Comparative Nutritional Studies of *Pythium* spp.

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SUMMARY

Nutritional experiments were carried out dealing with: (1) the utilization of sulphur compounds; (2) the action of different mineral salts and their interaction. The results showed that the species of *Pythium* used grew well at 28° buffered with Sorensen's salt to give an initial pH of 6.5 and incubated for 13 days for *Pythium afertile* and 10 days for the remaining species. Acid conditions below pH 5.0 and alkaline conditions above pH 8.0 were not tolerated. The best carbon and nitrogen sources are given. The best sulphur sources were Na_2S , $\text{Na}_2\text{S}_2\text{O}_5$, $\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$, $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$, $\text{Na}_2\text{S}_2\text{O}_5 \cdot 5\text{H}_2\text{O}$, $\text{K}_2\text{S}_2\text{O}_8$, molecular sulphur, DL-methionine, thioacetamide, L-cysteine, sodium thioglycolate, and DL-cystine. Factorial experiments were carried out in which the fungi were incubated at 28° for 13–14 days. Statistical analysis of the results showed that under the given conditions optimal growth measured as mg. dry wt. was obtained in liquid media containing glucose, 10.0 g./l.; KCl, 0.5 g./l.; with varying amounts of K_2HPO_4 , KNO_3 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ for the different species specified. There was a balance between K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ for *P. debaryanum* and there were significant interactions between the salts above taken two at a time for *P. debaryanum* and *P. ultimum* but the interactions for the remaining fungi were more variable. There was interaction between all three salts together for two of the species. *P. debaryanum* is more exacting in its nutritional requirements than the other species.

Some species of *Pythium* are purely saprophytic, others are normally parasitic but may be saprophytic. Thus *Pythium* would seem to be a good genus for comparative nutritional studies. Previous work at Newcastle upon Tyne (see Fothergill *et al.*) indicated that for some parasitic fungi the balance of the major inorganic salts in a culture medium was more important for the good growth of the mycelium than the concentration of individual salts, but in the case of some saprophytic species, the balance of salts was much less important. Results, however, over a range of fungi varied. The present experiments are a continuation of work designed to investigate these points. Excluding work done on *Pythium* as a plant pathogen, a few physiological investigations have been reported mainly dealing with the presence and function of pectinase in these fungi (Chona, 1932; Menon, 1934; Fernando, 1937; Ashour, 1954; Damle, 1952; Gupta, 1956; Wood & Gupta, 1958). The carbon, nitrogen, sulphur and vitamin requirements were investigated by Saksena (1942), Saksena & Mehrota (1949), and Saksena, Jain & Jaffri (1952), but Cantino (1955)

strongly criticized these works because of the lack of pH control during the experiments. No comprehensive nutritional investigation has yet been carried out on *Pythium*.

METHODS

The following organisms were used. *Pythium debaryanum* Hesse originally isolated from *Lepidium* sp., *P. ultimum* Trow from *Pisum sativum* L., *P. afertile* Kanouse & Humphrey and *P. torulosum* Coker & Patterson. All organisms were obtained from the Centraal Bureau voor Schimmelcultuur, Baarn. The first two species are parasites causing damping-off disease of seedlings, while the last two species are normally saprophytic soil fungi but they have been reported as growing on Gramineae (Middleton, 1948). Throughout this paper the following abbreviations are used: *P. debaryanum* is designated D; *P. ultimum*, U; *P. afertile*, A and *P. torulosum*, T.

Initially single tip isolates were used as inocula for each species on Difco potato-extract-glucose agar medium. Stock cultures were kept on slopes of this medium in 6 in. $\times$ $\frac{3}{4}$ in. test tubes and the cultures were stored at room temperature because Church & Scandiffio (1928) showed that cultures of *Pythium* live longer at room than at lower temperatures. Subcultures were made as required. No alteration of morphological and cultural characteristics of these fungi occurred during the period of the experiments. In all experiments cultures were grown in 30 ml. aliquots in 150 ml. Erlenmeyer flasks. After the growth period the mycelia were filtered on to tared Whatman no. 5 filter-papers and washed with distilled water. The mycelium with the filter-papers was then dried for 24 hr. at 85°, cooled in a desiccator and weighed; results are expressed as mg. dry wt. mycelium/flask average of 4 or 5 replicates. It was not found practicable to separate mycelium from filter-paper after filtration and as the filter-papers used lose 5% of their original weight on drying under the above conditions, appropriate allowance was made for this. For preliminary experiments the following basal defined liquid medium A was used: glucose, 10 g.; NH_4NO_3 , 1.0 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; KCl, 0.5 g.; water to 1 l. This medium was buffered with Sorensen's phosphate buffer ($\text{KH}_2\text{PO}_4 + \text{NaHPO}_4$) to give an initial pH of 6.5. Experiments showed that all the *Pythium* sp. grew well at this pH value. The parasitic species tolerated acid conditions, even at pH 5.0, better than the saprophytic species which gave very low mycelial yields at this pH level. None of the species would grow at pH 8.0.

Experiments were carried out to determine the best method of inoculation and it was found that agar disks gave the most consistent mycelial dry weights provided the disks were between 3 and 8 mm. in diameter. Disks of 4 mm. diameter cut from the edge of 48 hr. old cultures were used in all later experiments. Experiments also showed that 4 mm. disks did not significantly affect the nutritional results, that is, the nutritional effect of the Difco agar could be discounted and this was true also for the vitamins which may have been present in the agar. This latter point was tested for the presence of thiamin in the agar. *Pythium vexans* needs an exogenous supply of thiamin for growth (see Leonian & Lilly, 1945). This fungus was tested in (a) the basal medium alone, and in (b) the basal medium plus thiamin using agar inocula. The average weight of 5 replicates showed that in (a) growth was slight, but in (b) 71 mg. mycelium/flask were obtained. Further, the successful use of the agar

disk method in the determination of vitamin requirements of other *Pythium* spp. was shown by Leonian & Lilly (1939) and Saksena (1942). Addition or omission of the usual trace elements, Fe, Mn and Zn made no difference to mycelial dry weights and these may be present in sufficient quantities in the Analar reagents used throughout. Tests also showed that none of the fungi needed an exogenous supply of biotin, nicotinic acid, folic acid, sodium pantothenate, pyridoxine, riboflavin, *para*-aminobenzoic acid or ascorbic acid. Neither thiamin nor its moieties affected growth, which supports the findings of Verma (1944). Robbins & Kavanagh (1938), however, showed that *P. butleri* needed an external supply of this vitamin. The optimum temperature for mycelial growth was 28° but organism T had a rather slower growth rate than the other species. Optimum growth was reached after 13 days incubation for organism A and after 10 days for organisms D, U and T on basal medium A, and it was noted that in these preliminary experiments the mycelial yields of the parasites D and U were about twice those of the saprophytes A and T. Experiments on the effect on mycelial growth of autoclaving the medium were carried out in which the composition of basal medium A was varied by substituting $(\text{NH}_4)_2\text{SO}_4$ and asparagine respectively for NH_4NO_3 using U as the test organism. Autoclaving these media at 10 lb. pressure for 5 min. had no effect on the dry weight yields of this fungus but for periods above 15 min. dry weight was seriously reduced. Hence all media were subsequently autoclaved at 10 lb. pressure for 5 min.

RESULTS

Sulphur requirements

Preliminary experiments showed that all four isolates grew only slightly without sulphur in medium A. The sulphur requirement was then investigated by varying the sulphur source in this medium to give 0.5 g./l. sulphur and in which $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was omitted and KCl was replaced by 0.5 g./l. MgCl_2 . The final pH after autoclaving was between 6.5 and 6.6. The media were inoculated and then incubated at 28°. Dry weights of 4 replicate cultures were determined in all cases after 6, 9 and 15 days incubation. The final pH of the media were also determined. The results are shown in Table 1 which records the highest percentage yields expressed in terms of the yield from $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ as 100 % for the inorganic sulphur sources and from DL-methionine as 100 % for the organic sulphur sources. With $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ the actual highest yields were 88 mg. for organism D, 92 mg. for U, 78 mg. for A and 61 mg. for T, all after 9 days incubation, while with DL-methionine the corresponding highest yields were 78 (after 6 days), 87, 73 and 49 mg. The last three were all after 9 days incubation. In all cases, except two, with inorganic sources of sulphur the highest yields were obtained after 9 days incubation but with organic sulphur sources the highest dry weights varied with the organism and the incubation period.

For the inorganic sulphur sources all the compounds used, except sodium dithionate and ammonium sulphamate, gave good or satisfactory growth with all the fungi, but organism A only gave a moderate yield with molecular sulphur. These *Pythium* species are, therefore, euthiotropic (Volkonsky, 1933). Increasing the oxidation level of the sulphur atom had no significant effect on mycelial growth. Thus molecular sulphur (S), a thiosulphate ($\text{S}_2\text{O}_3^{2-}$), a metabisulphite ($\text{S}_2\text{O}_5^{2-}$),

a sulphite (SO_3^{2-}) and a sulphate (SO_4^{2-}) were all good sulphur sources for most of the species. Results using molecular sulphur, however, should be treated with caution. The results for the organic sulphur sources were more variable but, in general, the highest yields were obtained with DL-methionine, thioacetamide, L-cysteine (except for organism U), DL-cystine, sodium thioglycolate (except for organisms A and T). The remaining compounds used gave only moderate or poor growth responses. Sulphanilic acid, DL-ethionine, thiocyanide, thioacetanilide and thiohydantoin were only utilized to a small extent.

Table 1. *The utilization of inorganic and organic sulphur compounds by Pythium spp.*

Highest yields expressed as percentage of yields from A, $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$, and B, DL-methionine taken as 100. Final pH 6.2–6.6. (Mg. dry wt. mycelium/flask average of 4 replicates. 6 and 15 days incubation in brackets.)

Sulphur source	Species			
	D	U	A	T
A. $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ as 100 %, 9 days incubation				
Sodium sulphate	100	100	100	100
Sodium sulphide	102	90	79	67
Sodium metabisulphite	102	76	101	—
Sodium sulphite	98	84	103	92
Sodium thiosulphate	97	86	87	94
Sulphur	93	86	40 (6)	72
Potassium persulphate*	82	89	91	100
Sodium dithionate	31	35	8	18
Ammonium sulphamate	28 (6)	21	8	11
B. DL-Methionine as 100 %, 9 days incubation				
DL-Methionine	100 (6)	100 (9)	100 (9)	100 (15)
Thioacetamide	100 (15)	100	57	63 (15)
L-Cysteine	96	47	106	118 (15)
Sodium thioglycolate	90 (15)	67	47	36
DL-Cystine	85 (6)	98	97	84 (15)
Thiourea	56	40 (6)	25	34 (6)
Thiamin	58 (15)	54 (15)	28	38
Djenkolic acid	56 (15)	48 (15)	31 (15)	19
Taurine	29 (15)	21 (6)	47 (15)	104 (15)
Sulphanilic acid	22 (6)	24 (6)	11 (6)	10
DL-Ethionine	17	8	10	8
Thiocyanide	14	11	10	9
Thioacetanilide	14 (15)	33	26 (15)	13 (15)
Thiohydantoin	19	28	12	18 (15)
None	4	5	2	4

* Persulphate breaks down easily to sulphate.

— No growth.

The amino acids DL-methionine, DL-cystine, L-cysteine were very good sources for the species and in most cases these compounds were utilized more rapidly than any of the remaining compounds used. It may be that these compounds are absorbed and assimilated as intact molecules (see Steinberg, 1941). Djenkolic acid, also an amino acid, gave fair yields, but only after 15 days' incubation and it is worth noting that this compound is made up of two cysteine residues linked to a CH_2 group through

the sulphur atoms. Possibly djenkolic acid may have to be converted to cysteine before it is utilized. The poor utilization of thioacetanilide and sulphanilic acid supports Steinberg's conclusion (1941) that compounds containing sulphur attached to a benzene ring are unsuitable for use in assimilation by *Aspergillus niger*. Likewise thiohydantoin gave poor yields and it consists of a five-membered ring. DL-Ethionine was an extremely poor sulphur source for *Pythium*. Margolis & Block (1957) also showed that five species of yeast could not utilize this compound, while Schlenk & Tillotsen (1959) and Harris & Cohn (1959) showed that ethionine is an antimetabolite of methionine.

Factorial experiments

The object of these experiments was to determine the effect of varying the concentration of the mineral salts in the basal medium. The factorial design and statistical analysis of the results should indicate whether or not a balance between the salts is necessary for high mycelial yields under the given experimental conditions. The direct effect of the individual salts in the medium and the interaction between them is also determined. Previous experiments showed that basal medium A was a satisfactory medium for the four *Pythium* spp., but as it contained NH_4NO_3 as the nitrogen source it required buffering with Sorensen's salt. Haskins & Weston (1950), using *Karlingia rosea*, showed that when KNO_3 was used as a nitrogen source the pH of the culture medium remained approximately constant at pH 6.5 to 7.0. In the following experiments KNO_3 was substituted for NH_4NO_3 and after preliminary experiments basal medium B was evolved containing glucose 10 g.; KNO_3 , 2.0 g.; K_2HPO_4 , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; KCl, 0.5 g.; distilled water to 1 l. The pH of this solution was adjusted to 6.6 with 0.1 N-HCl. With this medium the pH value in the case of organisms D, U and T never fell below 5.35, while with A it never fell below 6.25. The cultures were incubated at 28° for 14 days with organisms D and T and for 13 days with organisms A and U. The concentrations of the salts were fixed on the basis of halving and doubling those in basal medium B. Thus KNO_3 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and K_2HPO_4 were each used at three concentrations and all possible combinations of them were set up giving a total of 27 variations. Each combination was done in quadruplicate; thus with any one species of fungus 108 culture flasks were incubated. Each 150 ml. Erlenmeyer flask contained 30 ml. medium. The results are the average mg. dry wt./mycelium of 4 replicates and the final pH values were determined in each case. The grouped results are shown in Table 2 and the grouped analyses of variance are given in Table 3. For abbreviations of the salt concentrations used in the text below see Table 2.

From the single salt analysis it is evident that there was no consistent result for all the organisms. This analysis showed that the differences between mycelial dry weights with KNO_3 were significant for all concentrations of the salt for organisms A and D. The highest mean weight for organism A occurred at the N_3 concentration and for organism D at the N_1 level of KNO_3 . For organism T significance between the N_1 and N_3 levels occurred with the highest mean dry weight at the N_1 concentration of salt. For organism U a significant difference occurred between the N_1 and N_2 levels with the highest dry weight at the N_2 concentration. At the N_3 level of KNO_3 with this organism there was a large decrease in mycelial dry wt. With K_2HPO_4

a significant difference was found between the P_1 and P_2 levels for organism A with the highest dry weight at the P_2 level and between the P_1 and P_3 levels for organism T with the highest dry weight at the P_2 level. For organisms U and D no significant differences in dry weights were found between any of the concentrations of K_2HPO_4 used, but for both organisms the highest dry weights occurred at the P_3 level of this salt. With $MgSO_4 \cdot 7H_2O$ the only significant difference in mycelial dry weight was

Table 2. *Growth responses of four species of Pythium to varying conditions of K_2HPO_4 , $MgSO_4 \cdot 7H_2O$ and KNO_3*

Concentration of K_2HPO_4 : $P_1, P_2, P_3 = 0.25, 0.5, 1.0$ g./l.; of $MgSO_4 \cdot 7H_2O$: $Mg_1, Mg_2, Mg_3 = 0.25, 0.5, 1.0$ g./l.; of KNO_3 : $N_1, N_2, N_3 = 1, 2, 4$ g./l. (Difference required between means for significance at odds of 99:1 for KNO_3 , K_2HPO_4 and $MgSO_4 \cdot 7H_2O = 5.8$ mg. for A and T, 6.2 mg. for U and 6.8 mg. for D)

A. <i>P. debaryanum</i> (=D) and <i>P. ultimum</i> (=U)																		
P ₁						P ₂						P ₃						
Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃		
D	U	D	U	D	U	D	U	D	U	D	U	D	U	D	U	D	U	
(Mean dry wt. mycelium mg./flask)																		
N ₁	167	168	162	175	160	168	168	172	171	175	168	170	153	163	181	169	172	175
N ₂	181	181	146	185	133	182	133	181	142	182	161	158	136	180	136	185	161	172
N ₃	149	154	133	151	127	149	139	159	129	146	116	148	127	170	123	151	139	176

B. <i>P. afertile</i> (=A) and <i>P. torulosum</i> (=T)																		
P ₁						P ₂						P ₃						
Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃		
A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	
(Mean dry wt. mycelium mg./flask)																		
N ₁	114	122	122	120	104	100	116	104	114	112	105	105	110	109	97	105	98	
N ₂	119	105	127	107	120	98	144	104	147	107	143	105	144	92	144	112	106	
N ₃	134	92	141	97	136	91	156	101	156	112	162	105	152	105	156	87	163	95

Table 3. *Analysis of variance (grouped) for Pythium spp. A, T, U, D*

Required 'F' and 't' values taken from Snedecor's tables (1934). Sums of squares and mean squares are omitted from table.

Variance	D.F.	F required odds					
		Found organism				99:1	19:1
		A	T	U	D		
Total	107	—	—	—	—	—	—
KNO_3	2	211.93	7.81	489.64	105.29	4.88	3.11
K_2HPO_4	2	32.54	7.46	3.03	0.42	4.88	3.11
$MgSO_4 \cdot 7H_2O$	2	1.69	5.53	1.84	1.00	4.88	3.11
$KNO_3 \times K_2HPO_4$	4	12.69	7.18	4.45	2.58	3.56	2.49
$KNO_3 \times MgSO_4$	4	3.10	2.45	5.51	7.48	3.56	2.49
$K_2HPO_4 \times MgSO_4$	4	2.16	3.11	3.20	5.25	3.56	2.49
$KNO_3 \times MgSO_4 \times K_2HPO_4$	8	0.1631	2.46	1.32	2.76	2.74	2.06
Residual error	81	—	—	—	—	—	—

found for organism T between the Mg_2 and Mg_3 levels with the highest dry weight at the Mg_2 concentration of the salt. For organisms A, U and D there were no significant differences between any of the levels of the salt. But for A the highest mean dry weight of mycelium was obtained at the Mg_2 level, for U at the Mg_1 level, and for D at the Mg_3 level of concentration of $MgSO_4 \cdot 7H_2O$.

Balance between K_2HPO_4 and $MgSO_4 \cdot 7H_2O$

The grouped analyses of variance showed that there was a significant interaction between K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ only for the parasitic organism D. This result also indicated that there was a physiological balance between these salts under the conditions of the experiments. Talley & Blank (1941) and Fothergill & Ashcroft (1955) have also shown that a physiological balance exists between these two salts and is important for the good growth of *Phymatotrichum omnivorum* and *Venturia inaequalis*. To confirm this result another experiment was designed to test the change in the amount of growth which might result when the concentrations of the salts were varied while still maintaining the balance between them. Thus the basic concentration of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ was 0.5 g./l. and 0.25 g./l. respectively; these concentrations were decreased to half in one set of solutions and increased twice and then 4 times in other solutions. This gave four treatments, each one having the same balance between the salts but the ratio of their concentrations was 0.5:1:2:4. Each of these treatments was tested singly with 0.5, 1.0, 2.0 and 4.0 g./l. of KNO_3 . The remaining ingredients of basal medium B remained constant. The initial pH of the media was adjusted to 6.5 prior to autoclaving and the cultures were incubated in 5 replicates at 28° for 14 days. The results expressed as mg. mean dry wt./mycelium are shown in Table 4, where treatments are numbered 1 to 16 in brackets.

Table 4. *Mean dry weights and growth responses of Pythium debaryanum after 14 days incubation at 28° on media with the same balance but with different concentrations of certain components*

Concentrations of salts as in Table 2 with following additions: K_2HPO_4 : P_4 = 2.0 g./l.; $MgSO_4 \cdot 7H_2O$: Mg_0 = 0.125 g./l.; KNO_3 : N_0 = 0.5 g./l. (The sixteen different solutions numbered in parentheses are referred to in the text as treatments 1 to 16).

	Mean dry wt. mycelium (mg./flask)				
	P_1 Mg_0	P_2 Mg_1	P_3 Mg_2	P_4 Mg_3	Mean N
N_0	122 (1)	110 (2)	108 (3)	106 (4)	111.5
N_1	140 (5)	147 (6)	162 (7)	143 (8)	148
N_2	158 (9)	182 (10)	164 (11)	172 (12)	169
N_3	158 (13)	163 (14)	164 (15)	157 (16)	160.5

General mean = 147 mg. dry wt. mycelium/flask.

The results showed that the mycelial yields for KNO_3 at N_0 and N_1 levels were significantly different from each other and from the P_2 and N_3 levels but these latter two levels were not different. To show the interaction of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ 't' tests were performed. There were no significant differences between: (a) treatments 2, 3 and 4 at the N_0 level of KNO_3 ; (b) treatments 5, 6, 7 and 8 at the N_1 level

of KNO_3 ; (c) treatments 10, 11 and 12 at the N_2 level; and (d) treatments 13, 14, 15 and 16 at the N_3 level. While there were significant differences between treatments 1 and 2 at the N_0 level of KNO_3 and treatments 9 and 10 at the N_2 level, this lack of significant differences at the respective levels of N confirms the conclusion that a balance exists between K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and that the concentration of either of these salts can be varied over a wide range without affecting mycelial yields providing that the concentrations of the other salts are varied in proportion. The significance obtained with treatments 1 and 9 at the lowest concentration of salts may indicate that the balance necessary for good growth only comes into operation above a certain minimum concentration of salts. A similar effect with *Venturia inaequalis* was noticed by Fothergill & Ashcroft (1955).

CONCLUSION

The analyses of variance also showed the first- and second-order interactions between the salts in the medium, and indicated some of the nutritional differences between the fungi investigated. For the fungi D and U all the first-order interactions (i.e. the interactions of all the salts taken two at a time) were significant at either the 99:1 or 19:1 level of odds. For the fungi A and T the results were more varied. For organism A the interaction of $\text{KNO}_3 \times \text{K}_2\text{HPO}_4$ and $\text{KNO}_3 \times \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were significant but not that between $\text{K}_2\text{HPO}_4 \times \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, while for organism T the interactions $\text{KNO}_3 \times \text{K}_2\text{HPO}_4$ and $\text{K}_2\text{HPO}_4 \times \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were significant but not that between $\text{KNO}_3 \times \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. In the second-order interactions only those for organisms D and T were significant.

In general the results of the factorial experiments showed that there was a considerable difference between the nutritional requirements of *Pythium debaryanum* (D) and *P. ultimum* (U). With the individual salts the greatest effect was produced by varying the concentration of KNO_3 . An absolute balance between all the nutrients was required by *P. debaryanum* (D) for good mycelial growth, but was not so necessary for *P. ultimum* (U). With the species, *P. afertile* (A) and *P. torulosum* (T) variation in the concentration of most of the individual salts produced large effects on mycelial growth and at the highest level of probability the interaction with KNO_3 was significant. Kitchell (1954), Fothergill & Yeoman (1957), and Fothergill & Jones (1958) also found that the concentration of the nitrogen sources and the interaction between these and the phosphorus source had a significant effect on the growth of *Mucor* spp., *Rhizopus stolonifera* and *Zygorhynchus* spp., all saprophytes. These results also support the findings of Fothergill & Jones (1958) who showed that there was a variation of balance in salt concentrations for different *Zygorhynchus* spp. Although each *Pythium* spp. has its own nutritional requirements D and U in general needed a more exact balance of mineral salts and showed less variation in their response to individual salts than did A and T here investigated. Differences in nutritional requirements between these fungi are statistically significant but they are not very large.

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