ISSN 0378 - 8024

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 27 Number : 1 January : 1998

TURKISH PHYTOPATHOLOGICAL SOCIETY

The Journal of Turkish Phytopathology is published by Turkish Phytopathological Society and issued twice or three times a year to from volume. The subscripton rate per volume is $ 21.00

EGE ÜNİVERSİTESİ BASIMEVİ
BORNOVA - İZMİR
1998

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 27 JANUARY NO. 1

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Two New Species for the Turkish Fauna: *Quinisulcius acutus* and *Scutylenchus stegus* (Tylenchida: Dolichodoridae)

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ABSTRACT

In this study, plant parasitic species of *Quinisulcius* and *Scutylenchus* in the tomato (*Lycopersicum esculentum* L.) fields in rotation with carrot in Beypazarı Ankara/district are examined in considering their two main aspects, namely faunistic and taxonomic. In 1991, during the summer months, when adult Tylenchida species are more abundant, soil and tomato samples were taken, from these samples, nematodes were extracted, prepared and the ones belonging to *Quinisulcius* and *Scutylenchus* were measured and identified. In this study, one species belongs to *Quinisulcius* genus as *Q. acutus* and also one species belongs to *Scutylenchus* genus as *S. stegus* were determined (Fig. 1, 2). The aim of this study is to add two new species for Turkish fauna. On the other hand, the male of *Q. acutus* was determined for the first time nematode fauna of the world.

INTRODUCTION

The nematode fauna of Turkey is poorly studied. One species belongs to *Quinisulcius* genus as *Q. acutus* and also one species belongs to *Scutylenchus* genus as *S. stegus* were determined. The aim of this study is to add two new species for Turkish fauna. Taxonomic status was given according to Allen (1995) and Thorne and Malek (1968). On the other hand, the male of *Q. acutus* was determined for the first time Nematode fauna of the world.

MATERIAL and METHODS

The main material of the study consists of plant parasitic nematode species belonging to *Quinisulcius* genus that are derived from soil and root examples taken from areas of tomato planting that rotate with carrot in Beypazarı district Ankara.

In this study, the areas of tomato planting that rotate with carrot in Beypazarı district Ankara were examined in August 1991 when the plant parasitic nematodes are so abundant, and examples of soil and tomato were taken.

The soil examples were taken as being told in nematological studies, according to the width of the area from approximately 20 separate points of each 5 decares (or less), mixing of soils that were taken 20 cm deep around plants roots being single example of 0.5 kg according to this in 1991, 9 soil and tomato examples were taken from Beypazarı (Ankara) district. Total number of areas of soil and tomato sampling represents the 2 % of tomato planting in the district.

In maintain active nematodes in soil, Cobb's (1918) sieve method developed by Cristie and Perry (1951) and Baermann's (1917) Sieve-Funnel methods known as Funnel method were used.

To prepare permanent nematodes, "Fixation and permanent preparation method", given by Seinhorst (1959) and developed by De Grisse (1969) was used. Measurements were done according to the formula cited by Siddiqi (1986).

In measurement and drawings for the diagnosis of species, all the straight and curved structures were measured by the "Curvimeter".

RESULTS and DISCUSSION

Order : Tylenchida
Suborder : Tylenchina
Superfamily : Dolichodoroidea
Family : Dolichodoridae
Subfamily : Tylenchorhynchinae
Genus : *Quinisulcius* Siddiqi 1971

Definition: Small sized (0.47-0.9mm), strongly curving when relaxed. Annules prominent. Lateral fields each with 5 incisures, smooth or areolated. Deirids absent. Cephalic region offset, rounded, finely annulated; labial disc indistinct; framework with light to moderate sclerotization. Stylet moderately strong, 12-24 µm long; knobs rounded, rarely cupped anteriorly. Median bulb well developed, oval, not offset from procorpus by a constriction. Basal bulb large, usually offset from intestine. Cardia prominent. Vulva at 51-59 %, lips not modified. Spermatheca round, axial or slightly offset, rarely functional. Ovaries paired, outstretched. Female tail conoid, usually ventrally arcuate, terminal annule enlarged, smooth or striated. Male rare or absent.

Bursa well developed, simple, enveloping entire tail. Spicules arcuate, with poorly developed distal flanges, 14-24 µm long. Gubernaculum modified, with proximal end directed dorsally.

Species: *Quinisulcius acutus* (Allen 1995) Siddiqi 1971 Syn. *Tylenchorhynchus acutus* Allen 1995 (Figure 1. A-H.)

Measurements:

Females (n=5): L=0.78-0.90mm; a=25.4-32.3; b=5.2-5.8; c=12.0-19.1; c'=2.4-3.0; V=53.9-60.8; Stylet=20-22µm; Tail=44-68µm; % MB=42.8-45.3; G_1=12.3-20.3; G_2=16.1-22.2; T/VA=0.1-0.2; Ran=23.-28.

Male (n=1): L=1.07mm; a=26.8; b=5.9; c=14.7; c'=2.6; Stylet=21 µm; Tail= 73 µm; % MB=45.6; Gub.=10 µm; Spic.=30 µm.

Definition female: After fixation body has gained open "C" shape. The head part is wide, hemispheric and it has fused with the body by making node slightly. The head bases is mildly hardened stylet is 20-22 µm long and it has moderate size, round stylet knobs with smooth anterior ends. The connus makes up of the half of the stylet length. Dorsal oesophageal gland opening is at 2-3 µm back of the stylet base. Median bulb is markedly-developed and it is oval, muscular with valves and its center is 64-65 mm far from the anterior end. Nervering is anterior to the isthmus. The excretion hole is at the base of the isthmus, 105-120 µm away from the anterior end. Hemizonid, in an unclear position, is 1-2 annules anterior to excretion hole. The annules are prominent and 1.7-2.3 µm wide at mid part of the body divided by longitudinal lines. The lateral area of 5 lines in cuticula continues decreasingly towards the anterior end. It has double ovaries. Although the anterior branch of ovary lies straight, the posterior branch has curves. Oocytes are of single-line. Spermatheca is mildly prominent, axional, small and round, and it has very few sperms or is empty. The tail is conical, mildly curved towards ventral, a smooth end. The part between anus and the tail has 23-28 annules. Phasmid is prominent and taken place slightly anterior to the middle of the tail.

Male: After fixation, body has gained open "C" shape. The head part with 6 annules is wide and hemispheric. It has fused with the body by making a node slightly. The head base is mildly hardened. Stylet is 21 µm long and has moderate size, round stylet knobs with smooth anterior ends. Connus makes up of the 46.61 % of the stylet length. Median bulb is markedly developed and it is oval, muscular with valves and its center is 82 µm far from the anterior end. The nervering is anterior to the isthmus. The excretion hole is at the base of the isthmus, 135 µm away from the arterior end.

Hemizonid, in an unclear position, is 2 annules anterior to excretion hole. The annules are prominent and 0.27-0.30 µm wide at the mid portion of the body divided by longitudinal lines. The lateral area of 5 lines in cuticula continues decreasingly to he anterior end. The testis is single and non-prominent. The tail is 73 µm long, phasmid is slightly anterior to the tail. Spicule is prominent and 30 µm long. Gubernaculum is curred, bow shape and 10 µm long.

Although the female individuals in the study fit the 1955 original definition of Allen from the aspects of measurement and general morphological characteristics, they have some differences. Longer (0.78-0.91 mm although 0.63-0.70 mm); "a" valve smaller (25.4-32.3 although 31-38); Stylet longer (20-22 mm although 16-17 mm); annule number in the tail is greater (Ran: 23-28 although Ran: 18); Althouh excreta ring opens to the base of the isthmus according to the original definition of Allen (1955) in this study the fixed populations' excretion hole opens to the mid of the basal bulb.

We have not seen a record saying that it existed in our country before, so it is a new record for Turkey.

Although in literature it is mentioned that there is "male" from of "*Q. acutus*", we have not met a record about its existence. In this study we have found only one "male". Its morphological and morphometrical characteristics are given in detail.

Order	: Tylenchida
Suborder	: Tylenchina
Superfamily	: Dolichodoroidea
Family	: Dolichodoridae
Subfamily	: Tylenchorhynchinae
Genus	: *Scutylenchus* Jairajpuri 1971

Definition: Small to medium sized, arcuate to curved when relaxed. Body cuticle marked by longitudinal striae or grooves. Deirids absent. Cephalic region offset, with six radial grooves and moderate sclerotization; labial disc inconspicuous. Median bulb near middle of oesohagus or more anterior. Vulva in a cavity or depression, with epiptygma. Vagina vera squarish in lateral view. Female tail conoid to subcylindrical with rounded tip; terminal tail cuticle not abnormally thickened; phasmids conspicuous. Spicules rather slender; gubernaculum crescent-shaped in lateral view.

Species: *Scutylenchus stegus* (Thorne and Malek 1968) Siddiqi 1979 Syn. *Tylenchorhynchus stegus* Thorne and Malek 1968 *Merlinius stegus* (Thorne and malek) Siddiqi 1979 (Figure 2. A-D).

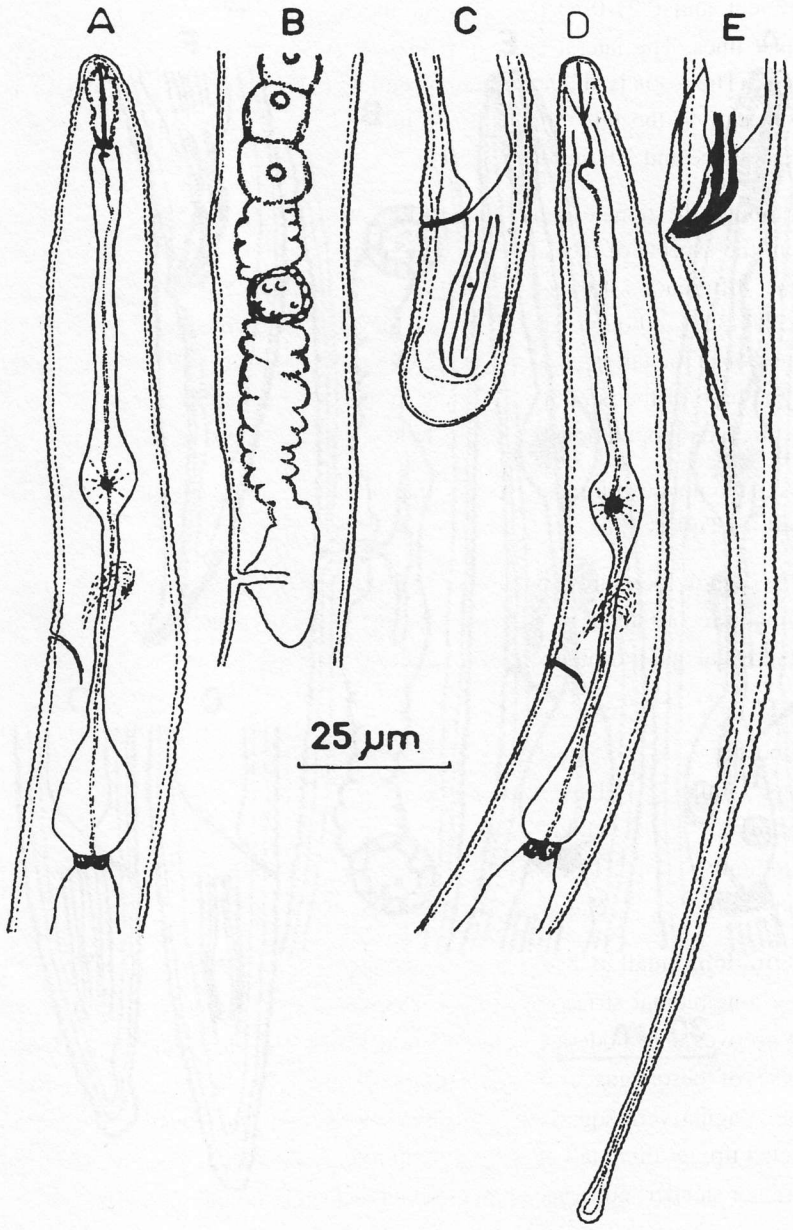

Figure 1. A-H. *Quinisulcius acutus*, A-E. Female. F-H. Male. A, F. Oesophageal region, D, G. Tail region. E, H. Entire female. B. Posterior gonad. C. Anterior gonad.

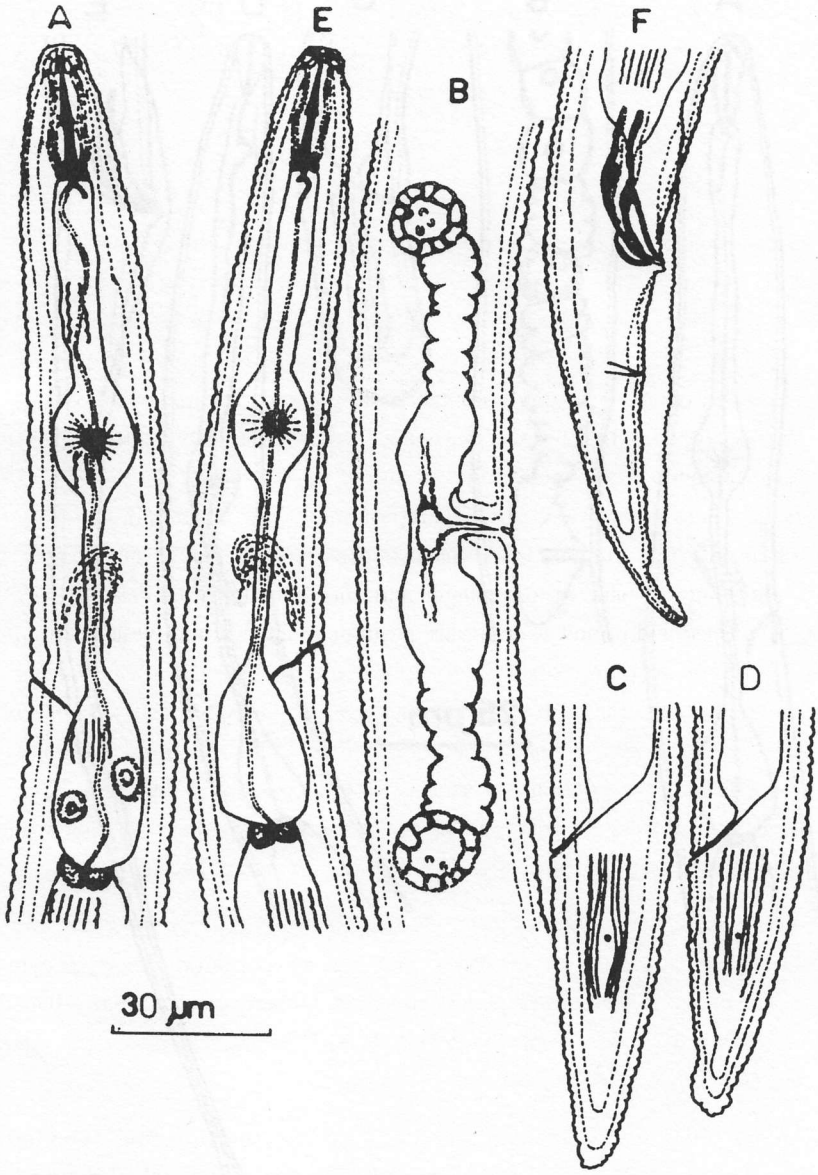

Figure 2. A-D *Scutylenchus stegus*, A, B. Female. C, D. Male. A, C. Oesophageal region, B, D. Tail region.

Measurements:

Females (n=1): L=0.87mm; a=28.1; b=6.3; c=17.0; c'=2.4; V=51.8; Stylet=20µm; Tail=55µm; %MB=42.0; m=46.5; O=9.3; G_1=18.3; G_2=17.1; T/VA=0.1; Ran=32.

Male (n=1): L=0.86 mm; a=24.7; b=5.7; c=12.5; c'=3.3; T=43.5; Stylet=22 µm; Tail = 69 µm; % MB=44.7; m=51.0; O=8.2; Gub.=13µm; Spic.=31 µm.

Definition female: After fixation, body has gained closed "C" shape. There are horizontal longitudinal striae on cuticula. The longitudinal striae are 24 in number. Head is round fused with the head forming slightly a node and has 4 annules. The head base is mindly hardened. Stylet is 20 µm long and has knobs with pitty anterior. Dorsal oesophageal gland opening is approximately 2 µm posterior to the stylet base. Median bulb is well developed, slightly oval, muscular, has valves and its center is 58 µm far from the anterior end. The excretion hole is in the middle of the basal bulb, 119 µm away from the anterior end. Hemizonid is not prominent, it is 2-3 annules anterior to the excretion hole and is 3 annules long. Basal bulb is well developed and pear shaped. Cardias at the point of fusion of oesophagus and intestine, are in the base of basal bulb markedly developed and round. Annules are prominent and 1.9 mm wide at mid part of the body. The lateral area has 6 lines and the number of the lines decrease from the basal bulb towars the anterior end. The reproduction system is didelphic, ovary is smooth and oocytes are single line. Spermatheca is non prominent, axional (=in the same axis with the ovaries) and does not contain any sperms. The tail is conical flat, with 32 annules and 2.8 times longer than the body width in the anus. The tail end is round with no annules. Phasmid is prominent and slightly anterior is the mid of the tail.

Male: General body shape, head area and oesophagus resemble the female's. Testicles lie to the anterior of the body and has no curration. Spermatocytes are double-lined. Spicule is well-developed 31 µm long. Gubernaculum is bow-shaped and 13 µm long. Tail is 3.2 times longer than the body width in the anus and width of 69µm. Bursa completely covers the tail. Phasmid is anterior to the tail.

Althought the *S. stegus* indivuals fixed in this study fit the 1968 original definition of Thorne and Malek, the spicule of the fixed male indivudual is found to be shorter (31 µm although 52 µm).

We have not seen a record saying that it existed in our country before, so it is a new record for Turkey.

ÖZET

TÜRKİYE FAUNASI İÇİN YENİ İKİ TÜR *Quinisulcius acutus* ve *Scutylenchus stegus* (TYLENCHIDA: DOLICHODORIDAE)

Bu çalışmada Beypazarı (Ankara) ilçesinde havuç ile münavebeye giren domates (*Lycopersicum esculentum* Mill.) ekim alanlarında bulunan *Quinisulcius* ve *Scutylenchus* cinslerine ait bitki paraziti türlerin faunistik ve taksonomik olmak üzere iki bölümde incelenmesi yapılmıştır. 1991 yılında ergin Tylenchida türlerinin çoğunlukla bulunduğu yaz aylarında toprak ve kök örnekleri alınmıştır. Alınan örneklerden elde edilen nematodların daimi preparatları hazırlanarak, *Quinisulcius* ve *Scutylenchus* cinslerine ait türlerin ölçüm ve teşhisleri yapılmıştır. Çalışmada *Quinisulcius* cinsine ait bir tür *Q. acutus* (Allen 1955) Siddiqi 1971 ve *Scutylenchus* cinsine ait *S. stegus* (Thorne and Malek 1968) Siddiqi 1979 bulunmuştur (Şekil 1, 2). Bu türlerin Türkiye faunası için yeni türler olduğu ayrıca *Q. acutus*'un erkeğinin literatür için yeni kayıt olduğu saptanmıştır.

LITERATURE CITED

ALLEN, M.W., 1955. A review of the nematoda genus *Tylenchorhynchus.* **Univ. Calif Pubs Zool. 61:** 129-166.

BAERMANN, G., 1917. Eine einfache Methode zur Affındung von Ankylostomum - (Nematoden) larven in Erdoprobem Geneesk Tijdschr. Ned.- Ind. **57:** 131-137.

CHRISTIE, J.R. and V.G. PERRY, 1951. Removing nematodes from soil. **Proc. Helminthol. Soc. Wash. 18:** 106-108.

COBB, N.A., 1918. Estimating the nema population of soil. Agric. Tech. Circ. U.S. Dept. Agric. 1, 48 pp.

DE GRISSE, A., 1969. Redescription on modifications de quelque tecniques utilisees dans l'etude des neematodes phytoparasitaires. Meded. Ritksfac. Landwet. Gent **34(2):** 351-359.

SEINHORST, J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. **Nematologica 4:** 67-69.

SIDDIQI, M.R., 1971. Structure of the oesophagus in the classification of the superfamily Tylenchoidea (Nematoda). Indian **J. Nematol. 1:** 25-43.

SIDDIQI, M.R., 1986. Tylenchida parasites of plants and insects. Farrnham Royal, UK: Commonwealth Agricultural Bureaux, 645 pp.

THORNE, G., and MALEK, R.B., 1968. Nematodes of Northern Great Plains, Part 1. Thylenchida (Nematoda: Secernentea). Tech. Bull. S.Dak. Agric. Exp. Stn. No: 31. Allen, M.W., 1955. A review of the nematoda genus *Tylenchorhynchus.* **Univ. Calif. Pubs Zool. 61:** 129-166.

J. Turk. Phytopath., Vol. 27, No. 1, 9-16, 1998 ISSN 0378 - 8024

Sensitive Detection of the Blackleg Pathogen *Ervinia carotovora* subsp. *atroseptica* by PCR

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ABSTRACT

The polymerase chain reaction (PCR), a sensitive tool for the identification of specific regions of DNA in smal quantities was used to detect *Erwinia catotovora* subsp. atroseptica strains from various sources of Turkey. Detection of *Eca* by PCR using genomic DNA extracts as template was found to be more sensitive than ELISA and IFAS tests based on monoclonal antibodies. 1 µl of DNA extracts from 2.5 x 10^2 *Eca* cells/ml and 1 µl cell lysates of 2.5 x 10^3 *Eca* cells were amplifield sufficiently to generate products visible on ethidium bromide stained agarose gels.

INTRODUCTION

Blackleg of potato is one of the most prominent bacterial diseases of the major seed potato growing areas (Bolu, Nevşehir and Niğde) of Turkey. Three year survey studies based on isolation soft rot *Ervinia* by inoculating CVP (crystal violet pectate) medium and differentiating them with biochemical tests chowed that both *Erwinia carotovora* subsp. *atroseptica (Eca)* and *Erwinia carotovora* subsp. *carotovora (Ecc)* were the causal agent of blackleg in the area (Benlioğlu and Bora, 1991). The same study also revealed that *Eca* was the predominant organism and isolated at high (71.3 %) frequencies while *Ecc* at 20 % and *Eca* + *Ecc* at 8.7 % of diseased stems. However, the percentage infestation of daughter tubers with *Eca, Ecc* and *Eca* + *Ecc* were 43.3, 39.7, 17.0, respectively.

Turkey produces annually about 4.000.000 t of potatoes and needs about 100.000 t of certified seed potatoes per year. Turkey annually imports about 8.000-10.000 t basic seed potatoes from abroad and this is propagated in the seed potato growing areas in order to meet farmer's demand. Potato seed certification programme is carried out by the extension services collaborating with The Plant Protection Research Institutes of The Ministry of Agriculture and Rural Affairs of Turkey. Inspections are mainly based on visual examination in potato fields and laboratory tests is also performed to detect soft rot *Erwinias* in or on seed tubers.

SENSITIVITE DETECTION OF THE BLACKLEG PATHOGEN *ERWINIA CAROTOVORA* SUBSP. *ATROSEPTICA* BY PCR

Exceeding demand for certified seed potatoes and high infestation level of tubers with *Erwinia carotovora* makes it necessary to apply reliable and rapid laboratory tests in potato certification procedure in Turkey. DNA based techniques particularly polymerase chain reaction (PCR) has been recently used for detecting plant pathogenic bacteria in potatoes as rapid, specific and sensitive diagnostic procedures (Seal et al, 1992; Xiang Li and De Boer, 1995; De Boer and Ward, 1995).

The aim of this study is to use a PCR based detection assay for the identification of *Erwinia carotovora* subsp. *atroseptica* and to compare PCR with two most currently serological assay enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody staining (IFAS) for the sensitivity.

MATERIALS and METHODS

Bacterial strains and media: All *Erwinia* cultures (Table 1) were freshly prepared from stocks stored at-80°C on protect beads (Technical Service Consultants, Ltd., Haywood, England) and routinelyl maintained on Nutrient agar (NA). All cultures were incubated at 25°C.

DNA extraction procedure: Total genomic DNA was isolated following two small scale procedures. In the first extraction protocol (De Boer and Ward 1995). 1 ml of cell suspension were pelleted in an Eppendorph tube by centrifugation at 14000 x g for 20 min and resuspended in 500 µl of Tris-EDTA buffer (pH8.0) containing 1% (W/vol.) sodium dodecyl sulfate and heated to 50°C for 1.5 h. Then one half volume of 7.5 M ammonium acetate was mixed with heat treated samples and centrifuged at 14.000 x g for 20 min in an Eppendorph centrifuge. DNA was precipitated from the supernatant fractions by adding 1 volume of isopropanol and centrifuged at 14.000 g for 25 min. Pellets were washed with 70 % ethanol and dried at 58°C for 10 min, dissolved in 50 µl of purified water, and heated to 50 to 55°C prior to PCR.

To prepare template DNA from cell suspensions, pelleted cell from 1 ml broth cultures were suspended in 20 µl of 0.1 % Triton x 100 by wortexing. Then 5 µl of 0.4 N NaOH were added and boiled for 1 min and then placed on ice for 5 min and added 5µl of 1 M Tris pH: 7.6. 1 µl of this was used for the template in the PCR reaction.

Cell lysates and DNA extracts were stored at -20°C for future use.

PCR assays: PCR amplification assays were routinely performed in a 20 µl reaction mixture containing 0.5 M each primer [Eca 1f 5'CGGCATCATAAAAACACG-3' and Eca2r 5' GCACACTTCATCCAGCGA-3' designed by De Boer & Ward (1995)]; 100µM each of deoxynucleotides dATP, dCTP, dGTP and dTTP; 2.0mM Mg^{++}, and 0.5 units of Taq DNA polymerase (BioCan Scientific, Missisauga ON Canada) in the buffer provided by the manufacturer. 1µl of DNA extracted from pure cultures or cell lysates was added to each mixture. 2 drop of mineral oil (molecular biology grade, Sigma) was added to prevent evaporation. PCR amplifications were performed in an Ericomp Easy Cycler (San Diego, CA).

Template DNA was denatured for 5 min at 95°C in the first PCR cycle and for 30 s at 94°C in subsequent cycles. Annealing was at 62°C for 45 s, and DNA extension at 72°C for 45 s. For the last cycle, the extension time was increased to 8 min. The amplified DNA frangments were electrophoresed in 1 % agarose gels in tris-borate buffer (Sansbrook, Maniatis, 1989) and visualised with ultraviolet light following ethidium bromide staining. A standard 100 bp DNA ladder (Gibco /BRL, Burlington, ON, Canada) was included on each gel.

Assesment of sensitivity thresholds: PCR analysis were compared with ELISA and IFAS using monoclonal antibody MAb 4F6 as described previously (de Boer and Mc Naughton, 1987) To determine the concentration of bacterial cells, from 24 h NA plate culture of *Eca (Eca-6)* strain, bacteria were suspended in sterile distilled water and were adiusted spectrophotometrically to 0.2 A_{590} nm (Approximately 1 x 10^8 cfu/ml), and serially diluted in 10-fold increments in distilled water and the concentration of *Eca (Eca-6)* was determined by a standard plate count procedure on nutrient agar. To asses the concentration of DNA extracts in relation to the number of colony forming units, bacteria from 1 ml of each dilution series were pelleted and processed through the Triton x 100 lysis or the DNA extraction procedure as described above. Dilution series were also tested by both ELISA and IFAS using monoclonal antibody. Three independent repetitions were conducted to determine the sensitivity thresholds.

RESULTS

Erwinia carotovora subsp. *atroseptica* and *E.c.* subsp. *carotovora* and other strains of *Erwinia* subspecies, and *Erwinia herbicola* were tested the specificity of PCR. All strains of *Eca* was yielded 690 bp DNA fragment with *Eca* 1 f and *Eca* 2r primers. No amplifield fragment was observed with strains of *E. c.* subsp. *carotovora, betavasculorum, odorifera, wasabiae* and *Erwinia herbicola* (fig 1 and table 1).

Tablo 1. Erwinia *carotovora* subsp. *atroseptica* strains and other bacteria tested in polymerase chain reaction (PCR) amplification.

Bacteria	Culture code	Host	Geographic origin	PCR
E.c.atroseptica	6	Potato	BC(Canada)	+
E.c.atroseptica	31	Potato	BC(Canada)	+
E.c.atroseptica	196	Potato	BC(Canada)	+
E.c.atroseptica	198	Potato	BC(Canada)	+
E.c.atroseptica	134	Potato(tuber)	Turkey	+
E.c.atroseptica	137	Potato(tuber)	Turkey	+
E.c.atroseptica	138	Potato(tuber)	Turkey	+
E.c.atroseptica	139	Potato(tuber)	Turkey	+
E.c.atroseptica	150	Potato(stem)	Turkey	+
E.c.atroseptica	151	Potato(stem)	Turkey	+
E.c.atroseptica	152	Potato(stem)	Turkey	+
E.c.atroseptica	153	Potato(stem)	Turkey	+
E.c.atroseptica	154	Potato(stem)	Turkey	+
E.c.atroseptica	155	Potato(stem)	Turkey	+
E.c.atroseptica	157	Potato(stem)	Turkey	+
E.c.carotovora	156	Potato(stem)	Turkey	-
E.c.carotovora	126	Potato(tuber)	Turkey	-
E.c.carotovora	127	Potato(tuber)	Turkey	-
E.c.carotovora	128	Potato(tuber)	Turkey	-
E.c.carotovora	129	Potato(tuber)	Turkey	-
E.c.carotovora	130	Potato(tuber)	Turkey	-
E.c.carotovora	131	Potato(tuber)	Turkey	-
E.c.carotovora	132	Potato(tuber)	Turkey	-
E.c.carotovora	133	Potato(tuber)	Turkey	-
E.c.carotovora	135	Potato(tuber)	Turkey	-
E.c.carotovora	136	Potato(tuber)	Turkey	-
E.c.carotovora	140	Potato(tuber)	Turkey	-
E.c.carotovora	141	Potato(tuber)	Turkey	-
E.c.carotovora	142	fresh water	Turkey	-
E.c.carotovora	143	fresh water	Turkey	-
E.c.carotovora	144	fresh water	Turkey	-
E.c.carotovora	145	fresh water	Turkey	-
E.c.carotovora	146	fresh water	Turkey	-
E.c.carotovora	147	fresh water	Turkey	-
E.c.carotovora	148	fresh water	Turkey	-
E.c.carotovora	149	fresh water	Turkey	-
E.c.betavasculorum	29	Sugar beet	Colorado	-
E.c.chrysantemi	340	Potato	Peru	-
E.c.odorifera	1840	chicory	France	-
E.c.wasabiae	SR91	Horseradish	Japan	-
Erwinia herbicola	LMG 2565	Cereal	Canada	-

Sensitivity thresholds: A 690 bp amplification product was obtained from DNA extracts and cell lysates of aqueous suspensions of pure culture. As little as 25 cells of *Eca* from DNA extracts, and 250 cells from lysates were detected on ethidium bromide-stained agarose gel after a 40 cycle PCR assay (fig. 2). Comparable sensitivity was obtained with IFAS and ELISA (table 2). But the threshold detection of ELISA was at ~ 2.5×10^5, and IFAS at ~ 2.5×10^4.

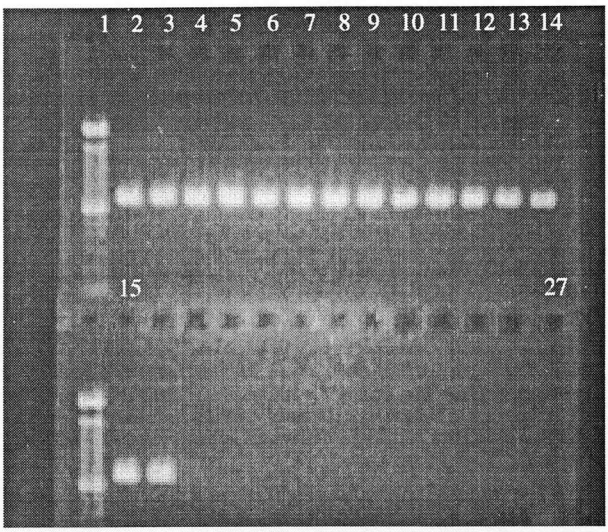

Figure 1. Ethidium bromide-stained agarose gel of polymerase chain reaction (PCR) products directed by *Eca* 1f and *Eca* 2r primers. Template DNA's were from *Erwinia carotovora* subsp. *atroseptica (Eca), Erwinia carotovora* subsp. *carotovora (Ecc)* and other *Erwinia* species. Lane 1, 10-kb ladder; lane-2 *Eca*-6; lane-3 *Eca*-31; lane-4 *Eca*-196; lane-5 *Eca*-198; lane-6 *Eca*-134; lane-7 *Eca*-137; lane-8 *Eca*-138; lane-9 *Eca*-139; lane-10 Eca-150; lane-11 *Eca*-151; lane-12 *Eca*-152; lane-13 *Eca*-153; lane-14 *Eca*-154; lane-15 *Eca* 155; lane-16 *Eca* 157; lane-17 *Ecc*-126; lane-18 *Ecc*-127; lane-19 *Ecc*-128; lane-20 *Ecc*-142; lane-21 *Ecc*-143; lane-22 *Ec. betavasculorum;* lane-23 *Ec chrysanthemi*; lane-24 *Ec odorifera*; lane-25 *Ec wasabiae*; lane-26 *Ec herbicola*; lane-27 negative control.

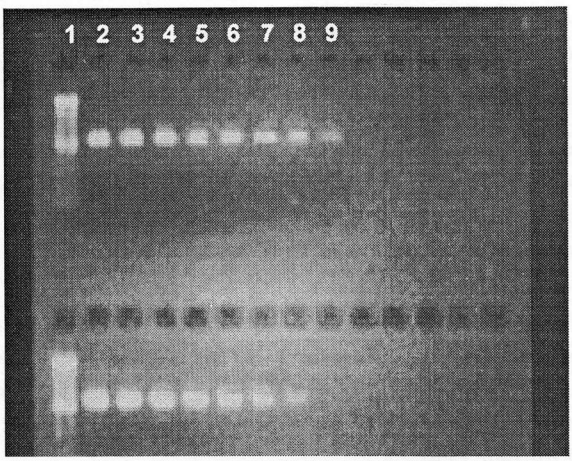

Figure 2. Ethidium bromide-stained agarose gel electrophoresis of amplified polymerase chain reaction (PCR) products from DNA extracts (top lanes) and cell lysates (bottom lanes) of a dilution series of *Erwinia carotovora* subsp. *atroseptica* strain *Eca*-6. Numbers lane 1, 10-kb ladder; lane-2 2.5 x 10^8 cfu; lane-3 2.5 x 10^7 cfu; lane-4 2.5x 10^6 cfu; lane-5 2.5 x 10^5 cfu; lane-6 2.5 x 10^4 cfu; lane-7 2.5 x 10^3 cfu; lane-8 2.5 x 10^2 cfu; lane-9 2.5 x 10 cfu.

Table 2. Comparison of polymerase chain reaction (PCR) with visual inspection, enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody staining (IFAS) techniques for detection of *Erwinia carotovora* subsp. *atroseptica.*

No. of bacteria in dilution series cfu/ml	IFAS[a]	ELISA[b]	PCR from DNA extracts[c]	PCR from cell lysates[c]
$2.5x10^8$	>50	1.754	++	++
$2.5x10^7$	>50	1.098	++	++
$2.5x10^6$	>50	0.524	++	++
$2.5x10^5$	19.7	0.226	++	++
$2.5x10^4$	6.0	0.083	++	++
$2.5x10^3$	0.2	0.079	++	++
$2.5x10^2$	-	0.073	++	+
$2.5x10^1$	-	0.076	+	-
St. Dist. Water	-	0.070	-	-

a. Average number of fluorescing cells per microscope field, based on 30 fields. ≥ 5 positive
b. Mean of triplicate readings at 405 nm threshold ≥3 x mean of negative control, positive
c. The intensity of the 690-bp PCR product in ethidium bromide stained agarose gel: +, weak band; ++, strong band; -, no band.

DISCUSSION

Two set of primers were specifically amplified a 690 bp DNA fragment of all *Eca* strains isolated from Turkey. But *Ecc* strains from various sources in Turkey did not produce any fragment with PCR. De Boer and Ward (1995) also revealed that all *Eca* strains having different serogroups from different geographic regions were identified specifically by using these set of primers.

We obtained similar results from two template DNA preparation for PCR assays. DNA extraction protocol had the sensitivity with 2.5 x 10^2 *Eca* cells / ml while 1 µl cell lysates prepared by boiling 2.5 x 10^3 cell/ml suspensions yielded PCR products. Seal et al. (1992) showed that target sequences from as few as five boiled bacterial cells of *Pseudomonas solanacearum* from pure culture were amplified sufficiently to generate products visible on agarose gels. Haas et al. (1995) also used boiled cell suspensions of *Agrobacterium tumefaciens* in PCR assays. In this study we tested pure bacterial cultures. However, De Boer and Ward (1995) were used DNA extracts from infected potato tissue and reported that sensitivity of detection by PCR in plant extraction fluids was similar to that for pure cultures.

Comparative studies of two serological assay (ELISA and IFAS) and PCR for detection of Eca indicated that PCR was 1000 times more sensitive than ELISA and 100 times than IFAS. Xiang Li and De Boer (1995) was found that detection sensitivity of

PCR was 500 to 5000 cfu per ml compared to 10^4 cfu/ml in immunofluorescence and 10^5 cfu/ml in ELISA for the detection of *Clavibacter michiganesis* subsp. *sepedonicus*. De Boer and Ward (1995) obtained PCR products from extracts of potato stems to which cells of *Eca* had been added at 450 cfu / ml.

PCR appears to be a promising technique for determining the phytopathogenic bacteria and detecting latent bacterial infections of plants (De Boer et al., 1996). In our studies PCR was found to be much more sensitive than two currently used serological techniques. However, PCR could be a useful and reliable tool to detect bacterial pathogens in seed certification and quarantine programmes in Turkey.

ÖZET

Türkiye'de çeşitli kaynaklardan izole edilen *Erwinia carotovora* subsp. *atroseptica (Eca)* izolatlarının tanılanması amacıyla küçük miktarlardaki DNA üzerinde spesifik bölgeleri tanımlayabilen ve çok duyarlı bir yöntem olan Polimeraz zincir reksiyonu (PCR) kullanıldı. *Eca*'nın saptanmasında; genomik DNA ekstraktlarının kalıp olarak kullanıldığı PCR yöntemi, monoklonal antiserum ile yürütülen ELISA ve Indirekt fluoresan antikor boyama testinden (IFAS) daha duyarlı olarak bulundu. Ethidium bromidle boyanmış agarose gel üzerinde görünebilir bantlar elde edilmesi için PCR ile çoğaltılabilecek 1 µl süspansiyon içindeki gerekli kalıp DNA miktarı; DNA ekstraktı kullanıldığında 2.5 x 10^2 Eca hücresi / ml, Lizat kullanıldığında 2.5 x 10^3 hücre / ml oranında bulundu.

ACKNOWLEDGEMENTS

This work was supported in the framework of the Turkish Scientific Research Council - NATO B-2 Program and, Agriculture and Agri-Food Canada.

LITERATURE CITED

BENLİOĞLU, K., ve T. BORA, 1991. Bolu, Nevşehir ve Niğde illerindeki patates üretim alanlarında *Erwinia spp*'nın yaygınlık oranları, tanılanması ve inokulum kaynakları üzerinde araştırmalar. Doktora tezi. Ege Üniversitesi, Türkiye.

DE BOER, S. H., and J.J. WARD, 1995. PCR detection of *Erwinia carotovora* subsp. *atroseptica* associated with potato tissue. **Phytpathology 85:** 854-858.

DE BOER, S. H. and M.E. Mc NAUGHTON, 1987. Monoclonal antibodies to the lipopolysaccharide of *Erwinia carotovora* subsp. *atroseptica* sergroup I. **Phytopathology 77:** 828-832.

DE BOER, S.H., D.A. CUPPELS, and R.D. GITATIS, 1996. Detecting latent bacterial infections, in S.H. De Boer (ed.), Advances in Botanical Research. Adademic Press, **23:** 27-57.

HAAS J.H., L.M. MOORE, W. REAM., and S. MANULIS, 1995. Universal PCR primers for detection of phytopathogenic *Agrobacterium* strains. **Appl. Environ. Microbiol. 61:** 2879-2884.

LI, X., and S.H. De BOER, 1995. Selection of polymerase chain reactions primers from an RNA intergeic spacer region for specific detection of *Clavibacter michiganesis* subsp. *sepedonicus.* **Phytopathology 85:** 837-842.

SEAL, S.E., L.A. JACKSON, and M.J. DANIELS. 1992. Isolation of *Pseudomonas solanacearum*-specific DNA probe by subtraction hybridization and construction of species-specific oligonucleotide primers for sensitive detection by the polymerase chain reaction. **Appl. Environ. Microbiol. 58:** 3751-3758.

J. Turk. Phytopath., Vol. 27, No. 1, 17-26, 1998 ISSN 0378 - 8024

Reaction of Some Onion Cultivars to *Aspergillus niger* Van Tiegham and *Fusarium oxysporum* Schlecht

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ABSTRACT

Five anion (*Allium cepa* L.) cultivars were screened for resistance to black mould and basal rot diseases caused by *Aspergillus niger* Van Tiegham and *Fusarium oxysporum* Schlecht respectively in in-vitro and in-vivo conditions. In in-vitro experiment, the pathogens were inoculated to seed and the reactions of cultivars by utilizing seed colonisation by these pathogens were determined. In in-vivo experiments, the pathogens were inoculated to seed and soil by using two infestation methods and the cultivars were compared to find out their reactions to damping-off and onion set rot which are different infections of black mould and Fusarium basal rot diseases depending on infestation methods. None of the cultivars were immune to infections of *A. niger* and *F. oxysporum*. The greatest percentage of seed colonisation by pathogens was obtained with cv. Texas Early Grano 502 at the end of in-vitro experiment. This cultivar was also consistently susceptible to pre-emergence damping-off and onion set rot infections caused by *A. niger* and *F. oxysporum* both by seed and soil infestations in vivo experiments. The cv. Banko was in the second range and it exhibited the same characteristics with Texas Early Grano 502 for *A. niger*, but it was more susceptible to *F. oxysporum* in the case of soil infestitation of pathogen. The cultivar from farmer populations named. Kantartopu was infected at high rate only in post-emergence stage as a results of soil and seed infestations with A. niger. The cv. Akgün 12 and Alex revealed more tolerance to infections caused by two pathogens than the other cultivars.

INTRODUCTION

Black mould and *Fusarium* basal rot diseases of onion (*Allium cepa* L.) are caused by *Aspergillus niger* Van Tiegham and *Fusarium oxysporum* Schl. f. sp. *cepae* (Hanz). Snyder et Hansen respectively (Rod, 1983; Hayden and Maude, 1992). These diseases are of economic significance primarily because of their causal agents are transmitted by contaminated seeds and soil to seedlings, onion sets and bulb (Hayden et al., 1994 a; Köycü and Özer, 1997).

Infected seeds, when used as planting material, can results in reducing germination and so increased pre-and post-emergence damping-off by serving as a source of primary inoculum. Furthermore the virulences of *A. niger* and *F. oxysporum* isolates obtained from different regions of Türkiye were determined as significantly different in the case of seed germination, pre-and post-emergence damping-off depending upon methods of inoculation (Özer and Köycü, 1997). Previous researches an cultivar susceptibility to basal rot have revealed different degress of tolerance for a number of commercial onion varieties (Lorbeer and Stone, 1965). In greenhouse and growth chamber tests in soils with different levels of artificial infestation with *F. oxysporum* f. sp. *cepae*, seedling of the four varieties generally were equally susceptible to infection as measured by emergence and stand (Abawi and Lorbeer, 1971a). Hybrid lines of yellow Spanish onion cultivars exhibited low incidence of basal rot (Thornton and Mohan, 1996).

Seed treatments with Prochloraz, thiram, benomyl+thiram control black mould and basal rot diseases (Hayden et al., 1994b; Özer and Köycü, 1998), however resistant onion basal rot diseases (Hayden et al., 1994b; Özer and Köycü, 1998), however resistant onion cultivars offer one of the best nonchemical means for controlling seeedborne and soilborne diseases. That is why commercial cultivars should have some degree of resistance to both *A. niger* and *F. oxysporum* pathogens. On the other hand, information about the susceptibility of onion cultivars to *A. niger* and *F. oxysyorum* is necessary to growers, making decisions regarding the disease menagement and could help to optimize the success of integrated control.

The purpose of this investigation was to study the reactions of some local and imported onion cultivars used in Turkey to infections caused by *A. niger* and *F. oxysporum* using by seed and soil infestations.

MATERIALS and METHODS

Plant Material

Five onion cultivars (Akgün 12, Alex, Banko, Kantartopu and Texas Early Grano 502) were used in the study to compare some local and imported onion cultivars. Among them, Akgün 12 was obtained from Yalova Horticultural Research Institute, Kantartopu was the local cultivar of farmer populations. Alex, Banko and Texas Early Grano 502 were the imported cultivars.

Fungal Materials

The experiments were conducted with an isolate of *A. niger* (most virulent by seed and soil infestation methods) and two isolates of *F. oxysporum* (A: most virulent

by seed infestation method, B: most virulent by soil infestation method) which were obtained from naturally infected onion seeds and sets (Köycü and Özer, 1997; Özer and Köycü, 1997). The isolates were cultured on petri plates containing Potato Dextrose Agar (PDA) at 20°C.

The reaction of onion cultivars to seed colonisation with pathogens in in-vitro conditions

The seeds of each cultivars were firstly surface-sterilized with sodium hypochloride (1 %) for 5 min and then washed 3 times with sterile distilled water and dried on sterile filter paper. These seeds were inoculated by soaking in a suspension of conidia containing 1 x 10^7 spores/ml of *A. niger* for 12 h (Hayden and Maude, 1992) and 5 x 10^3 spores/ml (Abawi and Lorbeer, 1971a) of *F. oxysporum* (A) for 15 min. Spore suspensions was prepared from 7 day-old cultures of pathogens. Four replicates of 25 seeds for each cultivar were placed onto PDA and on moistened filter paper (Blotter method). Six days later after inoculation, the number of seeds colonised by *A. niger* and *F. oxysporum* were recorded.

The reaction of onion cultivars to causal agents of pre-and post-emergence damping off and onion set rot in-vivo conditions.

Experiments were conducted in 1996 and 1997 using by two inoculation methods. Seeds belonging to each cultivar were infested with pathogens as mentioned above. In soil infestation method, the sterilized soil (mixture of 1/3 field soil + 1/3 manure + 1/3 sand) was infested with *A. niger* and *F. oxysporum* (B) according to Özer and Ömeroğlu (1995). 7 day-old cultures of pathogens grown on PDA in 8cm petri dishes were removed together with the agar and each culture was mixed in a seperate pot. Sama procedures were applied to control pot for each cultivar without pathogens.

Three replicates of 15 seeds of each cultivar were sown in plastic pots (12 x 10) and the pots were placed on greenhouse benches where temperature and relative humidity in 1996 and 1997, as monitored by hygrothermograph, ranged from 18.5 to 22.7°C and 67.0 to 55.3 % respectively. Photoperiod was around 12-14 hours day light. Plants were watered once a week with tap water.

In these experiments, a completely randomized block design was used. A month after sowing, incidence rate of pre-emergence damping-off and post-emergence damping-off were determined. Four months after sowing the seeds, incidence rate of onion set rot was recorded according to percentage of the control. Data were transformed to angles (arcsin transformation) before analysis of variance.

RESULTS

The reaction of onion cultivars to seed colonisation with pathogens

The percentages of seeds colonised by two pathogens were significantly higher in cv. Texas Early Grano 502 than those of other cultivars both on PDA and by blotter method (Table 1). The cv. Banko was in the second range when blotter method was used. There was always a greater percent of seed contaminaton by these pathogens on PDA plates for all cultivars.

Table 1. The rates of onion seeds colonised by *A. niger* and *F. oxysporum* with Blotter method and on PDA

Cultivar	The rates of seeds colonised by pathogens (%)[a]			
	Blotter		PDA	
	A. niger	*F. oxysporum*	*A. niger*	*F. oxysporum*
Akgün	16.0c[b]	6.0c	50.0b	64.0b
Alex	8.0c	7.0c	47.0b	56.0b
Banko	40.0b	33.0b	71.0b	49.0b
Kantartopu	17.0c	2.0c	51.0b	64.0b
Tex.Ear.Gra. 502[c]	75.0a	77.0a	96.0a	88.0a

[a] Each vaule is a mean of four replicates of 25 seeds

[b] Means within a column followed by the same letter are not significantly (p=0.05) different according to Duncans Multiple Range Test.

The reaction of onion cultivars to causal agents of pre-and post-emergence damping-off and onion set rot

The incidence rates of pre-and post-emergence damping-off and onion set rot developed after seed and soil infestations with *A. niger* and *F. oxysporum* were represented in Table 2. 3 and 4 respectively as a results of the experiments conducted in 1996 and 1997 years.

Significant differences among the cultivars were detected in 1997 for the pre-emergence damping-off caused by both *A. niger* and *F. oxysporum* (Table 2). However infestation methods were not significant for the reaction of the cultivars to both pathogens. The cv. Texas Early Grano 502 exhibited the highest incidence rate of this damping-off disease in the case of seed and soil infestations by two pathogens when it was compared to other cultivars. On the other hand, *A. niger* caused pre-emergence

damping-off at the high rate (for 1996 and 1997, 52.3 and 42.7 % respectively) in the cv. Banko following this cultivar when the pathogen was infested to seeds. In the case of soil infestation, the incidence rate of this disease in the cv. Banko was low, although statistically significant differences were not observed among infestation methods. The greater rates of pre-emergence damping-off was also obtained in the same cultivar by soil infestation of *F. oxysporum* in 1996 and 1997 years. The other cultivars (Akgün 12, Alex and Kantartopu) were effected from this damping-off at the lower rate (Table 2).

Table 2. The incidence rates of pre-emergence damping-off caused by *A. niger* and *F. oxysporum* after seed and soil infestations on five onion cultivars in 1996 and 1997

Cultivar	Infestation	The incidence rates of pre-emergence damping -off caused by (%)			
		A. niger		*F. oxysporum*	
Akgün 12	Seed	21.3[b]	29.3bc	24.3	14.7b
	Soil	29.2	16.0bc	33.3	12.0b
Alex	Seed	19.2	24.9bc	37.3	20.0b
	Soil	27.3	17.3bc	18.2	20.0b
Banko	Seed	52.3	42.7ab	24.7	33.3b
	Soil	19.0	22.7bc	47.6	36.0b
Kantartopu	Seed	17.3	12.1c	21.4	12.0b
	Soil	12.8	10.7c	20.5	12.0b
Texas Early	Seed	70.7	65.3a	66.7	74.0a
Grano 502	Soil	60.1	57.3a	89.3	66.7a

[a] Each vaule is a mean of four replicates of 15 seeds

[b] Means within a column followed by the same letter are not significantly (p=0.05) different according to Duncans Multiple Range Test.

There were also significantly differences among the cultivars in 1996 and 1997 years, for post-emergence damping-off disease occured by *A. niger* and *F. oxysporum* (Table 3). The highest percentage off post-emergence damping-off of seedlings by *A. niger* was observed in the cv. Kantartopu by seed and soil infestations of pathogen in both years. The cv. Akgün 12 and Banko followed this cultivar by significantly soil infestation of pathogen. Furthermore the cv. Banko developed also this damping-off disease at he higher rate in relation with other cultivars when *F. oxysporum* was infested to soil.

Table 3. The incidence rates of post-emergence damping-off caused by *A. niger* and *F. oxysporum* after seed and soil infestations on five onion cultivars in 1996 and 1997

Cultivar	Infestation	The incidence rates of post-emergence damping -off caused by (%)[a]			
		A. niger		*F. oxysporum*	
Akgün 12	Seed	2.7c[b]	12.0c	2.7c	12.0
	Soil	36.0a	20.0abc	16.0ab	29.3
Alex	Seed	5.3c	12.0c	4.0bc	24.3
	Soil	6.7bc	14.4bc	20.0ab	24.2
Banko	Seed	0.0c	10.4c	8.0b	15.1
	Soil	21.3ab	28.0abc	28.0a	24.0
Kantartopu	Seed	57.3a	42.7ab	12.0abc	18.7
	Soil	30.0a	53.0a	21.3ab	25.0
Texas Early	Seed	29.3a	12.0bc	6.7abc	18.1
Grano 502	Soil	4.0c	8.0c	10.7abc	13.3

[a] Each vaule is a mean of three replicates of 15 seedlings

[b] Means within a column followed by the same letter are not significantly (p=0.05) different according to Duncans Multiple Range Test.

As a results of the measurements of onion set rot which were caused by *A. niger* and *F. oxysporum* (Table 4), the cv. Banko had the highest incidence rate of onion rot by two pathogen in the case of their soil infestations. The cv. Texas Early Grano 502 could not developed onion set and the seedlings survived after post-emergence damping-off were very thin.

Table 4. The incidence rates of onion set rot caused by *A. niger* and *F. oxysporum* after seed and soil infestations on five onion cultivars in 1996 and 1997

Cultivar	Infestation	The incidence rates of onion set rot caused by (%)[a]			
		A. niger		*F. oxysporum*	
	type	1996[b]	1997	1996	1997
Akgün 12	Seed	4.0bc	1.3c	14.7	16.0ab
	Soil	0.0c	0.0c	4.0	4.0bc
Alex	Seed	14.7ab	10.7abc	2.7	16.4ab
	Soil	13.3ab	16.0ab	2.7	0.0c
Banko	Seed	12.0ab	20.0ab	12.0	18.7ab
	Soil	21.3a	22.7a	28.0	29.3a
Kantartopu	Seed	14.7ab	29.3a	12.0	28.0a
	Soil	5.3abc	10.7abc	8.0	14.7ab
Texas Early	Seed	Not	Not	Not	Not
Grano 502	Soil	developed	developed	developed	developed

[a] Each vaule is a mean of three replicates of 15 seedlings

[b] Means within a column followed by the same letter are not significantly (p=0.05) different according to Duncans Multiple Range Test.

DISCUSSION

Because of their practical and economical feasibility in comparison with the other control methods like soil fumigation and crop rotation (Abawi and Lorbeer, 1971a; Vik and Asstveit, 1984; Hess and Weber, 1988), the usage of resistant cultivars have been recommended for the control of those diseases on onion like basal rot, neck rot and pink rot. Susceptibility and resistance are assessed by measuring the degree of diseases such as incidence of infection, intensity of symptoms, extent of lesions and decay, degree of lowering viability (Neergaard, 1979). For the first time, in this study, the reaction of onion cultivars to black mould and basal rot caused by *A. niger* and *F. oxysporum* respectively were evaluated by several criteria such as seed colonisation, damping-off and onion set rot to determine resistant cultivars.

Seed health tests on the reaction to pathogen depends on the condition of incubation. In the standart laboratory procedure, test conditions must be included the fungi which are capable of producing sufficient mycelial growth and/or sporulation on agar media or in the blotter tests (Neergaard, 1979). The onion cultivars examined in this study exhibited higher incidence rate of seed colonised by pathogens on PDA media than blotter tests. However, cv. Texas Early Grano 502 had the highest rate of seeds colonised by pathogens in both tests. Germinating seeds of several plant species produce the antifungal compounds as other plant tissues in response to the presence of microorganisms (Keen, 1975). It is possible that some tolerant cultivar may produce such as antifungal compounds in our experiment.

Previous researches have showed that *A. niger* caused pre-emergence damping-off disease and that *F. oxysporum* increased post-emergence damping-off generally, although there were differences for disease severity among the isolates (Özer and Köycü, 1997). The tested onion cultivars had different incidence rates of the pre-and post-emergence damping -off and onion set rot caused by *A. niger* and *F. oxysporum*. At the same time, the susceptibility of them to pathogens also changed by seed and soil infectations of two pathogens. Among them, Texas Early Grano 502 was the most susceptible cultivar to infections occurred before emergence by seed and soil infestations of *A. niger* and *F. oxysporum*. This type of damping-off was very important for the early infection and for the inhibition of onion set development in this cultivar. The cv. Banko was also susceptible to *A. niger* and *F. oxysporum* but it exhibited variability in the stages of diseases caused by two pathogens according to infestation methods. The cv. Kantartopu as a representative cultivar of farmer populations had more susceptibility to post-emergence damping-off by both infestation methods of *A. niger*. The cv. Akgün 12 (Local cultivar) and Alex showed tolerance to diseases occurred by seed and soil borne *A. niger* and *F. oxysporum*. These findings are not agree with those

of Abawi and Lorbeer (1971 a and b) who reported that penetration and subsequent growth of pathogen in the tissues of susceptible and tolerant cultivar were always similar under the conditions employed which were favorable for the development of the disease in the susceptible cultivars.

It should be stressed that these are essentially tests and their validity compared with field tests remains to be demonstrated. However, this study will provide oppurtunities to investigate the reaction of different onion cultivars in the field conditions.

ACKNOWLEDGEMENTS

The author is thankful to the Directorate of Tekirdağ Viticulture Research Institute for providing their greenhouse facilities.

ÖZET

BAZI SOĞAN ÇEŞİTLERİNİN *Aspergillus niger Van Tiegham* VE *Fusarium oxysporum* Schlecht' A KARŞI REAKSİYONLARI

Bu araştırmada beş soğan çeşidinin in-vitro ve in-vivo koşullarda etmenleri sırasıyla *Aspergillus niger* Van Tiegham ve *Fusarium oxysporum* Schlecht olan siyah küf ve dip çürüklüğü hastalıklarına karşı dayanıklılık durumları incelenmiştir. In-vitro denemelerde, patojenler tohumlara inokule edilmiş ve çeşitlerin reaksiyonları tohumların patojenlerle kolonize olma durumlarına göre belirlenmiştir. In-vivo denemelerde ise patojenler tohum ve toprağa inokule edilmiş, çeşitlerin her iki hastalığın farklı infeksiyon şekilleri olan çökerten ve arpacık çürüklüğüne karşı reaksiyonları inokulasyon yöntemi dikkate alınarak karşılaştırılmıştır. Denemeler sonucunda, çeşitlerin hiçbiri *A. niger* ve *F. oxysporum*'un oluşturduğu enfeksiyonlara karşı bağışık olamamışlardır. In-vitro denemelerde, söz konusu etmenlerin kolonize olabildiği en yüksek tohum oranı Texas Early Grano 502 çeşidinde tespit edilmiş, bu çeşidin aynı zamanda *A. niger* ve *F. oxysporum*'un hem tohuma hem de toprağa bulaştırılması sonucunda oluşan çıkış öncesi ölüm ve arpacık çürümesine karşı belirgin derecede hassasiyet gösterdiği saptanmıştır. Banko çeşidi ise ikinci sırada yer almış ve *A. niger*'e hassasiyet yönünden Texas Early Grano 502 çeşidi ile benzer özellikleri sergilenmiş, ancak *F. oxysporum* enfeksiyonlarına karşı etmenin toprağa bulaştırılması sonucunda daha fazla hassasiyet göstermiştir. Yetiştirici populasyonunu temsil eden Kantartopu çeşidi *A. niger*'in her iki yolla bulaştırılması durumunda sadece çıkış sonrası dönemde yüksek oranda enfekte olmuştur. Akgün 12 ve Alex çeşitleri ise her iki patojen tarafından neden olunan enfeksiyonlara karşı diğer çeşitlere göre daha fazla tolerans göstermişlerdir.

LITERATURE CITED

ABAWI, G.S. and J.W. LORBEER, 1971 a. Reaction of selected onion varieties to infection by *Fusarium oxysporum* f. sp. *cepae.* **Plant Dis. Reptr. 55:** 1000-1004.

ABAWI, G.S. and J.W. LORBEER, 1971 b. Pathological histology of four onion cultivars infected by *Fusarium oxysporum* f.sp. *cepae.* **Phytopathology, 61:** 1164-1169.

HAYDEN, N.J. and R.B. MAUDE, 1992. The role of seed-borne *Aspergillus niger* in transmission of black mould of onion. **Plant Pathol. 41:** 573-581.

HAYDEN, N.J., R. B. MAUDE and F. J. PROCTOR, 1994 a. Studies on he biology of black mould (*Aspergillus niger*) on temperate and tropical onion. 1. A comparison of sources of the disease in temparate and tropical field crops. **Plant Pathol. 43:** 562-569.

HAYDEN, N.J., R.B. MAUDE and F.J. PROCTOR, 1994 b. Studies on he biology of black mould (*Aspergillus niger*) on temparate and tropical onion. 2. The effect of treatments on the control of seed-borne *A. niger.* **Plant Pathol 43:** 570-578.

HESS, W. M., and D.J. Weber, 1988. Assays for determining resistance and susceptibility of onion cultivars to the pink root disease. **Phytopathology 78:** 115-117.

KEEN, N.T., 1975. The isolation of phytoalexins from germinating seeds of *Cicer arietinum, Vigna sinensis, Arachis hypogeae* and other plants. **Phytopathology 65:** 91-93.

KÖYCÜ, N.D., and N. ÖZER, 1997. Determination of seedborne fungi in onion and their transmission to onion sets. **Phytoparasitica 25:** 25-31.

LORBEER, J. W., and K.W. STONE, 1965. Reaction of onion to *Fusarium* basal rot. **Plant. Dis. Reptr. 49:** 522-526.

NEERGAARD, P., 1979. Seed Pathology. vol:1. The Macmillan Press Ltd., London, UK.

ÖZER, N., and M. ÖMEROĞLU, 1995. Chemical control and determination of fungal agents of with disease of onion in Tekirdağ province. **J. Turk. Phytopath. 24:** 47-55.

ÖZER, N., and N.D. KÖYCÜ, 1997. The pathogenicity of *Aspergillus niger* and some *Fusarium* species on onion seeds and seedlings. Proceedings 10 th Congress of the Mediterranean Phytopathological Union, 1-5 June, Montpellier-Le Corum (France), 277-281.

ÖZER, N., and N.D. KÖYÇÜ, 1998. Evolution of seed treatments for controlling. *Aspergillus niger* and *Fusarium oxysporum* on onion seed. **Phytopathologia Medit, 37:** 33-40.

ROD, J., 1983. Fungus diseases of onion seed (*Allium cepa* L.). Bull. Vyzk. A Slecht. Ustavu Zelinarskeho, Olomouc, **27:** 4-11.

THORNTON, M. K., and S.K., MOHAN, 1996. Pesponse of sweet Spainsh onion cultivars and numbered hybrids to basal rot and pink rot. **Plant Dis. 80:** 660-663.

VIK, J., and K. AASTVEIT, 1984. Breeding for resistance to neck rot (*Botryts allii*) in onion (*Allium sepa*). **Euphytica 33:** 257-262.

J. Turk. Phytopath., Vol. 27, No. 1, 27-37, 1998 ISSN 0378 - 8024

The Potential Efficiency of Culture Filtrate of *Bacillus subtilis* AB-27 Against *Botrytis cinerea* and *Gloeosporium* sp. on Golden Delicious and Ankara Pear

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ABSTRACT

The potential efficiency of culture filtrate of *Bacillus subtilis* AB-27 isolate which is known an effective antagonist of some plant pathogenic fungi, was searched for *Botrytis cinerea* and *Gloeosporium* sp. on Golden Delicious and Ankara pear. Diseases caused by tested pathogens were considerably decreased on apple and pear fruit by the culture filtrate treatment comparing with those treated by Benomyl (300 µg/ml). The LD_{50} (Lethal Dose) of the culture filtrate on G.Delicious were 300.7 µl/ml for *B. cinerea* and 713.7 µl/ml for *Gloeosporium sp.* The LD_{50} of the culture filtrate on Ankara pear were 964 µl/ml for *B.cinerea* and 859.6 µl/ml for *Gloeosporium* sp.

INTRODUCTION

Apple and pear fruits are generally stored in cold storages for 6-10 months after harvest. The fruits are exposed to a great deal of pathogens during this long period. So far, more than 90 fungus species have been known as a potential causal agents of postharvest diseases. Despite modern storage conditions, postharvest losses have been estimated to be 20-25 % of the harvested crops (Rosenberger, 1991). The losses can be even greater in underdeveloped and developing countries (Wilson and Wisniewski, 1989).

Most of postharvest losses of apple and pear are due to rots caused by fungal pathogens belonging to genera of *Penicillium, Botrytis, Monilia, Nectria, Gloeosporium* and *Alternaria* (Pekmezci, 1976; Agrios, 1988; Gürer, 1989; Rosenberg, 1991). In addition to cultural and physical precautions to control postharvest diseases, in recent years, storage and transport under low oxygen (5 percent) have been used to suppress respiration of the host, the pathogen, and finally development of postharvest rots (Agrios 1988).

Gamma rays and radiation treatments are also useful in reducing some postharvest diseases of some crops (Agrios, 1988; Tiryaki, 1990; Tiryaki et al., 1994).

THE POTENTIAL EFFICIENCY OF CULTURE FILTRATE OF *BACILLUS SUBTILIS* AB-27 AGAINST *BOTRYTIS CINEREA* AND *GLOEOSPORIUM* SP. ON GOLDEN DELICIOUS AND ANKARA PEAR

Biological controls have been developed that are effective against some fungal and bacterial pathogens (Wilson and Pusey, 1985). Having some disadvantages such as potential oncogenic risks among other pesticides and appearance of pathogen resistance, fungicides are a primary means of controling postharvest diseases (Eckert and Ogawa, 1985; Gürer and Maden, 1990). Concerning the disadvantages of fungicide applications, a new alternative control technologies of controlling postharvest diseases that are perceived as safe by the people and have negligible risk to human helth and the environment need to be developed.

The objective of this work is to search the potential effeciency of culture filtrate of *B. subtilis* AB-27, an effective antagonist *in vitro* (Basım, 1990; Basım et al. 1991), against *B. cinerea* and *Gloeosporium* sp. on apple and pear fruits.

MATERIALS and METHODS

1. The pathogens: *Botrytis cinerae* (isolated from decayed apple tissue) and *Gloeosporium* sp. (provided by Prof. Dr. Salih MADEN, Ankara University, Faculty of Agriculture, Plant Protection Department) which are highly pathogenic on pear and apple fruits were maintained on potato-dextrose agar (PDA) and periodically transferred on PDA. To obtain inocula, *B.cinerae* was grown on PDA in petri plates for 14 days at 24±1°C and *Gloeosprium* sp. for 10 days at 24 ± 1°C with continuous light. Conidial suspensions were prepared by washing the colonies growing on PDA with 2 ml of sterile 0.05 % Tween 80. Aliquots were then collected and diluted with sterile deionized water to 1 x 10^5 conidia per mililiter as determined with a hemacytometer.

2. Preparation of culture filtrate: The soil-isolated *Bacillus subtilis* AB-27 (provided by Dr. İsmail ULUKUŞ, Biological Control Research Institute, Antalya) was grown on PDA slants tubes for 24 h at 30°C. Five mililiters of sterile tap water was pipetted onto the slant tube. The tube was gently shaken to wash the cells from the agar surface. The bacterial suspension (0.5 ml) was used to inoculate a flask of antibiotic production medium (Mc Keen and Pusey, 1986). The inoculated flask was incubated on a shaker at 170 rpm and 30°C for 3 days. The incubated production medium was first centifuged for 30 min at 5.000 rpm and then passed through membrane filter (0.45 µm). The doses of culture filtrate were prepared by diluting the culture filtrate with distilled water.

3. Spore germination: *In vitro* testing for spore germination was conducted in concave slide. The culture filtrate (undiluted) of *B. subtilis* AB-27, benomyl (300 mg/ml) and spore germination medium contained 2 g of KH_2PO_4, 0.1 g Na_2HPO_4, 5 gNa, K Tartarate, 75 g Dextrose, 1000 ml of deionozied water were examined for

inhibition of spore germination of. *B.cinerea* and *Gloeosporium* sp. The concentrations of the pathogens were 10^5 conidia/ml. Hanging drop slides were examined after 12 h. After additional 48 h incubation, hanging drop slides were examined second time. Each concave slide was used as a single replicate, and there were three replicates per treatment.

4. The fruits: Golden Delicious apples and Ankara pears used in tests were selected for uniformity in size and ripeness. Additional selection of the fruits were determined by Iodine test (Kupferman and Waelti, 1983) for uniformity in ripeness. Ripeness of selected fruits was in scale 4 for both apples and pears.

5. Wounding and inoculation of fruits: The surface sterilization of the fruits were by 70 % ETOH and rinsed with sterile deionized water. The fruits were wounded in 5 mm diameter and deep with a sharp cork-borer. The culture filtrate (20 µl) was transferred into wells on the fruits and then spore suspension (20 µl) containing conidia of *B. cinerea* and *Gloeosporium* sp. ($1x10^5$ conidia per mililiter) were added to the well containing culture filtrate. Sterilized deionized water (20 µl) and 20 µl of benomyl (300 µg per mililiter) were used as control and in order to determine the success of the culture filtrate, respectively. Each fruit was then placed into a polyethylene bag with the wounded face up. Five fruits were tested for each treatment, and each treatment was repeated two times. Fruits were incubated at 24 ± 1 °C for 7 days.

6. Disease assesment: At the end of the incubation period, diameters of lesions were measured perpendicular to the axis, and volumes of the disease tissues were determined by taking out disease tissue from inoculated fruits. Results were compared with both control fruits and fruits treated with benomyl. MSTAT statistic programme was performed for all calculations, and significance was P=0.05, using Duncan's Multiple range Test. The LD_{50} was determined based on the equation of regression analysis (Dimond et al., 1941).

RESULTS and DISCUSSION

The culture filtrate of *Bacillus subtilis* AB-27 controlled *Botrytis cinerae* and *Gloeosporium* sp. on Golden Delicious and Ankara pear. The concentration of the culture filtrate significantly (P=0.05) affected lesion development on both types of fruit inoculated with either pathogen (Table 1, 2). Rot caused by *Gloesporium* sp. was controlled by a lower culture filtrate concentration (LD_{50}: 859.7 µl/ml) than that of caused by *B. cinerea* (LD_{50}: 964 µl/ml) on pears. However, the culture filtrate controlled rot caused by *B. cinerea* on apples at lower concentration (LD_{50}: 300.7 µl/ml) than that of caused by *Gloeosporium* sp. (LD_{50}: 713.7 µl/ml). Perhaps, this might be related to presence of the basic differences in chemical composition of the fruits. The complete control of both diseases was obtained on Golden Delicious but not Ankara

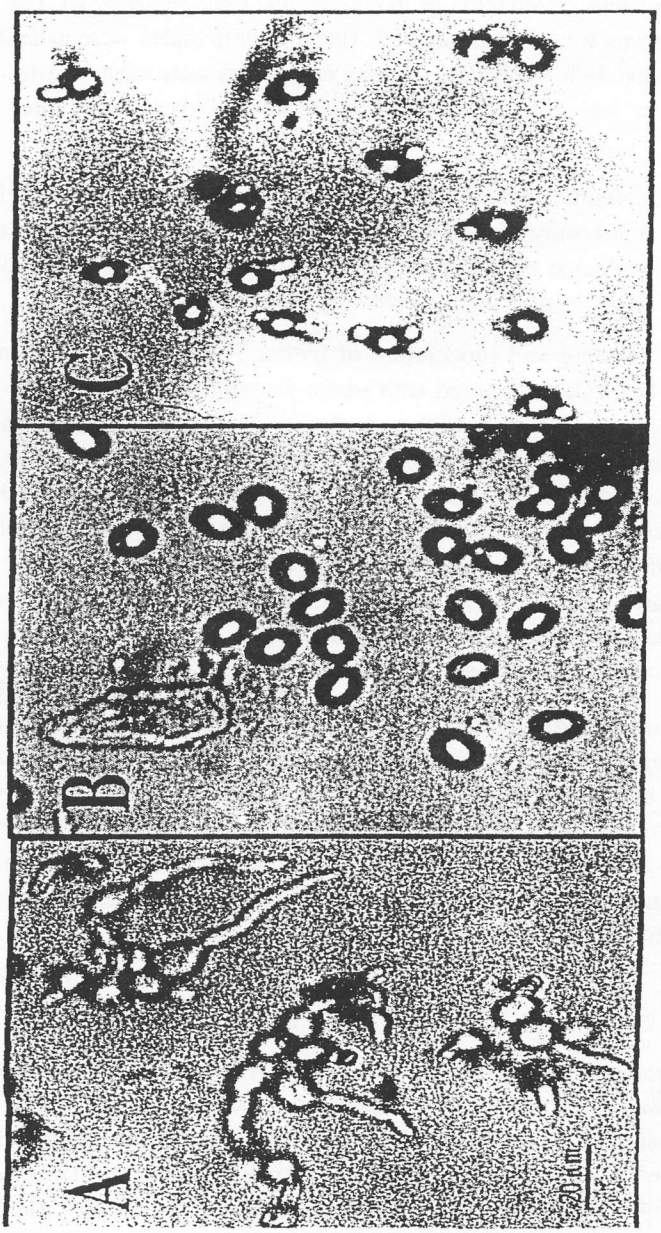

Figure 1. Effects of culture filtrate of *Bacillus subtilis* AB-27 and benomyl on conidia germination of *Bortytis cinerea* (**A**) Control. (**B**) Culture filtrate (undiluted). (**C**) Benomyl (300 µg/ml).

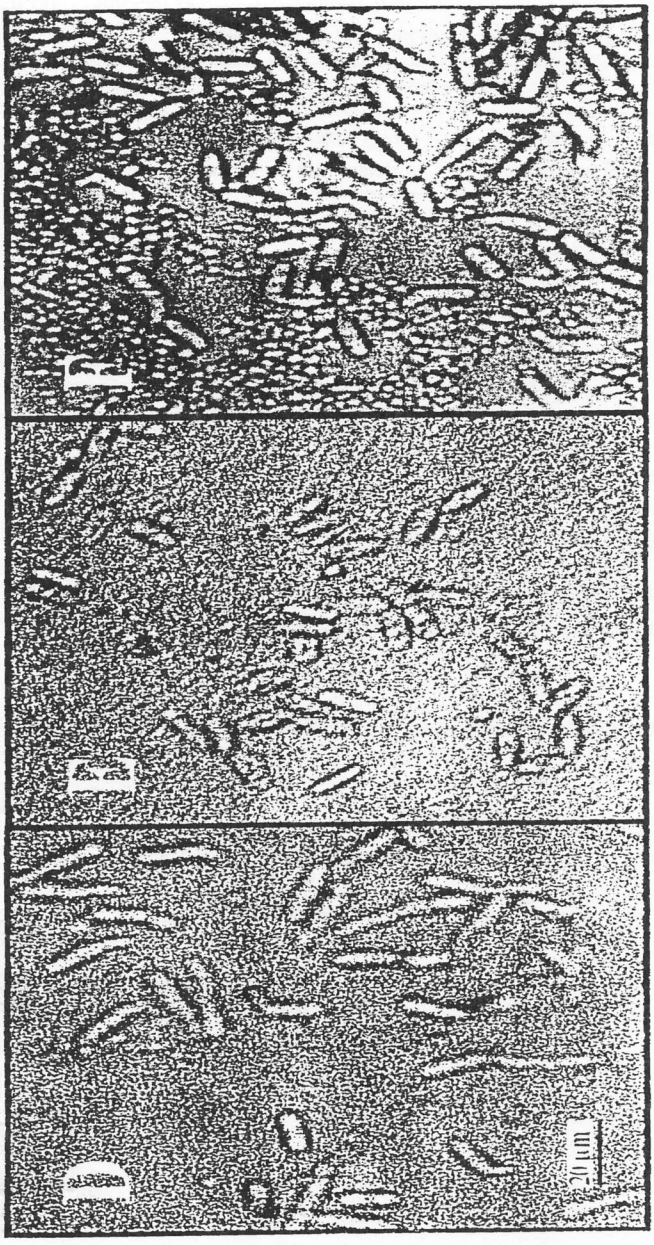

Figure 2. Effects of culture filtrate of *Bacillus subtilis* AB-27 and benomyl on conidia germination of *Gloeosporium* sp. **(D)** Control. **(E)** Culture filtrate (undiluted). **(F)** Benomyl (300 µg/ml).

Table 1. Effects of culture filtrate of *Bacillus subtilis* AB-27 and benomyl (300 µg/ml) against *Botrytis cinerea on* Golden Delicious and Ankara pear.

	Ankara pear		Golden Delicious	
Doses of culture filtrate (µl/ml)	Average rot* diameter (mm)	Average disease* volume (cm^3)	Average rot* diameter (mm)	Average disease* volume (cm^3)
Control	68.67A	43.75A	51.21A	65.57A
30	62.50AB	43.12A	35.42B	42.28B
167	59.00AB	41.16A	29.79C	38.76B
333	57.42AB	35.28AB	28.92CD	27.16C
500	53.33AB	35.28AB	24.67DE	24.43CD
667	46.33ABC	28.42ABC	22.00EF	23.55CD
833	43.25BC	28.41ABC	21.50EF	19.62CD
1000	41.83BC	20.58BC	18.63F	18.14D
Benomyl	25.67C	18.71C	0.00G	0.00E

* Values are means of five replicates. Values within one column followed by different letters are significantly different at p=0.05 (Duncan's Multiple Range Test).

Table 2. Effects of culture filtrate of *Bacillus subtilis* AB-27 and benomyl (300 µg/ml) against *Gloeosporium* sp. an Golden Delicious and Ankara pear.

	Ankara pear		Golden Delicious	
Doses of culture filtrate (µl/ml)	Average rot* diameter (mm)	Average disease* volume (cm^3)	Average rot* diameter (mm)	Average disease* volume (cm^3)
Control	39.92A	27.07A	36.58A	31.00A
30	38.96A	27.06A	35.83A	29.00B
167	36.54AB	21.75AB	35.12AB	21.27AB
333	36.08AB	19.58BC	34.83AB	21.27AB
500	34.04AB	17.88BCD	32.04AB	17.88BC
667	32.79B	15.71BCD	30.92AB	16.43BC
833	32.04B	13.53CD	28.33AB	14.02BC
1000	31.46B	12.57CD	25.21B	11.12C
Benomyl	25.29C	11.90D	0.00C	0.00D

* Values are means of five replicates. Values within one column followed by different letters are significantly different at p=0.05 (Duncan's Multiple Range Test).

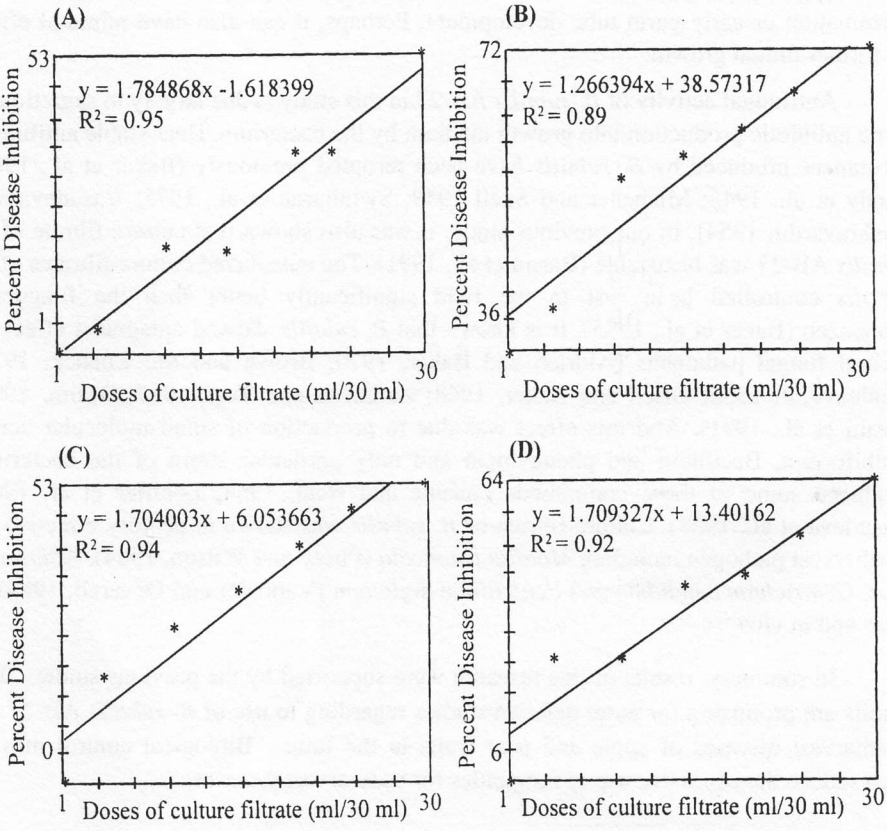

Figure 3. The linear relation on Golden Delicious and Ankara pear between doses of culture filtrate of *Bacillus subtilis* AB-27 and percent inhibition of diseases caused by *Botrytis cinerea* and *Gloeosporium* sp. **(A)** The linear relation on Ankara pear between doses of culture filtrate and percent inhibition of disease caused by *B. cinerea* **(B)** The linear relation on Golden Delicious between doses of culture filtrate and percent inhibition of disease caused by *B. cinerea* **(C)** The linear relation on Ankara pear between doses of culture filtrate and percent inhibition of disease caused by *Gloeosporium* sp. **(D)** The linear relation on Golden Delicious between doses of culture filtrate and percent inhibition of disease caused by *Gloeosporium* sp.

pears treated with benomyl at the concentration of 300 µg/ml. The highest concentration of the culture filtrate greatly reduced but did not completely control lesions on both fruits, whereas highest concentration of the culture filtrate completely inhibited the germination of conidia from *B. cinerea* and *Gloeosporium* sp. *in vitro* (Fig 1). Possibly, culture filtrate did not have the same stability and activity as culture filtrate tested *in*

vitro. Apparently, the mode of *B. subtilis* AB-27 activity on fruit is toward spore germination or early germ tube development. Perhaps, it can also have minimal effect on further fungal growth.

Antifungal activity of *B. subtilis* AB-27 in this study is due largely to segretion of some antibiotic production into growth medium by the bacterium. Heat-stable antifungal substances produced by *B. subtilis* have been reropted previously (Baker et al., 1983; Landy et al., 1948; Michener and Snell 1949; Swinburne et al., 1975; Vasudeva and Chakravarthi, 1954). In our previous study, it was also shown that culture filtrate of *B. subtilis* AB-27 was heatstable (Basım et al., 1991). The autoclaved culture filtrates of *B. subtilis* controlled bean rust in the field significantly better than the fungucide Mancozeb (Baker et al., 1985). It is known that *B. subtilis* showed antagonist effect on several fungal pathogens (Aldrich and Baker, 1970; Brown and Mc Conack; 1972; Dunleavy, J. 1955; Olsen and Baker, 1968; Zazzerini and Tosi, 1987; Basım, 1990; Basım et al., 1991). And this effect was due to production of small moleculer acids; Bulbiformin, Bacillicin and phengymcin and only particular strain of the bacterium produced some of these compounds (Asante and Neal, 1964; Loeffler et al, 1986; Vasudeva et al., 1958). Culture filtrate of *B. subtilis* was shown to be very effective on postharvest pathogen including *Monilia fructicola* (Pusey and Wilson, 1984), *Alternaria citri, Geotrichum candidum* and *Penicillium digitatum* (Vapinder and Deverall, 1984) *in vitro* and *in vivo.*

In summary, results of this research were supported by the previous studies. The results are promising for more detailed studies regarding to use of *B. subtilis* AB-27 for postharvest diseases of apple and pear fruits in the future. Biological control may at least reduce the excessive use of fungicides for postharvest diseases.

ACKNOWLEDGEMENTS

IThis work was supported by a fund from Akdeniz University.

ÖZET

Bazı bitki patojeni funguslara karşı antagonistik olduğu bilinen *Bacillus subtilis* AB-27 izolatından elde edilen kültür filitratının Golden Delicious ve Ankara armut'u meyvelerinde depo çürüklerine neden olan *Botrytis cinerea* ve *Gloeosporium* sp.' a karşı potansiyel etkisi araştırılmıştır. Kültür filitratının test edilen iki patojenin elma ve armut meyvelerinde sebep oldukları çürükleri önemli derecede azaltabildiği benomyl (300 µg/ml) ile muamele edilen meyveler ile karşılaştırılarak belirlenmiştir. Kültür filitratının LD_{50}'si G. Delicious üzerinde ve *B. cinerea* için 300.7 µl/ml ve *Gloeosporium* sp. için 713.7 µl/ml; Ankara armut'u üzerinde ise *B. cinerea* için 964 µl/ml ve *Gloeosporium sp.* için 859.7 µl/ml olarak belirlenmiştir.

LITERATURE CITED

AGRIOS, G., 1988. Plant Pathology. Academic Press, Inc. USA. pp 803.

ALDRICH, J. and BAKER, R., 1970. Biological control of *Fusarium roseum* f. sp. *dianthi* by *Bacillus subtilis.* **Plant Dis. Rep. 54**(5): 446-448.

ASANTE, G.S. and NEAL, A.L., 1964. Characterization of fungistatic substances produced by a *Bacillus* antagonistic to *Ceratocystis ulmi.* **Phytophology 54:** 829-822.

BAKER, C.J., STAVELY, J.R., THOMAS, C.A., SASSER, M. and MACFALL, J.S., 1983. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. **Phytopathology 73:** 1148-1152.

BAKER, C.J., STAVELY, J.R. and MOCK, N., 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. **Plant Dis. 69:** 770-772.

BASIM, H., 1190. Bazı *Bacillus subtilis* izolatlarının önemli bitki patojeni funguslara karşı *in vitro* koşullarda antagonistik etkilerinin araştırılması. Yüksek Lisans tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Bölümü, 74 s.

BASIM, H., MOMOL, M.T. and YEĞEN, O., 1991. *Bacillus subtilis* ile elma ve armutta hasat sonrası görülen *Gloeosporium* sp. ve *Botrytis cinerea*'nın biyolojik kontrolü. VI. Türkiye Fitopatoloji Kongresi, 7-11 Ekim 1991, İzmir, Türkiye Fitopatoloji derneği Yayınları, **6:** 223-228.

BROWN, G.E. and MCCORNACK, A.A., 1972. Decay caused by *Alternaria citri,* in Florida citrus fruit. **Plant Dis. Rep. 56:** 909-912.

DIAMOND, A.E., HORSFALL. J.G., HEUBERGER, J. W. and STODDARD, E.M., 1941. Role of the dosage-response curve in the evaluation of fungicides. Connecticut Agricultural Experiment Station Bulletin **451:** 635-667.

DUNLEAVY, J., 1955. Control of damping-off of sugarbeet by *Bacillus subtilis.* **Phytophathology 45:** 252-258.

ECKERT, J. W. and OGAWA, J.M., 1985. The chemical control of postharvest diseases: Subtropical and tropical fruits. **Ann. Rev. Phytopathol. 23:** 421-454.

GÜRER, M., 1989. Ankara yöresinde yetiştirilen Ankara armutlarında görülen depo çürüklük etmenleri ve bunların mücadelesi üzerinde araştırmalar. Doktora tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma bölümü, 111 s.

GÜRER, M. and MADEN, S., 1990. Chemical control of storage rots in Ankara pears. **J. Turk. Phytopath. 19:** 31-39.

KUPFERMAN, E. and WAELTI, H., 1983. Harvest edition. Postharvest Pomology Newsl., Wash. State Univ., Pulmann **1:** 9-11.

LANDY, M., WARREN, G.H., ROSENMAN, S.B. and COLIO, L.G., 1948. Bacillomycin: An antibiotic from *Bacillus subtilis* active against pathogenic fungi. **Proc. Soc. Exp. Biol. Med. 67:** 539-541.

LOEFFLER, W., TSCHEN, J. S.M., VANITTANAKOM, N., KUGLER, M., KNORPP, E., HSIEH, T.F. and WU, T.G., 1986. Antifungal effects of bacillicin and fengymycin from *Bacillus subtilis* F-29-3. A comparison with activities of other *Bacillus* antibiotics. **J. Phytopathology 115:** 204-213.

MCKEEN, C.D. REILLY, C.C. and PUSEY, P.L., 1986. Production and partial characterization of antifingal substances antagonistic to *Monilia fructicola* from *Bacillus subtilis.* **Phytopathology 76:** 136-139.

MICHENER, H.D. and SNELL, N., 1949. Two antifingal substances from *Bacillus subtilis* cultures. **Arch. Biochem. 22:** 208-214.

OLSEN, C.M. and BAKER, K. F., 1968. Selective heat treatment of soil and its effet on the inhibition of *Rhizoctania solani* by *Bacillus subtilis.* **Phytopathology 58:** 79-87.

PEKMEZCİ, M., 1976. Elma ve armutların soğukta ve kontrollü atmosferde muhafazası sırasında ortaya çıkan fizyolojik nedenli bozulmalar. Ç.Ü. Yıllığı Yıl: **7**(2): 77-93.

PUSEY, P.L. and WILSON, C.L., 1984. Postharvest biological control of stone fruit brown rot by *B. subtilis.* **Plant Dis. 68:** 753-756.

ROSENBERGER, D.A., 1991. Postharvest diseases. Pages: 53-54. In: Compendium of apple and pear diseases. (Eds. A. L. Jones and H.S. Aldwinckle), APS Press, USA, 100 pp.

SWINBURNE, T.R., BARR, J.G. and BROWN, A.E., 1975. Production of antibiotics by *Bacillus subtilis* and their effect on fungal colonists of apple leaf scars. **Trans. Br. Mycol. Soc. 65:** 211-217.

TİRYAKİ, O., 1990. Inhibition of *Penicillium expansum, Botrytis cinerea, Rhizopus stolonifer,* and *Alternaria teniussima* which were isolated from Ankara pears by Gamma irradiation. **J. Turk. Phytopath. 19:** 133-140.

TİRYAKİ, O., AYDIN, G. and GÜRER, M., 1994. Postharvest disease control of apple, quince, onion, and peach, with radiation treatment. **J. Turk. Phytopath. 23:** 143-152.

VAPINDER, S., and DEVERALL, B.J., 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. **Trans. Br. Mycol. Soc. 83**(3): 487-490.

VASUDEVA, R. S., and CHAKRAVARTHI, B.P., 1954. The antibiotic action of *Bacillus subtilis* in relation to certain parasitic fungi, with special reference to *Alternaria solani.* **Ann. Appl. Biol. 41:** 612-618.

VASUDEVA, R. S., SUBBAIAH, T.V., SASTRAY, M.L.N., RANGASWAMY, G. and IYENGAR, M.R.S., 1958. Bulbiformin an antibiotic produced by *Bacillus subtilis.* **Ann. Appl. Biol. 46:** 336-345.

WILSON, P. and Pusey, P.L., 1985. Potential for biological control of postharvest plant diseases. **Plant Dis. 69:** 375-378.

WILSON, C.L., and WISNIEWSKI, M.E., 1989. Biological control postharvest diseases of fruits and vegetables: An emerging technology. **Ann. Rev. Phytopathol. 27:** 425-441.

ZAZZERINI, A., and TOSI, L., 1987. Antogonistic effects of *Bacillus* spp. on *Sclerotinia sclerotium* sclerotia. **Phytopathology Medit. 26:** 185-187.

J. Turk. Phytopath., Vol. 27, No. 1, 39-46, 1998 ISSN 0378 - 8024

Incidence of Rhizomania Disease on Sugar Beet In Çorum, Kastamonu and Turhal Sugar Refinery Regions

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ABSTRACT

The dissemination of rhizomania in Çorum, Kastamonu and Turhal Sugar Refinery areas was investigated by collecting soil samples from these regions. Sugar beet cv. Fiona which is suspectible to the disease was sown as the trap-plant to the soil samples. The rootlets and leaves of the trap plants were tested by means of I-ELISA and the spread of NYVV was determined in the area investigated. The presence of Polmyxa betae was investigated by staining the rootlets with acid fuchsin in lacto phenol solution and the causal agent was observed by light microscopy.

Rhizomania was detected in sugar beet fields of Çorum, Kastamonu and Turhal sugar refinery regions at the rates of 77.39 and 66 %, respevtively. The dissemination of infected soil in these three sugar beet production areas are also given. *Polymyxa betae* was present at the rates of 91, 79 and 48 % in these areas, respectively. The ratios of non-viruliferous *Polymyxa betae* in Çorum, Kastamonu and Turhal were 14.9 and 13, respectively.

INTRODUCTION

Sugar beet is an important crop in Turkish agriculture. According to the 1995 statistics sugar beet production areas were 312.251 ha and the yield was 11.170.569 tons (Anonymous, 1995).

Rhizomania is one of the most important disease of sugar beet which limits its production in the whole world. This disease was first detected in Italy by Canova (Putz et.al., 1990) and because of the extensive formation of lateral roots it was called as rhizomania. In 1965 the association between the fungus, *Polymxa betae* Keskin and a virus was established. In 1966 the virus was named as Virus A by the same author (Putz et. al., 1990). Later on from the sugar beet leaves showing rhizomania symptoms a rod-

shaped virus was isolated and named as Beet Necrotic Yellow Vein Virus, BNYVV (Tamada and Baba, 1973) The relationship between the vector and the virus was studied by various researchers (Abe and Tamada, 1986; Putz et. al., 1990; Tamada and Baba, 1973) and the virus was included in the furovirus (fungus transmitted rod shaped viruses) genus (Abe and Tamada, 1986; Putz et al., 1990; Henry, 1989; Hill and Torrance, 1989; Payne and Asher, 1990).

Polymyxa betae is a biotrophic fungus and more common under high moisture conditions. It forms plasmodia in sugar beet roots and these plasmodia form cystosori (Keskin, 1964). The fungus survives in soil long years with the cystosori. Once the virus is acquired it is transmitted with the fungus (Gerik, 1992; Duffus, 1991). *Polymyxa betae* can cause stunting and necrosis in lateral roots. It can be easily detected in roots with staining (Gerik, 1992; Duffus, 1991). For the detection of the virus, studies were concentrated on the production and use of monoclonal and polyclonal antisera and application of serological tests (Barker, 1989; Koenig et. al., 1984; Torrance et. al., 1988).

BNYVV was first reported in Türkiye by Vardar and Erkan (1992). Erdiller and Özgör (1994), using diseased plant extracts, detected the virus in Adapazarı, Alpullu, Ankara, Eskişehir, Kastamonu and Turhal sugar refinery regions. In spite of the detection of the virus, There is no study about its vector in Türkiye.

The goal of this study is to find out the precalence of rhizomania and its fungal vector in Kastamonu, Çorum and Turhal Sugar Refinery areas which are important sugar beet growing regions in Türkiye. In this study a systematic and detailed soil sampling was accomplished in these sugar beet production areas. Sugarbeet cv. Fiona was grown in these soil samples under greenhouse conditions. The virus was detected by using I-ELISA and the presence or absence of fungal vector *Polymyxa betae* was also determined.

MATERIALS and METHODS

Soil samples were collected from sugar beet fields of Çorum, Kastamonu and Turhal Sugar Refinery regions. Each sample representing 200 ha production area was prepared by collecting 10 different samples from the field and mixed together and from the resulting mixture 1,5-2 kg. samples as a result were taken. Therefore 53 samples from Çorum, 52 from Kastamonu and 122 from Turhal Sugar Refinery regions totalling 227 samples were obtained and investigated.

Growth of Trap Plants

Soil samples were placed into polyethylene bags which contained double holes 0.5 cm in diameter at the bottom. The bags then were put into larger ones without holes in order to maintain high humidity and to protect from cross contamination. A 1 cm thick styrofoam plate was placed between the bags. Ten seeds of sugar beet cv. Fiona were sown to each soil sample. Plants were maintained in a greenhouse for 3 months with a temperature range of 25±7°C. Then they were harvested and roots were taken for determination of *Polymyxa betae.* Root and leaf samples were taken also for determination of BNYVV.

Determination of BNYVV

Detection of BNYVV in roots and leaf extracts were accomplished by I-ALISA according to the method of Koenig (1981) and presence of BNYVV was tested as the reaction against BNYVV IgG (Sigma). With a mortar and pestle 0.02 g rootlets were grounded in a 2 ml coating buffer. The sap was squeezed through cheesecloth and used in the assay (Abe and Tamada, 1986). Leaf extracts were prepared according to Koenig et al (1984). Leaf extracts were prepared according to Koenig et al (1984). Leaf material to be tested grounded in a mortar with ten fold of coating buffer. A French F3 (INRA-IBMP, Strasbourg, France) and an American isolate (Agri, Exp. sta., Texas, USA) were used as positive controls and a healty plant extract was used as negative control. In preliminary tests BNYVV IgG at 1 µg/ml and goat anti rabbit IgG at 1/4000 dilutions yielded good results, threfore, these dilutions were used throughout the ELISA tests. Results were obtained by Biotek ELISA reader at 405 nm. Absorbance values twice as much as the negative controls were considered as positive. The specimens bearing no hairy rootlets, no cystosori and showing negative values in I-ELISA test were considered negative for rhizomania.

Determination of *Polymyxa betae*

Root samples were maintained in 5 % formalin solution until microscopic examinations (Payne and Asher, 1990). Lateral roots were stained with lactophenol solution which contained 0.1 % acid fuchsin for observation of fungal structures of *P. betae* (Keskin) (Abe and Tamada, 1986). The root samples having *P. beate* cystosori and showing negative values in I-ELISA against BNYVV were considered as non viruliferous. But the root samples having cystosori and showing positive values in I-ELISA were considered as rhizomania infected.

RESULTS

Typical rhizomania symptoms on leaves and roots were observed on plants, growing under the greenhouse conditions after three months (Figure1). The number of samples which contain *Polymyxa betae* and reacted positively to BNYVV antiserum and the infection rates were presented in Table 1.

BNYVV infection was common in the three sugar refinery areas (Table 1). Plants having ELISA (+) values either in leaf or root samples, considered BNYVV positive. BNYVV positive plants yielded 96.45 % (+) ELISA in leaf samples, and 87,23 % in root samples. Only four of the infected samples, being 2,83 % at total, showed ELISA (+) results only in root while two of them, being 1,42 %, in leaf samples. Absorbance values obtained from roots and leaves ranged between 0.400-0.700, and 0.400-2.5, respectively. The percentage of leaves that showed high absorbance values was 34,55 %.

Prevalence of rhizomania was found to be the lowest in Kastamonu Refinery Region being 39%. The ratios were 66 and 77 % for Turhal and Çorum Regions, respectively.

Table 1. Incidence of *Polymyxa betae,* BNYVV and rhizomania infection in Çorum, Kastamonu and Turhal Sugar Refinery Regions

Location	Number of samples	Number of samples containing BNYVV*		Samples containing *P. betae*		Rhizomania infection
		Leaves	Roots	No.	%	(%)**
Kastamonu	52	20	3	25	48	39
Merkez	13	5	3	8	62	39
Bölge	12	7	5	7	58	58
Tosya	7	2	2	4	57	29
Boyabat	9	2	2	2	22	22
Taşköprü	11	4	4	4	36	36
Turhal	122	80		96	79	66
Bölge	28	18	19	24	86	75
Zile	30	21	19	27	90	70
Pazar	10	13	11	14	70	65
Niksar	20	7	7	9	90	70
Tokat	3	3	2	3	100	100
Aydınca	3	0	0	3	100	0
Artova	17	9	9	11	65	59
Çamlıbel	11	4	5	5	46	46
Çorum	53	41		48	91	77
İskilip	9	8	8	8	89	89
Kızılırmak	8	7	5	7	88	88
Merkez	12	6	5	9	75	50
Osmancık	4	4	3	4	100	100
Alaca	10	8	8	10	100	80
Sungurlu	4	4	4	4	100	100
Mecitözü	6	4	3	6	100	67
Total	227	136	123	169		

* Sample numbers showing positive ELISA values in leaves and roots.
** The percentage of BNYVV determined in leaves or roots.

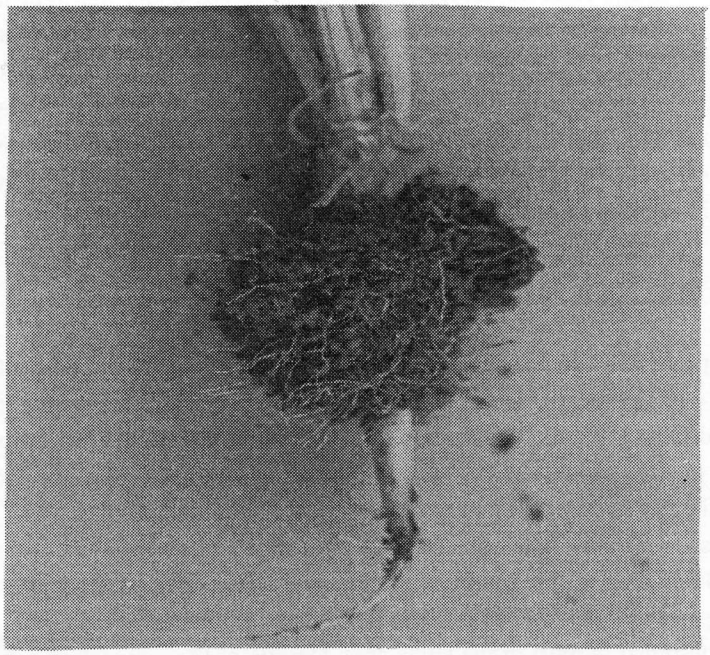

Figure 1. A sugar beet plant showing typical rhizomania symptoms.

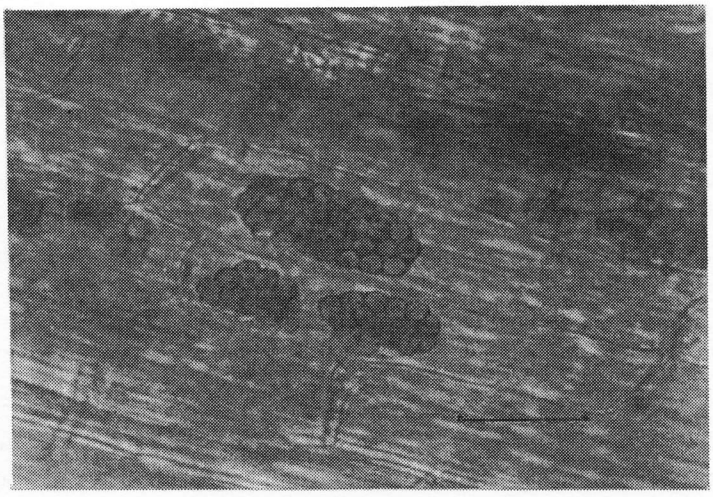

Figure 2. Cystorosi of *Polymyxa betae* observed on sugar beet rootlets. Bar = 50 µm.

Cystosori of *Polymyxa betae* were easily observed under the light microscope (Figure 2). *Polymyxa betae* was present in all three factory regions that were investigated. In Kastamonu region, approximately 50 % of the samples were found to be infected with *Polymyxa betae,* but these values were 79 and 91 % for Turhal and Çorum refinery regions, respectively, BNYVV was not detected in 18 samples from Kastamonu (5), Turhal (6) and Çorum (7) which had *Polymyxa betae.* This represents the presence of non viruliferous *Polymyxa betae* isolates.

DISCUSSION

It appears that rhizomania is common in the three sugar beet production areas. The presence of rhizomania in Kastamonu and Turhal regions was reported previously (Erdiller and Özgör, 1994). The disease also appears to be common in Çorum region. Occurrence of rhizomania was the lowest in Kastamonu region. This low incidence of rhizomania in this region could be attributed to the cultivation of sugar beet in non irrigated areas extensively. The occurrence of *Polymyxa betae* was also lower in this region. The elucidation of the reasons that *Polymyxa betae* does not exist in some areas could be important to determine the potential spread of the disease. *Polymyxa betae* was not very widespread in most of the study area. Despite of the presence of non viruliferous *Polymyxa betae* in some areas, virus was not detected, so these areas can be considered as suspectible to rhizomania infection.

Positive ELISA values were obtained from most of the leaf samples, but a few of the root samples showed positive values. As pointed by previous researchers (Putz et al., 1990). virus multiplication in leaves occurs more abundantly than in roots. On the other hand, the reason of observing positive ELISA values only in 4 of the root samples could be explained by missing or damage of the reciprocal leaf samples.

In Türkiye detailed studies on rhizomania are necessary. Disease infested areas should be determined. Quality of irrigation water and their sources and routes sholud be investigated. So these measures might be implemented to help in reducing the disease incidence.

ACKNOWLEDGEMENTS

The authors are grateful to Turkish Sugar Co. Inc. for supporting the study, to G. Heidel from Texas Agricultural Experiment Station for supplying BNYVV isolates and to O. Lemaire from INRA, France for supplying F3 isolate and BNYVV antiserum and IgG.

ÖZET

RHIZOMANIA'NIN ÇORUM, KASTAMONU VE TURHAL ŞEKER FABRİKASI EKİM ALANLARINDAKİ DAĞILIMI

Rhizomania'nın Çorum, Kastamonu ve Turhal Şeker Fabrikaları'nın ekim alanlarındaki yaygınlığı, bu bölgelerden toprak örnekleri alınmak suretiyle incelenmiştir. Hastalığa hassas şeker pancarı çeşidi olan Fiona, bu toprak örneklerine tuzak bitki olarak ekilmiştir. Tuzak bitkilerin kılcal kök ve yaprakları I-ELISA testine tabi tutulmuş ve bölgedeki BNYVV dağılımı araştırılmıştır. *P. betae*'nın varlığı ise, kılcal köklerin asit fuksin lakto fenol çözeltisinde boyanarak ışık mikroskobu altında incelenmiştir.

Rhizomania'nın Çorum, Kastamonu ve Turhal bölgelerindeki yayılış oranı sırasıyla % 77, % 39 ve % 66'dır. Bu üç fabrika alanlarındaki bulaşık toprak dağılımı da belirlenmiştir. *Polymyxa betae*'nın bu alanlarda bulunuş oranları ise, yine sırasıyla % 92, %79 ve %48'dir. Virus içermeyen *Polymyxa betae* ise sırasıyla % 14, % 9 ve % 13 oranlarında tespit edilmiştir.

LITERATURE CITED

ABE, H. and TAMADA, 1986. Association of Beet necrotic yellow vein virus with isolates of *Polymyxa betae* Keskin. **Ann. Phytopath. Soc. Japan 52:** 235-247.

ANONYMOUS, 1995. Tarımsal Yapı ve Üretim T.C. Başbakanlık Devlet İstatistik Enstitüsü. 557 pp.

BAKER, I. 1989. Development of a routine immunoassay for the Detection on Beet necrotic yellow vein virus using monoclonal antibodies. Sugar Beet Rhizomania Committee Paper. No. 2371, 27-34.

DUFFUS, J.E. 1991. Rhizomania (beet necrotic yellow vein virus) pages 29-30 in Compendium of Beet Diseases and Insects (E.D. Whitney and J.E. Duffus, eds.) APS Press, 76 p.

ERDİLLER G. and ÖZGÖR, O. 1994. Rhizomania diseaseof sugar beet in Türkiye. 9th Congress of Meditarrenean Phytopathological Union, Kuşadası, Aydın. 443-446.

GERİK, J.S. 1992. Zoosporic obligate parasites of roots. Pages 18-24 in: Methods for Research on Soil-borne Phytopathologic Fungi (L. L. Singleton, J. D. Mihail and C.M. Rush, eds.) APS Press, St. Paul. MN.

HENRY, C. 1989. Report of the investigation of bait seedling methods for the detection of rhizomania in soil, Sugar Beet Rhizomania. Committee Paper. No: 2371, 13-22.

HİLL, S.A. and L. TORRANCE. 1989. Rhizomania disease of sugar beet in England. **Plant Pathology 38:** 114-122.

KESKİN, B. 1964. *Polymyxa betae* nsp., ein parasit in den Wurzeln von *Betae vulgaris* Tournefort, besonders wahrend der Jugendent wicklung der Zuckerrübe. **Archive für Mikrobiologie, 49**: 348-374.

KOENIG, R. 1981. Indirect ELISA methods for broad spesific detection of plant viruses. **J. Gen. Virol. 55:** 53-62.

KOENIG, R., D.E. LESEMAN and W. BURGERMEISTER, 1984. Beet necrotic yellow vein virus: Purification, preparation of antisera and detection by means of ELISA, immunosorbent microscopy and electroblot immunoassay. **Phytopathologische Zeitschrift 111:** 224-250.

PAYNE, P.A. and M.J.C. ASHER. 1990. The incidence of *Polymyxa betae* and other fungal root parasites of sugar beet in Britain. **Plant Pathology, 39:** 443-451.

PUTZ, C., D. MERDINOGLU, O. LEMAIRE, G. STOCKY, P. VALENTINE and S. WEIDEMAN. 1990. BNYVV causal agent of sugarbeet rhizomania. Rev. of **Plant Pathology 69**(5): 247-254.

TAMADA, T. 1975. Beet Necrotic Yellow Vein Virus. CMI/AAB. Descriptions of Plant Viruses. No. 144, 4pp.

TORRANCE, L., M.T. PEAD and G. BUXTON. 1988. Production and some characteristics of monoclonal antibodies against beet necrotic yellow vein virus. **Ann. Appl. Biol. 113:** 519-530.

VARDAR, B. and S. ERKAN. 1992. The first studies on detection of BNYVV in Turkey. **J. Turk. Phytopath. 21**(2-3): 74-76.

J. Turk. Phytopath., Vol. 27, No. 1, 47-57, 1998 ISSN 0378 - 8024

The Effect of Virus Diseases on The Yield of Grapevine in Gaziantep and Kilis Provinces in Türkiye

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ABSTRACT

The effect of viruses on the grapevine yield parameters were insvestigated in Gaziantep in Türkiye. In order to determine yield losses, The criteria Weight of one cluster (gram); The total number of berries in the cluster (number), Weight of the total berries in the cluster (gr); Weight of 10 berries in the cluster (gr) and Weight of an average berry in an average cluster (gr). parameters were compared among the healthy and infected stocks of grapevine in different locations.

Obtained results revealed that virus infections cause statistically important yield losses in grapevine production in Gaziantep. It is also determined that yield reductions are higher in the newly introduced grapevine variaties than local varieties.

INTRODUCTION

Gaziantep and Kilis provinces are an important grapevine production area of Türkiye. In particular, this region is known for its late ripening table grape varieties, raisin production and especially the grape molasses (Antep pekmezi). More than 80 local varieties have been growing in this region for centuries. The region has also an important trade with 270.870 ton /year grapevine production (Anonymous, 1996) and exportation of raisin and grape molasses.

Due to the effects of virus diseases grapevine production in this area is decraesing year by year. Strawberry Latent Ringspot Virus (SLRSV) (Martelli et. al. 1987), Grapevine Fanleaf Virus (GFLV), Arabis, Mosaic Virus (ArMV), Grapevine Leaf Roll Virus (Type I and III), Grapevine Fleck Virus (GFkV) (Özaslan 1995) were reported on grapevine in this region. Virus diseases are causing, yellowing. leaf rolling, mosaic, vein banding, vein browning, leaf deformation, shortening of internodes, formation of double nodes, zigzag growth of the flushes and branches, flattering of the main branches, leaf rugose, unvigorous growth, deyaling of budbreak, dwarfing and finally dieback of the plant (Martelli 1993). These symptom expressions are induced by

the interactions of the environment, variety and viral strain. However, the affect of viral diseases on grapevine in terms of the quality and quantity is not yet determined in this region. According to the literature viruses causing 20-80 % yield losses on world basis Bovey et al., 1980; Martelli 1992, Walter and Martelli 1996). In the case of our country, this type of work was only conducted in Kahramanmaraş region and approximately 45 % yield losses were estimated (Özaslan 1993).

This work was carried out in order to find the effect of grapevine viruses on some yield parameters under the field conditions.

MATERIALS and METHODS

Plants were selected due to their infection with several different viruses; Grapevine Fanleaf Virus (GFLV), Grapevine Leaf-roll Associated Virus "Type I and Type III" (GLRaV-I and GLRaV-III), Grapevine Fleck Virus (GFkV) and Arabis Mosaic Virus (ArMV). The viruses were previously determined by ELISA test and indexing with vitis indicators by Özaslan (1995). This research is based on the comparision of the yields of infected and non-infected (with any of above mentioned viruses) grapevine plants growing under the some condition in the different locations of Gaziantep province.

The region contains 5 different redistricts namely; Gaziantep central, Nizip, Yavuzeli-Araban, İslahiye-Oğuzeli and Kilis-Karkamış-Elbeyli. Cultivation techniques used and ecological riches are different in these locations, therefore each location was considered individually.

Samples were collected from the late of September until mid of October in 1995 and again in 1996. Samples were collected from 37 different locations in the region. Two vineyards were investigated in each location. Four plants (two plants were positive with at least one of the viruses incolved, two plants were free of those viruses) were used from one vineyard. Three clusters were collected from each plant. In total, 888 clusters were obtained from 296 plants growing in 74 vineyard in the 37 different locations of Gaziantep and Kilis porovinces (Table 1).

Following yield parameters are determined: A-Weight of one cluster (gram), B-The total number of berries in the cluster (number), C-Weight of the total berries in the cluster (gram), D-Weight of 10 berries in the cluster (gram), E-Weight of an average berry in an average cluster (gram). The measurements were made in the laboratory of the local Agricultural service of the Ministry of Agriculture and Rural Affairs in Gaziantep.

The avareges of the different yield parameters were compared and evaulated according to the t-test.

Table 1. Surveyed districts, locations, vineyard and previously reported viruses in Gaziantep

Area	No.of. Location / No. of Vineyard / No.of plants	Determined viruses
Central	10/20/80	a,b,c,d,e
Nizip	5/10/40	a,b,c,d,e
Kilis, Karkamış		
Elbeyli,	10/20/80	a,b,c,d,e
Yavuzeli, Araban	5/10/40	a,b,c,d,e
İslahiye, Oğuzeli	7/14/56	a,d,e
Total	37/74/296	a,b,c,d,e

a: Grapevine Fanleaf Virus b: Arabis Mosaic Virus c: Grapevine Fleck Virus d: Grapevine Leaf Roll Virus (type-I) e: Grapevine Leaf Roll Virus (type-III)

RESULTS

In order to explain and discuss the data obtained the criteria A, C, D and E which are based on the weihgt of cluster and berries in gram were investigated together. The criterion B which is the total number of berries in a cluster was considered seperately.

The differences between healthy and infected plants were found as 37.88 % in A, 39.9 % in C, 43.2 % in D and 45 % in E in the Gaziantep and Kilis provinces. All these are significantly different at P 95.0 % LOG(x) (table 2). Each location in this table was given different response against to the each parameter.

According to these results the viruses cause reduction in some yield parameters in Gaziantep province of Turkey. The highest yield losses were obtained mainly in central and Nizip districts where the newly introduced and imported varieties are cultivated. The differences between healthy and infected plants in both location were about 43.8-49 % for criterion A, 48-49 % for criterion C, 51-46.4 % for criterion D and 50-45 % for criterion E, respectively.

The yield losses were lower in Kilis-Karkamış-Elbeyli location where the very old and totally local cultivars are grown for centuries with traditional practices. The differences between healthy and infected plants were 26.5 %, 27.2 %, 30.8 % and 33 % in criteria A,C,d and E respectively. Data obtained in the criteria related to the weight are statistically important in all the surveyed locations. On the other hand criterion B is given different results in each location. The differences against to this criterion were 8.9

% in central, 0.3 % in Nizip, 6.1 % in Yavuzeli-Araban, 13,2 % in Kilis-Karkamış-Elbeyli and 8,8 % in Islahiye-Oğuzeli districts and the average was about 8 %, in the region. During the analyzes the data with t-test at P 95.0 % LOG (x), results obtained from Kilis-Karkamış-Elbeyli and Yavuzeli-Araban location were not statistically important but the rest were important (Table 2). It revealed that viruses effect on the number of the berries in an avarge cluster is not as much as the loss of weight. This findings was induced to compare to the varieties. What are the response of the old and new varieties against to determined yield parameters? For this purpose, 9 new and 9 local (old) varieties were selected and the data were compared against to criteria A, B, C, D and E, results are summarized in the Table 3.

Table 2. Location and the effect of viruses of grapevine in some yield Parameters in Gaziantep and Kilis Provinces.

Location		A	B	C	D	E
Central	H	258,8±55,584	122,2±20,779	244,8±55,103	20,2±1,637	2,0±0,163
	I	145,6±35,493***	110,9±19,908*	125,9±34,028***	9,5±0,857****	0,9±0,097****
Nizip	H	342,3±63,541	139,8±26,484	309,9±84,711	28,1±1,856	2,8±0,192
	I	173,6±28,964***	136,6±23,739*	157,9±26,709***	15,0±1330****	1,5±0,132****
Kilis,Karkamıs Elbeyli	H	430,3±44,425	176,7±17,632	410,8±44,532	23,9±1,370	2,4±0,138
	I	320,5±39,488**	153,5±14,361	299,2±39,014**	16,5±1,552***	1,6±0,154***
Yavuzeli Araban	H	317,7±99,939	154,8±37,139	303,8±99,966	19,8±1,774	1,9±0,177
	I	189,8±62,684**	145,4±34,569	168,1±59,713**	10,9±1516***	1,0±0,170***
Islahiye, Oğuzeli	H	410,0±60,324	170,5±27,777	387,7±59,547	27,2±1,638	2,7±0,206
	I	266,1±40,179**	156,8±25,051*	245,3±41,029***	15,6±1,341****	1,5±0,142****
Avarages	H	351,8±31,195	152,8±9,985	331,4±30,149	23,8±1,717	2,3±0,179
	I	219,1±32,266***	140,6±8,215 *?	199,3±31,629***	13,5±1388****	1,3±0,144****
P(95,0%)LOG (X)		(H>I)=0,0096	(H>I)=0,4006	(H>I)=0,0089	(H>I)=0,0011	(H>I)=0,0012
Differences (%)		37.88	8	39,9	43,2	45

A-Weight of one cluster (gram) B - The total number of berries in the cluster (number)
C-Weight of the total berries in the cluster (gr.) D-Weight of 10 berries in the cluster (gr)
E- Weight of an avarage berry in an avarge cluster(gr.) H; Healthy I; Infected
* = < 0,5 ; ** = < 0,1 ; *** = < 0,01 ; **** = < 0,001

Results showed that new varieties are setting small berried clusters. Their weight are also less than old and local varieties in both situation either in healthy or infected. The average of the total weight of a healthy clusters in the new varieties was about 266,2 gr. but it was 495.7 gr. in local one, in case of infected the average were 143.8 and 378.2 respectively (Table 3). Both were statiscally important at the base of t-test.

When compare the parameters; A, C, D and E, the differences between healthy and infected in new and old varieties were determined as 46-23.8 %; 48.7 -25.3 %; 52-29 % and 52.8-28.6 % respectively. These are statically important.

Table 3. Data on some yield parameters of diversing grapevine varieties infected with certain viruses.

New Varieties		A	B	C	D	E
Razaki	H	219,43±26,417	132±10,056	211,6±25,310	219,7±1,877	2,0±0,178
	I	137,51±11,817	129±8,153	128,6±11,86	10,5±1,065	1,1±0,107
Cardinal	H	309,2±26,417	110±10,056	291,2±25,310	27,4±1,877	2,8±0,178
	I	128,19±11,817	106±8,153	114,1±11,86	10±1,065	0,9±0,107
Uslu[1]	H	116,38±26,417	76±10,056	104,6±25,310	14,6±1,877	1,5±0,178
	I	74,85±11,817	74±8,153	53±11,86	5,5±1,065	0,7±0,107
Sultani[2]	H	341±26,417	102±10,056	324,9±25,310	31,99±1,877	3,1±0,178
	I	142,19±11,817	116±8,153	134±11,86	16,9±1,065	1,7±0,107
Unknown[3]	H	213,6±26,417	100±10,056	190±25,310	18,75±1,877	1,9±0,178
	I	194,9±11,817	103±8,153	172,7±11,86	9,6±1,065	0,9±0,107
Unknown[3]	H	285±26,417	105±10,056	263,6±25,310	24,8±1,877	2,4±0,178
	I	129,81±11,817	100±8,153	114,03±11,86	12±1,065	1,1±0,107
Unknown[3]	H	219,2±26,417	123±10,056	204,7±25,310	18,5±1,877	1,8±0,178
	I	142,28±11,817	109±8,153	122,6±11,86	9,8±1,065	0,9±0,107
Unknown[3]	H	349,9±26,417	185±10,056	324,1±25,310	23,6±1,877	2,3±0,178
	I	189,11±11,817	164±8,153	160±11,86	9,8±1,065	0,9±0,107
Unknown[3]	H	343,4±26,417	120±10,056	320±25,310	28,5±1,877	2,8±0,178
	I	156,14±11,817	104±8,153	160,1±11,86	15,3±1,065	1,5±0,107
AVERAGES	H	**266,2±26,417**	**117±10,056**	**248,3±25,310**	**23,093±1,877**	**2,28±0,178**
	I	**143,8±11,817******	**111,6±8,153***	**128,7±11,86******	**11,16±1,06******	**1,07±0,107******
P(95,0%)		H>I=0,000315	H>I=0,342920	H>I=0,00028	H>I=0,000022	H>I=0,000013
Differences%		**46**	**4,7**	**48.7**	**52**	**52.8**
Old Varieties						
Antep Karası	H	605,2±35,098	240±16,563	590,1±35,815	25,1±0,968	2,5±0,099
	I	400±29,844	210±12,448	382,4±29,732	19±1,232	±1,90,124
Tahannebi	H	576,4±35,098	214±16,563	552±35,815	26,7±0,968	2,7±0,099
	I	498,6±29,844	196±12,448	473,6±29,732	25,02±1,232	2,5±0,124
Dımışkı	H	459±35,098	200±16,563	438,6±35,815	20,6±0,968	2±0,099
	I	400±29,844	185±12,448	381±29,732	18,1±1,232	1,8±0,124
Dökülgen	H	503,6±35,098	218±16,563	490±35,815	23,55±0,968	2,36±0,099
	I	399,6±29,844	200±12,448	371±29,732	17±1,232	1,7±0,124
Devegözü	H	362,6±35,098	153±16,563	341±35,815	23,13±0,968	2,3±0,099
	I	219,91±29,844	141±12,448	199,6±29,732	11,2±1,232	1,1±0,124
Maharabası	H	600±35,098	260±16,563	580±35,815	25,3±0,968	2,5±0,099
	I	460,4±29,844	213±12,448	428,6±29,732	20±1,232	1,98±0,124
Unknown[4]	H	590±35,098	248±16,563	560,3±35,815	29,6±0,968	3±0,099
	I	429,6±29,844	203±12,448	406±29,732	16±1,232	1,6±0,124
Unknown[4]	H	435,8±35,098	160±16,563	410,4±35,815	27,3±0,968	2,7±0,099
	I	320±29,844	151±12,448	298,2±29,732	20,01±1,232	2±0,124
Unknown[4]	H	329,43±35,098	111±16,563	298,3±35,815	29,03±0,968	2,9±0,099
	I	276,3±29,844	105±12,448	243,1±29,732	19±1,232	1,9±0,124
AVERAGES	H	495,7±35,098	200,4±16,563	473,4±35,815	25,59±0,968	2,56±0,099
	I	378,26±29,844**	178,2±12,448**	353,7±29,732**	18,3±1,232****	1,83±0,124****
P(95,0%)		H>I=0,01069	H>I=0,14969	H>I=0,01025	H>I=0,00014	H>I=0,00015
Differences%		**23.8**	**11.1**	**25.3**	**29**	**28,6**

* = < 0,5 ; ** = < 0,1 ; *** = < 0,01 ; **** = < 0,001

1, 2 ; Uslu and Sultani are also Turkish varieties but not local for Gaziantep and Kilis. .

3 ; Imported varieties , names are unknown 4 ; Lokal varieties, names are unknown.

As it is shown in the table 3, the difference or the yield losses regarding to criterion A was 23.8 % in the old varieties but it is increased up to 46 % in the new varieties. It is more or less two times more reduction of yield. In case of other parameters the situation is very close. It revealed that viruses were causing less yield losses in the old varieties than in the new varieties in Gaziantep province.

Althouhg the yield parameter related to the weight are has big difference and importance, the criterion B was determined also important statistically. In new varieties the difference was not so important with 4.7 % (117 in healthy and 111.6 in infected plants) but in the old varieties it was two times more and increased up, to 11.1 %, It means that virus infections are not so much effecting the setting of the berries in new varieties. On the other hand old varieties are producing less number of berries under the virus infection.

DISCUSSION

Türkiye is the 8th. biggest grapevine producer in the world (Anoymous 1995). The origin of *Vitis vinifera* as anatolia and more than 300 local *V. vinifera* cultivars are grown in Türkiye. Although the traditional self-rooting system has been using for centuries, modernized or high system plantation is beign conducted to spread from western to eastern part of anatolia. The main goal of grape production is still adopted to the table grape production for fresh consumption, exporting, raisin and grape molases purposes.

Vineyard farming in the districts of Gaziantep and Kilis provinces are vary depending of the cultivated varieties, cropping practices and environmental conditions. As mentioned before, new varieties (imported from, France, Italy, Iran and the other parts of Turkiye) are mainly cultivated in Gaziantep central, Nizip and some part of İslahiye-Oğuzeli districts. In those locations grovers are utilizing the new cultural practies including the use of rootstock and pergola system.

On the other hand, Kilis-Karkamış-Elbeyli, Islahiye, Oguzeli and most of Yavuzeli, Araban locations are still using the traditional self-rooting system with the very old and local cultivars.

As like as all other crop grapevine has a lot of problems which are limiting production and decreasing the yield and its quality. Among them Phyloxera is the major one which is an important pest in the all vineyards of the world. There are also many diseases caused by fungi, bacteria, nematodes, viruses and virus-like organisms in Turkish vineyards. More than 40 virus or virus like diseases reported in grapevine in the

world (Martelli 1993). Therefore the quality and quantity of the yield is decreased due to these pathogens. It is reported that grapevine viruses are responsible for 20-80 % of yield losses in different locations in the world (Martelli 1992; Bovey et al. 1980; Walter and Martelli 1996; Digiaro et al. 1998) In fact this figures are showing just the measurable yield losses.

Özaslan et. al (1991, 1993) reported the yield losses caused by viruses in Kahramanmaraş region where the neighouring province of Gaziantep. They pointed out that the difference of the total number of an average cluster between infected and healthy plants were not convincing. But in case of the differences obtained through all other parameters (Criteria A, C, D, E) considerably important and reliable. Finally virus diseases caused approximately 45 % yield losses in Kahramanmaraş province.

In the case of Gaziantep and Kilis provinces, 371 location were surveyed to determine the yield losses caused by previously reported (Özaslan 1995) viruses (Table 1).

In general virus diseases reduced 37.88 % of in criterion A. This is the average of all the investigated locations in Gaziantep province.

If one have a look at each regions, the highest value was obtained in Nizip with 49 % (Table 2), and the lowest one was in Kilis, Karkamış and Elbeyli with 26.5 % (Table 2). This differences could be due to cultivated varieties. The ancient grape varieties are grown in Kilis, Karkamış and Elbeyli locations. The main purpose of the plantation in those areas is addressed to the raisin and grape molase production. On the other hand, in Nizip region varieties are newly introduced and, are mostly cultivated for table grape and fresh consumption. So that tested viruses and varieties have been present in Kilis, Karkamış and Elbeyli region for centruies. They possibly could have an interaction which could be a positive point for yield production and finally, viruses caused acceptable yield losses in this ecosystem compairing with the other surveyed locations in this research. But in Nizip, due to introduction of new varieties, either new virus strains could be also introduced or the peresent virus strains become more destructive.

More over in central location of Gaziantep province, varieties are also newly imported and the difference in this criterion (A) is increased up to 43.8 % (Table 2).

It is concluded that this situation is the one of the negative, effect of the broken ecosystem and introduction of the uncontrolled propagation material.

The differences at the total number of berries in an average cluster (criteria B) was found as 8 % in general. It was convincing the phenomena that viruses causing

acceptable damages on the number of berries. This results are similar to the report of Özaslan (1993). But an interesting point was observed. This value is decreased down to 0.3 % in Nizip (where the highest value for criterio A obtained), and it is increased up to 13.2 % in Kilis-Karkamış and Elbeyli regions (Table 2). This data indicated that viruses on the newly introduced tablegrape varieties inducing to set more berry but they stay small. In case of the varieties for molasses and raisin production, the total number of berries in infected plants are lower than healthy ones (Table 3), but the weight of clusters are not so much lower as compare as the table grape varieties (Table 3). Finally, it is seems to be obvious that the ancient varient varieties are much more effected than newly introduced table grape varieties in the point of the total number of berries an average cluster in evaluated regions (Table 2 and 3).

The differences of the weight of total berries in an average cluster (Criterion C) was estimated as 39.9 % in general (Table 2). This criterion was also the lowest in Kilis Karkamış and Elbeyli regions with 27.2 % (Table 2).

The values of criterion C is increased up to 49.9 % in Nizip, 48.6 in central, 44.7 % in Yavuzeli-Araban and 37 % in İslahiye-Oğuzeli regions. In comparision of values of criterion C in the locations where the newly table grape varieties grown (Nizip and Central) and the locations where the mainly ancient varieties cultivated (Kilis-Karkamış-Elbeyli and Islahiye-Oğuzeli), above mentioned idea is getting stronger.

The weight of ten berries in an average cluster (Criterion D) for overall of Gaziantep province was found 13.55 gr. in infected plants and 23.86 gr. in healthy plants (Table 2). In case of criterion D, highest value was obtained in central region with 51.1 % differences, and lowest value was also observed in Kilis-Karkamış and Elbeyli regions with 30.8 % (Table 2). This results also supported the above mentioned idea.

In general, the weight of an average berry (Criterion E) is given similar results to the that of other criteria based on the weight (criteria, A, C and D). The average of differences in Gaziontep province against to criterion E was about 45 %. This results more or less same to the report of Ozaslan et al. (1993).

In conclusion; SLRSV, GFLV, ArMV, GLRaV (Type I and III). GFkV are reported viruses of Gaziantep Province (Martelli et al. 1987; Özaslan 1995). It does not mean that there is no other viruses in the region. This viruses are reported because they are tested either with serological or biologically. If a deatiled survey carried out some other virus and virus like diseases also could be identified in this region. The other important thing is healthy plants in this research are free from the above mentioned viruses so they could also be infected some other viruses. This resarch is based on the rough determination of virus diseases effect on some yield parameters under the field

conditions and the selected parameters are addressed directly to the number of berries (criterion B) and fresh weight of berries and cluster. The results are obtanied from the healthy and infected plants growing under the same conditions so the data is reliable for field condition only. During the evaluation of each location and comparing them each other, it is revealed that local varieties (in particular, favorable for molasses and raisin) are setting low number of berries but the weight of total cluster and even the average weight one berry is higher than that of other varieties especially the newly introduced ones.

It is concluded that there is a balance between local varieties and present viruses in some location. But where the balance interrupted with introduction of new varieties yield losses due to virus diseases is increased. For instance, in central region of Gaziantep province, the local office of Ministry of Agriculture and Rural Affairs imported some French and Italian varieties and distributed to the grovers. This practice resulted the highest yield losses (Table 2 and 3). If one have look to the detail, location name of a village in the central called "Sarısalkım" its means yellow-cluster, and this village is a well-known location with its table grape production in Southeastern Anatolian and Eastern Mediterranean regions of Türkiye. In the begining of 1980 s. some Italian and French varieties were introduced by the govermental and commercial companies. After seven to ten years, many problems including virus diseases are observed and grovers to give up the vineyard farming or to replace with other new varieties. It is determined that the yield losses in Sarısalkım location was 42 %, 47 %, 52 % and 50 % in criteria A, C, D and E, respectively. Similar results obtained in location "Arıl" in Nizip district. The stroy is more or less same and results too.

In fact this numbers are showing just the measurable losses. On the other hand, some other negative effects of virus (i.e diseases and the loss of quality, decrasing the productivity and rantable age of plants) are also present and open to research.

ÖZET

GAZİANTEP VE KİLİS BAĞLARINDA SAPTANAN VİRÜS HASTALIKLARININ VERİM ÜZERİNE ETKİLERİ

Gaziantep ve Kilis Bölgesi Güneydoğu Anadolu ve Doğu Akdeniz bölgelerinde geçci sofralık, kuutmalık ve özellikle de pekmezlik-şıralık üzüm üretimiyle ünlü bir bölgedir. Bu bölgede daha önce yapılan çalışmalarda, Çilek Latent Halkalı Leke Virüsü, Asma Kısaboğum Virüsü, Asma Yaprak Kıvırcıklık Virüs Hastalığı (Type-I, III) Arabis Mozayik Virüsü ve Asma Flek Virüs'lerinin bölge bağlarına zarar veren virüsler olduğu, serolojik ve biyolojik indekslemelerle saptandığı bildirilmiştir (Tablo 1).

Bu araştırmanın amacı da bölge bağlarında saptanan bu virüslerin bazı verim parametreleri üzerine olan etkilerinin araştırılmasıdır. Yapılan çalışmada, toplam salkım ağırlığı, salkımdaki dane sayısı, toplam dane ağırlığı, 10 dane ve 1 dane ağırlıkları verim parametreleri olarak alınmıştır. Virüslerle bulaşık olan ve olmayan asmalardan bu parametreler açısından elde edilen değerler t-testiyle analiz edilmiş ve sonuçlar değerlendirilmiştir.

Araştırma sonucunda virüslerin ortalama olarak % 37.88 civarında verim kaybına neden olduğu saptanmıştır. Ancak bu kaybın toplam dane sayısı açısından bölge ortalaması olarak % 8'e kadar düştüğü bir dane ağırlığı açısından % 45 civarına kadar çıktığı bulunmuştur (Tablo 2).

Gaziantep bölgesinde yetiştirilen bağlar ve kullanılan yetiştiricilik teknikleri arasındaki farklar dikkate alındığında daha çok eski ve şıralık çeşiterin yetiştirildiği bölgelerle yeni ve sofralık çeşitlerin yetiştirildiği bölgeler ayrı birer grup altına alınmış ve değerlendirilmiştir. Bu değerlendirmeden de eski çeşitlerde toplam salkım ağırlığı açısından ortaya çıkan kaybın yeni çeşitlere oranla daha az olduğu ortaya konmuştur. Diğer taraftan bu yerel çeşitlerde virüsle bulaşık bitkilerde dane tutumunun bölgeye yeni getirilen çeşitlere oranla daha az olduğu belirlenmiştir (Tablo 3).

LITERATURE CITED

ANONYMOUS, 1994. D.İ.E. Tarımsal yapı ve üretim yıllığı. T.C. Başbakanlık Devlet İstatistik Enstitüsü, Ankara.

ANONYMOUS, 1995. FAO production yearbook. FAO statistics series no: 71 Rome.

BOVEY, R. GARTEL W., HEWITT, W.B. MARTELLI, G.P. and VUITTENEZ A. 1980. Virus and Virus Like Diseases of Grapevines. Editions Payot Lausenne. ISBN 2-601-000341-1 Payot Lausanne, Switzerland. 181 pp.

DIGIARO M.D. BOSCIA, V. SIMONEA and V. SAVINO. 1998. Gravi casi di accartocciamento fogliare su cultivar ad uva da tavola di recentee introduzione in puglia. **Informatore Fitopatologico 48** (1-2), 76-79.

MARTELLI, G.P. (Ed) 1993. Graft transmissible diseases of Grapevines. Handbook for detection and diagnosis. FAO Publication Division, Rome, 263 pp.

ÖZASLAN, M., S. BALOĞLU ve M.A. YILMAZ. 1991. Kahramanmaraş Bölgesinde Lokal Olarak Yetiştirilen Üzüm Çeşitlerinde Virüs Hastalıkları. VI. Türkiye Fitopatoloji Kongresi Bildiriler, (401-406 s.), Türkiye Fitopatoloji Derneği.

ÖZASLAN, M., S. BALOĞLU and M.A. YALMAZ, 1993. The Effect of Virus Diseases on Grape Production in Kahramanmaraş Region in Türkiye. 11. th Roc of ICVG Montreux, Switzerland. No: 33 CP. 70-71.

ÖZASLAN, M. 1995. Adana, Tarsus, Gaziantep, Şanlıurfa ve Adıyaman Bölgelerinde Yetiştirilen Bağlara Zarar Veren Virüs Hastalıklarının Biyolojik ve Serolojik Yöntemlerle Saptanması. Doktora Tezi. Ç.Ü. Fen Bil. Enst. 1995. Kod No: 292.

WALTER and G.P. MARTELLİ. 1996. Selection clonale de la Vigne; Selection sanitaire et selection pomologique. Inflience des viroses et qualite. 1 ere partie; Efets des viroses sur la culture de la vigne et ses produits. Bullteinde L'OIV, 69, 945-971.

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