UDK 632.9

YU ISSN 0372–7866

INSTITUT ZA ZAŠTITU BILJA I ŽIVOTNU SREDINU - BEOGRAD
INSTITUTE FOR PLANT PROTECTION AND ENVIRONMENT - BELGRADE

# ZAŠTITA BILJA
# PLANT PROTECTION

VOL. 50(1), N°227, 1999.

Zaštita bilja Vol. 50 (1) No 227 (str.1–90 ) Beograd, 1999.

Zaštita bilja, Vol. 50(1), N°227: 1–90, 1999, Beograd

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*(Primljeno: 24.03.1999.)*

*(Prihvaćeno:20.06.2000.)*

## IDENTIFICATION OF PLANT PATHOGENIC BACTERIA BELONGING TO THE GENERA *PSEUDOMONAS AND ERWINIA*, THE PATHOGENS OF LETTUCE AND CAULIFLOWER

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Summary

*Bacterial rot* of lettuce leaves and cauliflower heads was frequently observed in Yugoslavia during the last several years.

Symptoms of dark brown and black rot of leaves were noticed on lettuce heads in the greenhouse as well as in the open-field production. One or more, mostly outer leaves of lettuce were affected. Later on, under favourable temperature and humidity condition sspreading of the infection in the inner parts of lettuce head resulted in softening of leaves and complete degradation of the tissue, causing a great yield losses (Fig. 1).

*Brown and black spots*, irregular in shape, were observed on the cauliflower heads during harvest time and on the market. The spots spread over the head surface as well as in the inner parts, covering large area and resulting in rotting of the cauliflower head tissue under favoruable conditions. (Fig, 2a, b, c). Apart from this type of symptoms "V" shaped necrosis of cauliflower leaf tissue and black rot of vascular tissue were noticed along the leaf edge (Fig. 2 d, e).

Since the affected plants of lettuce and cauliflower were not marketable, this disease has become a serious problem in the production of these vegetable species in this country.

Recently regarding severity and economical importance of the disease, we decided to investigate its ethiology and characteristics of the pathogen isolated.

*Numerous samples* of the diseased lettuce and cauliflower plants were collected from different growing areas in Yugoslavia during the last several years. In order to isolate the pathogen, samples were processed in the laboratory by applying the standard phytobacteriological procedures (Arsenijević 1988, 1997).

*Pathogenicity* of the isolated bacterial strains was checked by prick-inoculation of detached lettuce leaves and cauliflower head fragments, using bacteriological needle dipped into the bacterial suspension of $10^8$ cfu/ml. Inoculated plant material was placed on wet filter paper in Petri dishes (20 cm) and kept in humid chamber at the temperature of 20°C. Results were observed after five days.

*In order to differentiate genera* of the phytopathogenic bacteria some preliminary tests were carried out. Such as: Gram reaction, flagellation, growth on nutrient agar, production of fluorescent pigment on King's medium B, pectolytic activity, oxidase production, glucose metabolism, starch hydrolysis and nitrate reduction. Computerized BIOLOG test was also used as a fast way for preliminary characterization of unknown bacterial phytopathogens. Species identification was done by studying physiological characteristics of the isolated bacteria according to the methods given by Fahy and Persley (1983), Lelliott and Stead (1987), Schaad (1988), Klement et al., (1990) and Arsenijević (1997) besides, the isolated strains were serologically tested by applying ELISA test.

*Host range* of lettuce and cauliflower pathogenic bacteria was investigated by inoculating the pepper and tomato fruits, carrot and parsley slices, cabbage leaf fragments, onion bulbs and *Chrysanthemum* sp. *plants*. The bacterial suspension of $10^8$ cfu/ml was used. The suspension of $10^7$ cfu/ml was used for tobacco hypersensitivity test (HR).

From the samples of lettuce and cauliflower diseased tissue showing the symptoms of dark brown and black rot, numerous bacterial strains were isolated. The obtained results indicated that several pathogenic bacteria, belonging to different genera, could be the causal agents of the disease. Therefore, 25 strains belonging to the genus *Pseudomonas*, 11 to *Erwinia* and 4 to *Xanthomonas*, were studied in more detail (Table 1).

*Results of pathogenicity* testing showed that all 25 *Pseudomonas* spp. strains possessed wide host range (Table 3). They were Gram-negative, rod-shaped, motile with one or more polar flagella, and produced fluorescent pigment of King's medium B.

This group of fluorescent *Pseudomonas* showed heterogeneity regarding the characteristics manifested in the LOPAT tests (Table 4).

According to the obtained results strains Sa-01, Sa-02, Sa-1, Sa-4, Sa-16, Sa-38, Sa-40, Sa-62, Kf-09, Kf-55, Kf-104 and Kf-105 belonged to the II LOPAT group, including the bacterium *Pseudomonas viridiflava*.

*Oxidase and HR positive strains* (Sa-06, Sa-07, Sa-35, Kf-01, Kf-03, Kf-08, Kf-12, Kf-13, Kf-22) were determined as members of the III LOPAT group (*P. cichorii*).

*The remaining four fluorescent Pseudomonas* spp. strains produced levan, oxidase, protopectinase and arginindihidrolase. Therefore, they belong to the IVa LOPAT group, i.e. they posses characteristics similar to the bacterium *P. marginalis* (Table 4). Such differentiation of our *Pseudomonas* spp. strains, into three groups, was also confirmed by

the results of other biochemical tests as well as by BIOLOG and ELISA tests (Table 5).

*The non-fluorescent strains* (Sa-29, Sa-30, Sa-31, Sa-32, Kf-010, Kf-017, Kf-021, Kf-029, Kf–110, Kf-111, Kf-112), possessing pectolitic ferments and oxidative and fermentative metabolism of glucose, were identified as members of *Erwinia carotovora* group. In pathogenicity tests, these strains caused soft rot of inoculated plant material, typical for pectolytic bacterium of the genus *Erwinia* (Table 6). These rod-shaped bacteria, with peritrichous flagellation, formed light brown shiny colonies on NA. They were oxidase negative but catalase positive; reduced nitrates, hydrolyzed gelatin and grew at 37°C and in the presence of 5% NaCl; phosphatase and lecithinase activity were negative as well as acid production from α-methyl glucoside and dulcitol, but positive from lactose and trehalose. These results, as well as the results of BIOLOG and ELISA tests, indicated that those non-fluorescent strains, isolated from lettuce and cauliflower, belonged to the bacterium *Erwinia carotovora* subsp. *carotovora* (Table 7, 8).

Apart from the bacteria, belonging to the genus *Pseudomonas* and *Erwinia*, causing dark brown and black rot of lettuce leaves and cauliflower heads, *Xanthomonas campestris* pv. *campestris* was isolated and identified as a causal agent of cauliflower leaf yellowing and black rot of vascular system (Table 9).

*Key words*: bacterial rot; lettuce; cauliflower; *Pseudomonas cichorii*; *P. marginalis* pv. *marginalis*; *P. viridiflava*; *Erwinia carotovora* subsp. *carotovora*; *Xanthomonas campestris* pv. *campestris*.

*(Received: March 24, 1999.)*
*(Accepted:, June 20, 2000.)*

Plant Protection, Vol. 50 (1), No, 227; 1–60 1999, Belgrade

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*(Primljeno: 13.08.1999.)*
*(Prihvaćeno: 20.06.2000.)*

## INCIDENCE OF SENN PESTS (*Eurygaster spp.*) DURING 1994-1997

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S u m m a r y

In our country, senn pests (several species of the genera *Eurygaster* and *Aelia*) have been continuously monitored for the past 34 years. The data for the first 30 years (1964-1993) have been published in an earlier paper (Stamenković, 1994), while the present study reports the changes that occurred in the populations of these pests between 1994 and 1997. The incidence was monitored at the overwintering sites in the forests of the Fruška Gora and Deliblato Sand. During the four years of study, 800 samples were collected and 4,371 imagoes analyzed.

The average incidence of imagoes was 21.9, ranging from 16.8 to 32.6 adults per meter square (Tab. 1). The most common species were *Eurygaster austriaca* (68.9%) and *E. maura* (27.9%) - (Graf. 1). The female to male ratio was 1 : 0.9 in the former and 1 : 0.8 in the latter species (Tab. 2). The average mortality of *E. austriaca* was 1.6% on the Fruška Gora and 1.8% in Deliblato Sand. In *E. maura* the average mortality was 1.1% on the Fruška Gora and 0.9% in Deliblato Sand (Tab. 3).

*Key words:* Senn pests, *Eurygaster austriaca*, *E. maura*, overwintering sites, incidence, abundance, female to male ratio, mortality.

*(Received: August, 13, 1999.)*
*(Accepted: June 20. 2000.)*

Plant Protection, Vol. 50 (1), No 227: 61–67 1999, Belgrade

# REDOX REACTIONS OF FUNGI IN THE PRESENCE OF ANTHRAQUINONES

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Summary

The influence of chemical structure of anthraquinone added to standard nutrient agar in the amount of 0,1 mg/ml on redox reactions of the fungi A. *niger, T. viride* and *D. stemonitis* was investigated.

It was found out that the range of oxido-reducing reactions during vegetative period was reduced in the first half of the period and intensified in the second half, whereby the level of activity was completely determined by anthraquinone chemical structure, duration of vegetative period and the type of culture.

*Key words:* anthraquinone, redox potential, *Xanthoria parietina, Aspergillus niger, Trichoderma viride, Doratomyces stemonitis.*

*(Received: September 29, 1998)*
*(Accepted:,June 20, 2000)*

Plant Protection, Vol. 50 (1), No 227: 69–78 1999, Belgrade