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Antifungal activity of plant latex towards certain fungal organisms

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The paper describes the effect of latex from seven plant species on conidial germination and growth of germ tubes as well as on mycelial growth and sporulation of several fungi comprising of both saprophytes and plant pathogens. Latex from *Jatropha gossypifolia. J. tanjorensis*, and *Carica papaya* were found to be highly fungitoxic. The latex from *J. gossypifolia* inhibited condial germination in *Helminthosporium oryzae* and *Alternaria brassicicola* by 100%. Towards other test fungi also, the latex was significantly inhibitory. A fair level of fungitoxicity of this latex was retained even after 25-fold dilution. Latex from *J. gossypifolia* when tested on *H. oryzae* with respect to growth and sporulation on agar medium was found to suppress both the processes significantly.

Key Words : *Latex, Carica papaya, Calotropis gigantia, Alstonia scholoris, Euphorbia terucalli, Nerium odorum, Jatropha tanjorensis, Jatropha gossypifolia, antifungal activity*

INTRODUCTION

A large number of plants produce latex which contains flavonoids, saponins, cyanogenic glycosides, alkaloids, terpenoids proteins, resins, amino acids and other minor compounds (Kretovich, 1966; Rizk, 1986). Latex from a number of plants are of great economic importance and commercially exploited for production of rubber and fuel (Nielson, 1977; Weisz, 1979). Latex of several plants has been shown to possess antifungal activity (Saxena and Saksena, 1981; Gourinath and Monohararchary, 1988). The objectives of the present investigation were i) to examine the fungitoxic activity of latex of some plant species on pathogenic and non-pathogenic fungi and ii) to study factors affecting the antifungal activity of latex.

MATERIALS AND METHODS

Latex was collected from seven species of plants viz. *Carica papaya, Calotropis gigantia, Alstonia scholaris, Euphorbia terucalli, Jatropha tanjorensis, J. gossypifolia and Nerium odorum.* A 10% aqueous solution of latex formed the standard solution and it was designated as 'X' from which further dilutions were made as required by adding requisite amounts of sterile water. Fungitoxicity of latex solution were tested by the slide germination bioassay technique (APS, 1943), using propagule suspensions of five fungi : *Helminthosporium oryzae, Alternaria brassicicola Fusarium udum, Aspergillus niger* and *Penicillium digitatum.* Five microliters of spore suspensions were placed in groves of cavity slides on which 0.1 ml of latex solutions were applied. Spore germination and germtube length were recorded after 24 h incubation at 28°C. Spores taken in distilled water served as controls. Percent inhibition was calculated by comparing the values obtained in the control, for spore germination and germ tube growth.

The effect of latex on mycelial growth of fungi was tested by incorporating filter-sterilized solutions of latex into agar medium dispensed into sterlized petridishes. These were inoculated at the centre by placing mycelial discs (5 mm diam.) of test-fungi. The inoculated agar medium without any latex solution served as control. Radial growth of fungal colony was measured after 4 days of incubation at 28°C. Percent inhibition of mycelial growth was calculated using the formula of Vincent (1927). The extent of sporulation in fungi growing on latex-ammended agar medimum and in control was tested by taking 10-mm mycelial discs from 8-day old sporulating culture, suspending them in 2 ml of distilled water and vigorously shaking the water. From the resulting spore suspensions, 0.01 ml aliquots were examined microscopically to enumerate the number of spore per microscopic field in low power magnification using a haemocytometer.

RESULTS AND DISCUSSIONS

An initial experiment showed that latex from selected plants were highly toxic and therefore for comparing the antifungal activity, the standard solutions (10% or 'X') of latex were diluted 4-fold (x/4) and bioassayed. From bioassay results (Table 1) it was evident that latex from *C. papaya*, *J. tanjorensis* and *J. gossypifolia* were highly inhibitory to condial germination and germ tube growth of *H. oryzae* and *A. brassicicola*. From a comparison of inhibitory activity it was clear that latex from these three plants caused 100% inhibition of conidial germination and germ tube growth of *H. oryzae* and *A. brassicicola.* From a comparison of inhibitory activity it was clear that latex from these three plants caused 100% inhibition of conidial germination at 1:4 dilution. It was also noted that latex from these species exerted less inhibitory effect on *F. udum, A. niger* and *P. digitatum*, nevertheless the fungitoxicity towards these fungi was substantially high (56-69% inhibition in germination and 43-62% in germ tube length). Latex from *C. gigantia, A. scholaris* and *E. terlucalli* were however mildly fungitoxic since they caused 10-22% inhibition of spore germination and 6-12% in germ tube length. Results further indicated that latex of *J. gossypifolia* was most fungitoxic and that *H. oryzae* was most sensitive to latex from this species.

Table 1. *In vitro* screening of latex from several plants for their antifungal activity measured in terms of inhibition of conidial germination and germtube lengths in some fungal organisms

Source of latex[a]	Inhibition of conidial germination and germ tube length (% over control in water)									
	HO		AB		FU		AN		PD	
	G[c]	GT[c]	G	GT	G	GT	G	GT	G	GT
Carica papaya	100[b]	100[b]	100	100	56	62	69	52	66	43
Jatropha tanjorensis	100	100	100	100	66	39	41	33	45	39
J. gossypifolia	100	100	100	100	78	45	32	25	47	42
Calotropis gigentia	19	12	22	17	9	7	10	6	16	9
Alstonia schloaris	21	10	19	12	10	8	12	5	13	7
Euphorbia tirucalli	17	8	15	11	12	6	9	7	13	6

a A 10% aqueous solution of latex was considered as 'X' from which four-fold (X/4) dilution was made and bioassayed using conidia of fungi indicated. HO = *H. oryzae*, AB = *A. brassicicola*, FU = *F. udum*, AN = *A. niger* & PD = *P. digitatum.*

b Data based on average of 50 observations for conidial germination and 25 for germ tube length. Percent inhibition values were calculated with respect to germination and germ tube lengths recorded in water as control.

c G = Germination and GT = Germ tube length.

Latex contains a variety of plant metabolites. Those which have been reported as fungitoxic belong to the classes of flavonoids, coumarins, steroids, alkaloids, terpenes, cyanogenic glycosides (Mitra *et al*, 1984). The inhibitory activity of latex in the present study may be attributed to the presence of such compounds of diverse chemical nature in latex, in high level in two species of *Jatropha* under the present study. *Jatropha* spp. have been reported to contain phenolic compounds like lignans, coumarins and steroids (Mitra *et al.*, 1984) which have distinct antifungal activity. Besides these, purine alkaloids have been detected in *Jatropha*. Thus the strong supression of spore germination and germ tube growth of test fungi in *Jatropha*-latex may result from the combined inhibitory effect of several such compounds present in latex of two spp. of *Jatropha* under the family Euphorbiaceae in which plants in general are known to be extremely toxic (*Rizk*, 1987).It was interesting to note that *Jatropha* latex was fairly less inhibitory to *F. udum*. This was perhaps due to its ability to detoxify some of toxic metabolites present in latex like *F. avanaceum* which was shown to degrade avanacin A to its non-toxic form (Luning *et al*, 1978).

Latex from papaya fruits was previously shown to be inhibitory towards several pathogenic and non-pathogenic fungi (Saxena and Saksena, 1981) and our results confirm the earlier report. Fungitoxic property of papaya latex may be attributed to the presence of quite high level of proteolytic enzyme besides other compounds. Latex from *A. scholaris*, *E. terucalli* and *N. odorum* caused mild inhibition of spore germination and germtube growth in all the test fungi and this probably resulted from the presence of low levels of fungitoxic substances in their latex or to differential sensitivity of the fungi towards such substances.

Table 2. Percentage inhibition of conidial germination in *H. oryzae* and *A. brassicicola* in aqueous solutions of plant latex as a function of dilution

Source of latex	Dilutions of latex	Bioassayed on conidia of			
		H. oryzae		*A. brassicicola*	
		G[c]	GT[c]	G	G.T.
Carica papaya	X/8[a]	37[b]	43[b]	30	11
	X/16	17	21	12	7
	X/20	5	9	5	0
	X/25	0	0	0	0
Jatropha	X/8	100	100	100	
tanjorensis	X/16	95	89	81	72
	X/20	77	69	56	49
	X/25	19	34	12	30
Jatropha gossypifolia	X/8	100	100	100	100
	X/16	100	100	89	78
	X/20	89	82	52	49
	X/25	22	43	14	36

a A 10% aqueous solution of latex was designated as 'X' and X/8 dilution indicates 8-fold dilution and so on.

b Figures represent percentage inhibition with respect to condial germination and germ tube length in distilled water (control). Each figure is an average of three replicate observations on 100 conidia and 50 germ tubes.

c G = Germination, G.Tt. = Germtube length.

Since the screening of latex from the selected plant species showed that those from *C. papaya*, *J. tangorensis* and *J. gossypifolia* were extremely antifungal, an experiment was done to find out the dilution upto which their latex could retain fungitoxic effect. For this, the standard solutions (x) of latex from the three species were diluted with water from x/8 to x/25 and aliquotes were bioassayed against conidia of *H. oryzae* and *A. brassicicola*. Results (Table 2) showed that with gradual increase in dilution, the inhibitory effect of latex decreased progressively so that at X/20 dilution, only small supression of spore germination (5%) and germtube length (0.9%) were observed, in most sensitive bioassay organism, *H. oryzae*, in papaya latex. The inhibitory activity of *Jatropha* latex decreased considerably, but substantially higher level of inhibition was still noted.

Even at X/25, the latex of *J. gossypifolia* showed 22% inhibition in spore germination and 43% in germbube length. Results with respect to the effect of *Jatropha* latex on mycelial growth and sporulation of *H. oryzae* (Table 3) showed that latex was inhibitory towards vegetative growth as well as to sporulation at all three dilutions tested, recording 18 to 49% reduction over the control for the former and from 16-63% for the latter. Therefore the results were fairly comparable to those of spore germination studies. As already stated, the presence of toxic plant metabolites in latex might probably be the inhibitory activity on both mycelial growth and sporulation.

Table 3. Inhibition of mycelial growth and sporulation in *H. oryzae* on agar medium containing Latex of *J. gossypifolia*

Final dilution of latex in agar medium	Bioassayed on *H. oryzae*	
	Colony diameter (MM)	Sporulation (Spores/Sq.mm.
X/4[a]	25.5[b] (-49)[d]	1120
X/8	33 (-34)	1920
X/16	41 (-18)	2560
Water (Control)	50	3040

a A 10% aqueous solution of latex was considered as 'X' from which make calculated amounts were taken in petridishes so as to make final dilutions as indicated in solidifield potato dextrose agar (PDA) medium.

b Colony diameters (in mm.) recorded after 4 days' growth on PDA at 28°C in dark

c Mycellial discs (8-mm dia.) taken from sporulating agar cultures after 6 days' growth were immersed in 2 ml water, Shaken vigorously for 1 minute and then 10 ml aliquots taken out for microscopic observation. Figures represent the average number of conidia observed per sq.mm on agar medium

d Figures in parentheses indicate percent intibition with respect to control

An experiment was done to test the effect of temperature on inihibitory effect of latex from *J. gossypifolia*. Diluted (x/16 and x/20) latex solutions were maintained at 40, 60, 80, 100 and 120°C for 20 minutes, cooled and then bioassayed in the usual way using conidia of *H. oryzae*. From the results (Table 4) it appeared that temperature upto 80°C had not much adverse effect on inhibitory property of the latex. Treatment of 16 fold dilute latex at this temperature caused 94.8% inhibition of spore germination and 90% inhibition of germtube growth. However, beyond this range, antifungal activity was considerably lowered. The results thus indicated that the fungitoxic compounds present in latex of *J. gossypifolia* were fairly thermostable and possibly was not enzymatic in nature.

Table 4. Effect of temperature on antifungal activity of latex from *J. gossypifolia* as measured in terms of conidial germination and germ tube length of *H. oryzae*

Temperature (°C)	Dilutions of latex	Bioassayed on conidia of *H. oryzae* for their	
		Germination (%)	Germ tube length (mm)
40	X/16	0 (-100)	0 (-100)
	X/20	12 (-87)	8 (-95)
60	X/16	0 (-100)	0 (-100)
	X/20	11 (-88)	10 (-94.0)
80	X/16	5 (-94.8)	12 (-93)
		12 (-87.7)	14 (-91.0)
100	X/16	41 (-58)	32 (-81)
	X/20	52 (-47)	36 (-78)
120	X/16	51 (-48)	41 (-75.0)
	X/20	60 (-38.7)	49 (-70)
Latex at 28°C	X/16	0 (-100)	0 (-100)
(Control)	X/20	12 (-87.5)	9 (-94.6)
Water		99	168

Legends as in Table-2

Table 5. Effect of pH on the antifungal activity of *Jatropha* latex towards *H. oryzae* measured in terms of conidial germination and germ tube length

pH	Bioassayed on *H. oryzae* for			
	Conidial germination (%)		Germ tube length (mm)	
	Buffer [a]	Buffered Latex (X/16)[b]	Buffer	Buffered Latex (X/16)
4	99	0	170	–
5	100	0	165	–
6	98	12(-87.7)[c]	172	52(-70)
7	99	11(-88.8)	169	60(-65)
8	98	37(-62.2)	145	79(-46)
9	100	51(-49)	133	86(-35)

a For pH 4 & 5 (.005 M) citrate, for pH, Phosphate (.005M) & for 8 & 9, Tris-HCl (.005M) buffers were used.

b A 10% aqueous solution of latex from *Jatropha gossypifolia* was dessignated as 'X' and X/16 indicates 1:15 dilution of 'X' made with the buffers mentioned above.

c Figures in parentheses indicate percent inhibition of germination and germ tube lengths as compared to control in buffers indicated.

The effect of pH on antifungal activity of the latex from *J. gossypifolia* was tested. The standard solution (x) of latex was diluted using several buffers of low molarity as diluent, such as citrate for pH 4 and 5; phosphate for pH 7 and Tris-HCl buffer for pH 8 and 9.

Same buffers without latex acted as contrds. From the result (Table 5) it was evident that the latex exerted strong inhibitory activity towards *H. oryzae* under acidic condition at pH 4 and 5 with slight losss of activity at pH 6 and 7. In the alkaline range of pH 8 and 9, the latex solutions recorded rather considerable decrease in inhibitory property towards spore germination and germ tube growth.

Further works are in progress regarding extraction and purification of chemical components in the latex of *J. gossypifolia*. The physico-chemical and biological properties of latex from this plant species warrant deeper study.

REFERENCES

APS (1943) American Phytopathological Society, Committee on Standardization of Fungicidal Tests, 1943 : The slide-germination method of evaluating protectant fungicides. *Phytopathol.* **33** : 627-634.

Gourinath, A and Monoharachary, C. (1988). Effect of latex on four pathogenic fungi. In "Research in Plant Sciences and its relevance to future", International Conference, March 7-11, 1988, Deptt. of Botany, Delhi University, Delhi.

Kretovich (1966). *Principles of Plant Biochemistry*. Pergamon Press, London.

Luning, H.U., Waiyaki, B.G. and Schlosser, E. (1978). Role of saponins in antifungal resistance VIII. Interactions *Avena sativum Fusarium avenaceum*. *Phytopathol. Z.* **92** : 338-345.

Mitra, S.R., Choudhuri, A and Adityachaudhury, N. (1984). Production of antifungal compounds by higher plants - a Review of Recent Researches. *Pl. Physiol and Biochem.*, **11** : 53-88.

Nielsen, P.E., Nishimura, H., Otovos, J.W. and Calvin, M. (1977). Plant crops as source of fuel and hydrocarbon-like materials. *Science*, **198** : 942-944.

Rizk, A.M. (1987). The chemical constituents and economic plants of the Euphorbiaceae. *Botanical Jour. Linnean Soc.* **94** : 293-326.

Saxena, A.K. and Saksena, S.B. (1981). Effect of papaya latex on the germination of conidia of certain fungi. *Ind. Phytopath.* **34** : 305-306.

Vincent, J.M. (1927). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, **159** : 850.

Weisz P.B. and Marshall, J.F. (1979). High grade fuels from biomass farming : Potential and Constraints. *Science*, **206** : 24-29.

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