ibs GRAD 2âz) for weighing cassava roots,
- A sprinkler for product application.

2.4. Methods:
The work was carried out both in a protected environment (in a greenhouse) away from potential insect vectors of ACMV and in plantations [9-10].

First experiment: Greenhouse tests

Experimental setup:
The experimental design was a randomized Fisher block with three replications and three treatments.
Preparation of culture substrates:
To carry out this experiment, a sterile soil with a sandy texture was mixed with potting soil in a 60/40 ratio. This soil-potting soil mixture was then distributed in pots at a rate of 300 ml per pot.
Sowing of cuttings and growth of young plants:
Two (2) cassava cuttings of 25 cm in length and comprising 4 to 6 nodes were planted per culture pot; this corresponds to 6 cuttings per replication and per treatment. The preparations were then transferred to a 0.8 mm thick, airtight, transparent greenhouse. The pots were watered every two days with 300 ml of tap water, until the end of the experiment on day 63rd.
Treatment of cassava cuttings:
In order to study the effect of virucide on cassava plants, several treatments were used:
- Treatment T0: untreated cassava virus cuttings as a control;
- Treatment T1: healthy untreated cassava cuttings as a positive control;
- Treatment T2: Virused cassava cuttings treated with Stark virucide and Herbagreen.
The virucidal solution was prepared extemporaneously by mixing 3 ml of Stark + 3 ml of Herbagreen Fluisan + 4 g of Herbagreen Z20 in a 1 L volumetric flask and making up to 1 L with distilled water.
The resulting solutions were applied to the leaves 21 days after sowing the cassava cuttings using a calibrated sprinkler.

Experimental device:
The experimental design is a randomized Fisher block with three replications and five treatments:
T0: Untreated diseased plants;
T1: Sick plants treated with the recommended concentration of Stark and Herbagreen (1.8ml Stark + 1.8ml Herbagreen liquid + 12g Herbagreen Z20, all dissolved in 400ml distilled water).

The resulting solutions were applied to the leaves 21 days after sowing the cassava cuttings using a calibrated sprinkler.

Parameters assessed:
To evaluate the effect of the different treatments on the growth of cassava plants, two morphological parameters were measured every 21 days for 63 days. These are: stem height and infection rate of the plants by ACMV.

Stem height was determined using a tape measure, by measuring the interval between the ground and the last leaf fork, for stems whose point of insertion on the cutting is underground. For the stems emitted on the aerial part of the cuttings, we considered the height from the point of connection of the stem on the cutting to the last branch of the plant. Leaves showing symptoms of ACMV and asymptomatic leaves were counted every 21 days and the infection rate was calculated according to the following formula:

\[ TI(\%) = \frac{NFM}{NFT} \times 100 \]

- NFM: number of leaves showing symptoms of ACMV
- NFT: total number of leaves

Second experiment: Plantation tests
Site preparation and transplantation:
The preparation of the land consisted of cleaning with a daba. Two days later, the cassava cuttings were transplanted on the flat land. The distance between the plants was 1 m.

Experimental device:
The experimental design is a randomized Fisher block with three replications and five treatments:

- T0: Untreated diseased plants;
- T1: Sick plants treated with the recommended concentration of Stark and Herbagreen (1.8ml Stark + 1.8ml Herbagreen liquid + 12g Herbagreen Z20, all dissolved in 400ml distilled water).
- T2: Diseased plants treated with a concentration below the recommended concentration of Stark and Herbagreen (0.6 ml Stark + 0.6 ml Herbagreen liquid + 12 g Herbagreen Z20, all dissolved in 400 ml distilled water)
- T3: Diseased plants treated with the concentration above the recommended concentration of Stark and Herbagreen (3 ml of Stark + 3 ml of Herbagreen liquid + 12 g of Herbagreen Z20, all dissolved in 400 ml of distilled water)
- T4: Healthy untreated plants

Six plants were evaluated per treatment and per replication.

Application of products:
Three weeks after transplanting, the first application of the products was made as a foliar spray using an adjustable rate sprayer. The second application was made 4 days later. After the second application, the third, fourth and fifth applications were made at 15-day intervals.

Parameters assessed:
The following parameters were assessed before the first application of the products. These were plant height, number of diseased leaves, total number of leaves and degree of disease severity using the Cours scale (Cours, 1950; Fargette, 1987). Plant height was measured every 21 days with a tape measure as previously indicated. The number of diseased leaves and the total number of leaves per plant was determined by counting. The degree of disease severity (ACMV) was determined using the Course 1950 scale.

- Degree 0: No symptoms.
- Degree 1: Yellowish spots covering 1/5 of the leaf blade
- Degree 2: Spots covering half the leaf blade, appearance of leaf deformations.
- Degree 3: Affected leaves deformed, partially curled, reduced vegetative apparatus.
- Degree 4: Almost all leaf blades curled up, reduced vegetative apparatus
- Degree 5: Leaves reduced to 1/10 of their surface, atrophied branches, the plant withers and dies within a few months.

At harvest, the number of tubers per plant was counted. The mass of cassava tubers from each plant was determined by weighing with a Roberval scale.
3. Results and Discussion:

3.1. Effect of greenhouse treatments:

3.1.1. Effect of Stark and Herbagreen on height growth of diseased plants:

All plants grew continuously throughout the experiment regardless of treatment. However, the plants treated with the virucide Stark and Herbagreen (T2) had the greatest height with 92 cm on day 63 compared to 70.5 cm for the untreated healthy plants (T0) and 50 cm for the untreated virus-infected plants (T1).

3.1.2. Effect of STARK and Herbagreen on disease incidence:

Figure 2 shows the effect of the different treatments on the infection rate of cassava plants with ACMV. Untreated virus-treated plants (T1) had the highest infection rates at 30.7%. Plants treated with the virucide Stark and Herbagreen had a lower infection rate of 4.56%. The healthy plants remained healthy during the experiment.

3.2. Effect of treatments under natural conditions (planting):

3.2.1. Effect of STARK and Herbagreen on height growth of diseased plants:

The growth in height of cassava plants was a function of time for all treatments (Figure 3). However, there was a marked statistical difference between treatments. Plants treated with the virucide Stark and Herbagreen (T1, T2 and T3) obtained the greatest height on day 84 of the experiment with 132.75 cm. These plants were followed by the untreated healthy cassava plants (T4) which had a height of 75.45 cm. The virus-infected and untreated plants (T0) had the lowest heights with 51.33 cm.

3.2.2. Effect of STARK and Herbagreen on the evolution of the degree of severity of ACMV:

The degree of severity of ACMV was a function of time and treatments Figure 4 shows the degree of severity of ACMV as a function of time. The healthy plants (T4) did not show any symptoms of ACMV during the whole experiment. The degree of severity of ACMV remained more or less constant with an average of 2. The degree of severity of ACMV dropped with the use of different concentrations of virucide and Herbagreen. Thus, for the treatment (T3) with a concentration above the recommended concentration, the degree of severity dropped from 3 on day 21 to 0 on day 84 of the experiment. For the treatment (T2) with the concentration below the recommended concentration, there was also a drop in the degree of severity.

Figure 1. Effect of STARK and Herbagreen on the height growth of cassava plants of the Yace variety as a function of time.

Figure 2. Effect of different treatments on the rate of infection of plants by ACMV.

Figure 3. Effect of STARK and Herbagreen on the height growth of cassava plants of the variety "Yacé" in plantation as a function of time.

Figure 4. Effect of STARK and Herbagreen on the evolution of the degree of severity of ACMV.
severity of ACMV from 2 on day 21 to practically 0 on day 84. At the recommended concentration (T1), the degree of severity also dropped from 2.25 on day 21 to almost 0 on day 84.

### 3.2.3 Effect of treatments on the infection rate of cassava plants affected by ACMV:

In planting, the infection rate of cassava plants was a function of the treatments (Figure 5). Healthy untreated plants (T4) remained symptom-free throughout the experiment. The infection rate of the untreated virus-affected plants was statistically the highest, at over 30%. The three treatments with the use of Stark virucide and Herbagreen had identical infection rates but lower than the infection rate of the T0 control.) Thus, the infection rates for treatments T1, T2 and T3 were 8.2, 14.52 and 4.56% respectively.

![Figure 4. Effect of STARK and Herbagreen on the degree of severity of ACMV as a function of time.](image)

![Figure 5. Rate of infection of cassava plants by ACMV according to the different treatments.](image)

### 3.3. Effect of Stark and Herbagreen on production:

#### 3.3.1. Number of tubers:

The effect of Stark virucide and Herbagreen on the number of tubers of cassava plants is shown in figure 6. The number of tubers was a function of the treatments. After statistical analysis, three classes are presented. Thus, treatments T1, T2 and T3 which represent the different doses of virucide and Herbagreen obtained the highest number of tubers with respectively 4.53, 4.75 and 5.08 tubers and represent class 1 as they are not statistically different from each other. Class 2 was obtained with the treatment (T4) with 3.56 cassava tubers. Class 3 was obtained with the T0 treatment consisting of untreated virus-infected plants which obtained 2.7 cassava tubers.

#### 3.3.2. Tuber mass:

Figure 7 shows the effect of Stark and Herbagreen on the tuber mass of cassava plants. After statistical analysis, three classes were presented. Class 1 is represented by treatments T1, T2 and T3, these treatments obtained the highest tuber masses with 3.058, 2.72 and 3.59 kg respectively. The untreated healthy plants constituted by treatment T4 constitute the second class with a mass of 2.46 kg. Class 3 was constituted by the untreated virus-infected plants (T0) which had less than 1kg.

![Figure 6. Effect of STARK and Herbagreen on the number of tubers of cassava plants of the variety "Yacé.](image)

![Figure 7. Effect of STARK and Herbagreen on tuber mass of cassava plants of the variety "Yacé.](image)
3.4. Discussion:
The trial conducted to evaluate the effect of the virucide STARK coupled with the biological fertilizer Herbagreen on the growth and development of cassava plants infected by the ACMV virus showed a highly significant positive action of the products used, both in a controlled environment under glass and in a farmer plantation [11]. The coupled application of STARK virucide and Herbagreen resulted in the rapid growth of parameters such as internode elongation, length and even tuberous root yield of cassava plants affected by ACMV. This effect would result from the combined action of the virucide STARK and the calcite and zeolite which constitute the active ingredient of the fertilizer Herbagreen. The combined action of the virucide and the fertilizer resulted in faster and healthier plant growth [12]. The application resulted in the elimination of the proportion of diseased leaves, a decrease in the severity and incidence of the disease in affected plants. Also, the emission of new healthy leaves was possible after the application of STARK and Herbagreen. This is explained by the vigour of the plants due to the action of calcite, zeolite and STARK which allows them to resist the disease a little more. This renewed vigour of the plants would support a good yield due to an increase in photosynthetic activity [13].

There is little work on curative control of African cassava mosaic. The few works done have used genetic control methods while making the choice of plant material resistant to the disease and cultural control methods. This was illustrated by Muneer et al. (2011) [14] who mentioned that the control of African cassava mosaic is essentially preventive. It is still in this vein that said that there are three possible approaches to reduce production losses caused by African mosaic on cassava. These are reducing the proportion of virus infected plants, delaying infection by destroying them at an advanced stage of cassava vegetative growth, pre-inoculating the plant with a moderate strain of the same virus to protect it from infection with virulent strains [15].

Cassava obtained from contaminated cuttings (i.e. before planting) loses much more production (55 to 77%) than a plant contaminated by the whitefly (i.e. after planting), even if the contamination occurs early (35 to 60%). If vector contamination occurs after 100 days after planting, there is no further effect on production [16]. This is a general phenomenon for viral plant diseases, with an even greater effect when inoculation is early. It should be noted that these results highlight the beneficial effect that the application of a sanitation method could have. Phytosanitation is one of the methods of controlling this virus. This term refers to the improvement of the sanitary state of the propagation material and the elimination of inoculum foci from which further contamination with the virus could take place through the activity of the vector fly [17].

This study which focused on the curative control of one of the viral diseases of cassava (African Cassava Mosaic Virus (ACMV)) was carried out on the "Yacé variety" by using a virucide (Stark) and an organic fertilizer (Herbagreen). In general, treatment of diseased plants with Stark and Herbagreen significantly improved vegetative growth and fresh tuberous root production of cassava compared to controls. In the controlled environment, the average stem length was 97.4 cm at the concentration of 5ml/L water of Stark and Herbagreen with a low infection rate of cassava plants. On the farm, the average stem length was 130 cm for all the plants that underwent the different treatments while with the control, the average length recorded was 56.5 cm on the last day of the evaluations [18]. As for the average severity of ACMV, it progressively dropped with the different applications until it was cancelled with the higher concentration (5ml/L of STARK water, and Herbagreen) during the 4th evaluation corresponding to the 84th day after the 1st application. The treatment corresponding to the concentration of 5ml/L of STARK and liquid Herbagreen gave the best yields with an average number of 5.08 tubers per plant, and an average mass of 3.59 Kg per plant. The virucide Stark and the bio-fertilizer Herbagreen could be used in cassava cultivation to improve the yield of cassava tubers in case of ACMV infection; However, the popularization of the use of these products also requires testing [19-20].

4. Conclusion:
This study which focused on the curative control of one of the viral diseases of cassava (African Cassava Mosaic Virus (ACMV)) was carried out on the "Yacé variety" by using a virucide (Stark) and an organic fertilizer (Herbagreen). In general, treatment of diseased plants with Stark and Herbagreen significantly improved vegetative growth and fresh tuberous root production of cassava compared to controls. In the controlled environment, the average stem length was 97.4 cm at the concentration of 5ml/L water of Stark and Herbagreen with a low infection rate of cassava plants. On the farm, the average stem length was 130 cm for all the plants that underwent the different treatments while with the control, the average length recorded was 56.5 cm on the last day of the evaluations. As for the average severity of ACMV, it progressively dropped with the different applications until it was cancelled with the higher concentration (5ml/L of STARK water, and Herbagreen) during the 4th evaluation corresponding to the 84th day after the 1st application. The T3 treatment corresponding to the concentration of 5ml/L of STARK and liquid Herbagreen gave the best yields with an average number of 5.08 tubers per plant, and an average mass of 3.59 Kg per plant.
References:


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