Content of capsaicin extracted from hot pepper (*Capsicum annuum* ssp. *microcarpum* L.) and its use as an ecopesticide

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Abstract

The latest world trends in scientific research are directed towards the production of secondary metabolites, their use and application. Capsaicin, the pungent principle of hot peppers is one of the best-known natural compounds. Nowadays, research has been focusing the influence of capsaicin on physiological and biochemical processes of humans, animals, and recently plants as a biopesticide. Phytochemical studies of *Capsicum annuum* L. increase the application of secondary metabolites in pharmacy, food technology and medicine. In this paper, the possibilities of utilization of *Capsicum annuum* ssp. *microcarpum* L. for extracting capsaicin and its use as a biopesticide against the green peach aphid *Myzus persicae* Sulz. in pepper culture are summarized. The content of capsaicin was evaluated spectrophotometrically, and the ability of capsaicin for acting as biopesticide was calculated according to Abbott. Results showed that oleoresin from *Capsicum annuum* ssp. *microcarpum* L. and its dilution 1:20 are the most efficient as a biopesticide. From these results we can say that this kind of peppers can be used as a raw material for extraction of capsaicin, because of its high concentration and efficiency.

Keywords: capsaicinoids, ethanol extraction, oleoresin, biopesticides.

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Secondary plant metabolites represent a significant economic group used in different areas such as production of food additives, pigments, pharmaceuticals and biopesticides [1]. The most important components in the group of secondary metabolites, derived from the biologically active components of the species *Capsicum annum* L. are the group of alkaloids-capsaicinoids.

Capsaicinoids are derivates of benzylamin. Differences within their structure depend mainly on their acyl moieties, and three structural elements are involved: first, the length of the acyl chain (C8-C13), then the way it terminates (linear, iso or anteiso-series), and the presence or absence of unsaturation at the ω-3(capsaicin type) or ω-4 carbon atom (homocapsaicin type I and II) [2,3].

Capsaicin, a homovanillic acid derivative (8-methyl--*N*-vanillyl-6-nonenamide, Figure 1), is an active component of the red pepper. The level of the capsaicin in the seasonal pepper is around 0.025%, and in the hot pepper around 0.25% [4,5].

It is an extraordinarily versatile agent, and its use in a variety of fields ranges from pharmacology and nutrition to chemical weapons and shark repellence. Capsaicin is represented with 69% in the group of capsacinoids; dihydrocapsacinoids with 22%; nordihydrocapsacinoids with 7%; homocapsaicin and homohydrocapsaicin takes only 1% in the group of capsaicinoids. Capsaicin and dihydrocapsaicin being approximately twice as pungent as nordihydrocapsaicin and homocapsaicin and they are responsible for the hotness of the pepper. The pungency of capsaicinoids and pepper containing preparations can be expressed in Scoville Heat Units (SHU) and the human palate can detect it even diluted in 1:17 000 000 ratio [2,3,6].

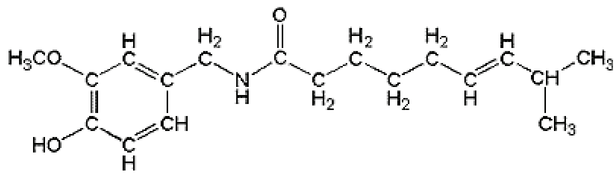

Figure 1. Structural formula of capsaicin.

Because of the antimicrobial capacity of the capsaicin, Walter (1995), for the first time, suggested a protective medium that contains capsaicin, as a base component in the product that belongs to the group of biochemical pesticides. From 1995 onwards, a lot of products have been registered in the EPA (Environmental Protection Agency, USA), insecticides and rodenticides based on capsaicin. In the end of 2001, the EPA registered around 195 active materials as biopesticides and 780 products [7].

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Because of great interest and the complex nature of the research aimed to examine the character of the biopesticides, they are categorized into three major classes: microbial pesticides, protective elements, and biochemical pesticides. Biopesticides are natural substances made from herbal extracts or from pheromones from insects, which in the control of the pests have no toxic effect. Capsaicin belongs to the third class of biopesticides [8–10].

It is important to note that capsaicin-containing products have primarily been used to repel insects since ancient times. Literature survey has revealed that capsaicin has lethal and antifeedant effects on various invertebrates, which is another reason why organic farming is directed toward the production of biopesticides [9,11]. The aim of this experiment is to examine the relationship between the concentration of capsaicin and its activity as a biopesticide.

EXPERIMENTAL

Plant material for extraction of capsaicin

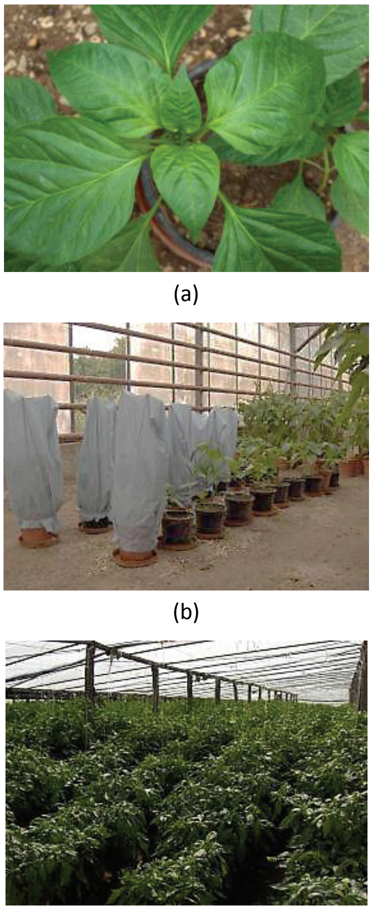
Dried fruits of hot pepper *Capsicum annuum* ssp. *microcarpum* L. were used for extraction of capsaicin (Figure 2).

Figure 2. Habitus of hot pepper, Capsicum annuum ssp. microcarpum, in the phase of fruiting.

The seed of the hot peppers from the breed "Bonbona" was taken from the gene bank at the Faculty of Agricultural Sciences, Strumica, Macedonia. The peppers were grown in an open field area, which was situated in the region of Strumica (41°26'15'' NGW and 22°38'35" EGL). They were collected in late September, in the phase of botanical maturity [12]. The fruits were dried in a Binder dryer at a temperature of 50 °C until constant weight. The dried material was powdered in a blender (Gorenje SIC400B).

Plant material for testing the efficiency of capsaicin extraction

The examinations for determining the efficiency of capsaicin as biopesticide were made in closed conditions on another type of pepper culture from the breed Inferno, which was infected with the plant louse of *Myzus persicae* Sulz (Figure 3).



(a)

(b)

(c)

Figure 3. Pepper Capsisum annuum L. breed Inferno grown in a greenhouse under controlled conditions. a) Apical bud of pepper host plant infected with Myzus persicae Sulz.; b) Infected host plants covered with trap bag; c) Pepper production breed Inferno.

The infection was formed on the apical buds of the host plant right before the phase of initial flowering. The initial infection on the hosting plant is given in Figure 3a. In order to enable better and faster development of the plant louse, and also to prevent the spreading of the infection to the other plants, the infected samples were covered with trap bags (Figure 3b). The procedure for controlling the efficiency of the extract of capsaicin was repeated three times on the same infected plants. Infected plants were treated in the period of 14 days after the initial infections. Treated plants were in the phenological phase of full flowering, when the eggs of the plant louse and adult forms of the parasite were noted on the leafs.

In the other case of growing peppers, the presence of plant-louse of *Myzus persicae* Sulz. was also noticed in the early fruiting phase on pepper fruits. These plants were infected without any artificial infection.

The same treatment was used in this case, where capsicum oleoresin and variant 1 and 2 (Table 1) were used as the treatment solution.

Table 1. Capsaicin concentration in oleoresin and its dilutions

Variant	Rate of dilution	Capsaicin concentration, mg/ml
Oleorasin	–	12.2375
1	1:2	6.1187
2	1:10	1.2237
3	1:20	0.6118
4	1:50	0.2447
5	1:125	0.0998
6	1:625	0.0167

Methods of work

Capsicum oleoresin can be prepared from hot peppers using a variety of organic solvents, but ethanol is the only one suitable for obtaining pharmaceutical grade material [3]. The dried and smashed material from hot pepper *Capsicum annuum* ssp. *microcarpum* L. was kept into desiccators and this material was used for obtaining the capsicum oleoresin. Extraction was performed with 96% (v/v) ethanol from dry plant material (0.1–0.5 g of powdered plant material was taken for extraction), in a water bath using a temperature of 40 °C, within a period of 5 h. Then, water vacuum filtration was included in the experiment for obtaining an ethanol extract of capsaicin. The obtained oleoresin had a concentration of 12.712 mg capsaicin/ml extract.

After obtaining the basic oleoresin, six dilutions were made for treatment of the plants (Table 1) with the aim of determining the effects of different concentration of capsaicin in the diluted samples. Dilutions were made ex tempore, before the treatment of infected plants, and sterile distillated water was used as a control.

Capsaicin and analogs were detected at 100 ng level by UV monitoring at 279 nm [8]. The absorbance of capsaicin, in the proper dilutions of the ethanol extract, was measured spectrophotometrically (UV/Vis Varian 50) at a wave length of 281 nm [13].

The standard curve (Figure 4) was made with standard dilution (0.02–0.1 mg/ml) of capsaicin (Sigma), and the coefficient of the linear correlation (Figure 5) was $R^2 = 0.998$ ($y = 9.7734x + 0.1409$).

Testing of the effectiveness of capsaicin as an ecopesticide

The second part of this study, aimed to determine the relationship between content of capsaicin and it use as a biopesticide, was conducted on pepper from the breed Inferno, which was infected with aphis *Myzus persicae* Sulz. Three replicates of the infection were maintained for each concentration along with the control. The evaluation of the efficiency of the active material was based on the number of infected leaves with aphis. The results of the efficiency of the capsaicin as biopesticide were measured in 24 hours, and calculated according to Abbott's formula [14]:

$$\text{Efficiency by Abbott (\%)} = \frac{\text{Test mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}}$$

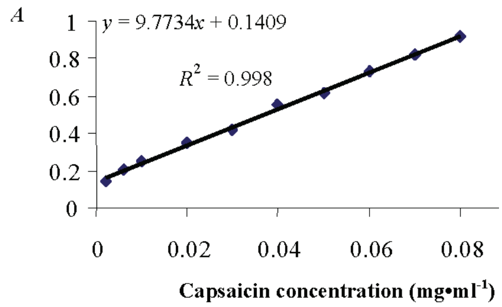


Figure 4. Capsaicin standard curve at 281 nm.

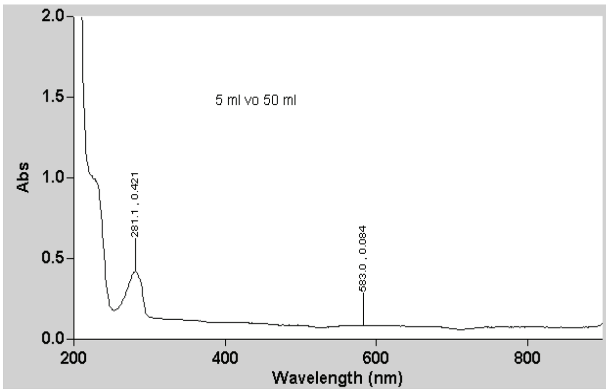


Figure 5. UV/Vis spectra of capsaicin standard (Sigma) with typical peak at 281 nm.

The efficiency of capsicum oleoresin and variant 1 and 2 on the accidentally infected plants in the phase of fruiting was also evaluated as a demonstration of their activity as biopesticides.

RESULTS AND DISCUSSION

The content of capsaicin in the oleoresin dilutions is given in Table 1. As expected, the results confirmed the highest concentration of capsaicin in the oleoresin, and a 700 times lower concentration in the last diluted sample.

The intensity of the aphis attack on the pepper was high, with a large number colonies formed. The treatment of the pepper, with all the research variants, depending on the concentration of the capsaicin in the dilution, gave a different effect (Table 2).

Table 2. Efficiency of the capsaicin in appropriate dilutions according to Abbott after 24 hours of the treatment on the pepper

Variant	No. of leaves infected with Aphids	No. of Aphids before treatment	No. of Aphids after treatment	Control	Efficiency by Abbott, %
Oleorasin	3	132	2	78	97.4
1	3	118	6	66	90.9
2	3	76	5	51	90.1
3	3	77	13	48	72.9
4	3	38	21	36	41.7
5	3	49	13	21	38.1
6	3	37	20	21	4.8

The results obtained in this experiment, once again confirmed concentration/dose dependent increase in larvicidal activity, according to the literature [15]. The largest efficiency in the repression of the aphis on the pepper is observed at oleoresin with 97.4%, where the capsaicin concentration is 12.2374 mg/mL. Its activity drops gradually until the last dilution.

LC_{50} = 0.2934 mg/mL is concentration that is achieved with dilution of 1:50. This means that the concentration of capsaicin in oleoresin and first two dilutions (variants 1 and 2 with efficiency from 97.4–90.1%) is high enough to kill 90% of the insects, and in third variant the concentration is enough to kill 50% of parasites. In the next three variants, dilution is very high and the concentration of the capsaicin is in the range of 0.2447 to 0.0167 mg/mL, so the smallest effect of this dilution is completely understandable. The highest concentration, in contrast with the smallest, is 20.3 times more efficient. From the results it is obviously that capsaicin showed high efficiency with larvicide and adulticide capacity, but only if it is in proper concentration. We can say that the dose and efficiency are linearly dependent (Figure 6) for the first three concentration of capsaicin.

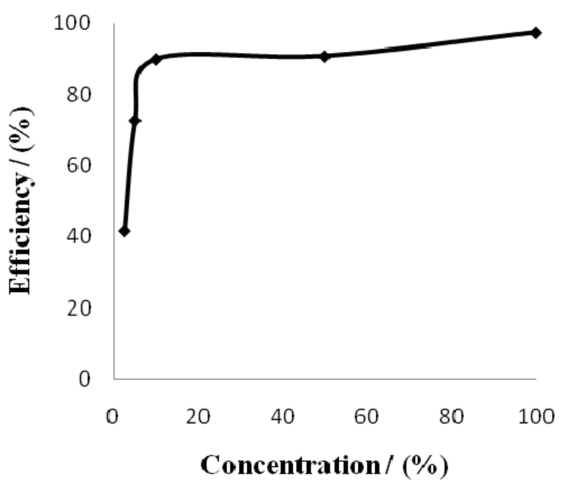


Figure 6. Concentration dependent efficiency of capsaicin against Mayzus persicae Sulz.

CONCLUSION

The everyday use of different types of pesticides makes the aphis *Myzus persicae* Sulz. more resistant to today's products. On the other hand, the written records point to the use of new products as biopesticides in control and repression of pests, especially popular in organic production. This resulted in efforts to find a new natural and safety way to protect plants from insects and parasites.

The experimental results confirmed that the examined pepper contains a high concentration of capsaicin. It can be widely used as material for extracting capsaicin. Its oleoresin can be used as an effective biopesticide along with its dilutions even to the rate of 1:20.

The aim of this study was to make a chemical and insecticide characterization of oleoresin extracted from *Capsicum annuum* ssp. *microcarpum*, giving an emphasis on quantitative information about the concentration of capsaicin in different variants, and correlation with its activity as a biopesticide.

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IZVOD

SADRŽAJ KAPSAICINA U LJUTOJ PAPRICI (*Capsicum annuum* ssp. *microcarpum* L.) I NJEGOVA PRIMENA KAO BIOPESTICIDA

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(Stručni rad)

Alkaloidi dugo vremena predstavljaju predmet istraživanja u organskoj hemiji i farmakologiji, zbog svoje biološke i fiziološke aktivnosti uslovljene hemijskom strukturom. Kapsaicinoidi su grupa alkaloida, koji se javljaju kao kompleksne mešavine acil konjugata na vanilamin u rodu *Capsicum*. U ovom eksperimentu je korišćena vrsta paprike *Capsicum annuum* ssp. *microcarpum* koja predstavlja jednu od najljućih sorti koje se proizvode u Makedoniji. Iz paprike je izolovan kapsaicin, čija je koncentracija određivanja spektrofotometrijski. Uzimajući u obzir da je u poslednje vreme malo podataka o kapsaicinu kao biopesticidu, u radu je ispitan uticaj kapsaicina, kao i njegove koncentracije, na larve i adultne parazitske organizme *Myzus periceae* Sulz. Rezultati istraživanja su pokazali da je capsaicin efikasan biopesticid protiv *Myzus periceae* Sulz., jer je LC_{50} = 0,2934 mg/ml, i da je njegova aktivnost direktno zavisna od koncentracije. Najveća aktivnost kapsaicina kao biopesticida je u opsegu koncentracija od 1,2237 do 12,2375 mg/ml, pri kojima se postiže efikasnost od 90,1-97,0%. Dobijeni rezultati opravdavaju upotrebu ove vrste paprike kao sirovine za ekstrakciju kapsaicina, kao i upotrebu kapsaicina kao biopesticida za navedenu vrstu organizama.

Ključne reči: Kapsaicinoidi • Ekstrakcija etanolom • Oleoresin • Biopesticidi