CHAPTER 3

MATERIALS AND METHODS
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3.1 Site description

The experimental site is located at Jalukbari which is on the south bank of river Brahmaputra. Geographically the site is located at 26°12'N latitude and 91°50'E longitude at an altitude of 62 meters above the mean sea level. The experimental materials comprised of seed sample of *Aijung* rice. The variety was chosen for its present popularity amongst the rice farmers of most of the districts of Assam as monsoon paddy (*Sali* rice) in low land aquatic rice ecosystem. Seeds of *Aijung* rice variety were obtained from the local farmer of Jalukbari area. The variety has average life cycle duration of 150 days. The variety exhibited active vegetative stage of growth with vigorous growth of root system and other crop characters contributing to higher yield performance. The experiments were carried during the years 2006-2008. Soil samples were collected aseptically at monthly interval and in different growth stages of rice plant from the experimental plots during the course of investigation. Fertilizers were not used during the period. The pot culture experiments were laid out in the Botanical garden of Gauhati University. All equipments, glass wares and chemicals were provided by department of Botany, Gauhati University for the study.

3.2 Climatic condition of the sampling site

The place occupies a unique position amidst complex geologic and physiographic make-up, characterized by hilly areas and high lands and low lying aquatic sites. The area in general forms an integral part of the South- east Asiatic Monsoon lands, under varying climatic conditions and geomorphic processes. The low hill ranges with hot and humid climate plays a dominant role exhibiting local weather phenomenon and its climatic individuality. The area lies in the regime of monsoon climatic of sub-tropical zone
characterized by heavy summer rainfall, winter drought and high humidity. Some of the factors determining the climatic pattern of the area are:-

- Its location and physiography
- Presence of extensive water bodies and rivers
- Seasonal variation in the pressure condition over Bay of Bengal and North-western Indian landmass
- Tropical oceanic air-masses of South-west monsoon
- Local mountain and valley winds and
- Luxuriant vegetation.

The above-mentioned features affect the aerial distribution of rainfall. Based on various distinctions regarding the trend, tendency, distribution of temperature, rainfall, rainy days, fogs and thunder storms the weather of the area may be divided into four primary seasons:-

i. **Pre-Monsoon:** this season is short, begins in the early part of March and continues up to the end of May. From the month of March the land surface is steadily heated and the temperature rises but the western disturbances brings moderate rainfall to heavy rainfall and reduces the shooting temperature. The month of April and early part of May is characterized by disappearance of morning fogs and occasional thunderstorms. From the meteorological data it is seen that average maximum temperature of both the year ranges from 30.7°C to 33.7°C (Fig 1a). The relative humidity ranged from 63 to 77% with an average of 72% during the period. Rainfall ranges from 18.7 mm to 290.3 mm in 2006 and 29.8 to 286.6 mm in 2007 during this Pre-Monsoon period (Fig.3.1). The rainfall amount during this period was 489.5 mm and 412.6 mm respectively, which is 37.8% and 24.5% of the total rainfall of the year.

ii. **Monsoon:** Monsoon season starts during the later part of May and it last up to the first part of October. Widespread low clouds and high humidity (75 to 85%) maintains more or less uniform temperature (31.4 to 34.1°C) throughout the
period. Rainiest months of the period are May (290.3 mm) and July (247.2 mm) in 2006 and June (294.2 mm) and July (286.8 mm) in 2007 when more than 82 to 85% of the total annual rainfall occurs. This is the most important season for Sali rice cultivation. The warm and sultry weather is oppressive, but most congenial for wet cultivation. In fact this is the season of dominant agricultural operation in Assam.

iii. Retreating Monsoon: The southwest monsoon withdraws sometimes in between the last part of September and first part of October and continues up to the middle of November, when fog commonly occurs. The maximum temperature recorded during the period is 32.6°C in month of September and minimum temperature of 17.9°C in the month of November. The period is characterized by dry and cold climate with rainfall amount rapidly tapering towards November (15.9 to 32.2 mm). From the month of October the weather becomes progressively drier. With the advance of the season the ground cooling begins and the morning fog appears. The weather progressively cleans up and the fair, sunny days prevail till the end of November.

iv. Dry winter: The winter season begins in the middle of November (27.9 to 29.8°C) and continues up to the end of February (25 to 28.8°C). The season is characterized by very little amount of rainfall with an average of 2.5% to 7.6% of the total rainfall of the year. January and December was found to be the driest month. Relative humidity remains fairly constant during the period.

From the above-mentioned four seasons, highest temperatures were recorded towards July (33.7°C) to August (34.1°C) in the year 2006 and May (33.7°C) and August (34°C) in 2007. May recorded the highest rainfall (290.3 mm) in 2006 and June (294.2 mm) in 2007. January recorded the lowest minimum temperature (11.1°C and 9.9°C) in 2006 and 2007 respectively without any rainfall.

During the course of investigation meteorological data such as rainfall, temperature and humidity (Fig. 3.1-3.3) were taken from the Indian Meteorological Station, Borjhar, Guwahati, Assam.
Fig. 3.1 Monthly variation of temperature (°C) at the study site

Fig. 3.2 Monthly variation of relative humidity (%) at the study site

Fig. 3.3 Monthly variation of rainfall (mm) at the study site
3.3 Soil Analysis for physico-chemical characteristics

Soil samples were collected aseptically from the experimental rice field at monthly interval during the period from January-December 2006. Five soil samples were collected randomly from 0-20cm depths and the collected soil samples were mixed thoroughly to prepare a composite sample, air dried and sieved through 1mm sieve before analysis. Physico-chemical analyses of the collected soil samples were carried out by standard procedure.

3.3.1 Soil particle size and textural class analysis

The particle size analysis was done by Buoyoucos hydrometer method (Buoyoucos, 1962; Gee and Bauder, 1986). Aliquot of an air-dried and sieved sample (40g) was taken in a beaker to which 100 mL of 2% sodium hexa-metaphosphate solution were added. After 18 hr, the suspension was shaken for 10 min and then volume was made to 850 mL with distilled water. The initial temperature ($t_1$) and hydrometer reading ($h_1$) of the suspension were noted after 4.5 min and final temperature ($t_2$) and hydrometer reading ($h_2$) were noted after 120 min. The percentage of clay, silt and sand were calculated by the following equations:

\[
\% \text{ Clay} = \frac{(h_2 + t_2) \times 100}{\text{soil weight (g)}}
\]

\[
\% \text{ Silt} = \frac{(h_1 + t_2) - (h_1 + t_1) \times 100}{\text{soil weight (g)}}
\]

\[
\% \text{ Sand} = 100 - (\% \text{ Clay} + \% \text{ Silt})
\]

From the percentage of sand, silt and clay the textural class of soil was analysed using textural triangle.

3.3.2 Estimation of soil pH

Soil $p^H$ was determined by an electrical digital $p^H$ meter in a 1: 5(w/v) soil-water suspension. 5g soil sample was taken into labelled 50 mL plastic tubes to which added 25 mL of distilled water. The soil-water suspension was shaked for 1h using the mechanical
shaker. The soil was allowed to settle for a few minutes and the pH was measured after a two point (pH 4 and pH 7) calibration of the pH meter.

3.3.3 Estimation of soil moisture

Soil moisture was estimated by Gravimetric method. 100 g of fresh soil sample was taken in an aluminium moisture box and kept in the oven at 105°C for about 24 hr until all the moisture is driven off. After removing from oven, they are cooled to room temperature and weighed again. The difference in weight was considered to be moisture contained in 100 g soil sample. The percentage moisture content was calculated as follows-

\[
\text{Moisture (\%)} = \frac{\text{Loss in weight}}{\text{Oven dry weight}} \times 100
\]

3.3.4 Estimation of soil temperature

Soil temperature was recorded with a soil thermometer at the time of sampling.

3.3.5 Estimation of water holding capacity (WHC)

Water holding capacity was determined by the method of Veihmeyer and Hendrickson (1931). A uniform plot measuring 5 m x 5m was selected and filled with water to completely saturate the soil. The plot is covered with a polythene sheet to check evaporation. After 24 hr of saturation, soil sample was collected in a moisture box from the centre of the plot and the percentage of moisture in the soil was calculated.

\[
\text{WHC} = \frac{Y-Z}{Z-X} \times 100
\]

Where,

- \(X = \text{Weight of empty box}\)
- \(Y = \text{Weight of moisture box + moist soil}\)
- \(Z = \text{Weight of moisture box + oven dry soil}\)
3.3.6 Estimation of soil organic carbon

Soil organic carbon was analysed by Walkley and Black’s rapid titration method (Jackson, 1973; Walkley and Black, 1934). 1 g of air dried soil was transferred to 500 mL Erlenmeyer flask to which added 10 mL of 0.167 M \( \text{K}_2\text{Cr}_2\text{O}_7 \) solution (prepared by dissolving 49.04g of dried \( \text{K}_2\text{Cr}_2\text{O}_7 \) in water and diluted to one L) using a pipette. To these added 20 mL of concentrated \( \text{H}_2\text{SO}_4 \) using a dispenser and the beaker was swirled to mix the suspension. The beaker was allowed to stand for 30 minutes. The suspension was diluted to 200 mL with water and to which added 10 mL of 85% \( \text{H}_3\text{PO}_4 \) and 0.2 g of \( \text{NaF} \). About 10-15 drops of ferroin indicator (prepared by dissolving 3.71 g of o-phenanthroline and 1.74 g of \( \text{FeSO}_4.7\text{H}_2\text{O} \) in 250 mL of water) was added just prior to titration. The solution is titrated with 0.5M \( \text{Fe}(\text{NH}_4)_2(\text{SO}_4).6\text{H}_2\text{O} \) (prepared by dissolving 196 g of ferrous ammonium sulfate in 800 mL of water containing 5 mL of concentrated \( \text{H}_2\text{SO}_4 \) and diluted to 1L) until the colour changes from violet-blue to green. Two blanks were prepared containing all the reagents but without soil. The percentage oxidizable organic carbon and finally the total organic carbon (\( \text{C}_{\text{tot}} \)) were calculated as follows.

\[
\% \text{ Oxidizable Organic Carbon (w / w)} = \frac{(V_{\text{blank}} - V_{\text{sample}}) \times 0.3 \times M}{Wt}
\]

To convert oxidizable organic carbon to total carbon the above result is multiplied by a constant factor 1.33.

\[
\% \text{ Total Organic Carbon (w / w)} = 1.33 \times \% \text{ Oxidizable Organic Carbon}
\]

Where,

- \( M \) = Molarity of ferrous ammonium sulfate solution (approx. 0.5M)
- \( V_{\text{blank}} \) = Volume of ferrous ammonium sulfate solution required to titrate blank (mL)
- \( V_{\text{sample}} \) = Volume of ferrous ammonium sulfate solution required to titrate the sample (mL)
- \( Wt \) = Weight of air-dry soil (g)
- 0.3 = \( 3 \times 10^{-3} \times 100 \), where 3 is the equivalent weight of C
3.3.7 Estimation of total nitrogen

Total nitrogen was estimated by Kjeldahl method (Jackson, 1973). 1g of soil sample was taken and placed in Kjeldahl flask. To this added 0.7g of CuSO₄, 1.5g K₂SO₄ and 30mL H₂SO₄. The mixture was heated until frothing ceases. The solution was allowed to boil until it becomes clear and digestion was continued for at least 30 min. The flask was then removed from the heater and allowed to cool and transferred to distilling flask by addition of 50mL of water. 20 mL of 0.1M H₂SO₄ was taken in the receiving conical flask and 2-3 drops of methyl red indicator added to it. Enough water was added to cover the end of the condenser outlet tubes. 30 mL of 35% NaOH was added in the distilling flask in such a way so that the contents do not mix. The content was heated to distil the ammonia for about 30-40 minutes. The flask was then removed and the outlet tube into receiving flask is rinsed with a small amount of distilled water. The excess acid in the distillate was titrated with 0.1M NaOH (prepared by dissolving 4g NaOH in 1L water and standardized against 0.1N H₂SO₄). Blanks on reagents were determined using same quantity of standard acid in a receiving conical flask. Percent nitrogen was calculated as follows.

\[
\% \text{Nitrogen} = \frac{1.40 (V_1 M_1 - V_2 M_2) - (V_3 M_1 - V_4 M_2)}{W} \times 100
\]

Where,

\(V_1= \text{mL of standard acid taken in receiving flask for samples}\)
\(V_2= \text{mL of standard NaOH used in titration}\)
\(V_3= \text{mL of standard acid taken to receiving flask for blank}\)
\(V_4= \text{mL of standard NaOH used in titrating blank}\)
\(M_1= \text{Molarity of standard acid}\)
\(M_2= \text{Molarity of standard NaOH}\)
\(W= \text{Weight of sample taken in g}\)
3.3.8 Estimation of available phosphorus

For the determination of available phosphorus Brays method no.1 method was used (Bray and Kurtz, 1945).

1. Preparation of standard curve: Standard stock solution was prepared by dissolving 0.1916g of pure KH₂PO₄ in 1L of distilled water. This solution contains 0.10 mg P₂O₅/mL. 10 mL of this solution was added to 1L of distilled water. This solution contains 1µg P₂O₅/mL. From this, 1, 2, 4, 6 and 10 mL of solution was taken separately in 25 mL flasks. To each flask, added 5 mL of Bray’s extractant no.1 (0.03M NH₄F in 0.025 HCl), 5 mL of molybdate reagent (prepared by dissolving 1.5 g of (NH₄)₂MoO₄ in 300 mL of distilled water followed by addition of 350 mL of 10M HCl solution and the final volume diluted to 1L) and the mixture was diluted to about 20 mL with distilled water. 1mL of dilute SnCl₂ was added and diluted to 25 mL mark. After 10 min, the blue colour of the solution was read on spectrophotometer at 660 nm. The absorbance reading was plotted against µg P₂O₅.

2. Extraction: 50 mL of Bray’s extractant no.1 was added to the 100 mL conical flask containing 5 g soil sample. The solution was shaken for 5 min and then filtered.

3. Development of colour: 5 mL of the filtered soil extract was taken in a 25 mL measuring flask to which added 5 mL of molybdate reagent and diluted to 20 mL with distilled water. 1 mL of dilute SnCl₂ was added and diluted to 25 mL mark. After 10 min, the blue colour of the solution was read on spectrophotometer at 660 nm with the blank prepared similarly but without the soil. The amount of available P (kg/ha) was calculated as follows.

\[ P (\text{kg/ha}) = \frac{A}{1000000} \times \frac{50}{5} \times \frac{2000000}{5} = 4A \]

Where,

Weight (g) of the soil taken = 5 g
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Volume (mL) of the extract \* = 50 mL
Volume of the extract taken for estimation = 5 mL
Volume made for estimation (dilution = 5 times) = 25 mL

Amount of P observed in the sample on the standard curve = A (µg)

Weight of 1 ha of soil up to a depth of 22 cm is taken as 2 million kg.

3.3.9 Estimation of available Potassium

Potassium was estimated by flame photometric method (Jackson, 1973). The molar neutral ammonium acetate solution was prepared by dissolving 77g of ammonium acetate in 1L of distilled water and pH adjusted to 7 by either ammonium hydroxide or acetic acid as per the need. Standard potassium solution was prepared by dissolving 1.908g pure KCl in 1L of distilled water. This solution contains 1 mg K/mL. 100 mL of this solution was taken and and diluted to 1L with ammonium acetate solution. This gives 0.1 mg K/mL as stock solution. For preparation of working standard solutions, 0.5, 10, 15 and 20 mL of the stock solution was taken separately in 100 mL volumetric flask and diluted to 100 mL with the M ammonium acetate solution. These solutions contain 0.5, 10, 15 and 20 µg K/mL respectively. 5g of soil sample was taken in a 150 mL Erlenmeyer flask and 25 mL of neutral N ammonium acetate was added to it. The above mixture was put in a reciprocating shaker for 5 min and filtered through Whatman No.1 filter paper. K in the extract was estimated in a flame photometer using K filter after necessary calibration of the instrument.

\[ K \text{ (kg/ha)} = A \times \frac{25}{5} \times \frac{200000}{100000} = 10A \]

Where,

A = content of K (µg) in the sample, as read from the standard curve.

Weight of 1 ha of soil up to a plough depth of 22 cm is approximately 2 million kg.
3.4 Quantitative determination of soil microflora at different depth of rice crop field

3.4.1 Collection of soil samples

Soil Samples from the rice crop field were collected aseptically at monthly intervals from the experimental plot during the course of the investigation. During the study chemical fertilizers were not used. All the equipments such as conical flask, beakers, Petri plates and pipettes were sterilized prior to the experiment. Soil samples were collected throughout the cropping season (July-December 2007). The soils were collected randomly from ten different spots of the experimental field at three depths: (0-10 cm), (10-20 cm) and (20-30 cm) using a sterilized hand auger. The soil samples were collected in polythene bags and are sealed immediately following collection to get rid of any sort of external microbial contamination. In dry season considerable quantities of post-harvest materials like straw and dead roots were found to occur in the rice field soil during the crop season. The collected soil samples were mixed thoroughly and the composite samples were sieved through 1mm sieve to separate the soil debris and soil clod. For chemical analysis, samples were air dried and sieved through 0.2 mm sieve.

3.4.2 Analysis of physico-chemical properties of soil

The pH of the soil samples were measured with an electrical digital pH meter in 1:5 (w/v) soil-water suspensions. Soil moisture content was determined by drying 10 g of fresh soil in a hot air oven at 150°C for 24 hr. Soil organic carbon was analyzed using rapid titration method of Walkley and Black (1934), as described by Jackson (1973). Total nitrogen was estimated by Kjeldahl method (Jackson, 1973). For the determination of available phosphorus Brays method no.1 method was used (Bray and Kurtz, 1945). Potassium was estimated by flame photometric method (Jackson, 1973).

3.4.3 Quantification of soil microflora

1g soil sample has been dissolved in a 100 mL of distilled water taken in a 250 mL conical flask. Suitable dilutions were prepared from the soil suspension for
determination of soil microflora. For bacteria and actinomycetes counts were carried out at $10^{-5}$ dilutions respectively, and for fungal counts $10^{-4}$ was used. Prepared soil suspensions were carefully poured on plates containing Czapek’s Dox Agar media for estimation of fungi. Streptomycin (5 mg/100 mL) was used to check bacterial growth. Nutrient Agar media was used for bacterial growth and Starch Casein Medium for actinomycetes. The plates were incubated at 27±1°C. Colony counts were made on the 4th day of incubation for bacteria, on the 8th day of incubation for actinomycetes and on 7th day of incubation for fungi. Plate counts were expressed as colony forming units (cfu)/g dry soil, using the equation:

$$\text{cfu/g dry soil} = \frac{\text{Avg. no. of colonies}}{\text{Dry weight of soil}} \times \text{Dilution factor}$$

For qualitative studies, the pattern of each fungal species was measured using the formula

$$\text{Occurrence} = \frac{\text{Average number of colonies of a species}}{\text{Average number of colonies of all fungal species}} \times 100$$

The following media were used during the investigation period.

- Czapeck’s Dox Agar medium was used for isolation and culture of fungi.
- Nutrient agar medium was used to isolate bacteria.
- Starch-casein medium was used to isolate actinomycetes.

### Table 3.1 Chemical composition of media used for microbial enumerations

<table>
<thead>
<tr>
<th>Czapek’s Dox Agar Medium</th>
<th>Nutrient Agar Medium</th>
<th>Starch Casein Agar Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>g/L H₂O</td>
<td>Materials</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.0g</td>
<td>Peptone</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>3.0g</td>
<td>Beef-extract</td>
</tr>
<tr>
<td>MgSO₄. 7H₂O</td>
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<td>NaCl</td>
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<td>0.5g</td>
<td>Agar</td>
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<td>FeSO₄.7H₂O</td>
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<td>Sucrose</td>
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<td>MgSO₄. 7H₂O</td>
</tr>
<tr>
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<tr>
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<td></td>
<td>Agar</td>
</tr>
</tbody>
</table>
3.5 Quantitative and qualitative analysis of rhizosphere and non-rhizosphere microflora of rice crop at different stages of growth and their activities

3.5.1 Quantitative Analyses of rhizosphere and non-rhizosphere microflora at different growth stages of rice crop

The Aijung variety of rice was cultivated in an experimental field, to study the rhizosphere and non-rhizosphere microflora of rice crop at various growth stages. The crops were sampled at regular intervals considering five different stages of growth of the crop viz,

1. Young seedling stage
2. Active tillering stage
3. Booting stage
4. Flowering stage
5. Maturity stage

The crops were sampled for over a period of 150 days for collection of rhizosphere soils and the root system of the crop. The root systems were aseptically taken to the laboratory in sterile polythene bags. During sampling excess soils were shaken off from the root system. The root system together with the adhering soil particles was placed into 250 mL conical flask containing 100 mL sterile water. The roots were subjected to constant washing in sterile water following the method described by Harley and Waid (1955). The root free of surface soil was then transferred from the original dilution flask to petri dishes containing desired media for the isolation of root surface microflora. Likewise soils from the non-rhizosphere zone was also collected and analyzed for comparison. Estimation of microflora was carried out at different stages of plant growth.

Suitable dilutions (serial dilutions) were prepared from the soil suspension by using the serial dilution technique suggested by Warcup (1951). Various dilutions ranging from $10^{-2}$ to $10^{-6}$ were made from the initial suspension. For bacteria and actinomycetes counts, plating were carried out at $10^{-5}$ dilutions respectively. For fungal counts $10^{-4}$ was
used. Plating was done on Czapeck's Dox agar Medium for fungi, Nutrient Agar Medium for bacteria and Starch Casein Medium for actinomycetes. Plates were then incubated at 27±1°C. Fungal colonies were counted and identified on the 7th day of incubation and the total numbers of bacteria were counted on the 4th day of incubation. Colony counts for actinomycetes were made on 8th day of incubation. Total plate counts were expressed as colony forming units (cfu)/g dry soil.

After recording the estimates of rhizosphere and non rhizosphere microflora, the R/S values of bacteria, actinomycetes and fungi were individually calculated as follows-

$$R/S = \frac{\text{Organisms in the rhizosphere soils (R)}}{\text{Organisms in the non-rhizosphere soils (S)}}$$

3.5.2 Influence of edaphic factors on rhizosphere and non-rhizosphere microflora

Studies on the influence of certain edaphic factors on the rhizosphere and non-rhizosphere microflora was carried out by pot culture experiment (Mishra and Srivastava, 1970). The materials and methods used were as follows-

- Virgin soil was taken in three pots
- Rice field soil was taken into three pots
- Lime was added to virgin soil in such a way so as to make $pH$ of soil around 8. These alkaline soils were taken in three pots.
- Virgin soil was treated with HCl so as to make $pH$ of soil around 2.5. These soils were taken in three pots.
- In another set $pH$ of the virgin soil was adjusted to 7. These soils were taken in three pots.
- To increase the moisture content of the soil by 10%, 1200 mL of sterilized water was added to 12 kg of soil. These soils were taken in three pots.
- To increase the moisture content of the soil by 20%, 2400 mL of sterilized water was added to 12 kg of soil. These soils were taken in three pots.
Cow dung and virgin soil were mixed thoroughly in equal proportion and were taken in three pots.

Equal amount of sand and virgin soil were mixed thoroughly and taken in three pots.

Twenty seven young seedlings of same age were potted in different pots containing prepared soil types. The watering was done in every alternate day for 10% and 20% moisture pots, while in others, once a week. pH was checked and adjusted to the requirement on a weekly basis. Influence of edaphic factors on rhizosphere (R) and non-rhizosphere (S) microflora of rice crop was analysed using the procedure mentioned above.

3.5.3 Effect of edaphic factors on growth of rice seedlings

Four days after germination, five seedlings were transplanted into each pot, which contain above mentioned soil preparations. At harvest maximum root and shoot length, root and shoot dry weight and nutrient contents were assessed. Maximum root length was measured as the longest undamaged root. Maximum shoot length was measured from the base of the stem to the tip of terminal leaf blade. Root and shoot dry weights were measured after drying at 70°C for 48 hr. Shoot and root samples were analyzed for N (Kjeldahl method), P (wet digestion method) and K content (flame photometric method) (Ryan et al., 2001).

For estimation of nitrogen content 1g of finely ground dried plant material was taken in a 100 mL digestion tubes, to which added 3g of catalyst mixture (K$_2$SO$_4$-Se, 100:1 w/w) and 10 mL concentrated H$_2$SO$_4$. The mixture was stirred well and placed in a block digester at 100°C for 20 min. The tubes contents were thoroughly agitated and adhering materials on the tube surfaces were washed with the same concentrated H$_2$SO$_4$. The mixture was finally digested at 380°C for 2 hr. After completion of digestion, tubes were cooled and the volume was adjusted to 100 mL with distilled water. 10 mL of aliquot was pipette into 100 mL distillation flask to which added 10 mL of 10N NaOH. The distillate was collected and titrated with standardized 0.01N H$_2$SO$_4$. Each batch of
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samples used for digestion contained on reagent blank, one chemical standard (0.1g EDTA) and one standard plant sample. N (%) in plant material was calculated as follows.

\[ N(\%) = \frac{(V - B_1) \times N \times R \times 14.01 \times 100}{Wt_2 \times 1000} \]

Where,
- \( V \) = volume of 0.01 N \( \text{H}_2\text{SO}_4 \) titrated for the sample (mL)
- \( B_1 \) = Digested blank titration volume (mL)
- \( N \) = Normality of \( \text{H}_2\text{SO}_4 \) solution
- \( R \) = Ratio between total digest volume and distillation volume
- \( Wt_2 \) = Weight of dry plant material (g)
- 14.01 = Atomic weight of N

For estimation of phosphate content in the plant material, 22.5g of ammonium molybdate was dissolved in 400 mL of distilled water, and 1.25g of ammonium vanadate was dissolved in 300 mL of boiling distilled water. The vanadate solution was added to the molybdate solution in a 1L volumetric flask and the mixture was allowed to cool at room temperature. To this mixture 250 mL of concentrated nitric acid was added and diluted 1L with distilled water. Phosphate standard solution was prepared by dissolving 0.2195g of potassium dihydrogen phosphate in distilled water and the volume was adjusted to 1L. This solution contains 50 ppm P (Stock solution). A series of standard solutions was prepared from the stock solution. 1g of dried and ground plant material was taken in a 50 mL procelain crucibles and the sample was digested using wet oxidation procedure, as described in Kjeldahl procedure. The digested plant material was filtered with Whatman No. 44 filter paper in 100mL volumetric flask and 10 mL of ammonium-vanadomolybdate reagent was added to it. The solution was diluted to volume with distilled water. For preparation of standard curve, 0, 1,2,3,4 and 5 mL of standard stock solution was pipette to 50 mL volumetric flask and 10 mL of vanadomolybdate reagent was added to each flask. The reagent blank was prepared with 10 mL of ammonium-vanadomolybdate reagent. The absorbance of the blank, standards and samples was read after 30 min at 410 nm wavelength. The P concentration of unknown sample was calculated using the standard curve. P (%) in plant material was calculated as follows.
\[ P(\%) = \text{ppm P (from calibration curve)} \times \frac{R}{Wt} \times \frac{100}{10000} \]

Where,
\[ \begin{align*}
R & = \text{Ratio between total digest volume and the digest volume used for measurement} \\
Wt & = \text{Weight of dry plant material (g)}
\end{align*} \]

The plant sample for K estimation was digested by diacid using 9:4 HNO₃: HClO₄. 1g of plant material was placed in 100 mL volumetric flask. To this, 10 mL of acid mixture was added and the content of the flak was mixed by swirling. The flask was placed on low heat hot plate in a digestion chamber. Then, the flask was heated at higher temperature and the contents were evaporated until the volume was reduced to about 3 to 5 mL. the completion of digestion was confirmed when the liquid become colourless. After cooling the flask, 20 mL of distilled was added and the volume was made up with distilled water. The solution was filtered through Whatman No.1 filter paper. The samples are then read in flame photometer at 548 nm wavelength.

3.5.4 Qualitative Analysis of rhizosphere and non-rhizosphere mycoflora rice at different stages of growth

Experiment was carried out by growing rice plants in pots for the study of rhizosphere and non-rhizosphere mycoflora at various growth stages of rice crop. The crops were sampled at regular intervals for collection of rhizosphere soils considering five different stages of growth of the crop viz, young seedling stage, active tillering stage, booting stage, flowering stage and maturity. The procedure is similar to as mentioned in unit 3.3.1.

3.5.5 Study of rhizoplane mycoflora

Root surface microfloras were isolated employing the method of Harley and Waid (1955) as discussed above in unit 3.3.1. Roots collected from the rhizosphere were weighed and washed thoroughly 10 times with sterile distilled water. All of the washing solution from the root samples was collected in a 100 mL flask and serially diluted. Three categories of root was cut separately into small pieces of about 1 cm in length. These are
tip zone (1/3rd of the total length of the root), intermediate zone (1/3rd of the total length of the roots between the tip and crown zone) and Crown zone (1/3rd of the total length of the roots). Five bits of each category of root were transplanted to each Petri plates containing CDA medium for isolation of fungi. For each category of root, three sets of Petri dishes were prepared. The percentage occurrence of rhizoplane mycoflora at different stages of growth was calculated. Fungi were identified using standard literatures (Barnett and Hunter, 1972; Gilman, 1957).

3.5.6 Soil microbial biomass and enzyme activities

3.5.6.1 Estimation of soil microbial biomass

Soil MBC and MBN were determined by the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Two sets of triplicated soil samples (20 g on a dry basis) were moistened to 55% WHC and incubated at 25°C for 21 d prior to the biomass measurements. One set is fumigated with alcohol-free CHCl₃ and the other set is kept without fumigation. For fumigating soil samples, the beakers with soil are placed in a large vacuum desiccator that is lined with moist filter paper. A beaker containing 50 mL of alcohol-free CHCl₃ is also paced in the desiccator. The desiccator is then evacuated with the help of vacuum pump till the CHCl₃ starts boiling. The CHCl₃ is allowed to boil for 1-2 min and then the desiccator is sealed and incubated for 24 hr at 25°C. After chloroform removal, soils with or without chloroform fumigation were extracted with 0.5M K₂SO₄ (1:4 soil: extractant) for 1 hr. The extracts were filtered through Whatman no. 1 filter paper and stored at 4-5°C till further assay. For estimation of biomass C, an aliquot of the K₂SO₄ soil extract (fumigated and non-fumigated) is used for measuring organic C by digestion with K₂Cr₂O₇ and back-titrating with 0.2M ferrous ammonium sulphate (19.625 g ferrous ammonium sulfate dissolved in 300 mL distilled water containing 20 ml conc. H₂SO₄ and diluted to 10:100 mL) with 1, 10 = phenanthrobine ferrous sulphate as the indicator. MBC was calculated as: MBC = Eₓ / kₑc, where Eₓ = (organic C extracted from fumigated soils)-(organic C extracted from non-fumigated soils) and kₑc = 0.45 (Wu et al., 1990).
For estimation of biomass N, an aliquot of the K$_2$SO$_4$ soil extract (fumigated and non-fumigated) are used for measuring total N content. Total N is measured by modified Kjeldahl digestion method as described earlier under total soil N estimation. MBN was calculated as: MBC = $E_N/k_{EN}$, where $E_N = (\text{total N extracted from fumigated soils}) - (\text{total N extracted from non-fumigated soils})$ and $k_{EN} = 0.54$ (Brookes et al., 1985). The results presented are the arithmetic means of the measurements of triplicate samples and are expressed on an oven-dried basis soil weight basis (24 hr, 105°C).

3.5.6.2 Estimation of soil enzyme activity

Enzyme activities like amylase is assayed as described by Kelly and Rodriquez (1975), dehydrogenase by the method of Casida (1977) and acid phosphatase and alkaline phosphatase were assayed as per the method of Tabatabai and Bremner (1969).

3.5.6.2.1 Amylase activity

For estimation of amylase activity, two gram of soil was taken in 100 mL Erlenmeyer flask and 10 mL of 1M sodium acetate buffer (pH 6) and 5 mL of 1% soluble starch is added to it. The mixture is incubated at 37°C for 24 hours. A 1 mL portion of the reaction mixture was pipetted out and the reaction was stopped by the addition of 1 mL of dinitrosalicylic acid reagent. Estimation of the reducing sugars released was estimated using the DNS method (Gascoigne and Gascoigne, 1960). The result is expressed as mg of reducing sugar released kg$^{-1}$ soil d$^{-1}$.

**DNS- method:** 1 mL of the reaction mixture is added to 1 mL of DNS reagent (dissolved simultaneously 1g of dinitrosalicylic acid, 200mg of crystalline phenol and 50 mg of sodium sulfite placed in a beaker with 100 mL of NaOH by stirring). The mixture is then heated for 5 minutes in a boiling water bath. After the colour developed, 1 mL of 40% Rochelle salt is added when the contents of the tubes remain still wall. Then tubes were cooled under the running tap. The absorbance is measured at 575 nm. The amount of reducing sugar is measured using a standard prepared from glucose.
3.5.6.2.2 Dehydrogenase activity

Dehydrogenase activity was assayed under standard conditions by the method of reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) to formazon (Casida, 1977). Two gram of soil and 0.2 g of CaCO₃ (AR grade), 1mL of 2% sterile triphenyltetrazolium chloride (TTC) solution and a column (1cm) of distilled water was added to screw cap test tubes and incubated at 37°C for 24 hr(s). After incubation, the tubes were removed and contents were filtered through Whatman No. 2 filter paper with washings of methanol until a colourless filtrate was obtained. Then, the filtrate was made up to 100 mL with methanol in a volumetric flask and absorbance was read at 485 nm in a spectrophotometer (Spectronic-20) using methanol as a blank. The absorbance units were converted to concentrations of triphenylformazon (TPF) from a standard curve prepared from TPF and expressed as mg TPF kg⁻¹ soil d⁻¹.

3.5.6.2.3 Acid and alkaline phosphatase

For estimation of phosphatase, one gram of fresh sample were placed in a 50 mL Erlenmeyer flask and mixed with 0.25 mL of toluene and 4 mL of Modified Universal Buffer (pH 6.5 for assay of acid phosphatase and pH 11 for assay of alkaline phosphatase). 1 mL of 0.025M p-nitrophenyl phosphate (PNP) solution was added into it. The flask were stoppered, swirled for a few seconds and incubated at 37°C for 60 min. After incubation, the samples were cooled at 2°C for 15 min. After cooling1 mL of 0.5M CaCl₂ and 4 mL of 0.5M NaOH were added into the flask and mixed well for few seconds and supernatant was filtered through Whatman No. 2 folded filter paper. The yellow colour complex was measured using 1cm cuvette in the spectrophotometer at 420 nm. The amount of p-nitrophenol released was calculated by referring to a calibration graph. Controls were maintained following the same procedure described above but 1 mL of p-nitrophenol was added after incubation but before the addition of CaCl₂ and NaOH. Results were expressed as mg PNP kg⁻¹ soil d⁻¹.
3.5.7 Estimation of soil respiration in rice crop soil

Soil respiration is measured by the absorption and titration method of Me. Fadyen (1970). One kg of fresh soil sample (from which all root materials has been removed) was placed in a glass jar (15×15×20 cm). Then 100 mL glass beaker containing 20 mL of 0.1N KOH solution was kept inside each jar. The lid of the jar was then sealed by grease to make it air right and kept undisturbed for 24 hrs. The jar lid was opened after 24 hrs and CO₂ fixed by KOH was estimated by titrating the same with 0.1N HCl solution using phenolphthalein as an indicator. The same procedure is also used for estimation of soil respiration from rhizosphere and non-rhizosphere soil and respiration from different depth of rice crop field. While for the subtraction of atmospheric CO₂ a control was run by using sterilized sand instead of soil sample. Respiration was expressed as CO₂ evolved in terms of mg CO₂ evolved/kg dry soil/24 hrs on dry weight basis.

Calculation

CO₂ (mg) = (B-V) N+E

Where, B= Volume of acid titrate without the sample
V= Volume of acid titrate with the sample
N= Normality of the acid
E= Equivalent weight of CO₂ (E=22)

3.6 Analysis of seed mycoflora and its relationship with rhizosphere mycoflora

50 healthy rice seeds were tested without any pre-treatment and another 50 seeds were tested after pre-treatment with 0.1% mercuric chloride solution for 2 minutes, followed by washings with sterile water. For each treatment, 5 Petri plates containing Czapeck’s Dox Agar medium at a rate of 10 seeds/ plate was taken for assessing mycoflora composition.

For assessing the relationship of seed mycoflora with rhizosphere mycoflora three series of pots were taken. Seeds were sown in three series of pots such that each pot produces 5 seedlings. The three series of pots were taken as follows.
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a) Surface sterilized seeds in natural soil (to determine the mycoflora originating from soil).
b) Unsterilized seeds in sterilized soil (to determine the mycoflora of seed origin).
c) Unsterilized seeds in natural soil (taken as control).

The plants were regularly watered with sterile water to maintain the optimum soil moisture. Pots with the plants were covered with cellophane to prevent air-borne contamination. Small slits were made on cellophane for allowing the growing seedlings to protrude out through the slits.

The root systems were sampled at regular intervals of 7 days over a period of 35 days (5 weeks). Roots were carried in sterile polythene bags to the laboratory for the investigations. The roots were subjected to constant washing with sterile water as per method of Harley and Waid (1985) and the washed roots were cut aseptically into 0.5 cm segments. The root segments were plated in order from tip zone onwards on Czapeck's Dox Agar medium with added streptomycin (5 mg/100 ml medium). The plates along with the root segments were incubated at 27°C and examined regularly from 2nd day onwards up to 35 days.

For analyses of rhizosphere and non-rhizosphere mycoflora soil plate method (Warcup, 1951) was employed. The procedure is similar to as mentioned in unit 3.3.1.

3.7 Effect of root exudates of rice on germination of some dominant fungal spores

3.7.1 Root exudates assay

3.7.1.1 Cultivation of rice seedlings

In the root exudation tube (40 cm x 5 cm) a plug of glass wool was tightly placed 10 cm from the bottom. The portion below the glass wool was filled with distilled water and the tube was covered with cotton wool sandwiched between the two layer of aluminium foil and sterilized at 1.4 kg cm⁻² pressure for 30 min. After cooling, surface sterilized seeds were aseptically placed on the glass wool at the rate of five seeds per
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tube. The root exudates were collected 15, 30 and 50 days after sowing (DAS) and were analyzed for total carbohydrates, total free amino acids and total phenols.

3.7.1.2 Analysis of root exudates

Quantitative determination of total soluble carbohydrate was determined with anthrone reagent (Morris, 1948). Two g of anthrone was dissolved in 1L of 95\% H\textsubscript{2}SO\textsubscript{4} to form anthrone reagent. Five mL of exudates sample was mixed thoroughly with 10 mL of anthrone reagent. After 10 min, the colour was measured at 620 nm on a spectrophotometer against a blank containing water and reagent.

Quantitative estimation of total free amino acids was determined by ninhydrin method (Moore and Stein, 1948). To 500 mL of root exudates, added 5 mL of 80\% ethanol and centrifuged to 10,000 rpm for 10 mins. The extraction process was repeated twice and the all the supernatants were pooled and evaporated to dryness. To the dried supernatant 5 mL of distilled water was added. To 0.1 mL of this extract added 1mL of ninhydrin solution and the volume was adjusted to 2 mL by adding distilled water. The tube is heated in boiling water bath for 20 min. Then, 5 mL of 1:1 water-propanol diluents was added and the content was mixed vigorously. After 15 min the intensity of purple colour was measured in a spectrophotometer at 570 nm against a reagent blank. The reagent blank was prepared as above by taking 0.1 mL of 80\% ethanol instead of extract.

The analysis of total phenols was based on Mallick and Singh (1980). To 0.25 mL of sample, added 2.5 mL of ethanol and centrifuged at 10,000 rpm for 20 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 mL of 80\% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 5 mL of distilled water to the dried supernatant. To which added 0.5 mL of folins phenol reagent and 2 mL of 20\% sodium carbonate. The reaction mixture was kept in boiling water bath for 1 mins. The absorbance was measured at 650 nm against a reagent blank in a spectrophotometer.
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3.7.2 Effect of root exudates on germination of fungal spores

3.7.2.1 Fungal spore suspension preparation

Spores from 15-day old cultures, grown on potato dextrose agar (PDA), of the following eight fungi- Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, P. oxalicum, and Trichoderma viride, were used in the experiment. Spores were collected from the surface of fungal colonies by gently scraping with a loop and suspended in 10 mL sterile water containing 2 % (v/v) Tween 80. The suspension was mixed vigorously by vortexing for 15- 20 min. The spore suspension stock was diluted in sterile water to obtain a concentration of $5-7 \times 10^4$ spores/mL. The spore concentrations were enumerated microscopically using haemocytometer.

3.7.2.2 Spore germination

Root exudates obtained after filtration was considered to be 100 per cent concentrated. This was further diluted to 25, 50 and 75 per cent concentration. The spores were allowed to germinate in appropriate exudates at room temperature. 0.1% sucrose was used for germination of control. Aliquots of 1mL of root exudates were mixed with 1mL of spore suspension taken on a groove slides. The germination was checked after 24 hours of incubation by adding a loopful of lactophenol cotton blue and screened microscopically at 400x. A spore was considered germinated when the germ tube length exceeded the diameter of the spore. Germinated spores were counted and recorded as a percentage of total spore number.

$$\text{Percentage (\%) of spore germination} = \frac{\text{Total no. of spores} - \text{No. of germinated spores}}{\text{Total no. of spores}} \times 100$$

3.8 Effect of cultural filtrate of some seed borne fungi on rice seed germination

3.8.1 Microbial assay of seed mycoflora

Healthy rice seeds were collected from the local farmer of the study area.
Microbiological assay of the mycoflora associated with rice seeds were carried out using Standard blotter method (ISTA, 1976) and Nutrient agar plate method (Muskett, 1948).

3.8.1.1 Standard Blotter Method

Unsterilized as well as sterilized rice seeds were placed on three sets of moist blotters paper at the rate of 10 seeds/ Petri plate. The experiment was laid with three replications each for sterilized and unsterilized seeds. For analysis of internal seed borne fungi seed were surface sterilized with 2.5% sodium hypochlorite solution for one minutes followed by several washings (3-4changes) with sterile distilled water before plating, while unsterilized seeds were plated without treatment. The plated seeds were incubated at 25±2°C in an incubator for seven days. Sterile distilled water was sprayed aseptically on the Petri plates every third day in order to keep the blotters sufficiently moist. After incubation fungi were isolated by pure culture technique. Further slides were prepared and examined under a compound microscope for identification of the isolated fungi.

3.8.1.2 Agar Plate Method

The other set of experiment was carried out on potato dextrose agar medium. Similar to blotter method, three replicates were taken each for unsterilized and sterilized seeds at a rate of 10 seeds/ Petri plates. The seeds were placed on sterilized glass Petri plates containing 20 mL Potato dextrose agar medium. The plates were incubated at 25±2°C for seven days. Through constant observation during the incubation period, growth characteristics of fungal colonies were recorded. At the end of the incubation period, pure cultures of fungi growing out of seeds were prepared on suitable agar slants for further examination.

3.8.2 Effect of seed borne fungi on germination and seedling vigour

The effect of seed borne fungi on germination and seedling vigour was analyzed as per method of Singh and Swami (2004).
3.8.2.1 Seed inoculation method

Rice seeds were surface sterilized with 0.1% Mercuric chloride (HgCl₂) solution for one minute followed by several washings with sterile distilled water before plating. The plated seeds were inoculated by two-week old sporulating culture of each fungus. The seeds were then air dried for 72h at room temperature. Ten inoculated seeds were sown separately in 30cm earthen pots. After 14 days of sowing, observation on per cent germination, radical and plumule length and seedlings vigour were recorded. The seedling vigour was determined following the formula of Baki and Anderson (1972) as shown below.

\[
\text{Vigour index} = (\text{Mean of root length} + \text{Mean of shoot length}) \times \text{Percentage of Seed germination}
\]

3.8.2.2 Soil inoculation method

The isolated fungi were grown separately on autoclaved rice medium (10:5 rice/water) in 100mL conical flasks. The flasks were incubated at 25±2°C for ten days. Sterilized earthen pots (30cm) were filled with pre-sterilized soil. For inoculation, the upper 4 cm layer of soil was thoroughly mixed with rice medium for supporting the fungal growth. The pots were covered with polythene bags and left for 24h in green house. After 24h ten healthy sterilized rice seeds were sown in each pot. Equal number of healthy sterilized seeds sown in uninoculated pot served as control. After 14 days, observation on per cent germination and seedling vigour were recorded.

3.8.2.3 Seed submergence method.

The isolated fungi were grown separately in Richard’s broth medium and incubated for 21 days at temperature of 25 ± 2°C. Culture filtrates were obtained by filtering the contents through Whatman filter paper no.44. Healthy surface sterilized rice seeds were separately soaked in these culture filtrates for 24h and allowed to germinate in sterilized plates containing three layers of moist sterile blotter paper. Seeds soaked in sterile distilled water serve as control. Petri plates were incubated at 25 ± 2°C for 14 days. After incubation period, per cent seed germination and seedling vigor were recorded.
3.8.3 Effect of culture filtrate of seed borne fungi on rice seed germination

Surface sterilized seeds were soaked for 24h in different concentration (25, 50, 75, and 100) of 7, 14 and 21 day old culture filtrate of each fungus. Dilution was made with sterile distilled water. Three replicates of 10 seeds were taken for each concentration, days and fungus. Equal number of seeds soaked in sterile distilled water serves as control. The soaked seeds were plated on three layers of moist blotter paper and incubated at 25 ± 2°C for 14 days. After incubation period per cent germination was recorded.

3.9 Studies on the degradation of rice stubbles by soil fungi

3.9.1 Estimation of rice stubble associated fungi

The rice stubble inhabiting microfungi were studied by following the method of Sinha and Dayal (1983) and dilution plate technique (Warcup, 1960). The stubbles samples were crushed aseptically. From the crushing sample of rice stubble, 1g of the powder was suspended into 10 mL of sterile distilled water. After that it was thoroughly and vigorously shaken. From this mixture further dilution series were prepared and a final dilution of $10^{-4}$ was made. Five replicates with 1 mL from this final suspension ($10^{-4}$) were inoculated in petridishes containing 20 mL Czapeck’s Dox Agar medium with 100ppm streptomycin. The plates were incubated at 27±1°C for 7 days. Fungal colonies/g of air dried stubble was counted and pure culture was made for the identification of individual fungus.

3.9.2 Decomposition of stubbles

The second sets of experiments were carried out to study the microfungi which are capable of degradation of rice stubbles specially lignin and holocellulose under laboratory condition. Twelve dominating fungi of rice field soil isolated by dilution technique (Warcup, 1951) on the potato Dextrose Agar medium were taken for investigation. Streptomycin (100 ppm) was used to suppress bacterial growth. The isolated fungi were then inoculated separately to rice stubbles to study their efficiencies in bio-degradation.
Experiments were carried out under laboratory conditions for a period of two months.

Dried pieces (2.5 cm long) of rice stubbles (10 g) of Aijung variety were taken along with 100 mL of sterile distilled water in each 250 mL Erlenmeyer flask, plugged and sterilized for 1 hr at 15 lbs pressure. The stubbles pieces in the flasks were then inoculated with spore suspension (2 mL) of each fungus separately, and thoroughly shaken. For each fungus 9 replicates were prepared. Parallel sets of control (stubble + distilled water) were also maintained. These flasks were then incubated at 30°C for 20, 40 and 60 days with periodic shaking at two days interval to spread the fungus uniformly. Partially degraded stubbles were taken out from the flasks in triplicate and dried to constant weight at 80°C and finally powdered. Lignin and holocellulose constituents of rice stubbles were estimated quantitatively.

3.9.3 Estimation of lignin decomposition

Lignin content of rice stubble was estimated quantitatively following the procedure of Saeman et al., (1954). In this method the total carbohydrate of powdered stubble were dissolved by hydrolysis in sulphuric acid. The lignin was condensed to an insoluble residue which was determined gravimetrically. Air-dry stubble powder (400 mg) was chilled in ice water and then macerated first in 3 mL of ice cold 72.01 % (w/v) sulphuric acid for 2 minutes and then in a 30°C (± 0.2) water bath in a flask fixed to a shaker for one h to ensure complete penetration of the acid into all stubble particles. The acid strength was then diluted to 4% by adding water (84 mL) and then autoclaved at 121°C for 1 hr. After cooling, it was filtered through sintered glass filter and washed several times with distilled water to render the lignin free of acid. The lignin residue was then air dried to a constant weight.

3.9.4 Estimation of holocellulose decomposition

Holocellulose in the powdered stubbles was estimated quantitatively following the procedure of TAPPI standard (1954) and Cowling (1961). Holocellulose is taken as the residue remaining upon successive pre-extraction of powdered stubble with ethanol.
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benzene, ethanol and hot water to remove extraneous substances, followed by a succession of chlorination and monoethanol-amine extraction treatments to remove lignin. Air-dry powdered stubble (100 mg) moistened with ice water was taken in a glass crucible and chlorinated for 5 min(s). The chlorinated sample was freed from excess chlorine and hydrochloric acid with ethanol and subjected to two treatments each of 2 minutes duration with 3% solution by volume of ethanolic monoethanol-amine maintained at 75° C to 80° C. The excess monoethanol-amine was finally removed by repeated washing with ethanol and distilled ice water. This extraction process was repeated until the sample became completely white. The holocellulose preparation was washed several times in ethanol and ethyl ether and finally placed in an oven at 35 °C for 2 h to remove ether from the sample. The crucible and its content were finally dried to constant weight.

3.4.4 Statistical analysis

Data obtained from the respective experiments were pooled and the means were used in statistical analysis using SPSS version- 16. The microbial population data were statistically analysed by two-way ANOVA. Correlation coefficients (r) between various soil physico-chemical characteristics and population of bacteria, actinomycetes and fungi were analysed by using Pearson’s correlation coefficient. P values < 0.01 and < 0.05 were considered as significant. Variance components like coefficient of variations were also calculated. Means were separated with Duncan Multiple Range Test (DMRT) in respective tables. Relationship between soil physical and chemical properties, microbial biomass and enzyme activities was analysed using Pearson’s linear correlation coefficients. Linear regression was used to evaluate the relationships between organic carbon and biomass carbon and nitrogen and total nitrogen with biomass carbon and nitrogen. Two-way ANOVA was also used to analyse depth and month wise variation of soil respiration, and in decomposition of rice stubbles by soil fungi.