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Contribution to physiological and biochemical diagnostics of *Fusarium* taxa commonly isolated in Egypt

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Fusarium isolates belonging to 24 species and 8 sections were subjected to 11 growth, physiological and biochemical tests (166 isolates). All species were able to grow on 25% Glycerol nitrate agar (G25N), Mannitol medium, Czapek iprodione dichloran agar (CZID) and Dichloran chloramphenicol peptone agar (DCPA). Growth rate, colony colour and reverse on G25N and CZID were promising diagnostic criteria for separating species within sections. The growth rate and reverse on Mannitol agar was diagnostic not only for separation of *F. oxysporum* from *F. solani*, but also for separation of many other *Fusarium* species treated. The ability to grow on Tannin-sucrose agar could distinguish some species of sections *Liseola* and *Sporotrichiella*. Acid production on Creatine-sucrose agar distinguished some species of section *Liseola* and *F. trichothecioides* of section *Discolor*. Acetylmethylcarbinol production revealed for the first time that all isolates of *F. subglutinans* can produce this substance, while most remaining isolates lacked this ability. Tests for peroxidase, urease and pyrocatechol oxidase proved to be very useful. The phosphatase enzyme seems to be common in *Fusarium* species and therefore it is only diagnostic for *F. camptoceras* (negative results). Some physiological features could be used as criteria to distinguish some sections or species within the same section.

Key words: *Fusarium*, taxonomy, physiological features, acetylmethylcarbinol, diagnostic.

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Článek hodnotí využití fyziologických a biochemických metod pro identifikaci druhů rodu *Fusarium*. Bylo studováno 24 druhů z 8 sekcí tohoto rodu, celkově 166 izolátů. Některé zjištěné znaky lze využít pro rozlišení sekcí a druhů.

INTRODUCTION

Fusarium (*Hypocreales*, Ascomycota) is a taxonomically difficult genus. Its identification based on morphological features only is not reliable; thus the identification of different *Fusarium* species requires special culture media, some physiological criteria or molecular methods (see e.g. *Fusarium*-ID). The high variability in

species, especially under different environmental conditions, has led taxonomists to consider some special criteria for the classification of species (Booth 1975).

There are many isolation media for recovering and identifying *Fusarium* species, such as Czapek Dox agar (Raper & Thom 1949), Peptone-PCNB agar (Nash & Snyder 1962), Dichloran chloramphenicol peptone agar (Andrews & Pitt 1986) and Czapek iprodione dichloran agar (Abildgren et al. 1987). Also selective media for isolation and identification of *Fusarium* species were developed and found helpful, permitting rapid identification of *Fusarium* isolates on such media (Komada 1975; Andrews & Pitt 1986; Abildgren et al. 1987; Vázquez et al. 1993; Thrane 1986, 1996).

The use of physiological and biochemical criteria in *Fusarium* taxonomy after the classical works by Booth (1971), Gerlach & Nirenberg (1982), Nelson et al. (1983), and Leslie & Summerell (2006) was explored to some extent by Komada (1975), Švábová et al. (1980), Thrane (1986), Wasfy et al. (1987), Brayford & Bridge (1989), and Vázquez et al. (1993).

The goal of this study was to find any other criteria that might be useful in *Fusarium* species differentiation. The present study was designed to find some physiological and cultural features of *Fusarium* species commonly occurring in Egypt.

MATERIAL AND METHODS

This study included 166 *Fusarium* isolates belonging to 24 species from 4 sections with abundant microconidia (section *Liseola*, representing 5 species and 36 isolates; *Sporotrichiella*, 5 species and 33 isolates; *Elegans*, 1 species and 9 isolates; and *Martiella*, 1 species and 8 isolates) and 4 sections with abundant macroconidia and scarce microconidia (*Arthrosporiella*, representing 3 species and 20 isolates; *Discolor*, 4 species and 27 isolates; *Gibbosum*, 2 species and 12 isolates; and *Lateritium*, 3 species and 21 isolates). Most of these isolates (143) were isolated from different sources in Egypt (e.g. soil, air, seeds, grains, etc.) during the period 2004–2005, deposited in the Mycological Herbarium of the Department of Botany and Microbiology, Faculty of Science, Assiut University, and assigned MH numbers. All isolates were identified based on morphological and microscopic features within a year of isolation (Booth 1971, Nelson et al. 1983, Leslie & Summerell 2006). These isolates were subjected to some tests concerning physiology and growth behaviour on selected agar media. Ten isolates were kindly obtained from the culture collection of Assiut University Mycological Centre (AUMC) and 13 isolates from the Centraalbureau voor Schimmelcultures (CBS) (see Tab. 1).

Tab. 1. Numbers and origin of *Fusarium* isolates investigated.
MH: Mycological Herbarium of Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt
AUMC: Assiut University Mycological Centre, Egypt
CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Section	Species	No of isolates	Origin
Elegans (1 species)	*F. oxysporum* Schltdl.	9	Soil (rhizosphere MH 100, MH 101, desert MH 102, cultivated MH 103), stem of *Canola* (MH 104), maize grains (MH 105), lentil seeds (MH 106), watermelon root (AUMC 870), soil (AUMC 1403)
Martiella (1 species)	*F. solani* (Mart.) Sacc.	8	Soil (rhizosphere MH 107, desert MH 108, MH 109 cultivated MH 110), maize grains (MH 111), sesame seeds (MH 112), air (MH 113), human keratitis (MH 114)
Liseola (5 species)	*F. anthophilum* (A. Braun) Wollenw.	4	*Sorghum* grains (MH 115), lentil seeds (MH116, MH 117), *Zostera marina* (CBS 187.34)
	F. nygamai L. W. Burgess & Trimboli	8	Soil (salt marsh MH 118, desert MH 119, cultivated MH 120), *Sorghum* grains (MH 121, MH 122), lentil seeds (MH 123), air (MH 124), banana tissue (MH 125)
	F. proliferatum (Matsush.) Nirenberg	7	Soil (desert MH 126, MH 127, salt marsh MH 128), maize grains (MH 129, MH 130), *Dianthus caryophyllus* (CBS 115.97), soil (AUMC 3190)
	F. subglutinans (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas	8	Soil (rhizosphere MH 131, salt marsh MH 132, desert MH 133, cultivated MH 134), corn grains (CBS 215.76), sesame (MH 135, MH 136) and lentil (MH 137) seeds
	F. verticillioides (Sacc.) Nirenberg	9	Soil (desert MH 138, salt marsh MH 139, cultivated MH 140), sesame (MH 141), lentil (MH 142) and *Canola* (MH 143) seeds, *Sorghum* grains (MH 144), air (MH 145), *Matricaria chamomilla* (AUMC 2399)
Sporotrichiella (5 species)	*F. chlamydosporum* Wollenw. & Reinking	6	Soil (rhizosphere MH 146, cultivated MH 147, reclaimed MH 148), *Sorghum* grains (MH 149), sesame seeds (MH 150), CBS 145.25
	F. fusarioides (Gonz, Frag. & Cif.) C. Booth	6	Soil (desert MH 151, MH 152, MH 153 and cultivated MH 154, MH 155), *Hibiscus sabdariffa* (AUMC 3181)
	F. poae (Peck) Wollenw.	8	Soil (rhizosphere MH 156, salt marsh MH 157, MH 158, desert MH 159, cultivated MH 160, MH 161), air (MH 162, MH 163)
	F. sporotrichioides Sherb.	8	Soil (rhizosphere MH 164, salt marsh MH 165, desert MH 166, MH 167, cultivated MH 168, MH 169), repeatedly breeded buffalo (MH 170, MH 171)
	F. tricinctum (Corda) Sacc.	5	Soil (rhizosphere MH 172, MH 173, cultivated MH 174, salt marsh MH 175), *Hordeum sativum* grains (CBS 253.50)
Arthrosporiella (3 species)	*F. avenaceum* (Fr.) Sacc.	6	Soil (salt marsh MH 176, MH 177, desert MH 178, cultivated MH 179, MH 180), CBS 143.25
	F. camptoceras Wollenw. & Reinking	6	Soil (salt marsh MH 181, desert MH 182, MH 183, cultivated MH 184, MH 185), *Beta vulgaris* roots (CBS 245.61)
	F. semitectum Berk. & Ravenel	8	Soil (rhizosphere MH 186, salt marsh MH 187, MH 188, desert MH 189, cultivated MH 190), *Sorghum* grains (MH 191), air (MH 192), *Sorghum vulgare* (CBS 165.57)

Section	Species	No of isolates	Origin
Discolor (4 species)	*F. culmorum* (W. G. Smith) Sacc.	7	Soil (rhizosphere MH 193, salt marsh MH 194, desert MH 195, MH 196, cultivated MH 197), CBS 171.28, soil (AUMC 158)
	F. graminearum Schwabe	6	Desert soil (MH 189, MH 199), sesame seeds (MH 200, MH 201, MH 202), lupine roots (AUMC 473)
	F. sambucinum Fuckel	8	Soil (rhizosphere MH 203, salt marsh MH 204, desert MH 205, cultivated MH 206, MH 207), cotton root (AUMC 893), millet grain (AUMC 1405), CBS 118.13
	F. trichothecioides Wollenw.	6	Soil (desert MH 208, MH 209, cultivated MH 210), air (MH 211), repeatedly breeded buffalo (MH 212, MH 213)
Gibbosum (2 species)	*F. concolor* Reinking	6	Soil (salt marsh MH 214, MH 215, desert MH 216, cultivated MH 217, MH 218), *Hordeum vulgare* (CBS 183.34)
	F. equiseti (Corda) Sacc.	6	Soil (salt marsh MH 219, desert MH 220, cultivated MH 221, MH 222), air (MH 223), bean aphid (AUMC 757)
Lateritium (3 species)	*F. lateritium* Nees	8	Soil (salt marsh MH 224, MH 225, desert MH 226, cultivated MH 227), lentil rhizoplane (MH 228), air (MH 229), repeatedly breeded cow (MH 230), *Morus alba* (CBS 134.24)
	F. udum E. J. Butler	5	Cultivated soil (MH 231), maize (MH 232) and *Sorghum* (MH 233) grains, lentil rhizoplane (MH 234), seeds of *Canola* (MH 235)
	F. xylarioides Steyaert	8	Soil (desert MH 236, cultivated MH 237, MH 238), lentil (MH 239) and sesame (MH 240, MH 241) seeds, air (MH 242), trunk of *Coffea* species (CBS 258.52)

Growth on 25% Glycerol nitrate agar medium (G25N) (Pitt 1973), Ammonium salts agar medium (Mannitol medium) (Brayford & Bridge 1989), Czapek iprodione dichloran agar (CZID) (Pitt & Hocking 1997) and Dichloran chloramphenicol peptone agar (DCPA) (Andrews & Pitt 1986) was investigated. Plates of G25N, CZID, DCPA, and Mannitol agar were inoculated and after 7 days of incubation at 25 °C examined for growth rate and colony morphology characteristics. Plates of Tannin-sucrose medium (TAN) (Thrane 1986) were inoculated and incubated at 25 °C and examined for growth after 7, 10, 14 and 21 days of incubation. The isolates can be divided into two groups, a group which is able to grow on TAN agar (TAN+) and a group which is unable to grow on TAN agar (TAN–). Acid production on Creatine-sucrose agar plates (CREA) (Frisvad 1985, 1993) which were three-point inoculated and incubated at 25 °C in the dark was also examined after 7 and 10 days of incubation.

Acetylmethylcarbinol production (Voges-Proskauer test). The ability of *Fusarium* species to produce acetylmethylcarbinol from glucose was detected using Glucose phosphate broth (Harrigan & McCance 1966). Tubes containing medium were inoculated and incubated at 25 °C for 5–7 days. After the incubation period, 1 ml of culture was transferred to another tube, 0.6 ml of 5% (wt/vol) α-naphthol dissolved in

absolute ethanol was added, and mixed thoroughly. Then 0.2 ml of 40% aqueous KOH was added. The mixtures mixed well, and were incubated in a slanted position to increase the surface area of the medium (the reaction is dependent on oxygen). A positive reaction (after 15 and 60 min.) is indicated by a strong red colour which begins to develop at the surface of the medium.

Urease activity. We used the medium described by Paterson & Bridge (1994). This medium contains urea and bromocresol purple as a pH indicator. Agar plates were inoculated centrally and the results were recorded five days after incubation at 25 °C. Isolates capable of producing urease turned the yellow colour of the acidic medium purple-red or deep pink. The diameters of the colonies as well as the colour zone (in cm) were measured. The enzyme index for urease was expressed according to Ho & Foster (1972) and Ismail (2001) as follows: Enzyme index = Diameter of outer limit of clear zone / Diameter of fungal colony.

Peroxidase activity. The ability of *Fusarium* species to produce peroxidase was determined by a modification of the procedure described by Egger (1986). *Fusarium* species were grown on MEA (Malt extract agar, Pitt & Hocking 1997) in Petri dishes for 12 d and were cut with a flamed cork borer within the outermost 10 mm of the colony. One drop each of a freshly prepared 1% (w/v) aqueous solution of pyrogallic acid and 0.4% hydrogen peroxide was added to the well. Development of a golden yellow to brown colour indicates peroxidase activity (Egger 1986).

Phosphatase activity. The ability of *Fusarium* species to produce the phosphatase enzyme was detected using the Phosphatase medium described by Gochenaur (1984). Agar plates were inoculated centrally by *Fusarium* isolates and incubated at 25 °C for 5 days. After incubation, one drop of 30% ammonium hydroxide solution was added to the lid of the inverted plate. Hydrolysis of sodium diphosphophenolphthalein was considered positive if a deep fuchsia-coloured zone developed around the colony in the presence of ammonium hydroxide vapours.

Pyrocatechol oxidase activity. The ability of *Fusarium* species to produce the extracellular enzyme pyrocatechol oxidase was detected following the method described by Chandrashekar & Kaveriappa (1988). 2% MEA agar plates containing 0.17% tannic acid were inoculated centrally and incubated at 25 °C for 10 days. The presence of a dark brown zone around the colony indicates pyrocatechol oxidase activity.

RESULTS AND DISCUSSION

This research was aimed at establishing some physiological criteria for the identification of common *Fusarium* species within sections or distinguishing sections from each other. Eleven cultural, physiological and biochemical tests were

assessed in order to find any criteria which can be useful in such identification. These tests include growth features and pigmentation on some selected media as well as production of enzymes and other metabolites. One hundred sixty-six isolates belonging to 24 *Fusarium* species were used in the present work (Tab. 1).

Growth on 25% Glycerol nitrate agar medium (G25N)

All investigated strains grew on G25N medium and produced pigmented colonies. However from the results obtained, growth on G25N could be considered a promising diagnostic medium for separating species of *Fusarium* within individual sections. Based on this, species could be divided into two groups: species with a growth rate of less than 2 cm diameter and those with a growth rate of more than 2 cm diameter per 7 days. Only the isolates of 8 species produced colonies with variable diameters on G25N: *F. udum*, *F. xylarioides*, *F. nygamai*, *F. proliferatum*, *F. subglutinans*, *F. verticillioides*, *F. fusarioides* and *F. poae* (Tab. 2).

The colony colour and pigmentation on G25N medium were consistent for most species, which might make these characters diagnostic for these species. For example, some species showed an orange reverse while others were brownish. At the same time the colony colour (aerial mycelium) of all isolates of the three species within section *Arthosporiella* (*F. avenaceum*, *F. camptoceras* and *F. semitectum*) and *F. concolor* showed a yellowish aerial mycelium. The aerial mycelium of *F. culmorum*, *F. graminearum*, *F. trichothecioides*, *F. equiseti*, *F. lateritium*, *F. udum*, *F. proliferatum* and *F. solani* was orange, while that of *F. anthophilum*, *F. nygamai* and *F. fusarioides* was pinkish to pale red (Tab. 2). Growth and pigmentation on G25N agar is used not only for identification of *Penicillium* species (Pitt 1973) but also for identification of species of the genus *Phoma* (Montel et al. 1991).

Growth on Mannitol agar medium

The strains could be divided into two groups according to colony diameter: less than 4 and more than 4 cm diameter. All isolates of *F. avenaceum* (section *Arthrosporiella*), *F. concolor*, *F. equiseti* (both section *Gibbosum*), *F. udum* (section *Lateritium*), *F. anthophilum* (section *Liseola*) and *F. tricinctum* (section *Sporotrichiella*) created colonies smaller than 4 cm diameter, while those of *F. camptoceras* (section *Arthrosporiella*), *F. culmorum*, *F. graminearum*, *F. sambucinum*, *F. trichothecioides* (all section *Discolor*), *F. xylarioides* (section *Lateritium*) and *F. proliferatum* (section *Liseola*) created colonies larger than 4 cm in diameter (Tab. 2).

Tab. 2. Colony diameter (cm), colour and reverse of *Fusarium* isolates grown on 25% Glycerol nitrate agar (G25N), Mannitol agar and Czapek iprodione dichloran agar (CZID). Colony colour and reverse refer to the Methuen handbook of colour (Kornerup & Wancher 1978).

Species (number of isolates)	**G25N**			**Mannitol**			**CZID**		
	Diameter Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**	**Diameter** Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**	**Diameter** Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**
F. oxysporum (9)	1.6 ± 0.1 (1.5–1.9)	pale yellow or white	deep yellow, light yellow or pale yellow	4.2 ± 0.5 (3.2–4.7)	reddish white, pale red or white	brownish red, greyish red, reddish brown or red	5.1 ± 0.4 (4.6–5.8)	pinkish white, white or pastel pink	purplish grey or brownish grey
F. solani (8)	1.6 ± 0.2 (1.3–1.9)	orange white, pale orange or white	pale orange, orange-grey or yellowish orange	3.4 ± 0.7 (2.4–4.2)	yellowish white, orange-white, light yellow, light orange or pale orange	light orange or orange	4.8 ± 0.4 (4.2–5.3)	white	light yellow-brown or brownish grey
F. anthophilum (4)	1.8 ± 0.1 (1.7–1.9)	pinkish	greyish orange	3.1 ± 0.5 (2.7–3.8)	reddish white or pale red	greyish red or reddish brown	4.0 ± 0.1 (3.9–4.2)	orange-white, pale orange or white	greyish brown or orange-grey
F. nygamai (8)	2.0 ± 0.1 (1.9–2.2)	pale red or pinkish	orange-white or greyish orange	3.8 ± 0.3 (3.3–4.2)	reddish white or pale red	pastel red, red or dull red	5.3 ± 0.2 (5.1–5.7)	pinkish, white or pale red	greyish brown, reddish grey or pastel red
F. proliferatum (7)	1.9 ± 0.2 (1.7–2.2)	light orange or pale orange	greyish orange or light orange	4.4 ± 0.3 (4.1–4.7)	pale red, reddish white or white	red, dull red, greyish red or pastel red	5.4 ± 0.2 (5.2–5.6)	white or pastel pink	reddish grey, greyish red or greyish brown
F. subglutinans (8)	1.9 ± 0.3 (1.5–2.3)	white or pale red	pale orange or orange-white	3.5 ± 0.4 (2.9–4.1)	pale red, pastel red, reddish white or white	greyish red, reddish brown or red	5.7 ± 0.4 (5.1–6.3)	greyish rose, white or pinkish	greyish ruby, pinkish or reddish grey

Species (number of isolates)	G25N			Mannitol			CZID		
	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse
F. verticillioides (9)	2.1 ± 0.2 (1.9–2.4)	pinkish, white or pale red	greyish orange, pale orange or light yellow	4.0 ± 0.7 (3.1–4.9)	pale red, pastel red or dull red	brownish red, red, greyish red or reddish brown	5.5 ± 0.3 (5.1–5.8)	greyish rose, greyish red or pinkish	greyish ruby, reddish grey or greyish brown
F. chlamydosporum (6)	2.3 ± 0.2 (2.0–2.7)	pale yellow, white or yellowish white	yellowish orange, reddish yellow or pale orange	3.9 ± 0.3 (3.5–4.4)	light yellow, greyish yellow or orange-yellow	brownish orange or greyish orange	4.9 ± 0.4 (4.2–5.3)	yellowish white, pale yellow or white	greyish yellow, light yellow or deep yellow
F. fusarioides (6)	2.2 ± 0.3 (1.9–2.5)	pinkish or pale red	reddish yellow or light orange	3.8 ± 0.5 (3.3–4.4)	light yellow, reddish yellow or greyish yellow	brownish orange or golden brown	5.2 ± 0.3 (4.8–5.5)	reddish white or white-pale red	dull red, pastel red or deep red
F. poae (8)	2.1 ± 0.2 (1.8–2.4)	pale orange or pale yellow	light orange, orange or red-orange	4.5 ± 0.6 (3.7–4.9)	orange-yellow, light-greyish yellow or brownish yellow	greyish yellow or brownish yellow	5.0 ± 0.4 (4.5–5.8)	white-greyish red, reddish white or white-pale red	brownish red, reddish brown-orange, yellowish red or pastel red
F. sporotrichioides (8)	2.7 ± 0.3 (2.4–3.2)	orange white, light yellow or pale orange	golden brown or light brown	3.9 ± 0.5 (3.1–4.5)	orange-yellow, light yellow or reddish yellow	greyish orange, greyish yellow or reddish yellow	5.6 ± 0.3 (5.4–6.1)	white-pale red, white-pastel red or reddish white	reddish brown-yellow, pastel red or brownish red
F. tricinctum (5)	2.5 ± 0.1 (2.3–2.7)	white or pale red	light orange or greyish red	3.2 ± 0.4 (2.8–3.7)	light yellow, pale yellow or greyish yellow	reddish yellow, orange-yellow, greyish yellow or dark yellow	5.0 ± 0.2 (4.8–5.3)	reddish white, pale red or pastel red	deep red or brownish red

Species (number of isolates)	G25N			Mannitol			CZID		
	Diameter Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**	**Diameter** Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**	**Diameter** Mean ± SD (Minimum – Maximum)	**Colony colour**	**Reverse**
F. avenaceum (6)	1.4 ± 0.1 (1.3–1.5)	pale yellow or yellowish white	orange, dark orange or golden yellow	2.9 ± 0.5 (2.3–3.4)	pale yellow, dull yellow or yellowish white	light yellow or pale yellow	3.9 ± 0.3 (3.5–4.4)	white	light orange or pale orange
F. camptoceras (6)	3.1 ± 0.1 (2.9–3.3)	pale yellow or yellowish white	light yellow or pale yellow	4.8 ± 0.8 (4.0–5.9)	pale yellow or light yellow	greyish yellow or light yellow	3.9 ± 0.2 (3.6–4.2)	white	light to orange-brown, brownish orange or reddish golden
F. semitectum (8)	2.3 ± 0.2 (2.1–2.6)	yellowish white or pale yellow	light brown, light orange or brownish orange	3.9 ± 0.5 (3.4–4.9)	light yellow, pale yellow or yellowish orange	light yellow or greyish yellow	4.1 ± 0.4 (3.7–4.8)	white-pale orange, orange-white or white-greyish orange	light brown or pale to pastel red-brown
F. culmorum (7)	2.4 ± 0.1 (2.3–2.5)	pale orange, orange white or white	brownish yellow, golden yellow or dark yellow	5.1 ± 0.4 (4.4–5.6)	pale yellow or light yellow	light orange or orange	5.6 ± 0.3 (5.3–6.2)	yellowish white-pinkish or light orange-pinkish	brownish red, red or dark red
F. graminearum (6)	2.4 ± 0.1 (2.2–2.5)	light orange or pale orange	golden yellow or deep orange	5.3 ± 0.4 (4.9–5.9)	light-pale yellow, orange-white or pale orange	light orange, deep orange or greyish orange	5.8 ± 0.3 (5.5–6.2)	pale red or greyish red	reddish orange-brownish red or brownish red
F. sambucinum (8)	1.6 ± 0.1 (1.5–1.8)	white or yellowish white	yellowish white, orange-yellow or light yellow	5.1 ± 0.3 (4.7–5.5)	light yellow, yellowish white or orange-white	greyish orange, pale orange, orange or dark yellow	5.5 ± 0.2 (5.2–5.8)	white or yellowish white	light-dull yellow or yellowish brown

Species (number of isolates)	G25N			Mannitol			CZID		
	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse	Diameter Mean ± SD (Minimum – Maximum)	Colony colour	Reverse
F. trichothecioides (6)	2.3 ± 0.3 (2.0–2.7)	orange white or pale orange	brownish yellow or golden yellow	4.7 ± 0.5 (4.0–5.4)	pale yellow, pastel yellow or yellowish white	yellowish orange, light yellow or reddish yellow	5.3 ± 0.8 (4.6–6.7)	yellowish white, white or pall yellow	yellowish brown, dull yellow or greyish yellow
F. concolor (6)	2.4 ± 0.1 (2.3–2.5)	yellowish white or pale yellow	light yellow	3.3 ± 0.4 (2.6–3.8)	yellowish orange, light yellow or reddish yellow	light yellow or pale yellow	4.7 ± 0.4 (4.5–5.4)	yellowish white or reddish white	greyish orange or light yellow
F. equiseti (6)	2.5 ± 0.1 (2.4–2.6)	pale orange or orange white	pale orange or light orange	3.2 ± 0.6 (2.7–4.3)	yellowish white, pale yellow or light yellow	light yellow, reddish yellow or greyish yellow	5.5 ± 0.2 (5.2–5.8)	white-light orange, light orange or white-golden yellow	light orange-orange, light brown or orange
F. lateritium (8)	2.5 ± 0.1 (2.3–2.6)	greyish orange, light orange or pale orange	light brown, orange or brown	4.6 ± 0.6 (3.9–5.5)	reddish white or pale red	pastel red or dull red	4.7 ± 0.3 (4.2–4.9)	white, orange-white or pale orange	light orange or greyish orange
F. udum (5)	2.0 ± 0.2 (1.8–2.2)	pale orange or light orange	light brown or brown	2.9 ± 0.3 (2.5–3.3)	pastel red, pale red or white	brownish red, reddish brown or pale red	4.7 ± 0.2 (4.4–4.9)	pinkish white, pastel pink or white-brownish grey	purplish grey, brownish grey or greyish ruby
F. xylarioides (8)	2.1 ± 0.2 (1.8–2.3)	white or pale yellow	pale yellow or yellowish white	4.2 ± 0.2 (4.0–4.5)	reddish white	pastel red or dull red	4.4 ± 0.3 (4.1–4.9)	pinkish white, white or pastel pink	brownish grey or purplish grey

F. solani could be distinguished from *F. oxysporum* by its orange reverse on mannitol agar versus a red to brownish red reverse in *F. oxysporum*, confirming the earlier finding by Brayford & Bridge (1989). Moreover, all species from section *Arthrosporiella* (*F. avenaceum*, *F. camptoceras* and *F. semitectum*), *F. trichothecioides* (section *Discolor*), *F. concolor* (section *Gibbosum*) and *F. poae* (section *Sporotrichiella*) produced yellowish pigmentation. Orange pigmentation was produced by *F. culmorum*, *F. graminearum* and *F. sambucinum* (all section *Discolor*), *F. chlamydosporum* and *F. fusarioides* (both section *Sporotrichiella*), and *F. solani* (section *Martiella*). A reddish pigmentation was observed in all isolates of *F. oxysporum* (section *Elegans*), *F. lateritium*, *F. udum*, *F. xylarioides* (section *Lateritium*) and the 5 species of section *Liseola* (Tab. 2).

Brayford & Bridge (1989) also found that all isolates of *F. avenaceum*, *F. chlamydosporum*, *F. sporotrichioides*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. lateritium*, *F. longipes* and *F. pallidoroseum* (= *F. semitectum*) tested on ammonium salt agar containing either sorbitol or xylitol produced unpigmented colonies.

Mannitol medium was first used by Brayford & Bridge (1989) to distinguish strains of *Fusarium oxysporum* from those of *F. solani* by means of pigmentation. Orange pigments were commonly produced by isolates of *F. solani* and red ones by *F. oxysporum*. In the present work, growth and pigmentation on mannitol agar showed that this medium is promising as a diagnostic tool not only for differentiation of *F. oxysporum* and *F. solani* (Brayford & Bridge 1989), but also for many species treated in this research.

Growth on Czapek iprodione dichloran agar medium (CZID)

Colony diameters of most species of *Fusarium* proved to be diagnostic hence some species grow restrictedly (< 5 cm diameter per 7 days), while others grow rapidly (> 5 cm diameter per 7 days). Three species of section *Arthrosporiella* (*F. avenaceum*, *F. camptoceras*, *F. semitectum)*, the three species from section *Lateritium* (*F. lateritium*, *F. udum* and *F. xylarioides*), and one species out of five in section *Liseola* (*F. anthophilum)* created colonies smaller than 5 cm, while *F. culmorum*, *F. graminearum* and *F. sambucinum* (all section *Discolor*), *F. equiseti* (section *Gibbosum*), *F. nygamai*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides* (all section *Liseola*), and *F. sporotrichioides* (section *Sporotrichiella*) created colonies larger than 5 cm (Tab. 2).

A brownish reverse was revealed on CZID for all isolates of *F. camptoceras*, *F. semitectum* and *F. solani*, while *F. avenaceum* showed an orange reverse. The reverse of *F. culmorum*, *F. graminearum*, *F. proliferatum*, *F. fusarioides*, *F. poae*, *F. sporotrichioides* and *F. tricinctum* was red to brownish red. A fourth group was formed by species with a yellowish reverse. This group includes

F. sambucinum, *F. trichothecioides* (both section *Discolor*) and *F. chlamydosporum* (section *Sporotrichiella*) (Tab. 2).

The colour of aerial mycelium on CZID differed between species. The aerial mycelium of *F. avenaceum*, *F. camptoceras* and *F. solani* was white, in *F. graminearum*, *F. fusarioides*, *F. poae*, *F. sporotrichioides* and *F. tricinctum* it was reddish, in *F. semitectum* and *F. equiseti* orange, and in *F. oxysporum* and *F. udum* it was pinkish. Isolates of the remaining *Fusarium* species had mycelia of variable colour (Tab. 2).

Dichloran (2,6-dichloro-4-nitroaniline) is a well known chemical inhibiting rapid-growing moulds (King et al. 1979). Iprodione [3-(3,5-dichlorophenyl)-N-isopropylcarbamoyl-2,4-dioxoimidazolidine-1-carboxamide] is reported to inhibit *Alternaria*, *Botrytis*, *Didymella*, *Rhizoctonia* and *Sclerotinia* species. Czapek iprodione dichloran agar (CZID) was first developed by Abildgren et al. (1987) as a selective medium for the isolation of *Fusarium* species, which proved to be the most effective medium for this purpose in the work by Abdel-Hafez et al. (2009). The present work indicates another use for this medium: not only for isolation of *Fusarium* species but also for distinguishing these species.

Growth on Dichloran chloramphenicol peptone agar medium (DCPA)

DCPA was developed for the selective isolation of *Fusarium* species and dematiaceous hyphomycetes from cereals by Andrews & Pitt (1986). Here, in an effort to make use of this medium, isolates of *Fusarium* were grown and two categories of species could be observed according to their growth rates (less or more than 5 cm diameter). However, all isolates tested produced unpigmented colonies.

Fusarium lateritium, *F. udum*, *F. xylarioides* and *F. anthophilum* showed colony diameters less than 5 cm while 10 species represented by 65 isolates (*F. avenaceum*, *F. camptoceras*, *F. culmorum*, *F. graminearum*, *F. concolor*, *F. verticillioides*, *F. solani*, *F. chlamydosporum*, *F. fusarioides* and *F. tricinctum*) showed colony diameters of more than 5 cm. Other isolates of 10 species produced colonies of variable diameter on this medium. In this respect, Andrews & Pitt (1986) reported that isolates of *Fusarium graminearum* produced smaller colonies with heavier conidiogenesis on DCPA (27–43 mm), however a rapid growth (exceeding 5 cm diameter) of *F. graminearum* isolates on DCPA was reported in the present work (Tab. 3).

Growth on Tannin-sucrose medium

Eighteen species out of the 24 studied were able to grow on Tannin-sucrose medium while all isolates of the remaining six species were not able to grow on this medium. These were *F. culmorum*, *F. graminearum*, *F. sambucinum* and *F. trichothecioides* (section *Discolor*), *F. anthophilum* (section *Liseola*) and

F. poae (section *Sporotrichiella*). It is interesting that all species of sections *Arthrosporiella*, *Elegans*, *Lateritium* and *Martiella* were able to grow on tannin-sucrose medium while all species of section *Discolor* were not. Further, from section *Liseola* only *F. anthophilum* and from section *Sporotrichiella* only *F. poae* were not able to grow on tannin-sucrose. Hence, this test could be useful as a diagnostic tool for distinguishing sections and in some cases for distinguishing species within sections, e.g. in sections *Liseola* and *Sporotrichiella*.

In a study on 11 *Fusarium* species by Thrane (1986), eight species were able to grow on Tannin-sucrose agar, while 3 species were unable to grow on this medium. It is worth mentioning that our results were in complete agreement with those of Thrane (1986). *F. avenaceum* (6 isolates in the present work, 76 isolates in Thrane's work), *F. equiseti* (6 and 26), *F. oxysporum* (9 and 37), *F. solani* (8 and 17), *F. sporotrichioides* (8 and 18), *F. tricinctum* (5 and 21) and *F. verticillioides* (9 and 17) were able to grow on Tannin-sucrose medium, while *F. culmorum* (7 and 102), *F. graminearum* (6 and 9) and *F. poae* (8 and 46) were unable to grow on this medium (Tab. 3).

Acid production on Creatine-sucrose agar

All isolates of all species tested gave consistent results on Creatine-sucrose agar except those of *F. proliferatum*, which were isolate-dependent. Only three species were able to produce acid on Creatine-sucrose agar, *F. trichothecioides* (section *Discolor*), *F. nygamai* and *F. verticillioides* (both section *Liseola*), while the other 20 species were not (Tab. 3).

Creatine-sucrose medium was developed as a differential medium for *Penicillium* subgenus *Penicillium* (Frisvad 1981, 1985) and was one of the few media recommended also by Bridge et al. (1989, 1991). This medium was modified and used as a diagnostic medium for use in *Penicillium* and *Aspergillus* taxonomy avoiding pH adjustments by Frisvad (1993). In the present work this medium proved to be useful in distinguishing species within sections *Discolor* and *Liseola*.

Acetylmethylcarbinol production (Voges-Proskauer test)

Only a limited number of isolates (22 out of 166) were able to produce acetylmethylcarbinol in Glucose phosphate broth. Acetylmethylcarbinol was produced by all isolates of *F. subglutinans* (8 tested isolates), some isolates of *F. camptoceras* (4 out of 6 tested isolates), *F. trichothecioides* (2 out of 6), *F. nygamai* (2 out of 8), *F. proliferatum* (2 out of 7), *F. verticillioides* (2 out of 9) and *F. chlamydosporum* (2 out of 6) (Tab. 3). On the other hand, all isolates tested of the other 17 species lacked the ability of producing this product. There are no reports available on the production of acetylmethylcarbinol by species of *Fusarium* to compare our data with.

Tab. 3. Mean colony diameter (cm) on Dichloran chloramphenicol peptone agar (DCPA), growth on Tannin-sucrose agar (TAN), acid on Creatine-sucrose agar (AC), and acetylmethylcarbinol production (AMC) and enzyme production by different *Fusarium* species.

Species (number of isolates)	**Mean colony diam. ± SD** (Minimum – Maximum) **on DCPA**	**TAN**	**AC**	**AMC**	**Urease index Mean ± SD** (Minimum – Maximum)	**Peroxi-dase**	**Phos-phatase**	**Pyro-catechol Oxidase**
F. oxysporum (9)	5.2 ± 0.4 (4.7–5.8)	+	–	–	2.7 ± 0.2 (2.4–2.9)	+	+	– (7) + (2)
F. solani (8)	6.2 ± 0.5 (5.6–7.0)	+	–	–	0	+	+	+
F. anthophilum (4)	4.2 ± 0.3 (4.0–4.6)	–	–	–	3.1 ± 0.2 (2.9–3.3)	–	+	–
F. nygamai (8)	5.1 ± 0.3 (4.5–5.5)	+	+	– (6) + (2)	0	+ (6) – (2)	+	– (5) + (3)
F. proliferatum (7)	5.2 ± 0.4 (4.6–5.7)	+	– (5) + (2)	– (5) + (2)	3.6 ± 0.8 (2.9–5.2)	+	+	–
F. subglutinans (8)	5.1 ± 0.4 (4.6–5.9)	+	–	+	2.6 ± 0.4 (2.1–3.3)	– (5) + (3)	+	+ (5) – (3)
F. verticillioides (9)	5.4 ± 0.3 (5.0–5.8)	+	+	– (7) + (2)	2.1 ± 0.2 (1.8–2.3)	+ (5) – (4)	+	+ (7) – (2)
F. chlamydosporum (6)	5.9 ± 0.3 (5.6–6.4)	+	–	– (4) + (2)	0	+	+	– (4) + (2)
F. fusarioides (6)	5.7 ± 0.3 (5.3–6.3)	+	–	–	2.8 ± 0.2 (2.5–3.1)	+	+	+
F. poae (8)	5.0 ± 0.3 (4.5–5.8)	–	–	–	2.1 ± 0.2 (1.9–2.3)	+	+	– (5) + (3)
F. sporotrichioides (8)	5.2 ± 0.3 (4.9–5.9)	+	–	–	2.5 ± 0.3 (2.2–3.1)	+	+	+
F. tricinctum (5)	5.6 ± 0.4 (5.0–5.9)	+	–	–	0	+	+	– (3) + (2)
F. avenaceum (6)	5.5 ± 0.3 (5.1–5.9)	+	–	–	0	+	+	–
F. camptoceras (6)	5.8 ± 0.3 (5.4–6.0)	+	–	+ (4) – (2)	2.8 ± 0.5 (2.3–3.5)	+	–	–
F. semitectum (8)	5.0 ± 0.3 (4.7–5.6)	+	–	–	1.8 ± 0.2 (1.5–2.2)	+	+	+
F. culmorum (7)	5.9 ± 0.3 (5.5–6.5)	–	–	–	0	+	+	–
F. graminearum (6)	5.6 ± 0.2 (5.4–5.9)	–	–	–	0	+	+	+ (3) – (3)
F. sambucinum (8)	5.4 ± 0.3 (4.9–5.8)	–	–	–	1.7 ± 0.2 (1.4–2.0)	+	+	–

Species (number of isolates)	**Mean colony diam. ± SD** (Minimum – Maximum) **on DCPA**	**TAN**	**AC**	**AMC**	**Urease index Mean ± SD** (Minimum – Maximum)	**Peroxidase**	**Phosphatase**	**Pyrocatechol Oxidase**
F. trichothecioides (6)	4.7 ± 0.4 (4.1–4.9)	–	+	– (4) + (2)	0	+	+	+ (3) – (3)
F. concolor (6)	5.3 ± 0.3 (5.1–5.8)	+	–	–	0	+	+	–
F. equiseti (6)	5.1 ± 0.3 (4.6–5.3)	+	–	–	2.1 ± 0.4 (1.7–2.7)	– (4) + (2)	+	+
F. lateritium (8)	4.3 ± 0.3 (4.1–4.7)	+	–	–	2.6 ± 0.4 (2.2–3.4)	+	+	+
F. udum (5)	4.3 ± 0.1 (4.1–4.4)	+	–	–	0	+ (3) – (2)	+	–
F. xylarioides (8)	4.1 ± 0.3 (3.8–4.6)	+	–	–	2.2 ± 0.2 (2.0–2.5)	+	+	–

Acetylmethylcarbinol (acetoin) is a natural end product produced by some microorganisms in glucose fermentation. Glucose is metabolised to pyruvic acid, which is a key intermediate in glucolysis**.** The production of acetoin is one pathway for glucose degradation occurring in bacteria (Abd-El-Malek & Gibson 1948). In the present work we made use of this test in *Fusarium* species differentiation for the first time.

Urease activity

Ten out of the 24 tested species lacked the ability of producing the urease enzyme. Out of the three species in section *Arthrosporiella* only *F. avenaceum* did not produce urease, while in section *Discolor* only *F. sambucinum* was capable of producing urease. Also, *F. oxysporum* and *F. solani* could be distinguished from each other by this enzyme, since *F. oxysporum* produced it, whereas *F. solani* did not. Also *F. concolor* could be separated from *F. equiseti* (both section *Gibbosum*) by its ability to produce urease. One out of three species in section *Lateritium* and two out of five species in section *Sporotrichiella* and one out of five in section *Liseola* lacked the ability to produce this enzyme (Tab. 3). Urease production seems to be a promising diagnostic tool in species differentiation in the genus *Fusarium*.

Švábová et al. (1980) did not detect urease activity in all tested species, including *F. solani*, *F. poae*, *F. tricinctum*, *F. avenaceum*, *F. semitectum*, *F. sporotrichioides*, *F. moniliforme*, *F. oxysporum*, *F. lateritium*, *F. acuminatum*, *F. culmorum* and *F. sambucinum*. In accordance with our results, *F. oxysporum*

and *F. verticillioides* originating from human keratitis produced the urease enzyme (Gharamah 2006), but *F. solani* was positive in his work. Biochemical and physiological techniques may be useful for the characterisation of *Fusarium* isolates (Wasfy et al. 1987). Urease production is also a useful feature and diagnostic tool in the chemotaxonomy of the genus *Beauveria* (Mugnai et al. 1989) and *Penicillium* (Bridge et al. 1989, Hussein 1997).

Peroxidase activity

All isolates of 18 out of the 24 species proved positive for peroxidase production. These isolates included all species of sections *Arthrosporiella* (3 species), *Martiella* (1), *Sporotrichiella* (5), *Discolor* (4) and *Gibbosum* (2), while only two species of *Lateritium* and one of *Liseola* reacted positively. On the other hand, all isolates of *F. anthophilum* (4) failed to produce peroxidase. The isolates of the remaining five species showed variable responses towards peroxidase production (Tab. 3).

Zare-Maivan & Shearer (1988) found that all freshwater lignicolous fungi tested (10 ascomycetes, 7 deuteromycetes and 1 oomycete) were positive for peroxidase production. Peroxidase production seems to be common to a wide variety of fungi and thus has little value in identifying lignin-degrading isolates (Egger 1986).

Phosphatase activity

It seems that phosphatase production is common in species of *Fusarium*, since all species tested were able to produce phosphatase (Tab. 3). Gharamah (2006) found that 30 isolates of 16 fungal species tested were phosphatase producers, however at different rates.

Pyrocatechol oxidase activity

All isolates of six species out of the 24 species tested proved positive for pyrocatechol oxidase production, 9 species proved negative, while the isolates of 9 species were variable. The positive species are *F. semitectum* (section *Arthrosporiella*), *F. equiseti* (section *Gibbosum*), *F. lateritium* (section *Lateritium*), *F. solani* (section *Martiella*), *F. fusarioides* and *F. sporotrichioides* (both section *Sporotrichiella*), which could be distinguished from the other negative species in the same sections (Tab. 3).

In a study of the production of pyrocatechol oxidase by 8 aquatic hyphomycetes, only two species were capable of producing this enzyme (Chandrashekar & Kaveriappa 1988). However, there is no literature available on the production of pyrocatechol oxidase by *Fusarium*.

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