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Assessment of Organophosphorus and Pyrethroid Pesticide Residues in *Lactuca Sativa* L. and *Solanum Macrocarpum* L. Cultivated in Benin

By Prudence Agnandji, Lucie Ayi-Fanou, Magloire Acakpo Nonvignon Gbaguidi, Boris Fresnel Cachon, Mathieu Hounha, Micheline Agassounon Djikpo Tchibozo, Winfred Seth Kofi Gbewonyo, Fabrice Cazier & Ambaliou Sanni

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Abstract- Pesticides are harmful and toxic in nature. They are used for vegetable protection against pest attack. The present study aimed to determine the level of Organophosphorus and Pyrethroid residues in vegetables (Solanum macrocarpum L. and Lactuca sativa L.) Thirty one vegetable samples (16 samples of Solanum macrocarpum L. and 15 samples of Lactuca sativa L.) from four vegetable farms were collected. The samples were cleaned up before the extraction was performed. Analysis was then processed using the QuEChERS method. Residue analysis was performed using a GC-PFPD for Organophosphorus residues and GC-ECD for Pyrethroid residues. MedCalc Statistical Software version 15.0. was used for the statistical analysis. The results revealed that most of the samples are contaminated by Pyrethroid and Organophosphorus residues.

Keywords: levels contamination, organophosphorus, pyrethrinoid, vegetable crops, south of benin.

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Assessment of Organophosphorus and Pyrethroid Pesticide Residues in *Lactuca Sativa* L. and *Solanum Macrocarpum* L. Cultivated in Benin

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Keywords: levels contamination, organophosphorus, pyrethrinoid, vegetable crops, south of benin.

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I. Introduction

In Benin, vegetable farming is intensified in southern department's town such as Cotonou and Seme-Kpodji. Vegetables grown during this activity are most often vulnerable to attack by pests (weeds, bacteria, fungi and insect pests, etc.) [1]. A study carried out by Azandeme-Hounmalon *et al.* [2] in Southern Benin, on a population of mite fauna associated with local vegetables (*Amaranthus cruentus* L., *Solanum macrocarpum* L.) showed that several species of mites attack these vegetables. One of them is *Tetranychus evansi*. The consequences of these attacks are the recording of losses in the harvest yield and the decline in monetary income. To remediate these problems, vegetables farmers use pesticides [3,4,5,6]. There are several groups of pesticides including insecticides such as Pyrethroids (Pyr) and Organophosphorus (Op). They are the insecticides which replaced Organochlorine insecticides [7].

In southern of Benin, active ingredients of synthetic Pyr and Op frequently used in vegetable farming are cypermethrin, lambda cyhalothrin, deltamethrin, profenofos, chlorpyfos, malathion [8]. These active substances have a protective role for vegetable crops against pests such as *aphids*, *beetles and lepidoptera* [9]. Pesticides are widely used (in fruit and vegetables) because of their susceptibility to insect and diseases attack. Consequently, food safety is a major public concern worldwide. During the last decades, the increasing demand for food safety has stimulated research regarding the risk associated with consumption of fruits and vegetables as they constitute to many part of human diet, contributing nutrients and vitamins. Therefore, residues of pesticides could affect the consumers, especially when these commodities are freshly consumed. The total dietary intake of pesticides residues that remain on agricultural commodities are known as carcinogens/or toxins and therefore it is desirable to reduce these residues [10]. Despite the fact that Pyr and Op pesticides are used by farmers in Benin to protect their vegetables, no data exist about Pyr and Op contamination levels in vegetables grown in Benin. The present study was designed to evaluate the level of contamination of Op and Pyr residues in exotic and local vegetable crops (*Solanum macrocarpum* L. and *Lactuca sativa* L.) cultivated in southern Benin.

II. Material and Methods

a) Study Areas

Vegetable samples were collected in 2017, during March to July in two farms sites located in Cotonou (intra-urban area) and Seme-kpodji (Suburban area). The map of this study was described in a previous publication [11].

b) Sampling

A total of 31 vegetable samples were collected from the study areas including 15 samples of *Lactuca sativa* L. and 16 *Solanum macrocarpum* L. These vegetables were chosen because they are very common in Beninese diet. The sampling plan consisted of delineating a 10 x 10 m plot in the middle of the field of each farmer. Five primary samples were chosen along the diagonals. These samples were roughly cut and mixed in situ to obtain a final sample of 750 g. Each sample was packed in aluminium foil, plastic bag and ice box. The samples were sent to the laboratory and it was stored at -20°C until the moment of the analysis.

c) Reagents and Glassware

All glassware used for extraction and cleaning was rigorously washed with tap water and detergent and rinsed twice with distilled water. They were again rinsed with acetone and finally dried in an oven. All solvents and reagents used were of the analytical grade supplied by VWR Chemical. Individual pesticide standards were avaiable at Ghana Standard Authority (GSA) laboratories in Acra.

d) Extraction and Clean-up

Vegetable sample (15 ± 0.1 g) was weighed and milled in a mill (MOULINEX Soup & Co Lm 901b1 Blender) before being transferred to a 50 mL centrifuge tube containing 15 mL of acetonitrile (ACN) HPLC grade. The tube was closed and shaken vigorously on a shaker (STUART SA7, VITESSE FIXE) for 1 min. After shaking, a mixture of 4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogenocitrate sesquihydrate was added. The tube was closed and immediately shaken vigorously using a shaker for 1 min and centrifuged for 10 min at 3000 rpm. An aliquot (6 mL) of the extract was transferred into centrifugation tube containing the following sorbents: 150 mg of anhydrous magnesium sulphate, 50 mg of PSA, 50 mg of black graphite Carbon and 50 mg of adsorbent C18 for purification. We added formic acid at 10µL per mL to the tube of extract (1%) then the tube was shaken vigorously for 1 min and centrifuged for 10 min at 3000 rpm. Thereafter, 4 mL of the extract supernatant was concentrated with a dessicator containing silica gel activated by heating in oven at 105 °C connected with a pump LABCONCO 117 (A65312906) N038-500. The residue was collected with 1 mL of the mixture of formic acid in ACN (1%) and introduced in vial.

e) Analysis of Insecticide Residues Using Gas Chromatography (GC) Method and Quality Control

A Varian CP3800 gas chromatograph Electron Capture Detector (ECD) and Pulsed Flame Photometric Detector (PFPD) were used to detect and quantify respectively Pyr and Op residues, in the vegetable samples from different sites. For Pyr, samples were injected into the column of the GC with an autosampler at a temperature of 270°C. The compounds were partitioned through the stationery phase, a capillary column (30 m + 10 m EZ Guard, internal diameter (i.d.) 0.25 mm, fused with silica coating VF- 5 ms, 0.25 µm film) carried by solvents. The oven temperature was programmed at 70°C for 2 min, then 25°C/min till 180°C followed by 5°C/min up to 300°C. The carrier gas was nitrogen maintained at a constant flow rate of 1 ml/min. However, for the Op, the samples were injected into the column of the GC under the same conditions described in previously. The compounds were partitioned through the stationery phase, a capillary column (30 m x 0.25 mm internal diameter (i.d.) fused with silica capillary coating with VF- 1701 ms, 0.25 µm film) carried by solvents. The oven temperature was programmed at 70°C for 2 min, then 25°C/min till 200oC followed by 20°C/min up to 250°C. The carrier gases were nitrogen (1 ml/min), Air 1 (17 ml/min), Hydrogen (14 ml/min) and Air 2 (10 ml/min).

The molecules of the insecticide residues were differentiated at different rates through the gas. The ECD and PFPD was used to detect differently Pyr and Op compound present. The detection temperatures were 300°C for the ECD and 280°C for the PFPD. These detectors were linked to a computerized integrated system which counts and records the signals as peaks, which were used to quantify insecticide residues present.

The residue of pesticide was identified based on comparison of the measured relative retention times to those of known standards, indicated in Table 1. The residue levels of Pyr or Op pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincide with the standards were extrapolated on their corresponding calibration curves to obtain the concentration.

The quality was assured through the analysis of solvent blanks, procedure blanks. The method was optimized and validated using spiked (together) with the internal standard to evaluate the recovery of compounds. The recoveries of internal standards ranged between 71.4% and 110.9% for all the Op and Pyr pesticide. The limit of detection was 10 ppb for Pyr and Op pesticides.

Table 1: Retention times of the pesticide residues

Pesticides residues	Retention times (min)
Organophosphorus Residues	
Methamidophos	7.14
Ethoprofos	8.86
Diazinon	9.61
Fonofos	9.87
Dimethoate	10.46
Pirimiphos-methyl	10.65
Chlorpyrifos	10.87
Malathion	11.11
Fenitrothion	11.28
Parathion-ethyl	11.56
Chlorfenvinp	11.76
Profenofos	12.56
Pyrethroid residues	
Allethrin	16.52
Bifenthrin	22.70
Fenpropathrin	23.07
Lambda Cyhalothrin	24.62
Permethrin	26.38
Cyfluthrin	27.58
Cypermethrin	28.17
Fenvalerate	29.91
Deltamethrin	31.18

f) Statistical Analysis

Results obtained are expressed in mean ± standard deviation of the mean. Statistical analysis was performed by Student test unmatched to compare samples mean. The second test was Chi-square and it was used to determine the difference between the number of vegetable samples which the level in Pyr and Op residues are above the Maximum Residue Limits (MRLs). The statistical significance for each analysis was considered at $P<0.05$. MedCalc Statistical Software version 15.0. was used for the statistical analysis.

III. Results

a) Level of Pyrthroids Pesticides Concentration in Solanum macrocarpum L. and Lactuca sativa L.

Vegetables such as *Solanum macrocarpum* L. and *Lactuca sativa* L. are an important source of nutrition for human system and human diet. The contamination of those vegetables by pesticides could cause a threat to human health. Many studies about monitoring of Pyr pesticides shown their presence in vegetables. In our study we researched nine Pyr molecules in vegetables (*Solanum macrocarpum* L. and *Lactuca sativa* L). The mean total concentration of Pyr residues (0.076±0.248 mg/kg) found in *Solanum macrocarpum* L. is higher than the mean total concentration (0.070±0.280 mg/kg) found in *Lactuca sativa* L., but the difference is not statistically significant ($p > 0.05$).

b) Range, mean±SD of Pyrethroids Found in Solanum macrocarpum L. and Lactuca sativa L.

Table 2 shows minimum, maximum, mean ± standard deviation of Pyr found in *Solanum macrocarpum* L. and *Lactuca sativa* L.

High concentration (0.045 mg/kg) of allethrin was detected in *Solanum macrocarpum* L. samples with mean of 88×10-3±11.5×10-3 mg/kg.

Bifenthrin and fenpropathrin weren't detected in *Lactuca sativa* L. samples but in *Solanum macrocarpum* L. their concentration reached 0.02 and 0.007 mg/kg. Their averages are 2.50×10-3±5.45×10-3 and 0.56×10-3±1.79×10-3 mg/kg.

The highest concentration of lambda cyhalothrin found in *Solanum macrocarpum* L. was 2.13 mg/kg with an average of 523.7×10-3±564.1×10-3 mg/kg. Residues of permethrin and fenvalerate were detected in *Lactuca sativa* L. at maximum concentration levels of 0.027 and 0.049 mg/kg respectively, with averages of 2.33×10-3±6.96×10-3 and 15.4×10-3±14.6×10-3 mg/kg. The highest concentration of cyfluthrin found in *Solanum macrocarpum* L. is 0.098 mg/kg with an average of 21.1×10-3±22.3×10-3 mg/kg. That of cypermethrin found in *Solanum macrocarpum* L. is 1.025 mg/kg with an average of 186.3×10-3±260.3×10-3 mg/kg. Similarly, the maximum level of deltamethrin residues found in *Solanum macrocarpum* L. is 0.028

mg/kg with an average of 11.9×10-3±8.55×10-3 mg/kg.

c) *Frequency of Occurrence of Pyrethroid Residues in Samples of Solanum macrocarpum L. and Lactuca sativa L.*

Figure 1 shows the frequencies of appearance of Pyr residues in the vegetable samples analyzed. From the analysis of the data in Figure 1 it is noted that the residues of bifenthrin and fenpropathrin were not detected in the samples of *Lactuca sativa* L. On the other hand, these residues are found at frequencies of 25% and 12.5% respectively in *Solanum macrocarpum* L. samples. The residues of permethrin and allethrin are the least predominant in the two varieties of vegetables. They were detected at proportions of 6.25% and 25%, respectively, in the samples of *Solanum macrocapum* L. While in *Lactuca sativa* L. samples, these proportions were 26.67% and 33.33%.

Deltamethrin residues were found in proportions of 60% and 93.75%, respectively, in the *Lactuca sativa* L. and *Solanum macrocarpum* L. samples. Fenvalerate was found to be 86.67% in the *Lactuca sativa* L. samples. L. and 56.25% in samples of *Solanum macrocarpum* L. Cypermethrin, cyfluthrin and lambda cyhalothrin were found in all samples of *Solanum macrocarpum* L. and *Lactuca sativa* L. at frequencies greater than 73.33 %.

d) *Maximum Residue Limit of Pyrethroids residues in Solanum macrocarpum L. and Lactuca sativa L.*

Table 3 presents the Maximal Residue Level (MRL) and the percentage of the residues which the level are exceeded the limit.

Among Pyr residues analyzed, only three residues levels are above EU MRLs of European Union [12]. These residues are lambda cyhalothrin and cypermethrin in *Solanum macrocarpum* L. ; lambda cyhalothrin and fenvalerate in *Lactuca sativa* L. Among Pyr residues which the concentration levels were above MRLs, only lambda cyhalothrin concentration was detected in 7/16 case (43.75%) in *Solanum macrocarpum* L. and 7/15 case (40%) in *Lactuca sativa* L. Fenvalerate concentration found in *Lactuca sativa* L. was above MRLs in 3/15 case (20%).

Table 2: Pyrethroids Pesticides residues concentrations in the tested vegetables (mg/kg)

Pyrethroids Residues	*Solanum macrocarpum* L.		*Lactuca Sativa* L.	
	Range	Mean±SD	Range	Mean±SD
Allethrin	ND-0.046	$2.88\times10^{-3}\pm11.5\times10^{-3}$	0.003-0.011	$1.80\times10^{-3}\pm3.17\times10^{-3}$
Bifenthrin	0.001-0.020	$2.50\times10^{-3}\pm5.45\times10^{-3}$	ND	ND
Fenpropathrin	0.002-0.007	$0.56\times10^{-3}\pm1.79\times10^{-3}$	ND	ND
Lambda Cyhalothrin	0.067-2.127	$523{,}7\times10^{-3}\pm564.1\times10^{-3}$	0.017-1.713	$675.9\times10^{-3}\pm6.73\times10^{-3}$
Permethrin	0.003-0.014	$1.94\times10^{-3}\pm4.10\times10^{-3}$	0.001-0.027	$2.33\times10^{-3}\pm6.96\times10^{-3}$
Cyfluthrin	0.002-0.098	$21.1\times10^{-3}\pm22.3\times10^{-3}$	0.001-0.035	$8.73\times10^{-3}\pm10\times10^{-3}$
Cypermethrin	0.003-1.025	$186.3\times10^{-3}\pm260.3\times10^{-3}$	0.003-0.021	$45.8\times10^{-3}\pm103.5\times10^{-3}$
Fenvalerate	0.001-0.018	$3.75\times10^{-3}\pm5.08\times10^{-3}$	0.003-0.049	$15.4\times10^{-3}\pm14.6\times10^{-3}$
Deltamethrin	0.003-0.028	$11.9\times10^{-3}\pm8.55\times10^{-3}$	0.002-0.011	$3.27\times10^{-3}\pm3.69\times10^{-3}$

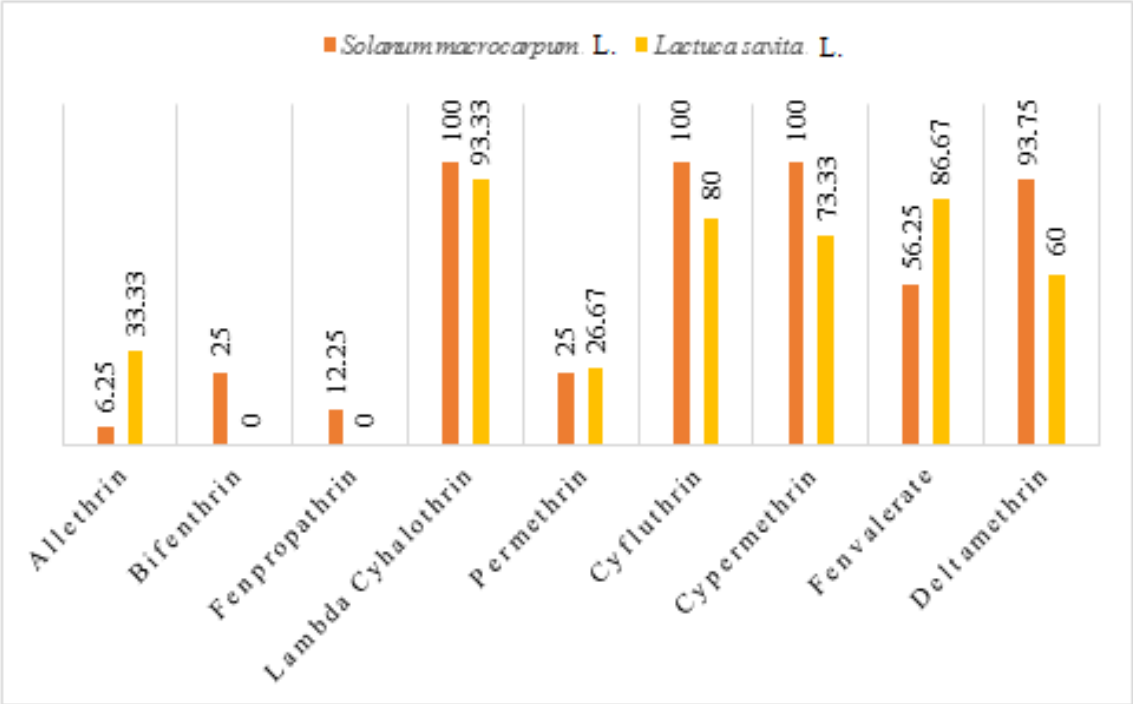

Figure 1: Frequency of Pyrethroid residues *in Solanum macrocarpum* L. and *Lactuca sativa* L.

Table 3: Maximum residue limits (MRLs) of Pyrethroids in vegetables samples and percentage of its above MRLs

Pyrethroids residues	*Solanum macrocarpum* L.		*Lactuca sativa* L.		X^2	*p-value*
	EU MRL mg/kg	% of *Solanum macrocarpum* L. > EU MRL	EU MRL mg/kg	% of *Lactuca sativa* L. > EU MRL		
Allethrin	--	--	--	--	--	--
Bifenthrin	--	--	0.01	0	--	--
Fenpropathrin	0.01	0	0.5	0	--	--
Lambda Cyhalothrin	0.2	43.75	0.5	40	0.023	0.879
Permethrin	0.05	0	0.05	0	--	--
Cyfluthrin	--	--	1	0	--	--
Cypermethrin	0.7	6.25	2	0	0.00108	0.974
Fenvalerate	0.02	0	0.02	20	1.624	0.202
Deltamethrin	0.2	0	0.5	0	--	--

-- = No determined.

e) Level of Organophosphorus Pesticides in Solanum macrocarpum L. and Lactuca sativa L.

As previously reported, the mean total concentration (0.065±0.360 mg/kg) of Op found in *Solanum macrocarpum* L. is higher than the mean total concentration (0.025±0.106 mg/kg) found in *Lactuca sativa* L., as indicated in Figure 2 . But the difference is not statistically significant ($p > 0.05$).

f) Range, mean±SD of Organophosphorus Found in Solanum macrocarpum L. and Lactuca sativa L.

Table 4 show minimum, maximum, mean ± standart deviation of Op found in *Solanum macrocarpum* L. and *Lactuca sativa* L.

For the Op, contamination levels ranged from non-determined (ND) to 4.809 mg/kg in the samples of *Solanum macrocarpum* L. and ND at 1.095 mg.kg in the *Lactuca sativa* L samples. These high concentrations were recorded for the residues of dimethoate and chlorpyrifos, respectively. The average residues of Op vary from 0.62×10-3 ± 2.50×10-3 mg/kg for fonofos at 1386×10-3 ± 178.9×10-3 mg/kg for Malathion in *Solanum macrocarpum* L. On the other hand, in *Lactuca sativa* L. These mean concentrations per residue vary from 0.78×10-3 ± 2.94×10-3 mg/kg for the diazinon at 203.6×10-3±305.4×10-3 mg/kg for chlorpyrifos.

In *Solanum macrocarpum* L. the two Op residues were not detected in all samples. These are diazinon and fonofos. In contrast, in *Lactuca sativa* L. residues of diazinon, ethoprofos, and parathion-ethyl were not detected in all samples. This finding shows that several Op molecules at varying doses are used on sites to control crop pests.

Figure 2: Mean of total concentration of Organophosphorus in Solanum macrocarpum L. and Lactuca sativa L.

Table 4: Pesticides residues concentrations in the tested vegetables (mg/kg)

Organophosphorus Residues	*Solanum macrocarpum* L.		*Lactuca sativa* L.	
	Range (mg/kg)	Mean±SD (mg/kg)	Range (mg/kg)	Mean±SD (mg/kg)
Methamidophos	0.011-0.014	$2.25\times10^{-3}\pm4.88\times10^{-3}$	0.008-0.016	$5.50\times10^{-3}\pm6.05\times10^{-3}$
Ethoprofos	0.006-0.011	$2.06\times10^{-3}\pm3.82\times10^{-3}$	ND-0.007	$10^{-3}\pm2.54\times10^{-3}$
Diazinon	ND-0.232	$14.5\times 10^{-3}\pm58\times10^{-3}$	ND-0.011	$0.78\times10^{-3}\pm2.94\times10^{-3}$
Fonofos	ND-0.01	$0.62\times10^{-3}\pm2.50\times10^{-3}$	0.009-0.012	$2.86\times10^{-3}\pm4.74\times10^{-3}$
Dimethoate	0.026-4.809	$310\times10^{-3}\pm1999.9\times10^{-3}$	0.025-0.034	$8.27\times10^{-3}\pm13.7\times10^{-3}$
Pirimiphos-methyl	0.009-0.028	$6.94\times10^{-3}\pm10\times10^{-3}$	0.008-0.014	$5.15\times10^{-3}\pm5.98\times10^{-3}$
Chlorpyrifos	0.015-0.704	$219.8\times10^{-3}\pm188.2\times10^{-3}$	0.025-1.095	$203.6\times10^{-3}\pm305.4\times10^{-3}$
Malathion	0.011-0.557	$1386\times10^{-3}\pm178.9\times10^{-3}$	0.017-0.266	$34\times10^{-3}\pm68.7\times10^{-3}$
Fenitrothion	0.021-0.03	$4.81\times10^{-3}\pm10.5\times10^{-3}$	0.023-0.025	$5.14\times10^{-3}\pm10.2\times10^{-3}$
Parathion-ethyl	0.01-0.015	$1.56\times10^{-3}\pm4.37\times10^{-3}$	ND	--
Chlorfenvinp	0.017-0.02	$2.31\times10^{-3}\pm6.34\times10^{-3}$	0.014-0.017	$2.21\times10^{-3}\pm5.66\times10^{-3}$
Profenofos	0.006-0.353	$77.6\times10^{-3}\pm109.9\times10^{-3}$	0.007-0.329	$27.3\times10^{-3}\pm84.3\times10^{-3}$

ND or -- = No determined.

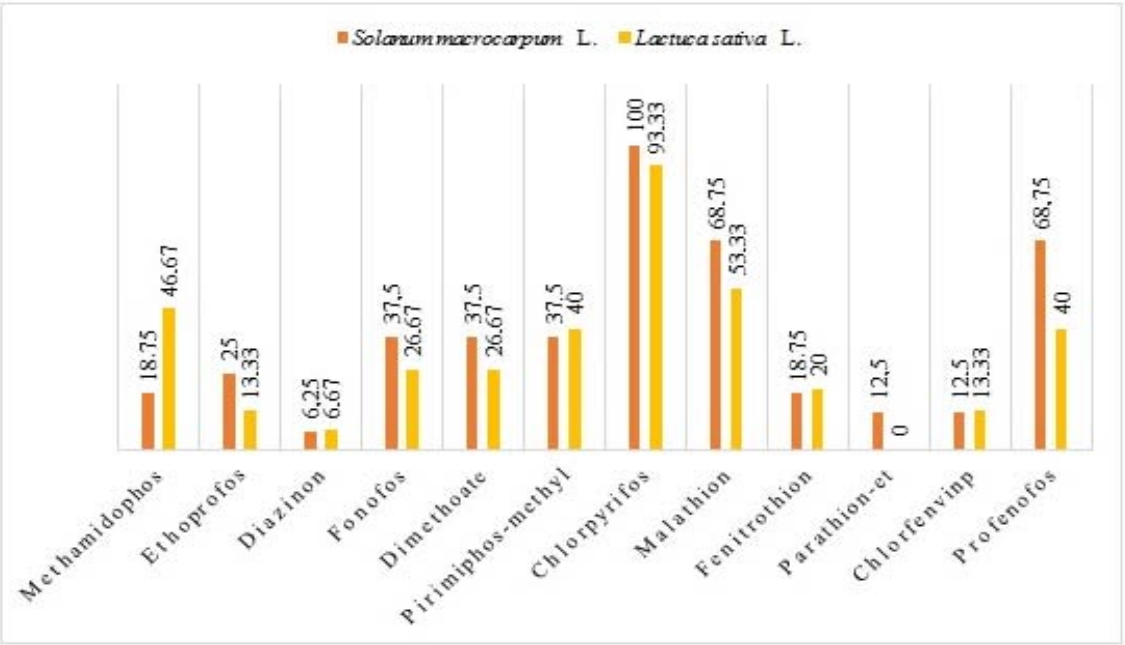

Figure 3: Frequency of organophosphorus *in Solanum macrocarpum* L. and *Lactuca sativa* L.

g) *Frequency of Organophosphorus in Solanum macrocarpum L. and Lactuca sativa L.*

A total of twelve Op molecules were searched in two varieties of vegetables as *Solanum macrocarpum* L. and *Lactuca sativa* L. Among the Op residues detected in the vegetable samples, parathion ethyl was not found in *Lactuca sativa* L. but it was found in low proportion in *Solanum macrocarpum* L. (12.5%). Similarly residues such as methamidophos ethoprofos, diazinon, dimethoate, fenitrothion, chlofenphos are also found in proportions of less than 30% in all the studied vegetables. On the other hand, residues of chlorpyrifos, malathion and profenofos are the most ubiquitous with proportions varying between 53 and 100%, as indicated in Figure 3.

h) *Maximum Residue Limits of Organophosphorus Residues in Vegetables and Percentage of Its above MRLs*

Table 5 presents the MRLs and the percentage of the Op residues which the level are exceeded the limite.

Among Op residues analyzed in *Solanum macrocarpum* L, only three residues level were below EU MRLs [13]. These residues are methamidophos, ethoprofos and parathion ethyl. The concentration of the rest was found at the level above EU MRLs at different proportion; diazinon (6.25%), chlofenvinp (12.5%), fenithrotion (18.75%), dimethoate, (25%), pirimiphos methyl (25%), profenofos (50%) malathion (56.25%) and chlopyrifos (75%).

Table 5: Maximum residue limits (MRLs) of Organophosphorus residues in vegetables and percentage of its above MRLs

	Solanum macrocarpum L.		*Lactuca sativa* L.		X^2	*p-value*
	EU MRL	% of Solanum macrocarpum L. > EU MRL	EU MRL	% of *Lactuca sativa* L.> EU MRL		
Methamidophos	0.01	0	0.01	26.67	2.813	0.093
Ethoprofos	0.02	0	0.02	13.33	0.606	0.436
Diazinon	0.01	6.25	0.01	0	0.00108	0.974
Fonofos	--	--	--	--	--	--
Dimethoate	0.02	25	0.02	26.67	0.093	0.761
Pirimiphos-methyl	0.01	25	0.01	26.67	0.093	0.761
Chlorpyrifos	0.05	75	0.05	53.33	0.782	0.376
Malathion	0.02	56.25	0.02	46.67	0.030	0.862
Fenitrothion	0.01	18.75	0.01	20	0.135	0.714
Parathion-ethyl	0.05	0	0.05	0	--	--
Chlorfenvinp	0.01	12.5	0.01	13.33	0.218	0.641
Profenofos	0.01	50	0.01	20	1.874	0.171

-- = no limit has fixed By EU.

For Op residues analyzed in *Lactuca sativa* L, only two residues level were below MRLs of EU [13]. These residues are diazinon and parathion ethyl. The concentration of the rest were found at the level above EU MRLs in different proportion; ethoprofos and chlofenvinp (13.33%); fenithrotion and profenofos (20%); dimethoate, pirimiphos methyl and methamidophos (26.67%); malathion (46.67%) and chlopyrifos (53.33%).

IV. Discussion

The investigation about Op and Pyr pesticides in two vegetables (*Solanum macrocarpum* L. and *Latuca sativa* L.,) grown in southern Benin, revealed that all the Op pesticides studied were found in *Solanum macrocarpum* L. leaves.

On the other hand, parathion-ethyl wasn't detected in *Lactuca sativa* L. The levels of Op residues ranged from no determined (ND) to 4.81 mg/kg in *Solanum macrocarpum* L. with mean of 0.065 ± 0.360 mg/kg. In *Lactuca sativa* L. the highest level detected is 1.1 ppm with mean of 0.025 ±0.106 mg/kg. The Op residues are methamidophos, ethoprofos, diazinon, fonofos, dimethoate, pirimiphos-methyl, fenitrothion, parathion-ethyl, chorfenvinphos, malathion, chloropyrifos and profenofos. Comparing our results with other studies, we note that diazinon was detected in vegetables in the range of 0.066 to 1.84 mg/kg [13].

These levels of contamination are superior to our finding which the ranges for this residue is ND to 0.232 mg/kg in *solanum macrocarpum* L. and ND to 0.011 mg/kg in *Latuca sativa* L. [13]. For these two studies, the levels of diazinon in vegetables exceed the EU MRLs of 0.01 mg/kg. Quintero *et al.* [14] also found similar results in vegetables. Contrary to our results, chlorpiryfos was found in Thailand at a concentration of 0.008 mg/kg in *Latuca sativa* L. [15]. This level of contamination is lower than the EU MRLs which is still 0.05 mg/kg. These findings show that Op pesticides are widely used in market gardening in the world. In the present study, chlorpiryfos, malathion and profenofos were detected at a high frequency with levels above the EU MRLs (Table 5). Our results corroborate with of Wanwimolruk *et al*. [16] who found in 34 samples of Chinese cabbage tested, chlorpyrifos (7.2-37.700 ppb), dimethoate (0.5-239 ppb) and profenofos (0.5-183.000 ppb). In this study, the proportion of samples with contamination levels above the MRLs is 29% [16]. In addition, the analysis of cauliflower samples revealed the presence of Op residues such as chlorpyrifos and profenofos. However, none of the samples contained residues above their respective MRLs [17]. The analysis of tomato from Ghana revealed the presence of malathion in 50% of the samples at an average concentration of 0.027 ± 0.021 mg/kg while dimethoate was found in samples at a rate of 15%. Its average concentration in the samples was 0.155 ± 0.113 mg/kg [18]. Malathion and dimethoate levels in the tomato samples were higher than the EU MRLs [18]. These levels of contamination, which are sometimes found below the MRLs, are due to the washing of the leaves of the vegetables concerned either during watering or during rain season and do not contradict the massive use of Op.

For Pyr, we studied allethrin, bifenthrin, fenpropathrin, lamda cyhalithrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin residues. The average concentrations found are respectively 0.076 ± 0.248 mg/kg in *Solanum macrocapum* L. and 0.070 ± 0.280 mg/kg in *Lactuca sativa* L. Allethrin has

no MRLs for the vegetable samples studied. Among bifenthrin, fenpropathrin and permethrin which have EU MRLs for the samples studied, they were detected in low frequency, without exceeding above EU MRLs. Akomea-Frempong *et al*. [13] also detected bifenthrin in vegetable (lettuce and cabbage) samples at an average concentration of 0.034 mg/kg. Bempah *et al*. [19] found permethrin in lettuce at a concentration range of 0.011 to 0.035 mg/kg with an average of 0.030 ± 0.009 mg/kg. This range of concentrations is higher than the level of permethrin we found in our samples of *Lactuca sativa* L. (0.001-0.027 mg/kg). For both studies, contamination levels of *Lactuca sativa* L. samples with permethrin are below the EU MRLs. Our levels of bifenthrin are lower than those found by González-Rodríguez *et al*. [20] who reported bifenthrin concentrations in lettuce of 0.02 to 0.05 mg/kg in Spain. Fenpropathrin was also detected in cucumber and tomato samples from Egypt at concentrations of 0.04 and 0.079 mg/kg, respectively (0.021-0.03 mg/kg) in *Solanum macrocarpum* L and (0.023- 0.025 mg/kg) in *Lactuca sativa* L. for this study [21]. These concentrations of fenpropathrin detected in the cucumber and tomato samples are higher than those we detected in our vegetables [21]. Permethrin, allethrin was found in eggplant in Ghana. Their respective mean concentrations are 0.003 mg/kg for Permethrin and 0.126 ± 0.018 mg/kg for allethrin [18]. In contrast, for the present study, Permethrin was detected in *Solanum macrocarpum* L. samples between 0.003 and 0.014 mg/kg. While in *Lactuca sativa* L. its concentration varies between 0.001 and 0.027 mg/kg. As for allethrin, it was detected between ND and 0.046 mg/kg in *Solanum macrocarpum* L. and 0.003 and 0.011 mg/kg in *Lactuca sativa* L. Permethrin was detected in 35% of the eggplant samples whereas in our case it was detected in 25% of the samples of *Solanum macrocarpum* L. and 27% in *Lactuca sativa* L. allethrin was detected in 100% of the samples [18]. In the present study, however, it is found only at 6.25% in the samples of *Solanum macrocarpum* L. and 33.33% in the samples of *Lactuca sativa* L. In contrast to our results, the residues of permethrin found in eggplant samples in Ghana were lower than the EU MRLs [19]. In contrast, consistent with our results, allethrin residues in the same samples exceeded the MRLs [18].

Our results are confirmed by Diop *et al*, [22]. who found lambda cyhalothrin in lettuce from different farms sites of Senegal. The mean of it concentration is 0.222 mg/kg and it range is ND and1.306 mg/kg. Less than 50% levels of lambda cyhalothrin found in lettuce samples are above EU MRLs.

All these observations show that Op and Pyr are massively used in market gardening in the world for pests control in farming. Their use is not without consequence on the sanitary quality of the vegetables whose contamination levels vary as well according to the residues and the cultures with contents sometimes exceeding the maximum limits in residues. From the nutritional point of view, the level of Pyr and Op residues found in *Solanum macrocarpum* L. and *Lactuca savita* L. samples at level above EU MRLs pose public health problem. Exposure to pesticides through a mouth is a major way for the people which aren't farm workers and don't live close to the vegetables fields. Many studies shown the health risk associated with the consumption of vegetables contaminated by pesticide residues [23,24,25,26].

This problem is especially encountered in poor countries by the proliferation of illegal trade in plant protection products, which are sometimes prohibited.

V. Conclusion

This study evaluates the contamination levels of Op and Pyr pesticides in vegetable crops in southern Benin revealed their presence at various concentrations showing their massive use in market gardening in southern Benin. The most detected residues are Pyr (lambda cyhalothrin) and Op (chlorpyrifos). By comparison of levels of residues with EU MRLs, lambda cyhalothrin, fenvalerate, diazinon, chlorfenvinp, fenitrothion, dimethoate, pirimiphos methyl, profenofos, malathion and chlorpyrifos were detected in samples of *Solanum macrocarpum* L. and/or *Lactuca sativa* L. at levels above the MRLs. These observations show that the use of pesticides in market gardening affects the sanitary quality of leafy vegetables such as *Solanum macrocarpum* L. and *Lactuca sativa* L. More studies should be done on quantifying these pesticide residues in other plants grown in Benin.

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Preliminary Investigation into the Periphyton Community of a Tidal Creek, Bonny River, Rivers State, Nigeria

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Abstract- In this report the periphyton community of a tidal creek in Bonny River was investigated, study site was demarcated to five stations where samples were collected for six (6) months. Laboratory analysis was based on standard methods and involved identification and enumeration of periphytic organisms. The periphytic community of the study area was composed of 83 species belonging to six classes and 61 genera. The most dominant class was Bacillariophyta represented by 32 species, followed by Chlorophyta with 18 species, Cyanophyta with 17 species, Pyrrophyta 7species, and Euglenophyta and Xanthophyta with 5 and 4 species respectively. The order of total class abundance contrasted with that of species richness, following in decreasing order – Bacillariophyta, Cyanophyta, Euglenophyta, Pyrrophyta, Chlorophyta and Xanthophyta. The increasing presence of Cyanophyta and Euglenaphyta in all stations suggests that study area is under stress which may have been caused by petroleum hydrocarbon.

Keywords: periphyton, cyanobacteria, bio-indicators, species richness, class abundance, dominance.

GJSFR-C Classification: FOR Code: 279999

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O. A. F. Wokoma [α] & U. Friday [σ]

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I. Introduction

Periphytons are a complex mixture of algae, cyanobacteria, heterotrophic microbes and detritus that are attached to submerged surfaces such as rocks, macrophytes etc. in most aquatic ecosystem (Ekhator, 2010). Periphytons are the dominant producers and sources of autochthonous organic matter in most standing water, since most lakes and reservoirs are predominantly small and shallow (Wetzel, 1996), similarly, when current velocity is appreciable nearly all primary production is from periphyton as phytoplankton are unstable and not abundant. Gaiser, (2008) observed that periphytons are a crucial and fundamental part of the food web, as the primary food source for small consumers such as fish and invertebrates. Through the physicochemical interactions with the biotic and abiotic environment, periphyton affects many biotic communities and ecosystem features such as nutrient circling, dissolved oxygen concentration and soil/sediment quality through photosynthesis to sustain much of the aquatic life in its surrounding (Gaiser, 2008; Vadeboncoeur and Steinman, 2002). According to Lowe and Pan(1996) periphyton contributes significantly to the biodiversity of these ecosystems.

Periphytons serve as an important food source for invertebrates, tadpoles, and some fishes (Finlay, *et. al*., 2002), and can absorb contaminants removing them from the water column and limiting their movement through the environment (Wikipedia, 2012). Periphyton due to their sedentary nature, species composition, community structure, succession and biomass are sensitive to changes in water quality and are often used as bio-indicators of ecological conditions and change in response to human and natural disturbance (Cascaller *et. al.*, 2003) and in classification of water ways (DeNicola, Eyto, Wemaere and Irvine, 2004). Their responses to pollutants or water quality changes according to Cairns, (2003), can be measured in a variety of scales or levels viz, physiologically, population, community etc levels. Their fast response to changes in the environment, naturally high species richness and their high level of tolerance/ sensitivity makes them ideal bio-monitors (Wikipedia, 2012).

Periphytons have a very high rate of reproduction, and in ideal conditions (sufficient supply of light and nutrients) their population can explode into blooms which can contribute to oxygen depletion, fish kills and aesthetic problems that can interfere with recreational use (DeNicola, et. al., 2004; Siva and John, 2002).

Despite its ecological significance, periphytic algae has received less attention from Hydrobiologists than planktons (phytoplankton and zooplankton), and in the study area there is no report on the periphytic community in the past. This study is therefore aimed at providing information to fill this gap.

II. Materials and Methods

a) *Description of the study area*

The study area – Kua-Kinabere creek, in Ogoni land is a mangrove wetland, an estuary of the Bonny River serving as transportation channel or route and is notorious for oil activities including illegal oil bunkering and or refining.

Prior to commencement of sampling, a reconnaissance visit was paid to the study area during which five (5) sampling stations were identified. Two

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replicate samples were collected from each station for six (6) months (March to August). The geographical location of the various sampling stations obtained with a hand-held Global Positioning System (G.P.S) instrument (GARMIN EXTREX) are, (STN 1) N 04040.017', E 007014.081'; (STN 2) N 04040.506' E 007013.577' (STN 3)N 04040.699' E 007014.800' (STN 4) N 04041.184' E 007014.199' and (STN 5) N 04041.169' E 007014.256'.

b) Field Methods

Periphyton samples were collected at low tide in each of the five sampling stationsin triplicates by randomly throwing a 2cm by 2cm quadrant on the surface of aquatic macrophytes or any other object and carefully scrapping off the quadrant area with a scalpel, which was then emptied into a properly labeled. To each of the container was then added a few drops of eosin solution to stain the tissues of the organisms and make them visible during microscopic analysis in the laboratory. The samples were then preserved in 10% formalin solution with few drops of eosin before transporting to the laboratory in an ice-chest cooler for identification and enumeration.

c) Laboratory Methods

In the laboratory samples were allowed to stand for a minimum of 24 hours before decanting the supernatant. The supernatant was removed carefully until a 50ml concentrated sample was achieved. The concentrated sample was then properly shaken and 1ml sub-sample was collected from it and transferred into a Sedgewick Rafter counting chamber using a stampel pipette. Identification and enumeration was carried out under a binocular compound microscope with magnification of 40 x 400. Three replicates of the sub-samples were analyzed. For each sample, each solitary cell was counted as one unit in a cell by cell basis. The result was then expressed in number of organisms per ml of sample.

Identification and characterization of the periphytic algae species was based on the descriptive keys and illustrations ofMaosen, (1978) and Durand and Leveque (1980).

III. Results and Discussion

The periphyton community in the study area was represented by 83 species, spread across 6 classes and 61 genera. Bacillariophyta had the highest number of species -32, followed by Chlorophyta with 18 species, Cyanophyta was represented by 17 species, Pyrrophyta, Euglenophyta and Xanthophyta, had 7, 5and 4 species respectively, as shown in Table 1. However, the total abundance of the periphytic community showed that the diatoms Bacillariophyta were the most dominant class accounting for 39.72% of the population, followed by the class Cyanophyta with 23.39%. The next is class Euglenophyta with 12.34%, Pyrrophyta with 10.93%, Chlorophyta with 8.35% while at the rear with 5.27% is class Xanthophyta, see figure 1. The most occurring species isthe diatom *Stauroneisacuta* with 5100 individuals followed by *Ophiocytium capitatum* of class Xanthophyta with 3200 individuals, following next with a population of 2700 is *Peridinium bipes* of class Pyrrophyta. However, the total abundance in decreasing order of dominance is Bacillariophya>Cyanophyta>Euglenophyta>Pyrrophyta >Chlorophyta>Xanthophyta.

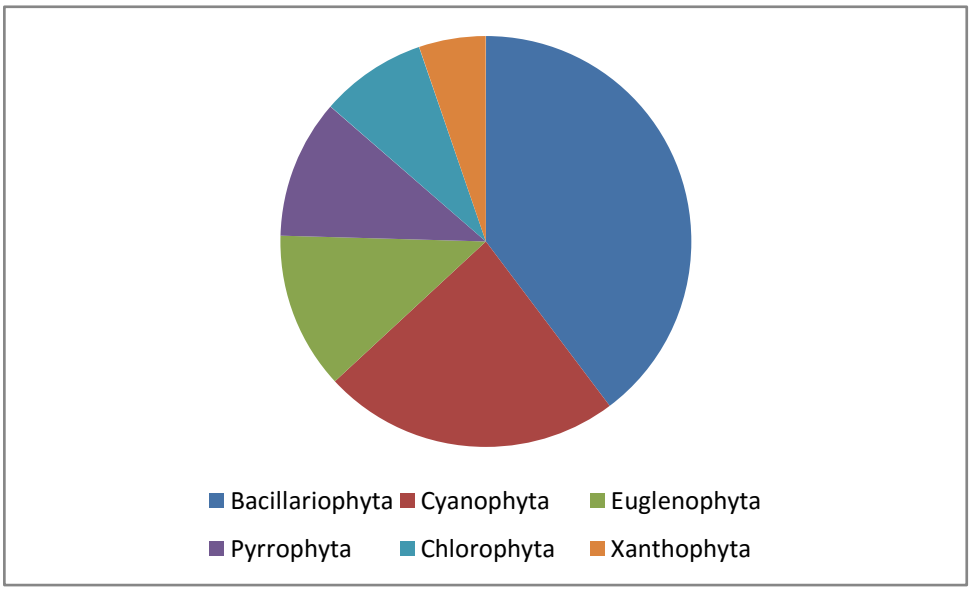

Figure 1: Periphyton Class Total Abundance in the study area

Table 1: Checklist of Periphytic algae in the study area

BACILLARIOPHYTA	*Achnantheshungarica*	*Aphanothececlathrata*
Melosiralistans	*Pinnulariabraunii*	*Oscilatorialacustris*
M. varians		*Spirulinasubtilssima*
M. distans	CHLOROPHYTA	*Raphidiopsiscurvata*
M. pusilla	*Closteriumdianae*	*Anabaenopsisraciborskii*
Cyclotellameneghiniana	*C. intermidium*	*Coelosphaeriumdubium*
C. comta	*C. moniliferum*	*Gomphosphaerialacustris*
C. operculata	*Carteriamultifilis*	*Gloeocapsaturgida*
Synedra ulna	*C. globosa*	*Gloeotrichiaechinulata*
S. vaucheriae	*Volvoxaureus*	
Cymatopleurasolea	*Gloeotaeniumloitlesbergerianum*	EUGLENOPHYTA
C.elliptica	*Asterococcuslimneticus*	*Euglena sanguinea*
Naviculacuspidata	*Schroederiasetigera*	*E. oxyuris*
N. radiosa	*Ankistrodesmusfalcatus*	*E. variabilis*
N. gracilis	*Radiococcusnimbatus*	*Trachelomonasdubia*
Stauroneisanceps	*Gonatozygonaculeatum*	*T. volvocina*
S. acuta	*Gymnozygamoniliformis*	*T. armata*
Gyrosigmaattenuatum	*Cosmariumgranatum*	*Colaciumcyclopicola*
Nitzschiadenticula	*Tetrallanthoslagerheimii*	
Surirellatenera	*Actidesmiumhookeri*	PYRROPHYTA
S. elegans	*Eudorinaelegans*	*Glenodiniumcinctum*
Cymbellahauckii	*Golenkiniaradiata*	*G. quadridens*
Epithemia zebra		*Gonyostonum semen*
Rhopalodiagibba	CYANOPHYTA	*Peridiniumbipes*
Flagilariacapucina	*Anabaena flos-aquae*	*Chiliomonasparamaecium*
Cocconeishustedtii	*Affinis*	
Cymatopleurasolea	*hassalii*	XANTHOPHYTA
Tabellariafenestrata	*Phormidiumvalderiae*	*Ophiocytiumcapitatum*
Pinnulariaappendiculata	*P. mucicola*	*O. cochleare*
Asterionellaformosa	*Microcystisaeriginosa*	*Tribonemaviride*
Stephanodiscushantzschii	*M. pulverea*	*Gloeobotryslimneticus*
	Nostocplanctonicum	

The 83 species of periphyton observed in this investigation is lower than the 149 and169 species gotten by Carrick and Steinman, (2001) and Chindah, (2004), respectively and even much lower than the 457 species recorded by Algarte, Siqueira, Murakami and Rodrigues (2009) in the Upper Parana River floodplain, but is comparable to the 77 species reported by Chindah, (1998) in the Upper Reaches of the New Calabar River and the 75 species recorded by Wokoma, Umesi and Edoghotu, (2010) in the Elechi Creek. It is however higher than the 30 species recorded by Onyema*et. al*.,(2010) in the shoreline of Lagosas well as the 54, 32 and 22 species reported by Wood, Kuluajek, Winton and Phillips (2011) in Rotoiti, Tikitapu and Okareka Lakes respectively. Several factors may account for the observed variation of periphyton species richness, such as differences in substrates from which periphyton was gotten, variations in nutrient concentration, habitat differences in water quality as well as changing conditions in some physicochemical parameters such as pH, Conductivity and Salinity gradient, (Chindah, 2004). The dominance of the periphytic algal community by the diatoms (Bacillariophyta) is a common feature in most Niger Delta water bodies. There was however a clear departure from this trend in the report of Algarte*et. al.,* (2009) where Zygnemaphyceae was observed as the most dominant class, followed by class Bacillariophyceae. Generally, the pattern of species richness in this study in decreasing order is (Bacillariophyta>Chlorophyta>Cyanophyta>Euglenophyta>Pyrrophyta>Xanthophyta) at variance with the trend reported by Chinda *et. al.* (2006) – (Bacillariophyta>Chlorophyta>Euglenophyta>Cyanophyta) and Wood *et. al.,* (2011) – Bacillariophyta >Cyanophyta>Chlorophyta>Euglenophyta>Xanthophyta = Chrysophyta.

The few species observed and even the low abundance of periphyton in this present investigation could be associated to the low nutrient content of the river. However, the order of specie dominance and community structure were slightly different.

The order of total abundance observed in this study showed Bacillariophyta as the most dominant class followed by Cyanophyta and Euglenophyta as the second and third most dominant classes. The high

number of Cyanophyta and Euglenophyta above Chlorophyta (with more species) indicates that the prevalent environmental conditions are favourable to them more than the others. This suggests that the study sites are contaminated with petroleum related wastes which may have enhanced their growth relative to the other classes. This is corroborated by the earlier reports of Wokoma et al., (2012) Chindah et. al., (2006) and Chindah, (1998) who concluded that cyanophyta thrives in oil contaminated environment.

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Stress Induced by Simulated Nitric and Sulphuric Acid Rain on the Nutrient Quality of *Cucurbita Moschata* (Duchesne Ex Poir)

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Abstract- Stress has been associated with several alterations in the physiological and biochemical composition of plants affecting their use in chemotherapy. Investigations were carried out to assess simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) stress on the medicinal quality of Cucurbita moschata. Results revealed that all the nutrients investigated had higher values at pH 6.0 than at pH 2.0, 3.0 and 4.0. The presence or absence of phytochemicals was not affected by SNAR and SSAR levels. Stress induced on ash, fat, fibre, carbohydrate and moisture caused significant (P=0.05) increase at pH 2.0 and 3.0 with decrease at pH 4.0 with the exception of leaf protein which showed increase at all levels of acidity compared to the control pH 6.0. Amino acids depicted a trend similar to that of protein. Potassium, sodium, calcium, magnesium, iron, copper, zinc, manganese and P were seriously affected by simulated acid rain stress. Stress caused an increase in vitamin A and the B-complex vitamins at pH concentration 2.0 with a decrease at concentration 3.0 and 4.0. While vitamin C was significantly reduced by simulated acid rain stress, vitamin K showed increase in content at all levels of acidity.

Keywords: *cucurbita moschata, medicinal quality, stress, simulated nitric and sulphuric acid rain.*

GJSFR-C Classification: *FOR Code: 069999*

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Keywords: cucurbita moschata, medicinal quality, stress, simulated nitric and sulphuric acid rain.

I. Introduction

Plants are constantly being exposed to both biotic and abiotic stress in their growing environment. Stress is induced by factors in the plant environment. Plant stress is defined as 'any unsuitable condition or substance that alters or hinders the growth and development of the plant'. Larcher referred to stress as 'changes in physiology' that occurs when species are exposed to unusual conditions that need not pose a threat to life but will cause an alarm response (Kranner *et al*., 2010). Abiotic stress is basically unavoidable and directly affects not only plant growth and productivity but also the chemical composition of crop plants. Prolong exposure of plants to acid rain is accompanied by oxidative stress as it is with other stressors in the plant environment. The negative effect of stress is often mediated by the oxidative damage initiated by reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Gill and Tuteja, 2010). Reactive oxygen species (ROS) production during environmental stress is one of the major causes for decreases in crop productivity, injury and death. Any situation in which cellular redox homoeostasis is impeded can lead to oxidative stress or reactive ROS generation (Asada, 1994). Stress is evident by the production of ROS.

Environmental stress is directly linked to increased accumulation of ROS. ROS production are increased by several environmental factors of stress, such as exposition to high levels of light, drought, heavy metals, salt concentrations, temperature extremes, air pollution, high irradiation (UV radiation), herbicides and pathogen attacks. Whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratão *et al*., 2005). The balance between production and removal of ROS may be altered by a number of biotic and abiotic factors, which may increase the intercellular levels of ROS (Apel and Hirt, 2004). When the level of ROS increase exceeds the cell defense mechanisms, the cell is in a state of oxidative stress (Sharma *et al*., 2012).

In plants, ROS act as secondary messengers in various key physiological processes and they induce oxidative damages under several environmental stress conditions when the balance between ROS production and elimination, necessary for normal cellular homeostasis, is disturbed. Reactive oxygen species are highly reactive and toxic by-product of aerobic pathways of metabolism, causing oxidative damages evidenced in the form of degradation of biomolecules such as pigments, proteins, nucleic acids, lipids, carbohydrates, and DNA, which result in plant cellular death (Gill and Tuteja, 2010; Kapoor *et al*., 2015; Singh *et al*., 2016). ROS play an important role of signaling in plants thus, controlling processes such as growth, development and response to biotic and abiotic environmental stimuli. The formation of reactive oxygen species has been described as being a result of several abiotic stresses, such as simulated acid rain Velikova *et al*. (2000), the application of herbicides (Song *et al*., 2007), pathogens (Mofunanya, 2015). Hippeli and Elstner (1996) observed that stress induced by acid rain components; SO_2 and NO_X resulted in the generation of reactive free oxygen radicals that causes inhibition of photosynthesis,

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enzyme degradation, damage to membrane, alterations in DNA, all leading to reduction in growth. Also observed was an increase in H_2O_2 level and MDA accumulation, an indication that oxidative stress has been induced in connection to membrane damage after a single spray of acid rain on bean plant (Velikova *et al*., 2000). Velikova *et al*. (2002) reported that stress induced by simulated acid rain treatment of pH 1.8 on *Phaseolus vulgaris* caused increase in H_2O_2 and MDA (malonyldialdehyde) which are end product of lipid peroxidation.

Cucurbita moschata (Duchesne ex Poir) is a member of the family Cucurbitaceae. Members of the family Cucurbitaceae are commonly called cucurbits which constitute a very large group with approproximately130 genera and 800 species. *Cucurbita moschata* is a squash native to Central America, northern and southern America. It is widely cultivated for its edible leaves and fruits in warm temperate and tropical areas. *Cucurbita moschata* is an indigenous vegetable in Nigeria. For a long time it has been an essential gradient in the diet of the rural communities as well as urban people in Nigeria. The leaves of *C. moschata* are sold in Nigerian markets as a vegetable and fruits used in various delicacies. It is a seasonal crop used traditionally as food for both human and animal (Doymaz, 2007). The young leaves and stems, flowers, young and ripe fruits of *C. moschata* are eaten as vegetable. The latter are also commonly used to prepare sweets and as fodder. The seeds are eaten whole, roasted or toasted and are ground into powder and used in different stews (Bates *et al*., 1990).

Nigeria is a country with a population of 198,000.000 people (NPC, 2018). Owing to this population increase, there is a corresponding rise in anthropogenic (human) activities which have given rise to acid rain precursor gases. Nigeria is the highest importer of used vehicles, with no guarantee on combustion efficiency. In accordance to the April 2006 edition of African Review of Business and Technology, London-based magazine survey, owing to power outages, Nigeria is also the largest importer of both petrol and diesel generators, estimated to the cost of $152 million per annum, with their attendant environmental and health implications. The acid rain precursors (SO_x and NO_x) that cause acid rain are transboundary pollutants. There are the same acid rain precursor gases anywhere in the World. In rural and urban cities in Nigeria, worn out tires are used to roast cow skin or hide, goat and poultry in abattoirs, which releases high quantities of SO_2 into the atmosphere. Oxides of nitrogen are (NO_x) are by-products of firing processes of extremely high temperature such as automobiles, utility plants and in chemical industries like fertilizer producing industries (Nduka *et al*., 2008). Due to these anthropogenic activities, cases of acidic rain are abound in Nigeria. Plants which are the primary producers are severely affected by acid rain.

Stress has been associated with several alterations in the physiological and biochemical composition of plants affecting their use in chemotherapy. Plant nutrients have been implicated in disease management. Plants have been in use for human welfare over the millennia in health promotion, drugs and for fragrance. The greater portions of the people in the rural areas of developing countries use plant-based traditional medicines for health care. *Cucurbita moschata* is a valued vegetable crop eaten by Nigerians. Considerable levels of acid rains have been determined in different parts of Nigeria on growth and yield of crop plants. Mofunanya and Egah (2017) studied the effect of simulated nitric and sulphuric acid rains on the nutrient content of *Amaranthus hybridus* in Nigeria. However, no literature exists in Nigeria on simulated acid rain effect on the nutrient quality of *C. moschata*. The present study seek to investigate stress induced individually by simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) on the medicinal quality of *Cucurbita moschata* leaf, stem and root.

II. Seeds and Materials Collection

Seeds of *Cucurbita moschata* were provided by a farmer in Akparabong, Ikom Local Government Area of Cross River State, Nigeria. Polyethylene bags of 16 mm in diameter were bought from the Ministry of Agriculture, Calabar. Nitric and sulphuric acids were purchased from a Scientific Shop all in Calabar, Nigeria. The Seeds were planted in poly bags, two seeds per bag. On germination, the seedlings were allowed to stay for a period of two weeks with regular application of distilled water. Simulated nutric and sulphuric acid rain application began after two weeks of germination. Planting and growth of *C. moschata* was carried out in the Department of Botany greenhouse, University of Calabar, Calabar, Nigeria (latitude 4.952'N and longitude 8.341'E) at 25±3°C to minimize pest infestation and uniformity of sunlight and water supply.

III. Simulated acid Rain Preparation and Application

Simulated nitric and sulphuric acid rain (SNAR and SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately prepared and a control pH of 6.0. Each pH concentration (HNO_3 or H_2SO_4) was prepared using different amount of acid. Thirty milliliters (30 ml) of each acid was used for pH concentrations of 2.0, 20.1 ml for pH 3.0 and 10.2 ml for pH 4.0 using a Deluxe pH meter and distilled water. Distilled water of pH 6.0 was used as control. A total of thirty five poly bags were used; fifteen bags for simulated nitric acid rain, fifteen bags for simulated sulphuric acid rain and five bags for the control that is, five bags for each pH concentration replicated three times were used in this study. Prior to

acid rain application, the poly bags were arranged in a completely randomized block design. Fifty milliliters (50 ml) of simulated acid rain concentration was applied at the initial growth period, this amount however, was increase with increase in growth. Spraying of simulated acid rain concentrations was done using a domestic hand-spraying unit on the plants as well as soil. Application was done at an interval of two days for thirteen weeks.

IV. Sample Preparation

At the end of thirteen weeks post simulated acid rain application, the whole plants of *C. moshata* grown at various pH concentrations were harvested and the plant parts (Leaf, Stem and Root) separated. The roots were washed in tape water to remove soil before sun-drying along with other plant parts for one week. The different parts were milled separately into powder in an electric mill (National Food Grinder, Model MK 308, Japan).The pulverized samples were used to analyzed the potential of simulated nitric and sulphuric acid rains stress on proximate, amino acids, vitamins and antinutrients contents of *Cucurbita moschata*.

V. Sample Analysis

Standard methods of plant samples analysis were employed. Crude protein was analyzed using the Kjeldahl method. Fat content was determined by AOAC (1995). Ash, fibre, carbohydrate and Vitamin contents of *C. moschata* were analyzed by the method of AOAC (2006). Amino acids content of samples was determined by the method of Speckman (1956; AOAC, 2006).

VI. Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science, Version 15.0 [SPSS, 2003]. Results were also expressed as percentage differences and differences between means were determined at 5% level of probability.

VII. Results

Table 1: Stress induced by simulated nitric and sulphuric acid rain on proximate nutrients of *Cucurbita moschata*

mg/100g								
Proximate nutrients	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Ash	Leaf	4.61± 0.01(18.2)	4.14±0.01(6.2)	3.95± 0.01(1.3)	3.27±0.01(16.2)	3.30 ±0.01(15.4)	3.40± 0.01(12.8)	3.90± 0.01
	Stem	4.70 ± 0.01(24.0)	3.99 ± 0.01(5.3)	3.81 ± 0.01(0.5)	4.84 ± 0.01(1.3)	3.95 ± 0.01(1.3)	3.79± 0.01(0.0)	3.79 ± 0.01
	Root	2.94± 0.01(37.4)	3.60± 0.01(23.4)	4.30± 0.016(8.5)	3.25± 0.01(30.9)	3.45± 0.01(26.6)	4.21± 0.01(10.4)	4.70± 0.01
Protein	Leaf	5.23 ± 0.01(54.7)	4.70±0.01(39.1)	4.10± 0.01(21.3)	5.16± 0.01(52.7)	4.54± 0.01(34.3)	3.78± 0.01(11.8)	3.38± 0.01
	Stem	6.01 ± 0.01(22.7)	4.18 ± 0.01(14.7)	3.84 ± 0.01(21.6)	5.80 ± 0.01(18.4)	5.28 ± 0.01(7.8)	4.14 ± 0.01(15.5)	4.90 ± 0.01
	Root	5.63 ± 0.01(28.5)	4.70± 0.01(7.3)	3.90± 0.01(11.0)	5.60± 0.01(27.9)	4.78± 0.01(9.1)	4.10± 0.01(6.4)	4.38± 0.01
Fat	Leaf	0.52 ± 0.12(36.6)	0.57 ± 0.02(30.5)	0.70 ± 0.15(14.6)	0.50 ± 0.02(39.0)	0.57 ± 0.01(30.5)	0.73 ± 0.01(11.0)	0.82 ± 0.02
	Stem	0.49 ± 0.01(38.8)	0.55 ± 0.01(31.3)	0.64 ± 0.01(20.0)	0. 51 ± 0.01(36.3)	0.62 ± 0.01(10.8)	0.75 ± 0.01(6.3)	0.80 ± 0.01
	Root	0.45 ± 0.01(22.4)	0.48 ± 0.01(17.2)	0.53 ± 0.02(8.6)	0.47 ± 0.02(19.0)	0.50 ± 0.01(13.8)	0.52 ± 0.01(10.3)	0.58 ± 0.01
Fibre	Leaf	5.30±0.01(51.9)	4.68± 0.01(34.1)	4.50 ± 0.01(28.9)	5.50±0.01(57.6)	4.75±0.01(36.1)	4.63±0.01(32.7)	3.49±0.01
	Stem	2.70±0.01(31.5)	2.85± 0.01(27.7)	3.25±0.01(17.5)	3.50±0.01(11.2)	3.54 ± 0.01(10.2)	3.90 ± 0.01(1.0)	3.94 ± 0.01
	Root	3.71± 0.01(28.4)	3.34± 0.01(15.6)	2.65± 0.01(8.3)	3.80± 0.01(31.5)	3.52± 0.01(21.8)	2.70± 0.01(6.6)	2.89± 0.01
Carbohydrate	Leaf	81.06± 0.01(4.2)	81.78 ±0.01(3.3)	81.78 ±0.01(2.6)	82.40± 0.01(2.6)	82.64± 0.01(2.3)	82.80± 0.01(2.1)	84.58± 0.01
	Stem	81.76 ± 0.01(3.3)	82.76 ± 0.01(2.2)	83.67 ± 0.01(1.1)	82.37 ± 0.01(2.6)	82.81± 0.01(2.1)	83.90 ± 0.01(0.8)	84.58 ± 0.01
	Root	85.10± 0.01(2.8)	84.22± 0.01(1.7)	83.90± 0.01(1.3)	84.51± 0.01(2.0)	84.72± 0.01(2.3)	84.80± 0.01(2.4)	82.82± 0.01

- Results are mean ± SE, n = 3 determinations, P=0.05, numbers in parenthesis are percentage difference
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and Simulated acid rain treated sample as a percentage of the control.

a) Stress induced by simulated nitric and sulphuric acid rain on proximate nutrients of Cucurbita moschata

Results analysis showed that proximate nutrients of *Cucurbita moschata* in the control plant parts were either higher or lower than the acid rain treated plant parts. Acid rain stress resulted in significant (P=0.05) increase or decrease in some proximate nutrients. Increase or decrease in proximate nutrients of *C. moschata* was in accordance with simulated acid rain concentration and amount in plant parts. Highest increase or decrease occurred at pH 2.0 and lowest at pH 4.0. Simulated sulphuric acid rain (SSAR) caused a decrease in ash content of leaf and root with an increase in stem content when compared to the control. At all acidity levels protein content of leaf increased significantly. Percentage increase of leaf protein showed values of 54.7%, 39.1%, 21.5% and 52.7%, 34.3%, 11.8%. Stem and root protein content was significantly increased at pH 2.0 and 3.0 with percentage increased values of 22.7%, 14.7% for SNAR and 18.1%, 7.8% for SSAR and 28.5%, 7.3%, SNAR and 27.9%, 9.1% for SSAR. A decrease in stem and root protein at pH 4.0 had percentage values 21.6%, 11.0% and 15.5%, 6.4% for SNAR and SSAR respectively. Decrease in leaf and root fat content with an increase in stem fat content due to acid rain stress was obtained. Simulated nitric acid rain (SNAR) stress led to significant increase in ash content of leaf and stem with decrease in ash content of root. SNAR and SSAR stress caused an increase in leaf fibre content at pH 2.0, 3.0 and 4.0 with percentage increase values of 51.9%, 34.1%, 24.6% and 57.6%, 36.5%, 32.7% respectively. Fibre in stem showed decrease in content at all levels of acidity with percentage decrease in values of 31.5%, 27.7%, 17.5% and 11.2%, 10.2% and 1.0% respectively for SNAR and SSAR. However, root fibre content depicted increase at pH 2.0 and 3.0 percentage increase values of 28.4%, 15.6% and 31.5%, 21.8% with decrease at pH 4.0 of 8.3% and 6.6% for both simulated acid rain. An increase in root fibre content of 28.4%, 15.6% and 31.5%, 21.8% at pH 2.0 and 3.0 and a decrease of 8.3% and 6.6% at pH 4.0 for SNAR and SSAR induced stress. SNAR and SSAR stress induced increase in root carbohydrate content compared to control (Table 1).

Table 2: Stress induced by simulated nitric and sulphuric acid rain on amino acids content of *Cucurbita moschata*

g/16 N								
Amino acid	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Histidine	Leaf	4.60±0.01(36.1)	4.35±001 (28.7)	4.10±0.01 (21.3)	4.45±0.01 (31.7)	4.20± 0.01 (24.3)	3.78±0.01(11.8)	3.38± 0.01
	Stem	5.50 ± 0.01(14.3)	5.29±0.01 (10.0)	4.36± 0.01 (9.4)	5.35 ± 0.01 (11.2)	5.19 ± 0.01 (7.9)	4.29 ± 0.01 (10.8)	4.81 ± 0.01
	Root	4.80± 0.01 (12.1)	4.50± 0.01 (5.1)	3.83± 0.01 (10.5)	4.60± 0.01 (7.5)	4.50± 0.01 (5.1)	3.67± 0.01 (14.3)	4.28± 0.01
Lysine	Leaf	6.66 ± 0.1 (28.8)	5.75±0.06 (10.6)	4.75 ± 0.03 (8.1)	6.18 ± 0.02 (19.5)	5.51 ± 0.01 (6.6)	5.20 ±0.02 (0.6)	5.17 ± 0.1
	Stem	4.70 ± 0.02 (20.2)	4.50±0.02 (15.1)	3.70 ± 0.03 (5.4)	4.52 ± 0.3 (15.6)	4.39 ± 0.01 (12.3)	3.57 ±0.01 (8.7)	3.91 ± 0.02
	Root	3.62 ± 0.03 (28.4)	3.48±0.02 (23.4)	2.68 ± 0.02 (6.0)	3.37 ± 0.02 (19.5)	3.23 ± 0.01 (14.5)	2.60 ± 0.01 (7.8)	2.82 ± 0.02
Arginine	Leaf	7.94 ± 0.01 (13.3)	7.45 ±0.01 (6.3)	7.19 ± 0.1 (2.6)	7.63± 0.01 (8.8)	7.41 ± 0.01 (5.7)	7.22 ± 0.01 (3.0)	7.01 ± 0.01
	Stem	7.02 ± 0.1 (10.2)	6.78 ± 0.01 (6.4)	6.10 ± 0.01 (4.2)	6.92 ± 0.02 (8.6)	6.62 ± 0.01 (3.9)	6.10 ± 0.01 (4.2)	6.37 ± 0.02
	Root	3.47 ± 0.01 (23.5)	3.05 ± 0.1 (8.5)	2.65 ± 0.01 (5.7)	3.35 ± 0.01 (19.2)	3.00 ± 0.01 (6.8)	2.49 ± 0.01 (11.4)	2.81 ± 0.02
Aspartic acid	Leaf	13.70± 0.02 (42.6)	11.55±0.02(20.2)	9.83 ± 0.02 (2.3)	13.47± 0.06 (40.2)	11.40± 0.01 (18.6)	9.75 ± 0.01 (1.5)	9.61 ± 0.06
	Stem	12.02± 0.01 (46.9)	10.64±0.01(30.1)	9.80 ±0.01 (19.8)	12.00± 0.01 (46.7)	10.46± 0.06 (27.9)	9.70 ± 0.01 (18.6)	8.18 ± 0.06
	Root	7.26 ± 0.01 (12.6)	7.03 ± 0.01 (9.0)	6.89 ±0.01 (6.8)	7.20 ± 0.01 (11.6)	6.98 ±0.01 (8.2)	6.75 ± 0.01 (4.7)	6.45 ± 0.03
Threonine	Leaf	4.96 ± 0.01 (16.4)	4.50 ±0.01 (5.6)	4.32 ± 0.01 (1.4)	4.71 ± 0.02 (10.6)	4.60 ± 0.01 (8.0)	4.30 ± 0.01 (0.9)	4.26 ± 0.1
	Stem	3.83 ± 0.02 (19.7)	3.79 ±0.01 (18.4)	3.06 ± 0.01 (4.4)	3.72 ± 0.01 (16.3)	3.40 ± 0.1 (9.4)	3.12 ± 0.01 (2.5)	3.20 ± 0.01
	Root	3.51 ± 0.06 (49.4)	2.86± 0.01 (21.7)	2.11 ± 0.02 (10.2)	3.32 ± 0.01 (41.3)	2.63 ± 0.02 (11.9)	2.20 ± 0.1 (6.4)	2.35 ± 0.01
Serine	Leaf	3.01± 0.01 (15.3)	2.80 ± 0.01 (7.3)	2.71 ± 0.01 (7.3)	3.28 ± 0.01 (25.7)	2.95± 0.1 (13.0)	2.80 ± 0.1 (7.3)	2.61 ± 0.03
	Stem	2.76 ± 0.2 (10.4)	2.60 ± 0.1 (4.0)	2.02 ± 0.01 (19.2)	2.85 ± 0.1 (14.0)	2.69 ± 0.01 (7.6)	1.98 ± 0.01 (20.8)	2.50 ± 0.01
	Root	1.78 ± 0.03 (26.2)	1.50 ± 0.03 (6.4)	1.20 ± 0.1 (14.9)	1.85 ± 0.01 (31.2)	1.55 ± 0.01 (9.9)	1.16 ± 0.1 (17.7)	1.41 ± 0.01
Glutamic acid	Leaf	13.73± 0.02 (12.6)	12.40± 0.01 (9.9)	12.31 ± 0.02 (1.0)	13.71± 0.02 (12.5)	13.24± 0.02 (8.6)	12.21± 0.02 (0.2)	12.19 ± 0.1
	Stem	13.48± 0.02 (10.3)	12.79± 0.01 (6.5)	12.45 ± 0.02 (4.0)	13.42 ± 0.02 (7.7)	12.64 ± 0.02 (5.6)	12.39 ± 0.02 (4.8)	12.10 ± 0.1
	Root	8.65 ± 0.01 (12.8)	8.17 ± 0.01 (6.5)	7.83 ± 0.1 (2.1)	8.45 ± 0.2 (10.2)	8.10 ± 0.02 (5.6)	7.78 ± 0.01(1.4)	7.67 ± 0.2
Proline	Leaf	3.93 ± 0.1 (38.9)	3.67 ± 0.1 (29.7)	2.90 ± 0.1 (2.5)	3.69 ± 0.1 (30.4)	3.46 ± 0.1 (22.3)	2.86 ±0.2 (1.1)	2.83 ± 0.06
	Stem	3.67 ± 0.1 (26.6)	2.95 ± 0.01 (9.7)	2.50 ± 0.1 (7.1)	3.40 ± 0.1 (26.4)	2.85 ± 0.1 (5.9)	2.50 ±0.1 (7.1)	2.69 ± 0.03
	Root	1.95 ± 0.1 (54.5)	1.71 ± 0.1 (34.6)	1.19 ± 0.1 (6.3)	1.80 ± 0.1 (41.7)	1.65 ± 0.1 (29.9)	1.11± 0.01 (7.9)	1.27 ± 0.03
Glysine	Leaf	5.83 ± 0.01 (23.3)	5.27± 0.01 (11.4)	4.88 ± 0.01 (3.2)	5.58 ± 0.01 (18.0)	5.07 ± 0.01 (7.2)	4.83 ± 0.01 (7.2)	4.73 ± 0.02
	Stem	2.95 ± 0.01 (28.3)	2.51 ± 0.01 (9.1)	1.97 ± 0.01 (14.3)	2.95 ± 0.01 (28.3)	2.46 ± 0.01 (7.0)	2.01 ± 0.01 (12.6)	2.30 ± 0.02
	Root	1.60 ± 0.01 (33.3)	1.45±0.01 (20.8)	0.99 ± 0.01 (17.5)	1.60 ±0.01 (33.3)	1.41 ± 0.1 (17.5)	1.05 ± 0.1 (12.5)	1.20 ± 0.02
Alanine	Leaf	4.30 ± 0.01 (22.9)	3.97 ± 0.1 (13.4)	3.62 ± 0.01 (3.4)	4.53 ± 0.01 (29.4)	3.99 ± 0.01 (14.0)	3.60 ± 0.01 (2.9)	3.50 ± 0.1
	Stem	3.79 ± 0.02 (16.3)	3.61 ± 0.1 (10.7)	2.95 ± 0.02 (9.5)	3.71 ± 0.01 (13.8)	3.50 ± 0.01 (7.4)	3.00 ± 0.01 (8.0)	3.26 ± 0.1
	Root	1.57 ± 0.02 (53.7)	1.25± 0.02 (22.5)	0.90 ± 0.02 (12.8)	1.54 ± 0.2 (51.0)	1.15 ± 0.02 (12.7)	0.94 ± 0.02 (7.8)	1.02 ± 0.1

Cysteine	Leaf	0.96 ± 0.1 (14.3)	0.89 ± 0.1 (6.0)	0.86 ± 0.1 (2.4)	0.95 ± 0.1 (13.1)	0.87 ± 0.1 (3.6)	0.86 ± 0.1 (2.4)	0.84 ± 0.01
	Stem	0.90 ± 0.1 (12.2)	0.85 ± 0.1 (6.3)	0.76 ± 0.1 (5.0)	0.90 ± 0.1 (12.2)	0.84 ± 0.1 (5.0)	0.74 ± 0.1 (7.5)	0.80 ± 0.02
	Root	0.60 ± 0.1 (20.0)	0.54 ± 0.1 (8.0)	0.45 ± 0.1 (16.0)	0.57 ± 0.1 (14.0)	0.55 ± 0.1 (8.2)	0.45 ± 0.1 (10.0)	0.50 ± 0.1
Valine	Leaf	4.88 ± 0.2 (12.2)	4.71 ± 0.03 (8.3)	4.72 ± 0.0 (3.9)	4.61± 0.1 (8.5)	4.20 ± 0.1 (6.0)	3.99 ± 0.1 (8.3)	4.35 ± 0.1
	Stem	4.96 ± 0.2 (12.5)	4.70 ± 0.1 (6.6)	3.79 ± 0.06 (14.1)	4.87 ± 0.1 (10.4)	4.65 ± 0.1 (5.4)	3.81 ± 0.1 (13.6)	4.41 ± 0.2
	Root	3.20 ± 0.1 (42.9)	3.03 ± 0.2 (35.3)	1.83 ± 0.2 (18.3)	3.11 ± 0.1 (38.8)	2.78 ± 0.1 24.1()	1.91 ± 0.1 (14.7)	2.24 ± 0.2
Methionine	Leaf	1.65 ± 0.02 (24.1)	1.51± 0.02 (13.5)	1.38 ± 0.02 (3.8)	1.79 ± 0.03 (34.6)	1.62 ± 0.2 (21.8)	1.42 ± 0.2 (6.8)	1.33 ± 0.01
	Stem	1.59 ± 0.02 (19.0)	1.47± 0.01 (16.7)	1.10 ± 0.02 (12.7)	1.65 ± 0.2 (31.0)	1.51± 0.2 (19.8)	1.10 ± 0.2 (12.7)	1.26 ± 0.01
	Root	1.16 ± 0.01 (39.8)	0.97±0.01 (16.9)	0.68 ± 0.01 (18.1)	1.22 ± 0.2 (47.0)	1.02 ± 0.2 (22.9)	0.70 ± 0.1 (15.7)	0.83 ± 0.01
Isoleucine	Leaf	4.71 ± 0.01 (6.3)	4.55 ± 0.3 (2.7)	4.48 ± 0.1 (1.1)	4.82 ± 0.02 (8.8)	4.60 ± 0.01 (3.8)	4.50 ± 0.01 (1.6)	4.43 ± 0.2
	Stem	5.20 ± 0.2 (8.3)	4.95 ± 0.2 (3.1)	4.66 ± 0.1 (2.9)	5.47 ± 0.01 (6.9)	5.03 ± 0.01 (4.8)	4.71 ± 0.1 (2.1)	4.80 ± 0.1
	Root	3.81 ± 0.2 (17.2)	3.64 ± 0.2 (12.0)	2.77 ± 0.1 (14.8)	3.86 ± 0.1 (18.8)	3.68 ± 0.01 (13.2)	2.80 ± 0.2 (13.8)	3.25 ± 0.1
Leucine	Leaf	7.71 ± 0.2 (9.5)	7.52 ± 0.01 (6.8)	7.11 ± 0.02 (1.0)	7.83 ± 0.1 (11.2)	7.61± 0.02 (8.1)	7.23 ± 0.1 (2.7)	7.04 ± 0.1
	Stem	8.80 ± 0.1 (5.6)	8.63 ± 0.1 (3.6)	8.19 ± 0.02 (1.7)	8.87 ± 0.1 (6.5)	8.69 ± 0.1 (4.3)	8.24 ± 0.2 (1.1)	8.33 ± 0.1
	Root	4.76 ± 0.1 (13.1)	4.54 ± 0.01 (7.8)	4.02 ± 0.02 (4.5)	4.85 ± 0.2 (15.2)	4.60 ± 0.2 (9.3)	4.00 ± 0.1 (5.0)	4.21 ± 0.2
Tyrosine	Leaf	3.87 ± 0.02 (12.2)	3.70 ± 0.2 (7.2)	3.55 ±0.03 (2.9)	3.76 ± 0.01 (9.0)	3.60 ± 0.01 (4.3)	3.49 ± 0.3 (1.2)	3.45 ± 0.13
	Stem	3.92 ± 0.01 (8.9)	3.75 ± 0.03 (4.2)	3.40 ± 0.02 (5.6)	3.85 ± 0.2 (7.0)	3.70 ± 0.1 (2.8)	3.37 ± 0.2 (6.4)	3.60 ± 0.02
	Root	2.95 ± 0.01 (18.0)	2.70 ± 0.01 (8.0)	2.36 ± 0.01 (5.6)	2.80 ± 0.02 (12.0)	2.67 ± 0.01 (6.8)	2.33 ± 0.01 (6.8)	2.50 ± 0.02
Phenylalanine	Leaf	4.56 ± 0.2 (16.6)	4.27± 0.2 (9.2)	3.97 ± 0.2 (1.5)	4.42 ± 0.02 (13.0)	4.18 ± 0.01 (6.9)	3.96 ±0.2 (1.3)	3.91 ± 0.3
	Stem	4.20 ± 0.2 (9.7)	4.01±0.1 (4.7)	3.69 ± 0.1 (3.6)	4.20 ± 0.02 (9.7)	3.98 ± 0.2 (3.9)	3.61 ± 0.01 (5.7)	3.83 ± 0.02
	Root	4.50 ± 0.1 (18.4)	4.11 ± 0.2 (8.1)	3.55 ± 0.2 (6.6)	4.19 ± 0.01 (10.3)	4.07 ± 0.2 (7.1)	3.55 ± 0.01 (6.6)	3.80 ± 0.1

- Results are mean ± SE, n = 3 determinations, P=0.05, numbers in parenthesis are percentage difference
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and simulated acid rain treated sample as a percentage of the control.

b) Stress induced by simulated nitric and sulphuric acid rain on amino acids content of Cucurbita moschata

Simulated nitric and sulphuric acid rain stress on amino acids depicted a trend of significant (P=0.05) increase in leaf samples at all pH concentrations, an increase in stem and root amino acids content at pH 2.0 and 3.0 with a decrease in content at pH 4.0 similar to protein. The quantities of amino acids in leaf, stem and root varied in control as well as in simulated acid rain treated parts. Increase in amino acids of *C. moschata* varied according to plant parts and level of acidity. Highest percentage increase in amino acids occurred at pH 2.0, followed by pH 3.0 and the lowest increase at pH 4.0 when compared to the control pH of 6.0. This trend was observed in all amino acids with the exceptions of aspartic acid, glutamic acids, proline and alanine which showed increased amounts in leaf, stem and root of simulated acid rain treated plants at all levels of acidity compared to the control plants. Percentage increases for leaf histidine (essential amino acid) at pH 2.0, 3.0 and 4.0 were 36.1%, 28.7%, 21.3% for SNAR and 31.7%, 24.3%, 11.8% for SSAR respectively. Percentage increase values for SNAR at pH 2.0, 3.0 were 14.3%, 10.0% for stem, 12.6%, 5.1% for root samples and percentage values decrease at pH 4.0 of 9.4% (stem) and 10.5% (root). Increase in leaf content of another essential amino acid threonine had values of 16.4%, 5.6%, 1.4% and 10.6%, 8.0%, 0.9% at pH 2.0, 3.0 and 4.0 respectively for SNAR and SSAR stress. Increase in threonine content at pH 2.0 and 3.0 in stem and root had percentage increase in values of 19.7%, 18.4% and 49.4%, 21.7% with percentage decrease in threonine content at pH 4.0 of 4.4% and 10.2% for SNAR stress. Associated values for SSAR were 16.3%, 9.4% and 41.3%, 11.9% with percentage decrease at pH 4.0 of 2.5% and 6.4%. SSAR stress led to higher percentage increases in methionine with values of 34.6%, 21.8% compared to values of 24.1%, 13.5%, for SNAR. Stem methionine had higher percentage increase values of 31.0%, 19.8% for SSAR as against values of 19.0%, 16.7% for SNAR. Methionine in root had higher percentage values of 47.0%, 22.9% for SSAR compared to values of 39.8%, 16.7% for SNAR. Non essential amino acids also depicted a similar trend of higher values according to acid levels for leaf in SNAR and SSAR treated, while stem and root had increase in content values at pH 2.0 and 3.0 and decrease in content of amino acids at pH 4.0. Percentage increase in leaf cysteine (non essential amino acid) content values induced by SNAR and SSAR at pH 2.0, 3.0 and 4.0 were 14.3%, 6.0%, 2.4% and13.1%, 3.6%, 2.4% respectively. Cysteine in stem and root had increase in content values of 12.2%, 2.5% and 20.0%, 8.0% at pH 3.0 and pH 4.0 with decrease in content values of 8.8% and 16.0% at pH 4.0 for SNAR stress. While SSAR stress caused percentage increase in content values of 12.2%,

1.3% and 14.0%, 10.0% with a decrease in content values of 12.5% and 12.0% for stem and root at pH 4.0. Corresponding values of 11.2%, 7.9% and 7.5%, 5.1% for stem and root for SSAR treated samples at pH 2.0 and 3.0 and percentage decrease in content values at pH 4.0 of 10.8% and 14.3%. Simulated acid rain stress induced significant (P=0.05) increase in aspartic acid (non essential amino acid) content in leaf, stem and root at all the pH tested. Percentage increase in aspartic acid had values of 42.6%, 20.2%, 2.3% for leaf samples, 46.9%, 30.1%, 19.8% for stem samples and 12.6%, 9.0%, 6.8% for root samples in SNAR at pH 2.0, 3.0 and 4.0. While values of 40.2%, 18.6%, 1.5% obtained for leaf, 46.7%, 27.9%, 18.6% for stem and 11.6%, 8.2%, 4.7% for root at pH 2.0, 3.0 and 4.0 respectively for SSAR stress. Values for other amino acids are shown in Table 2.

Table 3: Stress induced by simulated nitric and sulphuric acid rain on vitamins of *Cucurbita moschata*

Vitamins	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Vitamin A (β-carotene) (μg/dl)	Leaf	198.16±0.01(76.5)	184.56±0.01(64.4)	100.63±0.01(10.4)	191.71±0.01(70.4)	75.29±0.01(32.9)	90.81±0.01(19.1)	112.27±0.01
	Stem	260.22±0.01(33.4)	113.65±0.01(41.7)	146.23±0.01(25.0)	252.30± 0.01(29.4)	97.89 ± 0.01(49.8)	145.26±0.01(25.5)	195.04 ±0.01
	Root	259.73±0.01(40.6)	120.35±0.01(34.8)	151.47±0.01(18.0)	248.51± 0.01(34.6)	100.10±0.01(45.8)	138.49±0.01(25.0)	184.68 ±0.01
Vitamin B_1 (Thiamine) (mg/100 g)	Leaf	0.089 ± 0.01(78.0)	0.026 ± 0.01(48.0)	0.039 ± 0.01(22.0)	0.097 ± 0.01(94.0)	0.032 ± 0.01(36.0)	0.040 ± 0.01(20.0)	0.050 ± 0.01
	Stem	0.091 ± 0.01(33.8)	0.032 ± 0.01(52.9)	0.045 ± 0.01(33.8)	0.097 ± 0.01(42.6)	0.040 ± 0.01(41.2)	0.051± 0.01(25.0)	0.068 ± 0.01
	Root	0.057 ± 0.01(62.9)	0.020± 0.01(42.9)	0.027 ± 0.01(22.9)	0.060 ± 0.01(71.4)	0.023 ± 0.02(34.3)	0.030 ± 0.01(14.3)	0.035 ± 0.01
Vitamin B_2 (Riboflavin) (mg/100 g)	Leaf	0.957±0.01(529.6)	0.125 ± 0.01(17.7)	0.139 ± 0.01(8.6)	0.207 ± 0.01(36.2)	0.124 ± 0.01(18.4)	0.142 ± 0.01(6.6)	0.152 ± 0.01
	Stem	0.236 ± 0.01(38.0)	0.130 ± 0.01(24.0)	0.148 ± 0.01(13.5)	0.255 ± 0.01(49.1)	0.143 ± 0.01(16.4)	0.150 ± 0.01(12.3)	0.171± 0.01
	Root	0.181± 0.01(26.6)	0.123 ± 0.01(14.0)	0.130 ± 0.01(9.1)	0.190 ± 0.01(32.9)	0.131± 0.01(9.1)	0.135 ± 0.01(5.6)	0.143 ± 0.01
Vitamin B_3 (Niacin) (mg/100 g)	Leaf	0.812 ± 0.01(64.7)	0.261± 0.01(47.1)	0.331 ± 0.01(32.9)	0.985 ± 0.01(99.8)	0.299 ± 0.01(39.4)	0.401 ± 0.01(18.7)	0.493 ± 0.01
	Stem	0.992 ± 0.01(58.7)	0.314 ± 0.01(49.8)	0.562 ± 0.01(10.1)	0.999 ± 0.01(59.8)	0.437± 0.01(30.1)	0.570 ± 0.01(8.8)	0.625 ± 0.01
	Root	0.774 ± 0.01(58.9)	0.201± 0.01(58.7)	0.348 ± 0.01(28.5)	0.803 ± 0.01(64.9)	0.236 ± 0.01(51.5)	0.350 ± 0.01(28.1)	0.487 ± 0.01
Vitamin B_5 (Pantothenic acid) (mg/100 g)	Leaf	0.198 ± 0.01(18.6)	0.134 ± 0.01(19.8)	0.147 ± 0.01(12.0)	0.202 ± 0.01(21.0)	0.142 ± 0.01(15.0)	0.155 ± 0.01(7.2)	0.167± 0.01
	Stem	0.196 ± 0.01(17.4)	0.133 ± 0.01(20.4)	0.147 ± 0.01(12.0)	0.201± 0.01(20.4)	0.142 ± 0.01(15.0)	0.156 ± 0.01(6.6)	0.167 ± 0.01
	Root	0.195 ± 0.01(21.9)	0.128 ± 0.01(20.0)	0.131± 0.01(18.1)	0.211 ± 0.01(31.9)	0.141 ± 0.01(11.9)	0.151± 0.01(5.6)	0.160 ± 0.01
Vitamin B_6 (Pyridoxine) (mg/100 g)	Leaf	0.301± 0.01(17.6)	0.220 ± 0.01(14.1)	0.231± 0.01(9.8)	0.321 ± 0.01(25.4)	0.247± 0.01(3.5)	0.250 ± 0.01(2.3)	0.256 ± 0.01
	Stem	0.310 ± 0.01(19.2)	0.224 ± 0.01(13.8)	0.234 ± 0.01(10.0)	0.352 ± 0.01(35.4)	0.249 ± 0.01(4.2)	0.256 ± 0.01(1.5)	0.260 ± 0.01
	Root	0.183 ± 0.01(66.4)	0.081± 0.01(26.4)	0.099 ± 0.01(10.0)	0.198 ± 0.01(80.0)	0.088 ± 0.01(70.0)	0.101± 0.01(8.2)	0.110 ± 0.01
Vitamin B_9 (Folate or Folic acid) (μg/dl)	Leaf	37.06±0.01(12.2)	27.12 ± 0.01(17.9)	30.53 ± 0.01(7.6)	38.09 ± 0.01(15.3)	27.21±0.01(17.6)	31.31± 0.01(5.3)	33.04 ± 0.01
	Stem	37.05±0.01(12.2)	27.10 ± 0.01(17.9)	30.53 ± 0.01(7.5)	37.41± 0.01(13.3)	27.40±0.1(17.06.4)	30.89 ±0.01(6.4)	33.01± 0.01
	Root	11.52 ±0.01(40.7)	6.75 ± 0.01(17.6)	7.82 ± 0.01(4.5)	11.84 ± 0.01(44.6)	6.92 ± 0.01(15.5)	8.00 ± 0.01(2.3)	8.19 ± 0.01
Vitamin K (μg/dl)	Leaf	6.23 ± 0.01(35.7)	5.11 ± 0.01(11.3)	4.63 ± 0.01(0.9)	6.79 ± 0.01(47.9)	5.43 ± 0.01(18.3)	4.73 ± 0.01(3.1)	4.59 ± 0.01
	Stem	6.51± 0.01(44.7)	5.40 ± 0.01(20.0)	4.65 ± 0.01(15.0)	6.66 ± 0.01(48.0)	5.42 ± 0.01(20.4)	4.70 ± 0.01(4.4)	4.50 ± 0.01
	Root	3.90 ± 0.01(63.2)	3.40 ± 0.01(42.3)	2.48 ± 0.01(3.8)	3.98 ± 0.01(66.5)	3.42 ± 0.01(30.1)	2.61 ± 0.01(9.2)	2.39 ± 0.01
Vitamin C (Ascorbic acid) mg/100 g	Leaf	16.03 ± 0.01(18.7)	16.62 ± 0.01(15.7)	17.32 ± 0.01(12.2)	15.77 ± 0.01(20.0)	16.46 ± 0.01(16.5)	17.02 ± 0.01(13.7)	19.72 ± 0.01
	Stem	15.00 ± 0.01(15.0)	16.11 ± 0.01(8.8)	17.20 ± 0.01(2.5)	15.00 ± 0.01(15.0)	16.01± 0.01(9.3)	17.05 ± 0.01(3.3)	17.64 ± 0.01
	Root	9.84 ± 0.01(33.8)	11.56 ± 0.01(22.3)	13.95 ± 0.01(6.2)	9.51 ± 0.01(36.0)	11.21± 0.01(24.6)	13.38 ± 0.01(10.0)	14.87 ± 0.01

- Results are mean ± SE, n = 3 determinations, P=0.05
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and Simulated acid rain treated sample as a percentage of the control.

c) *Stress induced by simulated nitric and sulphuric acid rain on vitamins of Cucurbita moschata*

Results of increase and decrease in vitamins caused by simulated nitric and sulphuric acid rain are presented in Table 3. Screening of leaf, stem and root samples of *C. moschata* revealed the presence of vitamin A, C, K and the B-complex vitamins. The concentration of vitamins in plant parts varied in control as well as in simulated acid rain plant parts. Vitamins A, B_1, B_2 and B_3 in the control were higher in stem than in

leaf and stem, vitamin K and C were more in leaf than in stem and root. Vitamin B_9 did not differ significantly in leaf and stem while vitamin B_5 did not differ statistically in all plant parts. A general trend of highest increase in vitamins at pH 2.0, highest reduction at pH 3.0 and lowest reduction at pH 4.0 in vitamin content of leaf, stem and root of *C. moschata* was observed. Results revealed an increase in vitamin A and the B-complex vitamins at pH concentration 2.0 with a decrease in content at concentration 3.0 and 4.0. However, vitamin C was significantly (P=0.05) reduced by simulated acids rain stress while vitamin K showed increase in content at all levels of acidity. Highest reductions and increases in vitamins occurred at pH 2.0, followed by pH 3.0 and lowest at pH 4.0. Results revealed that simulated nitric acid rain caused more increases and decreases in all the B-complex vitamins and vitamin C while simulated sulphuric acid rain caused more increases and decreases in vitamins A and K. Results of an initial increase in vitamin A content at pH 2.0 had values of 198.16 ± 0.01, 260.22 ± 0.01 and 259.73 ± 0.01 for leaf, stem and root in simulated nitric acid rain, with decrease in content at pH 3.0 and 4.0 with values of 84.56 ± 0.01, 113.65 ±0.01, 120.35 ± 0.01 and 100.63 ± 0.01,146.23 ± 0.01, 151.47 ± 0.01 μg/dl respectively. Corresponding increase in values for simulated sulphuric acid rain of 191.71± 0.01, 252.30 ± 0.01, 248.51± 0.01 μg/dl and decrease in values of 75.29 ± 0.01, 97.89 ± 0.01, 100.10 ± 0.01 (pH 3.0) and 90.81 ± 0.01, 145.26 ±0.01, 138.49 ± 0.01 μg/dl (pH 4.0) for leaf, stem and root compared to control values of 112.27 ± 0.01, 195.04 ± 0.01, 184.68 ± 0.01 μg/dl respectively. Increase in vitamin B_1 content in leaf at pH 2.0 had value of 0.089 ± 0.01 with decrease of 0.026 ± 0.01and 0.039 ± 0.01 for pH 3.0 and 4.0 in simulated nitric acid rain stress. Simulated sulphuric acid rain had increase in value of 0.097 ± 0.01 (pH 2.0) with decrease in content of 0.032 ± 0.01 and 0.040 ± 0.01 mg/100 g. Increase and decrease in vitamin B_1 in stem for SNAR and SSAR had values of 0.091 ± 0.01, 0.032 ± 0.01, 0.045 ± 0.01 and 0.097 ± 0.01, 0.040 ± 0.01, 0.051± 0.01 mg/100 g compared to pH 6.0 value of 0.068 ± 0.01 mg/100 g. Vitamin B_1 in root had values of 0.057 ± 0.01, 0.020 ± 0.01, 0.027 ± 0.01 (SNAR) and 0.060 ± 0.01, 0.023 ± 0.02, 0.030 ± 0.01 mg/100 g (SSAR) respectively in comparison to 0.035 ± 0.01 mg/100 g for the control. Vitamin K content of leaf, stem and root of *C. moschata* was significantly (P=0.05) increased by SNAR and SSAR stress when compared to the control. Increase in mean values for vitamin K of leaf at pH concentrations 2.0, 3.0 and 4.0 were 6.79 ± 0.01, 5.43 ± 0.01, 4.73 ± 0.01 and 6.23 ± 0.01, 5.11 ± 0.01, 4.60 ± 0.01 respectively for SNAR and SSAR in comparison to control value of 4.57 ± 0.01 μg/dl. Increase in stem and root K and vitamins B_2, B_3, B_5, B_6, B_9 and C due to simulated nitric and sulphuric acid rain stress are as shown in Table 3.

VIII. Discussion

All forms of disturbances that causes alteration in the morphology, physiology and biochemistry of the plant is stress. The nutrients quality of *C. moschata* with great medicinal potentials were seriously affected by simulated nitric and sulphuric acid rain stress as revealed in this study. Results suggests that the leaf, stem and root of this *C. moschata* is a rich source of ash, protein, fat, fibre and carbohydrate (proximate nutrients), essential amino acids; histidine, lysine, methionine, leucine, isoleucine, threonine, phenylalanine, valine, tryptophane and non essential amino acids; arginine, aspartic acid, serine, glutamic acid, proline, glycine, cysteine, tyrosine, alanine and vitamins (Vit. A, B_1, B_2, B_3, B_5, B_6, B_9, C and K). From the nutritional point of view high consumption of *C. moschata* has various health benefits. The tea obtain from the leaves are used against stomach inflammation and jaundice. Presently, *C. moschata* is consumed as a vegetable and medicine in many countries like Yugoslavia, Argentina, India, Mexico, the United States, Brazil and Korea and Nigeria. In Korea, the plant is used traditionally to relieve edema during pregnancy and after delivery.

Acid rain has been reported to induce changes in the cellular biochemistry and physiology of the whole plant (Velikova *et al*., 2000). Increase and decrease in contents of phytonutrients occurred at different pH levels and varied according to acidity levels of simulated acid rain. SNAR and SSAR stress caused increase in leaf protein and amino acids content at all levels of acidity with the exceptions of glutamic acid, aspartic acid and proline which significantly increased in all plant parts. Stress is associated with increase or accumulation of compounds in plants. Increase in proline orchestrated by SNAR and SSAR in this study is in line with previous reports (Hare and Cress, 1997; Mofunanya et al. 2009) of proline accumulation in response to a wide range of biotic and abiotic stresses. This accumulation is commonly being encountered in natural environments. Exposure of tomato to simulated acid rain engendered accumulation of soluble phenols as an induced mechanism against SAR stress. These plant nutrients are important; Arginine, carnitine and cysteine significantly improve sperm quality and thus, male fertility. Amino acid cysteine, glutathione and carnitine are powerful antioxidants which protects cells from oxidative stress caused by free radicals therefore, anti-aging effects. Leucine, isoleucine and valine are anticancer agents. Arginine is used as a natural cure for impotence as they fight erectile dysfunction/impotence and improve sexual stamina. Arginine is also used in the management of menopause by reducing hot flushes.

Kumaravelu and Ramanujam (2004) studied the effect of exposure of green gram (*Vigna radiate* L. Wilczek cv. ADT-1 and Vamban to simulated sulfuric

acid rain (SAR) of pH 5.5, 4.0, 2.5 and 7.0 (control). They reported that acid showers at pH 5.5; soluble protein, reducing and total sugars and starch in both cultivars were higher and slowly decreased with increasing levels of acidity. A fluctuation in contents of proximate nutrients imposed by simulated acid rain stress was observed. Kausar and Khan (2009) documented that at pH 4.0 and 3.0 seed carbohydrate and seed protein of wheat plant was suppressed by simulated acid rain. Increase in protein induced by SNAR and SSAR stress is hazardous. Very high protein in vegetables and other foods are unsafe. High protein in foods promotes the intake of protein between 200 and 400 g per day equating to approximately 5 g/kg per day which is more than five times the Recommended Dietary Intake (RDI) (Australian Nutrient Reference Values NRVs, 2006). The problems of very high protein in vegetables and diets are that; very high protein promotes a very low intake of carbohydrates. If the body does not receive enough dietary carbohydrate, it will break down muscle tissue to make glucose (preferred body fuel) which causes muscle wastage, decrease in metabolism and a build-up of ketones. The heart may not function well if its major source of fuel is ketones. The liver and kidneys are put under strain because they have to detoxify and eliminate unusually high quantities of protein byproducts. In people with diabetes, kidney problems may be exacerbated. There is an increased risk of developing gout and gall bladder colic. High protein also causes greater losses of body calcium which increases the risk of osteoporosis. Increase amount of protein in diets can cause mild dehydration due to increased water loss through urine. Increased risk of dehydration puts the body under pressure (Australian Dietary Guideline, 2013). Decrease in protein induced by simulated acid rain stress caused deficiency symptoms to those who eat *C. moschata*. Sypmtoms such as wasting and shrinkage of muscle tissue, the build-up of fluids, particularly in the feet and ankles (oedema), the inability of the body to deliver sufficient amount of oxygen to the cells, usually caused by dietary deficiencies such as lack of iron (anaemia) and slow or gradual growth especially in children.

SNAR and SSAR stress caused increase and decrease in vitamin A and all the B-complex vitamins. However, vitamin C was significantly ($P=0.05$) reduced by simulated acids rain stress while vitamin K showed increase in content at all levels of acidity. These results partly agrees with previous report by Munzuroglu *et al*. (2005) that vitamin levels of plants sprayed with simulated acid rains decreased in respect of pH and time when compared to the control. Several reports on changes in some vitamin levels occasioned by various levels of stress abound (Havaux and Kloppstech, 2001). Mofunanya and Egah, 2017 reported a reduction in vitamin A, B_1, B_2, B_3, B_6, E and C in *Amaranthus hybridus* due SNAR and SSAR effects. Squash is grown in many parts of the world for use as medicine. Squash seeds have been used in Traditional Chinese Medicine (TCM) since at least 17th century. Apart from China, squash has also been used in the traditional medicine of many countries; Yugoslavia, Argentina, India, Mexico, America, Brazil and Korea. *C. moschata* is used in the prevention or control of diabetes and elimination or treatment of intestinal parasites (Caili *et al*., 2006). *Cucurbita moschata* serves as a staple food and doubles as medicinal component in folk medicine used for measles, insomnia, colic and treatment of amoebasis. The seed is vermifuge; it is eaten fresh or roasted for the relief of abdominal cramps and distension due to intestinal worm's parasite. It can also be used in treatment of bladder disorder, wounds and certain female reproductive complaints (Grosvenor and Smolin, 2002). Squash or pumpkin seeds have been used for the treatment of enlarged prostrate glands and parasites (Brown, 2001; Khare, 2003). Fu *et al*. (2006) documented that *C. moschata* possess antidiabetic, antihypertension, immune modulation, antibacteria, antihypercholestrolemia, antitumor, intestinal anti-parasitia, anti-inflammation and antalgic properties.

It is an excellent source of vitamin A carotenoids such as α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin which contribute to *C. moschata* claim as an anti-cancer agent (Bauman and Edwards, 2015). Vitamin A; α-carotene in *C. moschata* slows the aging process, reduces the risk of cataracts development thus, protecting the eye and prevent the growth of tumor. β-carotene reduces skin radiation damage and acts as an anti-inflammatory agent. Carotenoids in *C. Moschata* plant play a role in cancer prevention. One of its best known functions of vitamin A is its involvement in visual pigment formation in the retina of the eye thus, counteract night blindness and weak eye sight, aid in the prevention of macular degeneration, essential in bones and teeth development, builds healthy skin and mucous linings that act as a protective barrier against bacteria and viruses, promotes normal functioning of the male and female reproductive system. Retinol is necessary for epithelial tissues maintenance that lines both the internal and external surfaces, it is a powerful antioxidant that protects against cancer and cardiovascular disease by neutralizing free radicals in cells, required for the strengthening of the immune system against colds, flu and infection, promotes healthy hair and nails, may prevent skin problem like ache, promotes healthy skin free of wrinkle, and help remove eye age spots. Low levels of vitamin A could lead to night blindness, in cases of prolonged deficiency xerophthelmia (dry eyes) which ultimately lead to blindness. The health benefits of *C. moschata* is linked to its antioxidant activity has been reported and authors recommended the consumption of squash for it good taste, as a way to improve the nutritional deficiency of vitamin A and reduce diseases associated with it

(Gonzalez *et al*., 2001). Antioxidant activity of 41.66% was reported Tamer *et al*. (2010), but the squash cultivar was not specified.

Vitamin C content of leaf, stem and root of *C. moschata* is a strong water-soluble antioxidant that protects cells and tissues against damage resulting from toxic chemicals, pollutants and free radicals responsible for degenerate diseases and aging free radicals damage by donating electrons, and by producing other antioxidant like tocopherol (vitamin E) (Kim *et al*., 2012), helps in cancer prevention by blocking the formation of nitrosamines which are potential cancer-causing agents. Vitamin C is important in the synthesis of collagen in the body, a connective protein which act a ''cellular cement'' holding cells and tissues together. It helps the body immune system fight against infections and diseases. The vitamin helps in the absorption of iron in the body needed for the synthesis of red blood cell, also function in the reduction of bad LDL and in the increase of good HDL cholesterol, thereby helping in the prevention of atherosclerosis and diseases of the heart. It lowers high blood pressure, decreases susceptibility to allergens. Vitamin E is a powerful antioxidant that cancels the free radicals that cause tissue and cell damage. It acts as an antioxidant delaying degenerative diseases, protects skin from sun damage and ultraviolet radiation, delay the risk of skin cancer, may lower or delay the risk of cataracts or AMD (Age-related macular degeneration), help in the prevention of brain decline, neurological diseases like Alzheimer's disease, it lowers blood pressure and the body against heart disease and atherosclerosis. Vitamin E is also important in the formation of red blood cells, fertility and reproduction. Decrease the risk of prostrate and bladder cancer. It is an anti-aging vitamin, helps to boost the immune system functions in the body, vitamin E works in synergy with vitamin A to protect the lungs from pollution.

The flesh and seeds of pumpkin are rich in proteins, vitamins (Scarotenoids and tocopherols) (Stevenson *et al*., 2007) and minerals.

Butternut squash is rich in mineral but low in fat and calories. Though low in fat, does contain some healthy fats like alpha-linoleic acid which is a useful omega-3-fatty acid that the body cannot synthesize naturally used as an anti-inflammatory agent (Mateljan, 2007).The butternut squash is rich in complex carbohydrate and low in saturated fat and sodium, it is a good source of magnesium, manganese and potassium, iron, vitamin C, and riboflavin. These minerals and vitamins have both antioxidant and anti-inflammatory effect, regular intake of pumpkin can help to stabilize blood pressure and boost cardiovascular health, vitamin A helps to breakdown homocysteine which is dangerous by-product of metabolism and in protection of colon cells.

Results also revealed the presence of B-complex vitamins important to man. Vitamin B_1 (Thiamine) supports hydrochloride acid production for the digestive system which is critical for carbohydrate metabolism and energy production. Thiamine optimizes brain function and learning capacity, promotes mental alertness and memory, it fights depression, may slow atherosclerosis progression, needed for the formation of blood cell, important for a healthy nervous system, they coordinate nerves and muscles interaction, needed for normal heart, stomach and intestine muscle tone. It may also be associated with reduced risk of cataracts. Deficiency of thiamine causes beriberi or enlarged heart, loss of appetite, breathing difficulty and congestion in the lung, gastrointestinal disorders, causes stunted growth, Crohn's disease, forgetfulness or mental confusion; severe deficiency can result in brain damage and Wernicke-korsakoff syndrome (a form of dermentia), edema. Vitamin B_2 (Riboflavin) is essential for healthy skin, vision and to alleviate eye fatigue, may help to prevent cataracts, it helps in the conversion of carbohydrate to energy and therefore, play a key role in energy production, it is also essential for growth, and body tissues repair, required for the production of red blood cells and antibodies, required for vitamin B_6 and iron absorption, helps maintain the levels of other B vitamins in the body. Lack of vitamin B_2 causes ariboflavinosis; symptoms include cracks in the corners of the mouth or on lips, sore throat, shiny red-purple, inflamed or swollen tongue, sensitivity to sunlight, loss of taste, appetite, anemia, nerve damage (nervousness, irritability or depression), itching or burning eyes or bloodshot. Vitamin B_3 (Niacin) is an important vitamin for the conversion of food to energy. It is required for DNA production in cells, promotes the nervous system functioning, healthy looking skin, functioning of the digestive system, hydrochloride acid production for the digestive system, needed to slow the development of atherosclerosis and reduce the risk of heart attack. Vitamin B_3 may be useful in the treatment of osteoarthritis, may protect against Alzheimer's disease and mental decline related to age. Niacinamide may help in the treatment of age-related macular degeneration (AMD), may prevent diabetes, may reduce low density lipoproteins (LDL) cholesterol in high doses and triglycerides and increase in high density lipoproteins (HDL). Niacinamide deficiency could cause pellagra, a condition characterized by dementia, dermatitis and diarrhea. Other symptoms are dry, cracked and scaly skin, hostility towards others, lassitude (weakness), loss of appetite, indigestion or gastrointestinal disturbances, insomnia, depression, anxiety or mental confusion. Vitamin B_6 (Pyridoxine) is another B-complex vitamin which helps the immune system in the production of antibodies to fight diseases. It helps the body convert tryptophan to vitamin B_3, reduces the risk of stroke or heart attack by controlling

homocysteine level in the blood, reduces symptoms of premenstrual syndrome (PMS) and may aid in the prevention of oxalate kidney stones in women, and in the treatment of carpal tunnel syndrome. Pyridoxine helps in the degradation of fats and carbohydrates for energy production, helps in the regulation of blood sugar levels, it is essential for protein metabolism. needed for new cell formation and growth, promotes healthy skin and mucous membranes, essential for hemoglobin and red blood cell formation, important for the functioning of normal nerve and brain. It may also help in the treatment of depression. Insufficient amounts of pyridoxine could lead to various health conditions such as depression, abnormalities in mood or irritability, nausea or dizziness, chronic fatigue or muscle weakness, migraine headaches, asthma, increased susceptibility to infection, anemia, hypertension, nerve related problems such as convulsion and seizures, numbness of hands and feet, arm and leg cramps. Deficiency of vitamin B_6 also causes skin disorders such as dermatitis (eczema), sores on lips, mouth, inflammations of mucous membrane of the mouth or tongue.

Simulated nitric and sulphuric acid rain stress caused significant reductions and higher amount of nutrients resulting in fluctuation in quantity and quality in *C. moschata*. The increase in phytonutrients may be attributed to stress induced by SNAR and SSAR. Acid rain as well as other factors in the environment cause stress which leads to increase in free radical formation. Kong *et al*. (2000) reported that acid rain caused an increase in oxygen radicals and decrease in protein in their organ. Vitamins reductions in this study may be due to increase in free radical formation in the plant due to acid rain stress, and plant's use of the vitamins and other phytonutrients in order to resist the stress which results in a loss in nutrients. Reductions may also be due to the fact that in autotrophy crops metabolic ways formed by nutrients synthesis are somewhat inhibited by acidic conditions. It is an established fact that various crops exposed to stress change their metabolism. Decrease could also be due to the sensitivity of these nutrients to stress. The accumulation of these chemical constituents in simulated acid rain treated plant parts suggests that their synthesis is stimulated by stress induced by simulated acid rain as it is with other stress factors in the environment.

Cucurbita moschata should be protected from all forms of acidic rain to stabilize its medicinal quality since the poor populace in Nigeria and other developing countries depends on this vegetable crop plant to meet their health needs. Increase and decrease engendered by SNAR and SSAR stress on *C. moschata* nutrients implicated in medical practice should be given attention as too much or too little of these nutrients have great consequences to the health of the consumers of this vegetable. Simulated nitric and sulphuric acid rain affected the medicinal quality of *C. moschata*.

IX. Conclusion

Findings revealed significant increase and decrease in all phytonutrients in leaf, stem and root of *C. moschata* as a consequence of SNAR and SSAR stress. Individually simulated acid rain caused significant alterations in the medicinal quality of this vegetable.

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Study of Production Parameters of Bioethanol from Neem Fruit Pulp (*Azadirachta Indica*)

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Abstract- This study is a contribution to the valorisation of neem fruit pulp (Azadirachta indica) to produce bioethanol by alcoholic fermentation. During this study, a physical characterization of neem fruit and physicochemical neem pulp were performed. Also, the variation of the concoction pH of the fermentation tests was carried out in order to find the ideal pH for optimal production of bioethanol by the yeast Saccharomyces cerevisiae. The results obtained show that the pulp represents about 48% of the total mass of neem fruit. And also reveals that the neem pulp is very rich in total sugars (74% for Makabaye and 73% for Baoliwol) and that they can be converted into bioethanol by alcoholic fermentation. The unadjusted wort pH (pH = 5.4) resulted in a maximum bioethanol production of 5.1 mL/100g DM in 05 days of fermentation compared to other fermentation tests (pH = 4.3, 4.5 and 4.7). Also, the distillation of the fermented concoction allowed to obtain bioethanol with an alcohol content of 85% (v / v). This study has shown that neem fruit pulp can be used as an organic material rich in sugars, with a view to intensive production of bioethanol.

Keywords: *neem pulp, physicochemical characterization, concoction, alcoholic fermentation, bioethanol.*

GJSFR-C Classification: *FOR Code: 069999*

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Study of Production Parameters of Bioethanol from Neem Fruit Pulp (*Azadirachta Indica*)

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Abstract- This study is a contribution to the valorisation of neem fruit pulp (*Azadirachta indica*) to produce bioethanol by alcoholic fermentation. During this study, a physical characterization of neem fruit and physicochemical neem pulp were performed. Also, the variation of the concoction pH of the fermentation tests was carried out in order to find the ideal pH for optimal production of bioethanol by the yeast Saccharomyces cerevisiae. The results obtained show that the pulp represents about 48% of the total mass of neem fruit. And also reveals that the neem pulp is very rich in total sugars (74% for Makabaye and 73% for Baoliwol) and that they can be converted into bioethanol by alcoholic fermentation. The unadjusted wort pH (pH = 5.4) resulted in a maximum bioethanol production of 5.1 mL/100g DM in 05 days of fermentation compared to other fermentation tests (pH = 4.3, 4.5 and 4.7). Also, the distillation of the fermented concoction allowed to obtain bioethanol with an alcohol content of 85% (v / v). This study has shown that neem fruit pulp can be used as an organic material rich in sugars, with a view to intensive production of bioethanol.

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I. Introduction

The increasing demand of oil and the adverse effects such as the resulting climate change (Bunthita et al, 2016) have led to the search for alternative energy sources with very little impact on the environment (Siti et *al.,* 2017, Novidzro et *al.,* 2013). Thus, the depletion of crude oil reserves and soaring crude costs offer excellent prospects for bioethanol (Boulal et al, 2010), considered as an appropriate alternative to gasoline (Chamoumi, 2015). Bioethanol used as biofuel is mainly produced from plant reserve organs (Riess, 2012). However, the use of these reserves for the production of bioethanol competes directly with products intended for human consumption (Maria, 2012). To overcome these problems, research has focused on the valorization of inedible organic matter for the production of bioethanol. Indeed, the use of neem fruit is very interesting because its seed can produce oil and its pulp contains sugars. These sugars can be valorized by a biotechnological process for the production of a product with high added value, like bioethanol.

In Cameroon, the fight against desertification and climate change is a government concern. Faced with these challenges, neem is one of the most used trees for reforestation in the Sahelian regions (Foundoun, 1998). In March 2011, according to the Regional Delegation for the Environment and Nature Protection of the Far North, nearly 560 000 neem and Acacia plants have reforested about 1500 hectares in. Léré (Mayo Department). Kani). In addition, according to Tizé *et al*. (2016), about 45 500 neem trees are distributed in the city of Maroua (Cameroon). At maturity, neem produces an average of 50 kg of sweet fruit each year (Formad, 2013), whose pulp (48% of the total mass of neem fruit) remains unvalued. As a result, the neem pulp represents a significant amount of biomass, which requires energy recovery.

In order to give an added value to the fruit pulp of neem, the objective of this study is the physico-chemical characterization of the neem pulp for the production of bioethanol. Specifically, it is a question of making a determination of the total sugar content of neem fruit pulp, the specific production and the alcoholic content of bioethanol obtained from the fruit pulp of neem.

II. Material And Methods

a) Study area and plant material

The study was realized during the period February-March 2017 in the Far North region (Cameroon). Four sites of neen productions were selected for the collection of neem fruits: 02 sites in Mayo-Sava (Mora and Mora I) and 02 sites in the Diamaré (Makabaye and Baoliwol). Neem fruits were sorted on the basis of skin color (preferably yellow) and appearance (cool) to the touch, fallen from the tree between 24 and 48 hours. However, for the Mora I site the neem fruits were collected (yellow and firm to the touch) and were not subject to any selection criteria beforehand.

The plant material consists of neem fruits, collected in 04 sites in the Far North region

b) Biological material

The biological material is the dry yeast strain *Saccharomyces cerevisiae* (Lesaffre, Turkey), used for

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the alcoholic fermentation of the pulp juice of neem fruits.

c) Methodology

i. *Physical characteristics of neem fruits*

Parameters such as average weight, proportions of pulp and almonds were considered. The average weight was determined by weighing batches of 100 dried neem fruits using a scale (Compact Scale Electronic, USA). The masses of the fruit components were determined by weighing after coring (removal of the kernel) batches of 200g of dried neem fruit.

ii. *Process for obtaining the neem pulp powder*

Neem fruits were sorted at site collection and cleaned with water (Figure 1a). The fruits were distributed on a rectangular tray, placed on the roof to capture the maximum solar radiation for an average of 3 to 4 days. The fruits are returned from time to time to accelerate and harmonize the drying time (Figure 1b). Once this drying is complete, each fruit placed in the horizontal position is cut in half with a knife (Stainless Steel, China) and the kernel is removed. The pulp obtained was exposed to the sun for 6-8 days on average and the end of drying is observed when the pulps are firm at the finger pressure.

(a) Ripe and fresh fruits (b) Dried fruits

Figure 1: Driying of neem fruits

The obtained pulps were ground with a wooden mortar until the powder was obtained and the resulted powder was sieved using a sieve of mesh equal to 500 μm, the fine powder obtained was sent to the laboratory.

(a) Dried neem pulp (b) Neem pulp powder

Figure 2: Production of the powder from the dried pulp of neem

iii. *Physico-chemical characterization of neem pulp*

The dry matter was determined on a mass of 5 g of neem pulp, placed in an isothermal oven at 105 ° C to a practically constant mass (AFNOR, 1982). The total ash content was determined by calcining the test portion used for the dry matter, in a high temperature oven at 550 ± 15 ° C (AFNOR, 1982). The total lipid content was determined according to the Russian method (Bourely, 1982). The proteins were assayed according to the method of Devani et al. (1989) after mineralization according to the Kjeldahl method (AFNOR, 1984). Total sugars were assayed by the phenol-sulfuric method (Dubois et al., 1956).

iv. *Alcoholic fermentation of the juice of neem pulp*

a. *Preparation of the inoculum*

The inoculum was obtained according to the protocol described by Massengo *et al*. (2016). The yeast is pre-cultured by the introduction of 06 g the dry yeast strain Saccharomyces cerevisiae (Lesaffre, Turkey) in 100 ml of distilled water, containing 44 ml of a solution of 12% saccharose (v / v), with continuous stirring for 90 minutes and at a temperature of 27 ° C (Gauthier *et al*., 2005).

b. *Extraction of the neem pulp juice and preparation of the fermentation concoction*

Extraction of pulp juice from neem fruit was carried out according to the adapted method of Chniti (2015). A mass of 200 g of powder was diluted with distilled water at a 1/5 (w/v) dilution ratio is heated at 70 ° C. for 60 minutes (Chniti *et al.,* 2013), with continuous stirring. The juice is filtered using muslin after cooling. The resulting juice was heated at 85 ° C for 20 minutes to remove bacterial flora and cooled to room temperature (Diakabana *et al*., 2013, Massengo *et al*.,

2016). The medium is enriched with urea (NH_2CONH_2, 4g / L) to ensure optimal growth of yeasts and accelerate the kinetics of fermentation (Novidzro et al., 2013, Gbohaida et al., 2016) Inoculum in the ratio Innoculum/Concoction = 1/500 (V/V) was added to the fermentation concoction, with continuous stirring.

c. *Adjustment to different pH of the concoction of fermentation pulp*

From the obtained neem pulp concoctions, 04 alcoholic fermentation tests were carried out: a test where the pH of the concoction was not adjusted (pH = 5.4) and 03 tests with the pH of the concoctions were adjusted. at 4.3, 4.5 and 4.7 with dilute sulfuric acid solution (1.5N H_2SO_4). Once the desired pH is reached for the various adjusted concoctions (4.2, 4.5, and 4.7) and unadjusted concoction, they are transferred to 1L fermentors and anaerobically conducted for 120 hours (Ameyapoh et al., 2006), at a temperature of 30 ± 2 ° C (Boulal et al., 2013, 2010, Kaidi and Touzi, 2001). The device of the alcoholic fermentation is presented by figure 3.

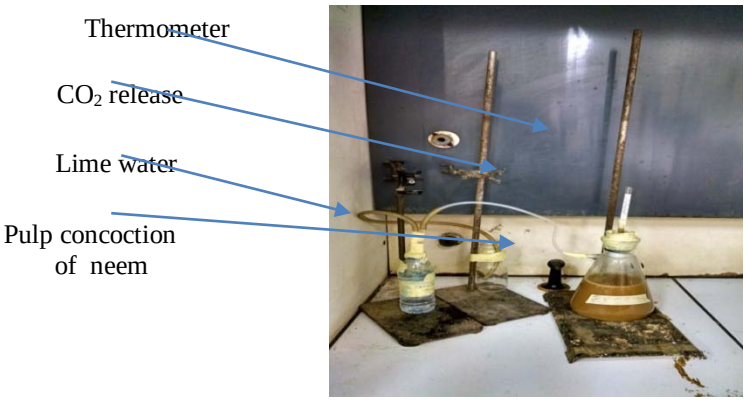

Figure 3: Dispositif de fermentation alcoolique (Labo ENSAI, Juin 2017)

The fermentation was monitored by taking 10 mL with a syringe every 24 hours and for 120 hours. The controlled parameters are: the pH, the temperature and the density of the fermentation must.

At the end of the alcoholic fermentation, the ethanol contained in the must is distilled off at a temperature of 78.5 ° C (Diakabana et al., 2016).

d. *Alcohol content of the bioethanol obtained*

The ethanol productivity was determined by directly measuring the volume of distillate obtained (after distillation) for each fermentation test. The alcoholic degree was determined after the distillation of the bioethanol mixture during the distillation of the different fermentation tests. The alcoholic degree was determined by the OIML method (1973).

d) Statistical analyzes

Each experiment was repeated 03 times for physicochemical analyzes of the pulp and the physical characterization of the fruit. Fermentation tests at different pHs were performed in duplicate. The results obtained were expressed in the form: M ± σ, with σ the standard deviation and M the average. A probability p <5% was considered as a non-significant difference in the data analysis.

III. Results And Discussion

a) Physical characterization of neem fruits

The physical characteristics of neem fruits are presented in Table 1.

Table 1: Physical characteristics of neem fruits

Sites Parameters	Makabaye	Mora	Mora I	Baoliwol
Average weight (g) [1]	260.25±2..34[a]	238.45±2.62[a]	240. 19± 0.90[a]	264.25±0.50[e]
Almond content (%) [2]	52.45±0.34[b]	51.35 0.34[b]	52.15±0.34[b]	52.21± 0.54[b]
Pulp content (%) [2]	47.55±2.50[c]	48.65±1.40[c]	47.85±1.70[c]	47.79±2.20[c]

Numbers with the same superscript letters on the same line indicate that these values are not significantly different at p <5%.

b) Physico-chemical characterization of neem pulp

The physico-chemical characterization of neem pulp is presented in Table 2.

Table 2: Physico-chemical composition of neem pulp

Sites Parameters	Makabaye	Mora	Mora I	Baoliwol
Dry matter content (g/100g)	90.27±0.30 [a]	87.33.30 [a]	87.67.30 [a]	89.00±0.40 [a]
Total ash (g/100g db)	06.31.13 [m]	08.10.16 [n]	07.57.19 [b]	07.26.69 [b]
Lipid content (g/100g db)	03.94.18 [i]	05.09.49 [c]	04.84.25 [c]	05.52.08 [j]
Protein content (g/100g db)	04.23.15 [d]	04.45.35 [d]	03.19.49 [k]	04,34.25 [d]
Total sugars (g/100g db)	74.63±0.60 [e]	57.56±0.10 [x]	20.17±1.5 [y]	73.59±2.08 [e]

Numbers with the same superscript letters on the same line indicate that these values are not significantly different at p <5%.

c) *Fermentation kinetics*

i. *pH*

The consumption of carbon and nitrogen substrates is accompanied by the production of acid metabolites and ethanol. This justifies the lowering of the pH of the various musts during alcoholic fermentation, represented by Figure 4.

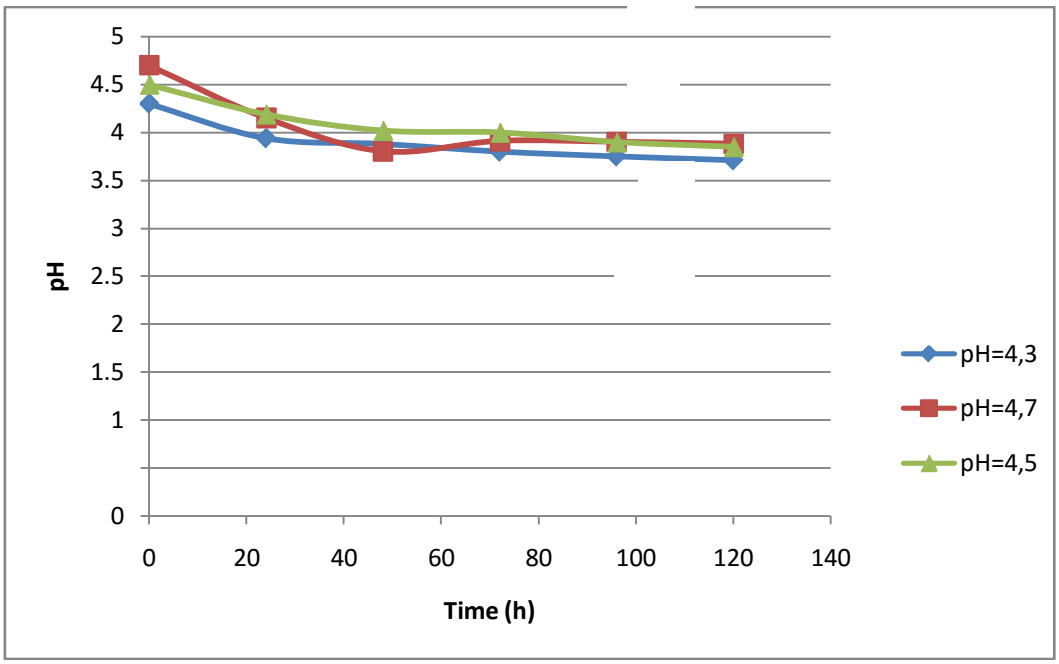

Figure 4: pH variation during alcoholic fermentation process of neem concoction

d) *Density of the fermented must*

The decrease in density (Figure 5) is observed for the different pH values (4.3, 4.7 and 4.5), which can be explained by the transformation of fermentable sugars into alcohol and the loss of mass under form of CO_2 (Gaillard et *al.,* 1995).

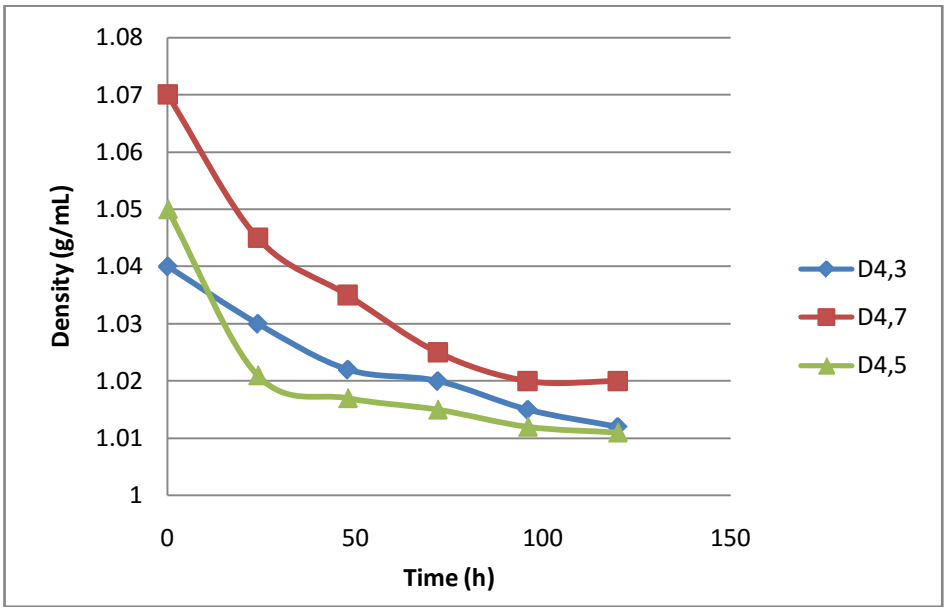

Figure 5: Variation of must density with time of fermentation

e) Bioethanol productivity and alcohol content

The production of bioethanol for the various alcoholic fermentation tests is shown in Table 3. The lowest value was observed in pH 4.5 and while the highest was observed at pH 5.4 in the unajusted must..

Table 3: Some physical parameters and Alcohol content of neem musts

pH		Alcohol content (mL/100 g de d.b)
Ajusted must	4.3 [i]	3.1 [a]
	4.5 [i]	3.0 [a]
	4.7 [i]	4.2 [b]
Unajusted must	5.4 [j]	5.1 [c]

Numbers showing the same superscript letters on the same column indicate that these values are not significantly different at $p < 5\%$.

IV. Discussion

The fruit pulp of neem with his high total sugar content (72-73%) is therefore an important source of bioethanol production. These levels are higher compared to the total sugar content of date must (50-60%) (Boulal et al., 2013). The low production of bioethanol from neem pulp is partly due to the loss of protein and nutrients during juice filtration. Indeed, these nutrients are necessary for the growth and development of microorganisms.

Low availability of sugars can also reduce the yield of bioethanol production. However, the use of enzymes such as pectinase (Ezoua et al., 1999) or pretreatment of the pulp beforehand (Alain, 2008) would make it possible to obtain significant yields of available sugars and possibly to improve ethanol productivity.

The high production of bioethanol at pH = 5.4 and pH = 4.7 compared to other tests (pH = 4.5 and pH = 4.3) is strongly related to the activities of the yeast *Saccharomyces cerevisiae*. The decrease in pH observed during the monitoring of fermentation tests, shows a microbial activity, source of production of acidic compounds (Ezoua et *al*., 2008). The latter appear to have an inhibitory effect on cell growth by causing a decrease in biomass production (Giannattasio et *al.,* 2005). This may justify the low production of bioethanol at pH = 4.3 and pH = 4.5.

At the beginning of fermentation, there is a rapid decrease of the pH and a sub-sequential increase of the acidity of the various fermentation tests. This phase could correspond to the growth phase of yeasts, resulting in the production of secondary metabolites. The increase in acidity could also be due to the production of CO2 or acidic compounds by the yeast during fermentation.

After 03 days of fermentation, pH stabilization was observed at the level of the different tests. This could correspond to the depletion of the medium in fermen-tescible sugars or the saturation of the media by

secondary metabolites likely to inhibit yeast growth or to slow down their fermentative activity (Novidzro et al., 2013). Also, Ouédraogo et *al*. (1999) showed that growth of Saccharomyces cerevisiae is optimal at pH = 5.

The fermentation of the different tests ends from the 3rd day while the stop is noticed on the 5th day. This observation is in agreement with that of Gbohaida et *al*. (2016).

In addition, the drop in density observed for the various tests is due to a loss of material (in the form of carbon dioxide) during the alcoholic fermentation (Kouakou et *al*., 1987) and the transformation of sugars into bioethanol (Ouédraogo et *al*., 1999).

V. Conclusion

Neem pulp is a very rich substrate of fermentable sugars, whose fermentative transformation is promising. This pulp represents about 48% of the total neem fruit mass, and is therefore an important source of biomass. The physico-chemical composition of neem pulp shows that total sugars are the major components (74% for Makabaye and 73% for Baoliwol). These sugars can therefore be converted by biotechnological processes into bioethanol. In the alcoholic fermentation tests of neem pulp at different pH values (4.2, 4.5, 4.7, and 5.4), the best bioethanol production rate was observed at pH = 5 , 4 (5.1 mL / 100g pulp MS) and pH = 4.7 (4.2 mL / 100g pulp MS). This study is the first to characterize neem fruit pulp and to determine its total sugar content for bioethanol recovery. Numerous perspectives stem from this present work, namely: the characterization of the sugars contained in the fruit pulp of neem and the optimization of the extraction of pulp sugars from neem fruits.

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Effect of Magnetic Field on Some Physical Properties of Tap Water

By Ashish Soni, Karishma Sharma & SS Verma

Abstract- Passing water through a magnetic flux has been claimed to boost chemical, physical and medicinal quality of water in many alternative applications. Though, the treatment method has been used for many years, it still remains within the realms of fallacy. If the claims of treating water with magnets are a unit true, the method offers enhancements on several of our applications of water in today's world. A big range of peer-reviewed journal articles have according contradictory claims concerning the treatment. A number of the foremost useful claimed water applications from magnetically treated water embody improvement in scale reduction in pipes and increased crop yields with reduced water usage. However, we are still unsure whether or not the technology works and researchers do believe it works and are still attempting to know the mechanisms of how it works. Several analysis papers are setting out to develop similar theories behind the mechanism of the treatment. The majority of the experiments performed throughout this analysis were determined to own deficient controls to provide conclusive results. The conclusions from this analysis were centred on coming up with improved experiments to produce additional conclusive results.

Keywords: *magnetic field, surface tension, conductivity, resistivity, electromagnet.*

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Ashish Soni [α], Karishma Sharma [σ] & SS Verma [ρ]

Abstract- Passing water through a magnetic flux has been claimed to boost chemical, physical and medicinal quality of water in many alternative applications. Though, the treatment method has been used for many years, it still remains within the realms of fallacy. If the claims of treating water with magnets are a unit true, the method offers enhancements on several of our applications of water in today's world. A big range of peer-reviewed journal articles have according contradictory claims concerning the treatment. A number of the foremost useful claimed water applications from magnetically treated water embody improvement in scale reduction in pipes and increased crop yields with reduced water usage. However, we are still unsure whether or not the technology works and researchers do believe it works and are still attempting to know the mechanisms of how it works. Several analysis papers are setting out to develop similar theories behind the mechanism of the treatment. The majority of the experiments performed throughout this analysis were determined to own deficient controls to provide conclusive results. The conclusions from this analysis were centred on coming up with improved experiments to produce additional conclusive results. While the experimental results weren't conclusive, the results earned backed the idea. Magnetically treated water doesn't do all that it's claimed it will. However, a number of the positive results obtained throughout this analysis recommend that the improved experiments developed from this analysis might give conclusive results on this moot topic.

Keywords: magnetic field, surface tension, conductivity, resistivity, electromagnet.

I. Introduction

Magnetized water is the water passed through a magnetic field. it's cheap, environmental friendly water treatment that incorporates a little installation fee and thus no energy necessities. The effects of magnetic field on water, is that the subject of disputable discussion. Several scientific journals and analysis claim that the magnetic field has no result on water and also the current successes haven't been able to be reproduced. Today, there square measures many reviewed papers and experiments that have done on magnetic water treatment with a considerable share attaining success within the treatment. Water could be a most tough substance to look at properties, because it carries the varied sort of particles within the type of small contaminants and alternative dissolved solids or liquid. This adds to the confusion regarding magnetized water with several claiming that bound chemicals within the water verify the success rates of the treatments. Round the world, in numerous laboratories, the water being treated varies from experiment to experiment, except once using water [1].

The development of water treatment with associate applied magnetic field has been familiar for several years and has been reported as effective in various instances however the Investigation on effects of magnetic field (MF) on water continues to be a disputable subject. Several articles and reports square measure out there in literature that reported that magnetic water treatment is helpful and magnetic water treatment has received attention from the scientific community. In nearly all cases researchers report some magnetic result, it absolutely was found that some properties of water were modified, and plenty of recent and strange phenomena were discovered when magnetization. Magnetic water is absolutely having magnetism. These results show that the molecular structure of water is extremely sophisticated, that wants to find out deeply.

a) Background

At now in time, magnetic water is classed by several to be pseudoscience whereas others are enjoying the advantages of this unknown science. The motivation for this study comes from the actual fact that such an easy technology will have helpful impacts on industries utilizing water. Water is one among nature's most significant gifts to human race. Essential to life a person's survival depends on drinking water. Water is one among the foremost essential components to physiological state -- it's necessary for the digestion and absorption of food; helps maintain correct muscle tone; provides gas and nutrients to the cells; rids the body of wastes; and is a natural air-con system. Health officers emphasize the importance of drinking a minimum of eight glasses of fresh water on a daily basis to take care of physiological state, water could be a key part in determinant the standard of our lives. Nowadays [2] folks square measure involved concerning the standard of the water they drink. Though water covers quite seventieth of the planet but only one of the planet water is accessible as a supply of drinking. Nevertheless our

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society continues to contaminate this precious resource. Water is understood as a natural solvent. Before it reaches the consumer's faucet it comes into content with many alternative substances as well as organic and inorganic matter chemicals and different contaminants. several public water systems treat water with Cl to destroy manufacturing contaminants which will be present within the water though medical aid is a very important step within the treatment of transportable water, the style and odour of Cl is objectionable and also the disinfectants that square measure accustomed prevents malady will produce by-products which can cause vital health risks/ nowadays potable treatment at the purpose of use isn't any longer a luxury it's a necessity customers square measure taking matters into their own hands and square measure currently determinant the standard of the water they and their families can drink by putting in a potable system that may offer them clean refreshing and healthier water.

b) Scope

This project is aimed at experimenting on the properties and applications to get an understanding of what is possible from magnetisation of water. Here is a list of some of the claims on magnetic water:

- The molecule increases in size and this increases the water solubility
- Magnetized water tastes better and is more clean
- Drinking of magnetic water may also promotes a more alkaline pH in the body
- The surface tension of water changes
- Conductivity of water changes.

From above properties in our project work the water to be used in the experiments includes:

- Surface Tension of water: tap water, water with salt and sugar.
- Conductivity of Water: tap water, water with salt and sugar.

c) Current Theories of Magnetised Water

The principle of this phenomenon is still not well understood and various contradictory hypotheses have been proposed. A journal article from explains the molecular makeup of water and its polarity. A molecule of water consists of one atom of oxygen and two atoms of hydrogen, H2O [7]. The covalent bond that holds each hydrogen atom to the oxygen atom results from a pair of electrons being shared. Because of the two hydrogen atoms sharing electrons on one end, the molecule possesses a positive charge on one end and a negative charge on the other. Some suggest this may cause the molecule to act similarly in some ways to a small bar magnet. This is referred to as the dipole moment of a molecule and is responsible for solubility of water. Figure 2.3 shows how the dipole moment of a water molecule is like a magnet. According to Quinn [8] the polar molecules attain different orientation under the influence of a magnetic field. The stronger is the magnetic field, the greater is the number of dipoles pointing in the direction of the field. The unusual properties of water can be attributed to extensive hydrogen bonding between its molecules.

In a magnetic field, magnetic force can break apart water clusters into single molecules or smaller cluster that contain equal number of water molecule. Therefore, the activity of water is improved. It should be noted that theories of water clusters are just that, theories and have not been proven yet [10]. Scientists are still unsure of the exact mechanisms by which treating water with magnets modifies its behaviours. There are numerous scientifically accurate theories, as well as several theories that apparently defy science as we know it. It is proper to point here that our current science isn't guaranteed 100% accurate and that we do not know everything about elements and molecules in the universe. We cannot throw new theories easily away just because they don't match with our past theories. We cannot throw new technology away either, just because we don't understand why it works [11].

II. Methodology

a) Measuring surface tension by capillary rise method

Surface tension molecular theory is very much a part of school physics curricula. Accordingly, a molecule well within a liquid is surrounded by other molecules on all sides. The surrounding molecules attract the central molecule equally in all directions, leading to a zero-net force. In contrast, the resulting force acting on a molecule at the boundary layer on the surface of the liquid is not zero, but points into the liquid. This net attractive force causes the liquid surface to contract toward the interior until the repulsive collisional forces from the other molecules halt the contraction at the point when the surface area is a minimum. If the liquid is not acted upon by external forces, a liquid sample forms a sphere, which has the minimum surface area for a given volume. Nearly spherical drops of water are a familiar sight, for example, when the external forces are negligible [12]. The surface tension 'γ' is defined as the magnitude 'F' of the force exerted tangential to the surface of a liquid divided by the length 'l' of the imaginary line on the liquid surface over which the force acts in order to maintain the liquid film.

$$\gamma = F/l$$

Capillarity is the combined effect of surface tension and cohesive & adhesive forces that causes liquids to rise in tubes of very small diameter. In case of water in a capillary tube, the adhesive force draws it up along the sides of the glass tube to form a meniscus. The cohesive force also acts at the same time to minimize the distance between the water molecules by pulling the bottom of the meniscus up against the force of gravity as shown in Fig. 1.1 and Fig. 1.2.

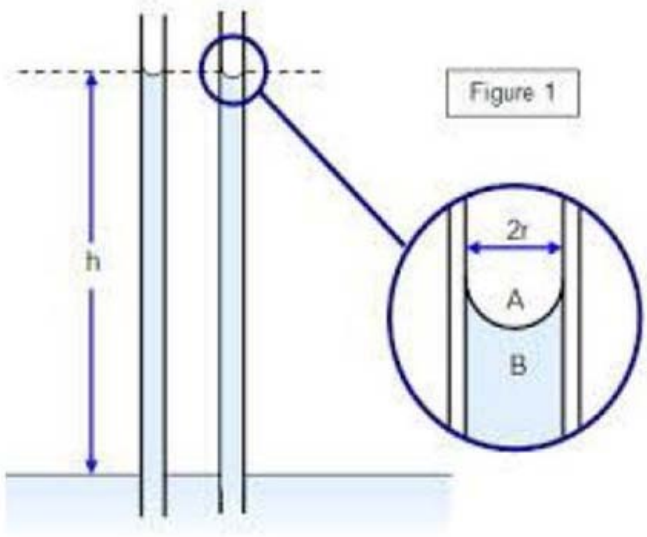

Fig. 1.1: Capillary rise

If the cross-section area of the capillary is circular and its radius is sufficiently small, then the meniscus is semi-spherical. Along the perimeter of the meniscus there acts a force due to the surface tension as:

$$f_1 = 2\pi r\gamma cos\theta$$

Where: r – the capillary radius,
γ – the liquid surface tension,
θ – the wetting contact angle.

The force f_1 is equalized by the mass of the liquid raised in the capillary to the height 'h', that is the gravity force f_2. In the case of non-wetting – it is lowered to a distance – h.

$$f_2 = \pi r^2 hdg$$

Where:
d – the liquid density (g/cm3) (actually the difference between the liquid and the gas densities),
g – the acceleration due to gravity.

In equilibrium (the liquid does not move in the capillary) that is $f_1 = f_2$, and hence:

$$2\pi r\gamma cos\theta = \pi r^2 hdg$$

Or

$$\gamma = \frac{rhdg}{2cos\theta}$$

If the liquid wets the capillary walls the contact angle $\theta = 0$, and Cos $\theta = 1$ then the surface tension can be determined as:

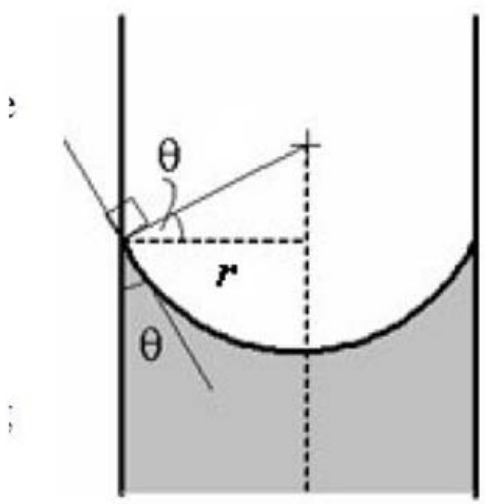

Fig. 1.2: Meniscus of water

$$\gamma = \frac{rhdg}{2}$$

Similar considerations can be made for the case of convex meniscus.

b) Conductivity of Water

Conductivity of a substance is defined as its ability or power to conduct and transmit the heat, electricity or sound. When an electric field is placed across a conductor, then the flow of its movable charges gives rise to an electric current and this property is called conductivity. Conductivity is a measurement of the ability of an aqueous solution to carry an electrical current. An ion is an atom of an element that has gained or lost an electron which will create a negative or positive state. For example, water molecule consists of hydrogen ions (H+) and oxygen ions (O-2) held together. The electrical conductivity can be expressed as mhos (reciprocal of ohms) or as Siemens. The conductivity of water is a measure of the ability of water to carry an electric current in most water is a the measure of ability of water to carry an electric current in most of the water the conductivity is very low so milli-Siemens or micro-Siemens are used as units foe conductivity. Pure water is bad conductor of electricity as it doesn't have any free ions that can circulate to pass electrical energy. If we add impurities, this becomes a good conductor of electricity as we get free ions that can pass energy. can conduct electricity because of these ions. The electrical resistivity ρ is defined as:

$$\rho = R\frac{A}{l}$$

Here R – The electrical resistance of a uniform specimen of the material, l-Length, A – The cross-sectional area of the specimen. Conductivity σ is defined as the inverse of resistivity: $\sigma = \frac{1}{\rho}$ or $\sigma = \frac{l}{RA}$ and has SI units of Siemens per meter (S/m).

III. Experimental Procedure

a) Apparatus used

i. *Magnetic field arrangement*

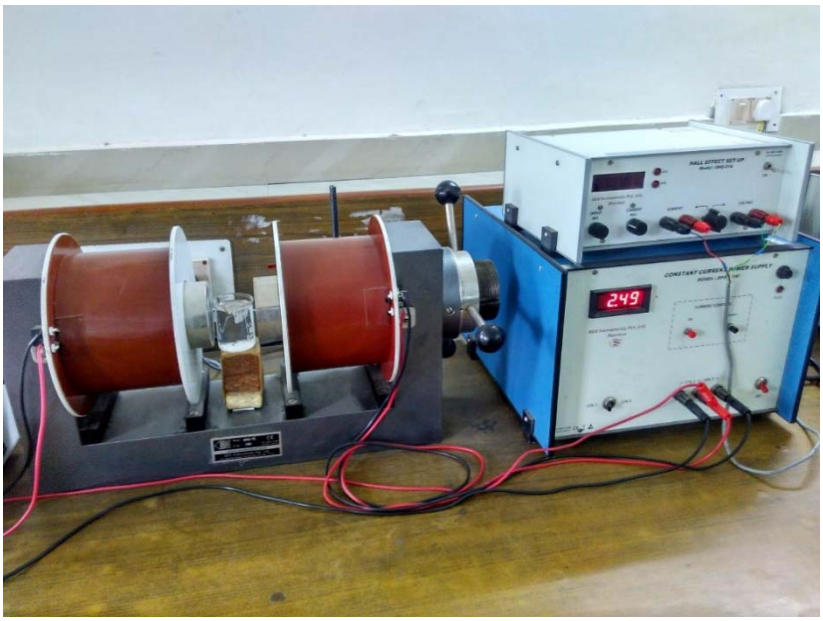

Fig. 2: Magnetic field arrangement

Fig, 1.2 shows the arrangement for magnetic field. We use the electromagnet which has power range from 0 to 3000 gauss. There is two pole of electromagnet between which there is an arrangement for keep the beaker of water so that we can magnetise the water and then measure the required properties of that magnetised water.

ii. *Surface Tension Arrangement*

To measure the surface tension, the arrangement is shown in fig 1.2 there is a stand and a capillary tube arrangement by which we can measure the surface tension at the different magnetic field.

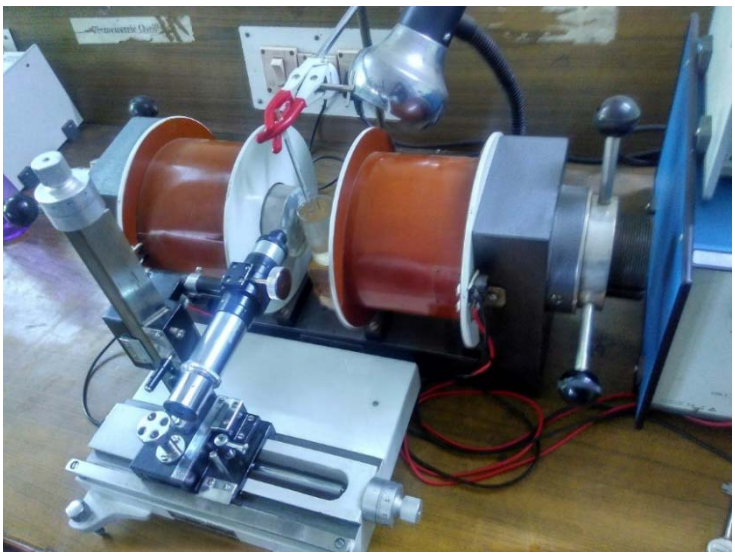

Fig. 3: Surface tension arrangement

iii. *Conductivity Arrangement*

To measure the conductivity of water we used a multimeter by which we can first measure the resistance of water, and further we calculate the conductivity.

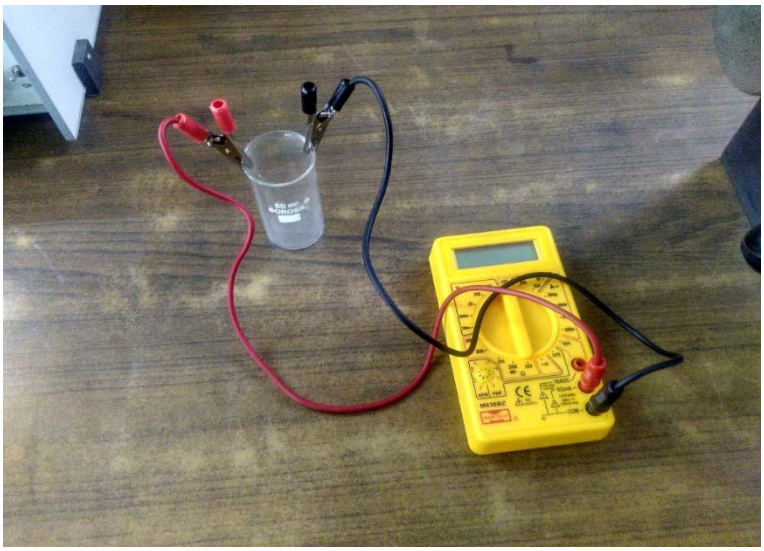

Fig. 4: Conductivity measurement arrangement

b) Calibration of the Electromagnet

i. *Magnetic field v/s current*

We use the electromagnet having range 0 – 3000 gauss to see the effect of the magnetic field on the water to do so we need to calibrate the values of the magnetic field at different values of current because in electromagnet we produce the magnetic field by applying current. So First of all I have note the digital gauss meter readings to calibrate the values of magnetic field i.e. the variation of magnetic field with current applied. If we take the values of the magnetic field at the centre of the pole of the electromagnet, the magnetic field is increases linearly with applied current. Graph shows the variation of magnetic field with increasing value of current.

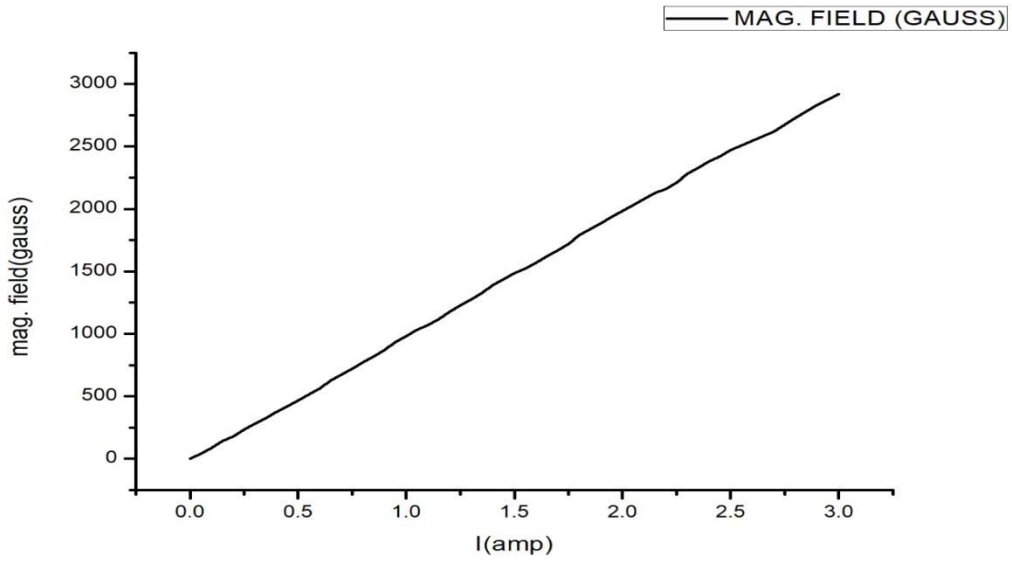

Fig. 5: Variation of the magnetic field with current

ii. *Magnetic field v/s distance*

Now we note the readings of magnetic field in the centre of the two pole of electromagnet with the change of the distance between the poles the variation of magnetic field with the distance is shown in figure.

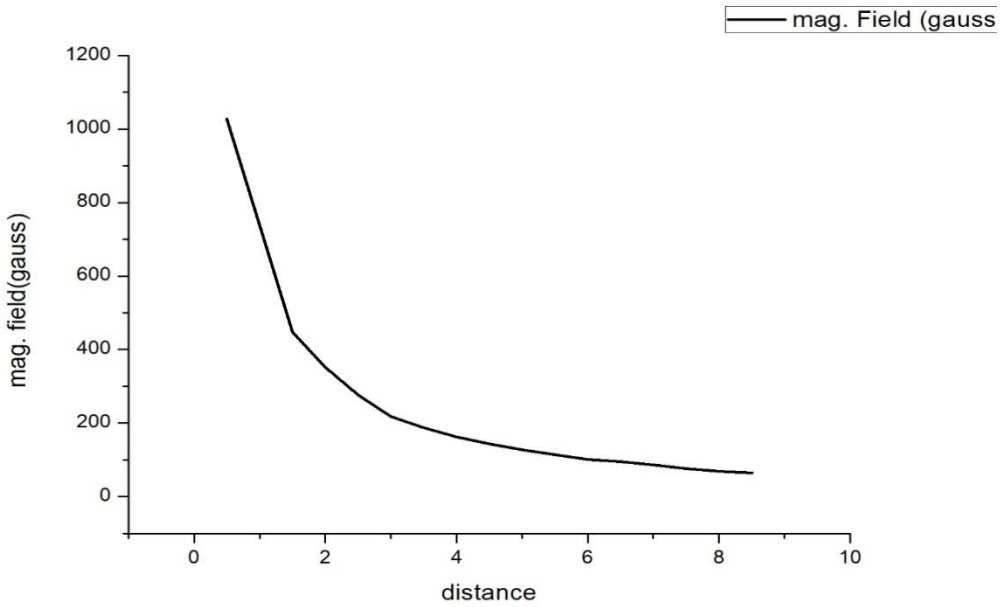

Fig. 4.5: Variation of the magnetic field with distance

c) Measurement of Surface Tension of Water at Different Magnetic Fields

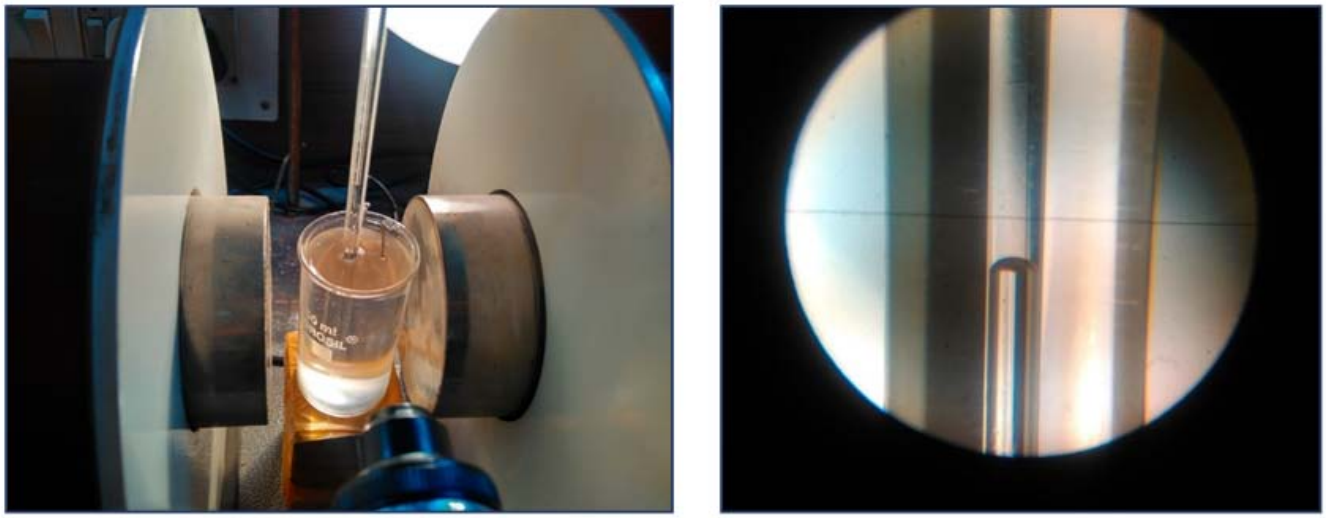

Fig. 6: Meniscus of water under microscope

- Firstly, to find the radius of the tube, fix the capillary tube in the stand horizontally.
- Focus the tube using travelling microscope to clearly see the inner walls of the tube. Let the vertical crosswire coincide with the left side inner wall of the tube. Note the reading of vertical diameter (say L1). Turn the microscope screws in horizontal direction to view the right side inner wall of the tube. Note down the reading of horizontal diameter (say R1). Thus, the radius of the tube can be calculated as ½(L1∼R1).
- Now for the Hight of water Fix the capillary tube in the clamp stand and stick a pin on the capillary tube for water reference level and check that the tube is perfectly vertical.
- Keep the beaker filled with water on the support base in between the poles of the electromagnets.
- Now bring the stand near the beaker. Let the tubes dipped in the water. Adjust the needle so that the lower tip of pin just touches the water surface.
- Determine the Vernier constant of the travelling microscope to be used.
- Focus the travelling microscope so that you can see the inverted meniscus of water. Adjust the horizontal crosswire to be tangential to the convex liquid surface. Note down the readings on the vertical scale.
- After noting the position of liquid surface for the tube, move the microscope further horizontally and

focus to the needle. Now move the microscope vertically and let the lower tip of the needle be focused at the point of intersection of the two cross wires. Note down the readings on the vertical scale.

- Thus, from the difference of the two readings noted above, the height of the liquid can be calculated.
- Finally calculate the surface tension by repeating the above procedure at different magnetic field and for different time period.

d) Measurement of Conductivity of Water at Different Magnetic Fields

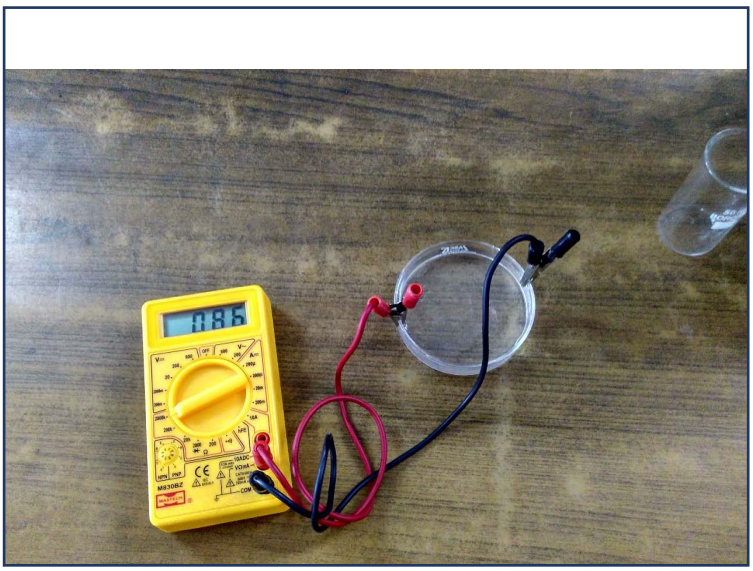

Fig. 7: Measurement of conductivity with multimeter

- Insert the red and black leads of the multi-meter into its positive and negative ports, respectively. The red lead represents positive, while the black lead represents negative.
- Now fill the beaker with water and put it in the magnetic field for some time (say t_1).
- Turn on the digital multi-meter and then change its measurement dial to the resistance setting. Resistance is denoted by the capital Greek letter Omega(**Ω**).
- Connect the leads at opposite ends of the longest dimension of the glass beaker filled with water.
- Measure the length, width and depth of the glass beaker in centimeters. The resistance in ohms that appears on the screen.
- Multiply the value of width by the depth to obtain the area of the sides of the glass dish in square centimeters.
- Divide the length by the product of the resistance and the area to arrive at the conductivity in units of Siemens per meter. The Siemens units equal one divided by the ohm.

IV. Results And Discussion

This chapter deals with the results of experiments carried out during the course of present study. We use the tap water, water mixed with salt and water mixed with sugar to see the effect of magnetic field on it.

As we see in the literature survey the Magnetic field can be affect many different properties of water like

- pH value
- Solubility
- Heat capacity
- Conductivity
- Surface tension

We focus our self only on last two properties that is the surface tension and the conductivity of tap water.

a) Results on surface tension test in normal tap water

The variation of surface tension with magnetic field at different magnetic field and for different time intervals is shown in the table below:

Table 1: Variation of surface tension with magnetic field using tap water

Time	Surface tension (N/m)			
	1030 gauss	1529 gauss	2030 gauss	2470 gauss
0 min	72.528	72.552	72.409	72.647
30 min	72.766	72.671	72.408	72.766
60 min	72.885	73.005	72.719	73.029
90 min	72.886	73.029	73.148	73.649
120 min	73.172	73.124	73.363	74.031

With the increasing magnetic field the surface tention of water shows very small changes at low magnetic field like at 1030 gauss the change is only one unit even for two hours but at higher magnetic field the change is comparetively large. It can be seen in the graph that slop of the magnetic field curve is more in the case of higher magnetic field and It is flat in the case of low magnetic field.

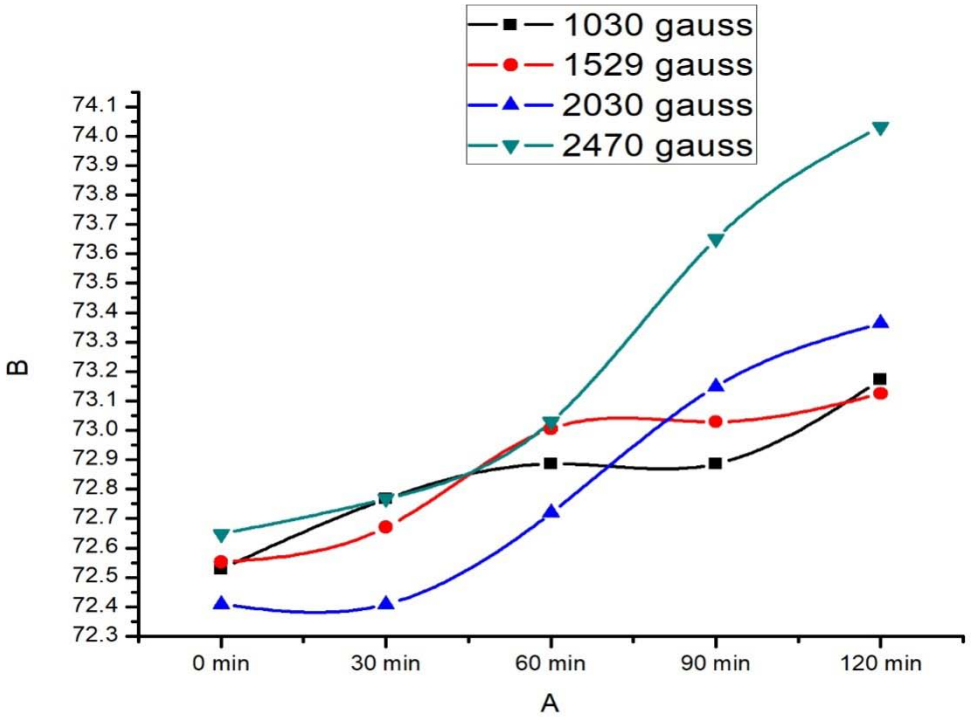

Fig. 8: Plot for variation of surface tension with the magnetic field using normal tap water

b) Result on conductivity test in normal tap water

Conductivity is also showing the variation with increasing magnetic field. We can see from the graph that the conductivity increases slowly for 60 minutes and after that, it increases rapidly. That is the effect of magnetic field is more if we put the water for a long time in the magnetic field.

Table 2: Variation of conductivity with the magnetic field using normal tap water

Time	Conductivity (Siemens per meter)			
	1030 gauss	1529 gauss	2030 gauss	2470 gauss
0 min	0.0162	0.016	0.015	0.017
30 min	0.0188	0.022	0.017	0.020
60 min	0.029	0.031	0.033	0.031
90 min	0.075	0.054	0.052	0.051
120 min	0.092	0.103	0.110	0.124

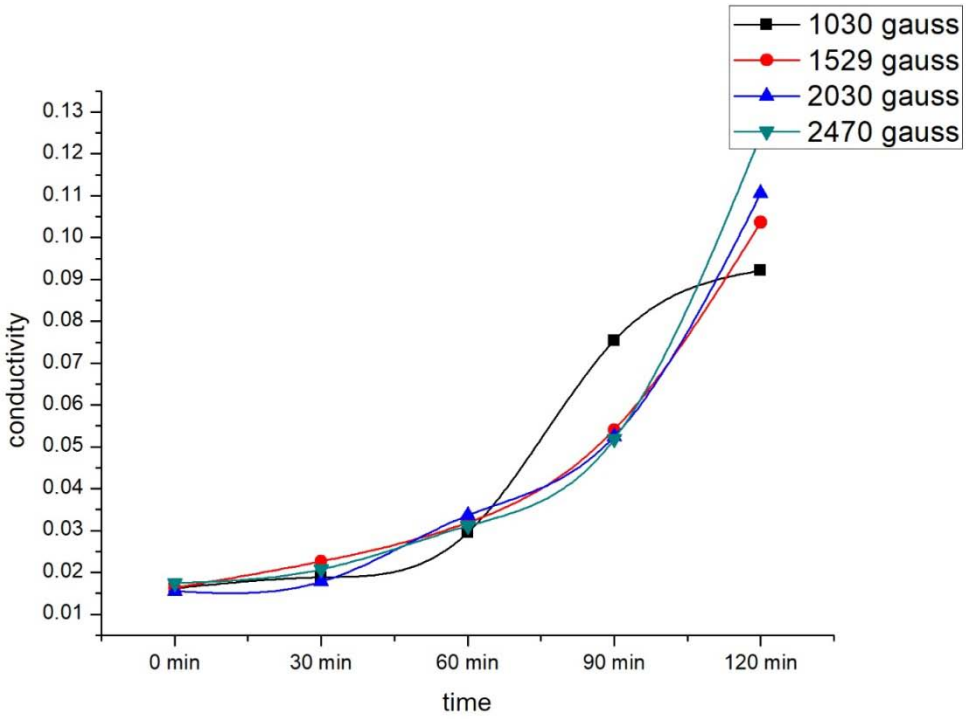

Fig. 9: Plot for variation of conductivity with magnetic field using normal tap water

c) *Results on surface tension of tab water mixed with salt*

Same experiment is done with the salted water and observes the variation of surface tension with magnetic field. The concentration of salt in water is 0.1 gram per ml of water. After adding the salt surface tension again increasing with increasing magnetic field. The variation of surface tension is shown in fig. 2.1

Table 3: Variation of surface tension with magnetic field using tap water mixed with salt

Time	Surface tension(N/m)		
	1030 gauss	1529 gauss	2030 gauss
0 min	72.838	72.361	75.893
30 min	73.172	75.057	76.513
60 min	74.890	77.778	78.899
90 min	77.801	79.281	79.281
120 min	79.49	80.403	79.615

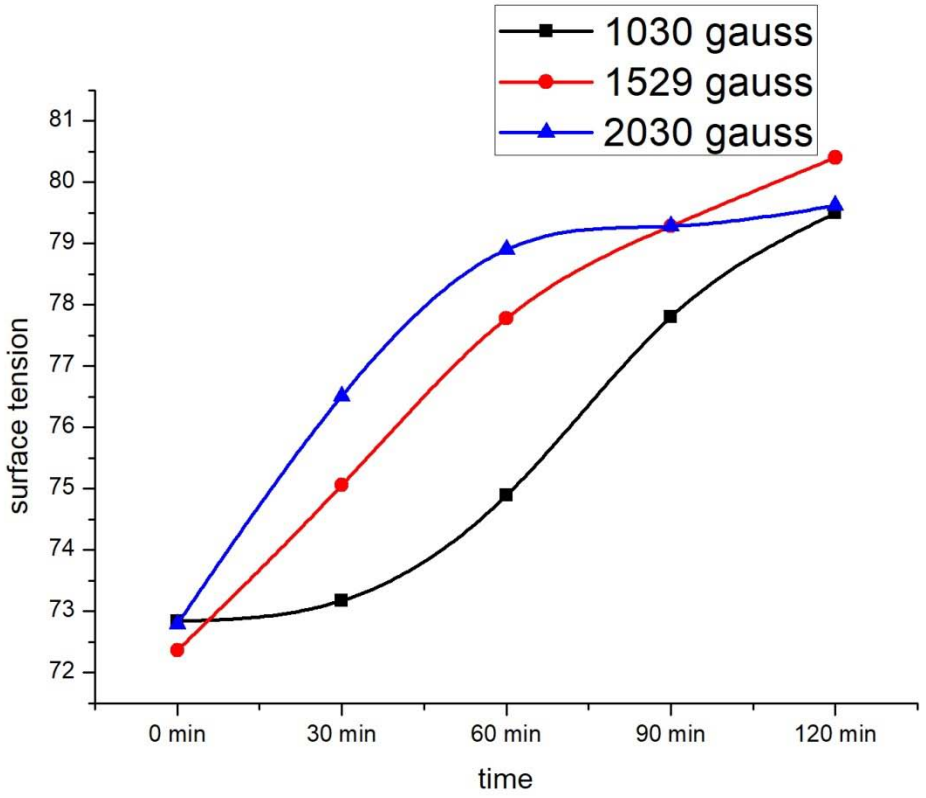

Fig.10: Plot for variation of surface tension with magnetic field using tap water mixed with salt

d) Results on conductivity test of tap water mixed with salt

In this case the conductivity of water is not so much varies but even it is increasing with very small value.

Table 4: Variation of conductivity with magnetic field using tap water mixed with salt

Time	Conductivity(Siemens per meter)		
	1030 gauss	1529 gauss	2030 gauss
0 min	0.131	0.138	0.134
30 min	0.142	0.155	0.155
60 min	0.146	0.165	0.155
90 min	0.146	0.165	0.155
120 min	0.146	0.165	0.155

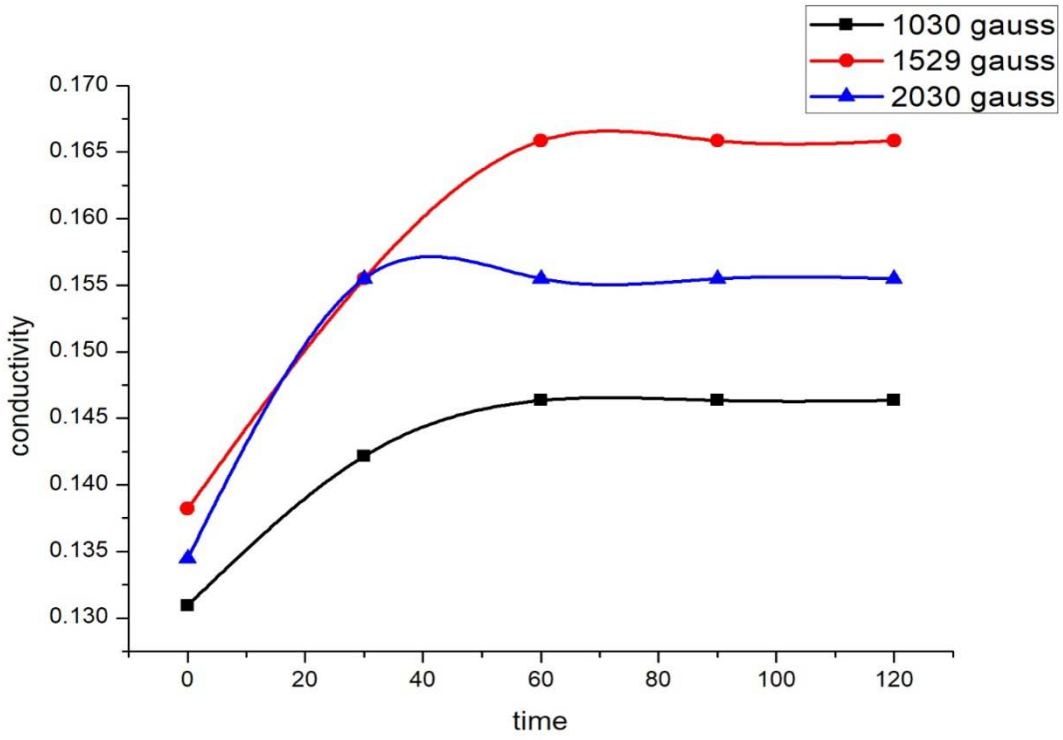

Fig. 11: Plot for variation of conductivity with magnetic field using tap water mixed with salt

e) *Result on surface tension of tap water mixed with sugar*

Now we have add sugar in water the concentration of sugar in water is 0.1 gram/ml. After adding sugar the surface tension of water again increasing with large variation in the values. The range of variation is from ~73 N/m to ~80 N/m. The measured values of surface tension are given in the following table 5.5.

Table 5: Plot for variation of surface tension with the magnetic field using tap water mixed with sugar

Time	Surface tension (N/m)		
	1030 gauss	1529 gauss	2030 gauss
0 min	74.747	73.220	72.695
30 min	74.890	74.460	75.17
60 min	79.138	79.281	79.925
90 min	80.259	80.832	81.190
120 min	82.908	85.172	82.956

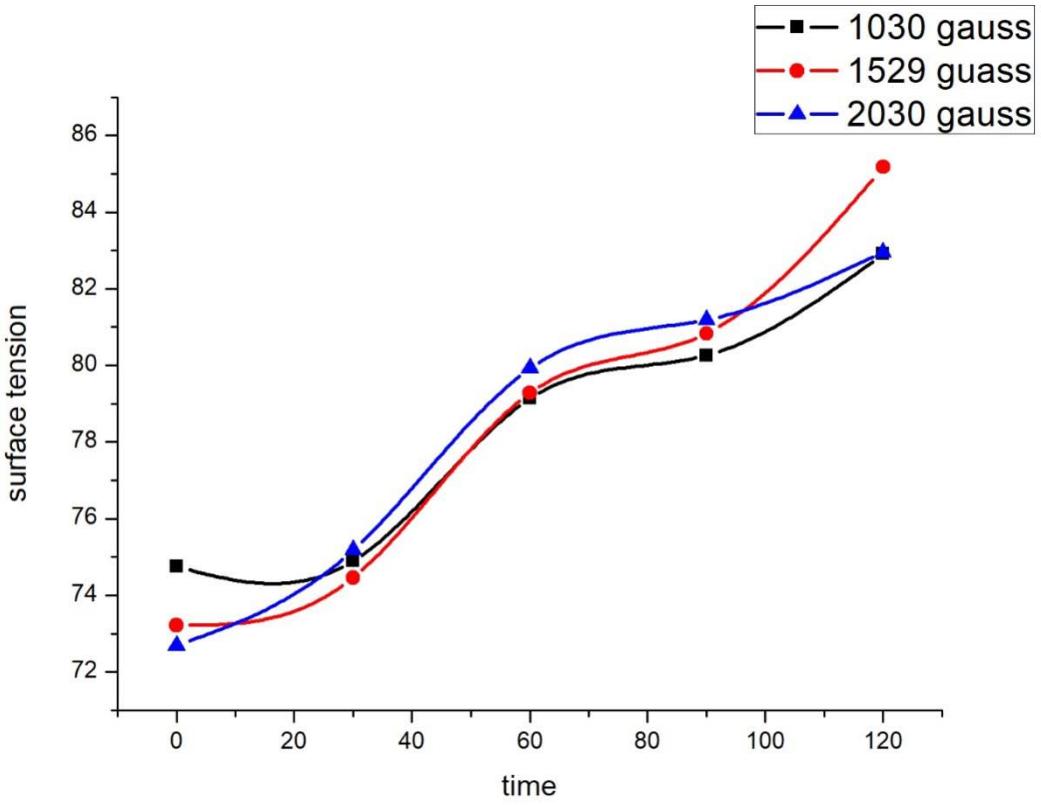

Fig. 12: Plot for variation of surface tension with magnetic field using tap water mixed with sugar

f) Result on conductivity of tap water mixed with sugar

Now, the same experiment is performed with the sugar addition in water and we found that there is also a change in conductivity but this time it decreases instead of increasing. So we can conclude that the conductivity can be decreased with the help of magnetic field. The values of conductivity are shown in the table. 5.6.

Table 6: Variation of conductivity with the magnetic field using tap water mixed with sugar

Time	Conductivity(Siemens per meter)		
	1030 gauss	1529 gauss	2030 gauss
0 min	0.071	0.071	0.065
30 min	0.064	0.065	0.061
60 min	0.061	0.062	0.052
90 min	0.055	0.054	0.049
120 min	0.051	0.051	0.047

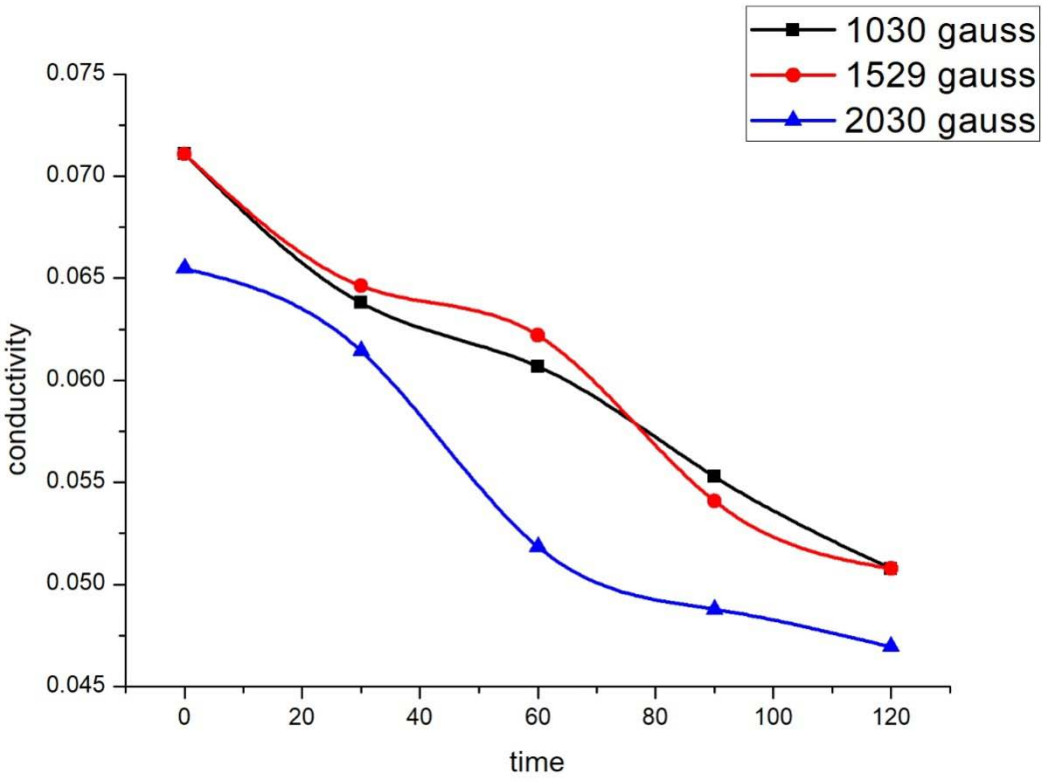

Fig. 13: Variation of conductivity with the magnetic field using tap water mixed with sugar

g) The brief summary of properties

S.No.	Type of Water	Surface Tension	Conductivity
1.	Normal tab Water	Increases	Increases
2.	Water mixed with salt (concentration 0.1 gram/ml)	Increases	Increases slightly and become constant
3.	Water mixed with sugar (concentration 0.1 gram/ml)	Increases	Decreases

V. Conclusions

In this work, it is found that the surface tension and conductivity of water changes in the magnetic field with time and for more time the effect is also more. Hence, we can change these properties of water using the magnetic field and thus magnetized water then can be used for any useful applications. The different findings of present investigations can be summarized as:

- After magnetization, the surface tension of tap water, water mixed with salt and water mixed with sugar has increased, this shows that the role of magnetization in water surface tension is working through water molecules. However, to the conclusion, we cannot rule out the effect of impurity atoms and the ion which still needs to be discussed in the further experiment.
- Surface Tension of water is related to the intensity of the magnetic field applied, for low magnetic fields like ~1000 gauss there is the minimum increase in surface tension while at a high magnetic field like ~2000 gauss there is comparatively large increment.
- Conductivity also changes with magnetic fields, and this is also directly related with the intensity of magnetic field.
- If we mix some amount of salt or sugar there is a change in the result as the surface tension in increasing at a high rate with the magnetic field. In tab water the range of surface tension is small (from 72n/m – 74 n/m) but after mix, the salt and sugar the range is increased (from 72n/m- 80n/m)
- For water and with salt the conductivity is found to be increasing, but after mix, some amount of sugar in water the conductivity is found to be decreasing.

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Utilization Pattern, Distribution, and Tree Diversity of Betalghat Block, Nainital District (Western Himalaya)

By Naveen Ch. Pandey, Neha Chopra, G. C. Joshi, Lalit M. Tewari &
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Abstract- The Himalayan region is the repository of socio-cultural and biological diversity with high valued plants. Participatory Rural Appraisal (PRA) tools were used to document the diversity, distribution and utilization pattern of trees in the Betalghat Block of Nainital District (Western Himalaya). A total of 148 species belongs to 109 genera, and 50 families were recorded from the study area. Maximum numbers of species were reported in the altitudinal zone 1001-1500m followed by the altitudinal zone, 500-1000m. Fabaceae, Moraceae, Rosaceae, Salicaceae, Malvaceae, Anacardiaceae, Lauraceae, Sapindaceae, Apocynaceae, Combretaceae, Euphorbiaceae, and Myrtaceae were the dominant families. For each species, botanical and vernacular names, multipurpose uses (Fuel, fodder, edible, medicinal, timber, agricultural tools, religious, ornamental, avenue, and fiber) were provided. For the conservation of economically viable plant species prioritization, reforestation, and forest rehabilitation is required.

Keywords: *trees, diversity, distribution, utilization pattern, PRA tools, betalghat.*

GJSFR-C Classification: *FOR Code: 069999*

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Strictly as per the compliance and regulations of:

Utilization Pattern, Distribution, and Tree Diversity of Betalghat Block, Nainital District (Western Himalaya)

Naveen Ch. Pandey [α], Neha Chopra [σ], G. C. Joshi [ρ], Lalit M. Tewari [ω] & Y. P. S. Pangtey [¥]

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I. Introduction

Vegetation is the most precious gift of nature which provided all kinds of essential requirements of the humans in the form of food, fodder, fuel, medicine, timber and oil, etc. (Anderson, 1986; Gaur, 1999). Species diversity is a component of biological communities and different methods are utilized to quantify it (Krebs, 1988). Floristic studies have required utmost importance in recent years in response to the need of developing and under developing countries to assess their plant wealth (Vediya and Kharadi, 2011; Patel et al., 2014). In the dry regions, trees play a role in maintaining an ecological balance and to enhance the life style of peoples. If we see the role of trees in rural landscape in a functional and expanded form then we can understood its importance (FAO, 1989a; Chavda and Mehta, 2015).

In the world, trees have always been associated with wisdom and immorality. Some trees also play a role in many of the world's mythologies. Woody vegetation plays an important role in prevention of soil erosion and increasing the water holding capacity, improving and securing the soil (Chavda and Mehta, 2015). Woody vegetation is used as fuel wood, food for humans and animals, medicine, dyes, fiber, and forage for livestock. Maximum wood of the forests is used as fuel in the world. Reduction of this resource can have negative effect on the prosperity of some populations (Chavda and Mehta, 2015). Forest is also very important in absorption of carbon, maintaining the oxygen balance in environment and losing of excess of water in the form of water vapours through transpiration (Sandhyarani et al., 2007; Mulia et al., 2010; Chavda and Mehta, 2015).

Uttarakhand is a part of Indian Himalayan Region (IHR) situated at latitudes $28^0$43'45"-$31^0$8'10" N and longitudes $77^0$35'5"- $81^0$2'25" E (Uniyal et al., 2007). Many workers have worked in this region ethnobotanically and floristically; i.e., Osmaston, 1927; Gupta, 1968; Kalakoti, 1983; Pandey, 1985; Pant, 1986; Pangtey and Rawat, 1987; Pangtey et al., 1988b; Gaur, 1999; Joshi and Joshi, 2001; Tewari et al., 2010; Kumari et al., 2011; Rawat and Vishvakarma, 2011; Sharma et al., 2011; Gangwar and Gangwar, 2011; Yadav and Bisht, 2013; Kanwal and Joshi, 2015; Pande et al., 2016; Pandey et al., 2016, 2017; Singh et al., 2017; Arya et al., 2018; Joshi et al., 2018. In recent years, documentation of traditional knowledge on floral diversity becomes a prerequisite to preserve traditional system of a region. Therefore, in the present study, a contribution has been made for diversity, distribution and utilization pattern of trees of Betalghat Block of Nainital district (Western Himalaya) with botanical name, vernacular name, and family and ethnobotanical uses for further research.

II. Materials and Methods

a) Geographical description of the study area

The study was conducted in twelve sites of Betalghat block (Nainital District) viz. Chimariya, Dhari-Kherni, Digthari, Simtaya, Phalyani, Padli, Bhowali, Kainchi, Dhunikhal, Kanda, Pankatara and Bhatrojkhan of Nainital district (during year 2016-2018) lies between $29^0$38'925" North latitude and $79^0$49'465" East longitude, covering an area of 256.33 Km^2 an altitudinal range varies from 700 to 1800 m asl (Fig. 1). The study area is

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bounded by Tarikhet, Bhikyasain, and Sult block of Almora district and Kotabag and Ramgarh block of Nainital district. The vegetation mainly comprises of the tropical, sub-tropical and temperate forest.

b) Climate

Long snowy winter and summer season is the characterized features of the climate of Nainital District. It is temperate and monsoon type (Singh and Singh, 1992) having four distinct seasons viz., monsoon (July to September), post-monsoon (October to November), winter (December to January) and summer (April to mid-June). Standard instrument was used to obtain the climatic data. The average rainfall is 1800 mm. The maximum temperature in the Nainital district is 42.2^{0}C and, the maximum is -5.4^{0}C.

c) Data collection and sample identification

Primary as well as secondary sources were used to collect the information on species diversity of trees and their economic uses and dependency of community on species. Participatory Rural Appraisal (PRA) tools (Silverman, 2005) were used to carry out the study. The study is based on the identification of tree species with the help of local/rural peoples, farmers, traditional knowledge holders to know the local names and multipurpose uses of the collected plants. The plant's specimens were collected and identified with the help of different floras and manuscripts, standard literature (Osmaston, 1927; Gupta, 1968; Gaur, 1999, Joshi et al., 2018) and matched with the herbarium specimen of Regional Ayurvedic Research Institute, (RARI) CCRAS, Ranikhet. The well-preserved plant specimens were deposited in the Department of Botany, Kumaun University, Nainital.

Fig. 1: Map of the study area

(Source- http://www.uttaranchal.org.uk)

III. Results And Discussions

a) Diversity

The people living in this region are totally dependent on the biological resources. The integral part of their economy and their livelihood are agriculture and livestock. The local inhabitants of the study area have a long tradition of using the plant resource for their various daily basic needs such as medicine, fodder, fuel, timber, agricultural implements, edible, religious and other uses. In present study, 148 species of trees were recorded i.e., Angiosperms (46 families, 103 genera and, 141 species) and Gymnosperms (four families, six genera and, seven species). Out of 50 families recorded Fabaceae (17 species) is the most diverse family, followed by Moraceae (14 species), Rosaceae (8 species), Salicaceae (7 species), Malvaceae (6 species), Anacardiaceae, Lauraceae, and Sapindaceae (5 species each), Apocynaceae, Combretaceae, Euphorbiaceae and Myrtaceae (4 species each) (Fig. 2). Total 148 species were recorded, out of which 30 Angiosperms, 4 Gymnosperms were recorded as cultivated and 111 Angiosperms, 3 Gymnosperms occurred in wild

conditions (Fig. 3). All the families are arranged in alphabetical order and updated with Tropicos (www.tropicos.org) (Table 1.)

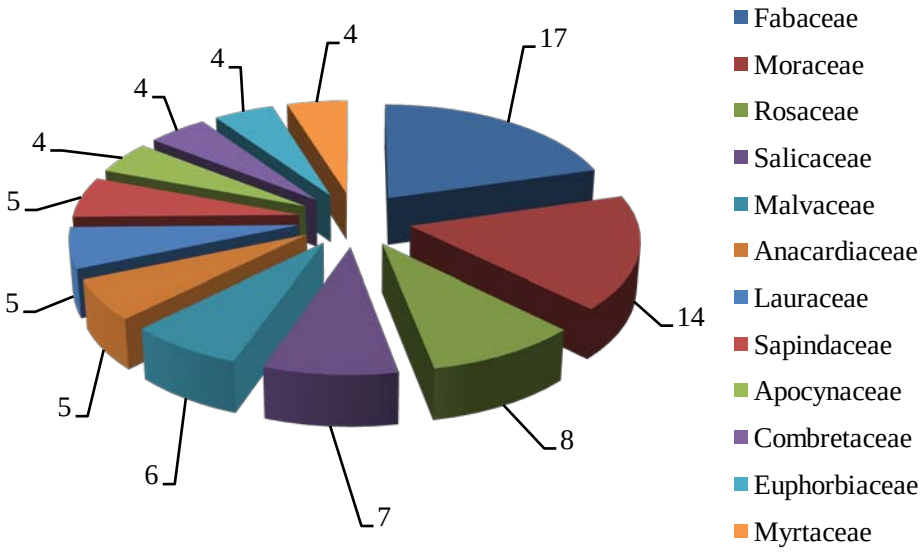

Fig. 2: Families representing the highest number of tree species

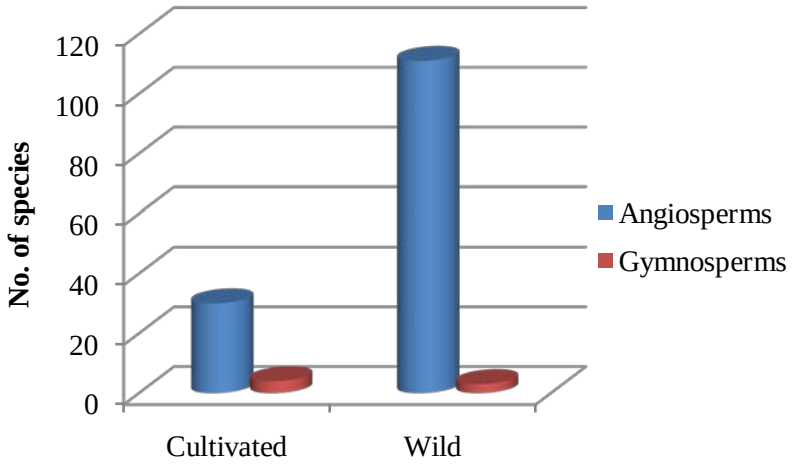

Fig. 3: Representation of cultivated & wild tree species

b) Altitudinal distribution

Along with an altitudinal gradient, altitudinal zone 1001-1500m showed the maximum number of species (Deciduous: 86, Evergreen: 45 spp.), followed by the zone, 500-1000m (Deciduous: 76, Evergreen: 33 spp.), 1501-2000m (Deciduous: 46, Evergreen: 38 spp.). Altitudinal zone, 1001-1500m considered as relatively a potential zone due to the representation of the maximum number of species. Overlapping of the species within the altitudinal zones were observed. Species distribution in different zones is presented (Fig. 4).

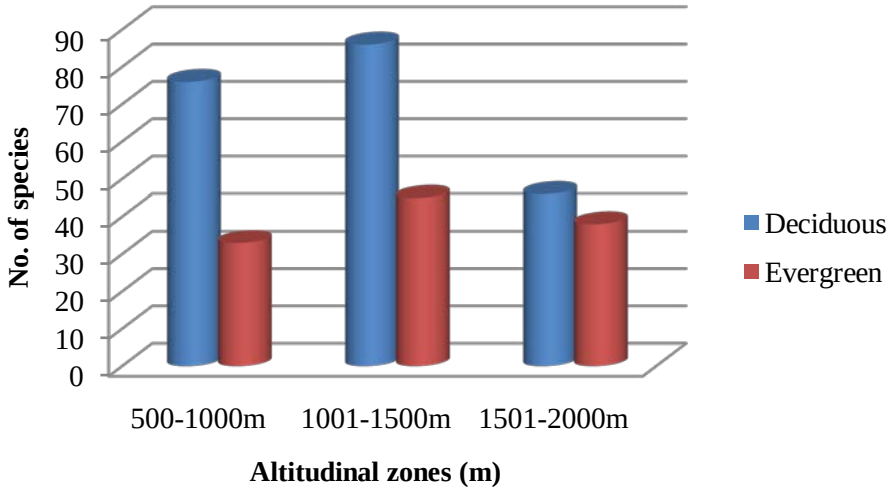

Fig. 4: Distribution of deciduous/evergreen trees along altitudinal zones

c) Economic importance

Of the total species, 26% species were used as fuel, 23% as fodder, 15% as edible, 12% as medicinal, 8% as timber, 6% as agricultural implements, 3% as ornamental, and religious, 2% as avenue, and fiber (Table 1 and Fig. 5). In the percent distribution of plant part of different species being used leaf were most commonly used part (34%) followed by wood (32%), fruit (16%), bark (9%), seed (3%), flower (2%) and root, latex, resin, and whole plant (1% each) (Fig. 6).

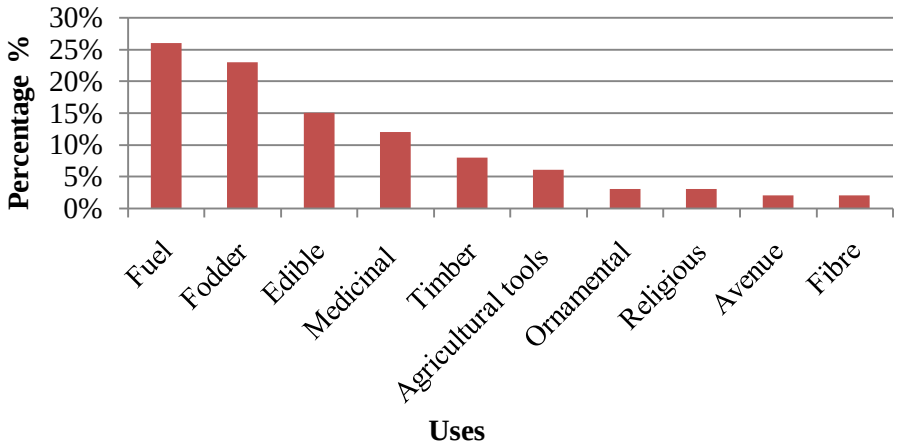

Fig. 5: Uses of tree species

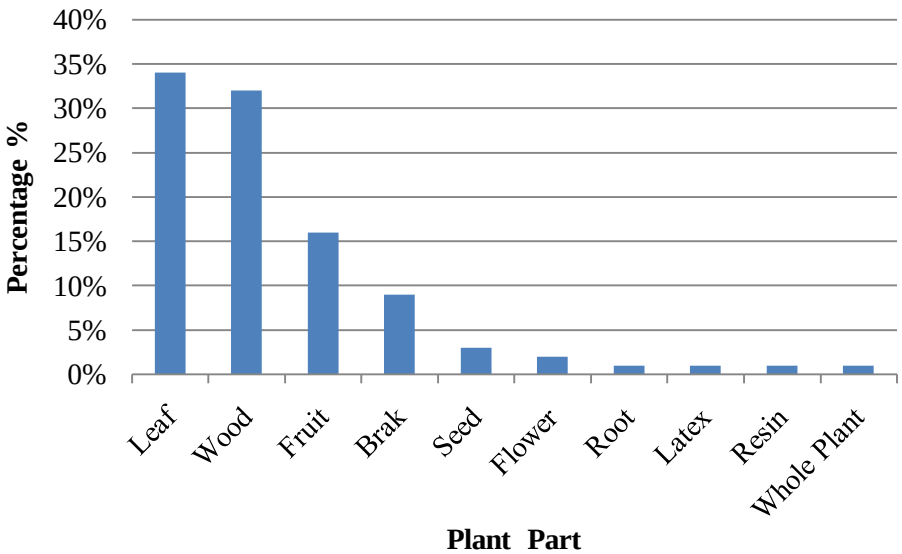

Fig. 6: Representation of plant part used

The present study recorded 148 species of trees from Betalghat Block, Western Himalaya. Various studies has been made i.e., Osmaston, (1927) reported 290 species of trees from Kumaon, Pant and Samant, (2010) recorded 97 species from Mornaula Reserve forest in Kumaun, Western Himalaya, Kumari, (2011) recorded 152 species from Almora district, Uttarakhand, Ballabha et al., (2014) recorded 73 species in Alaknanda valley, Garhwal Himalaya, Kishor, (2015) recorded 56 species in Munsyari Region, Kumaun Himalaya. Rawat et al., (2016) recorded 92 species from Montane zone, Western Ramganga valley, Uttarakhand, India and Bhatt et al., (2016) recorded 490 tree species from Western Himalaya, Pandey et al., (2017) reported 73 species of fodder trees in Betalghat Block, Nainital District, Kumaun Himalaya, Chauhan et al., (2017) recorded 39 species in District Tehri Garhwal (Western Himalaya) and Joshi et al., (2018) recorded 934 species from Ranikhet, West Himalaya.

IV. Conclusion

For the human existence and economic well being, ecosystem services and stability biodiversity is essential (Singh, 2002). The present study concluded that this region is a reservoir of an enormous natural floral diversity. The local inhabitants of this area living in the vicinity of the forests possess vast practical knowledge of biological resources and ethnobotanical uses. Most of the tree species were used as multipurpose species. The present study, concluded 148 tree species which belongs to 109 genera and 50 families. In the present study the species of *Pittosporum eriocarpum* Royle. *Oroxylum indicum* (L.) Kurz, and *Cinnamomum tamala* (Buch.-Ham.) Nees. & Eberm. are recorded under various threat categories by (IUCN, CAMP). This information is useful in developing strategies for the conservation and management of the species.

V. Acknowledgements

The authors are thankful to local people, informants for their immense help during the tenure of the study. We are also thankful to Director, Regional Ayurvedic Research Institute (RARI), CCRAS, Thapla, Ranikhet and Head, Department of Botany, Kumaun Universtiy, Nainital for providing infrastructure, facilities and encouragement. We are also thankful to IERP, GBPNIHESD, Kosi Katarmal, Almora.

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Table 1: Diversity, distribution and utilization of Trees of Betalghat Block, Kumaun Himalaya

Family/Species	Local Name	Altitudinal Range (m)	Fl & Fr time	Part Use	Uses	TH	SO
Anacardiaceae							
Lannea coromandelica (Houtt) Merr.	Jhingan	400-1200	Mar-Jul	Lf, Br, Wd	M, Fo, Fu	D	W
Mangifera indica L.	Am	200-1400	Mar-Jul	Fr, Lf, Wd	Ed, Re, Fu	E	C
Pistacia integerrima Sw.	Kakar	600-1800	Mar-May	Lf, Fr, Wd	Fo, M, Fu, Ti	D	W
Rhus wallichii Hk. f.	Akoria	1000-2400	May-Oct	Wd	Fu, At	D	W
Semecarpus anacrdium L.f.	Bhilwa	400-1100	Jan-Apr	Lf, Fr,Wd	Fo, Ed, Fu	D	W

Annonaceae							
Annona squamosa L.	Sarifa	400-1000	May-Jan	Fr, Lf, Br	Ed, M	E	C
Polyalthia longifolia (Sonn.) Thw.	Ashoka	300-1000	Mar-Sep	-	A, O	E	C
Apocynaceae							
Alstonia scholaris (L.) R.Br.	Chatiun	400-1200	Dec-Jun	Br, Lf	M	E	W
Holarrhena pubescens Wall. ex G. Don	Kwera	400-1300	Apr-Feb	Lf, Wd, Br, Lt	M, Fo, Fu	D	W
Thevetia peruviaria (Pers.) Schum.	Pili kaner	200-1200	Jan-Dec	-	O	E	C
Wrightia arborea (Dennst.) Mabb.	Dudhi	400-1400	May-Mar	Fr, Lf	Fo, Ed	D	W
Aquifoliaceae							
Ilex dipyrena Wall.	Kandela	1500-3200	Apr-Feb	Lf, Wd	Fo, Fu	E	W
Araucariaceae							
Araucaria bidwilli Hook.	Bunya tree	1200-2800	Sep-Nov	-	O	E	C
Araucaria columnaris (J.R. Forst.) Hook.	Bunya tree	1000-2000	Sep-Nov	-	O	E	C
Arecaceae							
Phoenix humilis Royle	Thakal	200-1400	Dec-Jun	Lf, Fr	Fo, Ed	E	W
Betulaceae							
Alnus nepalensis D. Don	Utees	1200-2900	Oct-Jan	Lf, Wd	Fo, Fu, At	D	W
Bignoniaceae							
Jacaranda mimosifolia D. Don.	Jacranda	500-2000	Mar-Oct	-	A, O	D	C
Oroxylum indicum (L.) Kurz	Tarulu	300-1200	Jun-Dec	Br, Lf, Fr, Wd	M, Fo, Fu	D	W
Boraginaceae							
Cordia obliqua Willd.	Bairala	600-1300	Mar-Jul	Lf, Fr, Wd	Fo, Ed, Fu, Ti	D	W
Ehretia acuminata R.Br.	Pudila	800-2000	Mar-Dec	Lf, Fr, Wd	Fo, Ed, Fu, Ti	D	W
Ehretia laevis Roxb.	Chamror	600-1500	Mar-May	Lf, Fr, Wd	Fo, Ed, Fu, At	D	W
Cannabaceae							
Celtis australis L.	Kharik	1300-2500	Mar-Oct	Lf, Fr, Wd	Fo, Ed, Fu, Ti	D	W
Celtis tetrandra Roxb.	Kharik	300-1800	Feb-Oct	Lf, Fr, Wd	Fo, Ed, Fu	D	W
Caricaceae							
Carica papaya L.	Papita	300-1200	Apr-Sep	Lf, Fr	Ed, M	D	C
Celastraceae							
Cassine glauca Kuntze	Dhebari	600-1500	Jun-Apr	Wd	Fu	D	W
Euonymus pendulus Wall. ex Roxb.	Bhambeli	1500-2800	May-Feb	Lf, Wd	Fo, Fu	D	W
Cephalotaxaceae							
Cephalotaxus griffithii Hook.f.	Cow tail pin	1300-3700	Sep-Nov	Fr, Lf, Sd	Ed, M	D	C
Combretaceae							
Anogeissus latifolia Wall.	Bakla	600-1400	May-Mar	Lf, Br, Wd	Fo, M, Fu, Ti	D	W
Terminalia alata Roth	Saij	350-1200	Jun-Apr	Lf, Wd	Fo, Fu, Ti	D	W
Terminalia bellirica (Gaertn.) Roxb.	Bahera	350-1200	Apr-Feb	Lf, Fr, Wd	Ed, Fo, M, Fu	D	W
Terminalia chebula Retz.	Harar	350-1400	Apr-Mar	Lf, Fr, Wd	Ed, Fo, M, Fu	D	W
Cornaceae							
Swida macrophylla (Wall.) Sojak	Khagsi	1300-2500	Apr-Feb	Lf, Wd	Fo, Fu	D	W
Swida oblonga (Wall.) Sojak	Gauntia	1400-2500	Sep-May	Lf, Wd	Fo, Fu	D	W
Cupressaceae							
Cupressus torulosa D. Don	Surai	900-3000	Jan-Nov	Wd	Fu, A, O	D	W
Thuja orientalis L.	Mor-pankhi	900-2800	Jul-Aug	-	A, O, Re	D	C
Daphniphyllaceae							
Daphniphyllum himalayense (Benth.) Müll. Arg.	Ratnali	1600-2800	Mar-Aug	Lf, Wd	Fo, Fu, At	D	W

Dipterocarpaceae							
Shorea robusta Gaertn. f.	Sal	300-1300	Mar-Jul	Rs, Lf, Wd	Fo, M, Fu, Ti	D	W
Ebenaceae							
Diospyros kaki L.	Kaku	1500-2500	May-Nov	Fr	Ed	D	C
Diospyros montana Roxb.	Tendu	200-1200	Apr-Dec	Wd	At	D	W
Ericaceae							
Lyonia ovalifolia (Wall.) Drude.	Anyar	1300-3500	Apr-Sep	Lf, Wd	Fo, Fu	D	W
Rhododendron arboreum Sm.	Burans	1200-3300	Mar-Nov	Fl, Lf, Wd	M, Ed, Fu, Re	D	W
Euphorbiaceae							
Bridelia retusa (L.) Spreng.	Kagroli	300-1000	Apr-Feb	Lf, Fr, Wd	Fo, Ed, Fu	D	W
Mallotus philippinensis (Lamk) Muell.-Arg.	Riyoni	300-1500	Sep-May	Lf, Fr, Sd, Rt, Wd	Fo, M, Fu, Ti	D	W
Sapium insigne (Royle) Benth.ex Hook. f.	Khina	300-2000	Feb-Mar	Lt	M	D	W
Sapium sebiferum (L.) Dum. Cours.	Charbi	800-2000	Jun-Dec	Lf, Wd	Fo, Fu	D	W
Fabaceae							
Acacia catechu (L. f.) Willd.	Khair	300-1300	May-Jan	Br, Lf, Wd	M, Fo, Ti, Fu	D	W
Acacia dealbata L.	Acacia	1200-2000	Feb-Aug	Wd	A, O, Fu	D	W
Albizia chinensis (Osbeck) Merr.	Siris	300-1500	Apr-Mar	Lf, Wd	Fo, Fu, Ti	D	W
Albizia julibrissin Durazz.	Kuneri	1200-2500	Apr-Dec	Lf, Wd	Fo, Fu, Ti	D	W
Albizia lebbeck (L.) Benth.	Siris	400-1500	Apr-Nov	Lf, Br, Wd	Fo, M, Fu, Ti	D	W
Bauhinia purpurea L.	Kwieyal	300-1600	Sep-Mar	Lf, Fl, Wd	Ed, Fo, Fu, At	D	W
Bauhinia semla Wunder.	Kandela	300-1700	Sep-Apr	Lf, Wd	Fo, Fu, At	D	W
Bauhinia variegata L.	Kanchnar	300-1700	Feb-Aug	Lf, Fl, Br, Wd	Ed, M, Fu, At	D	W
Cassia fistula L.	Amaltas	300-1400	Apr-Jan	Fr, Br, Lf, Wd	M, Fu, Ed, At	D	W
Cassia surattensis Burm. f.	-	300-1500	Apr-Sep	Lf	Fo	D	W
Dalbergia sericea G. Don	Ghogra	300-1650	Mar-Aug	Lf, Wd	Fo, Fu	D	W
Dalbergia sissoo Roxb.	Sisham	300-1500	Mar-Jun	Lf, Fl,Wd	M, Fo, Fu, Ti, At	D	W
Erythrina suberosa Roxb.	Rungar	300-1500	Mar-Jun	Lf, Br, Lf, Wd	M, Fu	D	W
Leucaena leucocephala (Lam.) De Wit.	Vilaiti baval	300-1200	Sep-Dec	Lf, Wd	Fo, Fu	D	C
Ougeinia oojeinensis (Roxb) Hochst.	Sandan	300-1600	Mar-Jun	Lf, Br, Wd	Fo, M, Fu, Ti, At	D	W
Robinia pseudocasia L.	Robinia	1200-2400	May-Oct	Lf, Wd	Fo, Fu, Ti	D	C
Tamarindus indica L.	Imali	200-1100	May-Apr	Fr, Lf, Wd	Ed, Fo, Fu, At	D	C
Fagaceae							
Castanea sativa Mill.	Chesnut	1500-2500	Mar-Aug	Fr, Wd	Ed, Fu	D	C
Quercus glauca Thunb.	Phalyant	800-2000	May-Aug	Lf, Wd	Fu	D	W
Quercus leucotrichophora A. Camus.	Banj	1200-2500	Apr-Oct	Lf, Wd	Fo, Fu, At	D	W
Juglandaceae							
Engelhardtia spicata Blume.	Garmahwa	600-1600	Mar-Jun	Lf, Wd	Fu, Fo	D	W
Juglans regia L.	Akhrot	1300-2800	Feb-Sep	Fr, Br, Lf, Wd	M, Ed, Ti, Fu	D	C

Lamiaceae							
Premna barbata Wall. ex Schaner.	Agniu	600-1300	Mar-Jul	Lf, Br,Wd	Fo, M, Fu	D	W
Tectona grandis L. f.	Sagon	300-1000	Apr-Aug	Wd	Ti, Fu	D	W
Lauraceae							
Cinnamomum tamala (Buch.-Ham.) Nees. & Eberm.	Tejpat	400-2100	Feb-Oct	Lf, Br	M	D	W
Neolitsea pallens (D. Don) Momiy. & Hara.	Chirar	1400-2500	Mar-Nov	Lf, Wd	Fo, Fu, Ti	D	W
Persea duthiei (King) Kosterm.	Kaula	1500-2800	Feb-Sep	Lf, Wd	Fo, Fu	D	W
Persea gamblei (King ex Hook.f.) Kosterm.	Kaula	500-1700	Mar-Aug	Lf, Wd	Fo, Fu	D	W
Persea odoratissima (Nees) Kosterm.	Kaula	700-2000	Feb-Jun	Lf, Wd	Fo, Fu	D	W
Lythraceae							
Lagerstroemia indica L.	Dhaura	500-1800	Mar-Oct	-	O, A	D	C
Lagerstroemia parvi flora Roxb.	Dhauri	350-1200	Apr-Jan	Lf, Wd	Fo, Fu, At	D	W
Magnoliaceae							
Magnolia grandiflora L.	-	300-2000	May-Oct	-	O	D	C
Michelia champaca L.	Champa	300-1600	Jun-Nov	-	O, Re	D	C
Malvaceae							
Bombax ceiba L.	Semal	300-1500	Jan-May	Lf, Fl, Fr, Wd	Ed, Fo, Ti, Fu, Fb	D	W
Firmiana fulgens (Wall. ex Masters) Corner	Budella	350-1300	Mar-Jun	Br, Lf, Sd	Fo, M, Ed, Fb	D	W
Grewia asiatica L.	Pharsniya	400-1500	Apr-Nov	Lf, Wd	Fo, Fu, Ed	D	W
Grewia optiva J. R. Drumm.ex Burret.	Bhimal	300-1400	Apr-Nov	Lf, Br, Wd	Fo, Ed, Fu, Fb	D	W
Kydia calycina Roxb.	Patha	300-1200	Jul-Feb	Lf, Wd	Fo, Fu, At	D	W
Stercullia villosa Roxb.	Budella	300-1400	Feb-Jul	Br, Lf, Sd	M, Ed	D	W
Meliaceae							
Azadirachta indica A. Juss.	Neem	350-1000	Mar-Jul	Br, Lf, Fr, Sd, Wd	M, A, Ti, At, A	D	C
Melia azedarach L.	Batain	300-1500	Mar-Feb	Lf, Br, Fl, Sd,Wd	M, Fo, Fu, Ti, At	D	W
Toona ciliata M. Roem.	Toon	600-1800	Mar-Jul	Lf, Wd	Fo, Fu, Ti, At	D	W
Menispermaceae							
Cocculus laurifolius DC.	Til phokar	300-1600	Apr-Jun	Lf, Wd	Fo, Fu	D	W
Moraceae							
Artocarpus heterophyllus Lam.	Kathal	300-1200	Feb-Jul	Fr	Ed	D	C
Ficus auriculata Lour.	Timil	500-1800	Feb-Jul	Lf, Fr, Wd	Ed, Fo, M, Fu, Re	D	W
Ficus benghalensis L.	Bargad	300-1400	Feb-Nov	Wp	Re, Ed, Fo	D	W
Ficus elastica Roxb.	-	400-1700	Apr-Sep	-	O	D	C
Ficus hispida L.f.	Totmila	300-1400	Mar-Jun	Lf, Fr, Br, Wd	Ed, Fo, Fu, Fb	D	W
Ficus neriifolia Sm.	Dhudhi	1200-2500	Mar-Sep	Lf, Fr, Wd	Fo, Ed, Fu	D	W
Ficus palmata Forsk.	Bedu	800-2000	May-Aug	Lf, Fr, Lt, Wd	Ed, Fo, M, Fu	D	W

Ficus racemosa L.	Gular	300-1400	Mar-Aug	Lf, Fr, Wd	Ed, Fo, Fu	D	W
Ficus religiosa L.	Pipal	300-1600	Oct- May	Wp	Re, M, Fo, Ed	D	W
Ficus semicordata Buch.-Ham. ex Sm.	Khunia	700-1500	May-Oct	Lf, Fr, Br, Wd	Ed, Fo, Fu, Fb	D	W
Ficus subincisa Buch.-Ham. ex Sm.	Chanchari	500-1600	Mar-Jun	Lf, Fr, Wd	Ed, Fo, Fu	D	W
Ficus virens Aiton	Pilkhan	500-1300	Feb-May	Lf, Br, Wd	Fo, Fu, Fb	D	W
Morus alba L.	Shatoot	300-2200	Mar-Jun	Fr, Lf, Wd	Ed, Fo, Fu	D	W
Morus serrata Roxb.	Kimu	1000-2800	Mar-May	Fr, Lf, Wd	Ed, Fo, Fu	D	W
Moringaceae							
Moringa oleifera L.	Sehjan	300-1200	Oct-Jun	Lf, Fr, Fl, Br, Rt, Wd	Fo, Ed, M, Fu	D	W
Myricaceae							
Myrica esculenta Buch.-Ham. ex D. Don	Kaphal	1500-2500	Oct-Jun	Lf, Fr, Br, Wd	Ed, M, Fo, Fu	D	W
Myrtaceae							
Callistemon citrinus (Curtis) Skeels.	Bottle brush	300-2500	Mar-Dec	-	O	D	C
Eucalyptus globulus Labill.	Eucalyptus	600-1600	Feb-Jun	Wd	A, Fu	D	W
Psidium guajava L.	Amrud	350-1600	May-Jun	Fr, Lf, Wd	Ed, M, Fu	D	C
Syzygium cumini (L.) Skeel.	Jamun	350-1400	May-Jul	Lf, Sd, Fr, Wd	M, Fu, Ti, Ed.	D	W
Oleaceae							
Fraxinus micrantha L.	Angu	1500-2800	Mar-Sep	Lf, Wd	Fo, Fu, At	D	W
Olea grandiflora Wall. ex G. Don	Garur	600-1800	Mar-Feb	Lf, Wd	Fo, Fu, Ti, At	D	W
Phyllanthaceae							
Glochidion assamicum (Mull. Arg.) Hook. f.	-	600-1100	Mar-Dec	Lf, Wd	Fo, Fu	D	W
Phyllanthus emblica L.	Amla	500-1600	Mar-Jan	Lf, Fr, Wd	Ed, M, Fu, Re	D	W
Pinaceae							
Cedrus deodara Loud.	Deodar	1500-3700	Sep-Dec	Wd	M, Fu, Ti, Re	D	W
Pinus rouxburghii Sarg.	Chir	700-2500	Feb-Jun	Rs,Lf, Wd,	M, Fu, Ti, At,	D	W
				Sd	Ed, Re		
Pittospraceae							
Pittosporum eriocarpum Royle.	Agni	1000-2200	Mar-Nov	Lf, Br, Rt	M, Fo	D	W
Proteaceae							
Grevillea robusta A. Cunn. ex R. Br.	Silver oak	400-2000	Mar-Aug	Lf, Wd	O, Fo, Fu, Ti	D	W
Rosaceae							
Prunus armeniaca L.	Khubani	1200-2500	Mar-Jun	Fr, Lf	Ed	D	C
Prunus cerasoides Buch.-Ham. ex D. Don	Padam	600-2600	Oct-May	Lf, Br, Sd, Fl, Wd	Fo, M, Ed, Fu, Re	D	W
Prunus domestica L.	Pulam	1200-2500	Mar-Jun	Fr, Lf	Ed, Fo	D	C
Prunus persica (L.) Batsch.	Aru	600-3000	Mar-Jul	Fr, Lf	Ed, Fo, M	D	C
Pyrus communis L.	Nashpati	600-2500	Mar-Sep	Fr, Lf	Ed, Fo	D	C

Pyrus malus L.	Seb	1400-2500	Mar-Jan	Fr, Lf	Ed, Fo	D	C
Pyrus pashia Buch-Ham.ex D. Don	Jangli mehal	700-2600	Feb-Dec	Lf, Fr, Wd	Fo, Fu, Ed, At	D	W
Stranvaesia nussia (D. Don) Decne.	Jangli garhmehal	1200-2200	Apr-Sep	Lf, Wd	Fo, Fu	D	W
Rubiaceae							
Haldina cordifolia (Roxb.) Ridsdale	Haldu	500-1100	Jun-Jul	Lf, Br, Rt, Wd	Fo, M, Fu, Ti, At	D	W
Wendlandia heynei (Roemer & Schultes) Santapau & Merchant.	Tirchunia	700-1600	Mar-Jun	Lf, Wd	Fo, Fu	D	W
Rutaceae							
Aegle marmelos (L.) Correa	Bel	300-1200	Feb-Aug	Fr, Lf, Rt, Wd	M, Ed, Re	D	W
Citrus jambhiri Lushington.	Jamir	1000-1800	Mar-Jun	Fr	M, Ed	D	C
Citrus medica L.	Nimboo	1000-2000	Apr-Dec	Fr	M, Ed	D	C
Citrus sinensis (L.) Osbeck	Santara	1200-2000	Apr-Feb	Fr	Ed	D	C
Sabiaceae							
Meliosma simplicifolia (Roxb.) Walp.	Coghsa	1200-2000	Apr-Nov	Lf, Wd	Fo, Fu	D	W
Salicaceae							
Casearia elliptica Willd.	Chilla	600-1200	Apr-Jul	Wd	Fu	D	W
Casearia graveolens Dalzel	Chilla	250-1600	May-Jul	Wd	Fu	D	W
Flacourtia indica (Burm.f.) Merr.	Kangu	600-1800	Jul-Dec	Lf, Fr, Wd	Fo, Ed, Fu	D	W
Populus ciliata Wall. ex Royal	Pahari papal	800-3200	Mar-Jun	Lf, Br, Wd	Fo, Fu, Ti, Fb	D	W
Salix babylonica L.	Majnu	1000-2200	Feb-Jul	Lf, Wd	Fo, Fu	D	W
Salix tetrasperma Roxb.	Gad-bhains	900-1200	Sep-Dec	Lf, Wd	Fo, Fu	D	W
Xylosma longifolia Clos.	Gardar	600-1500	Nov-May	Lf, Wd	Fo, Fu	D	W
Sapindaceae							
Acer oblongum Wall.ex DC.	Putli	1300-2100	Feb-Aug	Lf, Wd	Fu, Fo, Ti, At	D	W
Aesculus indica (Wall. ex Cambess.) Hook.	Jangli Pangar	1400-3200	May-Nov	Lf, Fr, Wd	Fo, M, Fu, A, O	D	W
Litchi chinensis Sonn.	Litchi	300-1200	Feb-Jul	Fr, Wd	Ed, Fu	D	C
Sapindus mukorossi Gaertn.	Ritha	600-1500	May-Feb	Lf, Sd, Wd	Fo, M, Fu	D	C
Schleichera oleosa (Lour.) Oken.	Kusum	300-1000	Mar-Aug	Lf, Fr, Sd, Wd	Ed, M, Fo, Fu, Ti	D	W
Sapotaceae							
Aesandra butyracea (Roxb.) Baehni	Chiura	900-1500	Nov-Jul	Fr, Sd, Lf, Wd	Ed, M, Fo, Fu	D	C
Madhuca longifolia (J. Koenig ex L.) J.F. Macbr.	Mahwa	300-1000	Mar-Jul	Fr, Wd	Ed, Ti	D	W
Symplocaceae							
Symplocos paniculata (Thunb.) Miq.	Lodh	1300-3000	Mar-Dec	Lf, Br, Wd	Fo, M, Fu	D	W

Ulmaceae							
Holoptelea integrifolia Planch.	Kanju	300-1000	Feb-May	Br, Lf, Wd	M, Fo, Ti, Fu	D	W
Urticaceae							
Boehmeria rugulosa Wedd.	Gheti	300-1700	Jul-Nov	Lf, Br, Wd	Fo, M, Fu	E	W

Abbreviation Used- Lf- Leaf; Fr- Fruit; Fl- Flower; Br- Bark; Rt- Root; Sd- Seed; Lt- Latex; Res-Resin; Wd- Wood; WP- Whole plant; Fl & Fr- Flowering and Fruiting; Ed- Edible; Fo- Fodder; Fu- Fuel; M- Medicinal; Ti- Timber; Re- Religious; At- Agricultural tools; O- Ornamental; A- Avenue; Fb- Fibre; TH- Tree Habit; So- Source; D-Deciduous; E- Evergreen; C- Cultivated; W- Wild.

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The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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