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Antiviral properties of essential oils of *Foeniculum vulgare* and *Pimpinella anisum* L.

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Summary — The antiviral activity of the essential oils of *Foeniculum vulgare* Mill. and *Pimpinella anisum* L. has been tested against PVX (potato virus X), TMV (tobacco mosaic virus) and TRSV (tobacco ring spot virus) on the hypersensitive host *Chenopodium amaranticolor*. Both the essential oils totally inhibit the formation of local lesions at a concentration of 3000 ppm.

antiviral activity – essential oils – phenols

Résumé — Les propriétés antivirales des huiles essentielles de *Foeniculum vulgare* et *Pimpinella anisum* L. L'activité antivirale des huiles essentielles de *Foeniculum vulgare* Mill. et *Pimpinella anisum* L. a été éprouvée quant à l'installation du PVX (virus x de la pomme de terre), TMV (virus de la mosaïque du tabac) et TRSV (virus des taches annulaires du tabac) sur un hôte hypersensible, *Chenopodium amaranticolor*. Les deux huiles essentielles inhibent totalement la formation de lésions locales à la concentration de 3000 ppm.

activité antivirale – huiles essentielles – phénols

Introduction

Green plants contain a reservoir of chemicals with unique biological activity (Farnsworth & Bingel, 1977). Essential oils extracted from higher plants have been extensively studied for their fungitoxic (Shukla & Tripathi, 1987 a, b), bactericidal (Narayan *et al.*, 1980) and insecticidal properties (Beye, 1978). Unfortunately such useful compounds have seldom been tested for their antiviral properties (Shukla *et al.*, 1985; Rao *et al.*, 1986). This study has therefore been undertaken with the view to examining the inhibitory activity of essential oils of *Foeniculum vulgare* and *Pimpinella anisum* against PVX, TMV and TRSV.

Materials and Methods

The cultures of different viruses, *i.e.* PVX, TMV, and TRSV were obtained from the National Botanical Research Institute, Lucknow, and maintained separately on the young plants of *Nicotiana tabacum* L. var. Turkish in a insect-proof glasshouse.

In order to extract the volatile constituents (essential oils), the fruits of *Foeniculum vulgare* Mill. and *Pimpinella anisum* L. were obtained from local stockists and subjected to hydrodistillation on Clevenger's apparatus (Clevenger, 1928). The essential oils were collected in the form of a water immiscible oil.

Standard inoculum of each virus, used as infective sap for inoculation in the present study, was prepared by macerating *Nicotiana tabacum* virus-infected leaves of the same age separately, with neutral phosphate buffer (pH 7.0, 0.1 M; 1 ml/g of leaf material), squeezing the crude extract through layers of muslin cloth and filtrate was centrifuged at 3000 g for 15 min. The supernatant was taken as standard inoculum for each assay.

Requisite quantities of each essential oil was added to 5% aqueous sterilized solution of acetone in order to get final concentrations of 1000 ppm, 2000 ppm and 3000 ppm of the oils separately after mixing the standard inoculum (1:1). For control standard inoculum and 5% aqueous sterilized solution of acetone (1:1) was used. The mixture of standard inoculum and inhibitor was vigorously shaken for 30 min and kept for another 30 min before inoculation in 20 healthy leaves of *Chenopodium amaranticolor* Costt et Reyn of the same age group, hypersensitive local lesion host, in randomised latin square pattern (Rao *et al.*, 1986). Each experiment had 5 replicates. The observations

were recorded on the seventh day, and the percentage inhibition of viruses was calculated using the following formula:

$$\text{Percentage inhibition of virus} = [(C-T)/C] \times 100.$$

Where: C = No. of lesions on control leaves;
T = No. of lesions on treated leaves.

The data were statistically analysed by test of comparison between the control and individual treatment sets (Shukla *et al.*, 1985).

Results and discussion

As is clear from table I, the essential oils of *F. vulgare* and *P. anisum* at 3000 ppm completely inhibited PVX, TMV, and TRSV infection. However, at 2000 ppm and 1000 ppm these oils only partially inhibited the infection (see also Table I).

The inhibition of plant viruses by chemicals was first tested by Allard (1914), and since then a large number of chemicals of plant origin, *i.e.* proteins, glycoproteins, colloids of higher molecular weight, phenolic tanins, etc., have been reported to possess antiviral activity (Bawden, 1954; Verma, 1973).

The oils of *Foeniculum vulgare* and *Pimpinella anisum* exhibited the presence of phenols (Shukla & Tripathi, 1987a, b). The inhibitory effect of these oils might be due to the presence of phenols in them, which are already known to be potent inhibitors of plant viruses (Joshi & Prakash, 1981). These phenols interact with virions *in vitro*, resulting in the loss of virus infectivity (Muftuoglu & Nienhans, 1976). The inhibitory effects of phenols present in these essential oils is also probably due to their effect on host susceptibility by blocking the infection sites on the leaf surface (Verma, 1973).

Further work on the isolation of active antiviral compound of the oil is in progress. These natural products will be useful in limiting the propagation

of these viruses and should become a good source of plant protectant. Nevertheless, the use of these oils at a commercial level must await the results of large-scale trials and the examination of application modalities.

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Table I. Effect of essential oils of *F. vulgare* and *P. anisum* on infectivity of PVX, TMV and TRSV.

Essential oils	Concentration (ppm)	Percent inhibition of virus*		
		PVX	TMV	TRSV
<i>Foeniculum vulgare</i>	1000	46.66	46.66	53.33 ^a
	2000	76.00 ^a	78.66 ^a	80.00 ^a
	3000	100 ^a	100 ^a	100 ^a
<i>Pimpinella anisum</i>	1000	73.33 ^a	66.66 ^a	60.00 ^a
	2000	86.66 ^a	76.00 ^a	80.00 ^a
	3000	100 ^a	100 ^a	100 ^a

* : Average on 20 leaves of *Chenopodium amaranticolor*.

a : Difference due to treatment with oils are significant at 1% level.

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