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Mycoflora of *Calendula officinalis* L. seeds

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Abstract

The seeds of *Calendula officinalis* harvested in the years 1985-1987 were investigated. Agar medium with nitrients was used to isolate the fungi. As a result of the mycological analysis, 3642 isolates belonging to 23 species and dark unsporulating mycelium were obtained. *Alternaria alternata* dominated among the isolated fungi. Moreover, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium avenaceum* and *Sclerotinia sclerotiorum* were obtained from the seeds.

INTRODUCTION

Calendula officinalis is an ornamental and curative plant. The manner of cultivation of the investigated plant is easy, as the seeds are sown directly on permanent plot (C h m i e l, 1984). The author of the present paper found only one information that this plant can be infected by Lycopersicum virus 3 (Brittlebank) Smith with caused mosaic spots on leaves. The virus is transferred by seeds (C z y ż e w s k i, 1975). Other reports on the role of *Calendula officinalis* seeds in transferrence of infections agents were not found in the Polish and foreign literature.

MATERIALS AND METHODS

The *Calendula officinalis* achenes from the harvest of 1985-1987 were the object of investigations, and the mycological analysis was carried out in February the following years. The examination material included 55 seeds samples. Fifty undesinfected and fifty surface – disinfected seeds were analysed from each sample.

The mycological analysis was carried according to Ł a c i c o w a et al., (1989).

In order to assing the fungi, monographs and keys were used during the investigation of bean plants (P i ę t a, 1981).

RESULTS

As a result of the mycological analysis of undisinfected seeds 2218 isolates of fungi from 21 species and unsporulating dark mycelium were obtained (tab. 1). From the disinfected seeds, however, 1424 isolates from 18 species and unsporulating mycelium were obtained (tab. 2). The fungi which was most ferquently isolated both from disinfected and undisinfected seeds was *Alternaria alternata* whose isolates constituted 48 % isolations of all the fungi.

Table 1

Fungi isolated from undisinfected seeds of *Calendulla officinalis*

Species	Number of isolates from seeds						Total number of isolates
	germinated			ungerminated			
	1985	1986	1987	1985	1986	1987	
Acremoniella atra (Corda) Sacc.	0	0	0	0	1	0	1
Alternaria alternata (Fr.) Keissler	0	12	208	129	365	332	1046
Aspergillus flavus Link	0	0	0	61	0	0	61
Aspergillus fumigatus Fres.	0	0	0	2	0	0	2
Botrytis cinerea Pers.	0	2	0	0	12	371	385
Chaetomium indicum Corda	0	0	0	7	0	0	7
Cladosporium cladosporioides Fres. de Vries	0	0	0	0	0	1	1
Cladosporium herbarum Link. ex Fr.	0	4	0	0	12	2	18
Fusarium avenaceum (Fr.) Sacc.	0	0	26	0	0	121	147
Fusarium culmorum (W. G. Sm.) Sacc.	0	6	3	0	45	21	75
Fusarium equiseti (Corda) Sacc.	0	0	6	0	0	13	19
Fusarium semitectum Berk. et Rav.	0	2	0	0	18	0	20
Gelasinospora reticulatispora Moreau	0	0	0	19	0	0	19
Penicillium cyclopium West.	0	2	3	4	2	4	15
Penicillium corymbiferum Bainier	0	1	0	0	1	0	2
Penicillium martensii Biourge	0	0	2	0	0	4	6
Penicilium vermiculatum Dang.	0	0	0	0	0	1	1
Penicillium viridicatum West.	0	0	0	0	0	2	2
Sclerotinia sclerotiorum (Lib.) de Bary	0	1	1	0	7	104	113
Trichothecium roseum Link	0	0	0	0	7	3	10
Trichoderma viride Pers. ex Gray	0	0	0	22	0	0	22
Dark coloured unsporulating mycelium	0	9	66	1	93	77	246
Total	0	39	315	245	563	1056	2218

In the case of undisinfected seeds, such pathogenic fungi as *Botrytis cinerea*, *Fusarium avenaceum* and *Sclerotinia sclerotiorum* were obtained. The above species were isolated mainly from ungerminating seeds (tab. 1). Moreover, *Fusarium culmorum* was often isolated from undisinfected seeds, particularly from the harvests of 1986 and 1987. Saprophytic mycoflora was represented by the species from *Aspergillus*, *Cladosporium* and *Penicillium* kinds (tab. 1).

Among the fungi isolated from disinfected seeds, the dominant pathogenic specimen was *Fusarium culmorum* (15 % isolates). Besides, *Botrytis cinerea* (78 isolates) was often isolated from the seeds harvested in the year 1986 and 1987. *Sclerotinia sclerotiorum* (33 isolates), however, was isolated only from seeds colected in 1987 (tab. 2). The above – mentioned species were most fequently isolated from ungerminating seeds. Both species and quantitative composition of saprophitic fungi from disinfected seeds was poorer than that from undisinfected seeds (tab. 2).

Table 2

Fungi isolated from disinfected seeds of *Calendula officinalis*

Species	Number of isolates from seeds						Total number of isolates
	germinated			ungerminated			
	1985	1986	1987	1985	1986	1987	
Alternaria alternata (Fr.) Keissler	1	19	21	41	318	394	784
Aspergillus flavus Link	1	0	0	0	0	0	1
Botrytis cinerea Pers.	0	4	0	0	11	63	78
Chaetomium indicum Corda	1	0	0	0	7	0	8
Cladosporium cladosporioides Fres.	0	0	0	0	1	4	5
Cladosporium herbarum Link. ex Fr.	0	1	0	0	4	0	5
Fusarium avenaceum (Fr.) Sacc.	0	0	1	0	0	8	9
Fusarium culmorum (W. G. Sm.) Sacc.	0	4	13	0	49	148	214
Fusarium equieeti (Corda) Sacc.	0	3	1	0	5	0	9
Fusarium oxysporum Schl.	0	0	1	0	1	0	2
Fusarium semitectum Berk. et Rav.	0	3	0	0	16	0	19
Gelasinospora reticulatispora Moreau.	0	0	0	11	0	0	11
Papularia sphaerosperma (Pers.) Höhn.	0	2	0	0	6	0	8
Penicillium cyclopium West.	2	4	0	4	16	1	27
Penicillium corymbiferum Bainier	0	0	0	0	6	0	6
Penicillium veridicatum West.	0	0	0	0	11	0	11
Sclerotinia sclerotiorum (Lib.) de Bary	0	0	0	0	0	33	33
Trichoderma viride Pers. ex Gray	0	0	0	3	0	0	3
Dark coloured unsporulating mycelium	1	10	10	26	94	50	191
Total	6	50	47	85	545	691	1424

DISCUSSION

The results of the investigations presented above point to a pathogenic threat of *Calendula officinalis* by *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Fusarium* sp. These pathogenic invade the seeds and cause anatomical, biochemical and physiological charges leading to the destruction of internal tissues (Czyżewski, 1975; Pirone, 1978). This phenomenon can serve as an explanation of frequent isolation of this fungi from ungerminating seeds. In the case of *Botrytis cinerea*, the main role in conidial spreading is played by pollinating insects, while sugars present in the nectar are favourable for the infection process (according to literature quoted by Borecka, 1973; Jarvis, 1977). Only very few raspberry fruits are infected directly by *Botrytis cinerea* conidia (1-1.4 %), and it is flowers which are mainly infected. I can be supposed that, similarly to raspberries, the population of *Calendula officinalis* achenes by *Botrytis cinerea* is a consequence of earlier flowers infection.

In the case of *Sclerotina sclerotiorum*, a primary infection is made by ascospores (Abawi et al., 1975) which ripen and are sown in the temperature 20-22°C, and this process can last 18-30 days most frequently in June and July (Rogosheva, Kochenkova, 1981), i.e. in the time of *Calendula officinalis* blooming.

The *Botrytis cinerea* and *Sclerotinia sclerotiorum* fungi infect plants and the relative air humidity of 90 % (Van den Berg, Lenz, 1968) and hence the vegetation periods abundant in rainfall may appear to be favourable for intensification of diseases caused by these pathogens.

Fusarium culmorum fungi as a polyphage, damages various plants and in the case of flowers, it also causes necrosis of seedlings of *Zinnia elegans* L. (Łacicowa et al., 1979) and necrosis of stalks in *Callistephus chinensis* Nees, *Dianthus* L., *Chrysanthemum* L. (Czyżewski, 1975). Polyphagic character, competitive abilities and as well as the dynamic growth and the formation chlamidospores caused that *Fusarium culmorum* is encountered in various habitats and on various agricultural crops. Population of internal tissues of *Calendula officinalis* achenes by *Fusarium culmorum* lowers their vitality which is proved by frequent isolation of this speciment from ungerminating, surface disinfected seeds. A similar remark can be made with reference to *Alternaria alternata*. Gomes and Dhingra (1983) stated a destructive activity of this specimen on lowering the vitality of bean seeds. Mycotoxins secreted by this specimen, such as alternariol and tenuazonic acid are particularly harmful for seeds (Sanchis et al., 1988). So far, *Alternaria alternata* has been known for infection of leaves of *Callistephus chinensis* Nees and *Pelargonium* L'Herit., causing maculation of these organs (Pirone, 1978). This specimen can be regarded as a necrophyte tending to conditional parasitism, particularly at high relative air humidity (Saad, Hagedorn, 1969).

The disinfection operation limited cinsiderably the number of the obtained isolates and fungi species, particularly of a saprophytic character like *Aspergillus*, *Cladosporium* and *Penicillium* which may a destructive effect on the seed mass in storage conditions (Łacicowa, 1989).

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Mikoflora nasion *Calendula officinalis* L.

Streszczenie

Przebadano materiał siewny *Calendula officinalis* zebrany w latach 1985-1987. Do wyosobniania grzybów zastosowano agarową pożywkę mineralną. W wyniku analizy mikologicznej uzyskano 3642 izolaty należące do 23 gatunków i ciemnej grzybni nie zarodnikującej. Wśród wyosobnionych grzybów dominował *Alternaria alternata*. Ponadto uzyskiwano często z materiału siewnego *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium avenaceum* i *Sclerotinia sclerotiorum*.