Indian J. mycol. Res., 18 (2), 57—66 (1980).

STUDIES ON CHANGES IN MICROBIAL POPULATION IN CONTINUOUSLY CROPPED RICE SOILS AT DIFFERENT FERTILITY LEVELS

BY

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Quantitative estimation of population of bacteria and fungi in soils continuously cropped with three crops of rice per year under different fertility levels for period of two years was undertaken. Crops of rice were aus (autumn rice)—variety tall indica Dular, aman (winter rice) and boro (summer rice)—variety dwarf indica IR8. Fertilizers were added in four different doses 1/3 optimum NPK, 1/3 optimum NP, 1/2 optimum NPK, optimum NPK—optimum in aus being 30 Kg N, 30 Kg P_2O_5 and 30 Kg K_2O per hectare. Three sets of control namely soil with plant without fertilizer, soil without plant, but with only fertilizer, and soil without plant and fertilizer. Soil samples were collected during vegetative and flowering stages of each crop as well as post harvest stages from remote soil and rhizosphere. Variations in populations were noted which were more related to stages of the crop, than to treatments which showed similar rrends. Intermediate fertility levels seemed to favour higher level of population. Influence of rhizosphere was evident in fungi, but not in bacteria. Fungal population attained higher level at the end of two years, but not bacterial. Studies on seven nutritional groups of bacteria, namely nitrifying, denitrifying, nitrogen fixing, sulphate reducing, sulphur oxidising, iron precipitating and cellulose decomposing showed that they were differentially present. Data on oxygen uptake in Warburg respiromerer showed decreased consumption of oxygen with increase in fertilizers, effect being more pronounced in remote soil than in rhizosphere. Variations in nitrogen phosphorus, and potassium content of the soil under different fertilizer treatments were noted. Nitrogen and phosphorus content had an influence on microbial growth, but potassium was not found to have any such effect.

INTRODUCTION

In recent years, an intensive programme of cultivation of rice has been taken up in the State of West Bengal. Wherever facilities of irrigation are

available rice is being grown continuously, two to three crops of rice being raised in the same land in a year. High yielding dwarf indicas, requiring higher dose of fertilizers are in cultivation. This practice of intensive cultivation of rice at high fertility level is likely to cause a change on edaphic conditions of rice plant. Altered physico-chemical conditions in the continuously cropped soil might bring about changes in microflora, both quantitatively and qualitatively. Chattopadhyay and Mukhopadhyay (1967) indicated change in microflora under such conditions in rice soil.

Present investigation has been undertaken to study soil microflora in relation to intensity of cropping and consequential changes in the major nutrients in the soil and their effect on soil microflora.

EXPERIMENTAL PROCEDURE

The plots for experimental study were located in the Faculty of Agriculture Farm at Haringhata. Three crops of rice, namely aus (autumn rice-sown in May, harvested in August) ; aman (winter rice crop-transplanted in August, harvested in December) ; and boro (transplanted in January and harvested in May) were grown varieties being Dular (tall indica) as aus, IR8 (dwarf indica) as aman and boro.

Soil was collected from four different places in each plot and homogenised in to one. Rhizophere and remote or outer soils were collected separately. From each homogenized lot four samples were drawn.

Population of bacteria and fungi were measured by dilution plate technique. For bacterial count 10^{-5} dilution and soil-extract agar medium (glucose, 1 gm., K_2HPO_4 1 gm., KNO_3 0·5 gm., Mg SO_4, $7H_2O$ 0·2 gm., $CaCl_2$ 0·1 gm., NaCl 0·1 gm., $FeCl_3$ 0·1 gm., distilled water-100 ml. Soil extract prepared by autoclaving 1 Kg. soil in 100 ml., of water at 15 lbs. pressure for 30 minutes. Solution was filtered after addition of a little $CaSO_4$. Volume was adjusted to 1000 ml. by addition of water. To 750 ml., of basal medium, 250 ml., of soil extract and bacto-agar-15 gm. were added and the entire content was sterilized at 15 lbs. pressure for 10 minutes) were used. For fungal count, 10^{-3} dilution, and Czapek Dox-agar medium ($NaNO_3$ - 2 gm., K_2HPO_4 - 1 gm., KCl 0·5 gm, Mg So_4, $7H_2O$ 0·5 gm., Fe SO_4 trace, sucrose-30 gm. agar agar-20 gm., distilled water 100 ml.) were used. Streptopenicillin at the rate of 30 unit per ml. was used to eliminate bacteria in determination of fungal population. Petriplates for study of fungi were incubated at 27°C for 120 hrs. and for bacteria at 34°C for 72 hours after plating. For purpose of calculation of microbial population from the soil solution per gm. of dry weight of the soil, 10 ml. of the stock solution was taken in an aluminium box and heated at 100°C in the oven until constant

weight was reached. Dry weight of the soil was determined and data expressed per gm. of soil.

Total nitrogen were determined following methods of Jackson (1962). Available phosphorus was determined after Fiske and Subba Rao (1925). For determination of available potassium, 5 gm. of soil sample was taken in an erlenmeyer flask containing 25 ml. of ammonium acetate solution (82 gm. of ammonium accetate dissolve in 1000 ml. of water) and pH adjusted to 7·0. The flask was shaken for 5 minutes, allowed to stand, and then filtered through Whatman No. 42 filter paper. To the filtrate, two drops of butyl alcohol were added and it was ready for flame photometric study. Quantity of potassium was determined from comparison with the standard curve.

Media used for study of nutritional groups were as follows :—(a) nitrifying bacteria—(i) K_2HPo_4 1·5 gm., distilled water 100 ml. (ii) $NaN0_2$ 1·5 gm., Na_2C0_3 1·5 gm., distilled water 100 ml. (iii) $MgS0_4.7H_20$ 0·45 gm., NaCl 0·75 gm., $Fe(S0_4)_3.9H_20$, 0·02 gm., distilled water 100 ml. (iv) bacto-agar 15 gm./100 ml. of distilled water. 1 ml. of each solution (i), (ii) and (ili) was added to agar solution. (b) nitrate reducing and denitrifying bacteria—(i) $KN0_3$ 1 gm., asparagine 1 gm., distilled water 250 ml., (ii) citric acid. 5 gm. or neutral sodium citrate 8·5 gm., K_2HP0_4 1 gm., $MgS0_4.7H_20$ 1 gm., $CaCl_2.6H_20$ 0·2 gm., $FeCl_3$—trace, distilled water—250 ml. Citric acid solution was neutralised with 10% NaOH solution. Two solutions were mixed and made up to 100 ml. to which 15 gm. of bacto-agar were added. (c) reduction of sulphate and sulphur compounds—asparagine 1 gm., K_2HP0_4 0·5 gm., $MgS0_4.7H_20$ 1 gm., $FeS0_4$ trace, lactic acid 4 gm, tap water 100 ml., bacto agar 15 gm. distilled water 900 ml. (d) Sulphur oxidising bacteria-sodium thiosulphate 5 gm., K_2HP0_4 0·1 gm., $NaHC0_3$ 0·2 gm., NH_4Cl 0·1 gm., bacto-agar 15 gm., distilled water-1000 ml. (e) Nitrogen fixing bacteria-mannitol 10 gm. K_2HP0_4 0 5 gm., $MgS0_4$, $7H_20$ 0·2 gm., NaCl 0·2 gm., $MnS0_4.4H_20$ trace, $FeCl_3$-trace, bacto-agar 15 gm., distilled water 1000 ml. (f) Cellulose decomposing bacteria-cellulose 2·5 gm., peptone 0·5 gm., K_2HP0_4 0·2 gm., $MgS0_4.7H_20$-0·2 gm., K_2C0_3-0·4 gm., $CaCl_2$ 0·02 gm., bacto-agar-15 ml., distilled water 1000 ml. (g) Iron precipitating bacteria-$(NH_4)_2S0_4$ 5 gm., $NaN0_3$ 0·5 gm., K_2HP0_4 0·5 gm., $MgS0_4.7H_20$ 0·5 gm., $CaCl_2$ 0·2 gm., bacto agar-15 gm., distilled water 900 ml., ferric ammonium citrate 10 gm. in 100 ml. sterilized separately and added before plating.

EXPERIMENTAL RESULTS

I. Quantitative estimation of soil microflora.

Quantitative estimation of population of bacteria and fungi in continuously cropped rice soil was undertaken for a period of two years. Fertilizer was added to the soil in different doses as follows :—

(i) With no fertilizer — Sample S_1

(ii) With 1/3 optimum dose of N, P and K — ,, S_2

(iii) with 1/3 optimum dose of N, P without K — ,, S_3

(iv) with 1/2 optimum dose of N, P and K — ,, S_4

(v) with optimum dose of N, P and K — ,, S_5

optimum dose for aus—variety Dular (tall indica) N-30Kg/ha ; P_2O_5-30Kg/ha ; K_2O-30Kg/ha ; for aman and boro I.R.8 (dwarf indica) N-90Kg/ha and P_2O_5-90Kg/ha. K_2-90Kg/ha.

Two different types of control namely soil without plants, but with fertilizers in optimum dose-S6, soil without plants and fertilizers-S7.

These seven treatments were replicated in randomized blocks with four replications. In one calendar year, seven samples were taken covering three growing crops of rice-aus, aman and boro - two samples each one at vegetative stage and another at flowering stage for three rice crops and one in the post-harvest fallow stage in between. In two years, samples were taken altogether at 14 different stages, details given in the Tables. Samples in respect of both types of control were taken in the second year. They were drawn from both rhizosphere and remote soil. Results together with statistical analysis are presented in Tables 1 to 5, each figure in the Table representing an average of 16 replicates (4 replicates in the experiment and 4 samples per replicate).

From data presented in Tables and Statistical analysis of the same, a distinct rise and fall in the population of bacteria could be noticed, and differences between the stages, as a whole were statistically significant.

Highest peak was noticed in the vegetative crop of second crop of boro and in the post harvest stages. Statistically significant difference was not observed between population of bacteria in remote soil and rhizosphere, Changes were more marked in remote soil, but pattern of variation was similar in both the cases.

A perusal of figures (Tables 5) in the control sets without plants (with or without addition of fertilizers) similar functuations could be seen in population of bacteria at different stages of observation. High level of population during the period coinciding with post-harvest fallow in January and vegetative stage of second boro crop point out that total size of fluctuations could not be entirely due to effect of crop, other factors pertaining to soil might be involved.

In different fertilizer treatments, similar trends in respect of fluctuations could be noticed but variations were noted. Statistically significant results relating

to increase in soil and in soil and rhizosphere were obtained at S3 (1/3 optimum N and P without K) as well as in S4 (1/2 optimum N, P and K). At the fertility level optimum for crop growth, bacterial population was comparatively low particularly in remote soil. Absence of potassium was not found to have any adverse effect, on the other hand a small stimulatory effect. In control sets without plants addition of fertilizers was found to have a marginally beneficial effect on bacterial population.

So far fungal population was concerned wide fluctuations in population were not noticed, as in bacteria, though differences at various stages could be observed. Fungal population did not show clearly distinct troughs or peaks as in bacteria, particularly in rhizosphere, though in remote soil the same were more marked. Population of fungi tended to attain progressively higher level with continuous cropping, in bacteria no such tendency was perceptible. Highest level was noticed in the vegetative stage of second boro crop and population was also high in the post-harvest fallow in January.

Fungal population tended to remain high in rhizosphere particularly at intermediate fertility levels. It was statistically significant than the same in remote soil. At no particular level of fertilizers, consistently more population of fungi could be recorded.

II. Determination of nutritional groupings in bacteria

For determination of groupwise bacterial populations needing specific requirements, bacterial populations of remote soil and rhizosphere at two specific stages of growth of rice, namely vegetative and flowering of second crop of boro were taken. These two stages are chosen, because highest population was recorded during the vegetative growth of second crop of boro which was followed by a sharp decline during the flowering period.

Bacteria were grown in specific nutritional media using dilution plate technique and number of colonies were recorded in each case. Seven different media were used for classification of bacteria into as many groups, namely (a) nitrifying bacteria, (b) nitrate reducing and denitritying bacteria, (c) bacteria reducing sulphate and other surphur compounds, (d) sulphur oxidising bacteria, (e) nitrogen fixing bacteria, (f) cellulose decomposing bacteria, and (g) iron precipitating bacteria. Data obtained were computed to the number per gm. of soil and percent distribution in terms of total population. Results are presented in summerised from in Tables 6 and 7, each figure represents an average of 16 replicates - 4 samples per replicate and 4 replicates.

From the data the following observations may be made. The most striking feature observed was that the sum total of populations recorded in different nutritional groups were always more that recorded in soil extract medium.

Nitrifying bacteria (Group A) were the least abundunt of all under all conditions studied. Some variations were however noted, namely population was comparatively higher in S_2 (1/3 N, P & K) and S_3 (1/3 N, and P) in comparison to S_1 (0 NPK). In the flowering stage, higher percentage was recorded in plots but control of the corresponding period did not show much variation. Differences in vegetative and flowering stages were not marked except at higher levels of fertilizers, namely $\frac{1}{2}$ N, P and K and optimal N. P and K.

Population of denitrifying bacteria (Group B) was also comparatively low. In the vegetative phase, wider fluctuations were noticed in comparison to flowering phase wherein population was comparatively higher. Similarly rhizosphere showed greater abundunce of the bacteria than remote soil.

Percentage of sulphur reducers (Group C) was much higher in comparison to nitrifying and denitrifying bacteria. This was true both in rhizosphere and remote soil, and in vegetative and flowering stages of the crop. Comparatively higher figures were noted in the flowering phase than in the vegetative and in rhizosphere than in remote soil. Variations were found to be much less.

Sulphur oxidising bacteria (Group D) were found to be moderately prevalent. They were found to be more in remote soil than in rhizosphere, and in the vegetative stage than in the flowering stage. Variations were observed, but they did not appear to be correlated with treatment.

Population of nitrogen fixers (Group E) was high under different fertility levels both in remote soil and rhizosphere. In the vegetative stage of the crop, it was highly more in rhizosphere than in remote soil, while in the flowering stage, the position was reverse. In general, more nitrogen fixers were recorded in the flowering stage than in the vegetative. Variations were noted among treatments. In control plots with fertilizers, but without plants, population was low, but in the same without plants and fertilizers, it was high. Presence of high level of unutilized nitrogen in the absence of crop may have a depressing effect.

Cellulose decomposing bacteria (Group F) were found to be fairly well distributed both in rhizosphere and remote soil, being more abundunt in the flowering stage.

Influence of rhizosphere was more noticable at lower levels of fertility where population was also higher. Usually low percentage was recorded at $\frac{1}{2}$ optimum NPK level.

Iron precipitating bacteria (Group G) were found to be in much higher number in the vegetative stage than in the flowering stage, both in rhizosphere and remote soil. Much less variation was recored in rhizosphere than in remote soil.

Nitrifying, denitrifying and sulphur-reducing bacteria were less abundunt in the soil in the vegetative stage of the crop, when the soil was water logged than in the flowering stage when soil was gradually drying up. Nitrogen fixing and cellulose decomposing bacteria were more in the vegetative stage, but differences were not marked. Sulphur oxidizing and iron precipitating ones were conspicuously more abundunt in the vegetative stage of the crop.

III. Study of activity of soil microflora by Warburg Respirometer

Katznelson and Roualt (1957) carried out studies in Warburg respirometer to determine metabolic activity in rhizosphere and remote soil by manometric methods.

Activity of microorganisms, as indicated by increased consumption of oxygen due to higher rate of respiration, in soils subjected to different fertility treatments in rhizosphere and remote soil was studied in Warburg respirometer. Soil samples collected were spread in thin layer on ordinary paper for one hour at room temperature, and then passed through 30 mesh sieve, and those passing through sieve were collected. Moisture content of each sample was determined separately. In all the samples, it was adjusted to seventy per cent water holding capacity. 7 gm of soil in the form of small pellets was introduced into each Warburg flask to which 3 ml of sucrose solution containing 50 μg/ml of sucrose was added. 2 ml of KOH was added in the central well. Vessels were attached to the manometers and placed in water bath at 30°C. After one hour equilibrium period manometers were closed and the fluid level was fixed at a set point. Manometric readings were taken after a period of six hours and the differences were recorded. Volume of oxygen uptake was determined by methods described by Umbreit et al (1957). Data are presented in Table 8. It would be seen that as judged from oxygen uptake, there was lowering of activity of microorganisms in the soil to which fertilizers were added. This effect was progressively pronounced with increased dose of fertilizers, which was more noticable in remote soil. In rhizosphere reduction in oxygen uptake was much less.

IV. Analysis of different chemical factor at various stages of crop growth in relation to microcial population

Nitrogen, phosphorus and potassium content of soils receiving different doses of these nutrients were determined at different stages of growth in three crops of rice grown for a period of two years as well as of post-harvest fallows in the way described before. Data are presented in Tables 9, 10 and 11. Correlation of changes in different nutrients to microbial population is given in Table 12.

Nitrogen

Considered over a period of two years and a fall in nitrogen was observed in all cases irrespective of treatments. Higher values were observed in the first post-harvest fallow between aman and boro followed by sharp decline in boro season with rise again in next kharif and subsequent fall. Different treatments showed similar trends, with minor variations.

Correlation studies showed that though not statistically significant, nevertheless a response with nitrogen level, which was conspicuous at optinum level. In case of fungi significant correlation was noticed at optinum level.

Phosphorus

Phosphorus content registered sharp alternate rise and fall followed by a sharp decline. Rises were noticed in the early stage and in the post-harvest stages in the first season.

There was a positive correlation with higher phosphorus content which was particularly evident in fungi. In soils receiving no treatment and low doses, there was however a negative correlation. Significant positive correlations were obtained with both fungi and bacteria at optinum level.

Potassium

Comparatively small rise and fall were noticed. Similar trends were observed in different treatments. Hardly any correlation was noticed between potassium and microbial population except in the optinum dose where fungal population was found to be have significant negative correlation.

DISCUSSION

Changes in microbial population both fungi and bacteria recorded in various treatments as well at different stages may be related to both physical and chemical conditions of the soil. Though definite relationships could not be established in all cases, yet it was observed that during the vegetative stage of growth of crop, lesser number of bacteria were recorded, while in flowering and post harvest stages, more number of bacteria were recorded. This fluctuation can probably be explained to some extent on the basis of competition between plant and microbes for nutrients. Besides in the vegetative stage, soil remains water logged in aman and boro or near saturation in aus whereas in the flowering stage it gradually dries up and water logged condition gradually disappears and water content is low. Data from control uncropped soil, where in similar trends were noticed indicate that apart from cropping, other factors might be involved in changes in microbial population.

In fungi, an overall increase in population was noticed at the end of two years, this might be due to certain advantages of soil fungi in their morphological features and methods of perennation through resting spores and ability to colonise and survive on dead tissues. It is however significant in view of the fact that in continuously cropped rice areas, there has been an increase in attack of *Sclerotium oryzae* and *Corticium sasakii*.

Rhizospheric influence on bacteria was not significant. This is in contrast to general findings and may be due to soil physical conditions—amorphous or structurless condition of the soil during puddling and water logged condition. These need to be investigated as previous works were exclusively on upland soils. In fungi, a positive response was however noticed.

Studies on nutritional groupings, indicate qualitative changes apart from quantitative ones. How far they are related to physical conditions peculiar to rice soils or nutrient status needs to be investigated.

Studies on microbial activity in Warburg respirometer present an interesting situation. While addition of fertilizers particularly in remote soil brings a gradual decline in oxygen uptake indicating decreased metabolic activity but a corresponding decrease in population is not noticed in soil. Rhizosphere did not always register increased population of bacteria as compared to remote soil, but activity was more as evident from much less diminution in oxygen uptake may be due to availability of amino acids and other nutrients through root exudates, as indicated by Rovira and McDougall (1967).

Of the nutrients, nitrogen and phosphorus contents were found to show variations, as well as definite influence on microbial growth. It was noticed that where residual nitrogen was high, namely in flowering and post harvest stages, bacterial population was high. At higher levels of phosphorus, a positive correlation was gradual decline in nitrogen and phosphorus status of the soil after continuous cropping of rice over a period of two years. This shows that nutrients are being removed at a greater rate than being replenished. Diminished microbiological activity either due to fall in population or otherwise will mean that recuperativa properties of the soil would be less. This situation will need in depth study and application of corrective measures.

It may be pointed out that a study over a period of two years may only be indicative of likely changes. Investigations over a longer period would be helpful in arriving at definite conclusions. Comparative findings on waterlogged and dry soils is likely to yield fruitful results.

Table 1. Data on population of bacteria ($X10^5$) per gram of soil under different treatment at different stages of growth of rice

Stages of observation	Fertilizer treatment									
	S_1 (0)		S_2 (1/3 opt, NPK)		S_3 (1/3 opt, NP)		S (1/2 opt, NPK)		S_5 (opt, NPK)	
	S*	R**	S*	R**	S*	R**	S*	R**	S*	R**
Flowering of aus 1st yr. July	228	213	43	100	122	151	81	280	91	78
Veg. phase of aman 1st. yr. Sept.	40	30	24	28	40	100	81	150	26	40
Flowering of aman 1st. yr. Nov.	172	114	173	80	141	121	381	256	131	57
Post-harvest fallow between aman and boro 1st. yr. Jan.	333	90	123	86	261	83	173	129	102	70
Veg. phase of boro 1st. yr. March	80	104	253	81	92	66	73	33	154	45
Flowering of boro 1st. yr. April	170	32	27	29	125	24	74	66	36	47
Post-harvest fallow between boro and aus 1st. yr. May	289	219	278	218	209	75	425	138	211	115
Veg. phase of aus—7 2nd. yr. June	64	151	168	213	100	67	295	127	98	100
Flowering of aus—8 2nd. yr. July	145	95	103	102	134	215	194	398	126	481
Veg. phase of aman—9 2nd. yr. Sept.	82	240	142	116	169	64	220	136	80	100
Flowering of aman—10 2nd. yr. Nov.	131	75	92	65	316	146	187	81	122	130
Post-harvest fallow—11 between aman and boro 2nd. yr. Jan.	111	60	253	152	208	133	196	111	273	154
Veg. phase of boro—11 2nd. yr. March	891	354	463	392	3600	16195	3650	576	544	636
Flowering of boro—13 2nd. yr. April	283	487	388	295	143	396	170	187	266	577

S*—Remote R**—Rhizosphere

Table 2. Statistical Analysis of the Data of Table 1.

Analysis of variance

Source	Degrees of Freedom	Sum of square	Mean square	F
Replication	3	471090.6	—	N. S.
Treatments	139	1416296079.1	—	—
Stages	13	372362243.3	28643249.6	60·02**
Soil Fertilizer	9	68890336.5	—	—
Fertilizer	4	63226100.2	15806525.1	33.12**
Soil (Soil and rhizosphere)	1	12852.6	12852.6	N. S.
Interaction Fertilizer X Soil	4	5651383.7	1412845.9	29.61**
Interaction Stage X Soil fertilizer	117	975043499.4	8333705.1	17.46**
Error	417	198971909.5	477150.9	—

**—Highly significant. N. S.—Not significant.

C. D. at 5% between stages—302.7220 ; S. E. (m)— ± 109.22.

C. D. at 5% between fertilizers—189.5712 ; S. E. (m)— ± 68.40.

C. D. at 5% between Soil—114.4248 ; S. E. (m)— ± 41.28.

Table 3. Data on population of fungi ($X10^3$) per gram of soil under different treatment at different stages of growth of rice

Stage of observation	Fertilizer treatments									
	S_1 (0)		S_2 (1/3 opt, NPK)		S_3 (1/3 opt, NP)		S_3 (1/2 opt, NPK)		S_4 (opt, NPK)	
	S*	R**	S*	R**	S*	R**	S*	R**	S*	R**
Flowering of aus 1st. yr. July	53	41	32	23	45	62	59	42	40	41
Veg. phase of aman 1st. yr. Sept.	7	2	2	63	7	68	1	63	8	10
Flowering of aman 1st yr. Nov.	35	7	11	20	29	14	25	67	37	13
Post-harvest fallow between aman and boro 1st. yr. Jan.	33	51	24	45	28	72	56	42	58	36
Veg. phase of boro 1st. yr. March	26	39	55	32	49	34	73	15	40	25
Flowering of boro 1st. yr. April	34	20	31	22	78	36	30	53	53	26
Posr-harvest fallow X between boro and aus 1st. yr. May	43	41	38	77	42	21	43	32	36	52
Veg. phase of aus—7 2nd. yr. June	45	37	52	70	53	38	47	48	39	33
Flowering of aus—8 2nd. yr. July	58	33	27	31	39	58	87	58	71	48
Veg. phase of aman—9 2nd. yr. Sept.	59	66	62	51	41	49	44	63	51	76
Flowering of aman—10 2nd. yr. Nov.	39	34	23	44	44	63	68	49	53	97
Post-harvest fallow—11 between aman and boro 2nd. yr. Jan.	62	40	30	42	57	26	69	63	48	41
Veg. phase of boro—12 2nd. yr. March	67	69	51	64	82	42	51	114	45	81
Flowering of boro—13 2nd. yr. April	45	100	34	43	48	63	36	66	39	49

S*—Remote soil. R**—Rhizosphere.

Table 4. Statistical Analysis of the Data of Table 3

Analysis of variance

Source	Degrees of Freedom	Sum of square	Mean square	F
Replication	3	425.3	141.76	1.16 (N. S.)
Treatment combination	139	215837.2	1552.78	12.81**
Stages	13	64105.1	4931.16	40.68**
Soil fertilizer	9	13118.4	1457.60	—
Fertilizer	4	10067.1	2516.77	20.67**
Soil (Soil and rhizosphere)	1	1017.1	1017.10	8.39**
Interaction stages X Soil fertilizer	117	138613.7	1184.73	9.77**
Error	417	50535.7	121.20	—

**—Highly significant ; N. S.—Not significant

C. D. at 5% between stages—15.3 ; S. E. (m)— ± 5.51,

C. D. at 5% between fertilizers—3.0 ; S. E. (m)— ± 1.09,

C. D. at 5% between soil—1.8 ; S. E. (m)— ± 0.66.

Table 5. Data on population of bacteria and fungi in control soil

Stages of observation	Soil without plant, but with fertilizer (S_6)		Soil without plant and fertilizer (S_0)	
	Bacteria ($X10^5$)	Fungi ($X10^3$)	Bacteria ($X10^5$)	Fungi ($X10^3$)
Veg. phase of aus 2nd. year June	55	38	102	32
Flowering of aus 2nd. year July	310	84	267	61
Veg. phase of aman 2nd. year Sept.	210	37	182	54
Flowering of aman 2nd. year Nov.	176	36	162	37
Post-harvest fallow between aman and boro, 2nd. year Jan.	145	37	310	63
Veg. phase of Boro 2nd. year March	359	64	317	63
Flowering of Boro 2nd. year April	413	32	324	20

Table 6. Data on the frequency of bacteria of different nutritional groups in remote soil and rhizosphere under different fertilizer treatments in the vegetative phase of boro (Population—figure X10^5)

Soil	Nutritional Media	A		B		C		D		E		F		G		T_1	T_2
	Fertility level	a	b	a	b	a	b	a	b	a	b	a	b	a	b		
REMOTE	S_1 (0)	5	0.2	122	4.9	535	21.5	598	21.8	428	16.8	248	9.9	613	24.1	2549	891
	S_2 (1/3 NPK)	1	0.09	7	0.61	181	15.2	172	15.1	137	12.6	145	12.7	491	43.8	1134	463
	S_3 (1/3 NP)	19	0.85	37	1.7	649	29.3	314	14.6	220	10.2	484	22.5	446	20.8	2169	3600
	S_4 (1/2 NPK)	14	0.17	31	0.35	1421	16.7	2545	30.0	361	4.3	79	0.93	4094	47.5	8495	3650
	S_5 (NPK)	8	0.55	34	2.15	290	18.7	257	16.5	250	16.1	338	21.4	380	24.3	1563	544
	S_6 (Control with NPK)	12	1.5	15	1.8	187	21.9	77	9.0	87	10.3	186	21.9	286	33.6	850	359
	S_7 (Control)	3	0.4	39	3.9	253	25.2	103	10.4	213	20.3	103	10.4	301	29.9	1014	317
RHIZOSPHERE	R_1 (o)	4	0.5	21	2.5	135	15.7	50	5.9	109	12.7	135	15.7	390	46.3	844	354
	R_2 (1/3 NPK)	12	1.4	12	1.4	240	28.0	68	8.1	84	9.8	95	11.2	285	34.0	745	392
	R_3 (1/3 NP)	3	0.22	12	0.88	78	5.2	19	1.3	498	33.1	303	19.8	603	39.8	1508	16195
	R_4 (1/2 NPK)	7	0.5	18	1.4	303	19.6	184	12.5	262	17.5	133	8.9	598	39.5	1505	576
		14	0.7	119	6.1	361	20.5	228	13.0	275	15.4	161	8.8	620	35.2	1764	636

a—Population of Bacteria ; b—% of Total Bacteria ; T_1—Grand Total ; T_2—Total in Soil extract agar

Table 7. Data on the frequency of bacteria of different nutritional groups in remote soil and rhizosphere under different fertilizer treatments in the flowering phase of boro (Population—figure X10^5)

Soil	Nutritional Media	A		B		C		D		E		F		G		T_1	T_2
	Fertility level	a	b	a	b	a	b	a	b	a	b	a	b	a	b		
REMOTE	S_1 (0)	9	0.63	81	5.6	341	24.0	128	8.9	344	24.2	214	11.1	294	20.6	1421	283
	S_2 (1/3 NPK)	100	4.3	181	7.8	564	24.4	516	22.3	510	22.0	277	11.9	163	7.0	2311	388
	S_3 (1/3 NP)	37	3.6	48	4.6	135	13.1	12	1.1	579	56.2	91	8.8	117	11.3	1029	143
	S_4 (1/2 NPK)	14	1.1	43	3.4	336	26.8	211	17.5	293	23.9	192	16.1	136	10.6	1255	170
	S_5 (NPK)	21	1.0	136	6.4	570	27.1	118	5.6	647	30.8	222	10.6	399	19.0	2113	266
	S_6 (Control with NPK)	27	1.39	132	6.9	437	23.1	302	14.3	261	13.4	463	23.6	330	17.2	1952	413
	S_7 (Contrcl)	9	0.54	86	5.1	232	15.1	117	0.9	612	36.6	246	18.1	317	19.2	1669	324
RHIZOSPHERE	R_1 (0)	8	0.30	155	6.6	538	25.8	175	7.2	395	18.6	376	17.4	520	24.1	2167	487
	R_2 (1/3 NPK)	4	0.4	177	8.3	490	39.9	88	6.9	204	15.8	231	18.0	154	11.9	1288	295
	R_3 (1/3 NP)	6	0.48	58	4.5	262	20.8	205	16.9	262	20.8	235	18.3	257	19.8	1277	396
	R_4 (1/2 NPK)	20	2.2	73	9.4	209	25.4	78	9.6	199	24.3	79	9.4	165	17.8	823	187
	R_5 (NPK)	66	3.2	183	9.1	420	20.9	177	8.8	344	17.7	404	20.1	413	20.5	2007	577

a—Population of Bacteria ; b—% of Total Bacteria ; T_1—Grand Total ; T_2—Total in Soil extract agar

Table 8. Data on activity of microflora of soil and rhizosphere under different fertility conditions as determined by oxygen uptake in Warburg Respirometer after a period of 6 hours

Soil type	Fertility level	Oxygen uptake	Relative ratios
Remote Soil	S_1 (Without NPK) (ON, OP, OK)	70.1	100
	S_2 (1/3 opt. NP) (30N, $30P_2O_5$ and $30K_2O$ Kg/ha)	47.5	67.8
	S_3 (1/3 opt. NP) (30N, $30P_2O_5$ Kg/ha)	44.8	64.0
	S_4 (1/2, opt. NPK) (45N, $45P_2O_5$, $45K_2O$ Kg/ha)	42.8	61.0
	S_5 (opt. NPK) (90N, $90P_2O_5$, $90K_2O$ Kg/ha)	6.1	9.1
Rhizosphere	R_1 (without NPK)	66.3	100
	R_2 (1/3 opt. NPK)	63 2	95.8
	R_3 (1/3 opt. NP)	58.9	89.0
	R_4 (1/2 opt. NPK)	54.2	82.0
	R_5 (opt. NPK)	52.4	79.8

Table 9. Data on pH of soil samples collected at different stages of growth of different crops of rice

Stage of observation	Fertilizer treatments						
	S_1 (no NPK)	S_2 (1/3 opt, NPK)	S_3 (1/3 opt, NP)	S_4 (1/2 opt, NPK)	S_5 (opt. NPK)	S_6 (opt. NPK, no crop)	S_7 (no crop, no NPK)
Flowering of aus 1st. yr. July	7.05	7 05	7.04	7 3	7.2	—	—
Veg. phase of aman 1st. yr, Sept.	7 0	7.0	7.0	7.0	7.0	—	—
Flowering of aman 1st. yr. Nov.	6.9	6.5	6 5	6 5	6.9	—	—
Post harvest fallow between aman and boro 1st. yr. Jan.	6.7	6.5	6 5	6.7	6.5	—	—
Veg. phase of boro 1st. yr. March	7.1	7.1	7.0	7.0	7.1	—	—
Flowering of boro 1st. yr. April	7.0	7.1	7.2	7.3	7.1	—	—
Veg. phase of aus 2nd. yr. June	7 5	7.5	7.4	7.5	7.4	7.4	6.7
Flowering of aus 2nd. yr. July	6.8	6.8	6.8	6.8	6 8	6.8	6.8
Veg. phase of aman 2nd. yr. Sept.	6.9	6.9	6.9	6.9	6.9	7.1	6.9
Flowering of aman 2nd. yr. Nov.	7.2	7.2	7.2	7.2	7.2	7.2	7.1
Post harvest fallow between aman and boro 2nd. yr. Jan.	6.8	6.9	6.8	6.8	6.8	6.9	6.9
Veg. phase of boro 2nd. yr. March	7.1	6.9	7.0	6.9	7.0	7.1	7.2
Flowering of boro 2nd. yr. April	7.5	7.4	7.5	7.6	7.6	7.5	7.7

Table 10. Data on available phosphorus content (ppm) l/6g. of soil under different fertility treatments

Stages of observation	Fertility treatments				
	S_1 (0)	S_2 (1/3 opt, NPK)	S_3 (1/3 opt, NP)	S_4 (1/2 opt, NPK)	S_5 (opt, NPK)
Flowering of aus 1st. yr. July	3200	3840	3840	6720	5460
Veg. phase of aman 1st. yr. Sept.	3400	1600	1600	1280	5920
Flowering of aman 1st. yr. Nov.	3200	2240	3200	3840	4160
Post harvest fallow between aman & boro 1st. yr. Jan.	3200	4800	9600	2560	7040
Veg. phase of boro 1st. yr. March	7680	6720	5120	4480	4480
Flowering of boro 1st. yr. April	5760	4480	5400	3840	6400
Veg. phase of aus 2nd. yr. June	10240	11420	7680	7360	9600
Flowering of aus 2nd. yr. July	2400	2640	2160	3360	1200
Veg. phase of aman 2nd. yr. Sept.	1920	1920	1760	2160	2160
Flowering of aman 2nd. yr. Nov.	920	1200	1200	1280	880
Post harvest fallow between aman & boro 2nd. yr. Jan.	1120	890	1360	1280	1340
Veg. phase of boro 2nd. yr. March	900	700	800	1100	950
Flowering of boro 2nd. yr. April	700	600	900	800	400

Table 11. Data on available potassium content μg/l/5g. of soil under different fertility treatments

Stages of observation	Fertility treatments				
	S_1 (0)	S_2 (1/3 opt, NPK)	S_3 (1/3 opt, NP)	S_4 (1/2 opt, NPK)	S_5 (opt. NPK)
Flowering of aus 1st. yr. July	17.6	17.2	17.2	16.4	13.6
Veg. phase of aman 1st. yr. Sept.	16.4	12.8	13.2	12.0	20.0
Flowering of aman 1st. yr. Nov.	18.4	17.6	15.6	18.2	19.6
Post harvest fallow between aman and boro 1st. yr. Jan.	12.0	12.0	11.2	20.0	20.0
Veg. phase of boro 1st. yr. March	19.2	18.8	17.2	18.4	19.0
Flowering ef boro 1st. yr. April	17.2	18.4	16.8	18.0	18.8
Veg. phase of aus 2nd. yr. June	17.2	20.4	19.6	19.2	17.6
Flowering of aus 2nd. yr. July	18.4	18.4	17.2	16.8	14.8
Veg. phase of aman 2nd. yr. Sept.	19.6	22.0	21.0	21.6	20.0
Flowering of aman 2nd. year	21.0	18.0	17.2	17.6	17.6
Post harvest fallow between aman and boro 2nd. yr. Jan.	17.2	18.4	17.2	16.8	14.8
Veg. phase of boro 2nd. yr. March	20.0	22.0	21.0	18.0	19.0
Flowering of boro 2nd. year April	15.0	19.0	18.0	14.0	16.0

Table. 12. Data on 'r' single correlation coeffecients between some chemical factors and microbial population at different fertility levels

Nature of Organisms	Fertilizer treatment	Chemical factors		
		Nitrogen	Phosphorus	Potassium
Bacteria	S_1	−.16	−.21	.04
	S_2	+0.25	−.13	.09
	S_3	+0.11	−.29	0.03
	S_4	−.10	.21	.12
	S_5	−.39	.55*	.006
Fungi	S_1	−.24	−.35	.02
	S_2	−.18	.29	.10
	S_3	−.11	.41	.003
	S_4	.06	.41	.029
	S_5	+.74*	.57*	−.77*

* Significant

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