ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 18(2) 2019, 193–203

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.2.17

ORIGINAL PAPER Accepted: 7.12.2018

FUNGI OCCURRED ON MARIGOLD (*Tagetes* L.) AND HARMFULNESS OF *Fusarium* SPECIES TO SELECTED CULTIVARS

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ABSTRACT

In Poland, cultivars belonging to three species of *Tagetes* are grown: *T. erecta*, *T. patula*, *T. tenuifolia*. Presented studies were conducted in 2015 in two localities of the Lublin region. Those studies included cultivars of *Tagetes* spp.: *T. erecta* (cv. 'Alaska', 'Hawaii', 'Mann Im Mond'), *T. patula* (cv. 'Bolero' and 'Carmen') and *T. tenuifolia* (cv. 'Lemon Gem' and 'Lulu'). Six weeks after the sowing and in flowering stage of analyzed marigold cultivars, the plants healthiness was assessed. The mean values of the disease index ranged from 16.25 to 29.0. The main cause of root infection at the seedling stage proved to be the species of *Fusarium* and *Rhizoctonia solani*. Proportion of plants with disease symptoms in flowering stage ranged from 1.25 to 5.5%. Regardless of the place of cultivation, species: *A. alternata*, *F. culmorum* and *F. equiseti* were the most often isolated from diseased plants in the flowering stage. Studies on the susceptibility of analyzed cultivars of marigold to infection by *Fusarium culmorum*, *F. equiseti* and *F. sporotrichioides* were conducted in a growth chamber. Significantly, the lowest value of the disease index of cultivar 'Bolero' (*T. patula*) allows to accept that cultivar as the least susceptible to infestation by *Fusarium* spp. under conditions of controlled temperature and humidity.

Key words: marigold, *Tagetes*, *Fusarium* spp., susceptibility, cultivars

INTRODUCTION

Marigold (*Tagetes* L.) is one of the most popular annual ornamental plant. In Poland, cultivars belonging to three species are cultivated the most often: *Tagetes erecta* L., *T. patula* L., *T. tenuifolia* Millsp. Marigolds belong to the standard assortment of summer planting. Their long-lasting flowering makes them very valuable plants in the garden, and their bright colors are an important visual accent. In addition, the flowers of marigold are suitable for garlands and floral decoration and give off a strong, characteristic aroma [Krause et al. 2004]. According to Gupta and Vasudeva [2012], various species of *Tagetes* are a source of biologically active substances, as follows: flavonoids, carotenoids and phenolic compounds. The essential oil of the leaves of *T. erecta* exhibited moderate antimicrobial activity against many species of pathogenic bacteria and fungi. In addition, the essential oils secreted by the roots of *T. erecta* and *T. patula* may limit the development of certain special forms of *Fusarium oxysporum* Schltdl. [Fan et al. 2010, Du et al. 2016]. Substances released into the soil by the roots of these plants inhibit the development of dangerous pests, including nematodes, and also repel aphids and other insects [Gupta and Vasudeva 2012].

Tagetes spp. and other plants from the *Asteraceae* family, at various stages of their development, can be infected by various fungi species. Dangerous pathogens of these plants include species of *Fusarium* genus, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Rhizoctonia solani* J.G. Kühn, which are the cause of the

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seedling blight and necrosis of the root neck [Łacicowa et al. 1979, Palacios et al. 1991, Kiecana and Mielniczuk 2010, Horst 2013]. *Fusarium* spp. are important plant pathogens that produce many metabolites of various phytotoxic activity. Toxins produced by fungi of *Fusarium* genus cause disturbances of physiological process of plants. Pathogenicity of *F. culmorum* and *F. equiseti* to plants is connected with production of trichothecenes, particularly deoxynivalenol. This metabolite causes a reduction of seed germination capacity, as well as roots and coleoptile development. It causes a number of changes at the cellular level as well, for example: inhibits the biosynthesis of protein and nucleic acids, affects the cell membrane permeability and mitotic cell divisions [Wiśniewska and Chełkowski 1994, Packa and Śliwińska 2003].

Marigold is also affected by *Botrytis cinerea* Pers. and *Alternaria* species, mainly: *A. alternata* (Fr.) Keissl., *A. tagetica* Shome & Mustafie, *A. zinniae* M.B. Ellis and *A. dianthi* J.V. Almeida & Sousa da Câmara. These fungi mainly cause leaf spot and flower blight [Tomioka et al. 2000, Wu et al. 2001, Qui et al. 2009, Chandel et al. 2010, Horst 2013, Aktar and Shamsi 2014]. *Alternaria tagetica* infects mainly *T. erecta* and *T. patula*, but its presence was not found on other species of *Tagetes* [Qui et al. 2009]. According to Tomioka et al. [2000], the disease symptoms caused by *A. tagetica* are usually stronger during periods of high humidity and temperature. This species produces two groups of phytotoxic metabolites; the first group includes hydrophilic and the second group lipophilic compounds. Both groups of metabolites participate in the pathogenesis of *T. erecta* leaf spots [Qui et al. 2009].

Among the fungi threatening the cultivation of marigold, *Septoria tageticola* Changsri & Weber, *Cercospora tageticola* Ellis & Everh. and fungi of the genus *Colletotrichum* are also mentioned [Horst 2013, Shukla and Thakur 2018].

Changing assortment of *Tagetes* cultivars recommended for growing and lack of information in the literature on the harmfulness of *Fusarium* spp. for these plants provoked research on the healthiness of this plant considering the susceptibility of selected cultivars to dangerous pathogens – *F. culmorum*, *F. equiseti* and *F. sporotrichioides*.

MATERIAL AND METHODS

Field experiments. Studies were conducted in 2015 in two localities of the Lublin region: in the vicinity of Krasnystaw (50°56'2"N, 23°21'4"E) and in Lublin city (51°13'21.9000"N 22°37'55.8480"E). Those studies included three species of *Tagetes*: *T. erecta* (cv. 'Alaska', 'Hawaii', 'Mann Im Mond'), *T. patula* (cv. 'Bolero' and 'Carmen') and *T. tenuifolia* (cv. 'Lemon Gem' and 'Lulu').

In all stands, the experiment was set up on 25 April 2015 with the previous crop for the cultivation of marigold being the vegetable. Cultivars of *Tagetes* were sown directly to the soil and achenocarps of selected cultivars were sown in four replications – on 4 plots with the area of 6 m^2 each, using 1 g of the sowing material per 1 m^2. No chemical treatment was used during the vegetation and the plots were manually weeded.

During the growing season, field observations were carried out twice. The first lustration was carried out 6 weeks after sowing (15.06.2015) and the second in full flowering stage of plants (25.07.2015).

Six weeks after sowing, the plants' healthiness was assessed. To this aim, 100 seedlings of each cultivar grown in the two analyzed locations were taken (25 seedlings from each replication). Disease symptoms on seedlings were classified using the following scale: 1° – ⅓ to ½ of the roots with necrosis, 2° – over ½ to ¾ of roots with necrosis, 3° – over ¾ necrosis of the roots and necrosis of the hypocotyl, and brown spots on the leaves, 4° – seedlings with total necrosis [Kiecana and Mielniczuk 2010]. The seedlings scored as above were counted and the disease index for each replicate was estimated according to McKinney's formula [Kiecana and Kocyłak 1999].

The obtained results were statistically analyzed by the analysis of variance. The mean values were compared by the smallest significant differences based on the Tukey test ($p = 0.05$). The statistical program ARSTAT, developed at the Department of Applied Mathematics and Informatics, University of Life Sciences in Lublin, was used for the calculations.

Seedlings with disease symptoms were subjected to mycological analysis. The Petri dish method was applied to isolate fungi colonizing the infected plants,

using a mineral medium: saccharose – 38 g, NH_4NO_3 – 0.7 g, KH_2PO_4 – 0.3 g, $MgSO_4 \times 7\ H_2O$ – 0.3 g, trace amounts $FeCl_3 \times 6\ H_2O$, trace amounts $ZnSO_4 \times 7\ H_2O$, trace amounts $CuSO_4 \times 7\ H_2O$, trace amounts $MnSO_4 \times 5\ H_2O$, Agar – 20 g on 1000 ml distilled water. One hundred fragments prepared from the roots and 100 fragments from the hypocotyl of the infected plants from each cultivar and each location were analyzed.

The fungi from *Fusarium* genus were identified according to Nelson et al. [1983] and Leslie and Summerell [2006], while the fungi from *Alternaria* genus were designated according to Simmons [2007]. Other fungi species were determined using monographs and keys cited in the paper by Mielniczuk et al. [2010].

During the second field observation (at fully flowering stage), the percentage of plants with symptoms of root and stem base necrosis was determined. Five such plants of each marigold cultivar grown in two localities were taken for the mycological analysis. From plants showing disease symptoms, in each combination of the experiment, 100 fragments from roots and 100 from the stem base were analyzed. The way of the mycological analysis was the same as in the case of seedlings.

Growth chamber experiment. The investigations of susceptibility of seedlings of seven marigold cultivars: *T. erecta* (cv. 'Alaska', 'Hawaii', 'Mann Im Mond'), *T. patula* (cv. 'Bolero' and 'Carmen') and *T. tenuifolia* ('Lemon Gem' and 'Lulu') to infection by three species from *Fusarium* genus: *F. culmorum* (Wm.G.Sm.), *F. equiseti* (Corda) Sacc. and *F. sporotrichioides* (Sherb.), were conducted in a growth chamber, at the temperature of 23–24°C and relative air humidity of 85%. Strains the pathogenicity of which had been previously tested in the laboratory using the method of Mishra and Behr [1976 according to Kiecana and Mielniczuk 2010] were used in the studies.

The fungal inoculum consisted of 14-days' cultures of the studied fungi strains: *F. culmorum* No. 43, *F. equiseti* No. 23 and *F. sporotrichioides* No. 89 growing on PDA medium in Petri dishes at the temperature of 22°C (plasters of medium with the sporulating colony of analyzed fungus strain). The achenocarps of the analyzed *Tagetes* cultivars the sprouts of which reached the length of 10 mm and were normally formed, were used for the studies. The selected material was placed on plasters of medium with the analyzed strain of each fungi species in plastic pots with the diameter of 10 cm filled with universal subsoil with an addition of sand in the proportion of 2 : 1, with pH 6.5, previously sterilized twice in an autoclave for 2 h at the temperature of 121°C, the atmospheric pressure of 1.21 atm. (0.12 MPa) [Mańka 1989]. The control consisted of pots where the caryopses that began to sprout were placed on plasters of medium without the fungus. All combinations of the experiment had four replication, with 25 plants in each. The plants grew for 24 days, after which the degree of the seedlings infection was established according to the above-mentioned scale in the field experiment. Then, the disease indices were calculated and they were submitted to statistical analysis such as in the case of plants growing in field conditions.

Ten seedlings with disease symptoms from each combination of the growth chamber experiment were taken for the mycological analysis. Fifty 3-milimeter-long fragments from the roots and the bottom part of seedling leaves were analyzed from each experimental combination. Appropriate methods were used to identify the fungi isolated from the diseased seedlings just such as in the case of plants growing in field conditions.

RESULTS

Field experiments: In field conditions, in the case of each localities and examined cultivar, seedlings with the symptoms of root necrosis, reduction of the root system and necrotic, brown spots on the hypocotyl, cotyledons and leaves of the seedlings, were observed. Besides, completely necrotized plants occurred. Statistical analysis showed that significantly highest values of mean disease index were observed in the case of cultivars: 'Hawaii' (*T. erecta*) – 29.0 and 'Lemon Gem' (*T. tenuifolia*) – 28.5, grown in the areas near Krasnystaw and in Lublin, whereas in the case of seedlings of cultivars 'Lulu' (*T. tenuifolia*) and 'Bolero' (*T. patula*) statistically the lowest values of the disease index were recorded – 16.25 and 16.50, respectively (Tab. 1).

Table 1. Values of the disease index for marigold seedlings cultivated in Lublin province

Species	Cultivar	Krasnystaw	Lublin	Mean
Tagetes erecta	'Alaska'	24.50 c	26.50 b	25.50 bc
	'Hawaii'	25.25 c	32.75 c	29.00 c
	'Mann Im Mond'	18.00 b	31.00 c	24.50b
Tagetes patula	'Bolero'	12.25 a	20.75 a	16.50 a
	'Carmen'	26.00 c	28.00 bc	27.00 bc
Tagetes tenuifolia	'Lemon Gem'	26.75 c	30.25 bc	28.50 c
	'Lulu'	13.00 a	19.5 a	16.25 a
Mean		20.82	26.96	23.89

Mean values in columns differ significantly ($P \leq 0.05$) if they are not marked with the same letter

Table 2. Fungi isolated from seedlings of analyzed marigold (*Tagetes*) cultivars

Species	Krasnystaw		Lublin		Total number of isolates (% of all isolates)
	root (% of all isolates)	hypocotyl (% of all isolates)	root (% of all isolates)	hypocotyl (% of all isolates)	
Alternaria alternata (Fr.) Keissler	10 (3.4)	70 (20.1)	11 (4.7)	79 (19.3)	170 (13.2)
Aureobasidium pullulans De Barry Arnaund	10 (3.4)	0 (0)	21 (9.0)	16 (3.9)	47 (3.7)
Botrytis cinerea Pers.	20 (6.8)	0 (0)	2(0.9)	18 (4.4)	40 (3.1)
Chaetomium globosum Kunze (Penz.) Penz. and Sacc.	0 (0)	2 (0.6)	0 (0)	0 (0)	2 (0.2)
Colletotrichum gloeosporioides Penz.	4 (1.4)	4 (1.2)	0 (0)	0 (0)	8 (0.6)
Epiccocun nigrum Link	0	2 (0.6)	0 (0)	0 (0)	2 (0.2)
Fusarium avenaceum (Fr.) Sacc.	10 (3.4)	14 (4.0)	6 (2.6)	21 (5.1)	51 (1.0)
Fusarium culmorum (Wm. G. Sm.) Sacc	38 (13.0)	108 (31.0)	24 (10.3)	92 (22.4)	262 (20.4)
Fusarium crookwellense Burgess. Nelson. Toussoun	9 (3.1)	4 (1.2)	0 (0)	0 (0)	13 (1.0)
Fusarium equiseti (Corda) Sacc.	46 (15.7)	38 (10.9)	48 (20.5)	59 (14.4)	191 (14.9)
Fusarium oxysporum Schltdl.	2 (0.7)	2 (0.6)	23 (9.8)	11 (2.7)	38 (3.0)
Fusarium sporotrichioides Sherb.	14 (4.8)	8 (2.3)	7 (3.0)	34 (8.3)	63 (4.9)
Humicola fuscoatra Traaen	36 (12.3)	10 (2.9)	1 (0.4)	6 (1.5)	53 (4.1)
Mucor hiemalis Wehmer	0 (0)	6 (1.7)	11 (4.7)	9 (2.2)	26 (2.0)
Papulaspora irregularis Hotson	0 (0)	32 (9.2)	17 (7.3)	11 (2.7)	60 (4.7)
Penicillium aurantiogriseum Dierckx	2 (0.7)	0 (0)	12 (5.1)	19 (4.6)	33 (2.6)
Pythium spp.	25 (8.5)	20 (5.8)	12 (5.1)	3 (0.7)	60 (4.7)
Rhizoctonia solani Kühn	51 (17.4)	4 (1.2)	18 (7.7)	22 (5.4)	95 (7.4)
Torula herbarum (Pers.) Link ex S.F. Gray	8 (2.7)	14 (2.0)	3 (1.3)	7 (1.7)	32 (2.5)
Trichothecium roseum Link	6 (2.0)	0 (0)	0 (0)	0 (0)	6 (0.5)
Trichoderma aureoviride Rifai	0 (0)	0 (0)	7 (3.0)	1 (0.2)	8 (0.6)
Non sporulating forms	2 (0.7)	10 (2.9)	11 (4.7)	2 (0.5)	25 (1.9)
Total	293	348	234	410	1285

As a result of the mycological analysis of seedlings of seven *Tagetes* cultivars grown in the area of Krasnystaw, 293 isolates from the roots and 348 from the hypocotyl were obtained. Colonies of those fungi belonged to 20 species. On the other hand, 234 fungal isolates from the roots and 410 from the hypocotyl, belonging to 16 species, were isolated from *Tagetes* spp. seedlings growing on the plots in Lublin.

In the case of the seedling roots of marigold grown in the vicinity of Krasnystaw, the dominating species proved to be *R. solani* – 17.4% of all colonies obtained from the roots. Among the species pathogenic to plants, *F. equiseti* and *F. culmorum* were also often obtained from the seedling roots of marigold grown near Krasnystaw. On the other hand, *F. equiseti*, the isolates of which constituted 20.5% of all root isolations, was obtained in the greatest amounts from the seedling roots of analyzed cultivars of marigold grown in Lublin (Tab. 2). Whereas *F. culmorum* was the most frequently obtained from hypocotyl of seedlings growing in two localities. Isolates of this fungus accounted for 31% in the case of plant growth near Krasnystaw and 22.4% in Lublin (Tab. 2).

Among seedlings growing in the vicinity of Krasnystaw and in Lublin, the species pathogenic to plants included: *B. cinerea*, *F. avenaceum*, *F. oxysporum*, *F. sporotrichioides* and *Pythium* spp. (Tab. 2).

In full flowering stage, in all analyzed cultivars of marigold plants with symptoms of root necrosis and stem base as well as leaf blotch and dying inflorescences and lower leaves were observed. In the vicinity of Krasnystaw, the share of diseased plants ranged from 1% (*T. patula* 'Bolero') to 6.5% (*T. erecta* 'Mann Im Mond'), while in Lublin from 1% (*T. tenuifolia* 'Lulu') to 5.5% (*T. erecta* 'Hawaii') (Fig. 1).

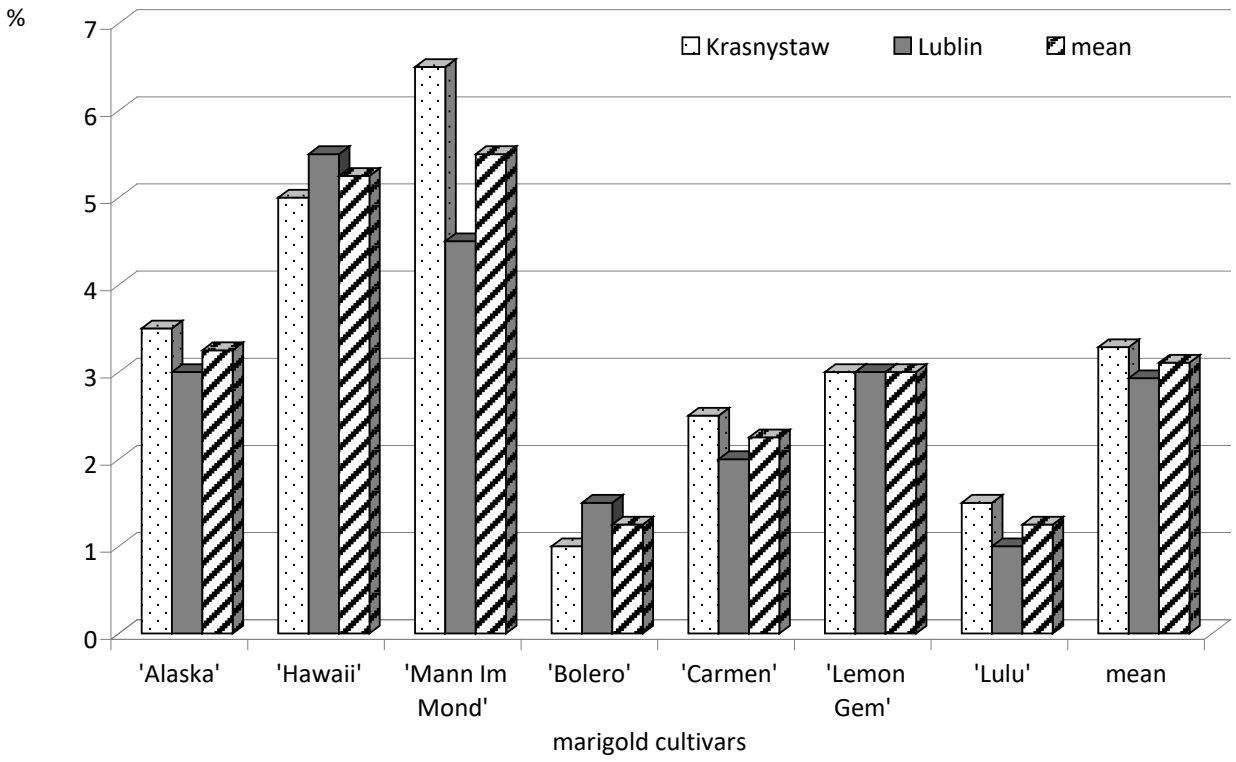

Fig. 1. Percentage of marigold plants with necrosis symptoms on roots and stem bases in flowering stage

As a result of mycological analysis of roots and the stem base of infected plants collected for the research in full flowering stage in the vicinity of Krasnystaw, a total of 912 fungal isolates were obtained, including 428 colonies from roots and 484 from the stem base (Tab. 3). In the case of plants grown in Lublin, 497 isolates of fungi from roots and 517 isolates from the stem base of the analyzed cultivars of marigold were obtained (Tab. 3). In both localities, colonies belonging to *A. alternata* were the most frequently isolated from the analyzed roots, and stem base. Isolates of this species accounted for 23.4% of total root isolations and 40.1% of all fungi isolated from the stem base of plants grown in the vicinity of Krasnystaw and 19.7% from roots and 38.9% from the stem base of plants grown in Lublin (Tab. 3).

Regardless of the cultivation place, from infected plants of the marigold in the full flowering stage, fungi of *Fusarium* genus were obtained. The most of isolates belonged to *F. culmorum* and *F. equiseti*, which constituted respectively 18.7% and 15.4% of all fungi from the roots of plants cultivated in the vicinity of Krasnystaw and 19.3% and 16.5% from the roots of plants growing in Lublin (Tab. 3).

However, among all the fungi obtained from the stem base of the analyzed cultivars of marigold grown in the vicinity of Krasnystaw, the isolates of *F. culmorum* and *F. equiseti* accounted for 13.0% and 8.9% of all colonies respectively, while in Lublin 19.5% and 11.4%, respectively. In addition, the species *F. avenaceum*, *F. oxysporum*, *F. poae* and *F. sporotrichioides* were isolated from infected plants of the marigold.

Among other pathogenic fungi, *R. solani* and *S. sclerotiorum* were also isolated from plants in full flowering stage (Tab. 3).

Table 3. Fungi isolated from roots and stem bases of analyzed marigold (*Tagetes*) cultivars in flowering stage

Fungus species	Krasnystaw		Lublin		Total number of isolates (% of all isolates)
	roots (% of all isolates)	stem base (% of all isolates)	roots (% of all isolates)	stem base (% of all isolates)	
Alternaria alternata (Fr.) Keissler	100 (23.4)	194 (40.1)	98 (19.7)	201 (38.9)	593 (30.8)
Aureobasidium pullulans De Barry Arnaund	20 (4.7)	0 (0)	11 (2.2)	3 (0.6)	34 (1.8)
Fusarium avenaceum (Fr.) Sacc.	10 (2.3)	15 (3.1)	8 (1.6)	21 (4.1)	54 (2.8)
Fusarium culmorum (Wm.G.Sm) Sacc.	80 (18.7)	63 (13.0)	96 (19.3)	101 (19.5)	340 (17.7)
Fusarium equiseti (Corda) Sacc.	66 (15.4)	43 (8.9)	82 (16.5)	59 (11.4)	250 (13.0)
Fusarium oxysporum Schltdl.	10 (2.3)	3 (0.6)	26 (5.2)	11 (2.1)	50 (2.6)
Fusarium poae (Peck.) Wollenw.	0 (0)	16 (3.3)	0 (0.0)	0 (0.0)	16 (0.8)
Fusarium sporotrichioides Sherb.	30 (7.0)	30 (6.2)	36 (7.2)	42 (8.1)	138 (7.2)
Mucor hiemalis Wehmer	0(0)	0(0)	12 (2.4)	9 (1.7)	21 (1.1)
Penicillium aurantiogriseum Dierckx	32 (7.5)	60 (12.4)	32 (6.4)	5 (1.0)	129 (6.7)
Rhizoctonia solani Kühn	10 (2.3)	0(0)	18 (3.6)	21 (4.1)	49 (2.5)
Sclerotinia sclerotiorum (Hedw.) Fuck	45 (10.5)	47 (9.8)	32 (6.4)	21 (4.1)	145 (7.5)
Microascus brevicaulis S.P. Abbott	5 (1.2)	0 (0)	11 (2.2)	6 (1.2)	22 (1.1)
Trichoderma aureoviride Rifai	0(0)	0(0)	15 (3.0)	6 (1.2)	21 (1.1)
Non sporulating forms	20 (4.7)	13 (2.7)	20 (4.0)	11 (2.1)	64 (3.3)
Total	428	484	497	517	1926

Table 4. Mean values of the disease index for seedlings of marigold obtained in growth chamber experiment with inoculation of subsoil with *Fusarium* spp.

Marigold species	Cultivar	Fungi strains			Mean	Control
		F. culmorum	*F. equiseti*	*F. sporotrichioides*		
Tagetes erecta	'Alaska'	91.50*b	87.00*b	92.50*b	90.33 abc	4.25
	'Hawaii'	100.00*c	99.75*d	100.00*d	99.92 c	4.25
	'Mann Im Mond'	99.50*c	98.25*d	92.75*b	96.83 c	7.25
Tagetes patula	'Bolero'	89.50*b	73.25*a	80.50*a	81.08 a	5.50
	'Carmen'	92.75*b	93.00*c	89.25*b	91.67 bc	5.25
Tagetes tenuifolia	'Lemon Gem'	100.00*c	100.00*d	96.00*c	98.67 c	9.00
	'Lulu'	84.25a	94.25*c	82.25*a	86.92 ab	2.50
Mean		93.93	92.21	90.57	92.24	5.43

* Mean values differ significantly compared to the control at $P \leq 0.05$
Mean values in columns differ significantly ($P \leq 0.05$) if they are not marked with the same letter

Growth chamber experiment: In the growth chamber experiment, seedlings showing disease symptoms occurred in all combinations with fungi inoculation. Plants in the experimental combination with *Fusarium* spp. were inhibited in their growth and they had a reduced root system. Necrotic streaks occurred on the hypocotyl surface of the seedlings. Necrotic spots with a chlorotic ring formed on the edge of the leaf blade were visible on the leaves. Dying of the whole seedlings was also observed. Disease indices for the seedlings in the experiment combination with *F. culmorum* were from 84.25 (*T. tenuifolia* 'Lulu') to 100.00 (*T. erecta* 'Hawaii', *T. tenuifolia* 'Lemon Gem') (Tab. 4).

Disease indices for all studied cultivars in the experimental combinations with *F. equiseti* and *F. sporotrichioides* ranged from 73.25 for *T. patula* 'Bolero' to 100.00 for *T. tenuifolia* 'Lemon Gem' and from 80.50 for *T. erecta* 'Bolero' to 100.00 for *T. erecta* 'Hawaii'. The control combination included single seedlings with symptoms of weak root necrosis. Values of the disease index for control seedlings ranged from 2.50 in the case of cultivar 'Lulu' to 9.00 in the case of cultivar 'Lemon Gem' (Tab. 4).

Statistical analysis of disease symptoms indicated significant differences as compared to the control in all cultivars of *Tagetes* spp. and in all analyzed fungi strains (Tab. 4). The statistical analysis also showed significant differences in the values of the disease index for seedlings in individual cultivars of marigold, as well. Statistically significantly, the lowest mean value of disease index characterized *T. patula* 'Bolero', and the highest – cultivars 'Hawaii' and 'Mann Im Mond' (*T. erecta*) and 'Lemon Gem' (*T. tenuifolia*).

Mycological analysis of seedlings with disease symptoms confirmed infection of the fungi used in the studies.

DISCUSSION

Investigations conducted in the field conditions pointed to the occurrence of marigold seedlings and older plants – in flowering stage, showing disease symptoms in the case of all studied cultivars growing in two different localities of the Lublin region.

In similar growing conditions, the percentage of infected *Tagetes* spp. seedlings and the values of disease index were higher than in the case of seedlings of *Zinnia elegans* Jacq., another species from the *Asteraceae* family [Kiecana and Mielniczuk 2010].

The main cause of *Tagetes* cultivars root infection at the seedling and flowering growth stages proved to be the species of *F. culmorum* and *F. equiseti*, commonly regarded as pathogens causing pre- and post-

emergence blight of seedlings as well as the rot of roots, bulbs and the stem base of a number of ornamental plants species [Łacicowa and Kiecana 1992, Pięta and Laskowska 2004, Kopacki and Wagner 2007, Kiecana and Mielniczuk 2010, Kiecana et al. 2014].

Results of experiments conducted in the field and in the growth chamber presented here also point to the role of species *F. culmorum* in the seedling infection of *Tagetes.* The harmfulness of this fungus for plants is larger at higher temperatures [Kiecana et al. 2003, 2009]. In the studies described by Kiecana and Mielniczuk [2010], *F. culmorum* in the growth chamber conditions was less pathogenic towards *Z. elegans* seedlings as compared to *F. equiseti* and *B. cinerea*, but more pathogenic than *F. avenaceum*.

The harmfulness of *F. equiseti* to the seedlings of marigold in phytotron experiment confirms its polyphagic character. This fungus is widely recognized as the cause of the seedlings blight, as well as root rot and the stem base of many ornamental and herbal plants species. Particularly high harmfulness of *F. equiseti* was previously attributed to seedlings of common daisy (*Bellis perennis* L.), zinnia (*Zinnia elegans* L.), thyme (*Thymus vulgaris* L.) and lemon balm (*Melissa officinalis* L.) [Łacicowa and Kiecana 1992, Machowicz-Stefaniak and Zalewska 2004, Zalewska and Machowicz-Stefaniak 2004, Kiecana and Mielniczuk 2010]. This fungus was characterized by the highest harmfulness to the zinnia seedlings among the four examined fungal species (*F. avenaceum*, *F. culmorum*, *F. equiseti* and *B. cinerea*) [Kiecana and Mielniczuk 2010].

Besides, *F. equiseti* was often obtained from roots and the hypocotyl of zinnia cultivated in the soil and climate conditions of the Lublin region [Kiecana and Mielniczuk 2010]. There are reports that this fungus can colonize ornamental plants seed material [Łacicowa et al. 1991, Kućmierz and Kuc 2006]. *Fusarium equiseti* is mainly regarded as a species colonizing the aging tissues and as a secondary colonizer of the host plant as well as a dangerous pathogen of some plants [Desjardins 2006].

The species *F. sporotrichioides* infecting seedlings and stems of older marigold plants is a polyphagous pathogen. In similar growing conditions, this fungus was obtained, among others, from the seeds of zinnia and thyme, as well as from the roots and stems of chrysanthemum [Kopacki and Wagner 2007, Kiecana and Mielniczuk 2010].

Under conditions of the phytotron experiment, this fungus showed lower harmfulness to the marigold seedlings, compared to *F. culmorum* and *F. equiseti*.

Similarly as in the case of china aster, chrysanthemum, zinnia and annual marigold in damaging seedlings, roots and the stem base of the *Tagetes* in full flowering stage, other species of the *Fusarium* genus participated: *F. oxysporum*, *F. poae* and *F. avenaceum* [Łacicowa et al. 1979, Kopacki and Wagner 2007, Kiecana and Mielniczuk 2010, Nawrocki 2013].

In the Lublin area, *F. avenaceum* was also isolated from the infected plants of marigold. This fungus is a well-known pathogen to ornamental plants [Łacicowa et al. 1979, Łacicowa and Kiecana 1992, Kowalik and Wendzel 2005, Nawrocki 2013]. Investigations of Kiecana and Mielniczuk [2010] conducted in growth chamber conditions revealed that *Fusarium avenaceum* proved to be considerably harmful to zinnia seedlings.

Alternaria alternata, which is considered to be the pathogen of the leaf and flower spot of *Tagetes* spp., *Z. elegans*, *Callistephus chinensis* and *Gerbera jamesonii* [Chandel et al. 2010, Kiecana and Mielniczuk 2010, Nagrale et al. 2012, Nawrocki 2013, Aktar and Shamsi 2014], proved to be the species frequently isolated from the marigold cultivated in analyzed locations of Lublin region. This fungus also colonized the sowing material of ornamental plants, including species of *Asteraceae* family [Wu et al. 2001, Szopińska and Tylkowska 2009]. Authors cited by Thomma [2003] suggest that endoglucanases, and exo-glucanases, are involved in *A. alternata* pathogenicity. The pathogenic character of this fungus is connected also with the formation of secondary metabolites, that have a character of phytotoxins. In *Alternaria* genus, many non-host-specific toxins have been identified. Tenuazonic acid, ten-toxin and zinniol are examples of toxins that are produced by

several *Alternaria* species. These fungi also produce host-specific toxins [Thomma 2003].

The species *S. sclerotiorum* belonged to the fungi frequently isolated from the infected plants of marigold in the full flowering stage. *Sclerotinia sclerotiorum* is an economically important necrotrophic fungal pathogen that produces a wide array of degradative lytic enzymes (endo, exo-pectinase, cellulase, hemicellulase, protease), which are believed to facilitate colonization and host cell wall degradation [Williams et al. 2011].

In the studies of Łacicowa et al. [1979] and Kiecana and Mielniczuk [2010], this fungus proved to cause the seedling blight, the rot of roots, the root neck and the stem base of *Z. elegans*. Nawrocki [2013] reported considerable harmfulness of *S. sclerotiorum* towards *C. chinensis*. This fungus can also cause yellowing of the leaves, rot of the stem base and dying out of different ornamental plants, as well [Gulya et al. 2006, Holcomb 2006, Kiecana and Mielniczuk 2010].

Frequent isolation of *B. cinerea* from diseased seedlings in two different cultivation environments, make it possible to consider this fungus the cause of the disease symptoms such as necrosis of roots and hypocotyls. *Botrytis cinerea* was also recognized as the cause of spots on leaves and flowers of zinnia and stems dying of this plant [Palacios et al. 1991]. Studies upon the harmfulness of selected pathogens towards *Tagetes* seedlings, in the conditions of a growth chamber experiment, made use of an inoculum of 14-days' trial cultures of the analyzed strains of *F. culmorum* No. 43, *F. equiseti* No. 23 and *F. sporotrichioides* No. 89 growing on the potato dextrose agar medium, like in the studies carried out by Mańka [1989]. The choice of the inoculum in the form of a fungus culture grown on PDA medium was based on the results obtained by Takegami and Sasaij [1970 according to Mańka 1989].

This is an easy way of obtaining large amounts of infectious material. In the studied conditions, this method proved to be very effective, because seedlings with symptoms of pre- and post-emergence blight of the examined cultivars of *Tagetes* spp. occurred in all experimental combinations.

CONCLUSIONS

Fusarium spp., in particular *F. culmorum* and *F. equiseti*, are of great importance in damage of marigold in different growth stages and conditions of cultivation.

Results obtained in the growth chamber experiment confirmed considerable harmfulness of the species *F. culmorum*, *F. equiseti* and *F. sporotrichioides* towards the seedlings of *Tagetes* spp.

Among the tested marigold cultivars, the lowest susceptibility to infection by *Fusarium* spp. was shown by cultivars 'Bolero' (*T. patula*) and 'Lulu' (*T. tenuifolia*) and the highest – by cultivars 'Hawaii' (*T. erecta*) and 'Lemon Gem' (*T. tenuifolia*).

ACKNOWLEGDEMENTS

The research was financed from funds allocated for the statutory activity (OKF/DS/2) and from own resources.

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