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# IN VITRO ACTION OF PLANT EXTRACTS ON *BOTRYTIS GLADIOLORUM*


**M. PÂRVU**



**Abstract**

M. PÂRVU, 1998, *In vitro action of plant extracts on Botrytis gladiolorum*_Not. Bot. Hort. Agrobot. Cluj, XXVIII.

The in vitro effect of extracts from *Berberis vulgaris* and *Chelidonium majus* was studied against *Botrytis gladiolorum* fungus isolated from *Gladiolus* spp. We evaluated in vitro the effect of total extracts from *Berberis vulgaris* and *Chelidonium majus* on fungal growth, sporulation, and sclerotia formation.. Plant extracts from *Berberis vulgaris*, containing 1% alkaloids, and *Chelidonium majus*, 0,25% alkaloids, were added to PDA at alkaloid concentrations of 25 to 250 µg/ml. Extract from both plants had increasing inhibitory activity against *Botrytis gladiolorum* fungus with increasing alkaloid concentration.



**Key words**: *Botrytis gladiolorum*, alkaloids



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Plant extracts from some cormophyte species exhibit antimicrobial action (Anonymous 1968; Ionescu-Stoian & Savopol 1977; Păun et al. 1986-1988). *Botrytis* spp. are among the most problematic fungal pathogens in agriculture and horticulture worldwide. Several approaches have been taken to control *Botrytis* including fungicides and biological control. Recently, plant extracts have been shown to have some inhibitory activity. Extracts from *Chelidonium majus* L. and *Pastinaca sativa* L. were highly active in vitro against grapevine isolates of *B. cinerea* reducing pathogen growth by 90% (Jiratko and Vesela 1992). Prior application of extracts from *Petroselinum hortense* Hoffm. and *P. sativa* completely inhibited growth of *B. cinerea* on detached haricot bean leaves, on the growing tip of *Lycopersicum esculentum* Mill., and on *Cinnamomum* spp. when pathogen inoculum was applied 72 h after plant extracts (Jiratko 1994). Compound BC-1000 from grapefruit seed extract at a concentration of 1,500 ppm had inhibitory activity against grapevine isolates of *B. cinerea* comparable to the fungicides vinclozolin and benomyl (Esterio et al. 1992).Total extracts from *Berberis vulgaris* and *C. majus* have antibiotic activity on a great number of pathogens (Craciun et al. 1976, Gheorghiu et al. 1969, Pârvu 1993; Pârvu, 1998). The active substances of these plants are berberin alkaloids and chelidonin, respectively ( Paun et al. 1986-1988, Tamas et al. 1987). Chelidonin occurs naturally at concentrations of 0.2 to 1.4% in roots and 0.012 to 0.8% in vegetative underground organs of *C. majus* (Gheorghiu et al. 1969). Activity of total alkaloids from *C. majus* has been tested on pathogenic bacteria from genera

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*Staphylococcus*, *Streptococcus*, *Escherichia*, *Pseudomonas* and *Candida* fungus pathogenic to man (Gheorghiu et al. 1969). In vitro action of plant extracts from *B. vulgaris* and *C. majus* were efficient against *Botrytis cinerea*, *B. tulipae* and *B. paeoniae* isolated from ornamental plants (Pârvu, 1998). In this study, we investigated the optimal dosage of total extracts from *B. vulgaris* and *C. majus* for inhibition of *Botrytis gladiolorum* isolated from *Gladiolus* sppp.

## MATERIALS AND METHODS

Pathogenic fungus used was *B.gladiolorum*. This fungus was isolated from *Gladiolus* spp. Total extracts from *B. vulgaris* and *C. majus* contained 1 and 0,25% alkaloids, respectively, and were obtained by the volumetric method from plants collected and processed according to standard methods (Anonymous 1968, Gheorghiu et al. 1969, Paun et al. 1986-1988). For obtaining the total extract from the *Chelidonium majus* species we collected the airy plant organs, called "Herba Chelidonii".

The airy parts were dried after collecting, then were macerated, and were introduced in an 70% ethyl alcohol solution. After a day, the total extract was collected.

In our experiments we used total extract without ethylic alcohol. The total extract from *Berberis vulgaris* was obtained in the same conditions, but, for this, we used the bark of stem. Extract were added to PDA after autoclaving to give alkaloid concentrations ranging from 25 to 250 µg/ml. Conidial suspensions ($10^{-5}$ /ml) of *Botrytis gladiolorum* were placed in the center of plates with extracts. Plates were incubated 12 days at 22° C, the optimal growth temperature for the fungi. Each treatment consisted of ten replicate plates. Antifungal activity was evaluated based on colony diameter (mm), sporulation and sclerotia formation, compared with growth on nonamended media. In *Berberis vulgaris* extract dominates the berberine alkaloid and in *Chelidonium majus* chelidonine. These alkaloids are active substances of total extracts. Data have been treated by analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

Total extracts from *C. majus* (Table 1) and *B. vulgaris* (Table 2) had antifungal activity against *B. gladiolorum*.Increasing alkaloid concentrations corresponded with increased inhibition. The highest active concentration of alkaloids varied with fungus and with plant origin of the extract. Total inhibition was observed at alkaloid concentrations of 250µg/ml. The antifungal properties of plant extracts against *Botrytis gladiolorum* can serve as starting point in establishing some elements of biological control.

**Rezumat**

Efectul in vitro al extractelor vegetale din *Berberis vulgaris* (1% alcaloizi) şi *Chelidonium majus* (0,25 % alcaloizi) a fost studiat împotriva ciupercii *Botrytis gladiolorum*, izolată de *pe Gladiolus* spp. A fost evaluat efectul extractelor totale asupra creşterii ciupercii, sporulării şi formării sclerotilor. Extractele vegetale au fost adăugate la mediul cartof-dextroză-agar(PDA) la concentrații de alcaloizi de la 25 la 250 µg/ml. Inhibiția totală a fost obținută la concentrații de alcaloizi de 250 µg/ml.



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**TABLE 1.** In vitro activity of *Chelidonium majus* extract on *Botrytis gladiolorum*

| Fungus | Alkaloid conc. µg/ml | Colony diam.[y] (mm) | Sporulation[z] | Sclerotia[z] | Inhibition % |
|---|---|---|---|---|---|
| *Botrytis gladiolorum* | 250 | 0 | - | - | 100.0 |
| | 225 | 5.0 | - | - | 92.8 |
| | 200 | 10.0 | - | - | 85.7 |
| | 175 | 18.0 | - | - | 74.2 |
| | 150 | 25.0 | - | - | 64.2 |
| | 100 | 45.0 | - | - | 35.7 |
| | 25 | 65.0 | + | ++ | 7.1 |
| | 0 | 70.0 | ++ | ++++ | - |

[y] Within a fungus, growth was significantly ($P<0.05$) reduced by all alkaloid concentrations compared with the nonamended control.

[z] For sporulation and sclerotial formation, respectively: - is absent; + is poor, 1-20 sclerotia/plate; ++ is moderate, 21-40 sclerotia/plate; +++ is dense, 41-60 sclerotia/plate; ++++ is abundant, >60 sclerotia/plate.

**TABLE 2.** In vitro activity of *Berberis vulgaris* extract on *Botrytis gladiolorum*

| Fungus | Alkaloid conc. µg/ml | Colony diam.[y] (mm) | Sporulation[z] | Sclerotia[z] | Inhibition % |
|---|---|---|---|---|---|
| *Botrytis gladiolorum* | 250 | 0 | - | - | 100.0 |
| | 225 | 6.0 | - | - | 91.4 |
| | 200 | 10.0 | - | - | 85.7 |
| | 175 | 18.0 | - | - | 74.2 |
| | 150 | 23.0 | - | - | 67.1 |
| | 100 | 45.0 | + | - | 35.6 |
| | 25 | 60.0 | ++ | + | 14.2 |
| | 0 | 70.0 | +++ | ++ | - |

[z]See legend for Table 1.

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# INVESTIGATIONS ON A METHODOLOGY OF TESTING RESISTANCE TO CHEMICAL STRESS OF HOP CULTURES *IN VITRO*

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**Abstract:**

ŞTEFANIA GÂDEA, SUCIU T., MUNTEAN L., HENEGARIU O., LILIA MACOVEI, 1998, Investigations on a methodology of testing resistance to chemical stress of hop cultures *in vitro*. (in English) Not. Bot. Hort. Agrobot. Cluj, XXVI - XXVII.

The purpose of this research was to determine an adequate basic media in order to test the influence of some chemical stress factors (NaCl) on the resistance of hop neoplantules generated by minicuttings and cellular suspensions.

The basic media was Murashige-Skoog (1962) with addition of phytohormones (auxins and cytokinins) and the chemical factor of stress was NaCl in three concentration (0,3%, 0,5% and 1%). At 1% NaCl we noticed an inhibition in growth. We selected the most vigurous plants, V1 and V2, which were relayered and passed on the same basic media. The operation was repeated several times in order to obtain forms highly resistant to salinity.

**Key words:** basic media, auxins, cytokinins, minicuttings, neoplantules, salinity

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## THE IMPORTANCE OF THE RESEARCH

This research is integrated into a wider theme regarding the resistance of some cultivated species to several chemical factors of stress (salinity, pH, herbicides a.s.o.) and producing of resistant forms.

The research aims at treating the following aspects:

- to determine an adequate basic media in order to test the influence of some chemical factors of stress (NaCl) on the resistance of hop neoplantules generated by minicuttings and celular suspensions;
- to identify proper sources to form appropriate calus;
- to obtain celular suspensions out of calus and to maintain the viability of the cells on environment of culture;
- to determine the time for treatment and the concentration of the chemical factors of stress.