RESULTS AND DISCUSSION

4. CULTURAL STUDIES

3.1 Selection of Suitable Culture Medium

For energy sources fungi relay upon their environment. Carbon, nitrogen, sulphur, phosphorus, sodium, potassium, calcium, magnesium, iron, manganese, zinc, copper, cobalt, molybdenum and vitamins are some of the important components for the growth of an organism.

A knowledge of the nutrition of the fungi is necessary for culturing them in laboratory or in industry. Thousands of years back man started cultivating fungi. In the beginning it was unintentional, lateron developed into an art in connection with the preparation of food stuffs and beverages. Natural materials were used in the early periods for culturing fungi in laboratories. In the last century many workers have tried to develop semisynthetic as well as synthetic media to cultivate fungi in laboratories. "There is no universal natural substrate or artificial medium upon which all fungi will grow" (Lilly and Barnett,1951). Even closely related forms may differ considerably in their nutritional requirements qualitatively as well as quantitatively (Arya, 1982).

The four Fusarium spp. under the present investigation were, therefore, grown in a number of media and their

mycelial growth, sporulation, and chalmydospore formation was studied, so that, a suitable basal medium could be selected.

Figure 2 depicts the combination of seven different media used in the present study and the results are summarised in tables 1 to 4 and figure 3.

A perusal of table 1 reveals that Richard's medium supported best growth of Fusarium udum. It was followed by modified Asthana and Hawker's medium 'A', Czapek's medium, Asthana and Hawker's medium 'A', potato dextrose medium, Nash and Snyder medium and Glucose Asparagine medium.

In the case of F. pallidoroseum growth was maximum on Czapek's medium followed by Richard's, potato dextrose, modified Asthana and Hawker's medium 'A'. Nash and Snyder and Glucose Asparagine media supported growth almost equally, while poor growth was obtained in Asthana and Hawker's medium 'A'.

The growth of F. oxysporum was maximum in Richard's medium. Czapek's, modified Asthana and glucose Asparagine, Asthana and Hawker's medium 'A' followed it.

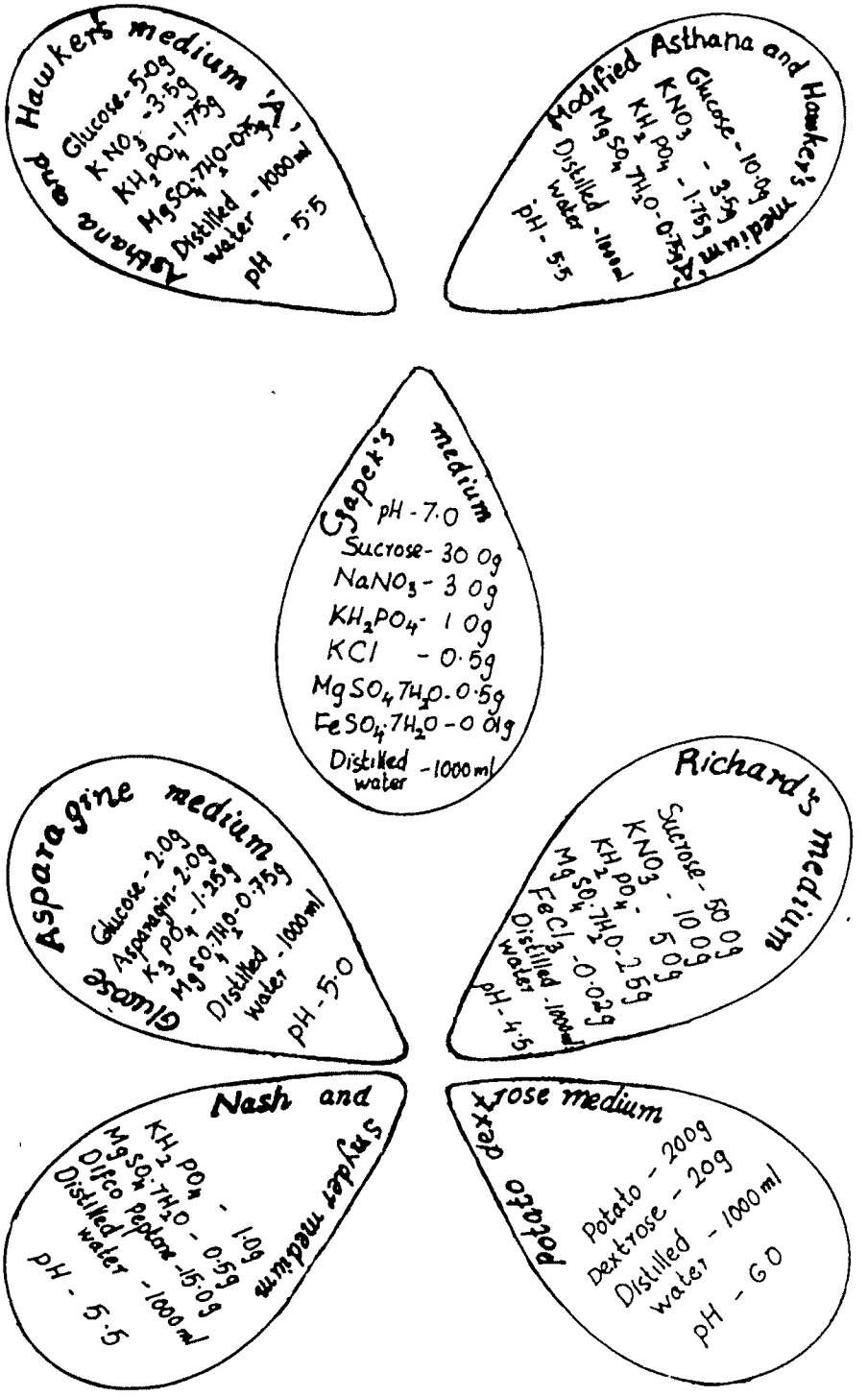

Hawker's medium 'A'
Glucose - 5.0g
KNO3 - 3.5g
KH2PO4 - 1.75g
MgSO4·7H2O - 0.75g
Distilled water - 1000ml
pH - 5.5
Asthana and Hawker's medium
Modified Asthana and Hawker's medium
Glucose - 10.0g
KNO3 - 3.5g
KH2PO4 - 1.75g
MgSO4·7H2O - 0.75g
Distilled water - 1000ml
pH - 5.5
Czapek's medium
pH - 7.0
Sucrose - 30.0g
NaNO3 - 3.0g
KH2PO4 - 1.0g
KCl - 0.5g
MgSO4·7H2O - 0.5g
FeSO4·7H2O - 0.01g
Distilled water - 1000ml
Asparagine medium
Glucose - 2.0g
Asparagin - 2.0g
K2PO4 - 1.25g
MgSO4·7H2O - 0.75g
Distilled water - 1000ml
pH - 5.0
Richard's medium
Sucrose - 50.0g
KNO3 - 10.0g
KH2PO4 - 5.0g
MgSO4·7H2O - 2.5g
FeCl3 - 0.02g
Distilled water - 1000ml
pH - 4.5
Nash and Snyder medium
KH2PO4 - 1.0g
MgSO4·7H2O - 0.5g
Difco Peptone - 15.0g
Distilled water - 1000ml
pH - 5.5
Potato dextrose medium
Potato - 200g
Dextrose - 20g
Distilled water - 1000ml
pH - 6.0

Fig.2.

Richard's medium supported maximum growth of Fusarium moniliforme. This organism attained good growth in Czapek's, potato dextrose, Asthana and Hawker's medium 'A'. Nash and Snyder and Modified Asthana and Hawker's medium 'A' supported moderate growth. However, poor growth was accomplished in Glucose Asparagine medium.

Excellent sporulation of all the four fungi were obtained in Richard's medium. It varied from excellent to good to fair in all other media, except Glucose Asparagine for all the four spp., and Nash and Snyder for Fusarium moniliforme, where poor sporulation was observed.

Chlamydospore formation was poor or failed to form in all the media, but in Nash and Snyder medium, F. moniliforme showed a fairly good response to chlamydospore formation.

At the end of incubation period pH of the medium shifted towards neutral or alkaline side in all the cases. On the contrary in Czapek's medium pH shifted to more acidic side.

Suitability of potato dextorse agar medium was earlier reported by Ramakrishnan and Damodaran (1954), Tandon (1960), Lapis and Deangkinay (1966), Hasija and Chowdhary (1980) in the organisms they studied. Among the various

synthetic media tried, Richard's medium exhibited best growth in all the species except F. pallidoroseum. Singh (1977) reported that F. acuminatum showed good growth on Czapek's medium than Richard's medium. Nash and Snyder medium supported good growth of F. oxysporum than the three other species under present study. The result was supported by the earlier report of Singh (1977).

Complex sugars have been hydrolysed during autoclaving of the medium. Acidity of the medium facilitate this phenomenon more. Medium should not have it's constituents in high concentration for general use (Leonian and Lilly,1940). Cochrane (1958) also suggested use of more dilute media than those often used in the studies of morphology and reproduction.

Although modified Asthana and Hawker's medium 'A' did not support good growth statistically, it supported sufficient growth and sporulation in all the Fusarium species under present study. Moreover, this medium is easy to manipulate with respect to the expected need for modifications and substitutions of its constituents. Hence, it is used in all subsequent cultural studies.

Table 1 : Average dry weight (in mg), Sporulation, Chlamydospore formation and change in pH by Fusarium udum on different culture media.

Sl. No.	Media	Dry weight	Sporulation	Chlamydospore formation	pH	
					Initial	Final
1.	Asthana & Hawker's medium'A'	55.14	Good	Poor	5.50	6.82
2.	Mod.Asthana & Hawker's medium 'A'	88.12	Excellent	Poor	5.50	6.84
3.	Czapek's medium	72.42	Good	-	7.00	6.60
4.	Glucose Asparagine medium	39.35	Poor	Poor	5.00	5.53
5.	Richard's medium	224.09	Excellent	-	4.50	6.12
6.	Nash & Snyder medium	44.35	Fair	-	5.50	8.04
7.	Potato dextrose medium	52.41	Good	Poor	6.00	6.44
	G.M.	82.27				

Summary of the dry weight results and conclusion at 5% level of P.

Treatment : Highly significant
Replicates : Non significant
S.E. : 4.11
C.D. at 5% level : ± 14.26

Dry weight results:

$5>2>3>\overline{1\ 7}>6>4$

Table 2: Average dry weight (in mg), Sporulation, Chlamydospore formation and change in pH by *Fusarium pallidoroseum* on different culture media.

Sl. No.	Media	Dry weight	Sporulation	Chlamydospore formation	pH	
					Initial	Final
1.	Asthana & Hawker's medium 'A'	23.92	Excellent	Poor	5.50	6.41
2.	Mod.Asthana & Hawker's medium 'A'	53.30	Excellent	Poor	5.50	7.24
3.	Czapek's medium	207.05	Good	-	7.00	6.79
4.	Glucose Asparagine medium	41.45	Poor	Poor	5.00	5.63
5.	Richard's medium	161.22	Excellent	-	4.50	6.83
6.	Nash & Snyder medium	42.25	Poor	Fair	5.50	6.01
7.	Potato dextrose medium	107.61	Excellent	Poor	6.00	6.44
	G.M.	90.97				

Summary of dry weight results and conclusion at 5% level of P.

Treatment	: Highly significant
Replicates	: Non significant
S.E.	: 3.41
C.D. at 5% level	: ± 11.83

Dry weight results :

$$3>5>7>2>\overline{6\ 4}>1$$

Table 3: Average dry weight (in mg), Sporulation, Chlamydospore formation and change in pH by Fusarium oxysporum on different culture media.

Sl. No.	Media	Dry weight	Sporulation	Chlamydospore formation	pH	
					Initial	Final
1.	Asthana & Hawker's medium 'A'	35.63	Fair	-	5.50	6.73
2.	Mod. Asthana & Hawker's medium 'A'	112.80	Excellent	Poor	5.50	6.83
3.	Czapek's medium	173.61	Good	-	7.00	6.29
4.	Glucose Asparagine medium	42.34	Poor	Poor	5.00	5.44
5.	Richard's medium	191.23	Excellent	-	4.50	6.27
6.	Nash & Snyder medium	60.23	Good	-	5.50	8.08
7.	Potato dextrose medium	92.39	Good	Poor	6.00	6.51
	G.M.	101.17				

Summary of dry weight results and conclusion at 5% level of P.

Treatments : Highly significant
Replicates : Non significant
S.E. : 2.00
C.D. at 5% level : $\pm$ 6.95

Dry weight results :

5>3>2>7>6>4>1

Table 4 : Average dry weight (in mg), Sporulation, Chlamydospore formation by Fusarium moniliforme on different culture media.

Sl. No.	Media	Dry weight	Sporulation	Chlamydospore formation	pH Initial	pH Final
1.	Asthana & Hawker's medium 'A'	70.04	Excellent	-	5.50	6.13
2.	Mod.Asthana & Hawker's medium 'A'	42.81	Good	Poor	5.50	6.49
3.	Czapek's medium	89.32	Good	-	7.00	6.75
4.	Glucose Asparagine medium	31.74	Poor	Poor	5.00	5.64
5.	Richard's medium	214.18	Excellent	-	4.50	6.32
6.	Nash & Snyder medium	45.45	Good	-	5.50	7.90
7.	Potato dextrose medium	75.92	Excellent	-	6.00	6.36
	G.M.	81.36				

Summary of dry weight results and conclusion at 5% level of P.

Treatment : Highly significant
Replicates : Non significant
S.E. : 3.10
C.D. at 5% level : $\pm$ 10.75

Dry weight results:

$5>3>7>1>\overline{6\ 2}>4$

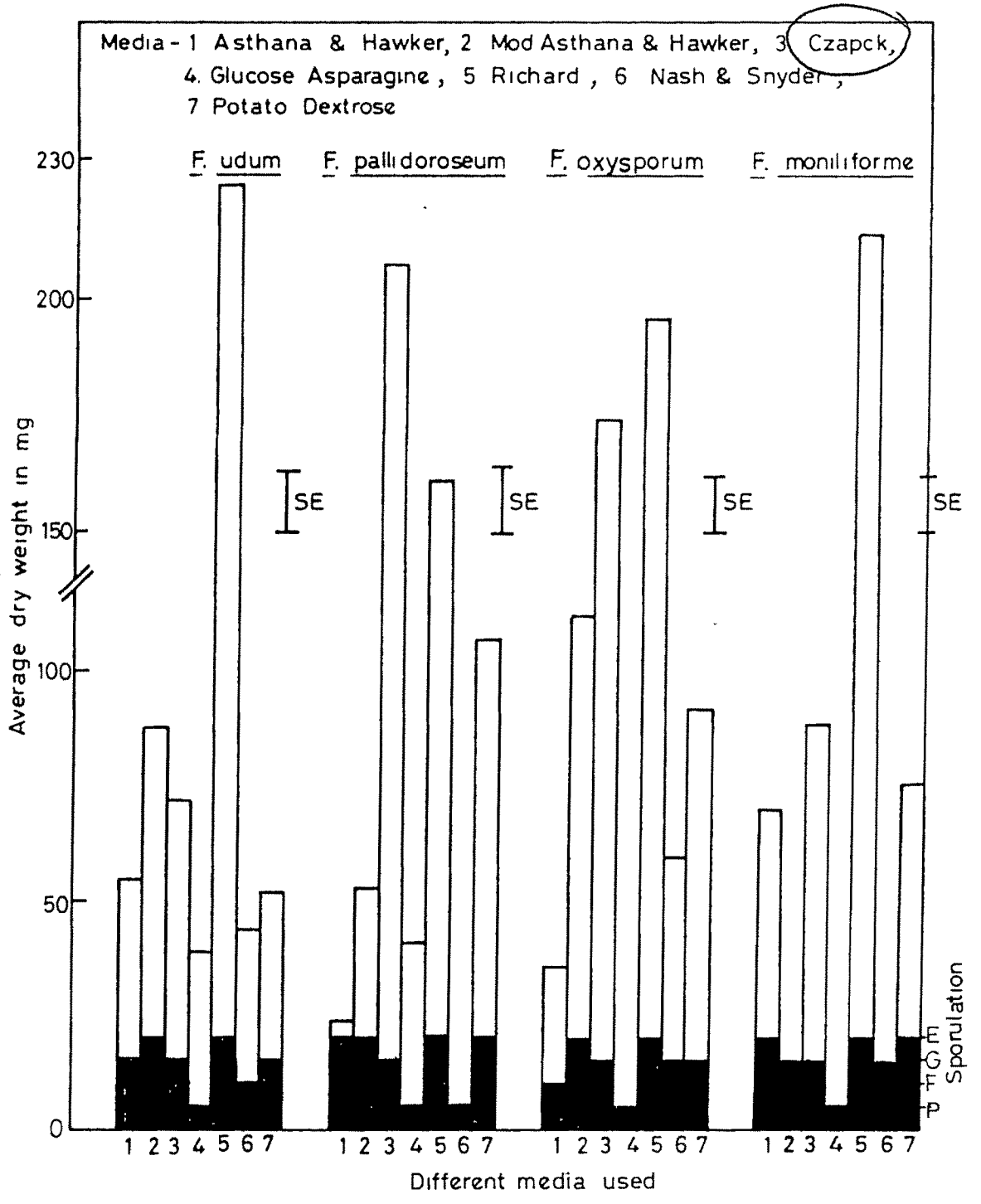


Fig 3. Average dry weight (entire bar) and sporulation (shaded) of four species of Fusarium on different culture medium

3.2 Influence of Different Hydrogen Ion Concentrations

Growth of fungi are known to be highly influenced by the hydrogen ion concentration of the medium. It affects permeability of protoplasmic membranes, uptake of minerals, entry of essential vitamins and organic acids into the cells, activity of enzyme systems, synthesis and stability of proteins and other similar life processes. The pH of the substrate also affect the processes like production of pigments, vitamins, antibodies and other metabolites. The pH of the host may play a definitive part in the severity of infection in many diseases. Incipient infection of Gleosporium psidii in the young green guava fruits was due to interaction of pH with other factors (Midha and Chohan 1968).

The organic catalysts have great influence on growth and sporulation patterns of an organism. It has been found by various phytopathologists that, enzymes have optima between pH 4 and 8. According to Lilly and Barnett (1951). "The chemical chagnes in media due to alteration of pH, whether imposed from outside or caused by the fungus, affects metabolic porcesses. The pH of a culture medium change during the growth of a fungus and these changes may affect the compsotion of the medium and thus the response of

the fungus". Yu (1954) reported good growth over a range of pH from 3 to 12 in Ascobolus magnificus. Phyllosticta cycadina grow between pH 3 and 9 (Bilgrami,1956). Botryodiopioidia theobromae and Hendersonula toruloidea could tolerate a pH range of 2.0-12.0 (Williamson 1964). Lal and Pathak (1970) reported Diplodia metalensis to grow between pH 3.5 and 8.5. However, a slightly narrow pH range (3.0 to 7.2) was observed for Phoma herbarum (Chung 1967). Agarwal (1955) found that pH range for the two strains of Fusarium coeruleum was between 3.4 and 11.0 and pH 6.4 was optimum for growth and sporulation. Rose (1960) reported that Fusarium culmarum, F. sambucinum var. coeruleum and F. oxysporum grew well between pH 5 and 8. Bhargava (1962) found the optimum range for the growth of Fusarium solani as 4.5 to 6.5. Malca et al. (1966) and Selvaraj (1971) reported that higher pH values upto 10.0 are better tolerated in Verticillium and Fusarium Spp.

In the light of all the above findings, it was decided to study the effect of pH of the medium on growth and sporulation of four species of Fusarium. The initial pH values of modified Asthana and Hawker's medium 'A' were adjusted from 2.0 to 10.0 (2.0, 2.5, 3.0, 4.0, 4.5, 5.0, 5.5, 5.8, 6.0, 6.5, 7.0, 8.0, 9.0 and 10.0). Results obtained are recorded in tables 5 to 8 and in figure 4.

Results indicate that all the four Fusarium spp. could grow and sporulate over a wide range of pH. The organisms failed to grow at lower pH i.e. 2.0. In the case of F.oxysporum, it could not even grow at pH 2.5.The maximum dry weight of the organisms was attained at pH 5.5. On the acidic side all the four Fusarium spp. showed significantly good growth rather than in alkaline side. Fusarium udum and F. pallidoroseum showed excellent sporulation over a pH range of 5.0 to 5.8. In F. oxysporum sporulation was excellent at pH 5.5 and 5.8. The pH range 5.5 to 6.0 was excellent for sporulation in the case of F. moniliforme. At lower pH from 2.0 to 3.0 all the Fusarium spp. except F. moniliforme were failed to sporulate. In F.moniliforme, a poor sporulation was noticed at pH 3.0.

Chlamydospore formation in F. oxysporum and F. udum was observed at various pH values between 3.0 and 10.0. But, in F.pallidoroseum and F. moniliforme it was observed between pH 2.5 and 10.0 and 4.0 and 10.0 respectively.

Yogeshwari (1948) reported that optimum pH for Fusarium vasinfectum, F.udum and F. moniliforme was 5.0. Joffe and Palti (1972) observed that in culture, isolates of F. solani and F. javanicum grew at pH 7.0 rather than 4.2. Cochrane and Cochrane (1971) observed that the proportion of chlamydospore fell linearly in the case of F. solani between

pH 4.0 and 6.5. Optimum growth and excellent sporulation of all the four organisms under present study were observed at pH 5.5.

The tables 5 to 8 clearly indicate that the pH of the medium of all the four Fusarium spp., tend to drift towards neutral point or slightly alkaline side at the end of incubation period. Hawker (1950) reported that the growth of Fusarium fructigenum on Richard's solution, which is normally acidic (pH 4.6), gradually increased the pH till the medium became strongly alkaline. Ramkrishnan (1942) worked on Colletotrichum falcatum observed that the final pH values of the nutrient medium are always neutral irrespective of the initial pH values. Similar results were observed by Muller (1962) for Gleosporium musarum and G. fructigenum and Agnihotri (1963) in Colletotrichum capsici.

The change in pH of the medium as a result of fungal metabolism is a common phenomenon. As stated by Lilly and Barnett (1951) "Four metabolic processes operate in the change of pH of a culture medium; (1) Utilization of cations;(2) Utilization of anions; (3) Formation of acid from neutral metabolites, especially carbohydrates and (4) Formation of bases, especially anions from amino acids and proteins. The net change in pH is the result of the interaction of all these processes". The rise in pH of the

culture medium has been attributed to the metabolic activities during growth resulting in adsoprtion of anion or production of ammonia from nitrogenous compounds. Lowering of pH in case of media with higher initial pH was, possibly due to absorption of carbon dioxide produced by the fungus during the process of respiration (Singh 1977).

The results obtained during the foresaid experiments confirmed the fact that best sporulation and maximum yield of the four species of Fusarium under present investigation were obtained at pH 5.5. Therefore in all the subsequent experiments the initial pH of the medium was adjusted to 5.5.

Table 5 : Average dry weight, sporulation, chlamydospore formation and final pH of Fusarium udum at various pH values

Sl. No.	pH of the medium	Dry weight in mg	*Sporulation	*Chlamydospore formation	Final pH	Drift in pH
1	2.00	0.00	-	-	-	-
2	2.50	10.75	-	-	5.12	+ 2.71
3	3.00	20.83	-	P	6.37	+ 3.37
4	4.00	38.55	P	F	6.51	+ 2.51
5	4.50	41.87	P	F	6.84	+ 2.34
6	5.00	54.62	E	P	6.86	+ 1.86
7	5.50	66.90	E	-	7.18	+ 1.68
8	5.80	58.21	E	P	7.60	+ 1.80
9	6.00	51.49	G	P	7.93	+ 1.93
10	6.50	50.14	G	P	8.06	+ 1.56
11	7.00	41.70	F	F	8.24	+ 1.24
12	8.00	35.63	P	F	8.29	+/ 1.29
13	9.00	31.56	P	P	8.61	- 0.39
14	10.00	22.33	P	P	9.01	- 0.99
	G.M	37.47				

* = Absent; E = Excellent; F = Fair; G = Good; P = Poor

Summary of dry weight results and conclusion of 5% level of P.

Treatment : Highly significant
Replicates : Non significant
S.E : 2.85
C.D. at 5% level : ± 8.13

Dry weight results : 7>8>6>9 10>5 11>4>12>13>14>3>2>1

Table 6 : Average dry weight, sporulation, chlamydospore formation and final pH of Fusarium pallidoroseum at various pH values

Sl. No	pH of the medium	Dry weight in mg	*Sporulation	*Chlamydospore formation	Final pH	Drift in pH
1	2.00	0.00	-	-	-	-
2	2.50	10.73	-	P	5.64	+3.14
3	3.00	16.94	-	F	5.92	+2.92
4	4.00	28.67	P	F	6.18	+2.18
5	4.50	37.33	F	F	6.45	+1.95
6	5.00	46.90	E	P	6.91	+1.91
7	5.50	56.18	E	-	7.00	+1.50
8	5.80	55.42	E	P	7.31	+1.51
9	6.00	46.49	G	P	7.83	+1.82
10	6.50	40.80	F	F	8.08	+1.58
11	7.00	36.95	P	G	8.73	+1.73
12	8.00	31.66	P	F	8.74	+0.74
13	9.00	29.61	P	P	9.26	+0.26
14	10.00	26.15	P	P	9.52	-0.48
	G.M	33.14				

* - =Absent; E = Excellent; F = Fair; G = Good, P = Poor

Summary of dry weight results and conclusion at 5% level of P.

Treatment	: Highly significant
Replicates	: Non significant
S.E.	: 2.44
C.D. of 5% level	: ±7.05
Dry weight results	: 7 8>6 9>10>5 11>12 13 4>14>3>2>1

Table 7 : Average dry weight, sporulation, chlamydospore formation and final pH of Fusarium oxysporum at various pH values.

Sl. No.	pH of the medium	Dry weight in mg.	Sporulation*	Chlamydospore formation*	Final pH	Drift in pH
1	2.00	0.00	-	-	-	-
2	2.50	0.00	-	-	-	-
3	3.00	12.76	-	P	5.61	+2.61
4	4.00	26.90	P	F	6.47	+2.47
5	4.50	40.35	F	F	6.73	+2.23
6	5.00	47.68	G	P	6.85	+1.85
7	5.50	64.81	E	-	6.94	+1.44
8	5.80	59.64	E	P	7.26	+1.46
9	6.00	56.19	G	P	7.81	+1.81
10	6.50	41.27	G	P	7.86	+1.36
11	7.00	40.41	F	F	8.40	+1.40
12	8.00	38.76	P	G	8.68	+0.68
13	9.00	26.14	P	P	8.67	-0.33
14	10.00	20.53	P	P	9.20	-0.80
	G.M	33.96				

* = Absent; E = Excellent; F = Fair; G = Good; P = Poor

Summary of dry weight results and conclusion at 5% level of P

Treatment : Highly significant

Replicates : Non significant

S.E. : 3.09

C.D. at 5% level : ±8.93

Dry weight results : 7>8>9>6>10 11 5 12>4 13>14>3>2>1

Table 8 : Average dry weight, sporulation, chlamydospore formation and final pH of Fusarium moniliforme at various pH values.

Sl. No	pH of the medium	Dry weight in mg	*Sporulation	*Chlamydospore formation	Final pH	Drift in pH
1	2.00	0.00	-	-	-	-
2	2.50	16.47	-	-	5.01	+2.51
3	3.00	31.02	P	-	5.83	+2.83
4	4.00	36.42	P	P	6.71	+2.71
5	4.50	43.70	F	F	6.92	+2.44
6	5.00	62.68	G	P	7.29	+2.29
7	5.50	74.48	E	-	7.50	+2.00
8	5.80	74.40	E	P	7.72	+1.98
9	6.00	71.81	E	P	7.96	+1.96
10	6.50	69.12	G	P	8.03	+1.53
11	7.00	64.94	F	F	8.05	+1.05
12	8.00	56.77	P	G	8.43	+0.43
13	9.00	26.47	P	P	8.62	-0.38
14	10.00	20.61	P	P	9.31	-0.69
	G.M	46.35				

* = Absent; E = Excellent; F = Fair; G = Good; P = Poor

Summary of dry weight results and conclusion at 5% level of P.

Treatment : Highly significant
Replicates : Non significant
S.E. : 3.71
C.D. at 5% level : ± 10.72

Dry weight results : 7 8 9 10>11 6>12>5>4>3>13>14>2>1

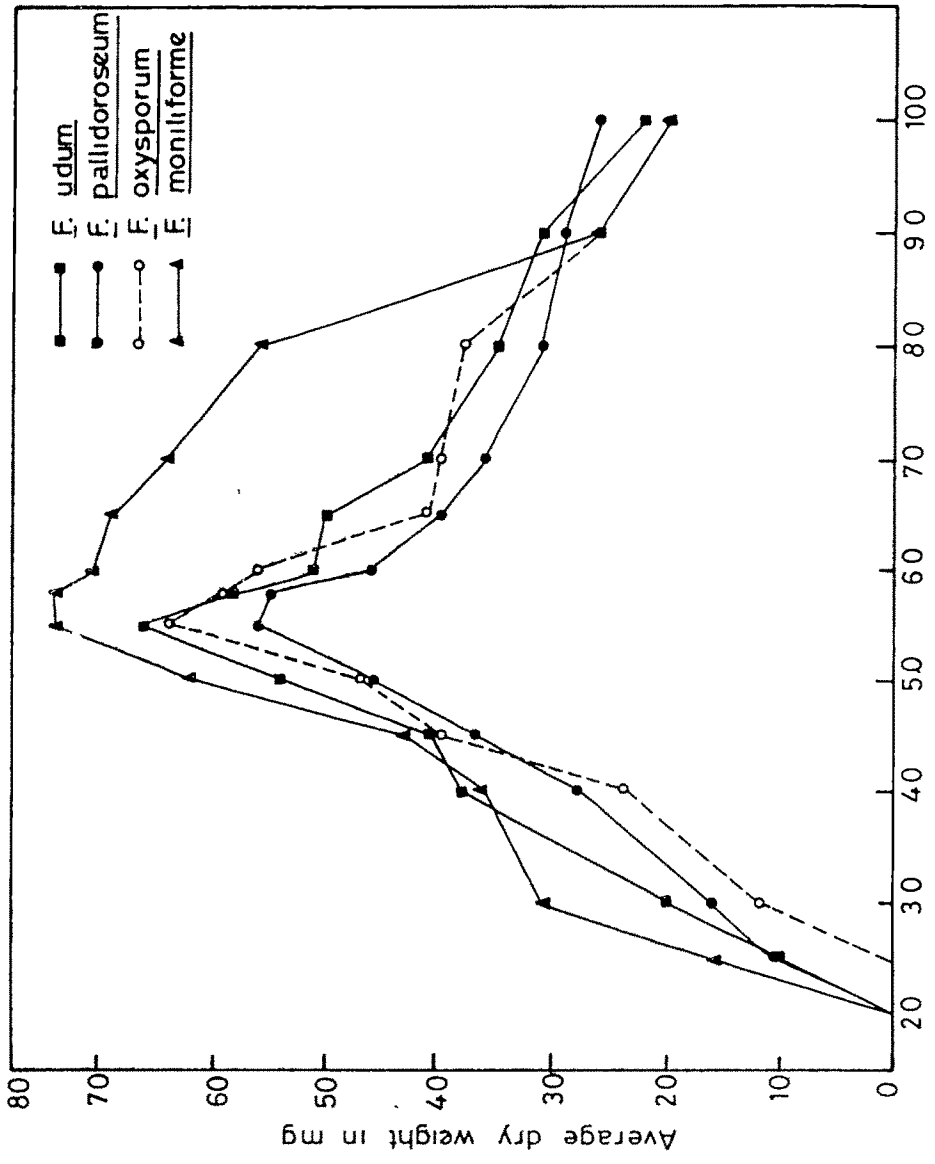


Fig 4 Effect of different hydrogen ion concentration on growth of four species of Fusarium

3.3 Influence of different temperatures

Temperature affects various activities of an organism such as mycelial growth, reproduction and spore germination. Thus, temperature determines various metabolic activities of fungi and even distribution of pathogenic fungi around different parts of the world. Disease can be avoid if the crops are grown at temperature in apposite to the pathogen, for example a rust disease will be less severe if the crop is sown a little late in December or early in October. The optimum temperature for fungal growth is between 20°C and 30°C. Below 0°C and above 40°C usually fungi do not grow, still though, certain fungi are reported to grow even below the freezing point. Broadfoot and Cormack (1941), Pehrson (1948) reported that some fungi are able to grow slowly at 0°C or slightly less. Even plants under snow may be infected by parasitic "snow mould" - Fusarium nivale (Dahl 1934). The other way round, there are certain organisms which have been found to grow at higher temperatures. La Touche (1948) reported the growth of Chaetomium sp. on straw at 62°C.

Agarwal (1955) reported that F. coeruleum could not sporulate at 8°C but the sporulation was best at 20°C-24°C and it decreased at higher temperatures. Ross (1960) reported that F. culmorum, F. sambucinum var. coeruleum and F.oxysporum grew best at 28°C. According to Joffe and Palti

(1972), in cultures, isolates of F.solani grew best at 24°C-32°C. Bhargava (1962) obtained best growth and sporulation of F. solani at 25°C. Bhatnagar (1967), observed a considerable variation in the production of micro and macrospore in two isolates of F. solani and also reported that, as the temperature increased the production of chlamydospores increased.

From the above mentioned facts it is evident that, before starting any physiological study, it is indispensable to have a thorough knowledge about the temperature requirement of the organisms concerned. Moreover, this would also give an idea about the most suitable environmental conditions for the survival of a pathogen in nature. Therefore, it was decided to determine the cardinal temperature, especially the optimum temperature for the growth and sporulation of the present isolates. Different temperatures such as 5°C, 10°C, 15°C, 20°C, 30°C, 35°C, 40°C and 45°C. were taken and the results are summarized in Tables (9 to 12) and Figure. (5).

An appraisal of the results clearly show that F. udum, F. pallidoroseum, F. oxysporum and F. moniliforme could grow a temperature range between 10°C and 40°C. In F. udum, there is a very weak growth at 45°C also. Higher temperature like

35°C support moderate growth as compared to the lower temperature (10°C). Best growth was in the range of 20°C to 30°C in all the 4 species and they failed to grow at low temperature i.e. 5°C.

All the four organisms exhibited maximum growth at 25°C. Similar results were reported earlier by Panwar and Chand (1968), Tandon and Bhargava (1962), Williamson (1964), Singh (1977) and Arya (1982). Brown and Wood (1953) stated subtropical and tropical strains have higher optimum temperature than the temperate ones.

Sporulation as well as chlamydospore formation of the present organisms were mainly influenced by the temperature variations. Sporulation varied from poor to excellent in all the 4 species, but F.udum and F.oxysporum failed to sporulate at 10°C. In all the 4 species of Fusarium, excellent sporulation was observed at the temperature range of 25°C to 30°C except in F.oxysporum, where good sporulation was obtained at 30°C. Divingaracia (1969) and Nash and Pieper (1972) reported that, temperature range of 20-25°C and 25-37°C are suitable for the production of spores. Chlamydospore formation was better in all the 4 species, when the temperature was unfavourable to growth. Good chlamydospore formation was observed in F.udum and F.

moniliforme at 10°C, while the other two species showed a fair development only at this temperature. At 35°C, chlamydospore formation was fair in all the species except which, F. udum showed a poor response. At 40°C chlamydospore formation was fair, poor, failed to develop and good in F. udum, F. pallidoroseum, F. oxysporum, and F. moniliforme respectively. A poor chlamydospore formation was observed at 45°C in F. udum only.

The final pH of the medium showed a drift towards neutral side after 15 days of incubation. This drift was proportional to the growth rate. Growth curve of the 4 species (Fig.5) showed almost similar trend as the typical temperature growth curve depicted by Cochrane (1958). In the present study, both sub optimal and supra optimal temperaures support only slow growth. In F. udum the temperature range for good growth is very narrow while it is broad in F. moniliforme, eventhough the growth rate is less.

Since the results evinced good vegetative and reproductive stages in all the four species of Fusarium at 25°C, it was selected for all subsequent experiments.

Table 9 : Average dry weight (in mg), sporulation, chlamydospore formation and final pH of *Fusarium udum* at different temperatures

Treatment Nos.	Temperature in °C	Dry weight	Sporulation	Chlamydospore formation	*Final pH
1.	5	0.00	-	-	5.5
2.	10	19.33	-	Fair	5.4
3.	15	39.51	Fair	Fair	6.7
4.	20	138.84	Good	Poor	7.2
5.	25	163.19	Excellent	-	7.4
6.	30	146.43	Excellent	-	7.4
7.	35	38.65	Fair	Fair	7.3
8.	40	11.17	Poor	Fair	6.4
9.	45	6.80	-	Poor	5.8
	G.M.	62.69			

* Initial pH = 5.5

Summary of dry weight result and conclusion at 5% level of P.

Treatment : Highly significant
Replicates : Non significant
S.E. : 1.06
C.D. at 5% level : ± 3.46

Dry weight results:

5>6>4>3 7>2>8>9>1

Table 10 : Average dry weight (in mg), Sporulation, Chlamydospore formation, final pH of Fusarium pallidoroseum at different temperatures

Treatment Nos.	Temperature in °C	Dry weight	Sporulation	Chlamydospore formation	*Final pH
1.	5	0.00	-	-	5.5
2.	10	30.13	Poor	Good	6.1
3.	15	57.86	Fair	Fair	6.9
4.	20	102.97	Excellent	Poor	7.4
5.	25	133.62	Excellent	-	7.2
6.	30	106.15	Good	Poor	7.2
7.	35	52.10	Fair	Good	6.8
8.	40	7.2	-	Poor	5.7
9.	45	0.00	-	-	5.5
	G.M.	54.45			

*Initial pH = 5.5.

Summary of dry weight result and conclusion at 5% level of P.

Treatment : Highly singificant
Replicates : Non significant
S.E. : 0.99
C.D. of 5% level : $\pm$ 3.25

Dry weight result :

5>6>4>3>7>2>8>$\overline{9\ 1}$

Table 11 : Average dry weight (in mg) Sporulation, Chlamydospore formation, final pH of Fusarium oxysporum at different temperatures

Treatment Nos	Temperature in °C	Dry weight	Sporulation	Chlamydospore formation	*Final pH
1	5	0.00	-	-	5.5
2.	10	22.47	-	Fair	5.9
3.	15	40.19	Poor	Poor	6.6
4.	20	96.90	Good	Poor	7.0
5.	25	124.63	Excellent	-	7.2
6.	30	120.25	Good	Poor	7.1
7.	35	35.68	Fair	Fair	6.8
8.	40	8.18	-	-	5.6
9.	45	0.00	-	-	5.5
	G.M.	49.81			

* Initial pH = 5.5

Summary of dry weight result and conclusion at 5% level of P.

Treatments : Highly significant
Relicates : Non significant
S.E. : 1.33
C.D. at 5% level : $\pm$ 4.35

Dry weight result :

$5>6>4>3>7>2>8>\overline{9\ 1}$

Table 12 : Average dry weight (in mg), Sporulation, Chlamydospore formation and Final pH of *Fusarium moniliforme* at different temperatures

Treatment Nos	Temperature in °C	Dry weight	Sporulation	Chlamydospore formation	*Final pH
1.	5	0.00	-	-	5.5
2.	10	28.73	Poor	Fair	5.8
3.	15	35.94	Fair	Good	6.8
4.	20	59.21	Good	Fair	7.0
5.	25	68.45	Excellent	-	7.1
6.	30	60.68	Excellent	Poor	7.0
7.	35	32.61	Fair	Fair	6.6
8.	40	25.1	-	Poor	5.7
9.	45	0.00	-	-	5.5
	G.M.	35.52			

* Initial pH = 5.5

Summary of dry weight result and conclusion at 5% level of P.

Treatments : High significant
Replicates : Non significant
S.E. : 1.42
C.D.at 5% level : ± 4.62

Dry weight result:

5>6>4>3>7>2>8>9 1

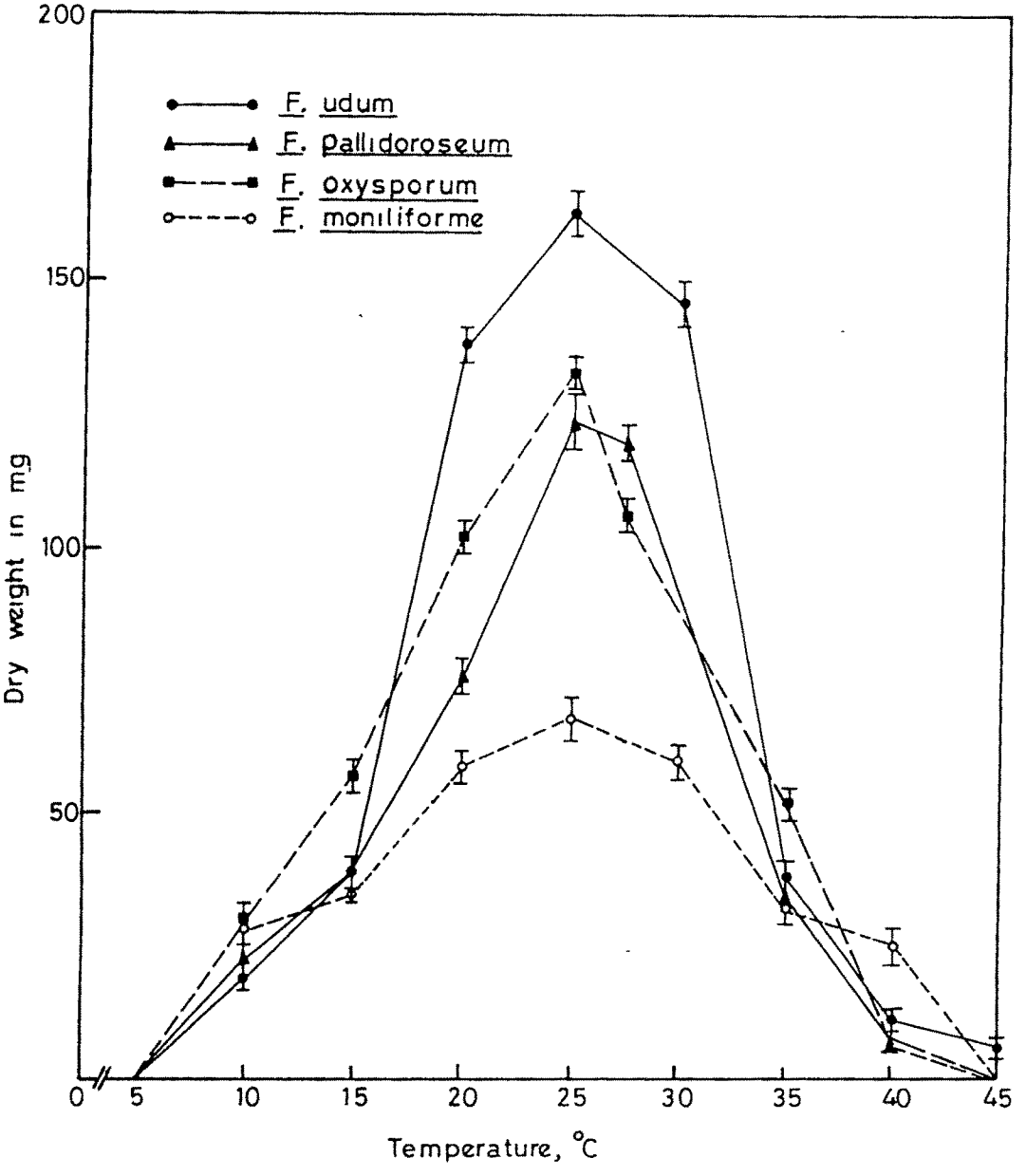


Fig 5 Effect of different temperature on growth of 4 species of Fusarium

3.4 Influence of Different Carbon Sources

Virulence of fungus is often equate with their growth rate (Gibbs et al.,1975; Grey, 1971) or sporulation rate (Wyllie and Devay, 1970a). Growth rate is in turn, profoundly influenced by the availability of the growth requirement like nutrients. In nature, soil fungi receive nutrients from root exudates, wound sap, and xylem sap. Organisms like Fusarium may also be able to exist as saprophytes.

Carbon occupies a very important place in the fungal nutrition. Carbohydrates are the chief source of carbon readly available to fungi. Out of this simple sugars are more common. A carbon source may be utilized by a fungus, while another source of similar chemical structure may prove useless for the same. Panwar (1972),Kapoor and Singh (1973) and Shreemali (1973) observed that not only different species but different isolates of a species also have specificity in their choices of carbon compounds. Fusarium can anaerobically catabolizes a variety of sugars by alcoholic fermentation (Wolf, 1955; Heath et al, 1956 and Gunner and Alexander,1964).

Unless and until all conditions are absolutely similar, a comparative work is not possible in fungi because, they

are highly sensitive to their environment.So any generalization in their behaviour may be wrong in lack of sufficient data.

As mentioned above, carbohydrates are the first choice of pathogenic fungi as carbon source (Arya,1982). Lilly and Barnett (1951) clearly stated that "there is no single sugar which supports the maximum amount of growth for all these fungi. All of these fungi utilize glucose although the maximum amount of growth was not always attained as this sugar". Tanakar (1956) and Papavizas and Ayers (1964) reported that glucose is preferred by many fungi than any other sugars. But Schade (1940) and Schade and Thimann (1940) reported that some organisms like Leptomitus lacteus was unable to utilize either glucose, fructose, galactose or sucrose. Carbon sources have greater influence in the sporulation of various fungi. Mathur et al., (1950) found, xylose give excellent sporulation in Colletotrichum lindemuthianum. On the contrary, Grewal (1957) observed that xylose not at all support sporulation in Gleosporium musarum.

In the present investigation growth, sporulation and chalmydospore formation of four species of Fusarium on different carbon compounds were studied. The carbon sources used are as follows:

Monosaccharides:

D-Arabinose, L(+)Arabinose, D-Ribose, Mannose, Sorbose, and D-Glucose.

Disaccharides:

Sucrose, Cellobiose and Lactose.

Polysaccharides:

Starch and Inulin.

The reponse of four Fusarium Spp. towards different carbon sources was recorded in terms of their vegetative and reproductive growth in the culture medium after 15 days of incubation. Results obtained are recorded in Table 13 to 16 and Fig. 6 to 9.

All the four Fusarium species under present study were found capable of growing in all the carbon sources. But, the amount of growth varied in individual carbon sources. Carbon was found indispensable for the growth, because none of the organisms could grow in its absence.

Both forms of Arabinose i.e. D-Arabinose and L(+) Arabinose were moderately supported the growth in all the 4 Spp. of Fusarium. Comparatively L(+) Arabinose provided more growth in all the four species. Similar results have been reported by Grewal (1957) for Gleosporium musarum, Fisano and Plucker (1958) for Cephalosporium longisporum, Lopez and

Fergus (1965) for Fusarium roseum, Rai (1982) for Phomopsis sapotae and Arya (1982) for Phomopsis psidii.

All species of Fusarium under present investigation exhibited good growth over D-Ribose except F.moniliforme which showed moderate growth. Not much study has been done with this sugar as a carbon nutrition of fungi, however, the available literature shows it to be a poor source. Thind and Randhawa (1957a), Bhargava (1962) and Bilgrami (1962) have reported this pentose sugar as a poor source of carbon for organisms studied by them. Singh (1977) on the contrary observed moderate growth of F. solani on D-Ribose.

Moderate growth was observed in F. oxysporum and F. pallidoroseum on Mannose. F. udum and F. moniliforme showed good and poor growth on this carbohydrate respectively. many workers like Bilgrami (1956), Thind and Rawla (1958), Tandon and Bhargava (1960) and Bhargava (1962) reported Mannose as a good source of carbon for fungi. But organisms studied by Chanda (1961), Williamson (1964), Singh (1977) and Arya (1982) performed a poor growth on this hexose.

Sorbose - a keto-hexose has commonly found to be a poor source of carbon (Tandon and Bilgrami, 1959a and Prasad 1963). In the present investigation, Sorbose supported growth very well in all the species of Fusarium. Among them

F. oxysporum showed excellent growth.Singh (1977) in his study reported that F. oxysporum obtained moderate growth over Sorbose. Simple sugars can be catabolized both aerobically and anaerobically. Cochrane (1958) considered that, Sorbose interferes with the respiratory path and only those organisms which have an alternate pathway are able to thrive well on Sorbose.

D-Glucose has been found to be a good source of carbon for the growth of fungi among hexose. This is evidented from the reports of Agarwal and Agnihotri (1970), Arya (1982) and Ho and Nawawi (1991). In the present study, D-Glucose provided an excellent growth for F.pallidoroseum and F. oxysporum. In F. udum and F. moniliforme, a moderate growth was observed on this carbohydrate. Saksena and Kumar (1962) reported moderate growth in Diplodia cajani and Botryodiplodia sp.

Sucrose is a common oligosaccharide associated with higher plants and in general a good source of carbon for fungi. Most of the fungi are able to hydrolyse sucrose into glucose and fructose. Thus it is assimilated through hydrolytic pathway. However, Mandels (1954) in Myrothecium verrucaria and Raizada (1957) in many members of Mucorales reported that these organisms are able to consume this sugar through non-hydrolytic pathway. Results of the present study

showed a wide difference of growth rate in all the four organisms after 10 days of incubation. This indicates that, all the organisms take about 10 days to hydrolyse the whole sucrose into its hydrolytic products. The results also lead to an inference that, these four species of Fusarium are able to produce sucrose or trans-fructosidase enzymes in adequate amount. Good growth of fungi on sucrose medium has been reported by Subramanian (1961), Misra and Mukherjee (1962),Lopez and Fergus (1965), Singh (1977) and Arya (1982). Leaphort (1956) on Ceratocystis pilifora and Williamson (1964) on Botryodiplodia theobromae, have reported poor growth of these fungi on sucrose medium.

Lactose, a mammalian milk sugar has not been reported to occur in green plants. This can be hydrolysed into glucose and galactose. The β-linkage between glucose and galactose can be split by the help of Lactase or β-galactosidase enzyme. Hydrolysis of lactose by fungi with the help of the enzyme in medium is taking more time rather than other disaccharides. That is why lactose is considered as a poor source of carbon (Lilly and Barnett,1953). In the present investigation, all the 4 species of Fusarium showed a moderate growth on this sugar. Good growth over lactose by fungi were noticed by Agarwal (1955), Misra and Mahmood (1960b) and Williamson (1964). Singh (1977) reported lactose as a poor source for F. accuminatum, F. oxysporum

and F. solani. Similar results were reported by Bilgrami (1956), Tanaka (1956),Durairaj (1956), Thind and Randhawa (1957a), Thind and Gill (1961), Bhargava (1962), Prasad (1963), Lopez and Fergus (1965) and Srivastava (1966).

Cellobiose, a disaccharide differs from maltose only in the nature of glycosidase linkage. This sugar found to be a good source for Fusarium udum, F. pallidoroseum and F. oxysporum but, only supports moderate growth for F. moniliforme. Lilly and Barnett (1953) stated that F. conglutinans, F. lycopersia and F. niveum attained a good growth on this sugar. Singh (1977) also reported a good growth of F. oxysporum on this disaccharide.

Inulin has been reported as a poor source of carbon for most of the fungi. It is probably due to failure of the fungi to secrete enough quantity of enzyme inulase (Thind and Randhawa, 1957a; Thind and Rawla, 1958; Matsushima and Klung, 1958; Chaturvedi, 1961 and Bhargava, 1962). Penicillium digitatum is not even able to utilize this sugar (Fergus 1952). In the present study, F. udum and F. moniliforme showed good growth on inulin. F. oxysporum showed moderate growth while F. pallidoroseum developed poorly. Good growth of organisms over this sugar was reported earlier by Srivastava (1966) and Singh (1977). It has been reported to be a moderate and poor source of carbon

for organisms studied by Matsushima and Klung (1958) and Chandra (1961).

In the present study, starch has been found to be a poor source of carbon for F. udum, F. pallidoroseum and F. moniliforme. But F. oxysporum showed moderate growth. Fungi which were unable to produce amylase, failed to utilize starch. According to the reports of Fergus (1952), Tanaka (1956), and Taler and Vining (1959), there were some fungi which showed poor growth on starch. But, contrary to this Wolf (1953) observed that Ustilago zeae failed to utlize starch.

All carbon sources are not equally suitable for fruiting of fungi. Those which favouring mycelial growth, may not favour sporulation. The influence of carbon source on the sporulation of fungi was clearly established by many workers like Timnick et al.,(1951), Tandon and Agrawal (1950), Misra and Mahmood (1960b), Chaturvedi (1961), Bhargava (1962), Srivastava (1960),Khanna (1974), Singh (1977) and Arya (1982).

"In general, it is found that oligosaccharides and polysaccharides supported more fruiting than do the simple hexoses" (Cochrane, 1958). This generalization however, fits partially with the Fusarium species under present investigation.

A mixed response was seen with pentoses in sporulation of all the four species of Fusarium. On the other hand, in the case of chlamydospore formation, it is not at all supporting any species. Excellent sporulation of F. udum and F. pallidoroseum was observed on D-Ribose, while it was good on D-Arabinose. On L(+) Arabinose, it was fair and good for F. udum and F.pallidoroseum respectively. F. oxysporum showed fair sporulation on all the three pentoses tested. F. moniliforme obtained good sporulation on D-Ribose, D-Arabinose and L(+) Arabinose. Arabinose has been reported to support good sporulation of fungi studied by Grewal (1957), Chandra (1961), Bhargava (1962),Prasad (1963) and Srivastava (1966).

With hexoses, excellent sporulation was observed in the case of F. udum. Sporulation was excellent on glucose and Mannose and good on Sorbose for F. pallidoroseum. In F.oxysporum, glucose and Mannose cherished good sporulation but Sorbose poorly supported it. Good sporulation was obtained in F. moniliforme over all hexoses tested. The favourite to Glucose as a carbon source for the sporulation of various fungi has been reported earlier by many mycologists like Das Gupta and Shoma (1960), Misra and Mahmood (1960b),Bhargava (1962), Srivastava (1960), Khanna (1974), Shivkumar (1975); and Singh (1977). Mannose support

excellent sporulation in Fusarium solani and Botryodiplodia ananasae (Bhargava,1962). B. theobrome (Prasad,1963) showed good sporulation over Sorbose. Chlamydospore formation was poor on glucose in all the 4 Fusarium Spp. under present study. F.udum, F. pallidoroseum and F. oxysporum developed chlamydospores poorly on Mannose. F. moniliforme failed to develop Chlamydospores in the presence of Mannose. F. udum and F. pallidoroseum developed chlamydospores poorly on Sorbose. On the other hand F. oxysporum and F. moniliforme failed to form chlamydospores on this sugar.

Sporulation of all the four species of Fusarium under present investigation was either excellent or good on disaccharides except on cellobiose where, fair sporulation for F. pallidoroseum and poor sporulation for F. oxysporum was observed. Sucrose has been found to be an excellent source for sporulation in the cases of F. pallidoroseum and F. moniliforme. Misra and Mahmood (1960b), Das Gupta and Shoma (1960),Bhargava (1962) and Srivastava (1966) also reported, sucrose as an excellent carbon source for sporulation. In F. udum and F. oxysporum sucrose supported good sporulation. F.udum poorly develop chlamydospores on sucrose media while other three species failed to develop it on the same. Lactose supported excellent sporulation in F.udum and F.pallidoroseum and good sporulation for F. oxysporum and F. moniliforme. According to Timnick et

al.(1951) and Misra and Mohmood (1960b) lactose supported good sporulation in the organisms they studied. Singh (1977) reported similar results on F. accuminatum and F. solani. Srivastava (1966) reported that lactose failed to induce sporulation of different isolates of Botryodiplodia theobromae. Chlamydospores were not developed in F. udum, F. oxysporum and F. moniliforme while it was poorly formed in the case of F. pallidoroseum,on lactose medium. Cellobiose supported only F.udum and F. moniliforme for good sporulation. Bhargava (1962), Williamson (1964) and Singh (1977) reported that, Cellobiose is a good carbon source for sporulation in fungi which they studied.

Although, the two earlier said disaccharides, poorly or not at all supported chlamydospore formation in the four species, of Fusarium studied. Cellobioase supported excellent chlamydospore formation in F.udum and F. pallidoroseum. Normally, chlamydospores are formed during the later stages of grwoth or at adverse conditions like lack of nutrients. Here the only explanation for chlamydospore formation at an early stage is catabolic repression (Bilgrami and Dube 1976). Cellobiose is hydrolysed into glucose residues in the early stages of incubation. When the amount of glucose increases in the medium it inhibits the synthesis of cellobiose degrading

enzyme and ultimately leads to a situation of lack of nutrient or carbon source. This induce the formation of chlamydospores.

Inulin as a carbon source supported good sporulation in F.udum and F. moniliforme. It poorly supported the sporulation in the case of F. pallidoroseum and F. oxysporum. Singh (1977) reported Inulin as an excellent supporter of sporulation in F. accuminatum and F. solani. However, Thind and Randhawa (1957a) found Inulin as a poor carbon source for sporulation in fungi. Chlamydospore formation was fair in all species of Fusarium except F. udum where it was very poor.

Starch - a common polysaccharide was not a supporter of sporulation in Fusarium Spp. under present study except in F. pallidoroseum, where it supported a good amount of sporulation. At the same time there was an excellent chlamydospore formation in all the species. Both Tandon and Agrawal (1951), Chandra (1961), Srivastava (1966) and Singh (1977) reported starch as a good carbon source for sporulation. Shreemali (1971) reported that, the organism which he studied was failed to sporulate over starch medium.

At the end of incubation period, media containing different carbon sources showed a drift in pH. The drift was

near to neutral side in many cases, but in few cases it almost reached the alkaline side.

The results clearly substantiate the fact that all the four species of Fusarium are specific in their growth and sporulation response on various carbon sources, and that there is no strict interaction between growth and sporulation. Although, in general these four species showed similar nutritional response like other members of moniliales, yet their behaviour was altogether different from each other within the group and even among other species of Fusarium studied so far by various investigators.

Table 13 : Average dry weight (in mg), Sporulation, Chlamydospore formation, final pH of *Fusarium udum* on different carbon sources.

Sl. No.	Carbon Source	Dry weight	Sporulation	Chlamydospore formation	Final pH
1	D-Arabinose	27.63	Good	Poor	6.2
2	L(+)Arabinose	54.67	Fair	-	8.1
3	Ribose	98.62	Excellent	-	8.2
4	Mannose	77.54	Excellent	Poor	7.5
5	Sorbose	84.35	Excellent	Poor	8.0
6	Glucose	91.68	Excellent	Poor	7.9
7	Sucrose	84.31	Excellent	Poor	7.3
8	Lactose	41.33	Excellent	-	6.9
9	Cellobiose	87.06	Good	Poor	7.2
10	Starch	22.60	Poor	Fair	7.9
11	Inulin	67.12	Good	Poor	8.1
12	Without carbon sources	0.00	-	-	5.5
	G.M.	61.41			

Summary of the dry weight results and conclusion at 5% level of P.

Treatments : Highly singificant
Replicates : Non significant
S.E. : 5.16
C.D. at 5% level : ± 14.8

Dry weight results :

$$3>6>9>\overline{5\ 7}>4>11>2>8>1>10>12$$

Table 14 : Average dry weight (in mg), Sporulation, Chlamydospore formation, final pH of Fusarium pallidoroseum on different carbon sources.

Sl. No	Carbon sources	Dry weight	Sporulation	Chlamydospore formation	Final pH
1	D-Arabinose	23.27	Good	Poor	6.5
2	L(+)Arabinose	63.25	Good	Poor	8.4
3	Ribose	99.73	Excellent	Poor	8.0
4	Mannose	60.48	Excellent	Poor	6.6
5	Sorbose	75.36	Excellent	Poor	7.9
6	Glucose	104.19	Good	Poor	8.0
7	Sucrose	77.92	Excellent	-	6.6
8	Lactose	51.01	Excellent	Poor	6.7
9	Cellobiose	76.34	Fair	Excellent	7.1
10	Starch	19.45	Good	Fair	8.0
11	Inulin	22.48	Poor	Fair	6.9
12	Without carbon sources	0.00	-	-	5.5
	G.M.	56.12			

Summary of dry weight result and conclusion at 5% level of P.

Treatments : Highly singificant
Replicates : Non significant
S.E. : 5.40
C.D. at 5% level : ± 15.59

Dry weight results :

6>3>7 9 5>2 4>8>1 11 10>12

Table 15 : Average dry weight (in mg), Sporulation, Chlamydospore formation, final pH of Fusarium oxysporum on different carbon sources

Sl. No	Carbon source	Dry weight	Sporulation	Chlamydospore formation	Final pH
1	D-Arabinose	22.23	Fair	Fair	6.4
2	L(+) Arabinose	61.91	Fair	-	7.9
3	Ribose	89.07	Fair	-	8.3
4	Mannose	57.34	Good	Poor	6.2
5	Sorbose	90.31	Poor	-	8.2
6	Glucose	96.75	Good	-	7.8
7	Sucrose	61.80	Good	-	6.9
8	Lactose	52.56	Good	-	6.7
9	Cellobiose	82.92	Poor	Poor	7.3
10	Starch	40.24	Fair	Excellent	7.8
11	Inulin	67.16	Poor	Fair	8.3
12	Without carbon sources	0.00	-	-	5.5
	G.M.	60.19			

Summary of dry weight result and conclusion at 5% level of P.

Treatments : High significant
Replicates : Non significant
S.E. : 4.67
C.D. at 5% level : $\pm$ 13.48

Dry weight results:

$$6>\overline{5\ 3}>9>11>\overline{2\ 7}>4>8>10>1>12$$

Table 16 : Average dry weight (in mg), Sporulation, Chlamydospore formation, final pH of Fusarium moniliforme on different carbon sources

Sl. No	Carbon sources	Dry weight	Sporulation	Chlamydospore formation	Final pH
1	D-Arabinose	39.32	Good	-	6.8
2	L(+)Arabinose	48.04	Good	-	7.6
3	Ribose	49.96	Good	Poor	8.4
4	Mannose	35.01	Good	-	6.2
5	Sorbose	49.64	Good	-	7.4
6	Glucose	72.55	Good	Poor	8.0
7	Sucrose	84.38	Excellent	-	7.5
8	Lactose	49.19	Good	-	6.4
9	Cellobiose	53.80	Good	-	7.0
10	Starch	27.84	Poor	Poor	8.1
11	Inulin	64.47	Good	Fair	8.3
12	Without carbon source	0.00	-	-	5.5
	G.M.	47.86			

Summary of the dry weight results and conclusion at 5% level of P.

Treatments : Highly significant.
Replicates : Non significant
S.E. : 3.52
C.D. of 5% level : ± 10.14

Dry weight result:

7>6>11>9>3 5 8 2>1>4>10>12

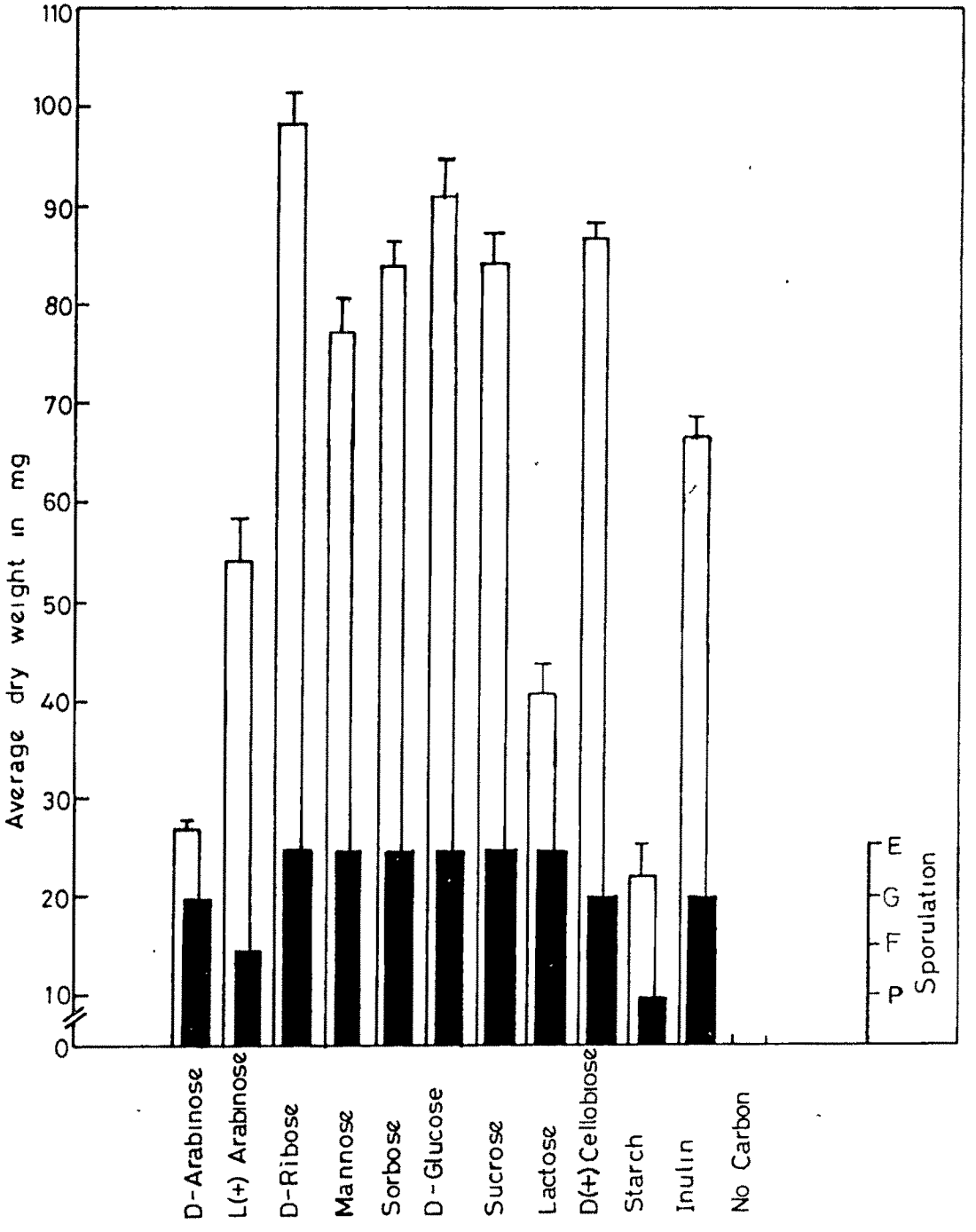


Fig.6 Average dry weight (entire bar) and sporulation (shaded) of Fusarium udum on different carbon sources

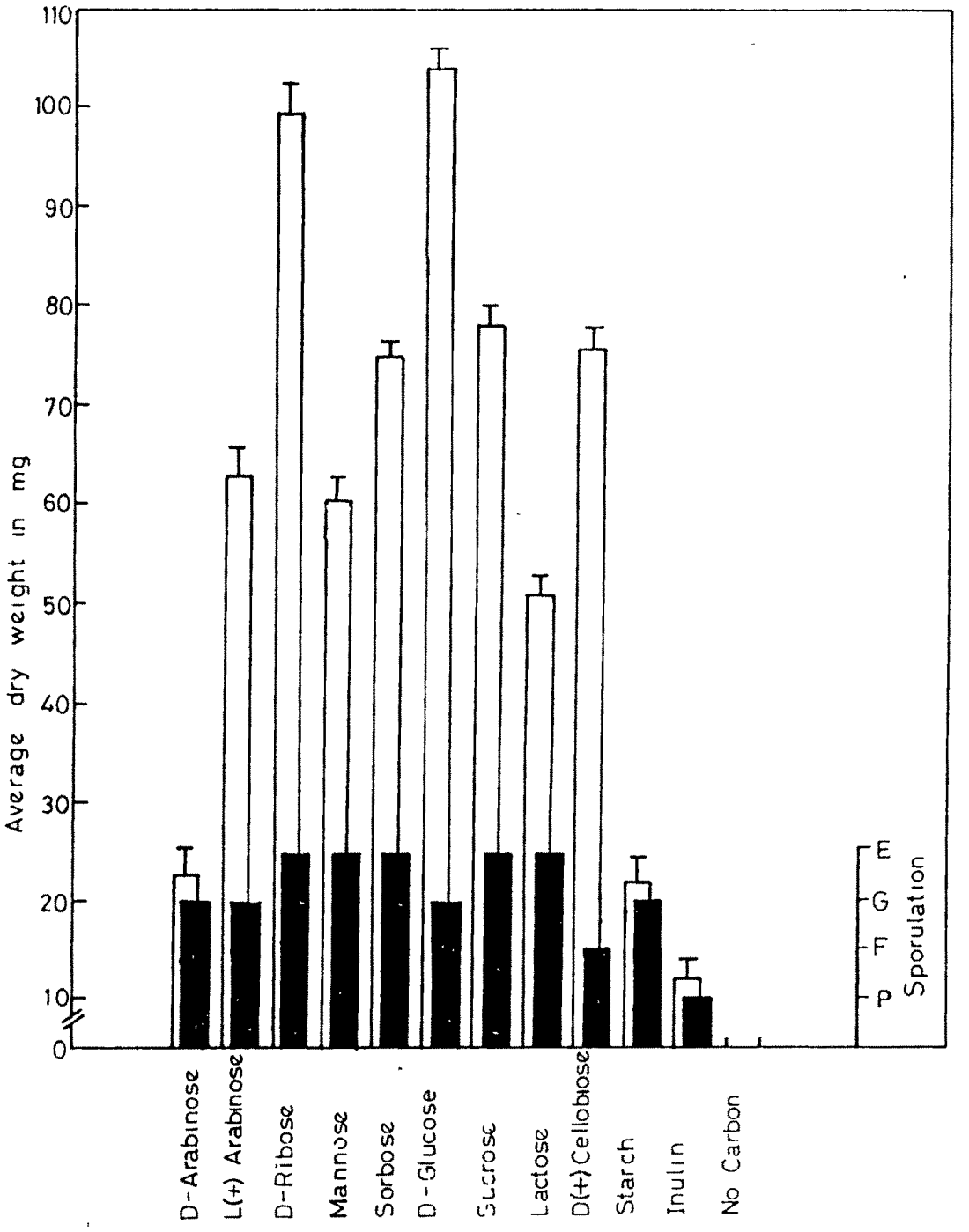


Fig 7 Average dry weight (entire bar) and sporulation (shaded) of Fusarium pallidoroseum on different carbon sources

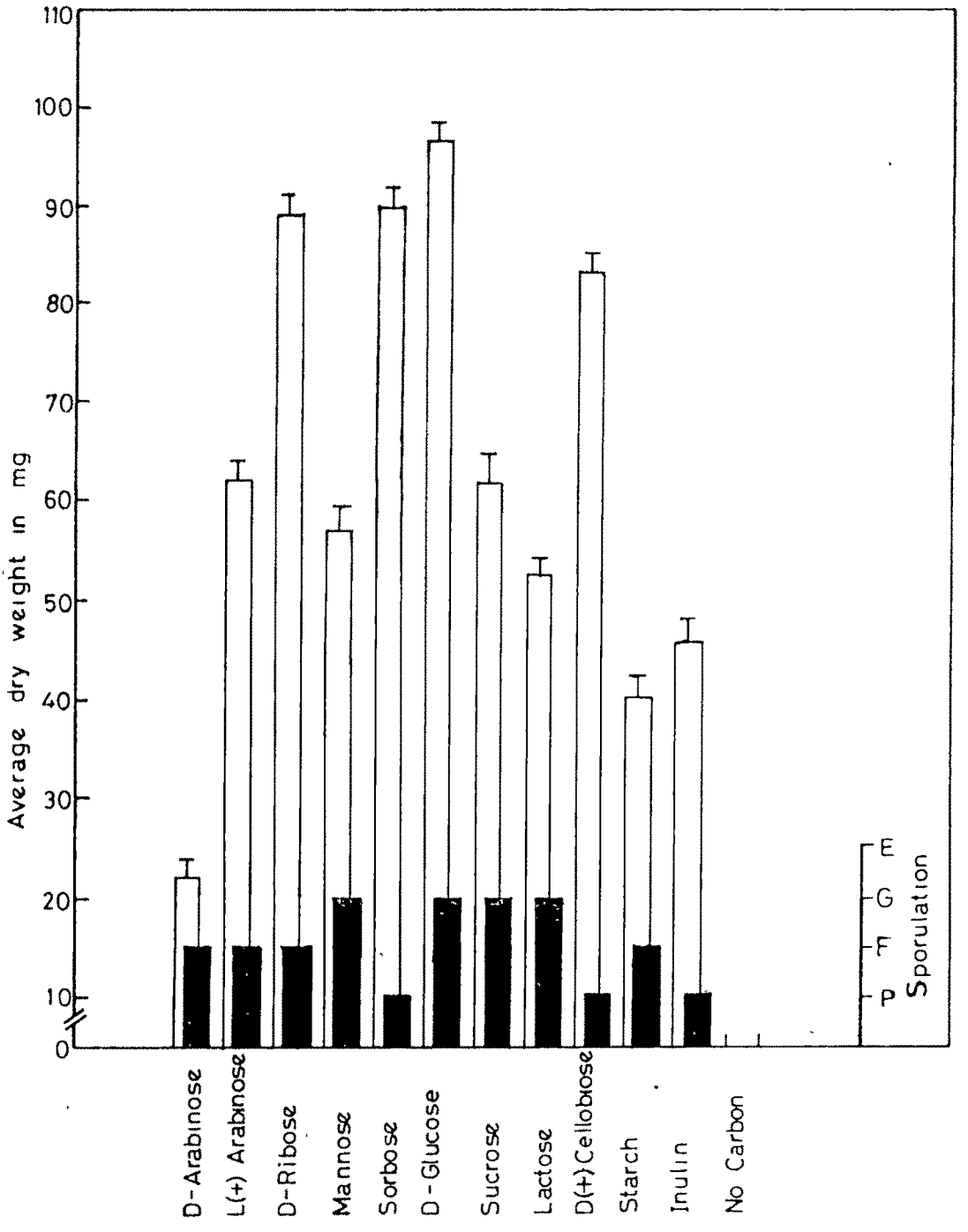


Fig 8 Average dry weight (entire bar) and sporulation (shaded) of Fusarium oxysporum on different carbon sources

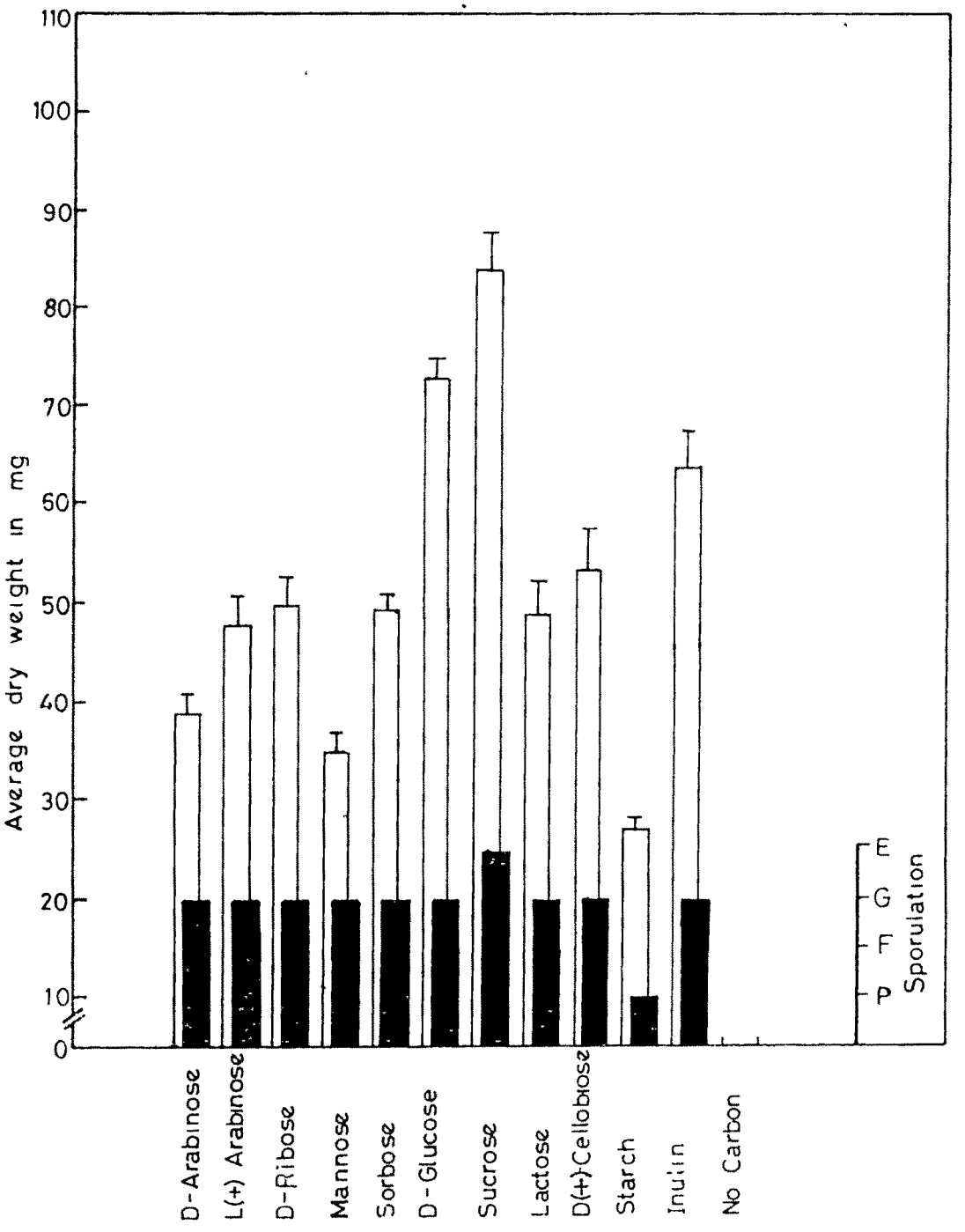


Fig 9 Average dry weight (entire bar) and sporulation (shaded) of Fusarium moniliforme on different carbon source.

3.4.1 Utilization of Various Carbohydrates

Chromatographic analysis of the medium was conducted daily upto 15 days to observe the utilization of various carbohydrates. The results are summarized in table 17 and figure 10. On the basis of this analysis, an attempt has been made to establish the pathway of utilization - hydrolytic (indirect) or non-hydrolytic (direct) of di and polysaccharides. The details of the methods used for the present study have been given in the chapter dealing with materials and method.

Monosaccharides play an important role in the carbohydrate metabolism of fungi. Complex carbohydrates usually first split-up into monosaccharide units or their derivatives, which subsequently enter into various metabolic pathways. Monosaccharides also take part in the synthesis of reserve food materials of fungal mycelium and various fungal polysaccharides are those which, consist of monosaccharide units such as glucose, mannose, galactose etc. (Arya, 1982).

D-Arabinose (Rf.0.70) occurs in nature in the form of arabans, a common constituent of plant polysaccharide and various gums especially gum arabica. Chromatographic

analysis of the medium showed that none of the Fusarium spp. could assimilate this sugar completely within 15 days of incubation. At the end of incubation, the pH of the culture medium remained in acidic side in all the cases.

L(+)Arabinose (Rf.0.70) has been found to be the more utilizable form than its D-form. In the present study this aldopentose was present in the medium upto 13 days in F. udum. Similar results are reported in Phomopsis viticola (Arya,1982) and Phomopsis sapotae (Rai,1982). In F. pallidoroseum and F. oxysporum this carbohydrate was consumed within 10 days. F. moniliforme took 14 days for complete utilization of L(+)Arabinose. Singh (1977) reported that F. oxysporum utilized this pentose sugar in 7 days of incubation. In all the four cases the final pH of the medium was shifted to alkaline side. Maximum shift was recorded in F. pallidoroseum.

Fusarium oxysporum and F.moniliforme were able to utilize D-Ribose (Rf.0.71) within a period of 6 and 4 days respectively. But, F. udum and F. pallidoroseum took more time and utilized it in 8 days. Singh (1977) reported F. acuminatum and F. solani were able to finish this sugar within a period of 4 days and F. oxysporum in

8 days. The final pH of the medium became alkaline in all the cases at the end of incubation.

Mannose (Rf.0.58), an aldohexose is a stereo isomer of D-glucose and galactose. It occurs in nature as a component sugar of the polysaccharide seminine. In the present investigation mannose was readily assimilated by F. udum on 5th day while F. pallidoroseum, F. oxysporum and F. moniliforme took 6 days to finish it completely. In all the cases the final pH of the medium remained in acid side, except in F. udum where it was slightly above neutral value.

Any of the four species of Fusarium studied during the present investigation were not able to utilize Sorbose (Rf.0.57) within the incubation period (15 days). Similar results were also reported by Chowdhury (1981), Rai (1982) and Arya (1982). However, F. oxysporum studied by Singh (1977) was able to utilize this ketohexose within 7 days of incubation period. During the present investigation, the final pH of the medium in all the cases was shifted to alkaline side and maximum drift was recorded in F.oxysporum.

Glucose (Rf.0.60) is an aldohexose found in various parts of plants. This hexose sugar is present in complex

carbohydrates like starch and cellulose and is also a component of many disaccharides like sucrose, lactose, maltose and cellobiose. Glycogen the most common reserve carbohydrate of fungi is composed of glucose units. In the present study all the four species of Fusarium readily utilized this sugar i.e. within 3 days. Singh (1977) observed that F. accuminatum and F. solani consumed this carbohydrate within a period of 4 days. The final pH of the culture medium drifted towards alkalinity in all cases.

The oligosaccharides are complex sugars composed of two or more monosaccharide units linked together by glycosidic bonds. They occur freely in nature or as the units of polysaccharides. These water soluble compounds yield monosaccharide components on hydrolysis. Two theories have been advanced regarding the utilization of oligosaccharides (Lilly and Barnett, 1953). Therefore two pathways are recognised in the utilization of oligosaccharides. In one case, oligosaccharides are hydrolysed into their component sugar and then utilized. This is known as indirect or hydrolytic pathway. The failure to utilize an oligosaccharide may be due to the incapability of the organism to synthesis the necessary hydrolytic enzyme. Oligosaccharides are also utilized by

pathways which do not require hydrolysis (Phosphorylation or oxidation) before being catabolised. This is called the direct or non-hydrolytic pathway. It has been found that different fungi assimilate individual oligosaccharides with varying degrees of efficiency. Albritton (1953) reported that fewer fungi were able to utilize oligosaccharides than the monosaccharides.

Sucrose (Rf.0.43) is of common occurrence in plants. A large number of workers have shown that most of the fungi are able to hydrolyse sucrose in to glucose and fructose and thus it is assimilated through a hydrolytic pathway. However, a few fungi like Myrothecium verrucaria (Mandels,1954) and members of Mucorales (Raizada,1957 and Sarbhoy,1965) were able to consume this sugar through a non-hydrolytic pathway. Results of the present investigation indicates that all the organisms studied were utilized this sugar through hydrolytic pathway and its rate of conversion varied within the organisms. This disaccharide was completely hydrolyzed and consumed by F. pallidoroseum and F. oxysporum within 10 days. F.moniliforme took only 8 days to utilize this sugar. But, F. udum took a prolonged period upto 12 days to finish this sugar and its hydrolic products. In F. pallidoroseum and F. oxysporum the final pH remained in the acidic side, while in F. udum and F. moniliforme it slightly drifted to alkaline side.

Lactose (Rf.0.31) is present in all mammalian milk, but not reported from plants so far. This β-galactoside glucose on hydrolysis gives a molecule each of glucose and galactose. In the present study none of the Fusarium species could utilize this sugar with in 15 days of incubation. Similar result was earlier reported by Tandon (1967), Singh (1977), Chowdhury (1981), Rai (1982) and Arya (1982). Though, there is an increase in pH of the medium in all cases, yet it remained in the acidic side only.

Hydrolysis and utilization of Cellobiose (Rf.0.35) by present Fusarium species varied considerably. F. udum and F.oxysporum consumed this disacchardide within 10 days of incubation. F. pallidoroseum took 14 days to utilize this sugar completely, while F. moniliforme failed to utilize it within the period of incubation. Singh (1977) reported that F. accuminatum was able to utilize it within 11 days, but F. oxysporum and F. solani were failed to utilize it completely within the incubation period (12 days). The drift in final pH of the medium was to neutrality in all cases in the present Fusarium species.

Polysaccharides are the carbohydrates of complex polymeric structure containing a large number of monosaccharide units. When all the units are of same

sugar, the polysaccharide is designated as homopolysaccharide, while those comprising two or more different monosaccharide units are called heteropolysaccharides. Polysacchardies occur in higher plants can be mainly grouped into two types. (1) Structural polysaccharides which are found in cell wall and other extraprotoplasmic inclusions and (2) the 'reserve' or 'nutrient' polysaccharides which are stored in the plant tissue to be used during the period of active metabolism. Cellulose, pectic substances and chitin are the most common structural polysaccharides and starch, dextrin, glycogen and inulin are the common reserve polysaccharides. Polysaccharides are usually available to most of the fungi but the rate with which they utilized depends on the nature and structural configuration of these carbohydrates as well as on the organism involved. Fungi convert these complex carbohydrates into soluble sugars of low molecular weight before utilization. The ability of an organism to utilize a polysaccharide depends upon its capacity to produce enzymes necessary for the conversion.

The utilization of starch and inulin - reserve polysaccharides were observed in the present investigation and results are summarized in table 17.

Starch is a principal reserve polysaccharide of plants and have an α-1,4-glucose polymer structure. On complete hydrolysis it yields D-glucose. Systematically the enzymatic hydrolysis of starch is as follows:

Starch ⟶ Dextrin ⟶ Maltose ⟶ D-glucose

This carbohydrate showed different rate of utilization among the four Fusarium species studied. F. udum and F. pallidoroseum were failed to utilize this carbohydrate within the incubation period. F. oxysporum utilized it within 12 days, and F. moniliforme took 14 dys for complete consumption of this polysaccharide. The final pH of the medium shifted to alkaline side at the end of incubation period in all the cases.

Inulin, a D-fructose containing polysaccharide consist of a sucrose unit linked to a large number of fructose residues. Fungi utilize this polysaccharide through enzymatic degradation and involves the enzyme inulase. This mechanism is known from very early findings of Borquelot (1893). It is expected that inulin would yield fructose and a small quantity of glucose on complete hydrolysis. The results obtained by Agnihotri (1963) indicated that during the utilization of inulin by four species of Aspergillus, no glucose could traced in

any case. However, neither glucose nor fructose was detected in any species of Fusarium under the present investigation. Similar result was also reported by Arya (1982) in four species of Phomopsis. The absence of these sugars in the medium during the utilization of inulin by Fusarium spp. may be due to the slow breakdown of inulin and simultaneous utilization of breakdown products from the medium. Final pH of the medium shifted to neutrality in F. pallidoroseum and to alkalinity in rest of the three.

It is evident from the present investigation that in cases where the rate of assimilation of sugar was slow, there was an increase in mycelial dry weight upto the end of the incubation period. On the other hand, the sugar utilization in the early period of incubation decreases the dry weight yield. This decline in the dry weight may be attributed to the autolysis or to the mobilization of reserve materials which enter the respiratory pool, when external supply of sugar was exhausted.

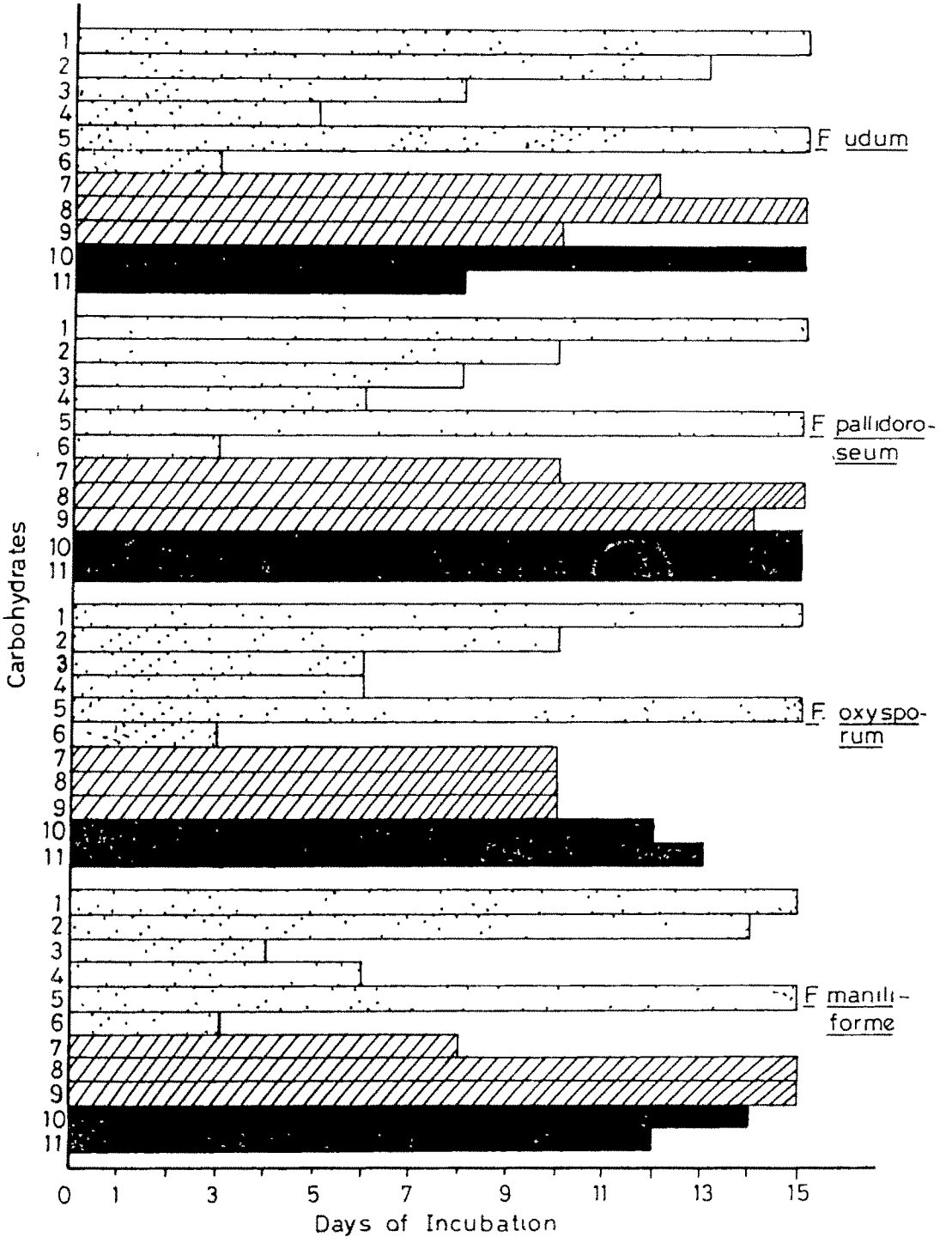
It has been reported by Giri et al. (1953 and 1954), Bilgrami (1964) and Ghosh (1966) that, a number of fungi synthesize oligosaccharides during their growth on different di- or polysaccharide medium. In the present

investigation no oligosaccharide was detected in the culture medium of four species of Fusarium.

Table 17: Rate of utilization of carbon source and drift in *final pH of culture medium in four species of Fusarium

Sl. No.	Carbon sources	F.udum		F.pallidoroseum		F.oxysporum		F.moniliforme	
		Rate (in days)	Drift in pH	Rate (in days)	Drift in pH	Rate (in days)	Drift in pH	Rate (in days)	Drift in pH
1	D-Arabinose	0-15	+0.7	0-15	+1.0	0-15	+0.9	0-15	+1.3
2	L(+)Arabinose	0-13	+2.6	0-10	+2.9	0-10	+2.4	0-14	+2.1
3	Ribose	0-8	+2.7	0-8	+2.5	0-6	+2.8	0-4	+2.9
4	Mannose	0-5	+2.0	0-6	+1.1	0-6	+0.7	0-6	+0.7
5	Sorbose	0-15	+2.5	0-15	+2.4	0-15	+2.7	0-15	+1.9
6	Glucose	0-3	+2.4	0-3	+2.5	0-13	+2.3	0-3	+2.5
7	Sucrose	0-12	+1.8	0-10	+1.1	0-10	+1.4	0-8	+2.0
8	Lactose	0-15	+1.4	0-15	+1.2	0-10	+1.2	0-15	+0.9
9	Cellobiose	0-10	+1.7	0-14	+1.6	0-10	+1.8	0-15	+1.5
10	Starch	0-15	+2.4	0-15	+2.5	0-12	+2.3	0-14	+2.6
11	Inulin	0-8	+2.6	0-15	+1.4	0-13	+2.8	0-12	+2.8

* Initial pH = 5.5



1 D-Arabinose, 2 L(+) Arabinose, 3 Ribose, 4 Mannose, 5 Sorbose, 6 Glucose, 7 Sucrose, 8. Lactose, 9 Cellobiose, 10 Starch, 11 Inulin

Fig 10 Rate of utilization of carbon source (in days) by four *Fusarium* species

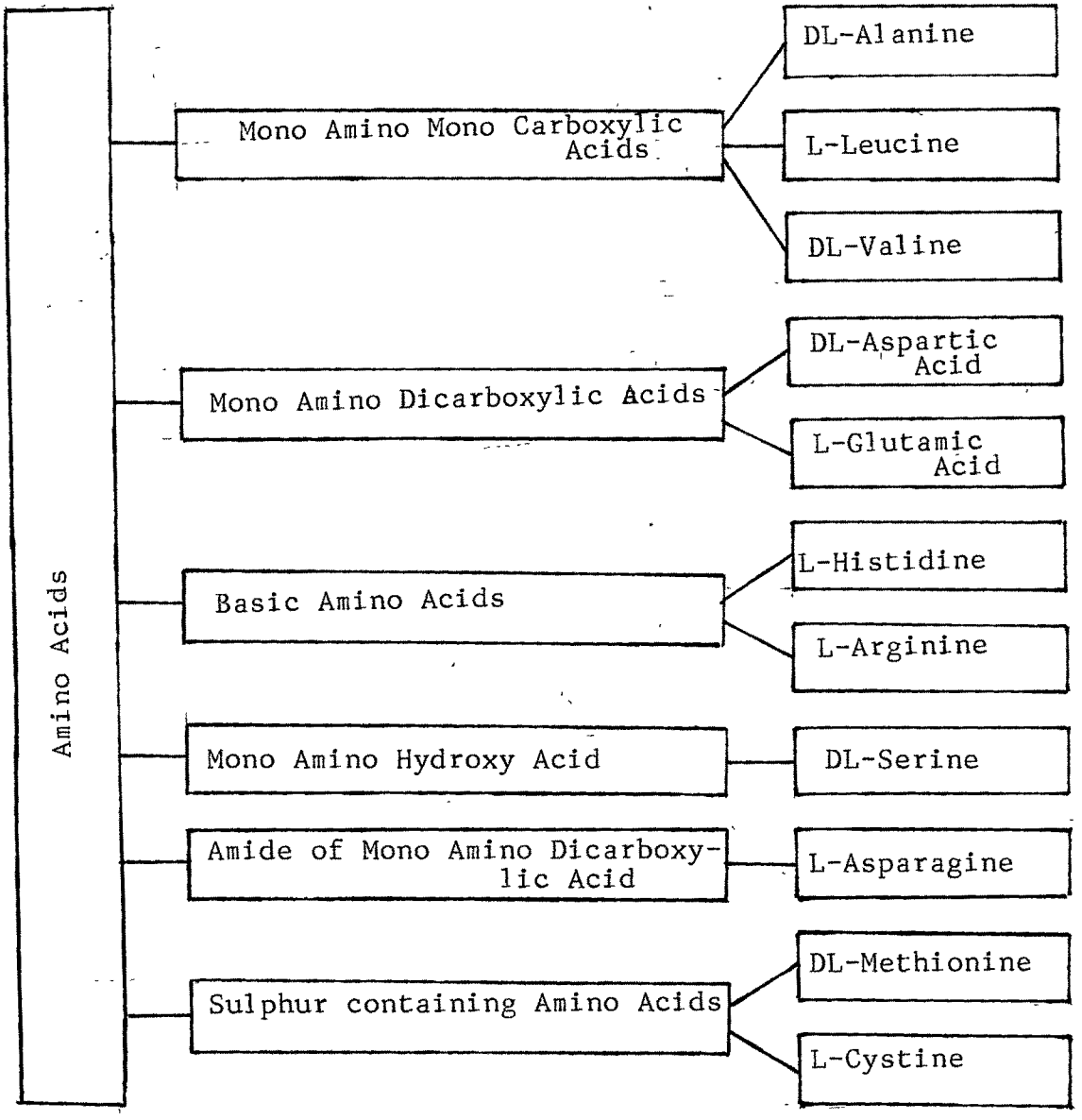
3.5 Effect of different Amino Acids as nitrogen source and their utilization

Nitrogen is an essential element for fungi. It has structural as well as functional importance and occurs in fungi in the form of complex organic nitrogenous compounds. According to Srinivasapai (1953), the nitrogen fraction of the hyphae of Fusarium species varies with the form of nitrogen available as nutrient. Both organic and inorganic forms of nitrogen are available to fungi from nature, but as far as utilization is concerned they intrinsically contradict from each other in their metabolic potentialities.

"No single pattern of nitrogen assimilation can be described to apply for all fungi" (Cochrane 1958). According to previous reports, the amino acids are converted into ammonia before their assimilation by fungi. Lilly and Barnett (1951) stated that nitrogen is liberated in the form of ammonia during the process of deamination and it is then utilized by most fungi.

In the present study, the effect of various amino acids on growth and sporulation of the four Fusarium species has been investigated. The utilization of amino acids was studied chromatographically by taking culture filtrates from

each set for 15 days (Figs 15 to 20). The following amino acids were used for the present investigation.



A set of medium was also prepared without any source of nitrogen as a control. Growth, sporulation and chlamydospore formation for 5, 10, and 15 days were recorded (Tables 18 to 21, figs.11 to 14).

It is apparent from the above table that the four Fusaria were incapable of growing on a medium devoid of any source of nitrogen even though they could assimilate a wide range of amino acids. Yet their individual efficiency varied considerably.

DL-Alanine was singificantly a good source for the growth of all Fusarium species. F. moniliforme attained maximum dry weight on 10th day among all the amino acids used in the present study. Similar results were reported by Bhargava (1970) in Fusarium solani and Singh (1977) in three species of Fusarium. The present organisms however, differ from the organisms studied by Kapoor (1968) and Singh (1968) on the ground of their moderate and poor growth on this amino acids. This aminoacid was assimilated by F.udum and F.pallidoroseum in 7 and 9 days respectively while, F. oxysporum and F. moniliforme took 10 days for the same. Jamaluddin (1973) reported that the two isolates of Aspergillus flavus utilised it within 7 days of time. Singh (1977) also reported that F. accuminatum and F. oxysporum

were able to utilize this amino acid within 7 days and 10 days respectively. F. udum attained maximum dry weight on 5th day, but in rest of the species it was on 10th day. Final pH of the culture filtrate showed a general trend to shift to neutral side at the end of incubation period. In F.pallidoroseum pH attained exact neutral value while in F. udum it was slightly above neutral value. In other two cases it was below neutral value i.e in acidic side.

L-Leucine produced moderate growth in all the organisms under present investigation. Similar result was observed by Singh (1977) in Fusarium Spp. including F. oxysporum. Tandon (1964) Singh (1968), Papavizas (1970) and Sehgal and Prasad (1971) have reported good growth in organisms studied by them.This mono amino monocarboxylic acid was completely utilized in 8 days by F. udum, F.pallidoroseum and F. moniliforme. It took 11 days for F. oxysporum to consume this amino acid completely. Singh (1977) also reported similar observation on three Fusarium species. It is evident from figure 15 that dry weights of present Fusaria on L-Leucine were maximum on 5th day, except in F. oxysporum, where it was on 10th day. The final pH of the culture filtrate drifted towards neutral side.

DL-Valine was found to be a good source of nitrogen for all Fusarium species. Similar observations were also

recorded by Bhargava (1970) for F. solani and Singh (1977) for F. accuminatum, F. oxysporum and F. solani. The present organisms differ from the organisms studied by Papavizas (1970) and Jamaluddin (1973) in their growth pattern.

In the case of F.udum and F. pallidoroseum DL-Valine remained in the culture medium up to 8 and 9 days respectively. Hasija (1970) reported that, the two species of Alternaria consumed DL-Valine in 8 days. F. oxysporum and F. moniliforme utilized this amino acid poorly and its presence was recorded up to the end of incubation period (Fig. 15). Singh (1977) reported that F. oxysporum could not utilize DL-Valine fully during the incubation period (12 days). According to Jamaluddin (1973) the amino acid was present till the end of incubation period (15 days) in the medium used by Aspergillus niger and A. flavus. The rate of growth decreased in F. udum and F. pallidoroseum, while it increased in F. oxysporum and F. moniliforme. The final pH of culture medium was in the acidic range in F. udum,and F. oxysporum. But it reached neutral in F. moniliforme. In F. pallidoroseum the final pH was slightly alkaline at the end of incubation.

DL-Aspartic acid as a nitrogen source, supported good growth in F. udum and F. oxysporum, while in F. pallidoroseum and F. moniliforme it supported moderate

growth only. Results of this kind was reported earlier by many workers like Strider and Wirstead (1960), Kurtz and Fergus (1964), Sehgal and Prasad (1971), Jamaluddin (1973), Singh (1977) and Arya (1982). However, Tandon and Chandra (1962), Singh (1968) and Arya (1982) found that, this amino acid is not a good source for Cercosporina ricinella, Aspergillus niger and Phomopsis sp. respectively. This amino acid was poorly utilized by the present species of Fusarium and was detected till the end of incubation period except in F. udum where it lasted up to 14 days only (Fig.16). An increasing growth rate was observed in all the cases. In F. pallidoroseum and F. moniliforme the final pH remained in the acidic range, but in F. oxysporum it attained neutrality and in F.udum it gone upto alkaline side.

L-Glutamic acid supported good growth in present Fusarium species. Tandon and Varma (1962), Thind and Maclan (1969), Bhargava (1970), Sehgal and Prasad (1971), Singh (1977) and Arya (1982) also reported that their organisms showed good growth on this amino acid. On the contrary, Chandra (1961) found it to be a poor source of nitrogen for the growth of Cercosporina ricinella. This mono amino dicarboxylic acid was found to be a satisfactory source of nitrogen for the four Fusarium species under study. However, they failed to consume it within 15 days (Fig.16). According

to Kapoor (1968) and Jamaluddin (1973), none of the fungi studied by them could assimilate L-Glutamic acid in 15 days. They also found it to be a satisfactory source for growth of fungi. The dry weight increased till the 15th day in all the cases. The final pH of the medium drifted towards alkaline side in F. udum, F. pallidoroseum and F. oxysporum, whereas, it became neutral in the case of F. moniliforme. Singh (1977) found that final pH of L-Glutamic acid medium drifted towards neutrality in F. oxysporum at the end of incubation period.

L-Histidine induced poor growth in all the four Fusarium species under present investigation. It has also been reported as a poor source of nitrogen by Jamaluddin (1973) and Khanna (1974). Absence of growth was recorded by Papavizas (1970) for Sclerotium cepivorum on this amino acid. Singh (1977) for Fusarium accuminatum, F. oxysporum and F. solani and Arya (1982) for Phomopsis gulabia and Phomopsis pedilanthi also found this amino acid as a poor source of nitrogen. Contrary to these reports Bhargava (1970) reported good growth of F. solani in this amino acid.

F. udum and F. pallidoroseum utilized L-Histidine within 8 and 10 days respectively, whereas F.oxysporum and F. moniliforme took a prolonged period of 14 and 13 days

respectively (Fig.17). Only F. oxysporum showed an increase in dry weight till the end of incubation period. In F. pallidoroseum and F. moniliforme an early increase and then a decrease was observed. But in F. udum the dry weight decreased on the 15th day as compared to 5th and 10th days of incubation. The pH of culture filtrate remained in acidic side in F. oxysporum and F. moniliforme, while it changed to alkaline in the case of F. pallidoroseum, and reached neutral in F. udum at the end of incubation period.

L-Arginine supported moderate growth of F. udum and F. pallidoroseum, while F. oxysporum and F. moniliforme grew poorly in this aminoacid. The latter two organisms thus resembled F. solani (Bhargava,1970), Gliocephalotrichum bulbilium (Jamaluddin,1973), and F. oxysporum (Singh,1977), and former two organisms resembled F. accuminatum and F. solani (Singh 1977).

L-Arginine was readily consumed by the present Fusarium Spp. than the other basic amino acid, L-Histidine (Fig.17). F.udum and F. pallidoroseum could utilize this amino acid within 7 days. F. oxysporum and F. moniliforme have taken 9 and 8 days respectively. The dry weight of mycelium of the present organisms decreased on 15th day as compared to 5th and 10th days. But in the case of F. oxysporum the maximum growth was recorded on 10th day. The final pH of the medium was acidic in all cases.

L-Serine supported good growth, only in F. moniliforme. In other three organisms it supported a moderate growth. In this respect F. moniliforme can be compared to Macrophomina Sp. and Drechslera australiense (Kapoor 1968), F. oxysporum and F. solani (Singh 1977). Jamaluddin (1973) recorded poor and moderate growth of two isolates of Aspergillus flavus. Out of the 4 species of Phomopsis studied by Arya (1982) one species showed moderate growth while others have shown good growth on this aminoacid. Papavizas (1970) reported that L-Serine was not utilized by Sclerotium cepivorum.

Comparatively the present Fusaria were able to utilize L-Serine in lesser time. F. udum and F. pallidoroseum could utilize it in 6 and 8 days respectively, while F. oxysporum and F. moniliforme took 10 and 11 days correspondingly (Fig.18). Similar results have been recorded by Jamaluddin (1973). While contradictory results were obtained by the studies done by Prasad (1963), Kapoor (1968) and Hasija (1970). The mycelial dry weight of three species i.e. F. udum, F. pallidoroseum and F. oxysporum decreased on 15th day than on the 5th and 10th days. In F. moniliforme an increase in dry weight was recorded on 10th day but it decreased on 15th day. The final pH of the medium was at neutral point in all the cases except F. udum,where it reached up to alkaline side.

L-Asparagine supported poor growth in all the Fusaria under study. F. oxysporum and F. moniliforme showed very poor growth in comparison with other two species. Similar results were recorded by Singh (1977). However, moderate growth of F. coeruleum (Tandon and Agarwal,1957) and two isolates of Aspergillus flavus (Jamaluddin,1973) were recorded on this amino acid. The present fungi also differed from the organisms studied by Sehgal and Prasad (1970) and Arya (1982).

Fusarium udum and F. pallidoroseum consumed L-Asparagine within 6 days of incubation time. F. oxysporum took 7 days for the same while F. moniliforme utilized it within 9 days (Fig.19). Similar results were recorded in three species of Fusarium studied by Singh (1977). The dry weight of mycelium in all organisms under study diminished on 15th day. The drift in pH was up to neutral in F. moniliforme, and slightly alkaline in F. pallidoroseum, but in other two cases it remained in acidic side only.

DL-Methionine has been found to be a poor source of nitrogen for F. udum, F. oxysporum and F. moniliforme, while in F. pallidoroseum it supported a fair growth. The behaviour of the former three fungi were similar to that of F. solani and Botryodiplodia ananasae (Bhargava, 1970),

Aspergillus flavus (Jamaluddin, 1973) and F. accuminatum and F. oxysporum (Singh,1977).

None of the four Fusarium species except F.udum could assimilate DL-Methionine within the period of incubation. F. udum utilized this amino acid completely within 13 days. Similarly fungi studied by Kapoor (1968) and Jamaluddin (1973) failed to consume this amino acid within 15 days of incubation period. Eventhough,this amino acid is a poor source of nitrogen for growth, the dry weight of mycelium increased in all the four species of Fusarium till the end of incubation period (Fig.20). The final pH of the medium remained acidic in all the cases.

Amongst the various amino acids used in the present study, L-Cystine was found to be the best source of nitrogen. Khanna (1974) and Singh (1977) reported this amino acid as a good source of nitrogen for various species of Alternaria and Fusarium. However, the organisms studied by Arya (1982) were grew only moderately over this amino acid. Like DL-methionine this sulphur containing amino acid was also present in the medium up to the end of incubation period (Fig.20). Dry weight of fungal mycelium was found to be increasing towards the 15th day. Khanna (1974) and Singh (1977) also reported similar results. The final pH of the

medium remained acidic in all cases except F.udum where it reached neutral point.

The nutritional conditions under which fungi sporulate are quite often different from those which are optimum for vegetative growth. In the present investigation all the organic nitrogen sources are not equally suitable for sporulation. Some which are favourable for mycelial growth do not favour sporulation. DL-leucine, DL-Valine, L-Glutamic acid and L-Arginine were found suitable for sporulation. Others viz. DL-Alanine, DL-Aspartic acid, L-Histidine, DL-Serine, L-Asparagine and L-Cystine induced poor to fair sporulation only.

Chlamydospore formation was poor or fair in almost all the cases. A good chlamydospore formation was recorded only in the case of F.udum on DL-Methionine. Same type of chalmydospore formation was earlier reported by Singh (1977).

Present Fusarium species lowered the pH of the medium in all the cases in the beginning of utilization. But later it shifted towards neutral or alkaline side. Similar results have been recorded by Saksena and Kumar (1962), Singh (1977), and Arya (1982). This phenomenon may be due to early utilization of ammonia formed during the process of

deamination, resulting in a decrease in the pH of the medium, which may be raised due to the accumulation of excess of ammonia in the later phase.

Table 18 : Average dry weight (in mg), sporulation, chlamydospore formation, final pH and utilization of different amine acids by Fusarium udum

Sl. No.	Amino acid	Days of incubation	Dry weight	Sporulation	Chlamydospore formation	Final pH	Presence of amino acids in days
		5	113.08	-	-		
1.	DL-Alanine	10	98.04	Good	-	7.3	0 - 7
		15	87.37	Excellent	-		
		5	97.93	-	-		
2.	L-Leucine	10	92.62	-	-	7.0	0 - 8
		15	81.09	Good	Poor		
		5	109.11	-	-		
3.	DL-Valine	10	96.78	Poor	-	6.9	0 - 8
		15	90.31	Excellent	-		
		5	81.92	-	-		
4.	DL-Aspartic acid	10	106.71	-	-	7.4	0 - 14
		15	113.26	Good	-		
		5	99.17	-	-		
5.	L-Glutamic acid	10	111.67	-	-	7.6	0 - 15
		15	125.83	Fair	Poor		
		5	71.06	-	-		
6.	L-Histidine	10	53.18	-	-	7.0	0 - 8
		15	49.64	Good	Fair		
		5	103.03	-	-		
7.	L-Arginine	10	96.84	Good	-	6.6	0 - 6
		15	87.16	Good	Fair		
		5	119.63	-	-		
8.	DL-Serine	10	98.17	-	-	7.3	0 - 6
		15	91.84	Fair	Poor		
		5	68.14	-	-		
9.	L-Asparagine	10	56.93	Fair	-	7.0	0 - 6
		15	49.45	Good	Fair		
		5	50.34	-	-		
10.	DL-Methionine	10	56.72	-	-	6.3	0 - 13
		15	59.31	Poor	Good		

Table 18 (contd.)

	5	114.70	-	-		
11. L-Cystine	10	134.23	-	-	7.0	0 - 15
	15	140.01	Fair	-		
	5	0.00	-	-		
12. Without nitrogen	10	0.00	-	-	5.5	-
(control)	15	0.00	-	-		

Summary of the dry weight results and conclusion at 5% level of P.

Treatments	: Highly significant
Replicates	: Non significant
G.M	: 83.43
S.E.	: 7.25
C.D at 5% level	: ± 21.12

Dry weight results after 15 days of incubation:

11>5>4>8 3>1 7>2>10>6 9>12

Table 19: Average dry weight (in mg), sporulation, chlamydospore formation, final pH and utilization of different amino acids by Fusarium pallidoroseum

Sl. No.	Amino acids	Days of incu-bation	Dry weig-ht	Sporu-lation	Chlamydo-spore formation	Final pH	Presence of amino acids in days
1	DL-Alanine	5	125.63	-	-	7.0	0-9
		10	113.27	-	-		
		15	98.31	Good	-		
2	L-Leucine	5	134.03	-	-	6.9	0-8
		10	93.64	Poor	-		
		15	87.18	Good	Poor		
3	DL-Valine	5	130.76	-	-	7.4	0-9
		10	126.24	Good	-		
		15	108.49	Excell-ent	-		
4	DL-Aspartic acid	5	76.33	-	-	6.9	0-15
		10	81.61	-	-		
		15	98.50	Fair	Poor		
5	L-Glutamic acid	5	96.21	-	-	7.3	0-15
		10	104.90	-	-		
		15	127.47	Good	Poor		
6	L-Histidine	5	53.67	-	-	7.2	0-10
		10	61.34	-	-		
		15	50.45	Poor	Fair		
7	L-Arginine	5	112.04	-	-	6.9	0-7
		10	101.92	-	-		
		15	89.17	Good	Fair		
8	DL-Serine	5	108.31	-	-	7.0	0-8
		10	98.48	Fair	-		
		15	86.95	Good	Poor		
9	L-Asparagine	5	73.11	-	-	7.2	0-6
		10	62.04	-	-		
		15	58.84	Poor	Fair		
10	DL-Methionine	5	39.78	-	-	6.6	0-15
		10	56.14	-	-		
		15	70.62	Fair	Fair		

Table 19 (contd.)

		5	108.73	-	-		
11	L-Cystine	10	119.15	-	-	6.4	0-15
		15	129.93	Poor	-		
		5	0.00	-	-		
12	Without nitrogen (control)	10	0.00	-	-	5.5	-
		15	0.00	-	-		

Summary of dry weight results and conclusion at 5% level of P.

Treatments	: Highly significant
Replicates	: Non significant
G.M.	: 85.64
S.E.	: 7.82
C.D. at 5% level	: ± 22.95

Dry weight results after 15 days of incubation:

$$11>5>3>\overline{4\ 1}>\overline{7\ \underline{2\ 8}}>10>9>6>12$$

Table 20 : Average dry weight (in mg), sporulation, chlamydospore formation, final pH and utilization of different amino acids by Fusarium oxysporum.

Sl. No	Amino acids	Days of incubation	Dry weight	Sporulation	Chlamydospore formation	Final pH	Presence of amino acids in days
1	DL-Alanine	5	90.01	-	-	6.8	0-10
		10	136.61	Poor	-		
		15	114.74	Fair	Fair		
2	L-Leucine	5	60.37	-	-	6.8	0-11
		10	101.77	Fiar	-		
		15	114.74	Fair	Fair		
2	L-Leucine	5	60.37	-	-	6.8	0-11
		10	101.77	Fair	-		
		15	91.68	Excellent	Poor		
3	DL-Valine	5	86.25	Poor	-	6.9	0-15
		10	95.81	Poor	-		
		15	111.02	Good	-		
4	DL-Aspartic acid	5	66.15	-	-	7.0	0-15
		10	93.60	-	-		
		15	125.43	Poor	Poor		
5	L-Glutamic acid	5	96.75	-	-	7.1	0-15
		10	114.68	Poor	-		
		15	121.07	Fair	-		
6	L-Histidine	5	16.01	-	-	6.4	0-14
		10	25.69	-	Poor		
		15	36.21	Poor	Fair		
7	L-Arginine	5	79.32	-	-	6.9	0-9
		10	88.69	-	-		
		15	60.43	Good	Poor		
8	DL-Serine	5	130.72	-	-	7.4	0-10
		10	118,85	Fair	-		
		15	93.47	Good	Poor		
9	L-Asparagine	5	57.06	-	-	6.9	0-7
		10	49.71	Poor	-		
		15	35.80	Fair	Fair		
10	DL-Methionine	5	29.26	-	-	6.0	0-15
		10	37.11	-	Poor		
		15	50.35	Poor	Fair		

Table 20 (contd.)

		5	100.76	-	-		
11	L-Cystine	10	119.83	-	-	6.1	0-15
		15	130.52	Poor	-		
		5	0.00	-	-		
12	Without nitr-	10	0.00	-	-	5.5	-
	ogen (control)	15	0.00	-	-		

Summary of dry weight results and conclusion at 5% level of P.

Treatment	: Highly significant
Replicates	: Non significant
G.M.	: 76.82
S.E.	: 6.54
C.D. at 5% level	: ± 19.07

Dry weight results after 15 days of incubation:

$$11>4>5>1>3>\overline{8\ 2}>7>10>\overline{6\ 9}>12$$

Table 21: Average dry weight (in mg), sporulation, chlamydospore formation, final pH and utilization of different amino acids by Fusarium moniliforme

Sl. No.	Amino acids	Days of incubation	Dry weight	Sporulation	Chlamydospore formation	Final pH	Presence of amino acid in days
1	DL-Alanine	5	129.61	-	-	6.7	0-10
		10	139.76	-	-		
		15	108.63	Fair	Poor		
2	L-Leucine	5	89.14	-	-	7.1	0-8
		10	83.06	Good	-		
		15	75.84	Excellent	Poor		
3	DL-Valine	5	78.39	Poor	-	7.0	0-15
		10	93.71	Good	-		
		15	101.08	Excellent	-		
4	DL-Aspartic acid	5	81.24	-	-	6.9	0-15
		10	89.16	-	-		
		15	96.74	Poor	-		
5	L-Glutamic acid	5	88,34	-	-	7.0	0-15
		10	99.75	-	-		
		15	115.61	Fair	-		
6	L-Histidine	5	44.67	-	-	6.7	0-13
		10	53.40	Fair	-		
		15	51.78	Good	Poor		
7	L-Arginine	5	90.32	-	-	6.7	0-13
		10	86.67	Poor	-		
		15	63.41	Fair	Poor		
8	DL-Serine	5	122.46	-	-	7.0	0-11
		10	127.39	Poor	-		
		15	104.17	Fair	-		
9	L-Asparagine	5	38,66	-	-	6.0	0-9
		10	38,31	Fair	-		
		15	26.84	Good	Poor		
10	DL-Methionine	5	40.28	-	-	6.1	0-15
		10	51.19	-	-		
		15	65.39	Fair	-		

Table 21 (contd.)

		5	99.02	-	-		
11	L-Cystine	10	103.65	-	-	6.8	0-15
		15	120.47	Poor	-		
		5	0.00	-	-		
12	Without	10	0.00	-	-	5.5	-
	nitrogen (control)	15	0.00	-	-		

Summary of the dry weight results and conclusion at 5% level of P.

Treatment	: Highly significant
Replicates	: Non significant
G.M.	: 77.73
S.E.	: 6.93
C.D. at 5% level	: ± 20.18

Dry weight results after 15 days of incubation:

11>5>1>8>3>4>2>10 7>6>9>12

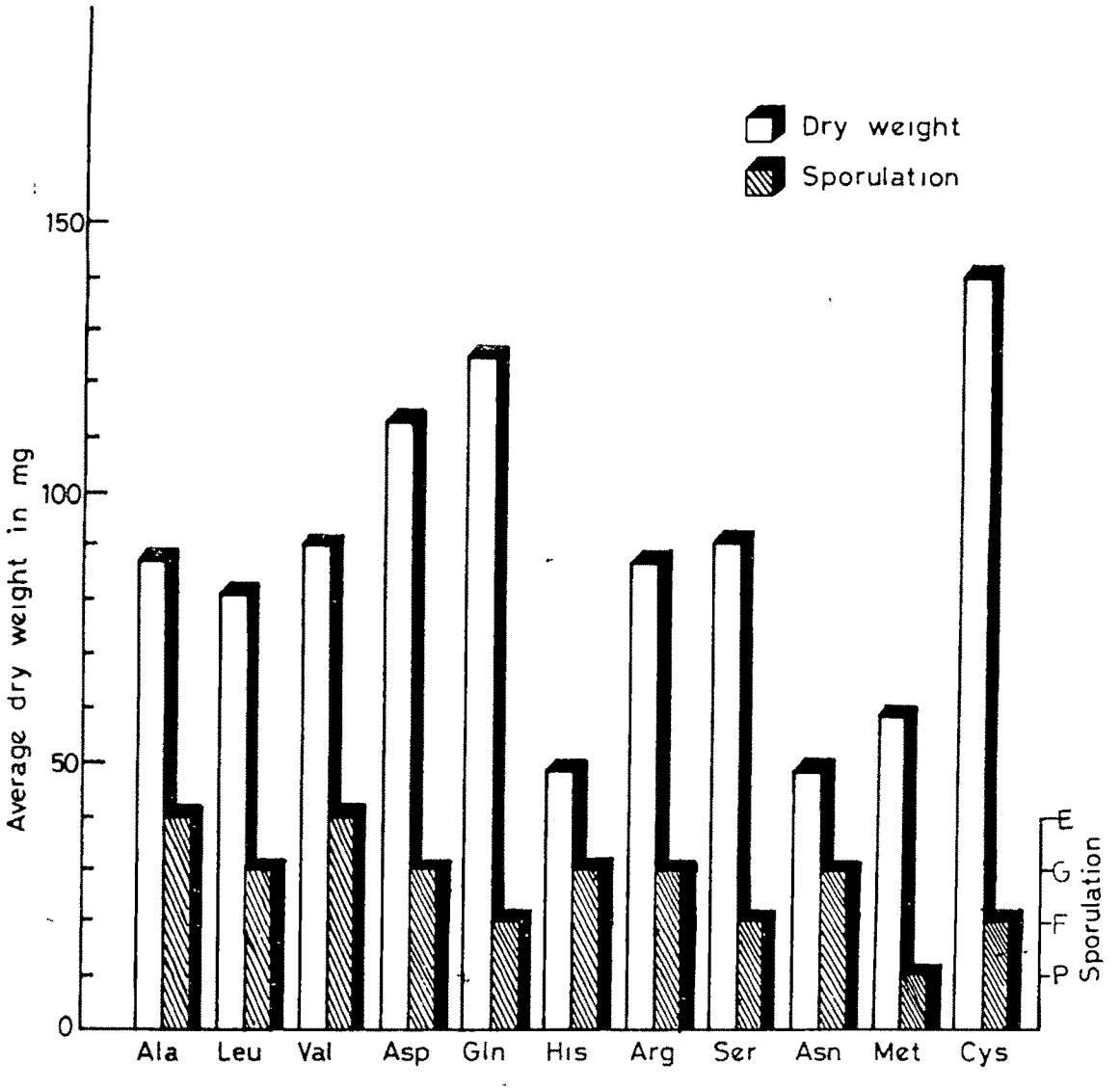


Fig 11 Average dry weight (After incubation period) and sporulation of Fusarium udum on different amino acids

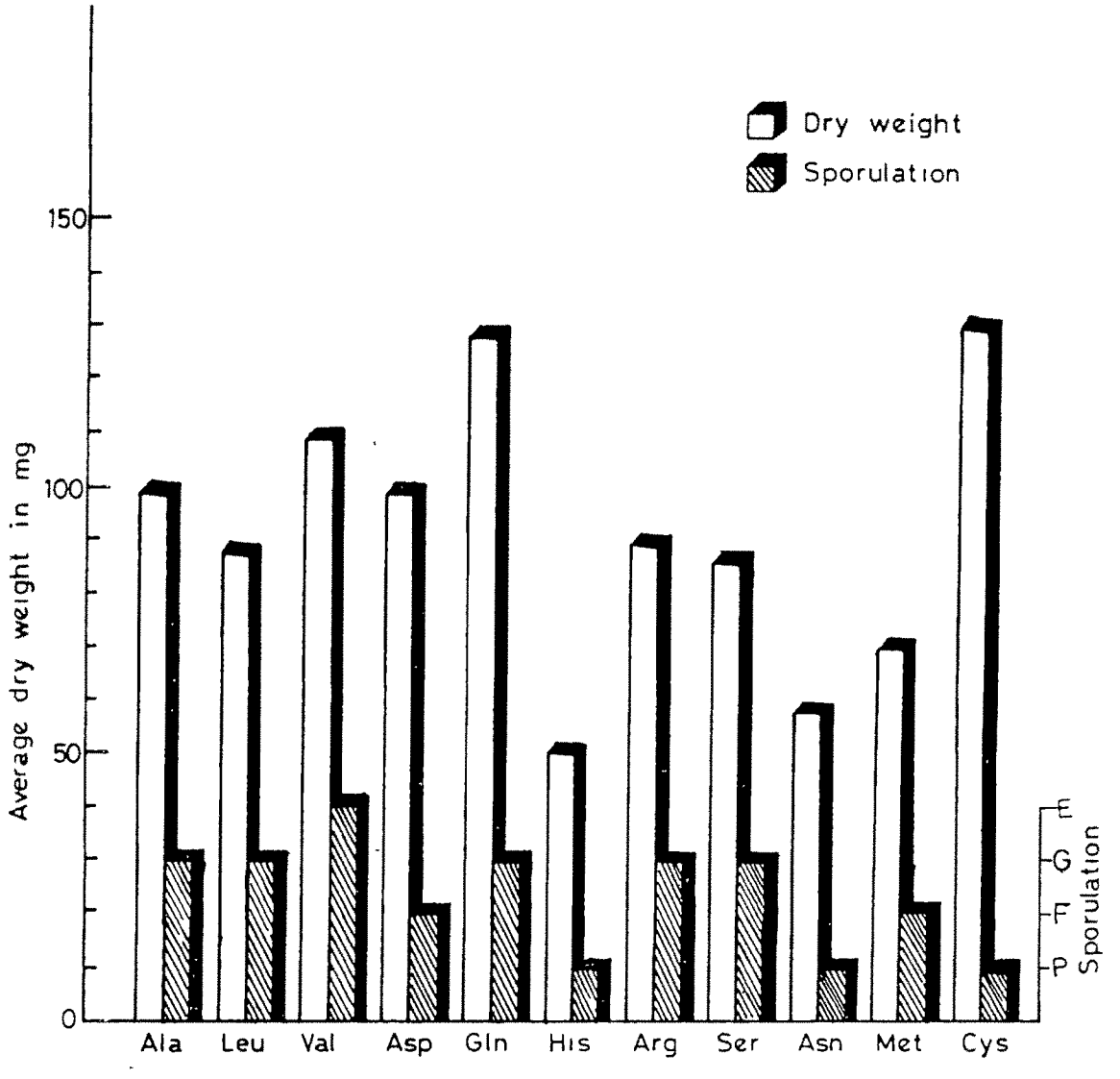


Fig 12 Average dry weight (After incubation period) and sporulation of Fusarium pallidoroseum on different amino acids

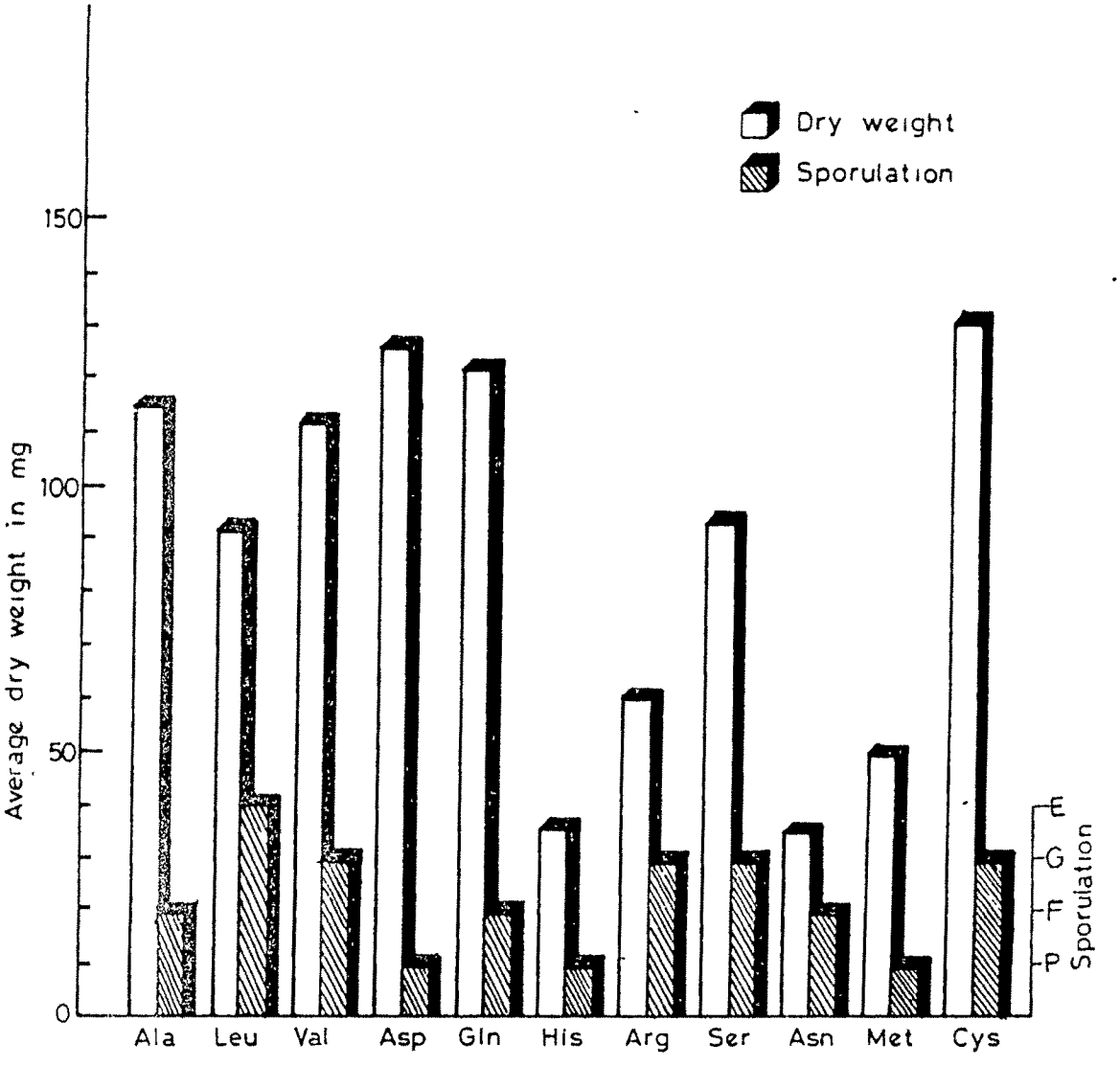


Fig 13 Average dry weight (After incubation period) and sporulation of Fusarium oxysporum on different amino acids

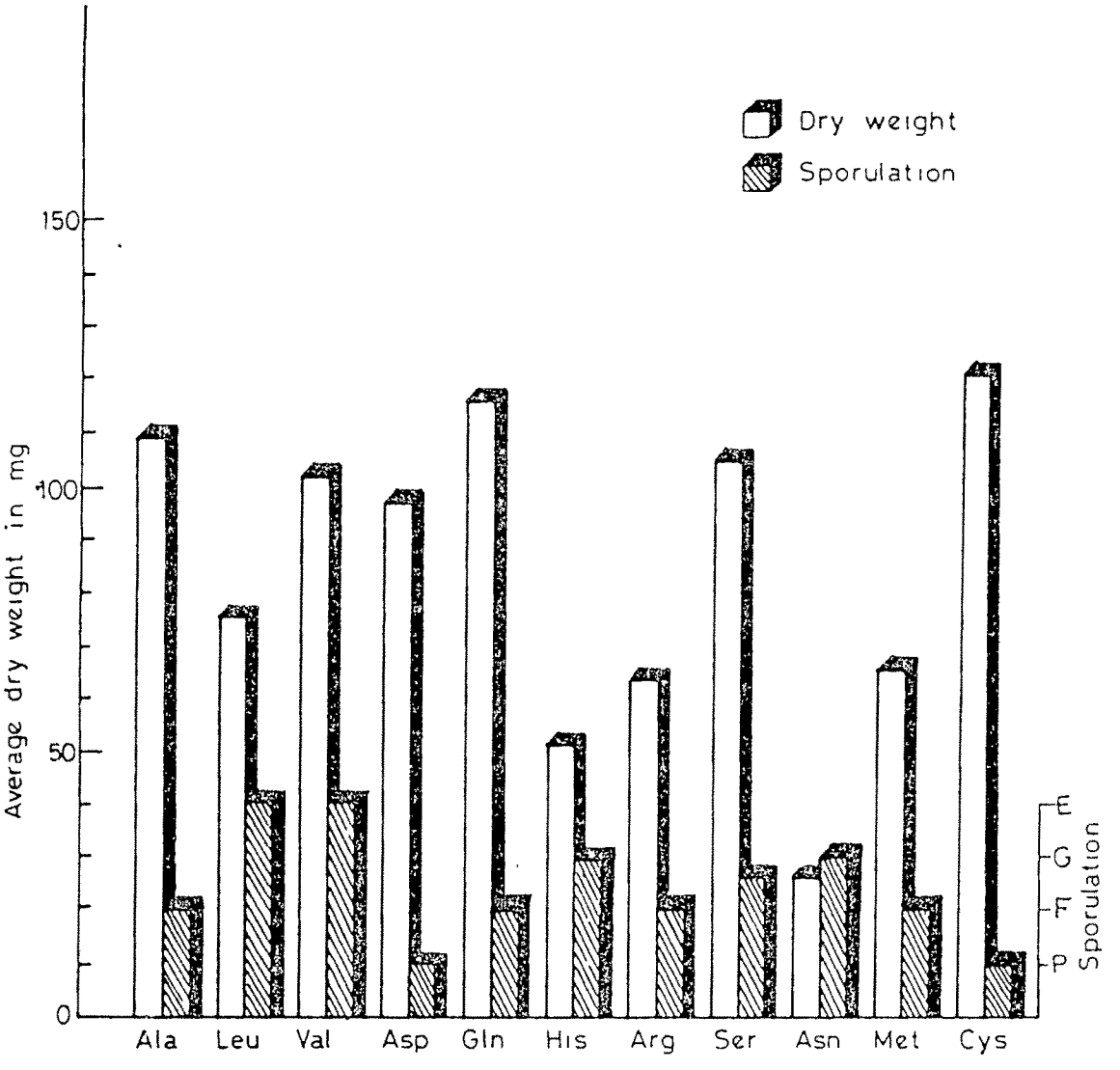


Fig 14 Average dry weight (After incubation period) and sporulation of Fusarium moniliforme on different amino acid

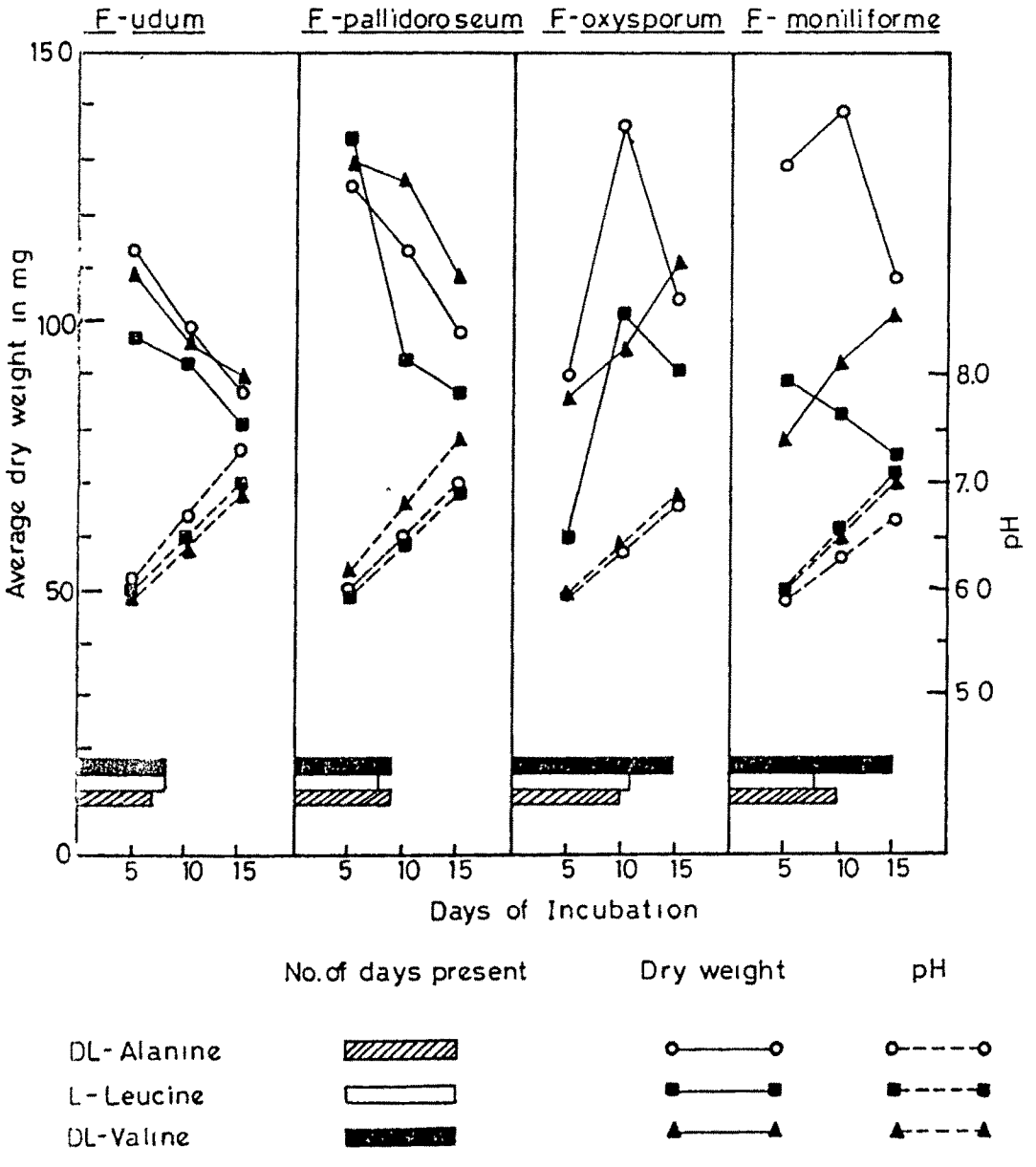


Fig 15 Average dry weight, pH change and utilization rate of mono amino mono carboxylic acids by four species of Fusarium

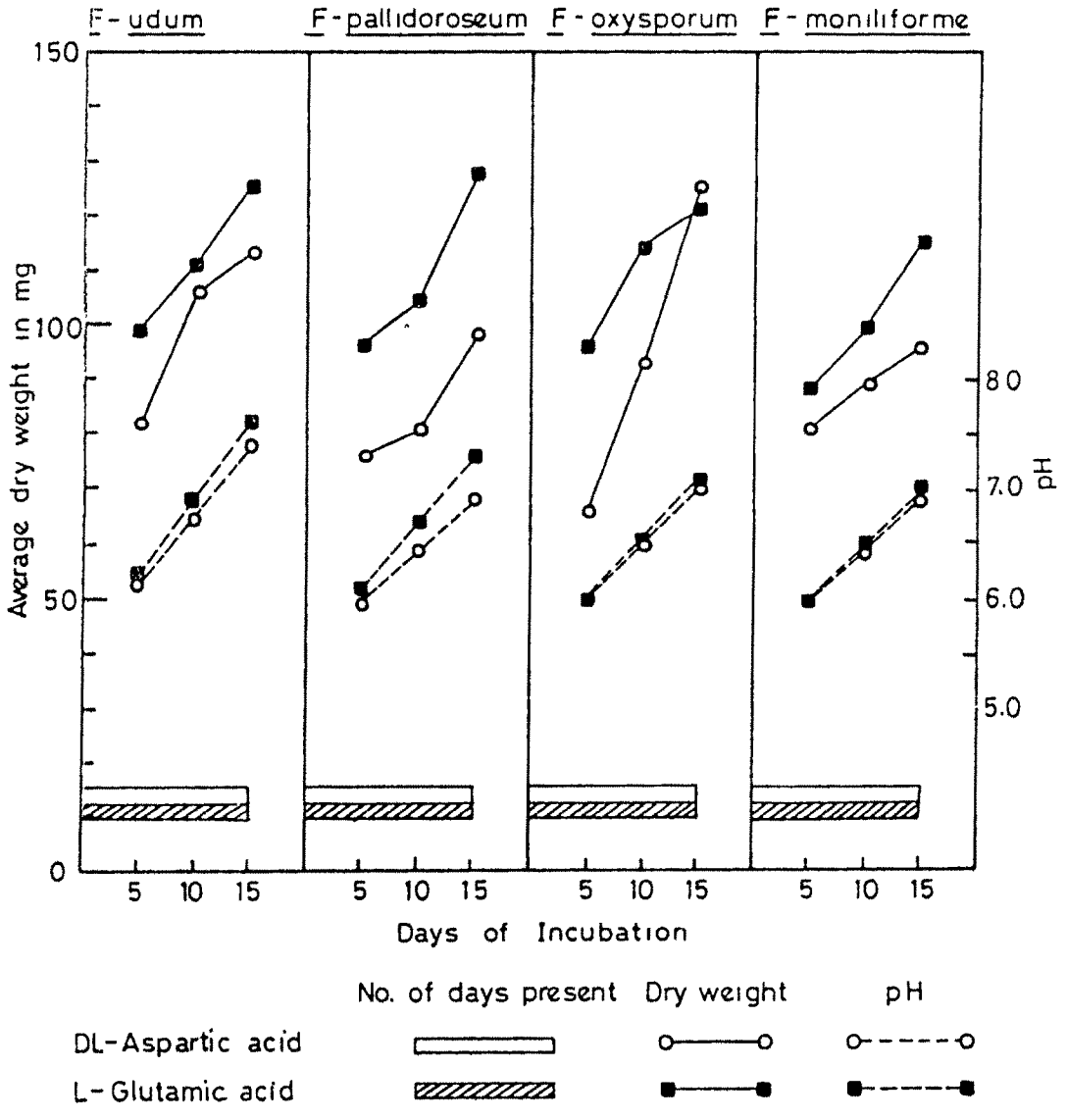


Fig 16 Average dry weight pH change and utilization rate of mono amino dicarboxylic acids by four species of Fusarium

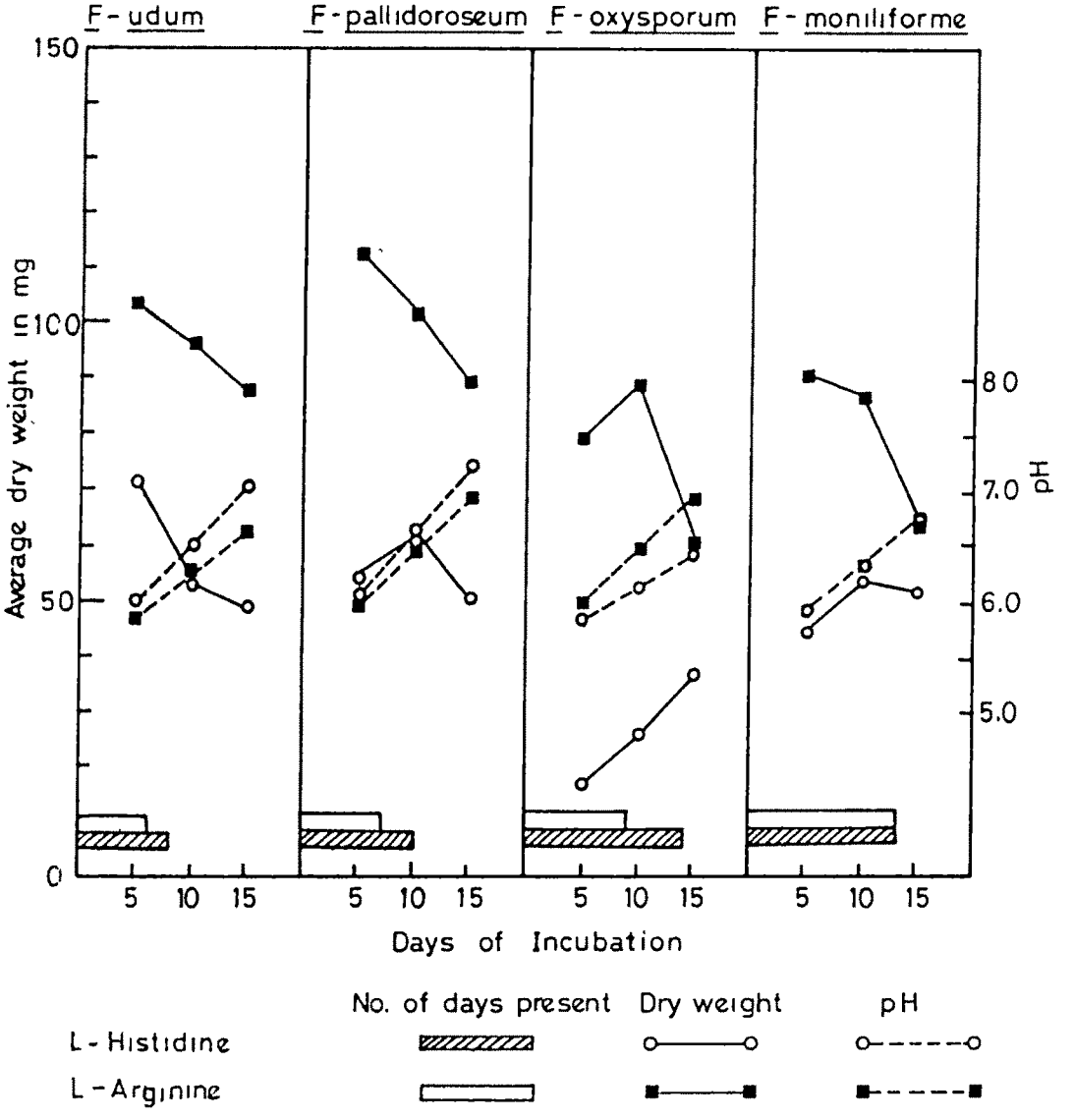


Fig 17 Average dry weight pH change and utilization rate of amino acids by four species of Fusarium

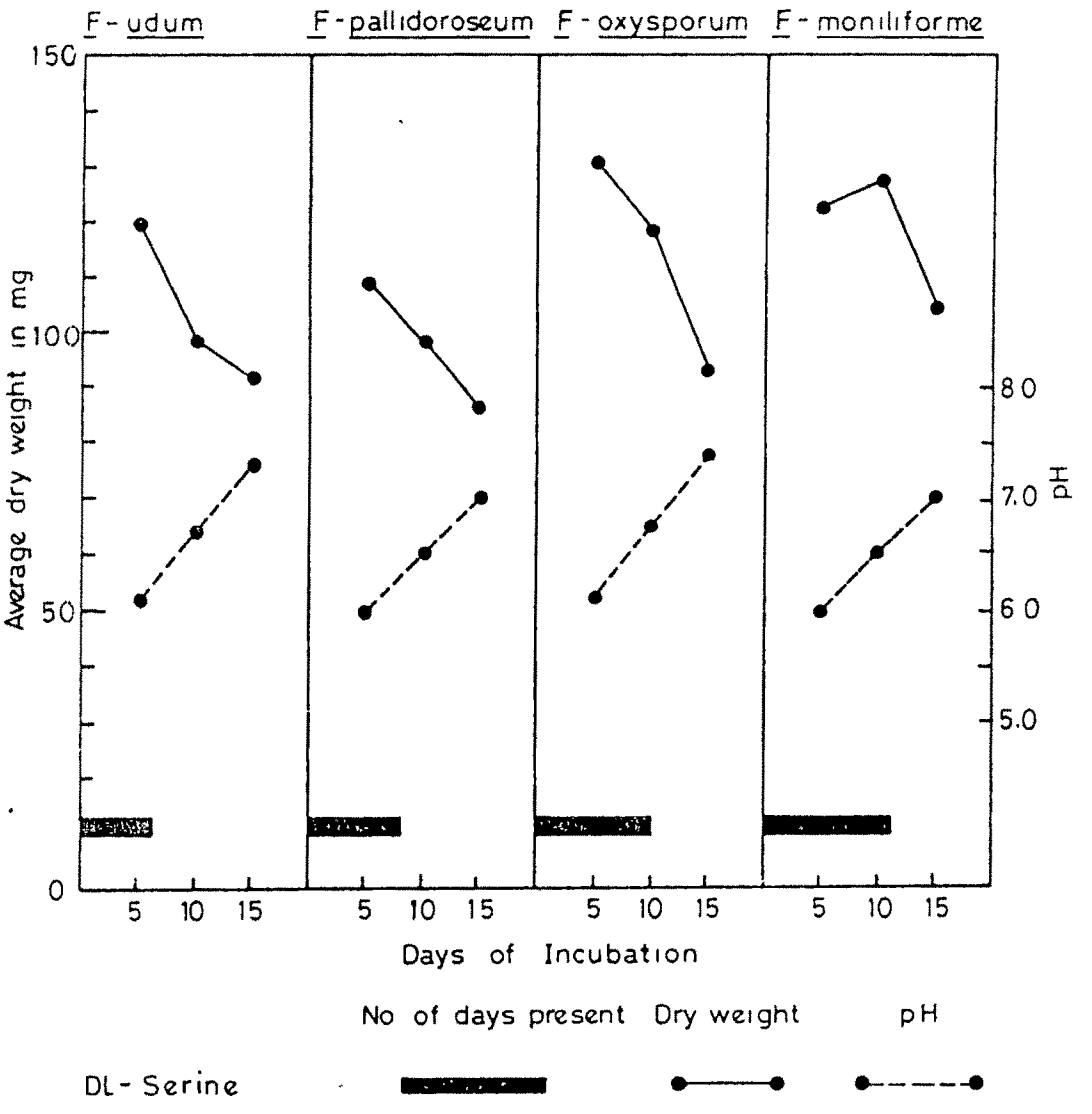


Fig 18 Average dry weight, pH change and utilization rate of mono amino hydroxy acid by four species of Fusarium

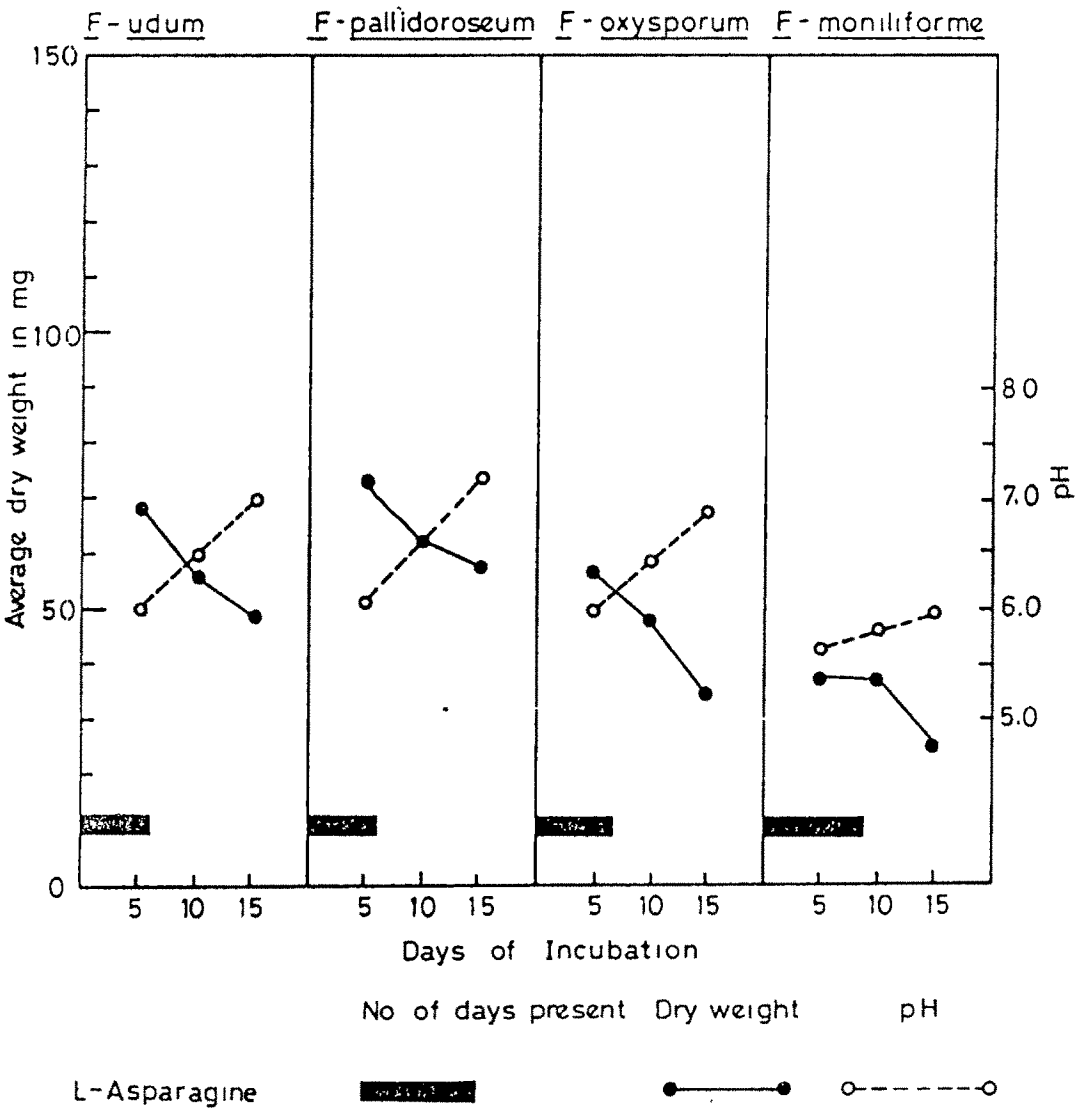


Fig 19 Average dry weight pH change and utilization rate of amide of mono amino dicarboxylic acid by species of Fusarium

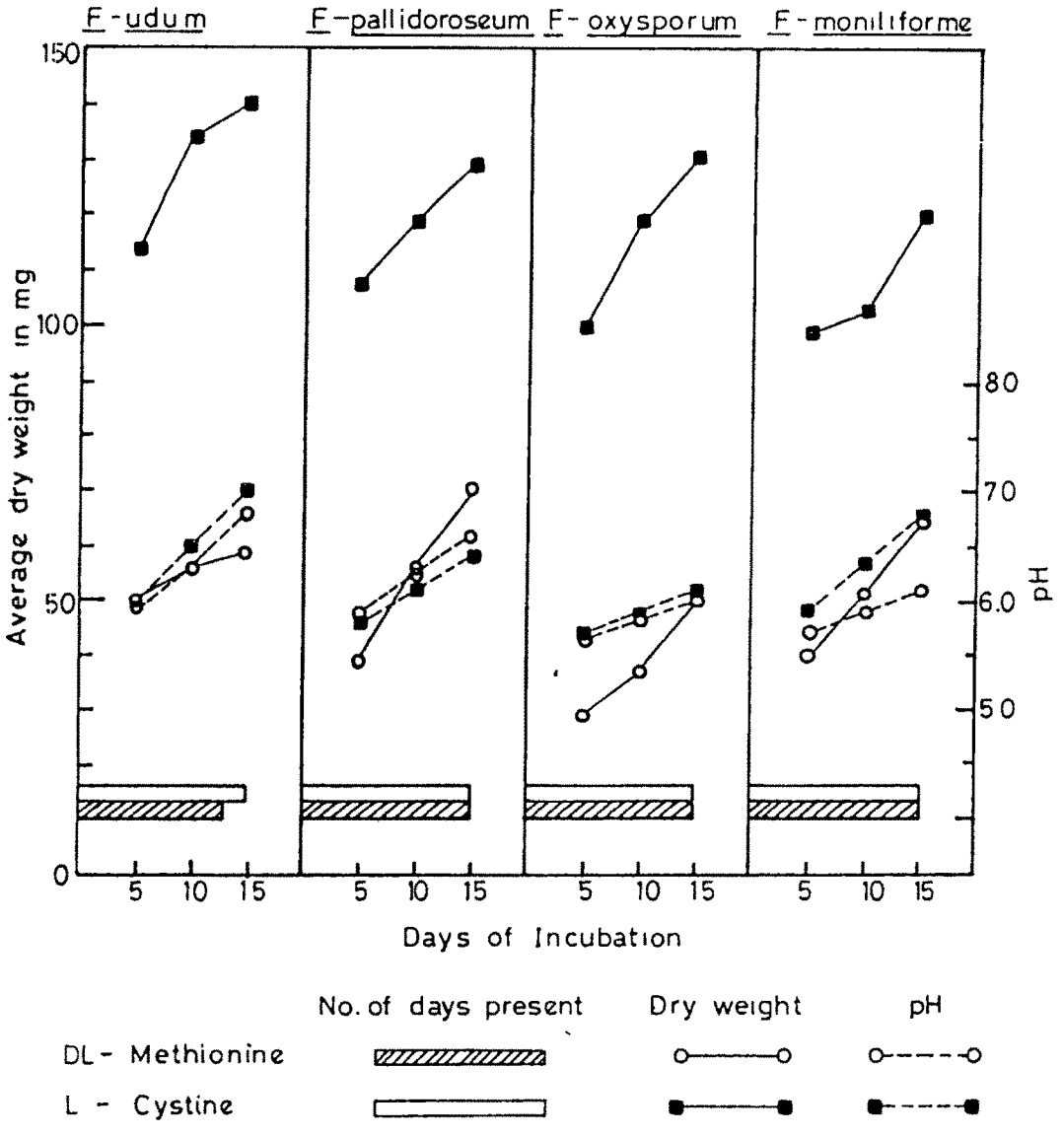
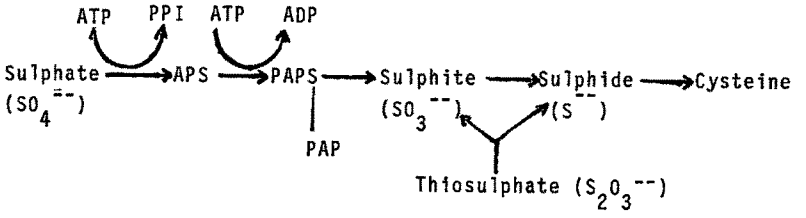


Fig 20 Average dry weight, pH change and utilization rate of sulphur containing amino acids by four species of Fusarium

3.6 Effect of Different Sulphur Sources

Sulphur, an important constituent of protein, play a significant role in the metabolic activities of fungus. It is an essential element for the biosynthesis of prosthetic (^{-}SH) group containing enzymes, coenzymes and vitamins, which are indispensable for many vital processes of the fungi (Lilly and Barnett, 1951; Cochrane, 1958; Tandon, 1961; Bhargava and Tandon, 1963). Challenger (1953) reviewed the importance of sulphur compounds, with special note to their role in biological methylation by fungi.

In comparison with general nutritional behaviour, fungi show variations in their choice and ability to utilize different sources of sulphur (Hawker,1950; Lilly and Barnett, 1951). Sulphides, thiosulphates, Sulphur containing amino acids, tio-urea and a variety of sulphur sources are easily utilized by many fungi. Some fungi can even utilize elemental sulphur (Sciarini and Nord,1954; Miller et al.,1953; Devey and Papavizas,1962). Cochrane (1958) stated that most fungi can supply all their needs for sulphur from inorganic sulphate,i.e. they reduce the sulphate and incorporate it in to organic molecules. Wilson (1962) suggested enzyme catalyzing sulphate activation and reduction pathway as follows (Bilgrami and Varma,1978).



Steinberg (1941) has made a comprehensive study of sulphur requirement of Aspergillus niger and has concluded that oxidized sulphur containing inorganic compounds were utilized, while sulphide and disulphides were unutilizable. Fisher distinguished two groups of microorganisms viz. Enthiotrophic and parathiotrophic on the basis of data obtained by Lwoff (1932) and Volkonsky (1933). Out of this the organisms belongs to the first group have the ability to utlize sulphate sulphur, while the organisms of the second group have the ability to utilize reduced sulphur only.

Many of the fungi appear to be more versatile as far as their ability to utilize sulphur source is concerned. For instance, Pencillium chrysoghnum (Hockerhull,1948), Pestalotia malorum (Tandon,1950) and Pythium Sp. (Saksena et al.,1953) are some of the fungi which can utilize a variety of sulphur sources, including inorganic and organic compounds. Our knowledge about the relative importance of different sulphur sources is still very

inadequate. It was, therefore considered desirable to study the influence of different sources of sulphur on the growth and sporulation of four species of Fusarium. The following inorganic sulphur sources were used for this purpose.

Ammonium Sulphate	$(NH_4)_2\ SO_4$
Calcium Sulphate	$CaSO_4$
Cobalt Sulphate	$CoSO_4$
Copper Sulphate	$CuSO_4.5H_2O$
Ferrous Sulphate	$FeSO_4.7H_4O$
Lithium Sulphate	$LiSO_4$
Magnesium Sulphate	$MgSO_4.7H_2O$
Manganese Sulphate	$MnSO_4.H_2O$
Nickel Sulphate	$NiSO_4$
Potassium metabisulphite	$K_2S_2O_5$
Sodium Sulphite	Na_2SO_3
Sodium Sulphide	Na_2S
Zinc Sulphate	$ZnSO_4.7H_2O$

A medium was also prepared without adding any source of sulphur so as to find out the effect of complete absence of sulphur on different fungi under study. The results are summarized in table 22 and figure 21.

The results revealed that all the sulphur sources were not equally suitable for the growth and sporulation

of the four pathogens under study. Magnesium sulphate supported good growth and excellent sporulation of all the organisms. Similar results have been reported for Phyllosticta cycadina and P. artocarpina (Bilgrami,1956), Botryodiplodia ananassae (Bhargava and Tandon,1963), Phomopsis sapotae and Chaetosphaeropsis truncata var. indica (Rai,1982), Phomopsis viticola, P. psidii, P. gulabia and Phomopsis pedilanthi (Arya,1982). Many workers like Lilly and Barnett (1951), Tandon and Bilgrami (1958), Agarwal and Ganguli (1960), Bhargava and Tandon (1963), Hasija (1969) and Mehrotra and Tandon (1970) reported that for most of the fungi, sulphate sulphur appears to be the most favourable source.

Ammonium sulphate induced moderate growth in all the four fungi under study. Bhargava and Tandon (1963) in Botryodiplodia ananassae and Rai (1982) in Chaetosphaeropsis Sp. observed similar results. At the same time, the above authors also reported that Machrophomina phaseolina (Bhargava and Tandon, 1963) and Phomopsis sapotae (Rai,1982) were produced poor growth on this sulphur source.Except F. pallidoroseum, all the other species of Fusarium studied here were excellently sporulated on Ammonium sulphate as in the case of Phomopsis Sp. (Arya, 1982) and Phomopsis Sapotae

(Rai,1982). In respect of sporulation F. pallidoroseum resembles Macrophomina phaseolina (Bhargava and Tandon, 1963) and three species of Phomopsis (Arya, 1982). Eventhough growth and sporulation were poor in the case of F. pallidoroseum, chlamydospore formation was excellent. But F.udum and F. moniliforme showed a poor chlamydospore formation. F. oxysporum was unable to produce chlamydospores on this sulphur source during 15 days of incubation.

Sulphates of Cobalt, Copper and Nickel were found toxic to all the four organisms. Similar results were also reported by Arya (1982). On the other hand Lithium and Zinc sulphates supported growth in all the species of Fusarium under study. F. pallidoroseum and F. oxysporum failed to sporulate on Lithium sulphate while F. udum exhibited a poor sporulation and F. moniliforme a good one. Good chlamydospore formation was observed in all the species except F. udum where, it was fair. F. pallidoroseum failed to grow on sinz sulphate while a poor growth was observed in F. udum. Poor growth on zinc sulphate was also reported by Arya (1982) and Rai (1982). Although moderate growth was recorded in F. oxysporum and F. moniliforme, the sporulation was absent and poor in both the species respectively. F. udum exhibited a feeble

sporulation on this sulphur source. Chlamydospore formation was poor, in all the species except F. moniliforme where, a fair chlamydospore formation was observed. The importance of metals in fungal nutrition is suggested by many workers, but these micrometallic elements are required in minute quantities. The toxic action of these metals may be based on the property of their ions to precipitate or denature the proteins, enzymes or catalysts. Copper compounds are generally used in fungicides and this fungistatic property of copper compounds may be attributed to their affinity to combine with 'sulphydryl' group of certain enzymes (Lilly and Barnett 1951).

Ferrous sulphate supported good growth in F. pallidoroseum and F. oxysporum and was moderate in F. udum. A poor growth was recorded in the case of F. moniliforme. Arya (1982) also reported similar result. Sporulation was excellent in F. udum (microconidia) and good in F. pallidoroseum (microconidia) and F. oxysporum. There was a fair sporulation in the case of F. moniliforme. A poor chlamydospore formation was observed in F. udum. In rest of the species it was good.

Magnesium sulphate yielded maximum dry weight among all the species studied except F. pallidoroseum where, the

maximum dry weight was attained over calcium sulphate. All the species exhibited good sporulation other than F. udum where, an excellent sporulation was perceived. F. pallidoroseum and F. moniliforme showed poor chlamydospore formation, while F. udum and F. oxysporum failed to develop it.

Like other sulphates Manganese sulphate also produced good growth in all the Fusaria under present investigation. Among them F. udum obtained maximum dry weight. However, it sporulate poorly. F. pallidoroseum and F. oxysporum attained good sporulation while, F. moniliforme exhibited a fair sporulation. Only F. moniliforme showed a feeble chlamydospore formation. Rest of the species failed to develop it.

Potassium metabisulphite was found toxic to F. pallidoroseum. Similar results were observed by Lal and Tandon (1974a,b) and Arya (1982). F. udum, F. oxysporum and F. moniliforme were produced good growth on this sulphur source. Arya (1982) also reported poor growth of Phomopsis psidii. Good, fair, and poor sporulation was observed in F. udum, F. oxysporum and F. moniliforme respectively. Chlamydospore formation was altogether absent in all the species.

A mixed response was observed in sodium sulphite for the four species of Fusarium. F. udum and F. moniliforme produced good growth, while in F. pallidoroseum and F. oxysporum it was poor. The former two organisms resemble Botryodiploidia ananassae (Bhargava and Tandon,1963) and later two are par with Phomopsis sapotae and Chaetosphaeropsis sp. (Rai 1982). As Phomopsis viticola of Arya (1982) F. udum also exhibited good sporulation over this sulphite. Sporulation was poor in F. pallidoroseum,fair in F. oxysporum and F. moniliforme (microconidia). A fair chlamydospore formation was observed in F. moniliforme, while it was poor in the case of F. oxysporum and absent in other two species.

As reported by Tandon (1950), Saksena et al. (1953) and Arya (1982), in the present investigation also sodium sulphide supported good growth in all the species except F. moniliforme. But F. miniliforme showed poor growth only. Arya (1982) also reported poor growth over this substance in Phomopsis gulabia and Phomopsis pedilanthi. Eventhough, good growth was observed on this sulphur source, the sporulation was poor in all the cases except F. oxysporum where, a fair one was perceived. Chlamydospore formation was good in F. pallidoroseum, but

it was poor in F. moniliforme and failed to form in the rest two species.

F. pallidoroseum and F. oxysporum were produced a feeble growth in the medium which was completely devoid of sulphur. On the other hand F. udum and F. moniliforme were unable to grow on this sulphur deficient medium. Earlier reports also indicate that different fungal organisms differ in their capability to grow without a sulphur source. However, Fusarium solani, Botryodiplodia ananassae, Macrophomina phaseolina (Bhargava and Tandon 1963) and some Mucorale members (Sarbhoy 1965) have been found to survive in complete absence of sulphur. Sporulation and chlamydospore formation was totally absent in the organisms under present study. So, sulphur was found essential for the growth and sporulation of all the organisms. (Lal and Tandon, 1974a,b; Rai 1982; Arya 1982).

A drift in final pH of the medium was observed towards neutrality. However, in a few cases it was more towards acidic side or alkaline side.

A perusal of the table and the above results, clearly reveals that all the four species of Fusarium were specific in their growth and sporulation responses towards

various sulphur sources and there was no close correlation between the growth and sporulation. It was interesting to note that when growth was good the sporulation varied from poor to excellent.

Table 22: Average dry weight (mg), sporulation, chlamydospore formation and final pH of 4 species of Fusarium on different sulphur sources.

Treatment	Sulphur sources	F.udum				F. pallidoroseum				F.oxysporum				F.moniliforme			
		Dry weight	Sporulation	Chlamydospore	Final pH	Dry wt.	Sporulation	Chlamydospore	Final pH	Dry wt.	Sporulation	Chlamydospore	Final pH	Dry. wt.	Sporulation	Chlamydospore	Final pH
1.	Ammonium sulphate	21.4	*E	P	5.0	26.2	F	E	5.9	17.1	*E	-	5.3	15.1	*E	P	5.0
2.	Calcium sulphate	62.2	P	-	6.4	57.1	*G	P	6.7	41.8	E	-	7.0	11.8	F	-	6.5
3.	Cobalt sulphate	0.0	-	-	5.5	0.0	-	-	5.5	0.0	-	-	5.5	0.0	-	-	5.5
4.	Copper sulphate	0.0	-	-	5.5	0.0	-	-	5.5	0.0	-	-	5.5	0.0	--	-	5.5
5.	Ferrous sulphate	22.6	*E	P	6.6	40.1	*G	G	6.0	38.9	G	G	6.5	18.0	F	G	6.0
6.	Lithium sulphate	7.2	P	F	6.0	14.5	-	G	6.3	6.2	-	G	6.0	8.3	G	G	6.1
7.	Magnesium sulphate	87.9	E	-	7.0	55.1	G	P	7.1	86.5	G	-	7.0	44.5	G	-	6.9
8.	Manganese sulphate	69.8	P	-	6.9	35.2	G	-	7.0	23.7	*G	-	7.2	24.5	F	P	7.0
9.	Nickel sulphate	0.0	-	-	5.5	0.0	-	-	5.5	0.0	-	-	5.5	0.0	-	-	5.5
10.	Potassium metabisulphate	10.1	G	-	6.3	0.0	-	-	5.5	18.2	F	-	6.8	11.4	P	-	6.4
11.	Sodium Sulphite	43.8	G	-	6.8	15.6	P	-	6.0	17.1	F	P	6.3	34.5	*F	F	6.5
12.	Sodium Sulphide	40.9	P	-	7.0	45.4	P	G	7.2	23.5	F	-	6.7	12.7	P	P	6.1
13.	Zinc sulphate	5.8	P	P	5.0	0.0	-	-	5.5	30.1	P	P	6.8	21.1	P	F	6.2
14.	No sulphur	0.0	-	-	5.5	6.7	-	-	5.8	3.1	-	-	5.8	0.0	-	-	5.5
	G.M	26.6				21.1				21.9				14.4			

* Microconidia, In all these cases macroconidia formation was poor or nil

Summary of dry weight result and conclusion at 5% level of P.

Treatments	Highly significant	Highly significant	Highly significant	Highly significant
Replicates	Non significant	Non significant	Non significant	Non significant
S.E.	4.45	3.27	3.51	2.04
C.D. at 5% level of P :	± 12.84	± 9.43	±10.14	± 5.88

Dry weight results

F.udum	7>8>2>11 12>15 1>10>6>18>3 4 9 14
F.pallidoroseum	2 7>12>5>8>1>11 6>14>3 4 9 10 13
F. oxysporum	7>2 5>13>8 12>10 11 1>6 14>3 4 9
F. moniliforme	7>11>8 13 5 1 12 2 10 6>3 4 9 14

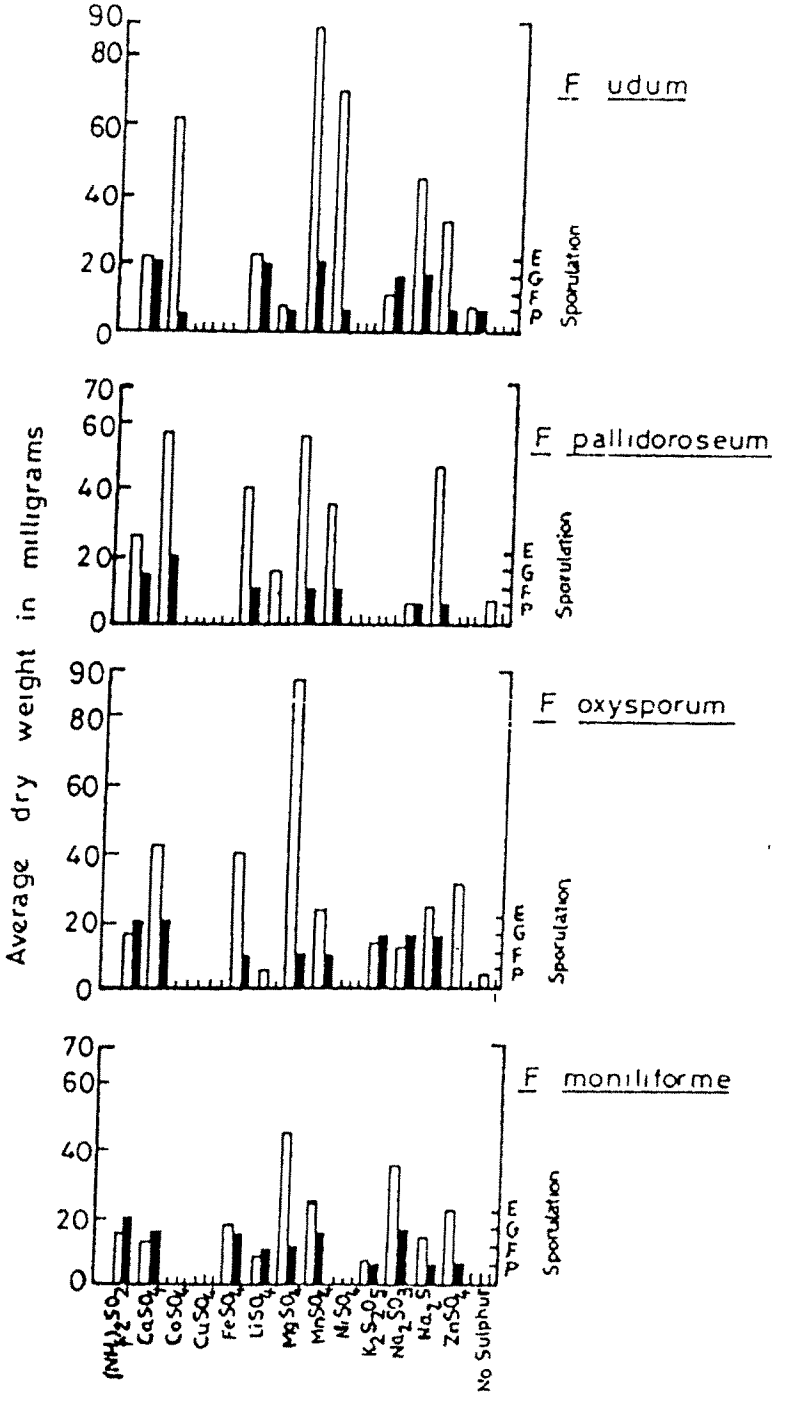


Fig 21 Average dry weight (entire bar) and sporulation (shaded) of four species of Fusarium on different sulphur sources

3.7 Effect of hydrogen-ion concentration on the utilization of Sodium nitrite

Many investigators including Cochrane and Conn (1950), Gordan (1950), Tandon and Bilgrami (1957),Chandra (1961), Hasija and Chowdhury (1980) as well as Rai (1982) have found the toxic effects of nitrite nitrogen on various fungi studied by them. The toxic effect of nitrite nitrogen is closely related to the hydrogen ion concentration and is greater in acidic range. In the present study the effect of pH of medium on utilization of sodium nitrite by four Fusarium Spp. has been investigated. Details of the procedure have been given in the chapter dealing with materials and methods. Results are summarised in table 26 and figure 22.

The outcome reveals that the toxicity of sodium nitrite is greater in acidic range for all the four Fusarium species. None of the organisms could grow in the medium which adjusted to ph 2.0 to 5.5. Arya (1990) also reported the same results in his study on four Phomopsis species. In Fusarium pallidoroseum pH range upto 5.5 recorded no growth, while F. oxysporum even not grew in the whole acidic range (Bhatnagar and Pathak, 1970; Singh 1977). The maximum growth of the present Fusarium

Spp. is obtained at pH 8.0. F.oxysporum yielded maximum dry weight of mycelium in alkaline range in comparison with other species of Fusarium studied.

Even though,F.udum showed a small amount of growth in acidic medium, the sporulation was poor. F.moniliforme failed to sporulate in acidic range. Good sporulations were recorded in F.pallidoroseum, F. oxysporum and F.moniliforme at pH 8.0. In F. oxysporum pH 9.0 also supports good sporulation. The rate of growth of the present organisms was decreased at pH 9.0 and 10.0 because of the high alkalinity of the medium rather than the toxicity of nitrite nitrogen. In highly alkaline medium accumulation of (OH^-) on the cell membrane prevents the passage of essential anions (Bilgrami and Varma,1957). Due to metabolic activity of the organisms the initial pH of the medium drifted towards alkaline side.

The present results resemble with the findings of Cochrane (1958), Shreemali (1971), Rai (1982) and Arya (1990). A perusal of table 26 reveals that initial pH has significant effect on the utilization of nitrite compounds. The utilization of sodium nitrite in alkaline medium was supported by various investigators in many organisms. Reports of Agrawal (1955) in Fusarium coeruleum

and Tandon (1967) in Botryodiplodia theobromae and Diplodia typhina revealed that these organisms are able to utilize nitrite nitrogen as the main nitrogen source. According to Tandon and Agrawal (1953) in F. coeruleum the hyphal yield was maximum when pH of the medium was in alkaline range. Thind and Duggal (1957) and Tandon and Srivastava (1963) also reported similar results. Tyagi (1976) observed that,if initial pH of the medium was adjusted to 7.5, Drechslera sorokiniana achieved good growth on nitrite nitrogen. Growth of F.moniliforme largely enhanced when pH of the medium was adjusted to 6.5 or more (Sahni 1967).

The toxic effect of the nitrite in acidic medium appears to be due to its form of undissociated nitrous acid, while the efficient utilization of nitrite at higher pH is due to the fact that the toxic effect of nitrous acid is prevented under such conditions. Brock (1951) also stated that the toxicity of nitrite to fungi is due to the acidity of the medium, and the alkaline medium which supports the utilization of nitrite compound is because of its free ionized acid rather than the toxic nitrite ion. According to Nord and Mull (1945) nitrite toxicity to Fusarium lini is due to the pyruvic acid accumulation in mycelium.

Table 23 : Effect of hydrogen ion concentrations on the utilization of sodium nitrite by four species of Fusarium.

Sl. No.	Initi -al pH	F. udum Dry wt.	Sporul ation	Final pH	F.pallidoroseum Dry wt.	Sporul ation	Final pH	F. oxysporum Dry wt	Sporul ation	Final pH	F.moniliforme Dry wt.	Sporul ation	Final pH
1	2.0	0.00	-	2.0	0.00	-	2.0	0.00	-	2.0	0.00	-	2.0
2	3.0	0.00	-	3.0	0.00	-	3.0	0.00	-	3.0	0.00	-	3.0
3	4.0	0.00	-	4.0	0.00	-	4.0	0.00	-	4.0	0.00	-	4.0
4	5.0	0.00	-	5.0	0.00	-	5.0	0.00	-	5.0	0.00	-	5.0
5	5.5	0.00	-	5.5	0.00	-	5.5	0.00	-	5.5	0.00	-	5.5
6	5.8	4.82	P	6.8	0.00	-	7.0	0.00	-	5.8	3.73	-	6.9
7	6.0	4.07	P	7.0	4.51	-	7.3	0.00	-	6.0	8.62	-	7.3
8	7.0	8.34	P	7.2	10.18	F	8.1	14.37	F	8.2	12.61	P	8.3
9	8.0	18.61	F	9.2	20.34	G	9.3	25.05	G	8.9	22.50	G	9.1
10	9.0	18.26	F	8.6	19.76	F	9.1	21.96	G	9.0	18.82	F	8.8
11	10.0	18.33	F	8.5	19.14	P	8.7	18.21	F	8.6	16.91	P	8.5
	G.M	6.58			6.72			7.23			7.56		

- = Absent, G = Good, F = Fair, P = Poor

Summary of dry weight result and conclusion at 5% level of P.

S.E.	1.36	1.50	1.74	1.47
C.D. at 5% level	±3.91	±4.34	±5.03	±4.25

Dry weight result:

F. udum : $\overline{9\ 11\ 10}>8>\overline{6\ 7}>\overline{1\ 2\ 3\ 4\ 5}$

F.pallidoroseum : $9\ \overline{10\ 11}>8>7>\overline{1\ 2\ 3\ 4\ 5\ 6}$

F.oxysporum : $\overline{9\ 10}>\overline{11}>8>\overline{1\ 2\ 3\ 4\ 5\ 6\ 7}$

F.moniliforme : $9>\overline{10\ 11}>8>7>6>\overline{1\ 2\ 3\ 4\ 5}$

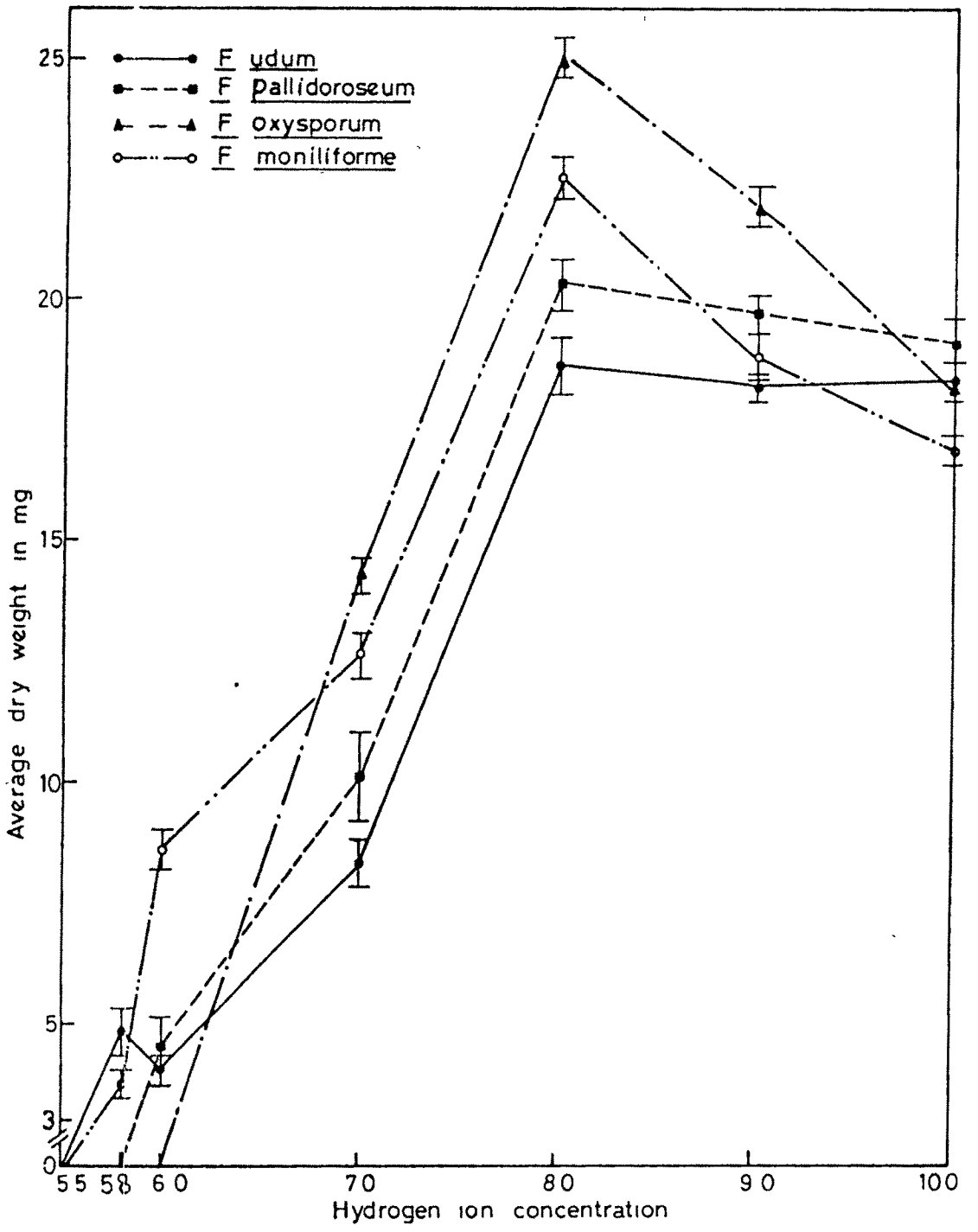


Fig 22 Average dry weight of four Fusarium species on the utilization of sodium nitrite at different hydrogen ion concentration

4. SOLARIZATION STUDIES

The solar heating method for disease control is similar, in principle, to that of artificial soil heating by steam or other means, which are usually carried at 60 to 100°C. There are, however, important biological and technical difference with soil solarization. There is no need to transport the heat from its source to the field. Solarization is carried out at relatively low temperature, as compared to artificial heating; thus, its effect on living and nonliving soil components are likely to be less drastic. In the present investigation, before starting solarization the following studies were conducted in the field.

4.1 Growth Study

Field experiments were conducted during the Pigeon pea growing season of the year 1989-90. These experiments were carried out to understand the rhizosphere microflora of four varieties of Cajanus cajan which were used in the present investigation. In addition, the suitability of the experimental plots to grow the four different varieties of Pigeon pea was also checked, because the land used to prepare the experiment plots was uncultivated. A number of plots (3.66 x 1.22 m) were prepared and four different

varieties of Pigeon pea were grown in it. The growth measurements were regularly recorded for 120 days i.e. upto the flowering stage. Readings of shoot length, root length, fresh weight of shoot system and root system were taken and are summarised in table 24 and figures 23 to 26.

Results show an apparent difference in the plant height from early growth stages (Fig.23). DPPA 85-5 variety attained maximum height at the end of 120 days after sowing. At the end of four months the height of the plants were 7 to 10 times higher than that of 20 days after sowing. The rate of growth in length of shoot was maximum during 60 to 80 days. Although, the plants showed growth in height, the rate of growth was diminished after 80 days. In the case of root length the results were recorded upto 100 days. During this period roots of all varieties attained about 0.91 m. length. The maximum root length was recorded in DPPA variety. In ICP 2376, the rate of root elongation was maximum on 80th day after sowing (Fig. 24). Local variety showed the least root elongation in comparison with all the other three varieties.

A perusal of table 24 and figure 25 revealed that, out of the four varieties used in the present investigation, the DPPA variety recorded maximum shoot fresh weight and

good growth vigour. ICP was the one which attained less fresh weight of shoot. According to figure 25, LRG-30 and local variety showed a perfect growth curve, while in DPPA and ICP the curves increased after a lag phase during 60 to 80 days after sowing. The maximum and minimum root fresh weight at the end of 100 days was recorded in DPPA and LRG varieties respectively. Upto 60 days the fresh weight of root was in the same range in all the varieties studied. Thereafter in DPPA the values increased steeply, while in others this took place only after 80 days (Fig.26). The rate of increase in root fresh weight was maximum on 80th day in DPPA and LRG varieties. But in ICP and local varieties the rate increased upto 100 days.

Table 24: Average shoot, root length and fresh weight of four varieties of Pigeon pea.

	Varie-ties*	Number of days after sowing					
		20	40	60	80	100	120
Shoot length in cms.							
	1	34.9	50.3	83.2	141.7	186.0	202.4
	2	23.2	41.5	78.3	126.4	152.1	168.8
	3	18.4	36.8	73.0	124.9	156.8	170.1
	4	15.6	35.2	71.9	122.5	149.3	165.1
Shoot fresh weight in gms							
	1	1.8	4.2	7.3	10.5	13.8	17.2
	2	1.5	3.6	6.0	8.4	10.9	13.5
	3	1.3	3.3	5.9	8.8	11.9	15.1
	4	1.2	3.5	6.4	9.5	12.8	16.1
Root length in cms.							
	1	10.0	23.8	36.5	57.3	81.6	-**
	2	9.3	20.6	37.0	54.1	77.2	-
	3	8.0	18.3	35.8	50.6	73.6	-
	4	7.0	19.1	34.9	49.0	2.87	-
Root fresh weight in gms							
	1	0.26	0.45	0.70	2.31	2.87	-
	2	0.20	0.40	0.62	1.26	2.42	-
	3	0.12	0.30	0.73	1.03	2.01	-
	4	0.09	0.34	0.78	0.95	2.41	-

* 1.- DPPa 85.5; 2. ICP 2376; 3 - LRG 30; 4 - Local variety

** - Not recroded beyond 100 days after sowing

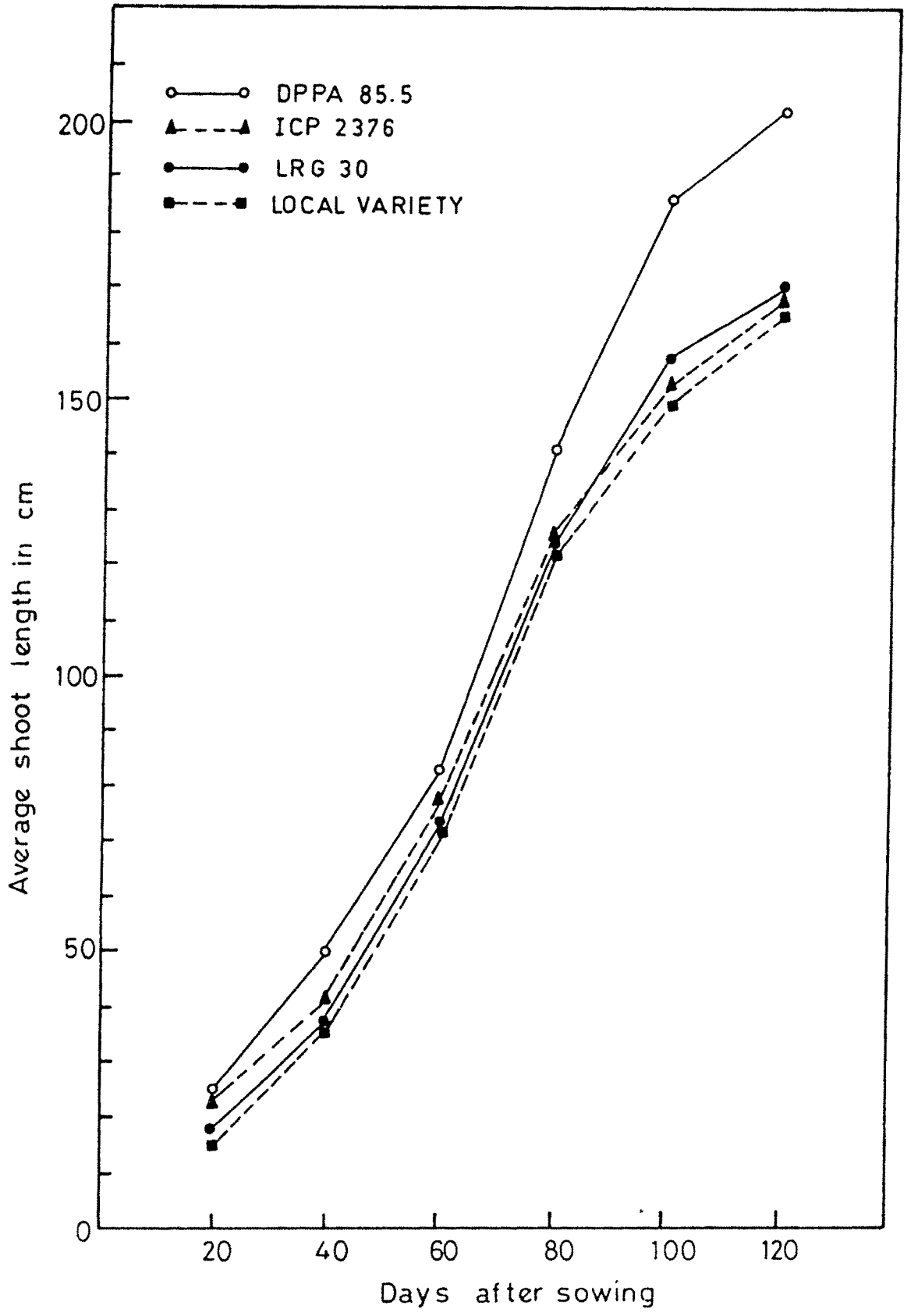


Fig.23 Average shoot length of four varieties of pigeon pea for a period of four months

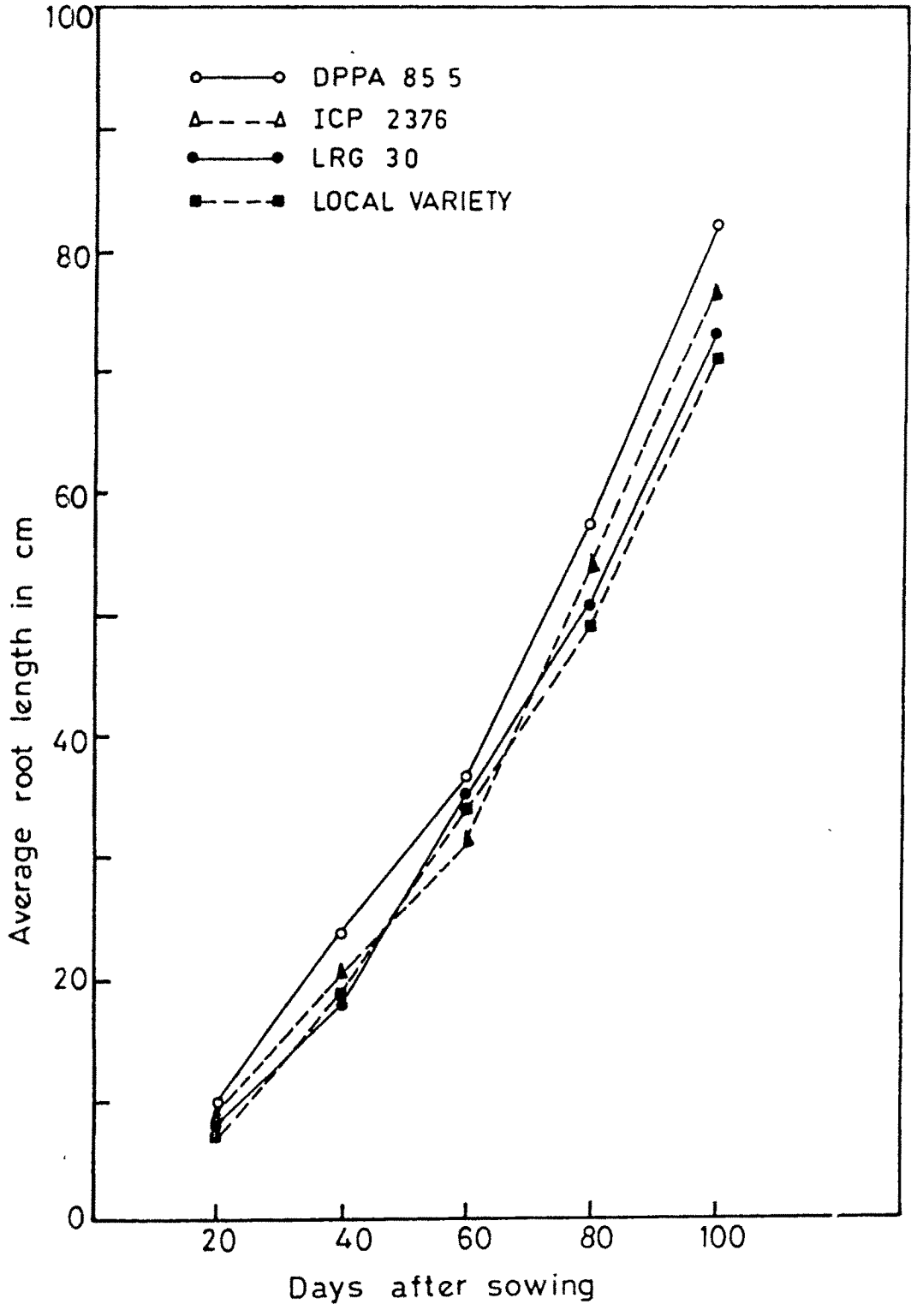


Fig 24 Average root length of four varieties of pigeon pea at an interval of 20 days

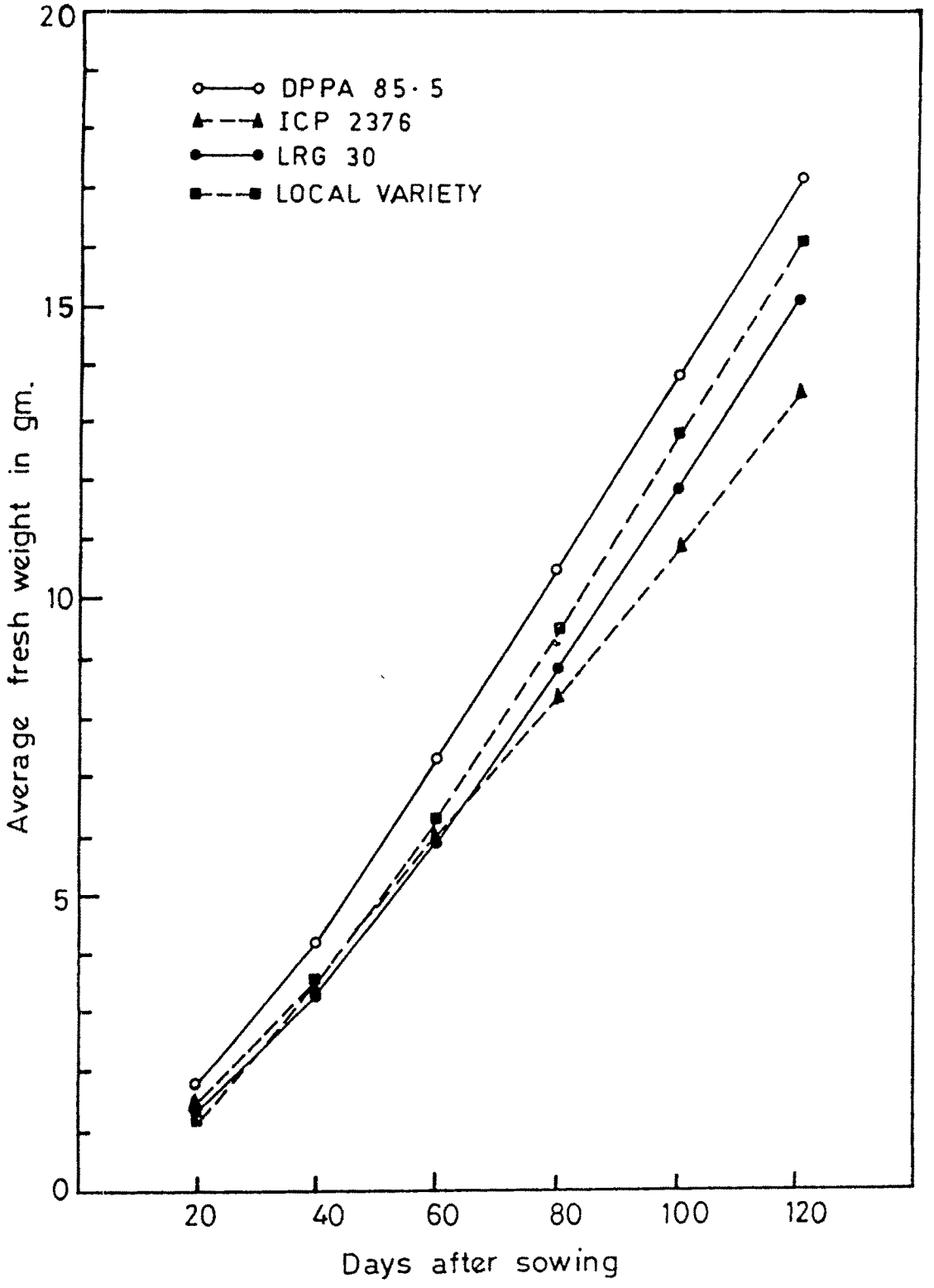


Fig 25 Average fresh weight of shoot in four varieties of pigeon pea.

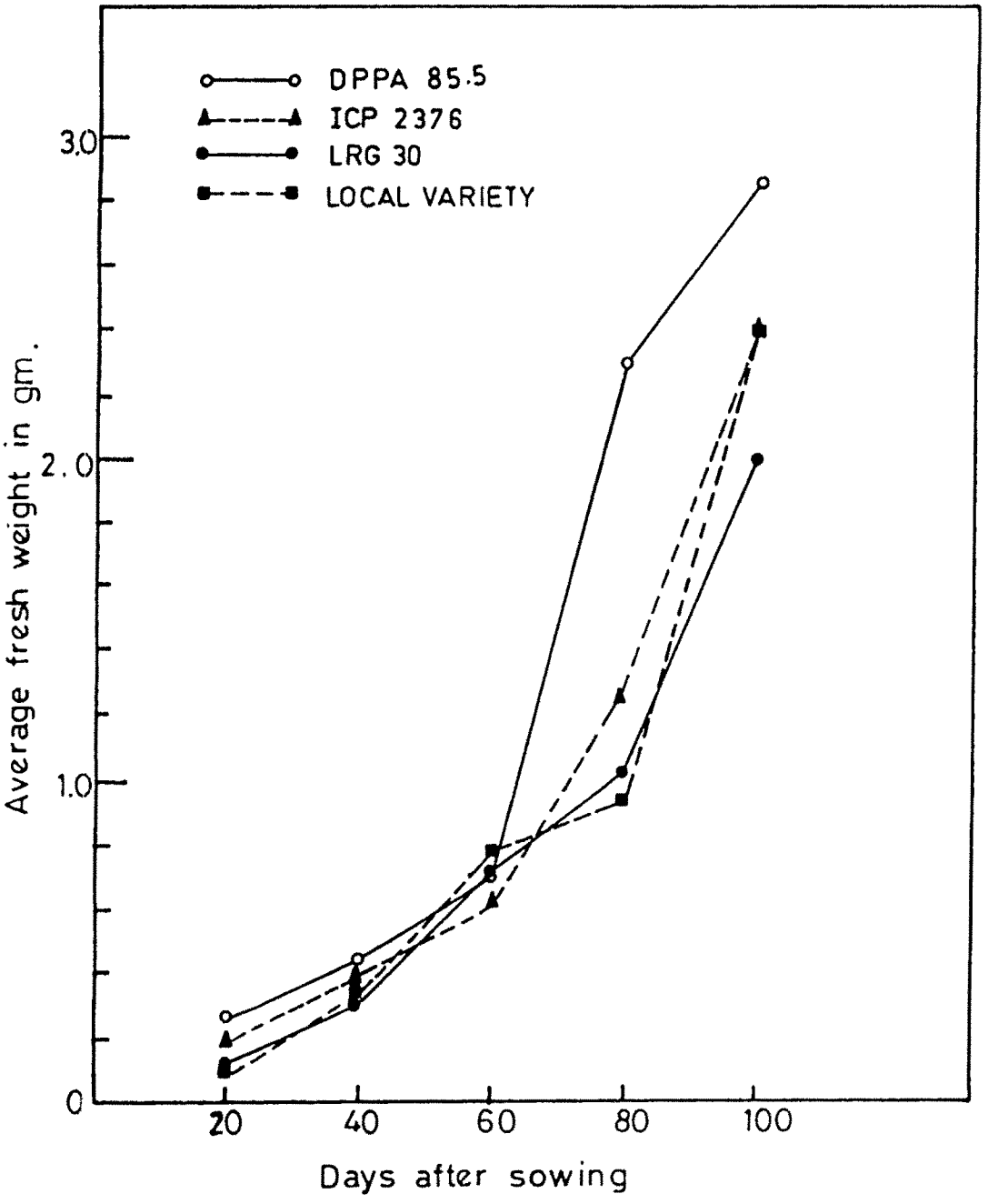


Fig.26 Average fresh weight of root in four varieties of pigeon pea.

4.2 Rhizosphere Study

In 1904 Hiltner (Warcup 1967) described how the surface of root was colanized by bacteria and he coined the term rhizosphere for the soil volume immediately influenced by the root. It is the root zone (rhizosphere) where microbes are most active in increasing the availability of nutrients for plants, and it is here also that they may exert their most injurious effects under certain conditions.

Plant rhizosphere has been studied with increasing interest during the last 30 years by soil microbiologists as well as plant pathologists. Kalznelson (1965) reviewed the available information. Soil environments,that influence plant growth and metabolism produce significant change in the rhizosphore effect,because the rhizosphere is controlled by metabolism of the plants (Khanna and Singh, 1975).

Microorganisms were isolated from non-rhizospheric and rhizospheric soils of four different varieties of Cajanus grown in the 'Arboretum' of the M.S.University of Baroda. The results are recorded in table 25. Rhizosphere and non-rhizosphere soils were harboured by Bacteria and Actinomycetes in significant amount. Normally, rhizosphere

region is the place where more microorganisms are found than non-rhizosphere soil. Khanna and Singh (1974 and 1975) reported similar results. But in the present study less number of fungi were isolated from rhizosphere soil. All the four varieties predominantly showed the presence of Aspergillus and Penicillium species. Out of the 14 species of fungi isolated, 10 were present in non-rhizosphere soil. They include, Aspergillus niger. Van Tiegh; A.fumingatus Fres; Aspergillus sp.1; Penicillium sp.1; Alternaria alternata (Fr.) kesslar; Fusarium udum E. Butler; Helminthosporium sp; Mucor hiemalis Wehmer; Phoma multirostrata Mathur; and Rhizopus nigricans Ehrenberg. Only four fungi were isolated from the rhizosphere of DPPA variety. Nine fungi each were isolated in the case of LRG and ICP, while in local variety only seven were isolated in the present study. Rhizopus nigricans was the only species present in both rhizosphere and non-rhizosphere soils of four varieties of pigeon pea. There is a great difference in quality of fungi present in the rhizosphere soil and non-rhizosphere soil. Among rhizosphere soil the quality as well as quantity of fungi differs according to the plant variety. Similar observations have been made by Manoharachary et al. (1977), Chandra and Raizada (1982). Both rhizosphere and non-rhizosphere soils harbour greater number of Aspergillus and penicillium spp. But Aspergillus was completely absent in the rhizosphere soil of LRG var. during the present investigation.

Table 25: Relative Proportion* of different micro-organisms isolated from non-rhizosphere and Rhizosphere of four different varieties of Pigeon pea before solarization

MICROORGANISMS	Pigeon pea varities				
	NR*	LRG-30	ICP 2376	DPPA 85.5	Local variety
Bacteria	+	+	+	+	+
Actinomycetes	+	+	+	+	+
Fungi					
Mucor hiemalis Wehmer	18.0	6.0	12.0	-	20.0
Rhizopus nignicans Ehrenberg	6.0	18.0	20.0	3.0	10.0
Choanephora curcubitarum (Ber.&Rav.) Thax.	-	10.0	-	-	-
Alternaria alternata (Fr.)Keisslar	4.0	15.0	15.0	-	-
Helminthosporium Sp.	6.0	15.0	-	-	6.0
Aspergillus niger Van Tiegh	20.0	-	10.0	42.6	20.0
A.fumigatus Fres	10.0	-	10.0	22.0	20.0
Aspergillus Sp 1	8.0	-	10.0	-	20.0
Aspergillus Sp 2	-	-	12.5	-	12.5
Fusarium udum E.Butler	2.6	-	-	-	3.0
Macrophomina phaseolina	-	2.0	-	-	-
Penicillium Sp 1	12.0	12.5	5.0	20.0	-
Penicillium Sp 2	-	10.0	-	-	-
Phoma multirostrata (Mathur)	4.0	2.0	5.0	-	-
Non sporulating forms	9.4	9.5	0.5	10.4	3.5

* Percentage in total population of Fungi

+ = Presence, - = Absence

* NR = Non rhizosphere

4.3 Soil Temperature Rise During Solarization Studies

Solarization studies were conducted during 1989-90 summer months as described in the chapter Materials and Methods. Solarization was done in the same plots, where 4 varieties of Pigeon pea were grown in the previous growing season. Immediately after solarization, the seeds of 4 varieties of Pigeon pea were sown. Care has been taken to sow the plots with the same variety which were grown in the previous season. Results were recorded in tables 26, 27 and figures 27, 28.

Solarization denotingly increase mean soil temperature over all depths (table 26). The maximum rise in temperature in comparison with non-solarized, non-irrigated soil to solarized and irrigated soil was 5,4,2°C for 0.03 m.m. (150 gauge), 0.06 m.m. (300 gauge) and 0.135 m.m. (1200 gauge) polyethylene sheet mulched soils respectively. Maximum temperature elevation was at 5 cm depth in all the treatments. This type of results were recorded previously by many workers like Katan et al.(1976), Mahrer (1979), Ashworth (1979), Pullman et al. (1979), Chen and Katan (1980), Elad et al. (1980). Mahrer and Katan (1980), and Arora and Pandey (1989). Katan (1981) recommended the use of possible thinnest polyethyelene tarp for efficient solarization. In the

present investigation, 0.03 m.m. polyethyelene sheet rendered maximum elevation in soil temperature (46°C). However, it is not recommendable for long term solarization because of its less durability. An appraisal of the table 26 reveals that, the thickest polyethylene sheet used in the present study (0.135 m.m) does not increased the soil temperature at deeper depths (20 cm).

Soil moisture is also recorded concurrently in each plots. The soil mulched with 0.03 m.m. thick sheet retain maximum soil moisture. Due to the exuberant condensation of moisture on the under surface of the sheet, the polyethylene sheets in the irrigated plots were found cling to the soil surface. This reduced tearing off of sheets by wind, rather than in the case of non-irrigated treatment. Eventhough desiccation and resultant condensation under the sheet was more in irrigated solarized plots, it lose much moisture content than non-irrigated solarized plots (Chauhan et al.,1988).

4.3.1. Effect of Coloured Polyethylene Sheet on Solarization

Table 27 shows temperature of mulched soil at a depth of 5,10,20 cm using coloured polyethylene sheets. Red

colour was not persistant, so it was excluded from the experiment. Black polyethylene tarp, though it is greatly heated by itself, is less efficient in heating the soil than transparent one (Katan,1981). In the present investigation also black coloured one shows the lowest temperature i.e. 35.5°C as compared to control (transparent) 44.5°C. Out of the 4 coloured sheets used, blue gave maximum elevation in soil temperature at 5 cm depth (fig.28). None of the coloured polyethylene tarped soils recorded temperature rise than control one.

Table 26 : Temperature and percentage moisture content of transparent polyethylene mulched soils at a depth of 5,10 and 20 cms.

Plot No	Treatment (covered with different guage)	Average Temperature C at a depth of			Percentage moisture at 10 cms depth
		5 cm	10 cm	20 cm	
1	Open (control)	420	40.0	39.0	15.0
2	150 guage sheet	46.0	43.0	41.6	49.0
3	300 guage sheet	45.0	41.0	38.0	22.5
4	1200 guage sheet	43.0	40.0	39.0	31.5

Table 27 : Temperature and percentage moisture of polyethylene mulched (different colours) soil at a depth of 5, 10 and 20 cms

Plot No	Treatment	Average temperature C at a depth of			Percentage moisture at 10 cm depth
		5 cm	10 cm	20 cm	
9	Green sheet	41.0	38.0	37.0	52.0
10	Black sheet	35.5	34.0	34.0	42.25
11	Blue sheet	42.0	40.0	38.5	35.25
12	Red Sheet*	-	-	-	-
	Control - Transparent 150 guage sheet	44.5	40.0	38.0	40.0

* Red colour was not persistant so reading are not taken

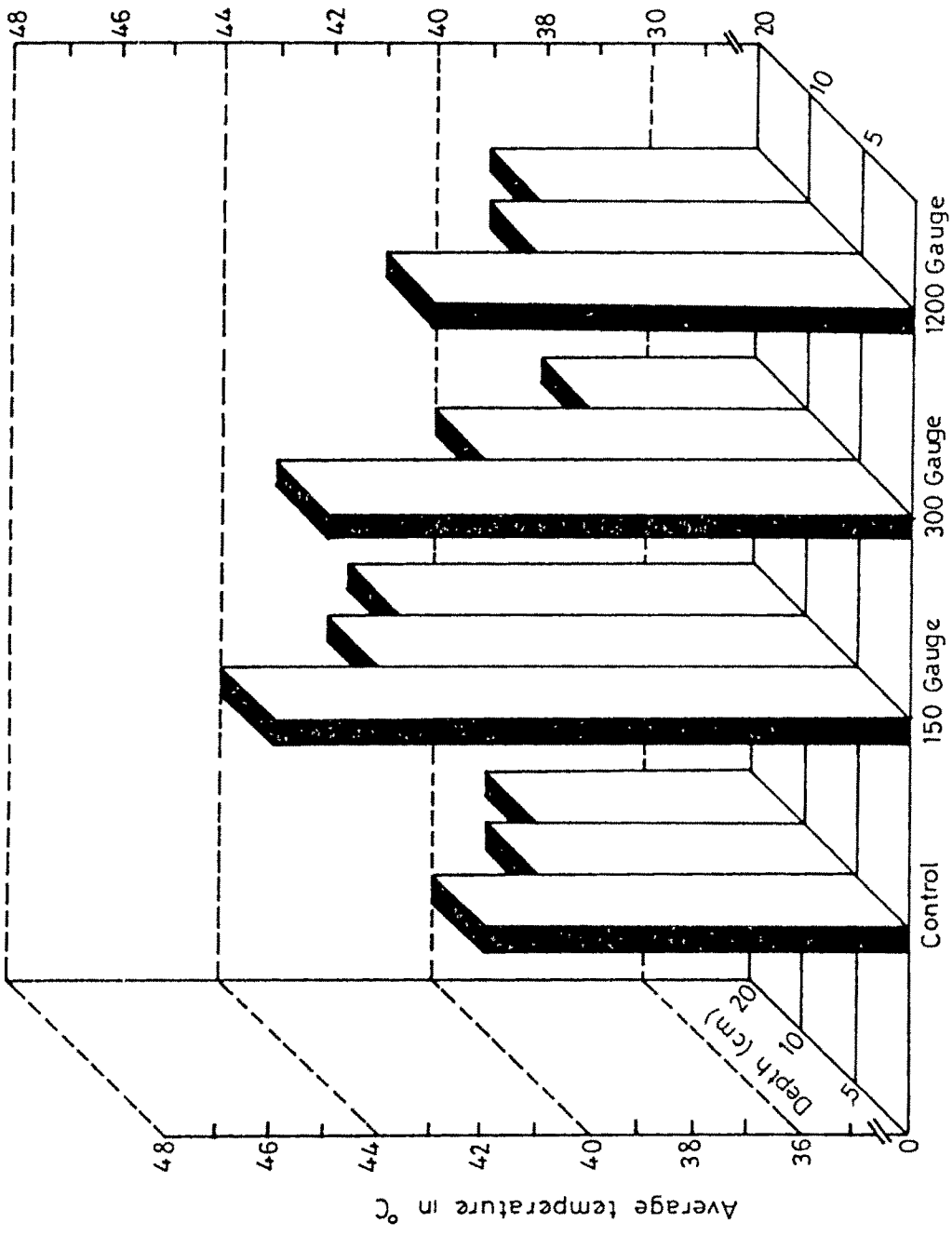


Fig 27 Maximum temperature of soil at a depth of 5, 10 and 20 cms in field subjected to solarization with different thickness of transparent polythene sheets

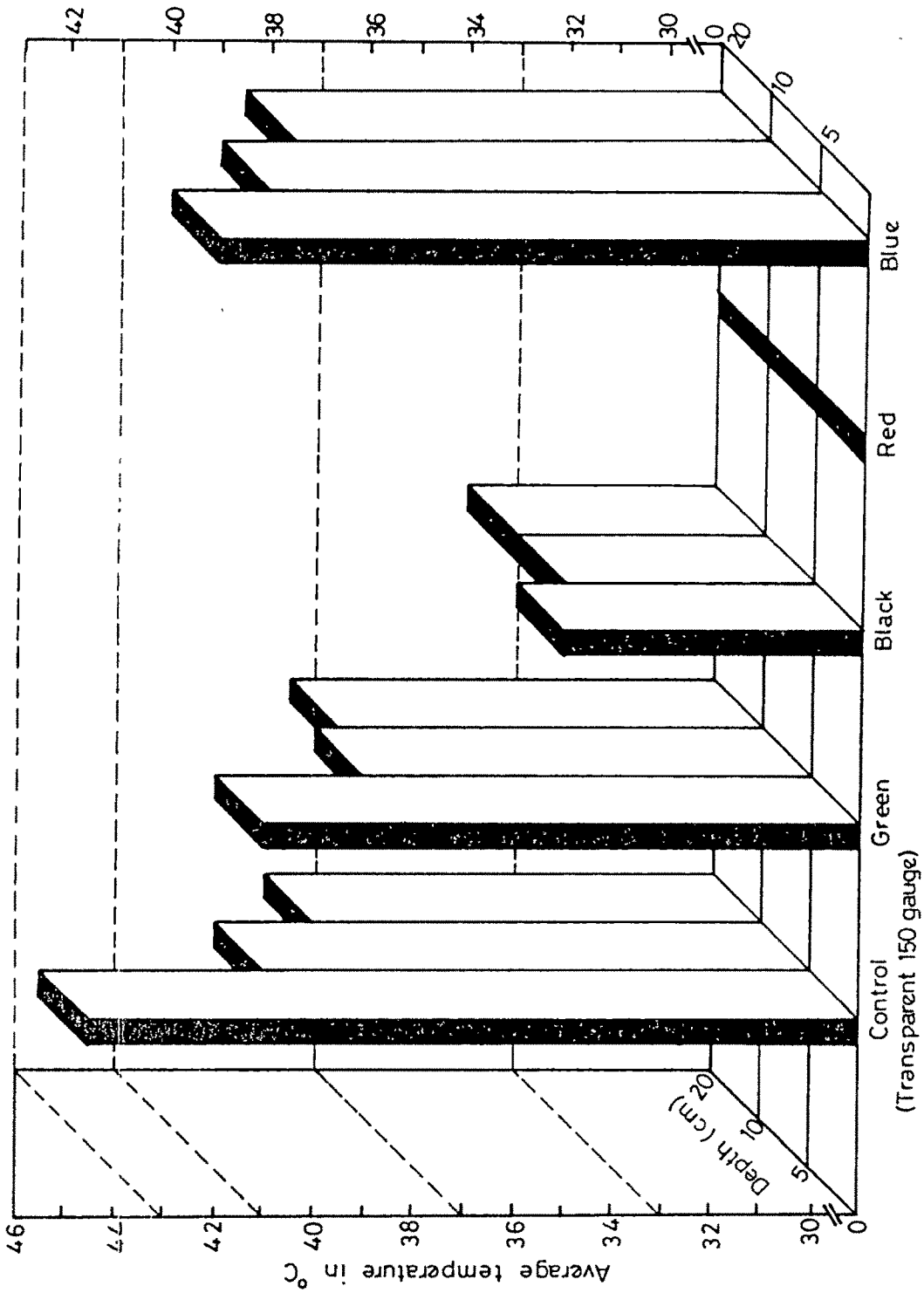


Fig28 Mean maximum temperature of soil at a depth of 5,10 and 20cms in field subjected to solarization with different coloured polythene sheets

4.4 Effect of Solarization on Rhizosphere and Non-rhizosphere Soil Microflora

Microorganisms were isoalted from non-rhizosphore and rhizosphore soils of 4 varieties of pigeon pea after solarization for a period of 45 days. Arora and Pandey (1989) reported that maximum elevation of soil temperature with solarization treatment was attained after 15 to 20 days of mulching and lowest propagules per gram dry soil was isolated on 45 days after solarization. In order to obtain a total decrease in population density of fungi from 90 to 85% Stapleton and DeVey (1982) mulched their study field with polyethylene sheets for 4-5 weeks only.

Table 28 shows the relative proportion of different microorganisms after solarization. Solarized soil also harboured good number of bacteria and actinomycetes as in the case of non-treated soil Choanephora cucurbitarum (Ber. & Rev.) Rhax., Helminthosporium sp., Fusarium udum E. Butler and one species of Penicillium which has been presented in the untreated soilw were totally absent after solarization both in non-rhizosphere and rhizosphere soils. Non-rhizosphere soil and rhizosphere soil of DPPA variety revealed the absence of Rhizopus nigricans Ehrenberg. Overall percentage in total population of fungi after solarization is greatly diminished as compared to

control soil (Non-solarized). In solarized non-irrigated soil the percentage population of Mucor hiemalis Wehmer decreased to more than half, while Apergillus and Penicillium Spp. increased. These results are consistent with previous reports of Warcup (1957) and Stapleton and Devay (1982). Only 7 genera of fungi were isolated from solarized soil, instead of 11 genera from untreated one. Two new genera - Chaetomium globosum Kunze and Cladosporium sp. which were absent in untreated soil were isolated after solarization. Population density of Alternaria alternata (Fr.)Keiss.,remain same in the non-rhizosphere soil after solarization. But rhizosphere soil of ICP variety recorded the absence and in LRG variety it become one fourth of untreated soil. After solarization treatment, Macrophomina phaseolina (Montl.)Ashby was isoalted from the rhizosphere soil of LRG variety only, while it was present in the rhizosphere soil of all varieties before solarization.

Eventhough solarized non-rhizosphere soil perceived an increase in population density of Aspergillus and Penicillium species, the rhizosphere soil of the 4 varieties of Pigeon pea observed a general decrease in their population.

Table 28 : Relative proportion* of different microorganisms isolated from non-rhizosphere and rhizosphere of 4 different varieties of pigeon pea after Solarization

MICROORGANISMS	NR**	Pigeon pea varieties			
		DPPA 85.5	LRG 30	ICP 2376	Local variety
Bacteria	+	+	+	+	+
Actinomycetes	+	+	+	+	+
Fungi					
Zygomycetes					
Mucor hiemalis Wehmer	6.0	-	6.0	12.0	20.0
Rhizopus nigricans Ehrenberg	-	-	18.0	20.0	10.0
Deuteromycetes					
Alternaria alternata (Fr.)Kerisslar	4.0	-	4.0	-	-
Aspergillus niger Van Tiegh	20.0	30.6	15.0	10.0	10.0
Aspergillus Sp 1	20.0	10.0	10.0	-	10.0
Aspergillus Sp 2	-	-	-	12.0	5.0
Chaetomium globosum Kunze	10.0	10.0	5.0	5.0	-
Cladosporium herbarum(Pres.)Link	-	-	-	10.0	15.0
Macrophomina phaseolina(Moubl.)Ashby	-	5.0	5.0	5.0	12.0
Penicillium Sp.	30.0	10.0	5.0	5.0	5.0
Phoma multirostrata(Mathur)Doe & Boer.	-	15.0	15.0	15.0	10.0
Sterile mycelium	10.0	19.4	17.0	6.0	3.0

* Percentage in total population of fungi

\+ = Presence, - = Absence

** NR = Non rhizosphere

4.5 Management of Wilt Pathogen by providing Organic Amendment during Solarization:

Soil amendment with organic materials such as manures is in practice since long. When green and dry parts of plants, saw dust etc. were tried and success has been achieved in suppressing the activity of soil borne pathogens (Linderman 1970, Singh and Singh 1970, Khanna and Singh 1975, Nauman and Lange-Dela Camp,1976 and Chandra et al.,1981).

Green leaves of three medicinally important plants i.e neem (Azadirachta **indica** A.Juess.), Calotropis (Calotropis procera R.Br.) and Nilgiri (Eucalyptus globulis Labill) , were incorporated in the soil before solarization and results obtained are depicted in table 29.

It is evident from the table 29 that after solarization bacterial colonies increased in Calotropis amended soil. Actinomycetes were present in all the three cases. Fungal colonies of eight members were obtained along with colonies of sterile mycelium. Although Fusarium solani was found in Eucalyptus amended soil,yet no colony of F udum was observed in any case. In neem and nilgiri

amended soil, the soil mycoflora decreased. The effect of amendment was clearly visible in the case of neem. Fungicidal property of neem leaf extract is earlier reported by Pandey et al., 1983 (See Indigenous Med.Plants) and Arya 1988. This may be due to presence of a chemical Ninibidin in the leaves of A. indica. The changes brought about in the microflora by organic amendments is due to their direct effect on the soil microflora, which may further alter the plant growth, metabolism and may create new exudation pattern which in turn may selectively modify the microflora and it needs further investigation.

Table 29: Microorganisms isolted after solarizatıon from Organıc amended soils.

MICROORGANISMS	Margosa	Calotropıs	Eucalyptus
Bacteria	+	++	+
Actinomycetes	+	+	+
Fungı			
Alternaria alternata(Fr.) Keisslar	-	+	-
Aspergıllus nıger van Tiegh	+	+	+
A. fumıgatus Fres.	-	+	-
Cladosporıum herbarum (Pres.)Lınk	-	+	+
Fusarıum solanı(Mart.) Sacc.	-	-	+
Mucor hiemalıs Wehmer	+	+	+
Penıcıllium Sp.	+	-	-
Rhızopus nıgricans Ehrenberg	-	+	-
Sterile mycelium	+	+	+

+ = Presence; ++ = Presence in more no.; - = Absence

5 PATHOLOGICAL STUDIES

Pathological studies were undertaken on four species of Fusarium obtained from soils of pigeon pea fields.

5.1 Morphological studies

In order to understand the morphological nature of the four species of Fusarium,light microscopic and electron microscopic (SEM) studies were conducted (Plate I and II). The methods employed for the fixing, slide preparation and specimen preparation are given in the chapter dealing with materials and method. Camera lucida diagrams were prepared to depict each organism and its different stages (Figs.29 and 30).

A. Fusarium udum E.Butler

The causal organism of the wilt disease. Synonyms are Fusarium butleri Wollenw (Wollenweber,1913); Fusarium uncinatum wollenw. (Wollenweber,1917); Fusarium lateritium f.sp.cajani (Gordon,1952) and Fusarium udum var. cajani Padwik (Padwick,1940). However, now the name Fusarium udum is widely accepted (Booth, 1971; Subramanian,1971; Booth et al.,1978; Gerlach and Nirenberg,1982; Upadhyay and

Rai,1989). There was a controversy among mycologists that, F. udum is a form of F. oxysporum. However discovery of the perithecial state of F.udum byRai and Upadhyay (1982) resolved this controversy. Detailed description of this organism has been given by wollenweber (1931), Subramanian (1955), Booth et al. (1978).

Colonies on PDA are moderately growing and at the beginning of growth the colonies are white in colour but later turning to off-white to brownish. Macroconidia falcate to lanceolate with a curvature in the thick central part and with a well developed pedicellate foot-cell. The macroconidia is bent outside and inner one is flattened towards inside, usually five septate (40-56 x 3.7 - 5.0 um), seldom 3 to 4 septate (18-42 x 3.5-4.0 um). Microconidia are usually single celled. Chlamydospores are terminal or intercalary, solitary or in chains, globose, ochraceous, thick walled and 7-9 um in diameter. Condiophore penicillately branched and compact.

B. Fusarium pallidoroseum (Cooke) Sacc.

This fungus was formerly named as F. semitectum Berk.& Rav. Singh (1988) described this organism as a causal agent of leaf blight in Pigeon pea. F.

pallidoroseum is considered as a weak parasite as well as secondary invader, but potential enough to cause rotting of plant parts. The fungus is seed borne in majority of the cases. In the present study also this fungus was first isolated from Cajanus seeds.

Colonies on PDA initially white in colour turning buff brown after long days of incubation. Microconidia or primary macroconidia are with a wedge shaped foot-cell arising from polyblastic cell and having one septum (6-20 x 2.5 um). Secondary macroconidia are also arising from a wedge shaped foot-cell and with a pointed beak, slightly curved, 3-7 septate (15-40 x 3.0-4.5 um). Chlamydospores are globose, single or in chains, mostly intercalary and 6-12 um in diameter.

C. Fusarium oxysporum Schlecht.

The fungus cause pre- and post- emergence mortality as well as foot and root-rot of plants. It infects the roots of many crop plants (cabbage, cotton, cowpea, potato, tomato, bean, watermelon) without causing any external symptoms. Plants of all age are susceptible to this organism. The pathogen is known to survive in plant debris and is seed and soil borne in nature. Some of the

synonyms are F. bulbigenus Cooke and Masee (Cooke and Masee, 1913), F. conglutinans Wollenw. (Woolenweber,1913) and F. bostrycoides Wollenw. and Reinkg. (Wollenweber and Reinking,1925).

Colonies fast growing on PDA, initially white turning to pale yellow, peach, vinaceous grey to purple violet after 20-25 days of incubation. Aerial mycelium white, brown at maturity, sometimes wrinkled but spreading. Microconidia are borne on simple short phialides arising laterally on the hyphae or from the sparsely branched conidiophores. They are variable in shape, oval to ellipsoidal, cylindrical, straight or curved and abundant (4.5-12.5 x 2.2-4.5 um). Macroconidia scattered,thin walled, borne on branched conidiophores, commonly 3 to 5 septate (three septate - 20 - 45 x 3.3-4.5 um and five septate 35- 55 x3.0-4.0 um). They are fusoid to subulate or pointed at both ends or hooked at the apex with pedicellate base. Chlamydospores abundant, terminal or intercalary or on short lateral branches smooth or rough, globose and 5 to 10 um in diameter.

D. Fusarium moniliforme J. Sheld.

F. moniliforme is a major parasite of several graminaceous crops such as rice, sugarcane, maize and sorghum. The fungus causes seedling blight, foot-rot,

stunting and hypertrophy of shoots of rice. The pathogen is soil and seed borne in nature. Gibberella moniliforme Sheld. (Sheldon,1924) and conidial state of Gibberella fujikuroi (Sawada) Ito ap. Ito and kimura are some of the synonyms.

Colonies growing on PDA are initially pale cream, lilac, turning to deep blue violet after 12-15 days of incubation. Aerial mycelium floccose to felted with or without powdery mass due to the formation of microconidia. Microconidia are formed in chains, fusiform to clavate with flattened base, some times are septate (4.5-13.5 x 1.5-2.5 um). Macroconidia are rare, fusoid, thin walled, 3 to 5 septate, apical cell elongated, curved and basal cell pedicellate (Three celled 23-35 x 2.5-3.5 um and five celled 32-58 x 2.5-4.0 um). Phialides simple, 20-30 um long or bearing 2-3 metulae.

5.2 Pathogenisity test

For this experiment 'Arhar' variety ICP 2376 which is susceptable to Fusarium wilt was used. Seedlings of same size and age (4 week old) were used for this purpose (Plate IV B). Two treatments viz. root injured and uninjured were employed. Details of experiments are given in the chapter materials and method. Re-isolation was made from the soil and from infected plant parts to confirm the

causal organism. Two sets of experiments were conducted, (a) Seedlings dipped in spore suspension (about 100 spores per lower field of compound microscope) and transplanted in sterilized soil, (b) keeping the seedlings in 20 days old culture filterate.

Table 30: Pathogenisity test and percentage wilting of 'Arhar' seedlings.

Organisms	Percentage of seedlings wilted		
	After one week		After five days
	Uninjured	Injured	In culture Filterate
F. udum	80	100	100
F. pallidoroseum	50	60	80
F. oxysporum	60	80	100
F. moniliforme	10	20	70
Control	-	10	-

Results from the above table clearly depict that, all the four species of Fusarium are capable of causing wilt of 'Arhar' seedlings (Domsch et al.,1980; Nene et al., 1985, and Singh, 1988). The main symptom of wilting was as if the sedlings have suffered from water shortage. The wilting was characterized by gradual withering, yellowing

and drying of leaves. Later on it was followed by drying of entire seedlings (Plate IV A). There was a higher percentage of infection in the case of seedlings with injured roots than those of uninjured.

F. udum was more pathogenic to pigeon pea seedlings than other three species. It showed a higher percentage wilting in both sets of experiments. F. oxysporum was also highly pathogenic to 'Arhar'. F. moniliforme very poorly induced wilt symptoms on seedlings in the first set of experiment. But in the second set, like other species F.moniliforme also induced wilting. In the first experiment the root injured seedlings showed a 10 percent wilting in control plants. This may be due to the injury. Except this insignificant wilting showed by the control plants, all remains healthy in both set of experiments. Thus from the above experiments it is very clear that F.udum and F. oxysporum cause more damage compared to two other species. Singh (1977) also found that F. oxysporum caused more damage to Pigeon pea. F. moniliforme failed to colonize in the root vasculature of the plant, it only induced a very feeble and weak infection on seedlings of the first set of experiment. In the second set all the four species were able to produce wilt symptoms. From this it is evident that, some toxic substances also play a role in causing wilting of Pigeon pea seedlings. An

attempt has been therefore made to detect the toxic substance (Fusaric acid) in the culture filterates of the four species of Fusarium.

5.3 Detection of Fusaric acid:

Fusaric acid was originally isolated from Fusarium heterosporum by Yabuta et al. (1934) and showed to be butyl picolinic acid. It is a non-specific vivotoxin. Its production in vitro by a number of fungi, all belonging to family Hypocreaceae have been demonstrated by Gauman (1957). The importance of fusaric acid to cause wilt symptoms has been worked out by many workers (Sandhu,1960; Kuo and Scheffer 1964; and Chandramohan and Mahadevan, 1968). Gauman (1957) and Sadasivan (1961) considered that Fusaric acid is the main cause of the wilt symptom but many other investigators (Kuo and Scheffer,1964; Mansour and Scheffer,1966; and Dimond,1970) are doubtful about its primary role and consider it to be of secondary importance only in the initiation of wilt symptom. Davis (1968 and 1969) studied about sixty six isolates of Fusarium oxysporum pathogenic to different plants and concluded that fusaric acid is one of the multiplicity of factors controlling host specificity and an apparent factor in the selective pathogenecity of F. oxysporum.

Fusaric acid was detected in all the four species of Fusarium. The amount produced by them, however varied. The method employed in this study is given in chapter 2. Visual comparison of the chromatograms, based on the size and colour intensity of the spots, the four species of Fusarium are graded as follows:

F. udum	++++
F.pallidoroseum	++
F. oxysporum	+++
F.moniliforme	++

5.4 Thermal death point:

The thermal death point is defined as the last temperature at which all the cells are killed in 10 minutes (Cochrane, 1958). The fungi can tolerate a limited temperature range. All their metabolic activities ceases after a certain temperature and coagulation of protein (Enzymes necessary for normal functioning of life) takes place, resulting into death of the organism.

In the present investigation, it was found that spores of Fusarium pallidoroseum and F. oxysporum were killed at 52°C and 50°C within 10 minutes respectively. It was 45°C for F. udum and 57°C for F. moniliforme.

A review of literature on thermal death point of other organisms indicated a similar temperaure range. The spores of Phomopsis sapotae (Rai,1982) were killed at 52°C Arya (1982) reported that spores of P. psidi and P. gulabia were killed at 57°C in 10 minutes. The temperatures 53°C and 54°C were found to be lethal to Hendersonala toruloidea and Botryodiplodia theobromae (Williamson,1964). Different isolates of some organism, shows difference in thermal death point. Spores of Botryodiplodia theobromae isolated from Sapodilla were killed at 54°C while isolates from citrus at 56°C and mango at 58°C (Srivastava,1966).