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Antagonistic effects of natural *Pseudomonas putida* biotypes on *Polymyxa betae* Keskin, the vector of *Beet necrotic yellow vein virus* in sugar beet

Antagonistische Wirkung natürlicher Biotypen von *Pseudomonas putida* gegenüber *Polymyxa betae* Keskin, dem Vektor des Rizomaniavirus

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Summary

This *in vivo* study investigated the ability of fluorescent *Pseudomonas* spp. to suppress *Polymyxa betae*, a vector of *Beet necrotic yellow vein virus* causing rhizomania in sugar beet fields. For this purpose, the *Pseudomonas putida* biotype A (PpA) and biotype B (PpB) were isolated from sugar beet growing areas in Samsun, Turkey and with indicated suppression of *P. betae* were used against *P. betae* as a biocontrol agent. Firstly, PpA and PpB were applied to the roots of sugar beet seedlings of rhizomania-susceptible (cv. Arosa) and partially resistant (cv. Leila) cultivars. Statistical differences were not significant among PpA, PpB, Leila and Arosa cultivars within rhizomania infested soil treatments, and there were also no significant differences among PpB and control groups ($P > 0.05$). In contrast, the partially resistant cultivar Leila with rhizomania free soil, PpB and negative control were significantly different from the other treatments ($P < 0.05$). Furthermore, numbers and diameters of the resting spores were significantly reduced by PpA and PpB treatments. In the treatments with PpA and PpB, a positive effect was observed on sugar beet weight and growth.

Key words: biocontrol agents, *Polymyxa betae*, *Pseudomonas putida* biotype A, *Pseudomonas putida* biotype B, rhizomonia

Zusammenfassung

Die antagonistische Wirkung natürlicher Biotypen von *Pseudomonas putida* gegenüber *Polymyxa betae* Keskin, dem Vektor des Rizomaniavirus der Rüben, wurde *in vivo* untersucht. Zu diesem Zweck wurden die Biotypen A und B von *Pseudomonas putida* im türkischen Zuckerrübenanbaugebiet von Samsun isoliert und ihr antagonistisches Potenzial gegenüber *P. betae* untersucht. Wurzeln Rizomania-anfälliger (cv. Arosa) und teilresistenter (cv. Leila) Zuckerrübensämlinge wurden mit den Biotypen A und B von *P. putida* inokuliert. Die Unterschiede zwischen den Biotypen A, B und den beiden Rübensorten waren in den Rizomania-Varianten statistisch genauso wenig signifikant ($P > 0.05$) wie die Unterschiede zwischen dem Biotyp B und der Kontrolle. Im Gegensatz dazu unterschieden sich die teilresistente Sorte Leila, der Biotyp B von *P. putida* und die negative Kontrolle in den Rizomania-freien Varianten signifikant ($P > 0.05$) von allen anderen Behandlungen. Die Anzahl und Größe der Dauersporen von *P. betae* waren darüber hinaus nach einer Behandlung mit den Biotypen A oder B vermindert, während die Wachstumsraten und das Gewicht der Zuckerrüben in den mit den Antagonisten behandelten Varianten erhöht waren.

Stichwörter: bakterielle Antagonisten, *Polymyxa betae*, *Pseudomonas putida* Biotyp A, *Pseudomonas putida* Biotyp B, Rizomonia

1 Introduction

Rhizomania is a worldwide serious disease of sugar beet (*Beta vulgaris* var. *saccharifera*) caused by *Beet necrotic yellow vein virus* (BNYVV) (Tamada 1975). This benyvirus is transmitted by zoospores of the soilborne protist *Polymyxa betae* Keskin (Keskin 1964). The symptoms of the disease vary, with some infected plants appearing healthy. General foliar symptoms are similar to water stress or nitrogen deficiency. Typical symptoms of the disease are characterized by a massive proliferation of lateral roots beneath an enlarged crown, constricted growth of tap root and severe stunting of the whole plant, resulting in great reduction in the sugar content. Resting spores (cystosori) of *P. betae* containing virus particles survive in soil for many years and this can raise difficulties in attempts to successfully control the disease (Rush and Heidel 1995).

Once a field becomes infested, crop rotation will not appreciably reduce disease risk because of the long-term survival of viruliferous cystosori. However, some soil fumigants, such as 1,3-dichloropropene, may kill enough cystosori to reduce disease development to acceptable levels. But fumigation treatments are very expensive, and research is being done to determine their efficacy and conditions under which they should be used. The use of soil-applied fungicides has not been effective for rhizomania control in infested fields (Wisler and Duffus 2000). Therefore, there is a worldwide effort to adopt the environmentally-friendly control practice strategies of sustainable agriculture to manage the disease, such as early sowing, proper irrigation, and breeding resistant varieties (Asher and Kerr 1996; Scholten and Lange 2000). Several plant breeding companies have developed cultivars partially resistant to BNYVV, originating mainly from the Rz1 gene (Holly-1-4) as described by Pelsy and Merdinoglu (1996) and in a later phase also Rz2 the wild beet accession WB42 from *B. vulgaris* subsp. *maritima* (Scholten et al. 1996). Some of these cultivars have shown a variable response in yield when grown in different countries. This could be caused by variation in pathogenicity due to differences in the RNA of BNYVV, as reported by Tamada et al. (1989).

Application of biocontrol agents for plant pathogens appears to be another promising alternative. The ability of certain saprophytic fungi (*Fusarium*, *Aspergillus* and *Trichoderma* strains) and bacteria (*Streptomyces*, *Bacillus* and fluorescent *Pseudomonas* strains) to protect plants against several plant pathogens through competition, parasitism and antagonism is well known (Utkhede et al. 1992; Alabouvette and Steinberg 1995; Siddiqui et al., 2001; Janisiewicz and Korsten 2002). Among those, fluorescent *Pseudomonas* spp. are soil microorganisms and important members of the microflora in the rhizosphere of many crop plants. Certain strains of fluorescent *Pseudomonas* spp. suppress plant diseases (*Fusarium* spp., *Pythium* spp., *Rhizoctonia* spp.) by inducing resistance, and protecting seeds or roots from infection by soilborne fungal and bacterial pathogens (Kloepper et al. 1980; Mukerji and Garg, 1988a; Mukerji and Garg 1988b; Keel et al. 1989;

Mahaffee and Kloepper 1997). Some strains of fluorescent *Pseudomonas* spp. produce mycolytic enzymes (β-1,3-glucanases, β-1,4-glucanases and lipases), siderephores (pyoverdin) and several secondary metabolites with antimicrobial activity, such as diacetylphloroglucinol, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin hydrogen cyanide, 2,4-diacetylphloroglucinol and pyoluteorin (Meyer et al. 1992; Zhang et al. 1998; Walsh et al. 2001; Raaijmakers et al. 2002).

The application of fluorescent *Pseudomonas* isolates in biocontrol may be advantageous or even desirable because they are adapted to specific environmental conditions. Equally important is the fact that native strains do not represent a foreign element potentially endangering local biodiversity (Mahaffee and Kloepper 1997; Raaijmakers et al. 2002). For this reason, the objectives of this research were to examine *Pseudomonas putida* biotypes with known activity against soilborne fungal pathogens for their efficacy in controlling *P. betae*, as well as to isolate potential new antagonists from the rhizosphere and roots of field grown sugar beet plants and screen them for their biocontrol capabilities. This research was conducted as a first step toward the development of effective biological control as a strategy for the management of *P. betae* transmission of sugar beet rhizomania.

2 Materials and methods

2.1 Soil sampling

A total of 30 soil samples was collected from sugar beet fields during soilborne virus surveys in the province of Samsun in Turkey in 2006 (Fig. 1). From five to 12 subsamples were randomly collected in each field and mixed. About one kg of soil sample was taken to a depth of 0–20 cm per location. The subsamples from a single sampling area were then thoroughly mixed and stored in sterile polyethylene bags at 4°C until processing (Grunewald et al. 1983). Later, these soil samples were used for the isolation of fluorescent *Pseudomonas* isolates.

2.2 Isolation of fluorescent Pseudomonas spp.

Stored soil samples were used to obtain fluorescent *Pseudomonas* isolates. Firstly, each soil sample was sieved through a 1 mm mesh sieve, mixed at a ratio of 1:10 with sterile distilled water and shaken thoroughly on a rotary shaker at 150 rpm at 24 ± 2°C for 60 min; then serial dilutions (10^3 –10^4) were prepared. Diluted samples were placed on King's B Agar (KBA) (Sands and Rovira 1970) and incubated at 24–26°C for 24–48 hours. Bacterial colonies were examined under a fluorescent light with a long wave length (366 nm) ultraviolet lamp for identification of fluorescent strains and further identification was based on colony morphology and fluorescent character, according to standard diagnostic methods (Lelliott and Stead 1987; Braun-Kiewnick and Sands 2001). At the end of this study, two fluorescent *Pseudomonas* isolates were identified by using the computer-assisted microbial identification system (MIS) which employs gas-liquid chromatographic analysis of bacterial fatty acids (Song et al. 2000).

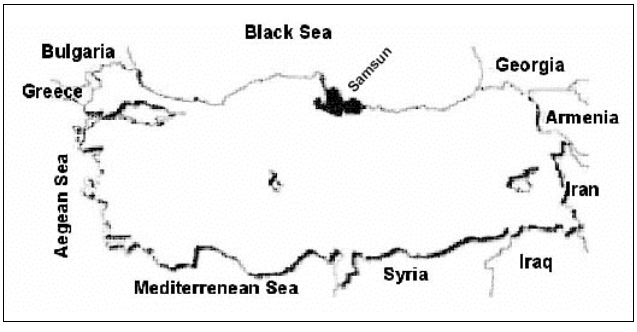

Fig. 1: Sugar beet survey area in Samsun Province of Turkey (dark colored). A total of 30 soil samples was collected from sugar beet fields during soilborne virus surveys in 2006.

2.3 Biocontrol activity

Natural fluorescent *Pseudomonas* isolates obtained from sugar beet production areas from Samsun province were used in biocontrol experiments. These isolates were grown on KBA at 24–26°C for 24–48 h. The whole culture suspensions of the isolates were diluted with deionised sterile water to produce a population of 10^9 colony forming units (cfu) per ml.

The treatments in test consisted of: (i) fluorescent *Pseudomonas* isolate number 2 (FPin2) + Arosa + BNYVV-infested soil, (ii) fluorescent *Pseudomonas* isolate number 5 (FPin5) + Arosa + BNYVV-infested soil, (iii) FPin2 + Leila + BNYVV-infested soil, (iv) FPin5 + Leila + BNYVV-infested soil, (v) Arosa + BNYVV-infested soil, (vi) Leila + BNYVV-infested soil, (vii) Arosa + non-infested soil, (viii) Leila + non-infested soil.

The lateral roots of sugar beet seedlings (4-6 weeks-old) of the rhizomania-susceptible cultivar (cv. Arosa) and partially resistant cultivar (cv. Leila) were pruned with a scalpel to facilitate entry of the isolates in order to induce systemic resistance. The roots of sugar beet seedlings were soaked in 50 ml of bacterial suspension containing 10^9 cfu ml^{-1} for 2 h at room temperature for the treatments 1 to 4. For the control treatments 5 to 8, the pruned roots of seedlings were soaked in 50 ml of deionised sterile water for 2 h. Afterwards, five sugar beet seedlings were planted into 250 ml pots containing a mixture of soil originating from a field heavily infested with BNYVV from Samsun province and sterile sand (1:1, soil:sand, by weight).

The pots were placed in sterilized plastic saucers spaced on greenhouse benches to avoid contamination by water splashing between pots. They were randomly placed into a growing chamber at 24 ± 2°C, relative humidity 70%, daylight 16 h and 8 h dark conditions, and watered with tap water as needed. After a growing period of 60 days, each pot was harvested separately, the roots of plants were washed with tap water and, total fresh plant and root weights were measured. Later, the root samples were divided into two parts: one was used to test for the presence of rhizomania by the DAS-ELISA test and the other for *P. betae*. These root samples were frozen at −20°C until used.

The experiment was carried out twice in a randomized plot design and five replications.

2.4 Serological tests

The roots of sugar beet plants were tested for the effects of fluorescent *Pseudomonas* isolates against the presence of rhizomania (BNYVV), using the double antibody sandwich ELISA (DAS-ELISA), according to Clark and Adams (1977), except that the extraction buffer included 0.1% nonfat dry milk instead of bovine serum albumin (Arif et al. 1994). The antisera for BNYVV determination were supplied by Loewe Biochemica, Sauerlach, Germany. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grödig/Salzburg, Austria). Samples were considered to be positive when the absorbance at 405 nm (A_{405}) values exceeded the mean of the negative controls by at least a factor of two (Meunier et al. 2003).

2.5 *Detection of* Polymyxa betae

Rootlet samples were taken from the bait plant test, washed to remove soil debris, and stained with lactophenol containing 0.1% acid fuchsin. The presence or absence of *P. betae* was determined by observing the amount of resting spore clusters in 10 pieces of the rootlets of each plant under a Leica light microscope and photomicroscope system (Type DMLS2) (40X objective) (Abe and Tamada 1986). To determine the numbers of resting spores in the lateral roots, plants were removed and bulked. The root samples of each treatment was ground with a pestle and mortar, suspended in 10 ml sterile distilled water containing 10 µl Tween 20 and passed through 320 µm nylon mesh to remove any remaining large root fragments. The number of *P. betae* resting spores in the suspension was determined using a haemocytometer counting slide. Because of variation in the number of component cysts per cystosorus, the total of number of individual cysts per 1 µl was counted. The resting spore numbers were counted with a haemocytometer and their diameter were measured using a light microscope (Asher et al. 2002).

2.6 *Statistical analysis*

All data were analysed using the General Linear models (GLM) procedure of SPSS 11.0 statistical software (SPSS Inc., Cary, NC, USA) using the following model, a completely randomized block design or Two-way ANOVA where $\hat{Y}_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$; ($\hat{Y}_{ijk}$: observation value; μ: means of population; α_i: effect of fluorescent *Pseudomonas* isolates; β_j: effect of plant variety; $\alpha\beta_{ij}$: interaction effect; e_{ijk}: residual error). Means were separated by using Tukey's multiple comparison. Significance was evaluated at $P < 0.01$ or $P < 0.05$ for all tests.

Table 1: Isolates of *Pseudomonas* used in biocontrol experiments

Isolate name	Origin/locality	Identified as
FPin2	Durakbasi/Carsamba/ Samsun	*Pseudomonas putida* biotype A (PpA)
FPin5	Yenikoy/Alacam/Samsun	*Pseudomonas putida* biotype B (PpB)

3 Results

3.1 *Detection of fluorescent* Pseudomonas *isolates*

From over 30 soil samples, two fluorescent *Pseudomonas* isolates were isolated from soil samples of Samsun province. Colonies of these isolates on KBA were whitish-grey, raised, and with diffusible yellowish-green pigment, and fluoresced blue under ultraviolet light (366 nm). Additionally, they were identified by using the computer-assisted microbial identification system (MIS) which employs gas-liquid chromatographic analysis of bacterial fatty acids. As a result of this study, they were confirmed as *P. putida* biotypes. One of the biotypes, *P. putida* biotype A (PpA) was coded as Fpin2 (fluorescent P. isolate number 2) and the other *P. putida* biotype B (PpB) was coded as Fpin5 (fluorescent *Pseudomonas* isolate number 5) (Table 1).

3.2 *Presence of BNYVV in sugar beet roots*

The test involved both the rhizomania-partially resistant cv. Leila and the susceptible cv. Arosa. ELISA values from roots infected by BNYVV were 6.47 times the mean of healthy partially resistant cultivar and 5.62 times the mean of healthy susceptible cultivar. ELISA values from BNYVV-infected roots were significantly reduced when plants grown in soil containing virus were compared with plants grown in BNYVV infested soil with PpA + rhizomania- partially resistant cultivar treatment. The exception was the PpB + rhizomania-partially resistant cultivar treatment, in which ELISA values of the other treatments for plants grown in soil containing the virus, while higher than values for soil non-infested with BNYVV, were not significantly different ($P < 0.05$). In contrast, ELISA values of PpB + rhizomania-partially resistant cultivar for plants grown in soil both infested and non-infested by BNYVV and negative control were lower than the other treatments, and were significantly different ($P < 0.05$). Besides these results, PpB was more effective in suppressing the occurrence of *P. betae* and the multiplication of BNYVV in the partially resistant cultivar. The average reduction of BNYVV multiplication by this isolate was 38.21% (Table 2).

3.3 *Effect of* Pseudomonas putida *biotypes on sugar beet*

In the treatments with PpA and PpB, a positive effect was observed on sugar beet plant weight and growth (Fig. 2).

In this test, total plant fresh weight was significantly decreased by infection with BNYVV. Although the plant

Table 2: ELISA A_{405} absorbance values of control and treatment groups for BNYVV

Treatments	n	Leila (Partially resistant cultivar)		Arosa (Susceptible cultivar)	
		Mean ± SEM	ELISA inhibition rates (%)	Mean ± SEM	ELISA inhibition rates (%)
PpA	5	0.622 ± 0.058^{a}	−1.1	0.471 ± 0.072^{ab}	10.8
PpB	5	0.380 ± 0.039^{bc}	38.2	0.480 ± 0.032^{ab}	9.1
NIS	5	0.095 ± 0.001^{d}		0.094 ± 0.101^{d}	
BIS	5	0.615 ± 0.004^{ab}		0.528 ± 0.031^{ab}	
Negative control	5	0.101 ± 0.001^{d}		0.010 ± 0.001^{d}	

SEM: Standard error of the mean.
PpA: *Pseudomonas putida* biotype A.
PpB: *Pseudomonas putida* biotype B.
NIS: Non-infested soil.
BIS: BNYVV-infested soil.

Fig. 2: (Left to right) a) Effect of *Pseudomonas putida* biotypes PpB and PpA on growth of Leila cultivar; L- BNYVV-infested soil, L- non-infested soil, L-PpB, L-PpA;. b) Effect of PpB and PpA on growth of Arosa cultivar; A- BNYVV-infested soil, A-non-infested soil, A- PpB and PpA. In the treatments with PpA and PpB, a positive effect was observed on sugar beet plant weight and growth.

weights in the PpA treatment were lower than for the PpB and NIS treatments, the difference was not statistically significant ($P < 0.05$). Plant weights for rhizomania-partially resistant and susceptible cultivars grown with PpA and PpB in soil both non-infested and infested by BNYVV were not significantly different, whereas weights of plants grown in soil infested by BNYVV without PpA and PpB were significantly lower ($P < 0.05$) than the other treatments (Fig. 3).

Regarding the effects of PpB and PpA on root weights, they also were significantly decreased by infection with BNYVV. Root weights with BNYVV-infection in PpA and PpB treatments were significantly reduced compared with control treatment (non-infection with BNYVV). The exception was PpB + rhizomania-partially resistant cultivar treatment, in which root weights of the other treatments for plants grown in soil containing the virus, while lower than root weights for soil non-infested with BNYVV, were not significantly different ($P < 0.05$). In contrast, root weights of PpB + rhizomania-partially resistant cultivar for plants grown in soil infested by BNYVV were significantly lower than for the non-infested soil treatment ($P < 0.05$) (Fig. 4).

3.4 *Effect of* Pseudomonas putida *biotypes on* Polymyxa betae

In the treatments with PpA and PpB, the growth of *P. betae* resting spores (cystosori) in the root tissues of sugar beet was inhibited. Furthermore, numbers of the resting spores were significantly reduced by PpA and PpB treatments. Although there was no significant difference between partially resistant and susceptible cultivars, there were highly significant differences among PpA, PpB and in partially resistant and susceptible cultivars grown with BNYVV-infested soil treatments ($P < 0.01$). The lowest number of resting spores was in PpB

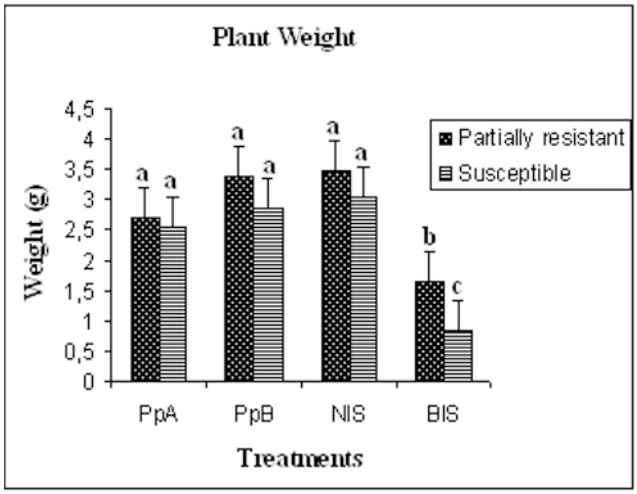

Fig. 3: The Effects of *Pseudomonas putida* biotypes PpB and PpA on total sugar beet plant weight. In this test, total plant fresh weight was significantly decreased by infection with BNYVV. Although the plant weights in the PpA treatment were lower than for the PpB and NIS treatments, they were not significantly different ($P < 0.05$).
PpA; *Pseudomonas putida* biotype A
PpB; *Pseudomonas putida* biotype B
NIS; Non-infested soil
BIS; BNYVV-infested soil%

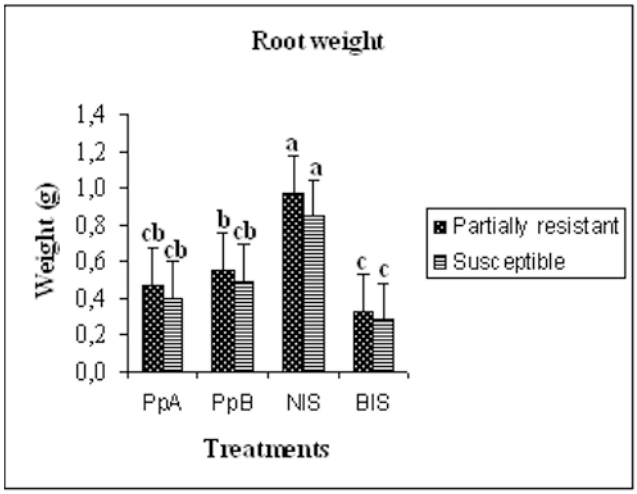

Fig. 4: Effects of *Pseudomonas putida* biotypes PpB and PpA on sugar beet root weight. Root weights of PpB + rhizomania-partially resistant cultivar for plants grown in soil infested by BNYVV were significantly lower than for the non-infested soil treatment ($P < 0.05$).
PpA; *Pseudomonas putida* biotype A.
PpB; *Pseudomonas putida* biotype B.
NIS; Non-infested soil.
BIS; BNYVV-infested soil.

treatment (Table 3), diameters of the resting spores were also significantly smaller than normal cystosori in PpA and PpB treatments. However, although there was no significant difference between PpA and PpB treatments, there were highly significant differences between PpA – PpB and partially resistant and susceptible cultivars grown with BNYVV-infested soil treatments ($P < 0.01$). The smallest resting spore diameter was in partially resistant cultivar grown with PpB treatment (Table 3). In addition, the cystosori had a dark colour and damaged membranes with PpA and PpB treatments (Fig. 5).

Table 3: Effects of *Pseudomonas putida* biotype A and B on *Polymyxa betae* resting spores

Treatments	Number of resting spores (spores per ml)	Diameter of resting spore (μm)
	Mean ± SE	Mean ± SE
LeilaPpA	$5.20 \times 10^7 \pm 0.653^{b}$	3.56 ± 0.165^{bc}
LeilaPpB	$1.70 \times 10^7 \pm 0.825^{c}$	3.35 ± 9.391^{c}
LBIS	$6{,}80 \times 10^7 \pm 0.800^{a}$	$5{,}70 \pm 0.180^{a}$
ArosaPpA	$5.10 \times 10^7 \pm 0.887^{b}$	4.42 ± 0.995^{b}
ArosaPpB	$1.60 \times 10^7 \pm 0.730^{c}$	3.92 ± 0.818^{bc}
ABIS	$7{,}60 \times 10^7 \pm 1.032^{a}$	5.70 ± 0.182^{a}
Significance	$P < 0.01$	$P < 0.01$

SE: Standard error.
PpA: *Pseudomonas putida* biotype A.
PpB: *Pseudomonas putida* biotype B.
ABIS: Arosa grown with BNYVV-infested soil.
LBIS: Leila grown with BNYVV-infested soil.

4 Discussion

The experiments focused on the ability of natural *Pseudomonas putida* biotypes to antagonize *Polymyxa betae*, the fungal vector of BNYVV. Antagonistic potential was assessed on partially resistant and susceptible cultivars in *in vivo* trials. Although *P. putida* biotypes were observed to be ineffective against BNYVV, they showed a positive effect on sugar beet weight and growth. Figures 3 and 4 show the results of partially resistant and susceptible cultivar weights with BNYVV-infected in *Pseudomonas putida* biotypes; treatments were significantly reduced compared with control treatment (non-infection with BNYVV).

We considered the suppression of *P. betae* cystosori in beet roots as an indication of the antagonistic efficiency of the *P. putida* biotypes. On the other hand, the ELISA A_{405} absorbance values of control and treatment groups for BNYVV in the roots could be evaluated statistically. Table 2 summarizes the results of ELISA inhibition rates of *P. putida* biotypes on mutiplication of BNYVV in infected partially resistant and susceptible cultivars. ELISA inhibition rates of the *P. putida* biotypes to suppress the multiplication of BNYVV varied between 9.1 and 38.2%. From the natural isolates, *P. putida* biotype B was more effective than *P. putida* biotype A in suppressing the occurrence of BNYVV in the partially resistant cultivar, Leila. The highest rate of ELISA inhibition on proliferation of *P. betae* by *P. putida* biotypes B was 38.2. In addition, growth of *P. betae* resting spores (cystosori) in the root tissues of sugar beet was inhibited. Results of this study are compatible with observations from NANDAKUMAR et al. (2001) and RAMAMOORTHY et al. (2002) who demonstrated induction of systemic resistance to a fungal pathogen disease by *Pseudomonas fluorescens*. Similarly, in this study, *P. putida* biotypes inhibited cystosori of *P. betae*, a fungal-like protist pathogen, in tissues. Furthermore, numbers of *P. betae* resting spores were reduced and they

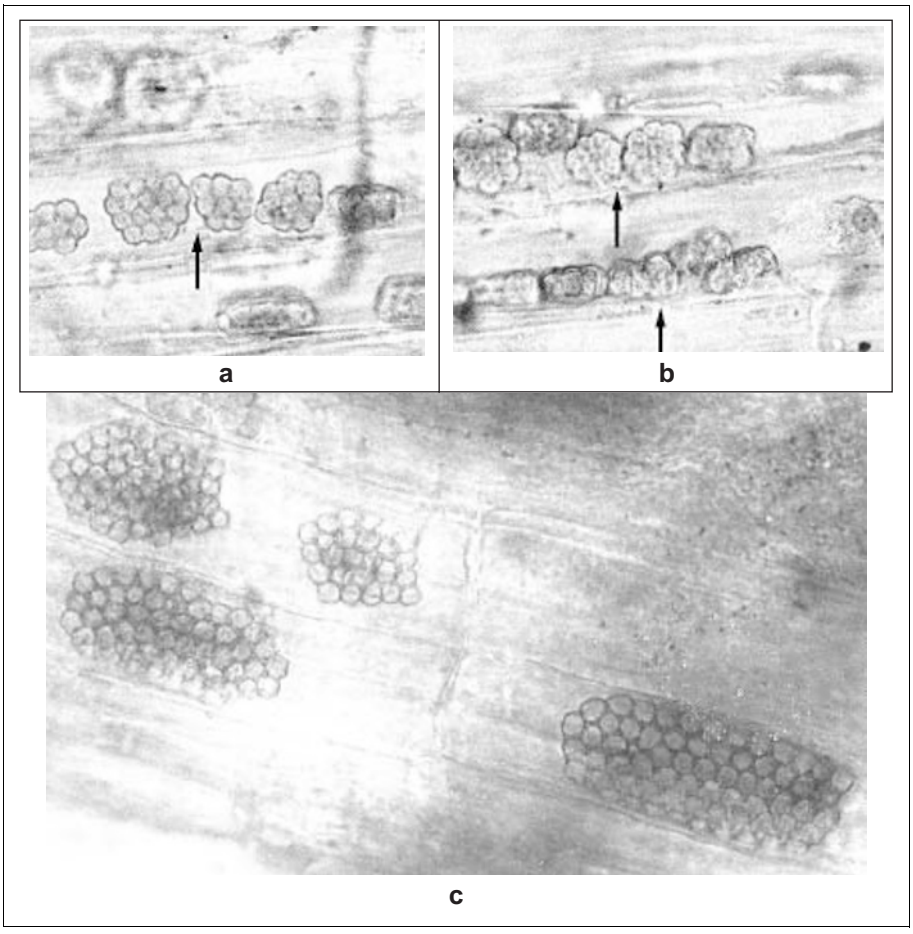

Fig. 5: Effect of PpB and PpA on cystosori of *Polymyxa betae*. a) Small diameter cystosori, b) damaged membrane of cystosori, c) normal cystosori (cystosori, stained with lactophenol containing 0.1% acid fuchsin and observed with a Leica light microscope and photomicroscope system) (Type DMLS2) (40X objective). The cystosori had a dark colour and damaged membranes with PpA and PpB treatments.

became smaller in diameter than normal cystosori; abnormal cystosori were dark in colour and had broken down membranes. Table 3 summarizes the results of the antagonistic effects of *P. putida* biotypes on resting spore numbers and diameters in infected partially resistant and susceptible cultivars. The highest antagonistic effect on numbers and diameters of *P. betae* resting spores was for *P. putida* biotype B treatment.

This is the first study from Turkey focusing on the antagonism of *P. putida* biotypes to *P. betae*. With respect to the long persistence of cystosori in fields, possibilities for controlling rhizomania are limited. Therefore, by using *P. putida* biotypes and resistant cultivars, plants' own inducible defence mechanisms may be enhanced against rhizomania. Further research is needed to develop a better methodology and to identify other strains of *P. putida* with greater efficacy against cystosori of *P. betae* and determine their potential as biological control agents.

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