Asian Research Journal of Agriculture

10(3): 1-18, 2018; Article no.ARJA.45761
ISSN: 2456-561X

Occurrence and Influence of Sudanese Onion Yellow Dwarf Potyvirus Isolates on the Common Bulbing Onion (*Allium cepa* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2018/45761
Editor(s):
(1) Dr. Rusu Teodor, Department of Technical and Soil Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Reviewers:
(1) Ferenc Bagi, Serbia.
(2) Filiz Ertunc, Ankara University Faculty of Agriculture, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/28060

Original Research Article

Received 30 September 2018
Accepted 14 December 2018
Published 02 January 2019

ABSTRACT

Further epidemiological studies on the Sudanese isolates of onion yellow dwarf (OYDV) and its effect on production of onion crops were carried out during two consecutive seasons under field conditions. Four OYDV isolates were recovered from four different localities in Khartoum and River Nile States where the common bulbing onion (*Allium cepa* L.) is the major crop. These isolates were named after their respective localities [viz., Gezira Islanj (GI), Wad Ramly (WR), Wad El basal (WB) and El Damar (D) isolates] and their characteristics were studied. Five different types of symptom patterns were encountered in the different onion growing seasons surveyed and hence could not be assigned to any specific OYDV isolates. Field surveys and "on growing" tests revealed that the true disease incidence was consistently substantially higher than the observed disease incidence and they were positively correlated ($r^2 = 0.63$). This may prove to have an important epidemiological consideration in disease forecast and disease management. The different OYDV isolates were found to reduce significantly the onion bulb yield as measured by bulb size, bulb weight and onion

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bulb quality (total soluble solids and dry matter content). In the field experiment, the various OYDV isolates were found to significantly reduce plant growth in terms of leaf width and leaf length. They also reduced significantly the seedstalk height and seedstalk diameter but not the number of seedstalks. The productive capacity in terms of number of days to anthesis, flowering percentage, umbel diameter, total number of florets per umbel and percent of abortive florets, were seriously affected by the different OYDV isolates. Consequently, the seed yield was drastically reduced and losses were 61.2, 65.3, 71.8 and 83.9% due to infection with WB, WR, GI-and D isolates, respectively.

Keywords: Onion yellow dwarf disease; OYDV; yield loss; OYDV-isolates; bulb crop; seed crop; true and disease incidence; Sudan.

1. INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops worldwide [1,2]. In Sudan, it is considered as the most popular and widely grown vegetable crop [3]. The season of production in the Sudan extends from August to May and the crop may be grown twice a year; an autumn crop from August to December, and a winter crop from December to May. Three kinds of onion crops are common in the Sudan; a green salad crop which could be raised from true seed or from small sized bulbs by splitting (locally known as Wad Haram), a dry bulb crop grown from tree seeds, and a seed crop raised from bulbs (locally known as cow crop) [4, 5,]. Many cultivars of onions are grown in the Sudan; imported or so-called local cultivars of red, yellow and white bulbs [6]. The farmers usually grow the local cultivars which are of local origin and take their names from either original localities or the name of the place where the cultivar seeds are usually produced.

OYDV which belongs to genus potyvirus [7, 8, 9] was first described by Bos [10] and Ploaie [11]. It is transmitted by more than 50 different species of aphids in a non-persistent manner [12,13].The disease has been reported to cause an injurious and massive effect on growth of onion plants and consequently on bulb or seed production [14-20]. OYDV was isolated for the first time in Sudan in 1982 and known locally as Karfass or Fargakh denoting the most conspicuous symptoms of leaves losing their turgidity and lop over [21]. The disease is usually very serious crippling and often attains an epiphytotic level that leads to serious yield losses [22,12]. Yield losses due to OYDV differ according to the crop. They were found to be very heavy on the bulb crop, but more serious on the seed crop. Both high and low levels of infection have been reported. Morgan [22] working in the Khartoum area reported a strikingly high incidence of infection (96%-99%) in onion plants grown from bulbs (seed crops) saved by farmers from the previous season's crop. On the other hand, she reported low levels of infection (29%). Salih [23] recorded a high incidence (55%- 95%) of OYD disease in onion fields grown from dry bulbs compared to fields grown from true seeds (8%-38%). Moreover, the problem of the disease seems to be aggravated by certain cropping systems especially in the main area of production like monocropping which leads to overlapping of onion crop and also by the continuous use of non-certified onion bulbs saved by the farmers from the previous season maintain a constant virus source and high inoculum pressure which result in heavy reductions in yields and quality of onions.

The available information about the actual effect of the virus and its various isolates on onion yield and seed production is very limited particularly under field conditions, so the scope of this study is to discover the occurrence of variability in OYDV isolates in Sudan and assess the influence of the different OYDV isolates on onion yield and seed production under field conditions. Moreover, to extrapolate the true disease incidence from the observed field incidence in an attempt to find a meaningful relationship between true and observed disease incidence that can be used in OYDV disease forecasts in the future.

2. MATERIALS AND METHODS

2.1 Field Survey

2.1.1 Symptoms expression of OYDV in the field

Noticeable symptoms variations were observed during the survey of onion commercial fields in Khartoum and River Nile States on plants naturally infected with onion yellow dwarf virus (OYDV). These visual symptoms were categorized into five different groups depending on apparent descriptive characters of the infected plants.

2.1.2 Effect of OYDV disease on onion seed crop

The main objective of this experiment was to assess the effect of natural virus infection on growth and yield components of onion seed crop. About 420 onion bulbs were randomly collected from the previous season's crop from three known infected areas in Khartoum State (viz. Wad Ramly, Wad El basal, Gezira Islanj) and one additional area in River Nile State (namely, El Dammar). The collected bulbs were planted in experimental plots in the University Farm at Shambat area. Each plot represented a location. All cultural practices were carried out as recommended and the plots received 4 sprays with systemic insecticides (Malathion and Metasystox) for the control of the thrips and aphids, respectively. The plant- to- plant method described by [24] and [25] was followed. Few weeks after emergence, ten plants showing typical OYDV symptoms were randomly selected in each plot and tagged. Plants chosen healthy and later developed symptoms were discarded. For each selected infected plant, an adjacent healthy one or the closest possible healthy plant was also tagged for comparison. Data were then recorded on the following growth and yield parameters: Leaf length (cm), Leaf width (cm), Number of seedstalks/plant, Length of main seedstalk (cm), Seedstalk diameter (cm) [(at the swollen region) using a vernier caliper], Umbel diameter (cm) (using a vernier caliper), Number of florets /umbel (including abortive and healthy florets), Percentage of abortive florets (AF). The abortive florets in the main umbel were counted and then the percentage was determined as follows:

$$AF\% = \frac{\text{Number of abortive florets/umbel}}{\text{Total number of florets/umbel}} X100$$

[12]

- Fertilization efficiency percentage (FE). This was calculated as follows: Thirty florets from each umbel were randomly selected and threshed. Their total number of seeds was calculated. The fertilization efficiency percentage was calculated according to the equation:

$$FE = \frac{\text{Number of seeds of 30 florets}}{180\ (6X\ 30)} X\ 100\ [12]$$

Where 6= Full number of seeds/florets

- Number of seeds/umbel and 1000-seed weight (g):

It was computed using a Mettler balance. Comparisons were then made between infected and non-infected plants using student's t-test [26].

2.1.3 True and observed OYDV incidence

This experiment was carried out to extrapolate the true disease incidence from the observed incidence. Surveys for OYDV in bulb crop were carried out in different onion growing areas in Khartoum and River Nile States. Towards maturity, five fields were randomly chosen in the selected areas. The mean observed disease incidence was determined by continuing the percentage of infected plants in 3m^2 towards the centre of each field after careful inspection based on visual characteristic foliage symptoms. All bulbs (healthy and infected) confined to the area examined in each field were harvested, placed separately in a jute sack and stored. After the end of the storage period a growing-on test was carried out in the second week of November. These bulb samples were planted in plots 2X3.5 m^2 consisting of 6 ridges each sample on a separate ridge and spaced at 25 cm apart. Four replications and randomized complete block design were used. Recommended cultural practices were carried out and 2 insecticides sprays using Metasystox were applied. The average infection percentage in each sample, based on characteristic visual symptoms, was determined 3-4 weeks after planting. This represents the "true" disease incidence at the respective locations. Correlations between "observed" and "true" disease incidence were calculated.

2.1.4 Bulb yield loss assessment due to OYDV infection in the field

The effect of the different OYDV isolates on bulb yield of naturally infected onion crop was studied in the field during two consecutive cropping seasons. The plant-to-plant method [24, 25] was followed as described before (Section 3.1.2). The experiment was carried out in the four main onion growing areas which are grown mainly with red onion cultivars, in Khartoum and River Nile States. Three of them were in Khartoum State, namely, Gezira Islanj (on the west Nike bank, in 25 km north of Khartoum), Wad Ramly and Wad El basal (60 and 70 km, respectively, north of

Khartoum on the eastern Nile bank). The fourth area was El Dammar district in River Nile State (310 km north of Khartoum). At maturity, bulbs from the infected and healthy tagged neighbor plants were harvested and placed individually in paper bags and the respective healthy and infected pairs of bulbs were marked by serial numbers. The following measurement and qualities were recorded for each bulb and statistically analyzed: bulb diameter (cm), bulb length (cm), bulb weight (g), % Total Soluble Solids (TSS) and dry matter content (%).

% Total Soluble Solids (TSS) was determined by using hand refractometer (0-50). Small pits of onion were drowned from the centre of each bulb sample using sharp knife and then crushed in a garlic crusher. The average of two readings taken from the extracted juice was recorded. While the dry matter content (%) was computed as follows:

The bulb fresh weight was first measured and then the bulbs were divided into small slices after the outer scales were removed by a sharp knife. Each bulb was weighed separately and dried in an oven at 72 C° for 48 hr. The percentage of dry matter content was calculated using the official methods of analysis [26]:

$$\%\ \text{Dry matter} = \frac{\text{Oven dry weight}}{\text{Fresh weight}} \text{X } 100$$ [12]

Comparisons were then made between the healthy and infected plants using student T-test [27]:

2.2 Field Experiments

2.2.1 Site description

The field experiments were conducted at the University Farm at Shambat. The site characterized by the semi-arid tropical climate, with hot summer, only part of which is rainy (during June-September). The field has a crackly, moderately alkaline soil (pH7.8-8.5). The farm is irrigated from the adjacent main Nile [4].

2.2.2 Effect of different OYDV isolate on red onion seed crop

The aim of this experiment was to study the effect of different OYDV isolate on growth and yield components of naturally infected onion seed crop.

2.2.2.1 Bulb collection

The naturally infected and healthy onion bulbs were collected from commercial onion farms in the four mentioned localities which were grown with red onion cultivars (Section 2.1.4). About 400 diseased and healthy onion plants were chosen and tagged after two months from planting during winter growing seasons. All onion plants that were chosen as diseased ones were showing typical foliage symptoms of OYDV. Healthy plants that later showed any kind of symptoms were discarded. At harvest, healthy and infected bulbs at each location were collected, cured, placed separately in jute sacks and stored. The infected bulbs collected from the four locations represented, respectively, the source of the four virus-isolates [Wad Ramly (WR), Wad El basal (WB), Gezira Islanj (GI) and El Dammar (D)].

2.2.2.2 Planting

The plot size was 3.5x4 m^2 with four ridges, 70 cm apart. The experiment consisted of eight treatments [(healthy vs. infected bulbs), four locations] arranged in a randomized complete block design with 4 replications, making a total of 32 plots. The stored infected and healthy mother bulbs were planted on the second week of November at spacing of 30 cm along the ridge. Insecticidal sprays were practiced every two weeks with Metasystox against aphids and with Malathion against thrips starting in January. One week after emergence, 5 plants out of 40 were selected randomly from two inner ridges of each plot and tagged; the two outer ridges were left as guards. Towards maturity of seed-heads (umbel), the following parameters were assessed:

leaf length (cm), leaf width (cm), diameter of main stalk (cm), length of main stalk (cm), number of seedstalks per plant, number of days to flowering (anthesis); was computed from planting date till the date of the opening of the first 3 florets in the main umbel], flowering percentage, number of florets per umbel, percentage of abortive florets, umbel diameter (by using a vernier caliper).

The flowering percentage was computed as follows:

$$\frac{\text{The number of flowering plants}}{\text{The total number of plants in each plot}} \text{X}100$$ [12]

The harvest was made on the first week of April after the signs of seed maturity were observed. It was repeated every 15 days to harvest the late maturing seed-heads. Umbel samples of each plot were cut and collected in separate paper bags and left to dry for 3-4 weeks before threshing and cleaning by hand. The average number of seeds per florets was then computed as follows: 30 florets from each umbel were selected randomly and threshed. Their total number of seeds was calculated and the average number of seeds per floret was determined. After harvesting and cleaning, the following attributes associated with seed production were measured:

- Number of seeds per umbel

Average number of seeds per umbel was determined by considering the seeds in total of 20 umbels, 5 from each plot.

- Total weight of seeds per umbel (g): this was computed using a Mettler balance.

- Weight of 1000-seeds (g)

A total of 1000 seeds was taken from yield of each umbel or umbels within the same plant and weighed on a Mettler balance to obtain 1000-seed weight.

- Final seed yield (ha^{-1})

The seeds from the designated area of each plot were weighed on a Mettler balance and from that final seed yield per hectare was calculated.

- Percentages of seed yield loss:

It was determined in reference to the yield of healthy plants in a unit area according to the following formula:

$$\%\text{Yield loss} = \frac{\text{Yield of healthy plants} - \text{Yield of infected plants}}{\text{Yield of healthy plants}} \text{X100}[12]$$

At the end of dormancy period, the following tests were carried out:

- Germination test in Petri-dishes (%)

50 seeds from infected and healthy plants were taken randomly from the stock of each of the4 localities and placed in 9-cm diameter Petri-dishes, over Whatman No.1 filter paper saturated with distilled water and then kept in an incubator at a constant temperature of 24 C° in the dark for 7 days. The percentage of seed germination was then computed. The experiment was arranged in a randomized complete block design with 8treatments and 3 replications. Each treatment consisted of 2 Petri-dishes and 50 seeds per dish.

- Germination test in the soil

The same germination test in Petri-dish was repeated in the glasshouse in plastic trays (27.5x15 cm) using sterilized soil. Fifty seeds were sown in each plastic tray cell. Each treatment was represented by two cells. Percentages of germination were then recorded after 30 days from sowing. The experiment was arranged in a randomized complete block design with 8 treatments and 4 replications.

- Seedling vigour

- Seedling fresh weight (g)

Seedlings in Petri-dishes were immediately weighed after removing from the germination incubator using a sensitive balance.

- Seedling dry weight (mg)

The onion seedlings were then dried in an oven (Memmert, Scientific Technical Supplies, Germany) at 72Cº for 48 h. The oven dry weight for each sample was recorded.

2.3 Statistical Analyses

All data of the experiment were subject to analysis of variance and significant differences between means were determined by Duncan's Multiple Range Test (DMRT) [28].

3. RESULTS

3.1 Field Survey

3.1.1 Symptoms variations

Considerable variations in disease expression were observed in leaves of infected onion plants grown from infected bulbs. These differences in symptoms were grouped into five different types (Fig. 1. A, B; 2. A, B and 3):

Type I: The leaves were semi flat, flaccid and curled with yellow green streaks altering with deep green background of the leaf in a form of bands. The whole plant was stunted with drooping leaves. Later, it produced a short twisted and very thin flower stalk. Umbels were very small in size when compared to those of healthy ones.

Type II: The leaves were characterized by very thin yellow streaks. However, the leaves were turgid and keeping their tubular form and size. The whole plant was stunted and later produced short and twisted stalks.

Type III: This type of symptoms seemed to be the most common and typical to OYDV symptoms. It consisted of leaves showing flattening, drooping with tallow thin streaks extending along the whole leaf. The leaves were oftenly dentate and had thick leathery texture. Plant stunting was common and seedstalks were small, short and twisted.

Type IV: The leaves were turgid, dentate and oftenly showing general yellowing or sometimes with yellow streaks. The seed stalks probably would not show up in some plants till the end of the season. The whole plant was also commonly stunted.

Type V: The plants were severely stunted; leaves were very small and flat. The leaves continued to become thinner with progressive infection to give very small thread-like leaves. Groups of yellow streaks were separated by distinct dark green bands running along the leaves from one side and pronounced dentations existed on the other side of the leaf. The seed stalks were very thin, very short and twisted carrying small malformed umbels. Severely infected plants might die before the end of the growing season.

No variation in symptoms were detected when inocula from these different categories of symptoms were sap inoculated on a unified selected range of onion varieties indicating that these variations in symptoms may not be due to different OYDV isolates. However, inocula from type I and II incited chlorotic local lesions on inoculated leaves of *Chenopodium amaranticolor* which later become necrotic. The possible existence of variability in OYDV was then sought in the different geographical regions of Khartoum and River Nile States, viz., Wad Ramly, Wad El basal, Gezira Islanj and El Dammar. Accordingly, the OYDV isolates were designated as WR, WB, GI and D, respectively.

3.1.2 Effect of OYDV on growth and yield of onion seed crop

3.1.2.1 Effect of OYDV on shoot and seed stalk growth

The data on leaf length, leaf width, number of seedstalks per plant, seedstalk height and seedstalk diameter are shown in Table 1. The results indicate that the virus infection significantly affected all these measured components with the exception of number of seedstalks per plan. The leaf length and width were reduced by 53.56 and 60.91%, respectively, whereas the number of seedstalks per plant, seedstalk height and seed stalk diameter were reduced by 15.12, 50.83, and 51.1%, respectively.

3.1.2.2 Effect of OYDV on flowering and some yield components

Table 2 shows the results of the virus infection on umbel diameter, number of total florets per umbel, percent abortive florets per umbel, fertilization efficiency, number of seeds per umbel and 1000-seed weight. The analysis of data revealed that virus infection significantly affected the reproductive capacity of the onion plants. Virus infection reduced umbel diameter by 42.91% and number of total florets per umbel by 62.77%. The percent abortive florets per umbel was increased due to infection to *Ca*. 2.5 times as compared to that in non-infected plants while the fertilization efficiency was reduced by 38.9% in the infected plants. Consequently, the number of seeds per umbel was reduced to only about one-third in the virus-infected plants compared to that produced by non-infected plants. Further, the virus also caused more than 45% reduction in1000-seed weight.

3.1.3 The correlation between the observed and true incidence of natural infection with OYDV-D isolate

The virus incidence was assessed by visual symptoms on the growing crop late in the winter season; this estimate represented the observed incidence. The true virus incidence, however, was calculated by considering the percentage of infected seedlings arising from the bulbs harvested from the plants previously assessed for visual symptoms. Table 3 shows the results of observed and true virus incidence which indicates that the latter is consistently considerably higher than the former. The correlation between the observed level of virus infection (independent variable x) and the true level of virus infection (variable y) was computed. The correlation coefficient (r^2) was 0.67 and the regression of the true virus infection level on the observed was represented by the equation:

$$Y=38.7+1.26X$$

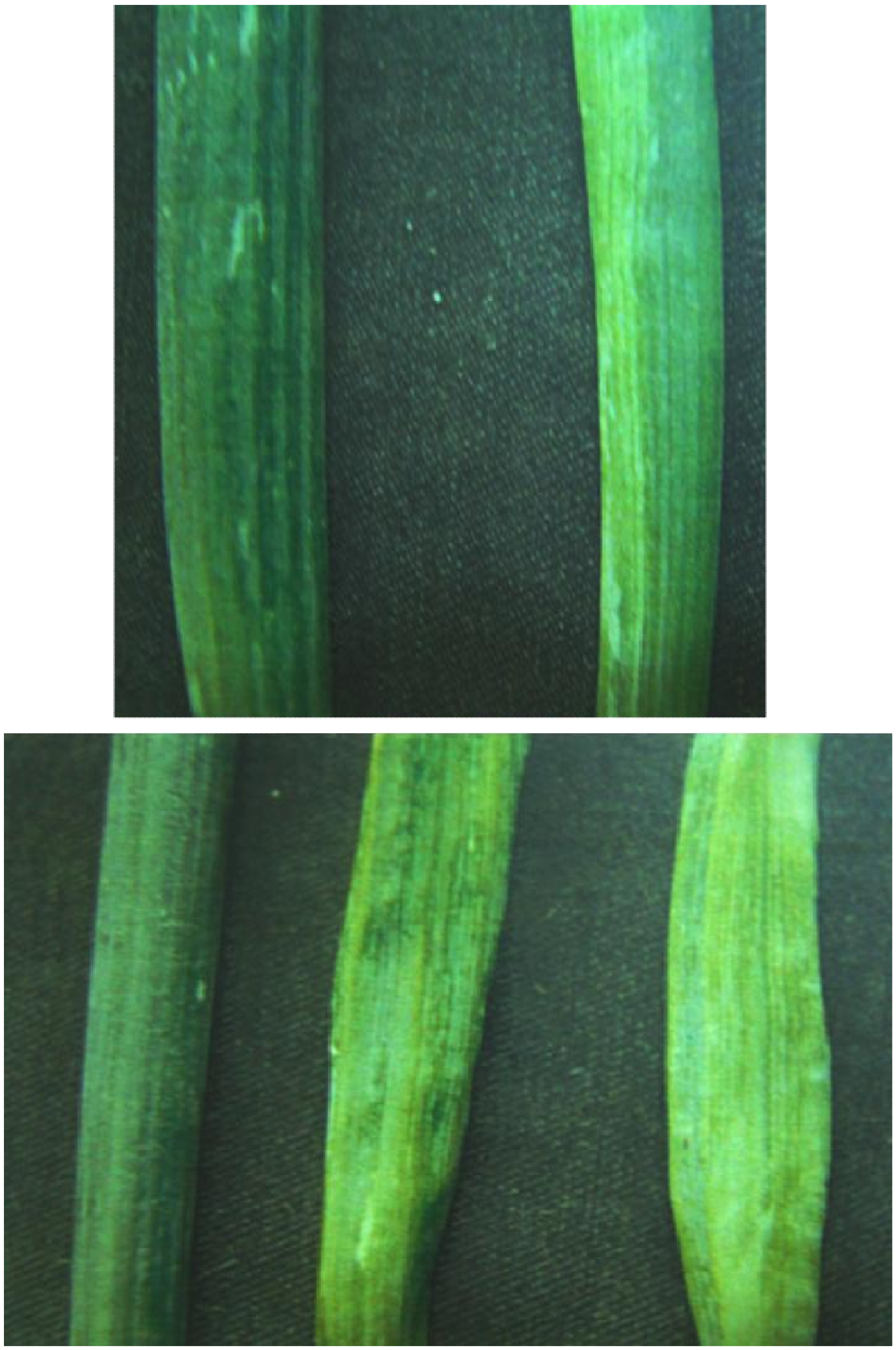

Fig. 1. Variations in OYD disease symptoms (A) Type I: Control (Healthy) (Left) & Showing streaking (bands), flaccidity and semi-flattening leaf (Right); (B) Type II: Control (Healthy) (Left) & Showing very thin yellow streaking, flaccidity and relatively tubular leaf form (Right).

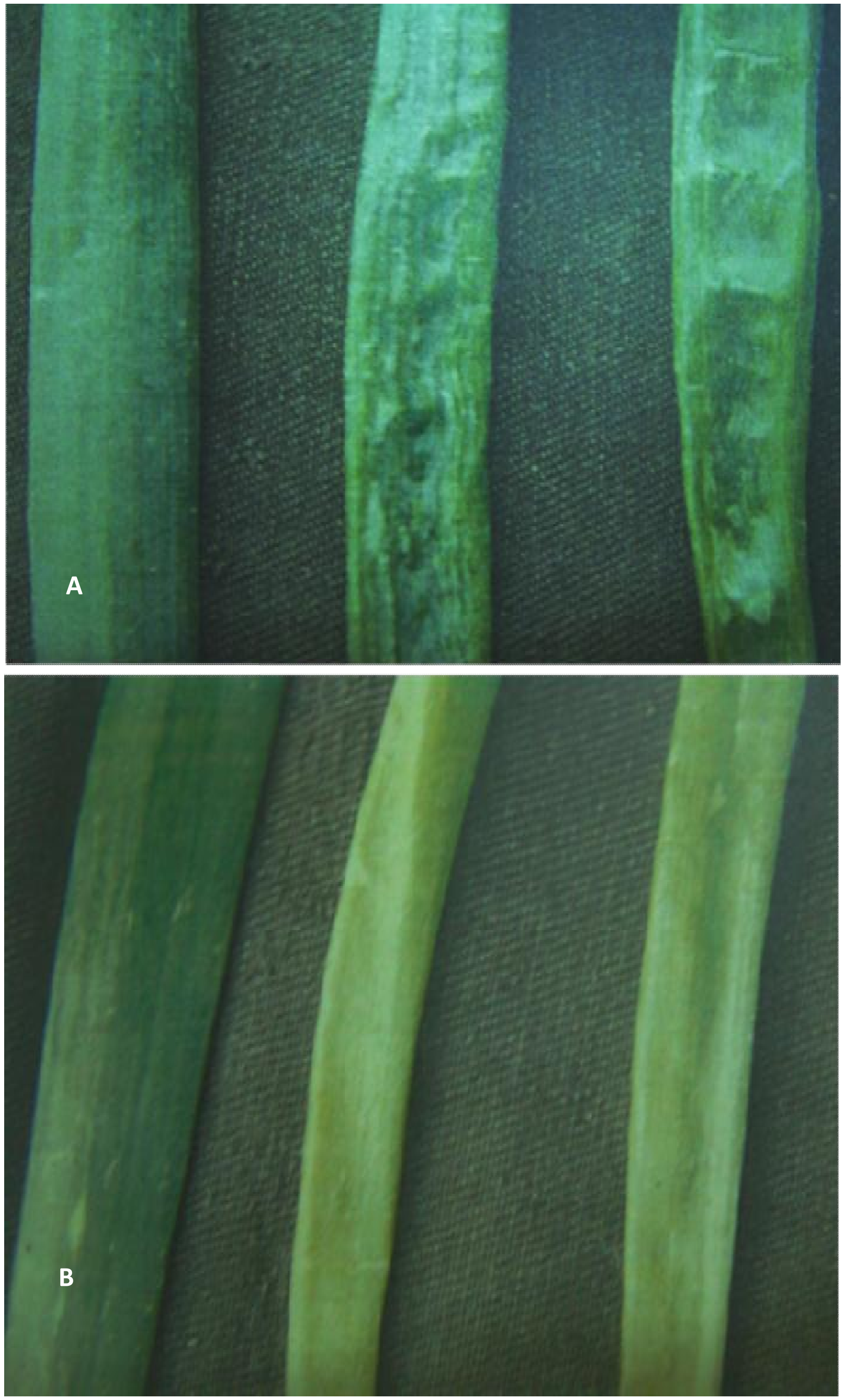

Fig. 2. Variations in OYD disease symptoms (A) Type III: Control (Healthy) (Left) & two sides of a leaf showing puckering pronounced streaking, flaccidity of leaves (Middle and Right); (B) Type IV: Control (Healthy) (Left) & the two sides of a leaf showing general yellowing, yellow streaking, flattening and reduced leaf size (Middle and Right).

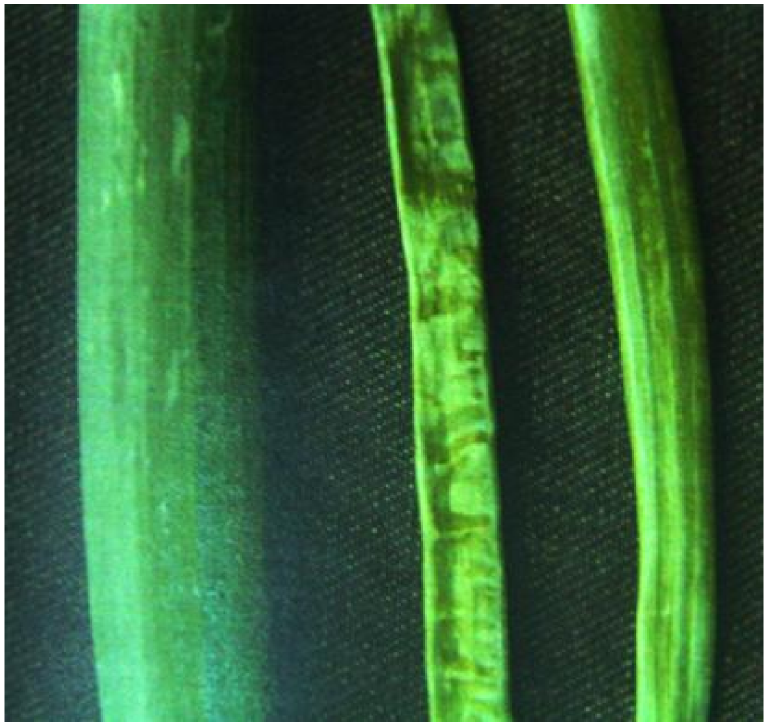

Fig. 3. Variations in OYD disease symptoms (Type V) showing Control (Healthy) (Left) & the two sides of a leaf showing thin yellow streaking (or bands), puckering, flattening and reduced leaf size (Middle and Right).

Table 1. Effect of OYDV natural infection on red onion shoot and seedstalk growth

Characters					
Leaf Length (cm)		**Leaf width (cm)**	**No. of Seedstalk/plant**	**Seedstalk Length (cm)**	**Seedstalk Diameter (cm)**
Healthy onion plant	58.74	2.20	8.6	93.62	2.25
Infected onion plant	27.28	0.86	7.3	46.03	1.10
% Change	-53.56	-60.91	- 15.12	- 50.83	- 51.10
5% LSD	4.526	0.272	3.24	10.27	0.291

- *The plant to plant method was used for comparison*
- *All figures are means of 60 onion plants*

Table 2. Effect of OYDV natural infection on flowering and seed yield

Characters						
	Umbel Diameter (cm)	**No. of Total Florets/Umbel**	**Abortive Florets /Umbel (%)**	**Fertilization Efficiency (%)**	**No. of Seeds/Umbel**	**1000-Seed Weight**
Healthy onion plant	8.11	940.30	14.84	68.20	2018.70	4.38
Infected onion plant	4.63	350.10	36.74	41.67	692.80	2.39
% Change	- 42.19	- 62.77	- 147.57	- 38.90	- 65.68	- 45.34
5% LSD	0.741	168.13	4.822	4.156	414.41	0.375

- *The plant to plant method was used for comparison*
- *All figures are means of 60 onion plants*

Table 3. The correlation between the observed and true disease incidence

Observed infection (%)	True infection (%)
1	39.58
10	66.17
15	68.15
20	70.31
30	75.51

3.2 Effect of Different OYDV Isolates on Bulb and Seed Yield

3.2.1 Effect of different OYDV on bulb yield and quality

The effect of the different OYDV isolates on bulb yield and quality was assessed using plant- to-plant method in the naturally infected fields at the respective locations Tables (4, 5).

3.2.1.1 Effect on bulb yield

The results assessed in two consecutive seasons demonstrated that healthy plants gave significantly higher bulb size and weight than OYDV-infected ones (Tables 4, 5). Some variations between the different virus isolates and between the seasons within the same virus isolates were also observed with respect to bulb diameter, bulb length and bulb weight. On the average, however, WR and WB isolates caused a similar percent reduction in the bulb diameter (25.4%) which was lower than the reduction due to G1 and D-isolates (30.67% and 31.1%, respectively). With respect to bulb length, the reductions were comparable and ranged between 14.25 to 17.92%. The percent reductions in the bulb weight were comparable for WR and WB (51.18% and 48.1%, respectively) and higher for G1and D-isolates which were comparable (56.31 and 58.55, respectively).

3.2.1.2 Effect on bulb quality

The different virus isolates were also found significantly reduce the total soluble solids (T.S.S) and dry matter content in both seasons as shown in Tables 4, 5. With respect to total soluble solids (T.S.S); all Khartoum isolates (WR, WB and D) produced on the average comparable effects (12.0-15.4), while the G1-isolate caused a considerably higher reduction (22.5%).

The dry matter content was more severely affected by the G1- and D-isolates (23.95% and 22.9%, respectively) in comparison with WR (15.5% reduction) and WB (14.6% reduction) (Figs. 4 and 5).

3.3 Effect of Different OYDV Isolates on Red Onion Seed Crop

3.3.1 Effect on growth

3.3.1.1 Leaf length (cm)

The results in Table 6 and Fig. 4 show that the different virus isolates significantly reduce the leaf length. The greatest effect was caused by D-isolate (51.3%) followed by GI (48%), WR (38.1%) and WB (33.6%).

3.3.1.2 Leaf width (cm)

The leaf width was significantly affected by the different virus isolates (Table 6 and Fig. 4). Higher reductions were caused by GI and D-isolates (58.82% and 50.23%, respectively) followed by WB (42.65%) and WR (39.88%).

3.3.1.3 Number of seedstalk per plant

The results in Table 6 indicate that none of the 4 isolates affected significantly the number of stalks per plant. However, clear reductions in the number of stalks were observed in plants infected with WR and D-isolates (14.4 and 33.9% reduction, respectively).

3.3.1.4 Seedstalk height (cm)

The data in Table 6indicate that infection with all OYDV isolates resulted in significant reductions in the height of the primary seedstalk compared to their respective controls. The reductions in seedstalk height were in the range of 30.3% to 42.4%, being greatest for D-isolate, followed by GI-isolate and least for WB-isolate.

3.3.2 Effect on flowering and some yield components (Fig. 5)

3.3.2.1Number of days to flowering (Anthesis)

The mean time to flowering was significantly affected by infection with the different OYDV isolates. Infection delayed the onset of flowering in the range of 7.65 to 12.45 days, being increasingly delayed in plants infected with D- and GI-isolates.

Table 4. Effect of four OYDV isolates on onion bulb yield and quality under field conditions (First season)

OYDV isolates	Bulb diameter (cm)		Bulb length (cm)		Bulb weight (g)		Total soluble solids (%)		Dry matter (%)	
	H	I	H	I	H	I	H	I	H	I
WR	6.98	5.26	5.26	5.04	113.66	67.97	16.40	13.00	18.40	15.24
% Change	- 22.20		- 4.18		- 40.19		- 17.70		-17.20	
WB	6.95	5.41	5.68	4.82	127.29	74.24	15.36	13.12	16.42	14.44
% Change	- 22.15		- 15.40		- 41.68		-14.60		-12.10	
GI	6.66	4.68	5.59	5.03	121.86	60.62	16.00	12.80	16.23	13.82
% Change	- 29.70		-10.02		-50.18		- 20.00		-14.80	
D	7.00	4.50	5.48	4.46	122.89	45.50	17.00	14.64	18.25	14.40
% Change	- 35.70		- 18.60		- 62.98		-13.88		-21.10	

- All treatments are significant (*P*=0.05)
- The plant to plant method was used for comparison
- All figures are means of 10 onion plants
- OYDV isolates are: WR= Wad Ramly; WB= Wad El basal; GI= Gezira Islanj; D=Damar
- H= Healthy, I=Infected

Table 5. Effect of four OYDV isolates on onion bulb yield and quality under field conditions (Second Season)

OYDV isolates	Bulb diameter (cm)		Bulb length (cm)		Bulb weight (g)		Total soluble solids (%)		Dry matter (%)	
	H	I	H	I	H	I	H	I	H	I
WR	6.09	4.92	6.42	4.82	149.76	56.67	15.28	13.28	15.20	13.96
% Change	- 28.70		- 24.40		- 54.52		- 13.10		-13.80	
WB	6.21	4.43	5.10	4.04	106.81	48.58	15.40	13.92	16.35	13.57
% Change	- 28.17		- 20.70		- 41.68		-9.60		-17.00	
GI	6.60	4.50	5.32	4.18	113.25	42.54	14.52	10.90	15.30	10.24
% Change	- 31.82		-21.43		- 62.44		- 25.00		-33.10	
D	6.72	4.94	5.24	4.72	121.96	55.96	16.44	13.92	16.47	12.41
% Change	- 26.49		- 9.90		- 54.12		-15.32		-24.70	

- All treatments are significant (*P*=0.05)
- The plant to plant method was used for comparison
- All figures are means of 10 onion plants
- OYDV isolates are: WR= Wad Ramly; WB= Wad El basal; GI= Gezira Islanj; D=Damar
- H= Healthy, I=Infected

Table 6. Effect of different OYDV isolates on red onion shoot and seedstalk growth

OYDV Isolates		Leaf length (cm)	Bulb Length (cm)	Bulb Weight (g)	Total Soluble Solids (%)	Dry Matter (%)
WR	H	54.35 a	1.78 b	10.40 a	95.80 a	2.24 b
	I	33.66 d	1.07 c	8,90 ab	59.50 bc	1.47 cd
WB	H	56.99 ab	2.11 a	8.50 a	96.35 a	2.35 ab
	I	37.58 c	1.21 c	8.35 ab	67.15 b	1.53 c
GI	H	55.97 ad	2.04 a	8.25 ab	90.10 a	2.24 b
	I	29.13 e	0.84 d	9.05 ab	58.95 c	1.23 d
D	H	58.49 a	2.17 a	6.55 bc	96.60 a	2.53 a
	I	28.48 e	1.08 c	4.40 bc	55.65 c	1.41d

- OYDV isolates are: WR= Wad Ramly; WB= Wad El basal; GI= Gezira Islanj; D=Damar
- H= Healthy I =Infected
- All treatments are significant (*P*=0.05)
- All figures are means of 20 onion plants
- Means followed by the same letter are not significantly different (P= 0.05) according to Duncan's Multiple Range Test.

Table 7. Effect of different OYDV isolates on flowering and some seed yield components of red onion

OYDV Isolates		No. of Days to Anthesis	Flowering Percentage	Umbel Diameter (cm)	Total no. of Florets/Umbel (%)	Percent Abortive Florets	No. of Seeds/Floret
WR	H	87.05 c	100.00 a	7.83 a	853.20 a	16.12 cd	3.25 a
	I	95.10 b	96.70 a	5,77 b	537.90 b	28.10 ab	2.72 d
WB	H	87.55 c	100.00 a	7.87 a	908.05 a	17.10 cd	3.09 b
	I	92.20 b	96.95 a	5.24 b	491.80 b	23.15 bc	3.05 bc
GI	H	89.45 c	100.00 a	7.89 a	920.70 a	17.42 cd	3.02 c
	I	101.60 a	88.75 b	5.26 b	467.20 b	27.18 ab	2.72 d
D	H	89.15 c	100.00 a	8.12 a	1002.15 a	13.05 d	3.12 b
	I	101.60 a	79.69 b	5.34 b	492.90 b	31.05 a	2.95 c

- OYDV isolates are: WR= Wad Ramly; WB= Wad El basal; GI= Gezira Islanj; D=Damar
- H= Healthy I =Infected
- All treatments are significant (*P*=0.05)
- All figures are means of 20 onion plants
- Means followed by the same letter are not significantly different (*P*= 0.05) according to Duncan's Multiple Range Test.

Table 8. Effect of different OYDV isolates on seed yield of red onion

OYDV Isolates		No. of Seed/Umbel	1000-Seed Weight (g)	Seed Yield/plant (g)	Computed Seed Yield/plant (ton/ha)	Seed Yield Loss (%)
WR	H	2272.0 b	3.69 b	47.36 a	1.35 a	
	I	1051.0 c	2.93 c	16.43 bc	0.469 b	65.26 B
WB	H	2326.0 ab	4.02 ab	48.12 a	1.33 a	
	I	1155.0 c	3.15 c	18.05 b	0.516 b	61.20 B
GI	H	2235.0 b	3.95 ab	43.99 a	1.26 a	71.75 B
	I	925.9 c	2.90 c	12.45 bc	0.356 bc	
D	H	2718 a	4.15 a	51.18 a	1.48 a	83.92 A
	I	1002.0 c	3.15 c	8.33 c	0.238	

- OYDV isolates are: WR= Wad Ramly; WB= Wad El basal; GI= Gezira Islanj; D=Damar
- H= Healthy I =Infected
- All treatments are significant (*P*=0.05)
- All figures are means of 20 onion plants
- Means followed by the same letter are not significantly different (*P*= 0.05) according to Duncan's Multiple Range Test.

3.3.2.2 Flowering percentage

With regard to flowering percentage, only GI-and D-isolate resulted in a significant effect, while the other virus isolates caused no detectable effect (Table 7). Reductions in flowering percentage were in the range of 3.1 to 20.3%, being greatest with D-isolate and least with WB-isolate.

3.3.2.3 Total number of florets per umbel

The total number of florets per umbel was significantly affected infection with any of the 4 isolates (Table 7 and Fig. 6). Reductions in the total number of florets per umbel was in the range of 36.95 to 50.82, being greatest with D-isolate, followed by GI- and WB-isolates and least for WR-isolate.

3.3.2.4 Umbel diameter (cm)

The size of the umbel was significantly affected by infection with any of the 4 isolates (Table 7) the size was reduced by nearly one-fourth to one-third of the control, being greatest with D, GI- and WB-isolate, respectively. The total number of florets per umbel followed the same trend (Table 7) and the reductions were in the range of 36.95 to 50.81%.

3.3.2.5 Percent abortive florets

The results in Table 7 demonstrate that the percent abortive florets was increased from 13.1-17.4% in the non-infected plants (control) to 23.2-31.1% in plants infected with the different OYDV isolates. The increase in the percent abortive florets was considerable. The same trend of the previous results was also maintained in this respect.

3.3.2.6 Number of seeds per florets

The average number of seeds per florets was significantly reduced in all infections except with WB-isolate (Table 7). The efficiency of fertilization was significantly interfered with WR- and GI-isolates compared to D-isolate.

3.3.3 Effect of onion seed yield

Table 8 displays the results on number of seeds per umbel, 1000-seed weight (g), seed yield per unit area (t/ha) and seed yield loss (%) as affected by the different OYDV isolates.

3.3.3.1 Number of seeds per umbel

The analysis of data revealed that all OYDV isolates under test caused significant reductions on the number of seeds per umbel. Although, there were no significant differences between these isolates. Yet some differences were clearly noticed (i.e. reductions of 50.3, 53,7, 58.6 and 63.13% by WB, WR, GI and D, respectively) (Table 8).

3.3.3.2 1000-seed weight

The weight of 1000-seed was found to be significantly affected by the 4 isolates. Again D- and GI-isolates showed higher reductions (24.1 and 26.6%, respectively) compared to WR-and WB-isolates but the differences were not significant.

3.3.3.3 Seed yield

The analysis of data showed that the different virus isolates reduced significantly the total seed yield per plants (Table 8 and Fig. 6). The greatest impact was noticed with D-isolate which was significantly different from WB-isolate but not from WR- and GI-isolate. Evidently, the overall effect of different OYDV isolates on the seed yield per unit area (t/ha) was significant. In this respect, the D-isolate had significantly more impact than the other isolates which were not significantly different from each other. The encountered seed yield loss caused by WB, WR,GI- and D-isolates was, respectively, 61.2, 65.26, 71.75, and 83.69%.

3.3.4 Effect on progeny seed germination and seedling vigour

Data on germination and seedling vigour as measured by germination percentage in Petri-dishes and in soil and seedling fresh and dry weights are shown in Table 8. The viability of the progeny seed was significantly affected by the different OYDV-isolates, whether it was assessed in the Petri-dishes or soil. GI-isolate caused the greatest loss in seed viability followed by D- and WR-isolates as assessed in the Petri-dishes. When assessed in soil, the same trend followed except that WR-isolate caused slightly less effect than WB-isolate. The reduction in seed viability caused by WB, WR, D- and GI-isolates in the Petri-dishes and in soil were (9.3%, 20.5%), (16.4%, 19%), (17%, 26.5%) and (20.3%, 31.75%), respectively.

The seedling vigour seemed to be slightly reduced by the infection of the mother plants as it was noticed from the fresh weight assessment. However, dry weights indicated significant effects of the different OYDV-isolates over the controls (Fig. 7). The greatest effect was caused by D-isolate (40.25%) followed by GI-isolate (37.7%), and WB-isolate (27.4%).

4. DISCUSSION

The results of the present study demonstrate the drastic effect of onion yellow dwarf potyvirus (OYDV) on production of both seed and bulb crops and indicate the existence of variability in Sudanese OYDV.

The various types of disease symptoms observed during the survey could not be associated with specific isolate since similar pattern of symptoms might exist in areas where each of the different isolates predominates. The variation in symptoms is, however, conceivable because of the possible existence of different variants in the virus population and also may be due to the suspected impurity of the local varieties of onion commercially grown in Sudan.

The disparity between the observed and true disease incidence recorded in this study supports the previous observation [19] that the latter is usually notably higher than the former. It is evident from the results that the observed and true disease incidence values are positively correlated (r^2=0.63). The analysis of accumulated data collected from various onion-growing sections over a considerable period of time is expected to provide epidemiologists with crucial information about the quantity and distribution of the initial inoculum that may be very useful in disease forecasts. The lower values of observed diseased incidence compared to true incidence reflect impairment of clear expression of symptoms in the current season when relying on visual symptoms alone [19]. This could be due to a very low virus titre in the plant tissue which most likely could be attributed to infection of relatively old plants in the field [29]. Alternatively, there could be a change in the climatic conditions during the season that causes masking of symptoms or produces very mild reactions that can be easily overlooked. Generally, several workers [19, 30, 31, 22] have concluded from numerous observations and experiments that plant age is a limiting factor behind any successful virus infection, and that younger onion plants are more vulnerable to infection than older ones.

In Sudan, both high and low levels of infection have been reported either in onion plants grown from bulbs (seed crops) saved by farmers from the previous season's crop(55%-99%) or in those grown from true seed (8%-38%) [22,23,12,4]. The difference between investigators might be due to virus strain, host cultivar, micro-environmental conditions or the presence of a latent virus [19].

The data revealed the drastic and deleterious effect of the four OYDV isolates on bulb yield and on growth and yield components of onion seed crop [12, 4]. The reductions in bulb weight and bulb quality were significant and varied according to the virus isolate and season of production (17, 32). While the first season reductions in bulb size and weight were relatively greater for the D-isolate, they were relatively so for Khartoum isolates (WR, WB and GI) in the second season. With respect to the bulb quality, the results appeared to be rather erratic due to the strong correlation between bulb size and bulb quality [33].

Fig. 4. General symptoms of OYD disease showing streaking, puckering, drooping and flattening of leaves.

Fig. 5. General symptoms of OYD disease showing streaking, puckering, drooping, flattening of leaves and pronounced stunting compared to apparently healthy plant (left).

Fig. 6. Effect of OYD disease on seedstalk and umbel diameter showing Control (Healthy) (Right); Showing straight seedstalk and large sized umbel full of fertilized florets (Left) & The remaining seedstalks show a range of malformed, small-sized umbels with delayed Anthesis.

Fig. 7. Effect of OYD infection (WR-isolate) on onion seed harvested from a single onion plant showing Control (healthy) (Left) & Seed harvested from a naturally infected plant (Right).

The variations due to variability in virus are conceivable, but the seasonal variations could be attributed to a variety of ecological and epidemiological factors. These may include inoculum density, aphid vectors distributions and efficiency and cropping pattern practices at different localities. The significantly high reductions in bulb weight recorded (40.2 to 63.0%) agree with the findings of several investigators [34-36]. The low storability of OYDV-infected bulbs observed by Bremer [34] has also been noticed in this study but no data have been collected. However, the reductions recorded on the bulb quality are interesting and confirm the previous findings by Morgan [22] in Sudan. In this regard, the data recorded in the present study and those reported by Morgan [22] appear to be the only available information.

The effect of OYDV infection on the seed crop is even more drastic. This was clearly demonstrated by: a) the pronounced stunting of the infected whole plant (>50% reductions in leaf and seedstalk growth) b) the significantly delayed anthesis (about a week to 12days), c) the substantially reduced reproductive potential of the infected plants which might not exceed a 16 to 39%; and d) the significantly poor quality of the progeny seed in terms of 1000-seed weight, germinability and vigour of emerging seedlings. Although the general trend among four isolates was similar exhibiting severe influence on all characters tested, yet the D-isolate appeared to have the greatest impact followed by G-isolate then WR and WB which seems to be comparable. Severe seed yield reductions that have resulted in serious losses have been reported in various countries by many workers [37-39,29,22,40,4]. Also, the data on quality characteristic of seed produced by OYDV infected onion plants reported in this study seem to be in accord with those reported by several investigators [41,29,22,4]. However, Rudolph [40] claimed that he could not observe any significant diminishing effect on seed viability. The disparity could be attributed to the aggressiveness of the virus isolate or strain, host cultivar and the development stage of the plant when it was infected [19].

The differences in severity of infection between the four isolates could be ascribed to: firstly, inherent pathogenic differences among the isolate; secondly, environmental conditions; thirdly, cropping procedures followed in each of the areas surveyed such as monocropping and overlapping of onion crops practiced in Gezira Islanj and to some extend in El Damar area. While only one crop of onion is cultivated in Wad Ramly and Wad El basal area as a late winter crop. Lastly, due to certain epidemiological considerations, the planting material (stored bulb) may carry high inoculum load which may be more deleterious than the small quantity of inoculum. The longer winter season in El Dammar area seems to possess more favourable factors for early establishment of infection in the different onion crops.

5. CONCLUSION

In conclusion, few perspectives emerge from this study. Firstly, the positive correlation between the true versus observed disease incidence has important epidemiological implication. This aspect can be utilized efficiently in disease forecasts and formulation of effective disease management programs. Secondly, the existence of variability in OYDV has been demonstrated; partly by symptoms variations observed in the field and by different effects on different crops of the common bulbing onion. This can be used, after further confirmation, as differential for local OYDV isolates. Thirdly, very drastic effects of OYDV on growth and yield of seed and bulb onion crops have been evident.

AKNOWLEDGEMENTS

The authors dedicate this research work to the soul of their late colleague Maha F. M. A. Gabbani, asking God forgiveness and mercy for her.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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