Uttar Pradesh Journal of Zoology

Volume 45, Issue 23, Page 187-196, 2024; Article no.UPJOZ.4426
ISSN: 0256-971X (P)

Seasonal Change in Quantity of Phyto-chemicals in Leaf of *Tiliacora acuminate* and Their Effect on Mortality of *Culex vishnui* Larvae

Someshwar Singha [a*]

[a] *Department of Zoology, Vivekananda Mahavidyalaya, Burdwan, West Bengal, India.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i234699

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4426

Original Research Article

Received: 17/10/2024
Accepted: 20/12/2024
Published: 23/12/2024

ABSTRACT

Japanese Encephalitis is one of the vectors borne disease created major public health problem in Indian peninsular and *Culex vishnui* is the key vector of this disease. Lots of plant extract were also reported for their mosquito larvicidal activity but correlation between season wise quantitative change in phyto-chemical and their effect on larvicidal potentiality was tried to establish though this research work. The study was conducted to established larvicidal activity of *T. acuminate* against *Cx. vishnui* larvae.

Crude extract of *T. acuminate* leaves were used for larvicidal bioassay against 3rd instars larvae of *Cx. vishnui* in three different seasons i.e. summer, rainy and winter. 0.5% concentration of crude extract show 100% mortality after 72 h in summer whereas 86% and 92% mortalities were recorded in rainy and winter seasons respectively against same dose and same time of exposure.

When crude extract with LC_{50} for 3rd instars larvae of *Cx. vishnui* at 72 h of exposure was tested against non target organisms i.e. *Chironomus* and *Daphnia sp* no mortalities were recorded.

**Corresponding author: Email: someshbio@gmail.com;*

Cite as: *Singha, Someshwar. 2024. "Seasonal Change in Quantity of Phyto-Chemicals in Leaf of Tiliacora Acuminate and Their Effect on Mortality of Culex Vishnui Larvae". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (23):187-96. https://doi.org/10.56557/upjoz/2024/v45i234699.*

Quantitative estimation of different phytochemicals of leaf of *T. acumination* was recorded in three prevalent seasons i.e. summer, winter and rainy.
The carbohydrate is raw metabolic substance which is used for generation of heat, to tolerate extreme temperature and it was found with higher concentration (7.91 mg/g) in winter. Starch is the non-structural carbohydrate which are assist in bud initiation, leaf maturation and flowering and it was found at highest dose in summer. (.81mg/g) Protein is used as building block of plant and highest protein content was recorded in summer. (132mg/g) The phenol is used as natural defence mechanism of plant and it was found at highest concentration in summer. (16.5 mg/g) Ash and moisture contents are used as senescence and decomposition of plant respectively.
Crude extract of leaf of *T. acuminata* show positive result for alkaloids, cardiac glycosides, saponins and flavonoids, in which alkaloids and cardiac glycosides are found with higher doses i.e. 0.72mg/g and 0.65 mg/g respectively. Numbers of phyto-chemicals are recorded at higher dose in summer. Plant phyto-chemicals are mainly used for their own defence mechanism by controlling different pest and pathogens population. Correlation between seasonal variation of plant phytochemicals (quantity) and mosquito larvicidal potentiality of *T. acuminate* leaves was established in this research work.

Keywords: Tiliacora acuminata; Culex vishnui; seasonal variation; phytochemicals.

1. INTRODUCTION

Mosquito is the major public health hazards having lethal capacity to kill more than million victims a year around the world (Vatandoost and Vaziri, 2001).

During blood feeding female mosquito transmit harmful pathogens from host to host i.e. malaria, dengue, Zika, Japanese encephalitis (JE) etc.

Gravid female mosquito sucked human blood as a source of protein and vitamins which are needed for development of their eggs. According to National Vector Borne Disease Control Programme, under the ministry of Health and Family welfare, Government Of India, *Culex vishnui* is the chief vector of Japanese encephalitis.

Culex vishnui(Theoblad), belong to the *Culex vishnui* group, is important vector of J.E in India (Lindahl et al., 2012).Distinct characteristics of legs, pals, proboscis and wings are used as key for identification of *Cx. vishnui*. These mosquitoes breed preferably in stagnant water of rice fields. J.E is mainly affected in rural and peri-urban area in India where human reside intimately with different vertebrate reservoir host.

In 1973, first major out beak of JE has been reported from Bankura and Burdwan.

Symptom of JE virus infection is moderate fever and headache but in severe condition it may characterize by high fever, headache, coma, paralysis and death. Incidence of Japanese encephalitis was increased dramatically with increase in rice cultivation (Banerjee and Chandra, 2004). According to World Health Organisation more than 3 million people under threat of JE infection in south East Asia and Western Pacific regions.

In the recent past plant derived products are used experimentally to control vector borne disease. Plants are rich source of different phyto chemicals which may be used for vector control programme.

Generally plant produced different phyto chemical which are used for their defence mechanism. Secondary metabolites of plants also have been reported for their insecticidal properties i.e alkaloids, tannins, flavonoids, cardiac glycosides, steroids etc (Shaalan et al., 2005). More than 200 plants belonging to family Rutaceae, Meliaceae, Brassicaceae, Labiates, Asteraceae, Solanaceaehave been reported for their mosquito larvicidal activity (Singh et al., 2001; Chowdhury and Chandra, 2007; Ghosh et al., 2008; Rawani et al., 2010).

Pupicidal, adulticidal and ovicidal activities of leaf extract of *Tiliacora acuminata* on *Culex vishnui* group was establisded previously (Singha, 2019). Leaf extract of *Holoptelea intrgrifolia* show seasonal variation in larvicidal potentiality on mortality of *Cx. vishnui* larvae (Singha and Chandra, 2022). 3-(2-Hydroxyphenoxy)-1,2-propanedial isolated from n-hexane extract *Mesua ferrea* leaves was recorded for larvicidal effect on *Cx. vishnui* (Singha, 2020).

n-alkane compounds from leaves of *Holoptelea integrifolia* showed repellence against *Cx. vishnui* adult mosquito (Singha and Chandra, 2022).

Tiliacora acuminata is commonly known as Bagmushda belongs to family Menispermaceae mainly distributed in India, Sri lanka and South East Asia.It is one type of climbing shrubs with alternate, ovate and lanceolate type of leaf. Inflorescence of this plant is axillary, panicle, drups on branched carpophores. It is mostly found along river bank and found in Indian subcontinent to Indo-china. Main identifying characteristic of this plant is its yellow fragment flower, long-stemmed woody vine and used tree for their vertical support. The flowering period of this plant is from April to December.

T. acuminata is ethno-medically used as antidote for snake bite (Chopra et al, 1956; Madhu and Ravindra, 2009). Leaf paste of this plant is applied on the bitten area for some kind of relief from snake bite (Sandhya and Seetharami, 2011). Crude and solvent extracts of *T. acuminata* flower have high dose of larvicidal activity against *Cx. quinquefasciatus* larvae (Singha et al., 2011).

Leaves of this plant are mainly used against snake bite, skin infections, jaundice, piles, ulcer, diabetes etc (Haridas et al., 2018). This plant also has antimicrobial, anti diarrheal, antioxidant, antiviral, anti inflammation activities. (Nagaraja et al., 2016; Nishantini et al., 2014; Uthirapathi et al., 2015; Hossain et al., 2013).

2. MATERIALS AND METHODOLOGY

2.1 Collection of Larvae

Present study was conducted at Department of Zoology, Vivekananda Mahavidyalaya, Burdwan, West Bengal. Larvae of *Cx. vishnui* mosquito were collected from rice field surrounding college campus. Plastic tray containing rice field water was used to acclimatization of mosquito larvae in laboratory condition and artificial food was added to the tray. Mosquito larvae were kept at 28^0C and 85% of relative humidity. They are collected with help of glass dropper and put into glass Petridis for bioassay test.

2.2 Preparation of Crude Extract

Leaves of *T. acuminate* were collected from bank of river Damoder, Polampur, Burdwa, West Bengal. The study has been conducted in order to establish seasonal variation in their larvicidal efficacy against *Cx. vishnui* mosquito larvae in three prevalent seasons i.e. summer, rainy and winter. Collected leaves are initially rinsed though water then chopped into small pieces followed by crushed by mortar and pestle. Required concentrations of crude extract were prepared by mixing of distilled water with stalk solution.

2.3 Bioassay with Crude Extract of Mature Leaf

Crude extract of mature leaf of *T. acuminata* was used as bioassay test at following test i.e. 0.1%, 0.2%, 0.3%, 0.4% and .05%. Required concentrations of crude extract were prepared following addition of required amount of distilled water. Prepared concentrations of crude extracts were transferred into glass Petri dish containing 100 ml of water. Twenty five 3rd instars larvae were exposed with different concentration of crude extracts. All experiments were repeated three times and larvae were considered died if they become motionless when gently probed with needle. Larval mortality was recorded after 24, 48 and 72 hour respectively and number of mortality was expressed by addition of mortality at 24 and 48 hour respectively.

2.4 Collection of Leaf for Biochemical Test

Mature leaves of *T. acuminata* were randomly collected from Burdwan during April 2022 to April 2023. At first leaves were properly cleaned with water to remove dust particles. Then leaves were soaked with paper towel before used.

2.5 Phytochemical Qualitative Screening

Collected *T. acuminata* plant leaves were ground into powder using grinding machine. To form aqueous extract, this powder was soaked in distilled water for 12 hours. Whatman filter paper (No: 42, bearing pore size 125 mm) was used for filtering the extract. Different biochemical tests were carried out according to protocol of Sofowarw, (1993), Treare and Evans, (1989) and Harbon(1973).

2.6 Alkaloids

10 ml of acidic alcohol was used to mix with 0.5 g of finely grounded plant powder. Then the mixture was boiled followed by filtered with Whatman 42 no filter paper. 5ml of filtrate was

diluted with 2 ml of ammonium solution. The mixture was added with 5 ml of chloroform and shaken gently. From the mixture chloroform portion was discarded by addition of 10 ml acetic acid. Then the solution was divided into two portions. The stalk solution was divided into two fractions in which one part was added with Mayer's reagent but next fraction was added with Dragondroff's reagent. If Dragendroff's reagent turned the solution brownish green Mayer's reagent created cream formation, it indicate presence of alkaloid's in plant sample.

2.7 Test for Tannins

0.5 g of leaf powder was put into test tube containing 20 ml of distilled water, followed by boiled it in water bath. Then the filtrate was added with few drops of (0.1%) ferric chloride and solution turned into brownish green colour, it indicate presence of tannins.

2.8 Test for Saponins

2 g of plant powder was put into test tube. 20 ml of distilled water was added with it followed by boiled the sample in water bath. When 5 ml of distilled water was mix with 10 ml plant extract and shaken it more powerfully. If bubbles with creamy mass appeared, it indicates presence of saponins.

2.9 Test for Flavonoids

According to Sofowara(1993) and Harbone (1973), part of aqueous extract was added with 5 ml of dilute ammonium solution followed by concentrated sulphuric acid. If yellow colour appeared and then disappeared after some time it indicates presence of flavonoids.

To confirm this test plant powder was boiled with ethyl acetate for 3 minutes and then filtered it. 4 ml of filtrate was added with 1 ml of diluted ammonium solution. Appearance of yellow colour confirmed presence of flavonoids.

2.10 Test for Steroids

0.5 g of methanol extract of plant powder was added with 2 ml of sulphuric acid and 2 ml of acetic anhydride. Presence of steroid was confirmed if the colour was changed from violet to green.

2.11 Test for Terpenoids (Salkowski Test)

5 ml of aqueous plant extract was added with 2 ml of chloroform followed by 3 ml of concentrated sulphuric acid. If reddish brown colour appeared at the interface, it indicates presence of terpenoids.

2.12 Test for Cardiac Glycosides (Keller-KillianiTest)

0.5 g of plant extract was collected in a test tube followed by addition of 5 ml of distilled water then 2 ml of acetic acid was added and finally mixed with one drop of ferric chloride to the solution. When the mixture was underplayed with 1 ml concentrate sulphuric acid, brown ring appeared at the interface. In the solution a ring like structure bearing three layer was appeared in which violet ring appear below the brown ring whereas green ring of acetic acid formed above the brown region and ultimately it was spread in brown region.

2.13 Quantitative Analysis of Some Phytochemicals

Quantitative analysis must be done against primarily positive phytochemicals i.e. alkaloids, cardiac glycosides, saponins, phenol and flavonoids.

2.14 Quantitative Analysis of Phenols

The amount of total phenol was determined according to procedure of Bray and Thrope (1954). The extract was put into test tube and added 1 ml of Folin-ciocalteu followed by 2ml of 20% sodium carbonate solution. The mixture was diluted up to 25 ml with distilled water and the amount of phenol was determined against blank reagent.

2.15 Quantitative Estimation of Alkaloids

At first 5 g of leaves of *T. acuminate* were dried properly and put into a beaker then added ethanol bearing acetic acid (Harbone,1973). 5 g of dried leaves were put into a beaker and added acetic acid containing ethanol. Plant leaves were soaked for 4 hours. After filtering, it was concentrated up to ¼ of its primary volume and added drop wise concentrated ammonium hydroxide until propitiation was complete. Total precipitation was washed with diluted ammonium

hydroxide and after filtered it was weight to determine amount of alkaloids.

2.16 Quantitative Estimation of Flavonoids

According to Bohm and Kocipai-Abyazam (1994),10 g of plant leaves were extracted with 100 ml of 80% methanol at room temperature. Whatman: 42 filtered paper was used to filter the extract. Filtrate was evaporated in water bath and residue was weighed to measure amount of flavonoids.

2.17 Quantitative Estimation of Saponins

According to Obadani and Ochuko (2001),20 g of ground plant leaf was put into conical flask containing 100 ml of 20% crude ethanol. Sample was heated over water bath at 55^0C temperature for 4 hours. At first the extract was filtered with filter paper and then the residue was collected. Residue was re extracted with help of 200 ml of 20% ethanol. Both extracts were added and their volume concentrated up to 40 ml in water bath containing 90^0C temperature. The extract was collected in a beaker then it was added with 20 ml diethyl ether. The mixture was shaken vigorously to confirm presence of saponins. The ether layer was discarded and the extract was repeatedly purified with n-butanol and 5% sodium chloride solution. Final extract was evaporated and dried residue was weighed.

2.18 Quantitative Estimation of Cardiac Glycoside

Cardiac glycoside of plant sample was quantitatively determined according to Tofighi et al. (2016) with some modifications. 10% extract of leaf of *T. acuminata* was mixed with 10 ml of prepared Baljet's reagent (95% of 1 ml picric acid + 5 ml of 10% NaOH). It was diluted with 20 ml distilled water and after 1 hour used spectrophotometer for absorption at 495 nm wave length. (Shimadzu spectrophtometer, model 160A Kyoto, Japan) Standard curve was prepared with help of different concentration; 12.5 to 100 mg/L of securidaside.

2.19 Seasonal Fluctuation of Primary Biochemicals

Summer (April to June), Monsoon (July to September) and winter (October to December) are three predominant season in West Bengal.

2.20 Preparation of Leaf Extract

1 g of mature leaves were chopped into small pieces and plunged in boiling ethanol for 10 minutes. They were centrifuged at 2000 rpm for 20 minutes. Supernatant and pellets were separated from centrifuged sample. The supernatant passed through charcoal powder and Whatman 41 filter paper.

2.21 Estimation of Total Soluble Carbohydrate

Total carbohydrate of plant leaf was measured according to protocol of Duboies et al. (1951) with some modification. 1 ml supernatant was treated with 4 ml of Anthrone reagent. To estimate carbohydrate content and standard curve was prepared by D-glucose and blank solution using UV-visible spectrophotometer with 630 nm wave length. Carbohydrate content was measured against this standard curve.

2.22 Estimation of Total Lipid

According to Folch et al. (1957), 1 g of fresh leave was homogenized in 20 ml chloroform: methanol (V/V) for 10 minutes. The extract was filtered and added with 25 ml chloroform: methanol (V/V) and stirred for 30 minutes. The mixture was passed through activated charcoal and shaken with 0.9% sodium chloride solution, to remove non-lipid content. Extracted lipids were dried in desiccators and weighed in electro-balance.

2.23 Estimation of Total Starch

The quantity of starch was estimated according to Duboies et al. (1951) with some modifications. 5 g of sample was homogenised in hot 80% ethanol. The residue was dried in water bath. It was added with 5 ml of distilled water and 6.5 ml of 52% perchloric acid. Finally 1 ml solution was prepared by mixing 0.2 ml extract and 0.8 ml of distilled water. It was added with 4 ml of Anthrone reagent. The amount of starch in final solution was measured by UV-vis spectrophotometer at 630 nm of wave length.

2.24 Ash and Moisture Content Estimation

1 g of mature leaves was placed in a hot air oven at 50^0C for 72 hours. The residue was removed from oven and weighed. Moisture content was

determined by difference between fresh and dried weighed of samples.

A portion of oven dried leaves were ash in a Muffle furnace at 450^0 C for 20 minutes. 2 ml distilled water was added with crucible to soak ash and dried at 110^0C temperature for 2 hours. Ash sample was finally weighed. Percentage of ash of leaves of *T. acuminate* was determined by this procedure.

2.25 Estimation of Total Protein

Lowary method was used to determine amount of total protein in plant leaves of crude extract (Lowry, 1951) Pellets from ethanol extract of leaf were separated and suspended its in 5%TCA solution at 0^0C ice bath for 10 minutes. 1 ml of suspension was put into centrifuged tube, followed by addition of 1 ml of 10% TCA solution. Then the sample was centrifuged at 5000 rpm for 45 minutes. The pellets were isolated again and re extracted with absolute ethanol. These processes were repeated for twice and finally pellets were treated with alkaline copper reagent [alkaline sodium carbonate: copper sulphate sodium potassium tertarate solution (50:1) V/V]. The amount of protein was determined against blank reagent and standard curved was prepared by Bovine serum albumins. Each sample were added with Folin-ciocalteu reagent and measured in UV-Vis spectrophotometer at 750 nm wave length.

2.26 Tests on Non Target Organisms

Different organisms which are generally share the common habitat of *Cx. vishnui* i.e. *Chironomus sp* and *Daphnia sp* were collected from rice field and they were acclimatised in laboratory condition and finally treated with LC_{50} concentration of crude extract of 72 h against these organisms. This experiment was designed to evaluate effect of crude extract of *T .acuminate* on non target organisms.

3. RESULTS

From the qualitative analysis of *T. acuminata*, it was found that mature leaves have alkaloids, saponins, phenols, flavonoids and cardiac glycosides. But tannins, saponins, steroids, terpenoids were absent from leaf *T. acuminata*.

Table 1. Qualitative analysis of secondary phyto chemicals from leaf of *T. acuminata*

Sl No	Alkaloids	Cardiac glycosides Saponins	Phenols	Flavonoids	saponins	Tannins	Steroids
1	++	++	+	+	+	-	-

+ = Presence of phyto chemical- = Absence of phyto chemical

Table 2. Quantitative estimation of secondary phyto chemicals from leaf of *T. acuminata* plant

Sl No	Secondary Phyto chemicals	Quantity (mg/g)
1.	Alkaloids	0.072± 0.001
2.	Cardiac glycosides	0.065 ±0.001
3.	Saponins	0.0041 ±0.00
4.	Phenolics	0.0039 ±0.00
5.	Flavonoids	0.0051±0.01

Table 3. Quantitative seasonalvariations of Primary Phyto chemicals

Sl No	Primary Phyto chemicals	Summer(mg/g)	Rainy (mg/g)	Winter(mg/g)
1.	Carbohydrate	5.63±0.87	6.85±1.01	7.91±.300
2.	Protein	132.13±7.39	101.71±4.31	89.39±11.49
3.	Lipid	6.23±.230	8.89±0.33	9.81±0.27
4.	Starch	.81±0.31	0.08±.02	.17±.051
5.	Phenol	16.5±0.21	15.09±0.38	16.21±0.67
6.	Ash	18.67±0.03	17.41±0.03	18.51±0.67
7.	Moisture	1.73%	1.91%	1.78%

Table 4. Seasonal variations in efficiency of crude extract of leaf of *T. acuminata* on *Cx. vishnui* 3rd instars larvae

Season	Conc. (%)	Mortality of *Cx. vishnui* mosquito larvae		
		24h	48h	72h
Summer	0.1	27.67±0.33	37.67±0.33	54.67±0.33
	0.2	41.67±0.33	54.67±0.33	73.33±0.67
	0.3	54.67±0.33	67.33±0.33	82.67±0.33
	0.4	83.33±0.33	85.33±0.67	91.67±0.67
	0.5	91.67±0.67	96.33±0.33	100±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Rainy	0.1	26.67±0.67	35.67±0.67	47.33±0.33
	0.2	37.33±0.67	41.33±0.33	50.33±0.33
	0.3	48.67±0.67	53.33±0.67	76.67±0.33
	0.4	67.33±0.33	73.67±0.67	76367±0.33
	0.5	72.33±0.33	74.33±0.33	86.67±0.33
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Winter	0.1	21.67±0.67	31.67±0.33	48.67±0.67
	0.2	33.67±0.33	37.33±0.33	52.33±0.67
	0.3	43.33±0.33	45.33±0.67	61.33±0.33
	0.4	61.33±0.67	65.33±0.67	71.33±0.67
	0.5	67.33±0.67	71.33±0.33	92.33±0.33
	control	0.00±0.00	0.00±0.00	0.00±0.00

Table 5. Effect of crude extract on non-target organisms

Non target organisms	Exposure of crude extract at LC_{50} dose against 3rd larvae of 72 h of exposure		
	24h	48h	72h
Chironomus sp	0.00±0.00	0.00±0.00	0.00±0.00
Daphnia sp	0.00±0.00	0.00±0.00	0.00±0.00

The quantitative analysis of phyto chemicals of leaves revealed that flavonoids are presents at least quantity but is rich in alkaloids and cardiac glycosides.

Season wise change in phyto chemical of leaves of *T. acuminata*, it was revealed that, quantity of carbohydrate, lipid, protein, starch and phenol are present at different quantities at different prevalent seasons i.e. summer, rainy and winter.

The highest concentration of carbohydrate was found during winter (7.91 mg/g) followed by rainy season (6.85 mg/g) and summer (5.63 mg/g).

But in case of starch content inverse relationship was found i.e. maximum density of starch was found in summer season (.81 mg/g).

Highest concentration of protein (132 mg/g) was found during summer due to high rate of plant growth, where as lowest quantity was found in winter (89 mg/g).

The lipid content was found at highest dose during winter (9.81mg/g) and lowest concentration was revealed in summer season (6.23 mg/g).

Ash content was recorded at level higher in summer (18.67mg/g) and lowest in rainy season (17.41 mg/g). But moisture content was recorded at highest dose in rainy season (1.91%) and lowest quantity recorded in summer season (1.73%).

Five different concentrations i.e. 0.1, 0.2, 0.3, 0.4 and 0.5% was applied separately against 3rd instars larvae of *Cx. vishnui*. These types of bioassay were conducted by leaves which were collected from different seasons of year i.e. summer, rainy and winter.

Cent percent mortality was recorded at 0.5% concentration in summer after 72 h of exposure where as 86% and 92% mortalities were founded for rainy and winter seasons respectively at 0.5% concentration on 3rd instars larvae after 72 h of exposure.

Highest mortality of *Cx. vishnui* larvae against crude extract of *T. acuminate* was founded in

summer where as moderate and lowest mortalities were recorded in winter and rainy season respectively.

LC_{50} concentration of crude extract against 3rd instars larvae of *Cx. vishnui* mosquito were applied against non target organisms but no death was recorded after 72 h.

4. DISCUSSION

Season wise variation in the quantity of secondary and primary phytochemicals in leaf of *T. acuminata* was noticed may be due to change of temperature, humidity, attacked by different pathogens and availability of water supply.

Carbohydrate is used as row material for metabolic respiration process through which energy is generated. High temperature and optimum solar radiation caused high rate of photosynthesis in summer and it was transported to growing region of plants. As a result sugar accumulation at mature leaves which is lower in summer.

In winter carbohydrate is mainly used as fuel to tolerate environmental temperature where as in spring it is used for new growth of leaves Dubois et al., 1951).

Starch, non-structural carbohydrate is mainly used for plant growth maintenance and energy production. In summer maximum starch content is found which are responsible for leaf expansion and maturation. Leaves of starch increase during bud initiation and flowering which is maintained till plant can enter into senescent phase (Lowry et al., 1951). During rainy season starch is converted into sugars.

Protein is the main building block of plant which can control growth and reproduction of plant. It's also indicates physiological state of plant (Wheeler et al., 1992; Ochiai et al., 1987). In *T. acuminata* highest level of protein content was found in summer where as minimum level was recorded in winter.

The amount of phenol content was increases in summer. It also assists in natural defence mechanism of plant and prevents severe defoliations.

The maximum amount of lipid content in leaves of *T. acuminata* was in winter. It is mainly used as storage of energy, membrane structure and hormone which are bound with cell wall. Unsaturation of acyl chain in lipid increase proportionately with increase environmental temperature and it also decrease metabolism (Jones and Harwood, 1993).

Ash content is positively correlated with plant senescence which increases with total crude fibre of plant leaves.

Highest moisture content was noticed in rainy season in leaf of *T. acuminata* which assist decomposition of plant leaves.

5. CONCLUSION

Quantity and quality of plant phytochemicals are responsible for different physiological functions as well as protect them self from attacked by different pest and pathogen populations.

Phytochemicals are varies in different season due to difference in temperature, humidity, light intensity, availability of water and pest attack. So in West Bengal, there are three prevalent seasons i.e. summer, winter and rainy. Quantities of primary and secondary phytochemicals were varied in different seasons as well as their mosquito larvicidal property also changed in related form. Due to higher quantity of phytochemicals of leaf of *T. acuminata* in summer, it show highest efficacy against J.E vector *Cx. vishnui* larvae.

So crude extract of leaves of *T. acuminata* was must be used effectively to control vector of Japanese encephalitis. This plant showed highest efficacy in summer due to presence of highest concentration of different phytochemicals. Limitation of this work is lake of connection between research invention and its industrialization and it used are restricted in research field only.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declares that generative AI technologies such as large language models (Chat GPT COPILOT etc) and text to image generations have not been used during the writing or editing of this manuscript.

DECLARATION

There are no any kinds of ethical issue because the target organism is medically important vector and their control is mandatory to prevent

spreading of communicable disease and material used is plant extract which has no any ill effect on non target and ecosystem.

ACKNOWLEDGEMENTS

Author is grateful to Dr. Animesh Debnath, TIC, Vivekananda Mahavidyalaya, for the instrumental support to carry out the work. Author extent their gratitude to the department of Zoology, Vivekananda Mahavidyalaya, for helping us throughout the work.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

Banerjee, A., & Chandra, G. (2004) Role of some factors on the breeding of JE vector Culex vishnui group. Journal of communicable disease, 36(4): 260-263.

Bohm, B.A., &Koupai-Abyazani, M.R. (1994) Flavonoids and condensed tannins from leaves of Hawaiian *Vacinium recticulatum* and *V. calycinum*. *Pacific Sci,*48, 458-463.

Bray, H.G., &Thrope, W.V. (1954) Analysis of phenolic compounds of interest in metbolism. *Methods in Biochem Anal,*1, 27-52.

Chopra, R.N., Nayar, S.L.&Chopra, I.C. (1956) Glossary of Indian Medicinal plants, Council of Scientific and Industrial Research, New Delhi, 244.

Chowdhury, N., &Chandra, G.(2007) Phytochemical sereening and effects of extracts of *Solanum Villosum* Mill. (Solanales:Solanaceae) on juveniles of *Culex quinquefasciatus* Say. *Biospectra,*2(2): 209-214.

Dubois, M., Gilles, K.A., Hamilton, J.K., Pebers, P.A.,& Smith, F. (1951) Colorimetric method for determination of suger and related substances*. Anal Biochem* 1951,28,350-356.

Folch, J., Lees, M., &Solane-stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem,* 226, 497-507.

Ghosh, A., &Chandra, G. (2005)Chemical analysis of *Cesturn diurnum* (L.) (Solanales: Solanaceae) and seasonal variation of some primary and sesondary biochemicals. *Adv Biochem Biotechnol,*1, 81-94.

Ghosh, A., Chowdhury, N., &Chandra, G.(2008) Laboratory evaluation of a phytosterold compound of mature leaves of Day Jasmine (Solanaceae : Solanales) against larvae of *Culex quinquefasciatus* (Diptera:culicidae) and non-target organisms. *Parasital Research,* 103, 271-277.

Harbone, J.B. (1973) Phytochemical methods: Chapman and Hall, London, 49-188.

Haridas,R., Murugeswaran, R., p Sumathi, P., s Thekkan, S., Thomas, B., &Kunhikannan, C.(2018) Phytoconstituent from friut parts of Tiliacora acuminata (Lam.) Hook.F and thoms and *Stephania wightii* (Am.) Dunn (Menispermaceae) with in vitro antioxidant activity. *Debagiri Journal Of Science*, 4(1), 96-109.

Hossain, M.A., Salim-AL-Raqmi, K.A., Al-Mijizy, Z.H., Weli, A.M., &Ai-Riyami, Q.(2013) Study of total phenol, flavonoids contents and phytochemicals screening of various leaves crude extracts of locally grown *Thymasd vulgaris*. *Asian Pacific Journal of Tropical biomedicine*, 3(9), 705-710.

Jones, A.I., &Harwood, J.L. (1993) Lipids and lipid metabolism in the marine alga *Enteromorpha intestinalis*. *Phytochem,*34, 969-972.

Lindahl, J., Chirico, J., Boqvist, S., Thuand, H.T.V., & Magnusson, U. (2012) Occurrence of Japanese Encephalitis Virus Mosquito Vectors in Relation to Urban Pig Holdings,*American Journal Tropical Medicine Hygiene,* 87(6), 1076–1082.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., &Randal, R.J. (1951)Protein measurement with Folin's phenol reagent. *Journal biological chemistry,* 193, 265-275.

Madhu, V.,& Ravindra Naik, D.S. (2009) Ethnomedicinal uses of leaf preparation in Andhra Pradesh, India, *Ethnobotanical leaflet*, 13, 1337-47.

Nagaraja,B., Kumar, S.V.C.A., Raju, D.S., Vikas, S., &Prasad, S.S. (2016). Evaluation of the antiulcer and thrombolytic activity of aqueous extract of *Tiliacora acuminata* bark in albino wistar rats. *Journal of Chemical and pharmaceutical research,* 8(4), 973-979.

Nishantini, A., Mohor, V.R., &Jeeva, S. (2014) Phytochemical, FT-Ir and GC-MS analysis of sdtem and leaf of *Tiliacoraacuminata* (Lam.). Hook and Thomas (Menispermaceae). *International Journal of Pharmaceutical Science and Research*, 5(9), 3977-3986.

Obadoi, B.O., &Ochuko, P.O. (2001)Phytochemical studies and comparative efficacy of the crude extract of some Homeostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci*,8, 203-208.

Ochiai, Y., Katsuragi, T., &Hashimoto, K. (1987) Proteis In three seaweeds: "Aosa" *Ulva latuca*, "Arame" *Eisenia bicyclis* ad "Makusa" *Gelidium amansii.Bull Japan Soc Sci Fish*,53, 1051-1055.

Rawani, A., Ghosh, A., & Chandra, G. (2010) Mosquito larvicidial activities of *Solanum nigrum* L. leaf extract against *Culex quinquefasciatus* Say. *Parasitol Research,*107, 1235-40.

Sandhya sir, B.,& Seetharami Reddi, T.V.V.,(2011) Traditional Phyto-antidotes used for snake bite by Bagata tribe of Eastern Ghats of Visakhapathanam district, Andhra Pradesh. *International Multidisciplines Research Journal*, 1(6),42-45.

Shaalan, E.A.S., Canyonb, D., Younesc, M.W.F., Abdel – Wahaba, H., &Mansoura, A.H. (2005) A review of botanical Phytichemicals with mosquitocidal potential. *Environ Int,*31, 1149-66.

Singh, S.P., Raghavendra, K., Singh, R.K., &Suboaroa, S.K. (2001)Studies on larvicidial properties of leaf extract of *Solanum nigrum* Linn. (family Solanaceae). *Curr Sci,*81, 1529-15301.

Singha, S. (2019) Dose dependent pupicidal, adulticidal and ovicidal activities of leaf extract of *Tiliacora acuminata* on japanese enceophalites vector *Culex vishnui* group. Journal of Advance Laboratoty Research in Biology, 10 (2): 35-40.

Singha, S. (2020) Control of japanese encephalites by active principle , 3-(2-Hydroxyphenoxy)-1,2-propanedial isolated from n-hexane extract of leaf of Mesua ferrea . International Journal of Tropical Insect Science, (41):1061-1067.

Singha, S., Banerjee, S., & Chandra, G. (2011) Synerjestic effect of *Croton caudatus*(fruits) and *Tiliacoraacuminata* (flowers) extracts against filarial vector *Culex quinquefasciatus*. *Asian Pacific Journal of Tropical biomedicine*, S159-S164.

Singha, S., & Chandra, G. (2022) Chemical characterization of n-alkane compounds in the leaves of *Holoptelea integrifolia*and its repellence against Japanese encephalitis vector .Entomon, 47(4): 473-476.

Singha, S., & Chandra, G. (2022) Seasonal variation in mosquito larvicidal potentiality of *Holoptelea integrifolia*. Utter Pradesh Journal of Zoology, 43(6): 55-60.

Sofowara, L.A. (1993) *Medicinal plants and traditional medicines in Africa* : Spectrum Books Ltd Ibaban : Harborne; 1993. p. 55-71

Tofighi, Z, Ghazi, S.N., Hadjakhoondi, A., &Yassa, N. (2016) Determination of cardiac glycosides and total phenol in different generation *Securigerasecuridaca* suspension culture. *Research Journal of Pharmacognosy*, 3(2), 25-31.

Trease, G.E., &Evans, W.C.X. (1989)Pharmacognosy : *a Physicians guide to herbal medicine* : 13thedn. Baillieri Tindal London.

Uthirapathi,M., Manohar, K., &Nilliah, N. (2015)Comparative investigation of antimicrobial and phyto chemical profiling of *Cyclea peltata* and *Tiliacora acuminata*. *Journal Applied Biology Biotechnology*, 8(3), 057-063.

Vatandoost, H.,& Vaziri, M. (2001) Larvicidal activity of neem extract (*Azadirachtaindica*) against mosquito larvae in Iran. *Pestology,*25(1), 69–72.

Wheeler, P.A., &Bj..orns..ater, B.R. Seasonal fluctuations in tissue nitrogen, phosphorus and N:P for five macroalgal spcies common in the Pacific northwest coast. *J Phycol*, 28, 1-6.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4426