Antiviral activity of coralloid root of *Cycas revoluta* extract against some viruses of tomato plant

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Summary. Crude coralloid root extract of *Cycas revoluta* showed significant antiviral activity against viruses of the tomato plant (PVX, PVY, TMV, TAV and TRSV) when applied 24 h before virus inoculation, or when mixed with different virus inocula before virus inocultion, in hypersensitive and systemic hosts. No such inhibition was observed when extract was applied 24 h after virus inoculation. TAV did not show any inhibitory response in a systemic host.
Key words. Viruses, tomato plant; antiviral activity; coralloid root extract.

Several workers have reported antiviral activity of juces from different parts of higher plants including seeds of *Capsicum annuum*[2], leaves of higher plants[3], latex[4] and root of *Boerhaavia diffusa*[5]. The present investigation is concerned with the antiviral activity of a crude extract of coralloid root of *Cycas revoluta*.

Tomato fruits are one of the best sources of protein, vitamins, calcium, phosphorus and other essential minerals for human beings. During the survey of viruses affecting the tomato crop in Eastern U.P. (India), potato virus X (PVX), potato virus Y (PVY), tobacco mosaic virus (TMV), tomato aspermy virus (TAV) and tomato ring spot virus (TRSV) were found to be most noxious. Therefore the search for antiviral agents for these viruses was an essential requirement for the protection of tomato plants.

Cultures of type strains of viruses (PVX, PVY, TMV, TAV and TRSV) obtained from the Indian Agricultural Research Institute, Delhi, were maintained on young actively growing tomato plants in an insect-proof glasshouse. The inoculum of each virus was prepared by grinding 1 g young diseased leaves with distilled water (1 ml/g of leaf material) in a pestle and mortar. The pulp was strained through 2 layers of muslin cloth and centrifuged at 3000 rpm for 15 min. The clear supernatant thus obtained was diluted 1:10 with distilled water and used for inoculation purposes.

The coralloid root of *Cycas revoluta* was crushed with an equal amount of distilled water (w/v 1:1) in a pestle and mortar. The pulp obtained was also filtered through 2 layers of muslin cloth. The filtrate was centrifuged at 3000 rpm for 15 min. The supernatant was diluted 1:1, 1:10, 1:100, 1:500 and 1:1000 with distilled water and used for experimental purposes.

Leaves of test plants were dusted with 600 mesh carborundum powder and virus inoculum was applied with the forefinger on the upper leaf surface. Unless otherwise stated, all 4 fully expanded leaves in hypersensitive hosts and 2 basal leaves in systemic hosts were inoculated.

Inhibition of viruses by coralloid root extracts of *Cycas revoluta*

Virus	Dilution of extract	Percent decrease in virus activity: Extract when mixed with virus before virus challenge		Effect when applied 24 h before virus challenge		Extract when applied 24 h after virus challenge	
		CA	LE$^+$	CA	LE$^+$	CA	LE$^+$
Potato virus X (PVX)	1:1	91[a]	82[a]	90[a]	83[a]	11	3
	1:10	83[a]	72[a]	83[a]	73[a]	8	–
	1:100	70[a]	56[a]	68[a]	53[a]	–	–
	1:500	39[a]	23[b]	30[b]	19[b]	–	–
	1:1000	9	–	–	–	–	–
Potato virus Y (PVY)	1:1	99[a]	93[a]	97[a]	89[a]	5	4
	1:10	96[a]	93[a]	93[a]	82[a]	–	–
	1:100	81[a]	75[a]	82[a]	70[a]	–	–
	1:500	46[a]	40[a]	42[a]	33[b]	–	–
	1:1000	13	4	11	–	–	–
Tobacco mosaic virus (TMV)	1:1	100[a]	94[a]	98[a]	87[a]	9	5
	1:10	74[a]	68[a]	66[a]	62[a]	–	–
	1:100	63[a]	52[a]	58[a]	42[a]	–	–
	1:500	43[a]	33[b]	37[a]	26[b]	–	–
	1:1000	–	–	–	–	–	–
Tomato aspermy virus (TAV)	1:1	78[a]	–	84[a]	–	6	–
	1:10	63[a]	–	72[a]	–	–	–
	1:100	51[a]	–	57[a]	–	–	–
	1:500	46[a]	–	52[a]	–	–	–
	1:1000	14	–	15	–	–	–
Tomato ring spot virus (TRSV)	1:1	100[a]	100[a]	100[a]	100[a]	11	9
	1:10	100[a]	100[a]	100[a]	97[a]	3	–
	1:100	86[a]	74[a]	87[a]	70[a]	–	–
	1:500	67[a]	62[a]	66[a]	59[a]	–	–
	1:1000	20	18	14	–	–	–

Differences due to treatment with extract are significant:
[a] at 1% level and [b] at 5% level. CA, *Chenopodium amaranticolor*, LE, *Lycopersicum esculentum* (tomato),
[+] the active virus from systemic host (tomato) was assayed on *C. amaranticolor*, 15 days after inoculation.

To detect viral inhibitory activity in the coralloid root extract three methods were used. (1) Extract was with different virus inocula in equal amounts and kept for 15 min. Then the mixture was inoculated on 10 leaves of *Chenopodium amaranticolor* plants (the control consisted of each virus mixed with equal volume of distilled water instead of the extract). (2) Extract was applied on 10 leaves of *C. amaranticolor* 24 h before virus inoculation. (3) Extract was applied on 10 leaves of *C. amaranticolor* 24 h after virus inoculation. Leaves of control plants were similarly rubbed with distilled water instead of the extract. Lesions were counted 7 days after virus inoculation in the case of PVX, TMV and TRSV; and on the 10th day after virus inoculation in the case of PVY and TAV.

All the experiments were performed in an insect-free glasshouse at about 24 ± 6 °C (63% humidity, 16-h-day and 18,000 lx). The data were analyzed statistically by the test of comparison between the control and the individual treatment to test for the significance of the activity of the extract[6]. Percent inhibition was calculated by the formula (C-T)/C × 100, where C is the number of lesions on control leaves and T is the number of lesions on treated leaves. The result of virus inhibition of tomato plants by the coralloid root extract are summarized in the table.

The results in the table indicate that extracts of coralloid root of *Cycas revoluta* showed significant inhibitory activity (19–100%) at different dilutions of extract when applied 24 h before virus challenge or when mixed with virus inoculum before virus challenge; no such inhibition was observed when the extract was applied 24 h after virus challenge in either hypersensitive or systemic hosts. The extract did not show any inhibitory activity in a systemic host (tomato) against TAV. The root extract was completely inactivated at 1:1000 dilution.

The average number of local lesions varies with the viruses viz. 82 ± 11.6, 68 ± 9.3, 83 ± 5.2, 96 ± 6.8, 76 ± 9.1 produced on *C. amaranticolor* by PVX, PVY, TMV, TAV and TRSV respectively in control sets of plants (without inhibitor). The 100% protected plants were found to be disease free.

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Effects of TA [4-ethoxy-1-(p-tolyl)-s-triazine-2,6-(1H,3H)-dione] on growth, antheridium differentiation and gibberellin uptake of gametophytes of *Anemia phyllitidis* L. Sw.

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Summary. Synergistic effects of TA on gibberellin-dependent reactions in spermatophytes are not detectable in gametophytes of the fern *Anemia phyllitidis* where gibberellin substitutes for antheridiogens with regard to induction of male sexual organs and dark germination. In this archegoniate TA caused a significant reduction in growth rate and morphogenesis of the gametophytes, inhibitions that were not abolished by simultaneous application of gibberellin.

Key words. Fern; *Anemia phyllitidis;* antheridium differentiation; TA (4-ethoxy-1-(p-tolyl)-s-triazine-2,6-(1H,3H)-dione); gibberellin uptake.

Some derivatives of isourea and triazinone respectively show synergistic activity with gibberellins in the rice seedlings test[1-3]. Since there is increasing evidence for a close relationship between the sexual pheromone system of schizaeaceous ferns and the gibberellin reactions in higher plants[4,5] it seemed of interest to investigate the mutual effects of gibberellins and triazinones in the *Anemia* bio-assays (method: ref. 6).

In the *Anemia* system TA shows no synergistic reaction with gibberellin A_3 (GA_3) either on antheridium differentiation or dark induction of spore germination (tables 1, 2). Instead of stimulating these processes, as it does in rice seedlings, the triazinone derivative cause a significant retardation of cell division in spores of *A. phyllitidis* when applied to the medium in concentrations ranging from 10^{-5} to 10^{-4} M (table 1). This inhibition was not reversed by simultaneous application of GA_3. In addition to a reduction of the rate cell division, TA showed morphogenetic effects in delaying the induction of two-dimensional growth leading to elongated gametophytes (fig. a + b). If one presupposes an identity of the primary reactions in the fern and spermatophyte system, the synergistic effect of TA

Table 1

TA (M)	day 12 cell number	d_{50}	CCN
0	16.4 ± 0.2	8	12.2 ± 0.1
10^{-5}	14.0 ± 0.2	8	12.1 ± 0.2
5×10^{-5}	10.8 ± 0.1	9	11.2 ± 0.1
10^{-4}	8.2 ± 0.1	11	11.5 ± 0.2

Effects of TA on cell division and of TA and GA_3 on antheridium induction and critical cell numbers (CCN) in *A. phyllitidis* (continuous white light; 22 ± 0.1 °C). d_{50}, days until 50% of the prothallia differentiate antheridia.

Table 2

TA (M)	% germination
0	32.8
10^{-6}	34.5
10^{-5}	27.6
10^{-4}	23.1

Effects of TA and GA_3 (10^{-6} g/ml) on dark germination of spores of *A. phyllitidis*. % germination after 10 days of culture (21 ± 0.1 °C).