Survey of *Fusarium* species in an arid environment of Bahrain II. Spectrum of species on five isolation media

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Five media, acidified potato dextrose agar (APDA), Nash-Snyder medium (NSM), Komada selective media (KSM), selective *Fusarium* agar (SFA) and *Fusarium* minimal medium (FMM) were employed with the soil dilution plate technique for the isolation of *Fusarium* species from soils of six noncultivated habitats and one cultivated soil of Bahrain Island. A total of 852 isolates were recovered on the above five media representing a total of 12 *Fusarium* species. NSM and SFA allowed to recover the 12 species from all the habitats and throughout the seasons. Significant interaction occurred between species recovered and media used for the various habitats. Qualitative and quantitative comparisons amongst media, based on recovery rate, microbial contaminants, colony appearance, sporulation, degeneration time, growth rate and initial identification indicated that NSM, SFA and KSM media were superior to FMM and APDA. Likewise, KSM, NSM and SFA gave the most similar results for all habitats, as measured by polar ordination. To enhance estimates of *Fusarium* abundance and distribution in soils, more than one medium should be used.

Keywords: *Fusarium*, arid habitat, media, recovery, soil.

Fusarium species are commonly associated with soil particles, organic matter and plant debris throughout the world (Lim & Varghese, 1977; Burgess, 1981). Most of the literature on *Fusarium* species, however, relates to cultivated soils. Collectively, species of *Fusarium* are amongst the most active and diverse members of soil-biotic microflora (Burgess & Summerell, 1992). They are not only parasitic and pathogenic (Cook & Bruehil, 1968) but they can be also involved in the various ecological functions relating to nutrient cycling and plant-soil-microbe interrelationships (Kreutzer, 1972; Kommedahl & al., 1987). In hot desert systems, these fungi colonise wide and complex ecological niches, influencing primarily the function, form and structure of the ecosystem (Stoner, 1981). The mechanisms of survival, where harsh climatic conditions prevail, are mainly governed by two biologically limiting factors, the capacity to decompose and utilize a wide spectrum of organic substrates (Abdel-

Hafez & al., 1990), and the ability to survive in the absence of suitable substrates (Smith & Snyder, 1975). *Fusarium* species may persist in soil as dormant chlamydospores (Nash & al., 1961; Nyvall, 1970), hyphae (Nyvall & Kommedahl, 1968), or conidia in plant residues (Kreutzer, 1972).

Tolerance of stressful environmental conditions (Khodair & al., 1991) and saprobic ability of *Fusarium* species (Warcup, 1960) are important attributes in determining their successful distribution and colonization of soil niches (Stoner, 1981). Fast-growing fungi and/or often antagonistic bacteria and actinomycetes with high colonization potential (growth rate) may, however, rapidly displace *Fusarium* species in the soil (Tsao, 1970).

To facilitate the recovery of a wide range of *Fusarium* species, it is often necessary to use different selective media (McMullen & Stack, 1983). The choice of a particular medium for isolation may influence the number of species recovered (Papavizas, 1967). Several selective media have been developed for isolation of one or more *Fusarium* species from the soil (Parmeter & Hood, 1961; Bouhot & Billotte, 1964; Papavizas, 1967; Denis & al., 1966; Komada, 1975; McMullen & Stack, 1983; Klotz & al., 1988). In addition, the effect of media on the recovery of *Fusarium* species from cultivated soils is well documented (Nash & Snyder, 1965). Such comparative approaches are not only important to evaluate species diversity within the genus *Fusarium*, but may improve understanding of soil-borne fungi, especially with respect to their saprobic behavior, population dynamics and mechanisms of survival under extreme environmental conditions (Griffin, 1972).

The objectives of this investigation were to survey the occurrence and distribution of *Fusarium* species in soils from various non-cultivated and cultivated habitats from the arid environment of Bahrain, and to compare the effect of five media on the recovery of *Fusarium* species from soil.

Materials and methods

Soil sampling locations and procedures

The climate of the main island of Bahrain is a typical Saharo-Arabian climate, characterized by hot, humid summers and mild winters with a low annual rainfall. Soil samples were collected from six locations representing seven habitats, including an agricultural site, all within the central and northern part of the main island (Fig. 1). The sampling locations and habitats, soil type, dominant plant community and corresponding soil chemical analysis information are

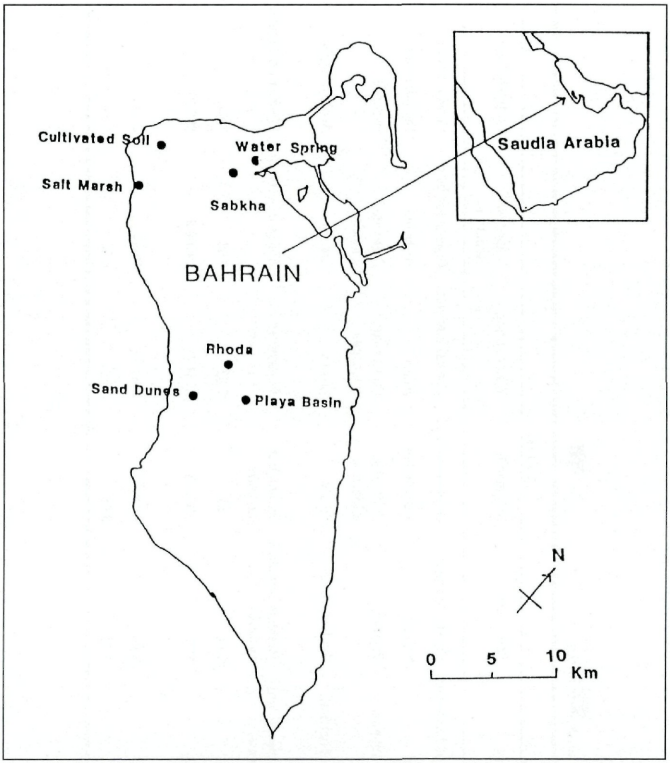

Fig. 1. – Location map of Bahrain representing the habitats where soil collections were made.

listed in Tab. 1. Each habitat was sampled once during the summer, in Sept. 1992, and once during the winter, in Feb. 1993.

At each habitat, three composite soil samples, each of 2 kg, were collected, approximately 20–30 m apart. Each sample consisted of about 10 subsamples (200 g), collected with a clean hand-trowel, approximately 4 m apart, and taken from the upper 15 cm of the soil profile. The subsamples were combined, placed in paper bags, labeled, transported to the lab, air-dried and stored at 5 C until processed, which occurred within one week (Burgess & Summerell, 1992).

Tab. 1. – Sampling sites with data on soil characteristics.

	Habitat						
	Playa Basin	Rhodat	Sand dune	Salt marsh	Water spring	Neglected plantation	Cultivated soil
Physiographic zone	Central depression	Central depression	Coastal lowland	Coastal lowland	Coastal lowland	Coastal lowland	Coastal lowland
Location	Um Jeder	Sakhir	Sakhir	Jannabiyah	Adari	As-Sehla	Bani-Jamrah
Soil Group	Raw minerals	Raw minerals	Regosols	Cultivated solonchak	Cultivated solonchak	Cultivated solonchak	Regosols
Soil Subgroup	Soils of interior basin	Soils of detrital fans	Aeolian sands	Sandy	Loamy	Loamy	Aeolian sands
Plant Community	*Zygophyllum*	*Prosopis juliflora qatarense*	*Panicum turgidum australis*	*Phragmites australis*	*Phragmites*	*Alhagi graecorum*	*Phoenix dactylifera*
Soil pH	7.89	7.86	7.66	7.64	7.62	7.36	7.65
Conductivity (μScm^{-1})	140.05	468.75	2963	5163.05	2754.7	19068.8	697.15
TSS	1.24	2.30	2.66	4.48	2.45	16.02	2.29
Organic Matter (%)	0.43	1.98	1.11	8.68	7.38	6.53	1.57

Although the soil samples were all taken at locations dominated by natural vegetation, samples were collected away from plant canopies or roots to avoid the rhizosphere effect.

For the assays, the composite soil samples were thoroughly hand-mixed in plastic bags under sterile conditions and divided in two parts. One part was stored at 5 C, the other was used for chemical analyses. Soil pH and electrical conductivity (μScm^{-1}) were determined in a 1:5 soil: water extract using a JENWAY Water Analyser (Model PW1). Organic matter content (%) was determined by ashing 100 g of air-dried soil in a furnace at 600 C for 1 hr, and calculating the difference in weight. Total soluble salts (TSS) were measured by mixing 20 g soil with 100 ml distilled water, filtering and evaporating the filtrate in an oven at 105 C. The dry residue was then weighed and the TSS calculated (Jackson, 1958). Tab. 1 present the average values of three soil replicates.

Isolation

The second part of the sample was further air-dried, homogenized and crushed when necessary using a mortar and pestle to a fine powder, and passed through a 0.5 mm soil screen to remove root fragments and other debris (Kreutzer, 1972).

The soil dilution plate technique (Johnson & al., 1960) was used to recover *Fusarium* species from the soil. Ten grams of each composite soil sample were suspended in 90 ml sterile distilled water and thoroughly mixed for 5 min. The soil suspension was allowed to settle for 30 sec. and a 1 ml aliquot of the 1:10 dilution was dispensed uniformly over the surface of three replicate plates per medium. Preliminary studies determined that this dilution ratio was the most suitable for soil from these habitats (15–20 *Fusarium* colonies/plate).

Isolation plates were incubated at 22 ± 2 C, with a 12 hr photoperiod, under cool white fluorescent lights for 10 days (Burgess & Summerell, 1992). The resultant colonies on each medium that were putative *Fusarium* species were transferred individually to standard 2% acidified potato dextrose agar medium (APDA) and incubated, as described.

Media used for isolation

Commercial acidified potato dextrose agar (Oxoid Ltd., Basingstoke, Hants, England) (APDA) for a nutrient rich medium; Komada selective medium (Komada, 1975; KSM); Nash-Snyder medium or peptone PCNB agar (Nash & Snyder, 1962; NSM); selective *Fusarium* agar (Tio & al., 1977; SFA); and *Fusarium* minimal medium (Papavizas, 1967; FMM) were used. The characteristics of each medium are summarized in Tab. 2. All media were autoclaved for

Tab. 2.– Characteristics of the isolation media used. APDA = acidified potato dextrose agar; KSM = Komada selective medium; NSM = Nash-Snyder medium; SFA = selective *Fusarium* agar; FMM = Fusarium minimal medium.

Medium	Chemical Status	Selectivity	Species spectrum	Selective inhibitory ingredients	Culture variability	Production of Pigmentation	Colony appearance	Total recovery	Levels of contamination
APDA	Natural	Nutrient rich	Narrow	Lactic acid	High	+	Distinct	High	High
KSM	Synthetic	Semi-selective	Broad	Oxgall, PCNB, Streptomycin sulphate	Low	+	Distinct	Medium	Medium
NSM	Synthetic	Selective	Broad	PCNB, Neomycin and Streptomycin sulphate	Low	–	Semi-distinct	High	Low
SFA	Synthetic	Selective	Broad	Streptomycin sulphate, Aureomycin sulphate, „Allisan" suspension	Low	+	Distinct	High	Low
FMM	Synthetic	Semi-selective	Narrow	Trace elements	Low	–	Indistinct	Low	Low

15 min at 120 C and allowed to cool to 45 C before the addition of antibiotics, adjustment of pH, or the addition of heat-labile ingredients. Fifteen ml of each medium were added to Petri dishes. Media were stored in the refrigerator for at least five days before use.

For identification purposes a natural potato dextrose agar (PDA) was employed.

Identification

Single spore or hyphal tip cultures for representative *Fusarium* species were prepared and maintained on PDA and further incubated under the conditions described above for seven days. Identifications were made according to Nelson & al. (1983). Representative sporulating structures of each species were mounted permanently with Amann's preservative on slides (Riddell, 1950) and were deposited in the herbarium of the Department of Biology, University of Bahrain.

Data analysis

To quantify fungal abundance and distribution at all habitats, media and seasons, relative abundance were determined. Relative abundance (%) of a species was defined as the number of isolates of a given species divided by the total number of the *Fusarium* isolates (x 100).

To measure the recovery of species by an isolation medium, percentage recovery rates were determined. Percentage recovery was defined as the number of species recovered using each medium, divided by the total *Fusarium* species (x 100).

One-way analysis of variance with an unequal number of replicates (Steel & Torrie, 1980) was employed to evaluate the most suitable and effective medium to recover *Fusarium* species from each habitat. As variations among habitats were insignificant, observations over all habitats were combined and comparisons (ANOVA) were made among media. When statistical significance among media was reached, means were separated by using Duncan's multiple range test at $P = 0.05$ significance level.

Chi-square tests were used to test homogeneity in contingency tables prepared using frequencies of occurrence (Steel & Torrie, 1980), to measure the independence of recovery of species by each medium, for all habitats and seasons.

To measure the dissimilarity among the isolation media (based on the number of species recovered by each media) at each habitat, a simple polar ordination method was used (Ludwig & Reynolds, 1988). Polar ordination is also expressed as an index of difference (ID), which was calculated as follows:

ID = 100 – Coefficient of similarity

Coefficient of similarity = 100 C / (A + B – C)

where A = total number species in medium A, B = total number of species in medium B, and C = number of similar species between the two media to be compared. Coefficient of similarity matrices were calculated from presence/absemce data. The position of each medium on the one-dimensional x axis (habitat) was calculated as follows:

$$x = L^2 + dAC^2 - dBC^2 / 2L$$

where x is the distance along the axis from the left end, L is the ID of the highest endpoint (e. g. media A and B), dAC is the ID between media A and C, and dBC is the ID between media B and C. An ID of 0 represents identity among media, while an ID of 100 represents a complete difference. Values of ID less than 50 were considered as close (approximate) associations.

Results

Fusarium species recovery and distribution

Distribution of *Fusarium* species among the various habitats using different media, during the two sampling periods, is shown in Tab. 3. The recovery of *Fusarium* species and isolates were higher in winter when compared to summer sampling. A total of 12 species were recovered from soil samples, of which the highest were from Rhodat (11 species) and the lowest from the neglected plantation (3 species). Rhodat, cultivated soil, and playa basin yielded more isolates (average 251) as compared to all other habitats.

The relative abundance of *Fusarium* species ranged from 50.7 to 0.46 % for *F. solani* and *F. reticulatum*, respectively (Tab. 3).

Isolation media

The percentage recovery of species on each medium was 83.4% for APDA, 91.7% for KSM, 83.4% for NSM, 66.7% for SFA, and 41.7% for FMM. The total recovery of *Fusarium* isolates for all the species, habitats and seasons was highly significant on SFA, followed by NSM, KSM and APDA and nonsignificant on FMM, as determined by Duncan's multiple range test at $P = 0.05$ (Tab. 4). No significant statistical variations, however, were observed among habitats for the recovery of *Fusarium* species by each medium.

Tab. 3. – Recovery of *Fusarium* spp. at the different habitats investigated on Bahrein island. P: Acidified potato dextrose agar); K: Komada selective medium); N: Nash & Snyder medium); S: Selective *Fusarium* agar; F: *Fusarium* minimal medium.

	Playa basin					Rhodat					Sand dune					Salt marsh					Water spring					Neglected plantation					Cultivated soil					Relative abundance (%)
	P	K	N	S	F	P	K	N	S	F	P	K	N	S	F	P	K	N	S	F	P	K	N	S	F	P	K	N	S	F	P	K	N	S	F	
F. oxysporum Schlecht emend. Snyd. & Hansen		+	+	+			+		+		+	+			+	+						+				+						+	+	+	+	16.31
F. solani (Mart.) Appel & Wollenweber emend. Snyd. & Hansen	+	+	+	+		+	+	+	+							+	+	+	+		+	+	+	+		+	+	+	+	+	+	+	+		50.7
F. tricinctum Corda	+	+	+	+		+				+		+														+					+	+	+	+		7.74
F. equiseti (Corda) Sacc. *sensu* Gordon						+																									+					0.7
F. sambucinum Fuckel				+		+	+	+	+			+		+			+				+											+	+		7.63
F. chlamydosporum Wollenw. & Reinking		+	+	+																																1.64
F. moniliforme Sheldon	+		+				+					+						+					+												1.87
F. compactum (Wollenw.) Gordon	+	+	+	+						+												+	+	+												9.62
F. reticulatum Mont.						+		+																							+					0.46
F. acuminatum Ellis & Ev. *sensu* Gordon						+																														0.47
F. pallidoroseum (Cooke) Sacc.						+																									+	+	+	+		1.05
F. lateritium Nees emend. Snyd. & Hansen			+				+																								+	+				0.8
Total species	9					11					4					4					5					3					8					
Total isolates	187					289					14					22					51					13					276					

Tab. 4. – Mean ± S. D. recovery or *Fusarium* isolates on five isolation media at various habitats on Bahrain island. A total of 105 plates were examined, representing 852 *Fusarium* isolates.

	Isolation media *				
Habitat	APDA	KSM	NSM	SFA	FMM
Playa Basin	13.2 ± 2.4	8.8±2.1	5.12 ± 1.91	8.16 ± 2.11	0.0 ± 0.0
Rhodat	4.4 ± 0.6	19.0 ± 4.2	9.0 ± 3.76	27.0 ± 4.38	1.0 ± 0.0
Sand Dune	3.0 ± 0.0	1.0 ± 2.8	0.0 ± 0.0	3.5 ± 0.54	0.0 ± 0.0
Salt Marsh	2.0 ± 1.2	4.0 ± 2.8	1.75 ± 0.21	3.0 ± 0.0	0.0 ± 0.0
Water Spring	3.5 ± 0.5	3.5 ± 0.5	5.5 ± 1.46	7.0 ± 3.73	1.0 ± 0.0
Neglecled plantation	3.0 ± 1.4	1.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
Cultivated Soil	1.0 ± 0.0	6.71 ± 3.1	22.0 ± 6.95	16.8 ± 2.68	3.0 ± 0.8
F–ratio**	1 04^{NS}	0.90^{NS}	0.34^{NS}	0.70^{NS}	1.16^{NS}
degrees of freedom (r_1, r_2)	(6,12)	(6,18)	(6,19)	(6,15)	(6,3)
Total *Fusarium* isolates***	106	206	231	294	15

* Media used were APDA (Acidified potato dextrose agar); KSM (Komada selective medium); NSM (Nash & Snyder medium); SFA (Selective *Fusarium* agar); FMM (*Fusarium* minimal medium).

**Numbers in each column are not significantly different (NS), according to Duncan's multiple range test (P=0.05)

*** A total of 105 plates were examined representing a total of 852 *Fusarium* isolates. Data represent ANOVA analysis at F-ratio 26.37** *with* 4, 37d.f. Numbers in the row followed by the same letters are not significantly different, according to Duncan's multiple rang test (*P*=0,05).

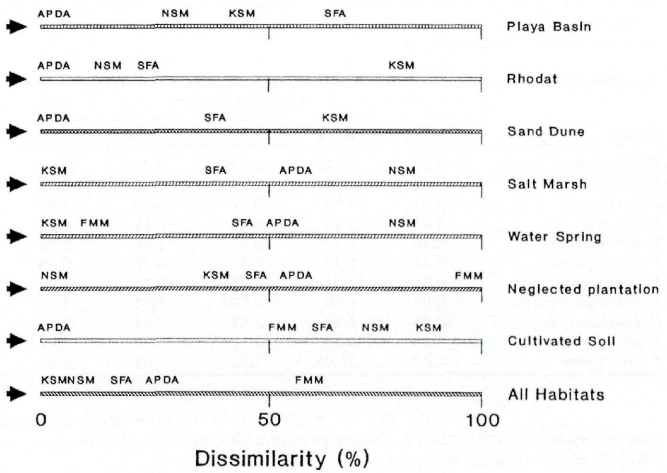

Fig. 2. – Single axis (x) polar ordination of five media at seven habitats, based on percentage dissimilarity.

Analysis of interaction between the recovery of species by each medium, over all habitats and seasons, was determined using the chi-square test (Tab. 5). A greater than expected recovery of *F. tricinctum*, *F. acuminatum*, *F. sambucinum* and *F. solani* occurred with APDA. The recovery of *F. oxysporum*, *F. sambucinum* and *F. lateritium* on KSM was greater than expected. More *F. solani* and *F. oxysporum*, *F. chlamydosporum* and *F. tricinctum* were isolated than expected on NSM. For FMM recovery of *F. pallidoroseum* was greater than expected. Similar significant chi-square values were obtained in the contingency table for species by media interaction, based on density values.

Although no medium tested was found to inhibit or favor any given species or isolates, *Fusarium* species sampled from the various habitats were not recovered independently of the isolation medium employed. As such, there was, primarily, a greater than expected recovery (significant interaction) of *F. oxysporum* and *F. lateritium* with KSM, *F. solani*, *F.oxysporum* and *F. chlamydosporum* with NSM, *F. tricinctum*, *F. solani*, and *F. acuminatum* with APDA, and *F. pallidoroseum* with FMM.

Distribution of isolation media (based on the recovery of *Fusarium* species) at each habitat, as determined by ID values, are presented in Fig. 2. The highest dissimilarity (%) among media

Tab. 5.– Contingency table for *Fusarium* species by media interactions. Data represent Chi-square statistic at *416.42* (40 d.f.). Species with less than 5 isolates were not included in the analysis.

	Isolation Media*				
Taxon	APDA	KSM	NSM	SFA	FMM
F. oxysporum	3.26	*26.28*	*27.10*	3.21	0.98
F. solani	*25.47*	0.36	*20.66*	0.48	0.45
F. tricinctum	*62.23*	4.38	*9.61*	0.07	4.90
F. equiseti	6.90	0.28	2.07	0.18	0.14
F. sambucinum	*8.36*	*8.10*	0.09	1.86	1.25
F. moniliforme	7.31	0.73	2.87	5.02	0.30
F. compactum	6.64	2.84	7.88	5.55	1.20
F. chlamydosporum	3.07	1.30	*27.78*	7.81	0.58
F. acuminatum	*52.95*	1.69	2.07	2.34	0.05
F. pallidoroseum	0.15	0.76	1.10	0.60	*26.42*
F. lateritium	1.27	*22.49*	1.82	4.02	0.24

* Media used were APDA (Acidified potato dextrose agar); KSM (Komada selective medium); NSM (Nash & Snyder medium); SFA (Selective *Fusarium* agar); FMM (*Fusarium* minimal medium).

occurred with the cultivated soil and the lowest from Rhodat. In all habitats, the lowest dissimilarities (%) were noticed with KSM, NSM, SFA, and APDA, respectively.

Discussion

The findings of the present study indicate the value of using different selective media for quantitative and qualitative recovery of *Fusarium* species from noncultivated soils. The estimation of abundance of particular species (especially a slow growing or rare species) (Wensley & McKeen, 1962), geographical distribution (Tab. 3) (Burgess, 1981), and the relative abundance of each species may be influenced by the media employed. Fungal species exhibit broad differential responses to nutrients and toxic selective agents, and have the ability to adapt to an ecologically wide selection of habitats (Kreutzer, 1972; Smith & Snyder, 1975; Abdel-Hafez & al., 1990). Therefore, in soil surveys of soil-borne *Fusarium* species (Kreutzer, 1972; Lim & Varghese, 1977; Burgess, 1981; Stoner, 1981; Kommedahl & al., 1987) or mycogeographical comparisons (Nash & Snyder, 1965; Burgess & Summerell, 1992), use of a single medium for recovery from intact soil particles may not necessarily reflect the natural occurrence

of individual *Fusarium* species (Papavizas, 1967; Tsao, 1970; Stoner, 1981; McMullen & Stack, 1983).

Comparative analyses to select the best media for the recovery of *Fusarium* species from natural soil revealed that a combination of NSM, SFA and KSM would recover the best species spectrum of *Fusarium* from soils of the desert environment of Bahrain.

The NSM medium was originally developed and used by Nash and Snyder (1962) to estimate, quantitatively, the inoculum density of *F. solani* f. sp. *phaseoli* in a field soil planted with bean. Selective inhibitory ingredients were the common soil fungicide pentachloronitrobenzene (PCNB) (750 ppm Terraclor; wettable powder, 75% a. i.) which inhibits the growth of undesirable fungi, and streptomycin (300 ppm) which inhibits bacterial contaminants. Since then, several modifications of the medium have appeared (Papavizas, 1967; Tsao, 1970; Kreutzer, 1972; McMullem & Stack, 1983; Burgess & Summerell, 1992). The medium has enabled researchers to isolate and quantify *Fusarium* species, especially the economically important species both from plants and soil (Nash & Snyder, 1965). In our study, several common soil inhabiting fungi, such as *Aspergillus*, *Penicillium* and *Trichoderma*, were frequently encountered on NSM, but their colonies were small and did not interfere with the recovery of colonies of *Fusarium* species. Sporulation was usually high, with dense clusters of macroconidia near the centre of colony. The growth of most species, however, was rather slow and colony development was somewhat irregular.

SFA is a modified Czapek-Dox medium containing the fungicide „Allisan" suspension (500 ppm, 50% w/w dichloran: 2,6-dichloro-4-nitroaniline) to inhibit growth of other fungi, especially zygomycetes. Burgess & al. (1988) have used the medium to recover *Fusarium* species from plant roots and soil debris in Australia. Colonies of most *Fusarium* species developing on SFA are distinctive and produce more pigmentation than on NSM medium. The total recovery and sporulation of the most common *Fusarium* species, i. e. *F. solani* and *F. oxysporum*, were usually high, but the medium was relatively less inhibitory to fungal and bacterial colonies than NSM.

KSM incorporates growth inhibitors and also chemicals that act as color indicators, when dissolved at a specific pH, to achieve a selective differentiation by pigmentation of *Fusarium* species among a variety of other fungi (Tsao, 1970). It is based on the fact that Fusaria can absorb, selectively, the substances from the medium (L-Asparagine and Fe-Na-EDTA at pH 3.8–4.0), to reduce specific secondary pigments. The medium is widely used to recover *Fusarium* species from soil on a large-scale basis, because isolates of the same species are easily grouped and identified on a color basis. Pigmentation produced on KSM, however, is merely an indication of

colonies belonging to the genus *Fusarium* and not typical of colors used as criteria for identification purposes. Colonies of *F. oxysporum* were distinct, well-delineated and sporulated heavily. The growth of some other *Fusarium* species was somewhat suppressed.

Of the five media used, FMM and APDA are not recommended because of several undesirable features (Papavizas, 1967; McMullen & Stack, 1983). FMM yields a low number of *Fusarium* species and isolates. Growth and sporulation of most *Fusarium* species is poor and colony appearance is indistinct, thus it appears to be toxic to some species. As expected, APDA favours rapid growth and often coalescing colonies of unwanted fungi, such as *Mucor*, *Rhizopus*, *Penicillium* and expanding colonies of yeasts that cover the small and slow-growing colonies of *Fusarium* species. The morphological variability among isolates is usually high and they tend to quickly degenerate.

The percentage recovery of *Fusarium* species was higher on NSM (83.33%) and KSM (91.66%), compared to SFA (66.66%), whereas the total recovery of *Fusarium* isolates was superior on SFA (Tab. 4). This suggests that the greater recovery of some *Fusarium* species from some soils could partly be due to the choice of media. No differences were reported when either general or selective media were used for recovery of *Fusarium* species from plant roots (Francis & Burgess, 1975). McMullen and Stack (1983) compared the differential isolation of *Fusarium* species from soil on KSM, NSM and Martin's rose bengal medium and concluded that the latter yielded maximum species recovery and was less inhibitory than the others.

Although it is difficult to assess the relative toxicity or stimulatory effect of a particular medium on the isolation of specific species of *Fusarium* from their natural habitat, the significant interaction between media and species recovered indicates that some media allow greater recovery of *Fusarium* species from soil (Papavizas, 1967; Tsao, 1970; Kreutzer, 1972). For instance, greater than expected recovery was observed on APDA (three species), KSM (two species), NSM (three species), and FMM (one species) (Tab. 5). The spectrum of species recovered on NSM, SFA and KSM was similar (Tab. 3). Polar ordination analysis, used to assess similarity among the media used for the recovery of *Fusarium* species from the various habitats (Fig. 2), indicated that species are more frequently isolated on NSM, SFA and KSM, followed by APDA. Papavizas (1967) compared several media and antimicrobial agents used in the recovery of nine *Fusarium* species from soil and indicated that NSM and Papavizas medium are both effective, and better than other tested media, for isolation and quantification of *Fusarium* colonies.

The findings that the use of only one medium may provide inadequate representation of the distribution and population of

Fusarium species in soils agree with those of McMullen & Stack (1983), and Papavizas (1967). Also, standardization of isolation methods, media preparation and incubation condition greatly minimizes variation among isolates of the same species and allows easy and rapid identification.

Although many isolation techniques are available to quantify fungi in soil, all have some limitations, especially the dilution plate method. The technique, however, reflects general distribution trends which might be associated with microbial activity and nutrient cycling processes, especially in stressful environments such as deserts (Kinsbursky & al., 1990). Ideally, more than one technique, each based on different principles, should be used for the isolation of *Fusarium* species from soil.

In the *Fusarium* soil-recovery system, significant interactions can be observed among the isolation technique, media, soil type, species, microbial population, and soil environmental factors (Papavizas, 1967; Stoner, 1981; Burgess & Summerell, 1992). Thus, one medium may not be suitable for all soils, or for the quantitative and qualitative recovery of all *Fusarium* species. Therefore, in surveys for soil *Fusarium* species, a multi-media approach for an effective recovery is recommended.

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References

Abdel-Hafez, A. I., M. B. Mazen & A. A. Galal (1990). Glycophilic and cellulose-decomposing fungi from soils of Sinai Peninsula, Egypt. – Arab. Gulf. J. Scient. Res. 8: 153–168.

Bouhot, D. & J. M. Billotte (1964). Recherches sur l'écologie des champignons parasites dans le sol. II. Choix d'un milieu nutritif pour l'isolement sélectif de *Fusarium oxysporum* et *Fusarium solani* du sol. – Ann. Epiphyt. 15: 45–56.

Burgess, L. W. (1981). General ecology of Fusaria. In: Nelson, P. E., T. A. Toussoun & R. J. Cook (eds.). *Fusarium*, Diseases, Biology, and Taxonomy. – Pennsylvania State Univ. Press, Univ. Park, Pennsylvania: 225–235.

——, C. M. Liddell & B. A. Summerell (1988). Laboratory manual for *Fusarium* research (2nd edition). – The Univ. of Sydney, Sydney, Australia, 156 pp.

——, & B. A. Summerell (1992). Mycogeography of *Fusarium*: Survey of *Fusarium* species in subtropical and semi-arid grassland soils from Queensland, Australia. – Mycol. Res. 96: 780–784.

Cook, R. J. & G. W. Brueil (1968). Relative significance of parasitism versus saprophytism in colonization of wheat straw by *Fusarium roseum 'Culmorum'* in the field. – Phytopathology 58: 306–308.

Denis, S., J. Snodgross, J. Charlotte, J. Koeburger & E. Elliott (1966). An azide-rose bengal medium for the isolation of *Fusarium* species from red clover roots. – Phytopathology 56: 584. (Abstr.)

Francis, R. G. & L. W. Burgess (1975). Surveys of Fusaria and other fungi associated with stalk rot of maize in eastern Australia. – Aust. J. Agric. Res. 26: 801–807.

Griffin, D. M. (1972). Ecology of soil fungi. – Chapman and Hall, London, 193 pp.

Jackson, M. L. (1958). Soil chemical analysis. – Constable and Co., London, 498 pp.

Johnson, L., E. Curl, J. Bond & H. Fribourrg (1959). Methods for studying soil microflora. Plant Disease Relationships. – Burgess Pub. Comp., Minneapolis, 178 pp.

Khodair, A. A., A. S. Ramadani & A. M. Aggab (1991). Occurrence and density of alkalophilic bacteria and fungi in saline soils of Makkah district, Saudia Arabia. – Arab. Gulf. J. Scient. Res. 9: 119–132.

Klotz, L. V., P. E. Nelson & T. A. Toussoun (1988). A medium for enhancement of chlamydospore formation in *Fusarium* species. – Mycologia 80: 108–109.

Kinsbursky, R. S., R. Degani, G. Baranest & Y. Steinberger (1990). Root microbial population dynamics in a soil profile under the canopy of the desert shrub *Zygophllum dumosum*. – J. Arid Envir. 19: 261–267.

Komada, H. (1975). Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. – Rev. Plant. Prot. Res. 8: 114–125.

Kommedahl, T., H. K. Abbas, C. J. Microcha, G. A. Bean, B. B. Jarvis & M. Guo (1987). Toxigenic *Fusarium* species found in roots and rhizospheres of *Baccharis* species from Brazil. – Phytopathology 77: 584–588.

Kreutzer, W. A. (1972). *Fusarium* spp. as colonists and potential pathogens in root zones of grassland plants. – Phytopathology 62: 1066–1070.

Lim, G. & G. Varghese (1977). *Fusarium* species from Malaysian soils. – Malays. Appl. Biol. 6: 45–57.

Ludwig, J. & J. Reynolds (1988). Statistical ecology. – John Wiley & Sons, 337 pp.

McMullen, M. P. & R. W. Stack (1983). Effects of isolation techniques and media on the differential isolation of *Fusarium* species. – Phytopathology 73: 458–462.

Nash, S. M., T. Christou & W. C. Snyder (1961). Existence of *Fusarium solani* f. sp. *phaseoli* as chlamydospores in soil. – Phytopathology 51: 308–312.

—— & W. C. Snyder (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. – Phytopathology 52: 567–572.

—— & —— (1965). Quantitative and qualitative comparisons of *Fusarium* populations in cultivated fields and noncultivated parent soil. – Can. J. Bot. 43: 939–945.

Nelson, P. E., T. A. Toussoun & W. F. O. Marasas (1983). *Fusarium* species – an illustrated manual for identification. – Pennsylvania State Univ. Press, Univ. Park, Pennsylvania, 193 pp.

Nyvall, R. F. (1970). Chlamydospores of *Fusarium roseum 'Graminearum'* as survival structures. – Phytopathology 60: 1175–1177.

—— & T. Kommedahl (1968). Individual thickenened hyphae as survival structures of *Fusarium moniliforme* in corn. – Phytopathology 58: 1704–1707.

Papavizas, G. C. (1967). Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. – Phytopathology 57: 848–852.

Park, D. (1963). The presence of *Fusarium oxysporum* in soils. – Trans. Brit. mycol. Soc. 46: 444–448.

Parmeter, J. R. & J. R. Hood (1961). The use of *Fusarium* culture filtrate media in the isolation of Fusaria from soil. – Phytopathology 51: 164–168.

Riddell, R. W. (1950). Permanent stained mycological preparations obtained by slide culture. – Mycologia 4: 265–270.

Smith, S. N. & W. C. Snyder (1975). Persistence of *Fusarium oxysporum* f. sp. *vasinfectum* in fields in the absence of cotton. – Phytopathology 65: 190–196.

Steel, R. G. & J. H. Torrie (1980). Principles and procedures of statistics. – McGraw-Hill, New York, 632 pp.

Stoner, M. F. (1981). Ecology of *Fusarium* in noncultivated soils. In: Nelson P. E., T. A. Toussoun & R. J. Cook (eds.). *Fusarium*, Diseases, Biology, and Taxonomy. – Pennsylvania State Univ. Press, Univ. Park, Pennsylvania: 276–286.

Tio, M., L. W. Burgess, P. E. Nelson & T. A. Toussoun (1977). Techniques for the isolation, culture and preservation of the Fusaria. – Austr. Plant. Pathol. Soc. Newsl. 6: 11–13.

Tsao, P. H. (1970). Selective media for isolation of pathogenic fungi. – Ann. Rev. Phytopathol. 8: 157–186.

Warcup, J. H. (1960). Methods for isolation and estimate of activity of fungi in soil. In: Parkinson, D. & J. S. Waid (eds.). The Ecology of Fungi. – Liverpool Univ. Press, Liverpool, England: 3–21.

Wensley, R. N. & C. D. McKeen (1962). A soil suspension–plating method of estimating populations of *Fusarium oxysporum* f. *melonis* in muskmelon wilt soils. – Can. J. Microbiol. 8: 57–64.

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