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ANTIVIRAL ACTIVITY IN EXTRACTS OF *PHYLLANTHUS FRATERNUS* WEBST (P. NIRURI)

D. V. R. SAIGOPAL, V. SIVA PRASAD and P. SREENIVASULU*
Department of Botany, S. V. University, Tirupati 517 502, India.

MANY natural products are known for their antiviral activity. Highly potent inhibitors of plant viruses have been found to occur in different parts of a large number of plants[1]. But active agents have been characterized in only a few plant extracts. The active agents may be carbohydrates, proteins, glycoproteins, tannins or phenolic compounds. They may act by modifying the test plant susceptibility, competing with virus for entry points, inactivating the virus after combining with it, modifying the host metabolism and/or inhibiting the virus replication. The effect of *P. fraternus* leaf and root extracts on the infectivity of tobacco mosaic (TMV), peanut green mosaic (PGMV)[2] and tobacco ringspot viruses (TRSV)[3] are reported here. *P. fraternus* is known to be used in curing jaundice (viral disease), a human ailment.

TMV, PGMV and TRSV maintained inside the insect-proof wiremesh house by frequent sap inoculation on *Nicotiana tobacum* var. Harrison Special, *Arachis hypogaea* cv TMV-2 and *Vigna sinensis* cv local, respectively, were used in the present work. *Chenopodium amaranticolor* (5–6 leaf stage), *Phaseolus vulgaris* cv local and *V. sinensis* (last two at 2-primary leaf stage) were used as test plants for TMV, PGMV and TRSV, respectively. *P. fraternus* in earthen pots 30 cm dia and all the other plants were raised in earthen pots 15 cm dia containing garden soil. The virus inocula were prepared by grinding in 1 TMV infected tobacco leaf disc (1 cm dia)/5 ml cold 0.01 M, potassium phosphate buffer, pH 7.0 (PPB), 1 g PGMV infected groundnut leaves/10 ml PPB and 6 TRSV local lesions from cowpea/ml PPB in separate mortars. The extracts were passed through two layers of muslin cloth and then used as virus inocula.

Freshly harvested *P. fraternus* leaves and roots were ground separately by using 0.05 M potassium phosphate buffer, pH 7.5 (2 ml/g), and then squeezed through two layers of muslin cloth. To detect the antiviral activity, extracts were mixed separately with different virus inocula in equal amounts, incubated at room temperature for 10 min, and then the mixtures were inoculated by using separate muslin cloth pads on respective test plant leaves dusted with 600-mesh carborundum. The controls consisted of each virus mixed with equal volume of PPB. On separate sets of test plants the leaf and root extracts were applied with cloth pads to the test plant leaves either 24 hr before or 24 hr after virus inoculation. The pretreated plants were washed with distilled water and then inoculated with virus inocula.

For determining the dilution end point of the inhibitors, the extracts were diluted to 1 : 1, 1 : 2, 1 : 5, 1 : 10, 1 : 25 and 1 : 50 with extraction buffer, mixed with equal volumes of virus inocula separately, and then inoculated to the respective test plant leaves. For controls, the virus inocula were mixed with an equal volume of PPB instead of extracts.

To determine the thermal inactivation of the plant extracts, 5 ml of the extracts were kept at room temperature (25–37°C) and at 40–70°C at 5°C interval for 10 min, cooled, mixed with an equal volume of virus inocula and applied to the leaves of the respective test plants.

The percent inhibition was calculated using the formula $(C - T)/C \times 100$, where C is the number of lesions on the control plants and T on the treated plants.

Both leaf and root extracts of *P. fraternus* inhibited the infectivity of the 3 tested viruses but the percent inhibition varied with the virus and the extract (table 1). Both pre- and post-inoculation treatment of leaf and root extracts reduced the infectivity of PGMV almost to the same level, whereas the infectivity of other two viruses varied with the time of application of the inhibitor. Post-inoculation treatment of the inhibitor resulted in higher percent inhibition of TRSV. In general, leaf extract was more inhibitory than root extract, but root extract was most effective when mixed with TMV and PGMV inocula. Among the 3 types of inhibitor treatments, inhibitor mixed with the inoculum was more effective, indicating that inhibitor is probably acting by forming complexes with virus particles and/or at the sites of virus entry besides

Table 1 *Effect of P. fraternus leaf and root extracts on the infectivity of viruses*

Virus	Plant part	Per cent inhibition*		
			Extract applied	
		Extract mixed with virus inoculum	24 hr before virus inoculation	24 hr after virus inoculation
TMV	Leaf	71	68	77
	Root	83	59	59
PGMV	Leaf	84	73	74
	Root	98	63	62
TRSV	Leaf	88	62	80
	Root	62	58	72

* average of three independent experiments.

modifying the test plants in establishing the virus infection. Verma *et al* tested the extracts of 17 medicinal plants on the infectivity of tobacco mosaic, sunhemp rosette, Gomphrena mosaic and tobacco ring spot viruses[4].

The inhibitory effect was not noticed when the crude sap of both leaves and roots was diluted to 1 : 50, and the percent inhibition decreased as the dilution increased with the tested viruses.

Temperature effect varied with the inhibitor source and virus. With PGMV the inhibitory effect of leaf extract was lost at 50°C, and of root extract at 70°C. Both extracts retained very little inhibitory effect even at 70°C against TMV and TRSV. The percent inhibition decreased as the temperature increased. These two physical properties support the presence of inhibitory agent in leaves and roots of *P. fraternus*. Further work is being carried on to characterize the active agent and its mode of action.

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A PHYSICAL METHOD FOR KILLING NOTONECTIDS IN NURSERY PONDS

M. V. MOHAN, M. J. SEBASTIAN
and D. M. THAMPY
College of Fisheries, Kerala Agricultural University, Cochin 682 506, India.

REARING of the spawn to fry stage in nursery ponds is affected by large scale predation due to aquatic insects, particularly the notonectids. The application of different types of emulsions[1-3] is effective to some extent in controlling the population of the air-breathing insects. But the technique cannot be employed during adverse weather conditions like strong wind and continuous rainfall and further, repeated use of chemicals in a delicate ecological system to check the repopulation of notonectids during the early stage of the rearing operation is not desirable. In the field it was observed that notonectids trapped in fry nets and unable to reach the water surface to respire were drowned in a few minutes. Hence, it was felt that some simple physical methods should be devised to check notonectids in small-sized nursery ponds without the ill-effects of chemical treatment.

Backswimmer (*Anisops* sp.) of two size groups 4–5 mm and 8–9 mm was collected from the nursery