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OSMOPHILIC FILAMENTOUS FUNGI IN WHEAT MEAL

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ABSTRACT

Twelve filamentous osmophilic fungi of local and foreign wheat meal were isolated and identified. Ten of the isolates belong to the genus ***Aspergillus;*** *one to* ***Penicillium*** *and one to* ***Paecillomyces.*** *Most of the isolated fungi could thrive high concentration of sucrose more that 60 % Only* ***Aspergillus candidus*** *and* ***Aspergillus ornatus*** *tolerate 80 % sucrose concentration. All the isolates exepet* ***A. quadricinctus*** *contain toxic materials in their mycelia and produce them externally. The action of untritional requirements, physical and chemical environments on growth were studied.*

INTRODUCTION

The term osmophily was proposed in (1912) by Richter. It was used to describe micro-organisms which grow and multiply in a solution of high osmotic pressure. Such organisms were separated into two categories, those which require solutions of high osmotic pressure for growth and those which grow over a wide range of concentrations (Scarr, 1951).

The ability of an organism to thrive in relatively dry environment is potentially determined by its response to the activity of the solute whether high or low and less obviously to the possible effects of osmotic pressure.
There are goups of micro-organisms which can thrive in dry environments which need not necessarily have a high ionic strength; examples of those organisms are the socalled "osmophilic" yeasts and "Xerophilic" moulds (Scott, 1956) ; Ingram, (1957) and Onishi, (1963).

A common method used in isolating **Aspergillus** species that invade stored seeds is to place the seeds on malt agar containing 7.5 - 10 % Nacl or on Czapek's medium with up to 80 % sucrose (Christensen, 1957).

Rapper and Fennel, 1965, showed that some members of *Aspergillus glucus* group have osmophilic properties. However , Kulik and Hanlin, (1968) , showed that other aspergilli may grow under halophilic conditions.

Little or no information are given in the literature about the physiological or biochemical basis of the tolerance of concentrated sugar solution by these organisms.

MATERIALS AND METHODS

Wheat grains:

Two samples of wheat grains were used in this investigation. One of them was imported, collected from Embaba Cereal Stores, Giza, Egypt. The second sample was local and obtained from Kafr Sakr, Sharkia, Egypt, after being stored for 12 months in the common farmer storage rooms.

Media:

1. Bunt and Rovira medium: It was used as deviced by Louw Webley, 1959 and modified as required by varying the carbon source supplied or its concentration.

2. Dox-rose bengal agar medium: It contained 2 % sucrose; 0.2 % Na_3 ; 0.1 % K_2HPO_4 ; 0.05%$MgSO_4$, $7H_2O$; 0.05 % KCl ; traces $FeSO_4$; 0.004 % rose be ngal and 2 % agar ; PH was adjusted at 4-4.5 after sterlization.

Standardized - spatula spray technique :

Fungi in the wheat meal were studied using the standarized-spatula spray technique as given by Elwan and El-Syed 1964. Surface sterilized wheat grains were ground thoroughly in a sterile mortar. The meal was passed through 0.2 mm sterile sieve under aseptic conditions. The load of a standardized spatula was evenly sprayed on the surface of Bunt & Rovira medium. The plates were incubated at 30°C.

Purification:

Spores or hyphal tips were detached and allowed to develop on Dox's rose-bengal agar. No difficulty was encountered in getting pure cultures of fungal isolates.

Identification:

Fungal isolates were identified using Gilman, 1956 , Barnett, 1960 and Raper & Fennell, 1965 , schemes.

R E S U L T S

Results indicate that twelve osmotolerent fungal species (Table 1) belonging to the three genera ***Penicillium*** , *Paceillomyces* and *Aspergillus* were isolated. *Paecillomyces elegans*, *A. melleus* and A. *ornatus* were recorded only in the foreign wheat sample. On the other hand, *A. petrakii*, *A. terreus* and *A. glucus* were detected only in the local wheat meal.

Percentages of the organisms as related to the total mycoflora on gradient sucrose concentration supplied to bunt and Rovira medium are shown in Table (2). *A. candidus* and *A. ornatus* tolerated up to 80 % sucrose concentration. **A.quadricinctus**, *A. fumigatus* and *A. meleus* tolerated upto 60 % sucrose concentration. *A. Japonicus* tolerated up to 50 % sucrose concentration. *Paecillomyces elegans*, *Penicillium* sp, *A. restrictus*, *A. glucus*, *A. petrakii* and *A. terreus* tolerated up to 40 % sucrose concentration.

Results of cultivating the isolates in gradient sucrose concentration supplied to Bunt & Rovira liquid medium are represented in Fig. (1) . The increase of sucrose concentration up to 80 % affected, but did not completely inhibit, the growth of *A. candidus* and *A. ornatus*, 70 % sucrose concentration did not completely inhibit the growth of the above *species and also A. japonicus*, *A. petrikaii*, *A. melleus*, *P. elegans* , *Penicillium* sp and *A. terreus*. All species grew on 60 % sucrose

Table 1: Fungal species from local and foreign meal of wheat grains.

Concentration	Fungal isolates from the local whoat meal	Fungal isolates from foreign wheat meal
30 %	*A. Japonicus, A. fumigatus, A. terreus, A. quadricinctus, A. restrictus, A. candidus, A. glaucus, Penicillium sp. & A. petrakii*	*Penicillium sp. Paecillomyces elegans , A. fumigatus, A. quadricinctus, A. Japonicus, A. restrictus, A. candidus A. molleus & A. ornatus*
40 %	*A. Japonicus, A. fumigatus, A. terreus, A. Petrakii, A. quadricinctus, A. restrictus, A. candidus, A. glaucus, and Penicillium sp.*	*Penicillium sp. , Paecillomyces elegans, A. fumigatus, A. quadricinctus, A. Japonicus, A. restrictus, A. candidus , A. melleus and A. ornatus.*
50 %	*A. Japonicus, A. quadricinctus, and A. candidus.*	*A. quadricinctus, A. candidus, A. melleus, A. japonicus, A. fumigatus and A. ornatus.*
60 %	*A. candidus, A. quadricinctus*	*A. candidus, A. fumigatus, A. melleus and A. ornatus.*
70 %	*A. candidus*	*A. candidus and A. ornatus*
80 %	*A. cardidus*	*A. candidus and A. ornatus.*

Table (2) : Percentages of the organisms as related to the total mycoflora on gradient sucrose concentration supplied to Bunt & Rovira medium using the standerrdized spatula spray technique.F, foreign wheat sample, L, local wheat sample.

	Sucrose Conc. (%)											
	30		40		50		60		70		80	
	Sample		Sample		Sample		Sample		Sample		Sample	
	F	L	F	L	F	L	F	L	F	L	F	L
A.candidus	3.1	2.4	8.0	14.0	20.0	61.0	46.4	84.0	62.3	100.0	56.0	100.0
A.fumigatus	8.0	36.0	14.0	24.2	12.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0
A.japonicus	11.1	21.1	1.3	16.1	8.0	14.0	0.0	0.0	0.0	0.0	0.0	0.0
A.quadricinctus	7.2	8.0	16.4	13.3	8.0	25.0	0.0	16.0	0.0	0.0	0.0	0.0
A.restrictus	9.2	8.3	2.1	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium sp	23.0	4.0	16.1	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.melleus	3.4	0.0	2.2	0.0	18.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
A.ornatus	4.0	0.0	7.0	0.0	33.0	30.0	0.0	37.7	0.0	0.0	44.0	0.0
Paecilomyces elegans	31.0	0.0	21.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.glaucus	0.0	5.2	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.petrakii	0.0	9.0	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.terreus	0.0	5.2	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

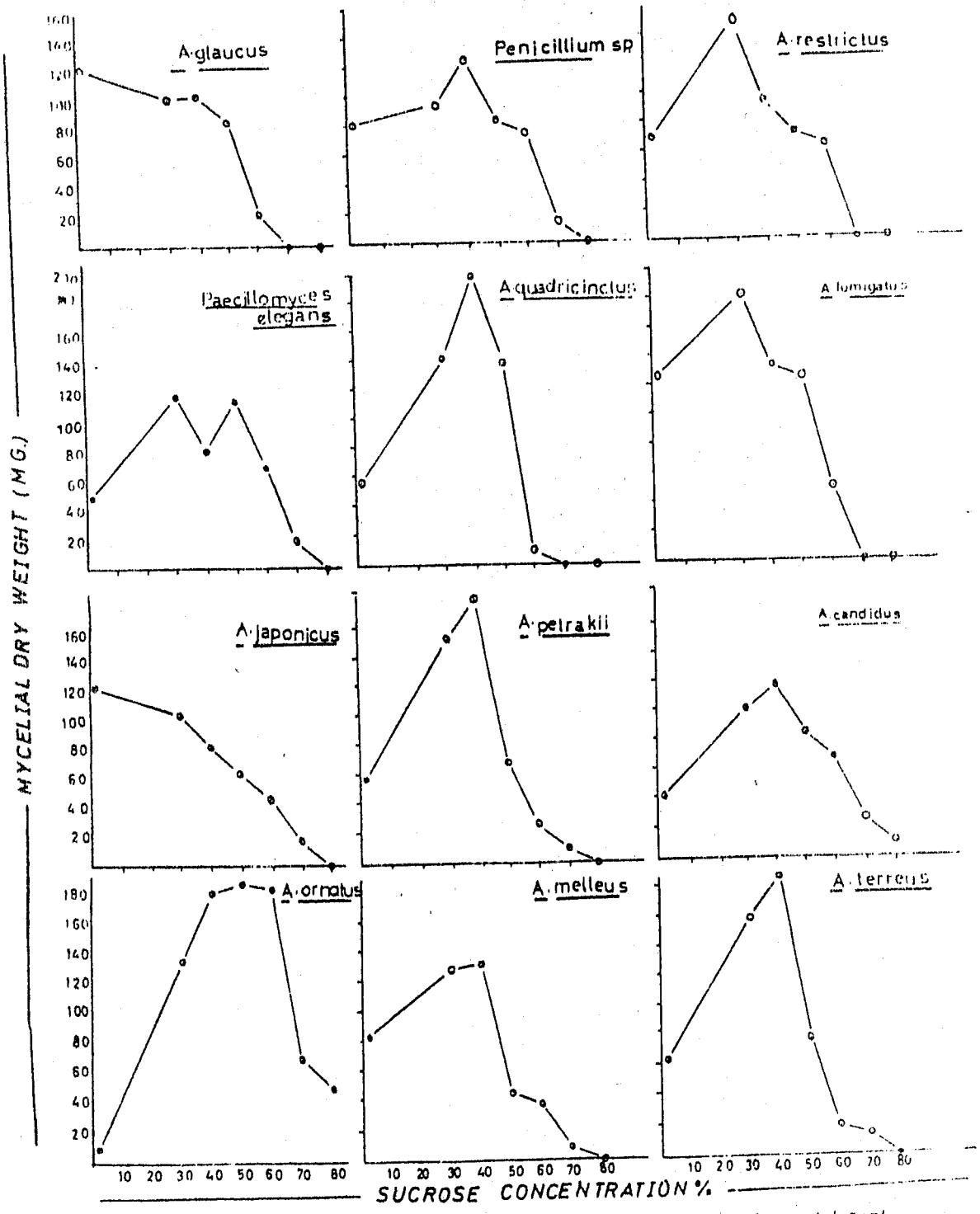

Fig. 1 Effect of gradient sucrose concentration on the growth of osmotolerant fungal isolates in B & R medium with 36% sucrose concentration

However, the optimal concentration for growth was 30 or 40 % sucrose accroding to the species (Fig. 1) . So 36 % sucrose concentration was used in further studies.

The minimum inhibitory concentrations (M.I.C.) of six caroon sources are given in Table (3), Results show clearly the M.I.C. value is a function of the used carbon source.

Fig. (2) shows that the species tolerance to 36 % sucrose varied greatly with PH variation. However, acidic PH favoured the osmotoleranoe by most of the isolates.

Optimum temperatures were found to be within the range of 25 - 30°C depending on the species; either the increase or the decrease in incubation temperature than that range minimized the osmotolerance of these fungi . Fig (3).

Results given in Table (4) indicate that all the dried fungal mycelia except that of *A. quadricinctus* showed lethal effect on the experimental mice. The growth filtrates of the isolates were toxic at different levels except those of *A. glucus* and *A. quadricinctus*.

Table (3) Minimum concentrations of different carbon sources inhibitory (M.I.C.) for isolated osmophilic fungi.

Fungal isolates	M.I.C. of different carbon sources (%)					
	Sucrose	Glucose	Fructose	Lactose	Maltose	starch
A. candidus	90	90	70	90	90	25
A. fumigatus	70	70	50	90	80	25
A. japonicus	80	80	80	80	90	30
A. quadricinctus	70	70	80	70	80	30
A. restrictus	70	70	90	90	90	25
Penicilium sp.	80	80	50	70	70	30
A. melleus	80	90	70	90	90	30
A. ornatus	90	90	70	90	90	25
Paecillomyces elegans	80	80	90	90	90	40
A. glaucus	70	70	30	70	80	25
A. petrakii	80	80	60	70	80	25
A. terreus	80	80	50	70	80	25

Table(4) : Toxicity tests of oven dried fungal mycelium and fungal growth filtrates as determined by the percentage mortality among albino mice Mortality percentage in control was zero.

Treatment Organism	Dried mycelium	Filtrate
	% mortality	% mortality
A. glaucus	100	0
A. terreus	100	50
A. fumigatus	100	100
A. quadricinctus	0	0
A. japonicus	100	100
A. restrictus	100	80
A. Petrakii	20	80
A. candidus	20	20
A. melleus	100	100
A. ornatus	100	100
Paecilomyces elegans	100	100
Penicillium sp.	100	80

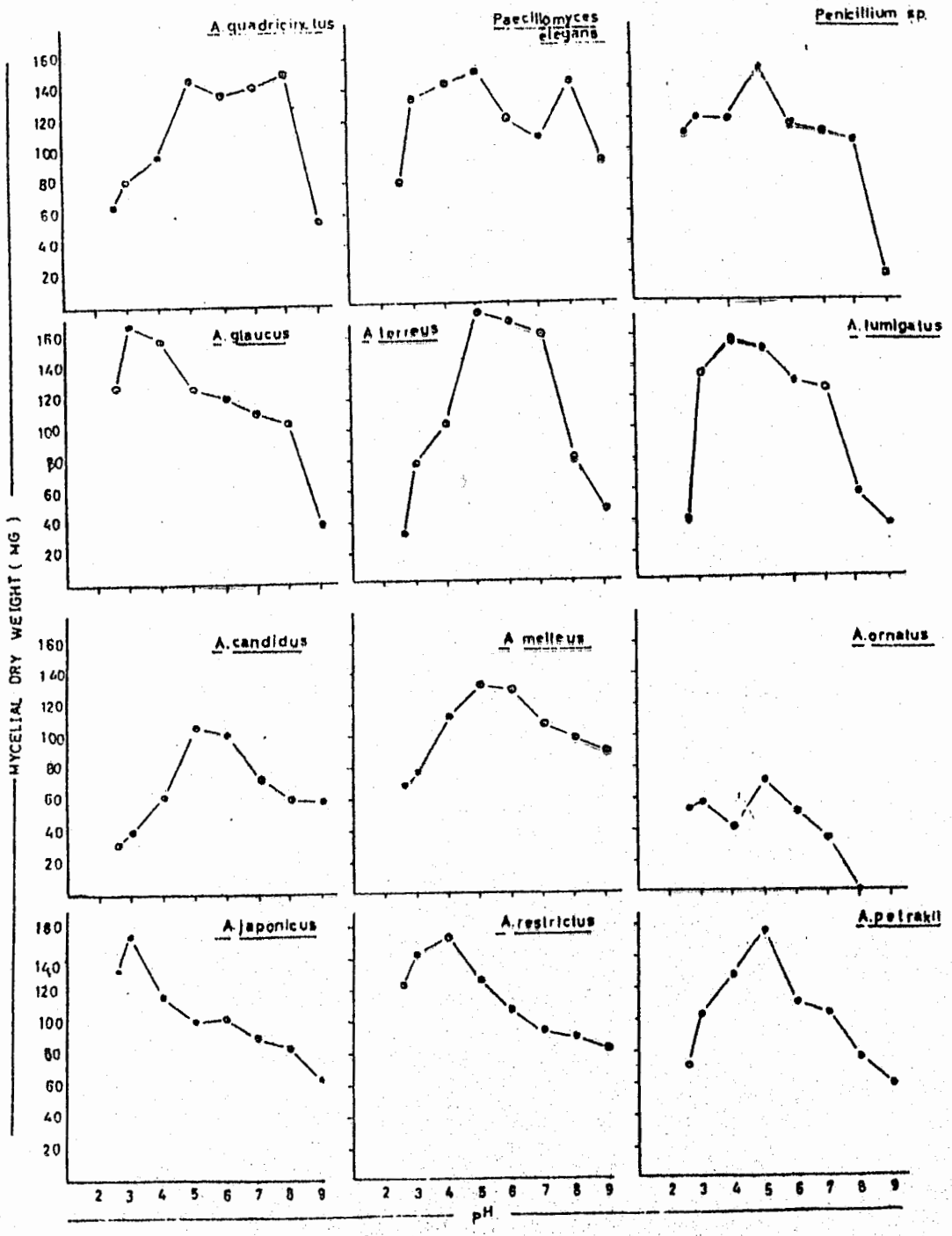

Fig. 2. Effect of pH ranges on the growth of osmotolerant fungal isolates in B & R medium with 36% sucrose concentration.

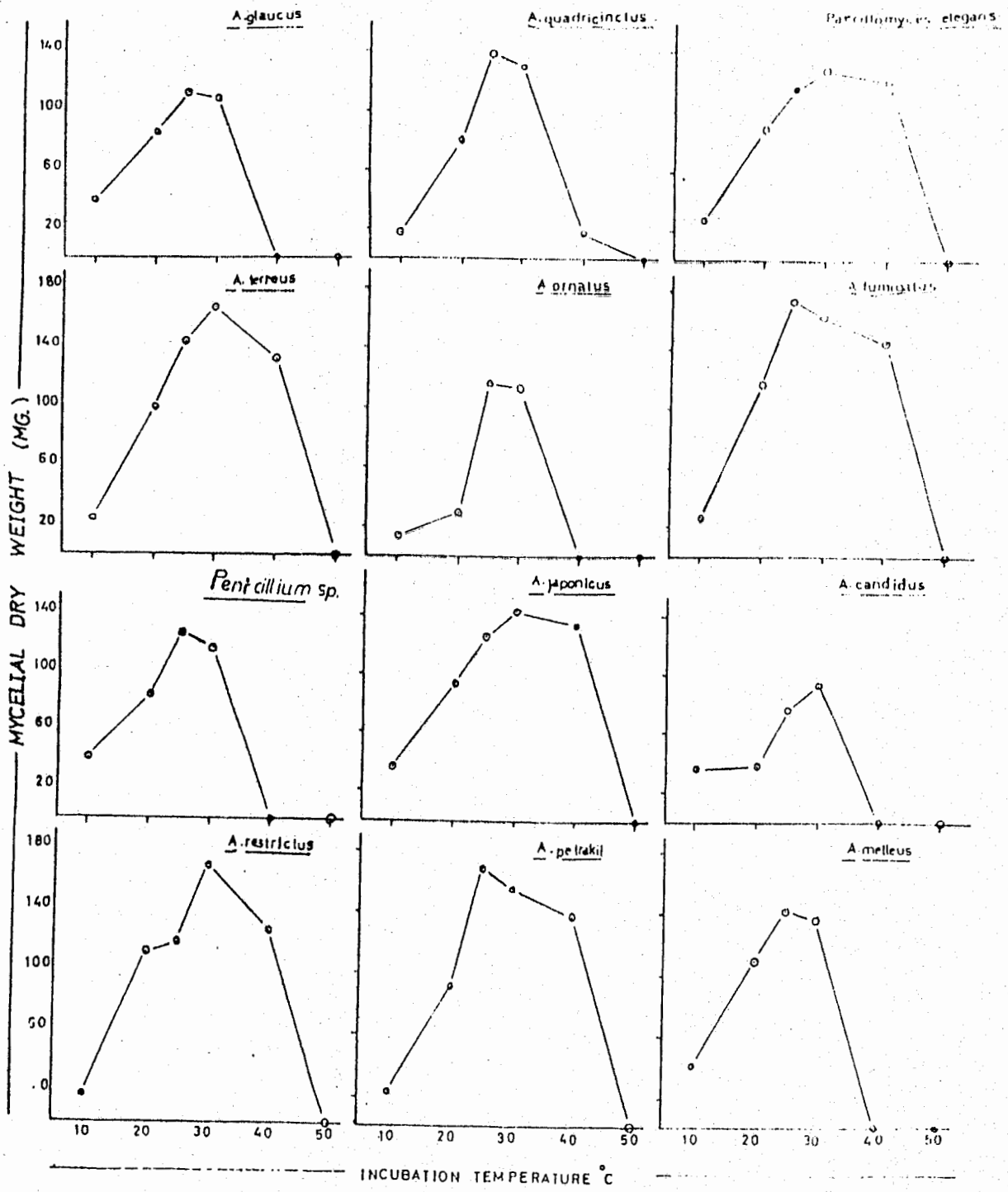

Fig. 3 Effect of incubation temperature on the growth of osmotolerant fungal isolates in B & R medium with 36% sucrose concentration.

D I S C U S S I O N

The ability of certain micro-organisms to proliferate in wheat grains or meal during storage is a problem which might not only affect the quality of this widely used food stuff but also might cause harm to the consumers. Microbial spoilage in storage would be due to various biotic entities among which fungi could be important.

Fungi which could thrive at high concentrations of carbonacious materials are considered osmotolerant and occasionally they might be osmophilic.

Twelve osmotolerant fungal isolates that developed on Bunt and Rovira medium receiving increasing gradient sucrose concentration (30 - 80 %) proved to belong to three genera namely *Penicillium*, *Paecillomyces* and *Aspergillus*. The recorded species were one each of *Penicillium* and *Paecillomyces* whereas ten species were found to belong to *Aspergillus viz: A. fumigatus, A. japonicus, A. quadricinctus, A. candidus, A. restrictus, A. glucus, A. petrakii, A. melleus, A. ornatus* and *A. terreus*. Aspergilli are thus the predominant osmotolerant isolates. Similar results were recorded by Christinse, (1972), who concluded that fungi are the causative of deterioration of stored grains and seeds.

Increasing sucrose concentration up to 60 % affected but did not completely inhibit the growth of the isolates. Isolates proliferate at normal as well as higher concentrations thus revealing a facultative osmophilic behaviour.

The minimum inhibitory concentration (M.I.C.) of the supplemented monosaccharides and disaccharides ranged from 30 - 90 %. However, the M.I.C. of starch (polysaccharide) was relatively low (25 - 40 %). The magnitude of differences between M.I.C. values of starch on one hand and all others (mono - & disaccharides) on the other hand would show the potential role of enzymatic effect. It would show that amylase synthesis in appreciable amounts is hindred by the increase of starch concentration whereas enzymes responsible for degrading sucrose, lactose and maltose are not equally affected.

With regard to the effect of PH, all the fungal isolates could grow in the range of PH 2.6 to 9.2 except *A. ornatus*. The tolerance to sucrose concentration varied greatly with pH variation. Generally the osmotolerance of the isolates increased in the pH range of 3 - 5.

Concerning the effect of incubation temperature, all the osmophilic isolates proved mesophilic and their osmotic tolerance decreased below 25°C and above 30°C . Temperatures of 50°C and higher did not help the microbial cells to profilerate. This would indicate that channels of keeping pace with thermal stress are different from those adapting the cell to osmotic stress.

The problem tackled in this work is concerned with the occasional spoilage of wheat meal. Needless to say that this is related to human welfare in various ways including nutritional and toxicological aspects. As expected no obligate osmophily was recorded, but facultative osmophiles were quite common. Their minimum inhibiting concentrations

indicate that increase in humidity in storage would lead to spoilate of the stuff material particularly when it is known that almost all the facultative osmophilic fungi were found (untabulated) proteolytic, amylolytic and lipolytic. Furthermore most of the isolates caused significant rates of mortality of mice indicating toxicity.

The present work helped to reveal the occurrence of osmotolerent fungi mainly belonging to the genus *Aspergillus* that could resist and proliferate at concentration of sucrose as high as 80 % and that these would be of practical. importance if conditions favourable for proliferation are represented in the environment.

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