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SEROLOGICAL DETECTION OF RNA VIRUSES ON FIELD-GROWN ONION IN NORTHERN NIGERIA

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Abstract. *Onion (Allium cepa) is commonly known as "Queen of the kitchen" due to its high valued, flavour, aroma and medicinal properties. Viruses are significant causal agents of plant diseases, leading to severe reduction in size and yields in onions. RNA viruses such as Tospoviruses, Allexiviruses Orthotospovirus and Carlaviruses affects global agriculture sustainability. Onion yellow dwarf virus (OYDV) is an important potyvirus that infect onions leading to 60% economic loss. A survey was initiated to determine the field disease incidence of OYDV using visual symptoms and serologically determine the occurrence of the virus using DAS-ELISA in four leading onion producing States in Nigeria. A total of 540 onion leaf samples were collected from 36 fields in 3 LGAs each of Kaduna, Kano, Zamfara and Kebbi. Assesses disease symptoms of irregular yellow stripping symptom was 29%, while chlorotic flattened leaves with downward curling was 16.3% on sampled onion plants. Kebbi state had the highest cumulative symptom incidence of 39.3% on infected onion fields, followed by Zamfara (35.86%) which was at par with kano (35.23) and the least was Kaduna (25.31%). Among the 12 LGAs surveyed, Jega LGA had the highest observed disease incidence of 13% followed by Shinkafi 11% whereas Sabon Gari had the least value 7%. DAS-ELISA, showed the presence of OYDV in Zamfara with 4% and Kebbi 7% disease incidence respectively. However, the virus is absent, Kaduna and Kano, as such it is safe to conclude that the symptoms noticed could be said to be those of other disease(s) that show varying degree of similarities or due to other factors. OYDV could be threatening if not managed appropriately. Farmers need to be enlightened on this disease and management strategies such as pest control and other measures to ensure the disease remains below the threshold level avoid higher level of occurrence.*

Keywords: *DAS-ELISA, Onions, OYDV, RNA virus, Serological detection.*

INTRODUCTION

Onion (*Allium cepa* L.) is a short duration bulb crop that belongs to the family *Alliaceae* under Order *Asparagales*). Onion is commonly known as "Queen of the kitchen" due to its high valued flavour, aroma and medicinal compound (JUNEJA *et al.*, 2023). It is a highly valued vegetable crop used to spice many dishes as matured bulb or green vegetable, when harvested early (ANYANWU, 2003). This important vegetable has been cultivated for thousands of years as rich source of different vitamins, minerals, proteins, carbohydrates and used as spice, flavoring agent in daily diet (BOUHENNI *et al.*, 2021).

Onion is the second most cultivated and consumed vegetable after tomatoes, with India (28.6%) and China (22.2%) as the largest producers, together accounting for 50% of the world's production. In 2023, the worldwide value of onion and shallot production was worth 111 million Mt, with a total production area of 5.9 million hectares (FAOSTAT, 2024). Africa is a significant producer of onions, Nigeria stands as a major player in onion cultivation in Africa and ranks 4th in Africa after Egypt, Algeria and Sudan with a production of 1,6 million tonnes in 2022, (FAO, 2022). In Nigeria, onion is grown mostly in Kano, Kaduna, Jigawa, Sokoto,

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Plateau, Bauchi and Kebbi States (KAKA *et al.,* 2022). Onion is an indispensable vegetable which is used as cooking condiment in almost every household in Nigeria. It can be eaten in its fresh/raw form, as ingredient in salads, processed to make onion paste, dehydrated onion flakes, onion oil and onion sauce (DIRECTORATE OF ONION AND GARLIC RESEARCh, 2021). They are useful in phyto-pharmaceutical preparations against various human and animal diseases, and additionally to treat diseases due to their bactericidal, anticarcinogenic, and hypoglycemic properties (GALMARINI, 2018), also antioxidant activity due to presence of polyphenols, flavonoids, tannins, quercetin and organosulphur (ZHAO *et al.,* 2021; KARAVELIOĞLU & HOCA, 2022) and manufacturing moth repellents (RAVI, 2016). Economically, onion production support both individual value-chain actors as well as the nation by way of exportation to other African countries.

Viruses are significant causal agents of plant diseases, leading to reduced yields in *Allium* species. Among the most common viruses affecting the *Alliaceae* family are *Potyviruses*, *Tospoviruses*, *Allexiviruses*, *Orthotospovirus* and *Carlaviruses* affecting global agriculture sustainability as stated by KING *et al.*, (2012). Among the virus groups, *Potyvirus* is the largest genus causing considerable economic loss that belongs to family *Potyviridae* having diverse host range including major crops like potato, tomato, sugarcane, banana, papaya, pepper etc (VISHWANATHAN *et al*., 2017). *Leek yellow stripe virus* (LYSV) and *Onion yellow dwarf virus* (OYDV) are significant potyviruses that infect *Allium* species causing severe reduction in plant vigor by inducing mosaic, pale color stripes, leaf streaks, water-soaked lesion, diamond shape eye spots, stunting and dwarfing, which results in reduced yield up to 60% annually (GILANI *et al.,* 2016; ŞAFAK & KÖKLÜ, 2024). OYDV is transmitted by over 50 species of aphid in a non-persistent manner; *Myzus ascalonicus, M. persicae, R. maidis* and *Acyrthosiphon pisum* being the most important virus vectors, vegetative production material and mechanical inoculation (CACIAGLI, 2008). OYDV has been reported in onion growing areas in the world, exhibits characters like dwarfing, stunting, irregular yellow striping to almost complete yellowing, reduction in number of flowers, seeds and impairment of seed quality which can lead to heavy yield losses up to 60% (WARD *et al.,* 2009).

This virus can induce both symptomatic and asymptomatic features indicating the possibility of latent infection. Since there is a constraint on virus detection-based on visual symptoms only (ABRAHAM *et al.,* 2019) therefore, detection methods with high accuracy are deployed, including serological-based and nucleotide-based detections. Serological detection such as Double Anti-body Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA) is a commonly used method for detecting viruses in *Allium* species. Therefore, objective of this research was to visually estimate field incidence of OYDV infection on onions and detecting the virus by probing collected plant samples using DAS-ELISA in four major onion producing States in northern Nigeria.

MATERIAL AND METHODS

1. Field survey and plant sample collection

This research was carried out during the dry season (late October to November) of 2013, when irrigation farming is commonly practiced. Onion production in North Western Nigeria is normally at its peak during this time of the year. The onion plant is usually planted in late September to mid-October and is ready for harvest by January and February of the following year. Three onion farms from three local government areas in Kaduna, Kano, Zamfara and Kebbi states were assessed. The local government areas visited were: Zaria, Sabon - Gari and

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Makarfi (Kaduna State), Rano, Kibiya and Kura (Kano State): Tsafe, Kaura - Namoda and Shinkafi (Zamfara State), Gwandu, Aleiro and Jega (Kebbi State).

The sampling was done using a 3 x 3m^2 quadrat at the five different positions on the field. From each quadrant, 3 onion leaves were collected, two symptomatic and one asymptomatic to carry out incidence survey of the virus and laboratory analysis. From the survey conducted, a total of 540 onion leaf samples were collected from 36 fields in 12 local government areas of Kaduna, Kano, Zamfara and Kebbi states. A total of 180 of the collected leave samples did not show symptom of the diseases (healthy sample), while the remaining 360 were collected from symptomatic plants. The healthy plants were sampled because plants have been known to carry viral diseases even when the symptoms do not appear. Weeds (87) in and around the fields that showing symptoms of the disease were also collected. The samples were collected into polythene bags and properly labelled were stored in an ice-pack and then transported to the Virology laboratory of the Department of Crop protection, Ahmadu Bello University, Zaria. The samples were stored in a refrigerator at a temperature of -20 ^{0}C for laboratory analysis. The corresponding results was converted to percentage by multiplying the incidence value by 100.

$$\text{Disease incidence} = \frac{\text{number of symptomatic plants in selected area}}{\text{total number of plants in selected area}} \text{ X } 100$$

2. Laboratory Detection of OYDV using DAS-ELISA

A total of 580 onion leaves and weed samples which were collected from the field surveys were tested serologically using OYDV DAS-ELISA AS-0447 1gG AP Dilute1:1000 and OYDV DAS-ELISA AS-0447 1gG Dilute 1000 positive control antigens (Manufactured by DSMZ GmbH). The DAS-ELISA buffers used for the experiment were prepared following standard procedures and measurements.

2.1 Coating buffer (CB) pH 9.6

Sodium carbonate (Na_2C0_3) 1.59 g, 2.93 g sodium bicarbonate ($NaHC0_3$) and 0.20 g sodium azide (NaN_3) were dissolved in 900ml of distilled water (H_2O) using FISCHER THERMIX magnetic stirring hot plate model 210T. The pH was adjusted to 9.6 by adding drops of aqueous hydrogen chloride (HCl). Jenway 3510 pH meter was used for determining the pH. at a temperature of 26^{o}C and slope of 100%.

2.2 Phosphate Buffer Saline (PBS) (pH 7.4)

Sodium chloride (NaCl) 8.0 g, 0.2 g monobasic potassium phosphate (KH_2P0_4), 1.15g dibasic Sodium Phosphate ($Na_2HPO^-_4$), 0.2 g Potassium Chloride (KCl) and 0.2g sodium azide (NaN_3) were all dissolved in 900 ml of distilled water (H_2O). FISCHER THERMIX stirring hot plate model 210T was used to facilitate the dissolution of crystals formed during the preparation. The pH was adjusted to 7.4 by adding drops of aqueous hydrogen chloride (HCl). Jenway 3510 pH meter was used for determining the pH. at a temperature of 26^{o}C and slope of 100%. Microtiter wells (64) were used, with one of the wells used for positive control and another used for the negative control. Dilution ratio was 11antibody into 1ml CB. 64 wells calculated for (in case of error) and thus 131 of antibody conjugate was added to 13 ml of CB.

2.3 PBS-Tween (PBST)

PBS+0.5 ml tween (20 ml per litre)

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2.4 Sample Extraction Buffer (SEB) (pH 7.4)

PBST+2% PVP (Sigma PVP - 40 polyvinyl pyrrolidone). The SEB was then adjusted to pH 7.4 by adding drops of NaOH (Temperature 27.4^{O}C, slope 100%), 500 ml PBST was taken and 10g PVP was added.

2.5 Conjugate Buffer

PBST +2% PVP + 0.2% egg albumin

2.6 Substrate Buffer

Diethanolamine 97 ml + 600 ml H_2O + 0.2g Sodium Azide (NaN_3). The substrate buffer was adjusted to pH 9.8 by adding drops of HCl and it was made up to 1litre by adding H_2O.

Leaves from the collected onions samples were harvested and put into mortars. The extraction buffer was added to each mortar containing harvested onions leaves and the samples crushed using pestle. 200 ml of antigen was added to each well containing 200 ml of the coating buffer. The CB mixture was incubated overnight.

3. Protocol for DAS-ELISA

Purified 1gG diluted in coating buffer and incubated at 37 ^{0}C for 4 hours. The plate was washed with PBS-Tween then soaked for few minutes followed by repeated washing. The plate was blotted by tapping upside down on tissue paper. 200 ul aliquots of the test sample (extraction in SEB) was added to duplicated followed by incubation overnight at 4 ^{0}C. The plate was then washed 3 times after which 200ul of anti-virus conjugate was added and incubated at 37 ^{0}C for 4 hours followed by washing. 200 ul aliquot of freshly prepared substrate (10 mg P-nitrophenyl phosphate (Sigma flake) dissolved in 10 ml of substrate buffer) was added to each well. This was then incubated at 60 minutes to obtain a clear reaction. Sorghum leaf was used as negative control and a positive control obtained from the DSMZ was used. The result was read by Spectrophotomeric measurement of absorbance at 405 nm. Samples that had a mean absorbance of 405 nm which is twice the value of the negative control is considered positive from the serological analysis using DAS-ELISA, and as such infected with *Onion Yellow Dwarf Virus*. Neem was used as negative control because neem has never been suspected to be an alternative host of OYDV as described by CLARK & ADAMS (1977) and ABRAHAM *et al. (*2024).

RESULTS AND DISCUSSION

Field Survey

The results of the field survey study, it was observed that infected onion plants showed onion yellow dwarf virus (OYDV) symptoms such as stunting of first-year onion (*Allium cepa*) plants, with the leaves showing irregular yellow striping to almost completes yellowing, downward curling, flattening, mosaic and yellow streak symptoms, striping, curling and distortion of flower, stems, reduction in the number of flowers and seeds, crinkling and flaccidity as shown in plate I and II. Similar symptoms were observed on onions infected with OYDV and reported by ABDEL WAHAB *et al.* (2009).

Irregular yellow stripping with curly leaves, accounted for 29% of the total symptoms noticed in the fields assessed. 78 samples collected showed the symptoms stated above.

Chlorotic leaves that were flattened and showed downward curling, accounted for 16.3% of the total symptoms noticed in the surveyed fields. Fifty-three samples collected showed these

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symptoms. Other symptoms such as mosaic, yellow streaking, flaccidity, crinkling and distortion of flower stem were also observed from the onion fields of all the survey states. This could imply that the onions may be infected with other viruses that were not probed in the present study. The survey on the visual incidence of OYDV based on symptom presented on the onion fields showed varying occurrence of the disease across the four states surveyed.

Results in Table 1 showed that all the farmers are cultivating on fragmented lands (<0.5ha) and majority sole cropping pattern and surrounded by mostly solanaceous crops. The disease was present in the three LGAs surveyed with Zaria (3.14%) and Hunkuyi (3.16%) having the highest average virus disease incidence while Sabon Gari had the least (2.13%).

Table 1

Field data showing observed farm size, cropping pattern and visual virus disease incidence in Kaduna State

LGA/farm	Farm size (ha)	Cropping pattern	Crops around field	Observed disease incidence (%)
Zaria a.	0.0103	Sole	Okra, sweet potatoes	5.36
b.	0.0116	Sole	Pepper	2.48
c.	0.0117	Intercropped with pepper	Maize, pepper, sweet potatoes	1.59
Hunkuyi a.	0.0097	Intercropped with okra	Okra, eggplant, pepper, tomatoes	3.83
b.	0.0091	Sole	onions, tomatoes	1.85
c.	0.0107	Intercropped with pepper	Pepper, onions, tomatoes	3.79
Sabon Gari a.	0.112	Sole	Tomatoes, onions, pepper	2.40
b.	0.0085	Sole	Eggplant, onions, pepper, tomatoes	1.64
c.	0.0091	Sole	Onions, tomatoes, pepper	2.37

Data from Table 2 connotes that all the farmers produce onions on small area of lands (<0.1ha). Over 70% of them practice sole cropping pattern and surrounded by mostly solanaceous crops. The disease symptoms were observed on all fields in the three LGAs surveyed with Kura (4.1%) and Kibiya (3.95%) having the highest average virus disease incidence while Rano had the least (3.72%).

Data from Table 3 reveals that all the farmers produce onions on very small area of lands (<0.1ha). All the farmers in this location practice sole cropping pattern and surrounded by mostly tomatoes and pepper. The highest average observed disease incidence was observed in Shinkafi (5.01%) followed by Tsafe (3.68%) while Kaura Namoda had the least (3.22%).

Data from Table 4 denotes that all the farmers produce onions on very small area of lands (<0.1ha) as a mono crop. The highest average observed disease incidence was observed in Jega (5.84) followed by Gwandu (4.03%) while Aleiro had the least average value (3.22%).

Table 2

Field data showing observed farm size, cropping pattern and observed virus disease incidence in Kano State

LGA/Farm	Farm size (ha)	Cropping pattern	Crops around field	Observed disease incidence (%)
Rano a.	0.0184	Sole	Eggplant, watermelon	3.99
b.	0.0320	Intercropped with pepper	Pepper, Tomatoes, Onions	3.82

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c.	0.0237	Sole	Onion, Tomatoes, pepper	3.34
Kibiya a.	0.0096	Sole	Onion, okra, pepper,	1.49
b.	0.0122	Sole	onions, tomatoes	6.05
c.	0.0087	Sole	Pepper, onions, tomatoes	4.28
Kura a.	0.0153	Sole	Maize, onions	3.94
b.	0.0079	Intercropped with pepper	Pepper, onions, tomatoes	4.77
c.	0.0181	Sole	Onions, okra, carrot	3.52

Table 3

Field data showing observed farm size, cropping pattern and observed virus disease incidence in Zamfara State

LGA/Farm	Farm size (ha)	Cropping pattern	Crops around field	Observed disease incidence (%)
Shinkafi a.	0.0090	Sole	Onions, pepper, lettuce	1.98
b.	0.0203	Sole	Onions	7.88
c.	0.0085	Sole	Onions	5.31
Kura Namoda a.	0.0092	Sole	Onions	3.33
b.	0.0138	Sole	Pepper, onions, tomatoes	3.59
c.	0.0088	Sole	Pepper, onions, tomatoes	2.74
Tsafe a.	0.0087	Mixed with carrot	Carrot, onions	2.13
b.	0.0091	Sole	Onions	4.12
c.	0.0100	Sole	onions, pepper, tomatoes	4.78

Table 4

Field data showing observed farm size, cropping pattern and observed virus disease incidence in Kebbi State

LGA/Farm	Farm size (ha)	Cropping pattern	Crops around field	Observed disease incidence (%)
Aleiro a.	0.0098	Sole	Onions	3.88
b.	0.0076	Sole	Onions, cassava	1.78
c.	0.0087	Sole	Onions	4.01
Jega a.	0.0173	Sole	Onions	3.91
b.	0.0093	Sole	Onions	4.88
c.	0.0134	Sole	Onions	8.73
Gwandu a.	0.0085	Sole	Onions	2.77
b.	0.0096	Sole	Onions	4.20
c.	0.0108	Sole	Onions	5.14

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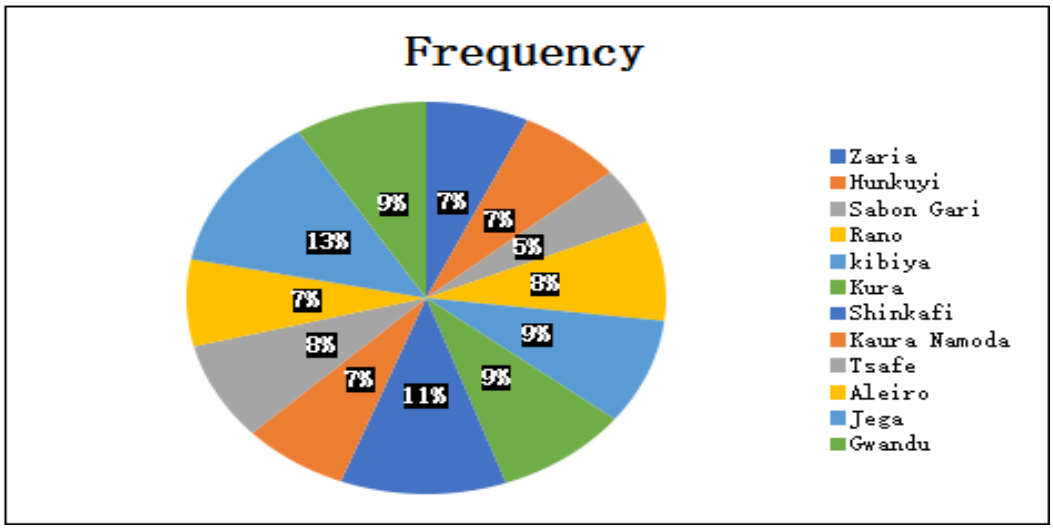

Figure 1. Observed virus disease incidence in the 12 LGAs surveyed (%)

The visual disease symptoms assessed were irregular yellow stripping with curly leaves and flattened chlorotic leaves with downward curling, Jega had the highest value of 13% followed Shinkafi 11% and then Aliero, Kibiya and Kura with 9% respectively as illustrated in the pie chart above. Although these specific symptoms are expressed on diseased plants, it cannot suffice to conclude the presence of OYDV in all these study locations.

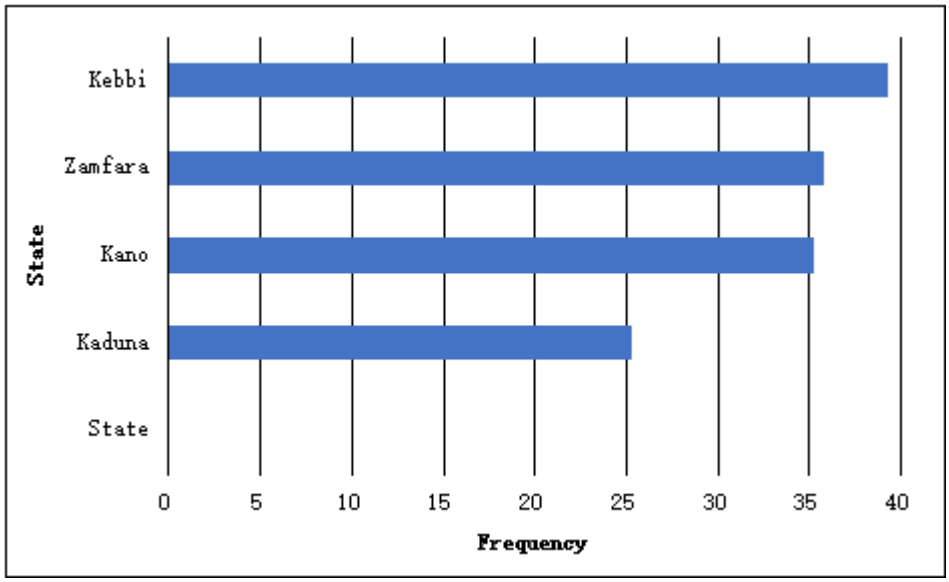

Figure 2. Observed disease incidence in the 4 states assessed (%)

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As shown in the bar chart, the cumulative observed disease incidence of OYDV on onions from the four states surveyed, Kebbi had the highest value with 39.3%, followed by Zamfara 35.9% which was slightly lower that Kebbi but almost the same with Kaduna 35.2%, while Kano was the lowest with 25.3%. All the values are within the safe levels as no state had up to 40% disease symptoms of irregular yellow stripping, flattened chlorotic leaves with downward curling which are typical of OYDV.

Serological Analysis (DAS-ELISA)

It can be deduced from the result in Table 5, after conducting laboratory analysis DAS-ELISA the virus was not found to be present in the samples collected from the fields in Kaduna State. The symptoms noticed could be said to be those of other disease(s) that show varying degree of similarities to OYDV, or it could be as a result of abiotic factors.

Table 5

Occurrence of OYDV in Kaduna State, Kano, Zamfara and Kebbi States

Location	Number of Samples Tested	Number of Positive Samples
Zaria	45	0
Hunkuyi	45	0
Sabon Gari	45	0
Rano	45	0
Kibiya	45	0
Kura	45	0
Shinkafi	45	0
Kaura Namoda	45	2
Tsafe	45	0
Aleiro	45	3
Jega	45	1
Gwandu	45	1

Table 5 above, depicts that no sample tested positive from DAS-ELISA laboratory analysis. This implies that the virus is not present in Kano state, thus, it could be suggested that the symptoms noticed could be said to be due to other diseases that induce symptoms related to OYDV or other possible conditions such as pest or inadequate plant nutrition.

The illustration of laboratory test conducted from samples collected in Zamfara State in Table 5, revealed two positive samples which is about 4% to be infected by OYDV in Kaura Namoda. The values obtained from these samples were twice that of the negative control. Although the virus is present in Zamfara state, the occurrence level is not alarming can be said to insignificant. Knowing that this disease is easily transmitted by aphids, farmers need to be enlightened on this disease and management strategies such as pest control and other measures to avoid higher level of occurrence

Out of 135 samples collected from onion fields in Kebbi state, a total 5 samples tested positive after DAS-ELISA. Aliero had 3 infected samples which is 7% and significantly higher than Jega and Gwandu with 1 sample each barely 2% which are at par. Despite, Kebbi state had the highest number of positive samples, the presence of the virus in the state is just about 4% of the total 135 samples and cannot be said to be significant this finding is in contrast with the work of Bagi *et al.,* 2012 where 30.5% of samples tested positive. However, there is need for famers awareness on OYDV disease symptoms (distortion of flower stems, reduction in the number of flowers and seeds, and impairment of seed quality), its negative effects on yields (infected plants are more susceptible to low temperatures while affected onion bulbs deteriorate

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during storage and show premature sprouting). Farmers should also encourage them to maintain healthy agronomic practices on their fields to avoid rapid disease spread and ensure the disease remains below the threshold level.

DAS-ELISA for Weed Samples

Infected weeds could can also serve as alternative hosts of the viruses or insect vectors within crop fields and also neighbouring crops, thereby making the management of viruses and their vectors difficult. A total of 87 weeds samples were collected from the various fields visited during the surveys and analysed using DAS-ELISA, the results are shown in the table below.

Table 6

Occurrence of OYDV in weed plants

Weed plants	Number of positive/Total number of samples
Cyperus difformis	0/11
Kyllinga erecta	0/4
Acanthospermum hispidium	0/7
Tridax procumbens	0/14
Ageratum conyzoides	0/9
Euphorbia hirta	0/5
Portulaca oleracea	0/9
Cyperus rotundus	0/7
Pennisetum pedicellatum	0/4
Stenotaphrum secundatum	0/9
Chromplaena odorata	0/8

Despite a large quantity of weeds (87) were collected for DAS-ELISA test for the presence of OYDV. There were no single positive samples in all the 36 farms surveyed in Kaduna, Kano, Zamfara and Kebbi states. Though OYDV was present in Kaura Namoda, Aliero, Jega and Gwandu LGAs, no weed from these infected fields has been infected so far this result does not agree with Kazinczi at al., 2004 and Chen *et al*., 2016 assertions that weeds could can also serve as alternative hosts of the viruses or insect vectors within crop fields.

CONCLUSIONS

At the end of this research, observed symptoms of irregular yellow stripping symptom was 29%, while Chlorotic downward curling was 16.3% on sampled onion plants. Based on LGAs; Jega had the highest value of 13% followed Shinkafi 11% and then Aliero, Kibiya and Kura with 9% respectively. While that of state was cumulative Kebbi had the highest value with 39.3%, followed by Zamfara 35.9%, Kaduna 35.2 %, and Kano was the lowest with 25.3%. The laboratory results from DAS-ELISA, Kaduna and Kano states show that the virus is absent, as such it is safe to conclude that the symptoms noticed could be said to be those of other disease(s) that show varying degree of similarities to OYDV or due to other factors. However, the disease is present in Zamfara and Kebbi states but at low levels. Farmers need to be enlightened on this disease and management strategies such as pest control and other measures to avoid higher level of occurrence and ensure the disease remains below the threshold level. Molecular identification should also be carried out in future as there were only few positives though symptomatic and no single positive obtained from 87 tested weeds during the study.

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