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Aspects regarding pathogen-enzymatic system interrelation at *Momordica charantia* naturalized in Romania

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Abstract

Momordica charantia is a medicinal plant species cultivated and naturalized in Romania that represents an important natural remedy in phytopharmaceutical preparations.

In healthy plant leaves of Momordica charantia the values of enzyme content were 0.154 U/g amylases, 0.89 U/g proteases and 150 U/g lipases (all analyzed by titrimetric method) and 58 μg /l lipases (analyzed with RQ-Flex apparatus).

Momordica charantia leaves from plants affected by Aspergillus niger (P1) were found to have the following values: amylases = 0.103U/g, proteases = 0.34 U/g and lipases = 120 U/g (analyzed by titrimetric method) and 28 μg/l (analyzed by RQ-flex method).

For Momordica charantia leaves from plants affected by Alternaria sp. (P2) the following values in enzyme content were determined by titrimetric method: amylases = 0.101 U/g and proteases = 0.61 U/g. The lipases content was analyzed by two methods: titrimetric – 123 U/g, and RQ-flex – 36 μg/L.

Determinations made from plant leaves, showed the existence of different amounts of enzymes in healthy and infected material. Also, the analysis performed here were different depending on the enzyme content and identified microorganisms.

In this paper we described some aspects of the interrelation pathogen-enzymatic system from naturalized species of Momordica charantia. Under laboratory conditions comparative measurements were made on enzymantic content of the leaves of Momordica charantia (both healthy and infected with pathogenic fungi Aspergillus niger and Alternaria sp.). Amylases, proteases and lipases content of the biological material were determined.

Key words: *Momordica charantia*, seeds, enzymes, phytopathogen

Introduction

The study of medicinal plants has been one of the most important human endeavours from the beginning of its existence. Over time, progress has made possible the isolation from plants and identification of the compounds with therapeutic properties. [1].

Nowadays, the attention is given to medicinal plants which are inexhaustible sources of raw materials to obtain drugs.

Many plants or selected parts of plants such as fruits, leaves, and seeds contain enzymes favourable for the functioning of the human digestive system or metabolism.

Medicinal plants containing such compounds are known like enzymatic plants. *Momordica charantia* (or bitter cucumber; bitter gourd) is a plant specific from southern Asia, but is also widespread in Africa and Australia. It is a climbing plant with lobed and serrated leaves (like vines). The flowers are small and yellow and grow at leaves' nodes. The fruit are fleshy, oblong or cylindrical shaped, covered with nodules, which give it a rough aspect, and it is orange when is ripe. Leaves are used in phytotherapy while the fruit and seeds are still subjects of intensive studies [8-10].

In *Momordica charantia* leaves several classes of phytotherapeutic compounds can be found. Previous studies on this plant tried to establish a relationship between the existing quantity of enzymes in the seeds and the pathogens affecting it and to identify some practical consequences of the antimicrobial potential it may have against some phytopathogenic agents [5-7].

In this research the laboratories from SC Hofigal Import-Export SA (company producing food supplements) had an important role, as they carried out studies on the biochemical composition of *Momordica charantia* leaves. An equally important contribution had the laboratories of Phytopathology from the University of Agricultural Sciences and Veterinary Medicine in Bucharest carried out research on microbiology, using common methods or adapted ones for specific investigations.

Material and Methods

We used samples of biological material from *Momordica charantia* leaves which were subjected to biochemical and microbiological tests in the research laboratories at SC Hofigal – Export-Import SA (food supplements) and at the University of Agricultural Sciences and Veterinary Medicine Bucharest.

The quantity of enzymes from leaves was observed in this study and it was found to depend on the pathogenic microorganisms infecting the plants.

To determine the micoorganisms we used leaves samples of *Momordica charantia* indicating an attack by some phytopathogenic agents. The fungi *Aspergillus niger* and *Alternaria sp*. were isolated, multiplied, and preserved in pure culture by repeated subculture of the vegetative mass on PGA culture medium (potato-glucose agar). The plates were incubated at room temperature (22 °C) for 12 days. During this time the number of colonies developed on plates was noted and each colony was characterised every three days [1-3].

A class of substances essential for normal functioning of the cells and present in the chemical composition of *Momordica charantia* seeds, is represented by the enzymes – proteins with significant catalytic properties of biomolecules.

The main groups of enzymes were determined, namely amylases, proteases, and lipases.

The methods used for these determinations were as follows:

1. Amylases content was determined according to the method of determining α-amylase described in the analysis manual of the Worthington Company. The reducing groups released from starch used as a substrate were determined by reducing the 3,5-dinitrosalicilic acid. The resulting yellowish-orange compound was read at double beam spectrophotometer UV-VIS JASCO, at a wavelength of 540 nm. A unit was represented by one micromole of reducing group (calculated in equivalent maltose) per minute, at 37 °C.

2. Proteases were dosed according to the classical method of endoproteinases dosing. This method consists on determining the enzyme activity by the method described by Drapeau in 1976, where casein is used as a cleavage substrate. A unit was represented by the amount of enzyme that releases acid-soluble fragments with an absorbance at 280 nm wavelength per minute, at 37 °C and 7.8 pH.

3. Lipases content – titrimetric method. Higher fatty acids, resulted from triglyceride hydrolysis of olive oil used as substrate and in the presence of lipase, are extracted with organic solvents and titrimetrically dosed with an alcoholic solution of NaOH in the presence of phenolphthalein as an indicator. The method of determining lipolytic activity was performed as described by Iordachescu D. and Dumitru I.F. [7]. One unit of lipolytic activity

is defined as the amount of enzyme reaction conditions indicated in 1 µmol fatty acid per minute at 37°C.

4. Lipases content – RQ-flex method. The lipases content of the vegetable samples was also determined by RQ-Flex technology which is a reflectometric method. The sample preparation method was the classical one.

Results and discussion

The leaves of *Momordica charantia* are utilised in phytotherapeutic supplements in order to achieve a hypoglycaemic and hypocholesterolaemic effect, while remineralising and vitaminising the human organism [12-15].

The enzymes from fresh plants have remarkable effects on the human body, normalising digestion, metabolism and tissue respiration. Also, this plant can be used in the treatment of degenerative diseases.

Fig 1. *Momordica charantia* – acclimatised plant affected by pathogens

For the healthy leaves of *Momordica charantia* the values of enzymatic content were the following: 0.154 U/g amylases, 0.89 U/g proteases, while for lipases they were 150 U/g and 58 µg/l, analyzed by titrimetric method and with RQ-Flex apparatus respectively.

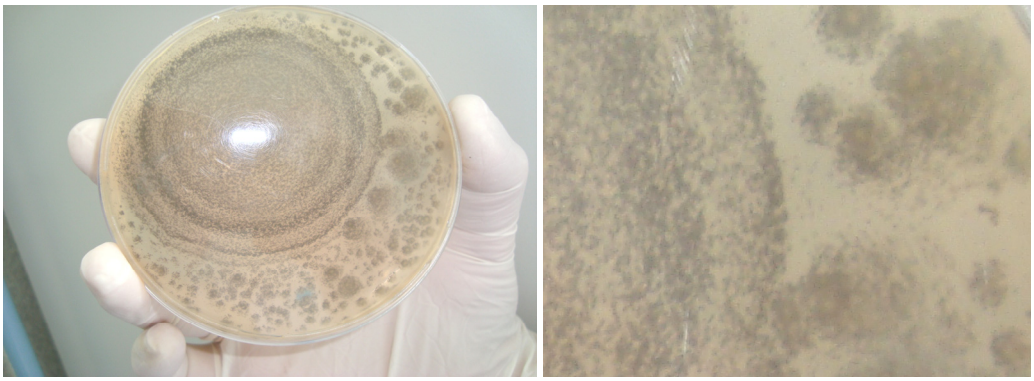

Fig. 2, 3. *Aspergillus sp* colonies. The isolated colonies are from *Momordica charantia* leaves

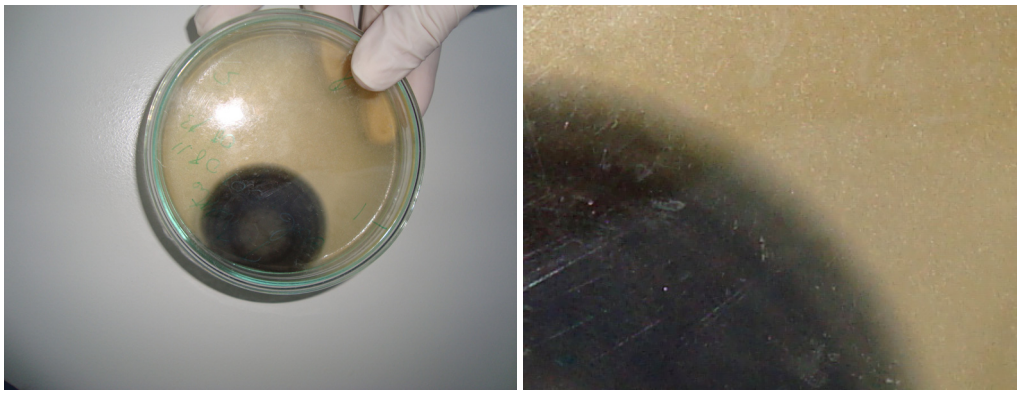

Fig. 4, 5 *Alternaria sp* colonies. The isolated colonies are from *Momordica charantia* leaves

The content in main types of enzymes was also determined for fresh plant material affected by *Aspergillus niger* (P1). It was found that the amylases content was 0.103 U/g, the proteases one 0.34 U/g, and that of lipases was 120 U/g and 28 µg/l, according to the method used – titration and RQ-flex respectivelly.

The *Momordica charantia* leaves from plants affected by *Alternaria sp.* were found to have slightly different values of the enzymatic content: amylases – 0.101 U/g, proteases – 0.61 U/g, and lipases – 123 U/g and 36 µg/l respectively for each of the two methods used (titration and RQ-flex).

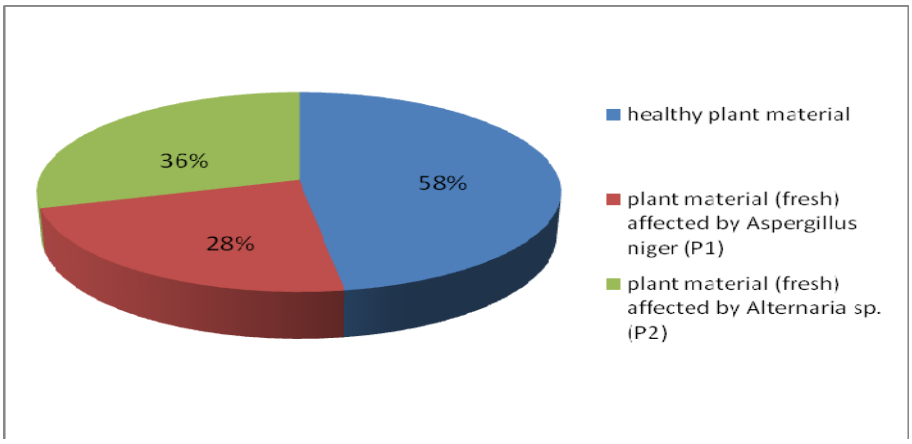

Fig. 6. Pathogenic attack in plant material

Tabel 1. Values of enzymes content from *Momordica charantia* affected or healthy

Plant material	**Amylases U/g**	**Proteases U/g**	**Lipases U/g**	**R-Q-Flex lipases µg/ L**
healthy material	0.154	0.89	150	58
P1	0.103	0.34	120	28
P2	0.101	0.61	123	36

The analysis of the enzymatic content revealed that there are significant differences between infected and healthy plants and shown us how usual pathogens (fungi) progressively

degrade plant enzymatic equipment as compared to healthy plants, resulting in the degradation of the plant and the loss of its vitality.

It is also notable that there are small differences between the values obtained for amylases and lipases content from the two infected samples (P1 and P2).

On the other hand the proteases content differs considerably for the two, with 0.34 U/g in fresh plant material affected by *Aspergillus niger* (P1) and 0.61 U/g in that affected by *Alternaria sp.* (P2). A possible explanation of these values is that the enzymes from plants affected by *Aspergillus niger* are degraded in a higher proportion by the loss of plants' proteins, therefore decreasing the proteases necessary.

In the next studies we will compare the amounts of proteins and proteases of the samples.

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