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EFFECTS OF ABAMECTIN ON PROTEIN AND RNA SYNTHESIS IN PRIMARY LEAVES OF *CUCURBITA PEPO* L. (ZUCCHINI)

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Summary. Abamectin is the active compound of the broad spectrum insecticidal and acaricidal preparation Lirosect which is produced in Biovet JSC – Peshtera. Spraying of intact 14-day-old seedlings of *C. pepo* with solutions of Lirosect 1.5 EK, applied at different concentrations, resulted in a marked stimulation of protein and RNA syntheses measured by the incorporation of [^{3}H]-uridine and [^{14}C]-lysine in total RNA and soluble protein, respectively. The most significant increase of protein and RNA syntheses was observed at 0.1% Lirosect. This stimulation was accompanied by an increase in leaf dry weight. These results could be interpreted in view of the ability of abamectin to act as a membrane-active complexone.

Key words: avermectins, abamectin, *Cucurbita pepo* L. (zucchini), RNA and protein synthesis.

Abbreviations: EDTA – ethylene diamine tetraacetic acid; RDW – ralative dry weight; TCA – trichloroacetic acid.

Introduction

Abamectin is a broad spectrum insecticide and acaricide with high pesticidal activity. Abamectin is a naturel product of the soil microorganism *Streptomyces avermitilis* and it is obtained through microbiological synthesis in biotechnological industry. It belongs to the family of macrocyclic lactones called avermectins, originally isolated as antipara-

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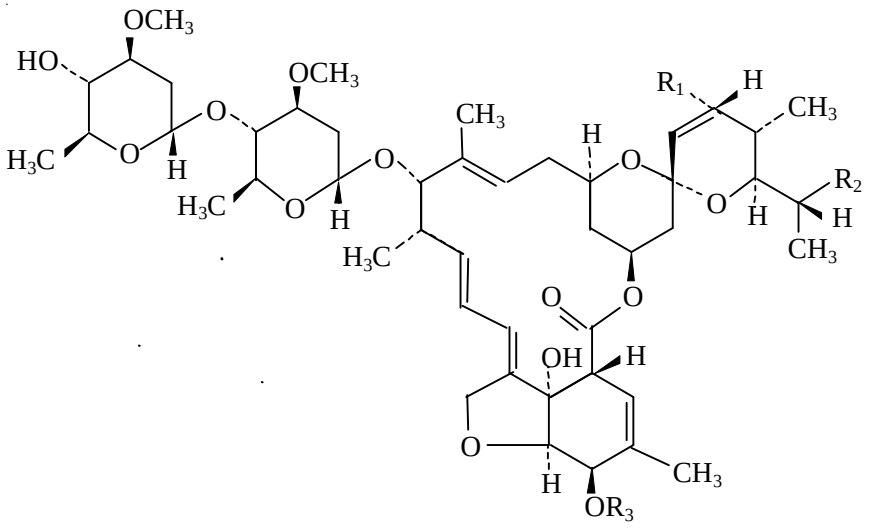
sitic agents whose chemical structure, mode of toxic action and high potencies for a broad spectrum of invertebrate pests have been thoroughly studied (Putter et al., 1981; Roslavtzeva, 1987, Bloomquist, 1993). Abamectin used in this work is produced in Biovet JSC-Peshtera as the active compound of the preparation Lirosect 1.5 EK which is a recent modification of the widely used for plant protection in Bulgaria Lirosect 2 EK. In experiments with glass-house grown tomatoes, was shown that abamectin applied as 2% aqueous emulsion at a concentration of 0.12%, displayed high insecticidal activity against the leaf-miner fly *Liriomyza huidobrensis* (Videnova et al., 1999). Furthermore, the effectiveness of abamectin against the leaf-miner fly was confirmed in assays with variety of glass-grown flowers (gerber, daisy, chrysanthemum (Markovski et al., 1999). It is well known that the mechanism of toxicity of abamectin is based on its specific action on γ-aminobutyric acid (GABA) thus blocking the nervous signal transmission at the neuromuscular junctions, leading to paralysis and death of insects. In general, avermectins block electrical activity in vertebrate and invertebrate nerve and muscle by increasing the membrane conductance to chloride ions (Bloomquist, 1993).

Avermectins have the following structural formula:



Avermectin	R_1	R_2	R_3
B_{1a}	–	C_2H_5	H
B_{1b}	–	CH_3	H

Where R_1 is absent, the double bond (=) is present.

Abamectin represents a mixture of avermectins B_{1a} and B_{1b} in a ratio of 80:20. Abamectin is characterized by a translaminar movement into treated leaves, oral activity against insect pests, and rapid breakdown in sunlight.

In comparison with the lot of data accumulated on the abamectin effect in animal cell, to our knowledge, there is no available data concerning the effect of abamectin preparations on the physiology of the plant cell. As judged by their outer look there are some observations that plants seem to benefit by spraying with abamectin solutions (leaves are deeply green and growth is stimulated) (Videnova et al., 1999).

The aim of the present work was to study the effects of the insecticide and acaricide preparation Lirosect produced in Biovet JSC – Peshtera, including abamectine as the active compound. The Lirosect preparation is applied at different concentrations and its effect is studied on two major biochemical processes – biosynthesis of proteins and RNA in primary leaves of zucchini seedlings. To our knowledge, the effects of abamectin on biosynthesis of proteins and RNA are reported for the first time in this work.

Material and Methods

Seeds of *Cucurbita pepo* L. (zucchini) cv. Cocozele, v. Tripolis were soaked for 4 h in tap water and germinated on a moistened filter paper for 4 days in darkness at 28 ºC. After that the etiolated seedlings were transferred to plastic pots and cultivated in a ½ Knop's solution under controlled light conditions: photon flux density 120 µmol.m^{-2}.s^{-1}, humidity 70 %, temperature 25 ºC and photoperiod 12/12 h for further 10 days. 14-day-old seedlings were then sprayed with solutions of Lirosect 1.5 EK (1.5 % aqueous solution of abamectin) applied at different concentrations – 0.05 %, 0.1% and 0.2 %. Five days after Lirosect treatment were cut discs with d = 1.2 cm from primary leaves of intact seedlings and were used in labelling experiments to estimate the rate of synthesis of RNA and total soluble proteins.

The dry weight analysis was performed after drying of 10 discs from primary leaves at 105 ºC.

In vivo labelling of proteins and total RNA

The incorporation of [^{14}C]- lysine in newly synthesized proteins was determined using the filter disc method according to Mans and Novelli (1961) with modifications. Discs from different variants with d = 1.2 cm^2 were incubated with 185 kBq cm^{-2} [^{14}C]-lysine in distilled water for 4 h and were used immediately or after freezing at -20 ºC for a couple of days. Frozen leaf discs were ground with mortar and pestle in precooled extraction buffer containing 20 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 20 mM KCl, 0.6 M sucrose, 30 % glycerol, 20 mM EDTA, 10 mM 2-mercaptoethanol. The homogenate was then centrifuged at 10 000 g for 30 min. Aliquots of the supernatants (0.1 cm^{-3}) were pipetted onto Whatman No1 filter paper discs and precipitated with cold 10% and 5 % TCA. Hydrolysis of aminoacyl-tRNAs was performed in 5 % TCA for 20 min

at 90 ºC and the discs were subsequently washed with cold ethanol, ethanol:ether (3:1) and ether. Radioactivity was counted in a liquid scintillation counter Beckman (USA).

In vivo RNA synthesis was determined using the procedure for estimation of RNA content according to Klyachko et al. (1979) with modifications. Leaf discs from different variants were labelled with 370 kBq cm^{-3} [^{3}H]-uridine (UVVVR, Praha, Czech rep.) for 4 h and homogenized in the extraction buffer. The homogenate was centrifuged for 30 min at 10 000 g and aliquots of the supernatants containing total cytoplasmic RNA were pipetted onto Whatman No1 filter paper discs and precipitated with cold 10% and 5% TCA. The discs were subsequently washed with cold ethanol, ethanol:ether (3:1) and ether. Radioactivity was counted in a liquid scintillation counter Beckman (USA).

Data are means of 3 separate experiments, each including at least 3 replications. Results are presented with SE.

Results and Discussion

The effects of Lirosect 1.5 EK applied at increasing concentrations on the rate of incorporation of [^{14}C]-lysine in newly synthesised proteins measured in the primary leaves 5 days after spraying of 14-d-old zucchini seedlings with the preparations tested are presented in Fig. 1. The results showed a marked increase in the incorporation rate at 0.05 (1.3-fold) and 0.1% Lirosect (1.8-fold) compared with the control whereas

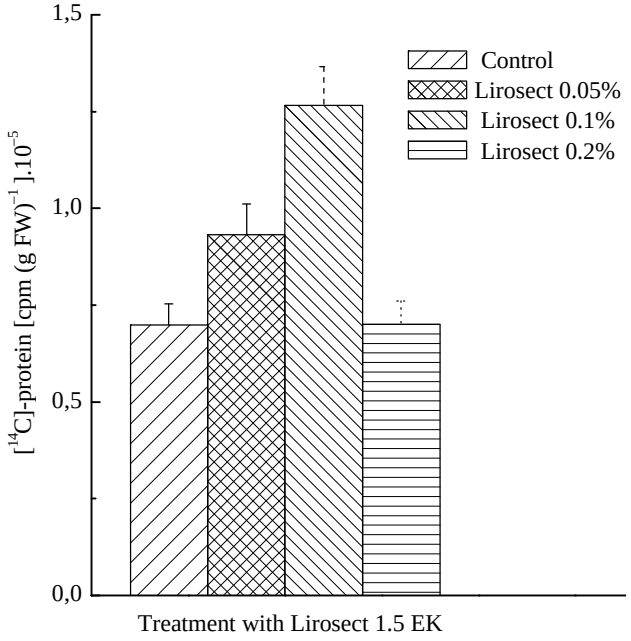


Fig.1. Effect of Lirosect on protein synthesis in primary leaves of *Cucurbita pepo* L. (zucchini) seedlings. 14-day-old zucchini seedlings cultivated on a ½Knop's solution under controlled light conditions were sprayed with solutions of Lirosect 1.5 EK applied at increasing concentrations. After 5 days the rate of incorporation of [^{14}C]-lysine in total soluble protein was determined. Data are presented with SE from three physiological experiments.

treatment with 0.2% Lirosect had no any significant effect on protein synthesis. Therefore, the stimulatory effect of abamectin was highest at a concentration of 0.1%. This concentration was proved earlier to be the most effective against the leaf miner fly (*Liriomyza huidobrensis*) on glass-house grown tomatoes (Videnova, 1999).

The effect of abamectin on RNA synthesis is shown in Fig. 2. As total RNA in the plant cell consists mainly of ribosomal RNA, the results on the total RNA synthesis reflect the effect of abamectin predominantly on the synthesis of rRNA. It is obvious

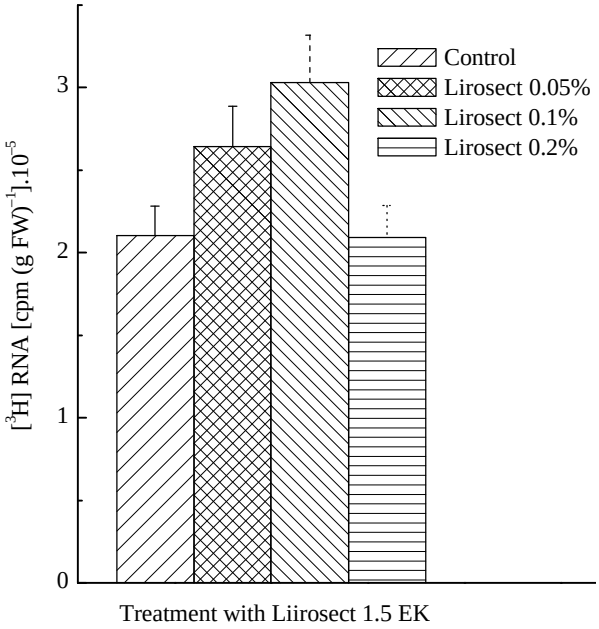


Fig. 2. Effect of Lirosect on RNA synthesis in primary leaves of of *Cucurbita pepo* L. (zucchini) seedlings. 14-day-old zucchini seedlings cultivated on a ½ Knop's solution under controlled light conditions were sprayed with solutions of Lirosect 1.5EK applied at increasing concentrations. After 5 days the rate of incorporation of [^{3}H]-uridine into total RNA was determined. Data are presented with SE.

that the rate of incorporation of [^{3}H]-uridine was stimulated in a similar manner as the rate of protein synthesis. Again, the highest increase (1.4-fold) was measured after treatment with Lirosect 0.1%. Similarly to protein synthesis, the highest Lirosect concentration applied (0.2%) did not affect the rate of incorporation of [^{3}H]-uridine – the values were close to the control.

The results on the effect of abamectin on the changes in dry weight of the primary leaves as a measure of biomass accumulation (Fig. 3) are in good agreement with its stimulatory effect on protein and RNA synthesis. The dry weight increased 1.5-fold at 0.1% Lirosect and diminished at 0.2% Lirosect.

It is worth noting that spraying zucchini seedlings with Lirosect did not lead to any side effects, exp. necrosis, chlorosis, etc. Similar results showing a good level of preservation of the main leaves which nourished the fruit (5th–16th leaf) were reported after spraying glass-house grown tomatoes with Lirosect (Videnova et al., 1999).

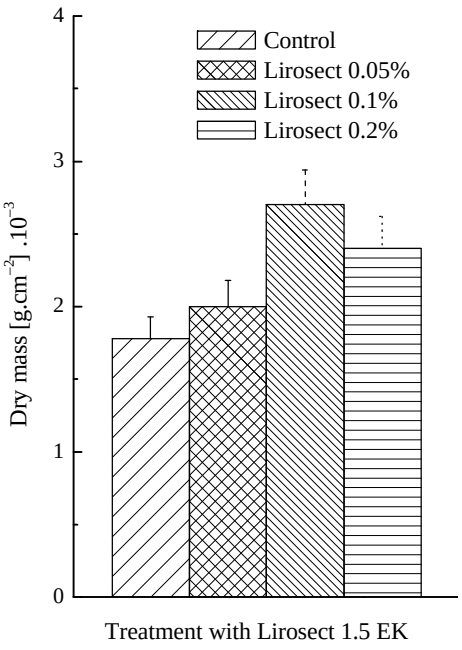


Fig. 3. Effect of Lirosect on dry mass of primary leaves of of *Cucurbita pepo* L. (zucchini) seedlings. 14-day-old zucchini seedlings cultivated on a ½ Knop's solution under controlled light conditions were sprayed with solutions of Lirosect 1.5 EK applied at increasing concentrations. Leaf dry mass was determined after 5 days. Data are means from three different experiments eached performed with 10 discs. Values are presented with SE.

The effects of abamectin on the protein and RNA synthesis described in this work could be discussed in view of the ability of a number of antibiotic substances to influence the transport through cell membranes thus modulating structure and function of cell organelles. A number of microorganism-derived compounds are known as membrane – active substances of antibiotic type which can increase the permeability of cell membranes including mitochondrial and chloroplast membranes, for different cations (Gale et al., 1975). Having in mind that abamectin is a streptomycete-derived substance with antibiotic activity, it can be assumed that the mechanism of its stimulatory effect on the protein and RNA synthesis is most probably based on its ability to act as a membrane-active complexone. Further experiments are needed to investigate the effect of abamectine on membrane permeability.

In conclusion, the results from the present study show that the insecticide and acaricide preparation Lirosect produced in Biovet JSC -Peshtera including abamectine as the active compound, applied at concentrations used in green-house practice (0.1%) can stimulate two major biochemical processes in plants – biosynthesis of proteins and RNA in the primary leaves of zucchini seedlings. These results could be interpreted in view of the ability of abamectin to act as a membrane-active complexone.

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