Tobacco Mosaic Virus Inhibition by Bark Extracts

Several plant virus inhibitors have been reported to be present in the different parts of plants[1] but no attention was paid to the inhibitory activity of juices from plant barks. This investigation is concerned with the inhibition of tobacco mosaic virus with the bark extracts of some plants.

Culture of tobacco mosaic virus used was maintained in systemically infected *Nicotiana tabacum* L. var. White Burley plants were kept in insect-proof cages. Inoculum was prepared by grinding diseased tobacco leaves in a mortar and the juice was expressed by squeezing the pulp through muslin cloth and diluted 1:10 with distilled water and kept at 0 °C. The inhibitor preparations were made by homogenizing small pieces of fresh and cleaned barks with an equal weight of distilled water in a waring Blender. Juices from these homogenates were expressed through several layers of muslin cloth and tested as inhibitors. The inhibitory effect of bark extracts was determined by comparing the infectivity of equal volumes of tobacco mosaic virus and distilled water (control) with equal volumes of tobacco mosaic virus and inhibitor, by the local lesion method. The mixtures were rubbed after 10 min of mixing on the *Chenopodium amaranticolor* Coste et Reyn leaves. 5 replicates were taken for each treatment. Each mixture was inoculated on 17 half-leaves and the half-leaves alloted to each treatment were distributed among plants so as to form a randomized block. Carborundum powder was dusted on leaves before inoculation and inoculations were made with fore-finger wet with inoculum. The results of tobacco mosaic virus inhibition by the bark extracts are indicated in the Table.

Effect of bark extracts on the infection by tobacco mosaic virus

Treatments of tobacco mosaic virus with bark extracts	Average No. of lesions/half-leaf
Artocarpus heterophyllus Lamk.	36.4
A. lakoocha Roxb.	0[a]
Azadirachta indica L.	0[a]
Butea monosperma (Lam.) Kuntze	61.5
Callistemon lanceolatus D C.	2.2[a]
Ficus bengalensis L.	1[a]
F. elastica Roxb.	0[a]
F. rumphii Blum.	0[a]
Eriobotrya japonica Lindle.	0[a]
Mangifera indica L.	35.6
Morus alba Bureau.	52.6
Plumeria rubra L.	2.7[a]
Psidium guayava L.	0[a]
Pyrus communis L.	2[a]
Syzygium jambolana F.B.I.	0[a]
Tamarindus indica L.	0[a]
Distilled water (control)	56.7

[a] Significant at 0.1 level.

Results of the Table indicate that the extracts from the barks of *Artocarpus lakoocha, Azadirachta indica, Ficus elastica, F. rumphii, Eriobotrya japonica, Psidium guayava, Syzygium jambolana, Tamarindus indica,* inactivates the activity of tobacco mosaic virus totally, while the extracts of other barks are also inhibitory up to a certain percentage. Further work on the nature and activity of inhibitors in the bark extracts is in progress[2].

Zusammenfassung. Die bekannte Hemmwirkung von Gewebeextrakten aus Pflanzenteilen auf die Virusentwicklung (Infektiosität?) wird ergänzt durch Untersuchungen über hemmende Wirkungen von Borkenextrakten. Es wurden Pflanzen mit und solche ohne hemmende Wirkung ihrer Borkenextrakte gefunden.

R. Singh

Department of Botany, The University, Gorakhpur (India), 24 July 1968.

[1] F. C. Bawden, Adv. Virus Res. *2*, 31 (1954).

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PRO EXPERIMENTIS

Detection of Nerve Cells by a Histochemical Technique

A special staining technique has been designed in order to obtain a specific and constant demonstration of nerve cells in the autonomic nervous system. This method is based upon the histochemical reaction for the detection of NADH-diaphorase activity[1] with nitro-BT as electron acceptor.

Thin-walled organs (e.g. mouse urinary bladder) or organs whose nervous structures are not far below the external surface (e.g. the intestinal canal of small animals, as regards the Auerbach's plexus) can be stained in toto. In order to get a thinner wall, it may be useful to stretch the organs during sampling by injecting saline solution under low pressure. After sampling, the organs must be frozen as soon as possible on dry ice for several minutes; in airtight vessels the samples may be stored a few days either at dry ice temperature or in a freezer at −70 °C. Afterwards the specimen is allowed to reach room temperature and kept stretched during thawing under saturated moisture. The organ is then dipped directly into the substrate solution[2], and incubated for 10–30 min at room temperature with gentle agitation. The reaction, which gives direct staining, may be visually

[1] A. G. E. Pearse, *Theoretical and Applied Histochemistry* (Churchill, London 1960).

[2] The simplest and one of the most suitable incubating medium was prepared as follows: Nitro-BT (0.5 mg/ml) 5 ml, 0.1 *M* phosphate buffer at pH 7.3 5 ml, H_2O 10 ml, NADH 10 mg. This must be prepared immediately before use.