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INFLUÊNCIA DE *Cladonia salzmannii* Nyl SOBRE FUNGOS MICORRIZICOS ARBUSCULARES E NO DESENVOLVIMENTO DE PLÂNTULAS DE *Genipa americana* L.

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Ata da defesa de dissertação da Mestranda **Flavia Pereira da Silva**, realizada em 22 de fevereiro de 2007, como requisito final para obtenção do título de Mestre em Bioquímica.

Às 10:40 minutos do dia 22 de fevereiro de 2007, foi aberto, no Auditório Prof. Marcionilo Lins/Depto. de Bioquímica, o ato de defesa de dissertação da mestranda **Flavia Pereira da Silva,** aluna do Curso de Mestrado em Bioquímica. Iniciando os trabalhos a Profa. Dra. Vera Lúcia de Menezes Lima, Coordenadora do curso supra citado, fez a apresentação da aluna, de seu orientador, o Prof. Dr. Nicácio Henrique da Silva, de suas co-orientadoras Profa. Dra. Adriana Mayumi Yano de Melo, da Universidade Federal do Vale do São Francisco-UNIVASF, e Profa. Dra. Eugênia Cristina Gonçalves Pereira, do Depto. de Geografia/UFPE, e da Banca Examinadora composta pelos professores doutores: Nicácio Henrique da Silva, na qualidade de Presidente, Vera Lúcia de Menezes Lima, ambos do Depto. de Bioquímica/UFPE, Maria do Socorro Bezerra de Araújo, do Depto. de Geografia/UFPE, e Fábio Sérgio Barbosa da Silva, do Depto. de Micologia/UFPE. Após as apresentações, o Sr. Presidente convidou a aluna para a apresentação de sua dissertação intitulada: **"Influência de *Cladonia salzmannii* na ocorrência de fungos micorrizicos arbusculares em rizosfera e desenvolvimento de plântulas",** e informou que de acordo com o Regimento Interno do Curso, o candidato disporia de até 50 (cinqüenta) minutos para apresentação do trabalho e o tempo de argüição para cada examinador, juntamente com o tempo gasto pelo aluno para responder às perguntas seria de 30 (trinta) minutos. A aluna procedeu a explanação e comentários acerca do tema em 35 (trinta e cinco) minutos. Em seguida, o Sr. Presidente convidou a Banca Examinadora para ocupar seus lugares e passou a palavra ao primeiro examinador, o Prof. Dr. Fábio Sérgio Barbosa da Silva, em seguida para a Profa. Dra. Maria do Socorro Bezerra de Araújo, e finalmente para a Profa. Dra. Vera Lúcia de Menezes Lima, os quais agradeceram o convite, fizeram alguns comentários, deram sugestões e iniciaram suas respectivas argüições. Ao final das mesmas, os referidos professores deram-se por satisfeitos. Em seguida, o Sr. Presidente usou da palavra para tecer alguns comentários, agradecer à Banca Examinadora e parabenizar a candidata. Finalmente, a sessão foi suspensa para proceder ao julgamento pela Banca Examinadora, a qual se reuniu na Secretaria do Curso. Após alguns comentários, a Banca decidiu, por unanimidade, conceder a menção "**Aprovada com Distinção**". Nada mais havendo a tratar, lavrei a presente ata que vai assinada por mim, Secretário, e demais membros da Banca Examinadora. Recife, 22 de fevereiro de 2007.

Vera Lucia de Lima.

Fábio Sérgio Barbosa da Silva

Maria do Socorro B. Araújo

Nicácio Henrique da Silva

A Jeová Deus,
o único digno de toda honra,
de toda glória e de todo poder.
OFEREÇO

A meus amados pais,
aos meus sobrinhos e irmãos
DEDICO

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A Jeová Deus, a fonte da vida de quem procede todo presente perfeito, por seu auxílio fortificante nas situações mais difíceis.

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RESUMO

Os liquens produzem substancias com atividade biológica, que podem ser lixiviadas ao solo e exercer ação alelopática sobre a microbiota. Objetivando estudar a influência do talo liquênico de *Cladonia salzmannii* sobre a atividade microbiana e sobre os fungos micorrízicos arbusculares (FMA) foram realizados dois experimentos. No 1º experimento avaliou-se o efeito do líquen na atividade microbiana em área de cerrado de Alhandra, estado da Paraíba. No 2º experimento foi avaliado o efeito do talo liquênico na associação micorrízica e no desenvolvimento de plântulas de *Genipa americana*. No campo, embora o solo sob o tapete liquênico apresentasse menor biomassa microbiana, evolução de CO_2 e hidrólise do diacetato de fluoresceina (FDA), e maior colonização micorrízica e esporulação de FMA em relação ao solo com ausência de liquens, não houve diferença estatística entre os tratamentos, fato que pode ser atribuído ao ácido barbático em baixas concentrações no solo. Para o experimento em casa de vegetação, o delineamento experimental foi do tipo inteiramente casualizado, com 4 tratamentos (controle, líquen, líquen+FMA e FMA) em 5 repetições. Após 4 meses as plantas foram avaliadas quanto a altura, diâmetro do caule, biomassa seca e fresca da parte aérea e radicular, densidade de esporos de FMA e colonização micorrízica, avaliou-se também a quantidade de ácido barbático no solo. Não houve diferença entre os tratamentos para altura e diâmetro do caule. As plântulas com líquen+FMA possuíam maior biomassa seca e fresca, tanto da parte aérea quanto radicular. Embora o ácido barbático tenha sido encontrado em baixas concentrações no solo, os tratamentos com liquens apresentavam maior concentração desse ácido do que os com líquen+micorriza.

Palavras chave: ácido barbático, *Cladonia salzmannii*, fungos micorrízicos arbusculares, alelopatia.

ABSTRACT

Lichens are know for being rich in substances considered asseing allelopathic or antimicrobial agents, which can be leached out from lichen by rainfall, and therefore the soil below is enriched by these compounds. Aiming to study the ecological role of these organisms, two experiments were performed. In the experiment 1 the effects of *Cladonia salzmannii* against microbial activity on sandy soils of Savannah-like ecosystem was evaluated. In the experiment 2 the effect of lichen thalli on arbuscular mycorrhizal fungi (FMA) and in the growth *Genipa americana* seedlings was analyzed. In the field, soils under lichen cover showed lower microbial biomass, CO_2 emission and activity of fluorescein diacetate hydrolysis, and heigher mycorrhizal colonization and number of spores than soil without lichen, but was no difference statistical, fact that could be related to the barbatic acid low concentrations. The experiment in greenhouse was in a factorial randomized design of 4 treatments (control, lichen, lichen+FMA and FMA) and 5 replicates. After 4 months the plants were analyzed with regard to the growth, stem diameter, dry biomass shoot, dry biomass root, number of spores and mycorrhizal colonization also was assessed presence of barbatic acid in soil. The growth parameters did not differ significantly among the treatments. The seedlings with lichen+FMA presented the highest dry and fresh of the biomass both shoot and root. The presence of lowest concentrations of barbatic acid in the soil was observed, although the lichen treatment had highest concentrations of this acid than lichen+FMA treatment.

Keywords: barbatic acid, *Cladonia salzmannii*, arbuscular mycorrhizal fungi, allelopathy.

Introdução

1. INTRODUÇÃO

1.1 **Generalidades**

Os solos são de fundamental importância à dinâmica da costa terrestre, tanto como suporte da vegetação natural dos ecossistemas, como para uso em cultivos. Em ambas as situações, a fertilidade natural é necessária para a manutenção das espécies vegetais e das que delas dependem (SILVA et al., 2003; KLIEMANN et al., 2003).

Para isso, nos ecossistemas naturais a ciclagem dos nutrientes orgânicos e inorgânicos possibilita a transferência de sais necessários à nutrição das plantas. No entanto, os fatores envolvidos nesta transferência dependem sobremaneira das condições climáticas, onde a disponibilidade de água e altas temperaturas são parâmetros ideais para as reações químicas de transformação mineral e transporte/síntese de produtos entre solo e planta (REICHARDT & TIMM, 2004). Por isso, no domínio intertropical, sobretudo áreas de florestas, tais mecanismos são mais evidentes e dinâmicos.

Por outro lado, antes da formação dos solos, os liquens exercem importante papel na pedogênese, visto que são capazes de habitar as rochas consolidadas, onde sais minerais não estão disponíveis para plantas que dele dependem.

O líquen caracateriza-se por ser uma associação simbiótica entre fotobionte (algas verdes e cianobactérias) e micobionte (fungos), na qual o micobionte protege fotobionte dos extremos de temperatura e umidade, que em contrapartida oferece os produtos da fotossíntese. Dessa associação resultam muitas substâncias liquênicas que participam ativamente da dinâmica microbiana no solo, como também possuem grande espectro de aplicação biotecnológica (NASH III, 1996).

Os liquens, tanto por ação física de suas rizinas como por ação química das substâncias liquênicas, contribuem na formação do solo e participam ativamente da ciclagem de nutrientes. Captam o que volatiliza do solo e, para ele liberam substâncias próprias de seu metabolismo, que funcionam de diversas formas na seleção da biota subjacente e/ou como quelantes de íons inorgânicos (LEGAZ, et al., 2006).

O conhecimento dos fatores envolvidos e o estudo da relação líquen/solo bem como a influência destes sobre os microrganismos do solo são de fundamental importância para a compreensão da função ecológica desempenhada pelos liquens sobre seu habitat.

1.2 Liquens e Solos

Os solos são originados de rocha, que por ação de processos físicos, químicos e biológicos de desintegração, decomposição e recombinação, se transformou, no decorrer das eras geológicas, em material poroso de características peculiares. São considerados complexos sistemas de componentes vivos: formado por raízes de plantas, populações de bactérias, fungos e animais e, não vivos: que inclui fragmentos de rochas, água, nutrientes minerais dissolvidos, espaços de ar e húmus (REICHARDT & TIMM, 2004; TAIZ & ZEIGER, 2004).

Dentre os processos de formação do solo a degradação química, que é a alteração/transformação de alguns materiais das rochas se constitui como um dos mais importantes. Ao longo do tempo organismos alteram a composição química do solo. Os primeiros organismos a se instalarem sobre as rochas aflorantes da superfície terrestre são os liquens (LEGAZ, et al., 2006).

Os liquens são organismos simbiônticos resultantes da associação estável e duradoura entre fungo (micobionte) e algas (fotobionte). Estima-se que existam cerca de 13.500 espécies de fungos liquenizados, correspondendo a 20% dos fungos conhecidos. A maioria das espécies fúngicas que participam da associação pertencem ao Filo Ascomycota ou, em menor proporção, Basidiomycota, cujos constituintes determinam a classificação dos liquens (HAWKSWORTH & HILL, 1984; NASH III, 1996). As algas são unicelulares, sendo comuns as clorofíceas e, o gênero *Trebouxia* presente em aproximadamente 70% das espécies. Além destas, também ocorrem espécies de *Coccomyxa* e *Trentepohlia*. Entre as cianofíceas, *Nostoc* e *Scytonema* são as mais freqüentes (MARCELLI, 2006). Visto se tratar de uma simbiose, algumas substâncias são sintetizadas pelo fotobionte, outras pelo micobionte ou podem ser resultante da associação.

O fotobionte produz carboidrato, que é transferido ao micobionte de forma rápida e em quantidade substancial para transformação e acúmulo. O micobionte é responsável pela síntese de metabólitos secundários. Esta síntese ocorre por três vias metabólicas principais: a do ácido chiquimico, do ácido mevalônico e do acetato-polimalonato, sendo a maioria dos metabólitos secundários formados por esta última via (HAWKSWORTH & HILL, 1984; HONDA, 2006). Desta forma, a liquenização pode ser considerada como uma estratégia onde o fungo pode satisfazer sua necessidade de carboidrato para respiração e crescimento. Além disso, em cianoliquens o micobionte ganha uma fonte de nitrogênio (NASH III, 1996). Para as algas, os benefícios são relativos à hidratação, onde o micobionte a protege da dessecação e

da intensa luminosidade. Dessa forma, fotobionte e micobionte têm se dispersado pelos mais diversos habitates, o que não aconteceria na condição de organismos de vida livre (HONDA & VILEGAS, 1998).

As substâncias produzidas pelos liquens são agrupadas, segundo sua localização no talo, em produtos intracelulares e extracelulares. Os produtos intracelulares (carboidratos, carotenóides e vitaminas, aminoácidos e proteínas) estão ligados à parede celular e ao protoplasto. São frequentemente hidrossolúveis e podem ser extraídos em água. Tais compostos ocorrem não apenas em liquens, mas em fungos e algas de vida livre, ou em plantas superiores (HAWKSWORTH & HILL, 1984, HONDA, 2006). Os produtos extracelulares também chamados metabólitos secundários, encontram-se na medula ou no córtex. Atualmente, cerca de 630 compostos provenientes do metabolismo secundário de liquens são conhecidos. São ácidos alifáticos, meta e para-depsídeos, depsidonas, ésteres benzílicos, dibenzofuranos, xantonas, antraquinonas, ácidos úsnicos, terpenos e derivados do ácido púlvinico, sendo a maior parte dessas substâncias sintetizadas exclusivamente por liquens (PIERVITTORI et al, 1994; ELIX & WARALAW, 1996). A concentração de metabólitos secundários pode variar de 0,1 a 10 % em relação ao peso seco do talo liquênico, embora em alguns casos a concentração possa ser mais alta (HONDA & VILEGAS, 1998).

No Brasil são encontradas diversas espécies de liquens, como *Cladonia salzmannii* (Figura 1) que tem sido registrada nos Estados da Bahia, Minas Gerais, Paraíba, Pernambuco e Sergipe. Neste último, é referida por AHTI et al. (1993) como abundante sobre solos arenosos. Apresenta estruturas escifóides e dilatadas, e tem como principal composto o ácido barbático (AHTI et al., 1993). Este ácido é um depsídeo formado por dois anéis aromáticos, interligados entre si por uma ligação éster (Figura 2). CULBERSON (1969) comenta que o ácido barbático também pode ser chamado de ácido alectórico, rizóico, rizônico, coccelico, coenomicina e cenomicina.

A síntese dos depsídeos ocorre pela via acetato-polimalonato, estes procedem de ciclações tipo orcinol. O processo biosintético inicia-se com a condensação de acetil-coA e de malonil-coA, a acetoacetil-coA resultante pode condensar com duas outras moléculas de malonil-coA em etapas sucessivas, formando um policetídeo de 8 carbonos. Este último cicliza por condensação aldólica produzindo orcinol, esta síntese ocorre sob a ação da enzima orselinato sintase. Os depsídeos são sintetizados a partir destas unidades monocíclicas mediante esterificação, várias esterases podem estar envolvidas nesse processo (Figura 3). Entretanto essas enzimas não foram ainda isoladas (HONDA & VILEGAS, 1998; MARCELLI, 2006).

Figura 1. *Cladonia salzmanii* ocorrente sobre solos arenosos tabuleiros costeiros (Alhandra, PB).
Fonte: autor

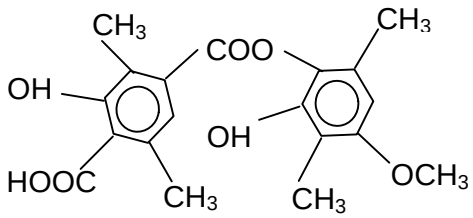

Figura 2. Modelo estrutural do ácido barbático.
Fonte: autor

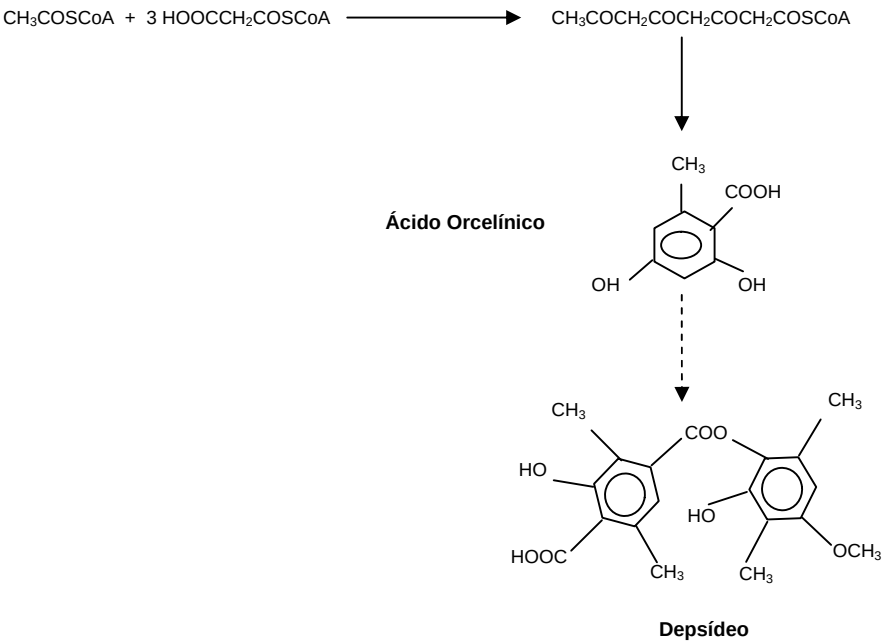

Figura 3. Reações biossintéticas de formação dos depsídeos
Fonte: autor

As substâncias liquênicas podem ser produzidas tanto no córtex quanto na medula, podendo ser lixiviadas para o solo e exercerem ação quelante sobre os minerais, extraindo cátions e dando origem a outros novos minerais. Períodos ocasionais de chuvas são suficientes para arrastar quantidades significativas de fenóis e percolá-los ao solo. Assim, a água da chuva vai extraindo progressivamente pequenas quantidades de substâncias liquênicas, visto que estas são pouco hidrossolúveis. Estudos comprovam que o ácido úsnico pode ser lavado do talo com a chuva ou umidade para a superfície da planta e para o solo (MALICKI, 1965). Desta forma, estes metabólitos secundários desempenham uma importante função ecológica, pois contribuem para a estrutura e composição da comunidade do solo (XAVIER-FILHO & RIZZINNI, 1976; LEGAZ et al., 1986).

Como pioneiros na colonização, os liquens degradam a rocha tanto por ação química de suas substâncias, como por ação mecânica por incrustação no substrato, favorecendo o processo de formação do solo. As substâncias liquênicas são conhecidas por sua habilidade de se complexarem com cátions do substrato mineral (PURVIS & HALLS, 1996). ADAMO et al. (1993) estudando o intemperismo de rochas sob a ação de seis diferentes espécies de liquens, atribuíram aos liquens a capacidade de alterar quimicamente rochas, em função do tipo e quantidade de metabólitos secundários.

A interface líquen-substrato é um local de considerável atividade química. Os ácidos orgânicos liberados pelo simbionte podem acelerar a decomposição de rochas (PIERVITTORI et al., 1994). BJELLAND et al. (2002) detectaram a presença dos ácidos lecanórico, divaricático e girofórico em substratos rochosos indicando assim que estes compostos podem estar em contato direto com minerais de rochas agindo como fonte de prótons, ou agente quelante, contribuindo para a degradação química das rochas.

À medida que o líquen decompõe a rocha, prepara um solo desenvolvido o suficiente para o estabelecimento de outros seres que requerem umidade e nutrientes, como os musgos e vegetais fanerogâmicos (HAWKSWORTH & HILL, 1984; LEGAZ et al., 1986; BANFIELD et al., 1999; SEDIA & EHRENFELD, 2005). Liquens do gênero *Cladonia*, embora não possuam uma associação com o substrato tão íntima quanto os liquens crustrosos, contribuem para a formação de húmus através da desintegração de fragmentos do talo (ASTA et al., 2001).

Quando comparado com regiões onde o solo apresenta vegetação escassa, o solo em áreas com liquens apresenta mais carbono e nitrogênio, umidade e nutrientes (BENALP & LANGE, 2003). EVANS & BENALP (1999) consideram os liquens organismos que influenciam a fertilidade e estabilidade do solo em regiões áridas.

A presença de substâncias liquênicas no solo e sua estabilidade com o tempo possibilitam a ocorrência de fenômenos alelopáticos, ou seja, o efeito direto ou indireto destes compostos em outros organismos, o que tem sido descrito amplamente na literatura (LEGAZ et al., 1986). Sugere-se que o ácido úsnico tem ação alelopática (YANO et al., 1999), mas pouco se conhece sobre seu mecanismo de ação. Além disso, os liquens, por meio das substancias liquênicas podem afetar o crescimento e reprodução dos microrganismos do solo, ou mesmo inibir a germinação de sementes localizadas no perfil do solo (FAHSELT, 1994; GIORDANO et al., 1999).

Por outro lado, STARK & HYVÄRINEN (2003) demonstraram que substâncias liquênicas produzidas por *Cladina stellaris*, como ácido úsnico e perlatólico, podem ser fontes de carbono para os microrganismos do solo, ao invés de exercerem efeito alelopático. Dessa forma, os liquens podem afetar a microbiota do solo de diferentes maneiras (SEDIA & EHRENFELD, 2005).

1.3 Fungos micorrízicos arbusculares (FMA)

O termo micorriza refere-se à relação simbiótica estável entre certos fungos do solo e raízes de plantas, caracterizada pela perfeita integração morfológica, bioquímica e funcional da associação (SMITH & READ, 1997). Estudos de raízes fossilizadas evidenciaram que as micorrizas surgiram há cerca de 400 milhões de anos, o que compreende o período do aparecimento das plantas terrestres (REMY et al., 1994; REDECKER et al., 2000).

Considerando a morfoanatomia das raízes colonizadas, as micorrizas são classificadas em ectomicorrizas, ectendomicorrizas e endomicorrizas. Estas se dividem em orquidóides, ericóides e arbusculares. Dentre elas, as micorrizas arbusculares são as mais comuns, amplamente encontradas na maioria dos ecosistemas, principalmente nos trópicos (SIQUEIRA, 1994). São formadas por fungos da divisão Glomeromycota, que colonizam raízes de plantas de quase todas as famílias de angiospermas e algumas gimnospermas, além de alguns representantes das briófitas e pteridófitas (QUILAMBO, 2003). Estima-se que 250.000 espécies de plantas no mundo são capazes de formar associações micorrízicas (XAVIER & GERMIDA, 1999; JOHANSSON et al., 2004).

Estes organismos são importantes componentes do ecossistema natural. Acredita-se que a condição micorrízica nas plantas seja regra, visto que cerca de 85% delas formam este tipo de associação, desempenhando destacado papel na composição e produtividade vegetal (van der HEIJDEN et al., 1998).

Os FMA são biotróficos obrigatórios, pois completam seu ciclo de vida apenas se estiverem associados a uma planta hospedeira, que fornece carboidratos e outros fatores necessários ao seu desenvolvimento e esporulação (SIQUEIRA et al., 2002). Não há evidências de especificidade hospedeira e não ocorrem alterações morfológicas macroscópicas nas raízes colonizadas. A presença da associação é apenas detectada por observações microscópicas de raízes clarificadas e coradas com corantes específicos.

Durante a formação da associação micorrízica a hifa do fungo penetra no córtex da raiz desenvolvendo micélio intraradicular e estruturas denominadas arbúsculos, que promovem aumento na superfície para trocas metabólicas entre a planta e o fungo. Alguns FMA também produzem vesículas; acredita-se que tais estruturas possuem função de armazenamento. Nessa simbiose, os fungos recebem fotossintatos produzidos pela planta hospedeira e esta se beneficia da melhoria do estado nutricional, pois a colonização de plantas por FMA aumenta a superfície para aquisição de nutrientes e, possivelmente de água pela planta, favorecendo o crescimento e, reduzindo efeitos negativos comuns em solos pobres em nutrientes (AYLING et al., 1997; XAVIER & GERMIDA, 1999; JOHANSSON et al., 2004; ANANTHAKRISHNAN et al. 2004).

A associação micorrízica promove vários benefícios às plantas, como o aumento no crescimento e na produção de matéria seca, maior tolerância a estresses de natureza biótica (pragas e doenças) e abiótica (seca, salinidade, etc.). Além disso, a micorrização promove alterações fisiológicas, aumentando a produção de exsudatos radiculares, e de compostos secundários, bem como as taxas de respiração, transpiração e fotossíntese (HARRIER, 2000). Dessa forma, os FMA constituem um fator importante na qualidade do solo devido ao seu efeito na fisiologia da planta, nas interações ecológicas do solo, além de sua contribuição para a agregação das partículas do solo, pois produzem uma glicoproteína chamada glomalina (RILLIG, 2004; RILLIG & MUMMEY, 2006; GADKAR & RILLIG, 2006).

As micorrizas arbusculares são de grande interesse para regiões tropicais, especialmente para o Brasil, onde face às condições ambientais, os solos são de baixa fertilidade. Entre os fatores que determinam direta ou indiretamente a formação, ocorrência e desenvolvimento dos FMA são citados: solo, ambiente, manejo e hospedeiro (SIQUEIRA, 1994).

A comunidade fúngica é controlada principalmente pelas condições edáficos e hospedeiro vegetal. Assim, características químicas do solo, como a presença de aleloquímicos, podem afetar a associação micorrízica, podendo atuar como inibidores ou

estimuladores da micorrização, interferindo na ocorrência e eficiência da simbiose (MALICKI, 1965).

1.4 Relação micorrizas/liquens

BROWN & MIKOLA (1974) verificaram o limitado crescimento de *Pinus silvestris* e *Picea abies* provocado pela ação do extrato aquoso do líquen *Cladonia alpestris*, que possui o ácido úsnico como um dos seus constituintes químicos. Os autores atribuíram esta limitação do crescimento à inibição dos fungos ectomicorrízicos associados a estas espécies. De modo similar, FISHER (1979) sugere que os liquens influenciam negativamente plantas, pois suas substâncias promovem mudanças na atividade metabólica de microrganismos e associações micorrízicas.

XAVIER-FILHO et al. (1985) ressaltam que as substâncias liquênicas medulares, após um período de chuva, são lixiviadas ao solo, o que consequentemente poderá ter função alelopática.

GOLDNER et al. (1986) reportaram em comunidades de campo abandonado de betume, onde o líquen *Cladonia cristetella* é muito abundante, ocorria inibição do crescimento de fungos ectomicorrízicos. Testando isoladamente o ácido úsnico, que era substância ocorrente em maior concentração, comprovaram que este é o responsável por esta atividade nas concentrações de 10, 25, e 50 mg/L.

MARKKOLA et al. (2002) destacam que a remoção do tapete liquênico contribuiu para o aumento na diversidade da comunidade ectomicorrízica, diminuindo a mortalidade da vegetação dela dependente. Em adição, OHTONEN & VÄRE (1998) atribuíram o alto índice de atividade microbiana observado no solo, sob a micota liquenizada, a grande produção de raízes finas em locais dominados por liquens.

1.5 Parâmetros de origem bioquímica e microbiológica para análise do solo

A biomassa microbiana é fundamental na transformação da matéria orgânica na ciclagem de nutrientes e no fluxo de energia, interagindo com as partículas do solo e participando de processos biológicos e bioquímicos essenciais à sustentação dos ecossistemas. Assim sua quantificação é importante em estudos sobre perda ou melhoria da qualidade do solo (CARDOSO, 2004). A avaliação da biomassa microbiana do solo permite obter informações rápidas sobre mudanças nas propriedades orgânicas do solo bem como avaliar os efeitos de substâncias alelopáticas. Dentre os diferentes métodos utilizados na quantificação da biomassa microbiana, à fumigação-extração é particularmente útil em solos ácidos e

orgânicos de florestas (GRISI, 1997). Esse método consiste na fumigação do solo com clorofórmio, que além de matar, rompe as células microbianas liberando o constituinte microbiano para o solo e assim permitido sua extração. Logo após a fumigação o material celular liberado é recuperado com um extrator fraco como o sulfato de potássio. A determinação do carbono nos extratos fumigados e não-fumigados é feita por dicromatometria a partir da retirada da alíquota do extrato (DE-POLLI & GUERRA, 1999). A atividade de algumas enzimas pode ser relacionada com a biomassa microbiana, auxiliando na interpretação da condição funcional da microbiota do solo.

Os diversos microrganismos no solo são responsáveis direta e indiretamente por processos microbiológicos e bioquímicos diversos, os quais exercem enorme influência na produtividade e sustentabilidade dos ecossistemas terrestres (SIQUEIRA et al., 1994). A atividade microbiológica inclui todas as reações metabólicas celulares, suas interações e seus processos bioquímicos mediados ou conduzidos pelos microrganismos do solo. Um método muito utilizado na análise da atividade microbiana do solo é a hidrólise de diacetato de fluoresceína (FDA), este se baseia na determinação colorimétrica da Fluoresceína, formada quando o FDA é hidrolisado por uma série de enzimas hidrolíticas (proteases, lípases e esterases) presentes no solo (SCHNÜNER & ROSSWALL, 1982).

Com o aumento da atividade metabólica dos microrganismos do solo, há uma correspondente fixação dos minerais por estes, na diminuição da atividade minerais anteriormente imobilizados são liberados, isto acontece principalmente após a morte dos microrganismos. Outro método utilizado na análise do metabolismo do solo é a respiração microbiana, onde o CO_2 emanado do solo é absorvido por uma solução de hidróxido de potássio e posteriormente quantificado por titulação com ácido clorídrico (GRISI, 1978).

A dinâmica e atividade dos microrganismos no solo são complexas, por isso é essencial o uso tanto de parâmetros bioquímicos como biológicos na avaliação do crescimento e atividade microbiana do solo.

2. JUSTIFICATIVA E RELEVÂNCIA

Os tabuleiros arenosos costeiros do nordeste do Brasil, com fitofisionomia de cerrado, abrigam espécies da família *Cladoniaceae* dispostas sobre o solo, em forma de tufos. Algumas delas são endêmicas do litoral brasileiro (AHIT et al., 1993) e extremamente influenciadas pelos fatores microclimáticos locais (LEGAZ et al., 1986), bem como reguladas pelos climas sazonais, liberando quantidades diferenciadas de suas substâncias para o solo (PEREIRA, 1989).

Com isso, as relações ecológicas entre liquens e suas substâncias e a microbiota do solo subjacente contribui para maior conhecimento da dinâmica de ecossistemas tropicais, a maioria deles com reduzida área de remanescentes.

3. OBJETIVOS

3.1 Geral

Avaliar o efeito do talo *in natura* de *Cladonia salzmannii* Nyl sobre a atividade microbiana em ecossistema natural de tabuleiro (cerrado) e sob condições experimentais em casa de vegetação sobre fungos micorrízicos arbusculares e o desenvolvimento de plântulas de *Genipa americana* L.

3.2 Específicos

- ✓ Verificar a percolação do ácido barbático, principal composto de *C. salzmannii* para o solo em condições experimentais
- ✓ Analisar parâmetros bioquímicos e biológicos do solo como indicadores do efeito de *C. salzmannii* na rizosfera
- ✓ Testar o efeito do talo liquênico de *C. salzmannii* no desenvolvimento de plântulas de *G. americana*, inoculadas com FMA
- ✓ Avaliar o efeito da presença do talo de *C. salzmannii* sobre a colonização e esporulação de FMA associados a *G. americana*

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Trabalhos a serem submetidos à publicação

CAPÍTULO 1

The influence of *Cladonia salzmannii* in soil microbial activity and mycorrhizal symbiosis

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The influence of *Cladonia salzmannii* Nyl. in soil microbial activity and mycorrhizal symbiosis

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Abstract

In the Brazilian Northeast it is common to find places with vegetation like Savannah whose sandy soils are replete of lichens. These symbiontc organisms produce phenolics, unique on this taxon, known for their biological activity. This paper aim to ascertain the microbial activity and mycorrizal symbiosis in the soil covered by lichens. The lichen covered soil presented lower values of CO_2 emission, C microbial biomass, fluorescein diacetate (FDA) hydrolysis rate than bare soil areas. In contrast, mycorrhizal colonization and number of spores was higher in lichen covered soil. Low barbatic acid concentration was detected in the soil.

Keywords: Arbuscular mycorrhizal fungi, barbatic acid, *Cladonia salzmannii*, lichen substance, microbial activity.

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1. Introduction

About 180 millions hectares of Brazilian area are occupied by cerrado vegetation, which expands over the Central-west, over part of the Southeast, over the North and Northeast regions of Brazil (Goedert, 1989). These soils are naturally acid, have low fertility, and low of organic matter activity. The occurrence of species such as *Cladoniaceae* (lichen) in the sandy soils of tableland (tabuleiro), with cerrado vegetation physiognomy, is common in the Northeast region of Brazil. In Alhandra, State of Paraiba, it is frequent the presence of *Cladonia verticillaris* and *C. salzmannii*, lichen species known for the production of substances such as barbatic and fumarprotocetraric acids, respectively, unique phenolics to this taxon.

The negative influence of lichen substances against fungi and bacteria has been shown in several *in vitro* growth experiments (Halama & Van Hauwin, 1997; Falcão et al., 2004). Generally, in one specimen it may occur one to three, or more, lichen substances. Their concentration may vary from 0.1% to 10% of lichen thalli dry weight, but in some cases the concentration can be higher. One of the most prevalents lichen substance in many lichen species is the barbatic acid, depside composed of two phenolic units an ester bound, but its biological activity has not been very much studied.

It is probable that occasional rainfall periods, or even the dew, can wash amounts of these elements and leach then out from lichen to the soil (Malicki, 1965). Thus, such substances play an important ecological role, contributing to the soil structure and composition, and may affect the soil microbiota (Legaz et al., 1986; Xavier-Filho et al., 2006).

After some time, the presence and stability of these substances may exert a direct or indirect effect on other organisms (Yano et al., 1999). It was observed an increase of ectomycorrhizal community diversity, after the removal of lichen cover, which caused a decrease in the mortality of vegetation dependent to this symbiosis (Markkola et al., 2002).

After assessing the microorganism activity in the soil under *Cladina stellaris,* Stark & Hyvärinen (2003) suggest that lichen substances, such as usnic and perlatolic acids, are C sources for the microbial community in the soil under lichen cover. Similarly, Ohtonen & Väre (1998) observed a high incidence of microbial activity in the soil under different species of *Cladoniaceae*.

Accordingly, one the most studied lichen substances is the usnic acid, but little is known about the barbatic acid effects on the soil microbial activity. Thus, the objective of this paper was to ascertain the influence of *C. salzmannii*, which contains barbatic acid as major phenolic, on the soil microbial activity and on mycorrhizal symbiosis.

2. Materials and methods

2.1. Collection and storage of lichen material

C. salzmannii samples were collected during the months of November and December (2005) from sandy soils of tableland (tabuleiro), in Alhandra county (Paraiba, NE of Brazil). In the laboratory the lichen material was separated from its substrate and stored in paper bags. After chemical and morphological identification, voucher specimens were deposited in the Herbarium UFP, Universidade Federal de Pernambuco (Nº: 44.143).

2.2. Soil analysis

Soil samples were collected from the Alhandra-PB vegetation like savannah (cerrado), whose sandy soils are replete of lichen colonies of *Cladoniaceae* family. Soil samples from beneath the *C. salzmannii* covers and from areas without lichen were collected. Ten samples were taken from each of the sampling sites: each sample consisted of three subsamples taken from depths 0 to 20cm and mixed. Soil samples were immediately placed in coolers until arrival in the laboratory. There the soil was separated from the roots, and stored at 4°C, until the analysis moment. The soil chemical and physical characteristics analysis took place at the fertility laboratory of the Empresa Pernambucana Agropecuária (IPA). The physical and

chemical characteristics of the soils from the different areas are described in table 1 and 2. There were differences among the chemical characteristics of the soils form the different areas assessed.

CO_2 evolution was determined using 100 g soil, moistened to 10% of its water-holding capacity, placed in hermetically sealed flasks and incubated for 15 d at 28°C. The CO_2 emitted was collected in 10 mL 0.1 M KOH and titrated with 0.1 M
HCl (Grisi, 1978).

The microbial biomass was determined by the fumigation-extraction method. Extractable organic C from soil samples, either fumigated or non-fumigated with chloroform, was extracted with 0.5 M K_2SO_4 and after oxidation with potassium dichromate ($K_2Cr_2O_7$), the C content was measured by titrated with 0.033 N $(NH_4)_2Fe(SO_4)_2$ (De-Polli & Guerra, 1997; Vance et al., 1987).

The FDA (fluorescein diacetate) hydrolysis was estimated as described by Swisher & Carrol (1980) an incubation period of 20 min at 25°C. Five grams of soil were incubated in 20 mL of 60 mM K-phosphate buffer pH 7.6. The reaction was started by addition of 0.2 mL of a FDA solution (2 $mg.mL^{-1}$ in acetona). After 20 min incubation, 20 mL of acetone were added immediately for stoping the reaction. The soil suspensions were filtered and measured at 490 nm. The concentration of fluorescein released was calculated by a calibration curve produced with standard quantities (0 - 400µg) of fluorescein.

The quantifying of glomalin (glomalin-related soil protein) was done according to Wright & Upadhyaya (1998) method. The 0.25 g soil samples were autoclaved with 2 mL of sodium citrate (20mM; pH 7.0) during 30 minutes, followed by centrifugation at 10000 g for 5 min and the absorbance of the supernatants was measured at 595 nm. The concentration of glomalin was calculed by calibration curve BSA (Bradford, 1976).

The roots were cleared with KOH (10%) and stained with Trypan blue (0.05%) (Phillips & Hayman, 1970). Naturally dark pigmented roots were cleared with 10% hydrogen peroxide for one hour before the staining with Trypan blue. The percentage of root colonization was calculated by the gridline intersect method (Giovannetti & Mosse, 1980). The number of arbuscular mycorrhizal fungi (AMF) spores was determined by wet sieving, followed by centrifugation in 40% sucrose (Gerdemann & Nicolson, 1963 e Jenkins, 1964) and counted under a stereoscopic microscope (40X).

The results were submitted to analysis of variance (ANOVA) and the averages were compared by the Tukey test ($p<0.05$). The mycorrhizal colonization values and the number of spores were transformed arcsen (x/100) and log (x+1), respectively.

2.3 Lichen substances detection in the soil samples

Soil samples (8 g) and lichen thalli (4 g) were submitted to successive extractions with 10 mL of the systems ether/ethyl acetate (65:35, v/v) and chloroform/acetonitrile (60:40, v/v). Both extracts were measured in spectrophotometer at 268 nm. The extracts were dried and used for Thin Layer Chromatography (TLC) assays.

2.4. Thin Layer Chromatography

The soil, lichen extracts and standard substance (barbatic acid) were submitted to TLC, carried out on silica gel plates $F_{254+366}$ MERCK, developed on A solvent system: toluene/dioxan/acetic acid, 180:45:5, v/v (Culberson, 1972). The spots were visualized under UV short and long wavelengths (254 e 366nm). The chromatograms had posterior spray of H_2SO_4 (10%), and heated at 100 ºC for color reaction of the spots.

The barbatic acid used as standard was extracted from *C. salzmannii* as described in Pereira et al. (1996) and the purification was done according to Asahina & Shibata (1954).

3. Results and discussion

Barbatic acid was detected in the soil samples (fig 1). The soil under lichens presented higher pH values, sum of bases (S), cationic exchange capacity (CEC), base saturation (V) (table 1). The tableland soils generally present low nutrient availability, due to lower pH values and high Al saturation in the soil (Lopes, 1984). The lack of Ca and Mg, many times related to the excess of Al in the superficial layers of the soil, diminish the root system development, making the plants susceptible to drought (Kliemann, 2003), in such a way that soils under lichen cover were more fertile.

The CO_2 emission, biomass and FDA values were higher for the areas where there was no lichen, but without statistical difference among the treatments (table 3). In the mycorrhizal assessment parameters the highest values of colonization and number of spores were observed in the areas with lichen (table 4).

Studies have shown the need to relate soil microbiology to enzymatic activity. Measures such as CO_2 emission and C microbial biomass, by their own, do not reflect the soil biological activity (Dick, 1997). In the case of respiration, the microorganisms may not be the only source of CO_2 emission, and the microbial biomass does not estimate the isolated microorganisms metabolic activity. As a measure of total microbial activity it was used in this paper the FDA hydrolysis, commonly used as an indicator to the activity of the soil hydrolytic enzymes (Taylor et al., 2002). However, the degree of enzymatic alteration may vary depending on the concentration and the way in which the studied element. D'Ascoli et al. (2006) stated that the soil enzymatic activity is more influenced by the quantity of C organic than the quantity Cu and Cr.

Using the FDA hydrolysis method as indicator of soil quality, in degraded and native areas in the Brazilian cerrado, Godoi (2001) observes that degraded soils going through revegetation with native species present chemical and microbiological properties similar to

preserved soil in native forest, and are higher than the soils reforested with *Eucalyptus sp*. Alencar & Costa (2000) used the same method for evaluating the impact using fungicides on microbial activity, and found out that the application of fungicides via water irrigation reduced the microbial activity in cultivated soils. In addition, Kähkönen et al. (2006) did not see meaningful differences in the FDA hydrolysis in a former sawmill area, which can be associated to the probable substitution of microbial species for tolerant species to those conditions.

Studying the effect of the salinity on soil microorganisms, Bing-Cheng at al. (2007) found low microbial biomass and CO_2 emission values, evidencing that salinity adversely affects the soil microbial community.

Marchiori Júnior & Melo (2000), analyzing the microbial C, did not find meaningful differences between 20-to-25 year-old pastures and natural forest. However, the same authors discovered in areas cultivated with cotton for 10 years, reductions higher than 60% in the microbial C value, compared to natural forest. Therefore, the soil organic matter and the microbial biomass can be altered with more or less intensively depending on the vegetation and the environmental conditions.

Ohtonen & Väre (1998), assessing the microbial activity in areas with lichens and in areas with bryophytes, did not notice differences in the microbial biomass, but areas with lichen presented higher qCO_2. The authors stated that the soil microbial activity depends on divers factors, such as climate, habitat productivity and ecological succession stage.

Comparing lichen, bryophytes, and grass effects on the soil, Sedia & Ehrenfeld (2005) highlighted that the lichens and the bryophytes clearly affect soil microbial properties.

Stark & Hyvärinen (2003) observed higher qCO_2 values, which should suggest that lichen substances such as usnic and perlatolic acid are sources of C to the microbial communities existing beneath the lichen covers.

Lichens are known for their biological activity of their substances, which have been widely studied in last years, especially in *vitro*. Such substances have proved to be active against fungi and bacteria (Pereira et al., 1991; Pereira et al., 1996), against tumors and carcinogenic cells (Pereira et al., 1994); and to have an alellopathic effect (Yano et al., 1999). Goldner et al. (1986) reported that the usnic acid at concentrations at 10, 25 and 50 mg L^{-1} it's responsible for the growth inhibition of ectomycorrhizal fungi.

The fact that there is no meaningful effect in the observed parameters can be the consequence of low concentration of the substance, an average of 3.9µg.mL^{-1}. Further studies about microbial activity, especially about arbuscular mycorrhizal fungi in soils beneath lichens is still necessary for a better understanding of processes and confirmation of the tendencies here observed.

4. Ackowledgements

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Table 1. Soil chemical characteristics in the lichen cover and bare soil areas.

Treatments	P	pH*	Ca	Mg	Na	K	Al	H	CEC	S	%	
	mg/dm^3		$cmol_c/dm^3$								V	m
lichen cover	2	5.11	0.85	0.45	0.04	0.03	0.55	5.22	7.1	1.4	19	29
bare soil	2	4.85	0.25	0.50	0.04	0.02	0.90	5.12	6.8	0.8	12	53

*H_2O S -sum bases; CEC-cation exchange capacity; V-base saturation; m- Al saturation.

Table 2. Physical soil characteristics in soil of tableland from Alhandra, Brazil.

Density (g/cm^3)		Soil texture (%)				Soil texture classes (%)	Umitidy (%)
Dap	Dr	Coarse Sand	Fine Sand	Silt	Clay		
1.51	2.63	57	28	15	0	Sandy	1.40

Table 3. Soil microbial and biochemical activity in lichen cover and bare soil areas.

Treatment	Respiration ($C\text{-}CO_2\ g^{-1}$ dw of soil d^{-1})	Microbial biomass (µg C g soil $^{-1}$)	FDA (g dw of soil h^{-1})
lichen cover	6.5a	1462.3a	146.5a
bare soil	7.3a	191842.7a	178.2a

Table 4. Mycorrhizal colonization and spores numbers in different treatments.

Treatment	Glomalin (mg/g soil)	Colonization	Number of spore
lichen cover	2.2	3.1	13.7
bare soil	2.1	2.5	8.5

The statistical signicances of the differences between treatments are assessed with the Tukey test ($P \leq 0.05$). The values were transformed arcseno x/100 (colonization) e log x+1 (Spores numbers).

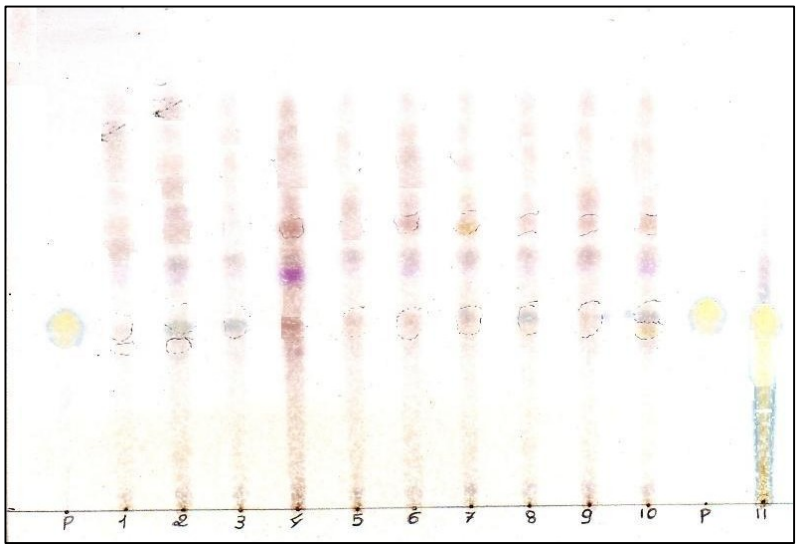

Figure 1. Thin Layer Chromatography of soil and lichen extracts. P=barbatic acid, 1-10= soil extracts, 11=*C. salzmannii.*

CAPÍTULO 2

Influence of *Cladonia salzmannii* on arbuscular mycorrhizal fungi and growth of *Genipa americana* seedlings

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The influence of Cladonia salzmannii on arbuscular mycorrhizal fungi and growth of Genipa americana seedlings

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Abstract

Lichens are well known for the important biological activity of their substances, which are accumulated either on the cortex or on the cell walls of medullary hyphae, and can be leached out to the soil by rainfall or dew. Thus, such substances can affect the soil microbiota in different ways. This paper aims at assessing the possible influence that Cladonia salzmannii may have on arbuscular mycorrhizal fungi and on the growth of Genipa americana. The inoculated plants either with lichens+mycorrhiza presented higher growth parameters, as well as a higher number of spores. It was detected in the soil the presence of barbatic acid, major compounds of C. salzmannii.

Keywords: Arbuscular mycorrhizal fungi, barbatic acid, Cladonia salzmannii, lichen substance.

1. Introduction

The fungi form symbiotic associations with algae (lichen) and with plant roots (mycorrhiza). These intimate and lasting associations among the organisms were and are of extraordinary importance in the establishment of phototrophs adapted to terrestrial ecosystems (Margulis, 1990; Jeffries & Young, 1994; Frank, 1995).

Those mutualistics associations have played an important ecological role. Lichens affect the chemistry of rocks during the soil formation (pedogenesis), and the arbuscular mycorrhizal fungi (AMF) interact directly with the plants, participating in the transfer of mineral nutrients and plant productivity (Pfleger et al., 1994; Legaz et al., 2006).

The weathering action of lichens on rocks involves both biogeophysical and biogeochemical process. Rhizine penetration and thallus expansion and contraction are the import mechanisms involved in biogeophysical weathering, whereas lichen substances are important in biogeochemical weathering due to the ability to complex metal cations from the mineral substratum (Purvis et al. 1996). The rainfall and dew can wash from the thallus to the

soil significant amounts of these substances, which may, after some time, result in the allelopathic effect on the soil microorganisms (Malicki, 1965; Legaz et al., 1986; Yano et al., 1999).

Accordingly, Brown & Mikola (1974) observed the limited growth of Pinus silvestris and Picea abies caused by the action of aqueous extract of the Cladonia alpestris, being the usnic acid major substance in this species. The authors associated that growth limitation to the ectomycorrhizal fungi inhibition.

The AMF are widespread in most ecosystems and colonize > 85 % of land plants (Siqueira, 1994; Xavier & Germida, 1999; Johansson et al., 2004). In these plant-fungal associations, the mycorrhizal fungi receive photosynthates produced by the plant and, in exchange, increase the plant capacity of mineral and water uptake (Azcón-Aguilar & Barea, 1997).

Among the factors that determine, directly or indirectly, the presence or development of the AMF the soil and the vegetation are to be highlighted. Thus, the presence of the allelochemicals in the soil, can affect the mycorrhyzal association, acting as inhibitors or stimulators that symbiosis. Therefore, the objective of this paper is to ascertain the possible influence of Cladonia salzmannii (lichen) on the arbuscular mycorrhizal fungi and on the development of Genipa americana L. (genipap) seedlings.

2. Materials and methods

2.1. Collection and storage of lichen material

C. salzmannii samples were collected during the months of November and December (2005) from sandy soils of tableland (tabuleiro), in Alhandra county (Paraiba, NE of Brazil). In the laboratory the lichen material was separated from its substrate and stored in paper bags. After chemical and morphological identification, voucher specimens were deposited in the Herbarium UFP, Universidade Federal de Pernambuco (Nº: 44.143).

2.2. Greenhouse experiments

The experiment was done in a factorial randomized design of 4 trataments (control, lichen, lichen+FMA and FMA) and 5 replicates, and it was carry out in the greenhouse conditions at room temperature (28 ºC ± 3ºC)

G. americana seeds were disinfected with 20% sodium hypochlorite, and sown in trays with 120 cells, containing sterilized vermiculite. Daily watering took place after the sowing.

After 3–4 leaves emerged, seedlings were transplanted into plastic bags (2 l) containing autoclaved soil from Alhandra-PB, and inoculated with two AMF species (Gigaspora albida Schenk & Smith and Acaulospora longula Spain & Sckenck). The inoculum was the mixture of spores, external hyphae and colonized root fragments in soil. After 20 days the bags were mulched with 33g of C. salzmannii. The plants were watered (50 ml) three times weekly.

Four months later the plants were harvested and analyzed. Height measurement was done, taking into consideration the distance between the collar and the apical region. The stem diameter was measured at the collar level. The plant components were separated in shoot (stem and leaves) and root, and dried at 50°C for 96h, then weighed for a dry mass assessment. The roots were cleared with KOH (10%) and stained with Trypan blue (0.05%) (Phillips & Haymann, 1970). Naturally dark pigmented roots were cleared with 10% hydrogen peroxide for one hour before the staining with Trypan blue. The percentage of root colonization was calculated by the gridline intersect method (Giovannetti & Mosse, 1980). The number of AMF spores was determined by wet sieving, followed by centrifugation in 40% sucrose (Gerdemann & Nicolson, 1963 e Jenkins, 1964) and counted under a stereoscopic microscope (40X).

The soil chemical and physical characteristics analysis took place at the fertility laboratory of the Empresa Pernambucana Agropecuária (IPA). The soil chemical and physical characteristics are described in tables 1 and 2, the soil was classified as sandy, it was observed lower pH values, sum bases (S), base saturation (V) and Ca, Mg, Na concentrations in the soil after the experiment.

The results were submitted to analysis of variance (ANOVA) and the averages were compared by the Tukey test ($p<0.05$). The mycorrhizal colonization values and the number of spores were transformed (arcsen (x/100) and log (x+1), respectively).

2.3 Lichen substances detection in the soil samples

Soil samples (8g) and lichen thalli (4g) were submitted to successive extractions with 10 ml of the systems ether/ethyl acetate (65:35, v/v) and chloroform/acetonitrile (60:40, v/v). Both extracts were measured in spectrophotometer at 268 nm. The extracts were dried and used for Thin Layer Chromatography (TLC).

2.4. Thin Layer Chromatography (TLC)

The soil and lichen extracts and standard substance (barbatic acid) were submitted to TLC, carried out on silica gel plates $F_{254+366}$ MERCK, developed on A solvent system: toluene/dioxan/acetic acid, 180:45:5, v/v (Culberson, 1972). The spots were visualized under UV short and long wavelengths (254 e 366nm). The chromatograms had posterior spray of H_2SO_4 (10%), and heated at 100ºC for spots color reaction.

The barbatic acid used as the standard was extracted from C. salzmannii as described by Pereira et al. (1996) and the purification was done according to Asahina & Shibata (1954).

3. Results

The treatments lichen and lichen+FMA presented a longer stem diameter in relation to the control, but there were no relevant differences between them. The same could be observed so as to the height of the plants (table 3).

The lichen+AMF treatment presented fresh and dry weights meaningfully higher than the other treatments. The plants inoculated only with lichen presented values lower than those for the plants inoculated with mycorrhizal fungi(table 3)..

The mycorrhizal colonization was considered low in all cases, but the lichen+AMF treatment was slightly higher compared to the others. The arbuscular mycorrhizal spore number was higher in lichen+AMF treatment (table 4).

The soil samples chromatography revealed the presence of barbatic acid (figure 1), however in a very low concentration (3.9µg/ml).

4. Discussion

Most of the studies about lichens evidence their negative influence on other organisms. Such influence can be related to the action of the lichen substances. Brown and Mikola (1974) mentioned the usnic acid, main component in the aqueous extract of the Cladonia alpestris, as the responsible for the limitation in the development of the Pinus silvestris and Picea abies. Fisher (1979) also suggests that lichens have a negative influence on higher plants through indirect effects mediated by changes in metabolic activity of soil microorganisms and mycorrhiza. In addition to that, Markkola et al. (2002) emphasize that the removing of lichen covers contributed to the increasing of the ectomycorrhizal diversity, diminishing the mortality of dependent vegetation. However, the present paper does not evidence that relation. This fact indicates that the chemical constitution of the studied species, as well as the concentration of its substances, is responsible for the activity that the lichen may exert on the tested organism.

The treatment that presented the highest vegetative growth values was the lichen+AMF, followed by the mycorrhiza treatment. The fact that seedlings colonized with AMF presented higher values than the control, in vegetative growth terms, is associated to its high capacity to acquire mineral nutrients from the soil, as the AMF plant colonization

enlarges the nutrient acquisition surface (Hayman,1970), and the water acquisition surface (Allen, 1991), enhancing the plant growth.

In most cases, fresh and dry weight, mycorrhizal colonization, and number of spores were higher for the lichen+AMF treatment. Besides, the plants inoculated only with lichens presented higher development than the control ones, and in some cases, like the fresh weight axis, similar values of the mycorrhiza treatment. The results aforementioned can indicate a potential lichen effect. According to Asta et al. (2001) Cladonia species may contribute to humus formation. In addition to affecting the soil temperature and humidity, lichens absorb the elements that volatilize from the soil, and use producing for lichen acids, which alter the substrate chemically (Nash III, 1996; Legaz et al., 2006). These factors may have contributed to the G. americana development in the plants with lichen as expected, for this happens frequently in forest formations located in humid flatlands (Lorenzi, 1992).

The mycorrhizal colonization was considered low, but the colonization and the response to inoculation can vary according to the different host, depending on the effectiveness of the AMF (Sanders et al., 1996), P availability in the soil (Miller et al., 1994) and the fungi-plant combination. According to Siqueira (1991), there is a great extent of functional compatibility which results in the establishment of symbiosis between plant and fungi, controlled by both organisms' genes and modulated by the environment. Zangaro et al. (2002) include the G. americana in the list of the species that normally have a very low incidence of mycorrhizal colonization (1 – 12%). In addition, many reports where different pH conditions were studied have shown root colonization to be reduced in lower pH (Silva et al., 1994; Nurlaeny, 1995).

The soil, after the experiment, presented pH increase. Ca, Mg, and Na concentrations also increased, as well as the CEC and the S values. It is known that the nutrient availability to the plants is affected by different soil features, such as pH, humidity and microbial activity.

Taking into consideration the fact that the presence of Al is one of the main factors that limit plant development in acid soils, it is important to mention that the Al values decreased after experiment, which is positive. Similarly, it was observed an important V increasing, mainly due to Ca and Mg concentrations increase, which contributes to soil fertility. It was also observed the presence of barbatic acid, in a low concentration (3.9µg/ml).

In addition, it was observed a slight presence of AMF spores in controls, perhaps due to some kind of contamination. The very low vegetative development ($P \leq 0.05$) proves that this probable contamination did not interfere in the obtained results.

Once that lichen substances are known for its biological activity, it is possible to suppose that the presence of barbatic acid in the soil may also be influencing the results either directly, by action on the plant, or indirectly by the effects on the AMF. Yano (1994) observed that the aqueous extract of Cladonia verticillaris stimulates the growth of Allium cepa, this fact relating to the presence of fumarprotocetraric acid. Thus, probably lichens exert ecological influence on the habitat, controlling somehow the surrounding vegetation.

5. Ackowledgements

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Table 1. Characteristics of the soil before and 4 months later the experiment. The seedlings G. americana inoculated with and without C. salmannii or AMF.

Soil	P	pH	Ca	Mg	Na	K	Al	H	S	CEC	%	
	mg/dm^3		$cmol_c/dm^3$								V	m
Before	3	4.60	1.25	0.25	0.05	0.03	0.40	5.45	1.6	7.4	21	20
After	2	5.06	1.75	0.30	0.75	0.01	0.15	4.13	2.8	7.1	40	5

S= sum bases; CEC=cation exchange capacity; V=base saturation; m=Al saturation

Table 2. Physical characteristics of soil used in experiment.

Density (g/cm^3)		Soil texture %				Clay %	Soil texture classes %	Humitidy %
Dap	Dr	Coarse Sand	Fine Sand	Silt	Clay			
1.51	2.63	57	28	15	0	4	Sandy	1.40

Table 3. Growth parameters of G. americana seedlings, cultivation in pots with and without C. salzmannii or AMF.

Treatments	Stem diameter (cm)	Growth (cm)	Axis		Roots	
			PF (g)	PS (g)	PF (g)	PS (g)
control	0.47b	8.9b	4.8c	1.3c	3.0d	0.7c
lichen	0.48ab	9.1b	5.5b	1.6b	3.9c	1.1b
mycorrhiza	0.5[a]	9.4a	5.0b	1.7b	4.9b	1.0b
lichen+AMF	0.6a	9.5a	5.2a	2.3a	7.3a	2.2a

The different letter describes statistical diferences ($p < 0.05$).

Table 4. Mycorrhizal colonization and spores numbers of G. americana seedlings growth with and without C. salzmannii.

Treatments	Esporulation	Colonization
control	1.1b	-
lichen	1.1b	-
mycorrhiza	1.5b	0.01b
lichen+AMF	2.5a	0.04a

The statistical signicances of the differences between treatments are assessed with the Tukey test ($P \leq 0.05$). The values were transformed arcseno x/100 (colonization) e log x+1 (Number spores).

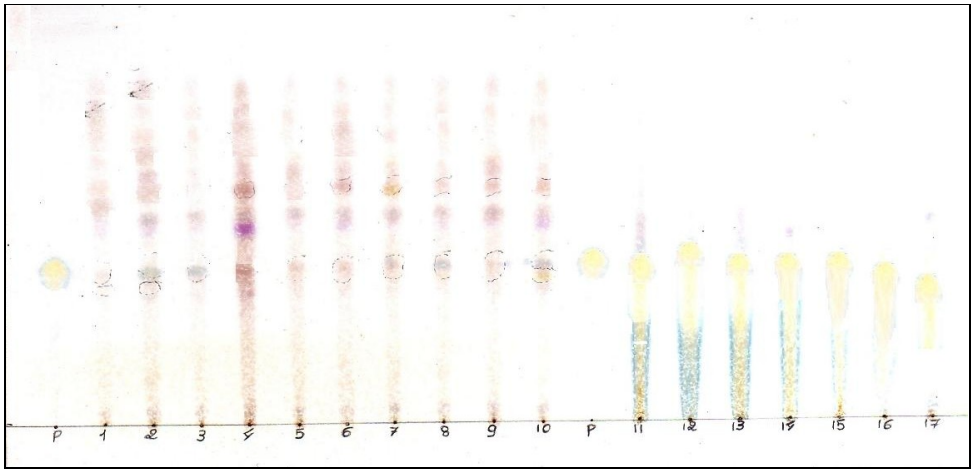

Fig 1. Thin Layer Chromatography of soil and lichen extratcs. P-barbatic acid, 1-10 soil extracts, 11-17 C. salzmannii.

5. CONCLUSÕES

Com base nos resultados obtidos neste trabalho, concluiu-se que:

- ✓ O fluxo de água pode arrastar substâncias do talo liquênico para o solo
- ✓ Concentrações muito baixas (3,9µg/ml) de ácido barbático não alteram significativamente a microbiota do solo
- ✓ Os liquens afetam as propriedades do solo e consequentemente desempenham alguma influência ecológica sobre seu habitat
- ✓ O número de esporos e a colonização micorrízica é maior na presença de *C. salzmannii*
- ✓ Os tufos de *C. salzmannii* influenciam o desenvolvimento de plântulas de *G. americana*, especialmente quando inoculadas com FMA.

Possíveis interações entre *C. salzmannii*, a vegetação adjacente e a microbiota do solo estão envolvidas, até certo ponto, no controle do padrão de distribuição da vegetação do seu habitat. Esta influência pode se dar pela da produção e liberação do ácido barbático no solo ou por meio de alterações químicas do solo.

Estudos em campo, realizados em diferentes períodos e que utilizem mais parâmetros bioquímicos e biológicos, como por exemplo, a atividade da fosfatase e da desidrogenase enzimas que desempenham um importante papel na ciclagem de nutrientes, são necessários para esclarecer como os liquens influenciam outros organismos.

Anexos

Sociedade Brasileira de Bioquímica e Biologia Molecular — SBBq
VIII Reunião Regional Nordeste da SBBq
3rd International Symposium in Biochemistry and Biotechnology
Hotel Praiamar, Natal, RN, 6 a 8 de Dezembro de 2006

INFLUENCE OF *CLADONIA SALZMANNII* ON ARBUSCULAR MYCORRHIZAL FUNGI AND GROWTH OF *GENIPA AMERICANA* SEEDLINGS

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A greenhouse experiment was carried out to evaluate the potential allelopathic effects of lichen substances on arbuscular mycorrhizal fungi (AMF) of soil. The experiment design was completely randomized, in 2(presence or absence of the lichen) x2 (presence or absence of the AMF), with 6 replications. The seedlings were transplanted and inoculated with 300 spores of AMF placed on the root, and 20 days later received 33g de *Cladonia salzmannii* (lichen). The plants were harvested 4 months after transplanted, and the following parameters were analyzed: growth, stem diameter, dry weight axis and root, number of spore and mycorrhizal colonization. All the samples (lichen and soil) were measured using thin-layer chromatography for detection of lichen substances. The lichen/mycorrhiza treatment had number of spores 3 times higher than others treatments, but slightly lower mycorrhizal colonization. The parameters stem diameter and growth were similar to treatments lichen (alt.10,7cm, dc1,05cm) and mycorrhiza (alt.11cm,dc.1,07cm) but higher in the lichen/mycorrhiza. In this treatment was observed higher values of both dry weight axis and root. Barbatic acid (lichen substance) was detected in the soil samples. This way, the difference sporulation was probably due to the presence this substance.

Key words: arbuscular mycorrhizal fungi, *Cladonia salzmannii*, barbatic acid, allelopathy.
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Please supply a short running title of up to 60 characters (including spaces).

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g) Acknowledgements can be made to such as funding agencies, colleagues who assisted with the work or the preparation of the manuscript, and those who contributed materials or provided unpublished data.

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FEMS Microbiology Reviews

The review should contain the items listed above, excepting that the *Materials and methods* and *Results* sections will not be relevant. The *Discussion* section is preferably replaced by *Concluding remarks*, which do not repeat the *Introduction* or main sections but may, for example, point to future directions.

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Guide for Authors

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