

BRIEF COMMUNICATION

Alleviation of plant virus infection by humic acids

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Abstract

K-humates, obtained from oxihumolites, alleviate infection of tobacco with tobacco mosaic virus both in mixture with virus inoculum and by spraying of leaves before inoculation. However, applications of K-humates after inoculation did not influence the virus infectivity.

Key words: *Nicotiana tabacum*, tobacco mosaic virus

Humic acids (HA) form a polydispersed, disordered heterogenous system of macromolecules of different composition and structure according to their origin and preparation. The presence of acid groups is responsible for their character of polyelectrolytes with the possibility to form complexes with different metals and organic compounds. Fractal geometry has been found to be a useful tool to describe their properties (Senesi 1992). They represent globular colloids with high surface activity (Ziechmann 1980, Pospíšil 1992). Their structure is pH dependent: at lower pH values the structures become more compact and less porous (Senesi *et al.* 1992).

Relatively unclear remains the impact of HA on metabolism and growth of plants despite number of reports documented both direct and indirect effects. Only one report deals with inhibition effect of oxidized phenolic acids, gallic acid and Na-humate on influenza virus A (Mentel *et al.* 1983).

The aim of our work was to find out possible effects of HA isolated from oxihumolites upon plant virus infectivity.

Oxihumolites were sampled from the mine "Hrabák" in Nord-West Bohemia. Humic substances were extracted with 0.1 M NaOH. Na-humates were centrifuged at 10 000 g, clear solution was acidified to pH 2.0 with diluted sulphuric acid. The coagulated humic acids were centrifuged, washed with water, and adjusted with

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0.1 M KOH to a pH 6.5 - 7.0 and then were mixed with cation-exchange resin as described earlier (Pospíšil 1971). Humic acids were adjusted with 0.1 M KOH to a pH 7.0. Resulting K-humates at the basic concentration of 2 g dm⁻³ were used in our experiments. Humic acids contained 6 - 10 % of ash, the absorbance ratio 400/600 nm was 5.4, -COOH content was 2.8 - 3.5 meq g⁻¹, total acidity > 3.5 meq g⁻¹.

Experiments were carried out with the common green strain of tobacco mosaic virus (TMV). The virus was maintained in *Nicotiana tabacum* L. cv. Samsun grown in sterilized soil in an insectproof greenhouse.

For experiments crude infectious sap clarified by low speed centrifugation (12 000 g) or purified TMV preparation made by polyethyleneglycol precipitation according to Gooding and Hebert (1967) suitably diluted with distilled water were used.

Quantitative assays of relative TMV infectivity were performed on leaves of *N. tabacum* cv. Xanthi nc. having hypersensitive response to TMV infection, forming defined countable local necrotic lesions.

The virus was inoculated in mixtures of virus suspension with K-humates (control with distilled water) or at various intervals before and after K-humates application by spraying. Each test was done on 30 mature leaves of 15 tobacco plants decapitated and darkened 24 h before experiment. The left-hand half of each leaf was inoculated with the control virus preparation, the right-hand half was used for testing the effect of K-humates.

K-humates in the mixture with virus suspension (1:1) inhibited infection in comparison with control virus suspension diluted with distilled water (Table 1). The

Table 1. Number of local lesions (mean \pm SE) developed after inoculation of mixtures of TMV with different concentrations of K-humate and control mixtures with distilled water.

K-humate	TMV+K-humate	TMV+distilled water	
2.0	148.28 \pm 17.49 ^x	336.07 \pm 38.81 ^x	**
	89.32 \pm 10.86	248.64 \pm 32.93	**
	60.24 \pm 9.96	170.00 \pm 21.78	**
1	159.34 \pm 14.01 ^x	232.87 \pm 22.86 ^x	**
	109.74 \pm 11.64	154.31 \pm 17.21	*
	85.10 \pm 11.06	126.15 \pm 17.32	*
0.5	199.28 \pm 18.74 ^x	243.67 \pm 22.48 ^x	N.S.
	148.34 \pm 14.67	164.20 \pm 18.05	N.S.
	124.41 \pm 11.99	151.36 \pm 16.11	N.S.

^x - infected with purified virus, * - differences statistically significant at $P < 0.05$,

** - differences statistically significant at $P < 0.01$

effect of low concentration of K-humates (0.5 g dm⁻³) was not statistically significant. This corresponds to the idea of interaction of viruses and K-humates by adsorption on surfaces or inner structures which could increase with increased K-humates concentration. This idea is also supported by results of experiments with application of K-humates (0.02 g for 30 leaf halves) onto leaf surfaces by spraying

them before inoculation (Table 2). On the other hand, treatment of leaves with K-humates after inoculation did not influence the virus infectivity at all.

Table 2. Effect of K-humate applied by spraying 24 h or 48 and 24 h before TMV inoculation on infection of tobacco leaves (mean number of local lesions \pm SE).

	K-humate	Distilled water	
24 h	124.23 \pm 16.53	214.00 \pm 31.19	**
	60.07 \pm 9.81	123.23 \pm 15.91	**
	70.16 \pm 10.76	200.22 \pm 29.73	**
48 and 24 h	43.25 \pm 10.41	198.21 \pm 30.56	**
	27.56 \pm 9.64	141.36 \pm 19.49	**
	32.87 \pm 11.22	150.03 \pm 21.28	**

** - differences statistically significant at $P < 0.01$

Therefore it is possible to suppose that certain low molecular fractions of K-humates after penetration into epidermal tissues do not influence regressive either the virus already present in the tissues or its reproduction within the tissues. The inhibition effect which occurs in mixtures and by treatment before inoculation should be possibly explained by inhibition of enzymes activities caused by HIA and namely by adsorption of viruses on HA surface and/or with respect to the fractal structure at pH 6.5 in pores of the macromolecules.

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