BIOLOGICAL CONTROL

Activity of Sesame Leaf Extracts Against the Symbiotic Fungus of *Atta sexdens* L.

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Atividade de Inibição de Extratos de Folha de Gergelim no Fungo Simbiótico de *Atta sexdens* L.

RESUMO - O fungo simbionte *Leucoagaricus gongylophorus* (Möller) Singer, sin. de *Rozites gongylophora* Möller, cultivado pela formiga cortadeira *Atta sexdens* L. teve seu desenvolvimento fortemente inibido por frações obtidas das folhas de gergelim, *Sesamum indicum* L. (Pedaliaceae). Total inibição foi observada na concentração de 2,5 mg/ml , sendo verificada inibição de 50% com algumas frações na concentração de 1,25 mg/ml. A análise cromatográfica do extrato hexânico revelou a ocorrência de uma mistura de ácidos graxos, onde os componentes majoritários foram os ácidos tetradecanóico, hexadecanóico, octadecanóico, icosanóico, docosanóico e 9,12,15 - octadecatrienóico. A separação desses compostos durante as várias etapas do fracionamento resultou em perda ou diminuição da atividade inibitória sobre o fungo, indicando que a inibição observada pode ser consequência da ação conjunta de alguns dos compostos presentes nas folhas e não de uma única substância.

PALAVRAS-CHAVE: Insecta, Hymenoptera, gergelim, fungo simbionte, formigas cortadeiras.

ABSTRACT - The symbiotic fungus *Leucoagaricus gongylophorus* (Möller) Singer (syn. *Rozites gongylophora* Möller) cultivated by the leaf-cutting ant *Atta sexdens* L. had its development strongly inhibited by crude extracts obtained from leaves of sesame, *Sesamum indicum* L. (Pedaliaceae). With most of the fractions from these extracts total inhibition was observed at 2.5 mg/ml, whereas inhibition of 50% was observed with some fractions at 1.25 mg/ml. Chromatographic analysis of the hexane extract showed that it was composed by a mixture of fatty acids, of which tetradecanoic, hexadecanoic, octadecanoic, icosanoic, docosanoic and 9,12,15 - octadecatrienoic acids were the major components. The separation of these compounds during the different steps of purification resulted in no or lower inhibitory effect for most of these frac-

tions, indicating that the inhibitory activity observed could be due to the joint action of these compounds present in the leaf tissue, rather than to the action of a single substance.

KEY WORDS: Insecta, Hymenoptera, sesame, mutualistic fungus, leaf-cutting ants.

Changing forest into agricultural environments has resulted in leaf-cutting ants becoming a major pest, which led to a decreasing in plant fitness (Marquis 1984, Ledig 1992). Leaf-cutting ants exhibit preferences (Bueno *et al*. 1989, Hebling-Beraldo *et al.* 1989) and select different substrates for the symbiotic fungus they culture (Cherrett 1968, Littledyke & Cherrett 1975, Rockwood 1976, Rockwood & Hubbell 1987). The pattern of preference and selectivity in the foraging activity of the leaf-cutting ants may be related to the metabolic requirements of each colony (Lewis *et al.* 1974). Ants prefer plants which are good substrate for the fungus development, avoiding those containing growth inhibitory compounds (Hubbel & Wiemer 1983, Hubbell *et al.* 1983).

Traditional control of ants with insecticides, in spite of its efficiency, is still a problem because of their non-selective toxicity (Loeck & Nakano 1982, Vilela & Howse 1988). There is a growing necessity for ant control without ecological injury, one that does not lead to selection of resistant populations, and that can be effective, specific and enduring (Diehl-Fleig *et al.* 1988, Silva & Diehl-Fleig 1988).

The use of plants toxic to the symbiotic fungus *Leucoagaricus gongylophorus* (Möller) Singer has been proposed as an alternative method of control. There are experimental evidences that secondary metabolites present in plants may be harmful to leaf-cutting ants and their symbiotic fungus (Howard *et al.* 1988, Bueno *et al*. 1990). Bueno *et al.* (1995) reported the death of *Atta sexdens* L. nests feeding on sesame (*Sesamum indicum* L.) leaves. Pagnocca *et al*. (1990) showed that crude extracts obtained from different organs of sesame inhibited the symbiotic fungus of *A. sexdens rubropilosa*. In the present investigation we have compared the effect of fractions, and subfractions obtained from sesame leaves (by a sequential process of extraction) on the development of the symbiotic fungus of *A. sexdens.*

Material and Methods

The symbiotic fungus was isolated from a nest of *A. sexdens* and is similar in all features to the ant-fungus first isolated and identified by Möller (1893) as *Rozites gongylophora* . The taxonomic position of these symbiotic fungi is not clear and it has been identified also as *Leucocoprinus gongylophorus* Heim (1957) or *Attamyces bromatificus* Kreisel, Powell & Stradling (1986).The current tendency is to refer to this fungus as *Leucoagaricus gongylophorus* Singer (Fisher *et al*. 1994). The culture medium A (Pagnocca *et al*. 1990) was used for both maintenance of the fungus and assay of growth inhibition. Its composition, in g/l was: glucose = 10.0; sodium chloride = 5.0; Bactopeptone = 5.0; malt extract = 10.0 and agar = 15.0.

Dry leaves of sesame were grounded in a Willey mill. The crude extracts were prepared by a sequential extraction with hexane, dichloromethane and methanol. The fractions obtained from the crude extracts were added to the medium until a final concentration of 5.0, 2.5 or 1.25 mg/ml. The subfractions from the ethyl acetate fraction were added in order to get a final concentration of 1.00 mg/ml, and the dichloromethane subfractions obtained from the dichlorometane fraction as follows: subfractions 1 and 2 = 0.80 mg/ml; subfraction

3 = 0.40 mg/ml; subfractions 4, 5, 6 and 7 = 1.00 mg/ml. Samples were solubilized preferentially in the same solvents employed for their extraction, and controls were prepared with the same solvents. Culture media were autoclaved during 10 minutes at 120 °C.

Some fractions,as indicated in table 1, were mixed with glucose (dry-mix) and then introduced in the medium A. This procedure was carried out with fractions which showed low solubility in organic solvents.

Thirty-day old cultures developed in 250 x 25 mm tubes containing 20 ml of solid medium were used. The mycelia present in five tubes were transferred asseptically to a glass tissue grinder (250 x 25 mm) containing 3.0 ml 0.1% (w/v) sterile peptone. After a slight fragmentation the suspension was diluted to 120 ml with 0.1% sterile peptone and inoculated as described (Pagnocca *et al.* 1990). Fungal growth was estimated on the basis of mycelial surface and density after 30-35 days of incubation at 25 ± 1°C, and referred as 100% (growth identical to the control), 80, 60, 40 and 20% or less of the control and [NG] = no growth. Each assay was run with five replicates and repeated once. The modal values were registered.

Results and Discussion

Extracts of sesame leaves were fractionated and the fractions obtained inhibited the fungal growth with variable intensity (Table 1). For a concentration of 2.5mg/ml, the best inhibition was obtained with both ethyl

Table 1. Fungal growth (%)[1] of *Leucoagaricus gongylophorus* in culture medium A containing different concentration [mg/ml] of fractions from hexane, dichloromethane or methanol sesame extracts. Control = (100).

Fractions	Extracts		
	Hexane	Dichloromethane	Methanol
Hexane	-	[1.25] / (90)	[1.25] / (60)
	-	[2.50] / (NG)	[2.50] / (NG)
	[5.00] / (50)	[5.00] / (NG)	[5.00] / (NG)
Dichloromethane	-	[1.25] / (50)	-
	-	[2.50] / (NG)	-
	[5.00] / (90)	[5.00] / (NG)	[5.00] / (10)
Ethyl Acetate	[1.25] / (50)	[1.25] / (50)	[1.25] / (70)[2]
	[2.50] / (NG)	[2.50] / (NG)	[2.50] / (NG)
	[5.00] / (NG)	[5.00] / (NG)	[5.00] / (NG)
Methanol	[1.25] / (90)	[1.25] / (70)	[1.25] / (50)[2]
	[2.50] / (NG)	[2.50] / (NG)	[2.50] / (NG)
	[5.00] / (NG)	[5.00] /.(NG)	[5.00] / (NG)
Acetic Acid	[1.25] / (100)	[1.25] / (100)	[1.25] / (100)[2]
	[2.50] / (40)	[2.50] / (NG)	[2.50] / (NG)
	[5.00] / (10)	[5.00] / (NG)	[5.00] / (NG)

[1]Thirty days incubation/25° C.
[2]Dry mix.
NG = no growth.

acetate and methanol fractions. Among the fractions obtained from dichloromethane extract those containing the dichloromethane or ethyl acetate fractions showed high toxicity at 2.5 mg/ml and also good activity (50% inhibition) in the concentration of 1.25 mg/ml. Similar results were obtained with the methanol fraction from the methanol extract and with the ethyl acetate fraction from hexane extract.

Since both fractions dichloromethane/dichloromethane and ethyl acetate/hexane showed good activity, they were further fractionated using a cromathographic column. Seven subfractions from the first and thirteen from the second were obtained and the action of each of them was evaluated separately. None of the subfractions derived from the dichloromethane/dichloromethane extract inhibited the fungal growth , and only two (number 5 and 6) obtained from the ethyl acetate/hexane extract were active (Table 2). Thus, with the same concentration of 1.0 mg/ml , the subfractions 5 and 6 showed similar activity (60% inhibition) while the subfractions 4 and 7 were responsable for a lower reduction (40% inhibition) in the fungal development. This fact along with the observation that all the subfractions prepared from dichloromethane/dichloromethane were inactive, are strong evidence that the inhibitory activity observed could be due to the cooperative action of these compounds present in the leaf tissue, rather than to a single substance .

Unfortunately the amount of material obtained in each purification step was too small to allow further identification of the active substances present in the ethyl acetate fraction derived from hexane extract, but there was evidence that they were fatty acids because they are the main compounds occurring in the hexane extract of sesame leaves. A preliminary assay showed the presence of several fatty acids in that extract such as tetradecadonoic, hexadecanoic, 9-12-15 octadecatrienoic, octadecanoic, icosanoic and docosanoic, and therefore it is possible that some, or even a mixture of them was responsible for the inhibitory effect observed. Efforts are being made in order to characterize the chemical composition of the most active

Table 2. Fungal growth (%)[1] of *Leucoagaricus gongylophorus* in culture medium A containing different concentration [mg/ml] of subfractions from dichloromethane or ethyl acetate sesame fractions. Control = (100).

Fraction	Subfraction	Concentration [mg / ml] / (% growth)
Dichloromethane	1 ; 2	[0.80] / (100)
	3	[0.40] / (100)
	4 ; 5 ; 6 ; 7	[1.00] / (100)
Ethyl acetate	1 ; 10	[1.00] / (100)
	13	[0.80] / (100)
	14	[0.50] / (100)
	2 ; 3 ; 8 ; 11	[1.00] / (80)
	16	[0.80] / (80)
	4; 7	[1.00] / (60)
	5 ; 6	[1.00] / (40)

[1]Thirty days incubation/25°C. Biological assay were performed with isolated subfractions.

fractions. It is well known that some fatty acids display antimicrobial activity specially against bacteria and fungi . It is possible that the death of laboratory nests feeding in sesame leaf as described by Bueno *et al.* (1995) could be due to the primary action of these compounds on the symbiotic fungus, and perhaps some of these toxic plant or even a compound isolated from them could be a useful tool for the control of this insect in the future.

Additionally, we investigated the possibility that sesamin, a lignan occurring in sesame's seed oil and reported as synergist insecticide, antisseptic, bactericide (Bedigian *et al.* 1985) and fungicide for this fungus (Pagnocca *et al.* 1996) could be responsible for the effect observed but attempts at its isolation from these extracts were unsuccessful.

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