Egyptian J. Virol. 1, 223-238 (2004)

Occurrence and Spread of Sugar Beet Rhizomania Disease Caused by *Beet Necrotic Yellow Vein Benyvirus* in Some Governorates of Egypt

S.Y.M. Mahmoud [1] and M. Hashem [2]

[1]Agricultural Botany Department (Microbiology), Faculty of Agriculture, South Valley University, Souhag, Egypt [2]Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.

Beet necrotic yellow vein Benyvirus (BNYVV) is the important soilborne virus disease in the production areas of sugar beet. In this study, field survey was carried out in sugar beet growing areas belonging to Kafrel-Sheikh, El-Behera, El-Dakahlia, El-Gharbia and El-Minia Governorates. The results of this survey revealed the presence of characteristic symptoms of rhizomania syndrome, i.e. stunted and constricted roots which developed a proliferation of rootlets. The foliar symptoms appeared as yellowing rounded patches in the field, including general chlorosis, wilting and necrotic vein yellowing. Samples of rhizomania-like symptom roots and its adjacent soil were taken from these areas and tested for the presence of BNYVV using indirect DAS-ELISA with specific polyclonal antibodies to C-terminal 60 amino acids of BNYVV coat protein and bait plants test, respectively. The results confirmed the presence of BNYVV in 46 out of 184 and 24 out of 50 root and soil samples, respectively. The virus was found with percentage of 65% in root samples collected from Kafrel-Sheikh. The transmission experiments indicated that BNYVV was mechanically transmitted to *Chenopodium amaranticolor*, *C. quinoa*, *Beta vulgaris* cvs. Pleno, Tripl and Gloria, *B. macrocarpa* and *B. maritema* inducing chlorotic local lesions spreads into the veins. The virus was also transmitted by a soilborne parasitic fungus, *Polymyxa betae* Keskin as a vector and by seed coating with viruliferous cystosori, the thick-walled resting spores of *P.betae*, as well as by root vortexing in virus inoculum containing carborundum. The virus is not adsorbed to the exterior of the fungal vector but is internalized. Our results indicated that BNYVV is a wide-spread in sugar beet in Kafrel-Sheikh Governorate, thus precaution methods must be taken for reducing virus transmitting to other free-virus regions.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is an important source of sugar in Egypt, whereas the crop are sown in autumn. Sugar beet is cultivated in many distinct geographic regions. The most intensive production is in Kafrel-Sheikh Governorate, to a lesser extent in El-Dakahlia, El-Behera and El-Gharbia but sporadically in El-Minia Governorate. Sugar beet production has suffered from many problems, especially from diseases and pests. There are previous studies in Egypt on viral infection. *Cucumber mosaic virus* (CMV) (Omar *et al.*, 1994 and Shaker, Maali, 2003), *Beet mosaic virus* (BtMV) (Abdel-Ghaffar *et al.*, 2003) and *Beet necrotic yellow vein virus* (BNYVV) Abdel-Salam and El-Shazly 2001) were observed once in Kafrel-Sheikh, Beni-Swief and Giza. Among several factors which have contributed to decline in sugar beet production in Egypt in recent years is rhizomania disease, which is considerably a devastation disease of sugar beet caused by *Beet necrotic yellow vein virus* (BNYVV)-the type member of the genus *Benyvirus* (Koening and Lesemann, 2000 and Van-Regenmortal *et al.*, 2000). BNYVV reduces yields of sugar beet more than 50% and sugar content of beets by 3-4% (Horvath, 1994 and Henry, 1996). Rhizomania caused by BNYVV is one of the most destructive viruses of all beets in many

regions of the world (Rush and Heidel, 1995 and Asher, 1999). Historically, the virus was first recovered in Italy (Canova, 1959) and then was identified in Japan (Masuda *et al.*, 1969). Subsequently, the BNYVV was found during the 1970s (Tamada and Baba, 1973) and early 1980s in more than 20 European countries. This virus has been revealed in China (Gao *et al.*, 1993), United States (Duffus *et al.*, 1984 and Harveson and Rush, 1993), Croatia (Juretic and Mamula, 1998), Kosovo (Taraku and Juretic, 1990), former Yugoslavia (Asher, 1993), Mediterranean region, Syria, Iran and Turkey (Varder and Erkan, 1992; Izadpanah *et al.*, 1996; Al-Chaabi *et al.*, 2000 and Mouhanna *et al.*, 2002) and has occurred more recently in Egypt (Abdel-Salam and El-Shazly, 2001). It is now widely distributed over most sugar beet growing areas in the world; whereas no sugar beet growing country is free of rhizomania or its vector *Polymyxa betae* Keskin (Gerik, 1989; Payne and Asher, 1990; Asher, 1993 and 1999 and Tamada, 2002). Russo *et al.*, (1981) and Fujisawa *et al.* (1982) mentioned that BNYVV has been isolated not only from sugar beet but also from spinach (*Spinacia oleracea* L.) and Swiss chard (*Beta vulgaris* var. Cycla L). BNYVV is characterized by rod-shaped particles, 20 nm in diameter and of four different model lengths 85, 100, 265 and 390 nm (Putz, 1977). It has been shown to contain four separate single-stranded genomic RNAs of 1467, 1774, 4612 and 6746 base pairs, respectively. The vector of BNYVV is a soil-borne fungus *Polymyxa betae* Keskin, one of the plasmodiophoramycetes (Ivanovic *et al.*, 1983; Abe and Tamada, 1986 and Wisler *et al.*, 1994). The fungus is an obligate parasite and has limited host range (Abe and Ui, 1986; Tuitert, 1994 and Kutluck *et al.*, 2000). Whereas rhizomania has spread to most sugar beet production areas of the world and the seeds cultivated in Egypt were imported from European countries, also, the occurrence of BNYVV in neighboring countries stimulated us to survey Egyptian sugar beet crop for BNYVV infection in Egypt. This study was performed by using symptomatology, different methods of transmission, test plants and serology. On the other hand, the relationship between BNYVV and *P.betae* was studied.

MATERIALS AND METHODS

Sampling

Sugar beet root samples were collected in April and May 2003 from 46 fields (4 subsamples per field) in the villages belonging to Kafrel-Sheikh, El-Dakahlia, El-Gharbia, El-Behera and El-Minia Governorates. 184 root samples were taken from October or November sown crops. The samples were collected from plants showing with typical rhizomania-like symptoms (Fig 1). Symptomless plants were collected from each locality for use as controls. If no symptomatic sugar beets were noticed in a field, samples were collected randomly within the field. The infection level in each locality was estimated from foliage symptoms in the fields. The estimation was conducted by counting the symptomatic plants among 10 randomly chosen plants in the tested fields. The roots were analyzed for the presence of root symptoms, *Polymyxa betae* and BNYVV by visually, light microscopy and indirect DAS-ELISA, respectively.

Soil survey for BNYVV

Soil samples taken from 15 localities were used in bait plant tests according to Taraku and Juretic (1990). Rhizosphere and soil attached with

Rhizosphere and soil attached with roots were collected from around beet plants considering the visual indications for the presence of rhizomania in field grown plants, such as yellow coloration of leaves and beard-like appearance of the roots. Fifteen soil samples were collected, 3 were taken from each locality and five were collected from waste in washing tank in sugar beet factory, Delta Sugar Company, Kafrel-Sheikh Governorate. The soil samples were air-dried for 3-4 weeks as described by Jones and Harrison (1969) and a layer 3 cm deep was placed in 10-cm diameter sterilized pots (3 pots per sample) sandwiched between layers of sterilized soil. Eight seeds of *B. vulgaris* cv. Pleno were sown in each pot. Pots were placed in plastic dishes and watering was done by topping-up each dish individually to prevent cross contamination by splashing from pot to another. Pots containing sterilized soil alone were used as control. After germination, the plants were thinned to 4 seedlings per pot. Sugar beet plants were grown for 12 weeks in the greenhouse at 23 ± 2 °C, adequate time to allow colonization by *P.betae* and infection by BNYVV if viruliferous *P.betae* was present in the soil as mentioned by Gerik (1992). Roots were harvested in each pot separately, washed free of soil and examined for rhizomania symptoms and *P.betae* then sap was extracted with hand press from the rootlets for detection the presence of BNYVV by indirect DAS-ELISA.

Detection of *P.betae*

Rootlet samples were taken from each plant and washed to remove soil debris, then preserved in 70% ethanol. Sections were stained with lactophenol containing 0.1% acid fuchsin then examined by light microscope according to Langenberg and Kerr (1982) for presence of the characteristic resting spores of *P.betae*. The fungal vector was identified in the Botany Department, Faculty of Science, Assiut, Assiut University. The density of *P.betae* resting spores in 10 pieces of the rootlets per plant (1 cm long) was estimated on a 0-5 scale and the mean value per plant was subjected according to Asher *et al.* (2002).

Viral inoculum preparation

Rootlets from plants which exhibit virus-like symptoms were checked microscopically for infection by *P.betae* and assayed by indirect DAS-ELISA for BNYVV infection then washed and allowed to air-dry. The dried hairy root tissues were pulverized and separated from soil and other foreign materials by series of fine meshed sieves. The resulting product consisted of powdered roots infested with viruliferous cystosori (resting spores). On the other hand, the crude inoculum for mechanical inoculation was prepared by grinding infected leaf tissues in mortar and pestle with 0.1 M Tris-HCl buffer, pH 7.2 containing 0.1% M sodium sulfite and 0.1% 2-mecaptoethanol. In case of hairy root samples, it was packed in a small tube in presence of 5 ml of extraction buffer, and then sonicated for 10 min in an ice bath. Tubes were frozen overnight, thawed, sonicated again and centrifuged (3000 rpm for 5min), and then the supernatant was used.

Biological identification

Crude inoculum which prepared from infected leaves or roots was used for mechanical inoculation. Twenty-five species and varieties belonging to five different families of test plants were inoculated with crude inoculum. Healthy test plants grown in sterilized soil were exposed to dark for 24 h before inoculation (Wisler *et al.*,

species were inoculated. The test plants included: *Beta vulgaris* L. cvs. Pleno, Tripl, Ras poly and Gloria; *B. vulgaris* L. var. Cicla; *B. macrocarpa* Guss; *B.maritema*; *Chenopodium amaranticolor* Coste & Reyn; *C. quinoa* Wild; *C. murale* L.; *Spinacea oleracea* L.; *Brassica nigra* Kock; *Capsella bursa-pastoris* L; *Capsicum annum* L. cv. Califorina; *Datura stramonium*; *D. metale*; *Lycopersicon esculentum* Mill cv. Peto 86; *Nicotiana glutinosa* L; *N. tabacum* var. White Burley; *N. tabcum* L. var. Samsun; *Petunia hybrida* Vilm.; *Cucumis sativus* L cv. Atlas; *Cucurbita pepo* L. cv. Eskandarani; *Phaseolus vulgaris* L.; and *Vicia faba* L. Equal number of test plants was rubbed with buffer as control. *C. quinoa* was used in back inoculation to determine if a plant was infected. Back-inoculations were made at least 5 weeks after the test plants had been inoculated. Plants recorded as BNYVV positive if they showed symptoms and back-inoculation positive. The BNYVV isolate was initially obtained by mechanical inoculation from infected roots and symptomatic leaf tissues collected from field samples on Kafrel-Sheikh onto leaves of *C. quinoa* Wild and *B. vulgaris* L. cv. Pleno plants. Our isolate derived from single local lesion was increased and maintained by continuous mechanical inoculation on *B.vulgaris* cv. Pleno.

Modes of inoculation and virulence evaluation under greenhouse conditions

This experiment was carried out by tow methods: First: seeds of sugar beet cv. Pleno were coated with powder of roots, which was prepared as mentioned above by using 2 % methylcellulose as a carrier. The inoculum rate was 1:10:10 (w/v/w). inoculum/methylcellulose/seeds. This batch of treated seeds was used for greenhouse study. Six seeds were sown in each pot (15-cm diameter), which contained sterilized mixture of sand and clay. Ten pots were sown with treated seeds and 5 pots were sown with untreated seeds as healthy controls. The pots were watered heavily in the first 3 weeks after planting to initiate early infection and thinned to 4 largest and most vigorous plants per pot. When the plants were established, they were watered daily. Second: seeds of sugar beet cv. Pleno were planted in sterilized sand in 15-cm pots in the greenhouse. Fifteen days old seedlings were uprooted and rinsed free of sand then placed by roots in 50 ml plastic centrifuge tubes and vortexed for 45 seconds in 5 ml of viral crude inoculum containing 0.15 g of 600-mesh carborundum according to method described by Hutchinson *et al.*, (1993). Inoculum consisted of 2 g of symptomatic *B. vulgais* leaf tissue infected with BNYVV that was ground in 5 ml of inoculation buffer. Seedlings were left in the inoculum for 5 minutes after vortexing then rinsed with sterilized distilled water. Four seedlings were planted in sterilized sand/clay in 15-cm pots and ten pots were used. Healthy controls consisted of seedlings vortexed in 5 ml of inoculum buffer containing 0.15 g of carborundum and seedlings transplanted without vortexing. Four weeks after planting, plants that survived was thinned to 4 largest and most vigorous plants per pot. In the two methods the replicates were arranged in randomized complete blocks on the greenhouse. Plants were harvested 22 weeks after planting, and then the roots were tested for BNYVV by biological assay on *C. quinoa* and symptoms while *P.betae* was detected by the presence of cystocori. On the

other hand, top and root dry weights were determined after drying.

Relationship between BNYVV and *P.betae* spores

To determine if the virus was just adsorbed to the exterior of the fungal vector spores or is internalized, we prepared resting spore solution taken from BNYVV infected roots according to Tuitert (1993). The resting spore solution was adjusted to 10^6 spore/ml approximately then divided into two parts: one of them was used to mechanical inoculation of *C. quinoa* directly and the other was sonicated for 10 min in small tubes with ice path then used to inoculation of *C. quinoa*.

Serological detection

The virus was serologically detected by an indirect DAS-ELISA method as described by Heidel *et al.*, (1997) with polyclonal antibodies to the C-terminal 60 amino acids of BNYVV coat protein. The antiserum was kindly supplied by Dr. S.E.Bouzouabae (Centre National of Research Scientific, IBMP, Strasbourg, France) and was diluted 1:2,000 (v/v) in TBST buffer (10 mM Tris-HCl buffer, pH 8.0, 150 mM NaCl, 0.05% Tween-20) as recommended by supplier. Sap was extracted with a handpress from rootlets and top roots. One hundred µl sap was diluted with 900 µl extraction buffer (PBS: 0.1 M NaCl, 0.01 M KH_2PO_4, 0.003 M KCl, PH. 7.4) containing 2% polyvinylpyrolidone, 0.2% ovalbumin and 0.05% Tween-20. The extract was kept frozen until analysis by DAS-ELISA. Samples determined to be positive if average of ELISA values reached three times when compared with the average of healthy readings. All root and leaf samples in survey, bait plants and host range experiments were tested by DAS-ELISA for BNYVV, but due to shortage of antiserum the other experiments could not be tested.

Statistical analysis

Data were subjected to analysis of variance and treatment means were separated by Duncan's multiple range tests (Duncan, 1965).

RESULTS AND DISCUSSION

Symptoms

In Egypt, rhizomania disease was first reported in El-Giza and El-Fayoum Governorates in 2001 (Abdel-Salam and El-Shazly, 2001), but beet necrotic yellow vein *Benivirus* (BNYVV), the causal of rhizomania, have never been investigated in the main regions of beet production (Kafrel-Sheikh, El-Behera, El-Dakahlia, El-Gharbia and El-Minia). So that, this regions were investigated in this study. In some localities, many sugar beet plants exhibited excessive crown growth and leaves grow in upright position than normal were noticed. Infected plants were more stunted with chlorotic leaves. Foliage on several infected plants readily wilted during the day even when soil moisture was adequate, but it regains turgid overnight. The necrotic yellow vein symptom, which the virus was named, is rarely seen under natural field conditions. Only beets grown in Kafrel-Sheikh, El-Behera and El-Dakahlia Governorates showed foliar symptoms as pale green leaves. It has elongation leaf petioles, narrowing and asymmetrical leaf blades. These narrower leaf blades are erect in the centre of plant and thus are easily observed. Infected plants were in circular groups. Root symptoms associated with infection were

variable. The tip of taproot was killed, resulted excessive lateral root proliferation. The new roots also became infected and eventually die. Lateral roots continue to develop giving the tap root "bearded" appearance from which the name of rhizomania (root madness) was derived. Also, small tumors were clearly visible at the base of root hairs as a result of proliferative cell division. These tumors are diagnostic of the disease caused by BNYVV. Microscopic examination of these small lateral roots revealed the presence of resting spores of *Polymexa betae* (Fig.1). The foliar and root symptoms were similar to previously recorded by Rush and Heidel (1995); Juretic and Mamula (1998); Asher (1999); Henry *et al.*, (1986) and Gerik (1994). BNYVV was isolated not only from sugar beet but also from spinach and Swiss chard as recorded by Russo *et al.*, (1981) and Fujisawa *et al.*, (1982).

Survey and detection of BNYVV

The appearance of rhizomania affected sugar beet plants differed greatly from healthy ones in the field at Kafrel-Sheikh. There was highly incidence of rhizomania in Kafrel-Sheikh, the main region of beet production in Egypt, whereas the soil is suitable for sugar beet production. Sugar beet plants in most of the fields showed conspicuous wilting and strong growth of lateral roots. The observable infection was 29.7%. Because of severe situation in this region, 40 samples from 10 fields in 6 localities were collected. Of these, 65% were infected with BNYVV (Table 1). In El-Behera, some fields in El-Hossain village showed rhizomania infection (observable infection was 25.0%), while the rest locations no symptoms were showed. Of the 40 samples taken from 10 fields in 3 locations, 12.5% were infected with BNYVV (Table 1). In El-Minia, whereas the weather is hot and dry, there were no symptoms of rhizomania. However, 24 samples were taken from 6 fields in 2 different locations in Abou-Kurkas and Malawy and although the plants showed no clear rhizomania symptoms, only one sample reacted with BNYVV antiserum (Table 1). In El-Dakahlia, whereas the temperature is similar to Kafrel-Sheikh, most visited fields on Belkas region showed rhizomania infection (12.0%). Of the 40 samples taken from 10 fields in one locality, 30.0% were infected with BNYVV. In El-Gharbia, although the temperature is suitable, no incidence of rhizomania was showed. Although many beets collected from fields showed no symptoms of rhizomania, indirect DAS-ELISA showed infection with rhizomania, whereas 5.0% from 40 samples collected from 10 fields in 3 localities reacted positively. We are surprised why the virus spreading is too low in El-Gharbia Governorate? Although, this regions adjacent to Kafrel-Sheikh. This may be due to the farmers use wide crop rotation. All root samples which have symptoms (beard-like appearance) give positive ELISA reaction, except on Ketoor and Abo-Kurkas regions, whereas some plants give positive reaction although root symptoms not detected.

Detection of BNYVV in soil samples using bait plant test

We tried to transmit the BNYVV by bringing the root systems of sugar beet plants into contact with field soil. In these experiments, sugar beet seeds were sown in soil taken from the fields of the five Governorates. Soil samples were collected from different 16 localities based on the symptoms caused by rhizomania in order to reduce the risk of missing BNYVV

infected plants. However, is sometimes difficult to detect infected sugar beet using such criteria. For this reason, soil samples were taken from around these plants. The results indicated that the virus was detected in many localities in Egypt. Of the 50 soil samples taken from 5 Governorates, 24 samples (48.0%) were infected with BNYVV. Characteristic structures of *P. betae* (plasmodia and cystosori) were found in all roots when examined by light microscope, except on soil taken from El-Minia Governorate (Table 2 and Fig. 2). On the other hand, the development of symptoms was confirmed by ELISA results. The incidence of BNYVV in Kafrel-Sheikh and Dakahlia was 100%. Five soil samples were taken from waste in Delta Sugar Company, (40%) of the samples reacted positively with BNYVV antisera. Nine soil samples were taken from different locations in El-Behera Governorate and it was found that only one sample (11.1%) from El-Hossain village was infected with BNYVV. On the other hand, 15 soil samples were taken from beet fields in El-Minia and El-Gharbia, neither samples from two Governorates were positively reacted with antisera of BNYVV (Table 2). We suggest that the wide-spread of virus in Kafrel-Sheikh and El-Dakahlia compared with other Governorates, is perhaps for the past history of sugar beet cultivation and the temperature is suitable for *Polymyxa betae* fungal infection as vector. Also, water is available and rains are heavy in this regions. These findings agree with results of (Gerik and Duffus, 1987, Blunt *et al.*, 1991, Mouhanna *et al.*, 2002 and Yilmaz *et al.*, 2004) they reported that this fungal vector is an obligate parasite and only infects the primary root tissues of young roots and the optimum temperature for infection is around 25 °C.

Host range and symptoms on mechanically inoculated plants

Beet necrotic yellow vein virus was easily sap transmissible to 11 of 25 species from 5 families, when Kafrel-Sheikh isolate was used for

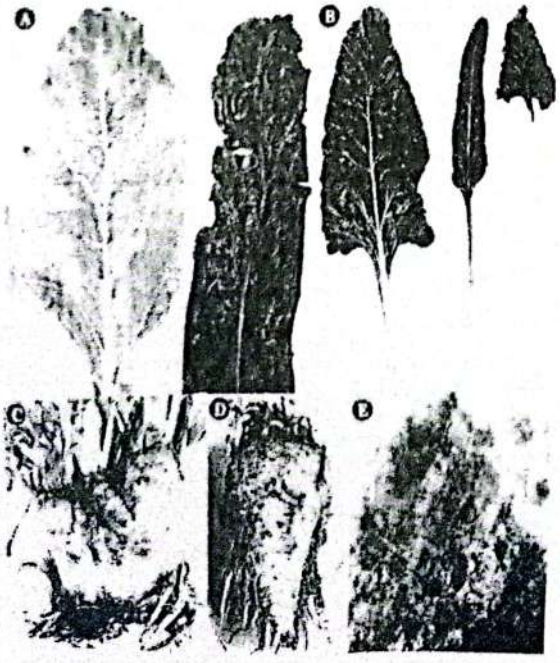

Fig. (1): Rhizomania disease symptoms in naturally infected plants. A, leaf with typical yellow and necrotic veins (on outer leaves only); B, Infected leaves showed narrow blades and long petioles compared with control, (left) (It is erect in the centre of plant); C, Foliar proliferation of small leaves in the root crown area and the tip of taproot is killed; D, Infected sugar beet root showing reduction root size, numerous hairs that form a dense hair-like network, tumors clearly visible at the base of root hairs and E, light micrograph of sugar beet lateral root cells infected with *Polymyxa betae* where cystosori were observed (x400).

inoculation. It infect the inoculated leaves only of most susceptible species although it infect *B. vulgaris* cv. Tripl and pleno, *B maritime* and *Spinacia oleracea* systemically (Table 3). BNYVV host plants and their symptoms were determined by mechanically inoculation to the following hosts. *B. macrocarpa* diffuse chlorotic spots developed in inoculated leaves about 6 days at 22 °C after inoculation. These spots were regularly shaped consisting of yellow local lesions which tend to spread along leaf veins (Fig. 3-1). Sometimes the lesions become fully necrotic, appearing as necrotic lesions. But in *B. vulgaris* cv. Triple, diffuse chlorotic blotches developed in leaves 7 days after inoculation. These lesions enlarged into irregularly shaped spots consisting of chlorotic rings, which tend to spread along leaf veins (Fig 3-2). While on *B. vulgaris* cv. Gloria, BNYVV caused circular green pale rings. It surrounded by yellow halos and spread along leaf veins only (Fig 3-3). At *B. vulgaris* cv. Pleno inoculated plants, the symptoms were characterized by yellow veins in systemically manner. Veins eventually turned to necrotic and leaf died (Fig 3-4). *B. vulgaris* cv. Ras poly showed chlorotic blotches in inoculated leaves. It consisting of green pale centre with yellow halos (Fig 3-5). Also, results indicated that, the virus isolate induced chlorotic local lesions in inoculated leaves of *C. amaranticolor*, which spread into the veins (Fig 3-6). At *C. quinoa*, local lesions developed in inoculated leaf as in *C. amaranticolor* but tend to more necrotic, red coloration sometimes appeared at their margins and they encounter leaf veins (Fig 3-7). Successful inoculations were further confirmed by back inoculation to *C.quinoa* or by positive reactions to the polyclonal antibodies against the C-terminal 60 amino acids of BNYVV coat protein in indirect DAS-ELISA tests. No visible symptoms or positive ELISA reactions were found with any the negative reactions of test plants. It is not clear why Bennett (1956) failed to mechanically transmit BNYVV whereas our isolate of BNYVV was relatively easily transmitted by sap inoculation. Successful inoculation was obtained by using root sap. These findings indicated that the collected samples were infected by BNYVV. These data are agreed with findings of Tamada (1975); Wisler, *et al.*, (1994); Horvarth (1994); Juretic and Mamula (1998) and Abdel-Ghaffar and Farrag, Eman (2004).

Inoculation methods and virulence evaluation

Two different methods of BNYVV transmission were tried. Data in (Table 4) indicated that 9 from 37 plants in seed coating treatment (24.3%) were positive when tested by bioassay and root symptoms. The virus was not detected in control plants which produced from uncoated seeds. These results can be confirmed our hypothesis, that the BNYVV might be entered to Egypt via seeds contaminated with viruliferus resting spores of *Polymyxa betae*, especially the seeds were imported from countries which had wide-spread viral infection. The vector of BNYVV is the soil-borne fungus *Polymymxa betae* belongs to Plasmodiophoramycetes and has a limited host range, primarily with the Chenopodiaceae, Amaranthaceae and Portulaceae (Abe and Tamada, 1986; Gerik and Duffus, 1987 and Asher, 1999). *P.betae* survives in the field soil as a cystosori. In the presence of host and proper environmental conditions, cysts give zoospores, which may be swim through free soil water until they contact a host root and encyst.

Encysted zoospores enter the host cell and became a plasmodium. The host cell became infected with BNYVV if *P.betae* is viruliferous. If a nonviruliferous zoospore infects a root cell containing BNYVV, the plasmodium can incorporate the virus. The plasmodium may develops into clusters of thick-walled resting spores (cystosori), that are stable for many years or differentiate into zoospores then repeat the infection cycle (Barr, 1988). Our results indicated that the virus was transmitted by seed coating with powdered roots infected with viruliferous cystosori. Also, 15 from 28 plants in root vortexing treatment (53.6%) were positive when tested by bioassay and root symptoms. The virus was not detected in control plants. On the other hand, no difference among treatments in top dry weights was found. Root dry weight in BNYVV treatments was significantly lower than those of control treatments. Based on these results we can consider that BNYVV is a great virulent.

Table (1): Survey of BNYVV at selected fields in sugar beet production areas of Egypt

Governorate, Village	Field Sampled	Foliage* symptoms %	Samples number		BNYVV infection %	Plants	
			Tested	ELISA positive		Root symptoms	*P.betae*
Kafrel-Sheikh							
El-Abassia	2	31	8	6	75	+	+
El-Raghama	2	33	8	4	50	+	+
Om-sen	2	23	8	5	62.5	+	+
Ahmd sliman	2	30	8	6	75	+	+
Ziedan	1	28	4	3	75	+	+
El-Abadiaa	1	33	4	2	50	+	+
Sub-total	10	29.7	40	26	65		
El-Behera							
El-Hossain	4	25	16	5	31.3	+	+
El-Emam	3	0.0	12	0.0	0.0	-	-
Mubark	3	0.0	12	0.0	0.0	-	-
Sub-total	10	8.3	40	5	12.5		
El-minia							
Abo-kurkas	3	0.0	12	1	8.3	-	-
Mallawy	3	0.0	12	0.0	0.0	-	-
Sub-total	6	0.0	24	1	4.1		
El-Dakahlia							
Belkas	10	12	40	12	30	+	+
Sub-total	10	12	40	12	30		
El-Gharbia							
El-Dawaklia	3	0.0	10	0.0	0.0	-	-
Kafrel-Abaida	3	0.0	15	0.0	0.0	-	-
Ketoor	4	0.0	15	2	13.3	-	-
Sub-total	10	0.0	40	2	5		
Total	**46**	**10**	**184**	**46**			

* Incidence of BNYVV was estimated by counting the symptomatic plants among 100 randomly chosen plants in the tested fields.

Table (2): Detection of BNYVV in soil samples by bait plants test

Governorate, Village	Samples number		Bait plants*	
	Tested	ELISA positive	Root symptoms	Mean *P.betae*** infection index
Kafrel-Sheikh				
El-Abassia	3	3	+	2.6
El-Raghama	3	3	+	2.1
Om-sen	3	3	+	2.3
Ahmd soliman	3	3	+	2.4
Ziedan	3	3	+	2.1
El-Abadiaa	3	3	+	2.2
Waste***	5	2	+	2.1
El-Behera				
El-Hossain	3	1	-	2.1
El-Emam	3	0.0	-	1.3
Mubark	3	0.0	-	1.1
El-minia				
Abo-kurkas	3	0.0	-	nd
Mallawy	3	0.0	-	nd
El-Dakahlia				
Belkas	3	3	+	2.2
El-Gharbia				
El-Dawaklia	3	0.0	-	1.4
Kafrel-Abaida	3	0.0	-	0.9
Ketoor	3	0.0	-	1.3
Control****	3	0.0	-	nd
Total	**50**	**24**		

* Plants grown in soils collected from around the symptomatic field grown sugar beet plants.
** Mean score for ten segments per plant according to Asher *et al.*, (2002).
*** Soils were collected from washing tank in sugar beet factory, Delta Sugar Company, Kafrel-Sheikh.
**** Sterilized soils.

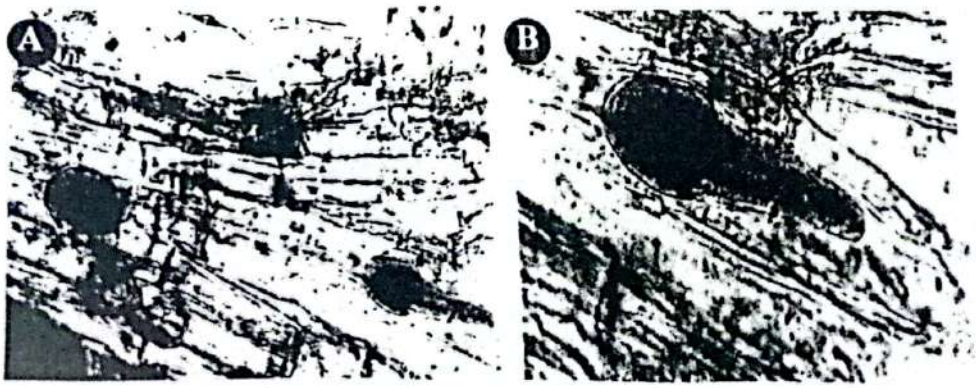

Fig (2): Light micrograph of sugar beet lateral root cells infected with *Polymyxa betae* A: cystosori with plasmodia in cortical cells (x400), B: plasmodia (x 1000).

Table (3): Symptoms and host range of *Beet necrotic yellow vein virus* (BNYVV).

Host plants	Symptoms	Back inoculation to *C. quinoa**	
		Inoculated leaves	Non-inoculated leaves
Fam: Chenopodiaceae			
Betae vulgaris L. cv Pleno	YV	+	+
B. vulgaris L. cv Tripl	CP	+	+
B. vulgaris L. cv.Gloria	CRS	+	-
B. vulgaris L. cv.Ras poly	CS	+	-
B. vulgaris L. var cicla	CS	+	-
B. macrocarpa Guss	YLL	+	-
B. maritema	CS	+	+
Chenopodium amaranticolor Coste & Reyn	CLL	+	-
C. quinoa Wild	CLL	+	-
C. murale L.	CS	+	-
Spinacea oleracea L.	SM	+	+
Fam: Solanaceae			
Capsicum annum L. cv. California	-	-	-
Lycopersicum esculentum Mill. cv. Peto 86	-	-	-
Datura stramonium L.	-	-	-
D. metale	-	-	-
Nicotana glutionsa L.	-	-	-
N. tabacum L. cv. White Burley	-	-	-
N. tabacum L. cv. Samsun	-	-	-
Petunia hybridae Vilm.	-	-	-
Fam: Fabaceae			
Phaseolus vulgaris L.	-	-	-
Vicia faba L.	-	-	-
Fam: Cucurbitaceae			
Cucumis sativus L. cv. Atlas	-	-	-
Cucurbita pepo L. cv. Eskandarani	-	-	-
Fam: Cruciferae			
Brassica nigra Koch	-	-	-
Capsell bursa-pastoris L.	-	-	-

YV=yellow veins, CP=chlorotic plotches, CRS=chlorotic ring spots, CS=chlorotic spots, YLL=yellow local lesions, CLL=chlorotic local lesions, SM=systemic mottling. +, exhibited symptoms and -, no symptoms.*=Back inoculation was conducted to *C.quinoa* as indicator host by using symptomatic and asymptomatic leaves taken from inoculated and non-inoculated leaves at the same plant.

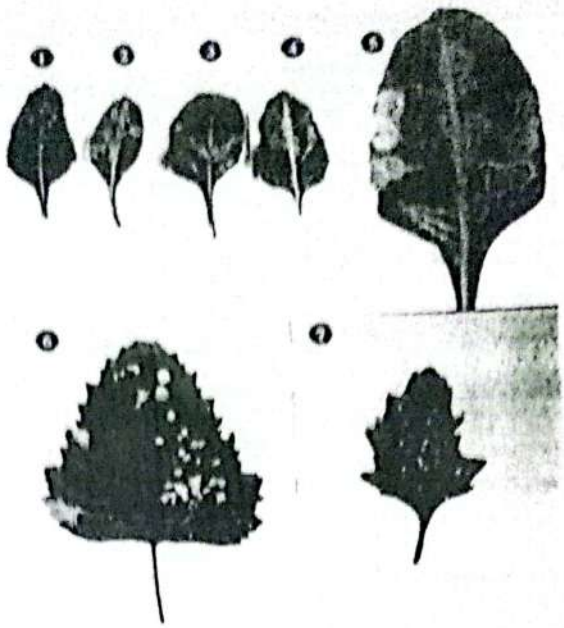

Fig (3): Symptoms induced in some test plants following inoculation with *Beet necrotic yellow vein Benyvirus* (BNYVV). (1) Chlorotic local lesions iduced in *Beta macrocarpa*. (2) Chlorotic blotches induced in *B.vulgaris* cv. Triple. (3) Circular pale green rings and surrounded by yellow halos in *B. vulgaris* cv. Gloria. (4) Yellow vein symptom in *B. vulgaris* cv. Pleno. (5) Close-up on inoculated leaf of *B. vulgaris* cv. Ras poly showed chlorotic blotches, it consisting of green pale centre with yellow halos. (6) Chlorotic local lesions in inoculated leaves of *C. amaranticolor*. (7) Local lesions developed on *C. quinoa* inoculated leaf.

Table (4): Efficacy of two transmission methods of BNYVV and effect on leaves and root dry weights in sugar beet under greenhouse conditions.

Treatments	Samples numbers		Infection %	Plant**		
	Tested	Positive*		*P.betae*	Weight (g)	
					Leaves	Root
Seed coating***	37	9	24.3	+	21.49 a	20.76 b
Control	20	0	0.0	-	23.72 a	38.34 a
Root vortexing****	28	15	53.6	+	19.59 a	18.26 b
Vortexed control	18	0	0.0	-	22.65 a	33.51 a
Transplanted control	20	0	0.0	-	23.66 a	36.94 a

* The presence or absence of BNYVV was confirmed by biological assay on *C.quinoa*.

** Means of the average of 9 single plant replications.

*** Sugar beet seeds were coated with *P.betae* and BNYVV infected root beet powders, while control one is uncoated.

**** Sugar beet seedlings were mechanically inoculated by root vortexing with mixture of carborandum and virus inoculum. Healthy controls consisted of seedlings vortexed with inoculums buffer and of seedlings transplanted without vortexing.

Also, from our results we can postulate that the virus is not adsorbed externally on its fungal vector spores but is internalized. These finding agree with Adams (1991). Also, virus identification was based on reactivity with specific polyclonal antibodies. Antiserum to C-terminal of the BNYVV coat protein was specific to BNYVV and did not cross react with the *Beet soilborne mosaic Benyvirus* whereas antisera to the core protein are cross-reactive (Wisler *et al.*, 1994). The present study has supported the occurrence of rhizomania by large-scale survey for all sugar beet

cultivated localities. We supported the epidemic spread of BNYVV and its vector (*P.betae*) in Kafrel-Sheikh Governorate. So that, the following are management practices that should be followed with vigilance once the disease is identified: by plant early (early planting helps to establish the crop before conditions are conductive for infection and disease spread); by deep tillage and solarization (for ensuring better soil aeration); by using plant resistant varieties (using plant resistant varieties as soon as the disease is identified in my own or neighboring fields); by avoid transplanting sugar beet into the fields (Blunt *et al.*,1992); by crop rotation (use a minimum 4 years crop rotation); by manage soil moisture (avoid excessive irrigation and ensure sugar beets are planted to fields that are well drained); and by site selection (investigate the field history before planting to ensure appropriate measures are taken).

ACKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to Dr. S.E.Bouzoubaa, Center National of Research Scientific, IBMP, Strasbourg, France, for donating of BNYVV polyclonal antibodies and Dr. M.H.Abdel-Ghaffar, Dept. of Agric. Microbiology (Virology Lab.), Fac. Of Agric., Ain Shams Univ., Cairo, Egypt, for assistance to kindly provides the BNYVV antibodies and for constructive comments on the first draft of this manuscript.

REFERENCES

Abdel-Ghaffar, M.H.; Salama, M.I. and Mahmoud, S.Y.M. (2003). Electron microscopy, serological and molecular studies on an Egyptian isolate of *Beet mosaic Potyvirus*. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo 11(2):469-484.

Abdel-Ghaffar, M.H. and Farrag, Eman, S.H. (2004). Detection and some characteristics of *Beet necrotic yellow vein Benyvirys* from rhizomania-affected sugar beet in Egypt. Egypt. J. Biotechnol. 18:215-232.

Abdel-Salam, A.M. and El-Shazly, Manal, A. (2001). Occurrence of rhizomania of sugar beet in Egypt associated with *Beet necrotic yellow vein Benyvirus* infection. Arab J. Biotech. 5(1): 135-150.

Abe, H. and Tamada, T. (1986). Association of *Beet necrotic yellow vein virus* with isolates of *Polymyxa beta* Keskin. Ann. Phytopathol. Soc. Jpn. 52: 235-247.

Abe, H. and Ui, T. (1986). Host range of *Polymyxa beta* Keskin strains in rhizomania-infected soils of sugar beet fields in Japan. Ann. Phytopayhol. Soc.Jpn. 52:394-403.

Adams, M.J. (1991). Transmission of plant viruses by fungi. Ann. Appl. Bio. 118:479-492.

Al-Chaabi, S.; Esmael, F.; Mando, J.; Darwesh, A.; Numan, S.; Matrod, L. and Al-Arabi, S. (2000). A survey of rhizomania disease on sugar beet and performance of mono embryonic cultivars to BNYVV infection in Syria. Arab J. Pl. Prot. 18: 1-8.

Asher, M.J.C. (1993). Rhizomania In: Cooke, D.A. and Scott, R.K.(Eds). The Sugar Beet Crop: Science into practice. Chapman and Hall, London. p. 311-346.

Asher, M.J.C.(1999). Sugar-beet rhizomania: the spread of a soilborne disease. Microbiology today 26: 120-122.

Asher, M.J.C.; Chwarszczynska, D.M. and Leaman, M. (2002). The evaluation of rhizomania resistant

sugar beet in UK. Ann. Appl. Biol. 141:101-109.

Barr, D.J.S. (1988). Zoosporic plant parasites as fungal vectors of viruses: taxonomy and life cycles of species involved. pp. 123-137 in: Development in Applied Biology 2, Viruses with Fungal Vectors, J.I.Cooper and M.J.C.Asher, eds. University of St. Andrews, UK.

Bennett, C.W. (1956). Sugar beet yellow vein disease. Plant Dis. Rept. 40: 611-614.

Blunt, S.J.; Asher, M.J.C. and Gilligan, C.A. (1991). Infection of sugar beet by *Polymyxa betae* in relation to soil temperature. Plant pathol. 40: 254-267.

Blunt, S.J., Asher, M.J.C. and Gilligan, C.A. (1992). The effect of sowing date on infection of sugar beet by *Polymyxa betae*. Plant pathol. 41: 148-153.

Canova, A. (1959). Appunti di patologia della barbabietola. Inform. Progr. Agri. 10:209-221.

Duffus, J.E.; Whitneg. E.D.; Larsen, R.C.; Liu, H.Y. and Lewellen, R.T. (1984). First report in Western hemisphere of rhizomania of sugarbeet caused by *Beet necrotic yellow vein virus*. Plant Dis. 68: 251. (Abstr.)

Duncan, B. D. (1965). Multiple range and multiple F test. Biometrics 11: 1-42.

Fujisawa, I.; Tsuchizaki, T. and Lizuka, N. (1982). *Bean yellow mosaic virus*, *Tabacco mosaic virus* and *Beet necrotic yellow vein virus* isolated from spinach. Ann. Phytopathol. Soc. Jpn. 48: 592-599.

Gao, J.L.; Geng, F.; Zabi, H.O.; Liang, X.S. and Lin, Y. (1993). The occurrence of sugar beet rhizomania caused by *Beet necrotic yellow vein virus* in China. Acta Phytopathol. Sinica, 13:1.

Gerik, J.S. (1989). Rhizomania: challenging problem faces California, Texas growers. Sugar prod. 15: 27-28.

Gerik, J.S. (1992). Zoosporic obligate parasites of roots. pp. 18-24 in: Methods for research on soilborne phytopathogeing fungus, (L.L.Singleton, J.D.Mihail and C.Rush, eds) American Phytopathological Soc., St. Paul MN., USA.

Gerik, J.S. (1994). Rhizomania-An update. Sugar beet update 4:10-12.

Gerik, J.S. and Duffus, J.E. (1987). Host range of California isolates of *Poymyxa betae*. Phytopathology 77: 1759. (Abstr.)

Harveson, R.M. and Ruch, C.M. (1993). An environmentally controlled experiment to monitor the effect of Aphanomyces root rot and rhizomania on sugar beet. Phytopathology 83:220-222.

Heidel, G.B; Rush, C.M.; Kendall, T.L.; Lommel, S.A. and French R.C. (1997). Characteristics of Beet soilborne mosaic virus, a furolike virus infecting sugar beet. Plant Dis. 81:1070-1076.

Henry, C.M.; Jones, R.A.C. and Coutts, R.H.A. (1986). Occurrence of soil-borne virus of sugar beet in England. Plant Pathol. 35: 585-592.

Henry, C.M.(1996). Rhizomania-its effect on sugar beet yield in the UK. Br. Sugar Beet Rev. 64:24-26.

Horvath, J. (1994). Beet necrotic yellow vein *Furovirus*.1. new hosts. Acta Phytopath. et Entomol. 29:109-118.

Hutchinson, P.J.; Henry, C.M. and Coutts, R.H.A. (1993). Mechanical inoculation of sugar beet roots with *Beet soil-borne virus* in the absence of *Polymyxa betae*. pp. 43-46 In: Proc 2nd Cymp.Intl. Work. Group plant viruses fungal vectors. C. Hiruki, ed. American Society of sugar beet Technologists, Denver, CO.

Ivanovic, M.; Macfarlane, I. and Woods, R.D. (1983). Viruses of sugar beet associated with *Polymyxa betae* pp. 189-190. Annual Report of Rothamsted Experimental station for 1982.

Izadpanah, K.; Hashemi, P.; Kamran, R.; Pakniat, M.; Sahandpour, A. and Masumi, M. (1996). Widespread occurrence of rhizomania-like disease of sugar beet in Fars. Iran. J. Plant Pathol. 32:155-157.

Jones, R.A.C. and Harrison, B.D. (1969). The behavior of *Potato mop-top virus* in soil and evidence for its transmission by spongospora subterranean (Wallr.)Lagerh. Annl. Appl. Biol. 63:1-17.

Juretic, N. and Mamula, D. (1998). First finding of *Beet necrotic yellow vein Furovirus* in Caroatia. Acta Bot. Croat. 55(56):1-6.

Koening, R. and Lesemann, D.E. (2000). Genus *Benyvirus*. In: Van Regenmortel, M. M. H. V.; Fauquet, C. M.; Bishop, D. H. L.; Carstens, E. B.; Estes, M. K.; Lemon, S. M.; Mariloff, J.; Mayo, M. A.; McGeoch, D. J.; Pringle, C. R.; Wickner, R. B. (Eds). Virus Taxonomy-Classification and Nomenclature of Viruses. (Seventh Report of the International Committee on Taxonomy of Viruses). Academic Press, San Diego, pp.917-922.

Kutluk, N.D.; Erkan, S. and Bicken, S. (2000). Weeds as hosts for rhizomania's agent. Z. Pflkrankh. Pflschutz. Sonderh. XVII, 167-171.

Langenberg, W.G. and Kerr, E.D. (1982). *Polymyxa betae* in Nebraska. Plant Dis. 66: 882. (Abstr.)

Masuda, T.; Kagawa, K. and Kanazawa, K. (1969). Studies on succession cropping of sugar beet.1. Some observations on the abnormal symptoms of sugar beet presumably due to succession cropping. Bulletin of sugar beet Research Supplement 11: 77-84.

Mouhanna, A.M.; Nasrallah, A.; Langen, G. and Schlosser, E. (2002). Survey for *Beet necrotic yellow vein virus* (the cause of rhizomania), other viruses and soil-borne fungi infecting sugar beet in Syria. J. Phytopathology 150:657-662.

Omar, R.A.; El-Kewey, S.A.; Sidaros, S.A. and Mahmoud, S.Y.M. (1994). Unusual strain of CMV affecting sugar beet in Egypt. Proc. 7th con. Phytopathol., 1-14.

Payne, P.A. and Asher, M.J.C. (1990). The incidence of *Polymyxa betae* and other fungal root parasites of sugar beet in Britain. Plant Pathol. 39:443-451.

Putz, C. (1977). Composition and structure of *Beet necrotic yellow vein virus*. J Gen. Virol. 35:397-401.

Rush,C.M. and Heidel, G.B. (1995). *Furovirus* diseases of sugar beets in untied states. Plant Dis. 78: 868-875.

Russo, M.; Martelli, G.P. and Franco, A.Di (1981). The fine structure of local lesions of *Beet necrotic yellow vein virus* in *Chenopodium amaranticolor*. Physiol. Plant Pathol. 19: 237-242.

Shaker, Maali, S. (2003). Effect of interaction between viral infection and fungal root-rot diseases on sugar beet plants. M. Sc. Thesis Fac. Agric. Cairo Univ. 116pp.

Tamada, T.(1975). *Beet necrotic yellow vein virus* CMI/AAB. Descriptions of plant viruses, 144:1-4.

Tamada, T. and Baba, T. (1973). *Beet necrotic yellow vein virus* from rhizomania-affected sugarbeet in Japan. Ann. Phytopathol. Soc. Jpn. 39: 325-331.

Tamada, T. (2002). Beet necrotic yellow vein virus. AAB Desciption

of Plant Viruses, No.291. [http://www.dpvweb.net/dpv/].

Taraku, N. and Juretic, N. (1990). Occurrence of *Beet necrotic yellow vein virus* in sugar beet in Kosovo. Acta Biol. Med. Exp. 15: 99-104.

Tuitert, G. (1993). Effect of conditions during storage of infested soil on infection of bait plants by *Polymyxa betae* and *Beet necrotic yellow vein virus*. Neth. J. of Pl. Pathol. 99:291-301.

Tuitert, G. (1994). Epidemiology of rhizomania disease of sugar beet. Ph.D. thesis, VIII-168pp. Wageningen Agricultural University, the Netherlands.

Van Regenmortel, M.H.V.; Fauquet, C.M.; Bishop, D.H.L.; Carstens, E.B.; Estes, M.K.; Lemon, S.M.; Maniloff, J.; Mayo, M.A.; McGeoch, D.J.; Pringle, C.R. and Wickner, R.B. (2000). Viruses Taxonomy: The classification and nomenclature of viruses. The seventh report of the international committee of taxonomy of viruses. Academic Press, San Dieyo. 1167 pp.

Vardar, F.B.; Erkan, S. (1992). The first studies on the detection of BNYVV in sugar beet in Turky. J. Turk. Phytopathology 21:71-76.

Wisler, G.C.; Liu, H.Y. and Duffus, J.E. (1994). *Beet necrotic yellow vein virus* and its relationship to eight sugar beet furo-like virus from the untied states. Plant Dis. 78:995-1001.

Yilmaz, N.D.K.; Yanar, Y.; Gunal, H. and Erkan, S. (2004). Effect of soil properties on the occurrence of *Beet necrotic yellow vein virus* and *Beet soil borne virus* on sugar beet in Tokat, Turkey. Plant pathol. J. 3(2):56-60