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Full Length Research Paper

Impact of soil salinity on fungal vector of rhizomania virus infecting *Beta vulgaris*

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***Polymyxa betae* Keskin is the only natural transmitting vector of the Beet necrotic yellow vein virus (BNYVV) among the cultivated sugar beet. This work aims to study the impact of salt stress on fungus-virus-host relationships. The fungal infected fine roots of sugar beet plant naturally infected with BNYVV were collected and treated with different salt concentrations (0, 2000, 4000, 6000 and 8000 ppm) of NaCl for one day prior to mixing it with a sterile soil. After virus symptoms appeared on leaves, disease severity has been determined, leave and roots tissues were collected for BNYVV detection. It was found that severe symptoms on sugar beet inoculated with treated roots by low salt concentrations (2000 and 4000 ppm) while at high salt concentrations, 8000 ppm less injury approximately as control treated with H_2O. On the other hand it was found the cystosorial colonization was increased in low salt concentrations (2000 and 4000 ppm) while decrease in high salt concentrations (6000 and 8000 ppm) especially in 8000 ppm. The same trend results were observed in virus concentration in roots where as the BNYVV concentration was increased in low salt concentrations (2000 and 4000 ppm) while decrease in high salt concentrations (6000 and 8000 ppm). So the relation between capacity of fungal vector to infect the plant by virus and salinity concentration are antagonistic. Soil salinity extremes most often lead to decrease infection of BNYVV via effect on fungal zoospore.**

Key words: *Polymyxa betae*, sugar beet, rhizomania, beet necrotic yellow vein virus (BNYVV), salinity.

INTRODUCTION

Polymyxa betae Keskin is an obligate parasite of sugarbeet roots and the plasmodiophorid vector of *Beet necrotic yellow vein virus* (BNYVV), which causes rhizomania disease. *P. betae* is found in almost all soils where sugarbeet is grown, spreading from plant to plant by means of motile zoospores and survive in the soil for many years for at least 15 years in the form of thicked-wall resting spores or cystosori (Davarani et al., 2013). Despite its ubiquitous distribution and parasitic habitat, *P. betae* is generally considered to cause relatively little damage in temperate climates, although it may be pathogenic in areas of the world where sugar beet is grown in warm soils (Blunt and Gilligan, 1991). *P. betae* can cause stunting and necrosis in lateral roots

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(Filiz et al., 1998) .In contrast, rhizomania disease causes severe economic losses in many countries and is spreading into new regions (McGrann, 2009). *P. betae* is not truly considered as pathogen but as vector of sugarbeet viruses, and it plays crucial role in the epidemiology of viral disease (Ourania et al., 2011).

The virus is carried internally by *P. betae* resting spores in the soil. *P. betae* Keskin has an important role in the transmission and distribution of rhizomania (Delbianco, et al. 2013). The relationship between inoculum potential and the actual inoculum density of viruliferous *P. betae* population is currently unknown (McGrann, 2009).

P. betae Keskin is the only natural transmitting agent of the rhizomania disease caused by *Beet necrotic yellow vein virus* (BNYVV) among the cultivated sugar beet (Safarnejad et al., 2013). Chemical control methods against the vector are either too expensive (methyl bromide soil disinfection) or ineffective. The only useful control measure is the growing of tolerant varieties (Richard-Molard, 1996).

Soil extremes most often lead to problems for plant growth and thus, indirectly for virus accumulation, a saline soil condition has been shown to specifically decrease the accumulation of *Beet necrotic yellow vein virus* (BNYVV) in sugar beets (Bartsch and Brand, 1998).

Liu et al. (2009) reported that soil extremes most often lead to problems for plant growth and thus, indirectly for virus accumulation, a saline soil condition has been shown to specifically decrease the accumulation of beet necrotic yellow vein virus (BNYVV) in sugar beets. Bartsch and Brand (1998) found that saline soil condition decreases rhizomania infection of *B. vulgaris* and concluded that it is still uncertain whether soil salinity operates directly on BNYVV multiplication or causes inactivation of vector zoospores or, influences endogenous plant factors such as root physiology and morphology.

The relationship between inoculum potential and the actual inoculum density of viruliferous *P. betae* population with salinity is currently uncertain, as well as whether soil salinity causes inactivation of vector zoospores or not. So the aim of this work was to study salinity potential on fungal vector of Rhizomania virus infecting *B. vulgaris.* The research work presented in this manuscript was conducted to evaluate effect of salinity potential on capacity of *P. betae* vector to infect *Beta vulgaris* by virus under different levels of salt (NaCl) in the greenhouse.

MATERIALS AND METHODS

Detection of fungal zoospore

Heavily roots from naturally BNYVV infected sugar beet plants were harvested from infested field kafr el-sheikh government (Egypt). Samples were obtained from soil surrounding sugar beet roots exhibiting rhizomania syndrome. These roots were cleaned from soil and washed with tap water then by calcium hypochloride solution (Clorox 5%), and sieved by 50 micro sieve. The roots were examined by optical microscope for the presence of cystosori of *P. betae* and the fungal structures. The tested roots were randomly selected and stained in lacto-phenol solution after boiling and examined with light microscope at 40x magnification.
Preparation of *P. betae* inoculums is as follows:

1. Fine roots of the naturally infected plant carrying zoospore were cut into small pieces 1.5 to 2.0 cm length consisting of the tap root or/and lateral roots attached to it and divided to five equal parts into 5 g.
2. The parts were then immersed in different salt concentration (0, 2000, 4000, 6000 and 8000 ppm) of NaCl in Petri dishes for one day.
3. Preparation of sterile soil was done by using 5% formalin for 2 weeks.

Experimental design

B. vulgaris var. Samba was obtained from Sugar Crops Research Institute, Egypt. Two grams of infected root were added to each pot of sterile soil. Five seeds of sugar beet were sown in a sterile soil in each pot. The relative humidity was approximately 70%. The plants were kept in greenhouse. After virus symptoms appeared on leaves, disease severity, tissue print immuno assay and dot plot immuno assay (TPIA and DPIA) were carried out for virus detection.

Detection of virus infection

Disease severity measurement

The percentage of infected plants and the severity of symptoms were examined using the following rating scale: For leaves (0 = no symptoms; 1,2 = chlorotic and mild mosaic; 3, 4 = Vein yellowing; 5, 6 = necrotic on leaf or petiole; 7,8 = leaf malformation). For roots [0 = no symptoms; 1,2 = root cracks; 3, 4 = root darkness by cutting (oxidation); 5,6 = root atrophy (small in size); 7,8 = root proliferation (beard shape)]. Disease severity (DS) values were calculated using the following formula according to Xicai et al. (1996):

$$DS\,(\%) = \frac{\sum(\text{disease grade} \times \text{number of plants in each grade})}{(\text{total number of plants} \times \text{highest disease grade})} \times 100$$

Serological technique

Dot plot and tissue print immunoassay

The systemic spread of BNYVV within sugar beet leaves were analyzed using DPIA and TPIA carried out exactly as described by Kaufmann et al. (1992) onto positively charged cellulose membranes. BNYVV were detected by specific antibodies with a chromogenic substrate reaction, leading to a bluish colour development on the blot surface if virus particles were present.

Root sampling

The remaining part of the roots was fixed in lactophenol after boiling for microscopic examination for detection of cystosorial.

Assessment of cystosorial colonization and infection development

The number of cyctosorial and infection percentages were

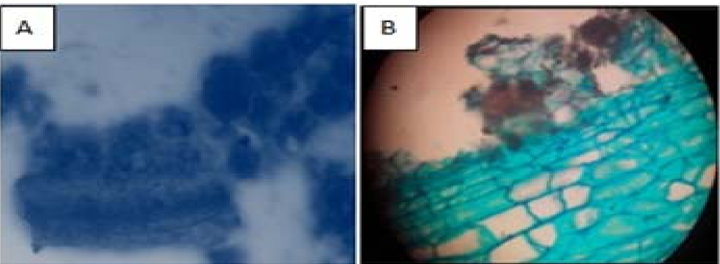

Figure 1. Cross section showing disease manifestation of sugar beet lateral roots upon infection with viruliferous *P. betae* Keskin (A) *P. betae* infected roots stained with lactophenol blue associated with cystosori (B) Transection of sugar beet root.

determined using modified method of (Duc 1989). The presence of cystosorial infection in sugar beet roots were estimated by observation of 10 cleared and stained 1 cm root segments under the microscope for every treatment. For the quantitative characterization of root cystosorial the following parameters were used: Colonization frequency (F%): The percent of roots with fungal structures (cystosori). Colonization intensity (M%): The percent of colonized cortex (cystosoria) in each root were determined according to Duc (1989).

Virus assay

Determination of BNYVV concentration in fungal infected roots. Some leaves of sugar beet plant of all treatments were taken and ground in a mortar containing 0.1 M phosphate buffer, pH 7.0 (1:2 w/v). The homogenate was filtrated through two layers of muslin. The leaves of healthy *Ch. quinoa* plants were dusted with carborundum and rubbed gently with a cotton swab previously dipped into the suspension of virus inoculum.

RESULTS

Virus and fungal incidence in naturally infected sugar beet

Naturally BNYVV infected sugar beet collected from infested field in Kafer El-Shek government exhibited disease syndrome typical to rhizomania virus where as leaves show narrow blade and long petioles. In some instances foliar proliferation of small leaves are observed in the root crown area.

Light microscopy examination cross section of infected sugarbeet roots were observed for presence of fungal cystosori associated with root cell death. Cystosori can be seen lined up on the external and internal layers (Figure 1).

Detection of disease elements in infected sugarbeets

BNYVV detection

Foliar symptoms: Infected sugar beet plants exhibited disease syndrome typical to rhizomania of sugar beet infected leaves show narrow blade and long petioles (Figure 2). In some instances foliar proliferation of small leaves are observed in the root crown area.

Root symptoms: Disease symptoms of infected roots vary greatly depending on the inoculums treatment. The invading fungus causes the killing of secondary roots and even the young tap roots lead to root proliferation and induce symptoms known as the bearded roots. This can be used as positive identification for rhizomania upon making longitudinal section in infected tap roots.

Serological detection of BNYVV: Infected sugar beet plants were confirmed serologically by DPIA and TPIA technique. The results were revealed as purplish blue color developed with specific polyclonal antibody of BNYVV compared with printing from healthy plants which remain green in the negative reactions (Figure 2).

The BNYVV infected sugar beet plants were confirmed by DPIA and TPIA technique. The results revealed different color intensity where *P. betae* infected roots without salt treatment give high density color. The color density was decreased in salt treatments by increasing level of NaCl where it revealed low color density with 8000 ppm revealed moderate color density at 4000 and 6000 ppm and revealed high color density with 2000 ppm (Figure 2).

Viral disease severity

It was observed different external symptoms. The plant treated with *P. betae* infected roots without salt treatment show severe symptoms as chlorosis, vein yellowing and vein necrosis with long petiole with disease severity 65% comparing to non sterile soil which give 10% only. The plant treated with *P. betae* infected roots treated with different concentration of salt show decrease in symptom by increasing concentration of salt Figures 2 and 3 with 58, 35, 27 and 20% for 2000, 4000, 6000 and 8000 ppm

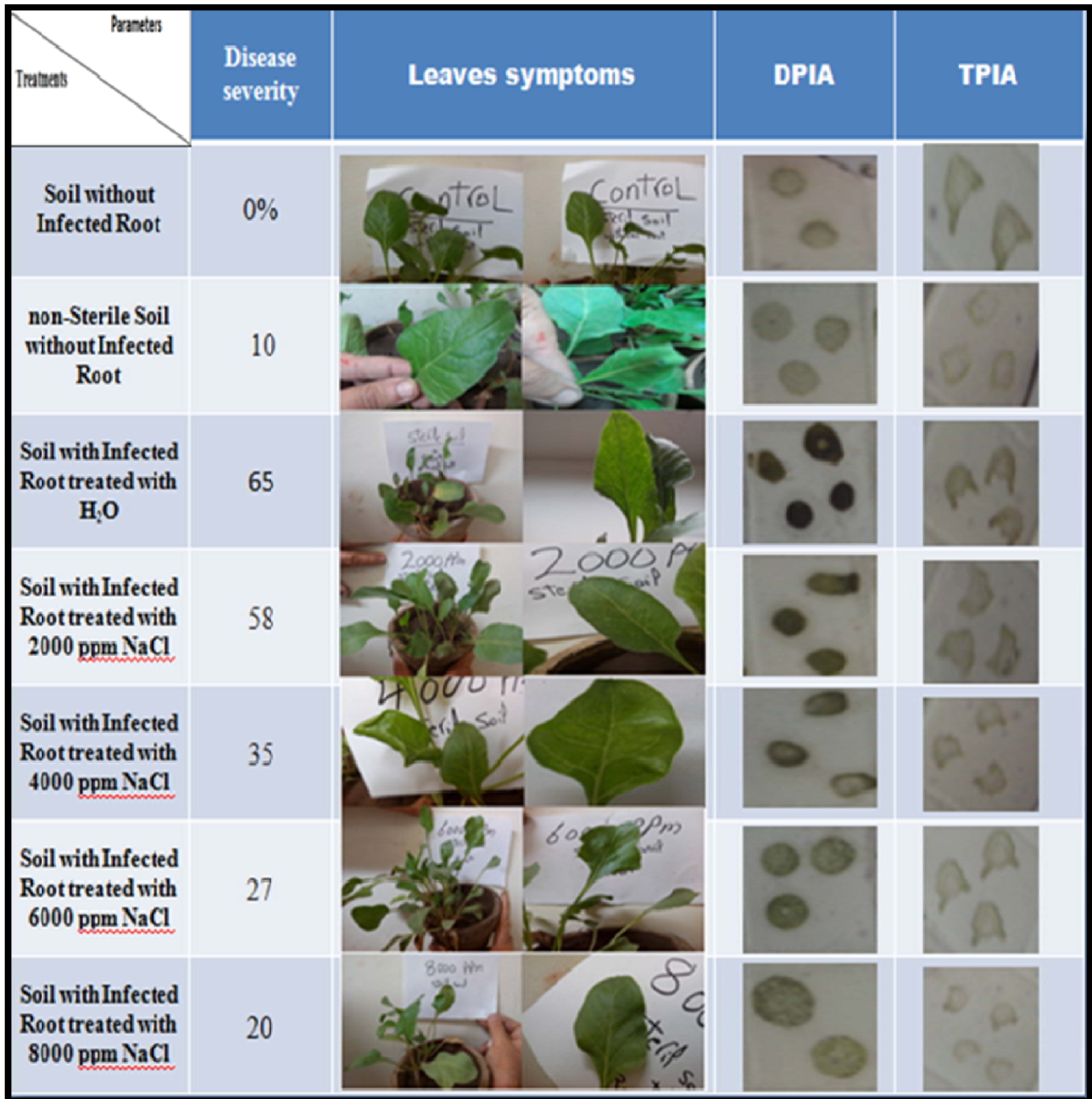

Figure 2. Disease severity percentage of BNYVV infecting sugar beet treated with different concentration of salt growing in sterile soils.

NaCl, respectively (Figures 2 and 4).

P. betae detection in roots of infected sugar beets

Microscopic examination of infected sugar beet roots from plants grown in inoculated soil with different fungal inoculums treatments revealed the presence of fungal cystosori (Table 1 and Figure 5). The degree of infection depended on the number of cystosori in the roots. The infection was greatest in the roots treated with water treatment followed by treatment of salt concentration 2000, 4000, 6000 and 8000 ppm.

The infection cystosorial frequency with *P. betae* were 65, 75, 54, 45, 35 and 8% for non-sterile soil without infected root, root treated with H_2O, root treated with 2000 ppm NaCl, root treated with 4000 ppm NaCl, root treated with 6000 ppm NaCl and root treated with 8000 ppm NaCl respectively (Table 1).

P. betae infection colonization intensity was changed with salt treatments. The P. *betae* infection was high percentage in infected root treated with H_2O with 64.6% while infected root treated with different concentrations of salt were revealed different level of infection 35.5 and 9.25% with 2000 and 8000 ppm, respectively.

The determination of the virus titer as a local lesion showed a continuous decrease in BNYVV content (by decrease in number of L.L with increase salt

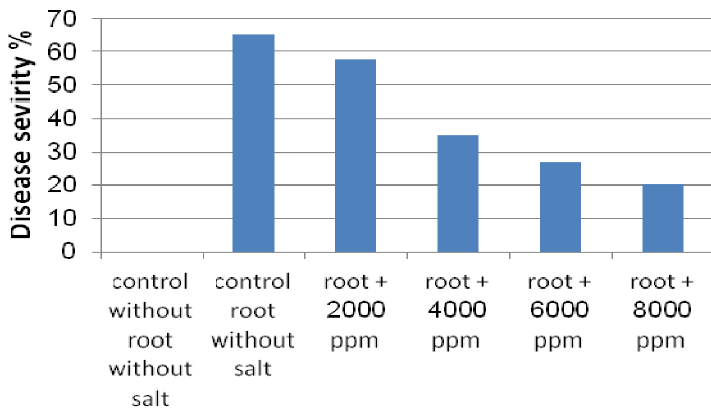

Figure 3. Histogram showing decrease in the disease severity of BNYVV infecting sugar beet with increasing NaCl concentration.

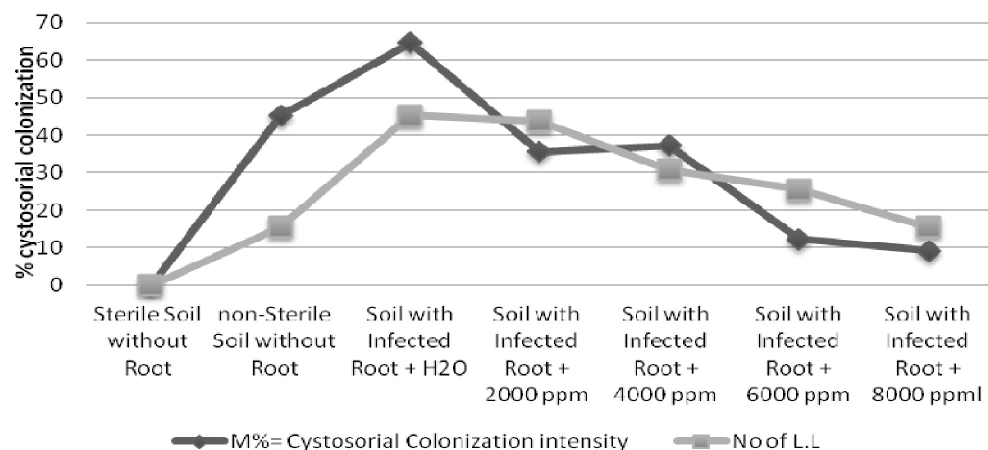

Figure 4. Relation between cystosorial colonization intensity and BNYVV concentration as a LL.

concentration) (Figures 4 and Table 2).

Relation between M%= Cystosorial Colonization intensity and BNYVV concentration as a L.L

It was found a relationship between cystosorial colonization intensity and BNYVV concentration as a L.L (Table 2 and Figure 4). The virus concentration and the fungal cystosorial density in roots treated with high salt concentration was decreased and reached the lowest level especially in case of 8000 ppm NaCl treatment. On the contrary, it was found that the cystosorial fungus density and virus L.L increase in the case of treatment of infected root without salt.

DISCUSSION

The study of the impact of soil salinity on fungal vector of rhizomania virus infecting *B. vulgaris* are very rare, despite the record of the disease in a number of Arab countries in Egypt (Mahmoud and Hashem, 2005), Syria (Mouhanna et al., 2002) and Lebanon (Choueiri et al., 2001). The study came to the importance of fungus spread in most of the land, especially governorates of Kafr el-Sheikh, Fayoum (Megahad, 2013). Naturally infected sugar beet harvested from infested field in kafer el-shek government exhibited disease syndrome typical to rhizomania of sugar beet infected leaves show narrow blade and long petioles. In some instances foliar proliferation of small leaves are observed in the root crown area.

Microscopic examination showed the infection of beet plants grown in soil collected from Governorate Kafr el-Sheikh by *P. betae*. The results were confirmed by serological tests.

Examination of infected lateral roots of sugarbeet plants showed the presence of cystosori of *P. betae* Keskin outside and inside root tissues. Root necrosis and tissue degradation were associated with the presence of cystosori as described by Rush and Heidel (1995).

Table 1. Effect of different concentration levels of salinity on *P. betae* infection of sugar beet growing in sterile soils.

Treatments	Parameter	
	F%	M%
Soil without infected root	0	0
Non-sterile soil without infected root	65.72	45.2
Soil with infected root treated with H_2O	75.75	64.6
Soil with infected root treated with 2000 ppm NaCl	54.25	35.5
Soil with infected root treated with 4000 ppm NaCl	45.72	37.25
Soil with infected root treated with 6000 ppm NaCl	35.2	12.25
Soil with infected root treated with 8000 ppm NaCl	8.22	9.25

F%= Cystosorial colonization frequency M%= cystosorial colonization intensity.

	No of infected plants	Disease severity	Fungal density	Root under microscope
Soil without Infected Root	0/20	0%	-	
non-Sterile Soil without Infected Root	2/20	10	±	
Soil with Infected Root treated with H_2O	20/20	65	++++	
Soil with Infected Root treated with 2000 ppm NaCl	15/20	58	+++	
Soil with Infected Root treated with 4000 ppm NaCl	12/20	35	+++	
Soil with Infected Root treated with 6000 ppm NaCl	8/20	27	++	
Soil with Infected Root treated with 8000 ppm NaCl	5/20	20	+	

Figure 5. Cystosorial colonization and disease severity under different concentration levels of salinity on *Polymxa betae* infection of sugar beet growing in sterile soils. **-:** There was no presence of the fungal cystosori. **+/-** : Presence of fungus is very low where it was observed with difficulty (at a rate of one cystosori in many fine roots). **+**: Low infection (at a rate of 1-2 cystosori in single fine roots). **++**: Moderate infection (at a rate of 3-6 cystosori in single fine roots). **+++**: High infection (at a rate of 7-10 cystosori in single fine roots). **++++**: Severe infection (the single fine root full with cystosori).

It was recorded infection by *P. betae* with the spread of rhizomania disease either directly or indirectly, as the presence of the disease considered as definitive evidence of the existence of *P. betae* in the soil, while the presence of the *P. betae* does not necessarily mean the presence of the virus, which infects beet plants (Eckart, 1997)

Here the results in this study indicate the possibility of transmission of the fungal cystosorial from infected beet roots in the soil to the healthy roots and these results are agreed with Abe and Ui, (1986), Gerik and Duffus (1987) and Goffart et al. (1989).

Microscopic examination showed that beet root after 10 weeks age proved to be infected by *P. betae*. The

Table 2. Determination of virus titre as a local lesions:

Treatments	Parameter	
	No of L.L	Development of L.L
Soil without infected root	0	
Non-sterile soil without infected root	15.5	
Soil with infected root treated with H_2O	45.3	
Soil with infected root treated with 2000 ppm NaCl	43.7	
Soil with infected root treated with 4000 ppm NaCl	30.8	
Soil with infected root treated with 6000 ppm NaCl	25.5	
Soil with infected root treated with 8000 ppm NaCl	15.5	

Number of L.L was calculated as a mean of five replicate.

number of infected plants were varied because of the density of cystosori of *P. betae* or may be due to some replicates did not have the sufficient inoculums to occur infection. The highest density of cystosori of *P. betae*, as possible in sterile soil with infected root treated with H_2O, where it is lowest in treatment of high salt concentration 8000 ppm.

The infection cystosorial frequency with *P. betae* were 65, 75, 54, 45, 35, 8% for non-sterile soil without infected root, root treated with H_2O, root treated with 2000 ppm NaCl, root treated with 4000 ppm NaCl, root treated with 6000 ppm NaCl and root treated with 8000 ppm NaCl respectively (Figure 5).

The frequency of virus infection depends on environmental conditions and inoculum densities of the viruliferous population of *P. betae* (Rush, 2003). The optimum temperature for infection with P. betae is 25°C (Asher and Blunt, 1987**).** It has been reported that where BNYVV reaches highest inoculums density in naturally infected soils there is usually greater colonization of the root system, and the virus that infects roots first usually reaches highest levels (Rush, 2003).

P. betae infection colonization intensity was changed with salt treatments. *P. betae* infection was high percentage in infected root treated H_2O with 64.6% while infected root treated with 2000 ppm NaCl was revealed high infection with 2000 ppm 35.5% compared to 9.25% with 8000 ppm.

From literature it was found that 31.4% of the roots were infested with aviruliferous *P. betae* cystosori while 14.3% of the roots did not contain any cystosori (Kutluk Yilmaz and Sokmen, 2010).

It was found that high salt concentration causing decrease in the cystosorial fungus density and virus concentration. It was also appeared to be a strong correlation between the number of attached zoospores and the virus content of the roots. Although this tends to indicate that attachment of viruliferous zoospores measurably and directly increase BNYVV-content, multiplication of the virus on its own cannot be excluded. According to Koenig and Stein (1990), once introduced the virus is able to spread throughout the plant without the aid of its vector. Nevertheless, these results shed new light on the role the vector plays in the course of the disease. To achieve a better understanding of the complex interactions between host, virus and vector further investigations are required.

The cystosorial colonization was increased in low salt

concentration (2000 and 4000 ppm) while decrease in high salt concentration (6000 and 8000 ppm) especially in 8000 ppm. The same result was observed in virus concentration in roots where as the virus concentration was increased in low salt concentration (2000 and 4000 ppm) while decrease in high salt concentration (6000 and 8000 ppm). So the relation between capacity of fungal vector to infect the plant by virus and salinity concentration are antagonistic. Soil salinity extremes most often lead to decrease infection of BNYVV via effect on fungal zoospore.

Conflict of Interest

The authors have not declared any conflict of interest.

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