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Existence of Antiviral Principles (AVPs) in Seed Extracts of *Harpullia cupanioides* (Roxb.) against Tomato Spotted Wilt Virus (TSWV), Rice Tungro Virus (RTV) and Cowpea Aphid Borne Mosaic Virus (CABMV)

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Seed extract of *Harpullia cupanioides* was tested against infection by Tomato spotted wilt virus (TSWV), Rice tungro virus (RTV) and Cowpea aphid borne mosaic virus (CABMV). Seed extract of *H. cupanioides* (10%) when sprayed on test plants 24 h before virus inoculation significantly inhibited virus infections. Percentage inhibition of TSWV in local lesion host by 10% seed extract of *H. cupanioides* was found to be 90.9. Pre-inoculation application of 10% seed extract of *H. cupanioides* provided 87.5% inhibition of CABMV infection. The seed extract (10%) sprayed rice plants recorded RTV infection of 48% as against 100% in control.

Keywords: Antiviral activity, plant extracts.

Antiviral factors have been detected in several plants including *Mirabilis jalapa* (Kubo et al., 1990; Takanami et al., 1990; Vivanco et al., 1999), *Chenopodium murale* (Srivastava and Verma, 1995), *C. amaranticolor* (Alberghina, 1976), *Vitis vinifera* (Erkan and Yorganci, 1982), *Bougainvillea spectabilis* (Verma and Dwivedi, 1984) and *Crotalaria juncea* (Velazhahan and Narayanasamy, 1991). These virus inhibitors are proteins (Taniguchi and Goto, 1976), glycoproteins (Verma et al., 1979) or polysaccharides (Singh et al., 1970; Worms and Nienhaus, 1975). An antiviral protein purified from *Mirabilis jalapa* is a basic type 1 Ribosome-inactivating protein (RIP) with a molecular weight of 24.2 kDa (Takanami et al., 1990). This Mirabilis antiviral protein (MAP) has been shown to inhibit the mechanical transmission of tomato mosaic virus in tobacco, tomato and pepper plants and cucumber green mottle mosaic virus in cucumber plants (Kubo et al., 1990). The genes coding for RIPs in *Phytolacca americana* have been cloned and the expression of RIP gene in transgenic potato and tobacco plants has been shown to increase resistance against broad spectrum of viruses (Lodge et al., 1993). Recently, we partially purified an antifungal protein from the seeds of *Harpullia cupanioides,* belonging to Sapindaceae

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family. The purified protein strongly inhibited the growth of several agronomically important fungal pathogens viz., *Rhizoctonia solani, Curvularia lunata, Colletotrichum musae* and *Alternaria alternata* (Bharathimatha et al., 2002). In the present study, the seed extract of *H. cupanioides* was tested for virus-inhibitory activity against Tomato spotted wilt virus (TSWV), Rice tungro virus (RTV) and Cowpea aphid borne mosaic virus (CABMV).

Materials and Methods

Preparation of H. cupanioides *seed extract*

Seeds of *H. cupanioides* were collected at the botanical garden of the Tamil Nadu Agricultural University, Coimbatore. Seeds were ground in a mixer and 1 g of the resulting ground seed was extracted in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0) for overnight at 4 °C. The homogenate was passed through two layers of muslin cloth and then centrifuged at 5,000 g for 15 min and the supernatant was used in this study.

Antiviral activity of H. cupanioides *seeds extract against TSWV*

TSWV isolate was maintained on cowpea (*Vigna unguiculata* L., cv. C-152) plants under glass house conditions. The virus was retrieved by macerating the cowpea leaves showing typical local lesions in a pre-chilled pestle and mortar with chilled 0.1 M phosphate buffer (pH 7.0) at the rate of 1 ml per 10 local lesions (Velazhahan and Narayanasamy, 1991). The extract was passed through muslin cloth and the filtrate was used as standard inoculum. Two dilutions of the *H. cupanioides* seed extract i.e. 5 and 10 per cent, were prepared and sprayed on the primary leaves of 7-day-old cowpea (cv. C-152) until run off. One day after spraying the seed extract, the cowpea plants were inoculated with the virus inoculum by gently rubbing the primary leaves previously dusted with 600 mesh carborundum. The plants were rinsed with distilled water shortly after inoculation to remove excess inoculum and carborundum. Plants sprayed with buffer served as control. In another set of plants, the virus was inoculated first followed by spraying of *H. cupanioides* seed extract at 24 h after inoculation. The development of local lesion was recorded 5 days after inoculation. Five plants in a pot formed one replication and each treatment was replicated five times. The experiment was repeated three times. The percent inhibition over control was calculated using the formula

$$I = \frac{(C–T)}{C} \times 100$$

Where
I = Percent inhibition of lesion formation over control
C = Number of local lesions in control
T = Number of local lesions in plants treated with *H. cupanioides* seed extract

Antiviral activity of H. cupanioides *seed extract against RTV*

The seed extracts of *H. cupanioides* were sprayed on 10–15 days old rice seedlings (cv. TN 1) with a glass atomizer until run off. After 24 h, the sprayed plants were inoculated with viruliferous *Nephotettix virescens* at the rate of 2 insects per plant (pre-inoculation application). In another set of plants, the virus was inoculated first followed by spraying of *H. cupanioides* seed extract at 24 h after inoculation. The number of plants infected and per cent infection were calculated 15 days after inoculation. Five plants in a pot formed one replication and each treatment was replicated five times. The experiment was repeated three times.

Antiviral activity of H. cupanioides *seed extracts against CABMV*

The culture of CABMV was maintained on cowpea (cv. C-152) plants. Virus inoculum was prepared by grinding virus infected young leaves in chilled 0.1 M phosphate buffer (pH 7.0) using a chilled pestle and mortar at the rate of 1 ml/g of infected tissue. The sap was squeezed through a thin layer of cotton wool and the supernatant was then used as the inoculum. The primary leaves of cowpea were inoculated with the inoculum as described previously for TSWV. Observations were made 15 days after inoculation for systemic infection of cowpea aphid borne virus. Five plants in a pot formed one replication and each treatment was replicated five times. The experiment was repeated three times.

Results and Discussion

The presence of antiviral priciples (AVPs) in plants against a number of viruses has been reported by several workers (Alberghina, 1976; Srivastava and Verma, 1995; Louis and Balakrishnan, 1996; Bharathi, 1999; Manickam and Rajappan, 1999). These inhibitors are reported to inhibit either virus infection or virus multiplication (Vivanco et al., 1999). Some plant extracts are known to induce virus-inhibiting agents (VIA) which act locally around the area of application (Verma and Baranwal, 1983) or induce systemic resistance in plants against viruses (Verma and Awasthi, 1979; Verma et al., 1979). Verma and Dwivedi (1984) reported that a highly active virus inhibiting agent developed systemically in a number of plants after treating their basal leaves with leaf extract of *Bougainvillea spectabilis* and the amount of the inhibiting agent reached the maximum 24 h after treatment. AVPs have been detected mostly in leaves (Srivastava and Verma 1995; Louis and Balakrishnan, 1996; Manickam and Rajappan, 1999) and roots (Verma et al., 1979). The results of the present study revealed that seed extracts of *H. cupanioides* were highly effective in inhibiting the infection of TSWV, CABMV and RTV at 10 per cent concentration. Percentage inhibition of TSWV in local lesion host by 10% seed extract of *H. cupanioides* was found to be 90.9. Pre-inoculation application of 10% seed extract of *H. cupanioides* provided 87.5% inhibition of CABMV infection. The seed extract (10%) sprayed rice plants recorded RTV infection of 48% as against 100% in control. Pre-inoculation application with *H. cupanioides* seed extract was found to be more effective in

Table 1

Inhibitory effect of *H. cupanioides* seed extracts against Tomato spotted wilt virus (TSWV), Rice tungro virus (RTV) and Cowpea aphid borne mosaic virus (CABMV)

Treatments	Concentration (%)	TSWV		RTV		CABMV	
		No. of local lesions	% inhibition over control	Per cent infection	% inhibition over control	Per cent infection	% inhibition over control
Pre-inoculation application	5	24.25^{c}	73.4	56.0b^{c} (48.46)	44.0	15.00^{c} (22.50)	85.0
	10	11.7^{d}	90.9	48.0^{c} (43.84)	52.0	12.50^{c} (20.46)	87.5
Post-inoculation application	5	89.25^{b}	26.6	72.0^{b} (58.36)	28.0	65.00^{b} (53.77)	34.9
	10	85.25^{b}	29.6	68.0b^{c} (55.83)	32.0	65.00^{b} (53.77)	34.9
Control		121.00^{a}	–	99.9^{a} (89.88)	–	99.99^{a} (89.85)	

Means with in the column followed by same letter are not significantly different (P = 0.05) by Duncan's multiple range test.
Figures in parentheses are arcsine transformed values

inhibiting the viruses than post-inoculation application. Similar results were reported by other researchers. Manickam and Narayanasamy (1996) reported that AVPs from aqueous leaf extracts of *Cocos nucifera, Sorghum vulgare, Prosopis chilensis* and *Croton sparsiflorus* when applied before challenge inoculation induced systemic acquired resistance in the test plant and increased the activities of catalase, peroxidase and phenylalanine ammonia-lyase. Verma and Mukerjee (1979) found that *Datura metel* leaf extract significantly inhibited TMV infection when applied 24 h before virus inoculation. Louis and Balakrishnan (1996) also reported that pre-inoculation application of *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* was better than post-inoculation application in inhibiting pumpkin mosaic virus (PMV) infection. Srivastava and Verma (1995) demonstrated that leaf extract of *Chenopodium murale* gave maximum inhibition of Tobacco mosaic virus (TMV) and Sunhemp rosette virus (SRV) in hypersensitive hosts when applied 1 h before inoculation. The present study suggests the possibility of the use of *H. cupanioides* seed extract for the management of TSWV, RTV and CABMV. Further studies on the physical properties of the inhibitors and characterization of AVPs are in progress.

Literature

Alberghina, A. (1976): The inhibitory activity of extracts of *Chenopodium amaranticolor* leaves on the infection by tobacco necrosis virus. Phytopath. Z. 87, 17–27.

Bharathi, M. (1999): Effect of plant extract and chemical inhibitors on cucumber mosaic virus of brinjal. J. Mycol. Plant Pathol. 29, 57–60.

Bharathimatha, C., Sabitha Doraiswamy and Velazhahan, R. (2002): Inhibition of fungal plant pathogens by seed proteins of *Harpullia cupanioides* (Roxb). Acta Phytopathol. Entomol. Hung. 37, 75–82.

Erkan, S. and Yorganci, U. (1982): Investigations on the inhibition of potato Virus X (PVX) infectivity by some plant extracts. J. Turk. Phytopathol. 11, 61–75.

Kubo, S., Ikeda, T., Imaizumi, S., Takanami, Y. and Mikami, Y. (1990): A potent plant virus inhibitor found in *Mirabilis jalapa* L. Ann. Phytopathol. Soc. Japan 56, 481–487.

Lodge, J. K., Kaniewski, J. K. and Tumer, N. E. (1993): Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. Proc. Natl. Acad. Sci., USA 90, 7089–7093.

Louis, V. and Balakrishnan, S. (1996): Effect of application of selected medicinal plant extracts on the incidence of pumpkin mosaic virus. Indian Phytopath. 49, 373–377.

Manickam, K. and Narayanasamy, P. (1996): Induction of resistance to tomato spotted wilt virus in mungbean [*Vigna radiata* (L.) Wilczek] by antiviral principles. Acta Phytopathol. Entomol. Hung. 31, 199–204.

Manickam, K. and Rajappan, K. (1999): Field efficacy of plant extracts and chemicals against greengram leaf curl disease. Indian J. Virol. 15, 35–37.

Singh, R. P., Wood, F. A. and Hodgson, W. A. (1970): The nature of virus inhibition by a polysaccharide from *Phytophthora infestans.* Phytopathology 60, 1566–1569.

Srivastava, N. and Verma, H. N. (1995): *Chenopodium murale* leaf extract, a potent virus inhibitor. Indian Phytopath. 48, 177–179.

Takanami, Y., Kuwata, S., Ikeda, T. and Kubo, S. (1990): Purification and characterization of antiviral protein from *Mirabilis jalapa* L. Ann. Phytopathol. Soc. Japan 56, 488–494.

Taniguchi, T. and Goto, T. (1976): Purification of an inhibitor of plant virus infection occurring in leaves of *Chenopodium amaranticolor.* Ann. Phytopathol. Soc. Japan 42, 42–45.

Velazhahan, R. and Narayanasamy, P. (1991): Inhibition of tomato spotted wilt virus (TSWV) by plant extracts. Sci. and Cult. 57, 141–142.

Verma, H. N. and Awasthi, L. P. (1979): Antiviral activity of *Boerhaavia diffusa* root extract and the physical properties of the virus inhibitor. Can. J. Bot. 57, 926–932.

Verma, H. N. and Baranwal, V. K. (1983): Antiviral activity and the physical properties of the leaf extract of *Chenopodium ambrosoides* L. Proc. Ind. Acad Sci. (Plant science) 92, 461–465.

Verma, H. N. and Dwivedi, S. D. (1984): Properties of a virus inhibiting agent, isolated from plants which have been treated with leaf extracts from *Bougainvillea spectabilis.* Physiol. Plant Pathol. 25, 93–101.

Verma, H. N. and Mukerjee, K. (1979): Induction of antiviral resistance in host plants by Datura leaf extract. Indian Phytopath. 32, 95–97.

Verma, H. N., Awasthi, L. P. and Saxena, K. C. (1979): Isolation of the virus inhibitor from the root extract of *Boerhaavia diffusa* inducing systemic resistance in plants. Can. J. Bot. 57, 1214–1217.

Vivanco, J. M., Querci, M. and Salazar, L. F. (1999): Antiviral and antiviroid activity of MAP-containing extracts from *Mirabilis jalapa* roots. Plant Dis. 83, 1116–1121.

Worms, G. and Nienhaus, F. (1975): Characterization and effect of virus inhibiting polysaccharide from cabbage plants. Phytopath. Z. 82, 224–245.