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Bacterial Diseases of Food Legumes in Aegean Region of Türkiye and Effectivity of Some Seed Treatments Against Bean Halo Blight

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ABSTRACT

*From the results of surveys carried out at two different stages of growth between the years of 1985-86, it was found that were a few bacterial brown spot (**Pseudomonas syringae** pv. **syringae** (van Hall) Young, Dye and Wilkie) and widely halo blight (**P.s. pv. phaseolicola** (Burhholder) Young, Dye and Wilkie) and common blight (**Xanthomonas campestris** pv. **phaseoli** (Smith) Dye)) on beans in Balıkesir. It was observed that **P.s. pv. syringae** and **P. viridiflava** (Burkholder) Dowson caused bacterial blights on peas. Bacterial blight (**X. c. pv. vignicola** (Burkh.) Dye causing blights on leaves, stems and pods on cowpea was only present in one growing areas. Bacterial pathogens from especially peas and cowpeas are isolated for the first time in Türkiye.*

Various seed treatments were effective at the rate of 0-79.22% to primary infections of bean halo blight disease. However, in general, all treatments were phytotoxic except mancozeb.

INTRODUCTION

Bacterial diseases which are of widespread occurrence causing economically important losses, have been known for a long time on food legumes. A great deal of extensive studies have been made with food legumes bacterial diseases and most of these diseases have been reported to be transmitted by the infected or infested seed (Taylor et al., 1979; Schuster, 1970; Schuster and Coyne, 1974; Zaumayer and Thomas, 1967; Young and Dye 1970; Wimalajeewa and Nancarrow, 1984; Taylor and Dye, 1972; Shekhawat and Patel, 1977; Karaca ve Demir, 1988).

Up to now, few researches were conducted in order to determine the diseases of bean in different parts of Türkiye (Sönmezalp, 1966; Zavrak, 1977; Karaca, 1977). But there were no studies conducted to determine the other food legumes diseases in Türkiye and therefore, the main purpose of this research was to survey and to determine the bacterial diseases of food legumes cultivation in Aegean Region. In addition, we have tried to achieve effectivity of some physical and chemical seed treatments for primary insections of haloblight of bean.

MATERIALS and METHODS

Survey and isolation of bacteria from diseased plants

The survey areas of this research were selected as Balıkesir, Çanakkale, Aydın, İzmir, Uşak and Denizli provinces which were mainly food legumes cultivation areas in the region. Surveys were performed at two different stages of growth (at seedling and flowering-podding) and samples were collected the infected parts of plants with bacterial diseases. As totaly 89 broad bean, 50 bean, 19 pea and 15 cowpea fields were surveyed.

Infected plant parts were surface-sterilized for 30s in 70% ethanol and rinsed in sterile distilled water. Small pieces were macerated in 5 ml of sterile distilled water. Each sample was plated on three plates each of King's medium B (King et al., 1954) Sucrose Nutrient Agar (SNA) and Yeast Dextrose Chalk Agar (YDCA). Single colony isolates were selected from the plates after 3 or 4 days of incubation at 24-28°C. The isolates were maintained at Nutrient Agar (NA-Difco) at +4°C.

Comparisons were made with the following cultures:

Pseudomonas syringae pv. **phaseolicola** (NCPPB 52), **P.s.** pv. **syringae** (NCPPB 1075), **Xanthomonas campestris** pv. **phaseoli** (NCPPB 1811), **X.c.** pv. **vignicola** (NCPPB 1838), **P.s.** pv. **pisi** (NCPPB 1366) and **P.viridiflava** (NCPPB 635) from the National Collection of Plant Pathogenic Bacteria Harpenden, England.

Pathogenicity tests

For these tests, the bacteria that had been stored in sterile tap water were transferred to SNA for pseudomonads and YDCA for xanthomonads and allowed to grow for 48-72 hr before use. Suspensions were prepared in sterile distilled water to give approximately 10^7 cells per milliter. Two wk old bean, broad bean, pea and cowpea plants were inoculated by spraying with bacterial inoculum with an atomiser. The atomiser was held at a distance of 20-30 cm. from the plants to prevent any further wounding and spraying was continued until the plants were completely wet. In addition test plants were inoculated by injecting 0.1 ml. of bacterial suspensions through the stem and petiole with a 1 ml syringae. After inoculation plants were held under conditions of high humidity at 24-28°C for 7-10 days. Three plant were inoculated for each inoculum. A control group was also included using sterile tapwater.

Morphological, physiological and biochemical properties

Colony colour was noted and the following tests were applied: For oxidase activity Kovacs method (Kovacs, 1956) was applied. To detect catalase activity, a loopfull bacteria of a 24-48 h NA culture was smeared into a drop of 10% H_2O_2. HS production was determined using Skerman's method (Skerman, 1967). Gelatin hydrolysis, nitrate reduction, starch dihydrolysis and anaerobic breakdown of L(+) arginine were detected by Lelliott et al. (1966). Hypersensitivity reaction for pseudomonads was determined in tobacco leaves (cv. white Burley) according to Klement (1963). Growth on carbon

sources was recorded on the Minimal Medium (MM) of Ayers et al. (1919). Sugars and other carbon sources likely to be decomposed by autoclaving were filter-sterilized (0.2 m Millipore) and included in MM (1 %W/V). The following substances were tested as carbon sources: glucose, mannose, sucrose, galactose, L-lactate, threhalose, glycerol, sorbitol and mannitol.

Seed treatment for the control of halo blight of bean

In all the experiments artificially inoculated seeds with **P.s.** pv. **phaseolicola** were used.

The following bactericides and some physical treatments at the given concentration were applied to artificially inoculated seeds: Hot water (at 52°C for 15 min); Dry heat (at 50°C for 3 hr; at 70°C for 2 hr); formalin (at 0.6% for 15 min), mancozeb (0.3 g/kg); streptomycin sulphate (2.5 gr/kg) and kasugamycin (0.25 g/kg).

After surface disinfection at drying at room temperature, the seeds were dipped in **P.s.** pv. **phaseolicola** suspensions (approximately 10^8 cell/milliter) for 2 hr. then dried at room temperature. Artificially infested seeds were treated with above mentioned chemicals and physical treatments. After the treated seeds were sown in the plots.

Experiments were designed to randomized plots with 8 characters (7 treatments + 1 control) and 3 replicates. 200 seeds were sown per plot.

Evaluations were done by counting the diseased and healthy plants per plot when the plants were at first true leaf stage. Efficacy of treatments were calculated according to Abbott. In addition percentage of seed germination were recorded separately in each treatment to determine phytotoxic effect.

RESULTS and DISCUSSION

During the surveys in bean cultivation areas of Balıkesir and Denizli 3 bacterial pathogens were found. No bacterial infections were detected in any field of survey areas at seedling stage in Denizli provinces. However, we collected samples of bean plants with symptoms of bacterial brown spot (at the rate of 0.5% and 0.7%) from the two fields of Çivril town. Two isolates obtained from diseased plants caused brown blight symptoms on inoculation sites 3 days after inoculation but red-brown lesions advanced and plants died 8-10 days after inoculation. Morphological, physiological and biochemical properties (Table 1) of these isolates were similar to reference strain (NCPPB 1075) and were identified as **P. syringae pv. syringae.** It was reported that **P.s. pv. syringae** caused reddish-brown, circular to angular lesions, often surrounded by a narrow yellow band on the leaves, stems and pods on bean (**Phaseoulus vulgaris**) (Harrison and Freeman, 1964; Vimalajeewa and Nancarrow, 1978; Baykal, 1970).

Twenty-six fields were surveyed in Balıkesir province and bean halo blight disease was determined in 8 survey areas at the rate of 0.5% - 21.2%. This incidence was increased to 8.5% - 63.6% at poding stage. Tabacco HR-positive isolates from diseased

plants caused halo lesions on leaves of bean by inoculation with handle atomiser. These isolates showed similar properties to reference culture (NCPPB 52) (Table 1) and were identified as **P.syringae** pv. **phaseolicola.** It was reported that **P.syringae** pv. **phaseolicola** caused crop losses under low temperature and high relative humidity conditions and infected or infested seeds with pathogen at the proportion to 5:10.000 induced epidemic (Schuster, 1970; Trigalet and Bidand, 1978; Katherman et al., 1980). This disease is also determined in bean growing areas in Türkiye (Karaca, 1977; Zavrak, 1977; Rudolph and Baykal, 1978).

Bean common blight (**X.campestris** pv. **phaseoli**) was observed in 5 fields (disease incidence changing from to 0.9% - 5.45%) at seedling stage. However at podding stage disease was found at 15% - 34% rate in 6 fields. Morphological, physidogical and biochemical properties of 13 isolates collected from diseased plants showed similarity to reference culture (NCPPB 1811) (Table 1). According to some researches **X. campestris** pv. **phaseoli** is seed-borne, survive for along time in bean seed and in case of using pathogen free seeds crop losses decrease (Schucter and Sayre, 1967; Weller and Saetler, 1980).

22 pea fields were examined for bacterial infections in Aydın (12) and Çanakkale (10) provinces. There was no bacterial disease in Çanakkale but two difrent bacterial blight disease were observed in 3 fields at seedling stage and 7 at podding at the rate changing from 0.5% to 12.5%.

Four isolates levan (+), Tobacco HR (+)) caused watersoaked lesions on inoculated pea leaves 3 days after inoculation by spraying with handle atomiser.

However it was not observed stomal infection on unwounded leaves. The same symptoms were obtained from reference culture **P.syringae** pv. **syringae.** On the other hand **P.syringae** pv. **pisi** together with the symptoms decribed above showed stomal infection 5-7 days after inoculation. These isolates gave the similar results with **P. syr.** pv. **syringae** and pv. **pisi** (Table 2). Both pathogens induce bacterial blight in peas and are seed-borne (Hunter and Cigna, 1981; Taylor and Dye, 1972). It is not possible to distinguish the two species by phisological and biochemical properties, but analysis of membrane structure, host range and faj sensitivity maybe able to contribute to it (Butler and Fenwick, 1970). It was concluded that our isolates were **P.s.** pv. **syringae** because of causing infection on bean plants after artificial inoculation no infection from **P. syr.** pv. **pisi** and differences in pathogenicity on pea leaves. During our observation seven isolates were obtained from other blighted plants and these isolates were identified as **P. viridiflava** (Table 2). Taylor and Dye (1972) reported increasing of **P. viridiflava** infections after damages from any fungal or bacterial infections or climatical factors.

In this study, cowpea bacterial blight caused by **X. campestris** pv. **vignicola** was found only in one area. According to morphological and biochemical properties of pv. **vignicola** and pv. **phaseoli** it is difficult to differentiate the two pathovars, whereas pv.

vignicola grows well on mannitol as carbon source (Gupta and Charavartı, 1981), (not pv. **phaseoli**) and this was confirmed in our study (Table 2).

Various seed treatments were effective at the rate of 0-79.22% to primary infection of bean halo blight disease. But these treatments were decreased the number of plants in the plots at the ratios changing from 0-72.56% (Table 3). Kasugamycin (78.2%), streptomycin sulphate (76.81%) and heat treatment (at 70°C for 2hr-71.24%) were seen to be effective. It was reported that streptomycin sulphate (2.5 gr/kg) and Kasugamycin (0.25 gr/kg) controlled the primary infection of bean halo blight at the rate of 98% (Taylor and Dudley, 1977). In general, except mancozeb, all treatments were phytotoxic. Due to the fact that formalin was not effective on the disease, hot water prevented the number of plants in the plots, heat treatment was both phytotoxic and ineffective and streptomycin sulphate was not economical and had disadvantage of antibiotics, it come to a conclusion that these treatments might not be suggested.

It will be useful the fact that seed production should be done under the arid regions, seed certification should be strict and tolerant varieties should be grown against both bean halo blight and common blight diseases.

ÖZET

EGE BÖLGESİNDE YEMEKLİK BAKLAGİLLERDE GÖRÜLEN BAKTERİYEL HASTALIKLAR VE BAZI TOHUM UYGULAMALARININ FASULYE HALE YANIKLIĞINA KARŞI ETKİLERİ

Ege Bölgesi bakla, bezelye, fasulye ve börülce ekim alanlarında sorun olan bakteriyel hastalıkların belirlenmesi ve bazı kimyasal ve fiziksel tohum uygulamalarının Fasulye Hale Yanıklığının primer enfeksiyonlarına karşı etkisinin ortaya konması amacıyla bu çalışma yürütülmüştür.

1985-86 yıllarında toplam 170 örnekleme alanında yürütülen surveyler sonucunda; fasulyelerde eseri oranda kahverengi leke (**P.s.** pv. **syringae**) saptanırken, özellikle Balıkesir ilinde önemli oranda hale yanıklığı (**P.s.** pv. **phaseolicola**) ve Yaygın yanıklık (**X. campestris** pv. **phaseoli**) enfeksiyonları saptanmıştır. Bakteriyel yanıklık etmenlerinden **P. syringae** pv. **syringae** ve **P. viridiflava**'nın neden olduğu enfeksiyonlar Aydın ili bezelye üretim alanlarında saptanmıştır. Survey çalışmalarının yürütüldüğü börülce tarlalarından 1 tanesinde (İzmir-Menemen) ise sürgün, yaprak ve genç kapsüllerde yanıklıklara neden olan Börülce Bakteriyel Yanıklığı (**X. campestris** pv. **vignicola**) hastalığının varlığı belirlenmiştir. Özellikle börülce ve bezelyelerde saptanan bakteriyel hastalıkların Türkiye'deki varlığı ilk kez bu çalışma ile ortaya konmuştur.

Çeşitli tohum uygulamalarının Fasulye Hale Yanıklığına karşı etkilerini belirlemeye yönelik olarak yürütülen denemelerden ise %0-79.22 arasında değişen oranlarda etki elde edilmiştir. Ancak bazı uygulamalar önemli oranda bitki çıkışını engellemiştir.

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Table 1. Characteristics of pathogenic bean isolates.

Characters	Isolates A (17)	B (2)	C (13)	**Psp** (NCPPB 52)	**Pss** (NCPPB 1075)	**Xcp** (NCPPB 1811)
Fluorescent pigment	+	+	-	+	+	-
Oxidase reaction	-	-	-	-	-	-
Levan type colonies	+	+	Yellow, mucoid	+	+	Yellow, mucoid
Potato rot	-	-	nd	-	-	nd
Arginine dihydrolase	-	-	nd	-	-	nd
Tobacco-HR	+	+	nd	+	+	nd
Nitrate reduction	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+
Aesculin hydrolysis	-	+	+	-	+	+
Catalase	+	+	+	+	+	+
Starch hydrolysis	-	-	+	-	-	+
H_2S production	-	-	+	-	-	+
Acid from						
sucrose	+	+	+	+	+	+
glucose	+	+	+	+	+	+
mannose	+	+	+	+	+	+
galactose	+	+	+	+	+	+
trehalose	+	+	+	-	+	+
sorbitol	-	+	-	-	+	-
mannitol	-	+	-	-	+	-
glycerol	+	+	+	+	+	+
L-lactate	-	+	nd	-	+	nd

(+) = positive reaction
(-) = negative reaction
nd = not determined

(A) = Psp
(B) = Pss
(C) = Xcp

Table 2. Characteristics of pathogenic pea and cowpea isolates.

Characters	Pea A (4)	Isolates B (6)	**Psp** (NCPPB 1066)	**Pv** (NCPPB 568)	Cowpea isolates (2)	**Xcv** (NCPPB 1868)
Fluorescent pigment	+	+	+	+	-	-
Oxidase reaction	-	-	-	-	nd	nd
Tobacco-HR	+	+	+	+	nd	nd
Levan type colonies	+	-	+	-	yellow, mucoid	yellow, mucoid
Arginine dihydrolase	-	-	-	-	-	-
Aesculin hydrolysis	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	+	+
H_2S production	-	-	-	-	+	+
Acid from						
glucose	+	+	+	+	+	+
mannose	+	+	+	+	+	+
galactose	+	+	+	+	+	+
trehalose	-	-	-	-	+	+
glycerol	+	+	+	+	+	+
mannitol	+	+	-	-	+	-
sorbitol	+	+	+	+	-	-

(+) = positive reaction (A) = Pss
(-) = negative reaction (B) = **P. viridiflava**
nd = not determined

Table 3. Effectivity of various seed treatments to primary infections of bean halo blight

Treatments	Mean Disease Rate (%)	Phytotoxic effect (%)	Effect (%)
Hot water (at 52°C for 15 min)	3.07[x]	72.56	79.22
Dry heat (at 70°C for 2h)	4.25	34.46	71.24
Dry heat (at 50°C for 2h)	8.59	10.43	41.88
Formalin	16.98	9.52	0
Mancozeb	5.80	0	60.73
Streptomycin sulphate (2.5 g/kg)	3.42	19.27	76.81
Kasugamycin (0.25 g/kg)	3.22	18.36	78.20
Control	14.78	-	-

(x) : Average of three replicates

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In vitro Effect of Fertilizers on Growth and Sclerotial Production of **Macrophomina phaseolina,** the Cause of Charcoal Rot of Sunflower

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ABSTRACT

*Mycelial growth of **M. phaseolina** to fertilizers varied greatly. Colony diameter was the lowest in urea (60 mg/l). Urea was followed by urea (60 mg/l) + triple super phosphate (40 mg/l). The effect of fertilizers on sclerotial production of **M. phaseolina** also varied considerably. The most effective treatments in inhibiting sclerotial production were also urea (60 mg/l) and urea (60 mg/l) + triple super phosphate (40 mg/l). Addition of other fertilizers to the medium significantly increased number of sclerotia. There were maximum sclerotial production in 15-15-15 (30 mg/l) + ammonium sulphate (30 mg/l) + triple super phosphate (10 mg/l) and 15-15-15 (30 mg/l) + ammonium nitrate (30 mg/l) + triple super phosphate (10 mg/l).*

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid is one of the most prevalent soilborne fungal pathogens of sunflowers grown in the Aegean Region of Türkiye (Onan et al., 1992). Effective and economical disease management strategies are still lacking on sunflower of the pathogen.

Management of charcoal rot requires integrated strategies that either reduce the population of microsclerotia in the soil or prevent infection. Chemical control methods are not completely effective. Because the plants may be attacked at any time between germination and flowering, but they remain free of symptoms for a long time, so it is difficult to decide whether or not to apply a fungicide. Host plant resistance is an economical means of disease management but is not available in sunflower cultivars commercially grown in Türkiye. Crop rotation, site selection, and land preparation are also important management tools.

As known, micro-organisms differ in their ability of utilizing nutrients. Some require nitrate and others nitrite as an inorganic source. Organic source sometimes prove better than inorganic source for different physiological functions. According to Abdou et al. (1982) sucrose proved to be the best for growth and dextrose for sclerotial formation of **M. phaseolina.** Some nutrients sources like ammonium chloride completely inhibited their formation. A decrease in C/N ratio favoured mycelial growth than sclero-

tial formation. Ghosh and Sen (1976) reported that four isolates of **M. phaseolina** utilized ammonium nitrate and organic nitrogen better than inorganic ones. Rehman et al. (1989) found that out of N, P, and K constituents in Czapek-Dox agar, NPK in combination proved better for **M. phaseolina.**

Fertilization program could provide an additional strategy for management of charcoal rot in sunflower production. Low microsclerotial density in soil typically results in decreased sunflower charcoal rot. This study was undertaken to determine the influence of fertilization programs applied on sunflower cultivation on growth and sclerotial production of **M. phaseolina.**

MATERIALS AND METHODS

The isolate of **M. phaseolina** used was recovered from a sunflower plant suffering from root and stem rot symptoms. After growth at 30C° stock culture on potato dextrose agar (PDA) were held at 5C° until it was used.

Treatments (a.i. mg/l) were as follows: T_0: control, T_1: ammonium sulphate (60 mg/l), T_2: ammonium nitrate (60 mg/l), T_3: triple super phosphate (40 mg/l), T_4: 20-20-0 (50 mg/l), T_5: 15-15-15 (30 mg/l), T_6: urea (60 mg/l), T_7: ammonium sulphate (60 mg/l) + triple super phosphate (40 mg/l), T_8: ammonium nitrate (60 mg/l) + triple super phosphate (40 mg/l), T_9: urea (60 mg/l) + triple super phosphate (40 mg/l), T_{10}: 20-20-0 (50 mg/l) + ammonium nitrate (15 mg/l), T_{11}: 15-15-15 (30 mg/l) + ammonium sulphate (30 mg/l) + triple super phosphate (10 mg/l) and T_{12}: 15-15-15 (30 mg/l) + ammonium nitrate (30 mg/l) + triple super phosphate (10 mg/l).

Effect of fertilizers on mycelial growth and sclerotial production of M. phaseolina

Fertilizer concentration was obtained by adding calculated amount of stock solution to autoclaved (15 psi/15 min.) PDA cooled to about 45C°. PDA without fertilizer served as check. Five, 9 cm culture plates were poured with PDA for each treatment. After the agar had solidified, 6 mm agar plugs containing **M. phaseolina** mycelium were cut with the help of sterilized cork borer from the margins of 5 days old culture. Each plug was placed in the centre of petri plate containing medium and incubated at 30 ± 2 C° for further observations. The mean diameter of mycelium for each treatment was recorded after 5 days of inoculation. With the help of a flame sterilized, 6 mm diameter cork borer, several discs were cut from each of the colony cultures of **M. phaseolina** for each treatments. The number of sclerotia on PDA discs were calculated under a stereomicroscope and average number of sclerotia/disc was calculated to visualize the effect of each fertilizer on sclerotial production of **M. phaseolina.**

RESULTS and DISCUSSION

Mycelial growth of **M. phaseolina** to fertilizers varied greatly (Table 1). Out of T_6, T_9, T_7 and T_{10}, the effects of T_1, T_2, T_3, T_4, T_5, T_8, T_{11} and T_{12} on mycelial growth

were statistically the same. Similarly, T_7 and T_{10} exhibited same effectiveness in reducing mycelial growth. Colony diameter was the lowest in T_6. T_6 was followed by T_9 (Table 1). This may probably be due to production of uric acid in the medium.

Table 1. Effect (in vitro) of various fertilizers on mycelial growth and sclerotial production of **Macrophomina phaseolina**

Treatments	Mean mycelial growth (mm)		Av. No. of sclerotia produced on/6 mm diameter agar	
T_0 (Control	90.0	a[x]	257.0	bc
T_1 (Ammonium sulphate)	87.4	ab	284.8	abc
T_2 (Ammonium nitrate)	87.0	ab	250.0	bc
T_3 (Triple super phosphate)	86.4	ab	328.8	ab
T_4 (20-20-0)	87.2	ab	244.0	c
T_5 (15-15-15)	87.2	ab	245.8	c
T_6 (Urea)	69.4	d	114.0	d
T_7 (Ammonium sulphate + Triple super phosphate)	85.0	b	304.2	abc
T_8 (Ammonium nitrate + Triple super phosphate)	86.8	ab	323.8	abc
T_9 (Urea + Triple super phosphate)	75.2	c	145.0	d
T_{10} (20-20-0 + Ammonium nitrate)	85.8	b	308.2	abc
T_{11} (15-15-15 + Ammonium sulphate + Triple super phosphatee)	87.0	ab	348.2	a
T_{12} (15-15-15 + Ammonium nitrate + Triple super phosphate)	87.8	ab	355.8	a

[x] Figures with same letter (s) do not differ significantly ($P<0,05$).

Like mycelial growth, the effect of fertilizers on sclerotial production also varied considerably (Table 1). The most effective treatments in inhibiting sclerotial production were T_6 and T_9. T_4 and T_5 were the lesser effective in inhibiting sclerotial production of

M. phaseolina. T_0 and T_2 statistically exhibited same effectiveness. Addition of T_1, T_3, T_7, T_8, T_{10}, T_{11} and T_{12} to the medium significantly increased number of sclerotia, and out of T_{11} and T_{12} there were no significant difference among the effectiveness of them. There were maximum sclerotial production in T_{11} and T_{12}. Triple super phosphate in combination with ammonium nitrate enhanced sclerotial production in **M. phaseolina.** Nutritional studies in vitro revealed that Czapek-Dox agar medium containing NPK constitutents favoured sclerotial formation (Rehman et al., 1987). They reported that presence of PK fertilizers in the medium increased sclerotial number while it decreased in urea. It could be said that these results are in agreement with those of Rehman et al. (1987).

Consequently, the most effective treatments in reducing two parameters i.e., mycelial growth and sclerotial production were urea and urea+triple super phosphate. The fact that there were minumum sclerotial production in urea (T_6) and urea + triple super phosphate (T_9) could provide an additional strategy for management of charcoal rot in sunflower production. So, it could be said that urea + triple super phosphate which is a fertilization program suggested on sunflower cultivation could be prefered to the other ones because of inhibiting sclerotial production of **M. phaseolina.**

ÖZET

AYÇİÇEĞİNDE Macrophomina phaseolina'NIN GELİŞİMİ VE SKLEROTIAL ÜRETİMİ ÜZERİNE GÜBRELERİN IN VITRO ETKİSİ

M. phaseolina'nın miselyal gelişimi, gübrelerin ilave edildiği ortamlarda farklılık göstermiştir. Koloni çapı ürede (60 mg/l) en düşük olmuştur. Bunu üre (60 mg/l) + triple super fosfat (40 mg/l) izlemiştir. Gübrelerin sklerotial üretim üzerindeki etkisi de önemli derecede farklı olmuştur. Sklerotial üretimi düşürmede en etkili uygulamalar üre (60 mg/l) ve üre (60 mg/l) + triple süper fosfat (40 mg/l) uygulamaları olmuştur. Diğer gübrelerin ortama ilavesi sklerot sayısını önemli derecede arttırmıştır. 15-15-15 (30 mg/l) + amonyum sülfat (30 mg/l) + triple süper fosfat (10 mg/l) ve 15-15-15 (30 mg/l) + amonyum nitrat (30 mg/l) + triple süper fosfat (10 mg/l) uygulamalarında sklerotial üretim en fazla olmuştur.

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Studies on Identification of Bacterial Microflora of Mushroom in Türkiye*

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ABSTRACT

In this research, it was investigated the determination and identification of pathogenic and saprophytic bacterial microflora isolated from mushroom growing houses located of Aegean, Marmara and Middle Anatolia Regions in Türkiye. Totally fifty two bacterial isolates were obtained from diseased materials. The whole flora isolated from diseased caps have composed of Fluorescent Pseudomonads. According to the results of different identification tests, it was determined that 52 fluorescent Pseudomonas isolates consisted of ***Pseudomonas tolaasii*** *(Paine) (18),* ***P. fluorescens*** *(Trev.) Migula (2),* ***P. putida*** *(Trev.) Migula (9),* ***P. gingeri*** *(Preece & Wong) (3) and mummy disease isolates (2). As the result of pathogenicity tests on caps, it was observed that* ***P. tolaasii*** *isolates were pathogenic on mushroom caps, while* ***P. putida*** *isolates were non-pathogenic, saprophytic bacteria. Also, it was determined that a few isolates of* ***P. fluorescens*** *were able to produce slihgt browning on caps when the environmental conditions were available for bacterial growth.*

All the pathogenic and saprophytic bacterial microflora isolated from mushroom growing houses during this research are the first record for Türkiye.

INTRODUCTION

Cultivated mushroom (**Agaricus bisporus** (Lange) Imb.) growing in Türkiye has started as subsistence cultivations 20 to 25 years ago (ERKEL, 1992; ERKAL, 1992). In our country, mushroom growing is widespread in Marmara, Aegean and Middle Anatolia regions, and the portion of the mushroom production of these three regions in total is about 85% (BORA **et al.**, 1994). In last eighteen years, some remarkable increases have observed on cultivated mushroom production. Mushroom production which was 80 tones in 1973 reached to 3052 tones in 1991 (ERKEL, 1992). Compared to the other mushroom growing countries, this increasement appears rather unimportant (ERKEL, 1992). The most important problem limiting the increasement of the mushroom production is the diseases and pests of mushroom (ERKAL, 1992). It was observed by BORA

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et al (1993, 1994) that the bacterial and fungal diseases of mushroom were the most serious problem of the mushroom growing houses located in Marmara, Aegean and Middle Anatolia Regions. There is not any research conducted on the mushroom bacterial diseases in Türkiye up to now. Therefore, determination and identification of the pathogenic and saprophytic bacterial microflora in the cultivated mushroom producing regions of Türkiye has been aimed at this study.

MATERIALS and METHODS

Some observations were performed in 87 mushroom growing houses in Marmara, Aegean and Middle Anatolia Regions, and the samples of mushroom caps showing the blotching, pitting, softening and abnormal growth were collected.

Isolation and Identification

After the diseased mushroom cap pieces were sterilized by Na-hypochlorite, these samples were homogenized with sterile destiled water and streaked on King Medium B (KB). It is known that the bacteria caused the disease on mushroom caps belong to fluorescent Pseudomonads. Thus, the colonies growing on KB were observed under UV light (360 nm) and the colonies showing fluorescense were purified. The various tests were performed including levan production, oxidase reaction, pectolytic activity on potato slices, arginine dihydrolase activity, hypersensitive reaction on tobacco leaves, gelatine hydrolysis, utilization of different carbon sources (sucrose, mannitol, sorbitol, M (-) tartarate), white line reaction with **P. reactans** (WONG and PREECE, 1979; ZARKOVER **et al.**, 1984) and 2-ketogluconate test for identification of fluorescent Pseudomonas complex. Then, the results were compared with findings of PALLERONI (1984), FAHY and PERSLEY (1983), LELLIOT and STEAD (1987).

Pathogenicity Tests

The pathogenicity of the bacterial isolates obtained from diseased mushroom caps were tested on freshly harvested caps with 2-3 cm diameter. A drop of a standart bacterial suspansion was put on each cap. Also sterile water and a suspansion of reference. **P. tolaasii*** isolate as reference were dropped on caps. Inoculated mushroom caps were kept at high relative humidity (90%) and 20°C temperature for 48 hours. Evaluations were made according to pitting and changing of the colour.

RESULTS and DISCUSSION

Totaly fifty two bacterial isolates were obtained from diseased material collected from different mushroom growing houses in Türkiye. The whole microflora isolated from diseased caps have composed of fluorescent Pseudomonads. Distinctive characteristics of fluorescent Pseudomonads isolated from diseased mushrooms are shown on Table 1.

* Reference **P. tolaasii** culture were obtained from Dr. K. Benlioğlu.

Table 1. Distinctive characteristics of fluorescent Pseudomonads isolated from diseased mushrooms.

Number of tested isolates	Fluorescense on KB	Levan	Oxidase	Pectolytic activity	Arginine dihydrolase	Tobacco HR	Gelatin hydrolysis	Utilizing from carbon sources: sucrose	Mannitol	Sorbitol	M (-) Tartarate	2-ketoglu-conate	White-line reaction	IDENTIFICATION
18	+	-	+	-	+	X	+	-	+	+	+	+	+	**P. tolaasii**
9	+	-	+	-	+	-	-	-	+	-	V	+	-	**P. putida**
11	+	+	+	+	+	-	+	+	+	+	-	+	-	**P. fluorescens** biovar II or IV
9	+	-	+	+	+	-	+	-	+	V	-	+	-	**P. fluorescens** biovar III
3	+M	-	+	-	+	-	+	+	+	+	V	-	-	**P. gingeri**
2	+	-	+	+	+	-	+	+	+	+	+	+	-	Mummy disease isolates

(+) = Clear reaction; (-) = No reaction; (X) = No hypersensitive reaction, but atypical greasy lesions then tissue collapse; (M) = Mucoidal growth; (V) = Variable reaction.

It is seen that 52 fluorescent Pseudomonas isolates consist of 18 **P. tolaasii,** 9 **P. putida,** 20 **P. fluorescens,** 3 **P. gingeri** and 2 mummy disease isolates in Table 1.

Our findings demonstrated that white-line reaction and hypersensitive reaction on tobacco leaves, besides some biochemical characteristics, were satisfactory for the identification of **P. tolaasii.** Also, WONG and PREECE (1979) and ZARKOWER **et al** (1984) advised the use of the white-line test in the identification of **P. tolaasii.** When bacterial strains isolated from diseased mushrooms were injected to tobacco leaves, only **P. tolaasii** isolates were able to induce no hypersensitive but atypical greasy lesions on the leaves. OLIVER **et al** (1978) reported that this reaction on tobacco leaves would be distinctive for **P. tolaasii.**

For the identification of **P. gingeri,** causal agent of ginger blotch, it was determinative for the bacterial isolates to grow mucoidal on KB and to give negative results in the white-line test and hypersensitive reaction on tobacco leaves.

The isolates of **P. fluorescens** yielded the positive results in the tests such as pectolytic activity, gelatin hydrolysis, 2-keto gluconate, utilizing from some carbon sources (eg; sucrose, sorbitol, mannitol, m (-) tartarate) and negative results at the tests of white-line and HR on tobacco.

The isolates given the negative results in the Arginine dihydrolase and gelatin hydrolysis tests were identified as **P. putida.**

It was determined that two bacterial strains isolated from caps showing mummy symptoms belonged to fluorescent Pseudomonads (Table 1). Although, the causal agent of mummy disease is known as a member of **P. fluorescens** complex, the causal agent of mummy disease couldn't definitely been identified (KALBARCZYK, 1985; GRIENSVEN, 1988).

The results of the pathogenicity on mushroom caps of some bacterial isolates are given in Table 2.

As shown in Table 2, all of **P. tolaasii** isolates caused the typical browning and pitting on mushroom caps 48 hours after inoculation, whereas **P. putida** isolates were not able to cause any symptom on caps. **P. putida** which is naturally found in casing soil or compost is a non-pathogenic and stimulating bacterium in the formation of pin-head of **A. bisporus** (KHANNA **et al.**, 1990; FLEGG **et al.**, 1985).

Table 2. The pathogenicity on mushroom caps of some bacterial strains isolated from diseased mushrooms.

Species of tested isolates	Number of tested isolates*	Typical Browning and pitting	Slight Browning	No Symptom
P. tolaasii	13	12	1	-
P. putida	7	-	-	7
P. fluorescens	4	-	2	2
Negative Control (Destiled water)		-	-	+
Positive Control (Reference **P. tolaasii**)		+	-	-

* Each isolate was tested on five separate caps.

According to the pathogenicity tests on mushroom caps, it was observed that **P. fluorescens** isolates were not primary pathogen on mushrooms, and only a few isolates caused the slight browning on caps. But, **P. fluorescens** may lead to the problem on mushroom growing houses where the environmental conditions are available for bacterial growth and disease incidence (BORA **et al.**, 1993; FLETCHER, 1989).

Symptomatological differences due to bacterial microflora determined in the mushroom growing houses are as follows: **P. tolaasii,** causal agent of bacterial blotch, causes the browning and pitting on mushroom caps (VEDDER, 1978; FLETCHER, 1989), while **P. gingeri** causes ginger coloured, superficial blotches on caps (WONG **et al.**, 1982; GUILLAUMES **et al.**, 1985). **P. fluorescens** biotypes are generally isolated from extensively browned and softened caps. Also, the causal agent of mummy disease leads to deformations of the caps (BETTERLEY and OLSON, 1989).

All the pathogenic and saprophytic bacterial microflora isolated from mushroom growing houses are the first record for Türkiye. Moreover, during this research, **P. tolaasii** the causal agent of brown blotch seemed to the most serious bacterial disease in the mushroom growing houses.

ÖZET

TÜRKİYE KÜLTÜR MANTARI BAKTERİYEL MİKROFLORASININ TANILANMASI ÜZERİNDE ÇALIŞMALAR

Bu çalışmada, Türkiye'de Ege, Marmara ve Orta Anadolu Bölgelerinde bulunan mantar üretim evlerinden izole edilen patojenik ve saprofitik bakteriyel mikrofloranın saptanması ve tanılanması araştırılmıştır. Hastalıklı materyalden toplam 52 bakteri izolatı elde edilmiştir. Hastalıklı şapkalardan izole edilen tüm floranın fluoresent Pseudomonas olduğu saptanmıştır. Çeşitli tanılama testlerinin sonuçlarına göre, 52 fluoresent Pseudomonas izolatının 18'i **Pseudomonas tolaasii** (Paine), 20'si **P. fluorescens** (Trev.) Migula, 9'u **P. putida** (Trev.) Migula, 3'ü **P. gingeri** (Preece & Wong) ve 2'si mumya etmeni olarak tanılanmıştır. Mantar şapkaları üzerinde patojenisite testi sonucunda; **P. tolaasii** izolatları şapkalar üzerinde simptom oluştururken, **P. putida** izolatlarının nonpatojenik olduğu gözlenmiştir. Ayrıca bazı **P. fluorescens** izolatlarının da üretim evinin koşulları bakteriyel gelişme için uygun olduğu zaman şapkalar üzerinde hafif kahverengileşmeye neden olduğu saptanmıştır.

Bu araştırma sırasında mantar üretim evlerinden izole edilen patojenik ve saprofitik bakteriyel mikroflora Türkiye için ilk kayıttır.

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Detection of **Xanthomonas campestris** pv. **phaseoli** and **Pseudomonas syringae** pv. **phaseolicola** by bacteriophages

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ABSTRACT

Xanthomonas campestris pv. phaseoli and Pseudomonas syringae pv. phaseolicola phages were isolated and their specifities were found out.

Two out of 16 bacteriophages obtained from X. c. pv. phaseoli and two of 3 X.c. pv. phaseoli "fuscans" phages, 1 of 13 P. s. pv. phaseolicola phages showed high degree specifity and they were found reliable in the detection of these pathogens.

INTRODUCTION

Bean (**Phaseolus vulgaris** L.) is an important crop in Türkiye.

Pseudomonas syringae pv. **phaseolicola** has been recorded in Ankara, Artvin, Bursa, Blacksea region, **Xanthomonas campestris** pv. **phaseoli** has been recorded in Adana, Amasya, Ankara, Antakya, Antalya, Denizli, İzmir, Samsun, Tokat and Uşak by many researchers up to now.

It is known that these pathogens are seedborne and only a few infected seed in 45 kg seed can cause serious crop losses in humid areas (Schuster and Coyne, 1981). For this reason, in the control of these pathogens, using healty seed and susceptible, reliable and fast methods for determination and identification of them are necessary.

Persley (1983) and Schaad (1982) emphasized that the bacteriophages were important, simple and rapid tools in the detection of the phytopathogenic bacteria. However their specifity and susceptibility have to be studied in detail.

Taylor (1970) isolated four phages of **P.s.** pv. **phaseolicola** and determined 3 of them to be specific while the other one lysed different bacterial species or pathovars.

Katznelson and Sutton (1951) and Katznelson **et al.** (1954) used **X.c.** pv. **phaseoli** phages in the direct method of the determination of the agent. Furthermore, they said that the phage hadn't lysed strain "fuscans" of **X.c.** pv. **phaseoli, X.c.** pv. **sojensis** and yellow pigmented saprophytic bacteria.

In our research, bacteriophages were isolated to use in the detection of **X.c.** pv. **phaseoli** and **P. s.** pv. **phaseolicola** exist in Türkiye, and their usefullness was evaluated.

MATERIALS and METHODS

In order to isolate bacteriophages, soil and plant samples were taken from bean fields in Ankara, Balıkesir, Eskişehir and Samsun in 1990.

59 Bacterial isolates of different genera and species were tested to determine specifity of the phages. The bacterial isolates were grown on YDCA (Yeast Extract Dextrose Calcium carbonate Agar) slants and kept at 4°C and transferred at the period of 2-2.5 months (Schaad, 1980).

Isolation of phages

Isolate 68 of **P.s.** pv. **phaseolicola,** isolate 138 of **X.c.** pv. **phaseoli** and isolate 148 of **X.c.** pv. **phaseoli** "fuscans" were used as hosts to isolate phages. The cultures were grown in 250 ml flasks containing 150 ml NGB (Nutrient Gliserol Broth) for soil samples or 30 ml NGB for plant samples. 75 g of soil samples or 5-10 g of plant samples were added into 48 h cultures grown in NGB. The mixture were incubated for 24 h and shaked occasionally. After incubation, 10 ml of mixture was taken and centrifuged at approximately 3000 rpm for 20-30 min. 5 ml of the supernatant was transferred into 25 ml screw-capped bottle. Chloroform was added 5% in the final concentration into the supernatant and vigorously agitated. They were kept at 4-6°C (Persley, 1983).

Determination and Purification of Phages

Bacterial suspensions of ~ 10^8 cells per ml were prepared from the cultures grown on KBA. 1 ml of bacterial suspension was added into the tubes containing 12 ml of NGA at 45°C and poured in steril petri dishes. Lids of petri dishes were opened until the surface of agar dries. 10-20 µl of the supernatant was spotted onto surface of the agar containing host bacterial cells and incubated at 25°C for 24 h. After this, when the place spotted by the supernatant became transparent, there were phage particules in the supernatant, and such samples were kept for purification.

The fluids containing phages were streaked onto NGA containing the host bacterial cells using an inoculation loop. After incubation at 25°C for 24 h isolated plaques were transferred into the bottles containing 3 ml of NGB. These phages were furified by means of selection of plaque and streaking of suspension for three times at least.

Preparation and Storage of Phage Stocks

1 ml of bacterial suspension of 10^8 cells/ml was added into tubes containing 30 ml NGA at 45°C, mixed and poured into petri dishes. 0.1 ml of purified phage suspension was spread onto NGA containing bacterial cells and rested for 4 h and then the fluid on the agar was withdrawn and transferred into the 25 ml screw capped bottles. Chloroform at 5% were mixed to phage suspensions and they were kept at 4°C.

Determination of Phage Specifities

Suspensions prepared from several bacterial isolates were added previously in NGA medium and then phage suspensions (having ten times of concentrated of dilution which gives barely confluent lysis on propagating strain) were spotted onto surface of

medium. The spots were allowed to absorb into medium for a short time and then incubated at 25°C for 24 h. After incubation, petri dishes were observed to determine plaque formation (Persley, 1983).

RESULTS AND DISCUSSION

13 **Pseudomonas syringae** pv. **phaseolicola** phages, 16 **Xanthomonas campestris** pv. **phaseoli** phages and 3 **X.c.** pv. **phaseoli** "fuscans" phages were isolated and their specifities were found out.

12 **P.s.** pv. **phaseolicola** phages lysed also different bacterial species or pvs., including **P. cichorii, P.s.** pv. **glycinea, P.s.** pv. **lachrymans, P.s.** pv. **syringae, P.s.** pv. **tabaci, P.s.** pv. **tomato** and strain NCPPB 1811 of **X.c.** pv. **phaseoli** (Table 1). One **P.s.** pv. **phaseolicola** phage showed high degree of specifity and lysed merely **P.s.** pv. **phaseolicola** isolates and did not react other bacteria to be tested. This phage (PP9) was found useful for the identification of **P.s.** pv. **phaseolicola.**

Taylor (1970) isolated four phages for **P.s.** pv. **phaseolicola.** Three of them lysed merely **P.s.** pv. **phaseolicola** while the fourth also lysed many related **Pseudomonas** spp.

X.c. pv. **phaseoli** phages except XP12 and XP13 lysed all **X.c.** pv. **phaseoli** isolates and formed unclear plaques on isolate Sinop 16 of **X.c.** pv. **phaseoli** "fuscans". XP12 and XP13 lysed only **X.c.** pv. **phaseoli** isolates while no lysis was determined on the other bacterial species (Table 2).

FP1, **X.c.** pv. **phaseoli** "fuscans" phage, lysed all isolates of **X.c.** pv. **phaseoli** "fuscans" and isolate NCPPB 1811 of **X.c.** pv. **phaseoli** and formed unclear plaques on 4 **X.c.** pv. **phaseoli** isolates. FP2 and FP3 lysed all isolates of "fuscans" but formed very small, seperate plaques on isolate NCPPB 1811 of **X.c.** pv. **phaseoli** (Table 3). These plaques can be easily distinguished from the clear ones. Furthermore the "fuscans" phages lysed none of other bacterial isolates. XP12 and XP13 didn't lyse **X.c.** pv. **phaseoli** "fuscans". FP2 and FP3 didn't also lyse **X.c.** pv. **phaseoli** and were specific to their hosts. These results were in accordance with Sutton and Wallen's (1967). They also obtained specific phages for **X.c.** pv. **phaseoli** and **X.c.** pv. **phaseoli** "fuscans". Therefore the detection of these bacteria might be achieved by using XP12 and XP13; FP2 and FP3. Our phages might be used for the detection of related bacteria and to confirm the results of other laboratory tests.

Table 1. Reactions of **Pseudomonas syringae** pv. **phaseolicola** phages on several bacterial isolates.

Bacteria (no of isolate)	Bacteriophages												
	PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP8	PP9	PP10	PP11	PP12	PP13
Agrobacterium tumefaciens (Yal. Chr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis (161)	-	-	-	-	-	-	-	-	-	-	-	-	-
Cur. f. subsp. flaccumfaciens (7)	-	-	-	-	-	-	-	-	-	-	-	-	-
Cla. m. subsp. michiganensis (3)	-	-	-	-	-	-	-	-	-	-	-	-	-
Erwinia amylovora (195)	-	-	-	-	-	-	-	-	-	-	-	-	-
E. carotovora subsp. atroseptica	-	-	-	-	-	-	-	-	-	-	-	-	-
E. carotovora subsp. carotovora	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas cichorii (51)	-	-	+	+	+	-	-	+	-	+	+	-	+
P. marginalis (11)	-	-	-	-	-	-	-	-	-	-	-	-	-
P.s. pv. glycinea (NCPPB 2411)	+	+	-	-	-	-	-	a	-	a	a	-	-
P.s. pv. lachrymans (204)	-	+	-	-	-	b	b	-	-	b	b	-	-
P.s. pv. phaseolicola (8 isolates)	+	+	+	+	+	+	+	+	+	+	+	+	+
P.s. pv. phaseolicola (158)	-	-	-	-	-	-	-	+	+	+	+	-	+
P.s. pv. phaseolicola (NCPPB 52)	+	+	+	+	+	+	+	+	+	+	+	+	+
P.s. pv. pisi (NCPPB 1366)	-	-	-	-	-	-	-	-	-	-	-	-	-
P.s. pv. syringae (120)	+	+	+	+	+	+	+	+	-	+	+	+	+
P.s. pv. syringae (121)	+	+	+	+	+	+	+	+	-	+	+	+	+
P.s. pv. tabaci (Bursa 89)	-/+	-/+	+	+	+	-/+	-	+	-	+	+	+	+
P.s. pv. tomato (35)	+	-	-	-	-	-	+	-	-	-	+	-	-
X. campestris pv. campestris (178)	-	-	-	-	-	-	-	-	-	-	-	-	-
X. c. pv. malvacearum	-	-	-	-	-	-	-	-	-	-	-	-	-
X.c. pv. phaseoli (9 isolates)	-	-	-	-	-	-	-	-	-	-	-	-	-
X.c. pv. phaseoli (NCPPB 3035)	-	-	-	-	-	-	-	-	-	-	-	-	-
X.c. pv. phaseoli (NCPPB 1811)	a	-	-/+	-	-	-	-	-/+	-	-/+	-	-	-
X.c. pv. phaseoli "fuscans" (3 isolates)	-	-	-	-	-	-	-	-	-	-	-	-	-
Fluorescent **Pseudomonas** sp. (106)	-	-	-	-/+	-/+	-/+	-/+	-/+	-	-/+	-	-	-
Fluorescent **Pseudomonas** sp.(9 isol.)	-	-	-	-	-	-	-	-	-	-	-	-	-
Yellow pigmented bacterium (6 isol.)	-	-	-	-	-	-	-	-	-	-	-	-	-

Description of the reactions:

clear plaque (+), unclear plaque (+), individual plaques in normal size (a) and individual plaques in small size (b)

NCPPB: National Collection of Plant Pathogenic Bacteria, Harpenden (UK).

Table 2. Reactions of **Xanthomonas campestris** pv. **phaseoli** phages on several bacterial isolates.

Bacteria (no of isolate)	Bacteriophages		
	14 phages	XP12	XP13
Agrobacterium tumefaciens (Yal. Chr.)	-	-	-
Bacillus subtilis (161)	-	-	-
Cur. f. subsp. **flaccumfaciens** (7)	-	-	-
Cla. m. subsp. **michiganensis** (3)	-	-	-
Erwinia amylovora (195)	-	-	-
E. carotovora subsp. **atroseptica**	-	-	-
E. carotovora subsp. **carotovora**	-	-	-
Pseudomonas cichorii (51)	-	-	-
P. marginalis (11)	-	-	-
P.s. pv. **glycinea** (NCPPB 2411)	-	-	-
P.s. pv. **lachrymans** (204)	-	-	-
P.s. pv. **phaseolicola** (9 isolates)	-	-	-
P.s. pv. **phaseolicola** (NCPPB 52)	-	-	-
P.s. pv. **pisi** (NCPPB 1366)	-	-	-
P.s. pv. **syringae** (120)	-	-	-
P.s. pv. **syringae** (121)	-	-	-
P.s. pv. **tabaci** (Bursa 89)	-	-	-
P.s. pv. **tomato** (35)	-	-	-
X. campestris pv. **campestris** (178)	-	-	-
X. c. pv. **malvacearum**	-	-	-
X.c. pv. **phaseoli** (9 isolates)	+	+	+
X.c. pv. **phaseoli** (NCPPB 3035)	+	+	+
X.c. pv. **phaseoli** (NCPPB 1811)	+	+	+
X.c. pv. **phaseoli** "fuscans" (148)	-	-	-
X.c. pv. **phaseoli** "fuscans" (149)	-	-	-
X.c. pv. **phaseoli** "fuscans" (Sinop 16)	- +	-	-
Fluorescent **Pseudomonas** sp. (10 isol.)	-	-	-
Yellow pigmented bacterium (6 isol.)	-	-	-

Table 3. Reactions of **Pseudomonas syringae** pv. **phaseolicola** phages on several bacterial isolates.

Bacteria (no of isolate)	Bacteriophages		
	FP1	FP2	FP3
Agrobacterium tumefaciens (Yal. Chr.)	—	—	—
Bacillus subtilis (161)	—	—	—
Cur. f. subsp. **flaccumfaciens** (7)	—	—	—
Cla. m. subsp. **michiganensis** (3)	—	—	—
Erwinia amylovora (195)	—	—	—
E. carotovora subsp. **atroseptica**	—	—	—
E. carotovora subsp. **carotovora**	—	—	—
Pseudomonas cichorii (51)	—	—	—
P. marginalis (11)	—	—	—
P.s. pv. **glycinea** (NCPPB 2411)	—	—	—
P.s. pv. **lachrymans** (204)	—	—	—
P.s. pv. **phaseolicola** (9 isolates)	—	—	—
P.s. pv. **phaseolicola** (NCPPB 52)	—	—	—
P.s. pv. **pisi** (NCPPB 1366)	—	—	—
P.s. pv. **syringae** (120)	—	—	—
P.s. pv. **syringae** (121)	—	—	—
P.s. pv. **tabaci** (Bursa 89)	—	—	—
P.s. pv. **tomato** (35)	—	—	—
X. campestris pv. **campestris** (178)	—	—	—
X. c. pv. **malvacearum**	—	—	—
X.c. pv. **phaseoli** (5 isolates)	—	—	—
X.c. pv. **phaseoli** (4 isolates)	−/+	—	—
X.c. pv. **phaseoli** (NCPPB 3035)	—	—	—
X.c. pv. **phaseoli** (NCPPB 1811)	+	b	b
X.c. pv. **phaseoli** "fuscans" (3 isolates)	+	+	+
Fluorescent **Pseudomonas** sp. (10 isol.)	—	—	—
Yellow pigmented bacterium (6 isol.)	—	—	—

ACKNOWLEDGEMENT

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ÖZET

XANTHOMONAS CAMPESTRIS PV. PHASEOLI VE PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA'NIN BAKTERİYOFAJ İLE TESPİTİ

Xanthomonas campestris pv. **phaseoli** için 16 adet, **X.c.** pv. **phaseoli** "fuscans" için 3 adet ve **Pseudomonas syringae** pv. **phaseolicola** için 13 adet faj izole edilmiştir. **X.c.** pv. **phaseoli** fajlarından iki tanesi, **X.c.** pv. **phaseoli** "fuscans" fajlarından iki tanesi ve **Pseudomonas syringae** pv. **phaseolicola** fajlarından bir tanesi oldukça yüksek özelleşme göstermişler ve ilgili patojenlerin tespitinde kullanışlı bulunmuştur.

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Rapid Detection of Tomato Mosaic Virus by Agarose Gel Electrophoresis

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ABSTRACT

Tomato Mosaic Virus (ToMV) was determined by agarose gel electrophoresis from purified preparations and crude leaf extracts. Purified preparations and crude leaf extracts produced two main band in gels but healthy tissue did not produce any band.

INTRODUCTION

Agarose gel electrophoresis separates and detects the agregated and partially stripped virus particles from purified preparation or crude extracts of plant infected with tobamovirus isolates (Asselin and Grenier, 1985; Reddick, 1989). Technique is useful to distinguish or separate viruses or strains of viruses (Asselin and Grenier, 1985; Reddick, 1989). We made some modification in these methods searching in purified ToMV and crude extracts infected with ToMV.

MATERIALS and METHODS

Virus isolate. ToMV was isolated from infected tomato plants in Mersin provience and characterized as a ToMV-M. Virus was propagated in **Nicotiana tabacum** "Samsun" and purified as described previously (Yılmaz and Davis, 1984). Concentration of virus was determined spectrophotometrically by using coefficient factor as 3 (Hollings and Huttinga, 1976).

Preparation of crude leaf extract. The crude extract used was prepared from tomato plants inoculated with ToMV-M. Twelve days after inoculation, young infected leaf tissue (0.5 gr) was ground in a steril pestle and mortar with 1 ml of 100mM sodium phosphate buffer, pH 7.2. The homogenate was filtrated and centrifuged at 8.000 rpm for 10 minutes with ALC micro centrifuge 4214. The supernatant was kept at -25°C for 1 hours. After melting of supernatant at room temperature, it was centrifuged at 10.000 rpm for 10 minutes and supernatant was used as a crude extract.

Agarose slab gel electrophoresis. Electrophoresis was performed by the modification of method suggested by Asselin and Grenier (1985). Purified and crude extract preparations were subjected to agarose slab gel electrophoresis in tris-glycin (25 mM Tris, 192 mM Glycin) buffer (pH 8.3-8.8) at 75 V for 45 minutes at room temperature with no circulation. Agarose (1.5%, w/v) was prepared in the same phosphate buffer.

Gel staining. Gels were stained in a solution of 0.1% Coomassie Brillant Blue R 250, 25% methanol, 65% water, and 10% acetic acid for 45 minutes at 40°C with agitation gently. Destaining was in a solution of 25% isopropanol, 65% water and 10% acetic acid for 45 minutes at 40°C with agitation gently and 10% acetic acid for 90 minutes at the same conditions.

RESULTS and DISCUSSION

As previously reported, it is possible to identificate the tobamoviruses by agarose gel electrophoresis and each isolate has individual profile (Asselin and Grenier, 1985; Reddick, 1989). When the purified ToMV-M isolate in a decreasing concentration (from 15 µg to 0.75 µg) was subjected to agarose gel electrophoresis, it showed that quantity of 0.75 µg virus could be detected. Electrophoresis profile produced two main band from purified preparations. The lower band corresponded to monomer particles and upper band was aggregated particles (Fig. 1).

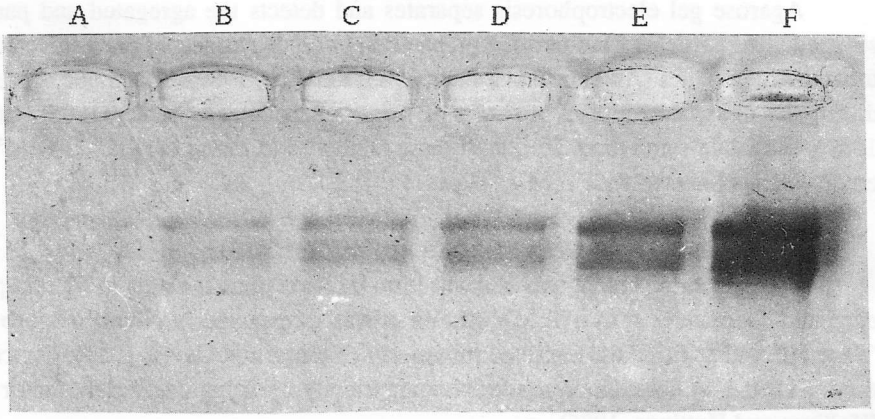

Fig. 1. Electrophoretic profile of purified ToMV-M in 1.5% agarose gel (60 ml). Electrophoresis was runned at 75 V in Tris-glycin buffer. F-A: decreasing quantities of ToMV-M (F, 15µg; E, 7.5 µg; D, 3.75 µg; C, 1.875 µg;ş B, 0.937 µg; A, 0.75 µg). 25 µl of samples was pipetted into the sample slot.

When the crude leaf extracts infected with ToMV were subjected to electrophoresis, the virus bands were easily observed at the same level that of purified preparation, and no band was observed in healthy tomato crude extract (Fig. 2).

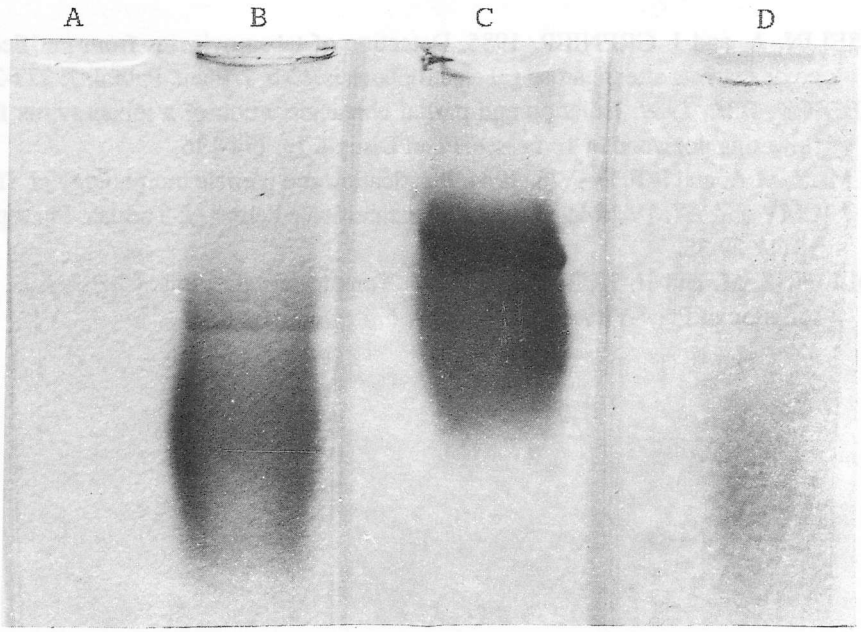

Fig. 2. Electrophoretic profile of purified ToMV-M, infected leaves and healthy extract. A, purified ToMV-M (0.75 µg); B, crude leaf extract of inoculated tomato; C, crude leaf extract of field infected tomato; D, crude leaf extract of healthy tomato.

There are several methods which have used for identification of plant viruses and need well equipped laboratory. Recently, Asselin and Granier (1985) reported to use agarose gel electrophoresis for detection of tobamoviruses from purified and crude extracts. ToMV (tomato mosaic virus) was detected up to 0.75 µg from purified preparations and infected plants by Agarose slab gel electrophoresis in 4 hours in this study.

ÖZET

AGAROSE JEL ELEKTROFOREZ YÖNTEMİ İLE DOMATES MOZAYIK VİRUSUNUN HIZLI TANILANMASI

Arıtılmış virüs preparasyonlarından ve domates mozayık virüsü ile enfekteli domates bitkisi ekstraktlarından domates mozayik virüsü agarose jel elektroforez yöntemiyle saptanmıştır. Arıtılan preparasyonlar ve ekstraktlar jellerde 2 bant meydana getirmiştir. Sağlıklı doku ekstraktlarında ise bu bantlar gözlenmemiştir.

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A Study on the Experimental Transmission Possibilities of Some of the Turkish Isolates of **Spiroplasma citri**

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ABSTRACT

Experimental transmission possibility and pathogenicity of different ***Spiroplasma citri*** *isolates, which cause stubborn disease in the East Mediterranean Area, have been studied by inoculating them to the* ***Euscelidius variegatus*** *(Kirsh) and* ***Circulifer tenellus*** *(Baker), which are known vectors and* ***Exitianus capicola*** *STAL, which is a possible vector in the same area.* ***S. citri*** *has been inoculated to the vector insects by the methods of microinjection and membrane feeding.*

In this study the survival and multiplication of ***S. citri*** *in the inoculated insects and also possibilities of reisolation of the pathogen transmitted to the test plants, have been studied. In the experimental transmission studies, in which* ***S. citri*** *isolates cultured from Washington, Navel, Valencia oranges and* ***Exitianus capicola*** *insects were used, the symptoms similar to* ***S. citri*** *have been observed on Madam Vinous Orange (****Citrus sinensis****), broad bean (****Vicia faba****), periwinkle (****Vinca rosea L.****), red clover (****Trifolium pratense****) and cabbage (****Brassica oleracea capitata****). The symtoms of the disease could not be observed on pea (****Pisum sativum****), onion (****Allium cepa****), London rocket (****Sisymbrium officinale****) and marigold (****Tagetes erecta****).* ***S. citri*** *could not be reisolated from those plants, whereas it has been reisolated from all of the symptomatically infected plants, which are mentioned above.*

INTRODUCTION

Citrus stubborn is a very well known disease all over of the world since 1915 (Calavan, 1968). The causal agent of this disease is a mycoplasma like organism (MLO) called **Spiroplasma citri,** which can be cultured artificial media diffrently than other plant MLO's (Fudl-Allah **et al,** 1972).

S. citri has been isolated from different citrus varieties such as oranges, mandarins, grapefruits and tangelos grown in east Mediterranean Area (Çağlayan and Çınar, 1990). Furthermore the pathogen has been also cultured from **Vinca rosea** L., which is an indicator plant for stubborn disease, and possible vector insects (Çınar and Çağlayan, 1988; Kersting and Şengonca, 1992). This situation indicates that this disease is naturally spreading in this area.

Because of **S. citri** can be cultured, Koch postulates can be aplied to this pathogen. **Euscelis plebejus** (Fallen), which is injected by spiroplasma culture, transmitted **S. citri** to the 2 citrus plants from 49 and the pathogen could be reisolated (Markham **et al,** 1974). Furthermore by using **Circulifer tenellus** (Baker) and other vector insects **S. citri** was transmitted from citrus to citrus and from citrus to periwinkle and clover (Markham and Townsend, 1974; Oldfield **et al,** 1976).

Spiroplasmas can be inoculated to the vector insects by the methods of microinjection and membrane feeding, **C. tenellus** and **Scaphytopius nitridus** (De Long) can acquire **S. citri** which is in concentrated suspension, in 10-20 minutes but they need 36 hours of feeding period to transmit it to the sweet orange seedlings (Rana **et al,** 1975).

In this study Turkish spiroplasma isolates were inoculated to the known vectors, **Euscelidius variegatus** (Kirsh), **C. tenellus** and to the **E. capicola,** which are collected from the field, by microinjection in order to observe if they are pathogenic to the different test plants. In addition it has also been studied that if candidate vector **E. capicola** can acquire and transmit East Mediterranean isolates of **S. citri** through membrane feeding. This part of study helps to prove how effective **E. capicola** is to transmit stubborn disease in the field.

MATERIALS and METHODS

This study has been done in John Innes Institute (JII) Norwich, U.K.As a vector **Exitianus capicola** STAL, which have been collected from the field of East Mediterranean Area, **Euscelidius variegatus** (Kirsh) and **Circulifer tenellus** (Baker), which were cultured in the laboratories of JII. For the pathogenicity work Madam Vinous orange **(Citrus sinensis),** broad bean **(Vicia faba),** periwinkle **(Vinca rosea L.)** red clover **(Trifolium pratense),** cabbage **(Brassica oleracea capitata),** pea **(Pisum sativum),** onion **(Allium cepa),** London rocket **(Sisymbrium officinale)** and marigold **(Tagetes erecta)** have been used as a test plants. The spiroplasma isolates, used in this study were Washington Navel (SPT-WN), Valencia (SPT-VAL) and insect (SPT-I).

Culture of insects and microinjection procedure has been done according to Markham and Townsend (1979) and Markham and Oldfield (1983).

The multiplication of spiroplasmas in insects have been recorded daily by culturing them after microinjection. Calculations of colony forming unit (cfu) of spiroplasmas in insects and survival experiments carried out as described before (Markham and Alivizatos, 1983).

For the pathogenicity work vector insects, which were injected by different Turkish spiroplasma isolates, fed on the host plants, mentioned above. This plants have been replaced by the new ones as needed. After the first symptoms appear, reisolation of pathogen has been done according to the method described by Saglio **et al.** (1973).

Membrane feeding has been carried out in order to study transmission and acquisition of spiroplasma isolates through membrane. The procedure has been followed as described before (Rana **et al,** 1975).

RESULTS

After the microinjection, all spiroplasmas could be cultured from the insects, injected by spiroplasma culture, whereas no spiroplasmas could be cultured from the insects, injected by sterile medium. The first day of injection the concentration of inoculum was $7x10^4$ cfu/insect, $1.5x10^5$ cfu/insect and $2.5x10^5$ cfu/insect for **C. tenellus, E. variegatus** and **E. capicola,** respectively. In 20 days it has reached to $7.5x10^5$ cfu/insect, $1.5x10^5$ cfu/insect and $2.5x10^6$ cfu/insect for **C. tenellus, E. variegatus** and **E. capicola,** respectively (Fig. 1).

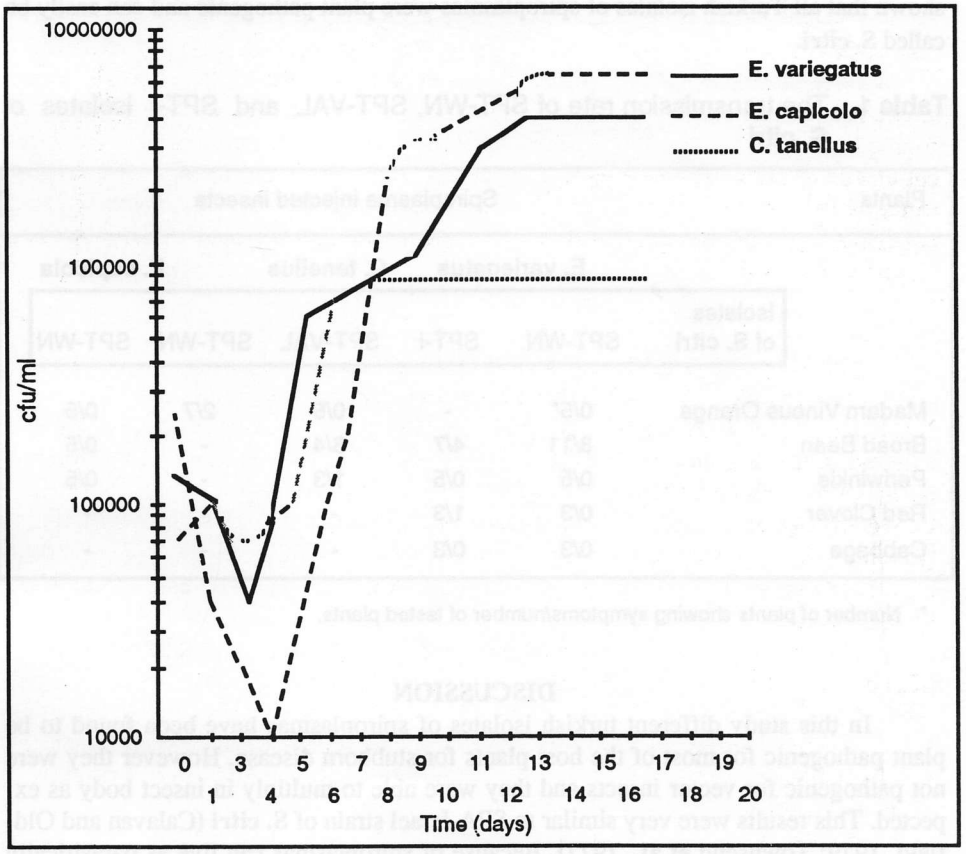

Fig. 1. Multiplication of SPT-WN isolates of **Spiroplasma citri** in **E. capicola, E. variegatus** and **C. tenellus.**

At the time of maximum titre between days 2 to 10, mortality was greater in SMC injected and healthy insects than in **S. citri** (SPT-WN) injected ones. Almost identical results were obtained for SPT-V and SPT-I (data not given).

Microinjected 11 of **E. variegatus** from 20 and 3 of **E. capicola** from 10 could transmit SPT-I isolate through membrane feeding 10 days after injection. Moreover **E. capicola** was found able to acquire Turkish spiroplasma isolate (SPT-WN) through membrane. All isolation attempts were unsuccesful until 4 days after feeding. After that spiroplasmas could be easily isolated from **E. capicola.**

According to the results of pathogenicity work the symptoms similar to **S. citri** have been observed on Madam Vinous orange, broad bean, periwinkle, red clover and cabbage. **S. citri** could be reisolated from all of those plants (Table 1). It has been shown that all Turkish isolates of spiroplasmas were plant pathogenic and can easily be called **S. citri.**

Table 1. The transmission rate of SPT-WN, SPT-VAL and SPT-I isolates of **S. citri**

Plants	Spiroplasma injected insects				
	E. variegatus		**C. tenellus**		**E. capicola**
Isolates of **S. citri**	SPT-WN	SPT-I	SPT-VAL	SPT-WN	SPT-WN
Madam Vinous Orange	0/5*	-	0/5	2/7	0/5
Broad Bean	8/11	4/7	3/4	-	0/5
Periwinkle	0/5	0/5	1/3	-	0/5
Red Clover	0/3	1/3	-	-	-
Cabbage	0/3	0/3	-	-	-

* Number of plants showing symptoms/number of tested plants.

DISCUSSION

In this study different turkish isolates of spiroplasmas have been found to be plant pathogenic for most of the host plants for stubborn disease. However they were not pathogenic for vector insects and they were able to multiply in insect body as expected. This results were very similar to SPA-Israel strain of **S. citri** (Calavan and Oldfield, 1979; Townsend **et al.,** 1977). Because of spiroplasmas can live as parasitically esp. on flowers (Daniels, 1979), the pathogenicity work is very important in order to prove new isolates as **S. citri.**

Recently natural incidence of citrus stubborn disease in East Mediterranean Area has been intensively investigated. The first evidences of natural transmission of the disease has been brought out by Çınar and Çağlayan, (1988). Following studies shown that only complex of **Circulifer haematoceps** from 7 leafhopper species, from which

S. citri was cultured, has been found as a natural vector of stubborn disease (Kersting **et al**). The same working group has also found that **Echium sp., Crepis echiodes** (L) and **Sesamum indicum** L. were natural hosts for **S. citri** and they are very important plants for natural transmission. In this study the possibilities of being a vector for **E. capicola** has been investigated. The positive points as a vector for this insect are as fallows: 1. **S. citri** multiplied to the expected titer (Townsend **et al.,** 1977) when injected with SPT-WN isolate. 2. They survived very well after microinjection of spiroplasma culture. 3. They can both acquire **S. citri** (SPT-WN) from and transmit it through membranes with reasonable efficiency. The negative point is that **E. capicola** showed poor survival on host plants of **S. citri** and they were not able to infect any of the host plants, used in this study (Table 1). Although **S. citri** has been detected in **E. capicola** by different workers (Bove **et al.,** 1979; Nhami **et al.,** 1980) it seems to be not a good vector candidate for natural transmission of citrus stubborn disease in the East Mediterranean Area.

ÖZET

SPIROPLASMA CITRI'NIN BAZI TÜRKİYE İZOLATLARININ DENEYSEL TAŞINMASI ÜZERİNE BİR ARAŞTIRMA

Doğu Akdeniz Bölgesinde turunçgil stubborn hastalığına neden olan farklı **Spiroplasma citri** izolatlarının patojenitesi ve deneysel taşınma olanakları, deneysel vektörler olan **Euscelidius varieagatus** (Kirsh) ve **Circulifer tenellus** (Baker) ile Doğu Akdeniz Bölgesinde olası bir vektör adayı olan **Exitianus capicola** STAL böceğine patojenin inokulasyonu yoluyla incelenmiştir. **S. citri** vektör böceklere mikroinjeksiyon ve membran beslenmesi yoluyla inokule edilmiştir.

Bu çalışmada inokule edilmiş böceklerde **S. citri**'nin yaşamını sürdürme ve çoğalmaları ile test bitkilerine taşınan patojenin geriye izolasyon olanakları araştırılmıştır. Washington Navel ve Valencia portakalları ile **E. capicola** böceğinden izole edilmiş olan **S. citri** izolatlarının kullanıldığı deneysel taşınma çalışmalarında Madam Vinous portakalı **(Citrus sinensis),** bakla **(Vicia faba),** Cezayir menekşesi **(Vinca rosea** L.), kırmızı yonca **(Trifolium pratense)** ve baş lahana **(Brassica oleracea capitata)** test bitkilerinde **S. citri**'nin oluşturduğu simptomlara benzer görüntüler gözlenmiştir. Bununla beraber bezelye **(Pisum sativum),** soğan **(Allium cepa),** bülbül otu **(Sisymbrium officinale)** ve dik kadife çiçeği **(Tagetes erecta)** bitkilerinde hiç bir simptom oluşmamıştır. Bu bitkilerden **S. citri** geriye izole edilemezken yukarıda tanımlanan ve simptom gösteren bütün bitkilerden patojen izole edilmiştir.

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Efficacies of Low Dosages and Repeated Applications of Some Herbicides Against Russian Knapweed (**Acroptilon repens** (L.) D.C.)

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ABSTRACT

*Application of 2,4-D isooctyl ester at flowering stage at the rates of 24 and 33.6 g (a.i.)/da did not control Russian knapweed (**Acroptilon repens** (L.) D.C.). Three applications of 2,4-D Triisopropanol amine + Picloram at the rate of 18.3 + 2.55 and 12.2 + 1.7 g (a.i.)/da gave 94.8% and 88.2% control on this weed respectively. Two and three applications of glyphosate at 240 g (a.i.)/da killed 97.0% and 96.2% of the weed respectively. Two and three treatment of the some herbicide at the rate of 168 g (a.i.)/da gave 93.5% and 96.5% effectivenes respectively. Citowett at the rate of 100 ml/100 l was added to all the spray mixtures.*

INTRODUCTION

(**Acroptilon repens** (L.) D.C.), known by different local names as kekre and acımık, has got very persistent deep and horizontally creeping roots (Muminov 1967, Kurhan 1964). The roots hava also got a high regeneration ability (Fisyunov and Ostanenko 1979). All these aspects of this noxious weed creates difficulty in its control.

Russian knapweed, observed almost all the crop growing areas of Türkiye, reached the intensity of 20-25 shoot/sq.m in some wheat fields in Ankara province (Sözeri and Erdiller 1991).

For the control of Russian knapweed, various herbicides, alone or in combination were used and different results were obtained. A lot of researcher found high dosages of dicamba and picloram were very effective against this weed; but they stated that these herbicides had a resudial effect and treated fields could be sown only after a long time following the applications (Gruzdev and Popov 1971; Mordovets and Nazarenko 1971; Berezovskii and Krumzdorov 1972; Krumzdorov 1974 a, b; Kıdrıshev and Styazhkovoi 1980; Khandusenko 1984). Besides dicamba and picloram, 2, 3, 6 TBA also was found effective and having resudial effects (Lysogorov **et al.**, 1971; Mordovets and Golovin 1974, 1976, 1983; Zharokova and Kıdrıshev 1984). It was also reported that 2,4-D ester dicreased populations of **A. repens** (Kurhan 1964; Khodorovskii 1969; Raskin **et al.** 1984; Khandusenko, 1984).

In a previous study picloram alone or mixtures of picloram+dicamba, picloram+ 2,4-D amine at high dosages controlled **A. repens** very effectively; while dicamba at 200 g/da was not effective. On the other hand glyphosate at high rates first killed the foliage of the weed but after a month the plants produced new shoots (Sözeri 1991).

In this work, effects of low dosage and repeated applications of 2,4-D ester picloram + 2,4-D amine, glyphosate were investigated.

MATERIALS and METHODS

This trial was carried out in an experimental field of the Department of Field Crops, Faculty of Agriculture, where homogenous and intensive Russian knapweed were present, in 1993-1994.

The herbicides used in the experiment, their rates and numbers of application is shown in Table 1.

Table 1. The herbicides used in the experiment, their active ingredients and percentages, rates and number of applications

Herbicides	Active ingredient and percentage	rate of application (a.i./da)	Number of application
2,4-D ester (Agro-D ester)	2,4-D isooctyl ester, 48	33.6	2
2,4-D ester (Agro-D ester)	2,4-D isooctyl ester, 48	24.0	3
Picloram + 2,4-D amine (Tordon 101 mixture)	2,4-D triisopro panol amine+picloram, 36.6+10.2	2.55+18.3	3
Picloram + 2,4-D amine (Tordon 101 mixture)	2,4-D triisopro-panol amine+picloram, 36.6+10.2	1.7+12.2	3
Roundup	Glyphosate, 48	240	2
Roundup	Glyphosate, 48	240	3
Roundup	Glyphosate, 48	168	2
Roundup	Glyphosate, 48	168	3

In all the treatments, Citowett at the rate of 100 ml/100 l was added to herbicide tank mixtures.

First treatment was done on 12.7.1993 when the Russian knapweed was average 25-30 cm in height and at flowering stage. Second and third applications were done with one week intervals. The experiment was set up in randomised experimental blocks with four applications. Sizes of the plotes was 3x2 m. Untreated stripes of 0.5 m among the parcels and 1 m among the blocks were left. The herbicides were sprayed by five litter capacity Simge made plastic sprayer.

Effectiveness of the herbicides was calculated one year after the treatment based on percent cover of Russian knapweed.

RESULTS

Effects of the herbicides after one year of the treatment on Russian knapweed is summerized in Table 2.

Table 2. Effectiveness of the herbicides against **Acroptilon repens**

Herbicides	application dosage (g(a.i.)/da	number of aplication	percent effecttiveness in the replicates				average* percent effectiveness	
			I	II	III	IV		
2,4-D ester	33.6	2	10	0	0	10	5.0	b
2,4-D ester	24.0	3	0	0	0	10	2.5	bc
picloram+2,4-D amine	2.55+18.3	3	97	90	99	93	94.8	a
picloram+2,4-D amine	1.7+12.2	3	90	90	93	80	88.2	a
Glyphosate	240	2	97	97	97	97	97.0	a
Glyphosate	240	3	99	97	99	90	96.2	a
Glyphosate	168	2	95	95	99	93	95.5	a
Glyphosate	168	3	95	95	97	99	96.5	a

* The difference between the values reciving the same letter are not statistically significant (P = 0.05).

As shown at table 2, 2,4-D ester was found ineffective. On the other hand three applications of picloram+2,4-D amine at the rates of 2.55+18.3 g/da and 1.7+12.2 g/da controled Russian knapweed 94.8% and 88.2% respectively.

For the first time glyphosate was tested in lower rates in this experiment and 240 g/da glyphosate was 97.0% and 96.2% effective for 2 and 3 applications respectively. It was also 95.5% and 96.5% effective at the rate of 168 g/da for 2 and 3 applications respectively.

The differences between various dosages and application times both for picloram+2,4-D mixture and glyphosate was not statistically significant (P/0.05). 2,4-D ester was ineffective.

DISCUSSION

Chemical control of **Acroptilon repens** with repeated applications at lower rates at flowering stage was taken into consideration for the first time in this investigation.

In this study 2,4-D ester did not control Russian knapweed and this was agreable with our previous work (Sözeri 1991). A similiar conclusion was stated by Kurhan (1964). On the other hand Khodorovskii (1969), Khandusenko (1984) and Raskın **et al.** (1984) also reported that 2,4-D ester reduced the populations of Russian knapweed but did not completely prevent its growth.

Along with the high rates of one applications of picloram (Mordovets and Nazarenko 1971, Krumzdurov 1974 a, b) we also found out that repeated applications of picloram at low rates could control **Acroptilon repens**. There might not be a resudial effect with this type of applications but it should be found out.

Glyphosate was first tested against **A. repens** by Sözeri (1991). In her study high rates of glyphosate was found to kill foliage of **A. repens** but not prevent regrowth of the roots. This effect might be atributed rapid kill of the foliage and prevention translocation of glyphosate to the roots. However in our work, repeated application of glyphosate at lower rates helped the herbicides to translocate to the roots of the weed and this gave a higher rate of control even after one year.

Glyphosate could be used both at non-crop lands and cereals at their ripening stage with this way of treatment since it might not cause a resudial effect.

ÖZET

BAZI HERBİSİTLERİN DÜŞÜK DOZLU VE TEKRARLI UYGULAMALARININ KEKRE (Acroptilon repens (L.) D.C.)'YE ETKİLERİ

Kekre (**Acroptilon repens** (L.) D.C)'ye çiçekli döneminde uygulanan 2,4-D izooctyl ester'in 24 g (a.m.)/da ve 33.6 g (a.m.)/da dozları bu yabancıotu etkilememiştir. 2,4-D Triisopropanolamin+picloram'ın 18.3+2.55 g (a.m.)/da ve 12.2+1.7 g (a.m.)/da dozlarının 3 kez uygulanması kekreyi sırasıyla; %94.8 ve %88.2 oranında kontrol etmiştir. Glyphosate'ın 240 g (a.m.)/da dozunun 2 kez tekrarlanması %97.0, 3 kez tekrarlanması ise %96.2 oranında etkili olmuştur. Aynı ilacın 168 g (a.m.)/da dozunun 2 ve 3 kez uygulamaları sırasıyla; %93.5 ve %96.5 oranında kekreyi etkilemiştir. Tüm uygulamalarda ilaç-su karışımlarına 100 ml/100 l oranında citowett katılmıştır.

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