

## EVALUATION OF SANITARY STATUS OF GRAPEVINES IN EGYPT

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### ABSTRACT

Incidence and distribution of viruses and virus diseases on grapevines (*Vitis vinifera*.) in different locations in Egypt were determined during March and April 2005 and 2006. Surveys for viruses were carried out at Grapevine areas in which international (imported) and local grapevine (native) cultivars and rootstocks are grown. A total of 446 symptomatic and 1896 a symptomatic leaf samples were collected from 19 different cultivars (native, imported and rootstock as well) were tested by enzyme-linked immunosorbent assay- double antibody Sandwich (DAS-ELISA) for the most commonly viruses found in grapevine trees: *Grapevine Fanleaf Virus* (GFLV), *Grapevine Fleck Virus* (GFKV), *Grapevine Virus A* (GVA), *Grapevine Leafroll associated Virus -1* (GLRaV-1), *Grapevine Leafroll associated Virus -3* (GLRaV-3), *Tomato Ring Spot Virus* (ToRSV) and *Peach Rosette Mosaic Virus* (PeRMV). GFLV, GFKV, GVA, GLRaV-1, GLRaV-3, ToRSV and PeRMV were found to be widely spread in grapevine propagated material and are considered as economically important grapevine viruses in Egypt. GLRaV-3 was the most widespread virus with (29.5 %) infection followed by GFLV (16 %) infection, GVA (15.9 %), GFKV (13.3%), GLRaV-1 (9.5 %) infection, then the infection rate of PeRMV 4.2 % in descending order. No viral infection was observed with ToRSV in imported and rootstock cultivars. The infection rate of imported grapevine cultivars and rootstocks were 5.7 % and 0.42 % respectively, while the infection rate of native cultivars was 36.02 %. 'Romy Ahmer' and 'Banaty Abiad', the two major Egyptian cultivars, recorded infection levels of 23.78% and 28.48%, respectively, Bez El-Anza showed 40.42% infection and Siwi Aswad recorded 45.22%

**Keywords:** Grapevine viruses, field survey, ELISA, virus testing.

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### INTRODUCTION

Grapevines are grown in Egypt in about 65000 Hectare which yielded 1.104.000 Tones. Part of the crop is consumed locally as a fresh product and the rest is processed and exported (\* FAO, statistical data). The most important governorates cultivated grapes are Behera, Kalubia, Munifia, Giza, Fayoum and Beni-Swef. Table-grape cultivars are by far the most widely grown, with a prevalence of the traditional local cultivars 'Banaty Abiad' and 'Romy Ahmer'.

In addition, a significant introduction of foreign cultivars mainly seedless (e.g. cvs. 'Flame', 'Superior', 'King's Ruby', 'Fantasy', etc.), have taken place in recent years (Ahmed et al 2004). More than 55 viruses or strains classified in 20 different genera are known to infect grapevine crops world wide (Martelli, 1993) and several substantially reduce yield and quality (Pearson and Goheen, 1988). *Grapevine Fanleaf Virus* (GFLV), *Arabis Mosaic Virus* (ArMV), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine Fleck Virus* (GFKV), GLRaV-1 and GLRaV-3 were detected in Czech propagation material of grapevine and are considered as

economically important grapevine viruses in the Czech Republic. ArMV and GLRaV-1 were found the most frequently viruses than 10% of examined vines. Small number of vines was found to be infected with GVA and GVB (Kominek and Holleinova, 2003). More than 30% of tested vines were positive to be at least one of five of the tested viruses. In Austeria, most spread was GLRaV-1 and GLRaV-3 (Flak and Gangl, 1994). However, about 15 viruses were recorded in grapevines in Hungary (Lehoczky *et al* 1992). *Arabis Mosaic Virus* (ArMV), *Tomato Ring Spot Virus* (ToRSV), and GFLV. GLRaV-1, -2, and -3 were detected in Washington and in Oregon States of USA (Martin *et al* 2005). GFLV, GFkV, GVA, ArMV, GLRaV-3, *Raspberry Ring Spot Virus* (RpRSV) and *Tobacco Ringspot Virus* (TRSV) were found in almost all Iranian vineyards examined (Rakhshandehroo, *et al* 2005).

The oldest known virus disease of *Vitis vinifera* caused by *Grapevine Fanleaf Virus* (GFLV) is fan leaf degeneration, which causes poor berry set and a yield loss, which can exceed 80% in some varieties.

This *Nepovirus* can infect almost all *Vitis* species (Paski *et al* 1983). Phloem restricted viruses of other virus genera are also known to infect grapevines.

They contribute to the aetiology of leafroll, rugose wood and fleck diseases that are widely spread in the Mediterranean and Near East regions (Digiario *et al* 2000). *Grapevine Leafroll associated Virus-3* (GLRaV-3) is the most widespread and economically important closterovirus and efficiently transmitted by some mealybug species (Habibi *et al* 1995; Peterson and Charles, 1997). Knowledge of incidence and distribution of grapevine viruses is crucial in developing sound diagnostic systems and appropriate control measures (Frison and Ikin, 1991). The sanitary status of Egyptian viticulture is little known, records referring to symptoms of leaf roll, rugose wood, and fan leaf observed in the field, mainly on vines of foreign origin (Martelli, 1988). Serology was the first method adopted in the evolution of rapid plant pathogen detection and identification (Clark and Adams 1977). This technique is based on the recognition of antigens with antibodies produced to them. In its initial application by plant virologists, serology had been used routinely to identify virus species and strains but was not amenable to high throughput assays. The enzyme-linked immunosorbent assay (ELISA) (Converse and Martin, 1990, Crowther, 2001) is based on a nearly decade earlier demonstration by Avrameas that glutaraldehyde cross-linked enzyme-antibody conjugates retained both the specificity of the Immunoglobulin G (IgG) molecule and the catalytic properties of the enzyme. ELISA allows qualitative and quantitative analysis, high throughput, and high sensitivity and was adopted rapidly and widely (Rowhani and Falk, 1995). ELISA has been developed for most of the economically important and widespread viruses characterized in grapevine (Boscia *et al* 1992).

This study was undertaken to determine the incidence and distribution of most wide spread viruses. External symptoms and serodiagnosis were carried out on *Grapevine Fanleaf Virus* (GFLV), *Grapevine Leafroll associated Virus-1* and 3 (GLRaV-1 and 3), *Grapevine Fleck Virus* (GFkV), *Grapevine Virus A* (GVA), *Peach Rosette Mosaic Virus* (PeRMV) and *Tobacco Ring Spot Virus* (TRSV) in Egypt during March and April (2005 and 2006).

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\* Website (FAO, statistical data 2004).

## MATERIALS AND METHODS

### Field surveys

In order to evaluate the phytosanitary status of Egyptian grapevines and frequencies of viruses and incidence diseases of Grapevine, a prospective study was aimed for survey was conducted during 2005 and 2006 covering significantly large geographical areas including different governorates. Samples collected during the two successive seasons from 6 governorates i.e., Behera (Nubaria), Kalubia, Minofia, Giza, Fayoum and Bani-Sweef, were consisted of mature canes from plants with symptoms, that suspected to be viral infections, and also from symptomless plants. The most frequent symptoms were a yellowish, mosaic and downwards rolling of the leaves, a poor coloration of the berry, low production, and a decline of the whole plant. For each sample, four leaves were collected, labeled, wrapped in plastic and stored at 4 °C until used for laboratory analysis.

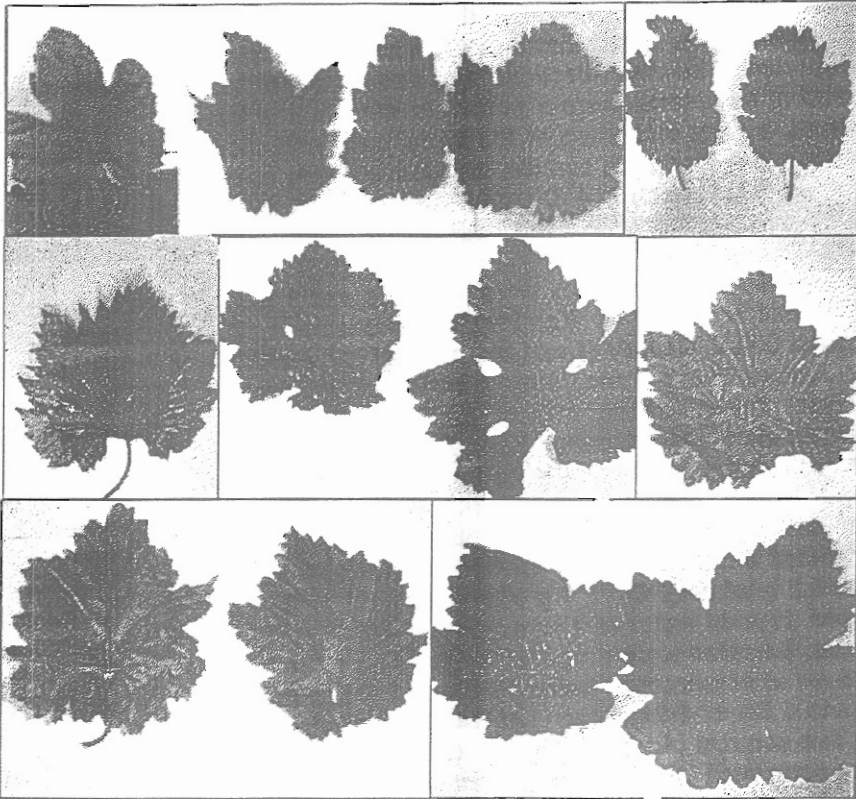
### DAS-ELISA

Grapevine viruses were detected using ELISA (Clark and Adams, 1977 and modified by Kominek and Holleinova, 2003). Commercial kits against GFLV, GLRaV (1 and 3), GVA, GFKV, PeRMV and ToRSV produced by Agritest, Valenzano, Italy were used in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) method according to instructions of manufacturer. Nunc Polysorp immunomicroplates were coated with Immunoglobulin G (IgG) to individual viruses diluted 1:1000 in coating buffer (1.59 g of Na<sub>2</sub>CO<sub>3</sub>, 2.93 g of NaHCO<sub>3</sub> and 0.2 g of NaN<sub>3</sub>, dilute to 1 L. with distilled water, pH 9.6). Reaction volume was 200 µl. Plates were incubated 4 hours at 37°C, washed 3 times with PBS (8 g of NaCl, 0.2 g of KCl, 0.2 g of KH<sub>2</sub>PO<sub>4</sub>, 2.9 g of Na<sub>2</sub>HPO<sub>4</sub>.12 H<sub>2</sub>O, 0.2 g of NaN<sub>3</sub>, 0.5 ml of Tween 20, add water to 1 L, adjust pH = 7.4) and samples (antigens) were added. Samples were prepared by grinding 0.5 g of leaves in 7.5 ml (ratio 1:15, w: v) of extraction buffer PBS with 2% of polyvinylpyrrolidone (PVP) K-40 and 0.2% of BSA, adjust pH 7.4. Commercially purchased negative and positive controls (Agritest, Italy) to individual viruses were used. All samples were performed in two wells.

Plates were incubated overnight at 4°C, then washed 3 times and added alkaline phosphatase conjugated antibodies to individual viruses diluted 1:1000 in extraction buffer. Plates were incubated for 4 hours at 37°C. All plates were then washed 3 times with PBS and added substrate buffer (97 ml of diethanolamine, 0.2 g of NaN<sub>3</sub>, adjust pH to 9.6 with HCl, dilute with distilled water to 1 L.) with 10 mg/ml of p-nitrophenylphosphate. Absorbances at 405 nm were measured in a BioTex-Elx808, BioTex, Highland Park, Winooski, VT, USA automatic reader that zeroed with an empty plate. After two hours, positive samples were considered when the mean of absorbance was at least two standard deviation units above the negative control (Clark and Adams 1977). Controls were included systematically and each sample was loaded in two different wells.

## RESULTS AND DISCUSSION

The most frequent symptoms of grapevine virus diseases collected from grapevine fields in different location in Egypt during March and April 2005-2006 in which native, imported and rootstocks grapevine tree cultivars were illustrated in (Fig.1) which appeared as a yellowish, mosaic and downwards rolling of the leaves, deformation and twisting on the leaves in addition to poor coloration of the leaves.



**Fig. 1: Different selected shapes of grapevine symptoms collected from native, imported and rootstocks grapevine fields in different locations in Egypt during March and April 2005, in which yellowish, mosaic downwards rolling, deformation and twisting leaves were recorded.**

All samples collected from different locations were analyzed through DAS-ELISA to detect GFLV, GVA, GFKV, GLRaV-1, GLRaV-3, ToRSV and PeRMV . By using the DAS-ELISA, virus infected grapevine were found in native and imported grapevine cultivars and rootstocks and in all investigated regions in Egypt (Table 1). With reference to the percentage of virus infection in different area, the distribution of virus infection was particularly high,

reached 29.5 % infection with GLRaV-3 followed by 16 % infection with GFLV, 15.9% infection with GVA, 13.3 % infection with GFKV, 9.5% infection with GLRaV-1 and 4.2 % infection with PeRMV, No virus infection were observed with ToRSV. The high infection levels were observed in Bani Sweef followed by Kalubia and Fayoum and there no virus infections were recorded in Minofia, Behera (Nubaria) and Giza.

**Table 1: Occurrence of viruses in imported grapevine propagated material and rootstocks**

Location	Sample tested	infected Samples	Percentage of samples reacted positively with each antiserum in DAS-ELISA*						
			GFLV	GVA	GFKV	GLRaV-1	GLRaV-3	ToRSV	PeRMV
Behera(Nubaria)	310	35	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kalubia	90	35	5.6	0.0	13.3	6.7	13.3	0.0	0.0
Minofia	60	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Giza	240	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fayoum	142	9	1.4	0.7	0.0	0.0	0.0	0.0	4.2
Bani Sweef	420	210	9	15.2	0.0	9.5	16.2	0.0	0.0
Total	1262	254	16	15.9	13.3	9.5	29.5	0.0	4.2

\*DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay).

*Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA),Grapevine fleck virus(GFKV), Grapevine leafroll associated virus -1 (GLRaV-1), Grapevine leafroll associated Virus -3 (GLRaV-3) , Tomato Ring Spot Virus (ToRSV) and Peach Rosette Mosaic Virus (PeRMV).*

Results in (Table 2) recorded that, in all samples tested the percentage of virus infection in grapevine cultivars and rootstocks analyzed by ELISA. GVA had the greatest infection levels (59.0 %), then GLRaV-1 and GLRaV-3 which recorded (55.6%), ToRSV (33.3%), PeRMV (20 %), followed by GFLV (14.8%), GFKV (14.8%) in native cultivars, while in the imported cultivars, GVA and GFKV were the dominant where recorded 9.7 % and 11.9 % respectively. GLRaV-1, GLRaV-3 and GFLV (6.5 %, 5.8 % and 4.9 % respectively) followed by PeRMV 1.1 % . No infections were observed with ToRSV. The sanitary condition of rootstocks was quite different, 192 samples tested from 7 different hybrids and species, PeRMV recorded (3.1%) of infection and GLRaV-3 was (2.1%). The number of rootstock samples tested was relatively low and it is interesting to note that some important viruses, like GFLV, GLRaV-1, GVA, ToRSV and GFKV, were totally absent.

The occurrence of grapevine viruses in native and imported grapevine varieties and rootstocks in Egypt using antisera specific to GFLV, GVA, GFKV, GLRaV-1, GLRaV-3, ToRSV and PeRMV revealed that the detection of viruses in grapevine was very reliable, even though the percentage of infected trees was low.

The obtained data presented in (Table 3) and illustrated in (Fig. 2), indicated that the imported (Superior Seedless, Thompson and Flame), and rootstock cultivars (Harmony, Freedom, Doge Ridge, Cabernete, LN33, ST. George and SO4) had average infection rates of 54.04 % of 1262 tested samples, while the native cultivars had average infection rates of 36.02 % of 1080 tested samples.

**Table 2: Percentage of virus infection in Egyptian grapevine cultivars and rootstocks**

Viruses	<i>Vitis</i> spp.					
	Native (1080 samples)		Imported (1070 samples)		Rootstock (192 samples)	
	Infected samples	%	Infected samples	%	Infected samples	%
GFLV	160	14.8	26	4.9	0	0
GVA	640	59.0	52	9.7	0	0
GFKV	160	14.8	64	11.9	0	0
GLRvA-1	600	55.6	35	6.5	0	0
GLRvA-3	600	55.6	31	5.8	4	2.1
ToRSV	360	33.3	0	0	0	0
PeRMV	216	20	12	1.1	6	3.1
Mean	36.02		5.7		0.42	

Also, data presented in Table 3 indicated that Romy 'Ahmer' and Banaty 'Abiad', the two major native cultivars, had average infection rates of 23.78 and 28.48, Bez El-Anza had 40.42% infection and Siwi Aswad had 45.22%. The most affected table grapes were Superior Seedless followed by Thompson and Flame cultivar, the average infection rates recorded 20.48, 19.92 and 12.9 respectively, the number of rootstock samples tested was relatively low, it is interesting to note that some important viruses, like GFLV, GLRaV-2 and GFKV, were totally absent.

The sanitary status of native Egyptian cultivars was poorer than that of imported cultivars for about 86% of 467 local vines tested were infected by at least with single virus, while the mixed infections by two or more viruses (57%) prevailing over single infections (29%) (Ahmed *et al* 2004).

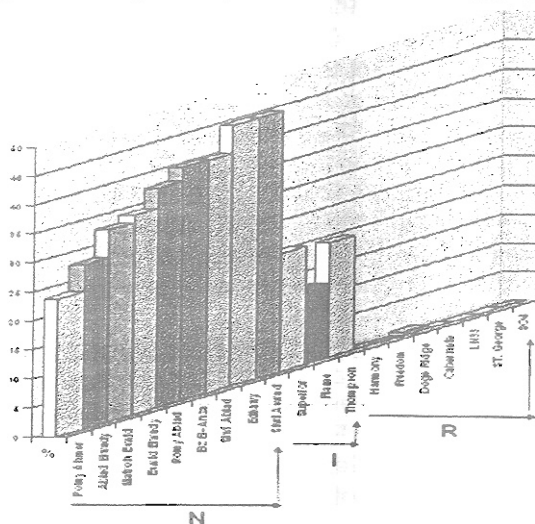
Finally, The results of field surveys and laboratory assays clearly show that the Egyptian grapevine industry does not enjoy a better sanitary condition than similar industries in other Mediterranean countries (Digiario *et al* 2000 and Ahmed *et al* 2004) and the sanitary status of Egyptian viticulture is little known records referring to symptoms of leafroll, rugose wood and fanleaf observed in the field, mainly on vines of foreign origin (Martelli, 1988), and to occasional recovery of the *Nepovirus* grapevine fanleaf virus (GFLV) from symptomatic vines (Tolba and El-Kady, 1991).

However, a number of unusual features distinguish the Egyptian situation from that recorded elsewhere in the region: (1) the apparently total absence of GFLV and the exceedingly low incidence of GFKV; (2) the very high incidence of GVA and GLRaV-3; (3) the low field incidence of leafroll symptoms and the apparent absence of rugose wood symptoms, notwithstanding the widespread distribution of some of the causal agent of these diseases (GVA and GLRaV-3) (Ahmed *et al* 2004). This study has provided a backdrop against which the direction of virus control can be more efficiently developed in Egypt and certification of planting material under state control is needed. A greatly expanded program to provide elite, virus-free propagation materials to registered nurseries in Egypt.

**Table 3: Occurrence of grapevine viruses in native, imported and rootstocks grapevine cultivars in Egypt, using antisera specific for GFLV, GVA, GFKV, GLRaV-1, GLRaV-3, ToRSV and PeRMV.**

Viruses Cultivars	Occurrence of grapevine viruses in native, imported and rootstocks*							Total frequencies
	GFLV	GVA	GFKV	GLRaV-1	GLRaV-3	ToRSV	PeRMV	
<b>Native cultivars</b>	0	16.6	0	33.2	0	50	66.7	23.78
Romy Ahmer								
Banaty Abiad	50	16.6	33.2	66.4	16.6	16.6	0	28.48
Matroh Eswid	50	50	16.6	50	16.6	50	0	33.31
Eswid Elwady	33	66.4	0	33.2	16.6	10	83.4	34.65
Romy Abiad	50	83.4	0	66.4	50	0	16.6	38.05
Bz El-Anza	50	66.6	0	66.4	83.4	16.6	0	40.42
Siwi Abiad	33.3	100	0	66.4	50	33.3	0	40.42
Edkawy	66.4	50	66.7	66.4	50	16.6	0	45.15
Siwi Aswad	50	83.4	16.6	50	100	16.6	0	45.22
<b>Imported cultivars</b>	20	1.5	60	24.6	15.4	0	21.9	20.48
Superior								
Flam	7.9	5.3	31.6	24.3	21.2	0	27	12.9
Thompson	27.3	0	72.7	27.3	12.2	0	0	19.92
<b>Rootstock cultivars</b>	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.07
Harmony								
Freedom	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Doge Ridge	0.0	0.0	0.00	0.0	2.1	0	2.1	0.60
Cabernete	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.07
LN33	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.00
ST. George	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
SO4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00

\*Percentage of samples reacted positively with each antiserum in DAS-ELISA



**Fig. 2: Incidence of virus infections in different native (N) imported (I) and rootstock (R) of grapevine cultivars in Egypt.**

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### تقييم للحالة الصحية لمحصول العنب في مصر

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قسم بحوث الفيروس والفيتوبلازما - معهد بحوث أمراض النباتات - مركز البحوث الزراعية

يعتبر العنب من أهم محاصيل الفاكهة في مصر حيث يزرع منه في العديد من الأصناف في مساحات واسعة في مختلف المحافظات. ونظرا لتعرضه للأصابة بالعديد من الأمراض الفيروسية فلقد أجريت هذه الدراسة لتحديد مدى وجود وانتشار هذه الفيروسات خلال موسم ٢٠٠٦/٢٠٠٥ وذلك في محافظات اللقنوبية والمنوفية والجيزة والنوبارية وبنى سويف والفيوم. ولقد اشتملت الدراسة على فحص عدد ٢٣٤٢ عينة بها أعراض شبيهة بالأعراض الفيروسية على أوراق شجيرات العنب بالإضافة الى عدد ١٨٩٦ عينة كانت سليمة ظاهريا دون ظهور أى أعراض عليها. تم إجراء الفحص باستخدام طريقة الأليزا في وجود الأجسام المضادة لأهم ٧ فيروسات من الفيروسات التي تصيب العنب. وقد أظهرت نتائج الفحص وجود فيروس التفاف الأوراق السلالة ٣- بنسبة ٢٩,٥% يليه فيروس الورقة المروحية في العنب حيث كانت نسبة الأصابة ١٦% ثم فيروس A العنب وفيروس ال GFKV وفيروس التفاف الأوراق السلالة ١- وفيروس التورد والموزايك في الخوخ حيث كانت نسبة الأصابة هي ١٥,٩% و ١٣,٣% و ٩,٥% و ٤,٢% على التوالي. كما أتضح من نتائج الفحص بأنه لا يوجد اي أصابات في العنب بفيروس التفاف الحلقي في الطماطم خاصة في الاصناف المستوردة والأصول . وقد وجد أن نسبة المنوية للأصابة في الأصناف المحلية كانت ٣٦,٠٢%. وكان صنفى الرومى الأحمر والبنساتى الأبيض هما أكثر الأصناف المحلية أصابة حيث بلغت نسبة الأصابة فيهما ٢٣,٧٨% و ٢٨,٤٨% على التوالي . في حين أن صنف بز العنزة فكانت النسبة المنوية للأصابة ٤٠,٤٤% وصنف الميسوى أسود كانت ٤٥,٢٢%.