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云 南 植 物 研 究 2005, 27 (4): 437~442
Acta Botanica Yunnanica

云南秋海棠挥发物抗微生物活性的研究*

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摘要: 对云南产的16种秋海棠属(*Begonia* L.)植物进行了挥发物抗微生物活性实验, 其中14种秋海棠为首次报道。结果表明, 在所研究的16种秋海棠中, 有8种秋海棠具有抗葡萄球菌(*Staphyllococcus epidermidis*)活性。而抗大肠杆菌(*Escherichia coli*)活性最强的是厚壁秋海棠(*Begonia silletensis* C. B. Clarke), 假厚叶秋海棠(*B. pseudodryadis* C. Y. Wu)和秋海棠(*B. grandis* Dryand.)。只有铁甲秋海棠(*B. masoniana* Irmsch.)和毛叶秋海棠(*B. rex* Putz.)对白假丝酵母菌(*Candida albicans*)有抗性。作为室内盆栽花卉被广泛应用的秋海棠, 不仅可以美化室内环境, 而且还可以作为药用和净化室内环境的生态产品进行推广应用。

关键词: 秋海棠; 抗微生物活性
中图分类号: Q 948.12, S 682.2 **文献标识码**: A **文章编号**: 0253-2700 (2005) 04-0437-06

Antimicrobic Activity of Volatile Emissions of Some Begonias from Yunnan, China*

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Abstract: Begonias are widely used as ornamentals for their attractive flowers and foliages. The substances of phytoncids have bactericidal, fungicidal and protistocidal activities as discovered by previous researches. Phytoncid activity of volatile emissions of species of the genus *Begonia* L. collected from various natural habitats in Yunnan Province, China, was studied. Sixteen species of *Begonia* were studied with 14 species being studied for the first time. The results indicated that eight species exhibited activity towards *Staphyllococcus epidermidis*. The most active towards *Escherichia coli* was *Begonia silletensis*, *B. psuedodryadis* and *B. grandis*. Only *B. masoniana* and *B. rex* were active towards *Candida albicans*. The species of the genus *Begonia* studied which exhibited antimicrobic activity may be recommended for the use of medicinal and ecological phytodesign.

Key words: *Begonia*; Antimicrobic activity

* Foundation item: This project was partly supported by Yunnan Natural Science Foundation (2001C0010Z & 2001C0060M)
Received data: 2004-11-15, Accepted data: 2005-03-16
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Introduction

In 1928, Russian scientist discovered substances produced by plants that have bactericidal, fungicidal and protistocidal activities (Tokin, 1957). These substances are called phytoncids.

The study of phytoncid properties of plants used in indoor environments is very important. As the exposure of humans to enclosed environments increases, so does their exposure to microorganisms increasing. Phytoncid activity is potentially of particular value for children's establishments and hospitals. The allergist Kaznacheeva (1990) indicated a high content of bacteria (a conditioned-pathogenic strain of *Staphyllococcus*), on the nasal mucosa of frequently sick children and adults. In conditions favorable for its growth, this strain may become pathogenic and provoke serious diseases. Widespread misuse of antibiotics has given rise to virulent strains of microorganisms resistant to antibiotics. Bacteria, fungi and protozoa, pathogenic to humans, do not develop resistance to plant phytoncids (Tokin, 1957; Gheikhman, 1986).

The composition of airborne microorganisms depends on the degree of air contamination by mineral and organic suspensions, temperature, humidity and other factors. In rooms occupied by people, the concentration of pathogenic and conditioned-pathogenic microorganisms can be especially high. The maximum quantity of microbes in air samples can increase to many tens of thousands per 1 m^3 (Kaznacheeva, 1990). A short stay of unifected people in close proximity to sick ones is sufficient for them to become infected by pathogenic bacteria and viruses, which can promote epidemics of serious diseases. Intensive study of the plants with phytoncid activity is currently being carried out at the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG, SB RAS). These plants are used for interior landscaping of children's establishments, hospitals and offices. Thus, it is possible to solve preventive and aesthetic tasks at the same time. This line of interior landscaping is called ecological and medicinal phytodesign (Tsybulya, 2001). Species of the genus *Begonia* L. may be promising for ecological phytodesign.

The genus *Begonia* is widely distributed in tropical and subtropical areas, except for Polynesia and Australia. More than 1400 species of this genus are described (Smith *et al*, 1986). Their life forms vary widely. Among them, there are perennial herbs, subshrubs with lignified stems, and stemless plants with leaves produced on the tuber hidden under ground. Leaf shapes and colors also vary widely.

Many species of *Begonia* are ornamental and undemanding plants. Most, especially species native to subtropical regions, withstand low humidity, low illumination and decrease of temperature without damage of leaf surface. Due to all this, begonias are widely used as indoor plants.

In the present paper, results are presented of a study conducted in 2001 of phytoncid activity in species of *Begonia* native to Yunnan province, China. The study was conducted at the Kunming Botanical Gardens. The plants used were accessioned from native habitats and are maintained in the permanent collections of the Garden.

Material and Methods

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This study considered 16 species of the genus *Begonia* L. from the collection of the Kunming Botanical Garden. The 16 species were *B. dryadis* Irmsch., *B. masoniana* Irmsch., *B. rex* Putz., *B. wangii* Yu, *B. prostrata* Irmsch, *B. silletensis* C. B. Clarke, *B. grandis* Dryand., *B. cathayana* Hemsl., *B. hemsleyana* Hook. f., *B. pseudodryadis* C. Y. Wu, *B. versicolor* Irmsch., *B. villifolia* Irmsch., *B. forrestii* Irmsch., *B. mengzzana* Irmsch., *B. palmata* var. *crassisetulosa* J. Golding et C. Kareg. and *B. tsaii* Irmsch. All the species were introduced from native habitat in Yunnan and cultivated in the Kunming Botanical Gardens. The experiments were performed in the laboratory of the Garden using a method developed in CSBG, SB RAS, Novosibirsk (Tsybulya, 1990, 2001).

Museum strains of organisms representing different groups: gram-positive (*Staphyllococcus epidermidis*), gram-negative (*Esherichia coli*) and fungus (*Candida albicans*) obtained from the Institute of Microbiology, Yunnan University were used. The plants were put in boxes of 50 cm × 50 cm × 85 cm volume. The design of the box volume is based on the size of *Begonia* plants planning to be tested. Sixteen *Begonia* species were experimented on three microorganisms and each test was repeated three times but two species were repeated six times to indicate the results obtained by previous research. The statistics were the mean figures of three experiments on each microorganism.

Overturned open Petri dishes with special-purpose agar nutrient medium were put on special holes in the box lid: nutrient agar for cultivation of microorganisms -for *E. coli*, nutrient medium for isolation of *Staphyllococcus* dry (elective soline agar) - for *S. epidermidis* and medium nutricium pro determincione cantaminacia siccum -for *C. albicans*. Necessary dilution (by consecutive hundred-fold dilutions) was pre-selected for obtaining isolated colonies of microorganisms. Petri dish culture was put into the thermostat at 37℃. A number of microorganism colonies grown were calculated 24–48 hours later. To estimate antimicrobic activity of volatile emissions of plants, relative decrease in microorganism number in the experiment is usually calculated compared to the control:

$$A = \frac{K-O}{K} 100\% \qquad (1)$$

Where A is activity of volatile emissions of plants studied; K is a number of control microorganism colonies; O is a number of experiment microorganism colonies.

To avoid methodic errors, when correlating phytoncid activity of different plant species or activity regarding different microorganism species, and calculating the magnitude of A, we conducted the experiments in comparable conditions: used boxes of the same size and established optimum exposure time for a concrete experiment, constant in subsequent experiments. Leaf area was calculated by the formula:

$$S = 0.5\ a\ b \qquad (2)$$

Where S is leaf area; a is leaf width; b is leaf length.

It is worth noting that we did not manage to select *B. prostrata* and *B. wangii* samples with approximately equal leaf area for the experiment as it was needed by the methods. It was difficult because of different habits of these species. Only one sample of *B. prostrata* with large leaves was used in the experiment. *B. wangii* has small leaves and we used 4 samples of it.

It is known that e emission of subtropical plant species is connected with seasonal rhythm (Antadze, 1960, Tsybulya, 1998). During the vegetative growth, the maximum volatility of phytoncids is explained by accumulation of certain substances (e.g., terpenes) and change in their composition (Akimov and Lishvanova, 1977). Young plant organs emit intensively the greatest quantity of volatile substances during active vegetative growth. Therefore, we only used plants at the stage of active vegetative growth in our experiments.

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Results and Discussion

Sixteen species of *Begonia* were studied and 14 of the species had not previously been studied for phytoncid activity. Data on the degree and specificity of phytoncid activity of the species studied are given in the Table 1.

Table 1 Degree of phytoncid activity (A, %) of *Begonia* species studied regarding the strains of some microorganisms

Begonia species under study	Wild Distribu-tion in Yunnan	Elevation (meter)	Leaf area of species under study (cm^2)	Degree of phytoncid, (A, %) to tested microorganisms		
				S. epidermidis	*E. coli*	*C. albicans*
B. dryadis	S & SE	600–1200	5980	22 ± 1	0	0
B. masoniana	S	170–220	4770	33 ± 4,3	0	32 ± 5
B. rex	S & W	990–1100	6029	16 ± 3,1	0	23 ± 1,5
B. wangii	SE	800–1825	1670	33 ± 6	0	0
B. prostrata	S & SW	1100–1500	11480	*	0	0
B. silletensis	SW	600–800	5280	0	50 ± 2	0
B. grandis	Most part	100–1380	4300	*	27 ± 1	0
B. cathayana	C & S	1200–1500	5610	30 ± 4	0	0
B. hemsleyana	S	1000–1300	6500	*	0	0
B. pseudodryadis	SE	1200–1400	6980	0	50 ± 5	
B. versicolor	SE & S	1200–1400	3103	*	20 ± 2	0
B. villifolia	S	1170–1300	3840	40 ± 7	0	0
B. forrestii	W	1200–3000	6962	34 ± 1,5	0	0
B. mengztzana	S	1700–2300	3970	0	0	0
B. palmata var. *crassisetulosa*	W & SW	1500–3200	7693	0	0	0
B. tsaii	S	1500	3718	27 ± 5	0	0

* – research has not been conducted

Of the sixteen species studied, *B. dryadis*, *B. masoniana*, *B. rex*, *B. wangii*, *B. cathayana*, *B. villifolia*, *B. forrestii* and *B. tsaii* exhibited activity towards *S. epidermidis*. The most active species towards *E. coli* were *B. silletensis* and *B. pseudoryiadis* but *B. grandis* and *B. versicolor* were also active towards *E. coli*. While only *B. masoniana* and *B. rex* were active towards *C. albicans*.

The data obtained regarding the species *B. masoniana* and *B. rex* correspond to those obtained in the experiments in CSBG, SB RAS (Fershalova and Tsybulya, 1998, 1999; Fershalova, 2000). We confirmed phytoncid activity of these species towards *S. epidermidis* and *C. albicans* and absence of activity towards *E. coli*. It is worth noting that species of the genus *Begonia* share the common property: species active towards *S. epidermidis* (gram-positive bacteria) are not active towards *E. coli* (gram-negative bacteria) and vice versa. This conclusion needs to be confirmed in further experiments.

One of the objectives of the study was to discern any correlation between phytoncid activity and the habitats of species. The issue of the association between antimicrobic activity and environmental conditions is of great interest. Study of mountain plants presents considerable opportunity for studying it. Some authors point to the fact that antibiotic plant properties increase as altitude of growing increases (Abdulayeva, 1953; Melkumyan, 1963; Pryzhnikov, 1966). Makarenko (1977), when

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studying the antimicrobic properties of medicinal plants of the Altaian flora, indicated the existence of a certain association between antimicrobic activity and species habitation. Species with more pronounced antimicrobic properties occur more often in the subalpine and alpine meadows, forest belt and grassland. Species growing in the desertificated steppe and high-mountain tundra belt are characterized by the lowest phytoncid activity.

We have not conducted research on the plants from the natural populations. But taking into account that plants used for this study in the Kunming Botanical Garden were collected from the wild, we decided to compare phytoncid activity of *Begonia* species from different natural areas.

From the data presented it is seen that exact dependence of the phytoncid activity on the habitat is not noted. Perhaps research conducted on the plants from natural populations will produce another result.

Association between phytoncid activity, taxonomic relationship and ecological type of plants still remains unanswered.

Conclusion

Research conducted on phytoncid activity of 16 species of the genus *Begonia* from the tropical and subtropical areas of Yunnan, China has shown that not all plants are active in the same way to gram-positive and gram-negative microorganisms. Volatile emissions of the *Begonia* species studied exhibited activity to microbiological test-objects of *S. epidermidis*, *E. coli* and *C. albicans*. For the most part volatile emissions of plants inhibit growth of gram-positive bacteria (*S. epidermidis*). To a lesser extent phytoncids of *Begonia* species studied affect fungus (*C. albicans*).

Additional study is needed to determine the interaction of phytoncid activity and ecological habitat of species. The species of the genus *Begonia* studied that display antimicrobic activity may be recommended for use in medicinal and ecological phytodesign.

Acknowledgements: Our thanks to Mrs LI Jing-xiu for her help of cultivating the plants used for this experiment, to the Institute of Microbiology, Yunnan University for supplying museum strains of organisms. Our special thanks to Mr. Paul Jones and Dr. Bruce Bartholomew for their detailed review of the manuscript and correction of English.

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