Chapter 3

MATERIALS AND METHODS

Research investigations were started in 2019, in the North Bank Plain Agro Climatic Zone of Assam, India, at Gotlong, an elevation of 235 feet above mean sea level. The typical temperature ranges from 32^0 to 35^0C in the summer and 15^0 to 20^0C in the winter, making it ideal for mosquito growth and development. Typically, the area gets two to three flood waves between May and September every year. The rainy season starts from March and extend upto September, however, there were aberrations in the recent rainfall trend, especially the intensity of rainfall declined towards the end of investigation period (i.e., 2022). The detail materials and methods of experiments under each objective are presented in the figure below:

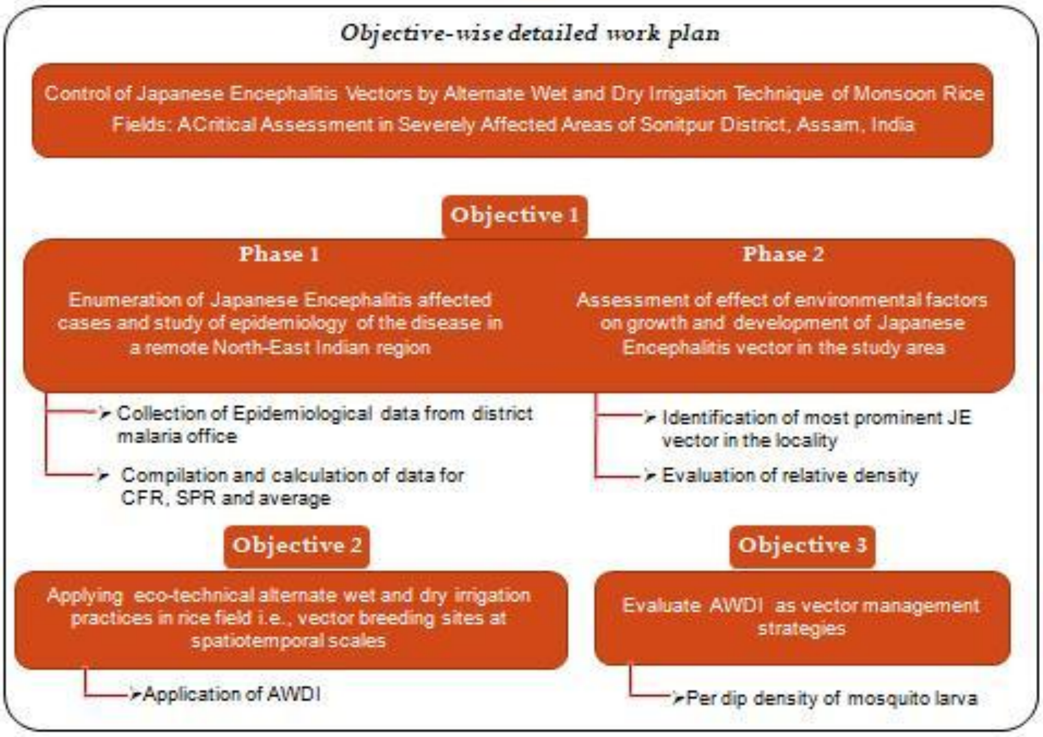

Figure 3.1. Schematic representation of experimental design

3.1 Objective 1

Epidemiological profiling of Japanese Encephalitis (JE) disease and evaluation of environmental factors influencing JE vector ecology in the targeted study area.

Objective 1 was fulfilled by conducting the following experiments in a systematic way:

3.1.1 Phase1: Enumeration of Japanese Encephalitis affected cases and study of epidemiology of the disease in a remote North-East Indian region

Experiment 1 Phase 1: Collection of Epidemiological data from sub centers in Sonitpur district

3.1.1.1 Geographical location, climatic condition and experimental site description

North Bank Plain Agroclimatic Zone, Tezpur, Assam is located at 26˚30´35” and 27˚02’11” North Latitudes and 92˚ 19’30” and 93˚47´13” East Longitudes with elevation between 73 to 75 m. Geographical location of the experimental site is shown in the Figure 3.2(c). A major groove of this research investigation is situated at a sub center named Bihaguri Block (26˚38’10” North Latitude and 92˚47’2” East Longitude), with strikingly high frequency of JE and AES incidences in 7 years (2013-2019). Investigation sites were also selected depending on their proximity to paddy fields in a village named Gotlong in Bihaguri Block Public Health Center in the central Brahmaputra valley. Map was prepared using Arc GIS 10.7 and toposheets (1:50,000) of Survey of India (Toposheet No. 83 A/12, 83A/16, 83B/5, 83B/6, 83B/9, 83B/10, 83B/13. 83B/14, 83 F/1, 83F/2, 83F/5, 83F/6, 83F/9 and 83F/10) and Google map to locate the co-ordinate of the study area.

Meteorological data of Sonitpur district were collected from Meteorological Department, Guwahati, North-East Regional Institute of Water and Land Management (NERIWALM), Tezpur for the period from 2019 to 2022. The meteorological data was broken down by month and includes information on temperature, humidity, and rainfall patterns. The temperature data of the district are presented as average maximum and average minimum in figure 3.2 (a). The average temperature of the district varies between the minimum temperature of 15.56°C and the maximum temperature of 32.31°C. The average minimum temperature recorded ranges between 11.4°C and 26.7°C in January and July respectively. The average maximum temperature ranges between 14.56° to 23.89°C in January and August. This indicates that the coldest month during winter is January and the warmest month during summer is August. The temperature range favorable for growth of mosquitoes in a given region is between

20°C and 40°C, which is one of the important factors for proliferation of mosquito. Other factors like rainfall, humidity, vegetation cover etc. are also important. The temperature graph as shown in figure 3.2(a) depicts the condition congenial for proliferation of mosquitogenic conditions prevailing in the district. Rainfall data were collected for the period 2019-2022 which are used to analyze the rainfall pattern in the district. The average annual rainfall in the district since 2019 to 2022 is about 2304.49 mm that clearly indicates the wet and humid conditions of the area which falls under the tropical type of climate. The rainfall data in the district show the irregularities of rainfall in different years. The year 2021 recorded lowest rainfall in the year, i.e. around 1680.09 mm, while the year 2020 recorded highest rainfall, i.e. more than 2742 mm. The change in the pattern of rainfall in recent years indicates a more frequent change from the average that suggests a sign of onset of climatic change. The average monthly variation of the rainfall shows that rainfall is more prominent from the month of April to September. As the district is located in tropical region, the relative humidity is usually high indicating higher percentage of water vapour content. The months from June to October recorded more than 82% of relative humidity which happen to be the rainy months in the district [Figure 3.2 (b)]. However, the percentage gradually decreases October onwards up to March.

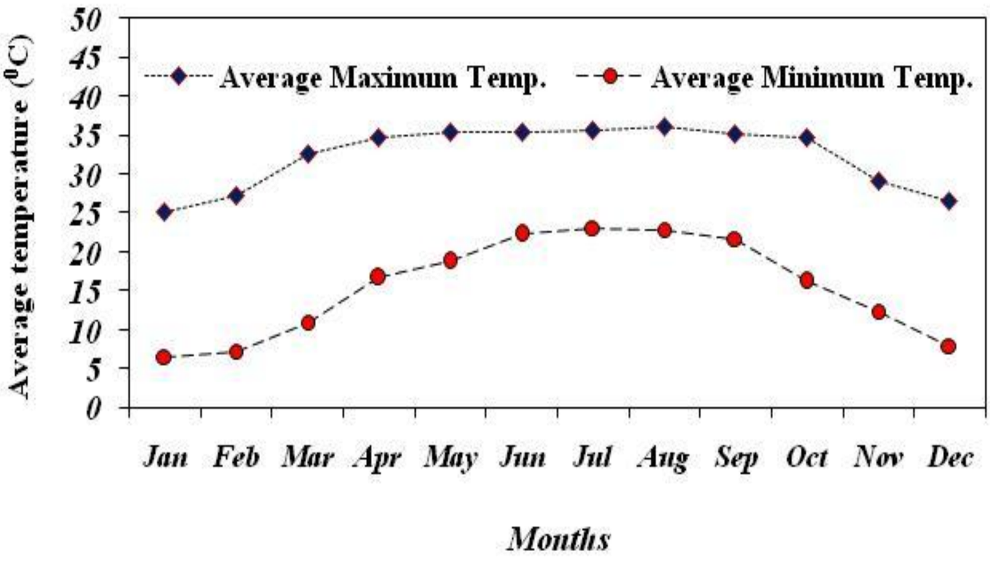

Figure 3.2 (a): Month wise average Minimum and Maximum temperature in Sonitpur district (2019-2022)

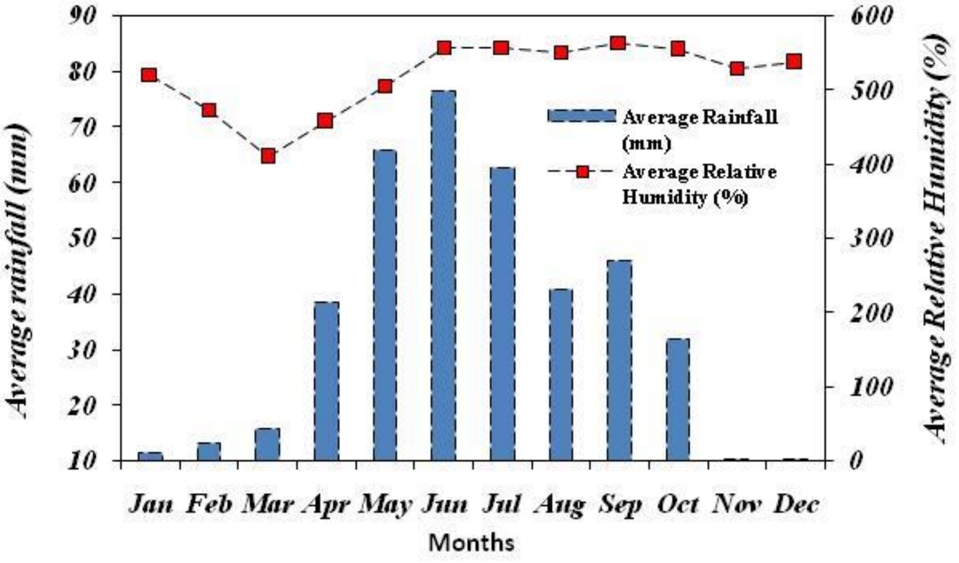

Figure 3:2 (b): Month wise average rainfall and relative humidity in Sonitpur district (2019-2022)

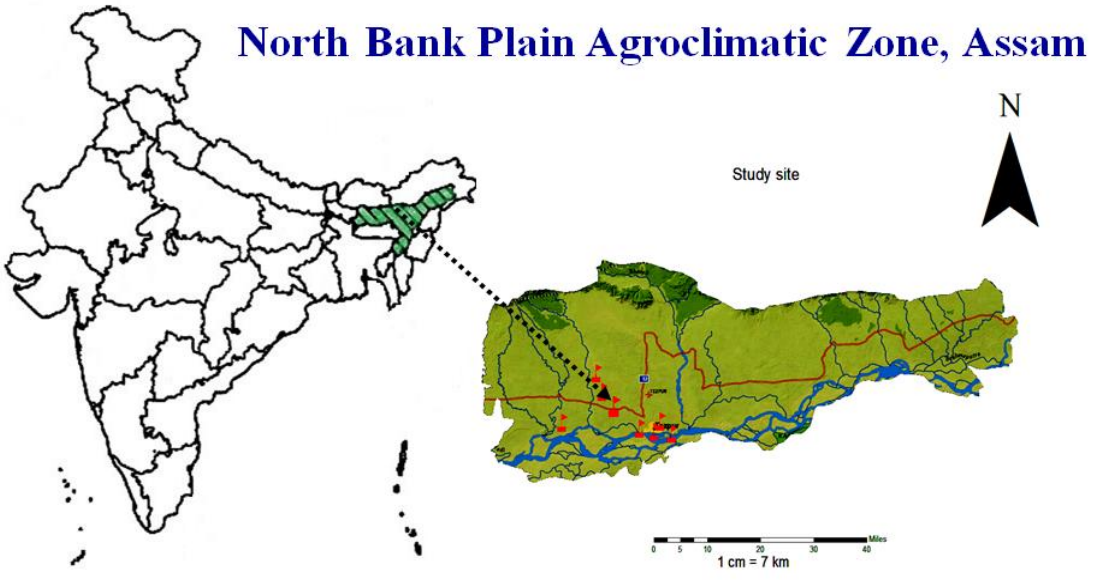

Figure 3.2 (c). Topographic map showing the investigation sites in the North bank plain agroclimatic zone, Sonitpur district, Assam

3.1.1.2 Literature survey

Extensive literature survey was accomplished using different search engines like Scopus, Google Scholar, Science Direct, and Research Gate to retrieve information from research investigations and download relevant information in online platform using keywords such as “alternate wet and dry irrigation,” “water management,” “aerobic rice cultivation,” “water saving potential,” “controlled irrigation,” “rice cultivates,” “water conservation,” “water saving,” “water use efficiency,” or “rice irrigation and agriculture,” “paddy,” “field water management”, “vector borne disease control” etc. both offline and online or electronic articles, reports, archives, and study material were reviewed to obtain relevant information on AWDI methods. Online materials included journal articles, e-reports, books and book chapters, official documents, bibliographic databases. An offline literature survey was performed by assembling information from books, magazines, and scholarly journals from remote libraries. Overall, review of literature was accomplished using a blended mode of information acquisition from both online and offline sources.

3.1.1.3 Collection of data

A pre-field survey was conducted to gather preliminary data and information about the research area before commencing with the fieldwork. It helped in the essential preparation to ensure successful fieldwork. Extensive fieldwork was conducted to acquire all essential and necessary data, as well as information and experience gleaned from close observation of ground reality. Secondary data were obtained from state health authorities. An attempt was made to create a good secondary database. References to earlier publications and available material were collected and thoroughly consulted.

Primary data collection is required to validate the legitimacy of secondary data and to make accurate conclusions. The data was gathered through rigorous field visits and surveys. Epidemiological investigations of JE and AES were conducted using the National Center for Vector-Borne Disease Control's standard questionnaire, AESF-4, in collaboration with the Integrated Disease Surveillance Program (IDSP) and the District Malaria Office (DMO), with the goal of identifying subcenters with high and low sensitivity to JE incidence.

3.1.1.4: Compilation of data and calculation of Case fatality rate (CFR), Sample Positivity Rate SPR and average:

3.1.1.4.1 Data compilation

Prior to data compilation, the primary and secondary data were comprehensively screened and a checklist of requirements for research analysis was prepared. The primary data was generated from laboratory and field investigations; secondary data was collected from National Center for Vector-Borne Disease Control (NCVBDC) office, Integrated Disease Surveillance Program (IDSP) center, District Malaria Office (DMO) and Indian Meteorological Department (IMD). From the primary and secondary data, high and low-sensitive areas prone to JE incidence were identified, and biological and physicochemical characteristics associated with the spread of JE were investigated and systematically analyzed. Data was carefully analyzed using descriptive and inferential statistical tools and compiled for obtaining meaningful research outcomes.

The number of significant JE vectors of *Culex* sp. which are known to breed in agricultural fields are collected and pooled and standardized to numbers per trap per night (12 dark hours) density. Pearson's correlation and linier regression are used in present study to statistically examine the interrelationship between meteorological and entomological variables. Regression analysis (SPSS) helped to understand the role of environmental factors in the seasonal abundance of different JE vectors mosquitoes in the study area. The dependent variables were mosquito abundance, and the independent variables were maximum temperature, minimum temperature, RF, no of rainy days, Relative humidity in morning and evening respectively.

3.1.1.5 Calculation of CFR, SPR and average

CFR estimates the proportion of deaths related to the total number of JE and AES cases. It is calculated using the following formula following Borah et al., (2018)[1].

$$\text{CFR \%} = \frac{\text{Number of deaths}}{\text{Number of cases}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

SPR is a method of enumeration of JE affected cases that accurately provide the rate of total JE and AES positive cases among total registered cases. It is expressed by following formula following Borah et al., (2018)[1].

$$\text{SPR \%: } \frac{\text{Number of cases whose sample were found positive for JE}}{\text{Number of cases whose samples were tested for JE}} \times 100 \ldots\ldots\ldots\ldots \ (2)$$

Average is the arithmetic mean and is calculated by adding a group of numbers and then dividing by the count of those numbers.

$$\text{Average} = (x_1+x_2+x_3+\ldots+x_n)/n \ldots\ldots\ldots\ldots \ (3)$$

3.1.2 Phase 2: Assessment of effect of environmental factors on growth and development of Japanese Encephalitis vector in the study area.

3.1.2.1 Experiment 1: Identification of most prominent JE vector in the locality

Research investigations were carried out at North Bank Plain Agroclimatic Zone, Tezpur, Assam, Gotlong located at 26˚65´25" North Latitudes and 92˚80´50" East Longitude. Entomological collection was done using John W. Hook Center for disease control (CDC) miniature light trap 6 VDC, following the guidelines for surveillance of acute encephalitis syndrome, National Vector Borne Disease Control Programme [2].

For mosquito sampling Mosquito traps were placed within 2 km diameter of human dwelling areas in selected study sites, i.e., areas having reportedly high JE incidences. Three Center for Disease Control (CDC) miniature light traps installed at three places each – cattle shed, residential huts and concrete residential houses depending on the feeding habits of female mosquitoes. Trapping was done for three nights in every month; for mosquito collection CDC light traps were used for duration of 12 dark hours. CDC Light traps are mounted at 1.5 m above the floors next to the foot of beds. Trapped mosquitoes were carefully brought to the laboratory, intoxicated with chloroform (purchased from Merck), and morphological characterization was performed in light microscopes (Olympus, 40x) following the specific taxonomic keys for mosquito species [3]. The details of female *Culex* mosquito for each subspecies that were collected from the trap are carefully recorded and labeled with their specific taxonomic keys.

3.1.2.2 Relative density

Mosquitoes were sampled and identified with the help of distinct morphological features characterized under light microscope (magnification 40X; lens 5X). Mosquito abundance or relative density were evaluated in four seasons – 1) cool and dry (January to March), 2) hot and dry (April –June), 3) hot and wet (July-September), and 4) cool and wet (October – December). The relative abundance or relative density of mosquito species was calculated using the formula suggested by Simsek, 2006[4]

$$\text{Relative density}=\frac{\text{No. of all specimens of each species collected during each period}}{\text{No.of specimens of all species collected during each period}}\times 100 \;\ldots..(4)$$

Five genera identified were: *Anopheles, Culex, Aedes, Armigeres,* and *Mansonia*. However, in this investigation, only *Culex* sp. was chosen because it is the most frequently occurring JE vector in the study area. *Culex* contains three JE subspecies: *Culex tritaineorhynchus, Culex vishnui*, and *Culex pseudovishnui*. These three important JE vectors of *Culex* sp. that breed in agricultural fields were gathered, aggregated, and standardized as numbers per trap per night (12 dark hours) density.

3.2 Objective 2

3.2.1 Application of eco-technical AWDI practices in indigenous rice cultivation systems to mitigate vector breeding across spatiotemporal scales.

3.2.1.2 Implementation of AWDI

Alternate wet and dry irrigation technique was implemented by using some important tools as briefly discussed below:

3.2.1.2.1. Efficient drainage and irrigation facility

AWDI was carried out by creating experimental lowland rice plots of 10 square meters each (Figure 4). Three plots were treated with the AWDI approach, whereas three plots were treated as controls, using the usual way of irrigation. Except for the

AWDI plots, the water level in the other five plots must be kept at 5 cm above the soil level throughout the study period.

3.2.1.2.2 Pani pipe or field water tube

It's a useful instrument for measuring water depth. A 30 cm long water tube was perforated up to 15 cm and planted in rice fields, keeping the perforated end below the soil and the imperforated end 15 cm above the soil and the water level was maintained at 15 cm below the soil surface before re-irrigation.

i) Rice variety resisting bacterial/fungal infection**:** Two varieties were selected for study depending on productivity, quality of grains and disease resistance potential. BB11 and Ranjit sub-1 were used for the experimental studies, BB11 can resist both bacterial and fungal infection and Ranjit sub-1 is stress resistant variety.

ii) Soil testing facility**:** Weekly monitoring of water quality, soil nutrient status, soil physico-chemical variables and soil organic matter.

3.2.1.3 Description of experimental sites

Experiments were conducted in the North Bank Plain Agroclimatic Zone, Tezpur, Assam (Figure 2), during the monsoon rice growing season (Jun to November) for three consecutive seasons, 2020, 2021 and 2022. Experimental sites chosen based on their relative proximity to rice growing fields in villages in Bihaguri sub center under the Block Public Health Center, Sonitpur.

3.2.1.4 Varietal description

High-yielding sali rice varieties B11 and Ranjit sub 1 were selected for this experiment. B11 is a bacterial blight and blast-resistant variety. It is a high tillering variety with a maximum plant height of 110-115 cm. Attain maturity within 110 to 115 days. The grain type is moderately long. Suitable for medium land, Ranjit sub 1 was developed by Assam Agricultural University. The date of notification was 2018. It is a submergence-tolerant variety. It can tolerate submergence for up to 2 weeks. It is suitable for lowlands. Thus it is a very popular and widely used variety of Sonitpur.

The average plant height is 115 cm, and it attains maturity within 150-155 days. The grain type is medium slender.

3.2.1.5 Experimental set up and sample collection

The experimental field was thoroughly ploughed, completely puddled to a depth of 15 cm, and leveled and 12 plots (7 m × 7 m) were constructed, with a 0.5 m space between each plot. 35-day-old seedlings were transplanted into the experimental plots in June 2020, 2021, and 2022 at a 16x15 cm spacing (plant x row) (Figure 3.3). Two irrigation systems were tested in the field in a randomized block design with three replications of each variety. The treatments were: 1) Irrigation at the prescribed application rate (control), which is the traditional technique for both B11 and Ranjitsub1. 2) Controlled irrigation using alternate wet and dry watering methods in both B11 and Ranjit sub1. A detailed experimental plot design and AWDI set up is given in Figure 4 below:

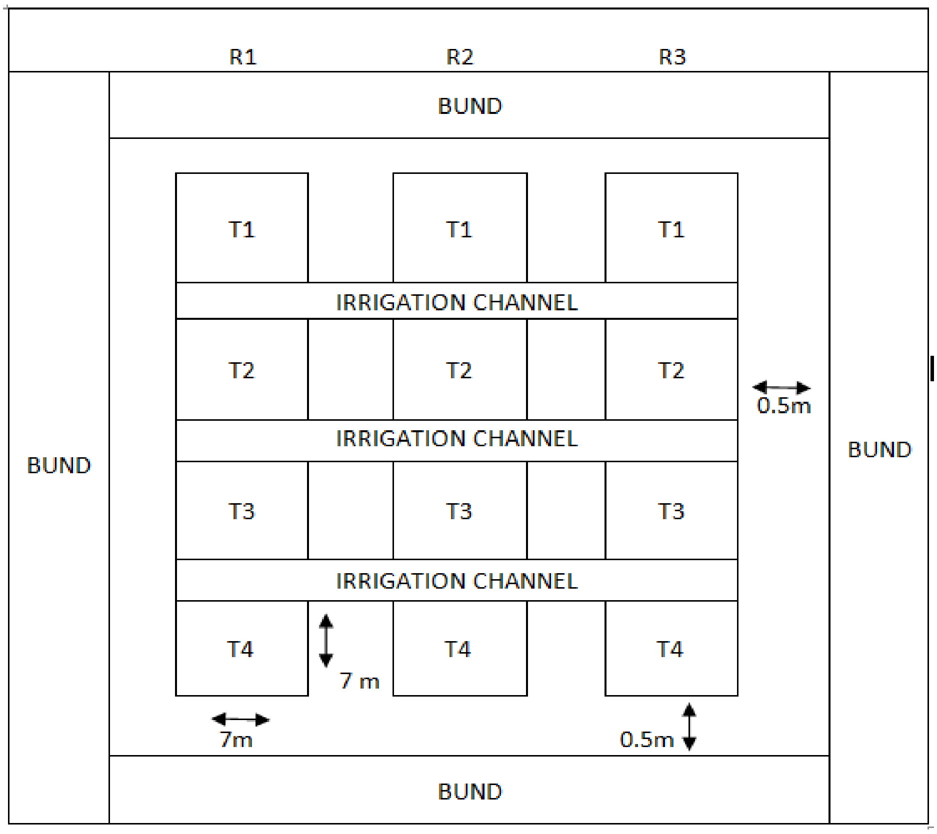

Figure 3.3. Field layout of experimental design: Randomized block design, plot size 7m×7m(*R: Replications, T: Treatment*) showing detailed and AWDI set up

3.2.1.6 Water management

Irrigation water was applied in the field under traditional process of cultivation to maintain 5±2 cm of standing water. During the crop-growing season there was total of 7 (mean) irrigation events, following the recommended package of practice for the cultivation of rice.Under AWDI treated plots, rice fields are treated with intermittent flooding and drying instead of keeping them in continuous submerged condition throughout the cropping season. To implement AWDI safely a “field water tube” is used to record the water level in the fields. A model field water tube was developed by International Rice Research Institute (IRRI), Philippines and Institute for Agro-Environmental Science (NIAES) using polyvinyl chloride (PVC) water tube having diameter of 10–20 cm, that invigilates water level above the soil surface[5]. These tubes were designed using PVC pipes, having an approximate diameter of 10−20 and 30 cm in length, half of the tube was perforated and remained under the soil and the non-perforated end remains above the ground and water was supplied to individual plots by stable irrigation tubes in each plot. In AWDI rice treatment, irrigation was scheduled according to water levels inside the inserted tubes, i.e., water was applied when soil water reached 15 cm soil depth. Immediately after plantation, the water level was maintained at 5 cm above the soil surface for up to 7 days in all the plots to reduce the transplanting shock. After 7 days of transplantation, excess water was drained out in AWDI and reirrigated up to 5cm when crakes appear in soil and water level goes below 15 cm soil depth. However, during rainy days the drains were kept open to drain out the excess rainwater. In a controlled condition, conventional method of cultivation was applied as it was practiced by the local farmers, where water table was constantly maintained at 5 to 7 cm, which is reirrigated when water table was seen below 5 cm (Figure 3.4). However, during panicle initiation stage the water level was maintained constantly at 5 cm level above soil surface, as it is susceptible/sensitive/ vulnerable stage to water stress. Mild water stress experienced during grain filling can promote the translocation of nutrients and carbohydrates to the grains. However, excessive or prolonged water stress can impede grain filling and reduce overall grain filling percentage. The timing and duration of dry periods during AWDI varies based on management practices. The timing of these dry periods relative to the growth stage of rice significantly impacts grain filling. It is crucial to manage dry periods effectively to minimize water stress during critical grain filling stages to optimize the grain filling percentage. During the crop growing season there were 4 to 5 irrigation events.

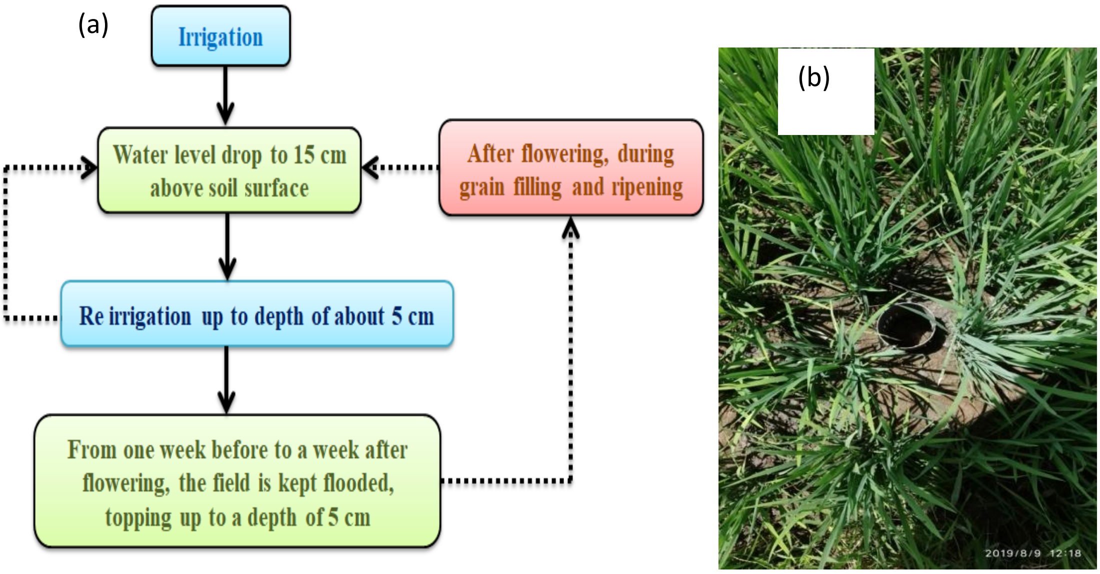

Figure 3.4: (a) Flowchart showing various strategies adopted in AWDI (b) Using pani pipe for AWDI

3.2.1.7 Soil physicochemical parameter analyses

Field soils were gathered using a soil auger from three randomly selected places. A composite sample of almost half kg was created by quartering method. The soil samples were air-dried under shade, ground and sieved using 2-mm sieve. The air-dried soil samples were used for testing different soil physicochemical parameters. Following AWDI treatment, soil samples were also collected randomly from different locations of the AWDI experimental plots from depth of 0-15 cm at different crop growth. All soil samples were collected in three replicates for every treatment and the samples were then brought to the laboratory in self sealing plastic bags to retain the soil moisture. 20 to 30 g fresh soil samples were used for determination of soil moisture content. The remaining soil was air dried for 7 days and sieved (2-mm) as described above and subsequently used for analysis of different parameters like pH, organic matter content, nitrate content

3.2.1.7.1 : Particle size analysis (sand,silt,and clay %)

Particle size or soil texture was determined by the International pipette method[6]. Approximately 10 g soil was taken in 500 ml beaker to which 6% hydrogen peroxide (H_2O_2) was added. The mixture was kept for 24 hours and after 24 hours again 30 ml of H_2O_2 was added. The mixture is then allowed to boil in water bath for one complete day. The solution is then transferred to a conical flask and 15 ml of 2.5 % sodium hexametaphosphate was added and placed the solution in mechanical shaker for 6 hours. It is then diluted making the volume up to 1000 ml and it was mixed by shaking content vigorously for about 1 minute. It was then allowed to stand without any disturbance, the was noted and the room temperature was recorded. The time of sedimentation of various soil particles was estimated from the decantation chart given in “method of analysis of soils”[7]

Silt and clay fraction

After the sand has settled down, the suspension containing silt and clay was pipette out and the content was transferred in to porcelain basin. The basin was kept overnight in an oven at temperature of 105^0C. It was then cooled and weighted. Silt and clay percentage was calculated as follows:

$$Silt\ and\ clay(\%) = \frac{Weight\ of\ sediment\ (g)\times 1000\times 100}{Volumn\ of\ content\ taken\ (ml)\times Weight\ of\ soil\ taken\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The remaining content was stirred and mixed after making the volumn and allowed to stand. 25 ml of the suspension containing only clay was pipette out and transferred into porcelain basin and kept overnight in oven. Next day after cooling the weight was taken. The clay percentage was calculated as follows:

$$Clay\ (\%) = \frac{Weight\ of\ sediment\ (g)\times 1000\times 100}{Volumn\ of\ content\ taken\ (ml)\times Weight\ of\ soil\ taken\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Now the silt and sand percentage were calculated as follows:

Silt(%)=Silt (%)- clay(%)

Sand (%)=100-silt and clay(%)

3.2.1.7.2: Bulk density (g cm^{-3})

Bulk density can be defined as the dry weight of soil per unit volume of sample.

Procedure

Weight of a empty glass bottle was recorded using the weighing machine and then the bottle was filled with the soil sample and again weight was recorded. Then the bottle was fully filled with water and the volume of the water was measured. All these three weights were recorded and put in the formula to get the bulk density of the collected soil samples[7].

Calculations-

Bulk density of the soil (g cm^{-3})= $\frac{W2-W1}{V}$… ……………………………………….(7)

Where, W_2= weight of the glass bottle with soil sample

W_1= weight of the empty bottle

V= volume of water required to fill the bottle

3.2.1.7.3: Particle density (g cm^{-3})

The weight per unit volume of the solid portion of the sample is called particle density. It is measured in gram per cubic centimetre.

Procedure:

Like bulk density, we need a glass bottle to measure particle density. First we have taken the weight of the empty glass bottle. Then the bottle was completely filled with water and the weight was measured. After it, weight of partially filled bottle with soil samples was taken.

Calculations-

Particle density (g cm^{-3}) = $\frac{W3-W1}{W2+W3-W1-W4}$…… ……………………………………….(8)

Where,

W_1= weight of the empty bottle

W_2= weight of the bottle filled with water

W_3= weight of the bottle with partially filled sample

W_4= weight of the bottle with partially filled sample + filled the remaining part with water

3.2.1.7.4: Porosity (%)

Porosity is the percentage of total pore space in the sample. Once the bulk density and the particle density were calculated; porosity can be measured from the following formula.

$$Porosity(\%) = \frac{Db \times 100}{Dp} \ldots\ldots (9)$$

Where, Db = bulk density; Dp= particle density

3.2.1.7.5: Soil moisture content (%)

Soil moisture content was estimated by gravimetric method which is the simplest and most widely used method for measuring moisture content of soil[8]. Equipment required for soil moisture estimation are moisture cans, automatic balance and drying oven. 20 gram of fresh soil was placed in moisture cans with tight fitting lids. The moist soil samples were weighted immediately and was allowed to dry in hot air oven at a temperature of 105^{0}C for 24 hours and reweighted after cooling in a desiccator. Tare weight of the dry moisture cans along with the lid was carefully measured. The soil moisture content was calculated by determining the loss in weight on drying and the weight of the oven dry soil as shown below[4]:

$$Soil\ misture\ content\ by\ weight(\%) = \frac{Loss\ of\ wt\ on\ drying}{wt. of\ oven\ dry\ soil} \times 100 \ldots\ldots (10)$$

Loss of wt on drying = wt. of wet soil- wt. of dry soil

Wt. of oven dry soil= Wt. of dry soil – tare

3.2.1.7.6 Soil pH

Soil pH was measured by Handaled Thermo Scientific multiparameter **S**ystem, Model-STARA3295, which had a single combined electrode. Before measuring the pH of the soil, the instrument was calibrated with standard buffer solution of known pH, 4.0, 7.0 and 9.2, which comes in ready to use solution in sachet. 1:2 soil to water ratio was prepared by adding 40 ml of distilled water to 20 gm of soil in a beaker. The suspension was stirred at a regular interval for 30 min and pH was recorded by immersing electrode in the suspension.

3.2.1.7.7 Soil organic carbon (SOC)(%)

Soil organic carbon was estimated using Modified Walkley & Black titration method. SOC was determined by digesting soil in chromic acid and sulphuric acid using the heat of dilution of sulphuricacid. Organic matter of soil gets oxidized, and excess of chromic acid remains unreduced by organic matter. The leftover chromic acid was determined by titrating with standard ferrous ammonium sulphate solution using diphenylamine as indicator.

1g of sample was taken in a conical flask and 10 ml potassium dichromate and 20 ml of concentrated sulphuric acid was added. The samples were heated until bubbles appeared. It was then kept for some time for cooling and 200 ml of distilled water was added. Afterwards, 1.5 ml of diphenylamine indicator was added following the addition of 10 ml of orthophosphoric acid. It was then titrated with ferrous ammonium sulphate solution. At the end point dark green colour appeared, SOC was calculated using the following equation [9]:

$$Soil\ Organiccarbon\ (\%) = \frac{Vk \times (1 - \frac{Vs}{Vb})}{W} \times \mathrm{Sk} \times 0.3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

Where,

Vk: Volume of $K_2Cr_2O_7$ solution

Vs: Titrant reading

Vb: Blank reading

Sk: Strength of $K_2Cr_2O_7$ solution

W: Weight of soil sample

3.2.1.7.4 Soil respiration($\mu g\ g^{-1}\ h^{-1}$)

Soil microbial respiration is a measure of cumulative respiration of both the aerobic and anaerobic microbe present in the sample that evolves due to microbial metabolism during the decomposition of organic matter present in the sample (FAO, 2023)[10]. Microbial respiration is measured by evaluation of CO_2 due to microbial metabolism. CO_2 evolved is absorbed in NaOH or KOH solution and then the solution is titrated with HCl in presence of phenolphthalein indicator.

10g fresh soil sample was taken in a beaker and placed it in the bottom of a jar. 5 ml 0.1N sodium hydroxide was taken in another beaker and placed it at the bottom of the jar. The jar was closed using rubber rings and two crossing pegs to ensure that it was airtight. Moisture content was predetermined using the procedure described in section 3.2.1.7.1. Blanks were prepared without soils and using the same chemical base. All jars were incubated at 25^0C. After incubation for 24 hours, the beakers with the trap solution were removed out and it was added with 5 ml of barium chloride (0.5M) along with few drops of phenolopthalein indicator. The solutions were then titrated with 0.05N hydrochloric acid under continuous stirring. The end point of the reaction was shown by changing pink colour to colourless solution. The burette readings were noted, and soil respiration was estimated using the following derivation suggested by FAO, 2023[10].

$$\text{Soil respiration } (\mu g\ g^{-1}\ h^{-1}) = \frac{(B-S)\times 100\times \delta}{(Mw-Md)\times t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(12)}$$

B= HCL volume used in titration of blank

S= HCL volume used in titration of sample

δ= Strength of HCl

M_w= Weight of the moist soil

M_d= Weight of the oven dry soil

t= Time of incubation

3.2.1.7.8 Estimation of exchangeable potassium (mg kg^{-1})

For exchangeable potassium (K), 20 ml of 1N ammonium acetate (pH 7) was added to 2 g of soil sample in conical flasks. The flasks were then loaded on a mechanical shaker and shaken for 30 minutes to obtain homogenized soil suspensions. The soil suspensions were then filtered through Whatman No. 1 filter paper. 5 ml of the filtrate was taken in a small tube and the K concentration was measured in a Flame photometer (Systronics Flame photometer 128,µC) and exchangeable K was calculated using the following equation following Black, 1965[11]:

Available K (mg kg^{-1}) = K concentration × dilution factor… ……………………... (13)

For example,

Weight of the sample = ‘W’ g

Dilution factor = $\frac{B}{W}$

Reading of flame photometer = ‘X’

ppm K obtained from standard curve corresponding to X = R

Available K (mg kg^{-1}) = $R \times \frac{B}{W}$

Available K (kg ha^{-1}) = $R \times \frac{B}{W} \times 4.493$.

3.2.1.7.9: Available Soil Nitrogen(mg kg^{-1})

Available soil nitrogen was measured by Alkaline permanganate method using Kel Plus Automatic Nitrogen estimation system (Kelplus, Kelvac VA). 5g of sample was loaded in distillation unit (Kelplus, Distyl-EM VA), outside hose of which was kept with 20 ml of 2% boric acid along with mixed indicator. Potassium permanganate along with sodium hydroxide solution was added automatically by distillation unit programme. The sample is then allowed to heat up by passing steam. The liberated ammonia was absorbed in 2% boric acid having mixed indicator in it. The solution turned to green as soon as ammonia got absorbed. The distillate was then titrated with 0.02N sulphuric acid and at the end end point of the reaction the solution regained its original shade. Blank samples were run simultaneously. Calculation was done using the following formula suggested by AOAC, 1995[12]:

Easily mineralizable N (mg kg^{-1}) $= \frac{(Vb-Vs)\times 0.28\times 1000}{W}$ ……………………………… (14)

Where,

W = Weight of the sample taken.

Vb = Volume of $\frac{N}{50}$NaOH solution consumed in the titration in the blank.

Vs = Volume of $\frac{N}{50}$NaOH solution consumed in the titration in the sample.

Easily mineralizable N in kg ha^{-1} = (Vb- Vs) ×125.44

3.2.1.7.10: Estimation of Available P (mg kg^{-1})

The estimation of available P was done by Olsen[13] extraction method when pH of the sample was more than 6 whereas for pH less than 6 the concentration was measured following Bray-Kurt's[14] method.

A. Estimation of available Phosphorus by Olsen extraction method

2.5 g sample was weighed in a 250 ml conical flask and 50 ml of extractant [0.5M sodium bicarbonate (NaHCO3) (pH 8.5)] solution was added. The flasks were loaded on a mechanical shaker and vigorously shaken for 30 minutes. After 30 minutes, the soil emulsion was filtered through Whatman (no. 42) filter paper. If yellow colored aliquot was observed then a spoon of P-free charcoal was added and filtered again. 5 ml of filtrate was transferred to a 50 ml volumetric flask and 4 to 5 drops of dinitrophenol, 4N ammonium hydroxide (NH_4OH) and 4N hydrochloric acid (HCl) were added sequentially. Subsequently, 5 ml of ammonium molybdate was added to each flask followed by 2-3 drops of (5 %) stannous chloride and then, volume was made up to 50 ml with distilled water. The solution was then mixed well, and its absorbance was read at 660 nm. Standard curve was prepared with 2 ppm of standard P solution as1, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 20.0 ml were pipetted in individual 50 ml volumetric flasks; available P was calculated using the equation mentioned below[13] :

Available P (mg kg^{-1}) = Absorbance × Slope × dilution factor……………………. (15)

B. Estimation of available P by Bray-Kurt's method

2.5g sample was weighed in a 250 ml conical flask and 25 ml of extractant 0.03N ammonium fluoride (NH_4F) solution was added. The flasks were loaded on a mechanical shaker and vigorously shaken for 30 minutes. After 30 minutes, the soil emulsion was filtered through Whatman (no. 42) filter paper. If yellow colored aliquot was observed then a spoon of P-free charcoal was added and filtered again.5 ml of filtrate was transferred to a 50 ml volumetric flask and 4 to 5 drops of dinitrophenol, 4N ammonium hydroxide (NH_4OH) and 4N hydrochloric acid (HCl) were added sequentially. Subsequently, 5 ml of ammonium molybdate was added to each flask followed by 2-3 drops of (5%) stannous chloride and then, volume was made up to 50 ml with distilled water. The solutions were mixed properly, and spectrophotometric (Make- Shimadzu, model UV-1700 Pharmaspec, UV-VIS Spectrophotometer) absorbance was read at 660 nm, and available P was calculated from the following equation suggested by Bray and Kurt, 1945[14]:

Available P (mg kg^{-1}) = *Absorbance* × *Slope* × *dilution factor*…………... ………(16a)

$$\text{Available P in kg ha}^{-1} = \frac{Absorbancy \times F \times Volume\ of\ extractant\ used \times 2.24}{Volume\ of\ filtrate\ used \times Weight\ of\ soil\ sample} \quad \ldots\ldots\ldots\ldots.(16b)$$

$$\text{Where } F = \frac{Concentration\ of\ P\ in\ ppm\ from\ standard\ curve}{Absorbancy from\ standard\ curve}$$

3.2.1.7.11 Determination of soil micronutrient content (Zn, Cu and Mn) (AAS and ICP-AES method)

To avoid contamination of soil, samples were collected very carefully in plastic bags using rust free wooden instruments, and samples were ground using wooden mortar and pastel. Soil samples were analyzed for bio-available metals following the diethylenetriaminepentaacetic acid (DTPA) extraction method of Lindsay and Norvell, (1978)[15] using Atomic absorption spectrophotometer (AAS) and ICP-AES.The diethylene triamine pentaacetic Acid (DTPA) test of Lindsay and Norvell, (1978)[15] is commonly used for evaluating fertility status with respect to micronutrient cations, i.e., Zn, Mn, and Cu. The DTPA method is an important and widely used chelating agent, which combines with free metal ions in the solution to form soluble complexes of elements. Working solution of 50 ppm for each metal was prepared out of the Prepared Standard solutions marketed by Merk. Then a series of standard solutions as 0.5, 1.0, 1.5, 2.0, and 2.5 ppm were prepared for each micronutrient.

DTPA extract was prepared by taking 1.97g of DTPA and 1.470 g $CaCl_2.2H_2O$ in a beaker and adding 20– 25 ml of double distilled water (DDW) along with 13.3 ml of tri ethanol amine (TEA) followed by 100 ml of Doubled Distilled Water. Then, make the volume up to 1 l. Adjust the solution pH 7.3 for extracting different trace elements remaining in bio-available forms. 20 ml of sample was taken in a 250 ml of conical flask. To it 40 ml of DTPA extract was added. Shaked for 2 hrs in the mechanical shaker and then filtered. The sample was ready to carry out the estimations of these elements with the help of AAS and ICP-AES.

3.2.1.9.1 Plant height (cm)

High yielding rice varieties were grown in this investigation - Sali, B11 and Ranjit sub -1. The rice plants were regularly monitored starting from the first day of implantation and morphological characters were systematically observed and noted. And Rice plant heights were measured in centimeters (cm) and were assessed from the base to the tip of the longest leaf of every rice plant.

3.2.1.9.2 Leaf area index (LAI)

LAI for each plant's individual leaves were computed from the length and breadth of each leaf. Leaf Area Index for each plant's individual leaves was computed by dividing total leaf area (m^2) by ground area (m^2) covered by plant[16]:

Leaf Area (LA) = length × width ×0.83…………………………………………….(17a)

LAI=Leaf Area/Ground Area………………………………………………….......... (17b)

3.2.1.9.3 Grain filling percentage

The grain filling percentage was calculated by dividing the number of filled grains in a panicle by the total number of grains in the panicle[17]. Five randomly selected panicles from each of the three replications were used to count the total number of grains in each panicle. For each panicle, the grains that were filled and those that were not counted independently.

$$Grain\ filling\ (GF) = \frac{Total\,number\,of\,filled\,grains\,per\,panicle \times 100}{Total\,grains\,in\,each\,panicle} \ldots\ldots\ldots\ldots\ldots.. \ldots\ldots\ldots\ldots.(18)$$

3.2.1.9.4 Grain yield(kg ha^{-1})

Mature plants were removed from a 1 m^2 area in replicas of each treatment, taking care to avoid the border row. The grain weight was measured and stated in kilograms per hectare following threshing and cleaning[17].The plant samples were utilized for chemical assay after being dried, ground, and sieved through a 2 mm stainless steel screen with the use of a grinder.

3.2.1.9.5 Carbohydrate (mg ml^{-1})

Carbohydrate of grain was determined by phenol disulphonic method of total carbohydrate estimation. Plant samples (grains) were grinded and 100 gm of sample taken in a boiling tube. It is mixed with 5 ml of 2.5N HCL and kept to hydrolyze in a boiling water bath for 3 hours. It was then cooled to room temperature and mixed with sodium carbonate to neutralize the solution. After making the volume upto 100 ml, the solution was allowed to centrifuge. 0.1 and 0.2 ml of sample solution in two separate test tubes. 1ml of phenol solution and 5 ml of 96% sulphuric acid was added to each of the tube and shake well. It was again placed at water bath at 25-30^{0}C for 20 minute and read the colour at 490 nm. Blank samples were run simultaneously using the same chemical base and same procedure as samples

Standard curve was drawn with glucose solution in a series of test tubes by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The volume was made upto 1ml in all the tubes including the sample tubes by adding distilled water. To it 5 ml of phenoldisulphonic is added in each tube. The solution was then heated for eight minutes in a boiling water bath. The contents were then cooled rapidly and the change in intensity of green to dark green colour was read at 490 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the

Y-axis. From the graph the amount of carbohydrate present in the sample tube was calculated[17]:

$$\text{Amount of carbohydrate present in the sample} = \frac{mg\ of\ glucose}{volume\ of\ test\ sample} \ldots\ldots\ldots(19)$$

3.2.1.9.6 Chlorophyll (mg g^{-1})

To execute this experiment, fresh leaves were cut in to similar sized. The pellets were weighed, transferred into a pestle-mortar with 80% acetone and grinded thoroughly. The ground samples were frozen at -18°C for 3 hours and centrifuged at 2500-3000 rpm for 20 minutes. The extract i.e., the supernatant derived after centrifugation was used to measure the absorbance of the samples was read at 664.2 nm and 648.6 nm for Chlorophyll A, Chlorophyll B respectively[9].

Calculation**:** Chlorophyll (mg chlorophyll g^{-1} fresh tissue)

Chlorophyll A = $(13.36 \times A_{664.2})$- $(5.19 \times A_{648.6})$……………………………………(20)

Chlorophyll B = $(27.43 \times A_{648.6})$- $(8.12 \times A_{664.2})$…………………………………....(21)

Total Chlorophyll = $(5.24 \times A_{664.2}) + (22.24 \times A_{648.6})$……………………………… (22)

Where

$A_{664.2}$ = Absorbance at 664.2 nm

$A_{648.6}$= Absorbance at 648.6 nm

3.2.1.9.7 Phosphorus and Potassium in plant (mg kg^{-1})

The P and K content in plant sample was done by the di-acid digestion method, using acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio 9:4. Initially, 1 g of dry ground grain and plant biomass (straw, leaf etc) was taken in conical flasks and 10 ml of the di-acid mixture was added to the flasks. The samples were then heated on a hot plate until the red fumes disappeared. Completion of digestion was confirmed when liquid become colorless. After cooling, 20 ml of distilled water was added to the digested sample, mixed thoroughly and the content was filtered. The content was directly analyzed in flame photometer for determination of K. For calibration of Flame photometer, standards of known concentration like 5, 10, 15, 25, 50 and 100 ppm were prepared by diluting stalk solution of 1000 ppm K following Black, 1965[17].

Calculation: $K\ content\ (mg\ kg^{-1}) = K\ concentration \times dilution factor$……………… (23a)

For analysis of P-content, 5 ml digested and filtered sample was mixed with 5ml of ammonium molybdate and the volume was made up with distilled water. Few drops of stannous chloride were added and finally the volume was made up to 25 ml and absorbance was read at 660 nm as suggested by Black, 1965[17].

Calculation: P content (mg kg^{-1}) = $Pconcentraion \times dilution\ factor$…………(23b)

Nutrient uptake was estimated by multiplying concentration (%) of particular nutrient of grain and straw with their respective yield (kg ha^{-1}) and finally the nutrient uptake by grain and straw was sum up to obtain total nutrient uptake by crop[18].

Calculation:

$$\text{Nutrient uptake by straw/grain (kg per ha)} = \text{Yield} \times \text{Nutrient content} \quad \text{……….(24a)}$$

$$Nutrient\ uptake\ by\ crop\ (kg\ per\ ha) = Nutrient\ uptake\ by\ grain + Nutrient\ uptake\ by\ straw \quad \text{………………(24b)}$$

3.2.1.9.8 Total Kjeldahl Nitrogen and crude protein (%)

Nitrogen in samples like plant and grains exists in a very complicated bonding structure. Therefore, the complicated structure must be broken into simple structures for estimation of total nitrogen and hence the crude protein. Therefore, determination of nitrogen in the plant leaves and grains included the process of digestion, where 0.5 g of plant sample was digested at high temperatures of 410-420°C in presence 10 ml of sulphuric acid. To speed up the process 5g of catalyst mixture consisting of potassium sulphate, copper sulphate and metallic selenium in the ratio of 50:10:1 was added. Digestion was carried out in Kjeldahl digestion tube using Kel Plus Automatic Nitrogen estimation system. The sample turned light green at the end of the digestion process and finally turned colourless on cooling down. The colorless digested samples are then were transferred to the Kjeldahl distillation unit, where the samples are heated by passing steam at steady rate, automatic addition 40 ml of 40% NaOH by the programmed distillation unit. The distillation process released ammonia which was absorbed in 20 ml of 4% boric acid containing a pre-mixed indicator. With absorption of NH_3, the pinkish colour of boric acid solution turned to green. In the last step, the green coloured distillates were titrated with 0.02N sulphuric acid. Simultaneously blank samples (without plant sample) were also treated similarly. At the end point of the titration, the pinkish colour was retained and the blank and the sample titer reading were noted down and calculated for total nitrogen content in percentage.

$$\text{Total Kjeldahl N }(\%) = \frac{(R \times Normality of the acid \times atomic weight of Nitrogen) \times 100}{W \times 1000} \text{……..…(25)}$$

$$= \frac{R \times 0.1 \times 14 \times 100}{0.5 \times 1000} \text{…………………………………………………………(26)}$$

$$= R \times 0.28$$

Where,

W= dry weight of the sample in gram.

R= sample titer- blank titer

The crude protein was determined using following formula:

$$Crude\ protein\ content(\%) = Micro - Kjeldahl Nitrojen content\ (\%) \times 6.25 \text{ ……...(27)}$$

The factor 6.25 was based on the assumption that nitrogen constitutes 16% of the protein suggested by AOAC, 1995[17].

3.2.1.10 Determination of plant micronutrient content (Zn, Cu and Mn) in soil (AAS and ICP-AES method)

Plant micronutrients were calculated by digesting the plant samples with a di-acid mixture, the digest obtained were diluted and treated following Section 3.2.1.7.8 i.e., estimation of soil micronutrients by AAS and ICP-AES method. A working solution of 50 ppm for each metal micronutrient was prepared out of a standard stock solution (add the name of the standard mentioned on the package). A series of standard solutions of 0.5, 1.0, 1.5, 2.0, and 2.5 ppm were prepared for each metal. 0.5g plant sample was taken in a conical flask. 12 ml of di acid mixture of percolic and nitric acid were added and digested the mixture was kept on hot plate till the plant sample residues turned colourless. The digests were cooled, diluted and filtered through Whatman No.1 filter paper. The filtrate volume was made upto 50 ml. The diluted solutions were analysed for micronutrients in Atomic Absorption Spectro-photometer following Lindsay and Norvell, 1978[17]. Micronutrient uptake by crop was estimated by using the similar formula described in 3.2.1.9.7, equation no 24a and 24b.

3.2.1.11 Nutrient harvest index (NHI)

Grain and straw samples of the rice plants were collected samples at the time of harvest and analyzed for total nitrogen following Micro Kjeldahl Method, while Total P and K were determined using Sulphuric-percholic acid digestion method demonstrated by Prasad, (1998). Nutrient removal was estimated by multiplying the NPK concentration (%) of grain and straw with their respective yield (kg ha^{-1}) and finally the nutrient uptake by grain and straw was sum up to obtain total nutrient uptake. Nutrient harvest index was computed using the formula given below[18]:

Nutrient harvest index (NHI) = $\frac{Uptake\ of\ perticular\ nutrient\ by\ the\ grain}{Total\ uptake\ of\ the\ nutrient\ by\ biomas} \times 100 \ldots \ldots$(28)

3.2.1.12 Water productivity index (WPI)

WPI is generally expressed as crop yield (kg ha^{-1}) per unit water use (m^{3} h^{-1}) ratios[19]. It includes all types of water supply such as rainfall, irrigation, and antecedent soil moisture and represented by the following formula[20]:

Water productivity index (kg/m^{3}) = $\frac{\text{Total grain yeild(kg/hac)}}{\text{Total water supply(m3/hac)}}$………………………………… (29)

3.3: Objective 3: Efficiency evaluation of AWDI as a vector management strategy in rice agroecosystems

3.3.1: Description of experimental sites

Experiments were conducted in the North Bank Plain Agroclimatic Zone, Tezpur, Assam, during the monsoon rice growing season (June to November) for two consecutive seasons, 2021 and 2022. The total rainfall experienced by the experimental field in 2021 was 699.67 mm with a total of 131 numbers of rainy days. Similarly in 2022 the total rainfall was 565.78 mm and total numbers of rainy days was 126 (figure 3.5). The daily rainfall and number of rainy days per month is represented in the figure: 3.3.1 experienced by the study site.

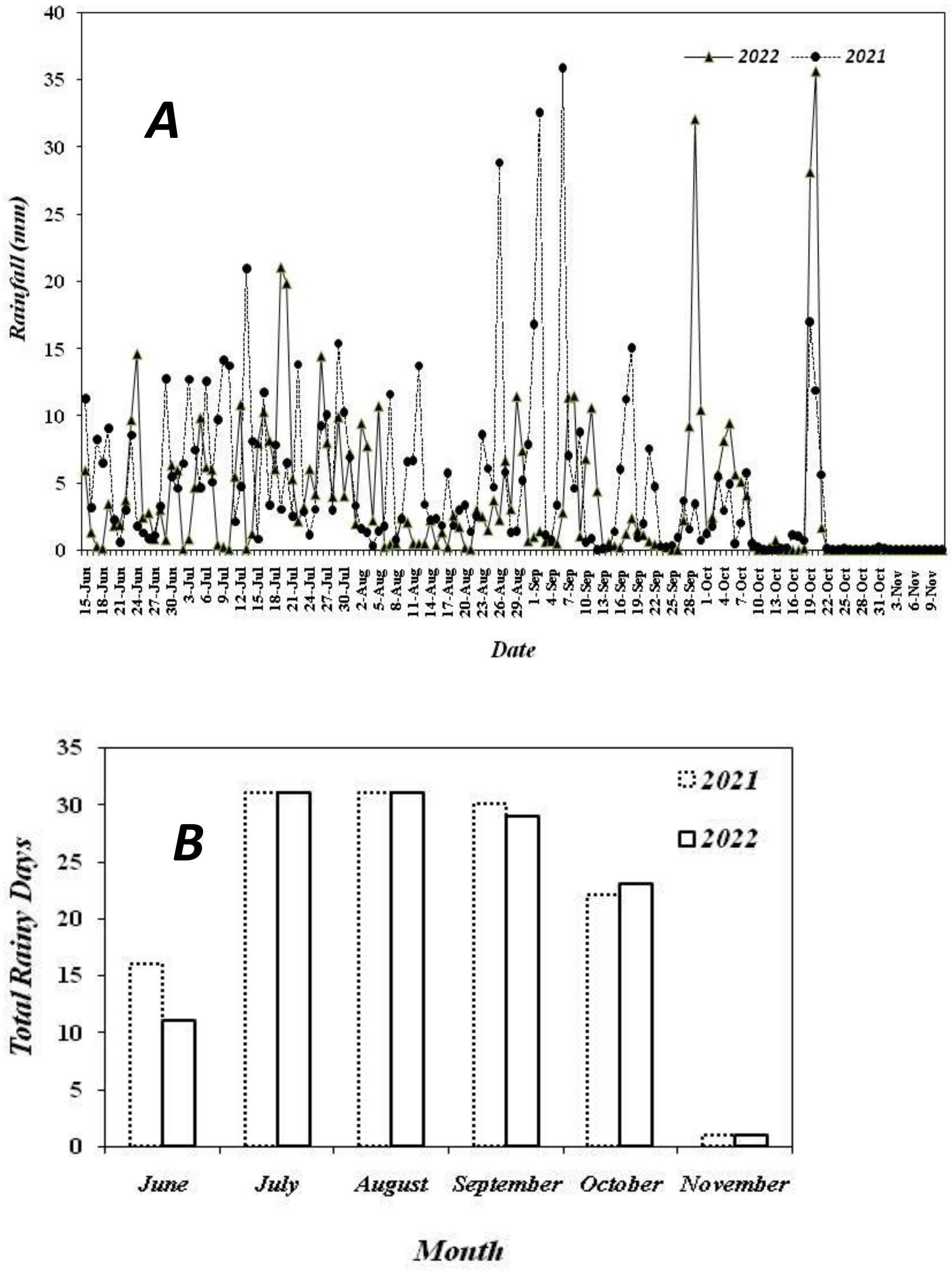

Figure 3.5: Distribution of rainfall events (A) and number of rainy days (B) during study period

3.3.2: Implementation of AWDI

Details methodology for implementation of AWDI in the study field and description of variety selected are described in **3.2.1.6** of chapter 3, page no. 52.

3.3.3 Water physicochemical parameter analyses

Water samples were collected using clean and sterile polyethylene bottles, by lowering a closed bottle to the bottom, opening and closing it there by hand and bringing it to the surface. Prior to sampling, the bottles must were thoroughly rinsed with the sample water even if it was precleaned.

3.3.3.1 Dissolved oxygen (mg L^{-1})

Dissolve oxygen (DO) was determined by Orion star RDO optical dissolved oxygen sensor and ThermoscientificOrion Star and Versa Star Meter. Before using the sensor it was calibrated by water saturated air calibration. After calibration the DO (mg L^{-1}) was measured following standard operating procedures for DO provided by ThermoscientificOrion Star and Versa Star Meter.

3.3.3.2 Nitrate (ppm)

Nitrate-N was measured using Nitrate ion selective electrode make of Thermoscientific ion Star and Versa Star Meter. Before measurement of nitrate, the electrode was calibrated and *Ionic strength adjuster* **(**ISA) for nitrate measurement was added in every sample to settle down other ions present in the solution. Following this nitrate-N was recorded as per the standard operating procedures for DO, provided by Thermoscientific Orion Star and Versa Star Meter.

3.3.3.3 pH

pH was simultaneously measured at the time of water sampling using Thermo Scientific Orion pH Meter with ROSS electrode. Readymade buffers of pH were used for calibration. After calibration pH of the water samples was measured following the instructions provided with the Thermoscientific Orion Star.

3.3.3.4 Temperature

Along with pH and electrical conductivity, temperature (˚C) was immediately measured during water sampling using a mercury thermometer.

3.3.3.5 Total Dissolved Solid (TDS) (mg L^{-1})

Total dissolved solid is determined as residue left after evaporation of the filtered sample. Water sampleswerefiltered through glass fiber filter paper and an evaporating dish was heated in a muffle furnace and weighted for evaporating the water samples.100 ml of the filtered sample was taken in the preweighed evaporating dish on a water bath having temperature not more than 98˚C. The filtrate residue for each water sample was heated at 103-105˚C in a hot airoven for one hour and the final reading was taken after cooling in a desiccator. TDS was calculated in mg L^{-1} using the following the formula[9]:

$$TDS\ (mg/L) = \frac{(A - B) \times 1000 \times 1000}{V} \quad \text{……………………………………………….(30)}$$

Where A= Final weight of the dish in gram

B= Initial weight of the dish in gram

V= volume of sample in ml

3.3.3.6 Salinity

Salinity was measured with Thermoscientific Orion Conductivity Meter by following the instructions supplied with the multiparameter electrode. Salinity was measured in parts per thousand

3.3.3.7 Total phosphorus (ppm)

Total phosphorus (P) was analyzed by digestion method following Trivedy and Goel, (1986)[9]. A suitable volume of water samples were taken in 100 ml Kjeldahl flask. 1 ml of sulphuric acid (H_2SO_4) and 5 ml of conc. Nitric acid (HNO_3) were consecutively added. Thereafter the samplesweredigested on ahot plateuntil the volume reduced to nearly 1ml and heated further till the solutions turned colorless, that indicated complete removal of nitric acid (HNO_3). The digests were diluted with distilled water and phenolphthalein was added to them. The acid content in the digested and diluted samples was neutralized by titrating it with NaOH. At the end point the solution turned pink and 2 ml of ammonium molybdate was added followed by addition 5 drops of $SnCl_2$ solution, the solutions turned deep blue and spectrophotometric reading was recorded at 690 nm, a distilled water blank was also prepared with the same amount of chemicals as the water samples and readings spectrophotometric reading were taken at 690 nm. For Standard curve, standard solution of phosphate of 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9 and 1.0 mgL^{-1} were prepared by dilution, from a starndard solution of 10 mgL^{-1} of phosphorus concentration. The standards were mixed with ammonium molybdate and stannous chloride and absorbance at 690 nm were recorded. Concentration of phosphorus was calculated after plotting graph between absorbance and concentration of standards and comparing absorbance of samples with unknown concentration of phosphorus[6].

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