THE EFFICIENCY ROLE OF AFRICAN NIGHTSHADES IN THE TRANSMISSION OF *CUCUMBER MOSAIC VIRUS* AND POTYVIRUSES IN KENYA (EAST AFRICA)

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ABSTRACT

Nightshades production is constrained by pests and diseases which severely impact the quantity and quality available in the value chain. The working hypothesis is that African Nightshades could be serving as a reservoir for *Cucumber mosaic virus* (CMV) and Potyviruses in Kenia. In each field, five suspicious plants that the age is between one to 2 months and 5 to 8 suspicious African Nightshades leaves were collected and putted paper bags and brought back to Germany. Suspicious African Nightshades leaves were propagated in Nicotiana benthamiana, Nicotiana rustica, Nicotiana samsun and Nightshade (Solanum scabrum) kept in greenhouse.. Nightshade (*Solanum scabrum*). Leaves with visible symptoms from inoculated test plants and the original material were tested by DAS-ELISA for CMV and by ACP-ELISA for Potyviruses characterization. In order to apply these estimates we must assume that the distribution of the values of replicate data points is normal around the mean of the values. The Standard deviation (SD) was calculed and basing on the calculation of cuf-off (cuf-off=(mean + 3SD)+10%); the lowest detectable analytic concentration gived a response that had a statistically significant difference from the response of the zero analytic concentration was the detection limit. In 28 surveyed farms, CMV (cuf-off= 0.24) and Potyviruses (cuf-off= 0.12) were detected in 20 farms.These results clearly demonstrated the epidemiology of CMV and Potyviruses in the farmlands in Kenya.

Mots clés : African Nightshade, Kenia, plant viruses

RÉSUMÉ

La production de légumes feuilles (Solanumsarrachoides) est limitée par les ravageurs et les maladies qui affectent gravement la quantité et la qualité disponibles dans la chaîne de valeur. L'hypothèse de ce travail est de vérifier si le Solanumsarrachoidespourrait servir de réservoir pour le virus de la mosaïque du concombre (CMV) et Potyviruses au Kenya. Dans chaque champau Kenya, cinq Solanumsarrachoides suspects ont été recoltésdont cinq à huit feuilles âgésd'un à deux mois, les échantillons ont été ramenés en Allemagne pour des analyses. Les feuilles de Solanumsarrachoidessuspectes ont été propagées sur Nicotiana benthamiana, Nicotiana rustica, Nicotiana samsunet Solanumscabrum maintenusen serre. Les feuilles présentant des symptômes visibles provenant des plantes d'essai inoculées et les échantillons d'origine ont été testés par DAS-ELISA pour le CMV et par ACP-ELISA pour la caractérisation de Potyvirus. Les moyennes, l'écart-type et le cuf-off (cuf-off = (moyenne + 3SD) + 10%)ont été calculés. La concentration analytique la plus faible détectée ayant donné une réponse differentestatistiquement significative par rapport à la réponse de la concentration analytique nulle était la limite de détection. Dans les 28 champs diagnostiqués , le CMV (cuf-off = 0,24) et Potyviruses (cuf-off = 0,12) ont été détectés dans 20 champs. Ces résultats ont clairement démontré l'épidémiologie du CMV et des Potyvirus dans les champs au Kenya.

Mots clés: Solanumsarrachoides, reservoir ,virus, Kenya

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Introduction

Food security has remained the primary agenda in Africa food policies and Kenya is not an exception. The attainment of food security in sub-Saharan Africa only be realized from increase in productivity through the use of sustainable good agricultural practices (GLOBALGAP) and prevention of losses caused by pathogens and pests in the field and along the value chain. Vegetables production as *African* Nightshade provides essential micronutrients lacking in the diets of millions of Africans (Oiye and al., 2009). Nightshade is rich in health promoting compounds (Abukutsa-Onyango, 2003; Shiundu and Oniang'o, 2007) and assist in combating micronutrient deficiencies and malnutrition. Nightshade also contribute to food security and income generation among the subsistence and semi-commercial farmers in Kenian(Abukutsa-Onyango, 2003; Shiundu and Oniang'o, 2007; Oiye and al., 2009). Nightshade production is constrained by pests and diseases which severely impacts the quantity and quality available in the value chain in the world (Alvarez *and al*., 2003;). The use of synthetic pesticides remains the primary means for controlling economical damage to crops, but this practice has come under scrutiny as it may pose potential oncogenic risks.

Nightshade has a phenomenal reproductive capacity, as a single plant can produce approximately 45,000 seeds (Blackshaw, 1991; Srinivasan *and al.*, 2013). Despite the availability of efficient herbicides, a mere one percent seed survival could translate into thousands of Nightshade plants per acre (Hutchinson, 2007). In addition to directly competing with potato, Nightshade also serves as a reservoir for an important virus with a cosmopolitan distribution, Potato leafroll virus (family Luteoviridae, genus Polerovirus), and its most efficient vector, the green peach) aphid, *Myzuspersicae* (Hemiptera: Aphididae) (Loebenstein, 2001; Thomas, 2002; Alvarez and Srinivasan, 2005; Srinivasan and Alvarez, 2008; Srinivasan *and al*., 2008). The hypothesis of this work was that African Nightshade is or not serving as a reservoir for *Cucumber mosaic virus*and Potyvirusesin Kenya.

2. Material and Methods

2.1 Samples collection

The geographical locations of Administration area of surveyed sites are represented in table 1. These sites have been considered for this study because of their high production in vegetables. In each Administration area, 5 to 8 suspicious samples were collected in differents farms in juin, 2014.

Table1: Geographical locations of surveyed sites in Kenya

Surveydareas	Location
Osorongai	00.3403N ; 034.5753E
Bukwa	00.3321N ; 034.5735E
Cheptonon	00.3322N ;034.5736E
Chepsaik	00.3308N ; 034.5614E
Mukuyuni	00.4521N ; 034.3556E
Chepkaka	00.4455N ; 034.3630E
Sikulu	00.74351N ; 034.65259E
Bituyi	00.76617N ; 034.70078E
Lutonyi	00.76328N ; 034.69988E
Madawa	00.27100N ; 034.52684E
Emangale	00.27148N ; 034.52871E

2.2 Inoculation and propagation of viruses

Kenian original materials (0.025g) were mechanically macerated in PBS (phosphate buffered saline). We had prepared PBS for 1 liter of distilled water (8.0g of Nacl; 0.2 g KH_2PO_4; 1.15g of Na_2HPO_4; 0.2g of Kcl; 0.20g of NaN_3 +Na2SO3+celite, pH to 7.4) and inoculated to biotest plants (*Nicotiana benthamiana, Nicotiana rustica, Nicotiana samsun* and the greenhouse grown Nightshade *S. scabrum)* to transfer putative viruses. The inoculated plants grown in an air conditioned glasshouse.

2.3. Detection of Virus

All Antibodies for DAS-ELISA (CMV: AS 0929-0929 IgG-AP) and ACP-ELISA (Potyvirus: AS0573-ACP/PTA –ELISA-RAM-AP) were provided by DSMZ (Germany). The first antibody for the detection of CMV was diluted in coating buffer (coating buffer for 1 liter of distilled water: 1.59 g of Na_2CO_3; 2.93g of $NaHCO_3$; 0.20g of NaN_3; pH 9.6) and 100µl were putted in each well of ELISA plate. After 4 hours at 37°C of incubation, the plate was washed three times as in step 3 with washing buffer (washing buffer for 1 liter of distilled water: 8.0g of sodium chloride (Nacl); 0.2 g KH_2PO_4; 1.15g of Na2HPO_4; 0.2g of Potassium chloride (Kcl); 0.20g of NaN_3; 0.5ml of Tween 20) and dry. Hundred µl of antigen extract (0.6 g plant material for 6ml of PBS + 2% of Polyvinylpirrolidone 400.000) were added to the first antibody and the plate was incubated overnight at 4°C. After washing and drying; the second antibody was diluted (1ul in 1000 µl) to conjugate buffer (conjugate buffer for 1 liter of distilled water: 8.0g of Nacl; 0.2 g KH_2PO_4; 1.15g of Na2HPO_4; 0.2g of Kcl; 0.20g of NaN_3; pH 7.4; 2% PVP; 0.2 % egg albumin: Sigma A-5253)and added to the plate. After incubating time (4 hours at 37°C), the plate was washed and dryed before adding the substrat (0.02g of p-Nitrophenyl Phosphate; 97ml of Diethanolamine; 600ml of distilled water; 0.20g of NaN_3; make up to 1 liter, pH 9.8) for luminofluorenscence detection. The optical density of the solution was measured at 405 nm after incubation overnight at 4°C.

For the ACP-ELISA the first step was the fixation of Antigen extract (coating buffer for 1 liter of distilled water: 1.59 g of Na_2CO_3; 2.93g of $NaHCO_3$; 1.12 g of DIECA. 0.20g of NaN_3; pH 9.6) and incubating overnight the ELISA plate at 4°C. After washing and drying, 100 µl of casein (2g skim milk into 100ml of PBS-Tween) were added to the antigen and incubate during 30 minutes at 37°C for blocking. After incubating time, the blocking solution was removed and the plate was dryied. The first and the second antibodies were diluted with conjugate buffer and the incubating time and measurement of optical density were the same as that used for DAS-ELISA.

2.4 Statistical analysis

Mathematically, the SD is the square root of the sum of the variances squared divided by the number of samples minus one. In order to apply these estimates we assumed that the distribution of the values of replicate data points is normal around the mean of the values. The Standard deviation (SD) was calculated using EXCEL 2010 and basing on the calculation of cuf-off ((mean + 3SD)+10%); the lowest detectable analytic concentration gived a response that had a statistically significant difference from the response of the zero analytic concentration was the detection limit.

3. Results

3.1 Symptom on biostest plants

The figures 1, 2 and 3 represented symptoms on biotest plants for the third transfer of *Cucumber mosaic virus*and Potyviruses in greenhouse.

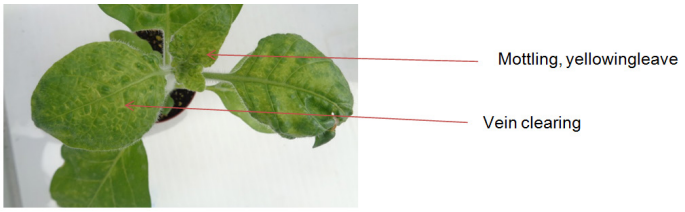

Fig.1:*Cucumber mosaic virus* symptom on *Nicotiana rustica*

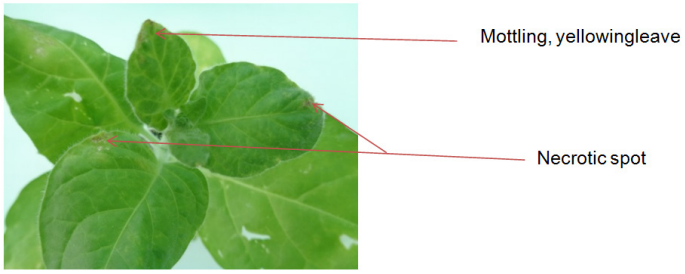

Fig.2:Potyvirues symptom on *Nicotiana rustica*

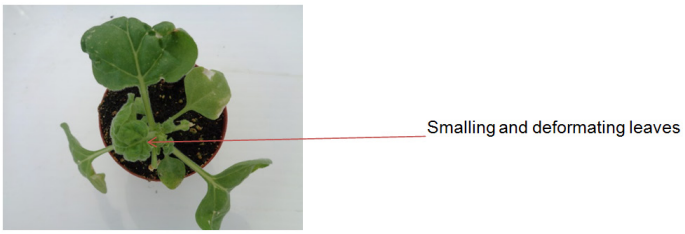

Fig.3:Potyvirues and *Cucumber mosaic virus* symptom on *Nicotiana benthamiana*

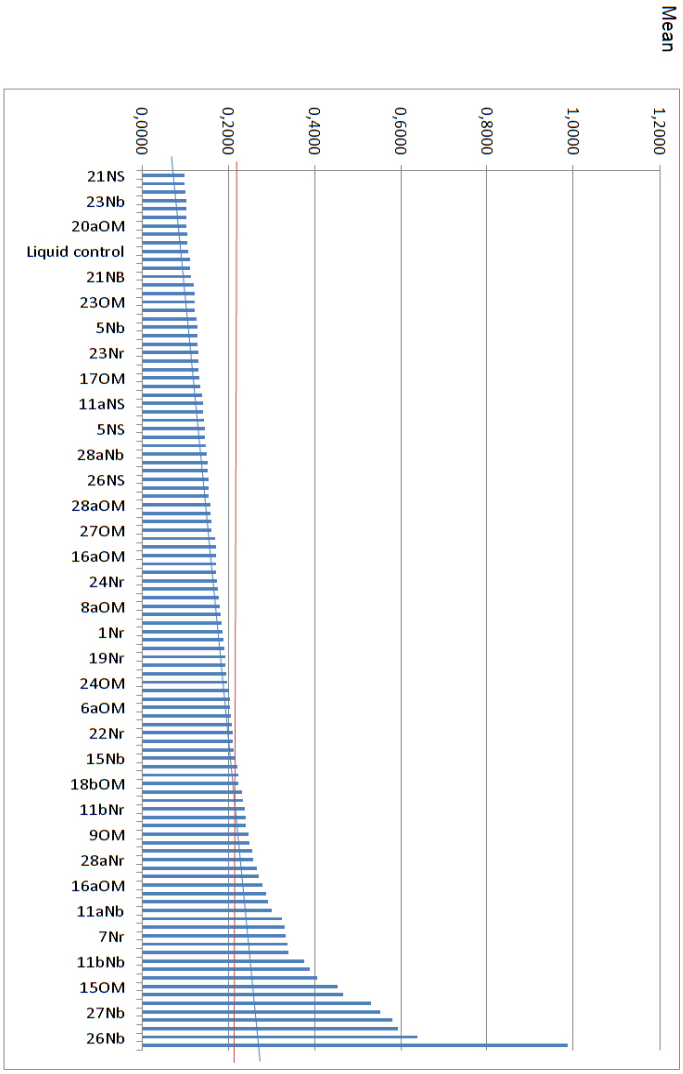

Fig.4: Detection of *Cucumber mosaic virus* on original material and biotest plants

Original Nightshade plant material (**OM**) collected at different Kenian farms and biotest plant material after mechanical inoculation with OM: *Nicotiana benthamiana* (**Nb**), *N. rustica* (**Nr**) and greenhouse grown Nightshade:*Solanum scabrum*'Olevolosi' (**NS**).

3.2Virus detection by ELISA in original materials and biotest plants

The figures 4 and 5 represented negative and positive samples according to calculedcuf-off for CMV (0.24) and Potyviruses (0.12). All values bevo the red line are postives and all values abow the red line are negative.

Discussion

The aim of this work was to examine infection of African Nightshade by different viruses.Two viruses were detected (CMV and Potyviruses).

CMV is one of the most important plant pathogens, can infect more than 1,200 plant species (Wang *and al*., 2011). There are many strains of CMV, and different strains can induce different symptoms in *Nicotiana tabacum* (Palukaitis *and al*., 1992). In this work, CMV induced local reaction as ringspots mottling, vein clearing and yellowing on *Nicotiana rustica* (fig. 2). According to plant virus descriptions such type of test-plant reactions is specific for CMV (Samuitienė and Navalinskienė 2008).

The family Potyviridae, which includes approximately 200 species of economically important plant viruses, causes

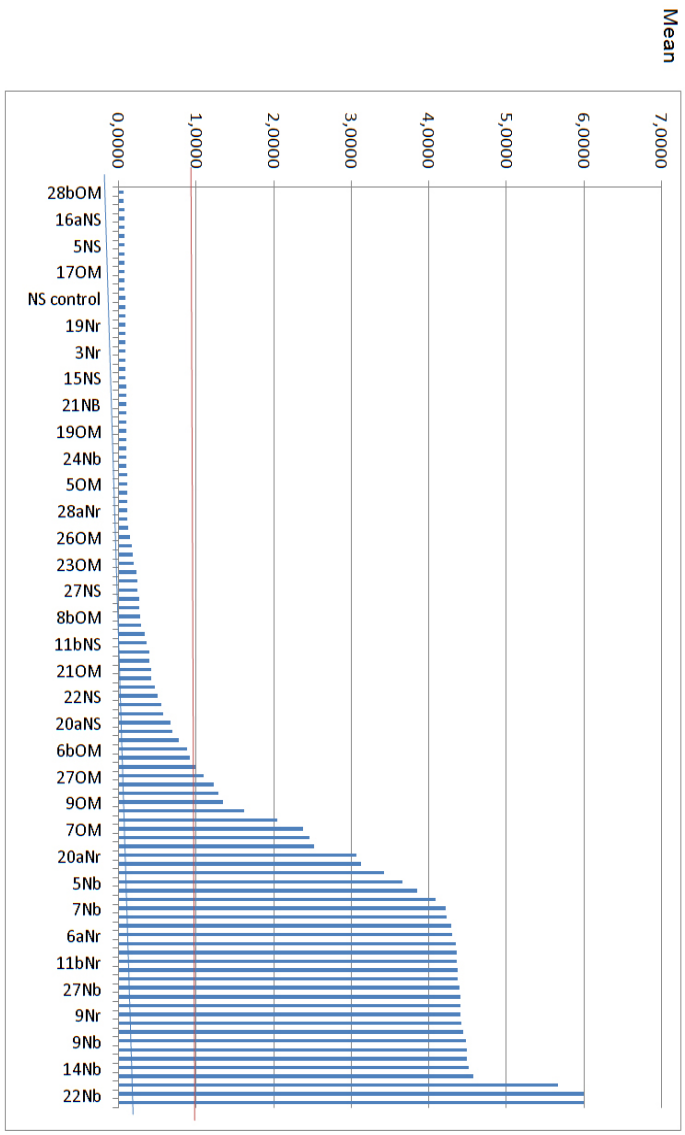

Fig.5: Detection of Potyviruses on original material and biotest plants

Original Nightshade plant material (**OM**) collected at different Kenian farms and biotest plant material after mechanical inoculation with OM: *Nicotiana benthamiana* (**Nb**), *N. rustica* (**Nr**) and greenhouse grown Nightshade *Solanum scabrum* 'Olevolosi' (**NS**)

significant losses in agricultural, pasture, horticultural and ornamental crops (Shukla *and al*., 1989; Wei *and al*., 2010). Potyviruses infection in *Solanum*

sarrachoides produced different symptoms (mottling, yellowing, necrosis) (Chrzanowska, 1992; Cervantes and Alvarez, 2010). In our case we observed different symptoms as mottling, necrotic spot (figure 3) on the third transfer for Potyviruses on *Nicotiana rustica*.

All of the Potyviruses are transmitted from plant to plant by several species of aphids. Aphids are able to transmit these viruses for very short periods of time called no-persistence transmission (Bosciaand *al*., 1997; Gibbs *and al*., 2008). Our study indicated that Nighshadeserve as reservoir for Potyviruses in Kenya. *Solanum saccachoides* is a recorded host for various strains of Potato virus Y (Cervantes and Alvarez, 2011). The presence of *S. sarrachoides* in the potato fields or nearby farmlands could contribute to more aphids on potato plants (Srinivasan *and al*., 2013). We clearly demonstrated the presence of Potyviruses in several farms samples from Kenya (table).

Conclusion

Based on the data of test-plant reactions, symptoms, positive reaction with CMV specific antiserum in DAS-ELISA test results and Potyviruses antiserum in ACP-ELISA it was ascertained, that Nightshade is serving as reservoir of CMV and Potyviruses in Kenia. But this result will be perform by the identification of the specific CMV strains and the specific Potyvirus by RT-PCR, PCR- RFLP.

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