academicJournals

Vol. 7(17), pp. 1138-1145, 3 May, 2013
DOI: 10.5897/JMPR12.173
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro effects of *Allium obliquum* extract on the growth and ultrastructure of *Botrytis paeoniae*

Marcel Pârvu[1], Alina Elena Pârvu[2]*, Lucian Barbu-Tudoran[3], Oana Roşca-Casian[4], Laurian Vlase[5], Maria Rozalia Danciu[6] and Ovidiu Pârvu[7]

[1]Department of Biology, Faculty of Biology and Geology, Babeş-Bolyai University, Romania.
[2]Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
[3]Electron Microscopy Center, Babeş-Bolyai University, Romania.
[4]A. Borza Botanical Garden, Babeş-Bolyai University, Romania.
[5]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
[6]Samus Technical High School, Cluj-Napoca, Romania.
[7]Faculty of Mathematics and Computer Science, Babeş-Bolyai University, Romania.

Accepted 26 July, 2012

The allicin and alliin contents of *Allium obliquum* L. ethanol extract were analyzed by liquid chromatography coupled with mass spectrometry method. Additionally, the ability of *A. obliquum* to inhibit the growth of *Botrytis paeoniae* Oudem. on the nutritive medium was assessed. The *in vitro* fungicidal activity of *A. obliquum* was studied by the ultrastructural changes induced in the conidia and sclerotia of *B. paeoniae* treated with the plant extract in minimum inhibitory concentrations.

Key words: Allicin, alliin, antifungal activity, electron microscopy, minimum inhibitory concentration.

INTRODUCTION

The *Botrytis* genus comprises over 20 species (Beever and Weeds, 2007) which produce the gray mold of many agronomically important plants (Beever and Weeds, 2007; Kirk et al., 2001). The members of this genus include *Botrytis cinerea* Pers., *Botrytis allii* Munn, *Botrytis fabae* Sardina, *Botrytis paeoniae* Oudem., and *Botrytis tulipae* (Lib.) Lind (Elad et al., 2007; Jarvis, 1977). The most studied species of this genus is *B. cinerea*, a plant pathogen attacking over 200 crop species worldwide (Webster and Weber, 2007). *B. cinerea* is difficult to control because it has a variety of modes of attack, diverse hosts as inoculum sources, and it can survive as mycelia and/or conidia or as sclerotia for extended periods in crop debris. In addition, *B. cinerea* produces a range of cell wall-degrading enzymes, toxins, and other low-molecular-weight compounds such as oxalic acid. New evidences suggest that the pathogen triggers the host to induce programmed cell death as an attack strategy (Williamson et al., 2007).

Since *B. cinerea* has developed resistance against almost all currently used fungicides (Giraud et al., 1999; Webster and Weber, 2007), finding new measures to control the gray mold is vital. The biological control of *Botrytis* species may be done via a variety of methods, including using microbial antagonists (Elad and Stewart, 2004), and different plant extracts (Choi et al., 2004;

*Corresponding author. E-mail: parvualinaelena@yahoo.com. Tel: +40364112682. Fax: +40364815349.

Miclea and Puia, 2010; Wilson et al., 1997) etc. Medicinal plants remain a rich source of novel therapeutic agents. Several studies regarding the action of plant extracts against some phytopathogenic fungi have been performed. However, many plant species remain chemically or biologically unevaluated with respect to their phytotherapeutic properties. In this respect, we studied *Allium obliquum* species, an edible plant and a very rare perennial which is located in a single region in Romania (on limestone rocks in Turda Gorges). This species is also native to south-east Russia, Siberia, and Central Asia (Ciocârlan, 2009).

Interestingly, our previous studies showed that the *A. obliquum* extract had an important antifungal effect against phytopathogenic fungi such as *Sclerotinia sclerotiorum*, *B. cinerea*, *Fusarium oxysporum* f. spp. *gladioli*, *Aspergillus niger*, and *Penicillium expansum* (Pârvu et al., 2010a). *B. paeoniae* attacks *Paeonia* plants, and it is widely distributed in Europe and America (Ellis, 1971). This fungus is found every year on *Paeonia* plants from Cluj-Napoca, Romania.

The aim of this study was to evaluate the antifungal activity of *A. obliquum* on *B. paeoniae* germination and growth and to examine the ultrastructural changes in the conidia and sclerotia of this fungus caused by the minimum inhibitory concentration (MIC) of the plant extract. Since the allicin found in *Allium* plant extracts is an important antifungal agent (Davis, 2005; Josling, 2003; Khodavandi et al., 2010), the second aim of the study was to perform a quantitative analysis of allicin and its precursor alliin obtained from *A. obliquum* extract.

MATERIALS AND METHODS

Plant

A. obliquum L. (Liliaceae) plants were grown in the Agrobotanical Garden of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania, from seeds collected in Turda Gorges, Romania. The plant was identified by Dr. Gheorghe Groza. A voucher specimen (CL 659564) was deposited at the herbarium of A. Borza Botanical Garden, Babeş-Bolyai University of Cluj-Napoca, Romania.

Preparation of alcoholic plant extract

Fresh *A. obliquum* herba (leaves, stems and flowers fragments of 0.5 to 1 cm) was harvested on 28th May, 2007 and was extracted with 50% ethanol (Merck, Bucureşti, Romania) in the Mycology Laboratory of Babeş-Bolyai University, Cluj-Napoca, Romania, by a modified Squibb's repercolation method. Briefly, 3 successive applications of the same menstruum were repercolated to the plant material. In each percolator, the plant material (150 g in the first, 90 g in the second, 60 g in the third percolator) was moistened with the menstruum, macerated for 2 days and then percolated at a rate of approximately 4 to 6 drops per minute for each 100 g of raw material. The first percolated fractions from each percolator were saved, and the next fractions were poured in the next percolator. Then, the saved fractions (60 ml from the first one, 90 ml from the second one, and 150 ml from the third one) were mixed and the resulting extract was 1:1 (w:v) (Pârvu et al., 2010a).

Preparation of fungal colonies

B. paeoniae isolated from *Paeonia officinalis* L. flowers obtained from plants grown in A. Borza Botanical Garden, Babeş-Bolyai University of Cluj-Napoca, Romania, was included in this study. Fungal colonies were obtained from the collection of the Mycology Laboratory, Babeş-Bolyai University of Cluj-Napoca, and were grown in Petri dishes containing Czapek-agar medium (BD Difco, Budapest, Hungary), following inoculation into the central point and incubation at 22°C for 5 days.

Determination of antifungal activity

The antifungal activity of the *A. obliquum* extract expressed as minimum inhibitory concentration (MIC) was determined by the agar-dilution assay, and was compared to the antimycotic drug fluconazole (2 mg ml^{-1}) (Krka, Novo Mesto, Slovenia) and control (nutritive medium and 50% ethanol). The percentage of mycelial growth inhibition (P) at each concentration was calculated using the formula:

$$P = (C - T) \times 100/C$$

where C is the diameter of the control colony and T is the diameter of the treated colony (Nidiry and Babu, 2005).

Statistical analysis

The results for each group were expressed as mean ± standard deviation. Data were evaluated by analysis of variance (ANOVA). Statistical differences were considered significant at the $p < 0.05$ level. The correlation analysis was performed by the Pearson test.

Quantitative analysis of alliin and allicin

The analyses of alliin and allicin from *A. obliquum* extract were performed using a newly developed liquid chromatography coupled with mass spectrometry detection (LC/MS) (Vlase et al., 2010). Briefly, an Agilent 1100 series high performance liquid chromatography (HPLC) system was used (Agilent Technologies, Darmstadt, Germany), coupled with an Agilent Ion Trap SL mass spectrometer equipped with an electrospray ion source. The mass spectrometer operated in a positive multiple reaction monitoring mode by using nitrogen as a nebulizing and dry gas.

The chromatographic separation of alliin was performed using a Zorbax SB-C18 100 mm × 3.0 mm i.d., 3.5 µm column (Agilent Technologies, Darmstadt, Germany). The mobile phase consisted 100% ammonium acetate, 1 mM in water, isocratic elution flow of 1 ml/min. The nebulizer was set at 70 psi, and the dry gas flow was 12 liters/min at 350°C temperature. The mass spectrometer was set to record the transition m/z 178 > m/z 88, which is specific to alliin (Sigma-Aldrich NV/SA, Bornem, Belgium). The retention time of alliin in the above described conditions was 0.64 min.

The chromatographic separation of allicin was completed in a Synergi polar column, 100 mm × 2.0 mm i.d., 4 µm (Phenomenex, California, USA). The mobile phase consisted of 100% aqueous

Table 1. Antifungal activity of *Allium obliquum* extract on *in vitro* germination and growth of *Botrytis paeoniae* fungus.

Extract of *A. obliquum* (µl/ml)	Colony[a] diameter (mm)	P[a] (%)	Flucona-zole (µl/ml)	Colony[b] diameter (mm)	P[b] (%)	Allicin (µl/ml)	Colony[c] diameter (mm)	P[c] (%)
C	60	0	C	60	0	C	60	0
10	56	11.67±0.11	20	50	16.66±0.14	40	58	3.33±0.05
20	32	48.33±0.32	60	24	60±0.48	60	46	23.33±0.21
40	14	73.33±0.46	100	5	91.66±0.87	80	28	53.33±0.46
50	4	91.67±0.93	120	0	100±0.92	100	5	91.66±0.87
60	0	100±0.57	-	-	-	120	0	100±0.9

[a] = The effect of *A. obliquum* extract; [b] = the effect of fluconazole; [c] = the effect of allicin. C = control (50% aq. ethanol). P = mycelial growth inhibition. Results are the mean of 4 experiments ± SEM.

ammonium acetate 1 mM, isocratic elution, at a flow rate of 0.6 ml/min. A 1 mM aqueous silver nitrate solution was added post-column by using a mixing tee with a flow rate of 10 µl/min. The nebulizer was set at 60 psi, and the dry gas flow rate was 12 liters/min at 350°C temperature. The mass spectrometer was set to record the transition m/z (449 + 451) > m/z (269, 271, 287, and 289), which is specific to the allicin-silver adduct. The retention time of allicin in the above-described conditions was 0.9 min.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) examinations of conidia and sclerotia

Samples containing the conidia and sclerotia of *B. paeoniae* isolated from culture obtained on Czapek-agar medium were incubated with *A. obliquum* extract at its MIC for 1 h. The grids were examined by scanning electron microscopy (SEM) with a JEOL JSM 5510 LV electron microscope and by transmission electron microscopy (TEM) with a JEOL JEM 1010 electron microscope (Japan Electron Optics Laboratory Co., Tokyo, Japan) (Hayat, 2000).

RESULTS

A. obliquum analysis by liquid chromatography/ magnetic separation (LC/MS) revealed 141.08 µg alliin/ml of the plant extract (Pârvu et al., 2010a) and 2.819 mg allicin/ml of the plant extract (Figure 1). The *A. obliquum* plant extract had a significant inhibitory effect on the mycelial growth of *B. paeoniae* in the nutritive medium, with a MIC of 60 µl/ml which is half of the value of the MIC of fluconazole and standard allicin (120 µl/ml) (Table 1).

The SEM micrographs showed that the control *B. paeoniae* conidia were ovoid and that they had 12 to 18 µm long/8 to 10 µm short dimensions. They had numerous, randomly positioned protuberances on the cell wall's surface (Figure 2). When treated with the MIC of the *A. obliquum* extract, the shape and size did not change, but the surface protuberances disappeared (Figure 3). The TEM micrographs of the control conidia showed the following features: a regular cell wall about 300 to 400 nm thick, with a two-layer structure; plasmalemma; cytoplasm matrix with nucleus and nucleolus; mitochondria, etc. The cell wall's external layer was thin and electrondense; the inner wall was thick, uniform, and less electrondense. The plasmalemma tightly adhered to the cell wall. The cytoplasm matrix (cytosol) was uniformly distributed, and the nucleus was ovoid or spherical. The mitochondria were numerous and usually ovoid and had an average electron density. The intracellular nutrient reserves were glycogen and lipid (Figure 4).

The TEM micrographs of the *B. paeoniae* conidia treated with the MIC of *A. obliquum* extract showed important ultrastructural changes. The cell wall had a slightly irregular outline, loosely distributed components, and was highly permeable. The cell wall's external layer was more electrondense than the control. The plasmalemma was mostly destroyed, and it did not adhere to the cell wall. Precipitation of the entire cytoplasm and destruction of organelles and nucleus were observed. Moreover, there was a reduced electrondense band (periplasmic space) between the altered cytoplasm and the cell wall (Figure 5).

The TEM micrographs of the *B. paeoniae* sclerotia showed the medullary hyphae components (cell wall, plasmalemma, cytoplasm, nucleus, lipid bodies, and glycogen) surrounded by a continous matrix of β-glucans. The plasmalemma

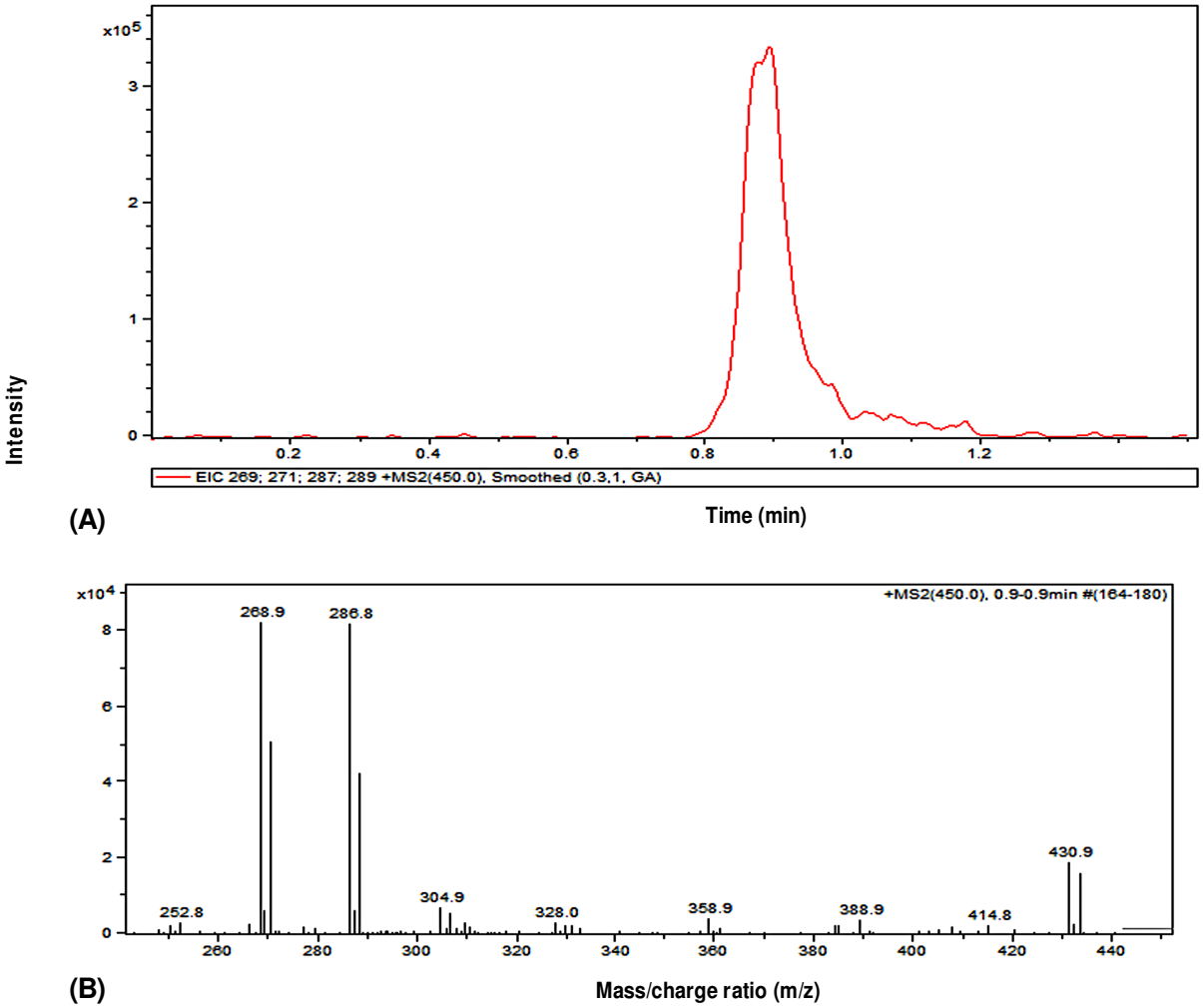

Figure 1. Chromatogram (A) and LC/MS (B) of allicin from *A. obliquum* extract.

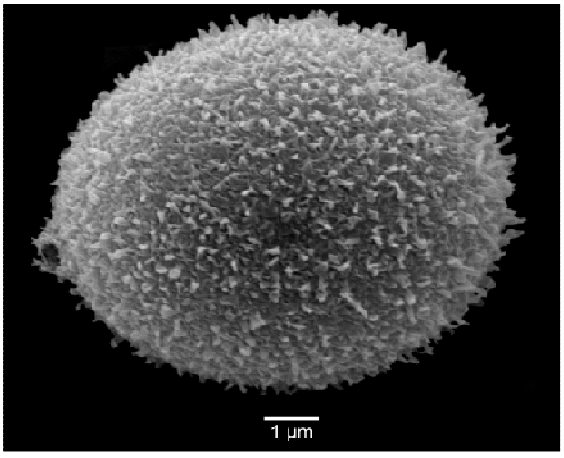

Figure 2. Scanning electron micrograph of a control *Botrytis paeoniae* conidium showing the surface with randomly positioned protuberances.

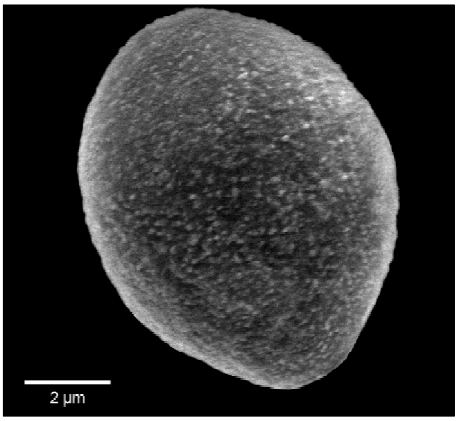

Figure 3. Scanning electron micrograph of a *Botrytis paeoniae* conidium treated with *Allium obliquum* plant extract at the minimum inhibitory concentration showing surface protuberance damage.

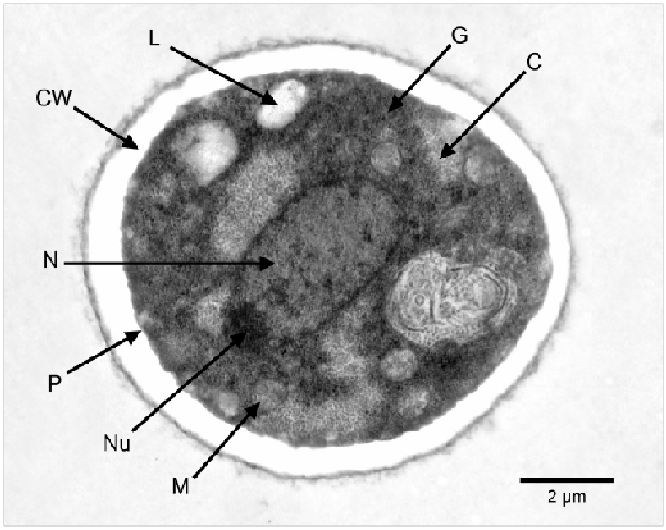

Figure 4. Transmission electron micrograph of an oblique section of a control *Botrytis paeoniae* conidium showing the ultrastructural components: cell wall (CW), cytoplasm (C), nucleus (N), nucleolus (Nu), plasmalemma (P), mitochondrion (M), glycogen (G), and lipid (L).

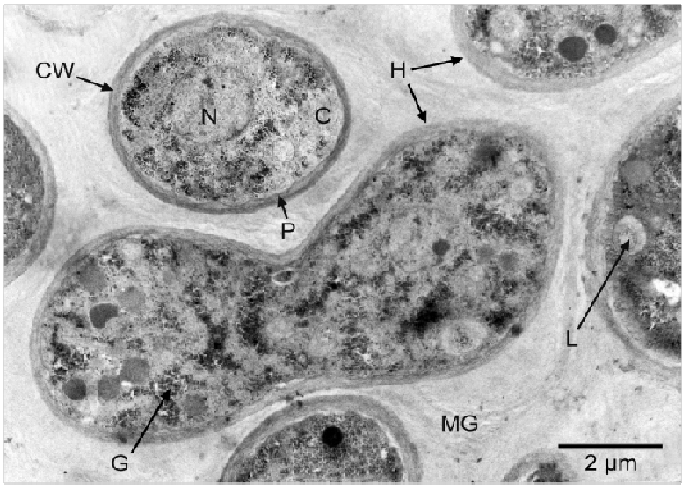

Figure 6. Transmission electron micrograph of an oblique section in the medullary hyphae of a control *Botrytis paeoniae* sclerotium, showing the ultrastructural components: hypha (**H**), matrix of glucan (**MG**), cell wall (**CW**), cytoplasm (**C**), nucleus (**N**), plasmalemma (**P**), glycogen (**G**), and lipid (**L**).

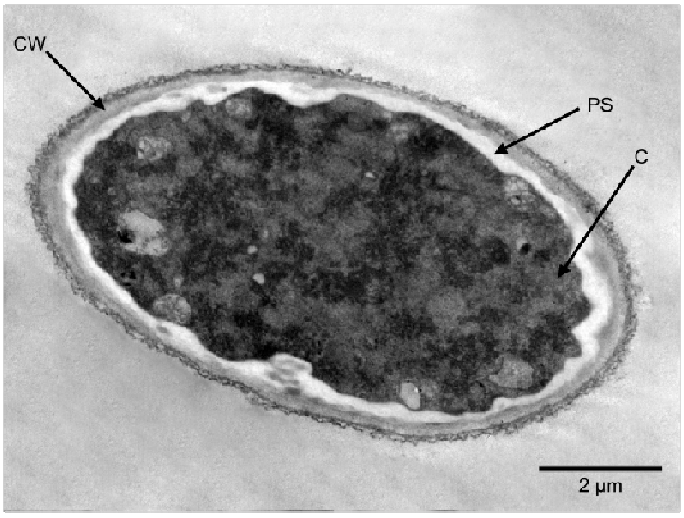

Figure 5. Transmission electron micrograph of an oblique section of a *Botrytis paeoniae* conidium treated with the minimum inhibitory concentration of *Allium obliquum* plant extract showing irreversible ultrastructural changes: cell wall (CW), cytoplasm (C), and periplasmic space (PS).

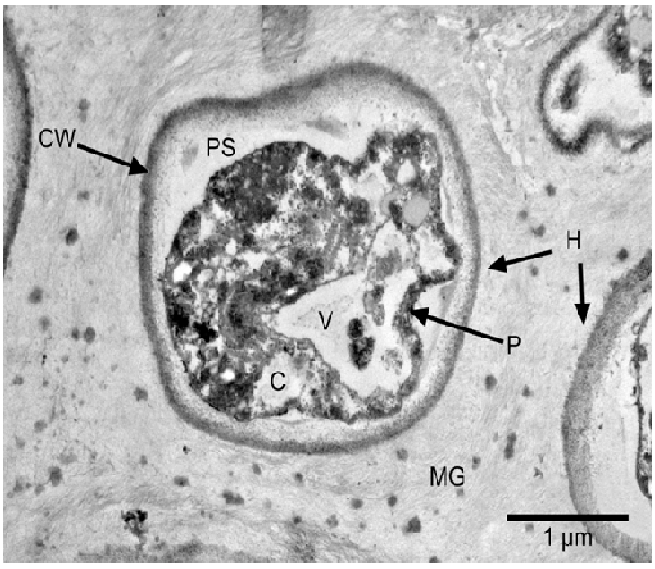

Figure 7. Transmission electron micrograph of an oblique section in the medullary hyphae of a *Botrytis paeoniae* sclerotium, treated with the minimum inhibitory concentration of *Allium obliquum* plant extract, showing irreversible ultrastructural changes: hypha (**H**), matrix of glucan (**MG**), cell wall (**CW**), cytoplasm (**C**), plasmalemma (**P**), vacuole (**V**), and periplasmic space (**PS**).

adhered to the cell wall (Figure 6).

The *A. obliquum* plant extract caused irreversible ultrastructural changes in the medullary hyphae of the sclerotia, as well as an alteration in the cytoplasmatic content, the rarefaction of cell wall, and glucan matrix. The precipitation of the entire cytoplasmic content and the destruction of the organelles and the nucleus led to the formation of a periplasmic space between the altered cytoplasm and the cell wall after treatment with plant extract at the MIC (Figure 7).

DISCUSSION

A. obliquum is a very rare perennial plant, and its antifungal properties have not been adequately studied previously. Furthermore, more studies need to be performed with *B. paeoniae* frequently found every year on flowers, leaves, and stems of peony plants from Cluj-Napoca. The phytotherapeutic properties of *Allium* species showed that different species have antifungal effects (Barile et al., 2007; Mahmoudabadi and Nasery, 2009; Shams-Ghahfarokhi et al., 2006). Therefore, we studied the action of *A. obliquum* extract on *B. paeoniae* growth to determine its fungicidal potential as a biological control of gray mold on peony plants.

Analysis by LC/MS showed that *A. obliquum* extract contains significant amounts of alliin and allicin. In *Allium* plants and extracts, different biologically active substances were identified, such as alliin and allicin (Josling, 2003), allicepin (Wang and Ng, 2003), ajoene (Ledezma and Apitz-Castro, 2006), saponins (Barile et al., 2007), steroids (Ren et al., 2010), flavones (Huma et al., 2009), fistulosin (Phay et al., 1999), and polyphenolcarboxylic acids (Pârvu et al., 2010b). The quality and quantity of the biologically active compounds from *Allium* species greatly depends on the species (Fritsch and Keusgen, 2006; Vlase et al., 2010), plant organ (Pârvu et al., 2011; Stajner et al., 2008), and the harvest time (Schmitt et al., 2005). Therefore, biologically active compounds must be identified and analyzed from each plant extract.

Alliin is the precursor of allicin formed by the action of the allinase enzyme. Ajoene is also a secondary substance resulting from alliin decomposition (Wang and Ng, 2003). Allicin is efficient against many fungal species, such as *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Fusarium laceratum*, *Microsporum canis*, *Mucor racemosus*, *Penicillium* spp., *Rhizopus nigricans*, *Saccharomyces* spp., *Trichophyton granulosum* (Josling, 2003), *Fusarium oxysporum* (Ogita et al., 2006), and other species (Davis, 2005; Khodavandi et al., 2010). Therefore, the allicin content in *A. obliquum* extract is important for the antifungal activity.

The *in vitro* inhibitory action of *A. obliquum* extract against *B. paeoniae* is stronger than that of standard allicin and the antifungal drug fluconazole. This can be explained by its complex chemical composition containing antimicrobial compounds, including allicin and polyphenols (Pârvu et al., 2010b). The *A. obliquum* plant extract had a significant inhibitory effect on the mycelial growth of *B. paeoniae* and completes our previous studies regarding antifungal activity of this extract against phytopathogenic fungi. The MIC of the plant extract varied between 50 and 80 µl/ml according to the fungal species (Pârvu et al., 2010a).

B. paeoniae can survive as mycelia and/or conidia and sclerotia in peony plants. Therefore, we studied the ultrastructural changes produced by the MIC of *A. obliquum* extract in the conidia and sclerotia of *B. paeoniae*. The *B. cinerea* conidia's cell wall has 2 layers and appears dark because of melanin which protects the spores from enzymatic action and probably ultraviolet light. Additionally, the *B. cinerea* conidia contain 3 to 18 nuclei (Epton and Richmond, 1980). The surface of dry *B. cinerea* conidia and other *Botrytis* species have many short protuberances (200 to 250 nm) which are visible under SEM and TEM. Hydration and redrying caused the disappearance of these protuberances (Doss et al., 1997). The *B. paeoniae* control conidia present numerous short protuberances and are similar to those of *B. cinerea* (Pârvu et al., 2008). When treated with the MIC of *A. obliquum* extract, the shape and size did not change but the surface protuberances disappeared.

Previous studies have shown that the morphofunctional integrity of fungal cell components is needed to maintain its viability and germination capacity. However, the precipitation of the cytoplasm and the destruction of the organelles and nucleus caused the loss of viability and germination capacity of *B. paeoniae* conidia treated with *A. obliquum* plant extract. Moreover, the plant extract caused irreversible changes that abolished the cell wall's barrier function and the possibility of activating enzymes bound to the cell wall (Isaac, 1992). All species of *Botrytis* form sclerotia which may, depending on the isolate and cultural conditions, differ in size and shape. Sclerotia are generally considered to be the most important structures involved in the survival of *Botrytis* species (Coley-Smith, 1980). The internal structure and histochemistry of *B. paeoniae* sclerotia are similar to those of *B. cinerea* and *B. fabae*. The rind walls contain melanin pigments, the medullary hyphae are surrounded by a continuous matrix of β-glucans, and the intracellular nutrient reserves are protein, glycogen, polyphosphate and lipid (Backhouse and Willets, 1984).

The antifungal activity of *A. obliquum* affected the structure of the medullary hyphae of *B. paeoniae* sclerotia and destroyed the glucan matrix, which may be utilized as carbohydrate source during sclerotium germination (Backhouse and Willets, 1985). The functional relationship between the cell wall and the cytoplasmic content in treated sclerotia was destroyed; as seen by the formation of a less electrondense band (periplasmic space) (Figure 7). The most important ultrastructural changes of sclerotia are the degeneration of cytoplasm and the destruction of the organelles and nucleus affecting the morphofunctional integrity of the fungal cells (Isaac, 1992). Thus, all these results revealed that the viability and germination capacity of the sclerotia were affected.

Conclusion

The *A. obliquum* plant extract exhibited strong antifungal

activity on *B. paeoniae*, and the MIC of the extract caused the conidia and sclerotia to lose viability due to severe ultrastructural changes. These data may be useful for the development of new natural antifungal products for the *in vivo* biological control of gray mold produced by *B. paeoniae* strains on peony plants, limiting the over-use of chemical fungicides.

ACKNOWLEDGMENTS

This work was supported by CNCSIS –UEFISCSU, project number PNII – IDEI code 2272/2008.

REFERENCES

Backhouse D, Willets HJ (1984). A histochemical study of sclerotia of *Botrytis cinerea* and *Botrytis fabae*. Can. J. Microbiol. 30:171–178.

Backhouse D, Willets HJ (1985). Histochemical changes during conidiogenic germination of sclerotia of *Botrytis cinerea*. Can. J. Microbiol. 31:282–286.

Barile E, Bonanomi G, Antignani V, Zolfaghari B, Sajjadi SE, Scala F,. Lanzotti V (2007). Saponins from *Allium minutiflorum* with antifungal activity. Phytochemistry 68:596–603.

Beever RE, Weeds PL (2007). Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds). *Botrytis*: biology, pathology and control. Springer, Dordrecht, The Netherlands. pp. 29–52.

Choi GJ, Jang KS, Kim JS, Lee SW, Cho JY, Cho KY, Kim JC (2004). *In vivo* antifungal activities of 57 plant extracts against six plant pathogenic fungi. Plant Pathol. J. 20:184–191.

Ciocârlan V (2009). Flora ilustrată a României *Pteridophyta et Spermatophyta*. Ceres Ed., Bucureşti, România.

Coley-Smith JR (1980). *Sclerotinia* and other structures in survival. In: Coley-Smith JR, Verhoeff K, Jarvis WR (eds), The biology of *Botrytis*. Academic Press, London. pp. 85–114.

Davis SR (2005). An overview of the antifungal properties of allicin and its breakdown products-the possibility of a safe and effective antifungal prophylactic. Mycoses 2:95–100.

Doss RP, Christian JK, Potter SW, Soeldner AH, Chastagner GA (1997). The conidial surface of *Botrytis cinerea* and several other *Botrytis* species. Can. J. Bot. 75:612–617.

Elad Y, Stewart A (2004). Microbial control of *Botrytis* spp. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds), *Botrytis*: biology, pathology and control. Springer, Dordrecht, The Netherlands. pp. 223–241.

Elad Y, Williamson B, Tudzynski P, Delen N (2007). *Botrytis* spp. and diseases they cause in agricultural systems – an introduction. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds), *Botrytis*: biology, pathology and control. Springer, Dordrecht, The Netherlands. pp. 1–8.

Ellis MB (1971). Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey, England.

Epton HAS, Richmond DV (1980). Formation, structure and germination of conidia. In: Coley-Smith JR, Verhoeff K, Jarvis WR (eds), The biology of *Botrytis*. Academic Press, London. pp. 41–83.

Fritsch RM, Keusgen M (2006). Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). Phytochemistry 67:1127–1135.

Giraud T, Fortini D, Levis C, Lamarque C, Leroux P, LoBuglio K, Brygooet Y (1999). Two sibling species of the *Botrytis cinerea* complex, *transposa* and *vacuma*, are found in sympatry on numerous host plants. Phytopathology 89: 967–973.

Hayat MA (2000). Principles and techniques of electron microscopy: biological applications. Cambridge University Press, London, UK.

Huma Z, Vian MA, Maingonnat JF, Chemat F (2009). Clean recovery of antioxidant flavonoids from onions: Optimising solvent free microwave extraction method. J. Chromatogr. A. 1216:7700–7707.

Isaac S (1992). Fungal-plant interactions. Chapman & Hall

Jarvis WR (1977). *Botryotinia* and *Botrytis* species; taxonomy, physiology, and pathogenicity: a guide to the literature. Research Branch Edition, Canadian Department of Agriculture, Ottawa, Canada.

Josling P (2003). Allicin the heart of garlic. NWI Publishing Callahan, Florida, USA.

Khodavandi A, Alizadeh F, Aala F, Sekawi Z, Chong PP (2010). *In vitro* investigation of antifungal activity of allicin alone and in combination with azoles against *Candida* species. Mycopathologia 169:287–295.

Kirk PM, Cannon PF, David JC, Stalpers JA (2001). Dictionary of the Fungi, 9th Edition. CAB International, Wallingford, UK.

Ledezma E, Apitz-Castro R (2006). Ajoene, el principal compuesto activo derivado del ajo (*Allium sativum*), un nuevo agente antifungico. Rev. Iberoam. Micol. 23:75–80.

Mahmoudabadi AZ, Nasery MKG (2009). Antifungal activity of shallot, *Allium ascalonicum* Linn. (Liliaceae) in vitro. J. Med. Plants Res. 3:450–453.

Miclea R, Puia C (2010). *In vitro* control of fungus *Botrytis cinerea* Pers. with plant extracts. Bull. USAMV Agric. 67:181–186.

Nidiry ESJ, Babu CSB (2005). Antifungal activity of tuberose absolute and some of its constituents. Phytother. Res. 19:447–449.

Ogita A, Fujita KI, Taniguchi M, Tanaka T (2006). Dependence of synergistic fungicidal activity of Cu^{2+} and allicin, an allyl sulfur compound from garlic, on selective accumulation of the ion in the plasma membrane fraction via allicin-mediated phospholipid peroxidation. Planta Med. 72:875–880.

Pârvu M, Pârvu AE, Crăciun C, Barbu-Tudoran L, Tămaş M (2008). Antifungal activities of *Chelidonium majus* extract on *Botrytis cinerea in vitro* and ultrastructural changes in its conidia. J. Phytopathol. 156:550–552.

Pârvu M, Pârvu AE, Roşca-Casian O, Vlase L, Groza G (2010a). Antifungal activity of *Allium obliquum*. J. Med. Plants Res. 4:138–141.

Pârvu M, Pârvu AE, VlaseL, Roşca-Casian O, Pârvu O (2011). Antifungal properties of *Allium ursinum* L. ethanol extract. J. Med. Plants Res. 5:2041–2046.

Pârvu M, Toiu A, Vlase L, Pârvu AE (2010b). Determination of some polyphenolic compounds from *Allium* species by HPLC-UV-MS. Nat Prod. Res. 24:1318–1324.

Phay N, Higashiyama T, Tsuji M, Matsuura H, Fukushi Y, Yokota A, Tomita F (1999). An antifungal compound from roots of Welsh onion. Phytochemistry 52:271–274.

Ren G, Qiao HX, Yang J, Zhou CX (2010). Protective effects of steroids from *Allium chinense* against H_2O_2-induced oxidative stress in rat cardiac H9C2 cells. Phytother. Res. 24:404–409.

Schmitt B, Schulz H, Storsberg J, Keusgen M (2005). Chemical characterization of *Allium ursinum* L. depending on harvesting time. J. Agr. Food Chem. 53:7288–7294.

Shams-Ghahfarokhi M, Shokoohamiri MR, Amirrajab N, Moghadasi B, Ghajari A, Zeini F, Sadeghi G, Razzaghi-Abyaneh M (2006). *In vitro* antifungal activities of *Allium cepa*, *Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia 77:321–323.

Stajner D, Popovic BM, Canadanovic-Brunet J, Stajner M (2008). Antioxidant and scavenger activities of *Allium ursinum*. Fitoterapia 79:303–305.

Vlase L, Pârvu M, Toiu A, Pârvu AE, Cobzac CS, Puşcaş M (2010). Rapid and simple analysis of allicin in *Alium* species by LC-CIS-MS/MS. Studia UBB. Chemia 55:297–304.

Wang HX, Ng TB (2003). Isolation of allicepin, a novel antifungal peptide from onion (*Allium cepa*) bulbs. J. Pept. Sci. 10:173–177.

Webster J, Weber RWS (2007). Introduction to fungi. Cambridge University Press, Cambridge, UK.

Williamson B, Tudzynschi B, Tudzynschi P, van Kan JAL (2007). *Botrytis cinerea*: the cause of grey mould disease. Mol. Plant Pathol. 8:561–580.

Wilson CL, Solar JM, El Ghaouth A, Wisniewski ME (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis. 81:204–210.