Chapter 6

Objective 3:

Efficiency evaluation of AWDI as a vector management strategy in rice agroecosystems

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6.1 Introduction

The primary mosquito vectors of Japanese encephalitis (JE) in India are *Culex tritaenorhynchus, Culex vishnui,* and *Culex pseudovishnui*, which predominantly breed in paddy fields[1]. These medically important species flourish in the complex agro-ecosystem of rice fields, characterized by standing water, high humidity, sunlight, and elevated temperatures throughout the crop growing season[2]. Other species of mosquitoes that breed in rice fields include *Aedes vexans* , *Culex vagans* , *Culex bitaeniorhynchus* , *Culex fuscocephala* , *Culex gelidus* , and *Culex whitmorei* [3]. However these species are not of medical importance[3]. There are noticeable changes in the physical and chemical characteristics of rice field water both during the day and throughout the crop cycle[4]. The relative abundance of mosquitoes breeding in rice fields is significantly impacted by these changes. Variations arise due to precipitation dilution, surface soil dispersal caused by agricultural techniques, natural events, and fertilizer application. The larval population is temporarily impacted by agricultural practices like as field drying and deweeding[5]. It was previously reported that applying nitrogenous fertilizer across rice fields increased the number of mosquito larvae[6]. In South India, the fields receiving the highest fertilizer dosage had the highest population of immature culicine[7].

Larval density and growth rate are reported to be influenced by temperature, pH, ionic composition, source and depth of water, temperature[8], and conductivity[9]. The well-known technique of managing water for water saving cultivation using intermittent irrigation is effective in preventing disease vectors from reproducing in rice fields[10][3]. The physico-chemical properties of water were reported to affect load of mosquito larvae and development in their nesting habitat[1][11]. Mosquito immaturities are generally positively impacted by temperature[12]. There was a definite negative correlation between the concentration of dissolved oxygen and culicines[12]. The dissolved oxygen content and pH have a positive relationship, and both tend to decrease with the age of the paddy plants[13]. Overall hardness of water was beneficial to the immature mosquitoes, but salinity doesn't seem to matter[13].

The three significant JE vectors adapt differently to the physical and chemical shifts in their breeding environments and the developmental stages of rice plants[12]. Adjusting irrigation techniques could lower the populations of *Culex tritaenorhynchus*, highlighting the need for a detailed understanding of the factors influencing mosquito abundance[14]. The present study intends to compare the effects of alternate wetting and drying irrigation (AWDI) methods on mosquito populations in India with those of conventional agricultural practices. This analysis seeks to elucidate the relationship between irrigation methods and mosquito vector dynamics, which is crucial for effective vector control strategies.

6.2 Methodology

The study was carried out for two consecutive years i.e. from 2021 to 2022. In this study, the investigation period was divided into five phonological stages (phases of growth) for the purposes –1) Initial (phase 1/P1) phase, 2) early vegetative3) late vegetative and panicle initiation phase (phase 2/P2), 4) flowering and grain filling phase (phase 3/P3) and 5) grain maturation and harvesting phase (phase 4/P4) (table 6.2).Phages were divided based on the growth and development of rice plants as we wanted to investigate *Culex* larvae in relation to paddy cultivation.

Table 6.2. Different growth phages of rice plant in the study field

Phonological stage	Number of days after transplanting(DAT)	Phage label
Initial	0	P1
Early vegetative	45	P2
Late vegetative and panicle initiation phase	105	P3
Flowering and grain filling phase	120	P4
Grain maturation and harvesting phase	135	P5

Table 6.3: Calendar of agronomic practice in the experimental fields

Agronomic practice	First year(2021)	Second year(2022)
Plantation	15th June	20th June
First AWDI	31st July	5th August
Second AWDI	12th September	17th September
Third AWDI	2nd October	7th October
Harvesting	11th November	16th November

6.2.1 Description of experimental sites

Experiments were conducted in the North Bank Plain Agroclimatic Zone, Tezpur, Assam, during the monsoon rice growing season (Jun to November) for two consecutive seasons, 2021 and 2022. 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) of chapter 3 .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. Investigation sites were also selected depending on their proximity to paddy fields in a village named Gotlong in Bihaguri Block Public Health Center in Sonitpur. The study site experienced a high rainfall during the study period. 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. The daily rainfall and number of rainy days per month is represented in the figure: 6.2.1 experienced by the study site

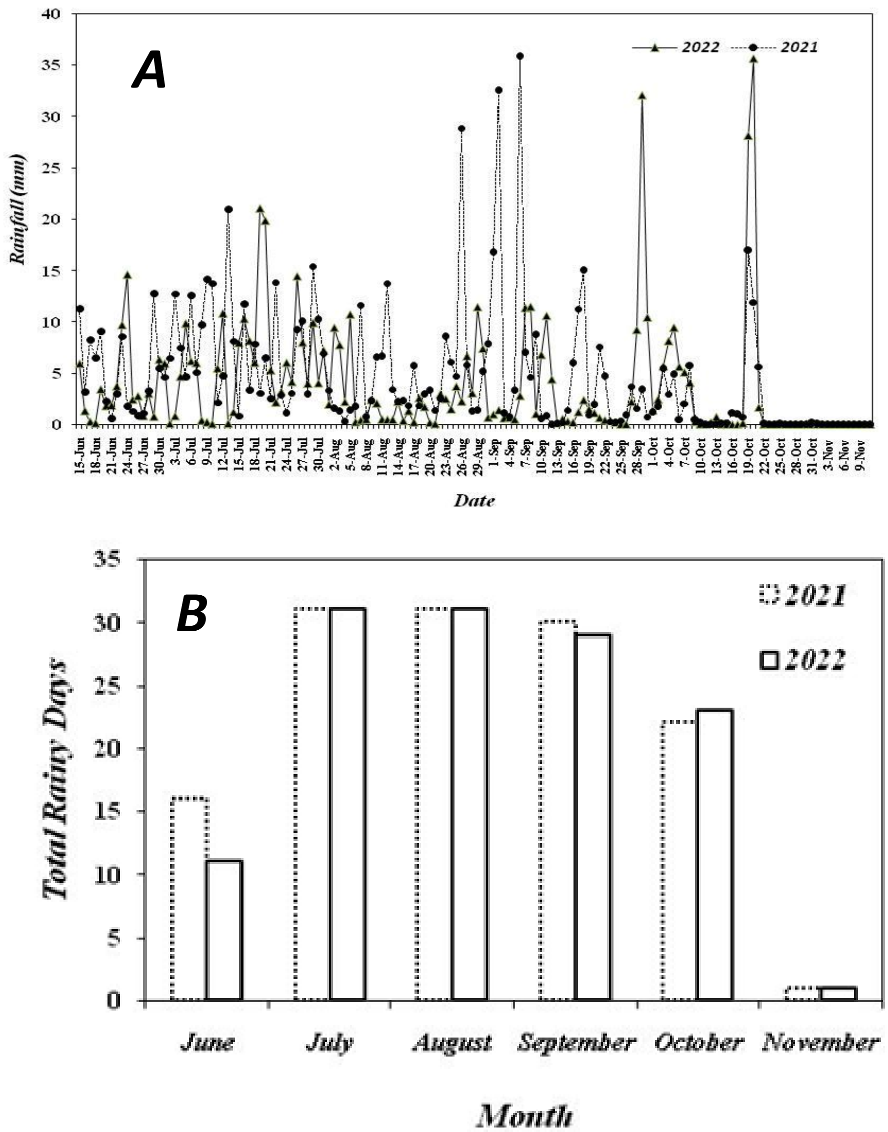

Figure 6.2.1: Distribution of rainfall events (A) and number of rainy days (B) during study period

6.2.2 Description of rice variety

High yielding rice varieties–Sali, B11 and Ranjit sub 1 were selected for this experiment. The B11 is bacterial blight and blast resistant variety. It is high tillering variety with maximum plant height of 110-115 cm. Attain maturity within 110 to 115 days. Grain type is moderately long. Suitable for medium land, Ranjit sub 1 was developed by Assam Agricultural University. Date of notification was 2018. It is submergence tolerant variety. It can tolerate submergence up to 2 weeks. It is suitable for low land. Thus it is very popular and widely used variety of Sonitpur. Average plant height is 115 cm and attains maturity within 150-155 days. Grain type is medium slender.

6.2.3 Water management

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. Here, the field remains continuously submerged throughout the cropping season. 2) Controlled irrigation using alternate wet and dry watering methods in both B11 and Ranjit sub1. 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[15]. 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 up to 7 days in all the plots, in order reduce the transplanting shock. After 7 days of transplantation, excess water was drained out in AWDI and reirrigated up to 5 cm 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. 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. However, during panicle initiation stage the water level was maintained constantly at 5 cm level above soil surface, as it is vulnerable stage to water stress. During the crop growing season there were 4 to 5 irrigation events.

6.2.4.1: Sample collection

Clean and sterile polyethylene bottles were used to collect water samples. Even if the bottles have been precleaned, they must be thoroughly rinsed with the sample water before sampling. Using a standard enamel dipper, we collected 1 L of water from each rice field where immature mosquitoes were collected, on each sampling occasion. A plastic container was used to transport water samples to the laboratory for immediate analysis. The water samples were analyzed according to the methods recommended by the American Public Health Association (APHA, 1976) [16] for Dissolve oxygen (DO), pH, Nitrate-N, Temperature, Total Dissolved Solid (TDS), Salinity and inorganic phosphorus. Leaf area index was calculated by standard procedure suggested by Chen, 1992[17]. Leaf area index was calculated at each growth phage of rice plant. Details methodology for water testing is described in Chapter 3.

6.2.4.2 Per dip density of mosquito larva

During different times of a study period and in different habitats, the per dip density measures the concentration of immature stages. To estimate the concentration of larvae and pupae in various breeding sites throughout a study period, per dip density is often computed. Observations were made throughout the growth phase of the paddy plant. Each field under conventional and AWDI was sampled randomly with a 350 ml dipper following a standard procedure suggested by Rajendran et al., 1995 [10]. Prior to returning the immature larvae to their field of origin, subfamily and genus of the larvae were recorded separately. Ballav et al., 2021[18] suggested the following formula to calculate larval density per dip.

$$Average\ Per\ dip\ density\ (APD) = \frac{Total\ number\ of\ immature\ collected\ from\ a\ habitat}{Total\ number\ of\ dips\ performed} \quad \ldots\ldots\ldots\ldots(33)$$

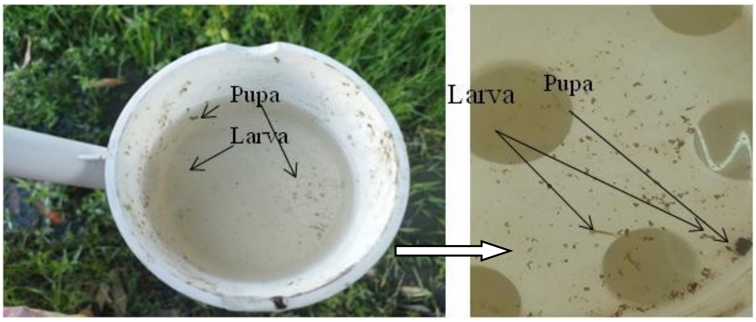

Figure 6.2.2: Picture showing collection of *Culex* larvae from study field with 350 ml dipper

6.2.4.3 Breeding index (BI)

Belkin (1954)[19] developed a simple index for determining larval densities which is represented as Breeding Index or BI. BI is calculated using following formula-

$$Breeding\ Index(BI) = \frac{SA \times PD \times ADP}{ND \times 100} \quad \text{...(34)}$$

Where, SA= Surface area of body of water serving as effective breeding site for mosquito

PD= Positive dips

APD= Average per dip

ND= Total number of dips

6.2.4.4 Statistical analysis

The larvae of significant JE vectors of Culex sp., known to breed in agricultural fields, are collected, pooled and standardised to average per dip density and breeding index. The present study uses Pearson's correlation and linear regression to examine the statistical interrelationship between field water parameters and entomological variables statistically. Regression analysis (SPSS) helped to understand the role of water chemistry with the abundance JE vector mosquitoes larvae in the study area. This was followed by Least Significant Difference (LSD) test to differentiate the efficiency of various treatments at $p < 0.05$ using SPSS.

6.3 Results and discussion

The fluctuation in values of the various abiotic factors showed characteristic patterns in present study, depending on the changes taking place within the ecosystem as a result of agricultural practices. The chemical composition of the field water depends on that of the irrigation water and the soil.

Table 6.4. Basic water physicochemical properties of experimental field during study period

Sl No.	Parameters	Year	
		2021	2022
1	**Temperature (°C)**	26	26.5
2	**Dissolved oxygen** ($mg\ L^{-1}$)	6.75	6.84
3	**Salinity (ppt)**	0.49	0.45
4	**Nitrate nitrogen (ppm):**	4.67	4.43
5	**pH**	7.2	7.22
6	**Total Dissolved Solids (ppm)**	25	26
7	**Inorganic Phosphorus (ppm)**	0.52	0.59

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

The mean concentration of dissolved oxygen in the water samples varied between 6.82 to 5.78 mg L^{-1}, which indicates minimal variation across different treatments in the study. This information highlights a stable but slightly declining trend in oxygen levels, pertinent for evaluating water quality. In 2021, the treatment had a statistically significant positive effect on dissolved oxygen (DO) levels across both varieties with a p-value of less than 0.05 (LSD = 0.08). Conversely, in 2022, a minor reduction in DO was specifically noted in treatment B11, also with a p-value of less than 0.05 (LSD = 0.18). The decrease in dissolved oxygen levels may be attributed to reduced photosynthetic activity, which occurs as algal biomass diminishes following canopy development[12]. This factor is crucial, as photosynthesis is a significant contributor to DO replenishment in aquatic environments. The study did not find a significant relationship between dissolved oxygen levels and *Culex* larval density, indicating that other environmental variables may be more influential on larval populations than DO concentrations (p_T >0.05, r=0.26).

The dissolved oxygen concentration in field water results from a dynamic equilibrium involving three main processes: production by photosynthetic aquatic biomass (PAB), diffusion between air and water, and consumption via respiration and oxidation[4]. This equilibrium is essential for understanding water quality and the health of aquatic ecosystems. Changes in dissolved oxygen levels are also influenced by algal blooms, particularly following nitrogen fertilizer application. During the crop growth period, oxygen values typically rise with the growth of PAB until approximately 20 to 40 days post-transplanting, after which the canopy's development leads to a decrease in photosynthetic activity and subsequently lower DO levels[12].

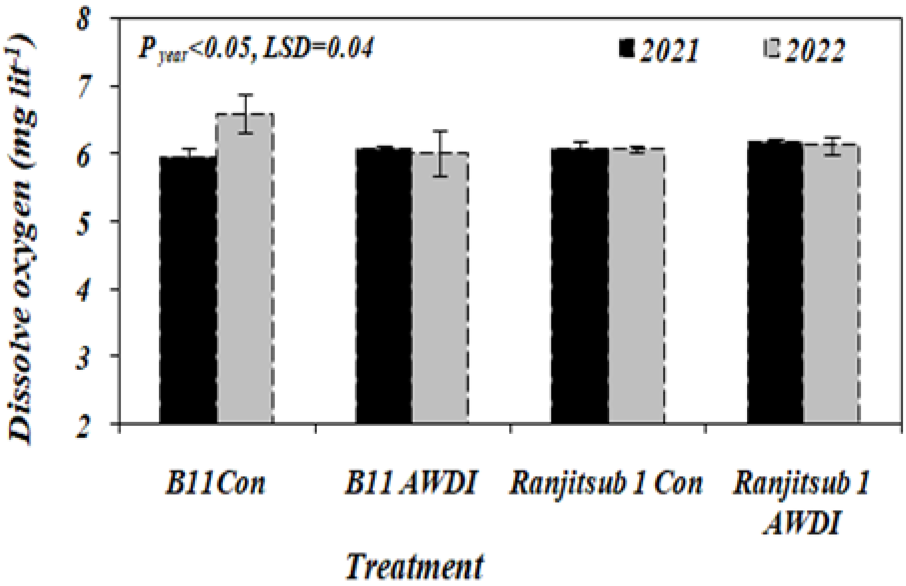

Figure 6.3.1: Temporal variation of Dissolved oxygen of water under two different agricultural practices in two different varieties. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice).

6.3.2 Nitrate nitrogen (ppm)

The mean concentration of Nitrate nitrogen (NO3-N) values ranged from 3.62 to 5.82 parts per million (ppm). An analysis of variance (ANOVA) indicated a significant difference in NO3-N content across treatments. A notable decrease in NO3-N due to treatment effects was observed ($p_T < 0.05$), with the Least Significant Difference (LSD) values recorded 0.58 and 0.15 for 2021 and 2022. The correlation study indicates a strong positive correlation between NO3-N concentration and mosquito immature stages, with a correlation coefficient of 0.92 ($p_T < 0.01$). A significant decrease in both NO3-N levels and larval densities in response to treatment effects observed in both varieties studied. Nutrients are primarily released into the water following land preparation, which is facilitated by microbial activity. Influencing factors include rainfall dilution, dispersion of surface soil due to agricultural practices, biological activities, and applications of fertilizers[5]. Decrease in nutrients may be due to the fact that release of nutrients is constrained by the AWDI process, as the drying of fields reduces water availability for nutrient mobilization

The study reveals a critical relationship between nitrate nitrogen levels and the dynamics of mosquito populations. Furthermore, it underscores the importance of various agricultural practices and environmental factors in nutrient availability and management. These insights could inform strategies for managing mosquito larvae in agricultural environments and optimizing nutrient use through effective land management practices.

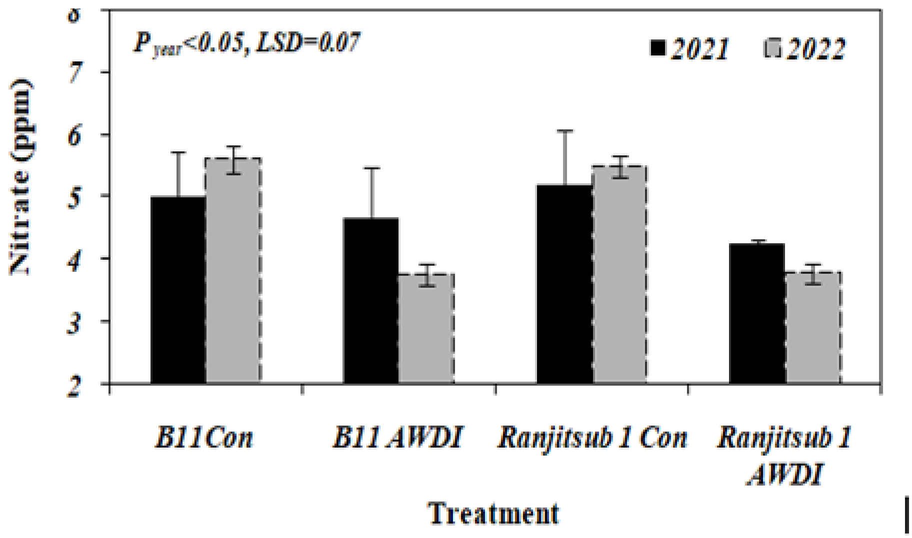

Figure: 6.3.2: Temporal variation of Nitrate-Nitrogen of water under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.3 Inorganic Phosphorus (ppm)

The analysis of variance (ANOVA) revealed a significant decrease in phosphorus levels due to treatment effects, with a p-value less than 0.05 and a least significant difference (LSD) of 0.89 in 2021 and 0.41 in 2022. Phosphorus content ranged between 0.42 to 0.75 ppm. This finding indicates that the treatments applied had a statistically significant impact on phosphorus concentrations. Additionally, a positive correlation was observed between the levels of inorganic phosphorus and the aquatic phage of mosquitoes in the present study, with a p-value less than 0.05 and a correlation coefficient of 0.865. This suggests that higher concentrations of phosphorus may promote the growth or development of mosquito populations in aquatic environments. The release of inorganic phosphorus into water systems is restricted, as indicated by the management practices implemented through the AWDI, which limits water levels in the field. Thus a decrease of phosphorus was noticed due to treatment effect. This restriction aims to mitigate the potential adverse effects of increased phosphorus levels in aquatic ecosystems[21].

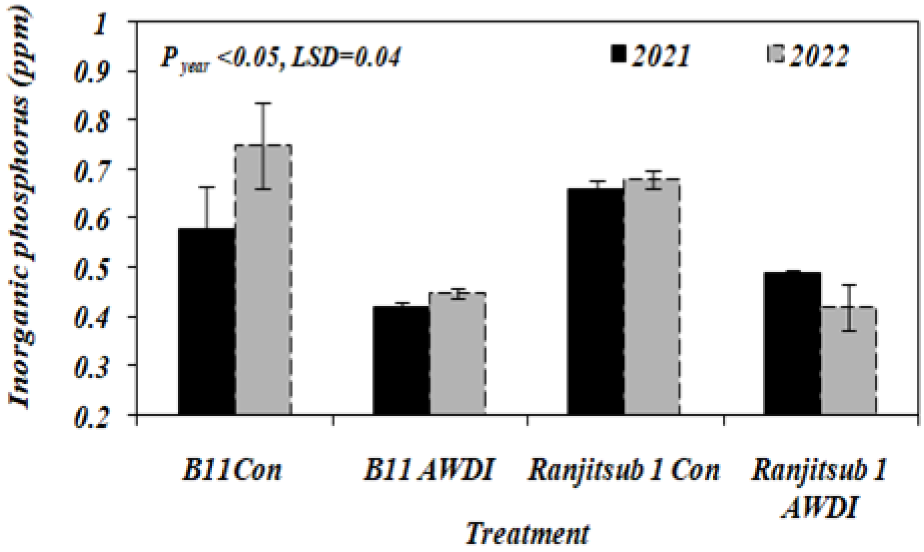

Figure 6.3.3: Temporal variation of Inorganic Phosphorus under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.4 Temperature of field water (°C)

The mean water temperature in the study varied between 19.26°C and 25.86°C, indicating a significant range that can affect aquatic ecosystems. A sharp decline in average water temperature due to treatment effects was observed in both years of the study, with statistical significance ($p_T<0.05$) indicated by Least Significant Differences (LSD) of 1.88 for 2021 and 0.18 for 2022. The water temperature was found to positively influence the abundance of immature organisms, supported by a correlation coefficient of 0.441 (p_T <0.05), underscoring the ecological dynamics at play. Floodwater temperature is influenced primarily by external factors such as air temperature, solar radiation, density of the rice canopy, and water depth, demonstrating the complex interplay of these elements within the ecosystem[22]. Previous study reported that low temperatures encourage the growth of eukaryotic algae, while higher temperatures favor blue-green algae, or cyanobacteria (BGA)[12]. Consequently, both extremes of temperature can adversely impact photosynthetic activity in floodwater, thus affecting the overall productivity of the aquatic environment[22]. Generally, increase in temperature was observed as the water levels decreased, indicating a likely link to water management practices. The recorded reduction in temperature within the AWDI field may be attributed to the frequent evacuation and influx of fresh water, as opposed to maintaining stagnant water throughout the cropping season along with strong wind speed to exert cooling effect to surface water[23].

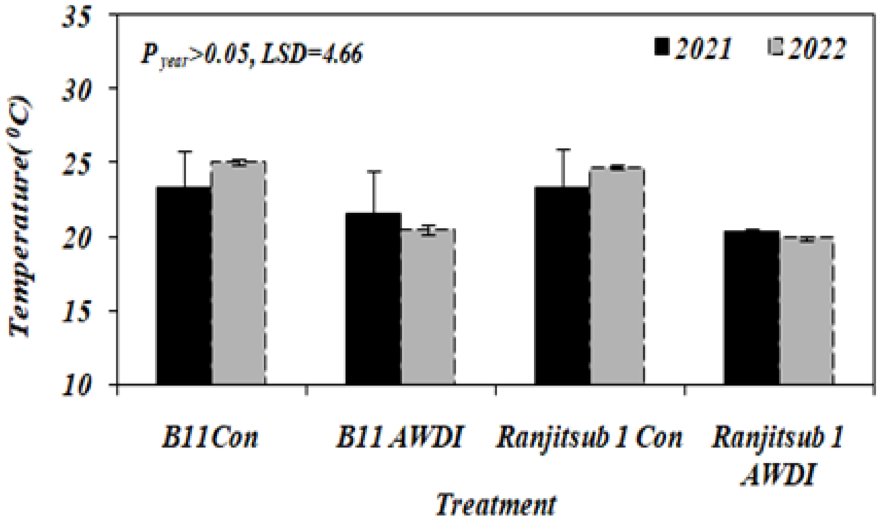

Figure 6.3.4:

Temporal variation of Temperature of water under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.5 Total Dissolved Solids (TDS) (ppm)

The average Total Dissolved Solids (TDS) ranged between 24 to 36.30 ppm, with higher concentrations observed in samples of B11 compared to Ranjit sub1. These values reflect the levels of various dissolved minerals in the water. TDS levels decreased in response to different treatments implemented during the study, showing statistical significance (p_T <0.05). The least significant difference (LSD) was recorded at 2.69 for 2021 and 0.73 for 2022, indicating meaningful changes over the two years. A significant positive correlation between TDS and larval density was found (p_T

<0.01), with a correlation coefficient of 0.879. This suggests that as TDS levels rise, the density of larvae also increases, highlighting the importance of mineral content in supporting aquatic life. The influence of treatments was particularly notable in Ranjitsub1, where both TDS levels and larval density were significantly affected. This indicates that the treatment methods employed may have had a more pronounced impact on Rnjit sub1 compared to B11. Additionally, a strong positive correlation between nitrate levels and TDS was observed (p_T <0.01, r=0.871). This correlation may suggest a connection to fertilizer use, as elevated nitrate levels can stem from agricultural runoff, which also contributes to increased TDS levels.

The findings underline the importance of monitoring TDS and its relationship with biological indicators such as larval density. These aspects are vital for understanding aquatic ecosystems and the influence of human activity on water quality. Thus, effective water management strategies, particularly that addressing fertilizer runoff, are essential to maintain balanced aquatic environments.

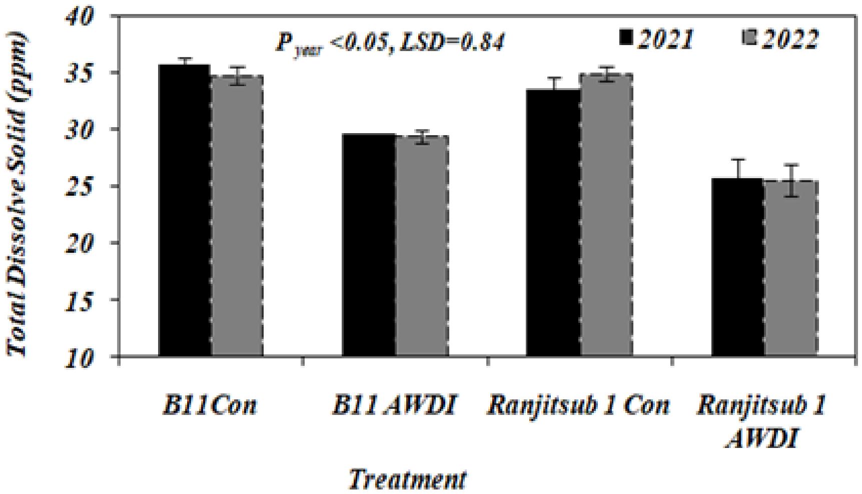

Figure 6.3.5: Temporal variation of Total dissolve solid (TDS) under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.6 Salinity (ppt)

The salinity levels during the rice growing period in 2021-2022 varied significantly, ranging between 0.31 to 0.92 parts per thousand (ppt).

This variation indicates responsiveness of salinity to different management treatments, and a statistically significant decline in salinity was observed as a result of these treatments ($p_T<0.05$, LSD: 0.17 for 2021and 0.44 for 2022). Among the treatments, B11 exhibited the highest efficacy in reducing salinity levels, highlighting its potential for effective salinity management in rice fields. This finding underscores the importance of evaluating treatment impacts in agricultural practices. Interestingly, the presence of salinity in irrigation water was found to positively influence larval abundance, as indicated by a strong correlation ($p_T<0.05$; r=0.707) between salinity levels and the population of larvae. This relationship suggests that increased salinity could enhance breeding conditions for certain mosquito species. The findings align with prior research by Sunish and Reuben, (2001)[12], which demonstrated that abiotic factors, including salinity, significantly affect the abundance of Japanese encephalitis vectors in rice fields across Tamil Nadu, India. Such studies emphasize the ecological implications of salinity in agricultural systems, particularly concerning vector dynamics. The treatments applied during the rice growing period have a notable impact on managing salinity levels, which in turn influences pest behavior and ecological balance within rice fields. Further research into the mechanisms behind these interactions would enhance our understanding of salinity's role in agricultural ecosystems.

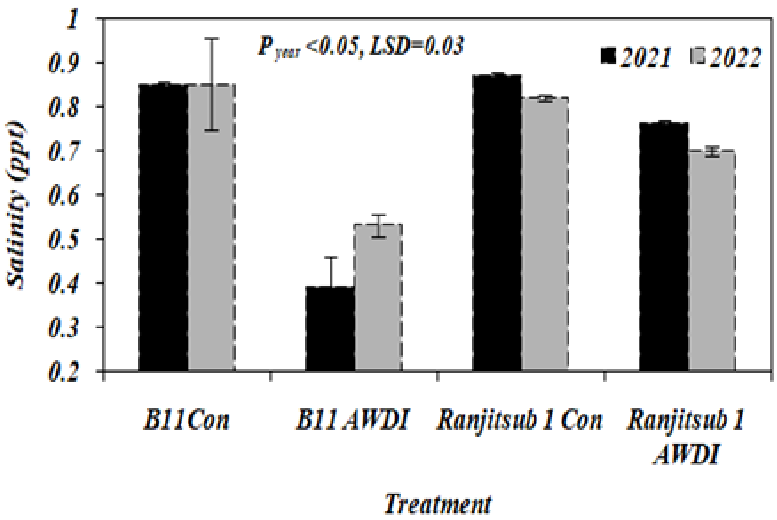

Figure 6.3.6: Temporal variation of Salinity under two different agricultural practices in two different varieties. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.7 Hydrogen-ion concentration (pH)

The study indicated that the hydrogen-ion concentration (pH) of paddy field water remained alkaline during the crop seasons. The pH values observed ranged from 7.23 to 7.88, highlighting a consistent alkaline condition across the observed seasons. The analysis showed no significant differences in mean pH values throughout the crop seasons, as indicated by the statistical result (p_T>0.05). The Least Significant Difference (LSD) values were calculated at 0.17 for 2021 and 0.71 for 2022, affirming the stability of pH levels over time. Furthermore, the study revealed that there was no correlation between pH levels and the presence of larvae, suggesting that factors other than pH may influence larval populations in the paddy fields, the pH levels in the paddy field water remained stable and alkaline over the observed period, with no significant seasonal variation or correlation to larval presence. These findings highlight the importance of continuous monitoring of water quality in agricultural practices while recognizing that pH alone may not adequately explain variations in aquatic life.

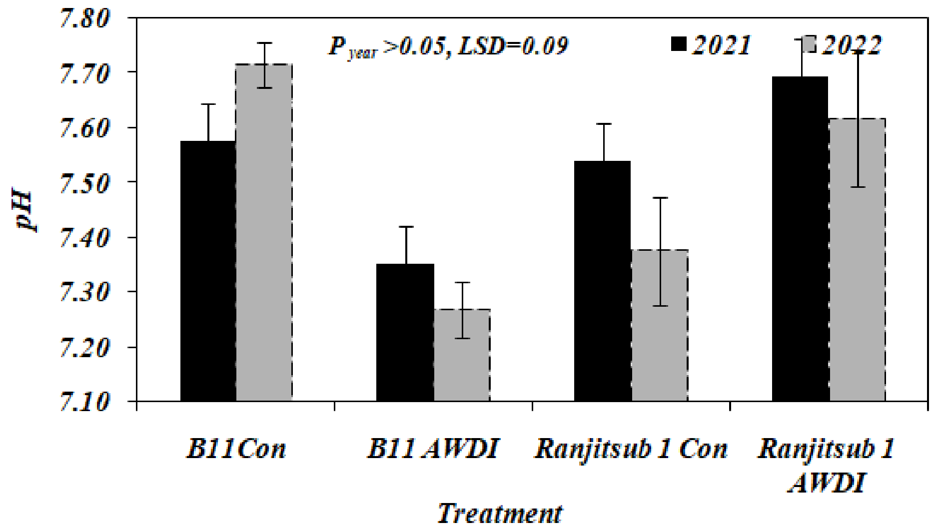

Figure 6.3.7: Temporal variation of pH under two different agricultural practices in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.8 Plant height (cm)

Height of the plant ranged between 98 cm to 130 cm at maturity of the plant. Significant decrease in height was observed due to treatment effect (p_T<0.05, LSD=0.44). Significant positive correlation of height with larval density and breeding index of *Culex* larvae at 0.01 levels was evident in our study. Maturation of paddy stands with well developed canopy may have provided more places for oviposition for adults by providing shade and maintaining favorable temperature, which resulted in increase in larval count. However, frequent drying of the fields in AWDI during the latter part of the rice growing season may also help to curtail the life cycle by destroying the larvae resulting in lesser numbers of mosquito immature at this time. Moreover, decrease in plant height due to treatment effect lead to lowering the oviposition for adults which in turn lowered the larval count under AWDI fields.

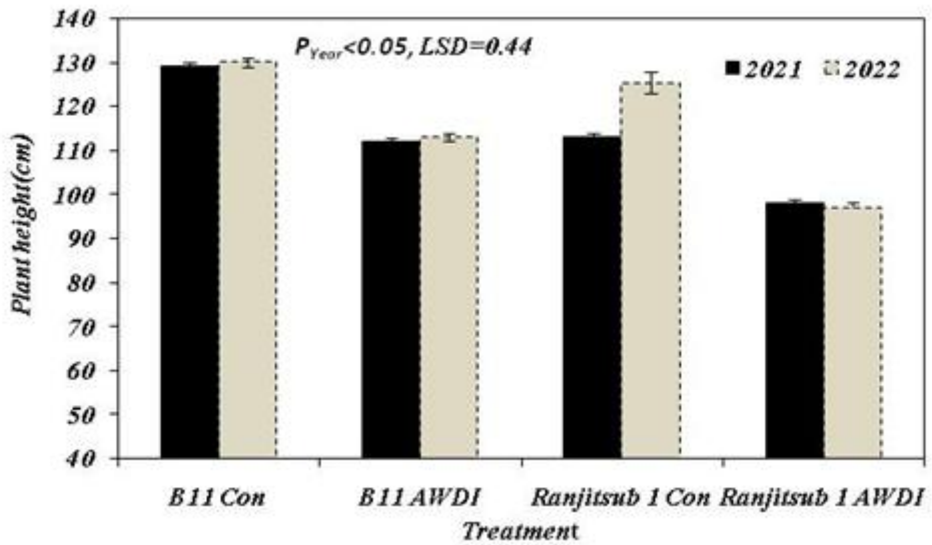

Figure 6.3.8: Temporal variation of plant height in cm under two different agricultural practices in two different varieties. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.9 Leaf Area Index (LAI)

The Leaf Area Index (LAI) serves as a critical physiological parameter, providing insights into light interception by crops and consequently influencing their growth rates. The findings from recent studies underscore the significant impact of various treatments on LAI, with a noticeable decrease observed when the AWDI method was employed. Statistical analysis indicated a significant effect of treatments on LAI, with a p-value less than 0.05 (LSD: 0.31 for 2021 and 0.76 for 2022). A diminished LAI was particularly noted under the AWDI method, suggesting its adverse effects on the crop during specific growth stages. The reduction in LAI appears to be more pronounced during the reproductive stage compared to the vegetative stage of rice growth. These observations highlight the need for further investigation to understand the underlying factors contributing to the variations in LAI across different growth stages. Throughout the study period, LAI values ranged from 2.68 to 4.28, indicating a healthy variation in leaf area coverage. Notably, the Ranjit sub 1 variety exhibited higher LAI values irrespective of the treatment applied. This finding suggests a potential genetic advantage of this variety in terms of leaf area development. Among the treatments evaluated, the B11 variety demonstrated a more pronounced effect on LAI compared to Ranjit sub 1. This difference warrants further exploration to determine the reasons behind the varying responses of these two varieties to different treatment methodologies. Despite the noticeable variations in LAI, correlation analysis revealed no significant relationship between LAI and *Culex* larvae. This finding suggests that other factors, perhaps environmental or management-related, may play a more crucial role in variation in LAI[24].

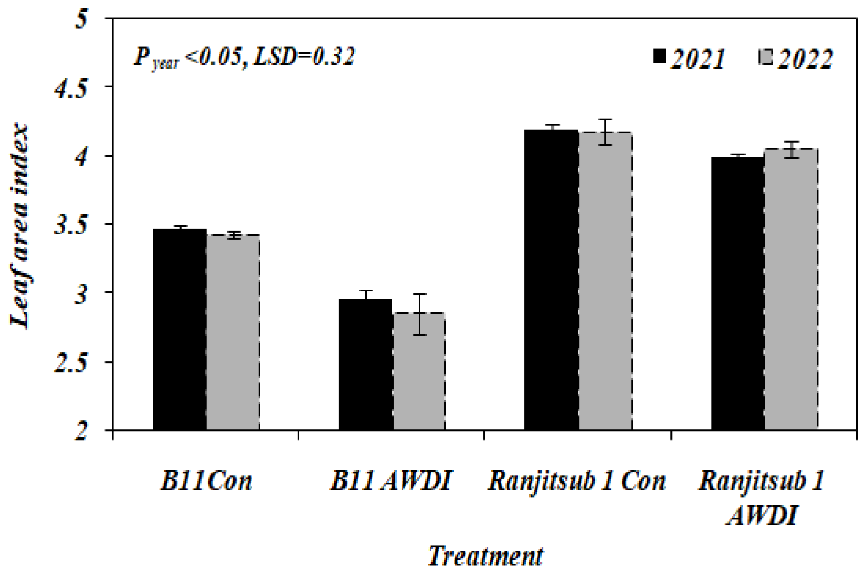

Figure 6.3.9: Temporal variation of Leaf area index (LAI) under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.10 Larval density (%)

The overall average larval density (APD) of *Culex* observed in various treatments ranged from 2.42 to 9.12 larvae per dip. This variability indicates a noteworthy fluctuation in larval populations across different environmental or treatment conditions. Statistical analysis revealed a significant decrease in larval density as a result of treatment interventions, with a significance level of ($p_T < 0.05$) and LSD values of 0.88 for 2021 and 0.69 for 2022. Such results imply that the implemented treatments effectively reduced *Culex* larval populations. In the year 2021, the treatment led to a 61.38% reduction in larval density at the B11 treatment site, while the Ranjit sub 1 exhibited a 69.77% decrease. For the year 2022, reductions were recorded at 68.31% in B11 and 72.57% in Ranjit sub 1. These findings underscore the varying effectiveness of treatments on larval density between the two treatment sites. The analysis of variance (ANOVA) indicated that the treatment effect was significantly more pronounced in larval density assessments compared to the B11 site. This suggests that the treatments may have been better suited or more impactful in the context of the B11 ecosystem. A significant positive correlation was observed between larval densities and various environmental factors, specifically NO_3-N (nitrate nitrogen), temperature, total dissolved solids (TDS), salinity, and phosphorus. This correlation highlights the role of environmental conditions in influencing *Culex* larval population dynamics.

APD of *Culex tritaeniorhynchus*, *Culex vishnui*, and *Culex pseudovishnui* ranged between 5.87% to 19.66 %, 2.73% to 16.40% and 0.93 % to 9.13% respectively during the study period. There was significant 45.16 % reduction of APD of *Culex tritaeniorhynchus* in B11and 64.91% reduction in Ranjitsub 1 in the year 2021(p_T<0.05, LSD= 1.81) while 64.70% reduction in B11 and 56.61% reduction in Ranjit sub 1 in 2022 (p_T<0.05, LSD=2.56). There was significant 86.58% reduction of *Culex pseudovishnui* in B11 in 2021 and 78.86% reduction in 2022 while 78.56% reduction in Ranjit sub 1 in 2021(p_T<0.05, LSD=1.63 for 2021 and LSD=1.84 for 2022) and 87.13% in 2022 while 75.12% reduction of APD of *Culex vishnui* was recorded in B11 in 2021 and 66.97% reduction in 2022 and 69.85% reduction in Ranjit sub 1 in 2021 and 83.35% reduction in 2022(p_T<0.05, LSD=1.55 for 2021, LSD=3.08 for 2022). Similar finding was reported by Cao et al. 2012. In their study in Jiangsu province, they found 72.14% reduction of *Culex. tritaeniorhynchus* in rice field due to intermittent irrigation. In the present study, under AWDI fields, after drying period, the fields are reflooded while there were still many pools of water in footprints and other depressions. Although other larvae were seen to be unable to make active efforts to migrate as the water withdraws, during the present study *Culex* larvae were often observed crawling short distances to reach the water in these pools. Thus few of them survived and that may be a reason for not removing 100% *Culex* larvae form AWDI field.

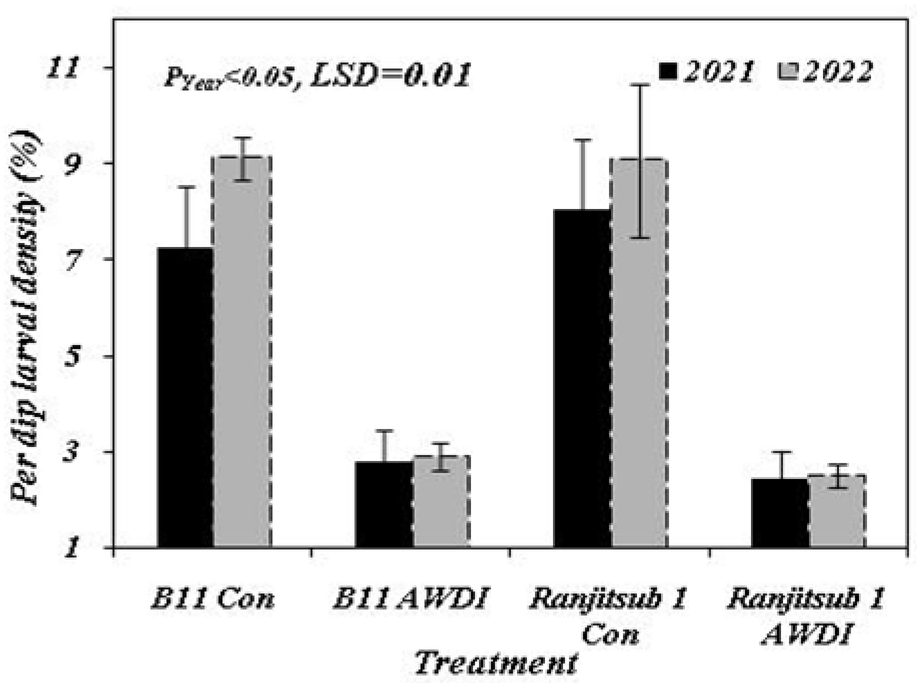

Figure 6.3.10: Temporal variation of Per dip larval density (%) under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

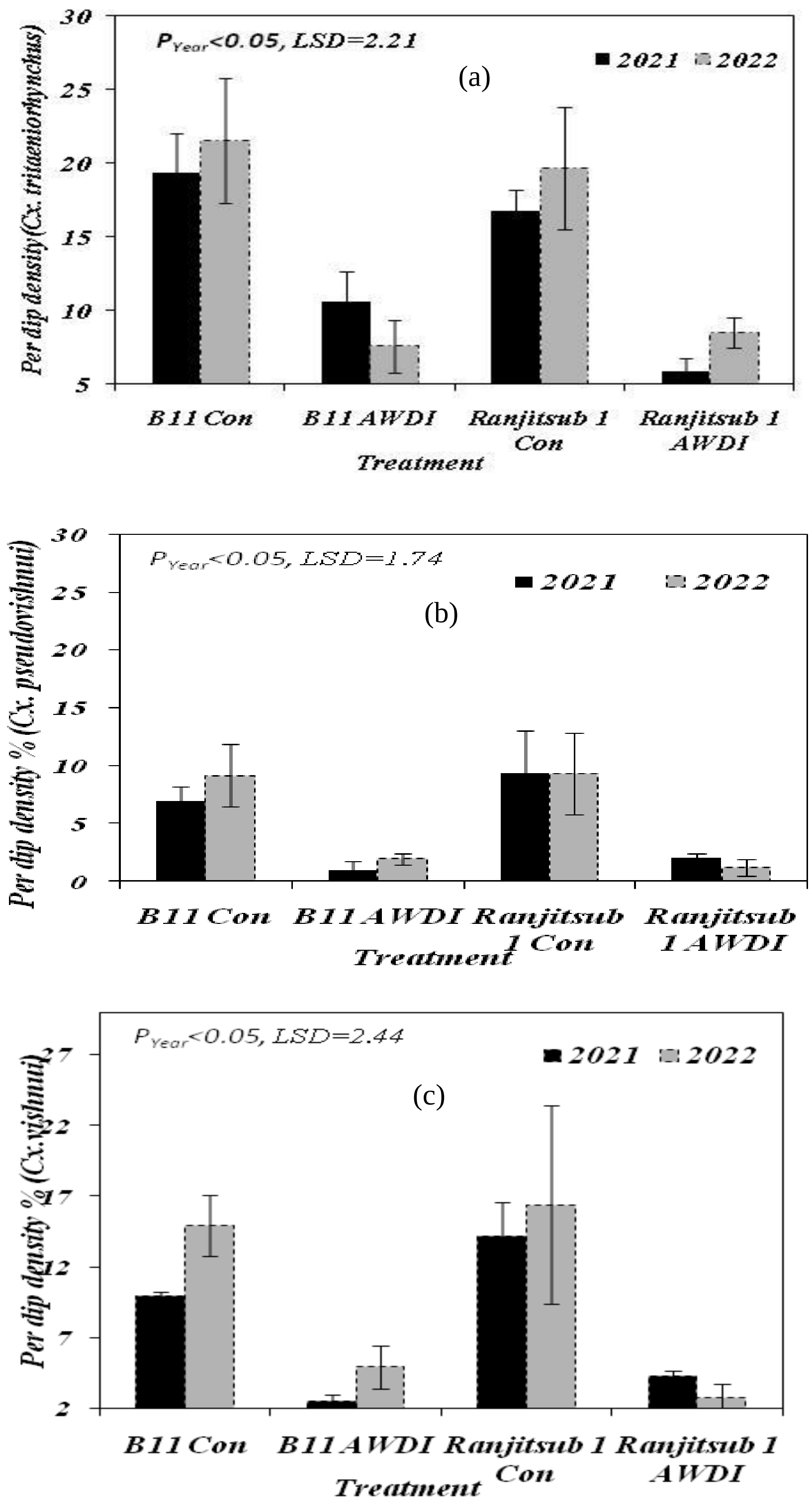

Figure 6.3.10(a),(b),(c): Temporal variation of Per dip larval density (%) of three different species under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

6.3.11 Breeding index

Significant change in overall breeding index of larvae was evident due to treatment effect (p_T<0.05, LSD=0.01). Significant lower BI was due to treatment effect observed in *Culex tritaenorhynchus (*p_T<0.05, LSD=0.79*)*, *Culex vishnui (*p_T<0.05, LSD=0.82*)* as well as in *Culex pseudovishnui* (p_T<0.05, LSD=0.19) in both the variety. However, the effect was more in Ranjitsub 1 than B11 (p_T<0.05). The per dip intensity identified the breeding sites of the *Culex* sp. in the rice fields; the overall average larval density (APD) of *Culex* was 2.42 to 9.12 larvae per dip i.e. 350 ml of water in the fields. This larval density specified the peak areas and period of breeding of *Culex* sp. in the rice fields; this is a very crucial indicator of early stage abundance of *Culex* sp. that will potentially convert to adults and contribute to active breeding and disease transmission.

Correlation study revealed that BI exert significant positive relation with NO3, temperature, TDS, salinity phosphorus at p value less than 0.01 level and with plant height at 0.05 level (Table: 6.5). In present study we have found decrease in salinity, TDS, temperature and NO_3 content in water due to implementation of AWDI. Between the two varieties, Ranjit sub 1 experiences more effect than B11. This may be the reason for finding much lesser larval count in Ranjit sub 1 than B11 under treatment.

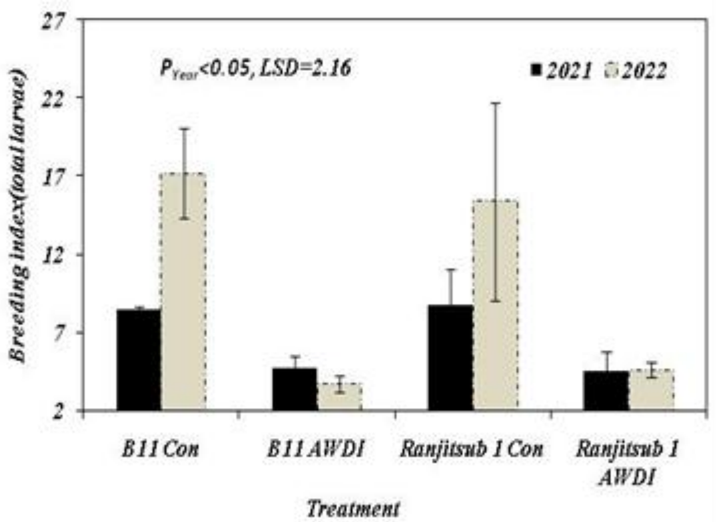

Figure 6.3.11: Temporal variation of Breeding Index of larvae (*Culex* sp.) under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

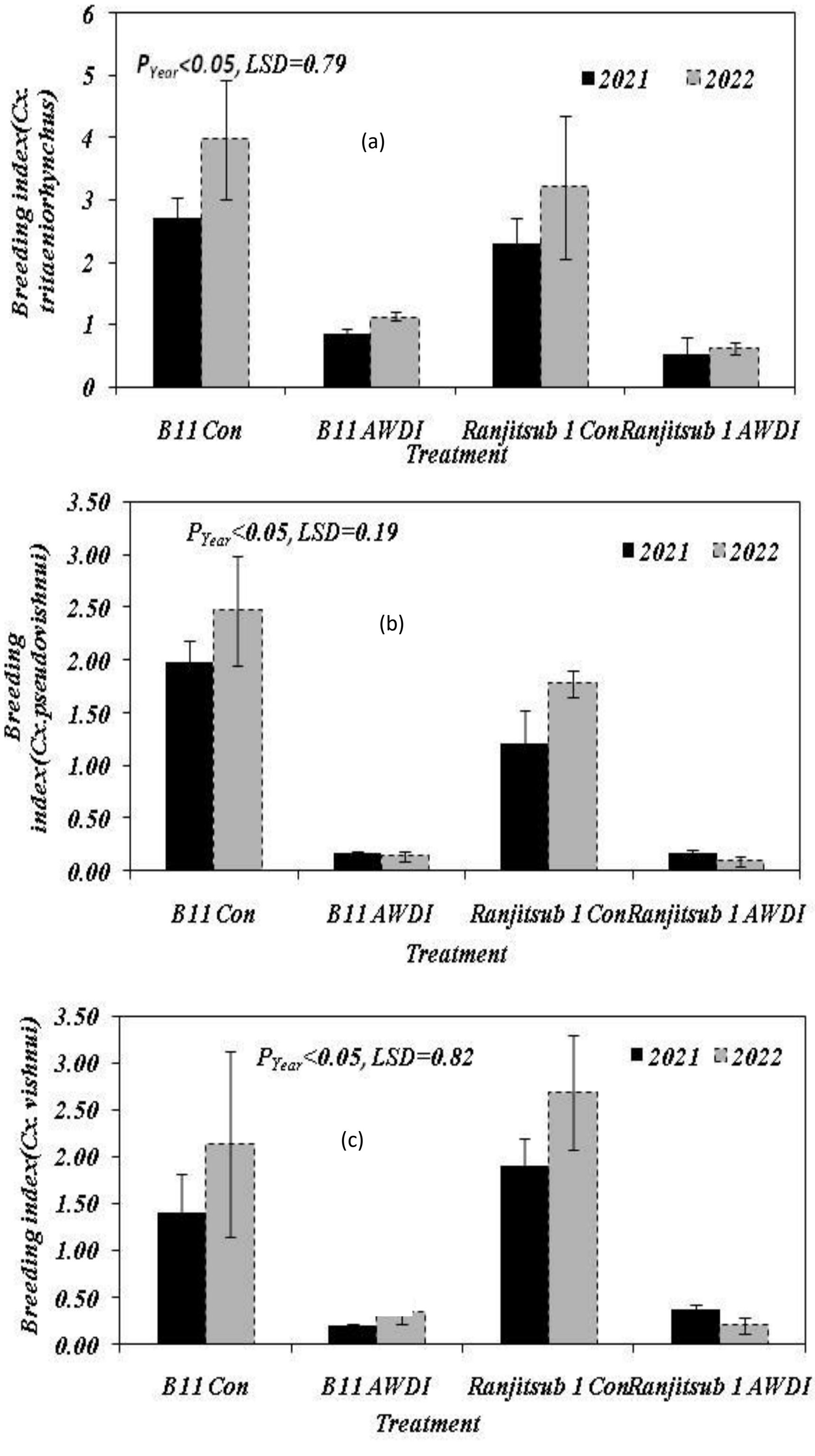

Figure 6.3.11 (a),(b),(c): Temporal variation of Breeding Index of three different species under two different agricultural practice in two different variety. Vertical bars represent standard error of mean (B11 Con, B11 Conventional practice; B11 AWDI, B11 Alternate and dry Irrigation practice, Ranjit sub1 Con, Ranjitsub 1 conventional; Rajit sub 1 AWDI, Ranjitsub1 alternate and dry irrigation practice)

Table 6.5: Pearson Correlations among physico chemical parameter of water and LAI with larval concentration

Parameters	Dissolved oxygen	NO3-N	Temperature	TDS	Salinity	Inorganic Phosphorus	LAI	APD of Culex larvae	pH	Paddy height	BI(Culex larvae)	APD Cx. tri.	APD Cx. pse	APD Cx. vis	BI Cx. tri.	BI Cx. pse
NO3-N	0.143															
Temperature	0.082	0.953**														
TDS	-0.027	0.871**	0.909**													
Salinity	0.143	0.752**	0.739**	0.501*												
Inorganic Phosphorus	0.411*	0.882**	0.870**	0.760**	0.748**											
LAI	0.026	0.359	0.3	0.002	0.681**	0.35										
APD of Culex larvae	0.17	0.913**	0.921**	0.875**	0.692**	.874**	0.306									
pH	0.349	0.222	0.196	-0.066	0.572**	0.269	0.332	0.155								
Paddy height	0.058	0.423*	0.544**	0.718**	0.099	0.419*	-0.588	0.488*	-0.066							
BI(Culex larvae)	0.342	0.752**	0.740**	0.714**	0.547**	0.758**	0.242	0.849**	0.17	0.405*						
APD Cx. tri.	0.104	0.846**	0.877**	0.869**	0.550**	0.739**	0.159	0.941**	0.158	0.549**	0.846**					
APD Cx. pse	0.212	0.851**	0.860**	0.758**	0.724**	0.884**	0.364	0.874**	0.177	0.387	0.580**	0.725**				
APD Cx. vis	0.177	0.846**	0.827**	0.789**	0.683**	0.836**	0.361	0.951**	0.106	0.397	0.870**	0.842**	0.773**			
BI Cx. tri.	0.36	0.762**	0.761**	0.738**	0.536**	0.758**	0.145	0.860**	0.236	0.498*	0.976**	0.889**	0.591**	0.844**		
BI Cx. pse	0.456*	0.846**	0.840**	0.774**	0.686**	0.944**	0.245	0.897**	0.245	0.484*	0.837**	0.796**	0.879**	0.839**	0.841**	
BI Cx. vis	0.327	0.744**	0.706**	0.694**	0.568**	0.764**	0.243	0.856**	0.16	0.393	0.970**	0.806**	0.590**	0.921**	0.946**	0.828**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

*APD, average per dip density;Cx., Culex;BI, breeding index;tri, tritaeniorhynchus; pse, pseudovishnui;vis, vishnui

6.4 Conclusion

The management of paddy water is the main cause of JE, and local agricultural practices' cycles are linked to the peak seasons of mosquito abundance. The ability of the soil to dry up is a significant characteristic of AWDI, as it limits the mosquito's life cycle from larvae and pupae to adulthood. AWDI, also known as intermittent irrigation, must be used during the full cropping season to all rice fields connected by irrigation canals across a substantial area in order to significantly reduce mosquito larvae. There is little literature on the advantages of AWDI in vector management programs. It is critical to recognize that strategies other than vaccination may play an important role in the prevention and control of JE, particularly in rural areas where vaccination coverage is sometimes low or there is no history of immunization against JE, as documented in Northeast India[26]. Isolated rural populations can be difficult to reach with vaccines, especially when it requires three doses to achieve appropriate neutralizing antibody levels. The management of vectors at its breeding sites may be beneficial in such circumstances. A literature survey on analyzing the effect of AWDI on JE vector densities found that there was a reduction of 14–91% in *Culex tritaeniorhynchus* immatures in rice fields using AWDI and a reduction of 55–70% in *Culex tritaeniorhynchus* adult population[27]. The effect this method may have on the incidence of JE needs further investigation. In our study, no immature was identified in to genus or species level, as local rice field are known to be dominated by *Culex tritaenorhynchus*, *Culex vishnui*, and *Culex pseudovishnui*[1]. Our study revealed a decrease in Culex vector due to implication of AWDI, which curtail the life cycle of the vectors by introducing a dry period in the field. Nevertheless, different field water parameters were studied to understand the factors influencing larval density, which is important from an agricultural perspective. These findings are critical for understanding the environmental variables and vector activity involved in JE outbreaks in this location, which may then be used to execute vector control and disease prevention strategies during peak risk periods. However, more research is needed to better grasp the intricate mechanism. This study can be considered as basis, depending on which vector management strategies can be adopted in state as well as national level.

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