Tropical Bryology 16: 11-15, 1999

Influence of *Cladonia substellata* Vainio extracts and usnic acid on germination and growth of *Allium cepa* L. seedlings

Adriana Mayumi Yano-Melo[1], Carlos Vicente[2] & Lauro Xavier-Filho[3]

[1] Departamento de Micologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Av. Moraes Rego, s/n, Cidade Universitária, 50670-420, Recife – Pernambuco, Brasil

[2] Laboratory of Plant Physiology, Faculty of Biology, Complutense University of Madrid, Madrid, Spain

[3] Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Caixa Postal 5009, 58051-970, João Pessoa – Paraíba, Brasil.

Abstract: The effect of lichen extracts obtained from *Cladonia substellata* and its main constituent, usnic acid, on the germination and growth of onion (*Allium cepa* L.) seedlings was investigated. No significant inhibitory effect of the lichen extracts and usnic acid on germination was found, except of usnic acid at the concentration of 726.7 μM. Growth inhibition of the radicle and of the hypocotyl of the seedlings was found for the total extract and for usnic acid in concentrations of 290.6 μM and 726.7 μM.

Introduction

A lichen from north-east Brazil, *Cladonia substellata* (Ahti *et al.*, 1993), is known to have the highest concentration of usnic acid among lichens, c. 98% of its chemical constitution, representing c. 7.3% of the thallus dry weight (Huovinen & Ahti, 1986). Usnic acid is a cortical substance broadly distributed among lichens (Rundel, 1978) and due to this fact, considerable research has been undertaken to show its efficiency in antineoplasic, antimicrobial and antimitotic activities (Henningsson & Lundstrom, 1970; Lima *et al.*, 1990; Pereira *et al.*, 1991).

Dalvi *et al.* (1972), Vicente *et al.* (1983) and Vavasseur *et al.* (1991) have shown that usnic acid is an inhibitor of oxygen catchment to some organisms, causing growth reduction and, in some cases, impeding the germination of seeds. However, decrease in germination and growth by usnic acid was not observed by Lawrey (1977) and Nishitoba *et al.* (1987) studying its effect on germination of moss spores and on growth of lettuce seedlings, respectively.

The present work evaluates the effect of pure usnic acid and extracts from *Cladonia sub-*

stellata on germination and growth of *Allium cepa* seedlings.

Materials and Methods

Onion seeds (cv. IPA-6) were supplied by the Agricultural Research Agency of Pernambuco (IPA), Recife-PE, Brazil.

The lichen material was collected in the region of Tabuleiro of Santa Rita, Paraíba State (Brazil) (7° 52' 45'' S and 35° 30' W), during the dry season (October/November) of 1991. Samples of 30g of thallus were dried and stored at room temperature (28° C), and pulverized for the preparation of extracts.

From this material aqueous, phosphate-buffered and total extracts, and pure usnic acid-solutions were prepared. The aqueous and phosphate buffered extracts were prepared using, respectively, distilled water and phosphate buffer 50 mM in pH 7.0. For the total extract, three solvents were used: ethylic ether, acetone, and ethanol/water (8:2 v/v). The solvents remained in a shaker with the thallial material for one hour. The solution of total extract for use in the germination test was prepared according to the methodology of Dalvi *et al.* (1972). The concentrations of the extracts were 166 mg/l, comparable to the second level (290.6 µM) of the pure usnic acid solutions.

To determine the lichen acid composition of the extracts, HPLC analyses were performed in a VARIAN 5000 apparatus under the following conditions: RP - C8 column of 40 x 0.4 cm of i.d.; mobile phase acetonitrile:acetic acid (80:20 v/v):water (98:2 v/v); flux 0.3 cm/min.; temperature 26° C; pressure 84 atmos. and UV detector at 280 nm (Table 1).

For the preparation of pure usnic acid solutions, the substance was isolated by the methodology of Asahina and Shibata (1954), utilizing the „Soxlet“ apparatus with the addition of 500 ml of ethylic ether as solvent, and extracting for 72 hours. The preparation of the usnic acid solution followed the same procedures as the total extract solution. For the tests three concentrations were utilized: 72.6 µM, 290.6 µM and 726.7 µM.

In the germination tests, onion seeds were placed in „gerbox“ plates containing filter paper (100 seeds in each „gerbox“ plate for each replication). Measurements were made on the sixth and tenth days when the following parameters were evaluated: germination rate, radicle and hypocotyl lengths. The experimental design was completely randomized, with four replications.

Results and Discussion

The *Cladonia substellata* extracts contained usnic, stictic and constictic acids in variable concentrations (Table 1). In the total extract of *C. substellata* usnic acid appeared to have the highest concentration (Table 1), which is in accordance with the observation of Huovinen and Ahti (1986). However, in the aqueous extract it had the lowest concentration while the highest concentration was of stictic acid. This is probably due to the low solubility of usnic acid in water (Vicente, 1975; Rundel, 1978; Hale, 1983), while stictic acid is highly soluble, as shown by Ascaso and Galvan (1976).

The *C. substellata* extracts did not inhibit the germination of *A. cepa* (cv. IPA-6) seeds, which exhibited a high germination rate (over 90%) (Table 2). Among the treatments, only 290.6 µM and 726.7 µM of usnic acid seemed to lower germination rates, germination decreasing with increasing concentrations of usnic acid. However, only the treatment at 726.7 µM concentration showed a germination rate below the mean which was statistically different from the control (Table 2).

Dalvi *et al.* (1972) had observed inhibition of germination of seeds of *Phaseolus mungo* and *Triticum aestivum* by usnic acid in concentrations of 290.6 µM and 726.7 µM. The results of the present work indicate that the inhibition effect depends also on the genotype of the seed that is being used.

The growth of *A. cepa* seedlings was strongly affected by treatments with usnic acid in concentrations of 290.6 µM and 726.7 µM and with the total extract of *C. substellata*. Affected were both the radicle and hypocotyl lengths (Tables 3 and 4). In the concentration of 72.6 µM usnic acid did not show an inhibitory effect on the growth of *A. cepa*. Likewise there was no effect from both the aqueous and phosphate buffered extracts.

The inhibiting influence of the total extract, contrary to the aqueous and phosphate-buf-

Table 1 – Lichen substance composition of the aqueous-, phosphate buffer- and total extracts of *Cladonia substellata*, as shown by HPLC.

EXTRACT	SUBSTANCES	RETENTION TIME (min.)	AREA (%)
Aqueous	stictic acid	1.12	60.45
	constictic acid	1.34	15.37
	usnic acid	3.53 - 7.98	22.16
Phosphate buffered	stictic acid	1.00	35.53
	constictic acid	1.40	31.02
	usnic acid	2.47 - 4.56	32.24
Total	stictic acid	0.83	25.67
	constictic acid	1.47	13.98
	usnic acid	2.10 - 6.0	59.58

Table 2 - Germination rates of *Allium cepa* (cv. IPA 6) seeds on usnic acid and *C. substellata* extracts.

TREATMENT	GERMINATION RATE (%)
Aqueous extract	95.3 a *
Total extract	93.5 a b
Control	93.5 a b
Phosphate buffered extract	92.0 a b
Usnic acid - 72.6 µM	91.6 a b
Usnic acid - 290.6 µM	87.1 b c
Usnic acid - 726.7 µM	81.2 c

* - values with the same letter do not differ statistically by the Tukey Test (0.05).

Table 3 - Mean length of *Allium cepa* radicle growth on usnic acid and *C. substellata* extracts.

TREATMENT	RADICLE LENGTH (cm)
Usnic acid - 72.6 µM	2.138 a *
Control	2.075 a
Phosphate buffered extract	1.761 a
Aqueous extract	1.718 a
Usnic acid - 290.6 µM	0.901 b
Total extract	0.698 b
Usnic acid - 726.7 µM	0.508 b

* - values with the same letter do not differ statistically by the Tukey Test (0.05).

Table 4 - Mean length of *Allium cepa* hypocotyl developed on usnic acid and *C. substellata* extracts.

TREATMENT	HYPOCOTYL LENGTH (cm)
Control	5.007 a *
Phosphate buffered extract	4.760 a b
Usnic acid - 72.6 µM	4.341 a b
Aqueous extract	4.157 b
Usnic acid - 290.6 µM	3.171 c
Total extract	2.786 c
Usnic acid - 726.7 µM	2.590 c

* - values with the same letter do not differ statistically by the Tukey Test (0.05).

fered extracts, suggests that usnic acid is the active agent: it constitutes around 60% of the total extract, while the other extracts contain much less (Table 1). This assumption is supported by the fact that the usnic acid-solutions have a similar effect in high concentrations, and none in low concentration.

These results are not in agreement with those of Nishitoba *et al.* (1987), but support the results of Goldner *et al.* (1986) and Lascève & Gaugain (1990). The decline in development of the root system may be explained by the observations of Vicente *et al.* (1983) and Vavasseur *et al.* (1991), that usnic acid plays a role in the respiratory process and thus affects growth.

Acknowledgements

The authors thank Dr Vânia Canuto (EMBRAPA/IPA, Brazil) for making available the facilities of the Seed Laboratory of the Agricultural Research Agency of Pernambuco for the germination tests, Dr Marçal de Queiroz Paulo (Dept. of Chemistry-UFPB, Brazil) for his criticism and suggestions, and Dr Eduardo Assis Menezes (Embrapa Semi-Árido, Brazil) and Dra. Leonora Costa Maia (UFPE, Brazil) for reviewing the manuscript.

Bibliography

Asahina, Y. & Shibata, S. 1954. Chemistry of Lichen Substances. Tokyo: Japan Society for the Promotion of Science, 240 pp.

Ascaso, C. & Galvan, J. 1976. Studies on the pedogenetic action of lichen acids. Pedobiologia 16: 321-331.

Ahti, T., Stenroos, S. & Xavier-Filho, L. 1993. The lichen family Cladoniaceae in Paraiba, Pernambuco and Sergipe, northeast Brazil. Tropical Bryology 7: 55-70.

Dalvi, R.R., Singh, B. & Salunkhe, O.K. 1972. Physiological and biochemical investigation on the phytotoxicity of usnic acid. Phyton (Buenos Aires) 29(1/2): 63-72.

Goldner, W R., Hoffman, F.M. & Mede, R.J. 1986. Allelopathic effects of *Cladonia cristatella* on ectomyco-rrhizal fungi common to bituminous strip-mine spoils. Canadian Journal of Botany 64: 1586-1590.

Hale Jr, M.E. 1983. The Biology of Lichens. 3rd ed. London, Edward Arnold. 190 pp.

Henningsson, B. & Lundstrom, H. 1970. The influence of lichens, lichen extracts and usnic acid on wood destroying fungi. Material und

Organismen 5: 19-31.

Huovinen, K. & Ahti, T. 1986. The composition and contents of aromatic lichen substances in the genus *Cladonia* section *Unciales*. Annales Botanici Fennici 23: 173-188.

Lascève, E. & Gaugain, F. 1990. Effects of usnic acid on sunflower and maize plantlets. Journal of Plant Physiology 136: 723-727.

Lawrey, J.D. 1977. Adaptative significance of methylated lichen depsides and depsidones. Lichenologist 9: 137-142.

Lima, R.C., Nascimento, S.C., Pereira, E.C.G. & Campos-Takaki, G.M. 1990. Atividade citotóxica e antitumoral de extratos liquênicos. Boletim da Sociedade Broteriana 63 (2): 339-348.

Nishitoba, Y., Nishimura, H., Nishiyama, T. & Mizutani, J. 1987. Lichen acids, plant growth inhibitors from *Usnea longissima*. Phytochemistry 26: 3181-3186.

Pereira, E.C.G., Campos-Takaki, G.M., Silva, N.H., Vicente, C., Legaz, M.E. & Xavier-Filho, L. 1991. Fractionation of *Cladonia substellata* crude extracts and detection of antimicrobial activity. Boletim da Sociedade Broteriana 64 (2): 173-186.

Rundel, P.W. 1978. The ecological role of secondary lichen substances. Biochemical Systematics and Ecology 6: 155-170.

Vavasseur, R., Gautier, H., Thiband, M.C. & Lascève, G. 1991. Effects of usnic acid on the oxygen exchange properties of mesophyll cell protoplasts from *Commelina communis* L.. Journal of Plant Physiology 139: 9094.

Vicente, C. 1975. Fisiologia de las Substancias Liquenicas. Madrid: Alhambra. 16 pp.

Vicente, C., Legaz, M.E. & Xavier-Filho, L. 1983. Estudio biologico y farmacologico de los antibioticos de origem liquenico. In: Simpósio Nacional de Farmacologia e Química de Produtos Naturais 2, Anais do Simpronat, João Pessoa, PB: UFPB, LTF, p. 319-343.