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SPONTANEOUS OCCURRENCE OF CUCUMBER MOSAIC VIRUS ON *ECHINOCYSTIS LOBATA*, *LEYCESTERIA FORMOSA* AND *SOLANUM MELONGENA* IN YUGOSLAVIA

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Introduction

Virus isolates were recovered from a cucurbitaceous plant (*Echinocystis lobata* /Michx./ T. et G.), a caprifoliaceous (*Leycesteria formosa* Wall.) and a solanaceous one (*Solanum melongena* L.) growing spontaneously or cultivated in the area of Zagreb. The isolates were studied primarily for purposes of identification. Experiments presented here have shown that the isolates belong to cucumber mosaic virus (CMV).

CMV ($R/1:\frac{1.1}{18}+\frac{1.0}{18}+\frac{0.7+0.3}{18}$:S/S:S/C, Ve/Ap) falls into cucumovirus group (Fenner 1976; cf. Harrison et al. 1971) together with tomato aspermy virus (Hollings and Stone 1971) and peanut stunt virus (Mink 1972). A possible member of the group is chrysanthemum mild mottle virus (Fenner ib.).

Plants from more than 40 families are susceptible to infection with CMV, including monocotyledonous and dicotyledonous species (Gibbs and Harrison 1970; cf. Thornberry 1966, Klinkowski 1968, Schmelzer and Wolf 1971, Smith 1972). Besides wild plants host range of the virus involves a number of cultivated herbaceous or woody species (cf. Smith ib.).

Spontaneous (natural) infections with CMV have been recorded on numerous plants including species from the three above-mentioned families. Out of those several cultivated species are mentioned here from the family *Cucurbitaceae* (*Citrullus vulgaris* Schrad., *Cucumis melo* L., *C. sativus* L., *Cucurbita* spp.) and *Solanaceae* (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Solanum tuberosum* L., etc.) (cf. Smith ib.).

Spontaneous infections of *E. lobata, L. formosa* and *S. melongena* have already been reported. CMV-infected *E. lobata* plants have been found in the USA, Czechoslovakia and Hungary (cf. Horváth and Szirmai 1973). Hollings (1961) and Schmelzer (1970) have found CMV in *L. formosa* in their respective home countries. Virus isolate recorded in 1961 by Savulescu and Ploaie in Rumania was considered by Schmelzer (ib.) also to be CMV. Infections with CMV in *S. melongena* have been reported in distant regions, e. g. in Trinidad (cf. Dale 1954), India (Seth et al. 1967), USSR (Vlasov 1970), Turkey (Tekinel et al. 1969), Italy (Rana and Vovlas 1972), etc.

In Yugoslavia *E. lobata* is a widespread plant growing frequently in the vicinity of water reservoirs (rivers, etc.). *S. melongena* is cultivated in many places, especially in eastern and south-eastern parts of the country. *L. formosa* is a moderately frequent ornamental bush in gardens and parks.

Material and Methods

Material

Three virus isolates marked E1, E2 and E3 were taken from three *E. lobata* plants growing several hundred meters apart from each other on the banks of the Sava river in the area of Zagreb. Somewhat stunted plants showed distinct symptoms on the leaves which included mosaic, variegation, vein banding (Fig. 1A), some necrosis and pointing of lamina parts (lobi).

In the Botanical Garden of Zagreb leaf symptoms in the form of chlorotic rings (simple or concentric), lines and sometimes oak-leaf pattern were detected on a *L. formosa* plant (Fig. 1C, D). To mark the isolate from that plant sign LF was used.

Many *S. melongena* plants cultivated in several plantations at the periphery of Zagreb displayed on their leaves vein banding, mottling and blistering symptoms (Fig. 1B). Virus isolates S1 and S2 were recovered from two of such plant specimens.

Fig. 1. Symptoms of isolates E1, S1 and LF in original host plants (A—D). A: Vein banding and variegation on *Echinocystis lobata* leaf (isolate E1); B: Mottling and blistering on leaf of *Solanum melongena* (isolate S1); C, D: Simple and ring-shaped chlorotic spots on *Leycesteria formosa* leaves (isolate LF). E: Serological reaction of isolates E1, E2 and S1 (S) with antiserum (A) to cucumber mosaic virus; three wells to the left contained leaf juice of healthy *Nicotiana megalosiphon* (N) or *Cucumis sativus* (c) plants. F: Pin point necrotic lesions on inoculated leaf of *Chenopodium amaranticolor* (isolate LF).

Sl. 1. Simptomi izolata E1, S1 i LF na izvornim biljkama-domaćinima (A—D). A: Vrpčasto obojenje uz žile i šarenilo na listu vrste *E. lobata* (izolat E1); B: pjegavi mozaik i mjehurasta ispupčenost na listu vrste *S. melongena* (izolat S1); C, D: jednostavne i prstenaste klorotične pjege na listovima biljke *L. formosa* (izolat LF); E: Serološka reakcija izolata E1, E2 i S1 (S) s antiserumom (A) od virusa mozaika krastavca; u tri bazena na lijevoj strani nalazio se sok zdravih biljaka *N. megalosiphon* (N) i *C. sativus* (c). F: *Ch. amaranticolor,* točkaste nekrotične lezije na inokuliranom listu (izolat LF).

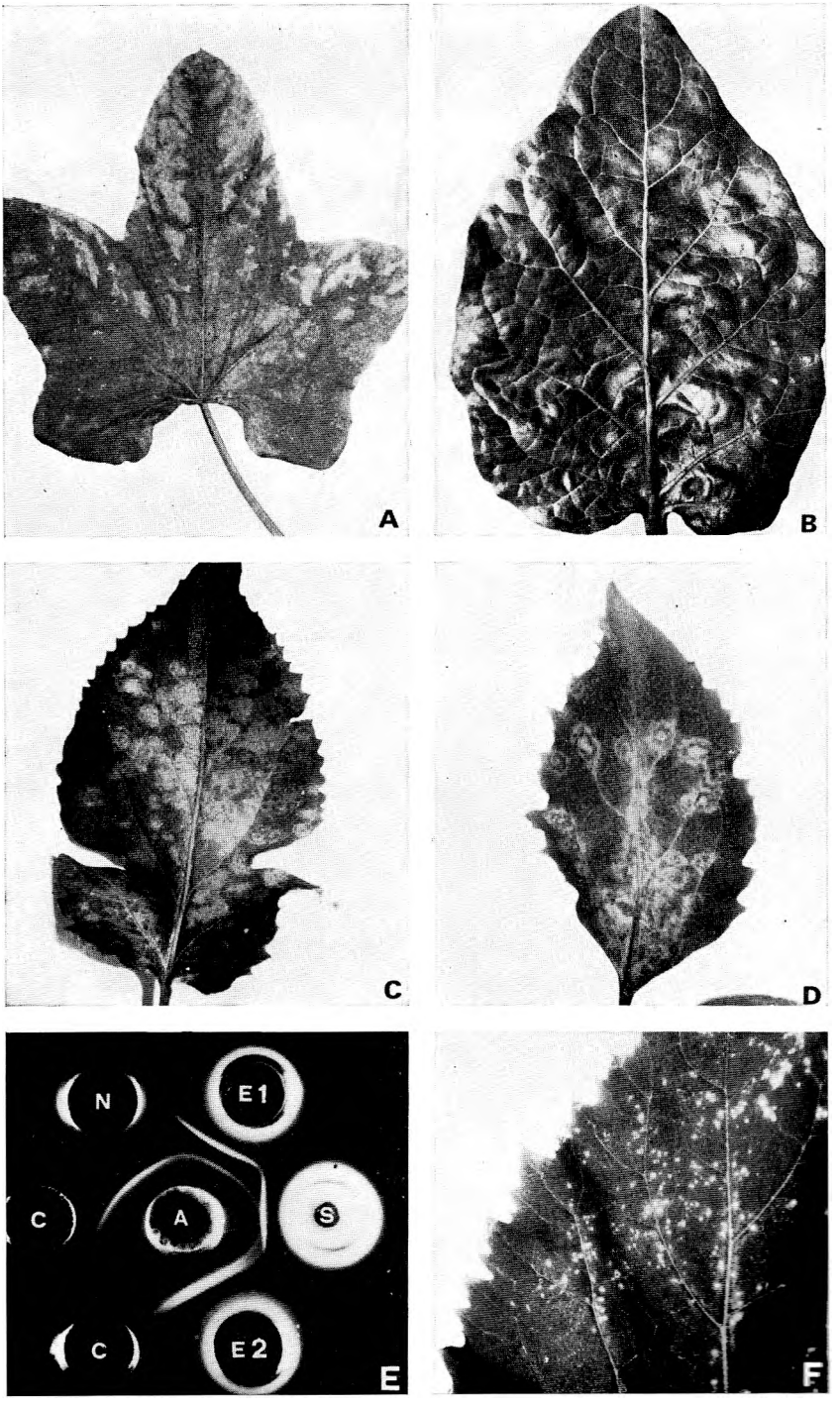

Fig. 1. — Sl. 1.

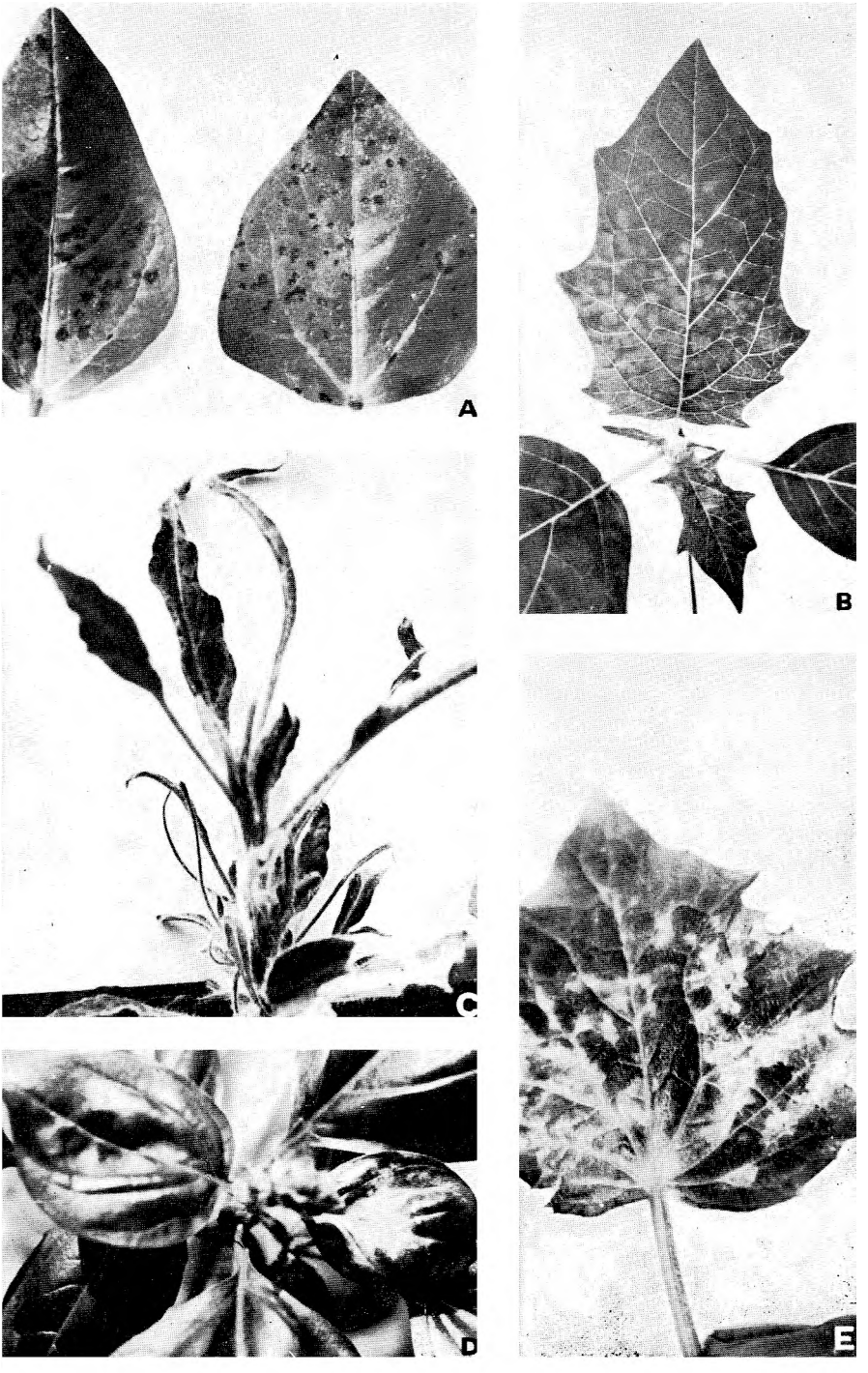

Fig. 2. — Sl. 2.

Methods

Isolates E1, E2, E3, LF, S1 and S2 were transmitted by mechanical inoculation of leaf juice of the original host plants (in isolate LF with addition of 0.06 M phosphate buffer containing 0.015 M Na-DIECA) to several common test plants including *Chenopodium* spp., *Nicotiana* spp., *Cucumis sativus,* etc.

In investigating the physical properties of the isolates juices of systemically infected leaves (*Nicotiana megalosiphon* Heurck. et Muell.), centrifuged at a low speed were used. *Ch. amaranticolor* and *Ch. quinoa* served as test plants.

In plant-protection experiments *Datura stramonium* was used as test plant. In those experiments W strain of CMV (cf. Horváth and Szirmai ib.) was administered for superinoculation.

Transmission experiments with *Myzus persicae* Sulz. were performed using *C. sativus* as source and test plant.

Serological experiments were carried out in 0.9% agarose gel which was prepared in distilled water. An antiserum against CMV (isolate from carnation) with a titre of cca 1/32 was at our disposal for that purpose. Basins for the reactants measured 3 mm in diameter. Leaf juices were centrifuged at low speed.

Results

Test plant reaction

Each of the isolates E1, E2, E3, LF, S1 and S2 was inoculated onto more than ten plant species. Symptoms which arose on several characteristic species are given in Table 1.

As visible in the Table, all the isolates were similar in general to each other according to symptoms they caused in test plants. However, no symptoms could be noticed in *V. sinensis* under the influence of isolates S1 and S2. In addition, the six isolates induced a severe systemic infection on *Nicotiana megalosiphon* Heurck. et Muell. plants (Fig. 2C). Severe signs of necrosis were often observed on leaves of that species.

Fig. 2. Symptoms of isolates E1, E2, and LF on test plants (A—E). A: Necrotic violet-brown lesions on inoculated leaves of *Vigna sinensis* (isolate E2); B—E: Symptoms of systemic infections; B: Chlorotic spotting in *Datura stramonium (isolate* LF); C: *N. megalosiphon,* narrowing of leaf laminae (isolate E1); D: Variegation in *Ocimum basilicum* (isolate LF); E: Variegation and malformation symptoms on a *C. sativus leaf* (isolate LF).

Sl. 2. Simptomi izolata E1, E2 i LF na pokusnim biljkama (A—E). A: Nekrotične ljubičasto-smeđe lezije na inokuliranim listovima vrste *V. sinensis* (izolat E2); B—E: Simptomi sistemičnih infekcija; B: *D. stramonium,* klorotična pjegavost (izolat LF); C: *N. megalosiphon,* sužavanje plojki (izolat E1); D: Šarenilo na vrsti *O. basilicum* uzrokovano infekcijom izolata LF; E: Šarenilo i deformacije na listu *C. sativus* (izolat LF).

Table 1. Symptoms provoked by isolates E1, E2, E3, LF, S1 and S2 on leaves of characteristic test plants

Tablica 1. Simptomi izolata E1, E2, E3, LF, S1 i S2 na listovima karakterističnih pokusnih biljaka

Test plant	Virus isolate		
	E1, E2, E3	LF	S1, S2
Chenopodium amaranticolor Coste et Reyn.	°L: pin point necrotic lesions	like E1 (Fig. 1F)	like E1
Ch. quinoa Willd.	L: yellowish lesions	like E1	like E1
Cucumis sativus L.	L: chlorotic spots; S: chlorotic spots, mosaic	like E1 (Fig. 2E)	like E1
Datura stramonium L.	S: chlorotic spots, pointing of lamina teeth	like E1 (Fig. 2B)	like E1
Nicotiana glutinosa L.	S: chlorotic spots, mosaic	like E1	like E1
Ocimum basilicum L.	—	S: variegation (Fig. 2D)	—
Vigna sinensis (Torner) Savi.	L: necrotic lesions (Fig. 2A)	like E1	no symptoms

°L: symptoms in inoculated leaves; S: symptoms of systemic infection; like E1: symptoms similar as with isolates E1, E2 and E3; —: not tested.

Physical properties

Isolates E1, S1 and LF were investigated with respet to thermal inactivation point (TIP) and dilution end point (DEP). Besides, isolate LF was tested for longevity in vitro (LIV). It was established that TIP of isolates E1 and S1 was in the range between 60° and 65° C. Isolate LF was inactivated between 55° and 60° C. DEP of isolates E1 and S1 was higher than 10^{-3} but below $2 \cdot 10^{-4}$. DEP of isolate LF was between 10^{-2} and 10^{-3}. Isolate LF remained infective after storage at 20°—25° C for a period of 24 hours but not after 48 hours.

Aphid transmission

Following a starvation period of 4 hours adult wingless aphids *M. persicae* were kept for 30 min. on systemically infected leaves of source plants which had been infected separately with isolate E1, LF and S1. Insects were replaced then immediately onto young healthy *C. sativus* plants which had no other leaves except cotyledons. Three to four insects were applied per a plant. Test feeding lasted 30 min. Finally the insects were removed from the plants, and the plants were sprayed with an aphicide.

As a result of the experiments 3 out of 10 test plants showed symptoms due to infection with isolate E1 or S1. With isolate LF aphids transmitted the infection to 2 out of 10 plants.

Plant-protection experiment

Three groups of plants each consisting of 10 *D. stramonium* specimens were used in the plant-protection experiment. The first and the second group were inoculated with the isolate LF. The third group was left uninoculated at that time, so that this group and the first one were controls. About fifteen days later plants from the second group displaying symptoms of systemic infection and the third group of plants were inoculated with strain W of CMV (cf. Material and Methods). After a while full development of symptoms caused by strain W was observed on the third group of plants. Those symptoms included a bright (yellowish) mosaic and variegation, which were clearly distinguishable from the symptoms on the two other groups of plants. It was nearly impossible to distinguish between the symptoms on plants from the two latter groups, i. e. from the first and the second group. Symptoms on both groups of plants were less bright than on the third group of plants. This indicated that infection by isolate LF protected the plants against infection with strain W.

Serological experiments

Undiluted or 1/2 diluted antiserum to CMV was first partially absorbed with the leaf juice of healthy *N. megalosiphon* plants, which was done directly in the central well (Fig. 1E). The virus containing juices and healthy juices were put into peripheral wells 1—2 hours after the respective well was charged with the antiserum. Juices containing separately isolates E1, E2, E3, LF, S1 and S2 gave fairly strong precipitation lines not found with healthy specimes. The position and curvature of the lines indicated specific virus-antibody precipitation (Fig. 1E). Some other comparatively strong lines appeared in experiments with virus containing sap and with the sap of healthy plants. Those lines were certainly due to the precipitation of normal plant constituents (proteins).

Discussion

CMV is a frequent pathogen in cultivated and wild plants in Yugoslavia where it has been found in about 20 plant species (cf. Juretić 1968, 1974, Plešе and Miličić 1974, Horváth et al. 1975, Taraku 1976). The present paper brings evidence on spontaneous spread of CMV in three additional plant species previously not recorded for that region.

CMV can sometimes be confused with other viruses, especially with tomato aspermy virus which has some properties in common with it (cf. Hollings and Stone ib.). With respect to this, distinct and strong serological reactions of isolates E1, E2, E3, LF, S1 and S2 with antiserum to CMV are worth mentioning, as well as the reaction of test plants, notably that of *C. sativus*. These results were of special value in identifying the six isolates.

Some difference could be found between our isolates. Isolates E1, E2, E3 and LF provoked conspicuous necrotic lesions on inoculated leaves of *Vigna sinensis* which could not be recorded with isolates S1 and S2

(cf. Klinkowski ib.). On the other hand, isolates E1 and S1 had a higher thermal inactivation point and were infective in a higher dilution than isolate LF. However, isolate E1 displayed a lower thermal inactivation point when compared with Hungarian CMV isolates from *E. lobata* (cf. Horváth and Szimai ib.).

According to the symptoms in test plants the six investigated isolates could perhaps be allied to Bhargava's spinach strain of CMV (cf. Gibbs and Harrison ib., Smith ib.).

CMV is well spread in the area of Zagreb in *E. lobata* and *S. melongena* plants. It is estimated that about 20% of *E. lobata* plants (occasionally more), and about 10—20% of *S. melongena* plants were affected by CMV. The estimate was made on the basis of symptoms in many field plants of both species which were similar to the symptoms on plants from which isolates E1, E2, E3 or S1 and S2, respectively were recovered. *E. lobata* is of special importance as a source for spread of CMV. There is evidence that CMV can be transmitted through seed of that species (cf. Horváth and Szirmai ib.). Besides, it is a plant with fairly large leaves which is often visited by aphids.

Summary

Symptoms suggestive of virus infection have been observed on numerous *Echinocystis lobata* and *Solanum melongena* plants in the area of Zagreb. From those plants several isolates were recovered, i. e. E1, E2, E3 *(E. lobata)*, S1 and S2 *(S. melongena)*. Besides, a further isolate (LF) was obtained from a *Leycesteria formosa* plant, which was cultivated in Botanical Garden of Zagreb.

All the six isolates gave in 0.9% agarose gel a distinct positive serological reaction with antiserum to cucumber mosaic virus (CMV). This and some other properties of the isolates (test plant reaction, physical properties, etc.) led to the conclusion that the six isolates belonged to CMV. Isolates E1, E2, E3 and LF caused necrotic lesions in inoculated leaves of *Vigna sinensis*. This was not found with isolates S1 and S2. Thermal inactivation point of isolates E1 and S1 was somewhat higher (60°—65° C) than in isolate LF (55°—60° C). According to symptoms in test plants the investigated isolates could be considered as similar to Bhargava's spinach strain of CMV.

E. lobata, *L. formosa* and *S. melongena* are reported herewith as spontaneous hosts of CMV unrecorded so far in the region of Yugoslavia.

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SADRŽAJ

SPONTANA INFEKCIJA VRSTA *ECHINOCYSTIS LOBATA, LEYCESTERIA FORMOSA* I *SOLANUM MELONGENA* VIRUSOM MOZAIKA KRASTAVCA U JUGOSLAVIJI

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Na velikom broju biljaka (20% i više) *Echinocystis lobata* koje su rasle uz rijeku Savu na području Podsuseda (Zagreb) pojavljivali su se u toku nekoliko godina simptomi virusne infekcije. Na listovima tih biljaka uočavali su se mozaik, šarenilo, vrpčasto obojenje uz žile (sl. 1A), te mjestimična pojava nekroze i ušiljenost režnjeva (lobi) plojke. Iz triju takvih biljaka izdvojeni su mehaničkom inokulacijom na pokusne biljke izolati E1, E2 i E3.

U manjim nasadima vrste *Solanum melongena* L. na području Zagreba opazili smo na oko 10—20% biljaka promjene u obliku pjegavog mozaika, vrpčastog obojenja uz žile i mjehurastih deformacija na listovima (sl. 1B). Iz dva primjerka te vrste izdvojili smo izolate S1 i S2.

Na ukrasnom grmu *Leycesteria formosa* koji se uzgajao u Botaničkom vrtu Prirodoslovno-matematičkog fakulteta u Zagrebu ponavljali su se u toku nekoliko godina simptomi na listovima u obliku jednostavnih i koncentričnih klorotičnih prstenova i pjega (sl. 1 C, D), te klorotične linije koje su ponekad pratile veće žile, pa je nastajao tzv. simptom »hrastova lista«. Iz te biljke izolirali smo virus koji smo označili kraticom LF.

Izolate E1, E2, E3, LF, S1 i S2 istraživali smo u prvom redu radi identifikacije. Pri tom smo proučavali promjene (simptome) koje su izolati uzrokovali na pokusnim biljkama, njihova fizička svojstva, tzv. reakciju unakrsnog zaštićivanja od infekcije, prijenos s pomoću lisnih uši i serološku pripadnost. Rezultati tih istraživanja, a prije svega rezultati seroloških pokusa i reakcija pokusnih biljaka, pokazali su da su istraživani virusi predstavljali izolate virusa mozaika krastavca (VMK). Svih šest izolata reagiralo je s antiserumom od VMK (priređen protiv izolata iz karanfila i dobiven ljubaznošću dra E. Luisonija, Torino) dajući specifične i jasne linije precipitacije u gelu. Precipitaciji virusa odgovaraju linije koje su u sl. 1E smještene najbliže bazenima s virusima. Ostale precipitacijske linije u slici nastale su kao posljedica serološke reakcije normalnih konstituenata biljke *(N. megalosiphon)* s odgovarajućim antitijelima. Gel je bio pripremljen od agaroze (0,9%) u destiliranoj vodi, jer standardni agarski gelovi nisu podesni za serološke pokuse s VMK (usp. Gibbs i Harrison 1970).

Reakcije pokusnih biljaka također su pokazale da izolati E1, E2, E3, LF, S1 i S2 pripadaju VMK. Posebno karakteristična bila je reakcija vrste *Cucumis sativus.* Sistemična infekcija na toj vrsti pokazala je da nijedan od naših izolata nije pripadao virusu besjemenosti rajčice (tomato aspermy virus), koji na toj vrsti uzrokuje promjene samo na inokuliranim listovima. Taj virus član je skupine kukumovirusi kao i VMK, pa neki njegovi sojevi mogu reagirati s antiserumima protiv određenih sojeva VMK (usp. Gibbs i Harrison ib.).

Fizička svojstva istraživanih izolata bila su u granicama vrijednosti za VMK (usp. Gibbs i Harrison ib.). Tako je točka termalne inaktivacije (TTI) izolata E1 i S1 bila između 60° i 65° C, a krajnja točka razređenja (KTR) između 10^{-3} i $2 \cdot 10^{-4}$. Horváth i Szirmai (1973)

utvrdili su nešto višu TTI za izolate VMK koji su nađeni u Mađarskoj na vrsti *E. lobata.* Izolat LF imao je TTI između 55° i 60° C, a KTR između 10^{-2} i 10^{-3}.

Izolatima E1, S1 i LF inficirali smo zdrave biljke *C. sativus* s pomoću lisnih uši *M. persicae* na neperzistentan način. Pri tom su ti insekti bili držani 30 min. na inficiranim biljkama i odmah zatim 30 min. na zdravim pokusnim biljkama.

Infekcija izolatom LF zaštićivala je biljke *(D. stramonium)* od infekcije sojem W VMK, što je u skladu s drugim navedenim rezultatima.

Prema tipu simptoma koje su uzrokovali na pokusnim biljkama istraživani izolati mogli bi pripadati Bhargavinom soju VMK iz vrste *Spinacia oleracea* L. (usp. G i b b s i H a r r i s o n ib.).

U radu su izneseni prvi podaci o spontanim infekcijama VMK na vrstama *E. lobata (Cucurbitaceae), L. formosa (Caprifoliaceae) i S. melongena (Solanaceae)* na području Jugoslavije.

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