VIRAL INFECTIONS OF THYME

THE EFFECTS OF VIRAL INFECTIONS ON THYME OIL ESSENCE

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Introduction

Medicinal herbs are subject to many parasites that attack both the roots and the shoots or epigeal part of the plant and these have negative effects on the plant's growth and effectiveness. The diseases are caused by fungi, bacteria, phytoplasms, viruses and organisms that are similar to viruses, like viroids, as well as the plants being open to attack and infestation by nematodes, insects and acaridae.

Health aspects are thus important to the financial viability of aromatic and medicinal herbs. Despite this fact plant disease studies in Italy have only been carried out to date on a sporadic basis.

Among the most dangerous and widespread of infectious pathogens are viruses, protein nucleus parasites that alter the host cell metabolism to their own advantage. In other words the virus parasites become an integral part of the cell's biochemistry. The resulting damage is mainly "quantitative" and a result of the morphological and structural changes in the individual organs caught up in the infection, whether these be the leaves, the stem, flowers, fruit or epigean organs. If we think of each tissue in a medicinal herb as being a chemical factory in which the natural elements are converted into active principles, or drugs, the possibility cannot be ruled out that the viruses could also be the cause of "qualitative" changes. Vegetable cells whose metabolism is determined by a foreign pathogenic agent could "manufacture" chemical principles that are different from those found in the healthy organism.

Aim of the study

In the light of the above, research was embarked on in 1992 in the form of a collaborative study between the Vegetable Disease Institute of the Agrarian faculty of the University of Bologna and the Giardino delle Erbe herb gardens of Casola-Valsenio (Ravenna) with the aim of identifying the most frequent viral infections come across in medicinal herb cultivation, not only in the above Garden but also in other areas in Emilia-Romagna and some other Italian regions such as Trentino-Alto Adige (Bellardi *et al.*, 1998a) and Liguria (Bellardi *et al.*, 1999).

Early research identified the most widespread and harmful viruses, determined their main epidemiological characteristics and assessed, on a case by case basis, the symptoms they presented themselves with in the medicinal herbs concerned. This early research thus regarded the "quantitative" aspect of the damage to production (Bellardi *et al.*, 1996, 1997,1998b).

Over the last few years, in view of the fact that many medicinal plants are essentially drugs and so grown for the obtaining of the chemical principles for their large-scale use in the pharmaceutical industry, an effort has been made to establish the possible effects of viral infections on both the quality and the quantity of the resultant drugs. Comparative studies have been carried out between the chemical principles extracted from healthy and infected plants, with particular regard to the "qualitative" aspect of such infections.

There is no record in the literature of similar research work so these are the first trials aimed at assessing the influence of viral infection on the composition of the drugs.

The species considered in the study were thyme (*Thymus vulgaris* L.) and salvia sclarea (*Salvia sclarea* L.) sage, the essential oils of which were examined. The results for thyme are given below.

Virological investigations

The thyme samples analysed were collected over a number of visits during the spring and summer of 1998 to the Giardino delle Erbe. Some plants were found to be less vigorous, with chloric or yellow leaves, early defoliation and reduced flowering.
Leaf samples were taken both from non symptomatic plants and those that were yellowish or suffering from dwarfism, to establish whether or not any virus was present.
Routine methods were applied in the virological investigations, including biological assays (with mechanical inoculation of herbaceous plants), the use of electron scanning microscopy ("negative colouration" and ultrastructural examination of resin-included tissue), and serum diagnosis (ISEM: immuno-absorbent electron microscopy).
Biological assays made it possible to isolate, from the symptomatic samples only, a virus that was transmitted to species belonging to the *Chenopodiaece* family. Electron microscopy investigation of leaf juice preparations from symptomatic thyme samples and artificially infected chenopodiace showed the presence of filamentous viral particles of a mean length of 436 nm.
Ultra thin sections of symptomatic thyme leaves included in resin showed viral particles to be present in the cytoplasm of the mesophyl.
The use of ISEM techniques with specific polyclonal anti-sera of filamentous viruses (*carlavirus* e *potexvirus*) did not make it possible to identify the virus but only permitted the establishment of a weak serological correlation with members of the *carlavirus*.

The extraction of essential oils

The extraction of essential oils from thyme samples collected in the field at the Giardino delle Erbe, was carried out by the "distillation in a steam current at atmospheric pressure" method.
The fresh thyme, in total quantities of about 4 kg, belonged to two separate batches. The first included non symptomatic plants while the second of plants with yellowish leaves and dwarfism. Before harvesting, all the thyme plants, with or without symptoms, were subjected to the particular virological investigation. The oil extracted from the two thyme batches was made up of about 4 ml in the non symptomatic (i.e. healthy) batch and about 2 ml in the symptomatic (i.e. infected) batch.
The two lots of essential oil were stored in a refrigerator in glass vials at about 4 °C until the time of the gas chromatography examination.

Mass Gas Chromatography (GC-MS)

These analyses were carried out with the aim of evaluating the percentage differences in the various components of the essential oil in the two thyme samples. They took place at the Pharmaceutical Sciences Institute of the University of Bologna.
The instrumentation details were as follows:

• **GC-MS system:**
- *Trace GC* "series 2000" coupled to a mass spectrometer *Thermo Quest* as source of ions.
- **Urto elettronico:** 70 Ev.

• **Column:** Rtx-5MS®, cast silica capillary column (Restek Corporation, Bellefonte, USA).
Dimensions: 30m x 0,25mm ID, film 0,25 µm, internally coated with *Crossbound*® (bonded phase: 5% diphenyl-95% dimethyl-polysiloxan).
• **Analysis parameters:**
GC:
- **Injection chamber:** *Split/Splitless* method column injection (splitting ratio: 1:50).
- **Injector temperature:** 250 °C.
- **Injected sample volume (without dilution):** 0,1 µl.
- **Carrier gas:** Constant flow helium (1ml/min.).
- **Oven:** linear T° increase programme:
- **start temperature:** 45 °C (per 10 min.).
- **temperature increase:** 2,5 °C/min.
- **final temperature:** 180 °C.

MS:
- ***Transfer line* temperature:** 250 °C.
- **ion source:** 200 °C.
- **bottom:** auto-controlled.

The mass spectrum is represented by a graph whose x-axis shows the retention times (the compound is in contact with the column) and the ordinates
the percentage abundance relative to the highest peak in relation to which the intensity of the other peaks is measured. (Chiarini and Fabbri, 1990).
The identification of the individual components (peaks) thus separated is based on a comparison of the retention times reported in the literature in similar analytic situations and, where possible, with *standard* retention times. The mass spectra were also compared with those recorded in the *mass spectra data bank* ("NIST", *terpeneoil-library*) and provided by the instrument's software.
From a comparison between the two mass spectra a quantitative difference of about 9% emerged between the thymol of the "infected" oil an the "healthy" oil (53,7% and 62,7% respectively).
Other substantial differences were found in the cymene content that, along with other components, differed by about 5% (at 19.9% in the "infected" sample and 14.4% in the "healthy" sample). Less significant differences were recorded also for terpinene, linalol, terpene and carvacrol *(table 1 page 108)*.

Discussion

The filamentous virus was found only in those thyme plants with symptoms of chlorosis, yellowish leaves and dwarfism and never in the symptomatic controls. It is therefore justifiable to assume that this was the etiological agent for the disease found in the field. The morphological and structural changes in the infected plants mean they strayed from the "normal" requisites of the finished product, a full, rounded and bushy plant. The symptoms correlated with the viral infection were therefore clearly in conflict with these requirements, influencing the quantity (yield) of the finished product and thus also the financial worth of the crop.
As regards the filamentous virus itself, investigations are still in progress given that there were insufficient elements available for its precise identification, its particles being "shorter" than those of *carlavirus*, the diameter smaller than the "rod" viruses: *tobamovirus*, *furovirus*, with the internal channel also not also being central etc. Only a molecular study could clarify the matter.
In the case of the essential oil a significance difference was recorded in the yield. With an equal starting weight the healthy sample produced twice as much oil as that extracted from the infected plant.
From the results of the mass gas chromatography it was observed that the only constituent part of the oil that was substantially less in the infected sample with respect to the healthy sample was the thymol (9%), the most important component of thyme oil. This is in fact the part that is of the greatest economic value in that it is used in the pharmaceutical, cosmetic and food industry fields for its anti-bacterial, fungicidal and spasmolytic properties (Marotti *et al.*, 1998).
In addition to this, for more than a decade now thyme oil has been used to inhibit the development of bacterial and fungal disease in plants grown for agricultural purposes, including poplar trees, potatoes, maize, vines and strawberries and so forth, both in Italy and abroad (Wood *et al.*, 1997). In Italy, and specifically in the island of Sardinia, thyme oil essence has been found to be effective in preventive treatment for the lemon tree against *Phytophthora citrophthora*, *Pseudomonas syringae and Penicillium digitatum* (Arras *et al.*, 1993; Arras, 1998).
In conclusion therefore, it emerges from these preliminary studies that viral infections in medicinal plants can have an effect on both the quantity and the quality of the resulting drug. In the specific case in hand the product oil yield and its quality suffered a percentage drop in thymol.

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TABLE. 1 – Changes in oil essence

Oil essence components	Symptomatic thyme	Non-symptomatic thyme
A-Tujene	0,4%	0,3%
A-Pinene	0,2%	0,1%
B-Pinene	0,1%	0,1%
B-Myrcene	0,6%	0,4%
A-Terpinene	0,9%	1,1%
P-Cymene	19,9%	14,4%
Limonene	0,3%	0,3%
1,8 Cineol	1,5%	1,4%
G-Terpinene	6,0%	6,5%
Linalol	2,9%	1,8%
Borneol	1,4%	1,0%
Terpin-4-ol	1,4%	0,5%
A-Terpineol	0,4%	0,5%
“methylether” thymol	0,3%	0,0%
“methylether” carvacrol	4,7%	3,5%
Thymoquinone	0,2%	0,6%
Thymol	53,7%	62,7%
Carvacrol	5,0%	4,8%

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