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# Influence of Tomato Black Ring Virus (TBRV) on Sugar Beet (*Beta vulgaris* L.) Yield and Quality

**Hanaa M.M. Hassan; M.R. Abdel-Latif and El-S. Abdou**

*Plant Pathol. Dept., Fac. Agric., Minia Univ., Minia, Egypt.*

Severe ring spots, mosaic, and stunted growth were observed on plantations of sugar beet in Minia governorate. This study revealed that the causal agent is a strain of tomato black ring virus (TBRV). This strain was tested to infect 44 plant species, cultivars, and varieties (belonging to 11 families). The virus was transmitted mechanically to all tested plants. Virus activity was lost in infectious sap exposed to 55°C for 10 min. It withstood a dilution of $10^{-4}$ and remained active for 7 days. The partially purified preparation of isolated virus gave one line of precipitate in gel-diffusion test and reacted positively in DAS-ELISA with OD 2.4 while the beet varieties, obtained OD ranged between 1.0 to 2.0. The infected Gazilla and Raspoly beets showed the highest reduction in shoot yield (35%), while Montpianko showed the lowest reduction in root yield (23%). The virus infection had less effect on sugar content in Montpianko and Oskarpoly varieties.

**Keywords:** Host range, ELISA, serology, sugar beet and tomato black ring virus.

Sugar beet (*Beta vulgaris* L.) was introduced into Egypt in the 1970s, but its growing, for sugar production on a large scale had begun in 1982 (Maareg *et al.*, 1998). Now it ranks as the second most important sugar crop after sugarcane in Egypt, as it is cultivated in more than 63 thousand hectares with an average production of 50 tons/h (Korayem, 2006)

During the last years, many pathological problems have been raised in sugar beet plantations (Srivastava, 2004). It was attacked by many phytopathogenic agents that catalogued to viruses, bacteria or fungi (Benada *et al.*, 1987). Sugar beet has been subjected to many virus diseases, *i.e.* beet mosaic virus (Nemchinov *et al.*, 2004), beet ring mottle virus (Zhang *et al.*, 1998), tomato chocolate virus (Verbeek *et al.*, 2010), beet western yellows virus (Xiang *et al.*, 2010) and beet necrotic yellow vein virus (Guilley *et al.*, 2009). Tomato black ring virus (TBRV), described by Smith (1946) for the first time, causes great economic losses in root yields and sugar beet content. This isolated and identified virus was belonging to the *Nepovirus*, an important genus of Comoviridae (Pringle, 1998).

During the growing season of 2006 a severe ring spots, curling, mosaic and stunted growth were observed on sugar beet in open field in Minia. Therefore, this work was undertaken to isolate and identify the causal agent(s), through symptomatology, host range, physical properties, partial purification, and serological tests of the causal virus. Also six imported varieties were used to study the effect of the virus on yields and sugar contents.

## Materials and Methods

*1. Virus isolation, propagation and inoculation:*

A naturally infected sugar beet cv. Gazilla showing ring spots and curling with mild mosaic was used as a source for the virus. Infectious sap was prepared in borate buffer pH 8.0 with 1:10 dilution (w/v) and used for mechanical inoculation (Josephine, 2000). The virus was propagated in *Chenopodium ammaranticolor* and *Cucumis sativus* cv. Beit Alfa.

The experimental work was conducted in air-conditioned insect proof greenhouse at approx. 27-30° C. All test plants were grown in plastic pots (12-cm) with sterilized mix soil (peat and sand 2:1) inside isolators in the greenhouse.

*2. Host range and symptomatology:*

Forty-four species, cultivars and varieties belonging to 11 families were tested by mechanical inoculation to determine the host range and record the symptom expression (Table 1 and Fig. 1). The inoculation was carried out at the 4-6- leaf stages. Local symptoms were recorded till two weeks after inoculation and the systemic symptoms were recorded till 4 weeks after inoculation. Samples of each plant were indexed back to *Ch. amaranticolor* to insure virus presence.

*3. Virus stability:*

Thermal-inactivation point (TIP), dilution-end point (DEP) and longevity (LIV) of the isolated virus were determined *in vitro* by using crude sap of artificially infected sugar beet cv. Gazilla and *Ch. amaranticolor* was used as an indicator host, showed local lesion symptoms.

*4. Partial purification of the isolated virus:*

The virus was purified according to the method described by Fritsch *et al.* (1984). Extraction of 100 g infected sugar beet cv. Gazilla leaves were extracted in borate buffer (0.1mM) pH 8.0 (1:10, w/v). The infectious sap was clarified by ascorbic acid (0.02 mM) and 2-mercaptoethanol (0.02 mM). The virus was concentrated using polyethylene glycol 6000 (8%) and sodium chloride (1%) for 3 times at 8000 rpm for 15 min each. The last pellet was re-suspended in borate buffer pH 8 and kept at -20°C till using. The virus concentration was examined in an ultraviolet-visible spectrophotometer (Milton Roy Spectronic 1201) and concentration was determined using an extinction coefficient of 10 $(mg/ml)^{-1}cm^{-1}$ at 260 nm (Scott and Barnett, 1991).

*5. Serological techniques for virus identification:*

The gel double-diffusion test was conducted using 0.75% agarose gels prepared with 0.03 M sodium phosphate buffer, pH 7.5, containing 0.85% sodium chloride and 0.02% sodium azide. Test samples were prepared in borate buffer pH 8.0 from infectious crude sap of sugar beet cv. Gazilla, *Ch. amaranticolor* and partially purified virus against the obtained diagnostic antiserum (Boehringer Mannheim, Germany).

Double-antibody sandwich (DAS-ELISA) described by Clark and Adam (1977) was carried out. The tested samples were prepared from partial purified virus and six beet varieties. Each sample was repeated four times. Values at least twice those

of healthy control or buffer, were considered as positive. ELISA test was repeated three times.

*6. Effect of virus infection on beet yield:*

Six sugar beet varieties distributed by the Egyptian Sugar and Integrated Industrial Company (E.S.I.I.C.) namely Montpianko, Gloria, Dimapoly, Oskarpoly, Raspoly and Gazilla were used in this investigation. Experiment was carried out in the greenhouse of Plant Pathol. Dept., Fac. Agric., Minia Univ., through 15th October of the two successive growing seasons of 2007/2008 and 2008/2009. Split plot design with four replicates each containing 10 of 50 cm-diameter pots were used. Sugar beet varieties were arranged in the main plots and the inoculation represented the sub plots. The inoculation was carried out on the 15th of December. On 20 April, the sugar beet was harvested and the yield of shoots and roots were estimated/plant.

*7. Effect of virus infection on beet quality:*

A sample of root of each variety was used to estimate polarity (Pol.), α-amino nitrogen (α-N), sodium ($Na^+$), and potassium ($K^+$) in the E.S.I.I.C. Laboratory.

Data were subjected to the proper analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Homogeneity of variance and differences among treatments were evaluated by the least significant difference test (LSD) at 5%.

## Results and Discussion

*1. Isolation, host range and symptomatology:*

The virus under investigation was isolated from naturally infected sugar beet cv. Gazilla. Forty-four species, varieties and cultivars belonging to 11 families were tested by mechanical inoculation to determine the host range and record the symptoms expression (Table 1 and Fig. 1).

The virus was transmitted mechanically to *Ch. amaranticolor* and from this host to other host plants (Table 1). Local symptoms ranged between chlorotic to necrotic lesions. Moreover, in 12 cases (*Beta vulgaris* cvs. Gazilla, Gloria and Raspoly, *Pnicum dichotomum*, *Brassica rapa*, *Raphanus sativus*, *Phaseolus vulgaris*, *Ligustrum vulgare*, *Nicotiana depandii*, *N. glutinosa*, *N. tabacum* cvs. White Burley and Petit Havana), the symptom appeared in the form of ring spots after 10-12 days of inoculation. These data are in agreement with those reported by Stobbs and Van Schagen (1984), Lehoczky and Burgyan (1986), Etienne *et al.* (1991) and Hassan (2005). Black rings appeared in inoculated *Vicia faba*, *Lycopersicum esculentum* and *Datura stramonium*, similarly as reported by Smith (1946) and Benada *et al.* (1987).

Systemic symptoms induced by the virus were varied according to the test host-plants. In most cases they appeared in the form of rings, mosaic, crinkling, vein necrosis and general necrosis. It appeared 12-28 days after inoculation alone or in combinations.

**Table 1. Main symptoms obtained with tomato black ring virus (TBRV), isolated from sugar beet (*Beta vulgaris* L., cv. Gazilla)**

| Host | | Symptoms* | |
|---|---|---|---|
| Family | Test plant | Local | Systemic |
| 1. Chenopodiaceae | *Ch. amaranticolor* Coste & Reyn | CL (8) | - |
| | *Ch. folisum L.* | - | M (16) |
| | *Ch. murale* L. | CrL (10) | - |
| | *Ch. quinoa* Wild | NL (11) | - |
| | *Beta vulgaris* L. cv. Gazilla | RS (9) | Cu, M (20) |
| | cv. Oskarpoly | CL(15) | St, D (20) |
| | cv. Gloria | RS (10) | M (15) |
| | cv. Dimapoly | - | Cu, M (15) |
| | cv. Mont Pianco | - | Vc,M,(13) |
| | cv. Raspoly | RS (12) | Y, M, (20) |
| 2. Compositae | *Lactuca sativa* L. | - | D+M(15) |
| | *Zinnia elegans* L | - | M (10) |
| | *Lantana amaranthus* L | CL(10) | Vc(20) |
| | *Pnicum dichotomum* L. | RS((10) | M(15) |
| 3. Crucifera | *Brassica rapa* L. | RS(10) | Mt(15) |
| | *Brassica oleracea* L. | CR(12) | M(20) |
| | *Raphanus sativus* L. | RS (10) | M(14) |
| 4. Cucurbitaceae | *Cucumis sativus* L. cv. Beit Alpha | CL (9) | VC (12) |
| | cv. Medina | - | D+M (15) |
| | *Cucurbita pepo* L. | NL(11) | Mt(15) |
| 5. Leguminosae | *Phaseolus vulgaris* L. cv. Giza 3 | RS((10) | M(15) |
| | *Pisum sativum* cv. Lincolen | CR (12) | R (20) |
| | *Vigna sinensis* cv. Azmerly | - | VN(22) |
| | *Vicia faba* L. cv. Giza 717 | BR(12) | D+Ro(15) |
| 6. Malvaceae | *Malva parviflora* L | - | M(20) |
| | *Hibiscus esculentus* L | - | M(25) |
| | *Gossipium barbadense* L. Giza 45 | - | Y(22) |
| 7. Oleaceae | *Ligustrum vulgare* L. | RS (10) | M(15) |
| 8. Rosaceae | *Rosa ordrat* L. | NL(9) | M(15) |
| 9. Sambucaceae | *Sambucus nigra* L. | - | M(15) |
| 10. Solanaceae | *Capsicum frutenscens* L. | - | M (12) |
| | *Datura stramonium* L. | BR(11) | - |
| | *Datura tatula* L. | - | Vc(10) |
| | *Lycopersicum esculentum* L. | | |
| | cv. Rutgars | BR (10) | Vb,D,(28) |
| | cv. Chilease | NR (12) | Y (25) |
| | cv. GSNima | - | M(24) |
| | *Nicotiana clevelandii* Gray | CS(12) | Vc(20) |
| | *N. debendyi* L. | RS (10) | Y(15) |
| | *N. glutinosa* L. | RS (12) | - |
| | *N. tabacum* cv. White Burley | RS (10) | - |
| | cv. Petit Havana | RS (11) | Mt(12) |
| | cv. Xanthi nc | CL(10) | N(25) |
| | *Solanum tubrosum* cv. Spunta | NL (10) | Cr(20) |
| 11. Umbelliferae | *Daucus carota* L. | CL (10) | Mt (15) |

* CL=chlorotic local lesions, St=Stunting, NL=Necrotic lesions, M= Mosaic, Vc= Vein clearing, Vb=Vein banding, BR= Black ring, D= Deformation, Mt= Mottling, CS= chlorotic spots, RS= Ring spots, R= rings, N= Necrosis, NS= Necrotic spots, Cu=curling, Cr= crinkling, Ro=rolling, SP= spots, and Y=yellowing. Figures between brackets refer to the time required for onset of the symptoms following mechanical inoculation

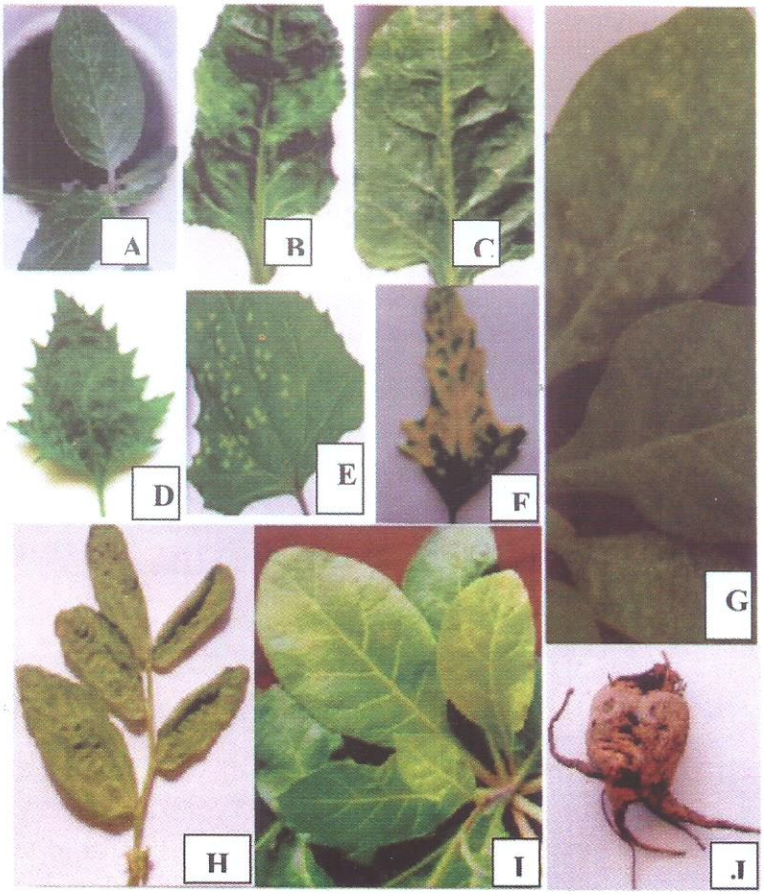


**Fig. 1. Symptoms of TBRV on: (A) *D. stramonium*, (B) Beet cv. Dimapoly, (C) Sugar Beet cv. Gazilla, (D) *Ch. murale* ;(E) *Ch. amaranticolor;* (F) Ch. folisum; (G) *N. tabacum* cv. Xanthi-nc; (H) *Vicia faba* cv. Giza 707; (I) *N. tabacum* cv. White burley. (J) Sugar beet root showing a "fungy" root system as affected by TBRV infection.**

*Persistence of infectivity in Plant Sap:*

Symptoms of systemic infection without any detectable local lesion were occurred on *Chenopodium folisum*, *Beta vulgaris* cvs. Dimapoly and Mont Pianco, *Lactuca sativa*, *Zinnia elegans*, *Cucumis sativus* cv. Medina, *Vigna sinensis* cv. Azmerly, *Malva parviflora* L., *Hibiscus esculentus* L., *Gossypium barbadense*, *Capsicum frutescens*, *Datura tatula*, *Sambucus nigra* and finally *Lycopersicum esculentum* cv. GS-Nima.. These data are in agreement with that obtained by Shimanski (1991) and Smarcha and Baburek (1992). In experiment of sugar beet varieties, symptoms were much more obvious on some roots of beet plant than on

other. Often the primary root is missing and the secondary roots are thickened to give a `fungy` root system (Fig 1, J). This result agreed with the early publication by Smith (1972). In the 50 diam-pots, sugar beet symptoms are much more obvious on some beet plants such as Dimapoly, Mont Pianco and Gazilla than others, and typically consist of a chlorotic blotchy mottle on one or more leaves.

Host range and symptomatology played a great role in identifying and classifying plant viruses (Hasan, 2004). But, sometimes different viruses caused similar symptoms in the hosts, also different climatic conditions and host genotypes can have vast effects on disease susceptibility. Therefore, from the symptomatology it was often very difficult to compare results observed in different laboratories. TBRV has been shown to have a wide host range and has spread all over the world. The hosts include important berry and fruit plants (*Rubus, Ribes, Fragaria* and *Prunus*), sugar beet, potatoes and different vegetables, *i.e. Allium*, *Brassica*, *Solanum* and *Phaseolus* (Uddin, 2010).

Table (2) shows that the virus was inactivated after 10 min exposure at 55°C. The virus withstood a dilution of $10^{-4}$ but not $10^{-5}$. It remained infectious in crude sap of sugar beet stored at room temperature (22°C) for 7 days. These results are in harmony with those recorded by Beemster and DeBokx (1987) but slightly varied with those of Smith (1972) and Hassan (2005).

**Table 2. Physical properties for isolated virus, *i.e.* Thermal inactivation point (TIP), dilution end point (DEP) and Ageing *in vitro* at 20°C (Ag), compared with the reported ones**

| Virus | TIP (°C) | DEP | Age (days) | References |
|---|---|---|---|---|
| Tested virus | 55 | $10^{-4}$ | 7 | |
| TBRV | 60-65 | $10^{-3}$-$10^{-4}$ | 14-21 | Murant, 1970 |
| TBRV | 55-70 | - | Few days | Harrison *et al.*, 1971 |
| TBRV | 58-62 | $10^{-2}$-$10^{-3}$ | 7 | Smith, 1972 |
| TBRV | 60-65 | $10^{-3}$-$10^{-4}$ | 12-21 | Beemster and deBokx, 1987 |
| TBRV | 60 | $10^{-4}$ | 10 | Hassan, 2005 |

*Partial purification:*

The isolated virus has successfully been partially purified with 2.1 mg/ml yield per 100 g infected sugar beet cv. Gazilla. The last pellet was highly infectious. The spectrum showed that the maximum absorbance was recorded at 260 nm and the minimum was at 242 nm. The average ratios of $A_{260}/A_{280}$, $A_{280}/A_{260}$ and $A_{max}/A_{min}$ were 2.10, 0.47 and 1.5, respectively, (Fig. 2). This data were typical for nepoviruses (Stace-Smith, 1984 and Rana *et al.*, 1987) and spherical viruses CMV (Abdelsalam *et al.*, 1989).

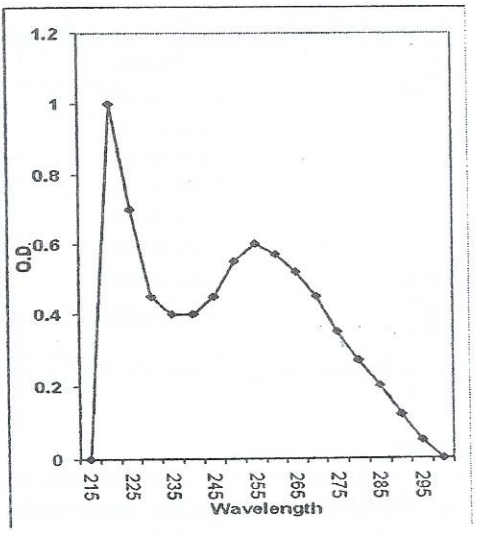


**Fig. 2. Ultraviolet absorption-spectrum for partially purified preparation (Sugar beet cv. Gazilla) of TBRV.**

*Serological techniques for virus identification:*

The partially purified virus preparation as well as artificially infectious crude sap of sugar beet cv. Gazilla and extraction of *Ch. amaranticolor* leaves reacted positively in Agarose double diffusion test with one line of precipitate in gel–diffusion tests (Fig 3).

In DAS-ELISA, the virus reacted positively with TBRV antiserum obtained from Boehringer Mannheim, Germany. The $OD_{405nm}$ ranged between 1 and 2.4 in the tested samples compared to 2.9 in positive control (Table 3). The highest absorbance value was recorded for partially purified virus (2.4) while the infectious sap of *Ch. amaranticolor* gave 2.1 $OD_{405nm}$. Among beet varieties, the highest value recorded was 2.1 in case of Gazilla, while the lowest one was in Raspolly. The results proved that the isolated virus was typically TBRV as observed by Stelmack (1985), Scott and Barnett (1991) and Smarcha and Baburek (1992).

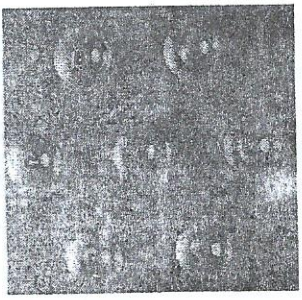

**Fig. 3. Reactions in agarose double diffusion test showing one line precipitation. The central well containing TBRV antiserum (A). The preferal wells contained: partially purified virus (B), crude sap of sugar beet cv. Gazilla (C) and infected extraction of *Ch. amaranticolor* (D). The other ones were negative controls.**

**Table 3. Values* of DAS-ELISA ($OD_{405nm}$) for different artificially infected beet varieties compared with partial purified virus and positive control one**

| Sample | Control | Values $_{405\ nm}$ |
|---|---|---|
| Montpianko | 0.02±0.003 | 1.8±0.15 |
| Gloria | 0.04±0.008 | 1.7±0.18 |
| Dimapoly | 0.05±0.007 | 1.3±0.10 |
| Oskarpoly | 0.03±0.006 | 1.0±0.19 |
| Raspoly | 0.02±0.006 | 1.6± 0.14 |
| Gazilla | 0.03±0.006 | 2.0±0.12 |
| Partial purified virus | 0.02±0.004 | 2.4±0.25 |
| *Ch. amaranticolor* | 0.05±0.002 | 2.1±0.17 |
| Positive control | - | 2.9±0.19 |

* Each value represented the mean of four replicates ± SD.

*Effect of virus infection on beet yield:*

Data in Table (4) show the effect of virus infection in root and shoot yield of sugar beet varieties. The shoot yield in healthy plants varied between 0.78 to 1.25 kg (in Raspoly and Montpianko, respectively). Both Raspoly and Gazilla are the most affected varieties by virus infection with 35% each. The lowest negative effect due to the infection was recorded in Montpianko (24%). This data was similar to those previously recorded by Mosen (2007).

**Table 4. Effect of TBVR infection on yield of some sugar beet varieties**

| Beet varieties | Shoot yield/plant (kg) | | | Yield root/plant (kg) | | |
|---|---|---|---|---|---|---|
| | Healthy | Diseased | Reduction(%) | Healthy | Diseased | Reduction(%) |
| Montpianko | 1.25 | 0.95 | 24 | 1.32 | 1.01 | 23 |
| Gloria | 1.02 | 0.71 | 30 | 1.17 | 0.86 | 27 |
| Dimapoly | 0.96 | 0.68 | 29 | 0.93 | 0.64 | 32 |
| Oskarpoly | 0.81 | 0.60 | 26 | 0.85 | 0.64 | 25 |
| Raspoly | 0.78 | 0.51 | 35 | 0.95 | 0.58 | 39 |
| Gazilla | 0.85 | 0.55 | 35 | 1.12 | 0.80 | 29 |
| LSD | 0.22 | 0.18 | | 0.25 | 0.13 | |

The root yield in healthy tested varieties was ranged from 0.85 (Oskarpoly) to 1.32 kg (Montpianko). The lowest reduction in root yield was recorded in variety Montpianko (23%), while the most remarkable losses were recorded in Raspoly (39%). Heijbroek (1988) reported that the yield reduction due to a virus yellows infection was estimated by 50%. Sugar beet is a biennial crop so yield is determined by the amount of radiation it captures within its first year of growth. In part of the world where there is an adequate rainfall or irrigation available to prevent drought stress, and the crop has a long growing season before winter, yields in excess of 200 adjusted tones of beet per hectare (32 t sugar/ha) have been recorded by (Anonymous, 2009).

*Effect of virus infection on beet quality:*

Data in Table (5) clarify the effect of virus infection on some quality parameters of sugar beet roots. Polarity value in healthy sugar beets varied between 18.9 (in Gazilla) to 21.1 (in Dimapoly) compared to 16.5 to 18.5 in infected ones. The highest decrease detected in Gazilla (12.7%), while the lowest one detected in Oskarpoly (6.3%).

Sodium contents in infected sugar beet roots (1.99) increased compared to the healthy ones (1.59). The most influenced variety was Gloria (35.1%) while the lowest one was Gazilla (10.6). Potassium contents also increased due to the virus infection. The most pronounced increase was detect in Gazilla (39.2%) while the lowest one was in Raspoly (8.2%)

The diseased sugar beet contained more α-amino than the healthy ones (2.11 and 1.81, respectively). Raspoly showed the most pronounced decreased (36.3%) while Montpianko showed the lowest one (7.8%). The increase in juice captions caused increase in molasses production which is disadvantages (Carlson *et al.*, 1997). The relationship between the lowest value of αN, Na and K and sugar losses of sugar beets were recorded (Kandil *et al.*, 2002). The reduction in sugar content was previously reported as affecting of many pathogens (Mosen, 2007). In 1990, the yellowing viruses caused reduction in root yield 23% and in sugar yield 40% (Smith and Hallsworth, 1990)

**Table 5. Effect of TBRV infection on some quality parameters* of sugar beet roots**

| Sugar beet varieties | Polarity | | | $Na^+$** | | | $K^+$** | | | N**α | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Diseased | Change (%) | Healthy | Diseased | Change (%) | Healthy | Diseased | Change (%) | Healthy | Diseased | Change (%) |
| Montpianko | 19.3 | 17.5 | -9.3 | 1.52 | 1.82 | +19.7 | 4.74 | 5.18 | +9.3 | 1.91 | 2.06 | +7.8 |
| Gloria | 20.1 | 17.9 | -10.9 | 1.68 | 2.27 | +35.1 | 4.79 | 5.34 | +11.5 | 1.92 | 2.12 | +10.4 |
| Dimapoly | 21.1 | 18.5 | -12.3 | 1.52 | 1.92 | +26.3 | 4.44 | 5.22 | +17.6 | 1.72 | 2.15 | +25.0 |
| Oskarpoly | 18.9 | 17.7 | -6.3 | 1.42 | 1.79 | +26.0 | 4.82 | 5.51 | +14.3 | 1. 93 | 2. 24 | +16.1 |
| Raspoly | 19.1 | 17.5 | -8.3 | 1.63 | 2.02 | +23.9 | 5.15 | 5.57 | +8.2 | 1.35 | 1.84 | +36.3 |
| Gazilla | 18.9 | 16.5 | -12.7 | 1.77 | 1.98 | +10.6 | 4.18 | 5.82 | +39.2 | 2.01 | 2.24 | +11.4 |
| Mean | 19.56 | 17.6 | -9.97 | 1.59 | 1.97 | +23.6 | 4.69 | 5.44 | +16.68 | 1.81 | 2.11 | +17.83 |
| $LSD_{0.05}$ | 1.83 | 1.59 | | 0.062 | 0.078 | | 0.53 | 0.61 | | 0.21 | 0.23 | |

* Data are mean of two seasons.

** Values are millequivalents/100g beet.

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# تأثير فيروس الحلقة السوداء في الطماطم (TBRV) علي إنتاجية وجودة محصول بنجر السكر

هناء محمد مرسي حسان ، مرزوق رجب عبد اللطيف ، السيد عبده السيد.
قسم أمراض النبات- كلية الزراعة – جامعة المنيا.

شوهدت بمحافظة المنيا أعراض بقع حلقية وموزايك وتقزم شديد على نبات بنجر السكر وأظهرت الدراسة أن هذه الأعراض ناتجة من الإصابة بسلالة من فيروس الحلقة السوداء في الطماطم.

لتحديد المدى العوائلي للفيروس تم استخدام ٤٤ نباتاً كشافًا مشخصاً ينتمي إلى ١١ عائلة نباتية مختلفة وتمت العدوى الميكانيكية لجميع النباتات بنجاح وعند دراسة الخواص الطبيعية للفيروس تبين أن درجة الحرارة المميتة كانت ٥٥ درجة مئوية عند تعرض العصارة المعدية لهذه الدرجة لمدة ١٠ دقائق وكانت درجة التخفيف النهائية للعصارة المعدية ١: $١٠^{-٤}$ وظل الفيروس نشطاً لمدة ٧ أيام في العصارة المعدية على درجة حرارة الغرفة.

تمت التنقية الجزئية للفيروس وأظهرت الدراسات السيرولوجية أن الفيروس المنقى أعطى خطاً واحداً في تجارب الانتشار المزدوج في الاجاروس كما أعطى تفاعلاً ايجابياً مع السيرم المضاد لفيروس الحلقة السوداء في الطماطم عند استخدامه في اختبار الاليزا بقيمة ٢،٤ بينما تراوحت درجة الكثافة اللونية لأصناف البنجر المختبرة والمعداة من ١،٠ إلى ٢،٠ .

أظهرت الدراسة أن المجموع الخضري لصنفي البنجر جازيللا وراس بولي كانا أكثر الاصناف انخفاضاً في الوزن نتيجة الإصابة بالفيروس (٣٥% لكل منهما) وكان الصنف مونت بيانكو أقل الأصناف انخفاضاً في وزن المجموع الجذري (٢٣%) وكانت العدوى الفيروسية أقل تاثيراً على محتوى السكر في الأصناف اوسكار بولي ومونت بيانكو.