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Affected by Nitrogen Source

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Discovering Thoughts, Inventing Future

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Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Response of Broiler Chickens to Graded Levels of Urea Treated Rice Offal. *1-5*
2. The Feasibility of using Natural Rocks as Sources of Iron, Manganese and Copper in Livestock Feeding in Ethiopia. *7-12*
3. The Isolation of, and Cytodifferentiation of a *Mucor* Species as Affected by Nitrogen Source and Elevated Temperature. *13-84*
4. Design Development of Spate Irrigation Structures in Raya Valley, Ethiopia. *85-91*
5. Nutrient Availability and Maize Growth in Soil Amended with Mineral Fertilizer and Pressmud Biocompost. *93-100*
6. Technical Efficiency of Ecologically Engineered Rice Production in the Mekong Delta of Vietnam: Application of SFA. *101-110*

v. Fellows and Auxiliary Memberships
vi. Process of Submission of Research Paper
vii. Preferred Author Guidelines
viii. Index

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Response of Broiler Chickens to Graded Levels of Urea Treated Rice Offal

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Abstract- A total of ninety (90) day-old unsexed broiler chickens averaging 50.0 grammes were utilized for the purpose of accessing their response to graded levels of urea treated rice offal. These were randomly allocated into 3 equal groups of 30 birds in each treatment replicated 3 times with each replicate having 10 birds. Three (3) diets designated I, II and III were formulated such that urea treated rice offal was included at 0, 7.5% and 15.0% respectively. The results show that there were no significant differences ($P>0.05$) in feed intake, weight gain and efficiency of feed utilization among birds fed the control diet and urea treated rice offal based diets. However, feed intake and weight gain were highest with birds fed 15.0% urea treated rice offal compared with those fed the control diet.

Keywords: *broilers, chickens, urea, rice offa.*

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Response of Broiler Chickens to Graded Levels of Urea Treated Rice Offal

S.O. Onuh [α], E.E. Idogah [σ] & E. Ameh [ρ]

Abstract- A total of ninety (90) day-old unsexed broiler chickens averaging 50.0 grammes were utilized for the purpose of accessing their response to graded levels of urea treated rice offal. These were randomly allocated into 3 equal groups of 30 birds in each treatment replicated 3 times with each replicate having 10 birds. Three (3) diets designated I, II and III were formulated such that urea treated rice offal was included at 0, 7.5% and 15.0% respectively. The results show that there were no significant differences (P>0.05) in feed intake, weight gain and efficiency of feed utilization among birds fed the control diet and urea treated rice offal based diets. However, feed intake and weight gain were highest with birds fed 15.0% urea treated rice offal compared with those fed the control diet. Finally, feed cost per unit weight gain significantly improved (P<0.05) with birds fed the diet containing 15.0% urea treated rice offal compared with those fed other diets. However, there were no significant differences (P>0.05) in feed cost per unit weight gain among birds fed the control diet and those fed the diet containing 7.5% urea treated rice offal. On the basis of the results obtained, it may be recommended that urea treated rice offal could be included at up to 15.0% of the diet of broiler chickens without any adverse effect on their performance.

Keywords: broilers, chickens, urea, rice offa.

I. Introduction

Nigeria has the potential to produce about 200,000 metric tonnes of rice offal from the 500,000 metric tonnes of rice produced annually (Wudiri, 1991). The offal, therefore, makes up about 40% of the parboiled rice and contains husk, bran polishing and small quantities of broken grains. In spite of its abundance, it has been neglected as animal feeds because it contains high level of fibre and low protein and energy (Oyawoye and Nelson, 1999).

Maikano (2007) reported the proximate composition of rice offal thus; 94.42% dry matter, 5.09% crude protein, 30.39% crude fibre, 3.40% ether extract, 16.67% ash and 46.10% nitrogen free-extract. Several workers (Dafwang and Shwarmen, 1996; (Abasiekong, 1997; Awesu *et al*., 2002) have reported that the high crude fibre (30 – 44%); mainly lignin and low protein contents have resulted in reduced voluntary feed intake and low utilization in poultry feeding. This high fibre concentration results in poor nutrient utilization and consequent poor growth performance due to the presence of non-starch-polysaccharides (NSP) and phytate when fed to broiler chickens without any form of treatment.

The use of rice offal to replace cereal grains in poultry diets have been studied (Dafwang and Damang, 1995; Carew *et al*., 2005) and has been successfully fed to broiler chickens at lower levels of inclusion (Amaefule *et al.,* 2006; Onuh, 2006; Maikano, 2007; Yakubu *et al*., 2007) order to reduce feed costs. Higher levels of inclusion may therefore necessitate the development of strategies to increase the value of this by-product in order to reduce its fibre content. Alkali treatments of various fibrous materials (Faniyi and Ologhobo, 1999) and urea treatment (Isikwenu *et al*., 2008; Onuh, 2011) have been reported to improve their nutritional qualities.

The study reported herein was conducted to determine the response of broiler chickens to graded levels of urea treated rice offal.

II. Materials and Methods

The study was conducted in the Poultry Unit of the Department of Animal Husbandry, Akperan Orshi College of Agriculture, Yandev-Gboko, Benue State, Nigeria.

The rice offal, containing mainly the husk, used in the present study was collected from Rice Mill in Gboko, Benue State, Nigeria.

The urea treated rice offal was prepared according to the procedures outlined by Isikwenu *et al*. (2008). It was then sun dried for 2 days. The proximate chemical composition of untreated and urea treated rice offal are presented in Table 1.

Table 1 : Proximate chemical composition of urea treated rice offal

Constituent	[a]Untreated	[b]Treated
Crude protein (%)	5.09	14.50
Crude fibre (%)	30.39	25.00
Ether extract (%)	3.40	7.37
Ash (%)	16.67	11.52

Sources: [a]Maikano (2007); [b]Yakubu et al. (2007).

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A total of ninety (90) day-old unsexed broiler chickens obtained from CHI hatchery, Ibadan averaging 50.00 grammes were utilized for the purpose of the study. These were randomly allocated into 3 equal groups of 30 birds in each treatment and brooded after a two day initial stabilization period on deep litter system. Each treatment was replicated 3 times with each replicate having 10 birds.

Three (3) diets designated I, II and III were formulated for broiler chickens such that urea treated rice offal was included at 0, 7.5% and 15.0% respectively. All diets were adequately fortified with vitamins and minerals. The compositions of the broiler starter diets are presented in Table 2 while those of finishing broiler diets are presented in Table 3.

Table 2 : Composition of broiler starter experimental diets

Ingredient	Dietary Treatments		
	I	II	III
	Control	7.5% UTRO	15% UTRO
Maize	45.00	40.50	33.00
Full-fat Roasted Soyabean	51.00	48.00	48.00
Urea Treated Rice Offal	0.00	7.50	15.00
Bone Meal	3.00	3.00	3.00
Mineral-Vitamin Premix+	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25
Common Salt	0.25	0.25	0.25
Total	*100.00*	*100.00*	*100.00*
Calculated Analyses			
Crude Protein (%)	23.43	22.97	23.39
Metabolizable Energy (Kcal/kg)	3213	2961	2706
Crude Fibre (%)	3.71	5.33	7.05
Methionine (%)	0.67	0.64	0.62
Lysine (%)	1.79	1.69	1.67
Methionine + Cystine (%)	0.93	0.88	0.86
Feed cost/kg (₦/kg)	74.65	69.63	64.75

UTRO = Urea Treated Rice Offal

+Vitamin-mineral premix provided the following vitamins and minerals per kg of diet: A 15,000 I.U; D3 3000 I.U; E 30 I.U; K 2.5mg, B , 2.0mg; B_2 6.0mg; B_6 4.0mg; Niacin 40mg; B_{12} 0.02mg; Pantothenic 10mg; Folic 1.0mg; Biotin 0.08mg; Choline Cl 500mg; Antioxidant 125mg; Mn 6mg; Zn 60mg; Fe 24mg; Cu 6mg; I 1.4mg; Se 0.24mg; Co 0.4mg. Product of Agricultural Technologies Nigeria Ltd. Marketed by S&D Farms Abeokuta.

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Table 3 : Composition of finishing broiler experimental diets

Ingredient	Dietary Treatments		
	I	II	III
	Control	7.5% UTRO	15% UTRO
Maize	46.00	42.50	40.00
Full-fat Roasted Soyabean	38.00	34.00	29.00
Urea Treated Rice Offal	0.00	7.50	15.00
Maize Offal	6.00	6.00	6.00
Palm Kernel Cake	6.00	6.00	6.00
Bone Meal	3.00	3.00	3.00
Mineral-Vitamin Premix[+]	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25
Common Salt	0.25	0.25	0.25
Total	*100.00*	*100.00*	*100.00*
Calculated Analyses			
Crude Protein (%)	19.88	19.13	18.10
Metabolizable Energy (Kcal/kg)	3105	2855	2605
Crude Fibre (%)	4.82	6.41	6.05
Methionine (%)	0.61	0.58	0.54
Lysine (%)	1.47	1.35	1.21
Methionine + Cystine (%)	0.85	0.80	0.74
Feed cost/kg (₦/kg)	68.84	63.76	58.64

UTRO = Urea Treated Rice Offal

[+]Vitamin-mineral premix provided the following vitamins and minerals per kg of diet: A 15,000 I.U; D3 3000 I.U; E 30 I.U; K 2.5mg, B , 2.0mg; B_2 6.0mg; B_6 4.0mg; Niacin 40mg; B_{12} 0.02mg; Pantothenic 10mg; Folic 1.0mg; Biotin 0.08mg; Choline Cl 500mg; Antioxidant 125mg; Mn 6mg; Zn 60mg; Fe 24mg; Cu 6mg; I 1.4mg; Se 0.24mg; Co 0.4mg. Product of Agricultural Technologies Nigeria Ltd. Marketed by S&D Farms Abeokuta.

The birds were held on a basal diet for the first 2 days and monitored for problems that may be associated with hatchery defects and other sources of variations that could cause reduced performance and death which were independent of dietary treatments. The birds were reared according to standard procedures outlined by Dafwang and Ogundipe (1982). The birds were fed each of starter diets for 28 days and thereafter fed each of finishing diets for 21 days. The birds in each treatment were fed weighted amounts of their group diet to appetite daily and fresh water was offered *ad libitum* throughout the period of the study while necessary prophylaxis and vaccinations for broilers were administered. The left over feed was collected and weighed before another days' feeding to determine actual intake. The birds were weighed weekly to determine weight changes. Feed conversion ratio was computed by dividing daily feed intake by the corresponding weight gain. Feed cost per unit weight gain was computed as a product of feed conversion ratio and feed cost per kg of each diet at the time of conducting the study. The dietary feed cost was obtained from the market prices of the different ingredients at the time and locality of the study. Data on feed intake, weight gain, feed: gain ratio and feed cost per unit weight gain were recorded on replicate basis weekly for 49 days.

Data on each parameter were subjected to the analysis of variance (ANOVA) for Completely Randomized Design (CRD) and where significant differences were indicated, the means were separated using Duncan's Multiple Range Test (DMRT) according to the procedures of the Statistical Package (SPSS, 2006).

III. Results

The summary of results of the response of broiler chickens to graded levels of urea treated rice offal is presented in Table 4.

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Table 4 : Response of broiler chickens to graded levels of urea treated rice offal

Parameters	Dietary Treatments			SEM
	I	II	III	
Average daily feed intake (g)	96.77	101.16	109.43	10.30^{NS}
Average daily weight gain (g)	45.27	43.03	49.53	4.95^{NS}
Feed conversion ratio	2.14	2.35	2.21	0.30^{NS}
Feed cost per unit weight gain (₦/gain)	184.43^{b}	167.09^{b}	139.87^{a}	19.76

[abc]Means followed by the same superscript in horizontal rows are not significantly different (P>0.05) from one another; SEM = Standard Error of Mean.

As the results have shown, there were no significant differences (P>0.05) in feed intake, weight gain and efficiency of feed utilization among birds fed the control diet and urea treated rice offal based diets. However, feed intake and weight gain were highest with birds fed 15.0% urea treated rice offal compared with those fed the control diet. Weight gain increased by 8.60% when 15.0% urea treated rice offal was fed compared with birds fed the control diet. Furthermore, feed cost per unit weight gain significantly improved (P<0.05) with birds fed the diet containing 15.0% urea treated rice offal compared with those fed other diets. However, there were no significant differences (P>0.05) in feed cost per unit weight gain among birds fed the control diet and those fed the diet containing 7.5% urea treated rice offal.

IV. Discussion

In the present study, it was observed that 15.0% urea treated rice offal increased feed intake compared with birds fed other diets. The result of the present study is not consistent with those of Iheukwumere *et al.* (2001) and Yakubu *et al.* (2007) who reported lower feed intake with birds fed urea treated rice milling waste when compared to those fed untreated rice milling waste. The high feed intake in the present study could be attributed to the effect of the urea treatment on the fibre content of rice offal. Weight gain at 15.0% level of inclusion of urea treated rice offal was highest compared with birds fed diets fed other diets. The results of the present study is consistent with the reports of Amaefule *et al*. (2006) who reported that broilers fed urea treated rice milling wastes had significantly higher final body weight and daily weight gain than those fed other diets. Efficiency of feed utilization in the present study of birds fed 15.0% urea treated rice offal was better than those fed the diet containing 7.50% urea treated rice offal. The results of the present study which showed that urea treated rice offal resulted in better feed utilization agrees with earlier reports of Abu *et al.* (1999) in rabbits and Iheukwumere *et al.* (2001) in broilers who reported an increase in weight gain and efficiency of feed utilization when birds were placed on diets containing urea treated rice-milling waste. It is known that the chicken is known to be especially sensitive to dietary energy concentration (Scott *et al*., 1982). In the present study, the energy content of the diets decreased with increasing levels of urea treated rice offal. Since energy intake is a productive function of feed intake, the higher feed intake of birds fed the urea treated rice offal in the present study could have been responsible for their higher weight gain.

On the basis of the results obtained, it may be recommended that urea treated rice offal could be included at up to 15.0% of the diet of broiler chickens without any adverse effect on their performance.

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The Feasibility of using Natural Rocks as Sources of Iron, Manganese and Copper in Livestock Feeding in Ethiopia

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Abstract- In Ethiopia, feed industries are widely using limestone as a cheap source of Ca without considering the source variability's and the amount of other minerals without adequate information on the bioavailability of its Ca content and the presence of other toxic minerals. This being the case, the present study was conducted to determine the iron, Manganese and copper content of samples of limestone , marble powder and gypsum collected from different parts of Ethiopia. Adequate quantities of lime stone, marble powder and gypsum were procured from different parts of Ethiopia and subjected to laboratory chemical analysis in triplicate. The results of this study clearly showed that the total ash content of all the materials analyzed in this study ranged between *81* and *99%*, indicating the potential use of these materials (limestone, marble powder and gypsum) collected from different part of Ethiopia as supplementary mineral feed source in very small amounts.

Keywords: *calcite powder; lime stone; livestock; marble powder; minerals.*

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The Feasibility of using Natural Rocks as Sources of Iron, Manganese and Copper in Livestock Feeding in Ethiopia

Abegaze Beyene [α] & Anne LeLacheur [σ]

Abstract- In Ethiopia, feed industries are widely using limestone as a cheap source of Ca without considering the source variability's and the amount of other minerals without adequate information on the bioavailability of its Ca content and the presence of other toxic minerals. This being the case, the present study was conducted to determine the iron, Manganese and copper content of samples of limestone , marble powder and gypsum collected from different parts of Ethiopia. Adequate quantities of lime stone, marble powder and gypsum were procured from different parts of Ethiopia and subjected to laboratory chemical analysis in triplicate. The results of this study clearly showed that the total ash content of all the materials analyzed in this study ranged between *81* and *99%*, indicating the potential use of these materials (limestone, marble powder and gypsum) collected from different part of Ethiopia as supplementary mineral feed source in very small amounts.

The samples of lime stone, marble powder and gypsum were procured from different parts of Ethiopia, which varied in Fe content *(ppm)* from *548.59 to 8238.67* with an average of *2797.69.* These values were very high when compared to the Fe content of calcium Carbonate and Calcite powder samples *(0.12 ± 0.00ppm).* Also the, Mn content(ppm) from *9.92-262.08* with an average *(91.02)* These values were low when compared to the Mn content of calcium Carbonate and Calcite powder of previous work which was *(233±2.33)* and Cu contents(ppm) from 3.17-*12.75* with an average (*7.73* ppm) the samples analyzed were almost the same (*8.00± 0.00ppm*) with those of calcite powder and Ca carbonate of previous work Abegaze Beyene (*2012*). the values of the Fe is much higher ,Mn and Cu content is comparable with common animal feed in Ethiopia. In summary the results of this study showed that lime stone and marble powder widely available in different parts of Ethiopia seems to have potential value as Trace elements supplement for livestock feeding. Testing the bioavailability of these materials with animal seems to be the future direction of research.

Keywords: calcite powder; lime stone; livestock; marble powder; minerals.

I. Introduction

Successful animal production depends on genetic and environmental factors including nutrition and management practices,of which nutrition plays an important role. It is believed that more than 50% of the farm expenditure or cost of animal production goes towards feeding of animals. Dietary nutrients promote programming and expression of the metabolic pathways that enables the animal to achieve its genetic production potential. All the nutrients (carbohydrate, proteins, fat, vitamins, and minerals) are equally important as deficiencies of one or more of these nutrients hamper the health status and productivity level of animals. Minerals may constitute a small fraction of the total ration but perform vital role in the body.

There is variation in the mineral content of different animal tissues. The concentrations of essential elements must usually be maintained within the narrow limits, if the functional and structural integrity of the tissues is to be safeguarded and the optimum growth, health and productivity status of the animal are to be maintained. Continuous ingestion of diets that are deficient, imbalanced or excessively high in a mineral, induce change of the normal mineral concentration of body tissues. In such circumstances the biochemical and physiological functions of the animals are affected which in turn may result in structural disorders. The developed structural disorders are variable with the mineral element concerned and its toxicity, the degree and duration of dietary deficiency, and the age, sex and species of animal involved (Chester and Arthur,(1988) Such a change could be prevented through the provision of balanced, palatable and adequate diet in desirable forms. According to McDowell et al (1993) mineral supplements differ in their bio-availability, one of the most important factors in mineral nutrition, which must be taken into consideration. Thus it is necessary to comparatively scan the available mineral supplements aimed at ensuring its adequacy and levels of toxicity incriminating minerals. This being the cases, the major objective of this research project was to study the feasibility of using natural rock as potential source Trace elements in livestock feeding in Ethiopia.

II. Materials and Methods

a) Sample Collection and Processing

Adequate quantities of Calcium carbonates, marble powder (both wet and dry), and gypsum and silica powder (silica powder analyzed for just curiosity

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only because it cannot be used as animal feed supplement) were collected from different locations as shown in Table1. Efforts were made to collect as many batch samples as possible during the field survey conducted. All the samples collected were transported to Jimma University college of Agriculture and Veterinary Medicine (JUCAVM). All the samples were dried at 100 ^{0}C and milled to pass through 1mm screen. The dried materials were stored in air tight contained until required for chemical analysis.

b) Chemical Analysis

All the laboratory chemical analysis was done in Canada at the Faculty of Agriculture of Dalhousie University. One gm of dried sample materials were taken into silica basin, charred to remove the smoke and ashed at 550^{o}C in a muffle furnace for two hrs. The ashed materials were transferred to clean and oven dried glass beakers, boiled with 20 ml of HCL acid for 5 minutes and filtered through what man filter paper No. 42 into 250 ml volumetric flask. The residue was washed with hot distilled water until free of acid and the volume was made to the mark with distilled water. This extract was used for analysis of different minerals using standard methods.

All the required standard solutions were prepared as shown in Table 2,and all the samples were analyzed in triplicate and Fe, Mn and Cu were estimated, according to AOAC, (2002), with the use of atomic absorption spectrophotometer (AAS) employing acetylene, air and specific hallow cathode lamps for the determination of individual mineral as the case may be.

Table 1 : Sources of calcium carbonate

Sr. No.	Date Of Collection	Name of Sample	Place of Collection
1	17/07/2013	**Marble powder(wet)**	Addis marble factory
2	17/07/2013	**Marble powder** (dry)	Addis marble factory
3	17/07/2013	**calcium carbonate(Lime stone)**	Amhara (Gojam) filiklik Abyssinia cement factory
4	17/07/2013	**calcium carbonate(Lime stone)**	Amhara (North showa) Jamma Abyssinia cement factory
5	17/07/2013	**gypsum**	Amhara(Go jam) filiklik
6	17/07/2013	**calcium carbonate(Lime stone)**	oromia (Durba) Mugger cement factory
7	17/07/2013	**silica powder**	oromia (Durba) Mugger cement factory
8	17/07/2013	**calcium carbonate(Lime stone)**	oromia (Durba) Durban cement factory
9	18/07/2013	**calcium carbonate(Lime stone)**	Hungshan cement factory Mojo Hirnna (Harar)

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Table 2 : Preparation of standard solutions for various elements

Element	Salt	Quantity in mg will be made to 100 ml with distilled H_2O	Yield	Standard range
Calcium	$CaCl_2.2H_2O$	40.76	100 ppm	1-20 ppm
Magnesium	$MgSO_4.7H_2O$	102.43	100 ppm	0.06-0.6 ppm
Copper	$CuSO_4 .5H_2O$	39.89	100 ppm	0.8-8 ppm
Zinc	$ZnSO_4.7H_2O$	44.235	100 ppm	0.4-2 ppm
Iron	$FeSO_4.7H_2O$	50.80	100 ppm	0.8-8 ppm
Manganese	$MnSO_4.H_2O$	31.39	100 ppm	0.5-5 ppm
Cobalt	$CoSO_4.7H_2O$	49.17	100 ppm	1.6-16 ppm
Lead	$(CH_3COO)_2Pb.3H_2O$	18.49	100 ppm	2.0-20 ppm
Cadmium	$CdCl_2$	16.81	100 ppm	0.6-6.4 ppm

III. Results and Discussion

a) Total Ash and Acid Insoluble Ash

The total ash, AIA, Fe, Mn and Cu contents of the limestone, marble powder, gypsum and silica collected from different part of Ethiopia are given in Table 3. According to Kabaija and Little (1993), the total ash content of most of the Ethiopian common animal feed is equal or lower than 12%. Total ash content of 10-12% and 4.6-8.7% was reported from range grasses and highland hays of Ethiopia respectively. The highest total ash content of 12% was reported from Chrysopogon *aucheri* grown in the highland of Ethiopia. According Table 3, total ash content of 99% was recorded from Addis Marble powder, Jamma Limestone (Abyssinia Cement), Durban Silica Mugger Cement, Durban limestone cement and from Hirna limestone hungshan cement, the value of which is very high compared to the others. The lowest total ash content of 81% was recorded from Durban Gypsum cement. The results of this study clearly showed that the total ash content of all the materials analyzed in this study ranged between 81 and 99% (on dry matter basis), indicating the potential use of these materials (limestone, marble powder and gypsum collected from different part of Ethiopia) as supplementary mineral feed source in a very small amounts.

Acid Insoluble Ash content of animal feed seems to receive adequate attentions. The BIS (2002) restricted Acids Insoluble Ash content to 2.5 to 3.0%in the final mineral mixtures as high levels of AIA lowers the utilization of nutrient and palatability. Ammerman et al (1984) reported that high levels of AIA in the ration of livestock depressed the utilization of P and certain other micronutrients. Kabaija and Little (1993), reported ADF ash content of 3-5% from common Ethiopian animal feeds. ADF ash content of range grasses ranged between 4.06 and 7.61%. It is reported that high levels of ADF ash in animal feed negatively affect digestibility. It is also reported that the high levels of ADF ash in animal feed could be attributed to the presence of large amounts of silica which in turn may seriously reduce digestibility van Soest, (1982). The result of this study showed that Durban Silica Mugger Cement contain 96 % Acid Insoluble Ash which makes it unfit as animal feed because of its insolubility. Jamma limestone, Durban gypsum Mugger and filiklik limestone Gojam contain 4.2-8.3% Acid Insoluble Ash, the values of which are high for the use as animal feed compared to the others. On the other side (Table 3) the Acid Insoluble Ash content of the others (Limestone Abyssinia cement factory(Jamma), Limestone Durban cemnt factory (Durba), Limestone Hungshan cement factory (Hirna)) ranged between 0.29 and 3.29%, the values of which are lower than that reported from the Ethiopian highland range grasses and straw based dry period roughage feeds. Therefore, the results of this study clearly showed that Limestone from durba, Limestone (Jamma) and Limestone (Hirna) could be used as mineral supplant in livestock feeding based on their percent composition of Acid Insoluble Ash.

b) Iron

Iron content of some hays from Ethiopian highlands and range grasses from Ethiopian Sidamo southern rangelands contains 191-974mg/kg an average of 485.14mg/kg and Range grasses contains 452-882 mg/kg an average of 697.3 mg/kg Kabaija, E., Little, D.A., ILRI (1988).in which in both cases it can be enough for the requirement of the animals.

Iron concentration in browses ranged (ppm) from 93 to 693 with a mean of 340±22.1 in the wet season and 51.3 to 188 with a mean of 97.8±22.1 in the dry season. The Fe content in all sampled browses was well above the recommended level for ruminants in feeds in both the wet and the dry seasons with higher concentration in the wet season than the dry season Temesgen and Y.K. Mohammed 24(3)2012. The forages in the study areas adequately supply the requirements of Fe for different classes of camels. Sousa et al (1981) reported high concentration of Fe in forages in both the wet and the dry seasons in northern Mato Grosso, Brazil. Most forage contains Fe concentration

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considerably in excess of the requirements of herbivorous animals McDowell (1992). McDowell (1992) reported that Fe contents of most feed ingredients is highly variable, reflecting differences in soil and climatic conditions as well as differences in variety or processing procedures. According to the result of this study (Table 3), the iron content of all the materials studied are very high except in lime stone from Abyssinia cement factory (Jamma), Marble powder Addis cement factory and lime stone from Durban cement factory which ranged from (548.59- 911.08ppm) while Limestone Abyssinia cement factory(Jamma) 2836.25ppmFe which is very high. Dietary Fe requirement for dairy is 50-100 ppm, for beef 50 ppm (NRC, 1984, 1985, 1988.1989.1994)Thus one kg of Addis Marble powder or Limestone Abyssinia cement factory(Jamma) can be enough to feed7 and 28 dairy cow or14 and 56 beef cows respectiely placed on iron free basal diet/day. In our finding the Acid Insoluble Ash content of the limestone studied in the current study ranged between 0.29 and 8.29% with mean value of 3.26%. Thus, the high content of iron and the low Acid Insoluble Ash content of limestone collected from all places seems suitable source of Fe supplement for livestock feeding under the current Ethiopian conditions. but those having more than 3.5% AIA it is difficult to use according The BIS (2002) restricted Acids Insoluble Ash content to 2.5 to 3.0%in the final mineral mixtures as high levels of AIA lowers the utilization of nutrient and palatability. Ammerman et al (1984) reported that high levels of AIA in the ration of livestock depressed the utilization of P and certain other micronutrients.

c) Manganese

Manganese content of some hays from Ethiopian highlands and range grasses from Ethiopian Sidamo southern rangelands contains 96-322mg/kg an average of 223.57mg/kg and Range grassescontains 45-72 mg/kg an average of 63 mg/kg Kabaija, E., Little, D.A., ILRI (1988).in whjch in both cases it can be enough for the requirement of the animals.

Manganese content in browses ranged from 9.52 to 371 ppm with a mean of 162±21.1 ppm in the wet season and 17.2 to 325 ppm with a mean of 82.8±21.1 ppm in the dry season . In the wet and the dry seasons, 5 and 40% of the sampled browses contained below the recommended concentration of Mn McDowell and Arthington (2005) in feeds for ruminants. The mean *concentration* of Mn in forage plants is well above the minimum requirement indicated in both wet and dry seasons with higher concentration in the wet than in the dry season . The mean concentration of Mn in browses is adequate to different classes of camels in the study areas in both seasons. Concentration of Mn in crops and forages is dependent on soil factors, plant species, and stage of maturity, yield, crop management, climate, and soil pH (McDowell 1992).

According to the result of this study (Table 3.), the manganese content of all the materials studied are reneges from 30.2 (ppm) limestone Abyssinia cement factory (jamma) which is very low while lime stone from Gojam (Filiklik)containing 262.08 (ppm) which is very high and having an average of 101.2 (ppm). Dietary Mn requirement for dairy is 40 (ppm), for beef also 40 (ppm) (NRC, 1984, 1985, 1988.1989.1994)Thus one kg of limestone powder Abyssinia cement factory (jamma) could be adequate to feed 3 dairy cow or 3 beef cow placed on iron free basal diet/day. In our finding the Acid Insoluble Ash content of the limestone studied in the current study ranged between 0.29 and 8.29% with mean value of 3.26%. Thus, the high content of manganese and the low Acid Insoluble Ash content of limestone collected from many places except samples collected from Abyssinia cement factory (Jamma). marble powder (wet)Addis marble factory are suitable source of Mn supplement for livestock feeding under the current Ethiopian conditions. the rest because of its high AIA it is difficult to use.

d) Copper

Copper content of some hays from Ethiopian highlands and range grasses from Ethiopian Sidamo southern rangelands contains 5.9--7.9mg/kg an average of 6.87mg/kg and Range grasses contains 3.6-5.6mg/kg an average of 4.35 mg/kg Kabaija, E., Little, D.A., ILRI (1988).in which in both cases it can be enough for the requirement of the animal.

Concentration of copper in sampled browses ranged (ppm) from 8.91 to 30.8 with a mean of 19.4±1.38 in the wet season and 3.64 to 23.9 with a mean of 12.2±1.38 in the dry season /In the wet and the dry seasons, 10 and 45%, respectively of sampled browses contained Cu in a concentration lower than the critical level suggested for ruminants McDowell and Arthington (2005). Based on the critical level of Cu in feeds established for ruminants, forages in the study areas could supply adequate amount Cu for camels of different classes in both the wet and the dry seasons. Copper deficiency is a severe limitation in grazing ruminants and has been observed in many parts of the world McDowell (1992). However, the current result and that of Woldu (1984) indicates that browse forages can supply adequate amount of Cu for browsing camels. This could be due to differences in soil factors, plant species and stage of maturity, climate, and soil pH (McDowell 1992). Copper concentration in the wet season was higher than that in the dry season.

Dietary Cu requirement for dairy is 10 (ppm), for beef 8 (ppm) (NRC, 1984, 1985, 1988.1989.1994).Thus

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According to the result of this study (Table 3), the copper content of all the materials studied are reneges from 3.17—12.75 (ppm)with an average of 8.2 ppm showing a beat less than the requirement of dairy cow but ,if the supplement is given from Addis marble factor (wet), limestone Abyssinia cement factory (jamma) it will meet the requirements of the dairy.

Table 3 : Total ash,AIA(on percent DM basis)and trace minerals content in gypsum,lime stone and marble powder (ppm)

SAMPLE NO	Place of collection	Dm%	AIA % of DM	Total ash	Fe ppm	Mn ppm	(Cu ppm
1	Marble powder wet # Addis marble factory	99.73	1.09	99.31	765.75	31.91	249.39
2	Marble powder dry #Addis	99.78	1.33	99.39	850.67	*39.50*	
	marble factory						
3	Lime stone Gojam (Filiklik)*Abyssinia cement factory	0.08	8.29	97.12	8238.67	262.08	239.54
4	Limestone Abyssinia cement factory(Jamma)	99.73	3.27	98.69	2836.25	122.58	249.38
5	Limestone Abyssinia cement factory(Jamma)*	82.07	4.24	77.16	548.58	30.25	205.13
6	Gypsum Mugger cement factory (durba*)	99.49	8.43	97.27	7823.25	211.33	248.78
7	silica Mugger cement factory (durba)*	99.96	95.72	99.58	1621.25	9.92	250.04
8	Limestone durban cement factory (dubra)*	98.40	0.29	97.70	911.08	53.83	246.88
9	Limestone hungshan cement factory (Hirna)*	99.89	3.29	99.20	1583.67	57.83	249.87

N.B. # Wet, Dry –While cutting the marble in the factory they are pouring water (wet)sample is taken, the other is without water (dry)

** This are local names where the respective factories are taking the row materials (lime stone ,gypsum or marble powder*

silica cannot be used as feed its content is analyzed just for curiosity only.

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IV. Conclusions

Samples of lime stone powder ($CaCo_3$) powder were collected from different parts of Ethiopia were subjected to laboratory chemical analysis in triplicates. The results obtained showed that the total ash content of all the materials analyzed in this study ranged between 81 and 99% (on dry matter basis), indicating the potential use of these materials (limestone, marble powder, gypsum and silica collected from different part of Ethiopia) as supplementary mineral feed source in a very small amounts. The Acid Insoluble Ash content of limestone from Abyssinia, cement factory (Jamma), Limestone Durban cement factory. (Durban), Limestone Hungshan cement factory (Hirna)) ranged between 0.29 and 3.29%, the values of which are lower with the exception of lime stone from Gojam (Filiklik). Gypsum Mugger cement factory (durba) and lime stone abysinia cement fctory (Jamma) which seems very high AIA than reported from the Ethiopian highland range grasses and straw based dry period roughage feeds. Therefore, the results of this study clearly showed that – those analyzed trace minerals having less than 3.5% (AIA) could be used confidently according The BIS (2002) and Ammerman et al (1984). (Silica from Mugger cement factory is not used as mineral supplement it is analyzed just for curiosity only). However, animal evaluation of the bioavailability of the tested materials seems to be the future direction of research.

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The Isolation of, and Cytodifferentiation of a *Mucor* Species as Affected by Nitrogen Source and Elevated Temperature

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Abstract- Vegetative differentiation leading to dimorphic switching by filamentous microorganisms has drawn intense attention from scientists for a long time because of the central role played in the attempt to understand life processes, as well as the serious impacts exhibited in medicine, agriculture and industrial processes. A mucoraceous isolate, *Mucor manihotis* (tentative), from an agricultural niche was found to exhibit dimorphism in minimal medium; induced were thalloarthric-, holothallic-, holoblastic conidia as well as polar budding globose yeast cells when supplemented with ammonium sulphate or peptone as nitrogen source. Boxplot construction showed that peptone enhanced growth, but elevated temperature had profound morphogenetic effect as ovoidal and spindle shaped yeast cells additionally induced.

Keywords: mucor isolate, minimal medium, box plot, cytodifferentiation, cytoplasmic membrane, neoplastic units, binary protoplasts, yeast ontogeny, elevated temp, yeast form variability.

GJSFR-D Classification : FOR Code: 960413p, 670602



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The Isolation of, and Cytodifferentiation of a *Mucor* Species as Affected by Nitrogen Source and Elevated Temperature

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Abstract- Vegetative differentiation leading to dimorphic switching by filamentous microorganisms has drawn intense attention from scientists for a long time because of the central role played in the attempt to understand life processes, as well as the serious impacts exhibited in medicine, agriculture and industrial processes. A mucoraceous isolate, *Mucor manihotis* (tentative), from an agricultural niche was found to exhibit dimorphism in minimal medium; induced were thalloarthric-, holothallic-, holoblastic conidia as well as polar budding globose yeast cells when supplemented with ammonium sulphate or peptone as nitrogen source. Boxplot construction showed that peptone enhanced growth, but elevated temperature had profound morphogenetic effect as ovoidal and spindle shaped yeast cells additionally induced. The simultaneous induction of different cell wall structures necessitated further examination of the early growth stage. The process of differentiation that resulted in yeast ontogeny rooted in cytodifferentiation, involving vanishing cell wall of germ cells and then sequentially generated cryptic forms, only membrane bound, until reappearance of cell wall in nascent yeast, which at maturity became polar budding. Simultaneous induction of wall-less entities on the one hand, and complex walled entities on the other prompted a re-examination of cell wall geometry as hinge for dimorphic switching. It was suggested that more attention should be given to cytoplasmic membrane as default candidate.

Keywords: mucor isolate, minimal medium, box plot, cytodifferentiation, cytoplasmic membrane, neoplastic units, binary protoplasts, yeast ontogeny, elevated temp, yeast form variability.

I. Introduction

Except for the yeasts, which exist in unicellular form, fungi are filamentous microorganisms which grow by tubular progression and may not or, have cross walls. Tubular coenocytic forms are the more primitive types. They belong to the Phylum Zygomycota which habours 2 classes: Zygomycete and Trychomycete, both of which unlike the higher fungi lack defined fruiting structures (White *et al*, 2006). Zygomycetous fungi reproduce asexually by the production of sporangiospores, arthrospores and chlamydospores. Septation is not a feature of this group of fungi except when aged (Kendrick, 1973), or as response to injury or to cut off older portion of hyphae (Gooday, 1973). In like manner, unicellular existence is not their growth habit. However, in certain conditions some of the genera habour some species which reversibly convert to a unicellular form. Examples include *Mucor, Mycotypha* and *Benjaminiella*. Of these genera, *Mucor* habour more species known to convert to the unicellular phase in modified environments (Ghomade *et al.*, 2012). These include *M. rouxii, M. circinelloides, M. pusilus, M. genevensis, M. hiemalis* and *M. racemosus*.

The alternative form of these species is globose yeast which multiply asexually by multipolar budding. This is perhaps why it is referred to as yeast-like cells. For normal yeast cells like *Saccharomyces cerevisiae* (Class, Ascomycota), or *Rodoturola* (Class, Basidiomycota), exhibit polar budding. For yeast-like cells for example, *M. rouxii*, the globose mothercell, which is multinucleate (Bartnicki-Garcia and Lippman, 1977; McIntyre *et al*., 2002) is comparatively larger than the normal polar budding yeast cells, but produce multiple buds by blastic action. Dimorphic *M. circinelloides* also produces yeastlike cells (Lubbehusen *et al*, 2003, McIntyre *et al*, 2002). This microorganism has also been shown to exhibit polymorphic existence in modified environments, for beside the normal tubular and coenocytic growth habit, forms recorded in defined environment include holoblastic-, holothallic-, thallo-arthric conidia, septate filament/hyphae with vesicular catenate conidia, and terminal budding yeast cells. (Omoifo and Omamor, 2005; Omoifo *et al*., 2006; Omoifo, 2006ab).

There was a preponderance of induced terminal budding yeast cells of *M. circinelloides* when cultivated in strictly defined medium (Omoifo, 2006ab; Omoifo *et al*., 2006). Because of this recurrent observation, a model was developed for its conversion (Omoifo, 2009), an intermediary form, protoplast, formed the central element to the transformative process; this subsequently converted to the yeast form which became terminal budding (Omoifo, 2003). It was also shown that potassium ions was needed in the multiionic medium for the formation of the protoplasts (Omoifo, 2013). Several transient phases were involved in the conversion process that involved intermedial circulation of ions (Omoifo and Awalemhen, 2012).

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Our medium design in which terminal budding yeast cells were induced, was thought to be responsible for the induction from sporangiospores, as it supplies the abiotic regulatory factors that are perceived, and intracellularly transduced so as to permit the genetic and physiological machineries to regulate its dimorphic switching. It has been used for the conversion of 3 different filamentous microorganisms to terminal budding yeasts. Firstly, *Dimorphomyces pleomorphis* (now invalid) was found to transit to terminal budding yeast cells at 15°, 20° and 37°C when the nitrogen source was $(NH_4)_2SO_4$ while preformed nitrogen supply, peptone, induced terminal budding yeast cells primarily at 15° and 20°C but multipolar budding yeastlike cells at elevated temperature, 37°C (Omoifo, 1996). Secondly, *Rhizopus stolonifer*, which was found to be incapable of converting to yeastlike cells under CO_2 atmosphere (Bartnicki-Garcia and Nickerson, 1962) converted to terminal budding yeast cells in a study that underscored the significance of calcium ion as agonist for the conversion process (Omoifo, 2011). Thirdly, several studies have hilighted the possible role of uracil, myoinositol, K^+, Na^+ and H^+ in the transformation of *M. circinelloides*; it was suggested that they play key roles in signal transduction and electrophysiological status in the conversion process (Omoifo, 2006ab; 2013; Omoifo and Awalemhen, 2012).

In the experimental design combining several environmental factors, assessment of the magnitude of growth was attempted using quadratic polymonial model procedure but this was unsuccessful as data obtained were inconsistent with the assumptions made therein. Explanation was therefore anchored on transmembrane-pH-gradient (Omoifo, 1996). This lent some credence to the modifications observed therein and gave full complicity to the growth phases evolved and consequently made possible explanation on the sigmoid growth pattern exhibited (Omoifo, 2011).

The study with *M. circinelloides* gave insight into the transport mechanism between the bulk and intracellular media and hilighted the important role played by Na^+ in stimulating the yeast form induction from protoplast in the timely upshot into the exponential growth phase (Omoifo, 2012). This was further emphasized as absolute congruence exhibited between ionic circulation and the sigmoid growth pattern (Omoifo, 2014).

Our studies have shown that transformation of sporangiospores to terminal budding yeast cells involved complex phenotypic modification exhibiting several transient phases until the stable form, which thereafter proliferate by budding. The present study aims to show how this process is affected by complex and inorganic nitrogen sources on the one hand, and elevated temperature on the other. Absorbance has been used for quantitation since a mixture of morphologies were formed especially as all are distinct from the filamentous growth habit, or the typical unicellular growth form.

II. Materials and Methods

a) Fungal strain isolation

The fungal strain used in this study was isolated from a wasted cassava stump (*Manihot esculenta* L.) in an old farm where cassava tubers were uprooted about 6months earlier. Pieces from sections of the stump, which had turned white, or black, were taken with a sterile forceps and deposited into sterile universal bottles which were then transported to the laboratory. The stripes of root were deposited unto sterile wash glass and further cut with a sterile scalpel and subsequently transferred 3 each unto PDA Petri dishes at equidistant and triangular points. These were incubated on the laboratory side bench and monitored 24 hourly such that outgrowths from samples were promptly isolated and purified on PDA. Pure cultures were stored in McCartney bottle PDA slants.

b) Microscopic examination and Fungal Identification

Slides were prepared from cultures of pure isolates and stained with lactophenol in cotton blue and viewed with AmScope Binocular microscope attached with 1.3MP USB camera. Specific morphological features of the pure isolate were captured. These were used for identification of the fungus based on description in literature including CAB (1971), Ellis (1976), Von Arx (1970) and Samson *et al*., (2000).

c) Inoculum preparation for growth studies

A bent sterile glass rod was used to rob the surface of the PDA culture so as to dislodge the spores into distil deionized water so as to make a suspension in the Petri dish. This was poured into sterile centrifuge tubes and washed at 5000rpm for 7min. After decanting the supernatant, the spores were further washed with 2 changes of sterile deionied distil water. Spore count of the isolate was taken with Neubauer Haemocytomer (BSS No 784 Hawsley, London, Vol. 1/4000) and was adjusted to $1x10^6$ spores per ml with sterile deionized distil water.

d) Reagents and culture media

The synthetic medium of Omoifo (1996) incorporated with uracil (Omoifo, 1997; 2006), myoinositol (Omoifo, 2006a, Omoifo *et al*., 2006), zinc (Omoifo and Omamor, 2005) and calcium chloride (Omoifo, 2011) was used. Briefly, it contained per litre 10g glucose (Fisher Scientiic Coy New Jersey), 5g K_2PO_4 (BDH, England), 5g KH_2PO_4 (Aldrich Chemical), 0.10g $FeSO_4$ (J.T. Phillipsburs), 0.20g $MgSO_4$ (BDH,England), 0.10g NaCl (Kermel), 0.075 $CaCl_2$ (BDH, England), 0.065g $MnCl_2$ (Reidd-DeHaen Ag, Hannover), 0.06g $CuSO_4$ (BDH, England), 0.06g $ZnSO_4$ (BDH, England), 0.10g uracil (Fluka AG Switzerland),

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0.250g myoinositol (Sigma), 1.83g Na_2HPO_4 (BDH, England), and 1.07g citrate (Sigma, USA). The nitrogen source was either 5g $(NH_4)_2SO_4$ (BDH) or 5g peptone (Biotech). Medium was prepared in 2000ml beaker and pH adjusted to 5.0 with 2N NaOH or 2N HCl using a hand held pH meter with glass electrode. Duplicate experiment was set up for each test. Each experiment contained 100ml broth in a 250ml conical flask. A 15μl of test isolate was inoculated into prelabelled flask using a micropipette in a sterile hood. After inoculation each broth flask was shaken for 30s and thereafter incubated at the set temperature regimes, viz: 15°, 28° and 37°C. At 24h intervals the broth flasks were brought to the sterile hood and 10ml withdrawn from each one with sterile pipettes one for each flask into factory sterilized universal plastic sample tubes. The broth flasks were thereafter returned to the set incubation regimes. The samples were kept at -18°C until analysis.

e) Biomass determination

Culture samples were thawed up to room temperature before biomass determination. The absorbance was obtained at 600 nm, using a Camspec M105 spectrophotometer Cambridge, UK).

f) Experimental design and analysis

Factors including nitrogen-source, temperature and time effect were incorporated into this design (Fig. 1). Thus absorbance values were subjected to exploratory box plot and to a 3-way analysis of variance to test for significance of factors and comparison between means made at $p < 0.05$ using GenStat Release 8.1 package. Time-course plots were made on Excel format.

III. Results

a) Preliminary morphological description

Fig.2a showed the specific cassava stump from which the initial isolation was made. Root-stalks of harvested cassava tubers, as well as underneath dry and cracked skin of the lower stem of the cassava stem turned black with white patches. Colonial growth of isolate: substrate level mycelium hyaline to white & irregularly circular, with conspicuous strands up to diam. 30mm in 24h with effuse and orange coloured aerial mycelium which lag 3-8mm behind the margin (Fig. 2b). At 72h substrate-level mycelium reach 90mm while 3-5mm of margin compact forming whitish mycelium (Fig. 2c). As from 120h after inoculation, aerial mycelium initially with distinct sporophores on forming compact mycelium reach the roof of the dish; the central diameter turn purplish on the A-side of the dish (Fig. 2d) while the B-side is reddish as blue strands/patches scatter all over the surface (Fig. 2e). The central core become purple with age and the whole mycelium gradually turn black (Fig. 2f). After 3days globose heads reveal as smooth walled sporangia borne on long sporangiophores (Fig.3a) apically septate thus cutting off the double walled sporangium in which spores are directly observed (Fig.3b&c). Spores may be short or long ellipsoidal, cylindrical, sausage roll or crescent shaped, which may also be multicelled (Fig. 3d-h). Apical columellum is globose (Fig. 4a) as sporophores arise from septate substrate level mycelia; the double walled septa irregularly delimit cytoplasm, which may be fragmented or become granular (Fig. 4b-d). Polarized germ cells may give rise to holothallic growth which may subsequently fragment, thus giving rise to arthrospores (Fig.4e).

Fig. 5a showed that sporophores terminating in globose columellum may also be curvy. These originate from septate mycelia that give rise to multiple germ tubes which anchor into the substratum with coenocytic apparent rootlets and subsequently ramify the medium (Fig. 5b,c). Cytoplasmic cleavages could occur in the cellular compartments thus giving rise to glistening bodies; these could be individually released (Fig. 5d,e). Release of such cytoplasmic contents as protoplasts become frequent as the culture aged (Fig.5f,g-h), so also is the formation of arthrospores (Fig.5i) and chlamydospores (Fig. 6). After 9days of growth, the double walled hyphal cells, septa and thick-walled chlamydospore become melanized (Fig. 6a-f).

b) Effect of N-source on Growth

Growth occurred in both inorganic and complex nitrogen sources. Total growth in the N-sources were shown in table 1, which also showed the morphological forms exhibited. These included discrete units and or determinate entities of various shapes and forms. Because of these varied forms, we sought to find out if the population had normal distribution. Parallel boxplots for absorbance values in Amm. sulphate and Peptone broths were shown in Fig. 7a. The Peptone broth median is above the 75th percentile of observations in the Amm. sulphate broth. Furthermore, the boxes which were positively skewed were not associated with outscores. These indicated that the populations which were distinct were normally distributed. On the graphic representation of cummulative estimator at the different temperature levels, performance was enhanced at 28°C but was least at 15oC (Fig. 7b).

When such plots were obtained for each nitrogen source at the different temperature regimes, the pattern display showed differences among the populations. The degree of dispersion among the Amm. sulphate populations at the different temp regimes were quite distinct (Fig. 8). It was optimal at 28°C where growth was symmetrical, an indication of an even dispersion in a normal population and also the first quartile was even more than the median in the 37°C regime, which in turn had its first quartile above the 15°C interquartile range. In contrast to the higher temp. regimes which were positively skewed with no outliers,

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the minimal observations at 15°C was negatively skewed and had an outlier at the upper extreme. This indicated that quantitative data in Amm. sulphate broth at this temp level was very low and also had very little dispersion, in contrast to that at higher temp regimes.

Fig 8 also showed group constructs in Peptone broth over the temp. regimes. The range at 15°C showed the least growth estimate. But this was greatly enhanced at 28°C being above the maximum in the aforementioned regime, and was also above the extreme value at 37°C. Thus the group display suggested significant differences in the estimator at the different temp. regimes. The boxes for the Peptone broth were positively skewed, having longer whiskers except for that at the elevated temp. where the negative skew had longer lower whisker, thus increasing the variability downward as most observations were concentrated on the high end; this was reflected in the larger interquartile range, and possibly suggested an interaction between medium type and elevated temperature on the growth estimator.

Now on comparing the Amm. sulphate- and Peptone broth graphics at each temp. regime, median for the former is absolutely lower than that in the Peptone broth. Similar comparison showed that the 2 interquartile ranges do not overlap along the vertical axis. Since half the observations in a distribution are between the upper and lower quartiles, that is, between the 25th and 75th percentiles, we see that half of the observation in Peptone broth is above the 75th percentile of the Amm. sulphate broth at any of the temp. regimes. These distinct characteristics suggested that the two population means (Amm. sulphate- and Peptone broth) differed beyond just random variation. We resorted to the analysis of variance procedure to validate or otherwise, this contention.

A 3-way analysis of variance showed that the impact of the individual factors on growth, including N-source, temp. and time, as well as the combined interactive elements, was significant p0.001 (Supplementary Table 1). Perhaps we should look at the behavioral trend of the microorganism in the cultures which may yield to understanding the specific effects of the factors.

c) Growth profiles

The deterministic nature of the observations evidence the fact that the study was parametrically based. Thus in a time-series manner, it was thought that trends could be established so as to lend data to further evaluation from which physiological relationship could be deduced. Growth profiles in the Amm. sulphate and Peptone broths were therefore obtained. These were shown in Figure 9.

Profiling growth of the microorganism at 15°C in Amm. sulphate medium did not give a sigmoid pattern. The hardly perceptible increase fell after 72h. But marginal increase occurred as from 96h till termination of experiment. This low profile exhibition seem to agree with the weakly presentation of the descriptive graphic earlier observed. Although not also sigmoid, a bell-view profile occurred between 24 and 72h in the Peptone broth, whereafter rapid growth ensued till termination of experiments.

Growth at the 28°C regime purviewed a logarythmic phase without inherent lag or log phase in Amm. sulphate broth. However, in Peptone broth an enhanced rise yielded to a more or less steady growth after 48h; but growth surged after 96h.

Profiling growth at 37°C was oustanding. In either media, a sudden rise in growth occurred but this leveled off after 48h. A resurgence occurred as from 72h and thereafter approached a stationary phase in Peptone broth. On the other hand, a truncated log phase was portrayed in Amm. sulphate broth.

Growth pattern in Peptone broth at all temp levels seem remarkable as a rise in growth up till 48h appeared to break off and a resurgence commenced after 72h. Interestingly, profile exhibition in Amm. sulphate broth at 37°C assumed similar pattern. These were not revealed in the graphic display of the boxplots. Perhaps, microscopic examination may be more revealing.

d) Ammonium sulphate-incorporated minimal medium

Growth occurred in all the broths incorporated with ammonium sulphate and at all temp levels tested. However, morphological expression varied.

e) Temperature 15°C effect on morphology

Commencement inoculum was sporangiospores which were of various shapes and sizes: globose, crescent, cylindrical or sausage roll-like, and ellipsoidal. These differentiated to spherical to germ cells and some had protruding germ tubes by 24h (Fig. 10a); further growth could occur with single or double germ tubes which could be oppositely borne on a single germ sphere and extended on each axis (Fig. 10b). These were however scanty. The predominant morphology was greenish spherical units which appeared to arise from cytoplasmic contents of growth spheres (Fig. 10c). Beside these were innumerable granular units. By the 2nd day clumps of, and dispersing granular units were copiously produced (Fig. 11a) and the hue of greenish spherical units became intensified (Fig. 11b). Similar observations were made after 72h of growth. But the sizes of the spherical units subsequently increased (Fig 12). Gradation in sizes was apparent: from granular units to ultimate spherical units. By day 4, clumps, which appeared to arise from implosion of the cytoplasmic contents were observed (Fig. 13a). These subsequently gave rise to structures, some of which could be short rods or cylindrical (Fig. 13b). Fig. 13c showed numerous formless units which could arise from this implosion. The emerging rods

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could be binary (Fig. 13d). The most outstanding morphology here was unicellular globose yeasts, which subsequently acquired budding capabilities (Fig. 13e). The daughter bud could adhere to the mother cell through a short sterigmata (Fig. 13f). By the 5th day, the spherical units were still numerous (Fig. 14a), but globose unicellular yeasts were predominant (Fig. 14b). These coexisted with enterothallic conidia (Fig. 14c) and numerous rod shaped single or binary structures that were protoplast-like.

f) Temperature 28°C effect on morphology

This intermediate temp. level elicited septate filamentous growth and holoblastic conidia in broth after 24h (Fig. 15). By 48h, numerous other morphologies were observed. These included beside the aforementioned types, thallic growth, neoplastic units, and protoplasts-possibles (Fig. 16a-f). These were very numerous at 72h after inoculation (Fig 17ab) and became the predominant morphology 96h after inoculation (Fig. 18a); they were coinduced with yeast cells (Fig. 18b). When walls of holoblastic conida ruptured (Fig. 19), and this was frequent , cytoplasmic contents were release either as granular or neoplastic units (Fig. 19). These were also co-induced with yeast cells.

g) Temperature 37°C effect on morphology

At 24h of growth clusters of differentiating neoplastic units (Fig. 20ai) were conspicuous. Some appeared to increase in size *in situ* (Fig. 20aii). The ultimate neoplastic units co-induced with yeast cells, which were polar budding (Fig. 20cd). Although not the most predominant, holoblastic conidia co-induced with varying sizes (Fig. 21a-c). These coexisted with globose and spindle shaped yeast cells (Fig. 21b,d) at 48h of growth. Spindle shaped and globose yeast cells became the most predominant morphologies at 72h after inoculation. This was shown in figure 22a. Even though holoblastic conidia were observed and appeared to dininsh in size and number, their cytoplasm appeared to differentiate *in situ* (Fig. 22bc) and also coexisted with the predominant globose and spindle shaped yeasts (Fig. 22d). Observation of the aforementioned morphologies continued after 96h from inoculation (Fig. 23a-f) with globose cells dominating (Fig. 23e). Although scantily, when thallic growth occurred and, or fragmented into arthrospores, cytoplasmic contents receded from cellular walls, thereafter differentiating (Fig. 23f) and on cell wall bursting, the cellular contents released as neoplastic units. At 120h after growth, at this elevated temp. regime all the aforementioned morphologies occurred. So also were ovoidal yeast cells encountered (Fig. 24a-f).

h) Peptone-incorporated minimal medium

Growth appeared more enhanced with the complex nitrogen source.

i) Temperature 15°C effect on morphology

After 24h of growth nondescript entities dispersed from the apparently imploded cytoplasm (Fig. 25a). This possibly gave rise to the clusters of protoplasts (Fig. 25b). Prevegetative cells or nascent yeasts were very conspicuous (Fig. 25c). These subsequently became polar budding (Fig. 25d). At 48h after cultivation, the ultimate neoplastic units were very numerous (Fig. 26) and coinduced with the other cryptic forms. When thallic growth co-induced with yeast cells, cytoplasmic contents could be released on rupture of cell wall (Fig. 27a), which assumed the characteristic features of true fungal wall (Fig. 27b). But the most predominant morphology at 72h after inoculation was globose unicellular cells (Fig. 27c). Numerous protoplasts could be seen transiting to nascent yeasts(Fig. 28a) and these abscised but subsequently became terminal budding (Fig. 28b). At 120h after growth, the protoplasts were robust (Fig. 29a). They coexisted with other thallic structures in which the cytoplasmic content also differentiated (Fig. 29bd). Fig. 29e exhibits form as binary protoplasts initiated nascent yeasts, which on disarticulation became prevegetative cell and these at maturity became globose unicellular yeast cells. These thereafter became polar budding (Fig. 29f).

j) Temperature 28°C effect on morphology

Various forms of thallic structures were induced at 24h after growth. These included holoblastic-, and thallloathric conidia (Fig. 30). Numerous neoplastic units and single rod and binary protoplasts co-induced and predominated the broth after 48h of growth (Fig. 31). By 72h, cytoplasm of all germ cells, holoblastic conidia and thalloarthric conidia were conspicuously granular and this on release converted to ultimate neoplastic units (Fig. 32). Protoplasts were also numerous (Fig. 32f) and could be observed even within the cellular compartments. Figure 33 showed that the growth cell cytoplasmic content (a) underwent apparent cytoplasmic differentiation (b) and subsequently lost the cell envelop, thus leaving only the neoplasm (c). This was subjected to apparent implosion (Fig. 33de) and thereafter dispersed as each nondescript entity increased in size (Fig. 33f). Cytoplasmic contents of thalloarthrospores (Fig. 33g), holothallic conidia (Fig. 33h) and fragmented spore (Fig. 33i) underwent similar processes of granulation, implosion, and on release of the individual units after cell wall rupture, nondescriptness and subsequent form development. Thus neoplastic units, protoplasts and yeast cells were abundant (Fig. 33jk). All the aforementioned forms were observed after 120h of growth, but neoplastic units, protoplasts and yeast cells predominated the medium. Fig. 34a showed units clinging to walls of thallic structures, extruded units from conidia (Fig. 34b) and numerous protoplasts (Fig.34c).

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k) Temperature 37°C effect on morphology

The morphology after 24h was predominantly yeast form. There were two types of yeasts- globose and spindle shaped (Fig. 35ab). These were of various sizes. Holoblastic-, holothallic- and thalloarthric conidia on the one hand, and transitory phases including neoplastic units and protoplasts on the other hand, as well as yeast forms-globose and spindle shaped- were observed as from 48h of growth (Fig. 36a-f). The observations and variability of forms at 72h after inoculation were not different from that at 48h, but conidia and the yeast cells appeared more robust (Fig. 37a-d). Co-induction of the various forms occurred at 96h. Thallic growth bearing blastospores were observed. True fungal filaments with double walled septa also debuted (Fig. 38b), although these were scanty and in each case, not very extensive. Similar representation occurred 120h after growth (Fig.39a), and further highlighted the variability of forms co-induced at this level. The yeast types occurred in a variety of sizes. Figure 39c showed a well matured spindle shaped yeast cell.

l) Primordial growth phase of Mucor manihotis in minimal medium

Preponderance of neoplastic units at the different temp. levels was outstanding and it was co-induced with the various morphological forms, including globose yeast cells, spindle shaped yeast cells, holoblastic conidia, holothallic conidia, thalloarthrospores. In some instance, neoplastic units appear to pinch off plastic neoplasm (Fig. 40a), or emerge from effused arthrosporal content (Fig. 40b) or after conidial rupture (Fig. 40c). In fig. 40de, the units foreground thalloarthrospores and holoblastic conidia (both out of focus), while Fig. 40f portrayed high numericity in ultimate neoplastic units. The difference in occurrence- from fluid neoplasm (Fig. 41a) or possible size increases of cytoplasmic granular units (Fig. 41b), prompted its re-examination at the primordial phase.

Fig. 42 showed cryptic forms sequential to globose yeast formation after 3h of growth in Amm. sulphate incorporated medium at 28°C. The growth of spore is accompanied with volume changes as it assumed spherical growth with the subsequent appearance of double wall of germ sphere. This yielded the spheroplast as cell wall is lysed. The cytoplasmic membrane subsequently disappear, leaving the intact cytoplasm and thereafter neoplasm which further differentiates, thus appearing as granular units. Therefrom, nondescript entities formed. As these nondescript provencal entities enlarged, individual units eventually asssumed shape, hence emerging as neoplastic units which ultimately appeared spherical. From the ultimate neoplastic units emerged single rod shaped protoplasts and subsequent binary protoplasts. These eventually gave rise to globose yeasts, which appeared in singles or doubles.

Microscopic examination after 6h of growth in Amm. sulphate incorporated medium appear to reveal more details in the formation of neoplastic units. This was shown in fig. 43. After the sphaeroplast formation, several stages were seen in the implosion of neoplasm until individualization of the initially apparently consistent neoplasm. These differrentiated, as granular component, asssumed individual life form *in situ* as provencal entities; therefrom becoming incipient neoplastic units. As they spread out through inherent conventional current, we could see primordial neoplasts changing into midmost neoplastic units. These subsequently assumed spherical shape as the ultimate neoplastic units.

Observation of broth after 9h of cultivation perhaps accord more recognition to the differentiating neoplasm, which yielded more cryptic incipient neoplastic units and the midmost neoplastic units as these became a little more conspicuous, until the ultimate neoplastic units were formed; the latter were spherical in shape. This was shown in fig. 44.

In the exposure of spores to organic nitrogen source, peptone, the process appeared to have been abridged as binary protoplast upshot from provencal entities occurred as early as 3h after growth; the steps: spheroplast-, neoplasm- and neoplastic units formation appeared subsumed. This was shown in fig. 45. Although the count was not obtained, visual observation indicated that protoplast population was higher in the Peptone broth in comparison with Amm. sulphate. However, ultimate neoplastic units were also observed in the Peptone incorporated broth. This was shown in Fig. 45.

Observation at 6h after cultivation in Peptone broth showed the evolving neoplasm undergoing several topological changes: from plastic form it became furrowed and, thereafter fragmentized into provencal entities. When the plastic neoplasm became amorphous, incipient or upshots of neoplastic units could be observed. Thus, midmost neoplastic units and ultimate neoplastic units were abundant. In contrast to cultivation in Amm. sulphate broth, the midmost neoplastic units were more robust and assumed varying shapes, some of which appeared flagellated. This was shown in fig. 46.

Subjective assessment showed that generation of neoplastic units or their upshot from imploded or fragmented neoplasm in Peptone incorporated broth was higher in number as compared to Amm. sulphate incorporated broth. Clusters of primal neoplastic units, midmost neoplastic units and ultimate neoplastic units were also more numerous in Peptone broth at 9h after cultivation (Fig. 47) in contrast to earlier sequential observation. Perhaps, this was as a result of time effect.

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In fig.48, we see that protoplasts also emerged from neoplastic units at 37°C in Amm. sulphate broth. This confirmed the fact that at whatever temp level used in this study, and whether inorganic or organic nitrogen source, the same process of differentiation occurred. That protoplast upshot from provencal entities, as shown in fig. 45, possibly meant that the rate of emergence of a particular form could be enhanced, hence a faster growth rate, a fact that could be extrapolated to the other growth entities. The unique effect of this is that the rate of achieving the optimum morphology of a unit is faster in the peptone medium. This could be reflected in the physiology, size, volume and form of the individual units. Fig. 49 illustrated the contrasting robustness of protoplasts induced in media incorporated in Amm. sulphate and Peptone.

It is pertinent to point out that the final morphological form induced in either medium is the same, embellished detail notwithstanding. Fig. 50 showed the topology of a spindle shaped yeast cell. Each javelin-like cell has an expanded central region, which may have several markings, and narrows down the apices. Although pointed at both ends, one apex appeared truncated. The markings may be restricted to one side of the approximately equally divided elongate yeast cell (Fig. 51).

m) Reversion to filamentous growth habit

At termination of experiments culture flasks were shifted to the laboratory side bench, temp. 28°C. Observation showed that mycelia matt formed on broth surface of the shifted cultures. This was shown in fig. 52. This indicated that the induced conidia and yeast cells, being the most predominant, as well as the transient forms including neoplasm, neoplastic units and protoplasts- were re-converted to the original growth habit, which is filamentous. Microscopic examination of such re-growth showed multipore sporangia, multitype sporangiospores, hyphal septation, thalloarthrospore formation, chlamydospore formation, and melanization of hyphal structures. This was shown in Figure 53.

IV. Discussion

That the fast growing isolate, at first whitish, then orange upright effussion of aerial mycelium, reproduce asexually by having large multispored globose sporangium enclosing distinct spheroidal columella borne at the apex of elongate sporangiophore, consign it to the Genus, *Mucor* (Family: Mucoraceae). Here, the smooth double walled sporangium enclosing multi-type aplanospores: globose, ellipsoidal, cylindrical, elongate, sausage roll-like and crescent shaped, which may have more than one cell against the spheroidal columellum with singular cross wall between its base and the coenocytic sporangiophore indicate an advanced species of the primitive Mucoraceae (Alexopoulos and Mims, 1979). This view is supported by the fact that its somatic structure approximates the characteristics of the higher fungi: multiple branching mycelium, the hyphae possessing more or less regular septa. This argument is re-inforced by the presence of thalloarthrospores and chlamydospores, characteristics of the hyphomycetes. In addition, hyphal and septal walls, are characteristically melanized with age. This is in sharp contrast to *M. rouxii* which produces multipolar budding yeastlike cells (in modified environments), coenocytic hyphal structures, but septum, whenever it occurs and sporangiophores that do not contain melanin pigments, except sporangiospores, i.e. 10.35% of spore dry weight (Bartnickii-Garcia, 1968). Although *Mucor* species exist which show cross walls right from commencement of growth (Hesseltine and Ellis, 1973), the presence of multiple type sporangiospores and melanized somal structures, make this a distinct *Mucor* strain. Baring a full taxonomic evaluation, including phylogenetic analysis, this isolate may tentatively be referred to as *Mucor manihotis*, taking into consideration the niche of first isolation (Davenport, 1980) and strictly for the purpose of this report.

The initial stages of growth of *M. manihotis* corresponded to stages 1 and 11 shown in the growth of *M. rouxii* (Bartnicki-Garcia and Lippman, 1977; Bartnicki-Garcia *et al*., 1968). Contrastingly, the primary thallic growth pattern gave rise to septate filaments of four different types, including holoblastic-, holothallic-, thalloarthric- and endoarthric conidia. However, none was extensive nor ramified the medium, but remained as discrete units. Hence it was possible to obtain optical density readings used for boxplot construction or pattern determination.

In thallic endoarthrospores/enterothallic conidia, cell wall appeared be formed within the existing hypha without disruption of existing hypal cell wall during septum formation (Fig. 27ab). This makes it similar to other zygomycete like *M. rouxii* (Barrera, 1983). On the other hand, it could be compared with the hyphomycete *Oidiodendron griseum* which exhibits determinate thallic growth followed by a backward production of spherical or subspherical arthrospores (Fig. 30d, 33g), or truncate arthrospores (Fig. 31, 39a) as found in *O. truncatum* (CAB, 1971; Ellis, 1973). On the other hand, it could be compared with *Geotrichum candidum* as filaments gave rise to oblong-rectangular or subglobose arthrospores (Fig. 30b). Of the holoblastic type, conidia were produced directly and successively from the growth initial by blastic action (Fig. 21ab, 22bc, 23a-d). Here, both the secondary wall and part of the thick primary (outer) wall entirely enclosed the spore, which in turn enclosed the cytoplasmic membrane; thus septum formation was between the outer walls of two conidia borne in succession (Carmichael, 1971; Kendricks, 1971). In the holothallic type, germ initial produced a germ tube; this converts to the short conidiophore as

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successive disarticulatable conidia were produced therefrom (Fig. 33hi).

Since dimorphism involves phenotypic switching from one growth habit to another in a culturally controlled situation, we see a change from the environmental mold morphotype to discrete units production in our minimal medium as exhibiting this phenomenon. Herein, conidiogenesis involved both persistent and nonpersistent conidia production. Persistent conidiogenesis include the various thallic forms (enterothallic-, holothallic-and holoblastic conidia) whereas nonpersistents involved the successive budding and transient discret units. These signify a change in shape from the natural tubular substratal growth and production of fertile aerial sporophores.

In an attempt to ellucidate the mechanism regulating hyphal tip growth, and hence responsible for dimorphic switching, Bartnicki-Garcia and colleagues (Bartnicki-Garcia *et al*., 1968, 1989) developed the concept of the vesicle supply center (VSC), where cell wall precursors and important enzymes are deposited in the subapical region, that is, at the ring of the cytoplasmic membrane immediately below the apex prior to exchange with the outside of membrane through exocytosis, (Dieguez-Uribeondo, 2003; Ghomade *et al*., 2012). Several workers have deduced that since it involved cell wall metabolism, apical growth, involving the change in integrity and or cell geometry could be attributed to the cell wall polymer deposition pattern (Bartnicki-Garcia, 1987; Orlowskii,1991; Chitnis and Desphade, 2002), wherein the enzymes chitin synthase and chitinase are involved in specific cell wall construction (Bartnicki-Garcia, 1973, 1987). Three such patterns have been suggested for fungi undergoing dimorphic switching: polarized hyphal tip growth for elongation; nonpolarized regulated growth for the yeast form which may appear globose or ellipsoidal, and deregulated polarized deposition pattern for the irregular configuration (Diophode *et al*., 2009).

If we consider the different types of discrete entities in our minimal medium, then the pattern distribution theory calls for further consideration. It is difficult to see how one cell polymer diposition pattern would induce the numerous shapes of this microorganism that occurred simultaneously. Now, take the determinate thallic growth where there is polarized growth, like the strictly prolongation on hyphal axis of *M. rouxii* (Bartnicki-Garcia and Lippman, 1969) resulting in coenocytic tubular filament. In *M. manihotis,* at the end of such growth, a retrogressive conidiogenesis occurs as backward centripetal growth of cytoplasmic membrane is followed by septum formation. What triggers an end to the forwardly directed chitosome - mediated cell wall biogenesis (Bartnicki-Garcia, 1981) and, then directly apposite resort to regressive internal spore formation in this type of conidiation, is not yet clear. As for the holoblastic conidia, is it a case of non-polarized deposition of cell wall materials followed by deregulated polarized deposition, then a repeat of non-polarized deposition with a continued reversion and continuation? This conidial type is a form in which the cytoplasm is encapsulated in its spore wall and this in turn is integumented by the thallic wall such that septum formation is by the inner thallic walls of adjacent conidia. How then is this structure regulated in contradistinction to thalloendoarthrospores where the primary thallic wall is not involved in septum formation? Still, within the same reaction medium are the more predominant populations of non-persistent conidia- polar budding yeast cells. If this falls within the non-polarized category, what about the other types of yeast shapes and the numerous transitory stages which were without geometry or shape?

The cell wall is integument of the cytoplasm and confers mechanical stability and shape. We find in this study numerous nondescript entities being released after conidial rupture. Unless by intussusception, these discernable entities could not have acquired an encompassing cell wall while within the conidium. Indeed microscopic examination at a magnification of 2500x showed granular units that only acquired cellular membrane with time, after several hop-stages. The germ cell was prime ontogeny for nondescript units, often after spheroplasting. From cleavages of neoplasm, a sequence of cryptic forms, with inherent changes in volume, additionally align in the differentiation process of this microorganism, each form different from its immediate precursor, but none appeared to be enclosed by a cell wall until the nascent yeast is initiated by the evolved binary protoplast. Thus, in our consideration the cell wall material deposition patterns do not explain the inherent cytoplasmic granulation and primeval entities emergent from the conidial structures nor the germ cell, neither the occurrence of neoplastic units or protoplasts which were considered only bounded by membrane. This implied that at this primordial phase sequential units no carbon moieties were being converted into mannans, glucans or chitin/chitosan, which are the polysaccharide structural components of the yeast cell wall (Arnold, 1981; Walker, 1998). The scheme shown in Fig. 54 possibly illustrates the lateral morphogenetic transformation (Omoifo, 2003) that occurred in our minimal medium.

Topological view showed that neoplasm, on differentiating from cytoplasm after lysis of membrane of spheroplast, underwent cleavages of content thus exposed to the medium. This inures population growth. On the other hand, granulation individualized the neoplastic content wherefore biophysical activities, as happens in a dynamic system like a broth, could enforce osmotic relations at its optimal level. This implied succinct development of membrane in order to regulate transverse movement of materials and energy transduction at this primary level. Suppose the fluid

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dynamics and stimulating factors permitted various epigenetic expressiveness of the innumerable offspring, what we might have could be reflected in the varying shapes and forms of incipient neoplastic units. Assuming there was vectorial energy transduction in a study conducted within a narrow range of pH, a concept that has been severally called upon in an attempt to explain an possible electrophysiological role in the dimorphic phenomenon (Omoifo, 1996, 2003, 2005, 2011b, 2012, 2014; Omoifo and Awalemhen, 2012; Omoifo *et al*., 2013), there could be a drive for directional physiological relationship that would perhaps give premium to specific biochemical reactions leading to expression of particular morphology. But witness the changing nature of morphological expressiveness, each state different from the precursor. Hence the various forms of neoplastic units and, sequentially differentiated to protoplasts; these initiated the nascent yeasts.

It was shown in the case of *Rhizopus stolonifer*, an organism said not to be dimorphic (Bartnicki-Garcia and Nickerson, 1962), that transformation to terminal budding yeast cells was achieved in synthetic broth at pH5.0 where growth was optimum and sigmoid pattern obtained (Omoifo, 2011b). Subsequent study using *M. circinelloides* showed that the early modifications that gave rise to the ultimate stable but nonpersistent morphology (Omoifo, 2013) were lag phase events during which phenotypic modification occurred in a system that permitted intermedial ionic flux (Omoifo, 2014). Subsequently, the induced proliferating yeast could be subjected to exponential growth (Omoifo, 2011b), only when the physiological process of glycolysis occurred (Omoifo *et al.,* 2013). At such primordial phase, a congruence occurred between the observed transient forms and Na^+-K^+ antiport movement (Omoifo and Awalemhen, 2012). Thus, in a 1.0g/l K^+: 0.10g/l Na^+ incorporated ionic broth, as used in this study, there was a time dependent intracellular K^+ accumulation as intracellular Na^+ simultaneously diminished, as germ cells sequentially transited through neoplasm to protoplasts and subsequently prevegetative cell prior to formation of obpyriform yeast cells of *M. circinelloides* (Omoifo, 2014). However, in the present study in which globose, ovoidal and spindle shaped yeast cells were induced, sigmoid growth pattern was not exhibited. Perhaps this was due to the presence of persistent morphological forms including holothallic-, holoblastic-, and thalloarthric conidia. Since these conidia were encased in geometrical walls of persistent nature, they remained permanently in broth after conidial wall burst and release of their differentiating primeval contents; their stability perhaps resisting the braisive effects of the dynamic reactions within the broth. This could impact strongly on the determined absorbance value of the broth at the early growth phase, although when such cell walls ruptured, which was frequent, the released primeval units were subjected to similar differentiating steps as those originating from germ cell.

The pathway to differentiation exhibited by the microorganism in both Amm. sulphate and Peptone incorporated broths was the same. The nature of vanishing and reappearance of the cell wall at the primordial phase of growth was unique. The two biophysical events were separated by a span where the intervening stretch domiciled numerous distinct forms, each of which was without a cell wall. The first event starts with spheroplast formation from the germ cell; then the cytoplasmic membrane disintegrates and only the cytoplasm is left. This converts to neoplasm, which occurs in various topological forms. This is followed by granulation and, or multiple cleavages subsequently viewed as nondescript entities. These provencal entities, in modified form, which then become incipient or pinched off as they change sizes/volume and shape, then changing through numerous and variously shaped middling stages become ultimately discernable as spherical neoplastic units. From these rod-shaped protoplasts form; they become binary through a denticle. A round protrusion appear to blast out from one end of the binary protoplast; it sutbsequently enlarges. Its integument contrasts sharply with the membranous protoplast that mothers it and its staining hue distinct from the protoplast. This is the nascent yeast initial. When it abscises, it becomes globose. This is the nascent yeast, or prevegetative cell. At maturity, it undergoes budding. Thus, daughter bud could be seen attached to the mother cell through a short denticle. Figures 54& 55 reflect this primordial sequential differentiation process.

In our view, the differentiation process through the sequential wall-less cryptic forms just described for nascent yeast formation originated from germ cell and the possible cytoplasmic membrane initiated conidial types tend to shift the emphasis on cell wall biogenetic possibility from the cell wall *per se*, to the cytoplasmic membrane default. Right from the time of spore inoculation, the bathing medium is the same for all derived growth spheres. Thus whatever changes inherent in the reaction medium, in terms of osmodynamic relationships, equally affect all growth spheres. Therefore, when cell wall of the conidial types ruptured, the primeval entities released into the commonwealth bathing medium subject to the same differentiating mechanism to nascent yeast initial as that arising from the germ cell. This is proof that the intracellular physiology that led to cytoplasmic granulation and ensuing differentiation thereby generating the neoplastic units within the conidial types was similar to what occurred within the germ cell which also generated primeval units. Since the pliable cytoplasmic membrane is the operational platform on which intermedial exchanges occur, especially through exocytotic and other transport activities in cell wall

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generation, it is possible that its specific orientation and available materials (substrate and ions) transport through it could detail alterations which streamline different programmes that impacted on the emerging but distinct morphologies. Witness in Fig. 56 the coexistence of a germ cell-generated neoplasm and emergent (young) holoblastic conidia, which were differentiating along two different lines. Similar observation was made by Omoifo and Awalemhen (2012) when it was found that a 1.10 g /l K^+ incorporation into multiionic broth led to induction of higher proportion of thallic conidial subtypes in comparison with 1.0 g /l K^+-medium which predominantly yielded terminal budding yeast cells of *M. circinelloides* Tieghem.

The lateral morphogenetic transformation outlined here presumes a remarkable intracellular coordination of differentiation. The observed sequences would be controlled by diffusable chemicals relaying signals in the long process of differentiation. The rate of reaction would possibly depend on the temperature level. As the graphic presentation above showed, the cummulative growth of transformed *M. manihotis* was higher at 28°C. This was followed by that at the elevated temp. 37°C and it was least at 15°C.

Following cytodifferentiation, the stable morphology was polar budding globose yeast cell. It is interesting to note that elevated temp caused morphological diversity of the yeast form. Thus additionally induced were spindle- and ovoidal shaped yeast cells at 37°C. This diversity probably resulted from the response of the induced form to stress imposed by the sublethal temperature level.

This study has shown how pristine granular units have evolved, then progressed through a sequence of cryptic entities, each different from the preceding one. What this entails is that a series of structural and functional adjustments occurred as one entity transited to the other until the final complex functional stable form occurred. Thus a germ cell converted into spheroplast after disappearance or lysing of the cell wall and, following the loss of the cytoplasmic membrane what we see was the cytoplasm from which neoplasm derived, an exhibition of differing topological changes whence imploding into innumerable neoplastic units in a directional transformation as each unit transited into individual protoplast, a precursor to the prevegetative cell. As we see, the changes that occurred during such differentiation apart from the size and shape, would include inherent metabolic activity, membrane potential as well as signal responsiveness. Thus inherently, each matured stable unicellular form had reproductive capabilities and, hence produced offspring. This trendline of daughter generation and or proliferation is distinct from the previously described germ sphere-derived population growth.

Therefore, primordial phase differentiation exhibited in this study did not involve the conventional mitotic division, which encompasses prophase-nuclear membrane disintegration, chromosome doubling, seggregation, cell division and daughter bud formation-activities which are DNA polymerase alpha mediated at the S-phase. In this study, daughter buds were formed after maturation of the nascent yeast initialized by the binary protoplast. It is reasonable to say that cytodifferentiation as expressed here involved differential gene expression that resulted in the multiple morphologies, though transient. But since the genetic constitution of the *M. manihotis* was not affected, it was possible to revert to the original filamentous growth habit, evidenced by the congruence of characteristics of the re-induced filaments at termination of experiments, with the original state of the isolate. Similar morphogenetic interconversion has been achieved in our laboratory using different microorganisms including the now invalid *Dimorphomyces* strains (Omoifo, 1996,1997), *M. circinelloides* (Omoifo, 2006ab, 2011ab, 2013, 2014; Omoifo and Awalemhen, 2012; Omoifo and Omamor, 2005; Omoifo *et al*., 2006) and *R. stolonifer* (Omoifo, 2011ab; Omoifo *et al*., 2013).

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- *Contribution.* The concept, design, analysis, literature search and write up was done by COO. NN was a project student who participated physically in this study.

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Table 1 : Total biomass, mean bomass, standard error and form of growth of *Mucor manihotis* cultivated in synthetic broth incorporated with organic and inorganic nitrogen sources at pH 5.0

Temperature (ºC)	Total biomass	Mean biomass	Standard error	Form of growth
		Ammonium sulphate		
15	0.211	0.021	0.004	Gs, Nu, Tg Yo, P, N, TY, Ec
28	2.088	0.208	0.068	Gs , Nu, Tg, y, Ec, Hc, Fs, P, B, Tg
37	0.808	0.080	0.022	Nu, Yo,Y, Yc, H, Yg, Tc, Yo
		Peptone		
15	0.912	0.091	0.417	Gs , `Nu, Tg, P, Y, N,
28	3.682	0.368	0.073	Tg, Hc, Nu, Tg, P, Pv, Ta
37	2.419	0.242	0.061	Nu, TgYs, Yg, B, Nu, To, Tg, Fs, Ta

Legend: Gs – growth sphere; Nu – neoplastic units; Tg – thallic growth; Yn – nascent yeast; P – protoplast; N – neoplasm; Y – polar budding yeast cells; Ec – enterothallic conidia; Hc – holoblastic conidia; Ta – thalloarthric conidia; Fs – septate filament; Pb – binary protoplast; Ys – spindle yeast cell; Yg – globose yeast cell; Yo – ovoidal yeast cell; Tc – thallic conidia; B – blastospore

Supplementary Table 1 : Analysis of variance of growth data of *Mucor manihotis* cultivated in minimal medium incorporated with Amm.sulphate and Peptone and incubated at different temp.levels at pH 5.0

Variate: Variate					
Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
BLOCK stratum	4	0.0000000	0.0000000	0.00	
BLOCK.*Units* stratum					
N-source	1	1.3926453	1.3926453	2113.62	<.001
Temp	2	2.9309735	1.4654867	2224.17	<.001
Time	4	2.0258522	0.5064630	768.66	<.001
N-source.Temp	2	0.1675792	0.0837896	127.17	<.001
N-source.Time	4	0.2373338	0.0593335	90.05	<.001
Temp.Time	8	0.6129923	0.0766240	116.29	<.001
N-source.Temp.Time	8	0.3096117	0.0387015	58.74	<.001
Residual	266	0.1752650	0.0006589		
Total	299	7.8522530			

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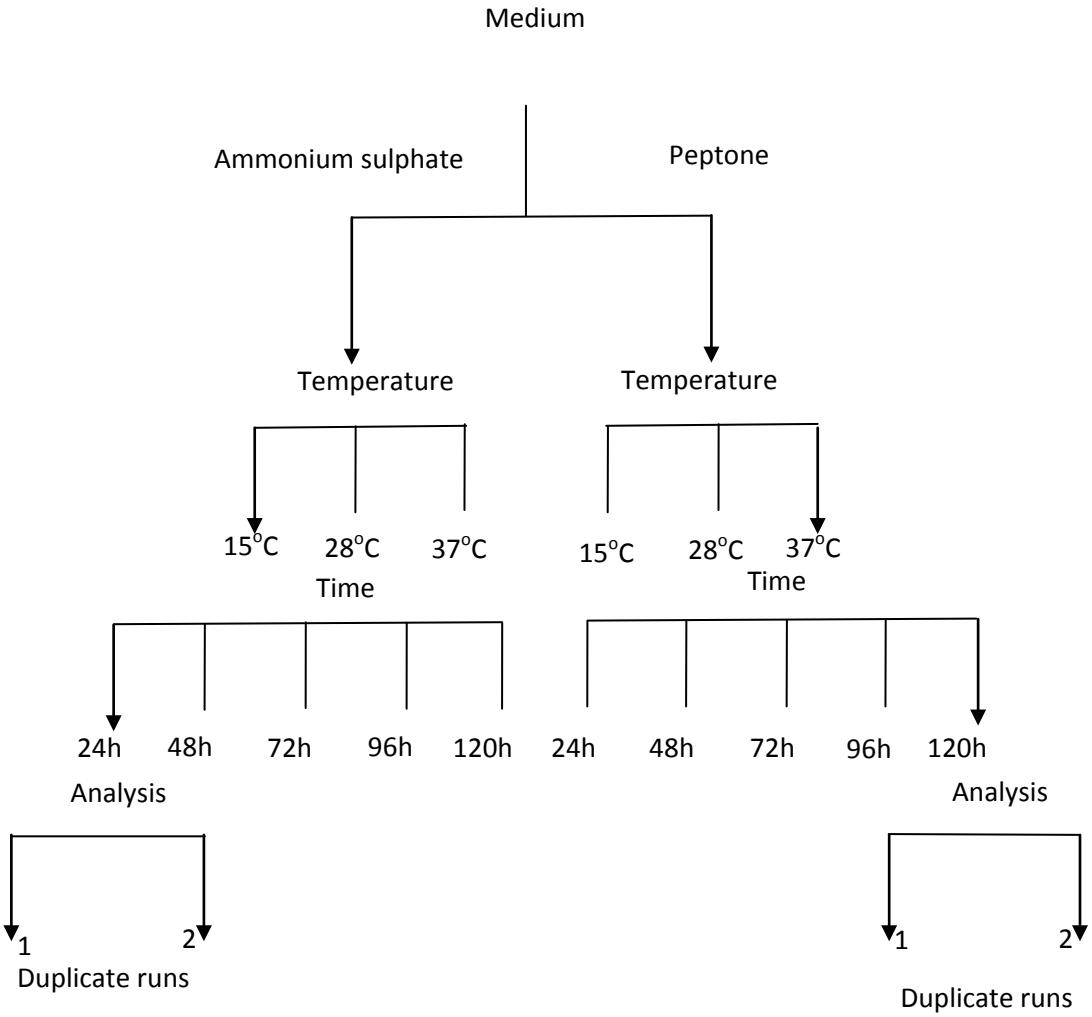


Figure 1 : Experimental design for growth and evaluation of *Mucor manihotis* used in this study

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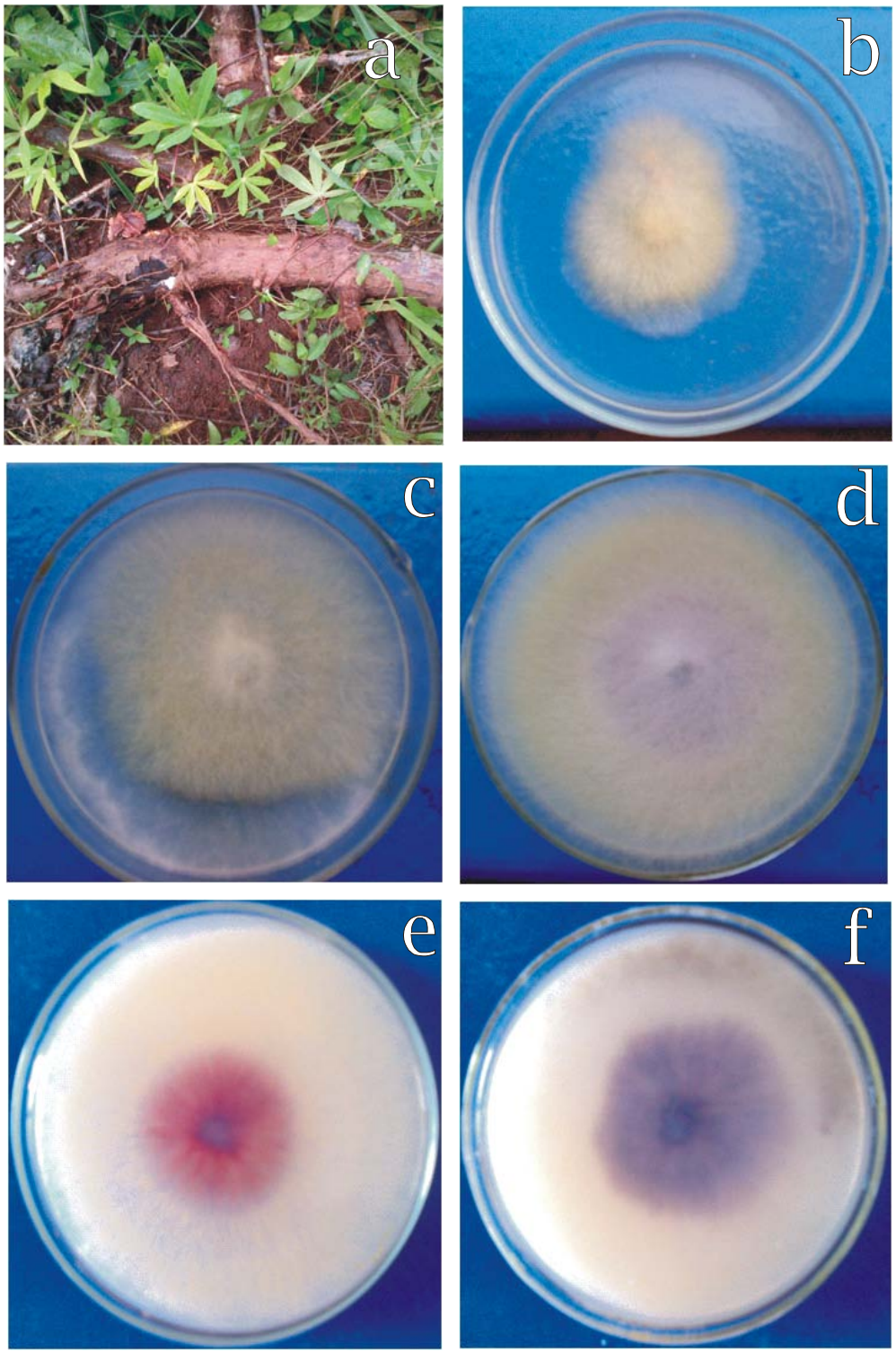


Figure 2 : PDA cultures of *Mucor manihotis;* **a**, specific point of sample isolation in the farm; **b**, culture at 1 day after inoculation; **c**, culture at 3 days from inoculation;, **d**, A-view at 6 days from inoculation, observe the colour change at the central region; **e**, B-view: the central region is reddish; **f**, 45 days from inoculation, the central region is purple while mycelium towards the edge turn black.

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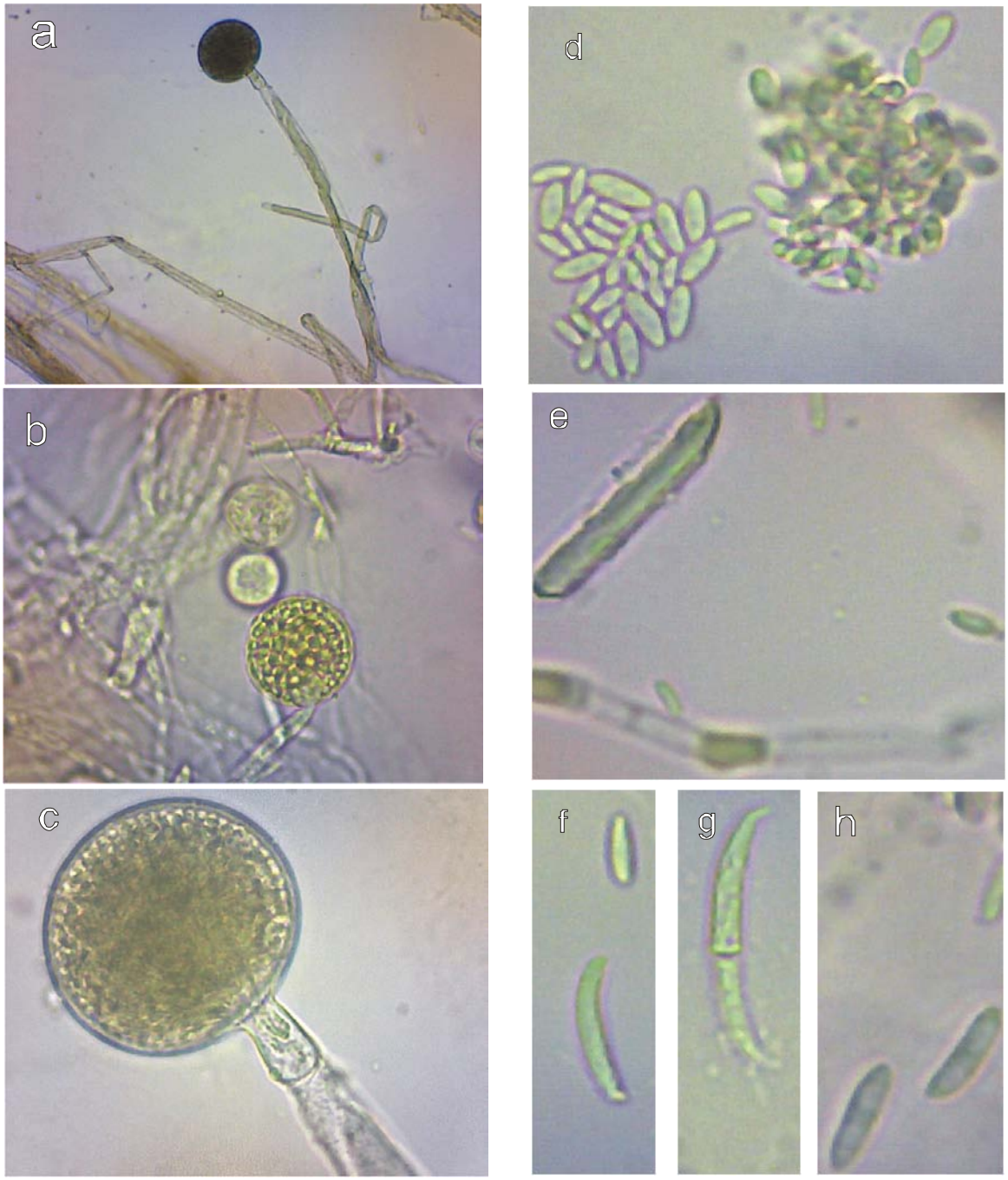


Figure 3 : Aerial mycelia of *Mucor manihotis;* ***a***, sporophore & sporangium; **b**, spores directly observed in the sporangium, x800mag; ***c,*** sporangium: observe septum cutting off columellum from sporophore, x1000mag; ***d,*** clusters of ellipsoid & cylindrical sporangiospores; ***e,*** cylindrical elongate sporangiospore; **f**, crescent shaped sporangiospore; **g**, double-celled crescent sporangiospore; **h**, sausage-roll sporangiospore; d e, f, g, h are at 2500mag.

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Figures 1b : The isolation of, and cytodifferentiation of a Mucor species as affected by nitrogen source and elevated temperature

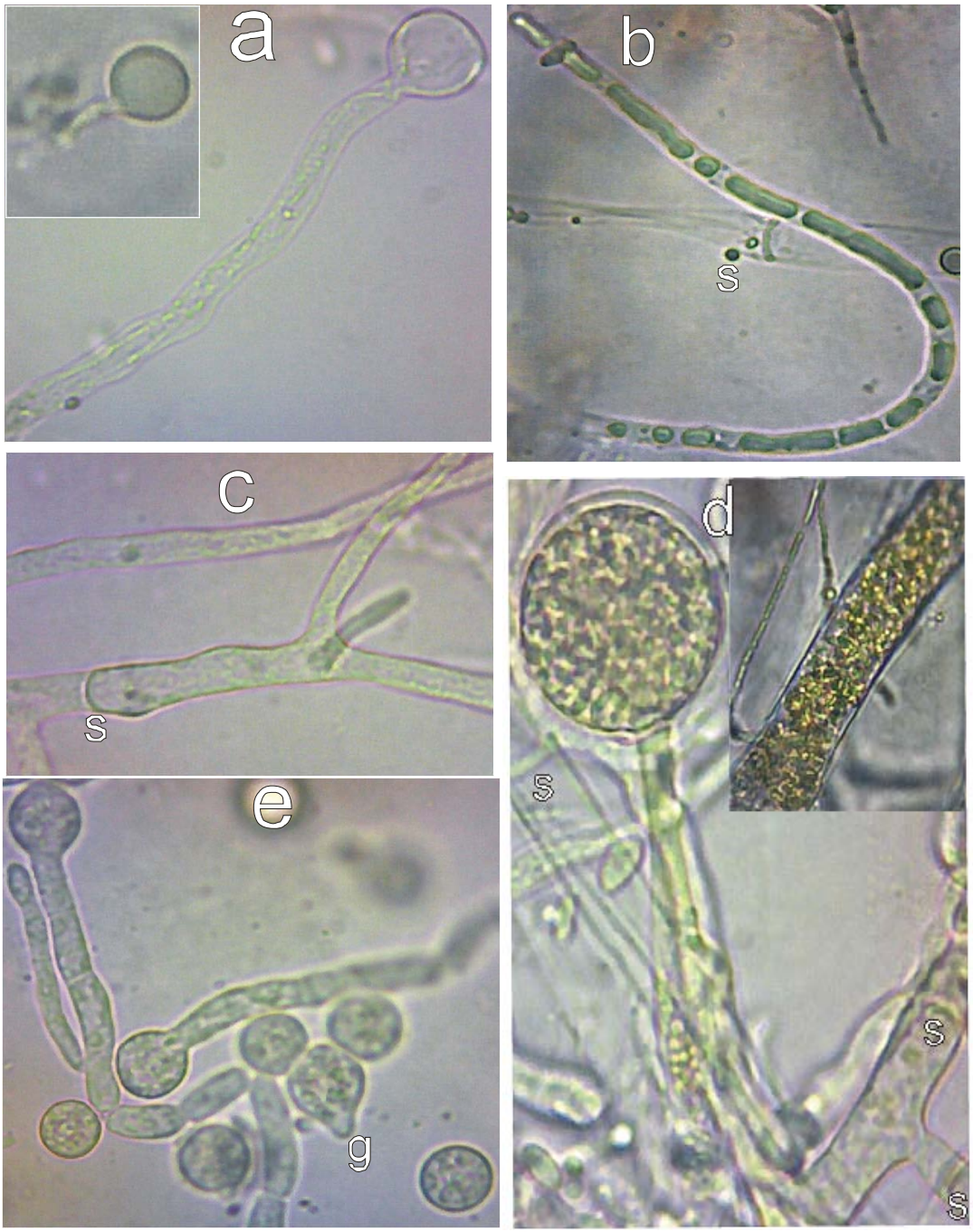


Figure 4 : *Su*bstrate-level mycelium of *Mucor manihotis* after 24h of growth on PDA; **a**, columellum of upright sporophore, inset: laterally borne columellum; **b**, thin filament showing early fragmentation of cytoplasm; **c**, multple branching mucelium; **d**, upright sporophore borne on septate mycelium, inset; mycelium with fragmented cytoplasm; **e**, thallo-arthric growth; g, germ tube, s, septum. Magnification: 2500x.

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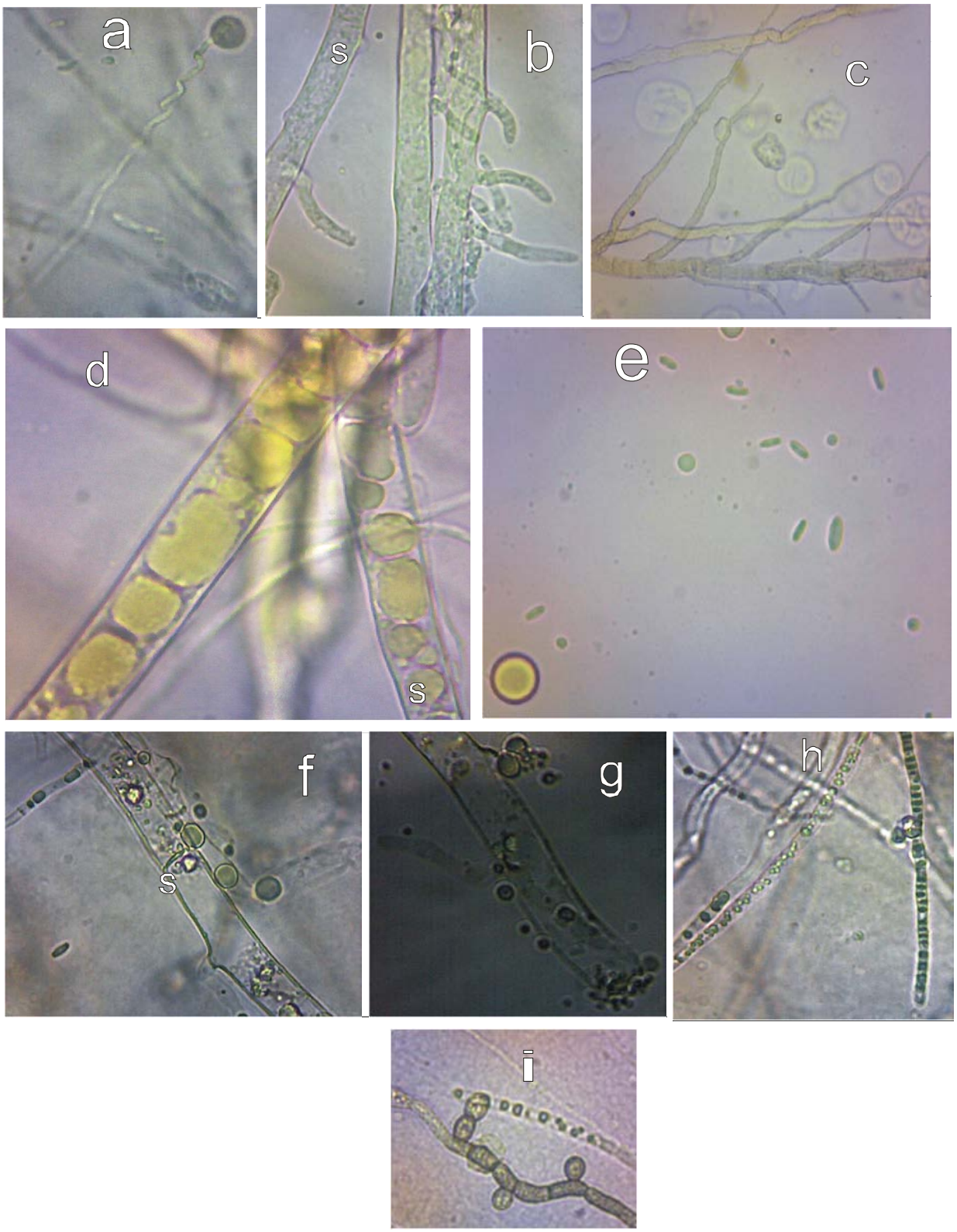


Figure 5 : Mucor manihotis: **a**, curvy sporophore terminating in globose columellum, x800; **b**, multiple germ tubes of septate mycelium, x1000; **c**, elongating hyphal branches forming mycelia, x800; **d**, hyphae with cytoplasmic cleavages, x1000; **e**, sporangiospores, x2500; **f**, septate hypha & protoplasts, x2500; **g**, hypha release protoplasts on rupture, x2500; **h**, protoplast formation within tubular compartments, x2500; **i**, internal & branch conidial formation of thallic growth, x1000; a-c @ 72h, d-g @ 120h, f-i @ 191h of growth

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2 a. Cytodifferentiation of Mucor species

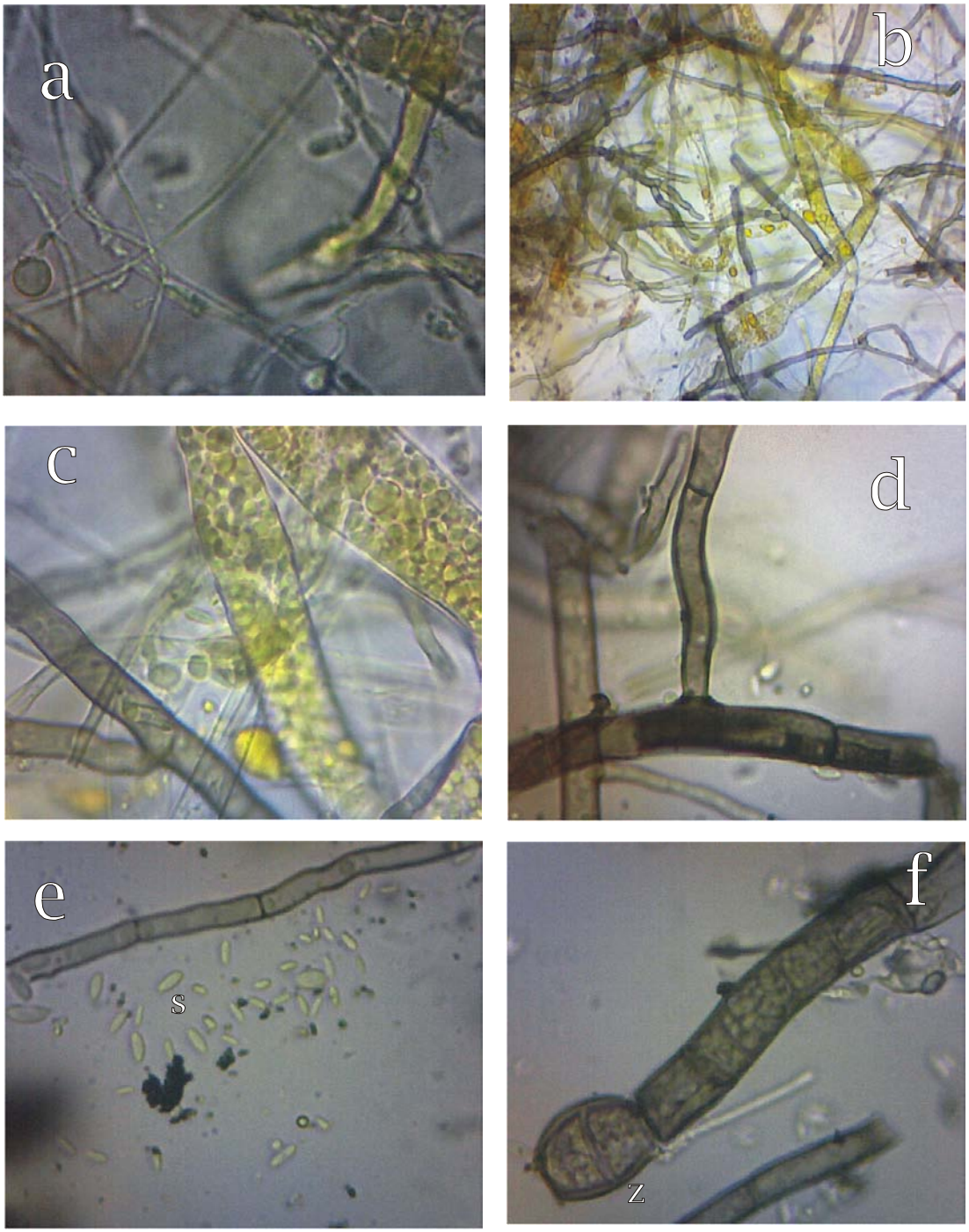


Figure 6 : Mucor manihotis PDA culture after 9days of growth; observe the melanized walls of filaments, a-f, and septa, d-f, s-sporangiospores; z- chlamydospore. Magnification, a, b -800x; c – f, 1000x

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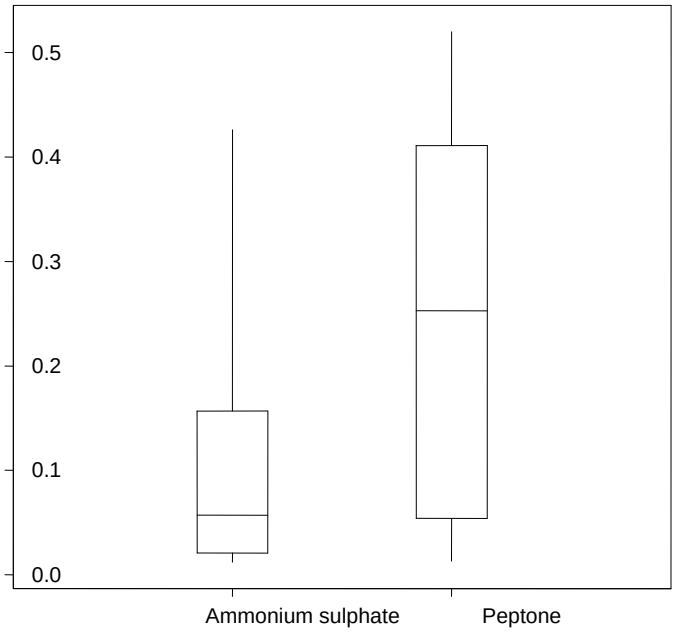


Figure 7a : Box plot of cummulative growth data of *Mucor manihotis* cultivated in minimal medium incorporated with organic or inorganic nitrogen source. Growth in the organic nitrogen source, peptone, was profoundly enhanced

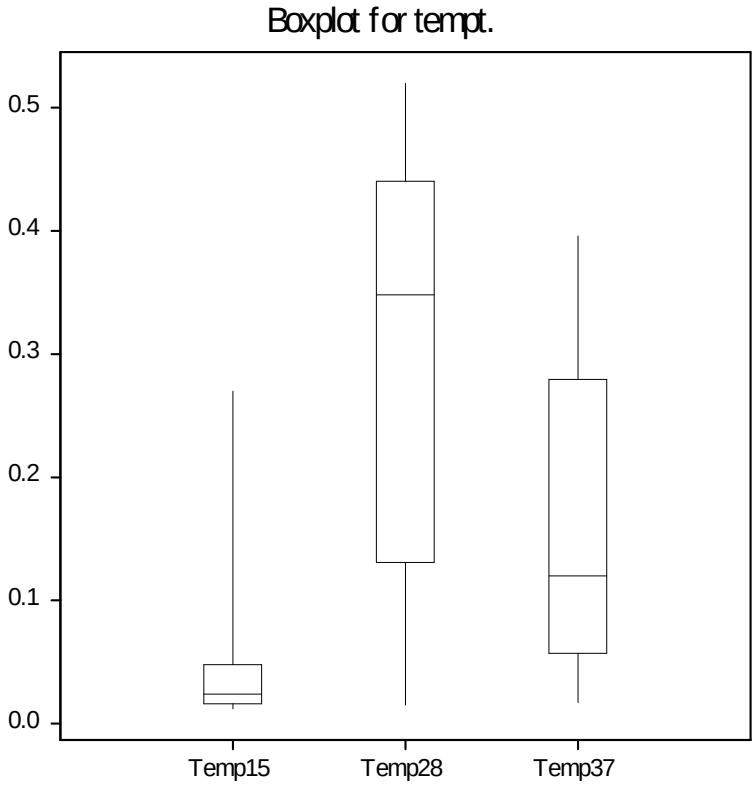


Figure 7b : Box plot of growth data of *Mucor manihotis* cultivated in minimal medium and incubated at the various temperature levels. At 28°C, growth was considerably enhanced and was least at 15oC where variablity was also greatest, represented by the extended whisker. However, it was pertinent to point out that in spite of the different cryptic forms induced, growth followed normal population dynamics

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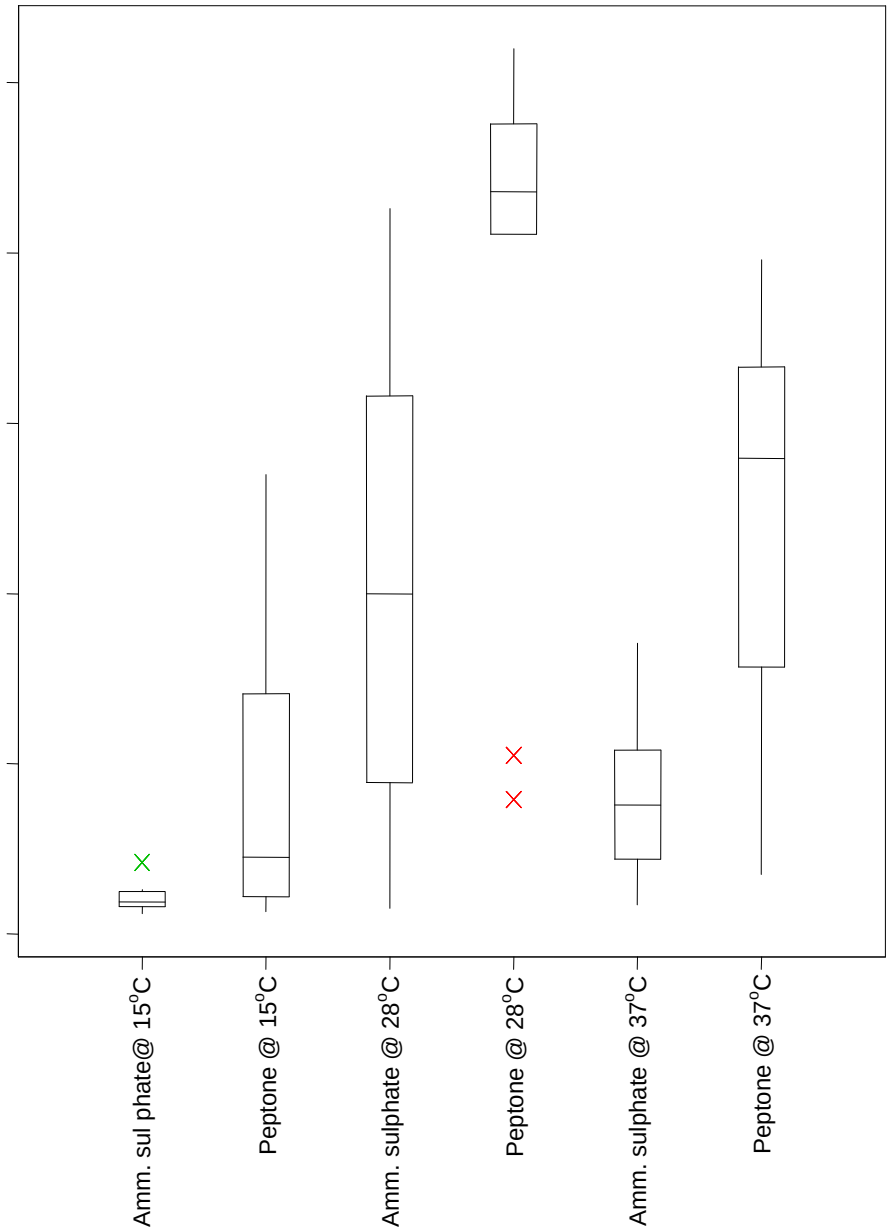


Figure 8 : Box plot of growth data of*Mucor manihotis* cultivated in ammonium sulphate and peptone incorporated minimal medium and incubated at the various temperature levels. At each temperature level growth in peptone incorporated broth was outstanding

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3 a. Cytodifferentiation of Mucor species

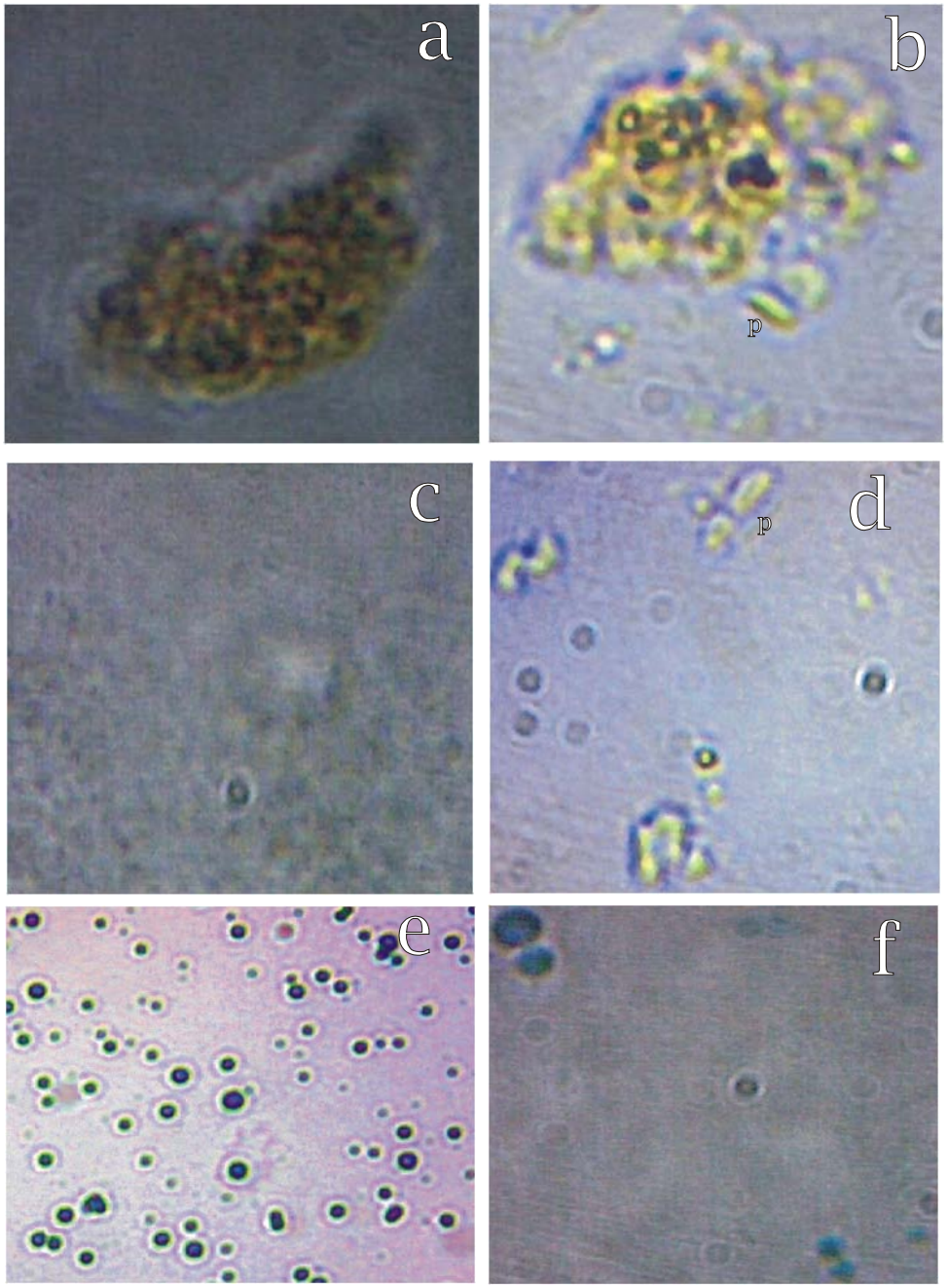


Figure 9 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 96h at temp 15°C; a, differentiating, x2500mag; b, individual neoplastic units culminating as protoplastic units (p, rod-shaped in this micrograph), x2500mag; c, dispersing formless neoplastic units; d, binary protoplast (p,), x2500mag; e, polar budding yeast cells, x1000mag; f, yeast cells, a short denticle separate daughter from mother cell. x2500mag

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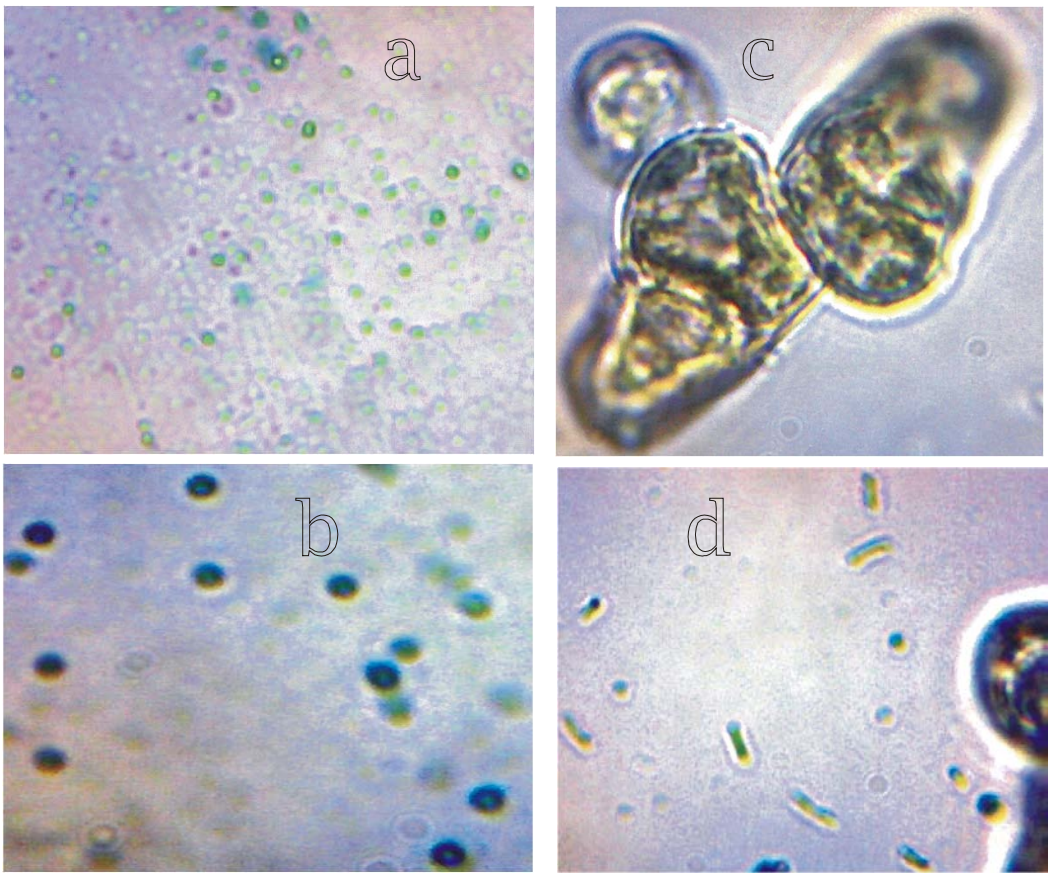


Figure 10 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 120h at temp 15°C; a, neoplastic units; b, globose yeast cells; c, enterothallic conidia; d, a lower elevation of (c) showing rod shaped & binary protoplasts. Magnification: x2500mag

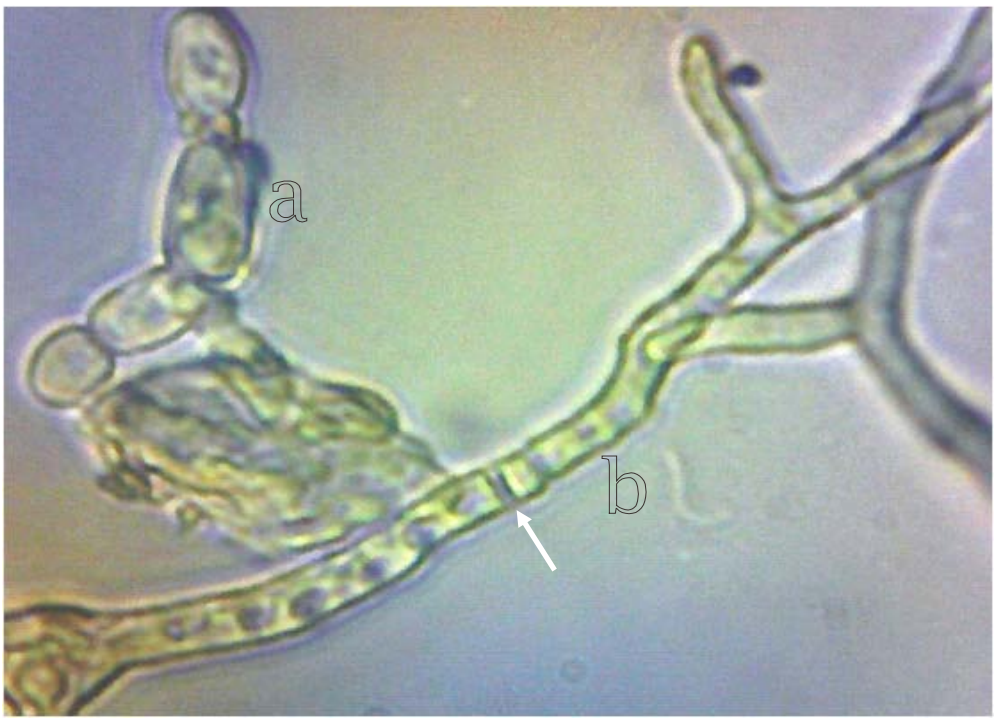


Figure 11 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 24h at temp 28°C; a, holoblastic conidia, x1000mag; b, septate fil aments; arrow, septum; x1000mag

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3 b. Cytodifferentiation of Mucor species

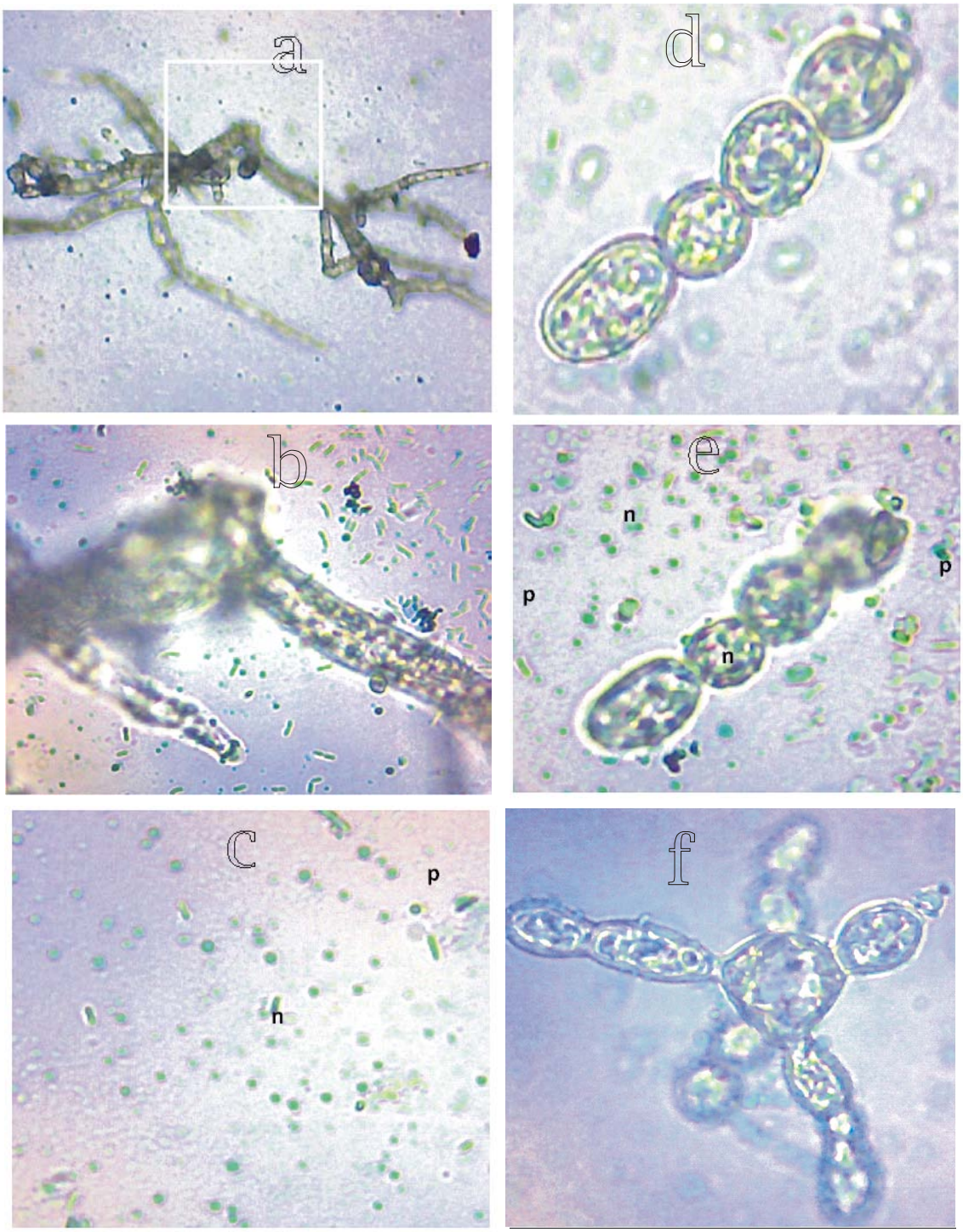


Figure 12 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 48h at temp. 28°C; **a**, thallic growth, x800mag; **b**, lower elevation of the section mark 'n' showing rod shaped & binary protoplasts, x2500mag; **c**, neoplastic units 'n' & rod shaped protoplasts, 'p', x2500mag; **d**, holoblastic conidia, x2500mag; **e**, lower elevation of (d) showing neoplastic units & protoplasts x2500mag; **f**, growth cell showing multiple germ tube production; x2500mag

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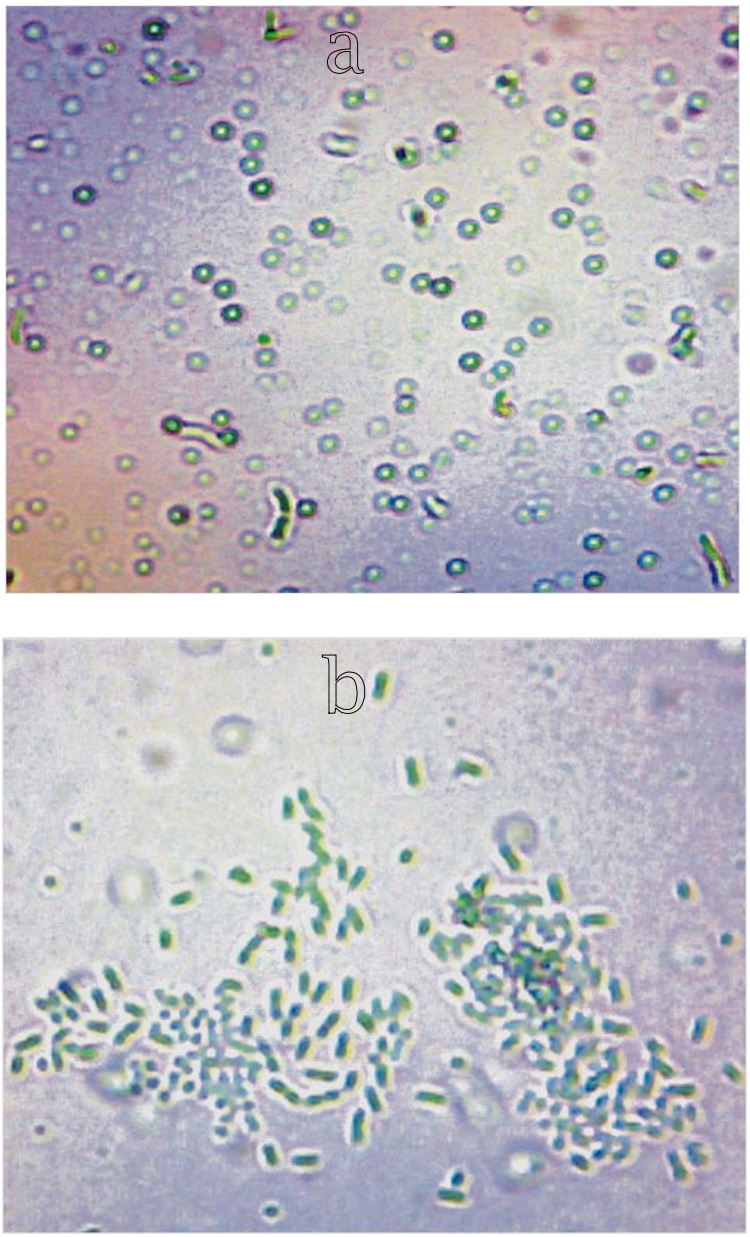


Figure 13 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 72h at temp 28°C; a, ultimate neoplastic units & binary protoplasts; b, rod shaped & binary protoplasts, x1000mag

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4 a. Cytodifferentiation of Mucor species

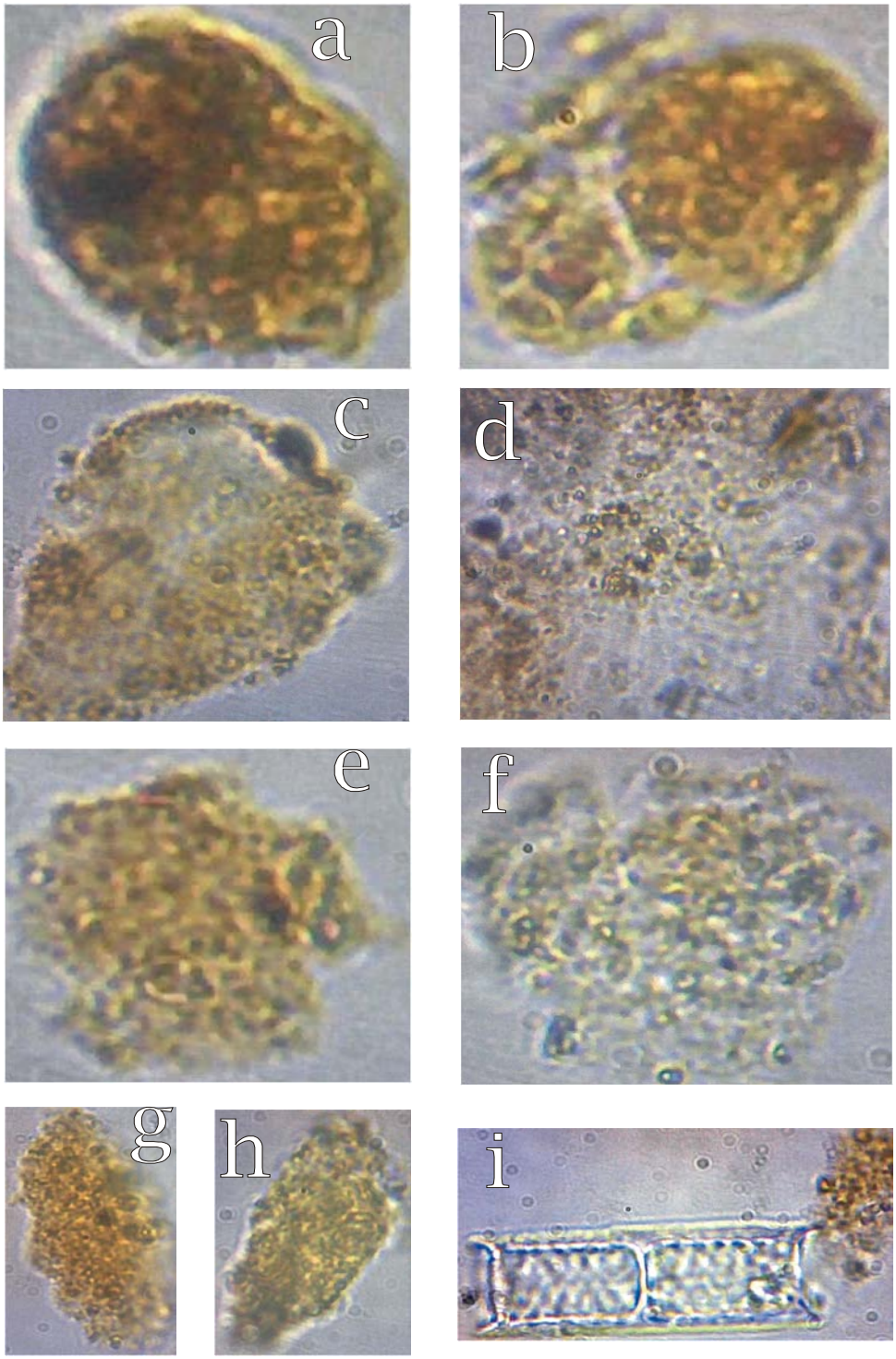


Figure 14 a- i : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 96h at temp 28° C; a, spheroplast - arising from loss of cell wall; b, cytoplasm, after lysis of cytoplasmic membrane; c-h; generation of provencal entities from neoplasm arising from lysis of germ cell envelop, and i, primeval units from rupture of endoarthrospore. Observe that the entities were at various stages of undefinition. Magnification, 2500x

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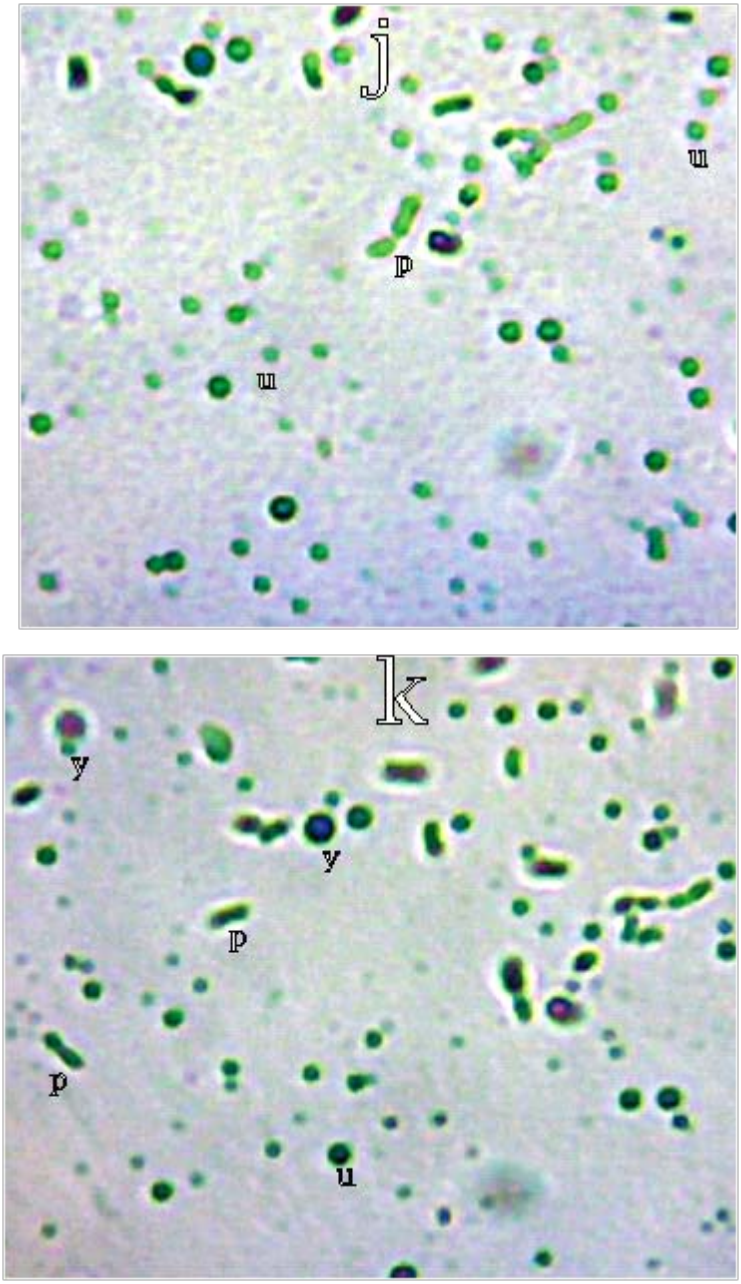


Figure 14 j k : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 96h at temp 28°C; **j**, ultimate neoplastic units, binary & rod shaped protoplasts; **k**, ultimate neoplastic units (u), single & binary protoplasts (p), and globose unipolar budding yeast cells (y); x1000mag.

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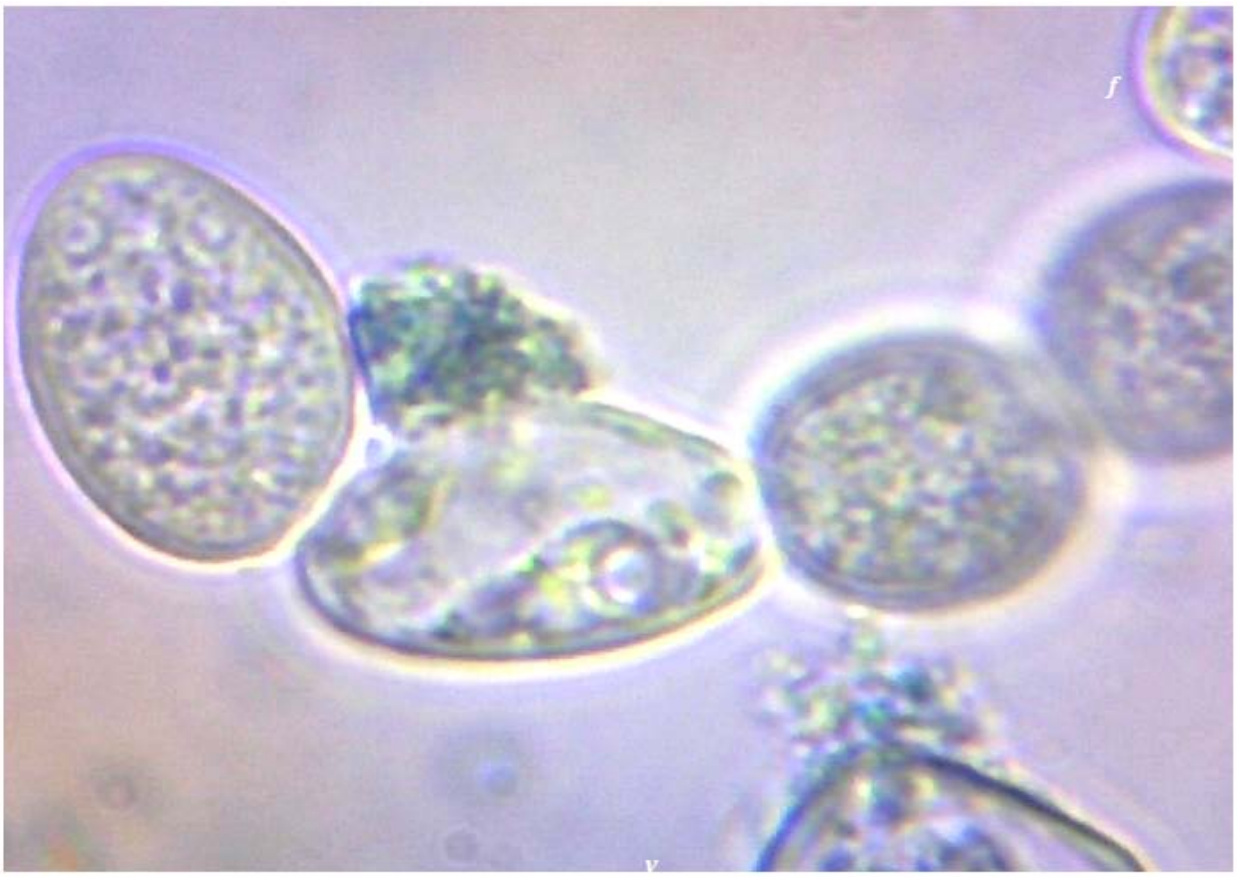


Figure 15 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 120h at temp 28°C; holoblastic conidia showing ruptured conidial wall (top & bottom) with the release of neoplastic units; this coexisted with protoplasts, unipolar budding yeast cells; x2500mag

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4 b. Cytodifferentiation of Mucor species

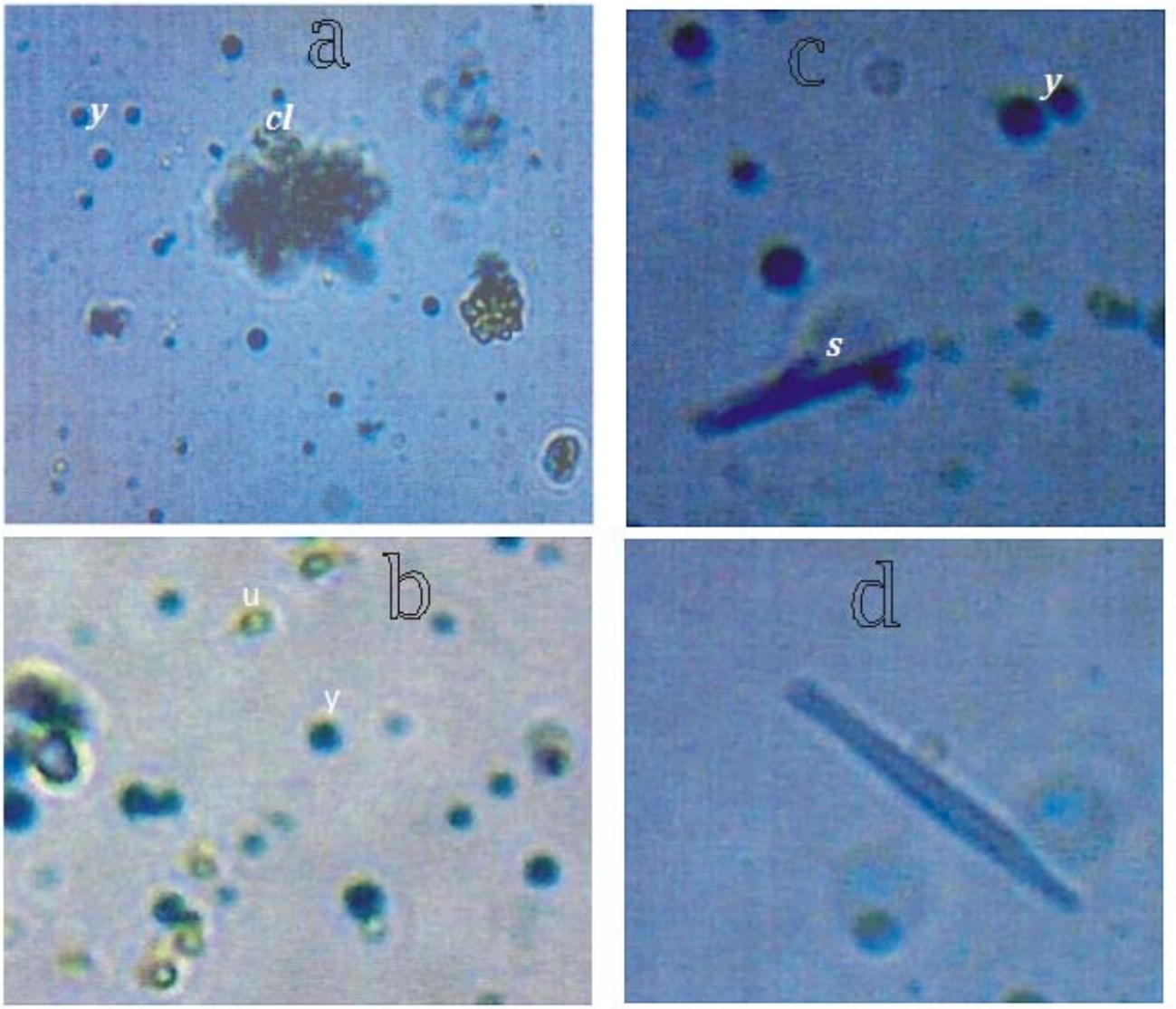


Figure 16 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 24h at temp 37°C; **a**, cluster of provencal entities (cl), differentiating units (y) & globose yeast cell; **b**, ultimate neoplastic units (u) & yeast cells (y); **c**, unipolar budding yeast cells (i) & spindle shaped yeast cell (ii); **d**, another elevation of the spindle shaped cell shown in 'c' above; x2500mag.

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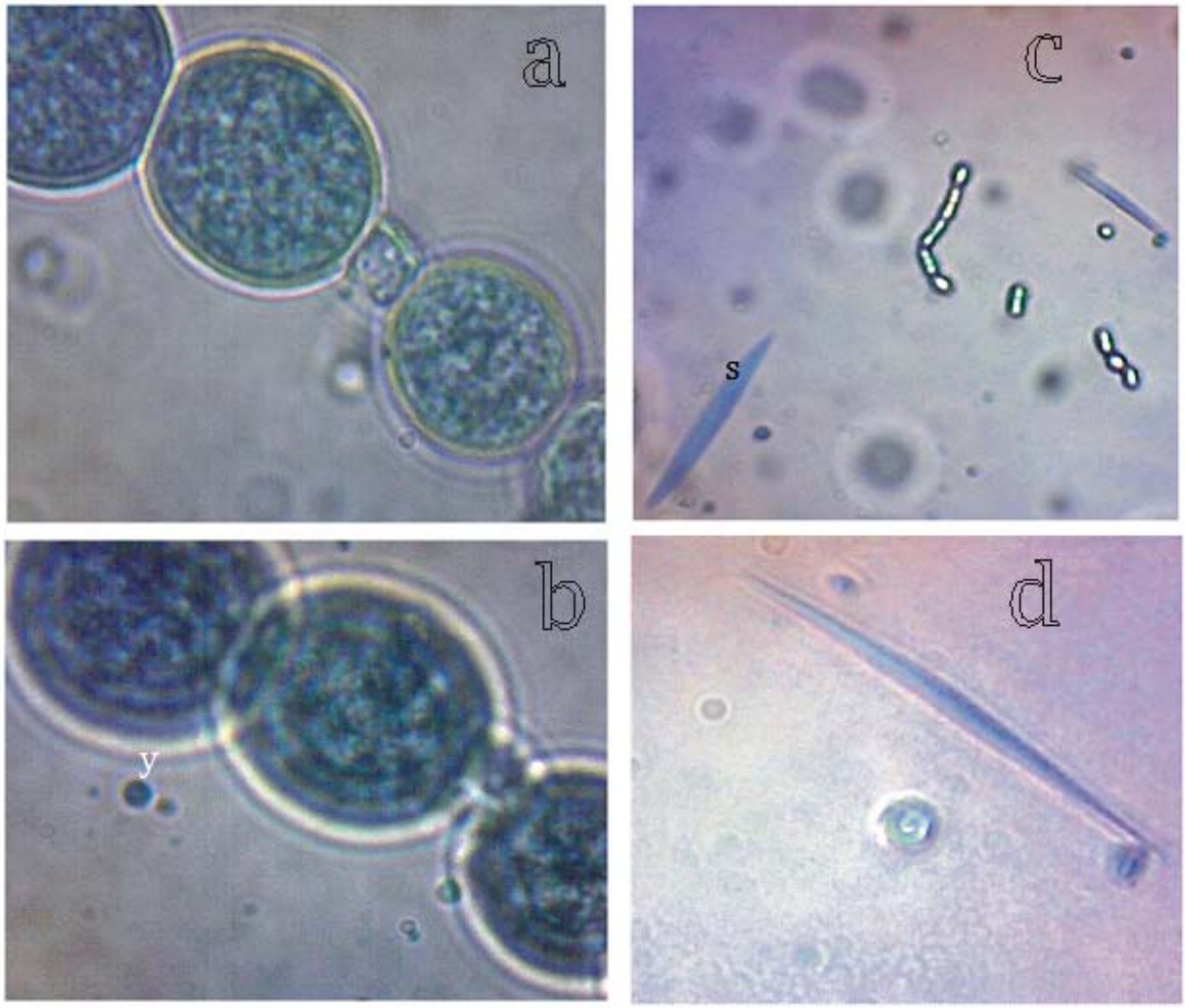


Figure 17 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 48h at temp 37° C; a, holoblastic conidia; b, another elevation of the holoblastic conidia revealing unipolar budding yeast cell (y); c, holoblastic conidia & spindle shaped cells (s); d, refocus on 's' above. All magnifications are at 2500x

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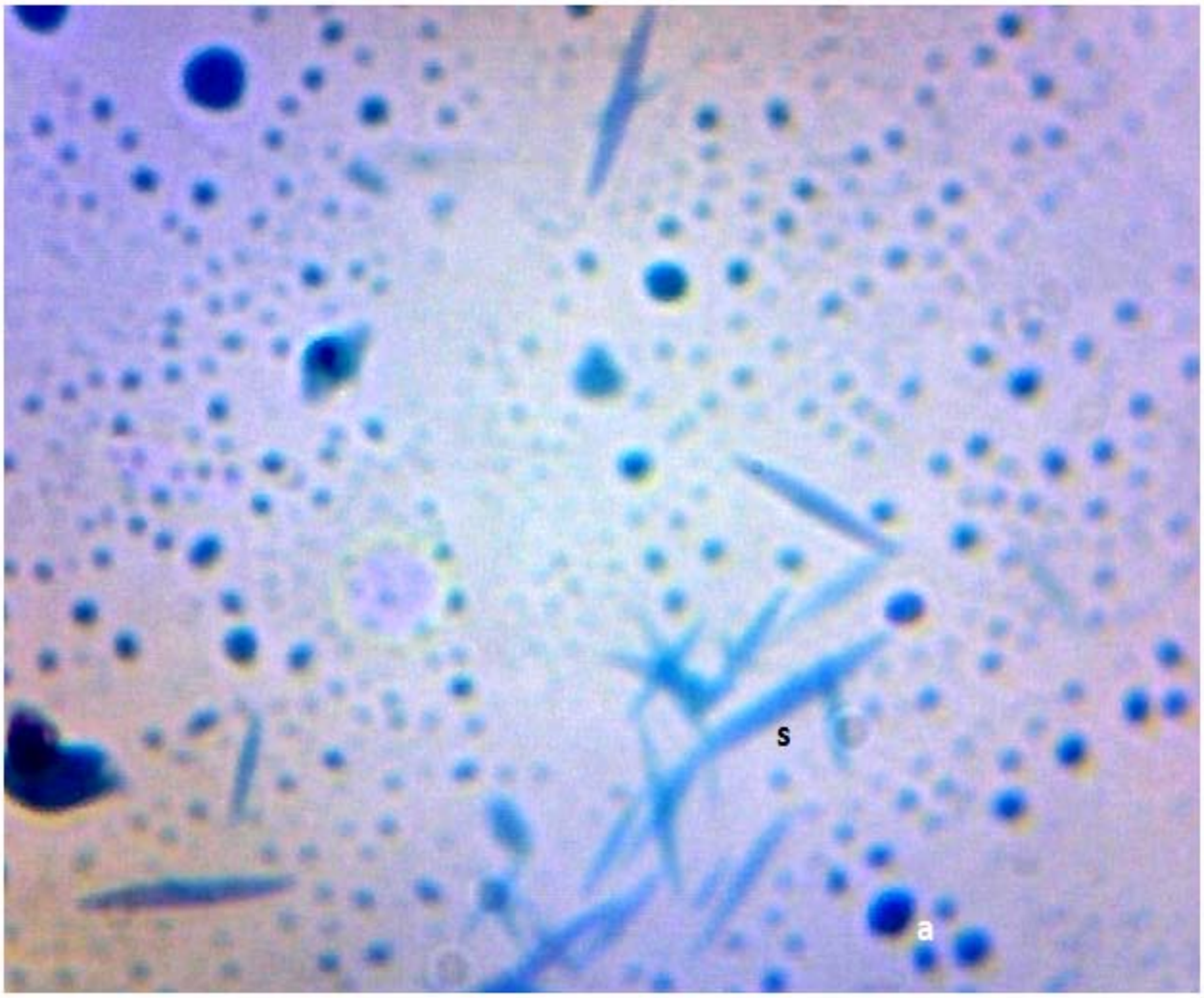


Figure 18a : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 72h at temp 37° C; a, globose yeast cells; b, spindle shaped yeast cells (s); mag; 2500x

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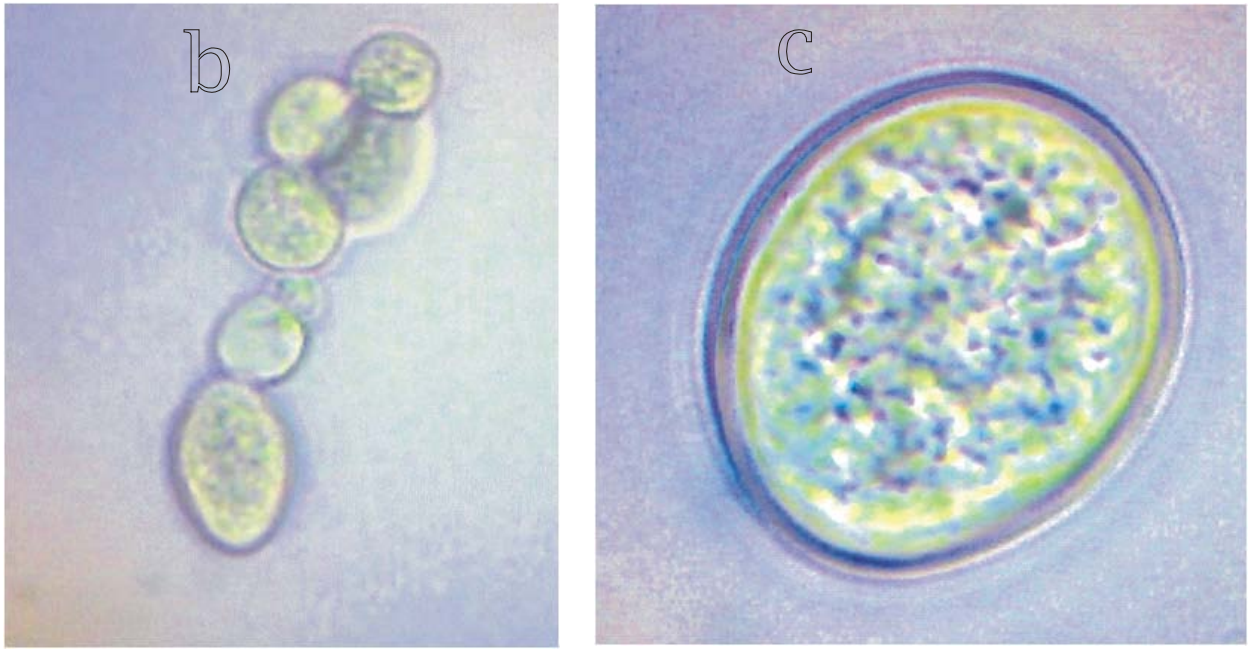


Figure 18 b c : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 72h at temp 37°C; b, holoblastic conidia, x1000mag; c, globose conidium with differentiated cytoplasm thus appearing a s granular entities, mag; 2500x

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5 a. Cytodifferentiation of Mucor species

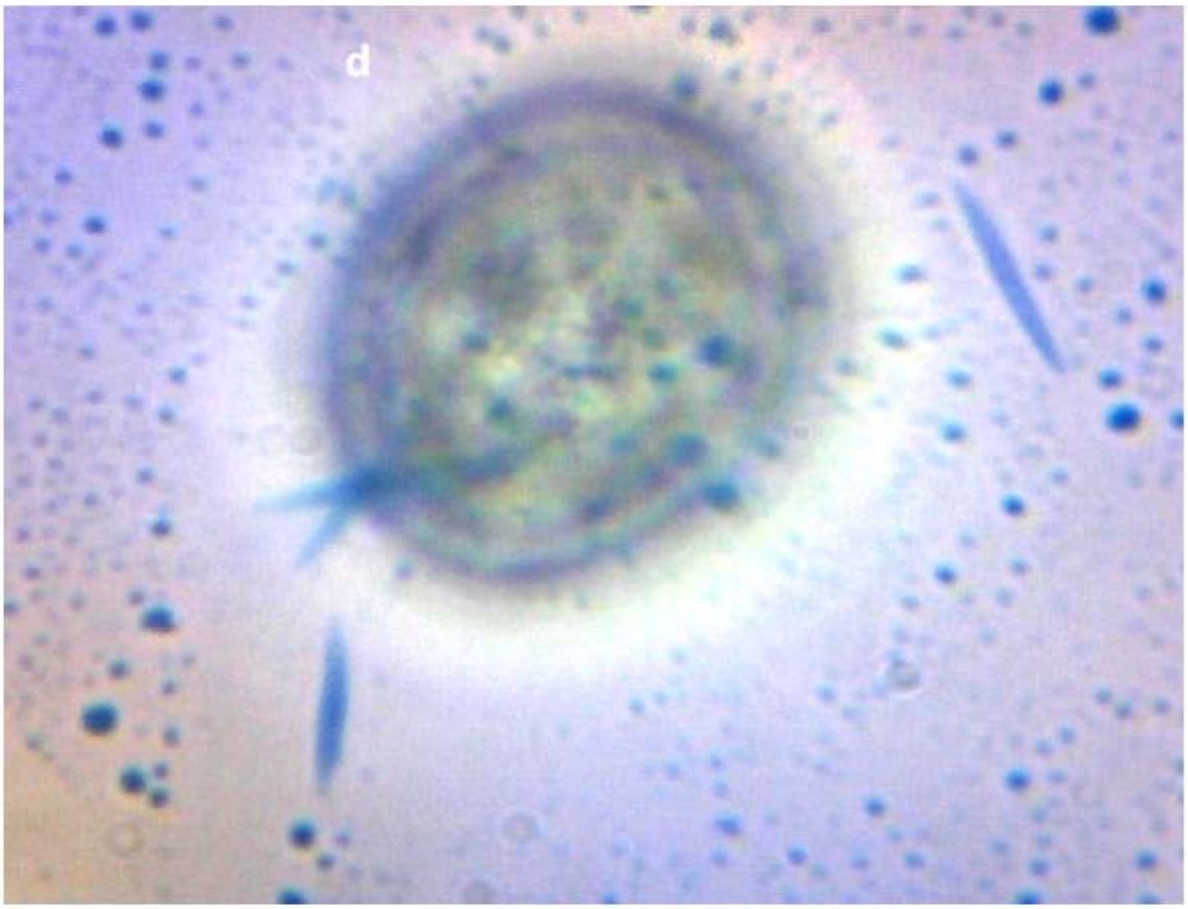


Figure 18d : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 72h at temp 37°C; another elevation of the conidium in fig 22b, showing globose & spindle shaped yeast cells; mag; 2500x

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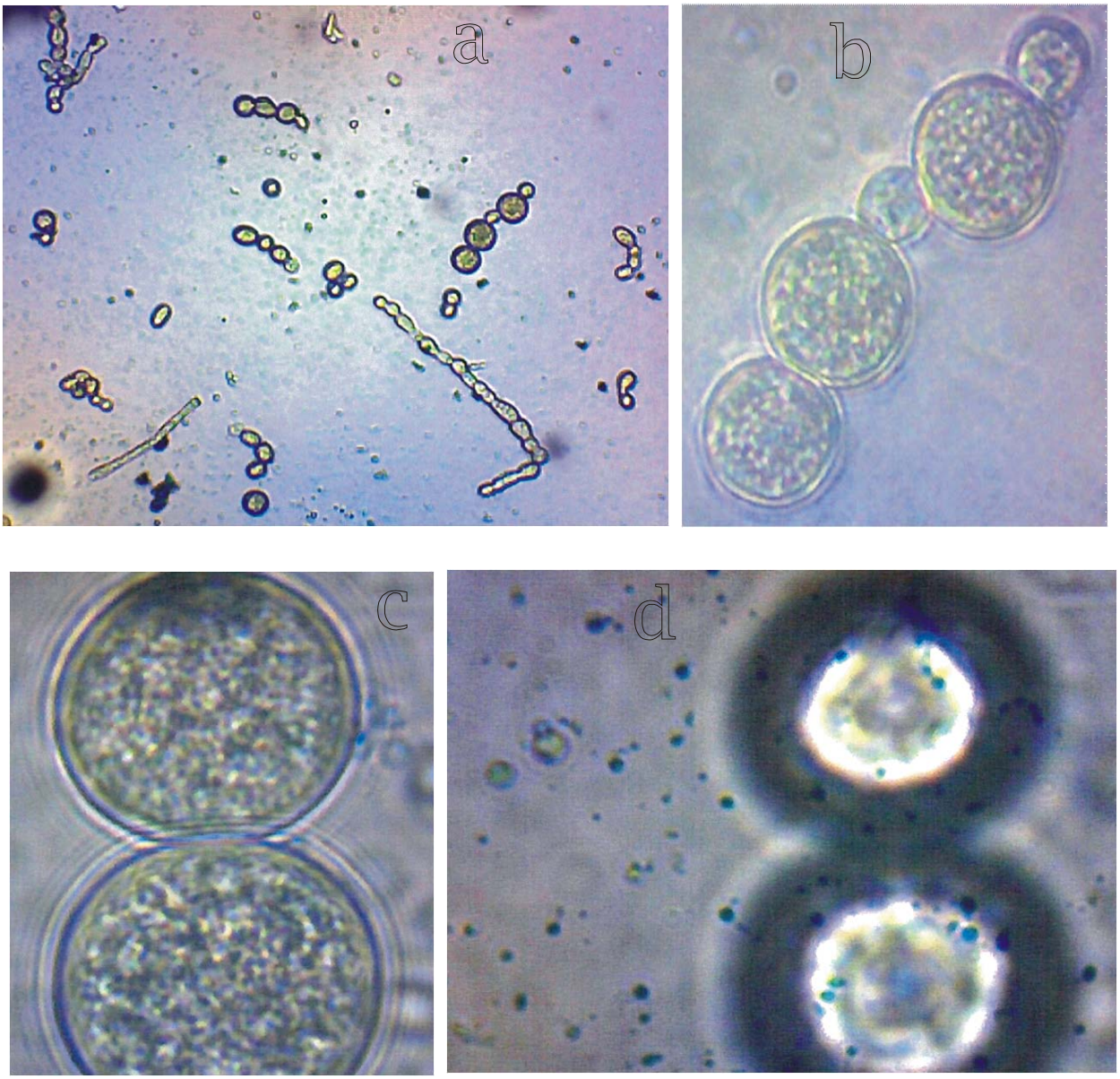


Figure 19 a-d : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 96h at temp 37°C; **a**, various forms of holoblastic conidia, x800mag; **b**, holoblastic conidia arrowed in 'a', x1000mag; **c**, holoblastic conidia in 'b' at x2500mag showing granular cytoplasm; **d**, another elevation of the conidia in fig 23c, showing coinduced globose & budding yeast cells; mag; 2500x

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5 b. Cytodifferentiation of Mucor species

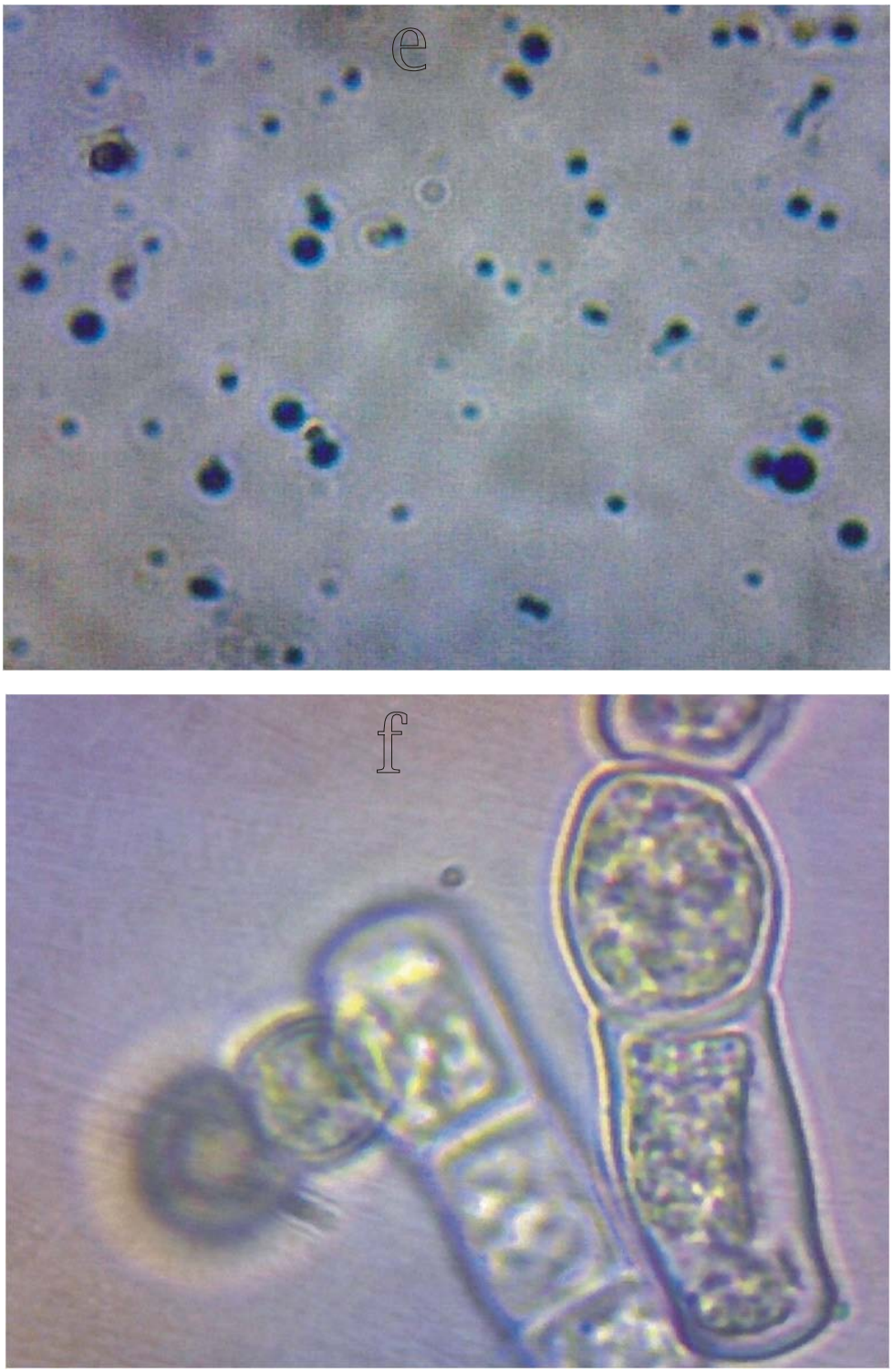


Figure 19 e-f : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 96h at temp 37°C; e, globose yeast cells – this was the most predominant form & co-existed with spindle shaped yeast cells, x2500mag; f, thallo-arthric conidia, this was however scanty, x2500mag

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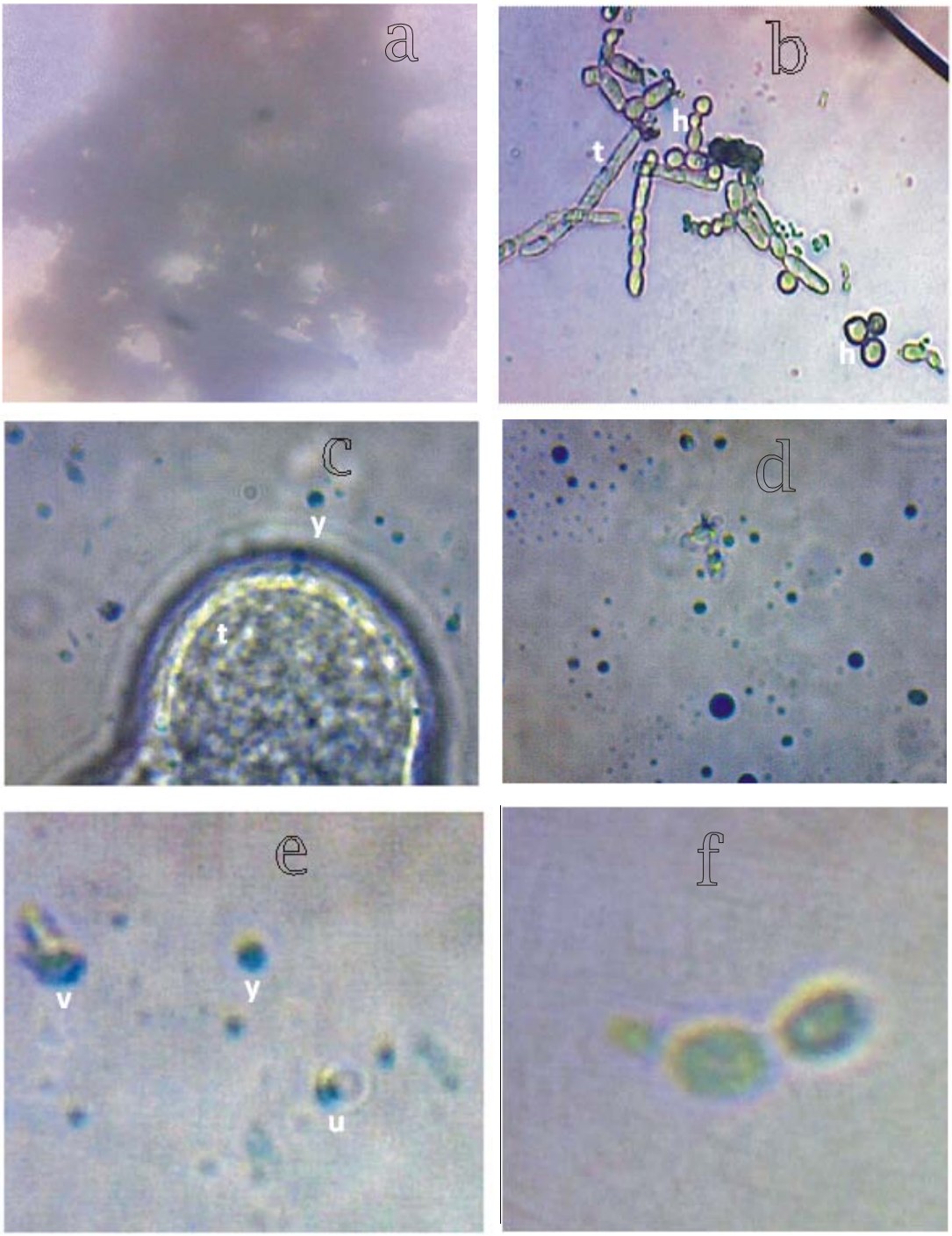


Figure 20 : Growth forms of *Mucor manihotis* induced in $(NH_4)_2SO_4$ incorporated minimal medium after 120h at temp 37°C; **a**, cluster of provencal neoplastic units (appeared finely grainy @ this mag); **b**, various forms of holoblastic, 'h' & thallic, 't' conidia; **c**, globose yeast cells, 'y', adjacent to thallic conidia; **d**, globose yeast cells; **e**, nascent yeast initial 'v' (out of focus); y (globose yeast), u (ultimate neoplastic unit); **f**, ovoidal yeast cells – these along with globose & spindle yeast cells were predominant forms coinduced with conidia in this medium. Magnification; a, c-f, x2500; b, x800.

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6 a. Cytodifferentiation of *Mucor* species

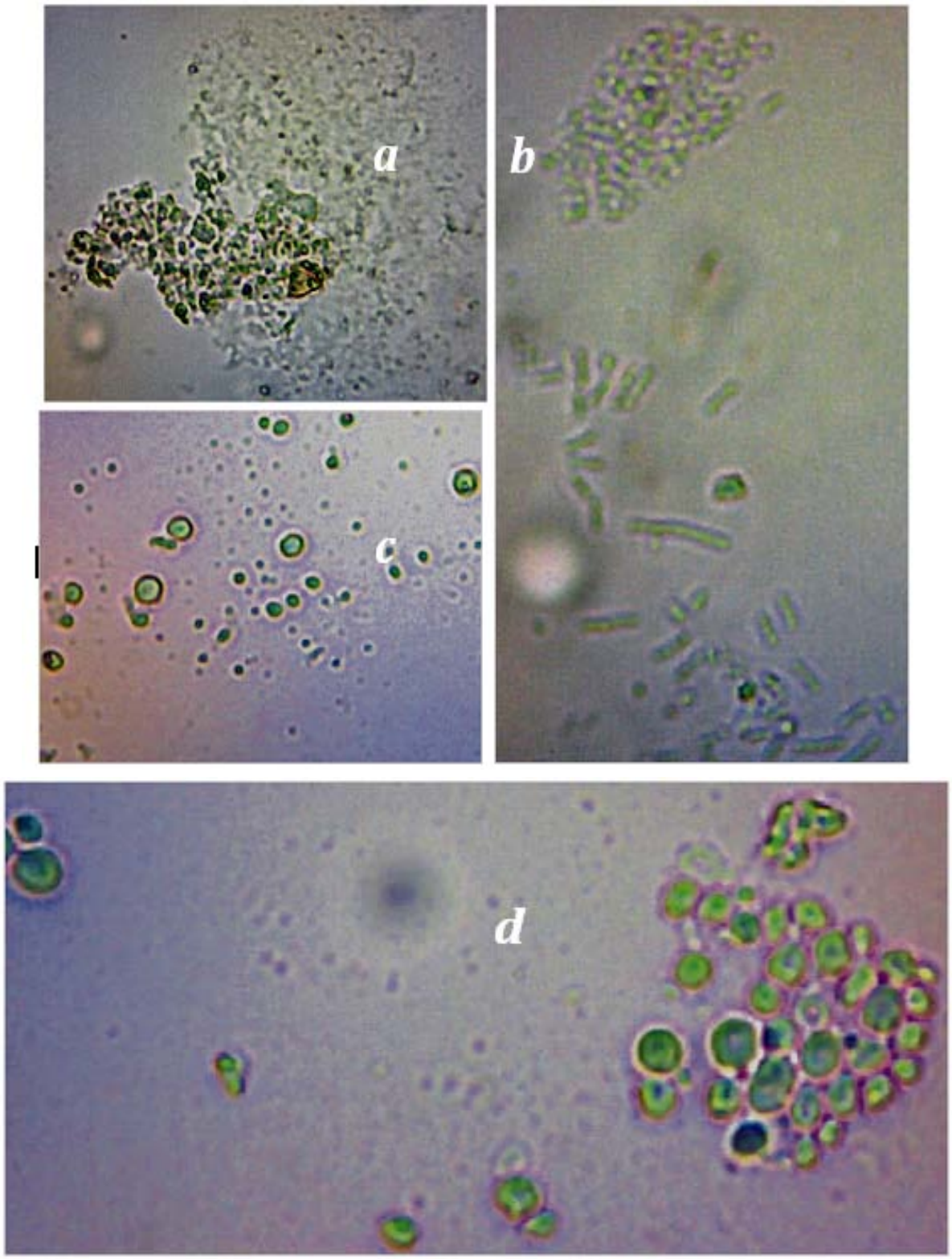


Figure 21 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 24h of incubation at temp 15°C; **a**, dispersing nondescript neoplastic entities; **b**, single rod-shaped & binary protoplasts; **c**, globose prevegetative cells; **d**, budding yeast cells. Magnification: 2500x.

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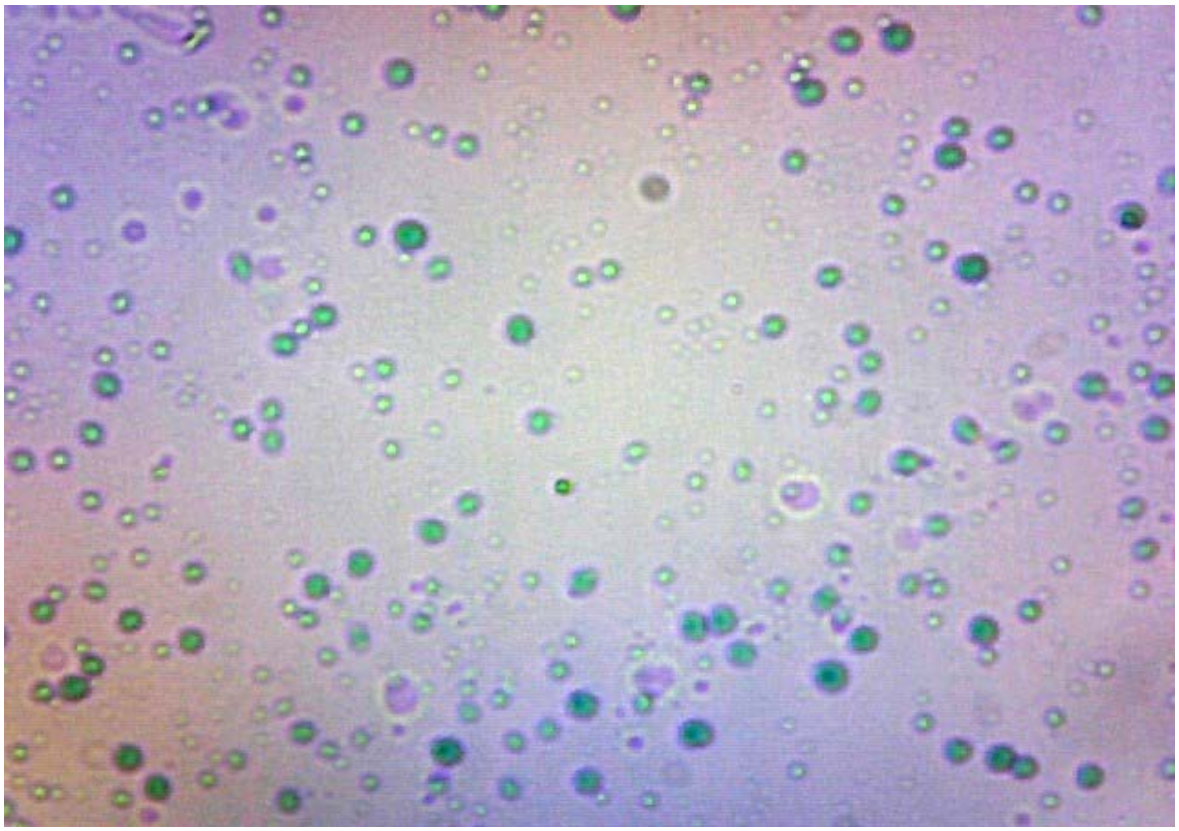


Figure 22 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 48h of incubation at temp 15°C showing numerous ultimate neoplastic units. Magnification: 1000x

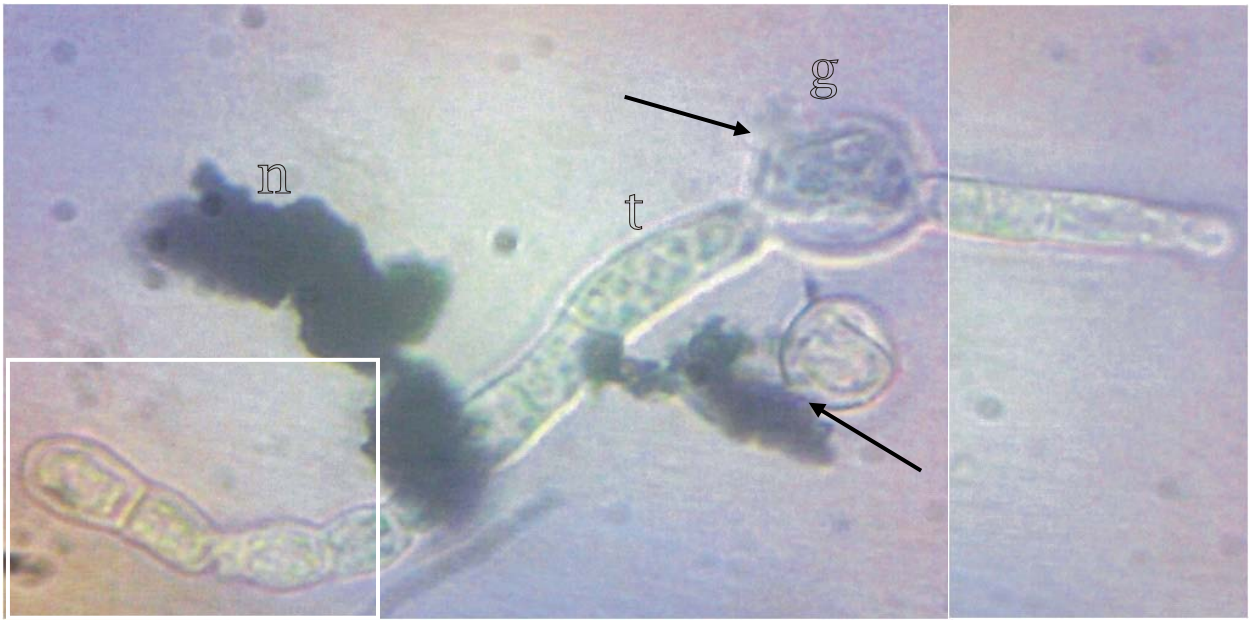


Figure 23 a : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 72h of incubation at temp 15°C showing thallic growth, 't' and neoplasm, 'n'; g, growth sphere. Note: cell wall of growth sphere ruptured (arrow) releasing clusters of granular units, but subsequently dispersed by broth conventional current. Magnification: 1000x

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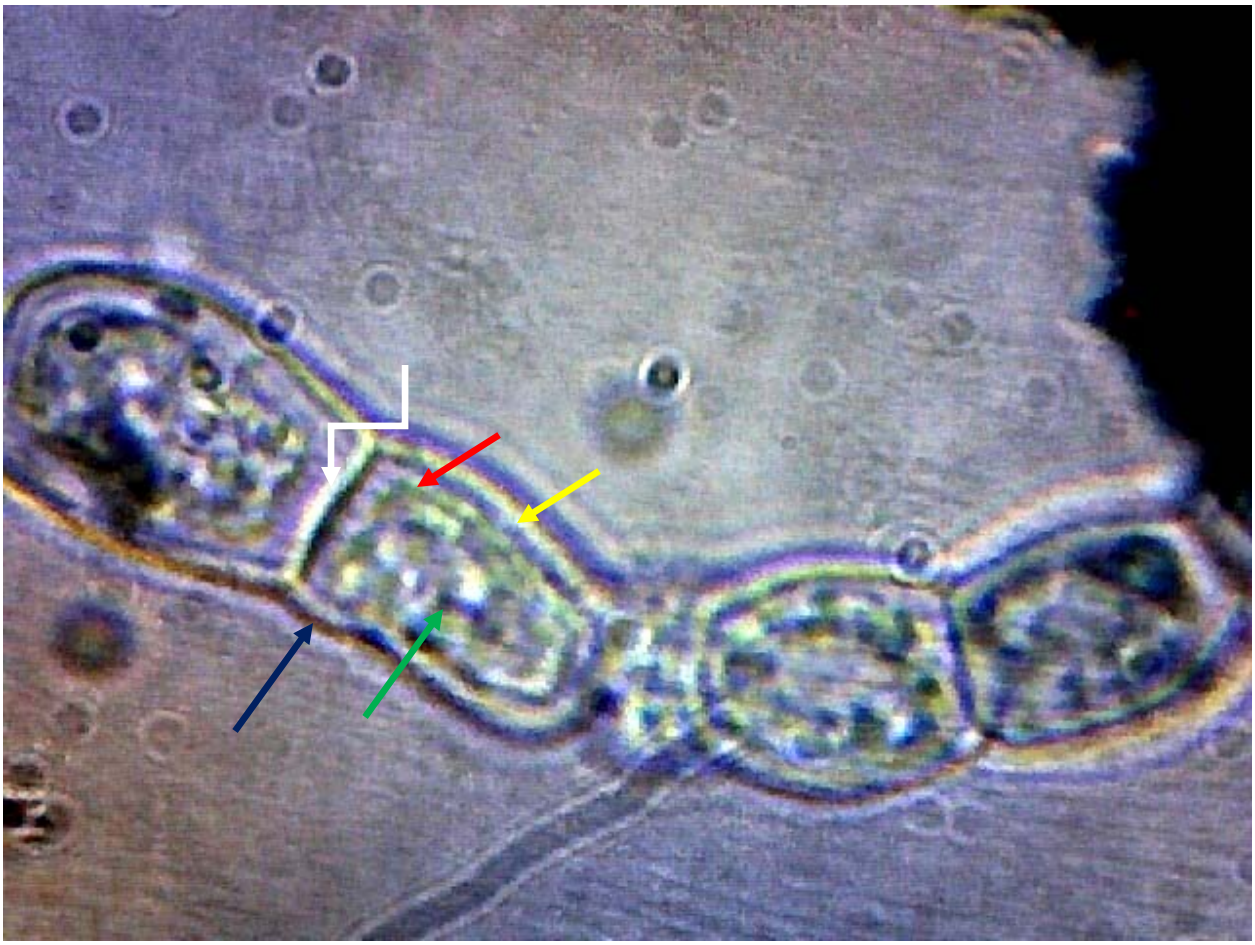


Figure 23 b : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 72h of incubation at temp 15°C showing part of thallic growth and neoplasm, box in fig. 27a. Arrows indicate characteristics of a true fungus: hyphal wall (blue); internal arthrosporal wall (yellow); septum (white); cytoplasmic membrane (red); cytoplasm (green). Magnification: 2500x

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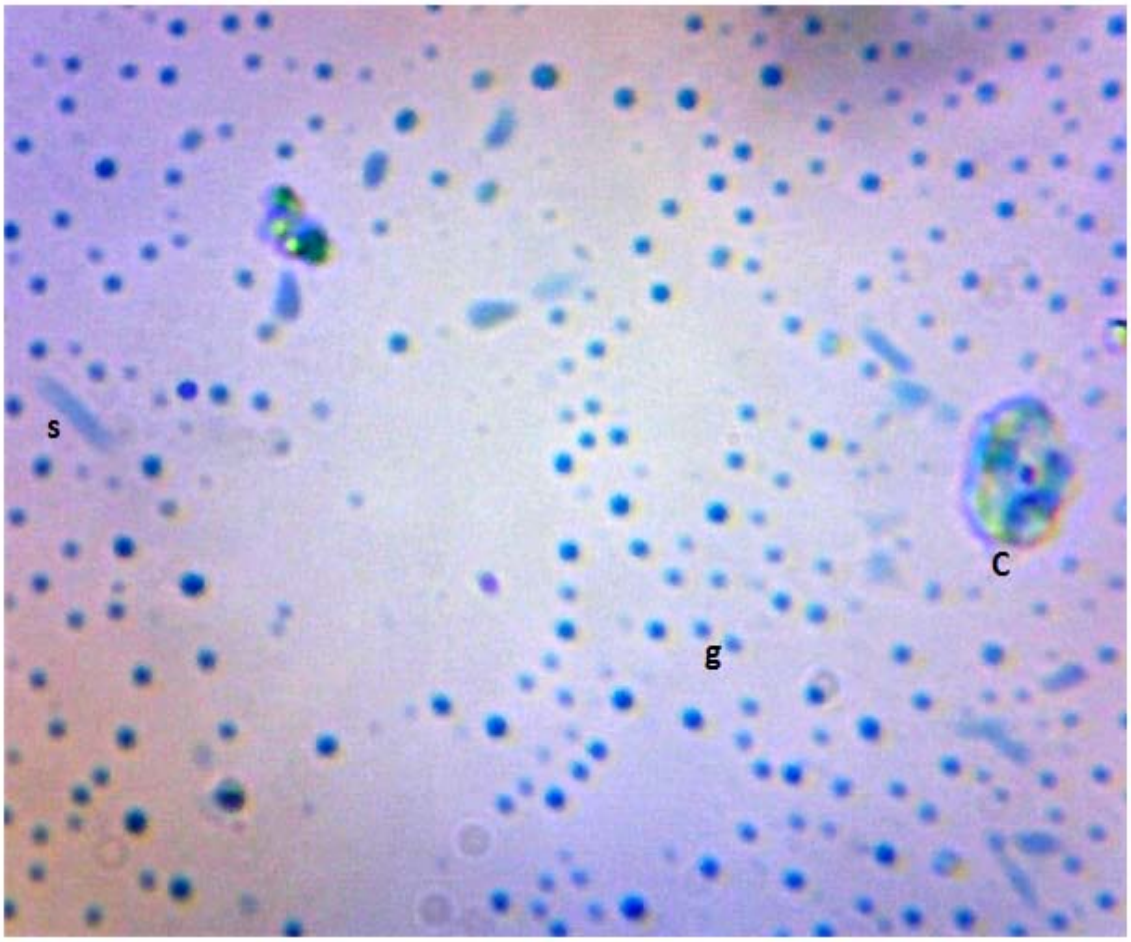


Figure 23 c : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 72h of incubation at temp 15°C showing cytoplasm (c), globose yeast cells (g) & spindle shaped yeast cell (out of focus). Magnification: 2500x.

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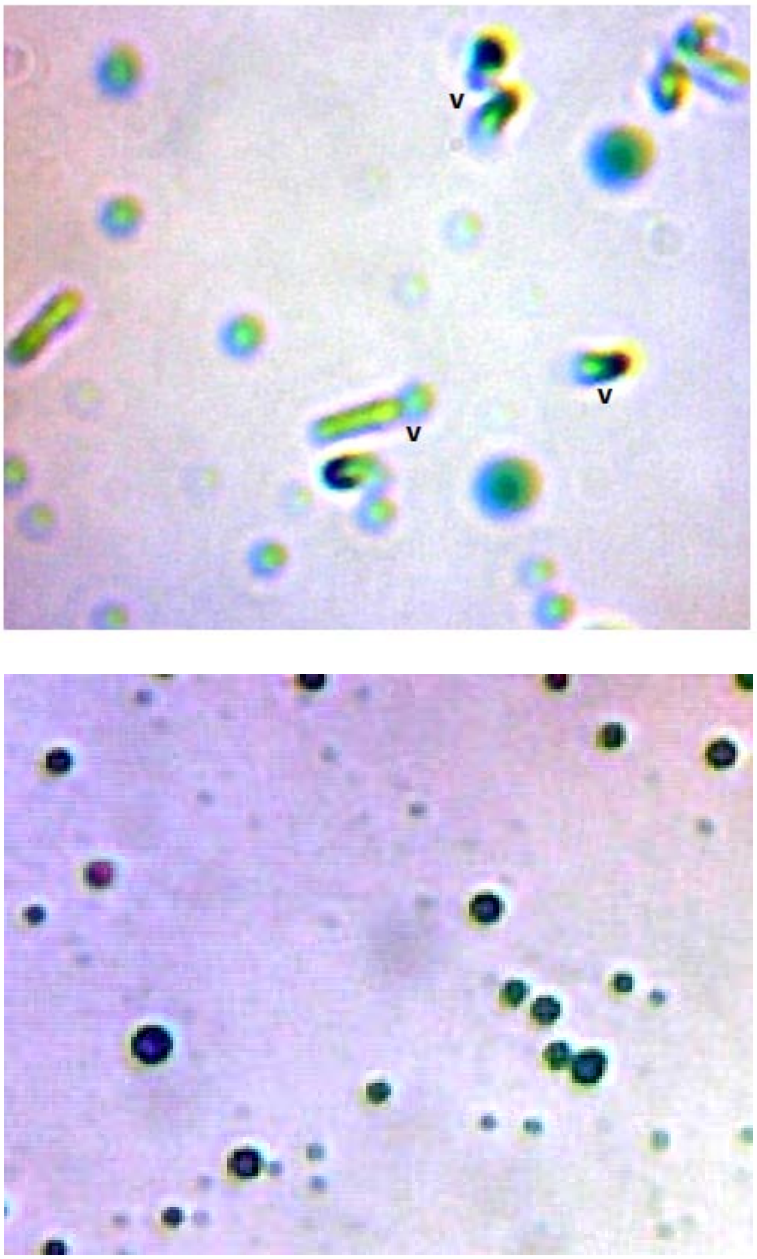


Figure 24 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 96h at temp 15° C; a, protoplasts with nascent yeast initial (v); b, budding yeast cells in singles and double. Magnification: 1000x

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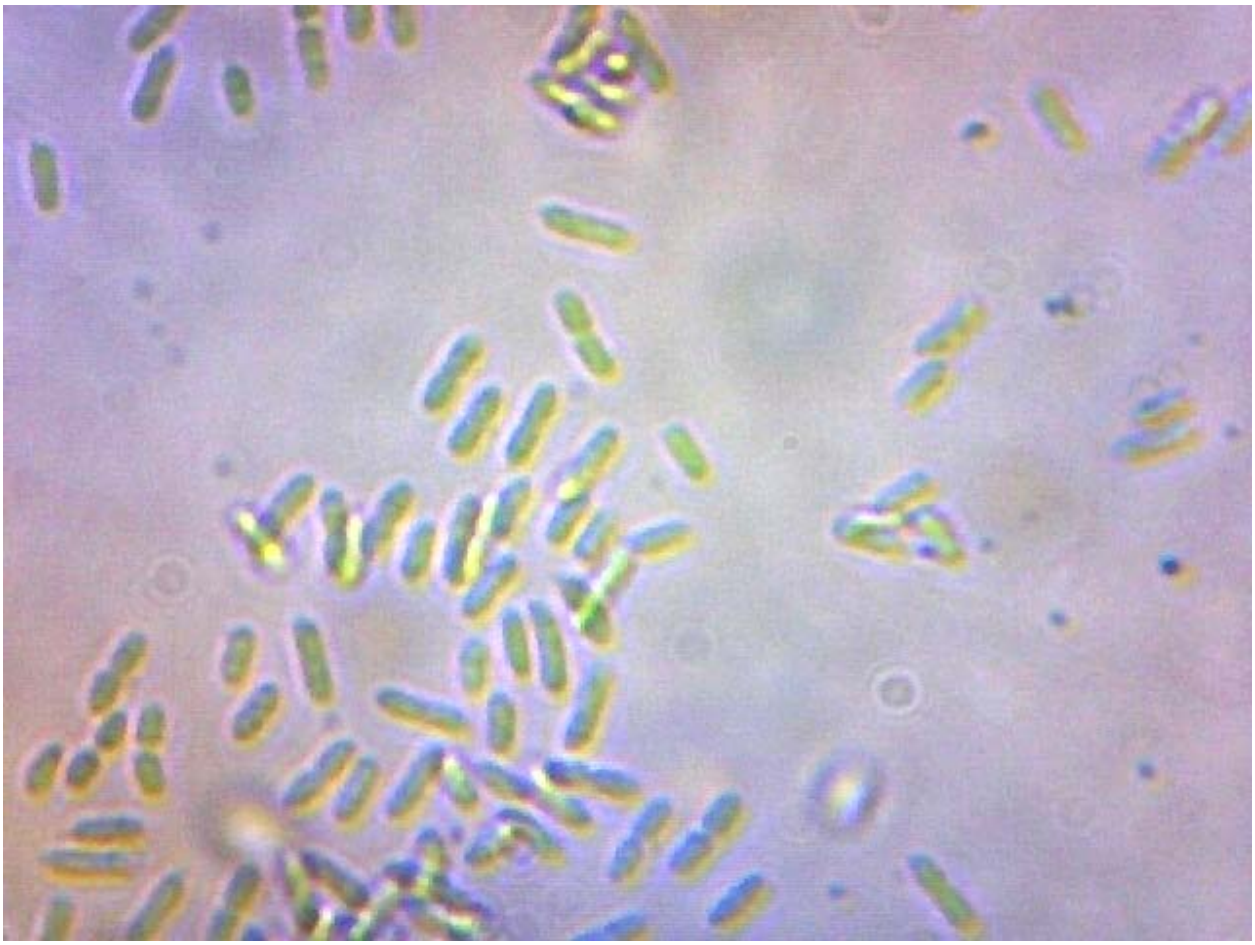


Figure 25 a : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C showing rod shaped & binary protoplasts. Magnification: 2500x

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6 b. Cytodifferentiation of Mucor species



Figure 25 b : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C showing protoplasts and thallic growth (out of focus). Observe the short denticle of the binary protoplasts (arrow). Magnification: 2500x

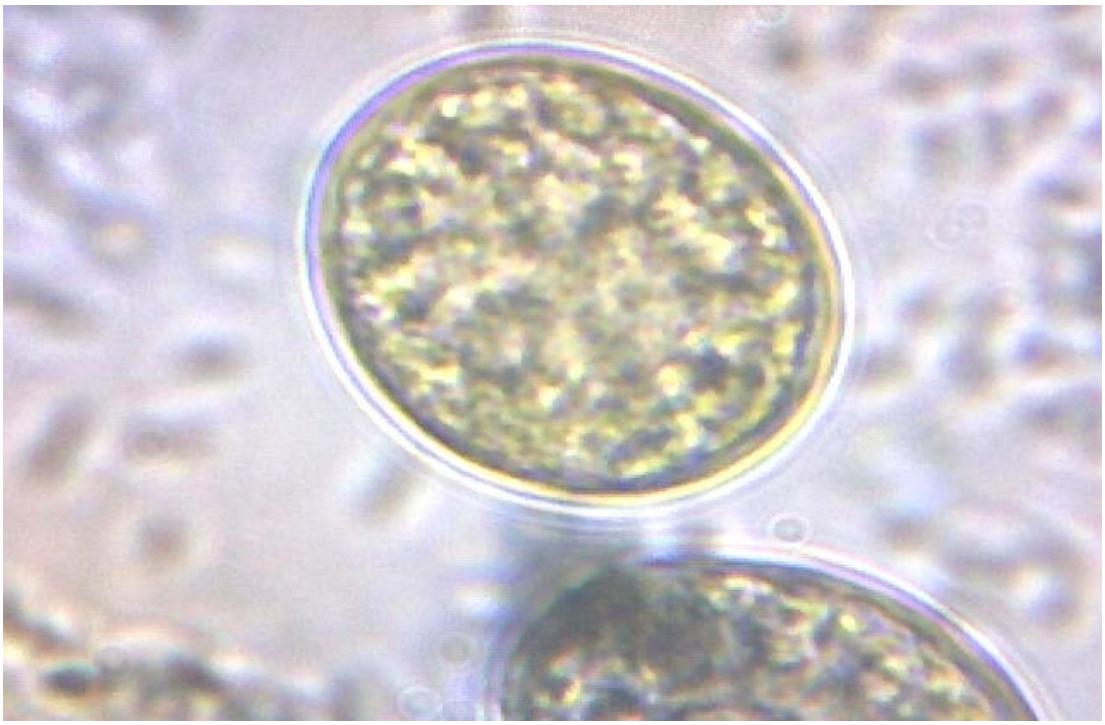


Figure 25 c : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C; protoplasm of conidia undergoing differentiation from apparent cytoplasmic consistency. Magnification: 2500x

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Figure 25 d : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C; another elevation of the conidia in fig. 29c showing numerous rod shaped and denticulated binary protoplasts. Magnification: 2500x

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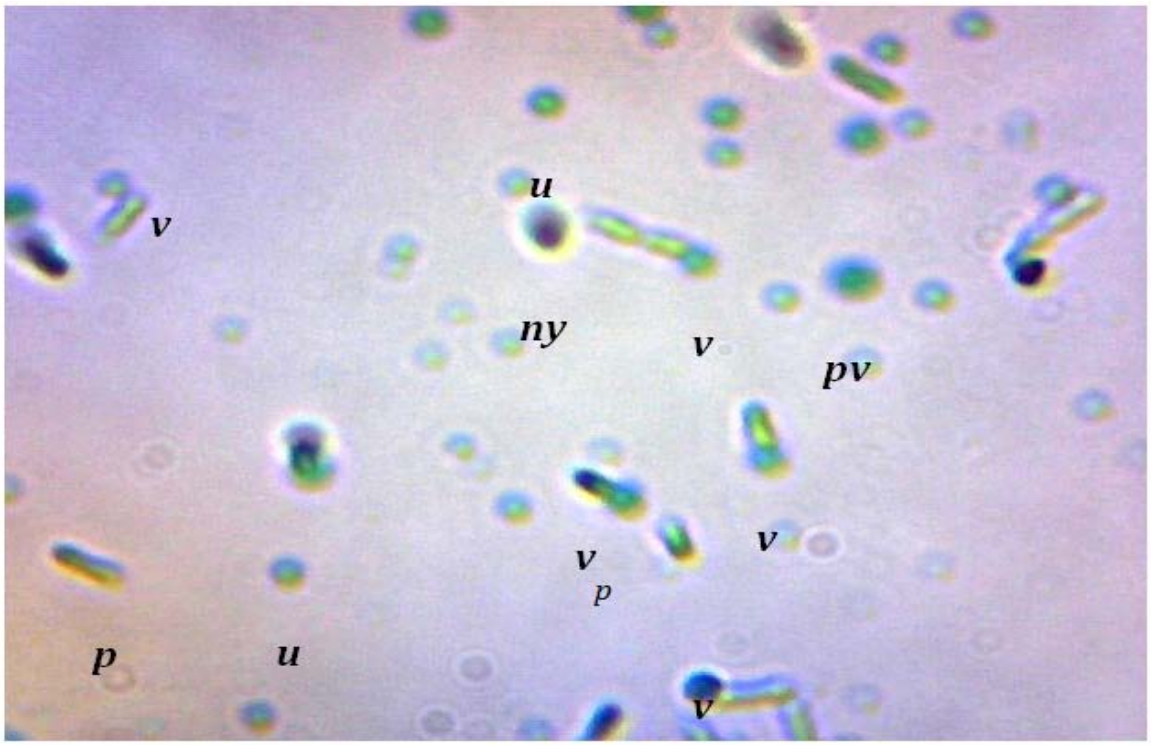


Figure 25 e : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C showing ultimate neoplastic units (u) & protoplasts (p); nascent yeast initial*, v, single or binary, generate prevegetative cell, pv, which* on ceding grow to maturity & subsequently become polar budding. Magnification: 2500x

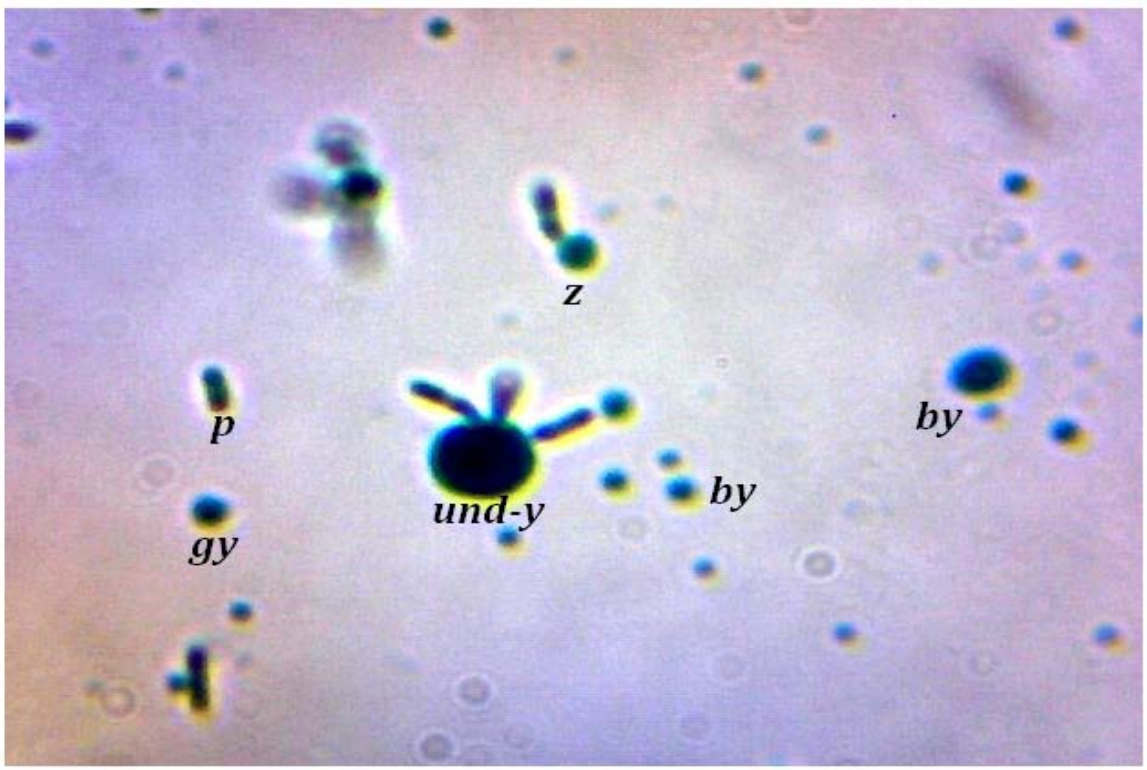


Figure 25 f : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 15°C showing rod-shaped protoplast (p), nascent yeast initial of binary protoplast (z), unipolar budding yeasts (by), globose yeast (gy) and undeclared yeasts (und-y). Magnification: 2500x

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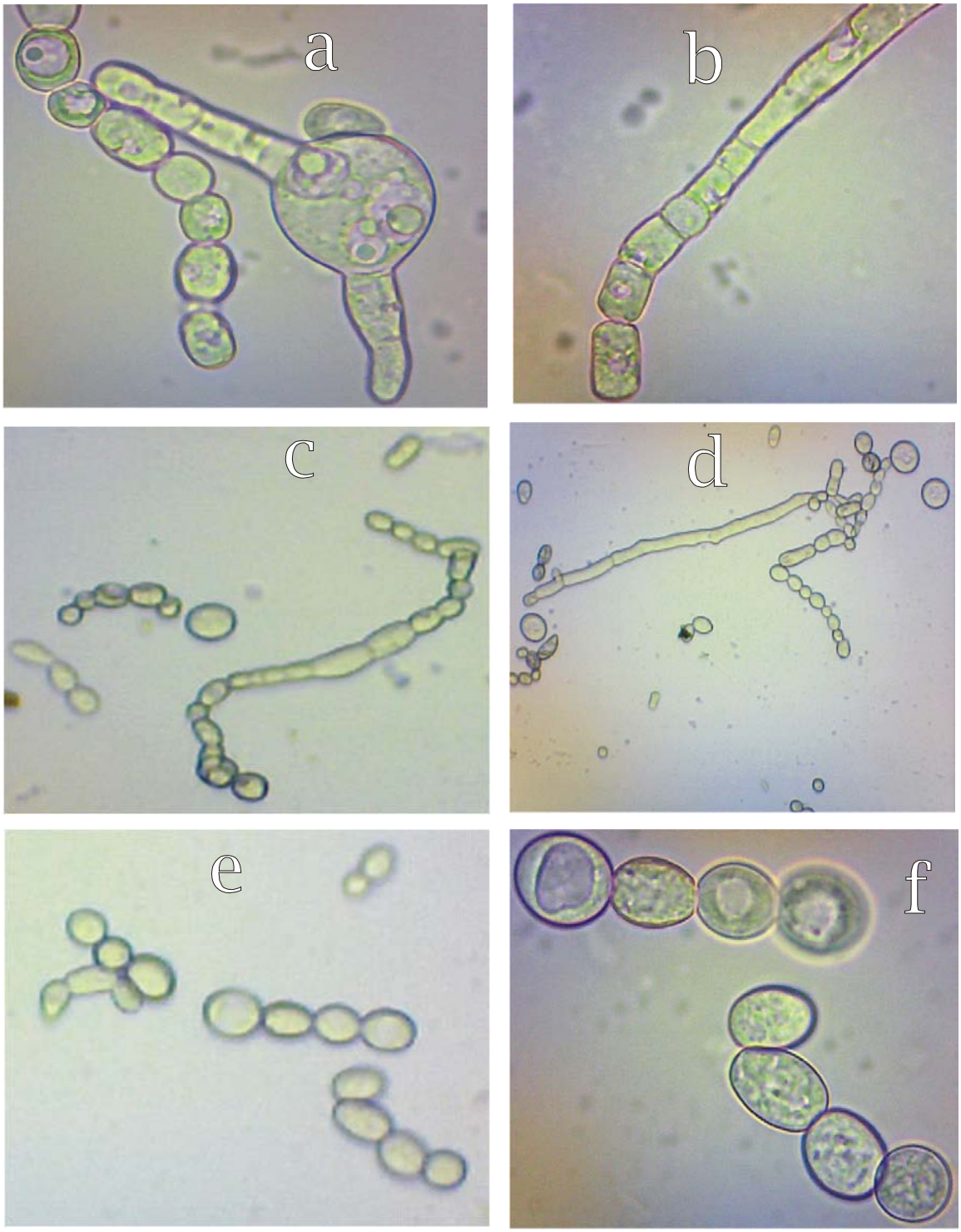


Figure 26 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 24h of incubation at temp 28°C showing various types of conidia; **a**, growth sphere with double germ tubes & aligned with holoblastic conidia; **b**, thalloarthric conidia ceding by fragmentation; **c**, types of holoblastic conidia; **d**, arthric conidia formed after determinate thallic growth; **e** forms of holoblastic conidia; **f**, holoblastic conidia 'e' at higher magnification. Magnification: a-b,f: 1000x; c-d: 400x; e, 800x

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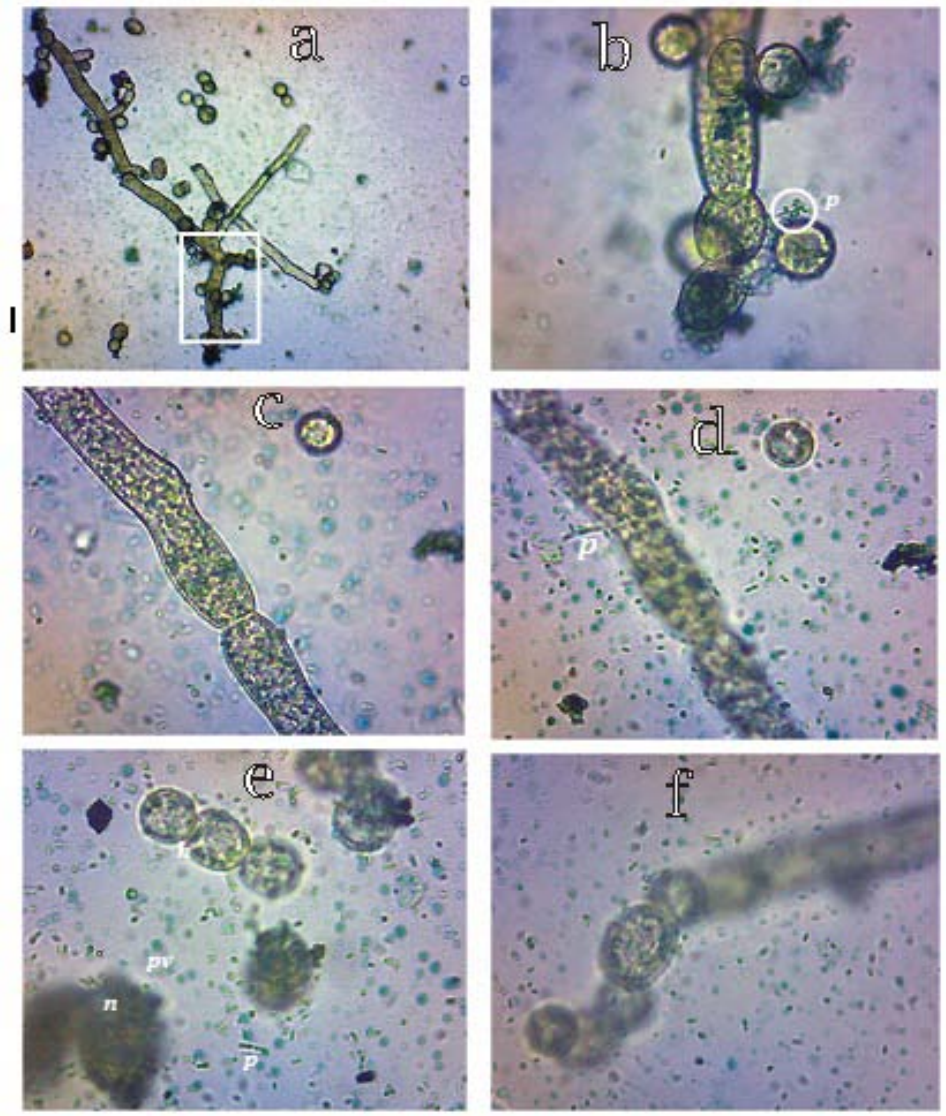


Figure 27 : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 48h of incubation at temp 28°C showing various types of conidia; **a**, thallic growth & holoblasstic conidia; **b**, boxed arthrosporal section in 'a'; observe the presence of clinging protoplasts, 'p'; **c**, septate thallic non-ramifying filament; **d**, a lower elevation of 'c' showing neoplastic units, rod shaped & binary protoplasts; **e**, holoblastic conidia 'h', cluster of neoplastic units 'n', protoplasts 'p' & prevegetative cells 'pv'; **f**, thalloarthric conidia formed by apical rounding up amidst neoplastic units & rod shaped protoplasts. Magnification: a, 400x; b-f, 1000x. *Except for neoplastic units, protoplasts & prevegetative cells cytoplasm of cellular structures appear granular*

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7 a. Cytodifferentiation of Mucor species

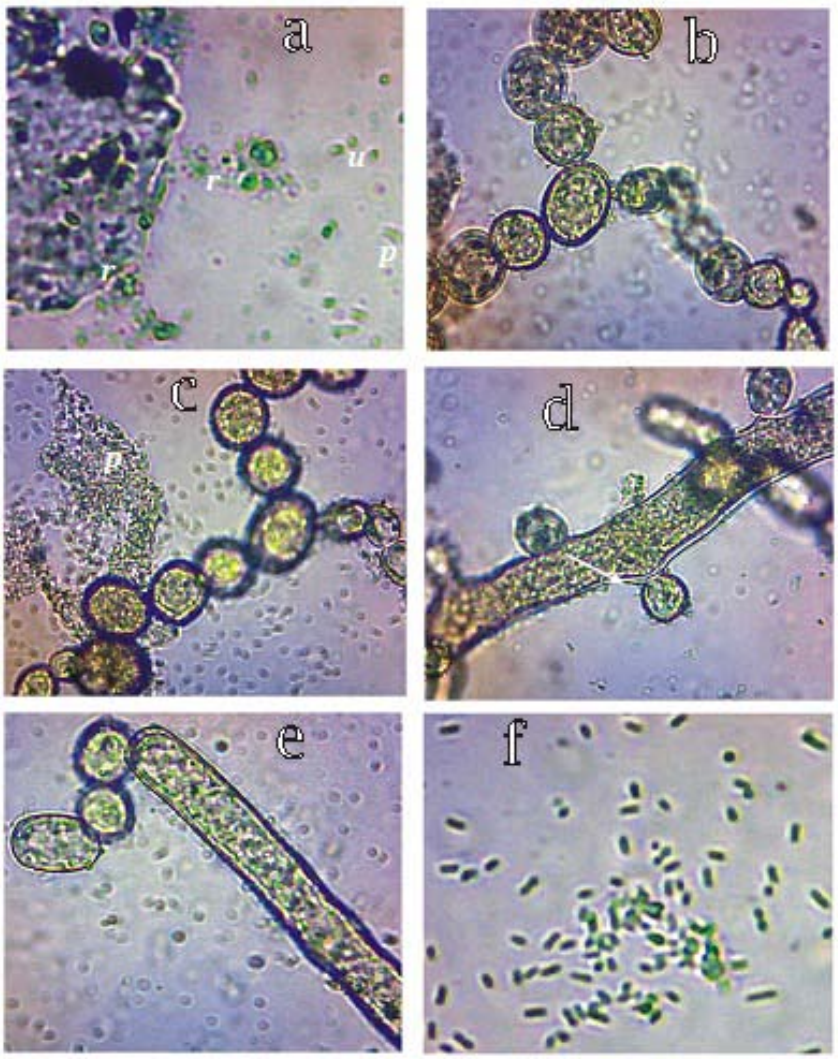


Figure 28 : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 72h of incubation at temp 28°C showing various types of conidia; a, clump of provencal neoplastic entities (n), primeval neoplastic units (r), ultimate neoplastic units (u) & protoplasts (p); b, holoblastic conidia with multilateral branching; c, lower elevation of 'b' showing numerous protoplasts (p); d, thallic growth with numerous laterally borne stumpy conidia; arrow indicate extruded protoplasts; e, determinate thallic growth with retrogressive arthrospore formation; f, protoplast (rod and binary). *In these thallic structures protoplasts were observed directly in the cellular compartments*. Magnification: a, 2500x; b-f. 1000x

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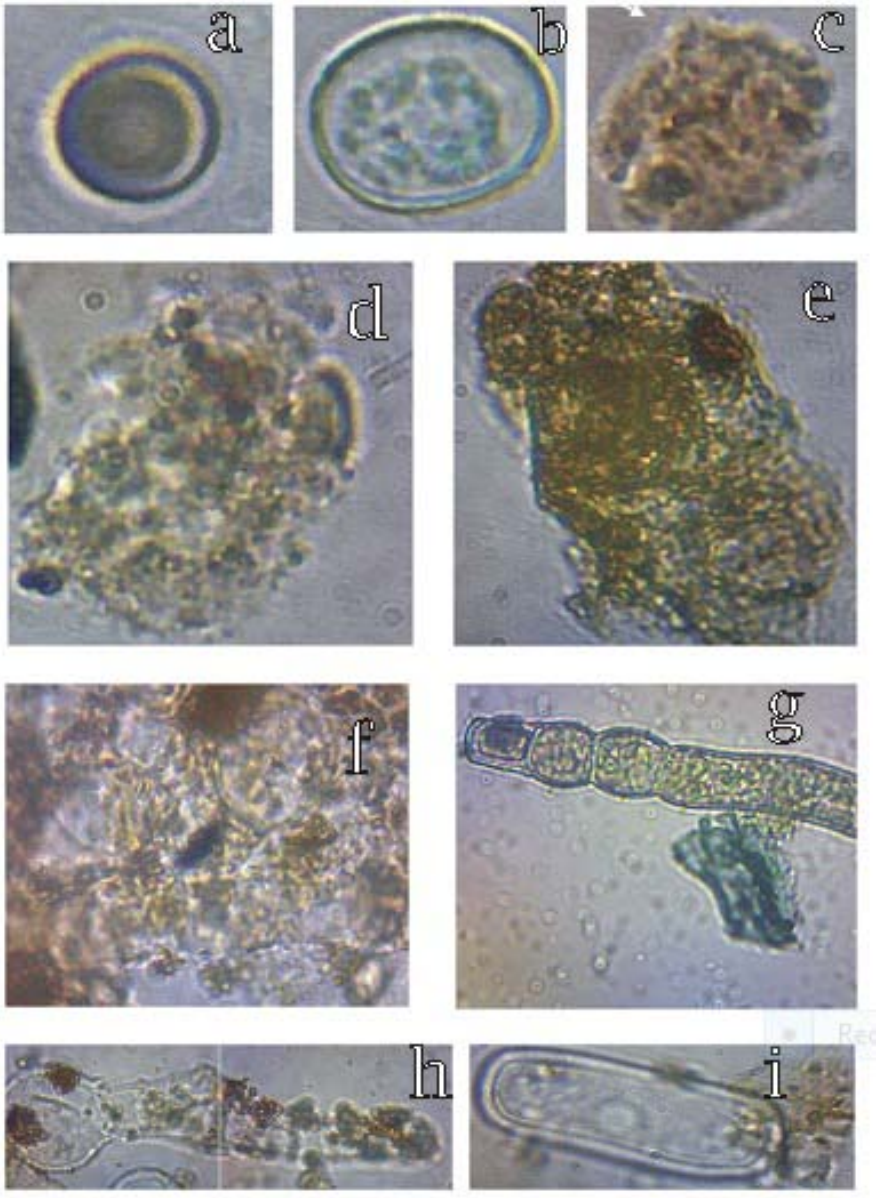


Figure 29 a-i : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 96h of incubation at temp 28°C; **a**, growth sphere; **b**, growth sphere with differentiating cellular contents; **c**; neoplasm; **d-e,** imploding neoplasm**; f,** dispersing provencal entities; **g**, conidiogenesis after determinate thallic growth, **h,** holothallic conidium & **i**, thalloarthrospore, on rupture spewed thier contents. All magnification except g (1000x) at 2500x

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7 b. Cytodifferentiation of Mucor species

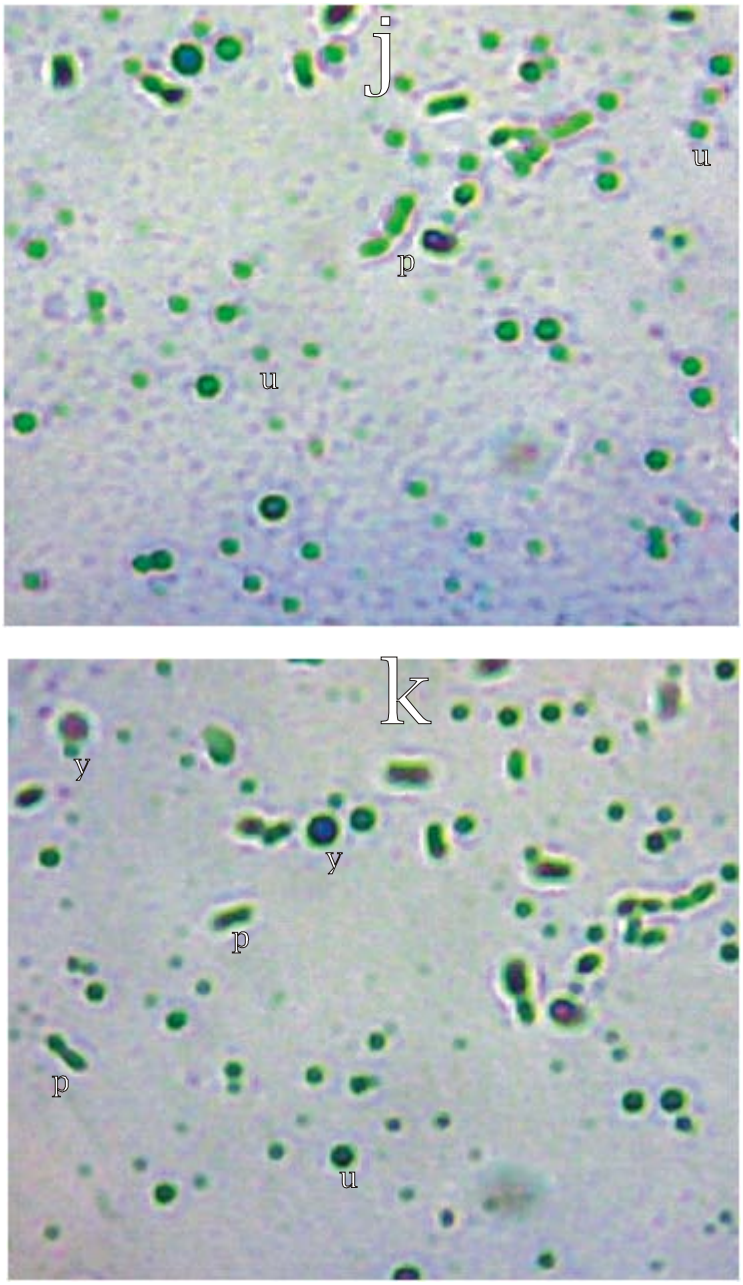


Figure 29 j k : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 96h of incubation at temp 28°C showing numerous ultimate neoplastic units (u) and protoplasts (p) and yeast cells. Magnification, x1000

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Figure 30 a : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 28°C showing a section of septate thallic growth with protoplasts clinging to the walls; background: unresolved protoplasts. Magnification: 1000x

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Figure 30 b : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 28°C showing protoplasts emerging form cluster of neoplastic units extruded from holoblastic conidium. Magnification: 1000x

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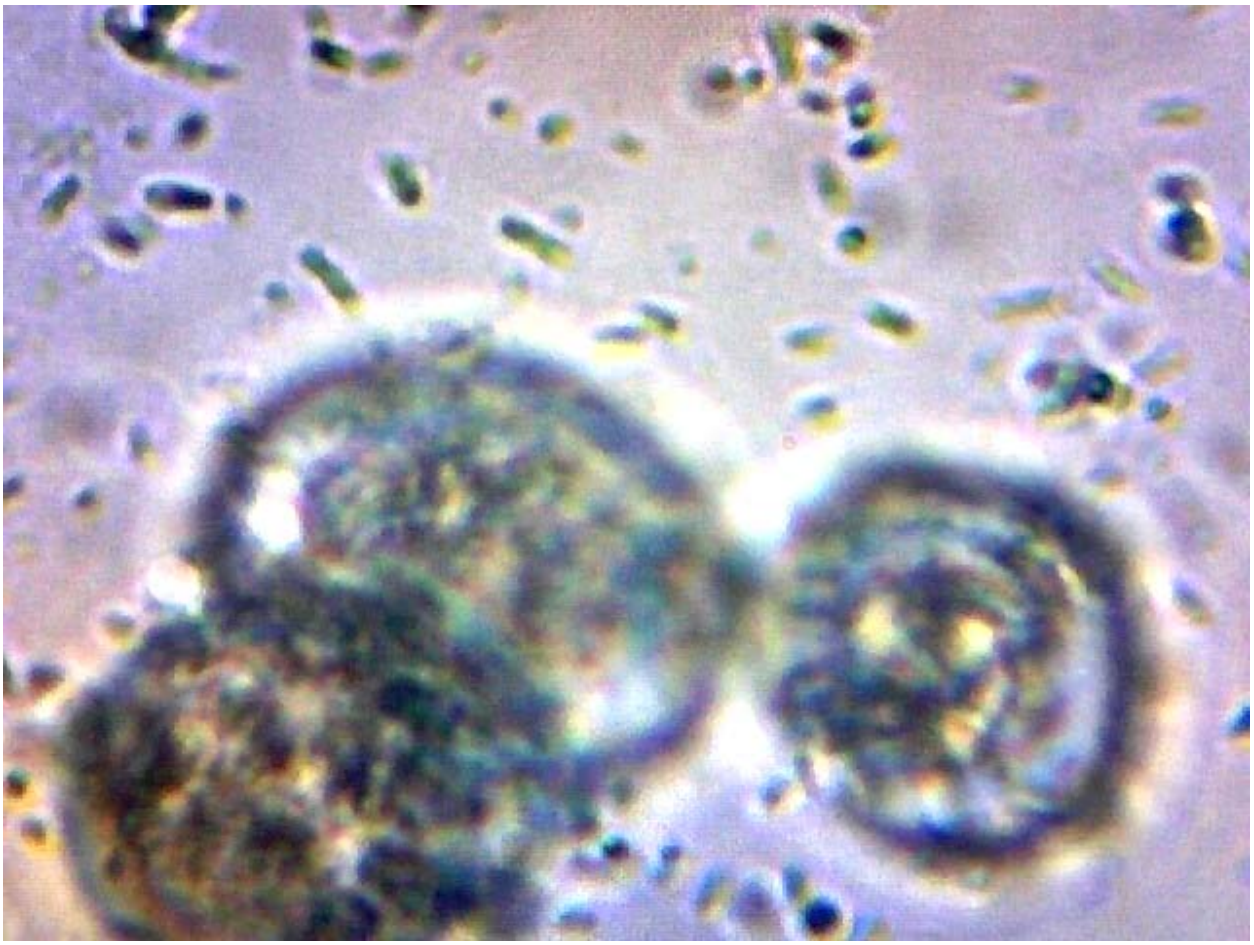


Figure 30 c : Growth forms of *Mucor manihoti*s induced in peptone incorporated minimal medium after 120h of incubation at temp 28°C showing protoplasts & holoblastic conidia (out of focus). Magnification: 2500x

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8a.Cytodifferentiation of Mucor species

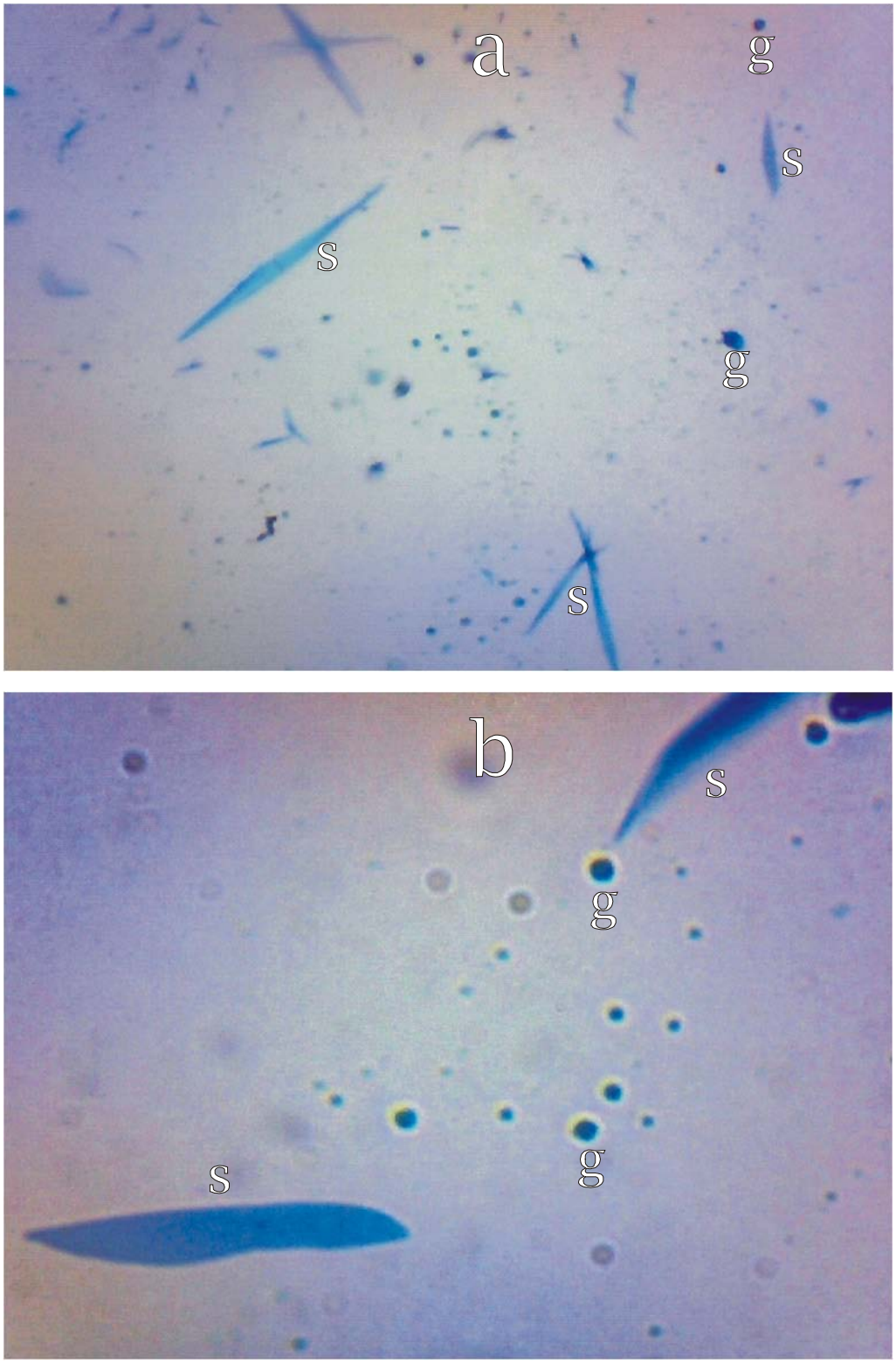


Figure 31 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 24h of incubation at temp 37°C showing globose- 'g' & spindle shaped 's' yeast cells. Magnification: a – 1000x; b - 2500x

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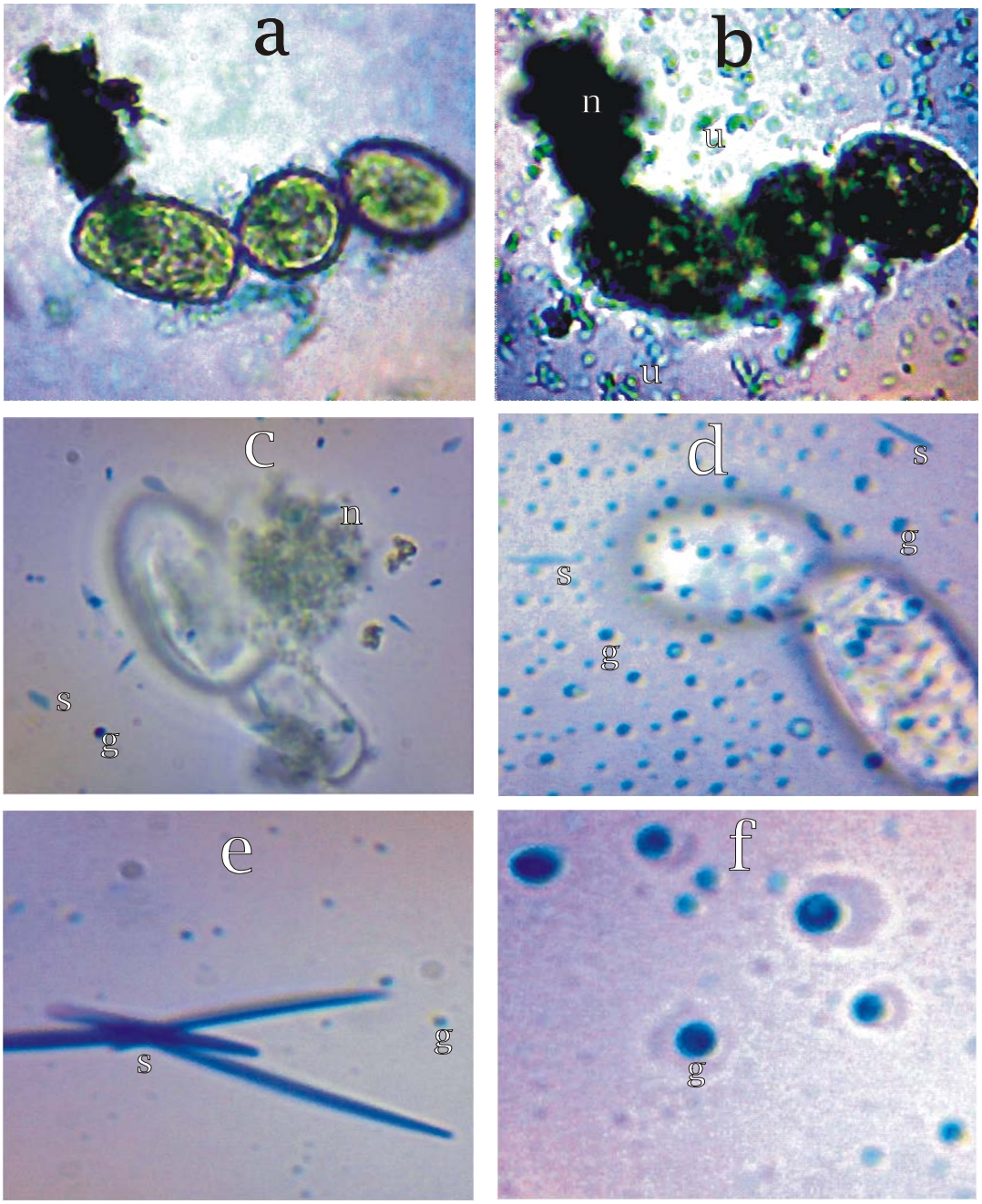


Figure 32 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 48h of incubation at temp 37°C; **a**, holoblastic conidia showing conspicuous cytoplasmic differentiation; **b**, another elevation of 'a' showing predominance of ultimate neoplastic units as primeval units 'n' are extruded by one conidium; **c**, this micrograph shows the co-occurrence of neoplastic units (n) conidia (h), globose (g) - and spindle (s) - shaped yeast cells on the same field; **d**, thalloarthric conidia are here out of focus, in the same field range with globose- & spindle yeasts; **e**, another field of the same slide showing elongated spindle shaped yeast cells, sizes of these cells varied greatly; **f**, globose yeast cells. Magnification: 2500x

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8b.Cytodifferentiation of Mucor species

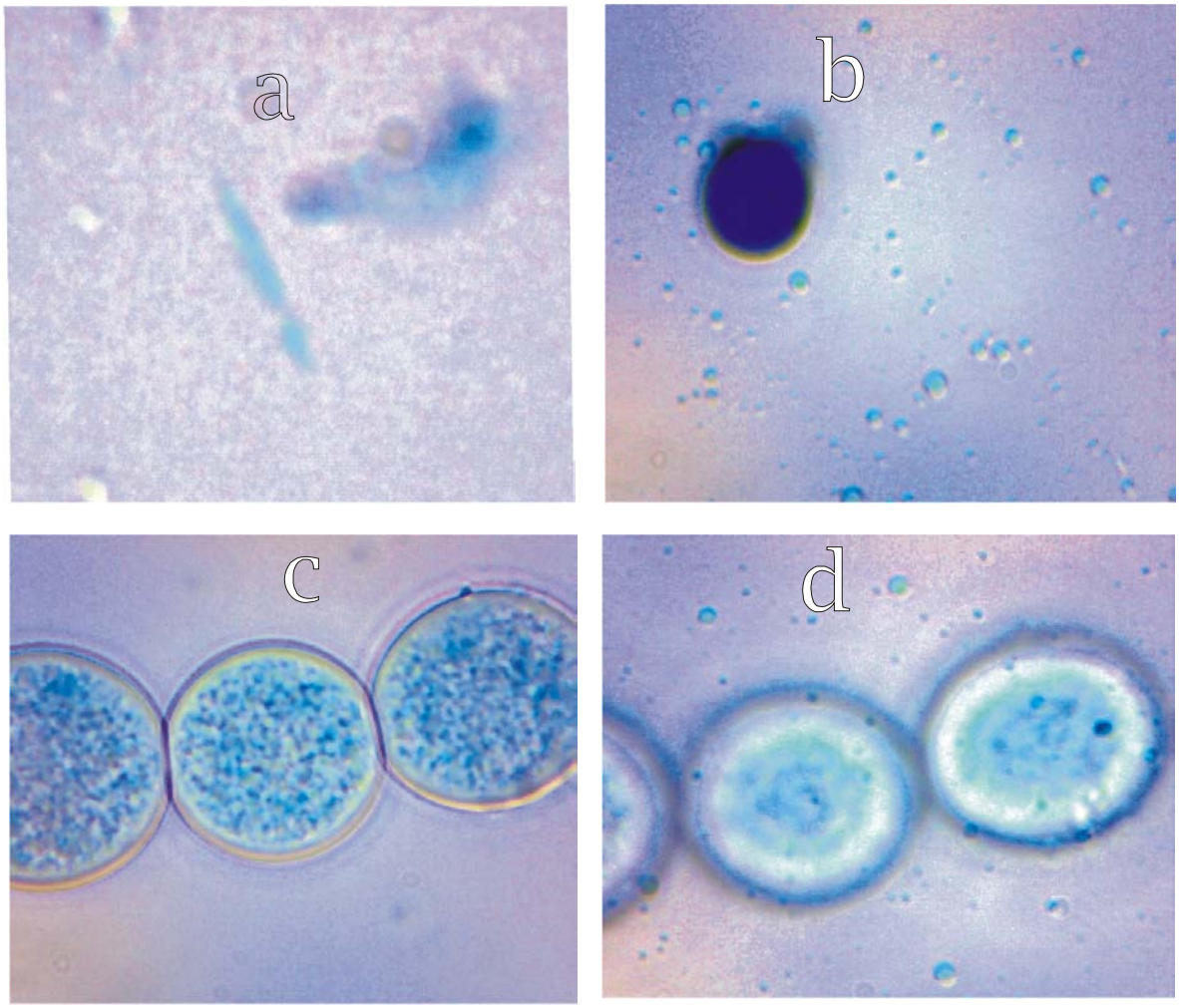


Figure 33 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 72h of incubation at temp 37°C showing **a**, spindle shaped yeast cell, **b**, globose yeast cell, **c**, holoblastic conidia with differentiated cytoplasmic content & **d**, another elevation of 'c' revealing globose neoplastic units. Magnification: 2500x

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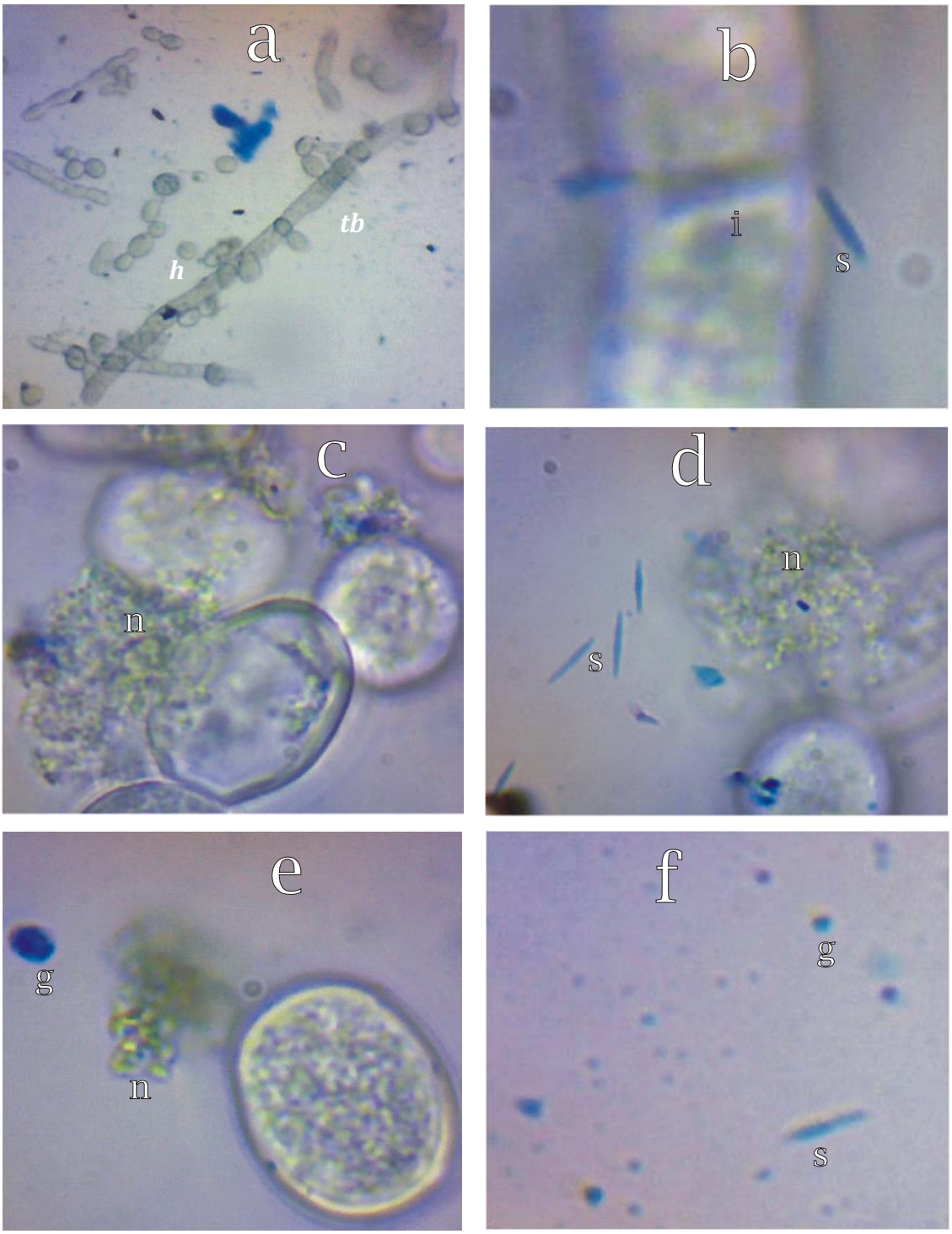


Figure 34 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 96h of incubation at temp 37°C showing **a**, thallic growth with blastospores 'tb' & holoblastic conidia 'h'; **b**, septate filament & spindle shaped yeast cell, 's'; **c**, conidial burst & release of neoplastic units 'n'; **d**, lower elevation of 'c' revealing spindle shaped cells; **e**, conidium, neoplastic units & globose yeast cell 'g'; **f**, globose & spindle shaped yeast cells; i=septum. Magnification: a - 400; b-f - 2500x

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9a.Cytodifferentiation of Mucor species

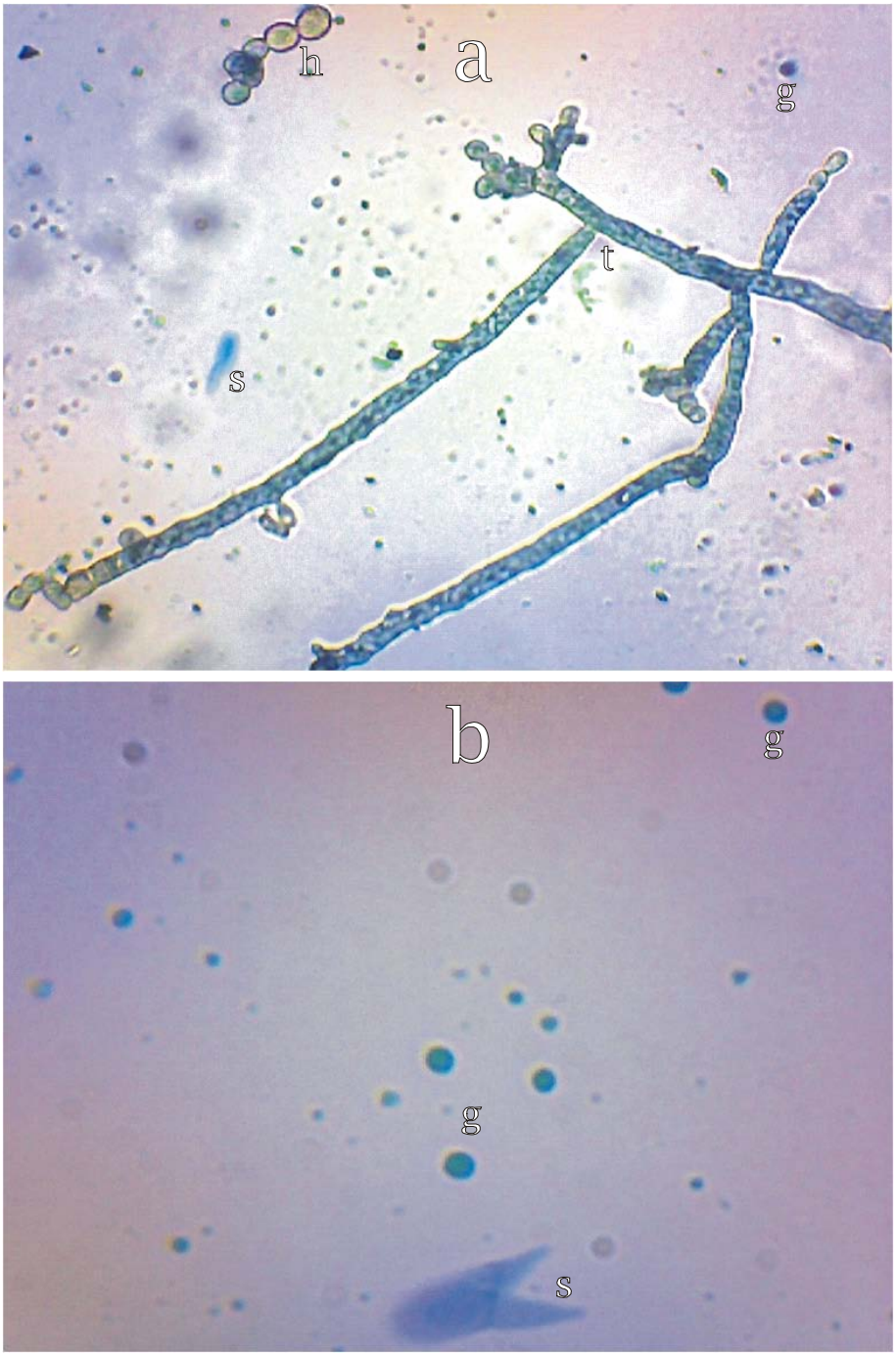


Figure 35 a b : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 37°C showing in **a**, thallic growth 't', thalloarthrospores 'ta' formed after determinate thallic growth, in co-occurrence with holoblastic conidia 'h', globose yeast cells 'g' & spindle yeasts cells 's' (out of focus) and in **b**, globose yeast cells & spindle yeast cells (out of focus). Magnification: a - 400; b - 2500x

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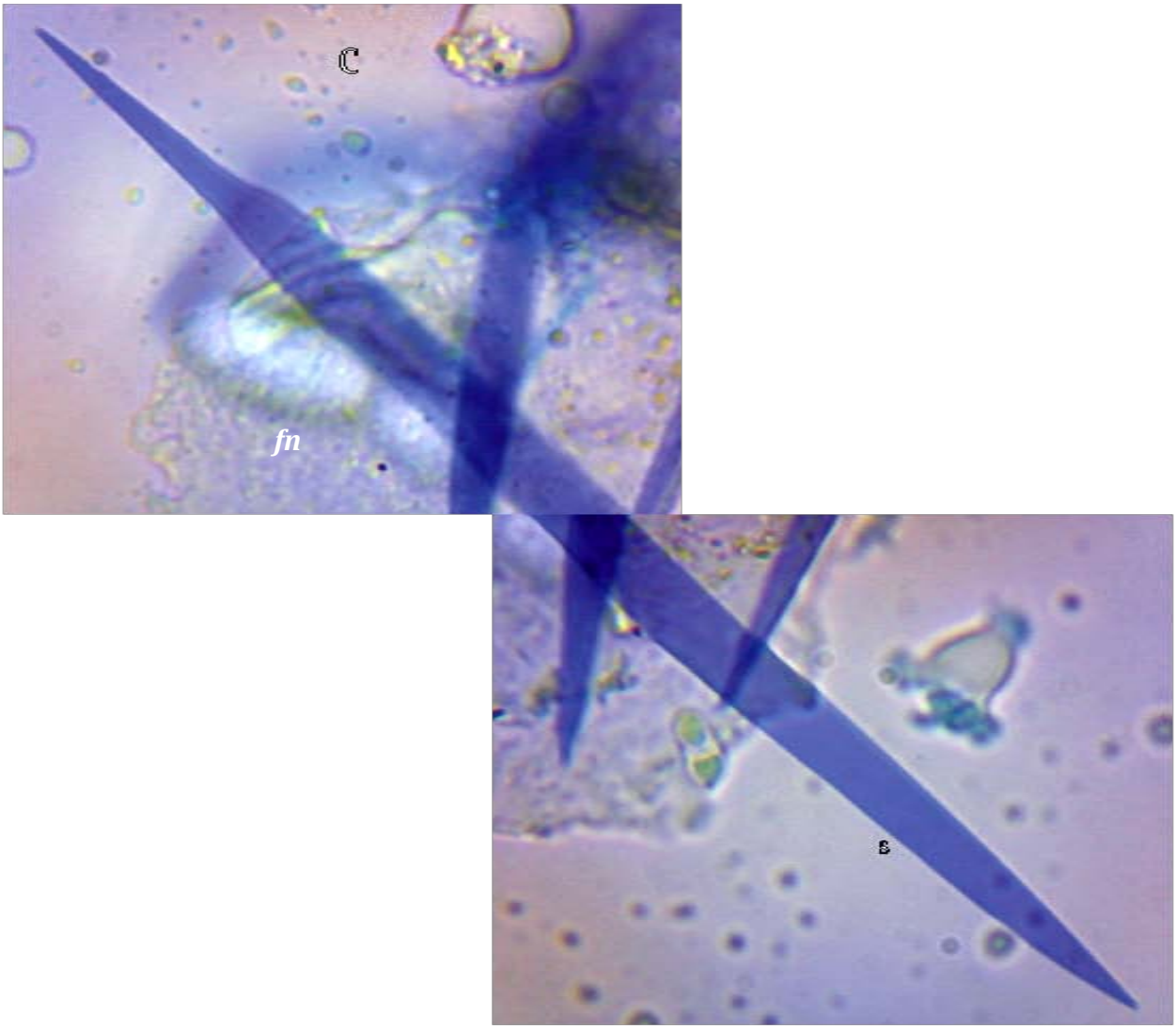


Figure 35 c : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 37°C showing in c, spindle yeast cells. Observe fluid neoplasm 'fn' at the background. Magnification, 2500x

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9b.Cytodifferentiation of Mucor species

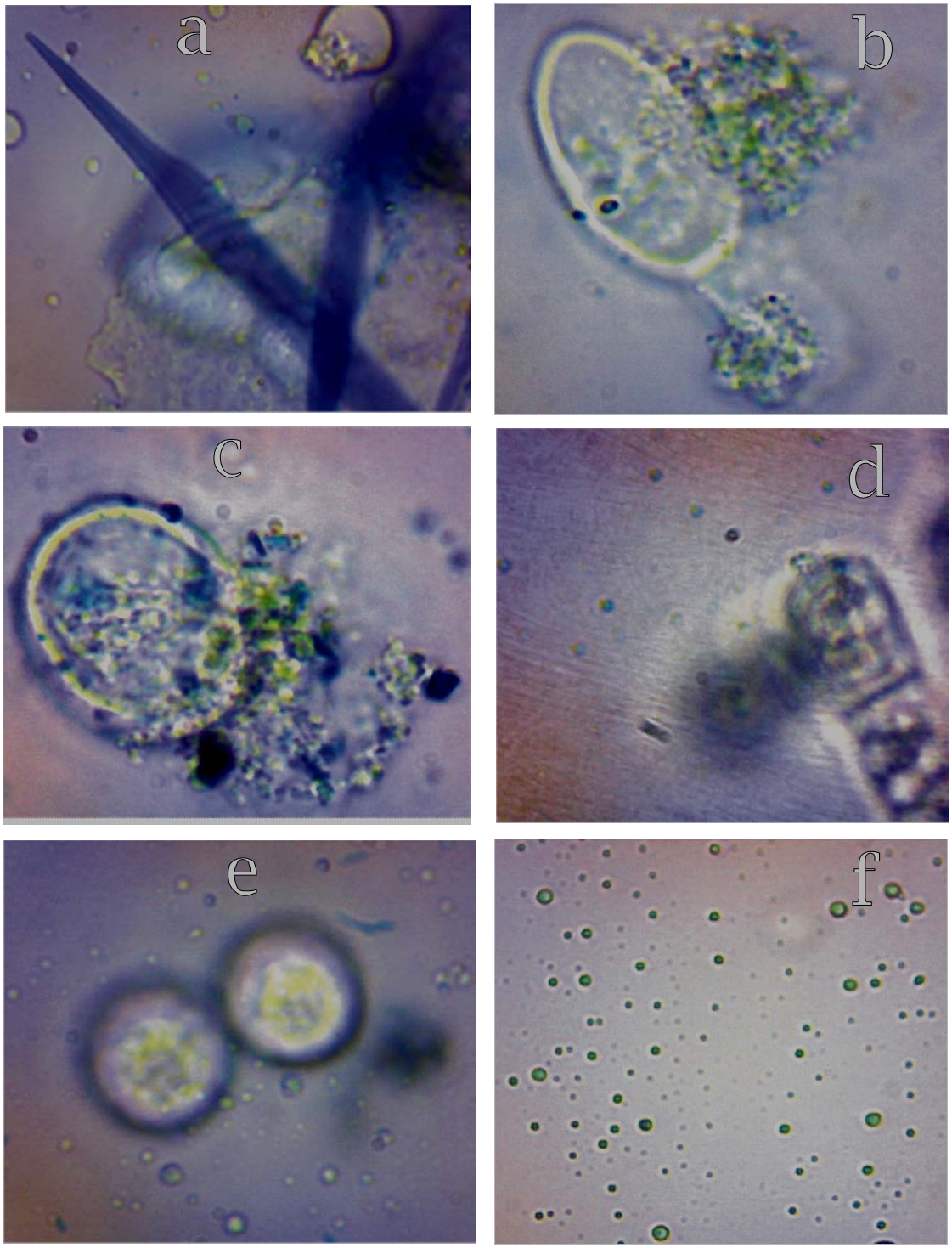


Figure 36 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 37°C & pH5.0; **a**, ultimate neoplastic units perhaps directly pinches off from the fluid neoplasm 'fn' at the background; **b,** numerous primeval entities released after arthrospore (out of focus) rupture; **c,** primeval entities released after conidium bursting; **d,** thalloarthric conidia (out of focus) & ultimate neosplastic units; **e,** ultimate neoplastic units & holoblastic conididia (out of focus); **f,** numerous ultimate neoplastic units. Magnification, 2500x

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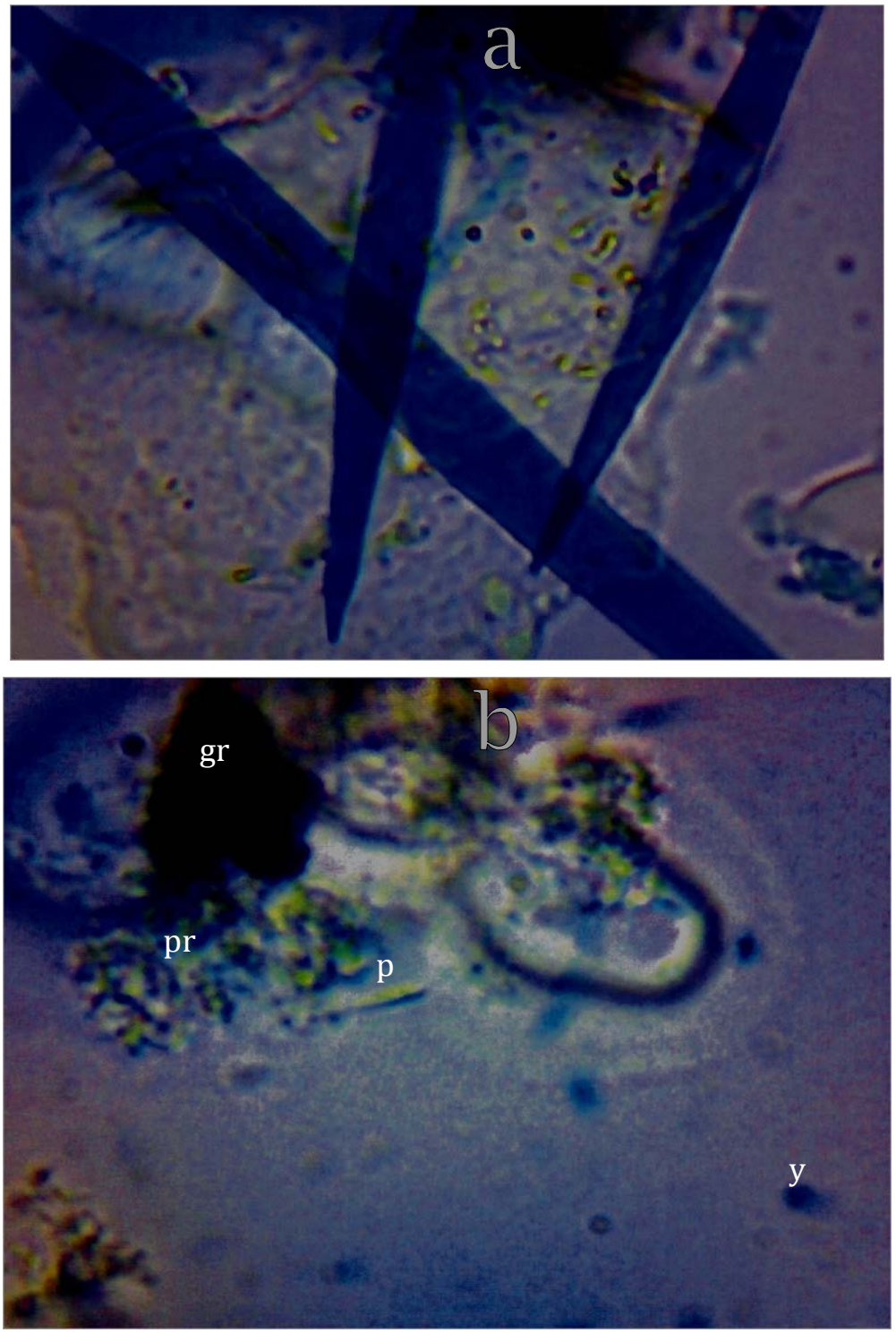


Figure 37 : Growth forms of *Mucor manihotis* induced in peptone incorporated minimal medium after 120h of incubation at temp 37°C & pH5.0 showing in **a,** apices of spindle shaped yeast cell against the background of plastic fluid neoplasm; **b,** cluster of granular units (gr), primeval entities (pr), protoplast (p), out-of-focus bursted arthrospore, & prevegetative yeast cell (bottom, dark blue). Magnification, 2500x

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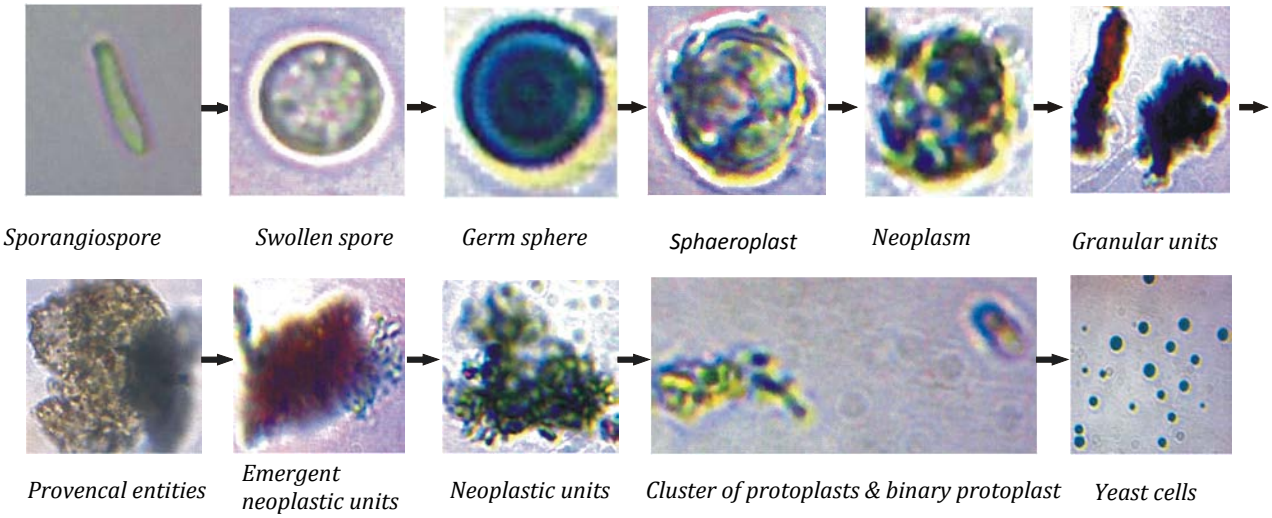


Figure 38 : Sequential induction of unicellular budding globose yeast cells of *Mucor manihotis* in Ammonium sulphate incorporated minimal medium after 3h of incubation at temp 28°C & pH5.0. Magnification, 2500x

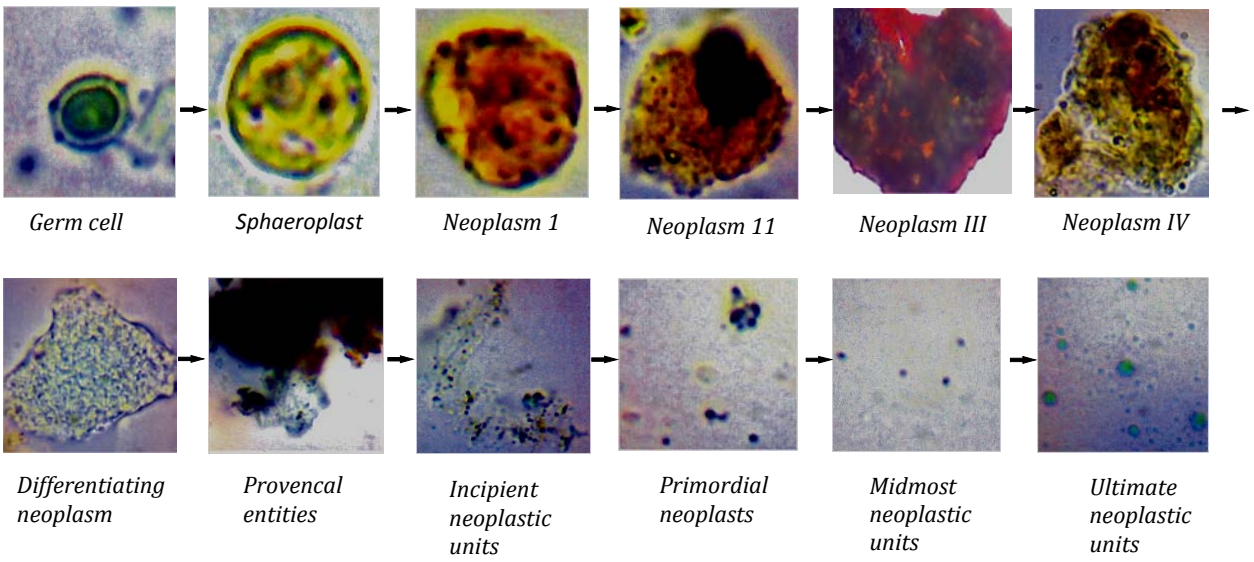


Figure 39 : Sequential induction of ultimate neoplastic units of Mucor manihotis in Ammonium sulphate incorporated minimal medium after 6h of incubation at temp 28°C & pH5.0. Magnification, 2500x

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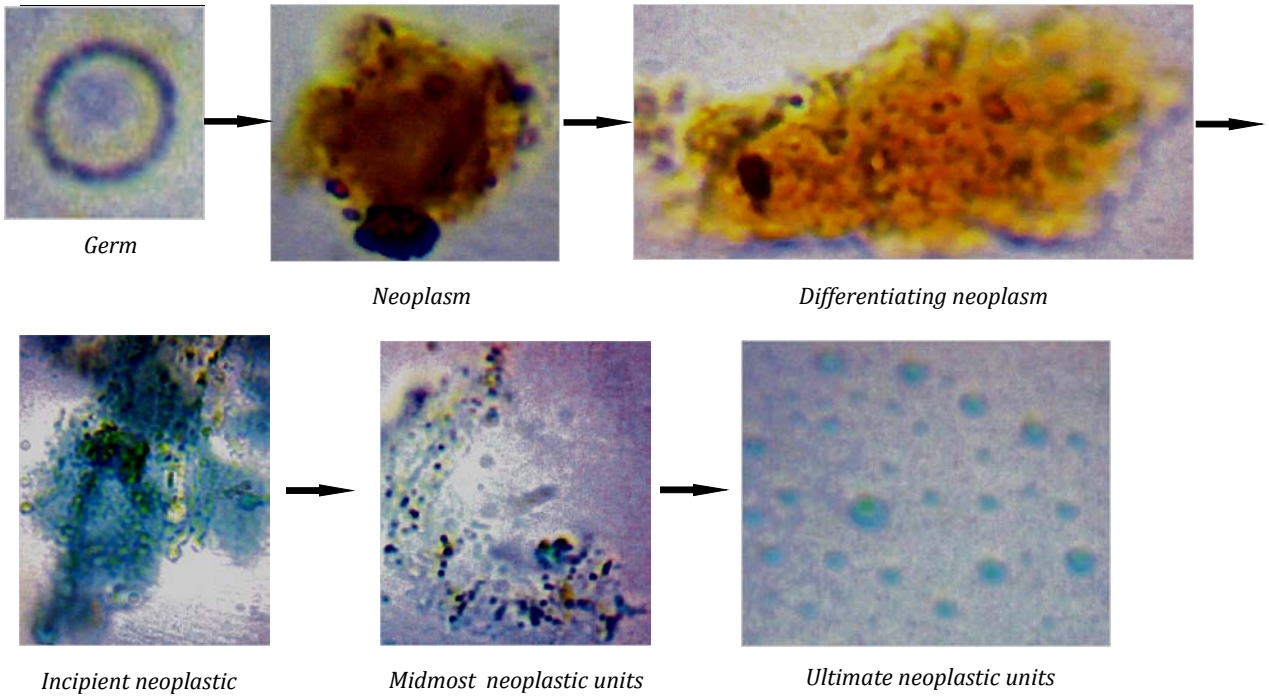


Figure 40 : Sequential induction of ultimate neoplastic units of Mucor manihotis in Ammonium sulphate incorporated minimal medium after 9h of incubation at temp 28°C & pH5.0. Magnification, 2500x

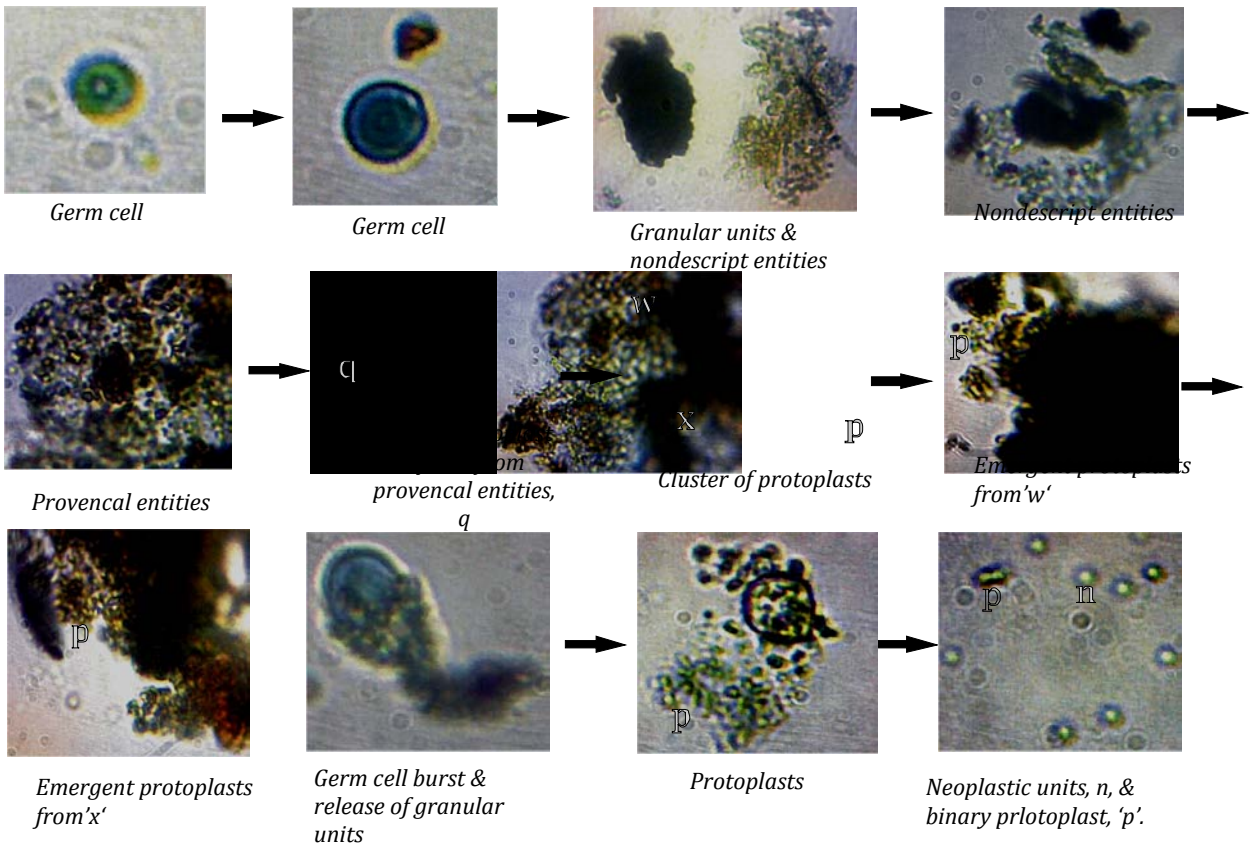


Figure 41 : Sequential induction of protoplasts of Mucor manihotis in Peptone incorporated minimal medium after 3h of incubation at temp 28°C & pH5.0. Magnification, 2500x. (Peptone enhancement of the generation of protoplasts: abridged transition from provencal entities to protoplasts)

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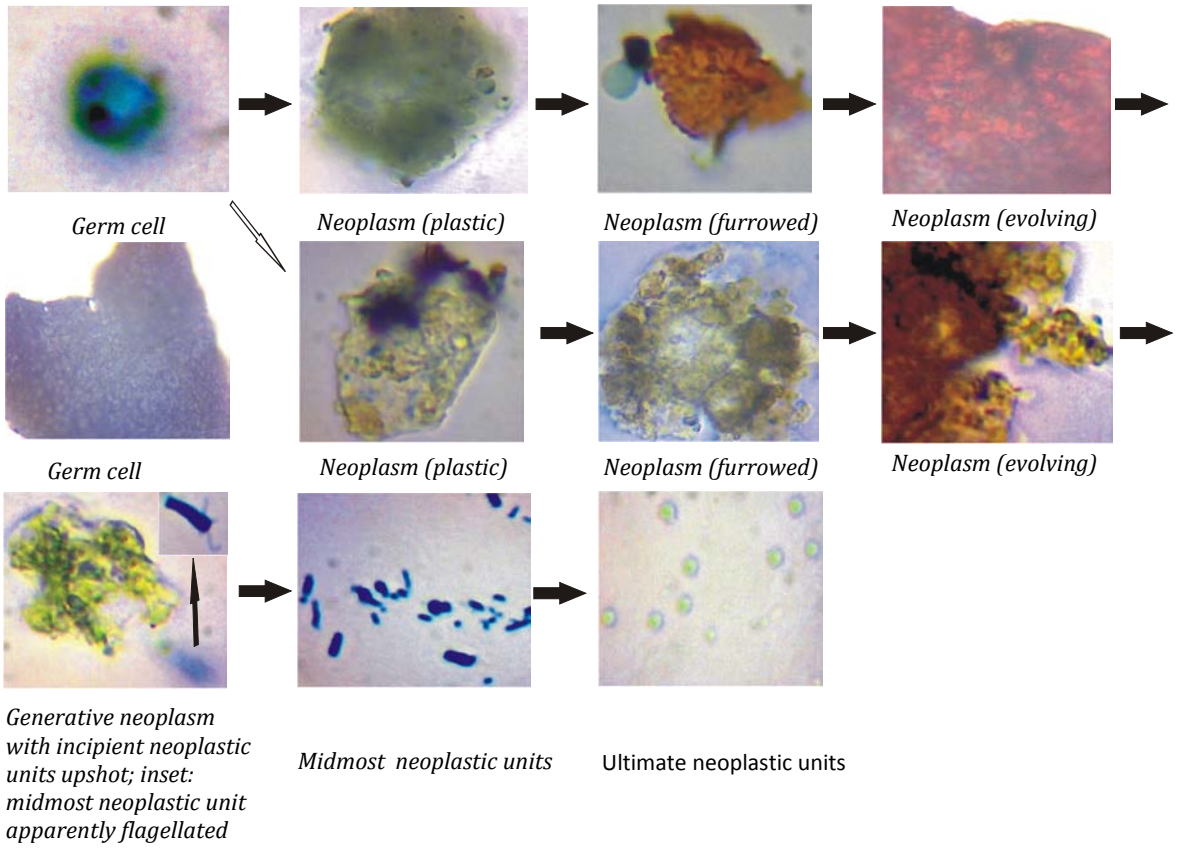


Figure 42 : Induction of neoplasm, nondescript & neoplastic units of *Mucor manihotis* in Peptone incorporated minimal medium after 6h of incubation at temp 28°C & pH5.0. Magnification, 2500x

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11a.Cytodifferentiation of Mucor species

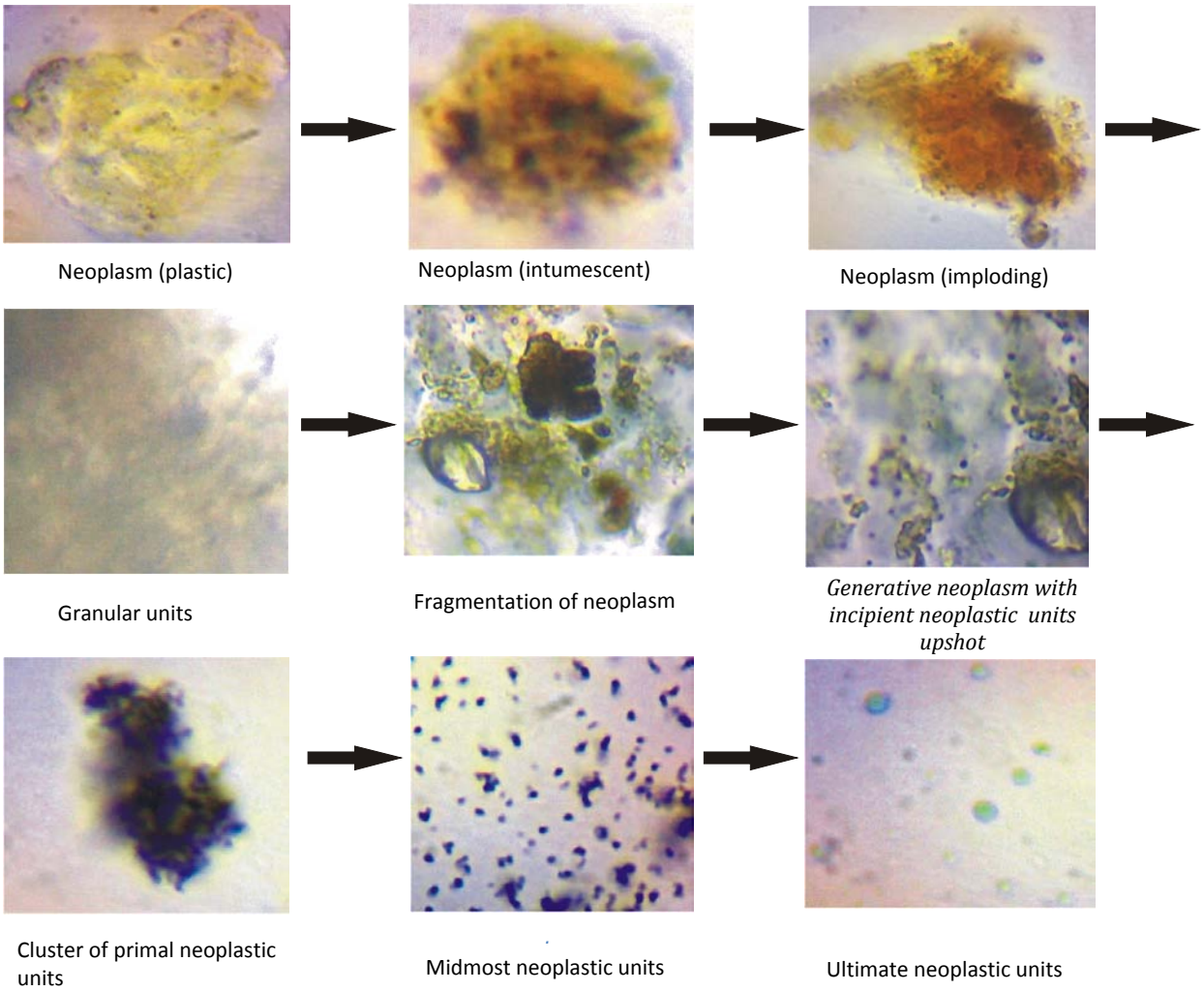


Figure 43 : Induction of neoplasm, nondescript & neoplastic units of *Mucor manihotis* in Peptone incorporated minimal medium after 9h of incubation at temp 28°C & pH5.0. Magnification, 2500x

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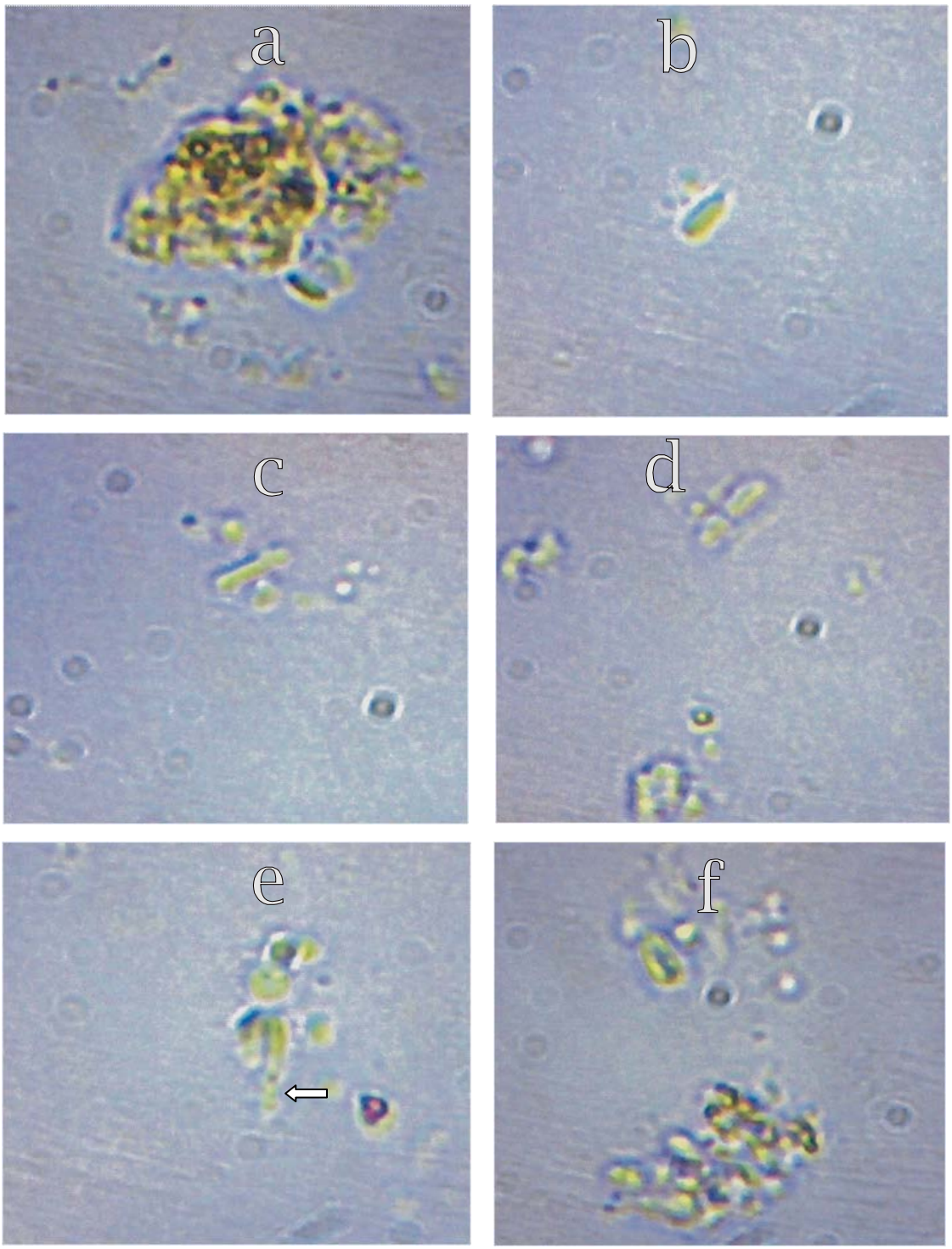


Figure 44 : Growth form of *Mucor manihotis* induced in Ammonium sulphate incorporated minimal medium after 96h of incubation at temp 37°C & pH5.0; **a**, cluster of neoplastic units & emergent protoplast; **b**, isolated protoplast; **c**, a doubling protoplast; **d**, binary protoplast; **e**, a protoplast (horizontal arrow) & a globose yeast cell with daughter bud (vertical arrow); **f**, cluster of protoplasts. Magnification: 2500x

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11b.Cytodifferentiation of Mucor species

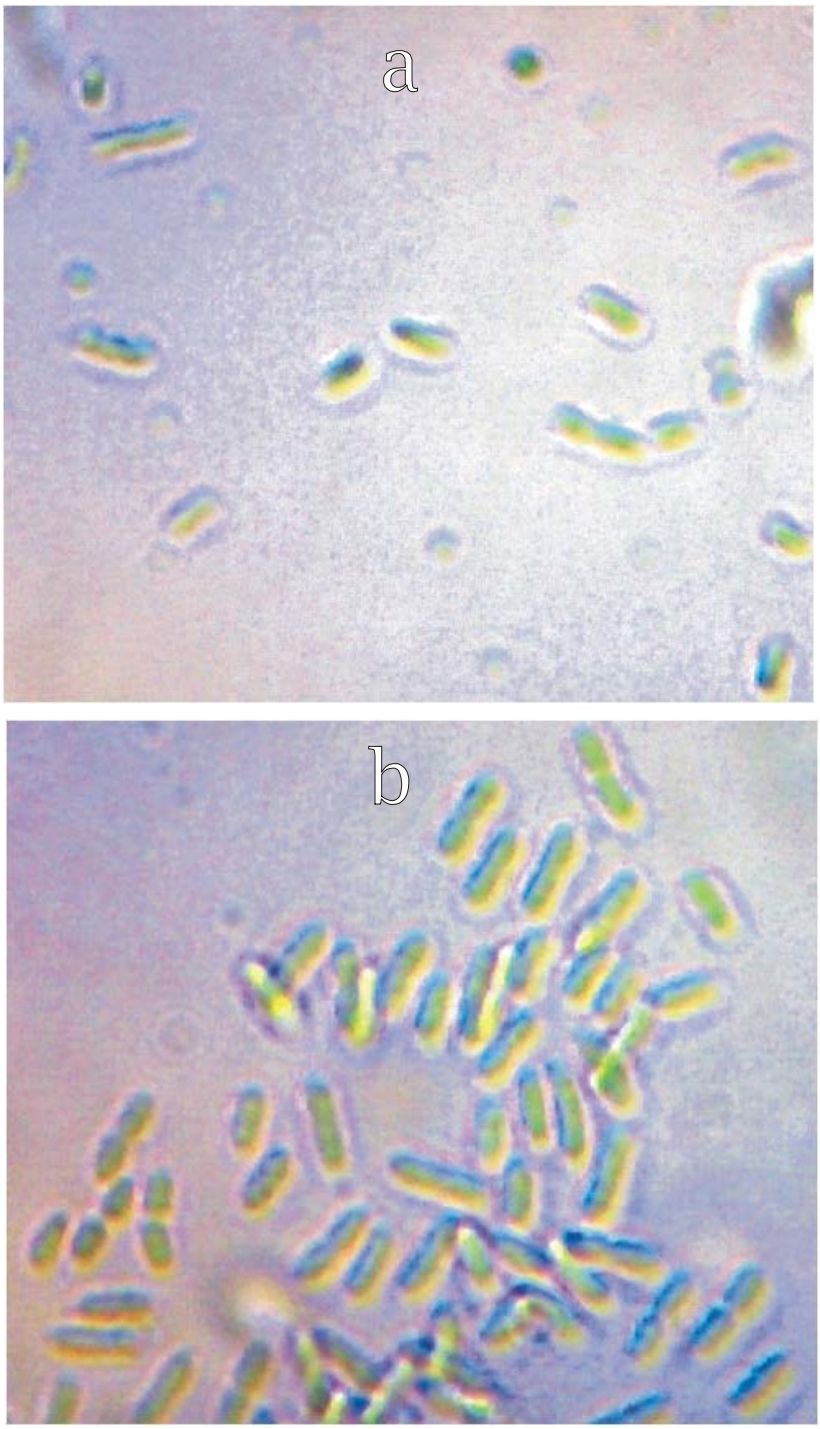


Figure 45 : Growth form of *Mucor manihotis* induced in Ammonium sulphate (a) - & peptone (b) - incorporated minimal media after 120h of incubation at temp 15°C & pH5.0; observe that the denticulate protoplasts in 'a' were less robust in comparison with those of 'b'. Magnification: 2500x

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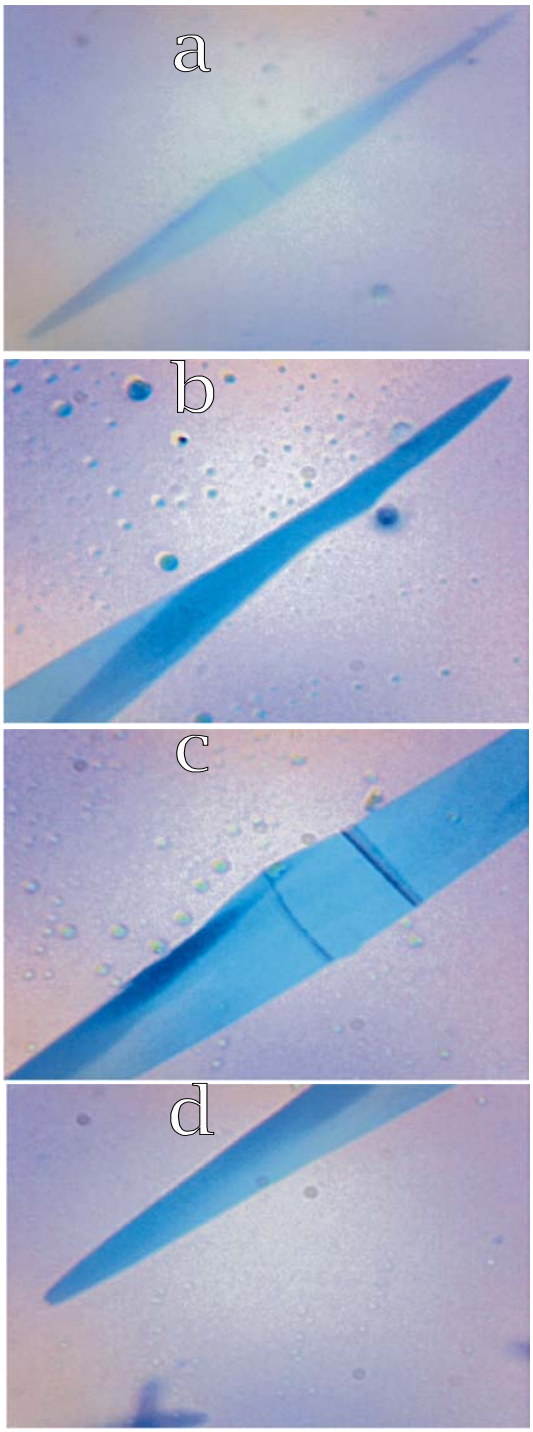


Figure 46 : Spindle shaped yeast form of *Mucor manihotis* induced in peptone incorporated minimal medium after 24h of incubation at temp 37°C & pH5.0; **a**, whole cell; **b**, upper segment; **c**, central segment with two cross walls; **d**, lower segment, observe the truncate tip. Note: the sorrounding cells are globose yeast cells. Magnification, 2500x

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12a.Cytodifferentiation of Mucor species

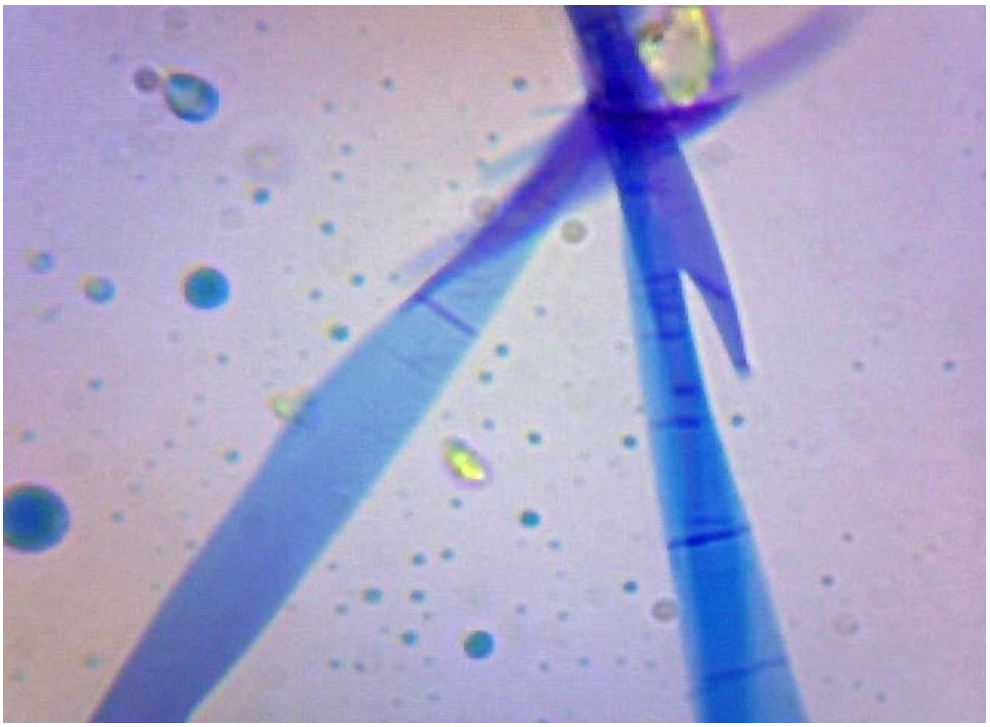


Figure 47 : Sections of Spindle shaped yeast & globose yeast form of *Mucor manihotis* induced in peptone incorporated minimal medium after 24h of incubation at temp 37°C & pH5.0; multiple cross walls are shown on the spindle yeasts; also observe the numerous prevegetative cells with various sizes, at the background. Magnification, 2500x



Figure 48 : Flask cultures of induced yeast cells of *Mucor manihotis* after two weeks of growth; *left*, Ammonium sulphate-incorporated culture; *right*, Peptone-incorporated culture. At termination of experiments conducted at pH 5.0 & temp. 15°, 28°, or 37°C, the flasks were left on the laboratory side bench at 28±1°C, ambient. The reversion to aerial mat that was re-induced in each of the flasks was observed following the *after-experiment* unperturbed period of incubation

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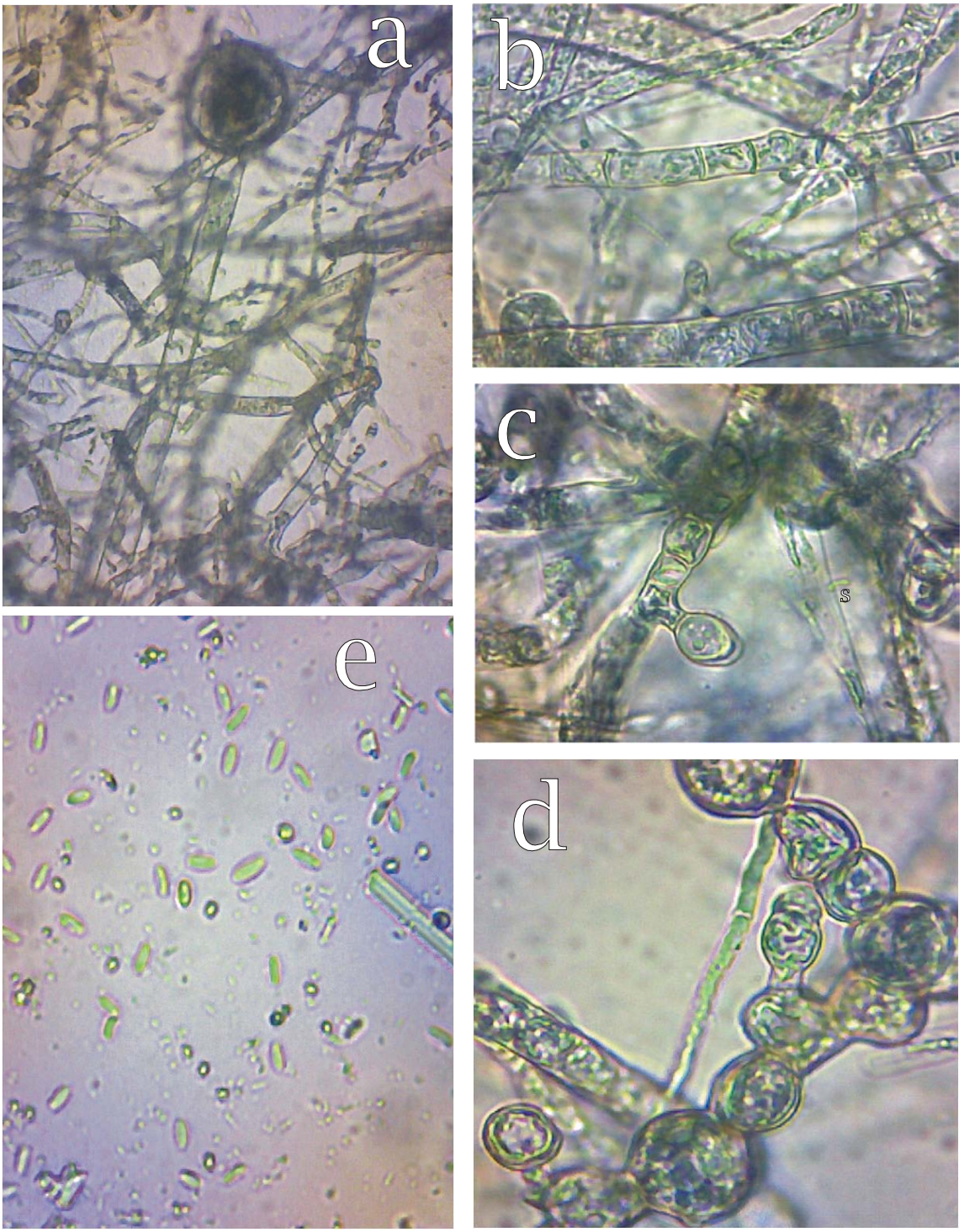


Figure 49 : Characteristics of *Mucor manihotis* after reversion to filamentous growth on termination of experiment; **a**, aerial mycelium showing sporangiophore & sporangium; **b-c**, broth surface mycelia showing septation of filaments; **d**, thallo-arthric growth; **e**, sporangiospores, s; a-d, $(NH_4)_2SO_4$-culture & d, Peptone- culture 9days after inoculation. Magnification, 1000x

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Fig. 50 : Lateral morphogenetic transformation of *Mucor manihotis* in minimal medium as observed in this study

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13.Cytodifferentiation of Mucor species

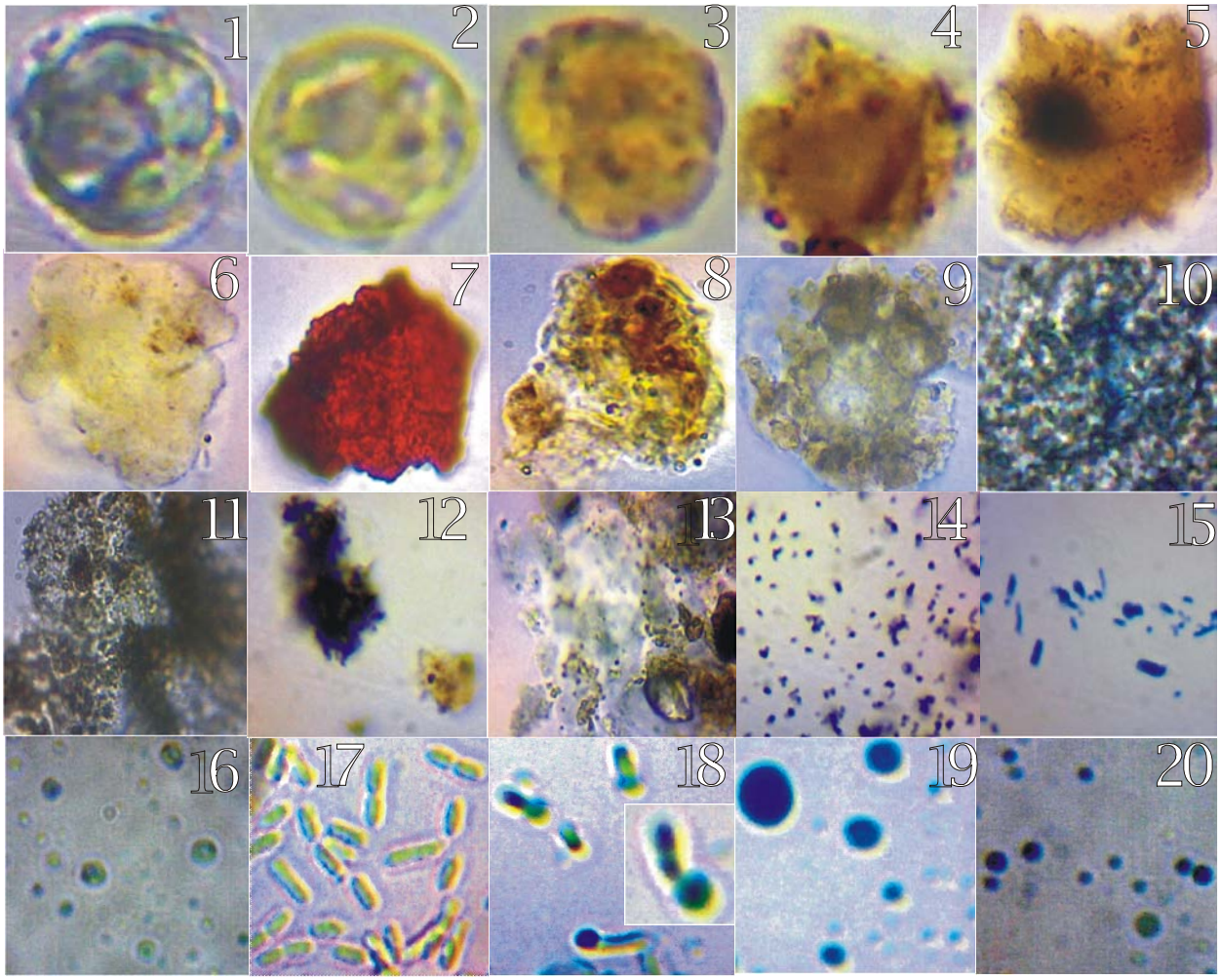


Figure 51 : Cytodifferentition of *Mucor manihotis* as observed in this study. 1, spheroplast; 2, cytoplasm; 3, neoplasm; 4-5, differentiating neoplasm; 6-9, imploding neoplasm; 10, provencal entities; 11, primeval units; 12-13, incipient neoplastic units; 14-15, midmost neoplastic units; 16, ultimate neoplastic units; 17, rod-shaped and binary protoplasts; 18, protoplast generation of nascent yeast initial (inset : optimum size nascent yeast initial); 19, prevegetative cell yeast cells; 20, polar budding yeast cells. Magnification, 2500x

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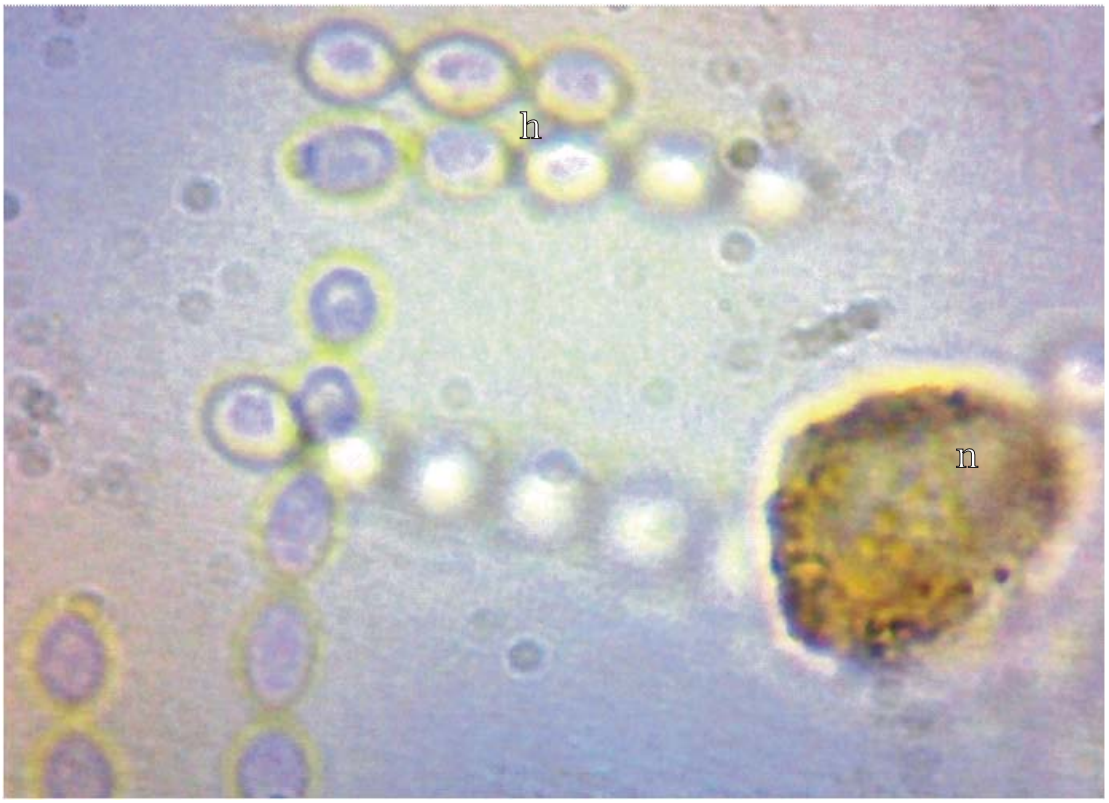


Figure 52 : Induction of neoplasm and young holoblastic conidia of *Mucor manihotis* in Peptone incorporated minimal medium after 6h of cultivation at temp 28°C & pH5.0. Magnification, 2500x. *As this study showed, two pathways exhibit here both leading to the same end-form: 1, when conidia mature cytoplasmic differentiation within each conidium occur but when conidial wall rupture primeval units expose in the medium & these subsequently differentiate to nascent yeasts; 2, when germ cell envelope lyse, priveval units expose in the medium & these subsequently differentiate to nascent yeasts*

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Design Development of Spate Irrigation Structures in Raya Valley, Ethiopia

By Hintsa Libsekal, Abrham Mehari, Charlotte de Fraiture, Tesfa-alem Gebre-egziaher & Atinkut Mezgebu

Tigray Agricultural Research Institute: Alamata Agricultural Research Center, Ethiopia

Abstract- Spate irrigation is a resource system, whereby flood water is emitted through normally dry wadi and conveyed to irrigable fields. Modernization of spate structures has been taking place in Raya valley since 1998 even though the efficiencies are not as intended. Initially the design standard was directly adopted from the conventional irrigation systems. Farmers were complaining that the implemented design standard was not appropriate with regards to their experiences. According to the professional's perception, the reason for schemes failure could be poor management and lack of maintenance. Spate irrigation design development in Raya valley shows significant changes through time; like widening of intake, increasing of deflection angle, excluding of rain fall during design and reduction of irrigation time. The spate schemes with relatively best performance still have problems like; sedimentation around intakes, less spate flow and low performances. Therefore, understanding of the experience, wisdom and tradition of farmers is necessary during design and construction of spate irrigation.

Keywords: spate irrigation, sedimentation, design development, intake, diversion structure, raya valley.

GJSFR-D Classification : FOR Code: 079901



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Design Development of Spate Irrigation Structures in Raya Valley, Ethiopia

Hintsa Libsekal [α], Abrham Mehari [σ], Charlotte de Fraiture [ρ], Tesfa-alem Gebre-egziaher [ω] & Atinkut Mezgebu [¥]

Abstract- Spate irrigation is a resource system, whereby flood water is emitted through normally dry wadi and conveyed to irrigable fields. Modernization of spate structures has been taking place in Raya valley since 1998 even though the efficiencies are not as intended. Initially the design standard was directly adopted from the conventional irrigation systems. Farmers were complaining that the implemented design standard was not appropriate with regards to their experiences. According to the professional's perception, the reason for schemes failure could be poor management and lack of maintenance. Spate irrigation design development in Raya valley shows significant changes through time; like widening of intake, increasing of deflection angle, excluding of rain fall during design and reduction of irrigation time. The spate schemes with relatively best performance still have problems like; sedimentation around intakes, less spate flow and low performances. Therefore, understanding of the experience, wisdom and tradition of farmers is necessary during design and construction of spate irrigation.

Keywords: spate irrigation, sedimentation, design development, intake, diversion structure, raya valley.

I. Introduction

According to FAO and UNDP, (1987) spate irrigation define as "an ancient irrigation practice that involves the diversion of flashy spate floods running off from mountainous catchments where flood flows, usually flowing for only a few hours with appreciable discharges and with recession flows lasting for only one to a few days, are channelled through short steep canals to bunded basins, which are flooded to a certain depth". Mehari et al. (2007) also defines spate irrigation in the simple way as "a resource system, whereby flood water is emitted through normally dry wadi and conveyed to irrigable fields". Moisture stress resistant crops, often sorghum and maize are grown in the spate irrigated agricultures and planted after the first flood irrigation water has occurred. In many areas crops can get matured and give reasonable yield using two or more floods depending on the water holding capacity of the soil.

According to Van Steenbergen et al. (2010) rough estimates, global spate irrigation coverage extends up to 3.3 million hectares even though uncertainty is there. According to the reference made by Mehari et al. (2011) spate irrigation is frequently practiced in the Middle East, North Africa, West Asia, East Africa and parts of Latin America. Although spate irrigation is uncertain type of investment economically it is very important practice in countries such as Yemen, Pakistan, Eritrea and Ethiopia where agriculture is a vital component of their economy (Ratsey, 2011). Even though spate irrigation contributes a lot for food security enhancement in the drought prone areas little concern and emphasis had been given in its developments.

In Ethiopia spate irrigation is a common practice in midlands as supplementary and in lowland area used as dominantly full irrigation. According to Van Steenbergen et al. (2011) in Ethiopia both farmer's initiative and public investments are the driving forces for spate irrigation development. Currently the cultivated areas under spate irrigation estimates to be 140,000 ha of which 20,000 ha is modern spate irrigation and 70,000 ha still need improvements and other 50,000 ha are under design and construction phases (Van Steenbergen et al., 2011).

Spate irrigation system in Ethiopia is increasing in arid areas particularly; south Tigray (Raya valley), Oromia (Bale, Arusi, West and east Hararghe), Dire Dawa Administrative Region, Southern Nations, Nationalities and Peoples Region (Konso), Afar and Amhara (Mehari et al., 2011)

Raya valley is one of the areas where spate irrigation is being practiced for long times. Farmers were diverting flood water to their farm land using traditional spate irrigation system. During the past decades many governmental and non-governmental organizations were trying to improve and modernize the traditional spate irrigation systems. Many traditional spate schemes were modernized while they did not perform as expected due to several problems. Among this problems are over sedimentation in diversion and canal, failure of structures, inappropriate design and poor participation of farmers during design and construction.

II. Methodology

a) Study area

The Raya Valley is located in the south-east part of the Tigray Regional State between 39022' to 39025' east longitude and 12017' to 12015' north latitude. It is bordered by Hintalo Wajerat Woreda to the north, Afar Region to the east, Endamekoni and Ofla woredas to the west and Amhara Region to the south. It comprises the total area of Raya Azebo and Alamata Woredas and

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some eastern high lands of Endamekoni and Ofla Woredas (REST, 1996). Figure 1 shows the location map of Raya valley. The total population of the Raya Valley Area is about 227,431 (136,039 for Raya Azebo and 85,359 for Alamata woreda).

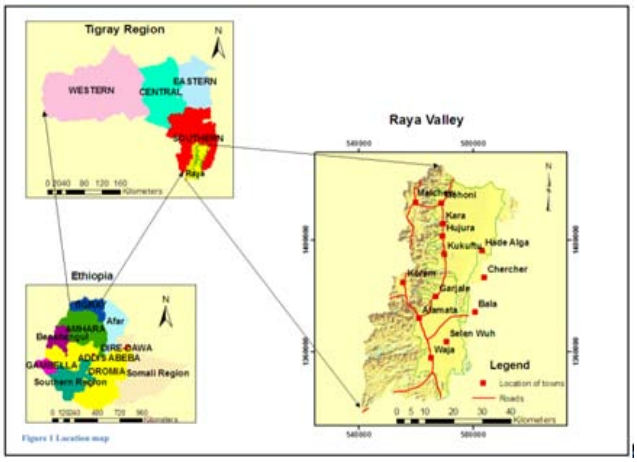


Figure 1 : Location map of Raya valley (source Gebreezgi A.H., 2010)

Topographically the Raya Valley is divided in to two major zones: low land areas with an altitude less than 1500 m.a.s.l which mostly covers large part of the central part of the valley; and the high land areas having altitude above 1500 m.a.s.l which covers the western and eastern edges of the valley. According to the moisture index criteria provided by REST, (1996) the Raya Valley area is classified as dry climates of semi-arid and arid types.

b) Data collection

Secondary data mainly study design report, design specification and scheme locations were collected from relevant organizations of Tigray Water Resources, Mines and Energy Bureau, Mekelle University, Raya Alamata and Raya Azebo weredas or districts. After having this secondary data rough evaluation on the design development in time was made and seven schemes namely Hara, Tirke, Fokissa, Beyru, Tengago, Dayu and Oda were selected for field observation and assessment. Hara, Tirke and Oda modern spate irrigation schemes did not have any report. Therefore analysis was made to this sites based on the current condition in the field and farmers perceptions.

An intensive scheme visit and observation was made for the seven selected schemes so as to envision the current situations in the ground. Headwork structures measurement was also made to Hara, Tirke, Fokissa, Beyru, Tengago, Dayu and Oda modern spate irrigation schemes. The field observation was aimed to measure the headwork structures and to observe the practical problems in the field. Structures like intake size, weir dimensions, sluice gates and main canals were measured. This data are used for comparison of design development with other scheme designs. Discussion with local farmers and experts were held in all visited schemes to determine the perception of the beneficiaries.

III. Result and Discussion

Modernization of spate irrigation schemes in Raya valley starts in 1998. Hara was the first modernized spate irrigation scheme in the area and that leads to many improvements in the designing and constructions of modern schemes in Tigray. Tirke spate irrigation scheme was also modernised in 2004 following to Hara scheme. In 2005 four schemes namely Fokissa, Beyru, Utu and Burka was designed and constructed while Ula-ula, Buffie, Tengago and Dayu schemes were constructed in 2006.

The design standard of Hara and Tirke was directly adopted from the conventional irrigation schemes which have low sediment concentration. The headwork of this two spate schemes has gated off take or intake with broad crested weir and all the structures were made up of concrete masonry. Hara and Tirke schemes were failed in one rainy season due to problem of sediment in both intakes and canal systems. Figure 2 shows the modernized headwork structures of Hara and Tirke spate irrigations.

According to the farmers perception the main reason for failure of these schemes was the inappropriate design structure of intakes. During construction the farmers were complaining about the size, shape and deflation angle of the gate. According to field observation the intakes of Hara and Tirke has 900 deflection angles from the river flow direction and less than one meter diameter of gate.

In 2005 when Fokissa, Beyru, Utu and Burka was designed and constructed the design engineers took key lesson from the failure of Hara and Tirke. They came to realize that the incoming sediment or bed material load was too high. Hence, they decided that gated intake, narrow canal and siphons cannot work as structures of spate scheme. At that time the designers tried to know the indigenous knowledge of farmers for sediment managements and they observed some traditional irrigation schemes in the valley.

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Intake structure of Hara scheme



Under sluice gate in Hara scheme



Crossing structure, Hara spate scheme



Orifice intake, Tirke scheme

Figure 2 : Headwork structures of Hara and Tirke schemes

The major findings of farmer's knowledge were wide open gate intake with an angle of deflection greater than 900 and wide size of canals. To some extent experts tried to understand and incorporate farmers traditional knowledge during design and construction. They took good lesson on size and deflection angle of intake and they tried to give attention for sediment problems.

The major changes of the design include;

- To change the gated intakes to open gate
- To increase the width of the intakes and canals
- Improving of diversion angle from 900 to 1200
- Avoiding of crossing structures

The main problems or limitations during these designs are:

- The crop water requirement (CWR) was calculated for 24 hours while flood occurrence is too short'
- Effective rainfall was considered during irrigation water requirement calculation (IWR) which leads to underestimation of net irrigation water requirement (NIWR) but rain fall is not reliable.
- As the width of the gates ranges from 1m to 3m depending to size of irrigable area but the farmers were still complaining as they were thinking even 3m gate is small.

In 2006 four spate schemes were designed and constructed namely Ula-ula, Buffie, Tengago and Dayu schemes. In addition to the design improvement takes place in 2005 some improvements were made based on recommendations of supervision. These improvements try to solve the limitations and problems occurred in the design of schemes made in 2005. Figure 3 presents the headwork structures of Tengago and Dayu modern spate irrigation scheme. The main design improvements for Ula-ula, Buffie, Tengago and Dayu schemes are;

- The calculation of crop water requirement was minimized to 4 hours
- Effective rainfall was neglected during net irrigation water requirement calculation
- The schemes design was limited to headwork and main canals.

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Diversion structure of Tengago scheme



Sluice gate blocked by gabion, Tengago



Diversion weir in Dayu spate



Silted intake, Dayu

Figure 3 : Headwork structures of Tengago and Dayu schemes

In 2011 Oda spate irrigation was designed with some improvements to traditional spate system, it was designed as a simple intake using gabion and only cut offs built to reduce the risk of bed level lowering around the river bed and intake (Embaye et al., 2012).

During the field visit to Oda spate irrigation it was found that the weir or cut off structure was completely destroyed by flood hazard. According to the farmers perception Oda scheme was failed before handed over to users just during completion of construction work. Now farmers are using in traditional way using forest and shrub embankment Figure 4 shows the failed weir axis and reconstruction of scheme in traditional systems.

From 2011 to 2013 there was no sound change in design development of spate irrigation schemes. Few schemes were constructing by the wereda or district of Raya Azebo and Raya Alamata bureau of water resources, mines and energy. Most of these schemes are simple and small structures and they are exposed to flood hazards. In 2014 two spate irrigation schemes were designed by Mekelle University. The headwork of these schemes was designed to have 50 centimeter high slant barrage across the river. This design was aimed to convey limited amount of water and during medium and high flood occurrences the flood will over flow above the barrage and sediments will flashed away. This spate scheme is still under construction and its performance and applicability was not assessed.

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Broken diversion structure, Oda



Embankment using forest and shrub, Oda

Figure 4 : Headwork structure of Oda spate irrigation scheme

The major design development made for spate irrigation system in Raya valley are summarised as shown in Table 1. The relatively best performed modern spate irrigation system in Raya valley was the one designed and constructed in 2006 namely Ula-ula, Buffie, Tengago and Dayu schemes. Renovations have been taking for these schemes to minimize the structural damages and sedimentation problems. Among this schemes Dayu spate irrigation scheme were found relatively best performing scheme. Therefore, this scheme was selected for further study.

Comparing Tengago and Dayu spate irrigation schemes Dayu is relatively best performing. The reason for this could be the difference of river flood discharge. As we can see from Table 2 the design flood discharge for Tengago is 50.0m3/s while 358.89 is flood discharge of Dayu. Even though the river discharge is small but Tengago was designed to irrigate 500 ha with two intakes in one diversion structure. The structures of Tengago are still in good conditions while there are accumulations of sediments around both intakes. Therefore designing of 500 ha to a river which has 50m3/s is not optimum and this could be the reason for poor performance.

a) *Current problems of schemes in relation to sediment management and spate flow*

Dayu spate irrigation scheme is the relatively best performed scheme in Raya valley while it is irrigating about half of the designed area.

The main structural problem of Dayu in relation to sediment management and spate flow are;

- Siltation problems both in intakes and main canal
- Diverted amount of water through in intake is small
- In small flood it is difficult to convey water through intake as too much sediments are accumulated in the intake than in weir.

b) *Causes of the problems*

i *Farmer's perception*

According to the farmer's perception the main cause of the structural failure to modern spate irrigation systems are;

- Narrow intake and canal width
- Angle of intake deflection
- Existence of sluice gate; it is not good because it can lost many floods.

Table 1 : Summary of spate structures design development

Parameters	Name of schemes					
	Hara	Tirke	Fokissa	Tengago	Dayu	Mersa
Year of construction	1998	2004	2005	2006	2006	2014
Design flood discharge	-	-	220.5	50.0	358.89	-
Weir length	35	34	35	23	29	-
Intake type	Closed gate	Closed gate	Open gate	Open gate	Open gate	Open gate
Gate size	0.8X0.8 both sides	0.9m diameter	3 m	2.5m right & 2m left	3 m	3 m
Deflection angle	900	900	1200	1200	1200	1200
Main canal system	Concrete	Concrete	Concrete	Concrete	Concrete	Concrete
Crossing structures	Available		Avoided	Avoided	Avoided	Avoided

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Assumed irrigation time	24 hrs	24 hrs	24 hrs	4 hrs	4hrs	4hrs
Effect of rainfall	Considered	Considered	Considered	Neglected	Neglected	Neglected
Designed ha	400	380	500	500	320	420
Current ha	0	0	100-150	<50	150	-
Over all status	Failed	Failed	Poor	Poor	Good	In construction

ii *Designers and experts perception*

According to the discussion held with designers and professional experts of spate irrigation system the main cause could be lack of good operation and maintenance in addition to lack of inappropriate design. As there is no known standard for spate irrigation system most the decisions for all structural design are by trial and errors. The experts are still not confident on the size and angle of intakes which they have been designing for years. In the other way round the experts are not convinced by the farmers complaining about the existence and functionality of sluice gate. Sluice gate is important parameter for sediment control. Opening of sluice gate during high flood helps to erode the accumulated sediments around intake. In low flood is must be closed so as to rise the water level and divert more water. Therefore the existence of intake could not be a problem but it needs care full management and frequent supervision.

c) *Remedial solutions for the problems*

i *From farmers point of view*

Based on the farmers indigenous knowledge most of traditional irrigation system are characterized as wide intake width up to 6 meter wide, the angle of deflection are greater than 1200 in some area they can make it near to 1800 which is parallel to the river flow and mostly they use temporary and small solid weir or barrage to clot the flow along the river and divert to earthen canal. For the modernized schemes the farmers put the following remedial actions;

- Width of intake have to be up to 5 meter
- Angle of deflection have to be more than 1200 deflected
- The weir must be without sluice gate

ii *From expert point of view*

The design experts of spate irrigation system are keen to know the impact of different deflection angle and width length on sediment management and spate flow. Therefore the possible remedial solution in relation to sediment management and spate flow could be;

- Width of intake 3m or 5m
- Deflection angle 1200 or 1500

IV. Conclusions

Modernization of spate irrigation was started in 1998. Hara and Tirke schemes were the first to modernize. The design parameter of these schemes was directly adopted from the conventional design system without consideration of the sediment income and extreme flood events. Nevertheless, these schemes were failed in one rain season and went out of use due to high sedimentations.

- Inappropriate design parameters of intakes and canals are the main cause of failure.
- Sedimentation and less spate flow are still the major problem in spate irrigation schemes.
- Farmers are complaining to experts for not considering their willing and construction of inappropriate designs. Experts were also complaining to farmers for their poor management and lack of maintenance.
- The design of main intakes has significantly improved over the past years. The intake dimensions were changed from closed intake, 900 deflection angles and narrow (90 cm wide) gates to 3 meters wide open intake with 1200 deflection angle and this improvement gives relatively good performance for modern spate irrigation schemes.
- The latest design of diversion structure is however, far below optimum. This design is irrigating about 50% of the intended area. The main reason for the poor performance could be lack of optimum intake designs
- Understanding the experience, wisdom and tradition is necessary during design and construction of spate irrigation.

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Nutrient Availability and Maize Growth in Soil Amended with Mineral Fertilizer and Pressmud Biocompost

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Abstract- This study was carried out to conform the Nutrient availability and maize growth in soil amended with mineral fertilizer and biocompost. Biocompost is prepared from sugarcane filtercake and other waste material of sugarmills. The biocompost samples were collected from Sugar Mill, Matiari and analyzed for macro nutrients. The pot experiment was conducted with mineral fertilizer and biocompost amendment for maize crop. The pot experiment was replicated three times in a completely randomized block design. Pot experiment results revealed that there were pronounced positive effects of addition of biocompost. Maize crop data showed that the effect of biocompost and mineral fertilizers was non-significant with respect to N, P and K contents. Data describing the soil physical and chemical properties at the end of pot experiment under mineral fertilizers and biocompost showed that the EC values of post harvest samples increased with the application of biocompost while pH was not affected.

Keywords: maize growth, nutrient availability, pressmud biocompost, physical and chemical properties.

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Nutrient Availability and Maize Growth in Soil Amended with Mineral Fertilizer and Pressmud Biocompost

Korai Punhoon Khan [α], Rajper Aftab Ahmed [σ], Baloch Shah Faisal [ρ] & Korai Shakal Khan [ω]

Abstract- This study was carried out to conform the Nutrient availability and maize growth in soil amended with mineral fertilizer and biocompost. Biocompost is prepared from sugarcane filtercake and other waste material of sugarmills. The biocompost samples were collected from Sugar Mill, Matiari and analyzed for macro nutrients. The pot experiment was conducted with mineral fertilizer and biocompost amendment for maize crop. The pot experiment was replicated three times in a completely randomized block design. Pot experiment results revealed that there were pronounced positive effects of addition of biocompost. Maize crop data showed that the effect of biocompost and mineral fertilizers was non-significant with respect to N, P and K contents. Data describing the soil physical and chemical properties at the end of pot experiment under mineral fertilizers and biocompost showed that the EC values of post harvest samples increased with the application of biocompost while pH was not affected. Soil organic matter, Olsen P and NH_4OA_c-K increased significantly with the application of biocompost, and mineral fertilizer application also increased Olsen P and NH_4OA_c-K contents in soil. The results of this study showed that biocompost can be used along with mineral fertilizers to increase maize growth and yield.

Keywords: maize growth, nutrient availability, pressmud biocompost, physical and chemical properties.

I. Introduction

Present is a rising apprehension among the scientific group of people, environmentalists and policy makers about the safe disposal of the large amounts of organic wastes produced worldwide. Urbanization, industrialization, increasing food demand for growing human population, intensive use of relatively easily available and inexpensive chemical fertilizers and economic force are adding to the production and buildup of large amounts of organic wastes. In Pakistan, a few organic wastes such as farm waste, city waste (sewage and sludge), poultry litter and industrial wastes (food, sugar, cotton and rice industry) are recycled back by applying back to agricultural land but a significant amount of organic wastes. As a result recycling organic wastes by applying on to agricultural land seems to be the only best option in such scenario (Zaman et al., 2002; 2004). However, soil may not be regarded as a dumping place for organic wastes (Cameron et al., 1997).

Organic waste such as pressmud or filter cake is a byproduct of sugar factories and characterized as a soft, spongy, amorphous and dark brown to brownish material. Pressmud is reported to be a valuable resource of plant nutrients and may therefore affect physical, chemical and biological properties of a soil (Rangaraj et al., 2007; Kumar and Verma, 2002; Jamil et al., 2008; Muhammad and Khattak, 2009; Nehra and Hooda, 2002; Ramaswamy, 1999). Razzaq (2001) reported that continuous land application of sugarcane filter cake to agricultural crops for 5-6 years is likely to improve soil health by adding sulfur (S) and organic matter to soil. Therefore land application of pressmud is becoming a common farm practice in the sub-continent countries of Pakistan and India.

Nutrients availability and maize growth yields increased with increasing nitrogen and pressmud rates (Bangar et al., 2000). Memon (2005) reported that the raw pressmud had depressing effect on dry matter yield of maize, and that the benefit of previously applied pressmud was obvious in the succeeding wheat crop. Viator et al. (2002) reported that filter cake increases cation exchange capacity (CEC) for thirty months after its application and its residual effect remains after four years.

Observance in view the significance of pressmud in the present scenario of agriculture and accessibility of nutrients, the purpose of the present study was to examine the influence of mineral fertilizer and biocompost amendments on maize growth and yield.

II. Materials and Methods

a) Pot experiment

This work was conduced in the Ware House of the Department of Soil Science. The soil was collected from Latif Experimental Farm of Sindh Agriculture University Tandojam. The soil was air dried and passed through 4 mm garden sieve. Ten kilogram soil was placed in each of the pots. The experiment was laid out with eight treatments with three replications in a randomized complete block design (RCBD). The treatments were factorial combination of four rates of

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mineral fertilizer (0-0-0, 150-0-0, 150-75-0 and 150-75-60 kg ha^{-1} N, P and K respectively) and two rates of biocompost 0 and 10 tons ha^{-1}.

b) Application of biocompost and fertilizers

The quantity of biocompost required for each treatment was calculated as per treatment plan and as per weight of soil per pot (10 kg/pot). Before placing soil in each pot, biocompost was thoroughly mixed with soil for the treatments involving biocompost application. Phosphorus and potassium were applied in the form of single superphosphate (18% P_2O_5) and sulphate of potash (50% K_2O) respectively before planting as per treatment plan. Fertilizers were thoroughly mixed with soil before filling it in the pots. Nitrogen application was made in the form of urea (46% N) in three splits starting from one week after germination of maize and followed by two fortnightly applications afterwards.

c) Maize Planting and agronomic observation

Ten seeds of maize (cv. Akbar) were planted in each pot. After germination, the seedlings were thinned to five per pot. The plants were irrigated as required and harvested after 7 weeks of growth. Before harvesting, height of each plant in each of the treatments was recorded from soil surface to the tip of the longest leaf extension. The plants were harvested at 1cm above the surface of soil, washed clean of any soil particles, and placed in paper bags by each treatment, and kept in oven at 70°C for drying. After achieving constant weight, the plants were weighted to determine dry weight of shoots by each treatment and replication.

d) Soil sampling and analysis

The soil sample from experimental plot was collected from a depth of 0 – 15 cm and air dried in shade. Then sample was ground using wooden pestle and mortar and passed through 4 mm sieve. The sieved sample was preserved in polythene bag for further analysis. The soil sample was analysed for various physical and chemical properties.

e) Biocompost and plant analysis

Biocompost used in this study and the plant samples drawn at harvesting from each treatment were analyzed for total N, P and K. Total N was determined by digestion with concentrated H_2SO_4 along with a mixture of selenium, $CuSO_4$ and K_2SO_4 in 0.1:1:10 ratios using Kjeltech Digestion System 20. The digests were distilled by using Kjeltech Distillation Unit 1002 as described by Winkleman et al. (1986). For P and K, the plant samples were digested in 1:5 $HClO_4$: HNO_3 mixture followed by analysis of the digest by vandomolybdophosphoric acid yellow colour method (Barton, 1954) for P and flame photometer for K (Jackson, 1958).

f) Calculation and statistics

The data were statistically analysed by using software Statistix 8.1 and the calculations were made using following formulas.

1. Standard Error for Difference between Means (S.E.D) was calculated using the following formula: S.E.D = (√2EMS/n) EMS= Error Mean Square. 2. Least
2. Significant Difference (LS.D) = S.E.D x t value for Error df at 5% probability level.
3. Coefficient of Variance (%) = (√MSE / Grand Mean) x 100.

III. Results and Discussion

a) Pot experiment on maize

i *Soil properties*

The soil used for the experiment was analyzed for some physico-chemical properties of soil sample (Table 1). The results revealed that the soil was a clay loam (31% clay) with EC 0.37 dSm^{-1}, and pH 7.83. It was low in organic matter (0.81%), and Olsen P (7.2 mg kg^{-1}), and adequate in NH4OAc – extractable K (330 mg kg^{-1}).

Table 1 : Physico-chemical properties of the soil used for pot experiment on maize

Soil properties	Values
Sand (%)	25.5
Silt (%)	40.5
Clay (%)	31.0
Textural Class	Clay loam
EC (1: 5 soil – water extract) (dS m^{-1})	0.37
pH (1:5 soil – water extract)	7.83
Organic matter (%)	0.81
Olsen P (mg kg^{-1})	7.2
NH_4OAc- extractable K (mg kg^{-1})	330

ii *Effect of biocompost and mineral fertilizer treatments on growth of maize*

a. *Plant height*

The data regarding plant height of maize as affected by mineral fertilizer and biocompost treatments are given in Table 2. Overall, the plant heights ranged from 34.13 to 55.87 cm. Addition of biocompost as well as mineral fertilizers showed significant increase in plant height. On an average, the plant height increased from 39.83 to 49.63 cm with the addition of biocompost which

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is equivalent to 24.6 % increase. When biocompost was applied alone to unamended soil, the plant height increased from 35.00 to 46.06 cm. Similarly, the application of N fertilizer also increased it significantly to 45.33 cm. On an average taken over biocompost treatments, the plant height increased significantly from 40.53 cm for unfertilized control to 50.60 cm for the treatment receiving N fertilizer only. Addition of P fertilizer did not help improve the plant height while addition of K fertilizer (NPK treatment) in fact resulted in reduced growth with average plant height of 39.13 cm.

Table 2 : Effect of biocompost and mineral fertilizer treatments on plant height (cm) of 7-week old maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/ha^{-1}	Fertilizer Mean
Control	35.00	46.06	40.53**b**
N	45.33	55.87	50.60**a**
NP	44.87	52.47	48.67**a**
NPK	34.13	44.13	39.13**b**
Biocompost Mean	**39.83b**	**49.63a**	**44.73**

CV %	*5.94*
S.E	*2.6*
L.S.D @ 5%	
Fertilizer	*3.29*
Biocompost	*2.32*
Fertilizer x Biocompost	*NS*

b. Dry weight of maize

The data in Table 3 showed that there was pronounced positive effect of addition of biocompost as well as mineral fertilizer treatments on dry weights of maize. Overall, the dry weights ranged from 17.23 to 29.93 g pot^{-1}. On an average, the dry weights increased by 28.2% from 19.93 to 25.55 g pot^{-1} with addition of biocompost. When biocompost was applied alone to unamended soil, the dry weight increased from 17.23 to 23.60 g pot^{-1}. Similarly the applications of N fertilizer also increased it significantly to 21.90 g pot^{-1} and to 29.93 g pot^{-1} when biocompost was also added. It was noted that there was no significant improvement in dry matter yield of maize due to P fertilization (NP treatment) and addition of K fertilizer (NPK treatment) to this treatment showed decline in dry matter yield. Thus the application of N fertilizer or biocompost were the most effective treatments in improving the dry matter yield of maize. Since the effect of fertilizer and biocompost treatments was similar in all combinations, the interaction between fertilizer and biocompost treatments was noted to be non significant.

Table 3 : Effect of biocompost and mineral fertilizer treatments on dry weight of 7-week old maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/ha^{-1}	Fertilizer Mean
Control	17.23	23.60	20.42**b**
N	21.90	29.93	25.92**a**
NP	22.33	26.26	24.30**a**
NPK	18.26	22.40	20.33**b**
Biocompost Mean	**19.93b**	**25.55a**	**22.74**

C V %	*13.49*
S.E	*3.07*
L.S.D @ 5%	
Fertilizer	*3.79*
Biocompost	*2.68*
Fertilizer x Biocompost	*NS*

b) Total N in maize

The results regarding the effect of biocompost and mineral fertilizer tretments are given in Table 4. The values ranged from 0.80 to 0.93%. The data showed that the application of either biocompost or mineral fertilizer treatments did not bring about a significant change in the total N content of maize. The values of total N with and without biocompost application were 0.85 and 0.86% resepectively. In case of fertilizer treatments, these values ranged from 0.83 to 0.88%. It was noted further that the interactions between biocompost and mineral fertilizer treatments were also nonsignificant.

Table 4 : Effect of biocompost and mineral fertilizer treatments on total N in maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/ha^{-1}	Fertilizer Mean
Control	0.83	0.82	0.83**a**

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N	0.86	0.90	0.88a
NP	0.93	0.83	0.88a
NPK	0.80	0.90	0.85a
Biocompost Mean	**0.85a**	**0.86a**	**0.86**

C V%	*8.75*
S.E	*0.08*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*NS*
Fertilizer x Biocompost	*NS*

c) *Total P in maize*

The data in Table 5 showed that total P in maize varied from 0.18 to 0.24% and averaged to 0.20% for all treatments. As the values varied within a narrow range, the effects of either biocompost or mineral fertilizer tretments were non significant. Similarly, the interaction between biocompost and fertilizer treatments was also nonsignificant.

Table 5 : Effect of biocompost and mineral fertilizer treatments on total P in maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/ ha^{-1}	Fertilizer Mean
Control	0.20	0.20	0.20a
N	0.21	0.21	0.21a
NP	0.23	0.24	0.23a
NPK	0.18	0.19	0.18a
Biocompost Mean	**0.20a**	**0.21a**	**0.20**

CV%	*14.60*
S.E	*0.03*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*NS*
Fertilizer x Biocompost	*NS*

d) *Total K in plant*

The total K contents of maize ranged from 1.12 to 1.23% as a result of biocompost and mineral fertilizer treatments (Table 6). The effects of either biocompost or mineral fertilizer treatments were found to be non significant. Similar was the case with the interactions between biocompost and mineral fertilizer treatments which were found to be nonsignificant.

Table 6 : Effect of biocompost and mineral fertilizer treatments on total K in maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/ h^{-1}	Fertilizer Mean
Control	1.13	1.15	1.13a
N	1.15	1.15	1.15a
NP	1.22	1.23	1.22a
NPK	1.12	1.15	1.14a
Biocompost Mean	**1.16a**	**1.17a**	**1.16**

CV%	*5.38*
S.E	*0.06*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*NS*
Fertilizer x Biocompost	*NS*

i *Physico-chemical properties of soil after harvest of maize*

a. Effect on soil EC

The data in (Table 7) showed that there was significant effect of biocompost treatments. The EC value was lowest (0.84 dSm^{-1}) for the control treatment not receiving either mineral fertilizer or biocompost. With addition of N, P and K fertilizer, the mean EC value increased from 0.84 to 1.05 dSm^{-1}, while the addition of biocompost increased it to 1.24 dSm^{-1}. Combined application of fertilizers and biocompost contributed to further increase in EC level to a maximum of 1.66 dSm^{-1}. Overall mean of EC values was 0.91 dSm^{-1} where no biocompost was applied. A highly significant increase in mean EC value, to 1.36 dSm^{-1}, was noted for the treatments receiving biocompost. In case of fertilizer treatments, mean EC values showed progressive increase from 1.04 dSm^{-1} for unfertilized control, through N and N+P treatments, to 1.35 dSm^{-1} for N+P+K treatment but the treatment differences were statistically non significant.

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Table 7 : Effect of biocompost and mineral fertilizer treatments on soil EC (dSm^{-1}) after harvest of maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/h^{-1}	Fertilizer Mean
Control	0.84	1.24	1.04b
N	1.24	1.11	0.94b
NP	0.96	1.54	1.20ab
NPK	1.05	1.66	1.35a
Biocompost Mean	0.91b	1.36a	1.13

C V %	*25.47*
S.E	*0.29*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*0.25*
Fertilizer x Biocompost	*NS*

b. Effect on soil pH

The data in (Table 8) showed that the pH values ranged from 7.20 to 7.47 for various treatments. There was slight increase in pH with the addition of either fertilizer or biocompost. However, the treatment differences were non-significant in both cases. Similarly the interactions between fertilizer and biocompost treatments were also non significant.

Table 8 : Effect of biocompost and mineral fertilizer treatments on soil pH after harvest of maize

Fertilizer treatment	No Biocompost	Biocomost 10t/h^{-1}	Fertilizer Mean
Control	7.20	7.37	7.28a
N	7.20	7.30	7.25a
NP	7.37	7.47	7.12a
NPK	7.43	7.47	7.45a
Biocompost Mean	7.30a	7.40a	7.35

C V %	*3.39*
S.E	*0.25*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*NS*
Fertilizer x Biocompost	*NS*

c. Effect on soil organic matter

Soil organic matter contents ranged from 0.70 to 0.93% as a result of mineral fertilizer and biocompost treatments (Table 9). There was no effect of fertilizer treatments on soil organic matter. However, application of biocompost increased it significantly fron 0.72 to 0.90%. This effect was similar for each fertilizer treatment and thus the interaction between mineral fertilizer and biocompost treatments was non significant.

Table 9 : Effect of biocompost and mineral fertilizer treatments on soil organic matter (%) after harvest of maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/h^{-1}	Fertilizer Mean
Control	0.70	0.93	0.82a
N	0.78	0.84	0.81a
NP	0.70	0.90	0.80a
NPK	0.71	0.93	0.82a
Biocompost Mean	0.72b	0.90a	0.81

C V %	*18.86*
S.E	*0.15*
L.S.D @ 5%	
Fertilizer	*NS*
Biocompost	*0.13*
Fertilizer x Biocompost	*NS*

d. Effect on Soil P (Olsen)

The data in (Table 10) showed that soil P contents ranged from 8.17 to 15.00 mg kg^{-1} due to biocompost and mineral fertilizer treatments. A highly significant increase in Olsen P was noted with the application of biocompost, whereby soil P increased, on an average, from 10.17 mg kg^{-1} to 12.65 mg kg^{-1}. Similarly, the effect of fertilizer application also produced highly significant increase in soil P from 10.08 to 13.50 mg kg^{-1}, on an average. Further, it was noted that there was interdependence between biocompost and mineral fertilizer treatments such that the interaction between

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mineral fertilizer and biocompost were highly significant. When no fertilizer was applied, the P contents increased with biocompost application. This was not the case when N was also applied. However, when N and P fertilizers were applied, there was significant improvement in soil P due to fertilizer as well as due to biocompost.

Table 10 : Effect of biocompost and mineral fertilizer treatments on soil phosphorus (Olsen, mg kg^{-1}) after harvest of maize

Fertilizer treatment	No Biocompost	Biocomost 10 t/h^{-1}	Fertilizer Mean
Control	8.17e	13.25b	10.71bc
N	9.50de	10.67cd	10.08c
NP	12.00bc	15.00a	13.50a
NPK	11.00cd	11.67bc	11.33b
Biocompost Mean	**10.17b**	**12.65a**	**11.41**

C V %	*7.96*
S.E	*0.91*
L.S.D @ 5%	
Fertilizer	*1.12*
Biocompost	*0.79*
Fertilizer x Biocompost	*1.59*

e. Effect on NH4OAC-extractable K in soil

The data in (Table 11) showed that soil K contents ranged from 333 to 500 mg kg^{-1} NH_4OA_C-extractable K due to mineral fertilizer and biocompost treatments. A highly significant increase in extractable K content was noted with the application of biocompost, whereby soil K increased, on an average, from 368 to 346 mg kg^{-1} NH_4OA_C-extractable K. Similarly, the effect of fertilizer application also produced highly significant increase in soil K from 363 mg kg^{-1} for control to 460 mg kg^{-1} where NPK treatment was applied. Further it was noted that the interactions between mineral fertilizer and biocompost were non significant.

Table 11 : Effect of biocompost and mineral fertilizer treatments on soil potassium (NH_4OA_C-extractable, mg kg^{-1}) after harvest of maize

Fertilizer treatment	No Biocompost	Biocomost 10t/h^{-1}	Fertilizer Mean
Control	333	393	363b
N	346	420	383b
NP	373	433	403ab
NPK	420	500	460a
Biocompost Mean	**368b**	**436a**	**402**

C V %	*12.57*
S.E	*50.59*
L.S.D @ 5%	
Fertilizer	*62.65*
Biocompost	*44.29*
Fertilizer x Biocompost	*NS*

We invesitigate the physical and chemical properties of mineral fertilizer and biocompost samples as well as maize growth. This study was conducted at the Department of Soil Science, Sindh Agriculture University Tandojam. The results of this study areas discussion below:

1. *Nutrient composition on pressmud*

The analytical data for the biocompost sample obtained from Matiari Sugar Mill, Matiari showed that the average values of total N, P and K contents were 1.8% N, 1.83% P and 0.9% K. Many studies have reported the nutrient composition of pressmud. Ibrahim *et al.* (1990) collected pressmud samples from five sugarmills of Punjab province of Pakistan and observed variable proportion of plant nutrients from one mill to another. The values ranged from 1.7-2.3, 1.0-1.3 and 0.6-0.8 % N, P and K respectively. Besides this, pressmud also contained sufficient amount of micronutrients, which ranged from 58-71, 4750-5904, 249-330, and 143-220 mg kg^{-1} Cu, Fe, Mn and Zn respectively.

2. *Pot experiment on maize*

The soil used for the experiment was a clay loam (31% clay) in texture, non saline (EC = 0.35 dSm^{-1}), alkaline in reaction (pH =7.87), low in organic matter (0.80%) and Olsen P (7.0 mg kg^{-1}) and high in NH_4OAc–extractable K (320 mg kg^{-1}). Application of biocompost alone increased maize plant height by 31.6% from 35.00 to 46.06 cm. similar increase (29.51%) was obtained with the application of N fertilizer. Application of biocompost along with N fertilizer increased the plant height further to 55.87 cm which corresponds to 59.6 % increase over control. And the interaction between

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mineral fertilizer and biocompost was non- significant. There was pronounced positive affect of addition of biocompost and mineral fertilizer alone or in combination on the dry weight of maize. Application of biocompost alone increased dry weight by 37% over control. Corresponding increase with N fertilizer was 27.1% whereas combined application of biocompost and N fertilizer increased it by 73.7% over control. And their interaction between mineral fertilizer and biocompost was found to be non- significant. The results of pot study on maize showed that there was pronounced positive effect of addition of biocompost on plant height and dry weights. Similarly, addition of fertilizer, particularly N, increased plant height and dry weights. However, similar study conducted by Memon (2005) revealed that their were pronounced positive effects of addition of fertilizers, particularly nitrogen on plant height and dry weights, and depressing effect of pressmud (5 t ha^{-1}) on maize growth and dry matter. However increase from 5 to 15 tons ha^{-1} slightly improved the growth and yield performance of maize. Drastic decline in maize dry matter yield was observed when the rate of pressmud was increased from 15 to 25 tons ha^{-1}. Plant analysis data revealed significant increase in N contents with the application of N fertilizer but P and K fertilization and pressmud did not significantly influence the N contents. It was hypothesized that the initial depressing effect of pressmud was related to presence of unrecompensed organic matter and the high rates of pressmud. Thus the benefit of pressmud was observed in a follow up experiment on wheat involving same soil and previously applied pressmud. These data therefore show that pressmud could be used in the fields for increasing crop production.

There was no effect of either biocompost or mineral fertilizer on the total N, P and K contents of maize. The interaction between mineral fertilizer and biocompost treatments were also non-significant.

3. *Physico-chemical properties of soil after harvest of maize*

The electrical conductivity of post harvest soil increased significantly with the application of biocompost, and also with combined application of fertilizer N, P and K. However, the interaction between biocompost and mineral fertilizer was non-significant. Post-harvest soil pH values did not show any effect of the mineral fertilizer biocompost treatments. There was no effect of fertilizer on the organic matter content of post-harvest soil. However the biocompost treatments showed highly significant increase in soil organic matter from 0.72 to 0.90 %, on an average. The interaction between mineral fertilizer and biocompost was non-significant. Olsen P content of post-harvest soil showed highly significant increase as a result of P fertilization (10.08 to 13.05 mg kg^{-1}) and biocompost (10.17 to 12.65 mg kg^{-1}) application. The interaction between mineral fertilizer and biocompost was also highly significant. Soil potassium (NH_4OA_C-extractable) increased significantly due to fertilizer from 363 to 460 mg kg^{-1}, on an average. In case of biocompost, a highly significant increase in soil K from 368 to 436 mg kg^{-1} was noted. The interaction between mineral fertilizer and biocompost was found to be non-significant. In one study Aziz *et al.* (2010) evaluated the beneficial effects of different sources of organic manures on soil physico-chemical properties and growth of maize. Organic manures viz. farm yard manure, poultry manure and pressmud were added in soil filled earthen pots at 10 t ha^{-1}. Results revealed that organic matter content, phosphorus and potassium bioavailability in soil and their uptake by plants were increased by organic manure application irrespective of the source. Likewise organic manure substantially improved the plant height, leaf area and shoots and root fresh and dry weights. Similarly shoot phosphorus and potassium contents were also improved by the application of organic manures.

IV. Conclusions and Recommendations

It is concluded that biocompost can be used as a soil amendment for improving soil organic matter and available nutrients and for increasing crop production. It is prepared to supplement this work through field studies on different crops for determining its real value to the former.

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Technical Efficiency of Ecologically Engineered Rice Production in the Mekong Delta of Vietnam: Application of SFA

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Abstract- An overuse of agro-chemicals in rice production has caused serious problems on biodiversity loss, water pollution, public health impacts and yield losses. Recently, the outbreaks of brown-plant hoppers was a great matter of concern. To deal with these issues, the use of ecological engineering was introduced in the Mekong Delta of Vietnam since 2009. However, there were no study on the potential benefits of the model in terms of technical efficiency. Hence, the objective of this study is to estimate and compare the technical efficiency of ecologically engineered rice farmers to those with traditional rice by using stochastic frontier analysis.

We have found that the eco rice farmers had higher input and output-oriented technical efficiency scores but insignificant compared to those with normal rice. The mean output-oriented technical efficiency of eco rice was 91.5%, which was 1% higher than that of traditional rice, 90.5%.

Keywords: *technical efficiency; stochastic frontier analysis; ecological engineering.*

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Technical Efficiency of Ecologically Engineered Rice Production in the Mekong Delta of Vietnam: Application of SFA

Vo Hong Tu [α] & Mitsuyasu Yabe [σ]

Abstract- An overuse of agro-chemicals in rice production has caused serious problems on biodiversity loss, water pollution, public health impacts and yield losses. Recently, the outbreaks of brown-plant hoppers was a great matter of concern. To deal with these issues, the use of ecological engineering was introduced in the Mekong Delta of Vietnam since 2009. However, there were no study on the potential benefits of the model in terms of technical efficiency. Hence, the objective of this study is to estimate and compare the technical efficiency of ecologically engineered rice farmers to those with traditional rice by using stochastic frontier analysis.

We have found that the eco rice farmers had higher input and output-oriented technical efficiency scores but insignificant compared to those with normal rice. The mean output-oriented technical efficiency of eco rice was 91.5%, which was 1% higher than that of traditional rice, 90.5%. Further, the mean input-oriented technical efficiency score of eco rice was 85% while only 83.5% for normal rice. The study suggests that the eco rice farmers need more efforts to expand output levels while the normal rice counterparts need to contract inputs level to improve productive efficiency and profits as well. The possible solution for the eco rice are not to use IR50404 variety. To improve the efficiency, the normal rice farmers need to cultivate three crops per year and to use OM6976 variety.

Keywords: technical efficiency; stochastic frontier analysis; ecological engineering.

I. Introduction

Increasing agricultural productivity, particularly in rice has been a long time and fist prioritized objective in the Mekong Delta of Vietnam (VMD), where is widely known as "rice bowl" of Vietnam, contributing annually more than 50% of total rice production (GSO, 2013). Technically, rice productivity in Vietnam has been increased steadily as a result of the application of new technology; for instance, hybrid rice varieties with shorter duration, higher yield and tolerance with diseases and the use of chemical fertilizers and pesticides. Such increased use of agro-chemicals (Thi Ut & Kajis a, 2006) is the source of environmental pollution, causing biodiversity loss, water pollution and public health impacts (Heong KL, 2009). Increasing demand on both quality (safer agricultural products in terms of lower use of agro-chemicals) and quantity (meeting population growth) has put more pressure on rice production –one of the staple food in Vietnam.

Owing to the importance of rice production in the VMD and its vulnerability to the outbreaks of brown-plant hoppers, the use of ecological engineering (abbreviated as eco hereafter), locally called as "*paddy field surrounded with flowers*" was first introduced in Tien Giang Province of the VMD since 2009 through the project which was technically coordinated by the international rice research institute (IRRI) and financially supported by Asian Development Bank (ADB). See the section of ecologically engineered rice production for more detailed information about ecological engineering. The model was then expanded to other provinces of the VMD thanks to its achievement in terms of much lower use of pesticide cost despite of slightly higher cost for flower planting. However, after more than four years from the first introduction, there have been no studies which concern about the efficiency of the model in the VMD have been conducted. As such it is crucial to estimate the benefits of the model in terms of the potential to reduce inputs so-called "input-oriented technical efficiency" and to increase an output level so-called "output-oriented technical efficiency".

So far, there have been two main approaches to estimate technical efficiency (TE) in the literature: data envelopment analysis (DEA) and stochastic frontier analysis (SFA). The results from these two methods differ slightly from each other. A reason for this difference is that SFA can separate noise effect apart from deterministic frontier while DEA can't. In addition, SFA is a parametric approach while DEA is non-parametric and based on mathematically programming. Depending on the purposes and the structure of data, we can choose one out of them or use both to estimate and compare the TE scores. In this study, we use SFA to estimate and compare the TE scores of eco rice farmers compared to those of traditional rice.

In 2004, Kompas estimated the TE scores of rice farmers in Vietnam by using panel data from 60 provinces and applying SFA. The study showed that the average TE was 78% for the Mekong Delta in 1999. Khai

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and Yabe (2011)also used SFA and the data from the Vietnam Household Living Standards Survey in 2006 to estimate such TE scores of 3,733 farmers. The study showed that the mean TE was 81.6%.However, neither of the two studies did consider about the stand-alone case of the VMD, particularly about eco rice.

Hence, it is essential to study the input-oriented and output-oriented TE of eco rice production as compared to traditional cultivation by using SFA and the factors affecting such efficiency scores.

The body of this paper is structured as follows. Section 2 describes the ecologically engineered rice production model in the VMD. Section 3 describes the analytical framework of using SFA to estimate the input and output-oriented TE and the data collection procedure. Following this, in section 4 we illustrate empirical results and discussions about TEs and the determinants affecting the efficiency for rice cultivation. Section 5 provides a summary and conclusions of the study.

II. Ecologically Engineered Rice Production in the VMD

Mitsch and Jørgensen (1989) were probably the first to define ecological engineering. The term has been adjusted during the implementation. Basically, they characterized ecological engineering as it: a) contributes to the restoration of ecosystems that have been substantially disturbed by human activities such as environmental pollution from rice production (i.e. overuse of agro-chemicals), and b) promotes the development of new sustainable ecosystems that have both human and ecological values (biological control).

Following this methodology, after the serious outbreaks of brown-plant hoppers in some Asian countries like China, Thailand, Philippine and Vietnam, the IRRI launched the project so-called "Rice Plant Hopper Project", which applied the ecological engineering to biologically control pests, particularly brown-plant hoppers. The project was financially supported by the ADB. In the VMD, the ecological engineering in rice was introduced in Tien Giang Province as a demonstration plot since 2009. Under the project, participated farmers were basically provided flower seeds and required to plant that kinds of flowers on bunds around the periphery of their paddy fields. The model was then expanded to other provinces thanks to its expected efficiencies. An Giang Province, the study site, adopted this model in 2011 and assigned the Department of Plant Protection to deploy and monitor the project using such method. Currently, hundreds of farmers have been adopting the ecological engineering in their paddy fields (PPDAG, 2012).

According to PPDAG (2012), the implementation process of the ecological engineering is illustrated within six main steps as follows:

- *Step 1:* Flower variety selection: flower varieties having colors of white and/or yellow are more preferable; they have fast growth rate, much pollen and nectar; and it is notable that flowering vegetables are also acceptable and encouraged. Indispensable flower varieties are cosmos, daisy and sesame.
- *Step 2:* Cultivation time of flowers: the flowers are normally planted two weeks before sowing rice. However, depending on flowering or blooming time of certain varieties of flowers, farmers should choose appropriate time.
- *Step 3:* Planting methods of flowers: depending on certain varieties we should choose the proper methods: transplantation, cut branches or sowing. Minimum area required for the model is 10 hectares, which must have at least one large bund as a main source of flowers or home to natural enemies.
- *Step 4:* Caring of flowers: the flowers require to be watered frequently. This is the main constraint for the diffusion rate of the model. It should be noted to take advantages of secondary plants, which are able to grow after harvesting.
- *Step 5:* Rice cultivation and caring: Applying "3 decreases, 3 increases" and "1 right, 5 decreases". Regarding to "3 decreases, 3 increases" technology, farmers will focus mainly on how to decrease seed amount, fertilizers and pesticides and increase yield, quality and profit. Similar to the nature of "3 decreases, 3 increases" technology, "1 right, 5 decreases" method requires more attention on reducing losses and inefficient use of resource. The term "right" stands for recognized rice varieties and 5 decreases contain reduces of seed, fertilizers, pesticides, water and post-harvest losses.
- *Step 6:* Flower harvest and seed selection for next rice crop: Collect flower seeds for next crops to save costs and take advantages of secondary flowers, which are able to grow after harvesting and cutting.

These six steps of the ecological engineering can be graphically described in figure 1. As shown from figure 1 that the use of ecological engineering or flower planting aimed at increasing the populations in terms of abundance and diversity, of natural enemies or beneficial organisms. Such increased populations of these natural enemies contributes to suppress vertically and horizontally pests populations leading to lower use of agro-chemicals. Theoretically, the output levels of the paddy fields with the ecological engineering are expected to be identical with that of normal rice fields despite of lower use of agro-chemicals. Together with the application of ecological engineering, farmers were also required to adopt new technologies – "3 increases, 3 decreases" and "1 right, 5 decreases" in their paddy fields. These methods were aimed at reducing

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production costs, including the reduction of agro-chemicals, which leads to higher profit and the protection of natural enemies as well. As consequences, paddy fields with ecological engineering have higher biodiversity and abundance of natural enemies as well as lower application of agro-chemicals as compared with traditional rice fields.

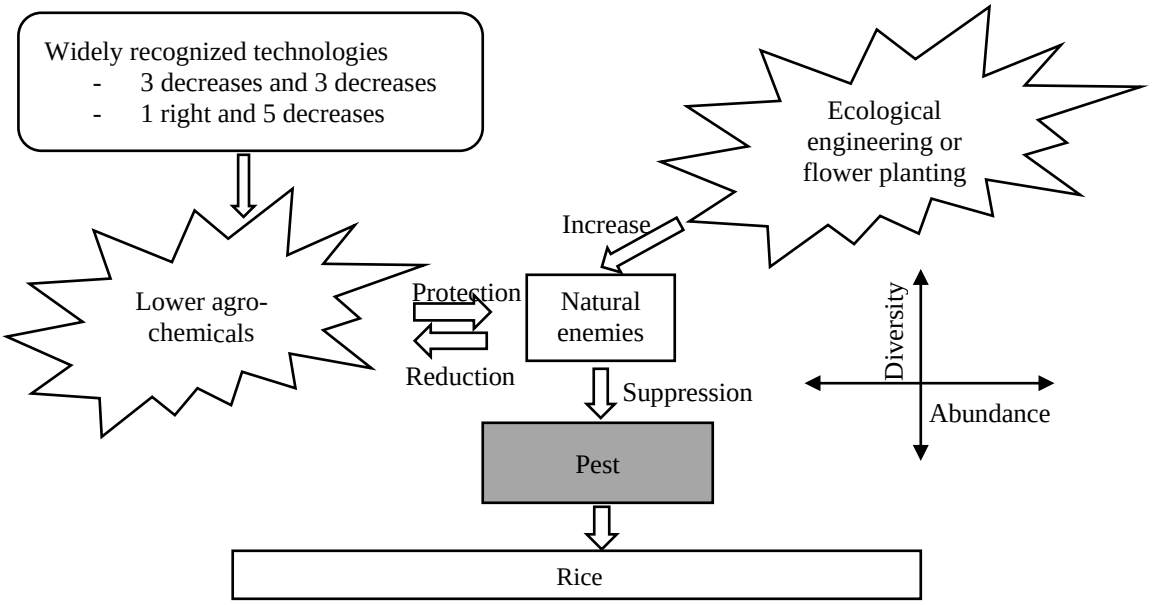


Figure 1 : The nature of the ecological engineering in An Giang Province

Source: the authors, 2015

III. Methodology

a) Measure of technical efficiency

In order to obtain the output-oriented TE, we assume a firm produces a vector of single output denoted as *Y*, with $Y \in R_+$ by using vector of inputs, which are denoted as *X*, with $X \in R_+$. In this study, we use Cobb-Douglas function form, which was commonly applied to estimate the stochastic production frontier in agricultural production (Battese, 1992; Bravo-Ureta & Pinheiro, 1993; Bravo- Ureta & Pinheiro, 1997; Coelli et al., 2005; Khai & Yabe, 2011). The stochastic production function of the *i-th* farmer in Cobb-Douglas form is given as follows

$$\ln Y_i = \beta_0 + \sum_{1}^{j} \beta_{ij} \ln X_{ij} + \varepsilon_i i \;=\; 1,2,\ldots,N \qquad (1)$$

where all farms are indexed with a subscript *i*; *j* is numbers of explanatory variables; β_j are parameters to be estimated; ε_i is a composed error term with $\varepsilon_i = v_i - u_i$, where v_i is symmetric, independently and identically distributed as ($v_i \sim N[0, \sigma_v^2]$), represents the exogenous effects such as impacts of adverse weather, natural disasters and acts of God, measurement errors and other statistical noises; and u_i is half-normal and nonnegative random error ($u_i \geq 0$), distributed as $u_i \sim N^+(0, \sigma_u^2)$, represents the technical inefficiency effect of the *i-th* farmer.

The output-oriented TE of the *i-th* farmer is obtained by multiplying e^{-v_i} on both sites of equation (1) and replacing the estimated parameters β with maximum likelihood estimates (MLE). This manipulation yields the measure of output-oriented TE as follows

$$OTE_i = \mathrm{e}^{-u_i} = \frac{y_i}{f(X_{ij}, \beta_j^*)e^{v_i}} \qquad (2)$$

According to Jondrow et al. (1982), u_i is predicted by the conditional expectation of u_i, given the value of random composed error variable ε_i The expression of u_i is given by

$$E(u_i|\varepsilon_i) \;= \sigma^* \left[\frac{\phi(\varepsilon_i \lambda/\sigma)}{(1 - \Phi(\varepsilon_i \lambda/\sigma))} - \left(\frac{\varepsilon_i \lambda}{\sigma}\right) \right] \qquad (3)$$

where $\sigma^* = (\sigma_u^2 \sigma_v^2/\sigma^2)^{1/2}$; and $\phi(.)$ and $\Phi(.)$ represent the standard normal density and cumulative distribution functions.

As regards the input-oriented TE, Reinhard et al. (1999); and Reinhard et al. (2000) proposed setting u_i=0 and replacing all inputs in equation (1) with $\Phi_i Z_i$, where Φ_i is the *i-th*in put-oriented TE score. This gives a new equation (4) as below

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$$\ln Y_i = \beta_0 + \sum_1^j \beta_j \ln \Phi_i X_{ij} + v_i \quad (4)$$

They then set equation (4) and (1) equally to estimate the input-oriented TE scores. The manipulation yields

$$\sum_1^j \beta_j \ln \Phi_i X_{ij} - \sum_1^j \beta_j \ln X_{ij} + u_i = 0 \quad (5)$$

Some manipulation of equation (5) yields the expression of the input-oriented TE as follows

$$\ln ITE_i = \frac{-u_i}{\sum_1^j \beta_j} => ITE_i = e^{\left(\frac{-u_i}{\sum_1^j \beta_j}\right)} \quad (6)$$

It is notablethat $\ln ITE_i = \ln ITE_i X_{ij} - \ln X_{ij} = \ln\left(\frac{ITE_i X_{ij}}{X_{ij}}\right)$.

The two measures of input and output-oriented TE are graphically illustrated in figure 2. The deterministic production function is described by the increasing, quasi-concave surface $OX_{2R}R_FX_{1R}$.Regarding to output-oriented TE, it is measured as the ratio of observed output level to maximum feasible output level that is reflected by $|OY_R|/|OY_Fe^v|$instead of $|OY_R|/|OY_F|$because SFA approach can separate noise effects apart from deterministic frontier, where Y_R and Y_Fe^v are the observed and maximum feasible output level, respectively, of farm R. The plane ABCR is the identical output quantity of farm R. As such, input-oriented TE showing the ability to contract inputs, holding output level constant, is measured as radial reduction of all inputs by$|Y_RB|/|Y_RR|$. According to Färe and Lovell (1978), the two measures are identical under constant returns to scale.

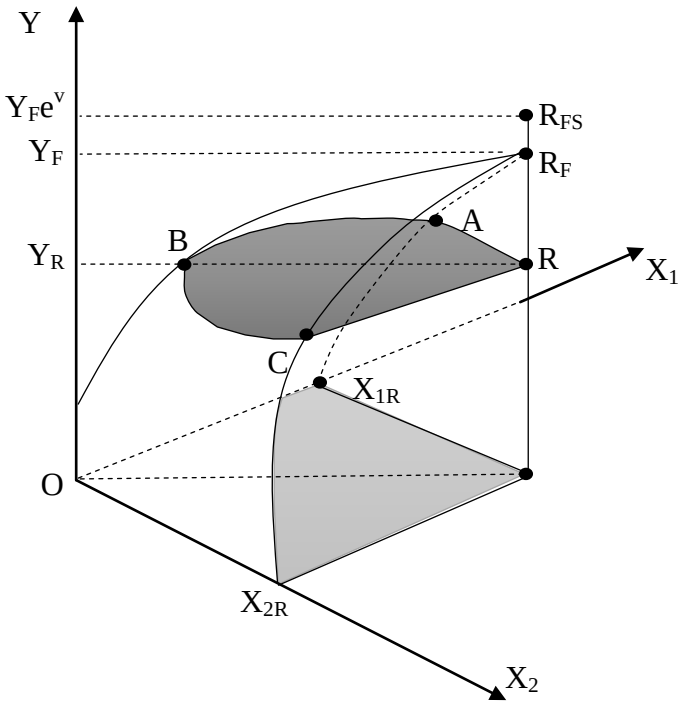


Figure 2 : Measures of output and input-oriented technical efficiency

Source: the authors, 2015

b) The factors affecting the efficiency

For policy implications and proper interventions, we use Tobit regression to identify the determinants of efficiency gaps, which was widely recognized as the second step of estimation in the literature (Bravo-Ureta & Pinheiro, 1993; Bravo-Ureta & Rieger, 1991; Bravo- Ureta & Pinheiro, 1997; Färe & Lovell, 1978; Khai & Yabe, 2011; Lee et al., 2009). The Tobit regression use efficiency scores as dependent variable having the scores censored at the maximum values. Independent variables are farm-specific characteristics such as farm size, family size and seed rice varieties.

Stata software version 12 was used to estimate stochastic production frontier and measure TEs as well as the determinants affecting such efficiency scores.

IV. Data Collection and Analysis

We conducted face-to-face interviews with 199 farmers, in which 74 of those are eco rice farmers and

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125 are normal rice counterparts. The survey was conducted in 2014 in An Giang Province of Vietnam, where was the second greatest rice producer in the VMD and adopted the ecological engineering since 2011(PPDAG, 2012). Based on the interviews with key informant panel of Provincial Center for Agricultural Extension An Giang, we selected 4 districts: Thoai Son, An Phu, Chau Doc and Chau Phu. These districts had performed the most successful application about the model. The main contents of the survey included production technology, from which we can estimates TEs and farm-specific characteristics, which are used for Tobit regression to investigate the factors affecting the efficiency.

Table 1 provides brief summary about the data set used for estimating production function.

Table 1 : Brief summary of the data set used for estimating production function

Indicators	Unit	Eco rice		Normal rice		T-value
		Mean	St.dev.	Mean	St.dev.	
Yield	Kg	7097,03	686,18	7147,79	672,73	-0.51
Nitrogen	Kg	101,73	21,53	112,54	23,03	-3.27***
Potash and Phosphorus	Kg	119,98	26,42	116,12	19,89	1.17
Pesticide	T.VND	3570,76	1216,21	4539,16	1527,9	-4.65***
Energy	T.VND	1511,11	567,71	1576,32	534,14	-0.81
Seed quantity	Kg	100,18	51,53	153,61	80,69	-5.11***
Labor	Days	261,02	31,22	245,76	39,64	2.83***
Capital	T.VND	893,6	560,96	3771,91	2295,02	-10.59***
Other expenditures	T.VND	6898.43	3140.73	4708.86	1344.38	6.81***

Source: Own estimates, data available from the authors

*Note: The numbers are the average values per hectare; *** indicates significant level of 1%*

Results from Table 1 show that the total amount of labor invested for eco rice were significantly larger than that for normal rice at the 1% level of significance, which is due to the higher requirement of labor for planting and caring flowers. The pure amount of potash and phosphorus fertilizer, energy and other expenditures incurred for eco rice were in significantly different from that for normal rice. However, nitrogen, pesticide, seed quantity and capital of eco rice were significantly lower at the 1% level of significance.

As mentioned in section "ecological engineering", eco rice was aimed at reducing pesticide use without compromising output level.In fact, the results from Table show that yield of eco rice was insignificantly lower than that of normal rice whereas pesticide use of eco rice farmers were significantly lower than those with normal rice at the 1% significant level. These results suggest that eco rice farmers achieved what they had expected from the adoption of ecological engineering.

V. Results and Discussion

a) Technical efficiency

According toBravo-Ureta and Pinheiro (1997); and Khai and Yabe (2011), before estimating the stochastic production function, ordinary least square regression (OLS) was used to identify the variables that significantly affect on the output. The estimates of the OLS function represents the "average" production function while the MLE yields the stochastic production frontier. The results of both OLS and MLE models were presented in Appendix A. Although the variable capital is one of main explanatory factors (Battese, 1992; Battese & Coelli, 1988; Bravo-Ureta & Pinheiro, 1997; Coelli et al., 2005; Khai & Yabe, 2011; Meeusen & Van den Broeck, 1977; Reinhard et al., 1999), it was excluded from the model in our study because of its insignificant correlation.

It is clearly shown in Appendix A that in case of OLS estimation, seed quantity was significant at the 10% level, labor and other expenditures were significant at the 5% level while the others were significant at the 1% level. In the case of MLE estimation, all parameter estimates were significant at the 1% level. The results from Breusch-Pagan test and Variance Inflation Factor (VIF) showed that neither multicollinearity nor heteroskedasticity was found in the model. According to Coelli et al. (2005), we could use either LR test or z-test to check the presence of technical inefficiency. Based on z-test, the z-value was 369.44 (i.e., 4.8766/0.0132), which exceeds the z-critical value of 3.09 at the 0.1% level of significance, suggesting that we reject the null hypothesis that there is no technical inefficiency.

In the Cobb-Douglas function, the coefficients show the proportional change in output when all inputs are changed. The sum of elasticities with respect to all

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inputs represents the production technology or returns to scale. According to Bravo-Ureta and Pinheiro (1997), the stochastic production function is the product of neutral upward shift of the average function, suggesting that the sum of elasticities of the both models (the OLS and MLE) are quite similar.

The sum of elasticities with respect to all inputs was approximately 0.52 in case of OLS and 0.54 in case of MLE (see Appendix A), which indicates that returns to scale are decreasing for rice farmers in the study sites. The computed F-statistic is 41.54, which exceeds the critical F value of 2.74 at the 1% level of significance, the null hypothesis of constant returns to scale therefore was rejected.

Now we turn to estimate the input and output-oriented TEs. Table 2 summarizes the TEs scores for both eco rice and normal rice.

Table 2 : Input and output-oriented TE scores of eco and normal rice

TE levels	Eco rice				Normal rice			
	Output		Input		Output		Input	
	Count	%	Count	%	Count	%	Count	%
≥90	46	62.2	24	32.4	80	64.0	47	37.6
80-90	28	37.8	26	35.1	31	24.8	37	29.6
70-80	0	0.0	23	31.1	12	9.6	23	18.4
≤70	0	0.0	1	1.4	2	1.6	18	14.4
Mean TE	91.5		85.0		90.5		83.5	
St.dev.	4.7		8.0		7.0		11.5	
Min	80.6		67.1		69.3		50.8	
Max	99.1		98.4		98.9		97.9	

Source: Own estimates, data available on request from the authors

As regards the output-orientation, the mean TE score of eco rice was 91.5%, which was about 1% higher than that of traditional rice, 90.5%. Further, the TE of normal rice had greater variation than that of eco rice with the former ranging from 69.3% to 98.9% while the latter falling in a range from 80.6% to 99.1%. These results suggest that eco rice farmers with minimum scores have the potential to increase the output level by 19%, while 30% for normal rice farmers, conditional on observed levels of inputs. As compared to those with the highest efficiency, the average eco rice farmers and normal rice farmers could realize to expand their output levels by approximately 7.7% (i.e., 1-[91.5/99.1]) and 8.5% (i.e., 1-[90.5/98.9]), respectively.

With regard to the input-orientation, as expected under the context of decreasing returns to scale, the input-oriented TE scores were smaller than the output-oriented TE scores. In fact, in the both cases of eco and normal rice, the input-oriented TE scores in the average was about 6.5% and 7.5%, respectively, smaller than the output-oriented TE scores. The mean TE score of eco rice (85%) was about 1.5% higher compared to those of normal rice (83.5%). Similarly, the input-oriented TE scores of normal rice had greater variation than that of eco rice, implying that eco rice farmers made use of inputs more efficiently, providing the observed level of output is constant.

Although eco rice farmers have joined the projects on the use of ecological engineering and received many technical training courses, which aimed at reducing production cost or inputs, it seems to be that the farmers focused much more on the production that the farmers focused much more on the production or output level than inputs contraction. A supported evidence for the statement is that 100% of the farmers had the output-oriented TE ranged above 80% while only about 67% in case of the input-oriented TE. Further, more than 30% of the farmers having the input-oriented TE scores distributed below 70%.However, as compared to those of traditional rice cultivation, the efficiency scores and distribution of eco rice was higher for all, suggesting positive and good signals of the use of ecological engineering and efforts of local extension workers.

Results from t-test, which was used to compare the significant difference in mean efficiency scores between eco rice and normal rice, show that t-values for input-oriented TE and output-oriented TE were 1.08 and 1.21, respectively. Although these results indicate that they were insignificantly different from each other at the 10% by using one-tail test, the differences in the accumulative distribution maybe reflects the potential benefits of eco rice. The detailed information about the differences in distribution of TEs scores between eco rice and normal rice is illustrated in figure 3.

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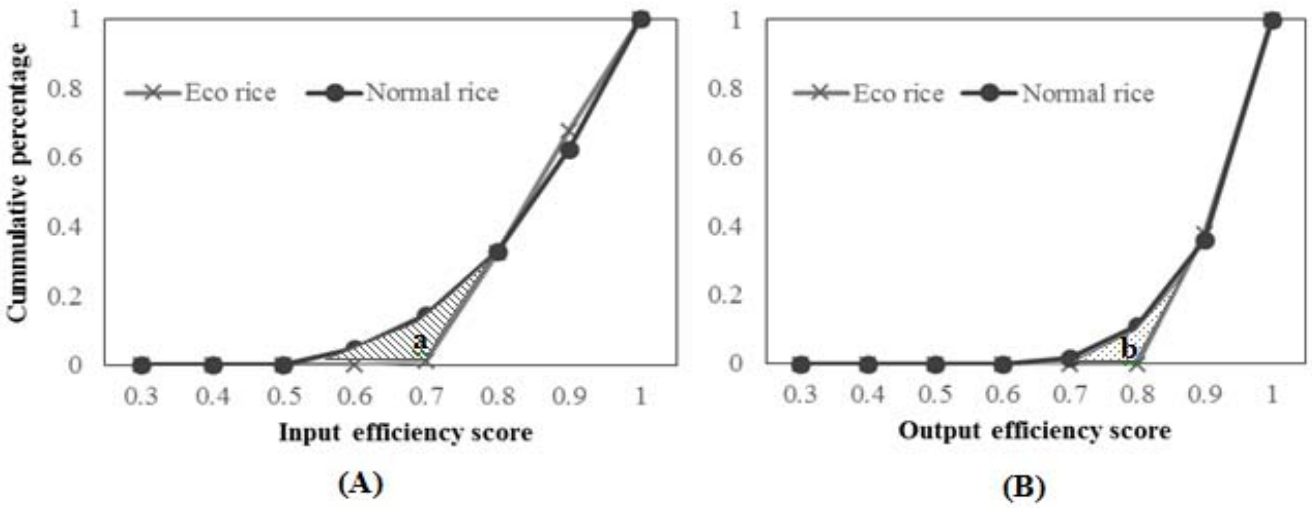


Figure 3 : Cumulative distribution of efficiency scores by groups of farmers

Source: the authors, 2015

As shown in figure 3 that the areas a and b indicate partially the potential losses of normal rice as compared to eco rice in both cases of input saving and output expansion, respectively.In fact, in case of input orientation, about 20% of normal rice farmers had scores distributed below 70% while 0% for eco rice counterparts, which reflects the positive effects of eco rice. According to figure 3 (A), as compared to a starting point of input-oriented TE of eco rice at 70%, those of normal rice with efficiency scores at 50%, 60% could realize to reduce about 29% and 14%, respectively, of their current use of inputs.

Similarly, in case of output-oriented TE, normal rice farmers with scores at 70% could recognize to contract about 12.5% compared with the starting point of eco rice. Moreover, in terms of efficiency, the potential losses of normal rice compared to eco rice were bigger in case of input-oriented TE than that of output-oriented TE (a > b).

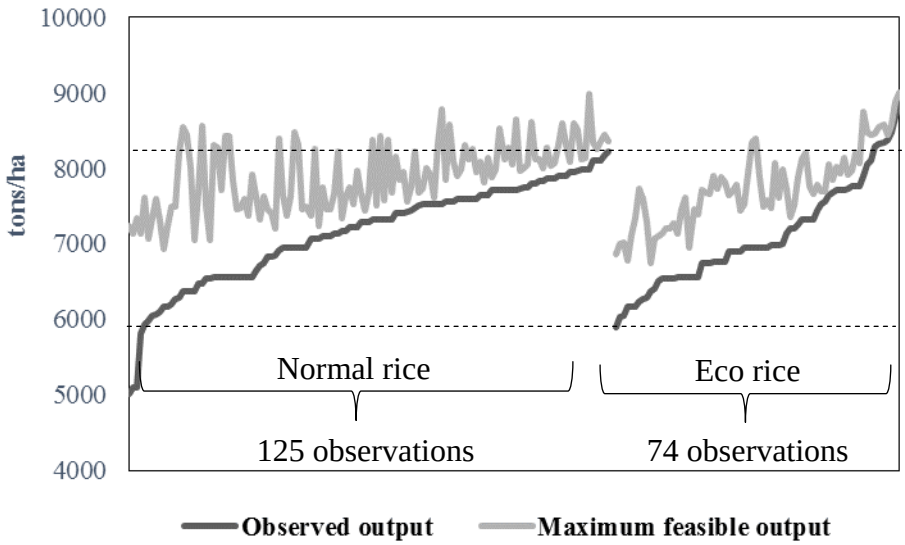


Figure 4 : Observed output and maximum feasible output

Source: the authors, 2015

For drawing better policy implications, the study also provide the general picture of observed output and stochastic frontier, which is illustrated in figure 4.

According to figure 4 and table 1, although the yields of eco rice and normal rice were not significantly different from each another, the distribution of observed output levels of eco rice was a bit higher as compared to that of normal rice; for instance, there were no eco rice farmers had output levels below 6 tons/ha. As regards stochastic production frontier, the farmers with

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normal rice had higher variation, particularly for those with observed output levels below 7.5 tons/ha, compared to those with eco rice. Under the context of decreasing returns to scale, a possible explanation is that those farmers extremely overused inputs levels. This result suggests that normal rice farmers need to pay more attention on the reduction of inputs to improve productive efficiency and profit as well.

On the contrary, the eco rice farmers need more efforts to consider the ways to improve output levels because the maximum possible output was approximately 9 tons/ha (figure 4). In the current situation, output expansion is one of the best solutions to attract more farmers to adopt the ecological engineering.However, output expansion based on inputs justification is not a profitable undertaking due to decreasing returns to scale. It is therefore essential to investigate the factors affecting the efficiency gaps.

b) The factors affecting the efficiency

So far, we estimated two kinds of technical efficiency: output-oriented and input-oriented TE for both eco rice and normal rice. Although the study suggest that the eco rice farmers should focus on output expansion while the normal rice farmers need more efforts to contract inputs, for broader options and different viewpoints of intervention, we will consider all kinds of efficiency in this section. In Tobit regression, all of them (kinds of efficiency) were considered as dependent variables to investigate separately the determinants of efficiency gaps.

The associated independent variables were farm-specific characteristics, including farm size (*farsize*), family size (*famsize*), numbers of crops per year (*crop*), sources of rice seed (*seed*), three main rice varieties (*IR50404, OM6976 and Jasmine*), pumping methods (*pump*) and eco rice (*eco*). These variables are those commonly incorporated in this step of estimation (Ahmad & Bravo-Ureta, 1996; Bozoğlu & Ceyhan, 2007; Bravo-Ureta & Evenson, 1994; Bravo-Ureta & Rieger, 1991; Bravo-Ureta & Pinheiro, 1997; Khai & Yabe, 2011). The detailed explanation of the variables are described in Table 3. The variables *farsize, famsize, sesource, OM6976, Jasmine and Eco* were expected to have significantly positive effects on the four types of efficiency scores while the variables *crop*, *IR50404* and *pump* have negative signs. The results of estimates with Tobit regression are presented in Table 3.

Table 3 : Tobit regression coefficients

Variables	Description	Eco rice		Normal rice	
		OTE	ITE	OTE	ITE
Farsize	Paddy area (ha)	0.0027**	0.0069**	-0.0006	-0.0010
Famsize	Number of members	0.0054	0.01290	0.0117***	0.0262***
Crop	1 = three crops/year 0 = two crops/year	-0.0120	-0.0286	0.0685***	0.1632***
Sesource	1 = verified source, 0 = otherwise	-0.0275**	-0.06992**	0.0012	0.0003
IR50404	1 = IR50404, 0 = otherwise	0.0197	0.0466	-0.0374**	-0.0800**
OM6976	1 = OM6976, 0 = otherwise	-0.0233	-0.0580	-0.0184	-0.0466
Jasmine	1 = Jasmine, 0 = otherwise	0.0411*	0.1017*	-0.0280	-0.0690
Pump	1 = self-pumping, 0 = co-operative	0.0441***	0.1098**	-0.0121	-0.0261
Constant		0.8742***	0.6728***	0.8129***	0.5386***
Sigma		0.0401	0.1010	0.0576	0.1328
Log-likelihood		129.9063	62.1061	176.9969	73.2951

*Note: ***, ** and * represent the significant levels of 0.01, 0.05 and 0.1, respectively*

OTE = output-oriented TE and ITE = input-oriented TE

Source: Own estimates, data available on request from the authors

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It is clearly shown in Table 4 that as a whole the variables that had positively significant impacts on the efficiency scores of eco rice was negatively significant in case of normal rice and vice versa. In the scope of this study, we had no explanation for this trend.

As regards eco rice farmers, the two variables *Crop* and *IR50404* had significant and negative impacts on the two types of efficiency (input and output-oriented TEs) at the significant level of 5%. The negative coefficients of *Crop* on both output and input-oriented TEs suggest that those who cultivated three rice crops per year had lower TEs scores as compared to those who cultivated only two crops annually. At first, the possible explanations are due to the overexploitation of soil fertile. However, this variable had positive and significant impacts on both TEs in case of normal rice. We could not make a plausible explanation for this adverseness.

Regarding to *IR50404*, the marginal effects of this variable in case of input-oriented TE and output-oriented TE were -5.1% and -8.7%, respectively, which indicates that the eco rice farmers who used IR50404 rice variety had lower TEs scores than those who used other varieties such as Jasmine and OM6976. In fact, the government has recommended not to use this rice variety massively because of its low quality.

Regarding to normal rice farmers, the variables *Crop* and *OM6976* had positive and significant connections with both input and output-orientated TEs at the 5% level of significance. The positive coefficient of *Crop* indicates that farmers cultivating three crops per year had higher TEs scores as compared to those producing annually two crops. As regards rice varieties, farmers who adopted OM6976 variety had higher TEs scores than those who used other ones.

VI. Conclusions

The study applied SFA to estimate and compare technical efficiency of 74 eco rice farmers to 125 normal rice counterparts in An Giang Province of Vietnam. We also investigated the determinants of efficiency gaps for all types of efficiency by using Tobit function.

We have found that returns to scale are decreasing. The farmers with eco rice had higher efficiency scores but insignificant as compared to those with normal rice. The mean output-oriented TE score of eco rice was 91.5%, which was 1% higher than that of traditional rice, 90.5%.The mean input-oriented TE score was also higher for eco rice (85%) than for normal rice (83.5%).Moreover, in terms of efficiency, the potential losses of normal rice compared to eco rice were bigger in case of input-oriented TE than that of output-oriented TE. As regards stochastic production frontier, the farmers with normal rice had greater variation, particularly for those with observed output levels below 7.5 tons/ha, compared to those with eco rice. Under the context of decreasing returns to scale, a possible explanation is that those farmers extremely overused inputs levels. The study also found that the maximum possible output was approximately 9 tons/ha.

The study suggests that the normal rice farmers need to pay more attention on the reduction of inputs to improve productive efficiency and profit as well while the eco rice farmers need more efforts to expand output levels. To improve the output-oriented TE for those of eco rice, results from Tobit regression show that the farmers should not use IR50404 variety and produce only two crops per year. To improve input-oriented TE for the normal rice farmers, besides new technology development, the study suggests that they need to cultivate three crops per year and to useOM6976 rice variety.

a) Competing interests

The authors declare that they have no competing interests.

VII. Acknowledgments

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Appendix A : Coefficients of production functions with OLS and MLE

Predictor	OLS		MLE	
	Coefficient	Std. error	Coefficient	Std. error
Nitrogen quantity	0.0899***	0.0305	0.1000***	0.0277
Potash and phosphorus	0.0937***	0.0304	0.0996***	0.0252
Pesticide cost	0.0639***	0.0176	0.0535***	0.0157
Energy cost	0.0622***	0.0167	0.0663***	0.0129
Seed expenditure	0.0133*	0.0078	0.0186***	0.0058
Labor	0.1343**	0.0633	0.1491***	0.0506
Other expenditures	0.0608**	0.0264	0.0534***	0.0204
Constant	5.6608***	0.4533	5.6901***	0.3601

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Function coef.	0.5181	Function coef.	0.5405	
F-test model	13.9200	λ	4.8766	0.0132
F-test CRS	41.5400	σ^2	0.0160	
R^2	0.3378	Log Likelihood	238.1771	

*Note: ***,**and * indicate statistically significant levels of 1%, 5% and 10%, respectively.*

Source: Own estimates, data available from the authors.

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"

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"

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2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

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Key points to remember:

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Approach:

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Content

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Approach:

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Abysinia · 20
Apically · 27

C

Chlamydospores · 22, 27, 35
Coexisted · 30, 31, 74
Columellum · 27, 34, 50, 52, 54
Conidiogenesis · 36, 116

D

Decanting · 25
Disarticulation · 31

E

Ellipsoidal · 27, 28, 34, 36
Enterothallic · 30, 35, 36, 44, 64
Epigenetic · 38

I

Incubated · 25, 26, 44, 58, 60
Integumented · 37
Intracellularly · 24

L

Lactophenol · 25

M

Micropipette · 26
Mucoraceous · 22
Mycelia · 27, 34, 50, 54, 158
Myoinositol · 24, 25, 26, 42, 44

N

Nondescriptness · 31
Nonpolarized · 36, 160

O

Offal · 1, 2, 4, 6, 8, 10, 12

P

Parboiled · 2
Phytate · 2
Pipettes · 26

S

Sphaeroplast · 33
Sporangiophores · 27, 35
Steenbergen · 166, 172, 174
Subapical · 36

T

Thawed · 26

U

Unsexed · 2, 4

Y

Yeastlike · 23, 24, 35



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