Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2024 Vol. 2(1): 62-66
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2024-0201-008*

Research Article

Incidence of *Citrus tristeza virus* in Zaria and Giwa Local Government Areas of Kaduna State, Nigeria

***Buhari Muhammad[1], Muhammad Aliyu Hamza[2], Abdullahi Auwal[2], and Aminu Magaji Bichi[2]**

[1]Department of Crop Protection, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University, Zaria
[2]Department of Forestry and Wildlife Management, Faculty of Agriculture, Ahmadu Bello University, Zaria
***Corresponding Author:** abuidris24@gmail.com, +2348034591888

Received: 27th February, 2024 **Accepted**: 12th March, 2024 **Published**: 31st March, 2024

ABSTRACT

Citrus tristeza disease is among the most devastating diseases of citrus worldwide. To ascertain the occurrence of the causal agent, *Citrus tristeza virus* (CTV) in Zaria and Giwa Local Government Areas of Kaduna State, samples were collected from four citrus orchards in September during the 2022 wet season. A total of sixty samples (n=60) were collected randomly comprising of both symptomatic and asymptomatic leaves, fifteen (n=15) from each field. The commonest symptoms observed included, yellowing of leaves, cupping, and leave vein clearing. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was employed in the detection of the virus from the leaves collected. The result shows the presence of the virus in the Local Government Areas (LGAs) with an incidence of 6.67% each. This study is the first to report the presence of CTV in Zaria and Giwa LGAs of Kaduna State. Thus, indicating the spread of the viral disease in the country-despite its low incidence- after it was first reported from the Southern part of Nigeria. This calls for an intensive survey to delineate the extent of the viral incidence in the country and embark on enlightenment campaigns for citrus farmers.

Keywords: Citrus, *Citrus tristeza virus*, prevalence, Kaduna, Disease

Citation: Muhammad, B., Hamza, M. A., Auwal, A., and Bichi, A. M. (2024). Incidence of *Citrus tristeza virus* in Zaria and Giwa Local Government Areas of Kaduna State, Nigeria. *Sahel Journal of Life Sciences FUDMA*, 2(1): 62-66. DOI: *https://doi.org/10.33003/sajols-2024-0201-008*

INTRODUCTION

Citrus is the largest genus in the family Rutaceae. It is used as a common name for different citrus fruits including oranges (*Citrus sinensis*), lemons (*C. limon*), limes (*C. aurantifolia),* grapefruit (*C. paradis*i), and tangerines (*C. tangerina* and *reticulata). Citrus* is believed to have originated from the southern slopes of the Himalayas, the entire north-eastern region of India, and adjacent China (Meena*et al*., 2018). Citrus fruits are a precious resource of phytochemicals that are beneficial for the human body, like vitamin C, Vitamins B, potassium, phosphorous, and other elements, and it is also used as anticancer, inflammation, antiviral, antibacterial, and antifungal substances (Abobatta, 2019). The citrus fruits were the second most produced fruit worldwide in 2021, accounting for 161.8 million tonnes produced in more than 10.2 million hectares (Pereira Gonzatto and Santos, 2023). The production of citrus fruits in Nigeria increased from 1.5 million tonnes in 1972 to 4.11 million tonnes in 2021; growing at an average annual rate of 2.20% (FAO, 2021). However, this production is confronted by several challenges including the problems of pest and disease infestation, premature fruit drop due to attack by nocturnal fruit piercing moths, termite damage to stem bark and tree roots and gummosis as well as

the death of budded seedlings (Christopher and Udoh, 2020). *Citrus tristeza virus* (CTV), a member of the family Closteroviridae is the most destructive viral pathogen of citrus from an economic point of view (Folimonova, 2020). The virus has killed, or rendered unproductive, millions of trees throughout most of the world's citrus-growing areas (Bar-Joseph and Dawson, 2008). Earlier studies have reported the occurrence of CTV on Citrus trees in Nigeria (Kareem*et al.,* 2013; Adediji *et al*., 2015). CTV possesses a 19.3 kilobase (kb) positive-stranded RNA genome, nearly twice as large as the average-sized RNA virus genome of 10 kb (Folimonova, 2020). The most common visual signs of CTV are seedling yellows, stem pitting, and quick decline, clearing of veins, cupping, chlorotic leaf flecking, and corky veins. According to the International Plant Protection Convention IPCC, 2016, using biological, serological or molecular amplification tests is the minimum requirement to detect and identify CTV. Enzyme-linked immunosorbent assay (ELISA) and direct tissue blot immunoassay assay (DTBIA) remain the most common because of their reliability, rapidity, and relatively low cost (Saponari*et al.,* 2008). Nucleic acid-based methods are also available for disease detection but are not easily affordable to the developing world (Want *et al*., 2022). The virus is disseminated only in infected bud wood, planting materials, and by several species of aphids (Olsen *et al.,* 2000). Measures to control CTV damage include quarantine and bud wood certification programmes, elimination of infected trees, use of *tristeza*-tolerant rootstocks, or cross protection with mild isolates (Monero, 2007). Virus symptoms mentioned above were observed in some citrus orchards from the study area, hence this study was conducted to determine the occurrence of CTV in Zaria and Giwa LGAs of Kaduna State to unravel the presence or otherwise of CTV in the two LGAs, which serve as a stepping stone towards effective surveillance of the disease and the virus and to sustain the productivity and yield of citrus in the country.

MATERIALS AND METHODS

Sample Collection

This study was carried out in two citrus farms in each of the Zaria and Giwa LGAs of Kaduna State in September 2022. The citrus farms were selected based on the symptoms observed earlier stated. Both symptomatic and asymptomatic plant leaves were collected. A total of sixty samples were collected randomly, fifteen (15) from each field. The samples were labeled serially, wrapped in polyethylene bags, and transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University Zaria for analysis. The samples were stored at about -20℃ before the diagnosis began. The neighbouring trees, crops as well as the sanitary condition of the farms and the farming system were noted. The average disease incidence of the virus in each LGA was calculated:

Disease Incidence = $\frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ examined} \times 100$

Enzyme-linked Immunosorbent Assay (ELISA)

The samples were screened for *citrus tristeza virus* using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using the protocol supplied by the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und ZellkulturenGmbh, Braunschweig (DSMZ), Germany and as described by Clark and Adams (1997). The plate was coated using the antibody of CTV and coating buffer in a dilution of 1:1000. A-200µlwas added into each test well of a microtitre plate using a micro pipette and incubated for 2 hours at 37 ^{o}C. After the incubation period, the plate was washed with phosphate-buffered saline Tween (PBST) three times using a wash bottle by soaking for 3minutes. The plate was blotted dry by tapping three times on a 3-layerd tissue paper. The leaf samples were homogenized using sterile mortar and pestle with sample extraction buffer at a ratio of 1:10 w/v. 200µlof the homogenate was discharged into each test well of the microtitre plate and incubated over night at 4^{o}C. The plate was then washed three times with PBST using a wash bottle by soaking for 3 minutes as described above. The enzyme conjugate was diluted in conjugate buffer at a ratio of 1:500 and 200µlwas added into each test well. The plate was covered and incubated for 3hours at 37^{o}C. At the end of the incubation period, the plate was washed as described above but in this step not tapped on tissue paper but rather cleaned gently. A-two hundred (200µl) of freshly prepared substrate (20g of p-nitro phenyl phosphate dissolved in 20ml of substrate buffer) was added to each test well. The plate was incubated for 60 minutes at room temperature for visual observation. Lastly, the absorbance in the wells was measured spectrophotometrically at 405nm with an ELISA plate reader (Biochrom EZ Read 400, Cambridge UK). Samples were considered positive if the A405nm value was twice that of the negative control (0.21).

Results

Incidence of CTV in Zaria and Giwa Local Government Areas of Kaduna State.

The result showed that two of the samples collected in both Zaria and Giwa LGAs with ELISA values (0.431 and 0.534) and (0.457 and 0.426) respectively tested positive for the virus while the remaining fifty-six appeared to be negative. The incidence of the virus in each LGA was calculated as 6.67%.

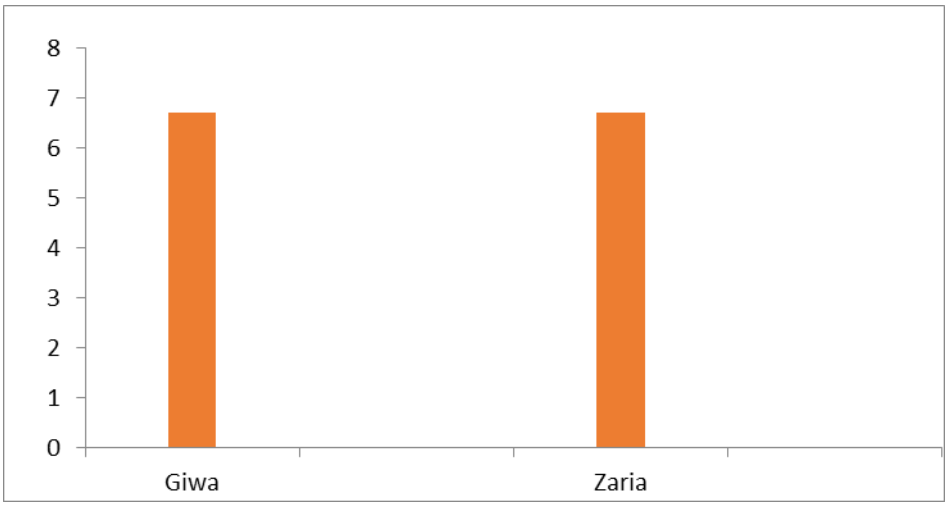

Figure 1: Incidence of *Citrus tristeza virus* (CTV) in Zaria and Giwa LGAs of Kaduna Stateduring the 2022 wet season

Plate1: Leaf samples of *citrus spp* showing CTV infection with different symptoms A) yellowing, B) necrosis, C) vein clearing and D) healthy in clearing and D) healthy

DISCUSSION

Based on the findings this study, it is evident that there was occurrence of *Citrus tristeza virus* from

both the symptomatic and asymptomatic leaf samples collected. Leaf vein clearing, yellowing, leaf cupping, and stunting were the major symptoms observed, which corroborated with what Figueroa *et al*. (2009) and El-Morsi *et al*. (2017) observed during their investigation of the disease. The four positive samples (1 and 6) from Zaria and (31 and 37) from Giwa showed almost all the symptoms of the disease including leaf cupping, vein clearing, temporary yellowing, and stunting of young leaves as described by Dawson *et al*. (2013). The remaining samples showed most of the obvious CTV symptoms such as yellowing, cupping, and vein clearing but there was no virus detected. The symptoms are being confused to be induced by other biotic and abiotic factors such as nutrient deficiencies, heat, or other insects and pathogens (Atta *et al*., 2012). However, to be confirmed with no equivocation, the Citrus clonal protection program (2002), stressed that the presence of CTV in a tree should be only determined by a pathogen detection method such as ELISA rather than by symptom expression, which is strictly adhered to in our findings. There was a 6.67% incidence of CTV in each of Zaria and Giwa LGAs in the samples collected. Most of the trees surveyed were young-aged trees. The low incidence of the virus could be judged based on the fact that the age of an orchard could be a factor in the high incidence of the virus (Dodds and Gumpf, 1991). However, the presence of the virus indicates that CTV is becoming a more important disease in Nigeria, building on the reports by Kareem *et al.* (2013) and Adediji *et al.* (2015). Hence, the an urgent need for the imposition of management practices to curtail its spread, particularly quarantine measures The low occurrence of the virus can also be attributed to the frequent spraying of insecticide, thereby reducing the aphids (Garzo *et al.,* 2020) that usually transmit the virus as the farms are all intercropped with annual crops. The low incidence and slow spread of the virus may also be attributed to the lower propagation of the Citrus in northern Nigeria because the virus is primarily spread via the propagation of infected plants and the propagation of virus-infected buds (Websh et *al*., 2019). It was observed that the farms where the samples were collected were properly managed especially by clearing weeds. This might also be the reason why there was a low incidence of the virus because the virus is managed by proper sanitation and constant insecticidal spray (Yakomi, 2017). The mild incidence of the virus may also be due to the young age of the orchards (Dodds and Gumpf, 1991), as all the farms visited are not more than 10 years old based on the farmers' response to our queries to that effect. This research is the first to report the presence of CTV in Zaria and Giwa LGAs and probably in Kaduna State to the best of our knowledge. Our questions to the farmers revealed that they mostly source their propagules from the Southern part of the country, hinting a possibility of the virus disease increase due to the introduction of cultivars and new varieties of citrus with no recourse to quarantine. This practice is attributable to the rapid spread of the disease across the globe (Broadbent, 1995; Moreno *et al*., 2008).

CONCLUSION

The study has shown the occurrence of the *Citrus tristeza virus* (CTV) in Zaria and Giwa Local Government Areas of Kaduna State. The virus was detected for the first time in the areas. It also shows that viral symptoms are strongly associated with the CTV. This development will pave the way for employing appropriate management strategies to prevent further spread of the virus in the areas and non-infected areas.

ACKNOWLEDGMENTS

We would like to sincerely express our appreciation to Mr AbdulMalik Zubairu, Mr. Jonathan O. Sedi, and Mrs. Hannatu Wakawa for their technical assistance during the conduct of this study.

REFERENCES

Abobatta, W.F. (2019). Nutritional benefits of Citrus Fruits. *American Journal of Biomedical Science and Research*, 3(4), 303-306.

Adediji, A.O., Atiri, G.I., and Kumar, P.C. (2015).Incidence, distribution, and first identification of *Citrus tristeza virus* by RT-PCR in citrus orchards in South-Western Nigeria. *Acta Horticulturae*, 12(1065), 759-766.

Atta, S.C., Zhou, Z., Yan, M.J., and Wang, X.F. (2012).Distribution and Research advances of *Citrus tristeza virus*. *Journal of Integrated Agriculture*, 11, 346-358.

Broadbent, P. (1995). Quarantine in relation to Australian citrus imports and exports. *Australasian Plant Pathology*, *24*, 145-156.

Christopher, I.C, and Udoh, E. (2020). An assessment of citrus family in Nigeria.*IJRRSSH*, 7(1): 10-15.

Citrus Clonal Protection Program (2002). Biocharacterization reaction of California *Citrus tristeza virus* isolates. Retrieved from http://capp.ucr.edu/diseases/ctvisolates.html. 24th August, 2023.

Dawson, W.O., Garnsey, S.M., Tanineni, S., Folimonova, S.Y., Harper, S.J., and Gowda, S. (2013). *Citrus tristeza virus*-host interactions.*Frontiers in Microbiology*, 4, 88.

Dodds, J.A., and Gumpt, D.J. (1991).*Citrus tristeza virus* in Central Africa.*Citrogr*, 76, 4-11

El-Morsi, A.A., Haroun, S.A., Hassan, A.M., Aseel, D.G., and Hafez, E.E. (2017). Characterization of *Citrus tristeza virus* isolated from Dakahlia Governorate, Egypt. *International Journal of Virology*, 13(1), 53-61.

FAOSTAT. (2021) http://faostat.org/site visited 6th July, 2022.

Figueroa, J., Foguet, L., Figueroa Castellanos, A., and Stein, B. (2009). Biological characterization of *Citrus tristeza virus* strains in lemon in Tucumán, Argentina. *Revista industrial y agrícola de Tucumán*, *86*(1), 37-41.

Folimonova, S.Y. (2020). *Citrus tristeza virus*: A large R.N.A virus with complex biology turned into a valuable tool for crop protection pathogens. *PLOS Pathogen,*16 (4):e1008416. https://doi.org/10.1371/jounal.ppat.1005416.

Garzo, E., Moreno, A., Plaza, M., and Fereres, A. (2020). Feeding behavior and virus-transmission ability of insect vectors exposed to systemic insecticides. *Plants*, *9*(7), 895.

IPPC. (2016). Diagnostic protocols for regulated pests.*https://IPPC/Site*

Kareem, K.T., Odu, B.O., Umeh,V., and Arogundade, O.(2013).Incidence and distribution of *Citrus tristeza virus* in Ibadan, Nigeria. *Journal of Applied Horticulture,* 15(3), 2-4.

Meena, A.K., Mingnam, F.D., Marak, C., and Meena, R.K. (2018). Citrus Decline.*Int. J Curr Microbiol. App. Sci,* 7(4), 2807-2815.

Monero, P., Ambros, S., Albiach-marti, M.R., Gherri, J., and Pena, L. (2008).*Citrus tristeza virus*: a pathogen that change of course of the citrus industry. *Molecular plant pathology,* 9(2), 251-268.

Olsen, M., Matheron, M., McClure, M., and Xiong, Z. (2000).Diseases of Citrus in Arizona. Document No. AZ-1154. Phoenix, AZ, 12pp.

Pereira Gonzatto, M.,and Santos, S.J. (2023). Introductory chapter: World Citrus Production and Research. intechopen.doi: 1057721 intechopen.110519.

Saponari, M., Marijonath, K., and Fakomi, R.K. (2008). Quantitative Detection of *Citrus tristeza virus* and aphids by real-time reverse transcription - PCR (Taqman). *Journal of Virological Methods*, 147, 43-53.

Wang, Y. M., Ostendorf, B., Gautam, D., Habili, N., andPagay, V. (2022). Plant viral disease detection: From molecular diagnosis to optical sensing technology—A multidisciplinary review. *Remote Sensing*, *14*(7), 1542.

Wubshet, Z., and Amare, D. (2019). Review on *Citrus tristeza virus*. *International Journal of Research Studies in Agricultural Sciences*, 5(4), 25-36.

Yokomi, R.K., Selvaraj, V., Maheshwari, Y., Saponari, M., Giampetruzzi, A., and Hajeni, S. (2017). Identification and characterization of *Citrus tristeza virus* isolates breaking resistance in trifoliate orange in California. *Phytopathology*, 107(7), 901-908.