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Synergistic Larvicidal Activities

Insecticidal Property of Jatropha Plant

Discovering Thoughts, Inventing Future

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Analysis of Zooplankton in Chenugonipally Pedda Cheruvu Gadwal, Telangana State. India

By J. Mahender, A. V. Rajashekhar, K. Ramesh & Md. Kaleem
Osmania University

Abstract- Zooplankton is an integral component of ecosystem and comprises of microscopic animal life that passively float or swim freely. This serves the functional role on the detrital spectrum in water. Chenugonipally Peddacheruvu tank is one of the irrigation tank. It is a small size tank, located between 16.23° N latitude and 77.8° E longitudes in Gadwal Mandal of Mahabubnagar district in Telangana state. Present work has been conducted on zooplankton analysis samples were collected at 30 days of interval between 7 am to 9 am for a period of two years from July 2012 to May 2013 and from July 2013 to May 2014. During the study period total 40 species of zooplanktons were identified belonging to 5 groups, among all 19 species were observed during the 1st year (2012-2013), while 21 species of zooplanktons were recorded during the 2nd year (2013-2014) study. At all the four stations during first year study the dominance of the zooplankton species as follows ;Rotifera > Copepoda > Cladocera > Ostracoda > Protozoa. Similarly during second year study the dominance of the zooplankton species as follows; Copepoda > Cladocera > Rotifera > Protozoa > Ostracoda.

Keywords: *chenugonipally pedda cheruvu, zooplankton, dominance.*

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J. Mahender [α], A. V. Rajashekhar [σ], K. Ramesh [ρ] & Md. Kaleem [ω]

Abstract- Zooplankton is an integral component of ecosystem and comprises of microscopic animal life that passively float or swim freely. This serves the functional role on the detrital spectrum in water. Chenugonipally Peddacheruvu tank is one of the irrigation tank. It is a small size tank, located between 16.23° N latitude and 77.8° E longitudes in Gadwal Mandal of Mahabubnagar district in Telangana state. Present work has been conducted on zooplankton analysis samples were collected at 30 days of interval between 7 am to 9 am for a period of two years from July 2012 to May 2013 and from July 2013 to May 2014. During the study period total 40 species of zooplanktons were identified belonging to 5 groups, among all 19 species were observed during the 1st year (2012-2013), while 21 species of zooplanktons were recorded during the 2nd year (2013-2014) study. At all the four stations during first year study the dominance of the zooplankton species as follows ;Rotifera > Copepoda > Cladocera > Ostracoda > Protozoa. Similarly during second year study the dominance of the zooplankton species as follows; Copepoda > Cladocera > Rotifera > Protozoa > Ostracoda.

Keywords: chenugonipally pedda cheruvu, zooplankton, dominance.

I. Introduction

Zooplankton is an integral component of ecosystem and comprises of microscopic animal life that passively float or swim freely. The principal components of zooplankton in lentic environment are represented by taxonomic groups Protozoa, Cladocera and Copepoda. In the reproductive biology of many fish, eggs and hatchlings pass through transient Planktonic life. Zooplankton incorporates primary and partly secondary micro faunal consumers operative system. This serves the functional role on the detrital spectrum in water. Zooplankton operations facilitate food web connectivity and cascading interactions in trophic structure of aquatic communities. The multitude of micro level transfer, transformations of biomass and energy mediated by zooplankton help to sustain stability and health of ecosystem. In trophic progression, zooplankton excretions provide nutrient pools in macro habitat for ready exploitation and proliferation by Cyanobacteria. In any prevailing milieu of aquatic pollution and anthropogenic impact activities, qualitative and quantitative changes in the diversity of zooplankton characterize plasticity and resilience and critical pollutant tolerance load of the focused ecosystem. The preferential distributions of species in zooplankton assemblages of saprobian systems provide a bio monitoring tool to maintain quality assurance in sewage treatment technology operations. Zooplankton is essential food for hatchlings and fingerlings in fish biology. The propensity of Rotiferans and Cladocerans is too built up rapidly high population densities under favourable environments. In culture fishery management, this offers opportunity to technology application for their mass culture to meet cost effective live food inputs. In toxicological studies on bio accumulation and bio magnification along food chains, zooplankton species are valuable experimental tools and tags.

II. Materials and Methods

a) Description of study area

Chenugonipally Peddacheruvu tank is one of the irrigation tanks in the Mahabubnagar district, about 90 Km away from the district headquarters and 180 Km away from the state capital Hyderabad. It is a small size tank, located between 16.23° N latitude and 77.8° E longitudes in Gadwal Mandal (Tehsil) of Mahabubnagar district in Telangana state. The present Chenugonipally Peddacheruvu tank receives water from the Jurala Project right canal and also receives sewage water directly from the Gadwal town. It is a perennial tank and the fishermen stocks advanced size carp fish seed every year in the month of July or August.

b) Collection of sample

Present work has been conducted on zooplankton analysis, for this 4 sampling sites of Chenugonipally Peddacheruvu tank was selected for the qualitative analysis of Zooplankton. Site 1 was fixed at near the Chenugonipally village, site 2 in the middle of the tank, site 3 was fixed near the outlet and site 4 was fixed near the Gadwal town side. Zooplankton were collected at 30 days of interval between 7 am to 9 am for a period of two years from July 2012 to May 2013 and from July 2013 to May 2014.

For the collection of zooplankton a known amount of water was filtered through the zooplankton net made up of nylon silk mesh size 55 μm for the precise collection of planktons net is towed horizontally and vertically. After collection of zooplanktons immediately transferred to plastic bottles and are preserved in 4 % formaldehyde.

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III. Results and Discussion

During the study on chenigonipally pedda cheuvu for a period of two years study (2012-13 & 2013-14), total 40 species of zooplanktons were identified belonging to 5 groups, among them 19 species were observed during the 1styear (2012-2013), while 21 species of zooplanktons were recorded during the 2ndyear (2013-2014) study. The number of organisms were tabulated according to their group wise in table no 1, 2, 3, 4, and 5. At all the four stations during first year study the dominance of the zooplankton species as follows

Rotifera > Copepoda > Cladocera > Ostracoda > Protozoa

Group Rotifera was recorded as the dominant group as it comprising of 5 species followed by Copepoda and Cladocera which both of these two groups comprising of 4 species each, which in turn followed by Ostracoda 3 spevies and protozoa 3 species. *Thirupathaiah. M et al (2012)* reported that diversity of zooplankton in Lower Manair reservoir, Karimnagar, AP, India. High rotifer species in the water body indicates enrichment due to direct inflow of untreated domestic sewage from adjacent areas, as it was suggested by *Arora (1966). Chandrashekhar (1998)* recorded diversity of rotifers to be influenced by the different water quality and other chemical factors. Rotifers dominance was also reported by *Solanki VR et al., (2015)* Pandu lake of Bodhan, Telangana State. The zooplanktons community fluctuates according to physico-chemical parameters of the environment, especially rotifer species change with biotic factors *(Karuthapandi et al 2013). Perumal and Santhanam (2002)* reported 37 species of zooplanktons in Vedanthangal lake, Tamilnadu.

Similarly during second year study the dominance of the zooplankton species as follows

Copepoda > Cladocera > Rotifera > Protozoa > Ostracoda

Group Copepoda was recorded as the dominant group during second year study comprising of 6 species followed by Cladocera comprising of 5species, Rotifera comprising of 4 species, which in turn followed by least dominance of Protozoa and Ostracoda comprising of 3 species each. The diversity patterns greatly depend on the water temperature and availability of food in the water body. *Avinash B et al., (2014)* suggested that species diversity indices of zooplankton from Sadatpur reservoir, Ahmednagar. The sufficient nutrient availability and other favourable conditions result in dominance of Copepoda. *Salve B et al., (2010)* observed that the diversity of Zooplankton in Wan reservoir, Nagapur (MS) India. The comparisons of size structure, fecundity and reproductive strategies of zooplankter's can indicate the nature and extent of pollutant loads.

Table 1 : List of zooplankton group Protozoa species obtained in two years of investigation

Group	Name of the Plankton	I Year	II Year
Protozoa	*Colpidium sp.*	√	√
	Arcella sp.	√	√
	Actinophyrus sp.	√	√

Table 2 : List of zooplankton group Rotifera species obtained in two years of investigation

Group	Name of the Plankton	I Year	II Year
Rotifera	*Brachionus sp*	√	√
	Cephalodella sp	√	–
	Trichocerca sp	√	√
	Keratella sp	√	√
	Euchlanis sp	√	√

Table 3 : List of zooplankton group Cladocera species obtained in two years of investigation

Group	Name of the Plankton	I Year	II Year
Cladocera	*Macrothrix sp*	√	√
	Bosmania sp	–	√
	cerodaphnia sp	√	√
	Dadaya sp	–	√
	Daphnia sp	√	√
	Chydorous sp	√	–

Table 4 : List of zooplankton group Copepoda species obtained in two years of investigation

Group	Name of the Plankton	I Year	II Year
Copepoda	*Cyclops sp*	√	√
	Diaptomous	√	√
	Eucyclops sp	–	√
	Mesocyclops sp	√	√
	Cyclopoid copepod sp	√	√
	Nauplius larvae	–	√

Table 5 : List of zooplankton group Ostracoda species obtained in two years of investigation

Group	Name of the Plankton	I Year	II Year
Ostracoda	*Cypris sp*	√	√
	Cyclocyprus sp	√	√
	Stenocypris sp	√	√

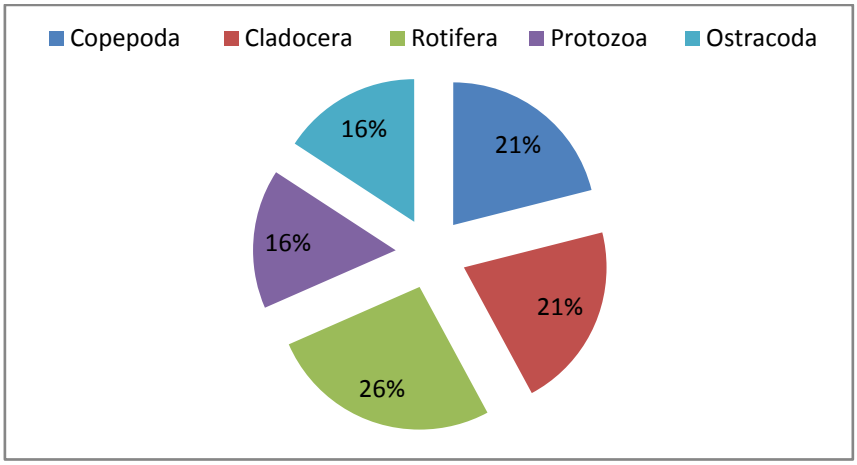

Figure 1 : Zooplankton composition during 2012-13

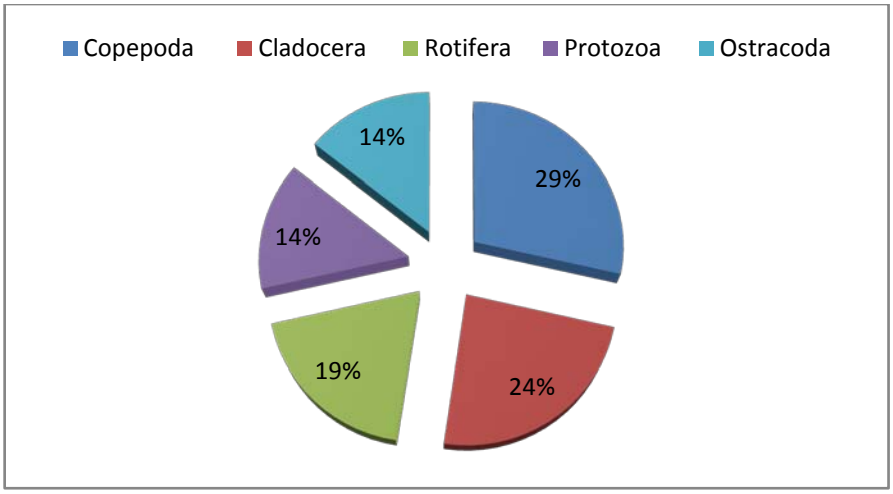

Figure 2 : Zooplankton composition during 2013-14

IV. Conclusion

Rotifer species are considered as good indicators of the trophic state of the water and exhibited high species richness and diversity. Rotifers respond more quickly to the environmental changes and used as a change in water quality. Copepoda appeared to be the most dominant community in the study second year study. Overall zooplankton diversity and abundance in Chenugonipally pedda cheruvu indicated that the water body is rich with nutrients and mesotrophic in nature.

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The Synergistic Larvicidal Activities of Three Local Plants on *Aedes aegypti*

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Abstract- The synergistic activity of extracts of *Lantana camara*, *Stachytarpheta indica* and *Allamanda blanchetii* against *Aedes aegypti* mosquito larvae were investigated. Ethanolic leaf extracts of these plants were tested separately and combined on *Ae. aegypti* larvae with concentration ranging from 5, 10, 20, 30 and 40mg/ml. In 72 hours bioassay experiment, mortalities were observed at the different time intervals but were highest at 40mg/ml concentration for the three plants independently, with the *L. camara* extract showing better larvicidal activity over the other plant extracts at 48 hours and 72 hours exposure. The LC_{50} of *L. camara* (6.08mg/ml), *S. indica* (8.15mg/ml) and *A. blanchetii* (6.44g/ml) indicates their ability to cause 50% larval mortality at such low concentrations. For the synergistic effects, all the concentrations exhibited high mortality at 48 hours and 72 hours exposure. The 40mg/ml showed the highest larvicidal activity (100% mortality) after 48 hours exposure with the L2:A1:S1 combination. Synergistic factor (S.F.) of 1.00, 1.27 and 0.94 were obtained for *L. camara*, *S. indica* and *A. blanchetii*. Synergism was recorded for *L. camara* and *S. indica*, while antagonism was recorded for *A. blanchetii*.

Keywords: *extracts, ethanol, synergism, mosquito, control, aedes.*

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Keywords: extracts, ethanol, synergism, mosquito, control, aedes.

I. Introduction

Mosquitoes are well known as annoying biting pests and vectors of disease-causing agents to humans and other animals. Mosquito alone transmit diseases to more than 700 million people annually (Someshwar *et al*, 2011). Though mosquito – borne diseases currently represent a greater health problem in tropical and subtropical regions, no part of the world is immune to this risk (Fradin and Day, 2002; Taubes, 1997). *Aedes* mosquitoes are widely distributed in Africa and can serve as vectors of yellow fever and dengue virus. When their distribution is combined with rapid population growth, unplanned urbanization, and increased international travel, extensive transmission of these diseases is more. Many African cities now have an increasing number of overcrowded, informal settlements, or 'shanty towns', characterized by low-grade housing, poor roads, inadequate water supplies, sanitation and waste management services and most people who live here have no access to running water and store drinking water in containers which often serve as breeding sites for *Aedes aegypti*, the primary vector of urban yellow fever. In addition, the lack of public sanitation services in many large cities prevent the removal of other artificial breeding sites such as metal cans, tires or derelict vehicles. According to the World Health Organization, there are currently 200,000 worldwide cases and 30,000 deaths from yellow fever per year, 90% of them in Africa, and as many as 50 million cases of dengue (WHO, 2009). The worldwide threat of arthropod transmitted diseases, with their associated morbidity and mortality underscores the need for effective control measures. Over the past decade, phytochemicals have been given progressively more attention as insecticidal alternatives. However, most studies on the synergistic and additive toxic effects of mixtures involving phytochemicals have been conducted on agricultural pests rather than vectors of diseases (Essam *et al*, 2005). Combined effect or synergistic effect of various control agents have proved very advantageous in the control of various pests (Caraballo, 2000; Pathak and Shukla, 1998). In this regard therefore, there is a need to also study vector control of various human diseases using a combination of botanicals, this will checkmate the menace posed by pests of public health importance. This work however tries to investigate the individual and synergistic larvicidal effects of *Lantana camara, Stachytarpheta indica and Allamanda blanchetii* on *Aedes aegypti* mosquito and to determine the active compounds that confer mosquitocidal properties on these plants and also to establish the use of these plants as alternative in the control of *Aedes aegyti* mosquito.

II. Materials and Methods

a) Plant collection

The plant materials used in this study are the fully developed leaves of *Lantana camara (Verbenaceae)*, *Stachytarpheta indica (Verbenaceae)* and *Allamanda blachetii (Apocynaceae)*. The mature leaves of *Lantana camara* and *Stachytarpheta indica* were locally collected in and around Ifakala Community in Mbaitoli Local Government Area of Imo State, South-

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East Nigeria, while the mature leaves of *Allamanda blanchetii* were collected from Amata Community in Ikeduru Local Government Area of Imo State, South-East Nigeria. The plants were identified by Dr. C. M. Duru, a botanist in the department of Biology, Federal University of Technology Owerri, Imo State, Nigeria and was later authenticated by a technologist in the department of Forestry and Wildlife Technology, Federal University of Technology Owerri, Imo State, Nigeria.

b) Sample preparation and extraction

The leaves of the plant specimens were washed with tap water and shade-dried at room temperature between 25-30°C for two weeks. The dried leaves were ground into fine powder from which the extracts were prepared. Ethanol extracts of the plants were obtained by taking 100g of the powdered-dried leaves in a container and 500ml of ethanol was added and kept for twenty-four hours with periodic shaking, then filtered and the filtrates were collected. The ethanol extracts were concentrated by rotary vacuum evaporator at 40°C and evaporated to dryness and stored at 4°C in air – tight bottle until use (Dong *et al*, 2005).

c) Test insect

The test insect used in this study is mosquito of the species *Aedes aegypti*. The eggs of *Ae. aegypti* were collected from the National Arbovirus and Vector Research Centre Enugu (NAVRC), Enugu State, South-East Nigeria. *Ae. aegypti* was obtained as egg colony on a white piece of cloth and reared in white basins containing tap water and maintained at 27±2°C. When the eggs hatched into first (1^{st}) instar larvae after two days, they were fed with yeast powder and biscuit powder in the ratio of 1:3. The larvae were reared until the required fourth (4^{th}) instar larvae emerged on the sixth (6^{th}) day.

d) Larvicidal bioassay

The bioassay were performed at a room temperature of 27±2°C, Relative humidity 70 - 85%, Photoperiod 12:12 (light : dark) and pH 7.0 of distilled water. Bioassays were set up according to a slightly modified version of the standard WHO larval susceptibility test methods (WHO, 1981) under similar conditions used for rearing. The test concentrations used for larvicidal bioassay were 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml and 40mg/ml. Each of the individual plant extracts were weighed according to required concentration and dissolved in 2ml of ethanol. 95ml of distilled water was measured with a 100ml measuring cylinder and poured into each of the containers to be used. The test concentrations dissolved with ethanol were introduced into the containers containing 95ml of distilled water. Then ten of 4^{th} instar larvae of the test insect were selected and counted using micropipette and placed into the small bottles and made up to 3ml mark which was introduced into the containers. For the combination of plant extracts, the same procedure was followed with the individual plant extracts weighed according to required amounts and mixed according to the required ratios for all the test concentrations. The larvae 4^{th} instar of *Ae. Aegypti* were subjected to various specified concentrations of the plant extracts *L. camara* (L100%), *A. blanchetii* (A100%) and *S. indica* (S100%) independently and in combinations in the ratios: L1:A1:S1; L2:A1:S1; L1:A2:S1 and L1:A1:S2 respectively. For each of the concentrations, three (3) replicates were maintained. A control was also maintained by adding 2ml of ethanol to 95ml of distilled water and ten (10) fourth (4^{th}) instar larvae in 3ml of distilled water introduced. The larvae were fed with yeast powder and biscuit powder at the ratio of 1:3 on daily basis (sprinkled on the surface of the water surface). The larval mortality were counted and recorded in percentages (%) at 24, 48 and 72 hours intervals. Dead larvae were removed to avoid decomposition. The median lethal concentration LC_{50} was determined using Levenberg-Marquardt algorithm(1964).The synergistic factor (SF) was calculated using the formula: SF = LC_{50} value of individual plant extract / LC_{50} value of plant with assumed synergist (Susan and Vincent, 2005)

e) Phytochemical analysis

i. *Test for Tannins*

About 0.5g of the plant extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 1% ferric chloride was added and observed for brownish-green or blue-black coloration. The presence of Tannins, Flavonoids, Saponins, Alkaloids, Cardiac Glycosides was noted and recorded.

ii. *Test for Flavonoids*

Dilute ammonia (5ml) was added to a portion of an ethanol filtrate of the extract. Concentrated sulphuric acid (1ml) was added and observed for yellow coloration that disappears on standing. The presence or absence of flavonoids was noted and recorded.

iii. *Test for Saponins*

5ml of distilled water was added to 0.5g of the extract in a test tube. The solution was vigorously shaken and observed for a stable persistent froth. The presence or absence of saponins was noted and recorded.

iv. *Test for Alkaloids*

1ml of plant extract was shaken with 5ml of 2% HCL and heated on a steam bath and filtered. 1ml of the filtrate was treated with o.5ml of Wagners reagent and observed for a reddish-brown precipitate. The presence or absence of alkaloids was noted and recorded.

v. *Test for Cardiac Glycosides*

1ml of lead sub-acetate was added to 2ml of plant extract, shaken and filtered. The filtrate was extracted in an equal volume of chloroform. The

chloroform layer was evaporated to dryness in a dish over water bath. The residue was dissolved in 3ml of 3.5% ferric chloride in glacial acetic acid and left to stand for 1 minute. 1ml of concentrated H_2SO_4 was run down the side of test tube and observed for a blue colour at the interface which is a positive test for deoxy-sugars (Evans, 2002). The presence or absence of cardiac glycosides was noted and recorded.

f) Statistical analysis

Data were analyzed using appropriate software (Microsoft Excel was used for calculating means and standard deviations and performing of analysis of variance (ANOVA). Table Curve 2D Systat, USA was used for mathematical modeling of data). The parameters were estimated by iterative minimization of least squares using Levenberg-Marquardt algorithm (Marquardt, 1964).

III. Results

The present study showed that the extract of the leaf of *Lantana camara Stachytarpheta indica* and *Allamanda blanchetii* expressed larvicidal activity against the 4th instar larvae of *Aedes aegypti*. The mosquito larvae exposed to the plant extracts showed significant changes in behavior. The most pronounced change in behavior observed in the *Ae. aegypti* larvae was the inability to stay at the surface of the water and also showed restlessness which finally resulted to death. These changes may be attributed to the presence of toxic compounds present in the plants. There was no change in behavior observed in the control.

Table 1 : Mean larval mortality of ethanolic leaf extracts of *Lantana camara, Allamanda blanchetii and Stachytarpheta indica* on the 4thstage larvae of *Ae. Aegypti*

Plant	Concentration (mg/ml)	Mean larval mortality (%) Time Interval (hours)		
		24	48	72
Control(with no extract)	0	0	0(0)	1(10)
Lantana camara	5	0(0)	1(10)	2(20)
	10	7(70)	8(80)	9(90)
	20	9(90)	10(100)	10(100)
	30	9(90)	10(100)	10(100)
	40	10(100)	10(100)	10(100)
Starchytarpheta indica	5	0(0)	1(10)	2(20)
	10	1(10)	3(30)	6(60)
	20	4(40)	7(70)	7(70)
	30	5(50)	9(90)	9(90)
	40	7(70)	10(100)	10(100)
Allamanda blanchetii	5	1(10)	3(30)	4(40)
	10	2(20)	7(70)	8(80)
	20	5(50)	8(80)	9(90)
	30	8(80)	9(90)	10(100)
	40	9(90)	10(100)	10(100)

Table 1 represented the dose dependent larvicidal effects of ethanolic leaf extracts of *L. camara*, *S. indica* and *A. blanchetii* on the larvae of *Ae aegypti* at 72 hours exposure. The table showed that the leaf extracts of *L. camara* recorded the highest percentage mortality at 48 hours and 72 hours of exposure followed by *A. blanchetii* and then *S. indica*. The result also showed that the 40mg/ml concentration recorded the highest percentage mortality on *Aedes aegypti* larvae at the various time intervals.

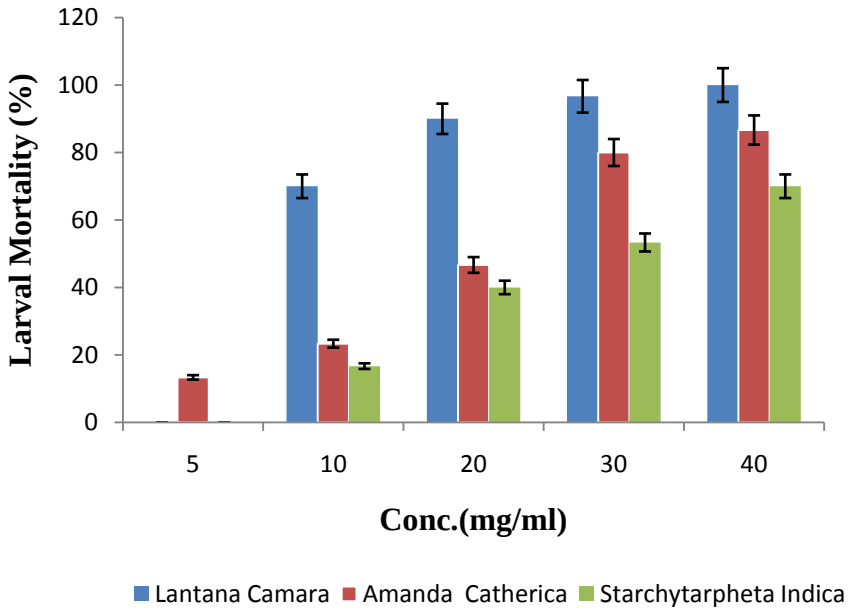

Figure 1 : Comparison of Mean larval mortality(%) of the ethanolic extract of the three plants: *Lanata camara*; *Allamanda blanchetii* and *Starchytarpheta indica* at different concentrations (mg/ml) against *Ae. Aegypti* 4th instar larvae

Table 2 : Mean Larval Mortality of combinations of ethanolic leaf extracts of *Lantana camara, Allamanda blanchetii and Stachytarpheta indica*_on 4th stage Larvae of *Ae. Aegypti*

Concentratiomg/ml	Mean larval mortality (%)		
	Time interval(hour)		
	24	48	72
Control(with no extract)	0(0)	0(0)	1(10)
5			
L1 : A1 : S1	3(30)	3(30)	5(50)
L2 : A1 : S1	4(40)	9(90)	10(100)
L1 : A2 : S1	4(40)	7(70)	8(80)
L1 : A1 : S2	3(30)	6(60)	7(70)
10			
L1 : A1 : S1	6(60)	8(80)	9(90)
L2 : A1 : S1	6(60)	9(90)	10(100)
L1 : A2 : S1	6(60)	8(80)	9(90)
L1 : A1 : S2	5(50)	7(70)	9(90)
20			
L1 : A1 : S1	7(70)	9(90)	10(100)
L2 : A1 : S1	8(80)	9(90)	10(100)
L1 : A2 : S1	7(70)	8(80)	9(90)
L1 : A1 : S2	6(60)	8(80)	9(90)
30			
L1 : A1 : S1	9(90)	9(90)	10(100)
L2 : A1 : S1	9(90)	10(100)	10(100)
L1 : A2 : S1	8(80)	9(90)	9(90)
L1 : A1 : S2	7(70)	8(80)	9(90)
40			
L1 : A1 : S1	9(90)	10(100)	10(100)
L2 : A1 : S1	9(90)	10(100)	10(100)
L1 : A2 : S1	8(80)	10(100)	10(100)
L1 : A1 : S2	7(70)	10(100)	10(100)

L = Lantana camara; A = Allamanda blanchetii; S = Stachytarpheta indica

Table 2 represented the dose dependent larvicidal effects of combination of the ethanolic leaf extract of *L. camara*, *A. blachetii* and *S. indica* on the larvae of *Ae. aegypti* at 72 hours exposure. The combination of the plant extracts at different test concentrations showed high percentage mortality even at low concentration at 48 and 72 hours exposure. The table also showed that the 40mg/ml concentration recorded the highest percentage mortality at 72 hours exposure while the L2:A1:S1 combination recorded the highest percentage mortality over the other combinations at 48 hours exposure. The high larvicidal activity showed by the ratio L2:A1:S1 gives rise to the *L. camara* plant extract being considered as the synergist in this present study.

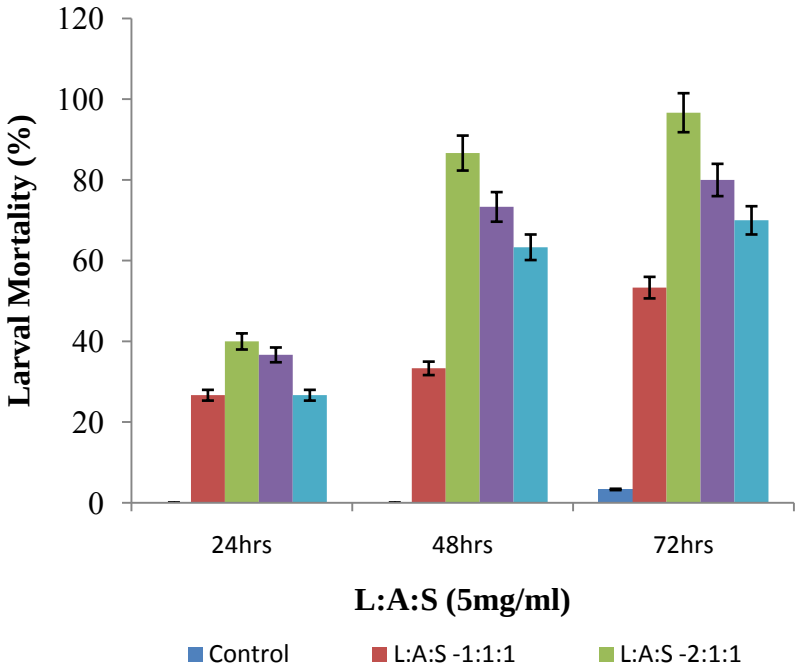

Figure 2 : Mean mortality (%) of the 5mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* 4th instar larvae

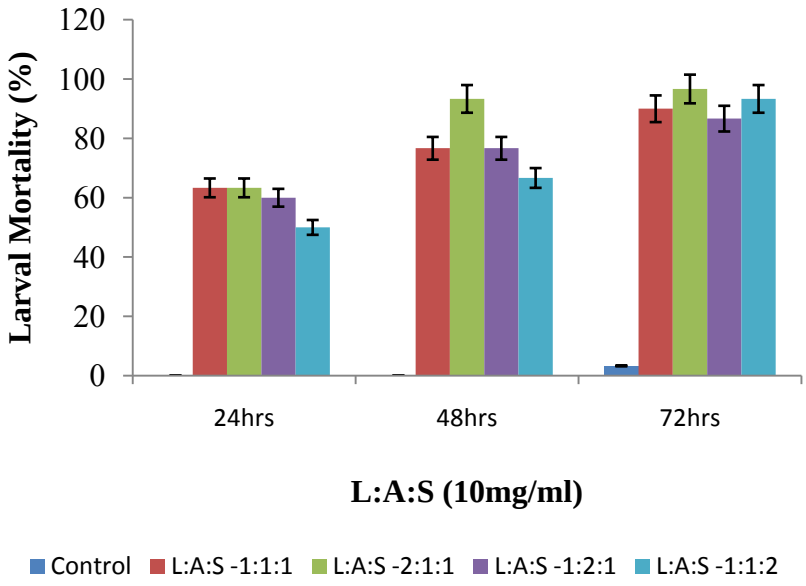

Figure 3 : Mean mortality (%) of the 10mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

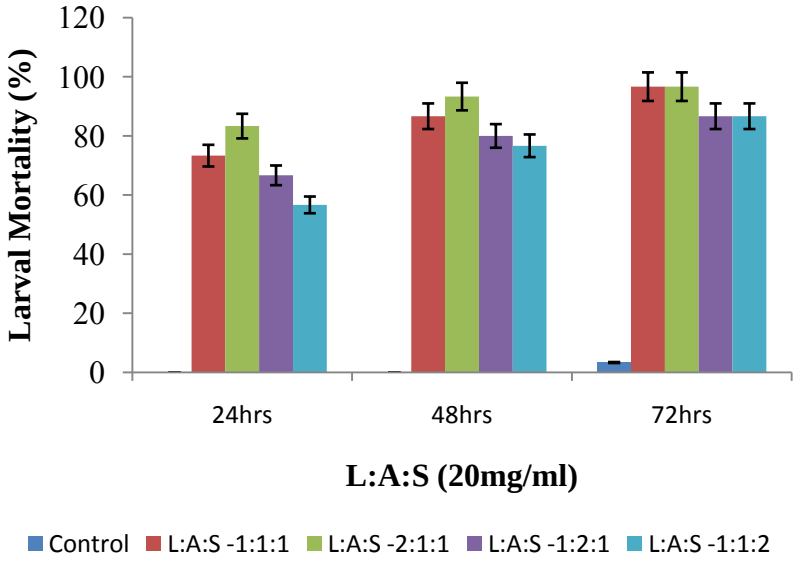

Figure 4 : Mean mortality (%) of the 20mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

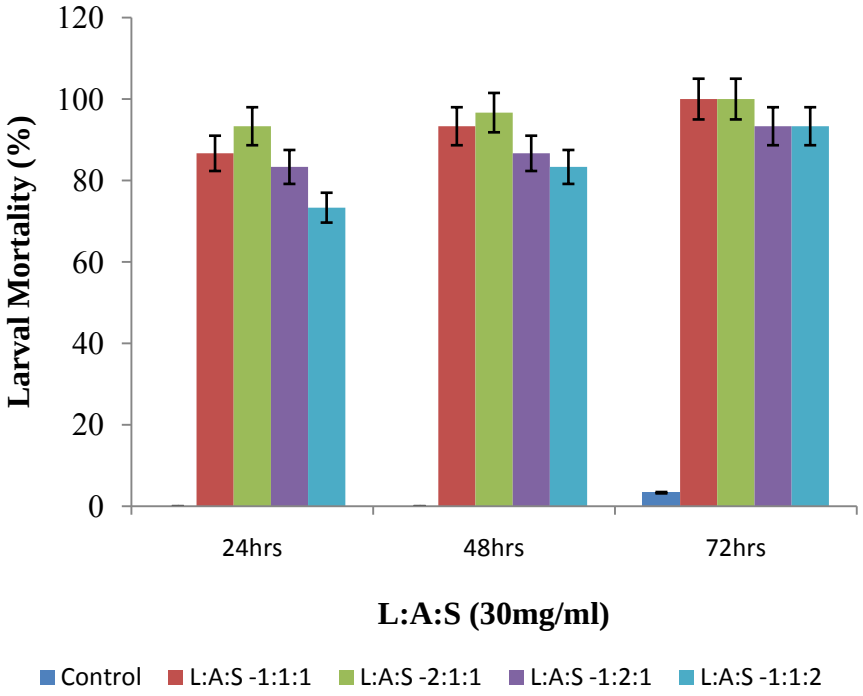

Figure 5 : Mean mortality (%) of the 30mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* 4th instar larvae

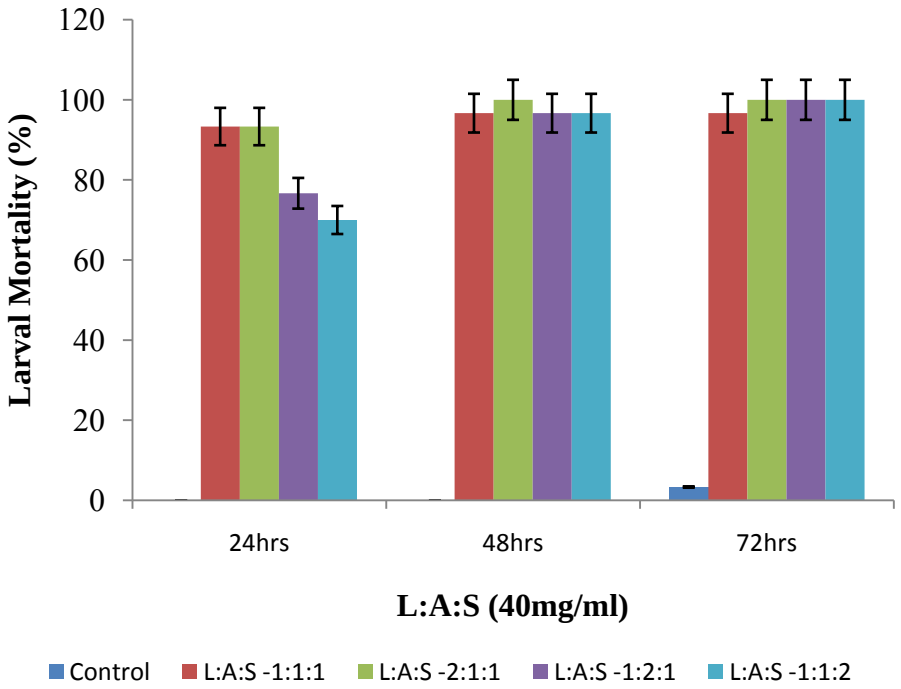

Figure 6 : Mean mortality (%) of the 40mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

Table 3 : Synergistic factor of ethanolic leaf extracts of *L. camara*, *S. indica* and *A. blanchetii* against the 4th larvae of *Ae. Aegypti*

Plant	Synergistic factor	Synergism	Antagonism
Lantana camara	1.00	*	
Stachytarpheta indica	1.27	*	
Allamanda blanchetii	0.94		**

** = synergism; ** = antagonism; ≥ 1 = synergism; ≤ 1= antagonism*

Synergism was observed between *L. camara* and *S. indica* while antagonism was noted between *L. camara* and *A. blanchetii* (Table 3).

Table 4 : Phytochemical analysis of leaf extracts of *L. camara*, *S. indica* and *A. blanchetii*

Phytochemicals	Plant		
	L. camara	*S. indica*	*A. blanchetii*
Flavonoid	+	+	-
Tannin	+	+	+
Alkaloid	+	+	-
Saponin	+	+	+
Cardiac glycosides	+	+	+

+ = present, - = absent

The phytochemicals present in the extracts of *L. camara*, A. *blachetii* and *S. indica* are represented in Table 4. The result showed that tannin, saponin and cardiac glycosides are present in all the three plants while flavonoid and alkaloid are present only in *L. camara* and *S. indica* and absent in *A. blanchetii*.

IV. Discussions

The ethanolic leaf extracts of *Lantana camara*, *Starchytarpheta indica* and *Allamanda blanchetii* were found to exhibit larvicidal activities individually and in combination indicating the mosquitocidal potentials of the leaves of these plants. The result of this study shows that mortalities increased with concentration ($P \leq 0.05$), this confirms the report of (Pelah *et al*, 2005) and (Mehra and Hiradhar, 2000) that there is a positive correlation between concentration and the percentage of the larval mortality.

However, on the individual basis, L. *camara* exhibited a higher larvicidal effect than *S. indica* and *A. blachetii* as was observed with their LC_{50}. The LC_{50} values of the three plants show that they can cause 50% larval mortality at 6.08mg/ml, 6.44mg/ml and 8.15mg/ml (which are low concentrations) which makes them preferable to synthetic insecticides. But combined effects or synergistic effects of various control agents have proved very advantageous in the control of various pests (Seyoum *et al*, 2002). Shaalan *et al* (2002) reviewed different mosquito larvicidal plant species with growth retarding, reproduction inhibiting, ovicides,

synergistic, additive and antagonistic action of botanical mixtures. Susan and Vincent (2005) reported the result of the mixture of *Pongamia glabra* and *Annona squamosa* extracts which exhibited synergistic effect against larvae of mosquitoes. A high larval mortality was recorded for the combination of *L. camara*, *S. indica* and *A. blachetii* at all test concentrations indicating synergism between the plant extracts. Antagonism was recorded for *A. blanchetii*. The 40mg/ml recorded the highest larval mortality after 48 hours exposure and the L2: A1: S1 combination was found to be the best combination over other combinations proposing *L. camara* as a good synergist in combination with other plants in the control of *Ae. aegypti* larvae. This combination exhibited relatively higher larval mortality at various concentrations as compared to others. The effects of these plant extracts on larval mortalities could be attributed to the various chemical components observed in these plants. Chiasson *et al* (2001) and Silva *et al* (2008) report that the bioactivity of the essential oil results from interaction among structural components, particularly the major constituents, the other compounds, even trace elements, which can also have a vital function due to coupled effects, additive action between chemical classes and synergy or antagonism. Several of these secondary metabolites are produced by some plants for their own defense from their enemies, and have been found to have good larvicidal activity such as steroids, essential oils, triterpenes, etc. (Chowdhury *et al*, 2008). Wiesman and Chapagain (2006) reported that saponin extracted from the fruit of *Balanites aegyptica* showed 100% larvicidal activity against *Ae. aegypti* mosquito larvae. A commercial saponin mixture extracted from *Q. saponaria* bark showed increasing toxicity (100% larval mortality) in *Ae. aegypti* and *Cx. pipiens* when both saponin concentration and duration of the experiment were increased (Pelah *et al*, 2005) while the apiperidine alkaloid from *Piper logum* fruits was found to be active against *C. pipiens* mosquito larvae (Lee, 2000) and cardiac glycoside exhibited acaricidal effect against larval and adult stages of the camel tick (Al-Rajhy *et al*, 2003). The results of this present study showed that tannin, saponin and cardiac glycosides are present in all the three plants (*L. camara, S. indica* and *A. blanchetii*), while flavonoid and alkaloid were found to be present in *L. camara* and *S. indica* but absent in *A. blanchetii*. The presence of these secondary metabolites may be responsible for the larvicidal activity against *Ae. aegypti* mosquito larvae, thereby proposing their use as control agents against *Ae. aegypti* mosquito. Someshwar *et al* (2011) observed that some secondary metabolites in combination may be responsible for better effect of larvicidal activity. This present study noted such observation and supports the use of the combination of the leaf extracts of *L. camara, S. indica* and *A. blanchetii* as a better control agent against *Ae. aegypti* mosquito. Susan and Vincent (2005) established that *Pongamia* extract acted as a powerful synergist with *A. squamosa* against mosquito larvae. The performance of combined application of *Neem* and *Karanja* oil cake was reported to be better against the mosquito larvae than their individual application (Shadia *et al*, 2007). According to Lokesh *et al* (2010) in their study, the results obtained showed the combination effects of *T. foenum* and *N. oleander* leaf extracts were much effective on mosquito larvae than the individual extracts. Someshwar *et al* (2011), established that 100% mortality of mosquito larvae was recorded when 0.2% crude extracts of *C. caudatus* fruits and *T. acuminate* flower at 1:1 combinations was applied and it was found to be the best. Though, the individual applications of plant extracts are good against mosquito, the synergistic effects is well established (Shadia *et al*, 2007). It has been observed that individual botanical insecticides are slow acting, time consuming, and active only at high concentration which makes them impractical and uneconomical for field application (Mohan *et al*, 2007; Narasimhan *et al*, 1998). However, the importance of proper selection of plant extracts as synergists in mixed formulations with different botanicals is being increasingly recognized in mosquito management (WHO, 1981). So use of these combinations in mosquito control can be of greater use (Lokesh *et al*, 2010).

V. Acknowledgement

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The Impact of Anthropisation on the Floristic Composition, the Structure and Ecological Characterization of the Ngaoundéré Cliff, Cameroon

By Tchobsala, Ibrahima Adamou, Dongock Nguemo Delphine & Nyasiri Jonathan
University of Ngaoundere

Abstract- A study was carried out on the impact of the anthropisation on woody vegetation of the Ngaoundere cliff. Its main objective was to assess the floristic composition, the structure and the ecological parameters of that vegetation. The research work was carried out on four formations of vegetation (fallows, fields, shrub savannahs, and arborescent savannahs). The floristic composition and structure vegetation were conducted on some plots of land covering 20m x 50m, giving a total of 24 ha on aggregate. The study of the data were done with the help of soft ware's like Excel, STATGRAPHICS plus 5,0 and XLSTAT all showed that the Ngaoundere woody vegetation shows an ''L'' structure under the influence of anthropic activities revealing a great number of waste and a small amount of mature stems. The specific richness is the most elaborate in savannahs planted with trees and the smallest in the fields and fallows lands. The Isoberlinia doka specie offers a comfortable ecological spectrum on the cliff even if it is running high risk of zooanthropic factors. The Cameroon Government should create and authenticate the cliff vegetation for a durable management.

Keywords: *anthropisation, floristic, structure, ecological characterization, ngaoundere cliff.*

GJSFR-C Classification : *FOR Code: 069999*

Strictly as per the compliance and regulations of :

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I. Introduction

The ecosystem deterioration represents today a biodiversity threat for the destruction of natural environments inevitably leads to scarcity, not to say the extinction of some species (Swaminathan, 1990). The populations growing needs in forestry shortages, some agricultural practices, the bush fires have considerably caused degradation which is more and more characterized by these habitats. This appears through the modification of floristic composition, the vegetation structure and poor natural regeneration of some species (Diatta *et al.,* 2009). Deforestation is one of the blights experienced by most of the forest around the world. It is mostly unbridled in tropical areas where the exploitation of the wood and agriculture are the main sources of wealth. The African continent is also affected by the extension of the forest (4 million of hectares per year). The convention on the biological diversity estimated in 2000 that 54,000 plant species and 5,200 animal species faced extinction, and this is mainly due to human action (SCBD, 2000). In Cameroon, the Adamawa region is the prone to natural and anthropic pressures. Real figures on the occupation of the Ngaoundéré soils showed that the savannah areas have diminished from 10.8% in 2001 in comparison with the one in 1951 (Tchotsoua, 2006). The main factors of that degradation are slash-and-burn cultivation (Zapfack, 2005), the combined effects of deforestation, the bush fires and pasture (Ntoupka, 1994, 1998; Tchobsala, 2011), the hydroelectric and mining exploitation and the population growth (Tchotsoua, 2006). The Ngaoundere cliff is under high anthropic exploitation, that is to say the intensification of farming, the over pasturage, the road constructions, camping and immigrant installations. All these threats contributed to the considerable reduction of the forestry surface of the area and consequently to the overall shape and to the specific resources of the vegetation. In view of this serious degradation of the environment in our planet the safeguarding of biodiversity remains the main tool of the 21st century (SCBD, 2000; UNSECO, 2009). It is for that reason that many research works have been carried out on the management methods, the conservation and the development of the savannahs throughout the Adamawa region (Tchotsoua 1996, 2006; Tchobsala 2011), but it is worth mentioning that no research work has been carried out on the impact of anthropisation of Ngaoundere cliff vegetation which is a non-conventional forested zone. This research has as main objective the study of anthropisation impact on the floristic composition, the structure and ecological characterization of the Ngaoundéré cliff so as to recommend its sustainable protection.

II. Material and Methods

a) Localization of the area of research

The locality of Wack, situated 50Km from Ngaoundere (picture 1) was chosen as the area of the investigation. The climate is a Soudano-Guinean type, mild and fresh with two seasons: the rainy and dry seasons (Yonkeu, 1993). The annual pluviometer

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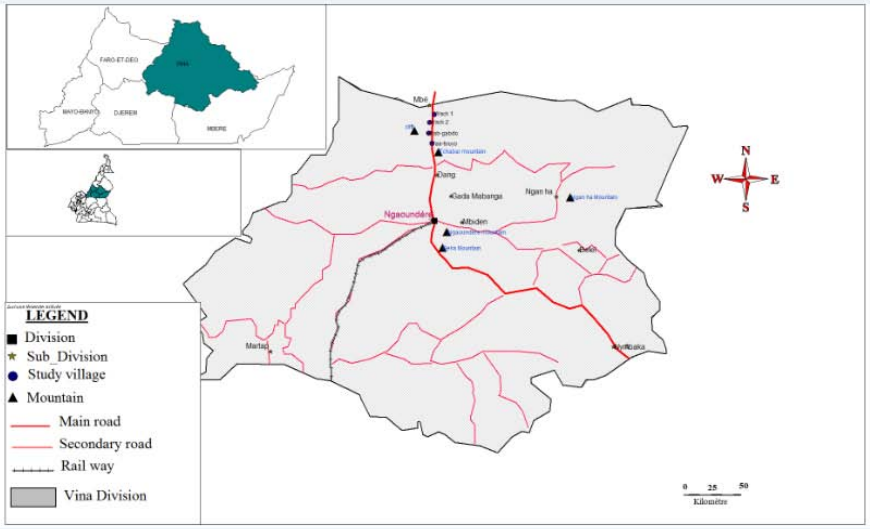
reaches 1479 mm with 9.8% of variation margins. Extreme temperatures vary between 5 and 7°C minima to 30 and 35°C maxima (Mope, 1997). In the Adamawa Region in general and in Ngaoundere in particular the vegetation is made up of very open shrub savannah, with the presence of some species such as *Adansonia digitata, Ziziphus mauritiana, Tithonia diversitfolia, Vitex dononia, Annona senegalensis, Piliostigma thonningii, Entada africana,* all streaming from lateral transition of the dense forest to the graminaceous and herbaceous savannah made up of species such as *Manihot esculenta, Cassia javanica, Annona squamosa, Hibiscus esclentus, Hibiscus sabdarifa, Arachis hypogea, Pennisetum purpureum* (Mopongmetsem, 2005).



Picture 1 : Area of research localization Map

b) Formula notices and ecological description of the vegetation

The collection of anthropisation indexes, dendrometric parameters of the environment were carried out on 24 areas, this is when one is either in or out of the cliff from four different formations of vegetation (fallow lands, farming fields, shrub savannahs, arborescent savannahs). They were carried out on every 300m on areas covering 20mx500m following six straight transects. The experimental system is a complete randomized group with four formations of vegetations (M_1, M_2, M3 and M_4), six transects (T_1, T_2, T_3, T_4, T_5, and T_6) and with four occurrences (R_1, R_2, R_3 and R4) (Table 1). All existing woody species found in small plates were systematically listed and counted. Those species were identified through their scientific names. The unidentified subjects were collected with the help of specialists and built up a herbarium in view of indentifying and confirming the Wakwa herbarium. Ecological depicted standard forms were filled simultaneously with the realization of thriving statement. In each small plate, all the trees species were taken down and for all the 1.30m subjects, the following dendrometric parameters were assessed; the height to draw up the structure of the population, the diameter of the tassel, the tour of the trunk of a tree which is 30cm from the floor to assess the surface of the ground and examine the ligneous distribution depending on the categories of circumferences. For the tassel measuring, two measurements were done at the foot of each tree in accordance with the cardinal points; North- South and East- West and this is the average of the two proportions which represented the diameter of the tassel. For mullicaul subjects, the circumferences of the biggest, the medium and the smallest stems were assessed for the medium circumference is worked out. The geographic details of stock taking thriving small plates were recorded thanks to the GPS

Table 1 : Experimental device

R= notice, T = transect, M = formation of vegetation, 1, 2, 3 and 4 respectively represent fallow land, field, shrub savannah and planted tree savannahs.

	T1	T2	T3	T4	T5	TS6
R1	M1	M1	M2	M2	M4	M4
R2	M1	M1	M3	M3	M3	M4
R3	M1	M2	M2	M3	M3	M4

c) Treatment and analysis of data

The basic knowledge exploited concern the appraisal of the frequency and the affluence of dominance. The frequency here represents the number of subjects of a species on the total absolute frequency of a species depicts the total number of projection where the species is actively involved. According to Braun- Blanquet (1932) training that species and the total amount of reports time 100.

FR (%) = A/B x 100 with, FR (%) = relative frequency, A= processing of the species while B= total number of processing.

This proportion or frequency is to determine the number of subjects made up of accidental species, incidental species, species which are frequent enough, frequent and very much frequent (Table 2).

Tableau 2 : Frequence indices of Braun-Blanquet (1932)

Indices	Frequency	Type of species
I	F < 20	accidental species
II	20 < F < 40	incidental species
III	40 < F < 60	enough species frequent
IV	60 < F < 80	frequent species
V	80 < F < 100	very much frequent species

Excess can refer to the total number of the subjects of each species. The excess of the species can be either absolute or relative. Absolute excess is the total number of subjects from a species out of the total number of subjects in the investigated site. Relative excess is the ratio between absolute excess out of the total number of the subjects of the community.

Dominance on the other hand is the collection of the subjects from each species and this is done with the help of percentage. Absolute dominance is the connection with the total earth's surface of the species (STTC) over the total earth's surface of the community time 100 DR = $\frac{STTe}{STTC}$x100

The relative Curtis value importance is the sum of the relative density, the relative frequency and the relative collection. IVCR (%) = FR+DR+DeR with IVCR: Curtis value Importance, FR=Relative frequency, DR=Relative dominance and DeR= Relative density. To get reliable results, we equally undertook to make some density calculations, by species and by the expansion stage of some species.

d) Relative density

The relative density deals with the following formula; D=N/S with N=number of the species in the research area and S= area occupied by the species. To this, the earth's surface was worked out.

e) Earth's surface

The earth's surface was worked out through the formula. Gi=TT D_H^2/4 where Gi is the earth's surface of the species i, D_H being the tassel diameter of the species.

f) Diversity and equitability indices

Specific diversity is tasted with the help of range indexes (Magurran, 1988; Kent and Coker, 1992). In fact, many types of mathematic formula's help to calculate these indexes. Among these are those which were selected and which are of common usage. These are the following;

g) The Shannon indices

The Shannon- Weaver also known as the Shannon – Wiener is an index which helps to assess biodiversity. This index indicates specific resources. The following formula illustrates it;

$$H' = -\sum_{i=1}^{s} Pi \ln Pi$$

H'; Shannon's biodiversity index, i; medium species; P (i): proportion of a species with regard to the total number of species (S) within the survey milieu (or specific diversity of the milieu) calculated as follows: P (i) = ni/N, where ni is the number of the subject of the species and N the total number of subjects, all species taken together.

The Shannon index is use to quantify the heterogeneity of the biodiversity of a survey area, hence to observe the rapid change in time. This theory should be associated with Simpson's.

h) The Simpson index

The Simpson index (D) is a method which helps to calculate the probability of two randomly selected subjects in a given area should be of the same species

$$D = \sum Ni\ (Ni-1)/N\ (N-1)$$

D = Simpson index; Ni= number of subjects of a given species; N= total number of subjects. The index will vary between 0 and 1. The more will get closer to 0, the more chances of getting different subjects of different species will be high. Besides these two indexes, we can work out the fairness of Pielou (E) which is the reverse of Shannon's index.

i) Jacquard's Similarity ratio

The Jacquard's similarity ratio (PS) (Floch, 2007) allows us to compare the different breeding grounds. It is shown through the following formula;

$$PS = \frac{c}{a+b-c} \times 100$$

Where a= the number of species from list a (breeding ground 1); b= number of species from list b (breeding ground 2); c= number of species common to the two breeding grounds.

The similarity between the housings is represented by the high merit of the index.

j) Hamming distance

The hamming gap proposed by Daget *et al.* (2003) quoted by the Floch (2007) is added to this index to compare the floristic statement. Its can shown through the following formula: H = 100 – PS where PS is the Jacquard's Similarity ratio. The deduced thresholds are divided up in Table 3.

Table 3 : Comparison threshold of floristic statement according to the hamming gap

Threshold	Comparison
H< 20	very low floristic difference
20<H<40	low floristic difference
40<H<60	medium- sized floristic difference
60<H<80	strong floristic difference
80<H	very strong floristic difference

k) Vertical structure of the Ngaoundere woody cliff

The woody subject distribution in terms of diameter and height class was conducted. For the diameter class distribution, Letouzey (1968) method was adopted. In this classification, the subjects are distributed in to four classes:

- the lower stratum made of shrubs with 0 to 10 cm of diameter.
- the stratum with small trees and with 10 to 20 cm of diameter.
- the stratum with mean trees and with 20 to 30 cm of diameter.
- the medium- sized stratum made of trees whose diameter is equal to or greater than 30 cm.

On the basis of result obtain from the height measurements, the subjects were afterwards simplified in greater classes: regeneration, future standard trees, standard trees and great trees.

l) Biological categories

They were worked out according to Raunkiaer (1934) classification, and adapted to tropical regions by Schnell (1971). The phanerophytes (PH) which are plants whose sprouting buds are located at a significant distance from the earth. According to their heights, they have:

a. Phanerophytes including

i. Megaphanerophytes (MgPh), whose heights are around 30 m;
ii. Mesophanerophytes (MsPh), whose heights range between 10 to 30 m;
iii. Microphanerophytes (McPh), whose heights range between 2 to 10 m;
iv. Nanophanerophytes (NnPh), shrubs with heigths under 0.4 to 2 m.
v. Phaneorophytes lianescents (Phgrv. Phgr); voluble plants with gimlets, cramps roots, crawling or supported;
vi. Phaneorophytes epiphytes (Phep).

b. Chameophyts (CH)

i. Chameophytes drawn up (Chd);
ii. Chameophytes prostrate (Chpr);
iii. Chameophytes crawling (Chrp);
iv. Chameophytes climbing (Chgr);

c. Geophytes (G)

Plants whose persistent growths or buds are sheltered in the ground during the bad season:

i. Rhizomateux geophytes (Grh);
ii. Tuberaux geophytes (GB);
iii. Geophytes climbing (Ggr);
iv. Geophytes epiphytes (Gep).

d. Hemicryptophytes (H)

plants which the growths are located at the short-nap cloth of the ground:

e. Theophytes (Th)

Annual plants or at very short growing period, deprived of persistent buds themselves and whose survival is ensured by seeds, they include:

i. Theophytes drawn up (Thd);
ii. Theophytes prostrate (Thpr);
iii. Théophytes scapeux (Thsc).

f. Hydrophytes (Hy); watery plants

Phytogeographical types

The principal phytogeographical types are those admitted for Africa (Lebrun, 1947). The recognized types are the species with broad geographical distribution including:

i. Cosmopolitans (Cos): Species found throughout the whole world;

ii. Pantropicales (Pan): Species known in Africa;
iii. Tropical-American and Asian;
iv. Afro American (AA): Species extended in tropical Africa and America;
v. Paleotropical (Pal): Species present in Africa and tropical Asia, Madagascar and Australia;
vi. Afromalgaches (AM): Species common to the islands of Madagascan and central African areas;
vii. Multi-regional African (PRA): Species whose surface of distribution covers several floristic African areas or two floristic areas which are not in contact.

We also have guineean and soudano zambezian species (G-sz) species including:

i. Sub-Omni guineo-congolese (GC): Species presented in all the floristic guinean area;
ii. Central (CG): Species whose surface of distribution goes from Cameroon to the Democratic Republic of Congo;
iii. Western guineans (WG): Species which are widespread of Western Africa in Western Cameroon;
iv. Cameroon-Congo (CaCo): Species only found in the Cameroonian solid mass and the Congolese basin;
v. Cameroon-Gabon (Ca-Gab): known species only of the forest solid mass Cameroon Gabon-Mayumbe.
vi. Cameroon (Camwood): Species only found in Cameroon.

m) Dissemination types of diaspores

The types of dissemination of diaspores were given according to the classification of Dansereau and Lems (1957). The various types are:

i. Pterochores (Ptéro): Small diaspores with aliform appendices;
ii. Pogonochores (Pogo): Diaspores with feathery or silky appendices;
iii. Slerochores (Scléro): Not fleshy and relatively light diaspores;
iv. Sarcochores (Sarco): Diaspores completely or partially fleshy;
v. Desmochores (Desmo): Diaspores hanging or adhesive;
vi. Ballochores (Ballo): Diaspores expelled by the same plants;
vii. Barrochores (Baro): Not fleshy diaspores;
viii. Pleochores (Pleo): Small diapores with a floating appendix.

n) Statistical analysis of the data

After going through and bringing together the data, a matrix known as the taking down of species was elaborated on the basis of the presence/absence of species on line and steering column for statements. The elaborated matrix was submitted to diversified analysis techniques with the view in aim of highlighting the species dispersion as well as the main plant formations that are brought out. Some variance analysis with the help of the XLSTAT and STATGRAPHICS plus 5.0 software were used to check the gaps between the number processing and the surface. In the presence of significant variance analysis, the medium- sized comparisons were conducted with the help of the Fisher Test. In addition, the difference in composition of the various environments (variety of a formation of vegetation) was tasted with multi varied variance analysis. Parameters such as height, denseness, ground surface and the diameter served as the Principal Component Analysis (PCA).

III. Results

a) The degradation indications of the Ngaoundere cliff vegetation

You can find in the Ngaoundere cliff oxen attached to pasture and some cow dung in the penning (photo1). These pasture indexes are clear signs that the vegetation of the cliff is in a very vulnerable position, both to native and non native animal's breeders. The cliff bordering population produce bush fire either to hunt or for pasture regeneration (early fire), or to get field ready for culture (late fire). Bush fire cause a lot of damages on woody species. Some inhabitants prefer to completely cut down trees which can serve as cultivating fields or for iron works (photo 2). House, path and road camping (photo3) created at the Ngaoundere cliff make up non negligible anthropisation indications for the rapid cliff degradation. The different anthropisation indication has its origin from the degradation of the Ngaoundere cliff.

Picture 2 shows the distribution of 24 ha for the surface used for experimentation. From this picture there is a clear evidence that 47,87% of 24ha of them are taken up by natural formation and 37,95% of fields found at the cliff take up a considerable portion of the destroyed areas. The setting of intensive farming is the main reason that contributes to the degradation of the culture found at the Ngaoundere cliff.

a) Babullock tied up for pasture b) Cow dung in penning

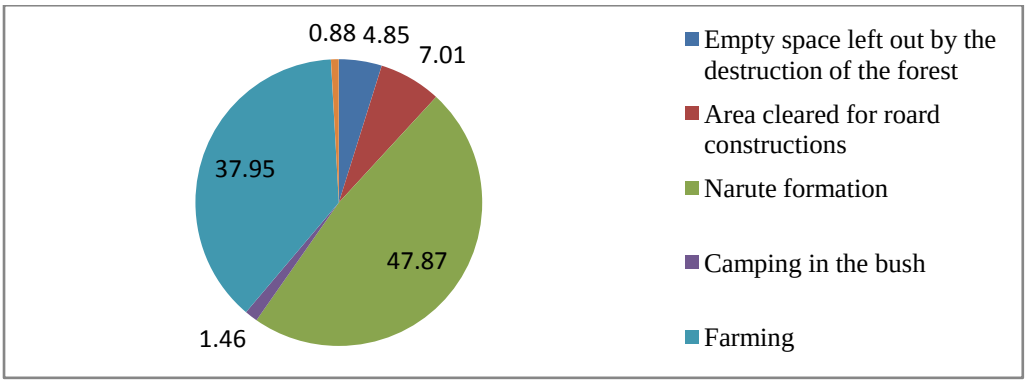
Photo 1 : Cown dung and penning at the Ngaoundere cliff

a) Isobelinia doka burnt b) Isobelinia doka cut down *c) Afzelia africana* cut down

Photo 2 : Burning and cutting down of wood at the Ngaoundere Cliff

a) Maize fields b) Camping c) paths

Photo 3 : Camping, fields, paths inside the Ngaoundere cliff (%)



Picture 2 : destruction of surfaces of the main occupying listed areas

b) Anthropisation indications of woody species in the Ngaoundere cliff

Wood logging, tree barking, animal tracks through dung, grass grazing, paths, bush fires and cultures burn are clear indications for anthropisation. Table 4 shows major indications for extinction species through human activities. They are; wood logging, bush fire and tree barking. Out of the 24ha studied, 113 species destroyed, 27 species burnt and 36 species barked were listed. As for the cut down which is impacted, the *Isoberlinia doka* (17 subjects), is the most solicited. It is followed by the *Grewia flavescens* (15 subjects), the *Daniellia oliveri* (3 subjects), the *Isoberlinia doka* (3 subjects), the *Psorospermum febrifigum* (2 subjects) and *Burkea africana* (1subject). The fallow practice with 40.74% is the most risky species. Concerning the tree barking 16 different types out of the 36 subjects were identified. They are; the *Pterocarpus erinaceus* (14 subjects), the *Piliostigma thonningii* (10 subjects), the *Hymenocardia acida* (8 subjects), the *Securidaca longepedunculata* (6 subjects), the *Grewia flavescens* (5 subjects), the *Phyllanthus muellerianus* (4 subjects), the *Isoberlinia doka* (4 subjects), the *Anogeissus leiocarpus*, the *Bridelia ferruginea*, the

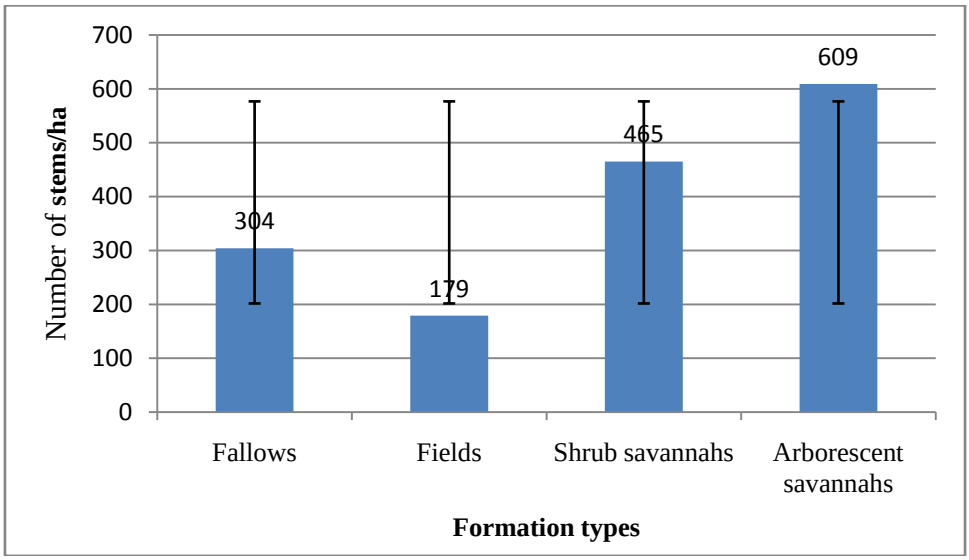
*Entadea africa*na, the *Pterocarpus lucens*, the *Sarcocephalus latifolius, Daniellia oliveri* (2 subjects), the *Sporospermum febrifigum* and the *Sterculia setigera* with one subject each (Table 4).

Table 4 : Indications of anthropisation of woody species in the Ngaoundere cliff

Species	Wood logging				Fire				Tree burking				Wood logging	Fire	Tree burking	total
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4				
Isoberlinia doka	9	2	4	2	1	0	1	1	4	0	0	0	17	3	4	24
Grewia flavescens	8	4	3	0	0	0	0	0	3	2	0	0	15	0	5	20
Daniellia oliveri	7	3	0	0	1	2	0	0	0	0	0	0	10	3	2	15
Piliostigma thonningii	5	0	0	0	0	0	0	0	4	6	0	0	5	0	10	15
Terminalia laxiflora	1	2	1	1	3	3	1	0	0	0	0	0	5	7	0	12
Bridelia scleroneura	4	2	0	1	0	0	0	0	0	0	0	0	7	0	3	10
Terminalia glaucescens	2	2	1	1	1	0	0	3	0	0	0	0	6	4	0	10
Hymenocardia acida	1	0	0	0	0	0	0	0	0	4	0	4	1	0	8	9
Securidaca longepedunculata	0	1	2	0	0	0	0	0	3	0	3	0	3	0	6	9
Afzelia africana	0	3	4	2	0	0	0	0	0	0	0	0	9	0	0	9
Sarcocephalus latifolius	2	1	1	1	0	0	0	0	1	2	0	0	5	0	3	8
Sterculia setigera	2	0	0	0	0	0	0	0	1	4	0	0	2	0	5	7
Vitellaria paradoxa	3	0	0	0	4	0	0	0	0	0	0	0	3	4	0	7
Anogeissus leiocarpus	0	0	0	0	1	0	2	0	0	3	0	0	0	3	3	6
Phyllanthus muellerianus	1	1	0	0	0	0	0	0	4	0	0	0	2	0	4	6
Pseudocedrela kotschyii	0	0	1	1	0	0	0	0	4	0	0	0	2	0	4	6
Psorospermum febrifugum	0	2	0	1	0	0	0	2	0	1	0	0	3	2	1	6
Bridelia ferruginea	3	2		0	0	0	0	0	0	0	0	0	5	0	0	5
Entada africana	1	1	0	0	0	0	0	0	2	1	0	0	2	0	3	5
Pterocarpus lucens	0	1	0	1	0	0	0	0	0	0	0	0	2	0	3	5
Pterocarpus erinaceus	1	1	1	0	0	0	0	0	1	0	0	0	3	0	1	4
Strychnos spinosa	1	1	0	2	0	0	0	0	0	0	0	0	4	0	0	4
Burkea africana	0	0	0	0	0	0	1	0	0	0	0	2	0	1	2	3
Steganotaenia araliacea	2	0	1	0	0	0	0	0	0	0	0	0	3	0	0	3
Lophira lanceolata	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Terminalia macroptera	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Stereospermum kunthianum	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Total	57	27	16	13	11	5	5	6	15	19	0	2	119	27	67	213

c) *The structure of the vegetation according to the population of vegetation densities*

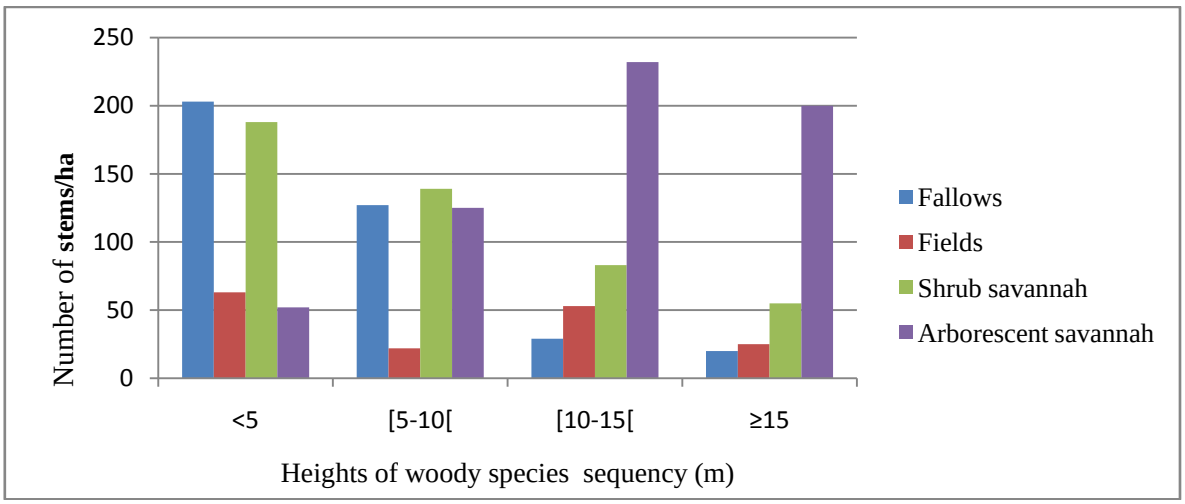
The global denseness of woody species varies from 179 stems/ha in fields which have 609 stems/ha in savannahs planted with trees and that shows subjects listed. There is an irregular distribution of woody species in the different formations of vegetation (picture 3).



Picture 3 : Denseness of different formation (stems/ha)

d) Vertical structure of different woody formations of vegetation

Picture 4 shows the distribution of woody plants with high class. The subjects whose height is inferior to 5m are more numerous in the vegetation. This explains a high regeneration of woody species in the area. This regeneration is highlighted with fallow lands and shrub savannah where the stems of the regenerations were 203 and 188 respectively. The woody plants whose height exceeds 15cm are less represented in the fallow lands (20 stems/ha) and shrub savannah (55 stems/ha), unlikely to areas planted with trees (200 stems/ha) where the species are well depicted. The study of the variance at threshold of 5% shows that there is a significant difference between the height of wood plants within the different experimentation milieu (0.023<0.05)

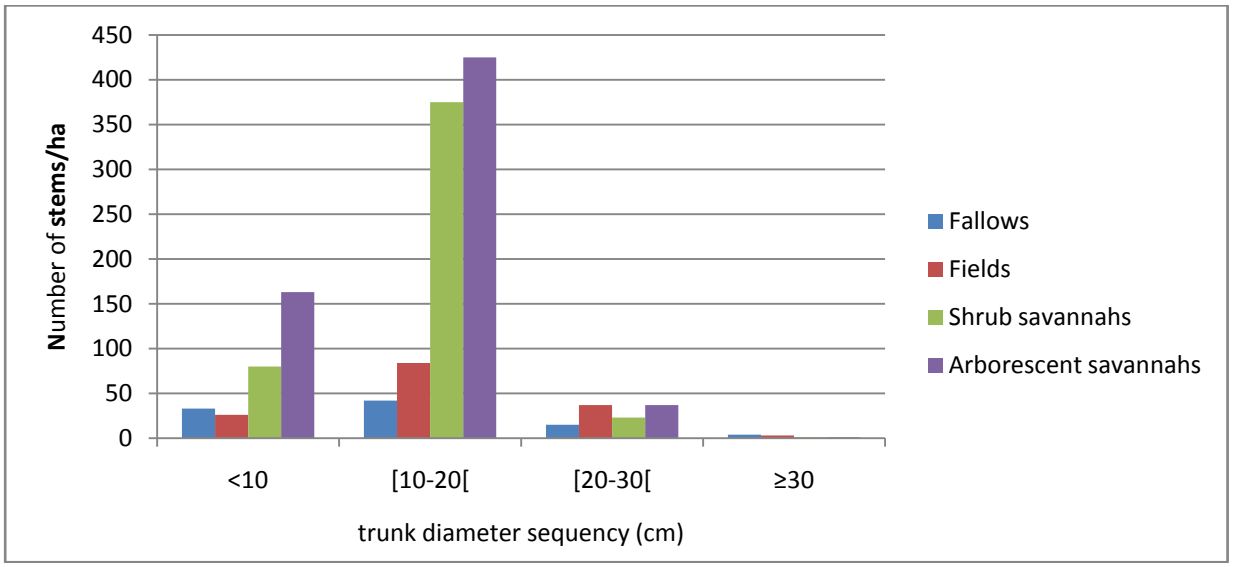


Picture 4 : Distribution of heights of stems species of different formations of vegetation

e) Distribution of horizontal structure of woody species of the different formations of vegetation

The vegetation is dominated by subjects whose diameter is between 10 to 20 cm. This category is highly presented in the planted trees savannahs (425 stems/ha and the shrub savannah (375 stems/ha). In the fallow lands and fields, the number of subjects in the category is 42 stems/ha and 84 stems/ha respectively. The high diameter woody species to 30cm are less important in the vegetation. Those whose diameter varies between 20 to 30 cm are mostly found in fields and in savannahs planted with trees (picture 5).

The threshold analysis of the variance of 5% has shown that there is no significant different between the diameters of woody species in the different formation of vegetation ($p < 5\%$) planted tree savannahs have medium diameter and the highest ones follow and then the fields. The fallow lands have medium and less diameters.



Picture 5 : Distribution of woody species according to the trunk diameter

f) Earth surface of the different formations of vegetation

As the other dendrometeric parameters, the highest earth surface can be noticed in the planted trees savannah (7830.29m^2/ha), followed by those of the shrub savannahs (7162.93m^2/ha). The fallow land occupy small earth surface, that is 4936.10m^2/ha; and this is due to the fact that it is mostly made up of discharges. The slow earth surface of the fields (5153.33m^2/ha) is due to the scarcity of trees destroyed to the benefit of corps (picture 6) statistic analysis reveal a highly significant different between the various formations of vegetation ($0.024<0.05$).

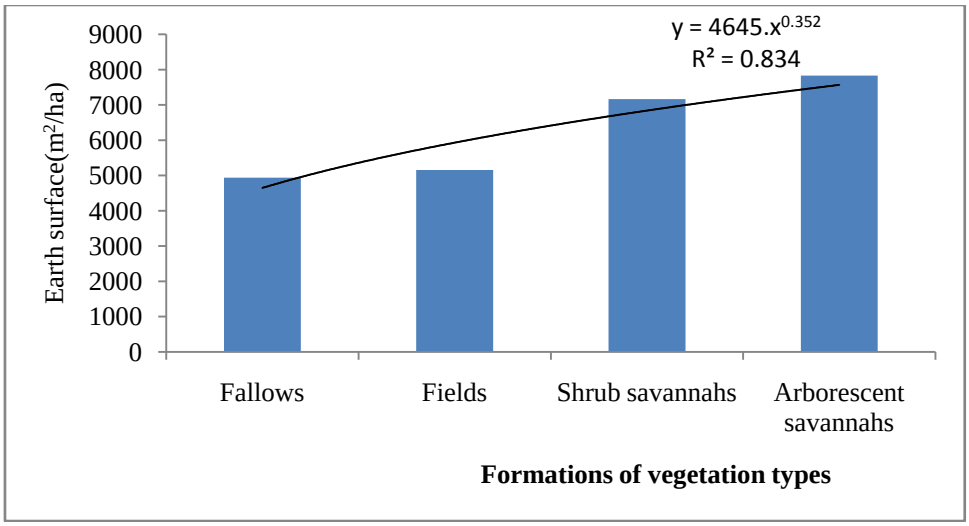


Figure 6 : Earth surface of the different formations of vegetation

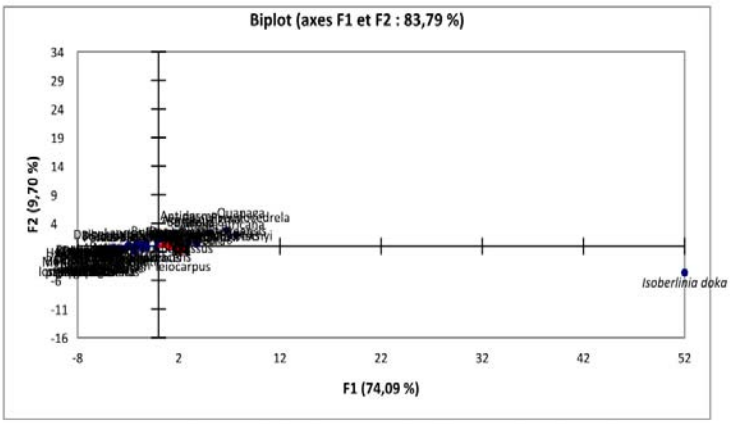
g) The correlation between the dendometric parameters and the cliff vegetation

The Principal Analysis in of component (PAC) between the tree heights, the chest rising height and the earth surface, shows a great correlation (R=0.948, P=0.05) between the earth surface of the fields and the fallow lands; between the chest rising height and the earth surface of fallow lands (R=0.927, P=0.05) (Picture 7). According to the height, the diameter and the earth surface, the species are classified as clouds vertically and horizontally. These species are represented at a 83.79% rate according to F_1 and F_2. Vertically the information's are concentrated in F1 with a high correlation rate of 09%. Horizontally, on the other hand (F2), there is only 9.70% correlation rate between the various species (Table 5). One can say through this analysis that the main constituent of the *Isolerlinia doka* specie shows height, diameter of the earth surface which is more important than the other species and this shows its isolation from the other species.

Table 5 : Analysis of the correlations between the height, the diameter and the earth surface of the species in the four formations of vegetation of the Ngaoundere cliff.

H: height, D: diameter and ST: earth surface; 1, 2, 3 and 4 respectively represent fallow land, field, shrub savannah and planted tree savannahs

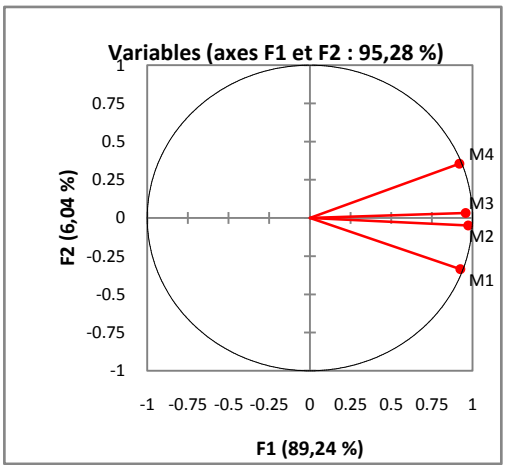
Variables	H1	H2	H3	H4	D1	D2	D3	D4	ST1	ST2	ST3	ST4
H1	1	0,634	0,698	0,753	0,709	0,662	0,662	0,665	0,631	0,654		0,653
H2	0,634	1	0,709	0,516	0,484	0,614	0,558	0,448	0,405	0,550		0,430
H3	0,698	0,709	1	0,644	0,591	0,616	0,755	0,532	0,494	0,551		0,535
H4	0,753	0,516	0,644	1	0,652	0,584	0,632	0,646	0,529	0,539		0,652
D1	0,709	0,484	0,591	0,652	1	0,902	0,869	0,791	0,927	0,899		0,862
D2	0,662	0,614	0,616	0,584	0,902	1	0,895	0,848	0,866	0,920		0,855
D3	0,662	0,558	0,755	0,632	0,869	0,895	1	0,849	0,784	0,837		0,818
D4	0,665	0,448	0,532	0,646	0,791	0,848	0,849	1	0,723	0,786		0,854
ST1	0,631	0,405	0,494	0,529	0,927	0,866	0,784	0,723	1	0,948		0,895
ST2	0,654	0,550	0,551	0,539	0,899	0,920	0,837	0,786	0,948	1		0,891
ST3	0,662	0,500	0,704	0,531	0,810	0,857	0,898	0,803	0,840	0,889		0,869
ST4	0,653	0,430	0,535	0,652	0,862	0,855	0,818	0,854	0,895	0,891		1

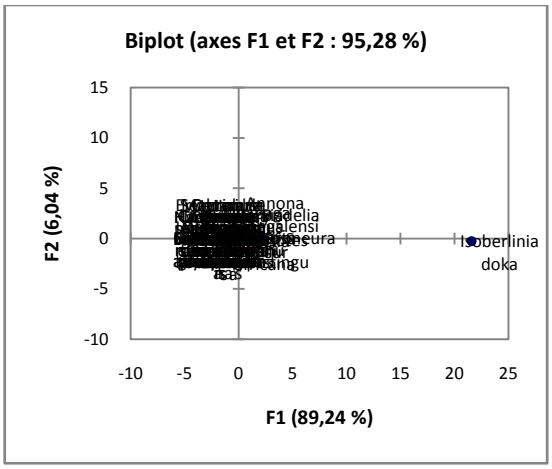


Picture 7 : Stat metric correlation (Person c_n) of the tree dendometric parameters

h) Dispersion modes of the Ngaoundere cliff woody species

Picture 8 shows the analysis in the main component of the species files/statement within the different formations vegetation. The analysis of the main element carried out on the species files statement helps to depict the characteristics of woody species in the different files according to their presence. The perception of the files and species on the factor analysis of axis 1 and 2 helps to merge the woody species with similar characters. The analysis relies on three axes F1, F2, F1 and F2 are valuable percentages and are as follows; 89.24%, 6.04% and 95.28% respectively. One notices that axis F2 helps for a better dispersion of areas of some species. The fallow lands (M_1) and the fields (M_2) have a high correlation; that is they show some species which are similar (Picture 13a). There is also a correlation, highly and significantly recognized between the shrub savannahs (M_3) and arborescent savannahs (M_2) ($p \leq 0.001$). The two formations of vegetation have similar species. The M_4 group shifted forward compared with the other formations of vegetation but close to shrub savannahs. It shows some similar species to shrub savannahs. This helps us to conclude that the shrub savannah are less affected by anthopisation and hence full in consideration in terms of species. As the species are put together at the beginning and form signs like clouds (Picture 13b). This shows that the species are less diversified in the different formation of vegetations. There are therefore and in most cases accidental species. However, the *Isobalinia doka* shows a dispersion which is shifted forward from the other species and this is justified through the abundance and high diversity in the different statements.





Picture 8A : Dispersion of the nations of vegetation *Picture 8B :* Dispersion of woody species

Picture 8 : Analysis in of component (PAC) of species dispersion in the different statements

i) Floristic composition of the cliff vegetation

24 notices were carried out different formation of vegetation of the cliff. For that purposes 1557 woody subject were divided into 73 species, 61 genders and 33 families were listed (Table 6). Arborescents savannahs are the most important groups in the biodiversity with 609 subjets, 64 species, 58 genders and 30 families. They are followed by the shrub savannah which has 465 subjects, 58 species, 56 genders and 28 families. As for the disrupted environment caused by man the biodiversity is poor. The fallow lands have 304 subjects, 51 species, 42 genders and 28 families. The fields have 179 subjects, 48 species, 53 genders and 28 families. This brings out the fact that the number of subjects in the arborescents savannahs is the double of the fields.

Table 6 : Floristic composition of the cliff vegetation

	Fallow	Fields	Shrub savannahs	Arborescent savannahs
Subjects	304	179	465	609
Species	51	48	58	64
Gender	42	53	56	58
Famillies	28	22	28	30

j) Ecological characterization of woody plant species of the vegetation

The analysis of the rich floristic species helps to indentify 73 species in the whole plates. Table 7 shows the exhaustive list of identified species, the frequencies, the densities, the affluences and the importance of the value of Curtis. The most frequent species are found in the area are: *Isoberlinia doka* and *Vitellaria paradoxa* which show an absolute frequency of 87.50% rate. Globally, many species are threatened. Among them we can name the *Breonadia salicina,* the *Ceiba Pentandra*, the *Combretum glutinosum*, the *Croton zambesicus*, the *Cussonia arborea*, the *Diospyros mespiliformis*. The area is dominated by the *Isoberlina doka* with a relative density of 3.98% the same specie shows the importance value of Curtis which reaches 50.91% of rate. It is followed by the *Pterocarpus lucens* (32.04%)

Table 7 : Number of subjects of species, frequency, density, dominance and importance value of Curtis

NI: Number of subjects; FRe: relative frequency; DeR : relative density; DR: relative dominance; IVCR: relative importance value of Curtis

Species	NI	FRe	DeR	DR	IVCR	Species	NI	FRe	DeR	DR	IVCR
Acacia ehrenbergiana	3	0,57	0,16	0,21	0,95	*Lannea acida*	15	1,52	0,47	0,89	2,87
Acacia polyacantha	15	1,52	0,74	0,89	3,14	*Lannea barteri*	13	1,7	0,85	0,48	3,03
Acacia tortilis	3	0,38	0,01	0,21	0,6	*Securidaca longepedunculata*	4	0,04	0,13	0,55	
Afzelia africana	33	2,84	1,69	2,19	6,73	*Lophira lanceolata*	14	0,19	0	0,13	0,32
Anogeissus leiocarpus	56	1,7	0,95	0,89	3,54	*Mangifera indica*	3	0,95	1,06	0,69	2,7

Antidesma venosum	13	1,33	0,25	0,76	2,33	*Maytenus senegalensis*	1	0,38	0,01	0,13	0,52
Annona senegalensis	12	2,84	0,24	2,13	5,2	*Mitragyna inermis*	36	0,19	0	0	0,19
Borassus aethiopum	23	1,33	0,32	0,48	2,12	*Monotes kerstingu*	1	1,52	3,21	2,54	7,26
Burkea africana	14	1,52	1,64	1,37	4,53	*Neocarya macrophylla*	2	0,38	0,18	0,13	0,69
Bombax costatum	19	1,52	0,97	1,1	3,59	*Nauclea gilletii*	22	0,19	0,03	0,07	0,29
Breonadia salicina	1	0,19	0	0	0,19	*Uapaga togoensis*	29	0,38	1,17	1,52	3,07
Bridelia ferruginea	50	2,08	0,18	1,1	3,37	*Parkia biglobosa*	22	2,84	2,38	1,65	6,87
Bridelia scleroneura	20	3,03	0,65	2,95	6,63	*Phyllanthus muellerianus*	30	2,08	0,09	1,04	3,21
Ceiba pentandra	3	0,19	0,32	0,21	0,73	*Piliostigma thonningii*	34	2,08	0,27	1,3	3,66
Combretum glutinosum	1	0,19	0,05	0,07	0,31	*Pseudocedrela kotschyi*	41	2,65	1,44	1,99	6,08
Cussonia arborea	8	0,19	0,2	0,35	0,74	*Pterocarpus erinaceus*	130	2,65	3,99	2,95	9,59
Croton pseudopulchellus	9	0,76	0	0,07	0,82	*Pterocarpus lucens*	66	3,22	20,64	8,17	32,03
Croton zambesicus	1	0,19	0,01	0,07	0,27	*Sarcocephalus latifolius*	2	3,41	2,49	3,84	9,74
Crossopteryx febrifuga	5	0,95	0,16	0,41	1,52	*Sporospermum febrifigum*	6	0,76	0,04	0,28	1,08
Dalbergia boehmii	11	1,52	0,33	0,63	2,47	*Steganotaenia araliacea*	28	1,89	0,25	1,24	3,38
Daniellia oliveri	20	2,08	0,9	1,04	4,02	*Stereospermum kunthianum*	10	2,08	0,71	1,65	4,44
Detarium microcarpum	10	0,57	0,26	0,54	1,37	*Sterculia setigera*	26	0,95	0,18	0,69	1,81
Diospyros mespiliformis	1	0,19	0,02	0,07	0,27	*Strychnos innocua*	3	0,38	0,01	0,21	0,61
Entada abyssinica	1	2,08	0	0	2,08	*Strychnos spinosa*	17	2,08	0,21	1,1	3,4
Entada africana	30	2,08	0,91	1,52	4,51	*Syzyguim guineense* var. *guineense*	6	0,57	0,12	0,41	1,1
Erythrophleum africanum	2	0,38	0,17	0,13	0,68	*Syzyguim guineense* var. *macrocarpum*	7	0,57	0,49	0,21	1,28
Ficus platyphylla	6	1,14	0,24	0,41	1,79	*Terminalia glaucescens*	124	3,41	3,6	7,48	14,49
Ficus synomorus	21	0,95	0,45	0,28	1,68	*Terminalia laxiflora*	158	2,65	5,68	9,47	17,81
Ficus sur	7	1,14	0,65	1,37	3,15	*Terminalia macroptera*	10		0	0	0,76
Ficus trichopoda	6	0,95	0,39	0,41	1,75	*Trichilia emetica*	3	0,57	0,15	0,21	0,93
Ficus vogelii	9	1,33	1,55	0,63	3,5	*Vernonia amygdalina*	7		0,01	0,35	1,31
Gardenia aqualla	17	1,89	0,24	0,89	3,03	*Vitelleria paradoxa*	96	3,98	3,61	5,63	13,22
Grewia flavescens	39	2,65	0,15	0,96	3,75	*Vitex donania*	19	1,89	0,73	1,04	3,66
Gyrocarpus americanus	1	0,19	0	0	0,19	*Vitex madiensis*	1	0,19	0	0	0,19
Hymenocardia acida	2	0,38	0	0,07	0,45	*Ximenia americana*	10	1,14	0,45	0,69	2,28
Isoberlinia doka	286	3,98	30,09	16,8	50,9	*Ziziphus mauritiania*	1	0,19	0	0,07	0,26
Khaya senegalensis	6	1,52	0,52	0,41	2,44	Total	1557	100	100	100	300

k) Ecological characterization of species according to the formations of vegetation

The Table 8 shows that the *Isoberlina doka* is present in the importance value of great necessity in almost all the four formations of vegetation. It respectively shows M1 (43.95%), M2 (67.63%), M3 (35.22%) and M4 (34.65%) in the fallow lands, the fields, the shrub savannahs and the arborescent savannahs. This species easily fits all the anthropic pressure thanks to its dull regeneration ability. Its relative density is 3.67% of subjects/ha for fallow lands, 31.43% of subjects/ha for fields and farming, 3.38% of subjects/ha for shrub savannahs and 4.19% of subjects/ha for the arborescent savannahs. This species has an important and relative collection in the cliff M1 (36.61%), M2 (31.4%) M3 (27.08%) and M4 (27.08%) in the fallow lands, the fields, the shrub savannahs and planted tree savannahs respectively.

Table 8 : Frequency, density, dominance and importance relative value of Curtis of species in the different formations of vegetation of the Ngaoundere cliff

ESpeciess	FR				DeR				DR				IVCR			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
Acacia ehrenbergiana	1,83	0	0	0,68	1,84	1	0,68	0	0,13	1	0	0	3,8	2	0,68	0,68
Acacia polyacantha	1,83	1,59	1,6	2,03	1,84	2,09	2,03	0,6	1,08	2,09	0,13	0,13	4,75	5,76	3,74	2,75
Acacia tortilis	0,92	0,79	0,8	0	0,92	0,04	0	0	0	0,04	0	0	1,83	0,87	0,79	0
Afzelia africana	1,83	3,97	4	2,03	1,84	1,86	2,03	3,59	1,5	1,86	1,57	1,57	5,17	7,69	7,57	7,19
Anogeissus leiocarpus	1,83	0	0,8	2,03	1,84	0,46	2,03	2,4	0,37	0,46	0,39	0,39	4,04	0,92	3,21	4,81
Antidesma venosum	1,83	0,79	0,8	0	1,84	0,5	0	2,99	2,15	0,5	0,2	0,2	5,82	1,79	1	3,2
Annona senegalensis	3,67	1,59	1,6	2,03	3,67	0	2,03	4,19	0	0	0,23	0,23	7,34	1,6	3,84	6,45
Borassus aethiopum	1,83	2,38	2,4	0,68	1,84	0,21	0,68	0,6	1,08	0,21	1,48	1,48	4,75	2,8	4,53	2,75
Burkea africana	0	2,38	2,4	0	0	0,03	0	3,59	0,15	0,03	0,02	0,02	0,15	2,45	2,4	3,62
Bombax costatum	0,92	0,79	0,8	2,7	0,92	0	2,7	1,2	1,21	0	0	0	3,04	0,79	3,5	3,9
Breonadia salicina	0	0,79	0,8	0	0	0,07	0	0	0,07	0,07	0,72	0,72	0,07	0,93	1,51	0,71
Bridelia ferruginea	3,67	2,38	2,4	1,35	3,67	0,65	1,35	1,8	0,6	0,65	1,42	1,42	7,94	3,67	5,16	4,57
Bridelia scleroneura	3,67	2,38	2,4	3,38	3,67	0,64	3,38	2,4	1,88	0,64	3,04	3,04	9,22	3,67	8,8	8,82
Ceiba pentandra	0	0	0	0,68	0	1,56	0,68	0	0	1,56	0	0	0	3,11	0,68	0,68
Combretum glutinosum	0,92	0	0	0	0,92	0,28	0	0	0	0,28	0	0	1,83	0,56	0	0
Cussonia arborea	0,92	0	0	0	0,92	0,38	0	0	0	0,38	0,36	0,36	1,83	0,76	0,36	0,36
Croton pseudopulchellus	0,92	1,59	1,6	0	0,92	0	0	0,6	0	0	0	0	1,83	1,59	1,59	0,6
Croton zambesicus	0	0	0	0,68	0	0	0,68	0	0	0	0,04	0,04	0	0	0,72	0,72
Crossopteryx febrifuga	0,92	0	0	2,03	0,92	0	2,03	0,6	0	0	0,61	0,61	1,83	0	2,64	3,24
Dalbergia boehmii	1,83	1,59	1,6	0,68	1,84	0,9	0,68	2,4	0,12	0,9	0,23	0,23	3,79	3,38	2,49	3,3
Daniellia oliveri	3,67	3,17	3,2	2,03	3,67	2,62	2,03	0	0,79	2,62	1,17	1,17	8,13	8,42	6,37	3,2
Detarium microcarpum	0	0	0	2,03	0	0,06	2,03	0	0	0,06	0,32	0,32	0	0,12	2,34	2,34
Diospyros mespiliformis	0	0	0	0	0	0	0	0,6	0	0	0	0	0	0	0	0,6
Entada abyssinia	3,67	2,38	2,4	2,7	3,67	0	2,7	0	0	0	0	0	7,34	2,38	5,08	2,7
Entada africana	3,67	2,38	2,4	2,7	3,67	0,16	2,7	0	0,23	0,16	2,46	2,46	7,57	2,7	7,55	5,17
Erythrophleum africanum	1,83	0	0	0	1,84	0	0	0	0	0	0,27	0,27	3,67	0	0,27	0,27
Ficus platyphylla	0,92	0,79	0,8	2,03	0,92	0	2,03	0,6	0,34	0	0,7	0,7	2,18	0,79	3,52	3,32
Ficus synomorhus	2,75	0	0	1,35	2,75	1,57	1,35	0	0,9	1,57	0,65	0,65	6,4	3,13	2	2
Ficus sur	0,92	0,79	0,8	2,7	0,92	1,75	2,7	0	0,39	1,75	0	0	2,23	4,29	3,5	2,7
Ficus trichopoda	0	0	0	2,03	0	0	2,03	1,2	0,07	0	0,41	0,41	0,07	0	2,44	3,64
Ficus vogelii	0	0,79	0,8	1,35	0	3,28	1,35	2,99	1,9	3,28	0,16	0,16	1,9	7,35	2,31	4,51
Gardenia aqualla	1,83	0,79	0,8	1,35	1,84	0,4	1,35	3,59	0,08	0,4	0,5	0,5	3,75	1,58	2,64	5,44
Grewia flavescens	3,67	2,38	2,4	2,7	3,67	0	2,7	1,8	0,12	0	0,49	0,49	7,46	2,38	5,58	4,99
Gyrocarpus americanus	0	0	0	0,68	0	0	0,68	0	0	0	0	0	0	0	0,68	0,68
Hymenocardia acida	1,83	0	0	0	1,84	0	0	0	0	0	0,02	0,02	3,67	0	0,02	0,02
Isoberlinia doka	3,67	4,76	4,8	3,38	3,67	31,43	3,38	4,19	36,61	31,4	27,08	27,08	43,95	67,63	35,22	34,65
Khaya senegalensis	0	2,38	2,4	0	0	0	0	3,59	1,1	0	0,91	0,91	1,1	2,38	3,29	4,51
Lannea acida	0,92	1,59	1,6	2,7	0,92	0,29	2,7	0,6	0,16	0,29	0,95	0,95	2	2,17	5,24	4,25
Lannea barteri	2,75	0,79	0,8	0,68	2,75	0,5	0,68	2,99	1,79	0,5	0,67	0,67	7,29	1,8	2,14	4,34
Securidaca longepedunculata	0,92	0	0	0,68	0,92	0	0,68	0	0,24	0	0	0	2,07	0	0,68	0,68
Lophira lanceolata	0	0	0	0,68	0	0	0,68	0	0,46	0	0	0	0,46	0	0,68	0,68
Mangifera indica	0,92	1,59	1,6	0,68	0,92	0	0,68	1,2	0	0	0,08	0,08	1,83	1,59	2,34	1,95
Maytenus senegalensis	0	0,79	0,8	0,68	0	0	0,68	0	0	0	0	0	0	0,79	1,47	0,68
Mitragyna inermis	0	0	0	0	0	1,78	0	0,6	5,37	1,78	2,91	2,91	5,37	3,56	2,91	3,51
Monotes kerstingu	0,92	0,79	0,8	0	0,92	0	0	4,19	0	0	0,11	0,11	1,83	0,79	0,9	4,3
Neocarya macrophylla	0	0,79	0,8	0,68	0	0	0,68	0	0,33	0	0,3	0,3	0,33	0,79	1,77	0,98
Nauclea gilletii	0,92	0	0	0	0,92	0	0	0	0	0	3,4	3,4	1,83	0	3,4	3,39
Ouapaga togoensis	0	0	0	0	0	2,29	0	1,2	1,23	2,29	5,02	5,02	1,23	4,59	5,02	6,22
Parkia biglobosa	1,83	3,97	4	2,03	1,84	0	2,03	2,99	0,19	0	0,07	0,07	3,86	3,98	6,07	5,09
Phyllanthus	0	2,38	2,4	3,38	0	0	3,38	2,4	0,5	0	0,4	0,4	0,5	2,38	6,16	6,18

muellerianus																
Piliostigma thonningii	2,75	3,17	3,2	2,03	2,75	3,65	2,03	0,6	0,59	3,65	1,61	1,61	6,1	10,47	6,81	4,23
Pseudocedrela kotschyi	0,92	3,17	3,2	2,03	0,92	1,73	2,03	4,19	2,62	1,73	8,16	8,16	4,45	6,64	13,37	14,38
Pterocarpus erinaceus	1,83	0,79	0,8	4,05	1,84	12,41	4,05	3,59	17,25	12,4	4,98	4,98	20,92	25,62	9,82	12,62
Pterocarpus lucens	1,83	3,17	3,2	4,05	1,84	1,34	4,05	3,59	1,68	1,34	2,32	2,32	5,35	5,86	9,55	9,97
Sarcocephalus latifolius	4,59	3,97	4	2,7	4,59	0	2,7	2,4	0	0	0,03	0,03	9,17	3,97	6,7	5,13
Sporospermum febrifigum	0	1,59	1,6	1,35	0	0,37	1,35	0	0	0,37	0	0	0	2,33	2,94	1,35
Steganotaenia araliacea	1,83	1,59	1,6	2,03	1,84	0	2,03	1,8	0,13	0	1,34	1,34	3,8	1,59	4,95	5,16
Stereospermum kunthianum	0	0,79	0,8	2,7	0	0,8	2,7	4,19	0	0,8	0,1	0,1	0	2,39	3,6	7
Sterculia setigera	0	0,79	0,8	1,35	0	0,58	1,35	1,2	0,35	0,58	0,53	0,53	0,35	1,96	2,67	3,08
Strychnos innocua	0	0,79	0,8	0	0	0	0	0,6	0	0	0,01	0,01	0	0,79	0,81	0,61
Strychnos spinosa	3,67	2,38	2,4	1,35	3,67	0	1,35	1,2	0,46	0	0,51	0,51	7,8	2,38	4,24	3,06
Syzyguim guineense var guineense	0	0,79	0,8	1,35	0	0	1,35	0	0	0	0,16	0,16	0	0,79	2,31	1,51
Syzyguim guineense var macrocarpum	0	2,38	2,4	0	0	2,51	0	0	0	2,51	0,02	0,02	0	7,41	2,4	0,02
Terminalia glaucescens	3,67	3,17	3,2	3,38	3,67	7,09	3,38	3,59	2,05	7,09	3,92	3,92	9,38	17,36	10,48	10,9
Terminalia laxiflora	4,59	3,97	4	1,35	4,59	5,76	1,35	1,2	8,54	5,76	11,94	11,94	17,71	15,48	17,26	14,49
Terminalia macroptera	0,92	1,59	1,6	0,68	0,92	0	0,68	0	0	0	0	0	1,83	1,59	2,26	0,68
Trichilia emetica	0	0,79	0,8	0	0	0	0	1,2	0	0	0,66	0,66	0	0,79	1,45	1,86
Vernonia amygdalina	0	1,59	1,6	1,35	0	0	1,35	0,6	0,14	0	0	0	0,14	1,59	2,94	1,95
Vitelleria paradoxa	3,67	3,97	4	4,05	3,67	6,59	4,05	4,19	0,37	6,59	3,69	3,69	7,71	17,15	11,71	11,94
Vitex donania	2,75	2,38	2,4	0,68	2,75	0	0,68	2,4	2,25	0	0,4	0,4	7,75	2,38	3,45	3,47
Vitex madiensis	0	0	0	0,68	0	0	0,68	0	0	0	0	0	0	0	0,68	0,68
Ximenia americana	0	0	0	2,03	0	0,36	2,03	1,8	0,44	0,36	0,13	0,13	0,44	0,73	2,16	3,95
Ziziphus mauritiania	0	0	0	0,68	0	0	0,68	0	0	0	0	0	0	0	0,68	0,68
Total	100	100	100	100	100	100	100	100	100	100	100	100	300	300	300	300

l) Composition and ecological characterization of the diversity of botanic Families

The species are divided into 33 families. Those families are neither equal nor share the same diversity. While some families are representative with one genus and only one species, some are on the other hand represented by many species. The Cesalpiniaceae and the Euphorbiaceae families have each 7 geniuses; the Mimosaceae and the Rubiaceae have each 6 genus; the Combretaceae and the Moracea have 5 genuses each. The table 9 recapitulates the families in the decreasing order and diversity. The fact that a family has many geniuses does not necessarily mean that it is highly diversified. The Meliaceae which has 2 geniuses and 2 species only have 9 subjects; that is a relative density of 0.01% rate. The Moraceae, with 2 genues and 5 species have 49 subjects with a relative density of 0.02% close that of Meliaceae. The Ebenaceae, Hermandiaceae, Hymenocardiaceae and Rhamnaceae are represented each by only one subject; the Cesalpiniaceae, with 7 genuses and 7 species are the most numerous at the cliff. Their relative density is 27.80% of rate.

Table 9 : Density, Dominance, importance value de Curtis and the number of subjects, species, geniuses and family.

Familles	NG	NE	NI	FR	DeR	DR	IVCR	Familles	NG	NE	NI	FR	DeR	DR	IVCR
Anacardiaceae	2	3	31	3,45	2,54	1,31	7,29	Hymenocardiaceae	1	1	1	0,94	0	0,13	1,08
Annonaceae	1	1	12	7,05	0,76	4,06	11,9	Loganiaceae	1	2	20	0,7	0	0,06	0,78
Apiaceae	1	1	28	4,7	0,81	2,36	7,86	Malvaceae	1	2	4	1,17	0	0,13	1,31
Araliaceae	1	1	8	0,47	0,66	0,66	1,79	Meliaceae	2	2	9	1,57	0	0,18	1,76
Arecaceae	1	1	23	3,29	1,02	0,91	5,22	Mimosaceae	4	7	115	8,14	4,6	4,15	16,94
Asteraceae	1	1	7	2,35	0,04	0,66	3,05	Moraceae	2	5	49	1,16	5,6	5,19	11,95
Bignoniaceae	1	1	10	5,17	2,27	3,15	10,6	Myrtaceae	1	2	13	5,36	0,9	25,45	58,68
Celastraceae	1	1	2	0,94	0,03	0,25	1,22	Ochnaceae	1	1	4	0,47	0	0,25	0,72
Cesalpiniaceae	7	7	399	19,4	27,8	1,72	5,94	Olacaceae	1	1	10	2,82	1,5	1,32	5,59
Chrysobanalaceae	1	1	2	0,94	0,58	0,25	1,77	Polygalaceae	1	1	4	0,94	0,1	0,25	1,31
Clusiaceae	1	1	6	1,88	0,13	0,54	2,54	Rhamnaceae	1	2	6	0,47	0	0,13	0,6
Combretaceae	3	5	349	5,54	1,26	2,63	9,43	Rubiaceae	6	6	55	2,84	14	13,14	30,19
Dipterocarpaceae	1	1	36	3,76	10,29	4,85	18,9	Sapotaceae	1	1	96	9,87	12	10,76	32,2
Ebenaceae	1	1	1	0,47	0,05	0,13	0,65	Sterculiaceae	1	1	26	2,35	0,6	1,32	4,23
Euphorbiaceae	5	7	123	4,97	2,48	2,7	10,2	Tiliaceae	1	1	39	6,58	0,5	1,83	8,87
Fabaceae	3	3	207	1,3	9,23	7,95	18,5	Verbenaceae	1	2	20	4,46	0,5	1,57	6,55
Hernandiaceae	1	1	1	0,47	0	0	0,47	Total	31	73	1557	100	100	100	300

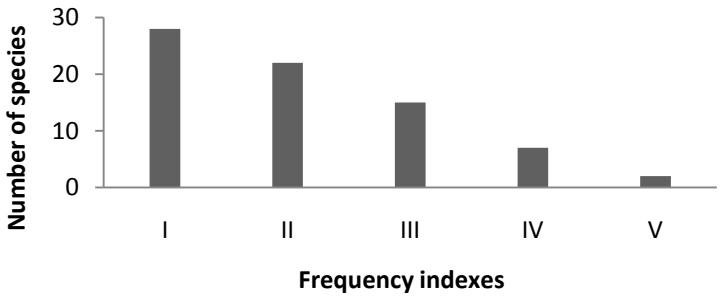
m) Distribution of ecological preference of plant species at the Ngaoundere cliff

The gauged frequencies help to understand the horizontal repartition of the species listed. Picture 9 illustrates the frequency histogram of woody species listed in the Ngaoundere cliff.

Out of the 73 species listed, 28 species that is 38.35% of the flora have a frequency indication equal to 1. Some among them are scare. As an example we have the *Breonodia salicina, Ceiba pendandra, Combretum glutinosum, Croton zambesicus , Cussonia barteri, Diospyros mespiliformis* To 2 frequency index. These are the one whose relative frequency varies from 20 to 40%. The species whose frequency index is equal to 3 represent 20.55% of the listed flora. Only 9.60% of the flora indicates frequency index equal to 4. It is represented by *Afzelia africana, Annona senegalensis, Parkia biglobosa, Bridelia scleroneura, Pterocarpus lucens, Sarcocephalus latifolius, Terminalia glaucescens.* The most frequent with a frequency index is equal to 5 are two of them with 2.77% the most of the listed flora. Those two species *Isoberlinia doka* and *Vitellaria paradoxa* have 87.5% frequency index and appear in the 21 of the 24 listed. This structure of the vegetation shows a high heterogenous frequency of the area and would be the consequence of a high anthropic pressure on the vegetation.

n) Shannon, Equitability of Pielou, Simpson diversities indexes of the Ngaoundere cliff

Table 10 shows the values of the different indexes calculated. The Shannon wearer calculated diversity indexes considerably vary from one group to the other. They are low in fallow plants (3.26) and the fields (2.81) as a result of the impact of anthropisation on the vegetation. In addition this index is higher in fallow lands than those of the fields and this is simply due to the fact that after the cutting down of trees there is a good reconstruction of the vegetation. The calculation of Pielou equitability theory associated with the Shannon confirms the fluctuation between index values of the diversity. In facts the value equitabilities are negligible in the fallow (0.54) and as in the shrub savannahs (0.74) and arborescent savannahs (0.85). Globally, there are 59% opportunities to equilibrium in the repartition of species in the area. Moreover, the Simpson index proves that the probability for subjects chosen and randon that is from different species is very high. The low values of the indexes of the fallow lands (0.13) and of the fields (0.18) show that they are negligible in biodiversity. This negligible diversity would be the consequence of human activities developed in the cliff by the resident population.



Picture 9 : Frequency of the species listed at the Ngaoundere cliff

Table 10 : values of the different indexes of calculated diversity

H= Shannon index, E= pielou equitability D = Simpson index

Indices / formations of vegetation	allows	fields	shrub savannahs	Arborescent savannahs	Mean
H'	3,26	2,81	4,05	4,77	3,72±0,87
E	0,54	0,23	0,74	0,85	0,59±0,27
D	0,13	0,18	0,26	0,35	0,23±0,19
1-D	0,87	0,82	0,74	0,65	0,77±0,09

o) The floristic similarity coefficients of Jaccard and the Hamming distances between the different

By applying the Jaccard test for the vegetation homogeneity or heterogeneity evaluation, the Table 11 values show that the floristic difference stronger between the fallow lands and the arborescent savannahs (67.82%), medium between the fallow lands and shrub savannahs (58.45%). This explains a relative heterogenerty between those environments. However, it is very poor between the follow lands and the fields (32.2%) the arborescent savannahs and the fields (30.3%), the fields and shrub savannahs (31.75%). The floristic difference is very poor between the shrub savannahs and arborescent savannahs (12.3%). The

poor floristic difference between the shrub savannahs and the arborescent savannahs conveys a great number of similar species they contain. This difference could due to the anthropisation degree on the survey zones and above all to the bioclimatic estate.

Table 11 : Floristic similitude coefficients of Jaccord and the Hamming distances between the different formations of vegetation

JP = Jaccard index H = Hamming distance

Lands	Fallow		Fields		Shrub savannahs		Arborescent savannahs	
	PJ	H	PJ	H	PJ	H	PJ	H
Fallow	100	0	67,8	32,2	41,55	58,45	32,18	67,82
Fields	67,8	32,2	100	0	30,3	87,7	69,7	30,3
Shrub savannahs	41,55	58,45	68,25	31,75	100	0	87,7	12,3
Arborescent savannahs	32,18	67,82	69,7	30,3	87,7	12,3	100	0

p) The influence of the natural resources on the biological types of distribution on the Ngaoundere cliff species

Table 12 shows the biological types of woody plants in various formations of vegetation. The mesophanero- phytes (Ms Ph) are far more important in the formations of vegetation; that is 38.59%. They represent 65.68% of woody plants of arborescent savannahs while in the shrub savannahs, the fields and fallow lands they respectively occupy 29.03%, 43.55% and 16.11%. The second biological type is represented by the nanophanerophytes (Nn ph) with 29.52% with dominant fallow lands of (58.22%) and very poor at the level of arborescent savannahs (3.28%). The megaphanerophytes (Mgph), with only (1.47%), are less abundant at the vegetation. Globally, the study of the variance at 5% threshold take into consideration the non negligeable difference between the types of the formations of vegetation and the biological forms found (p 0.01).

Table 12 : Distribution of biological types of plants at the Ngaoundere cliff

Mgph: Megaphaneropytes; Msph: Mesophanerophytes; Mcph: Microphanerophytes; Nnph: Nanophanerophytes.

Biological types	MgPh	MsPh	McPh	MnPh	Mean
Fallow	0	16,11	25,65	58,22	25,00±24,55[a]
Fields	0	43,55	17,31	29,05	22,48±18,43[a]
Shrub savannahs	0,64	29,03	42,79	27,52	25,00±17,63[a]
Arborescent savannahs	5,25	65,68	25,77	3,28	25,00±20,97[a]
Mean	1,47±2,54[a]	38,59±21,25[b]	27,88±10,70[c]	29,52±22,48[d]	24,37±1,26[a]

The figures assigned the same letters are not significanthy different from the threshold of 5%

q) The influence of anthropisation on the phytogeographic distribution of species at the Ngaoundere cliff

African multiregional species (PRA) represent 67.07% of the whole flora (Table 13). They are species whose distribution area covers many African floristic areas where only two are not physically in contact. The zoudano Zambian species (CZ), the cosmopolitans (Cos) and the pantropical species (Pan), representing respectively 0.42%, 2.10% and 1,53% are not found in their phytogeographic distribution areas. A high proportion of species with a substancial distribution may be a degradation index. Statistic analysis projects however the inexistancce of significant difference between the phytogeographic types and the formations of vegetation (p 5%).

Table 13 : phytogeagraphic distribution of vegetation species at the cliff (%)

AS = Afrotropical species; cos = cosmopolitan species; pal = paleotropical species, pan = pantropical species, PRA = African multiregional species; s1 = soudano Zambian species

Formations types/ phytogeagraphic	Fallow	Fields	Shrub savannahs	Arborescent savannahs	Mean
AS	8,33	11,11	15,09	16,66	12,80±3,79a
Cos	2,08	2,77	1,88	1,66	2,10±0,48b
Pal	16,66	19,44	13,20	15,00	16,08±2,65c
Pan	0,00	2,77	0,00	3,33	1,53±1,78b
PRA	72,91	63,88	69,81	61,66	67,07±5,20d
Sz	0,00	0,00	0,00	1,66	0,42±0,83a
Mean	16,66±28,29a	16,66±24,21a	16,66±26,88a	16,66±26,88a	16,66±25,54c

r) The influence of anthropisation on the dissemination of the diasporas species at the Ngaoundere cliff.

The dissemination of diaspore types varies according to the vegetation (Table 14). This comes out that the ballochores (Ballo) show a signifificant dissemination rate in the vegetation 26.63% of species insure themselves the dissemination of their own Diasporas in different formations of vegetation. The second element that contributes to the dissemination of the Diasporas is the wind. 21.85% of species are anemochores. The sclerochores (9.22%), the sarcochores (11.83%) and the barochores (12.67%) are less represented at the cliff vegetation.

Table 14 : dissemination of the Diasporas in the different formations of vegetation

diaspores types	Fallow	Fields	Shrub savannahs	Arborescent savannahs	Average
Anemo	20,83	23,91	22,64	20	21,85±1,76[a]
Ballo	27,08	28,26	24,52	26,66	26,63±1,56[b]
Barro	12,5	15,21	11,32	11,66	12,67±1,76[c]
Sarco	10,41	8,69	13,2	15	11,83±2,82[c]
Sléro	10,41	8,69	9,43	8,33	9,22±0,92[d]
Zoo	18,75	15,21	18,88	18,33	17,79±1,74[e]
Mean	16,66±6,72[a]	16,66±7,98[a]	16,67±6,25[a]	16,66±6,50[a]	16,66±0,00[a]

The figures assigned the same letters are significantly different from the threshold 5%

IV. Discussion

The exploitation of discussions of vegetative resources has immediate consequences on the biodiversity existence that is why the density and specific richness of vegetative species are more important at the level of wood savannahs which are less disrupted than other sectors like the fields. GIEC (2007) demonstrated that reduce the vegetative biodiversity of 20%. This demonstrations is similar to the one found at the level of the Ngaoundere cliff where the number of arborescent savannah subjects are the double of the fields. The vegetative resource exploitations are equally remarquable through the increase of scarce species and unpredictable with higher frequence index 1. One can observe the presence of scarce species such as. The intensification and the antropisation may be the cause of the scarcity of the four formations of vegetation of the cliff. The moose common species with an equal frequency "V" are at 2.74% of the listed flora. *Iberlinia doka* and *Vitellaria paradoxa* have a high frequency. In the same vein, Tchobsala (2011) in the periuban savannahs of Ngaoundere pointed out that the vegetation is made us of 37.67% species with frequent index1, 28% of species with frequent index equal to II, 23.18% of species with frequent index equal to III, 6.63% of species with frequent index equal to IV and 3.52% of species whose frequent index is equal to V. this vegetative structure explains a significant heterogeneity of the area and have appears to be as direct consequence of a significant anthropic pressure on the vegetation. This anthropisations is characterized by a high floristic difference between the fallow lands and planed tree savannahs with 67.82% rate. However, it not considerable between the fallow lands and the fields with (32.2%), the planted tree savannahs and the fields (30.3%) and the fields vs. the shrub savannahs (31.75%). The mesophanerphytes (Msph) are more considerable implanted tree savannahs and this shows that with this formation most of the species have trees whose height are superior to 30 m high compared with the shrub savannahs fields and fallow lands. Similar research works were carried out in carbon and showed that the mesophanerophytes are the more biological commontresss (Auberville, 1962). In addition, the megaphaneropytes are poorly depicted at the Ngaoundere cliff. Tchobsala and Mbolo (2013) research works at the Ngaoundere periubar savannhs also showed that the megaphanerophytes (2.75%) are poorly represented at the level of the Ngaoundere vegetation. At the phytogeographic level, the African multiregional species (PRA) represent 67.07% of the whole flora. They are species whose distribution area covers many African floristic regions where two floristique regions are not in constant contact. The soudano Zambian species (SZ), cosmopolitans cos and the pantropical species (pan) respectively representing (0.42%, 2.10% and 1.53%) are not found in their phytogeographic distribution area. A high species promotion with large distribution can be degradation index facto. The formations which were subjected to higher anthropisation. Like the fields and fallow land have high regeneration ability. The subjects' density is highly considered when the height is less than 5m. If the woody species whose height is superior to 15cm are less considered in fallow lands, fields and shrub savannah unlikely to planted tree savannahs. This testifies that the important species were destroyed by the population unaware of tree production in the fields. These results go in line with those of Tchobsala (2011) who proved that the vegetation in the Ngaoundere

periurban savannahs are dominated by shrubs; (result with high anthropisation rate). The fallow lands and the fields which underwent deforestation take up a negligible earth surface. This is due to the fact that it is mostly made up wastes in comparison with the planted tree savannahs. Some close similar research work were carried out by GIEC (2007) and revealed that some formations under some anthropisation pressures (wastes, fields, pastures) considerably reduce the tassel diameters and consequently reduced the earth surfaces of the formation of vegetation.

V. Conclusion and Perspectives

The survey on the anthropisation impact on floristic composition, ecological structure and characterization of the Ngaoundere cliff vegetation showed that global density and the floristic resources of woody species considerably reduce up to the threshold of 5% the fields, fallow lands, shrub savannahs and arborescent savannahs. The fields show negligible more floristic resources than the other formation of vegetation. The diversity index estimated to 3.25 shows that the anthropic disruptions are high influenced on the diversity of woody species. Besides, the conversion of the forestry arena of the cliff into a cultivation field by the deforestation phenomenon leads to a drastic reduction of specific resource. The cliff vegetation anthropisation deserve the putting in place of a sustainable development plan, a joint management and a certification of this measure forestry.

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Effect of *Withania Somnifera* as Feed Ingredient on Growth and Behavioral Changes of *Laebo Rohita*

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Abstract- The research was planned to study the effects of *withania somnifera* as feed ingredient on growth and behavioral changes of *Labeo Rohita.* For this purpose four aquarium were selected. Each aquarium was stocked with 15 fishes. Before stocking, fishes for each aquarium were treated with 5g/L sodium chloride (NaCl) to prevent fish from fungal infection. Fish sample were taken from Fish Hatchery which is situated at staina road. Two types of diets were given to fish one was normal diet which was given to fish and second was plant based diet which was obtained from *withania somnifera.* 1st aquarium was served as control while others three served as experimental. After every one week fish was collected at regular intervals to check the growth parameters. Growth parameters such as fresh weight, fork length, total length and standard length were monitored weekly. The result showed that final average body weight was found 24.5, 26.2 and 28 in *Withania somnifera*. Water quality parameters like DO, Alkalinity, Carbon dioxide, pH, Temperature and chloride ions was also checked by taking water samples from the aquarium. Data analyzed with the help of analysis of variance to reach some conclusions.

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I. Introduction

Due to increasing population, world is facing many problems in which most important one is shortage of food and undernourishment. Meat, milk and eggs are the source of protein which is obtained from animals. With the establishment of poultry and fish farming meat production is increasing to fulfill the requirements of proteins. Good quality protein is obtained with the development of fisheries (Sheikh and Sheikh, 2004).

Aquaculture is a type of agriculture which means to synthesize aquatic plants and animals in water instead of on land while agriculture is farming on land. Agriculture and aquaculture consist of same step for producing animals and plants. Due to continued expansion of shellfish and cultured fish species aquaculture has become a major component of animal health industry (Kolkovski and Kolkovski 2011).

Aquaculture requires continued research because it is a developing industrial sector (Alicia *et al* 2005). In the world most vastly growing food production is through aquaculture. Since 1984 aquaculture production rather than harvest from wild fisheries may become the great cause of providing fish in future (Wantanabe, 2002). The demand of high quality protein is rising due to increase in population. We acquire 16% of animal protein from fish. Antibiotics are used for the prevention of bacterial diseases in aquaculture (FAO, 2002).

Fish plays very important role in human nutrition as a protein source. In developed and developing countries it fulfills the requirement of protein but in under-developed countries it does not fulfils the requirement. The population is increasing as fast as 2.66% per annum and 66mg/person per day is standard amount of animal protein. In Pakistan only 2Kg/person/year fish meat is available. With the increasing income, improved diet and population growth demand of fish meat will continuously increases. As compared to other meat such as goat, cow, sheep fish meat can easily digestible in body. The ranges of its digestibility are between 85-90% (Rudolf, 1971).

Fish is an excellent substitute for red meat and excellent source of protein. Percentage of proteins in fish protein is high as compared to other protein based diet likewise 12% in eggs, 3.5% in milk, 6.6% in rice and 16-20% proteins in fish (Kumar, 1992). All the essential amino acids and minerals viz. copper, potassium, iron, iodine and vitamin A and D are included in fish meat (Gerking, 1966). Fish meat contains unsaturated fat contents and low concentration of carbohydrates. Fish meat contains anticancer properties and lessens the risk of heart diseases (Barlas, 1986). High quality protein diet increases the production of fish as compared to low quality protein diet (Virk and Sexena, 2003).

Fish meal can be replaced by grain and oilseed meals. The most commonly used protein source is soybean which has round about 48% protein and is frequently used in diet of aquatic and terrestrial animals. Substitution of fish meal is very less due to many reasons. Plant meals usually contain high levels of fiber, starch, non-soluble carbohydrates that influence on the growth of fish and its digestibility. Fishes perform an important role to providing food for human beings and also help in different actions. Now a day's main problem which is facing by fish nutritionals is that nutrients in which protein sources are low such as lipids and carbohydrate are required for extra protein sources. If less level protein energy sources given in fish diet they

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will be increased growth of fish. Recently non protein energy sources are used in diet of fish such as lipids and carbohydrates. Several studies shows that how much nutrition is important in aquaculture Adiukwu, 1999, Fasakin *et. al.,* (2003). Carnivorous fishes used less amount of carbohydrate as compared to herbivorous and omnivorous fishes Kaushik *et al.*, (1989) but they badly consume carbohydrate at both metabolic and digestive leaves Lovell, (1989). If fatty fish is producing its means lipid amount is greater in diet. Increase of lipid levels may reduce fish growth and produce fatty fish. In production of carnivorous fish only fish meal is sufficient for fed which fulfill the protein and lipid requirement.

Herbs are well-suited with body of fish because they have fewer side effects and also used as medicine due to nature of plants Borimnejad, (2008). So flourishing freshwater fish culture environment friendly inexpensive and ayurvedic plants should be used. The roots of herb *Asparagus racemosus* which is commonly called shatavari is used in different medicinal purposes. Roots of this herb contain saponins Sharma *et al.,* (2000), Jameela *et al., (*2011).

The commonly used herbal extracts are from *Withania somnifera* (Ashwagandha*), Ocimum sanctum* (Tulsi) and Amlaki with the dealing of Immunosuppressive conditions for animals and humans Devasagayan (1997). If Macrobrachium rosenbergii given ImmuPlus at the rate of 1g/kg for three weeks immunity level will definitely increased Kumari Jaya, (2004). ImmuPlus is an indian herb that consists of Indian plants extracts Jadhav and Bhutani (2006), Jaya Kumari *et al.,* (2007).

In Ayurvedic drugs basic source is Plant alkaloids. Due to their beneficial effects these drugs are used in medicines. Different quantity of plants is used in medicines and for treatment. Different drugs are being prepared from plants which are useful in the field of medicine (Singh *et. al.,* 2003, Tiwari and Singh 2004,).It is roughly estimated that total number of plants on earth is 2,50,000 in which eighty thousand are used as medicinal plants (Kumar and Joshi1987).

II. Materials and Methods

The present project was carried out to determine the effect of feed (25%) which is sunflower, wheat bran, rice polish based and *Withania somnifera* feed (30%, 35%, 40%) which is also plant based diet on the growth of *Labeo rohita* fingerlings. This experiment was conducted in Laboratory department of zoology Sargodha University Jaranwala campus Faisalabad.

a) Experimental Fish

Labeo rohita, fingerlings were taken from the Government Fish seed Hatchery satiana road Faisalabad. Each aquarium stocked with 15 fishes. Before start the experiment *Labeo rohita* fingerlings were bathed with 5g/L NaCl to ensure them free from fungal infection and ectoparasites. Experimental diets were given to fish twice a day. Other water parameters like dissolved oxygen, temperature and pH were also checked with digital meter. Air was supplied in the aquarium so that level of oxygen should maintain through air pumps.

III. Results and Discussion

a) Gain in Average Body Weight

Analysis of Variance demonstrated highly significant differences in different fortnights and treatments. The interaction between fortnights and treatments was also highly significant.

Table 1 : Analysis of variance table for weight

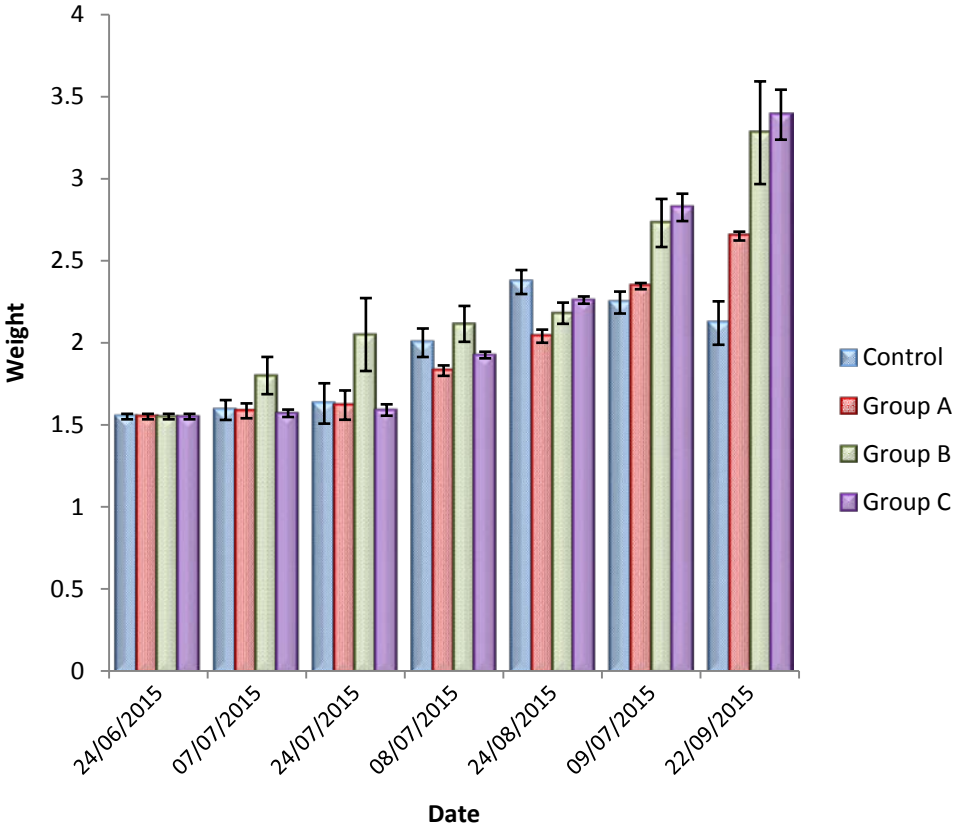
Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Date	6	58.0637	9.6773	90.60**
Group	3	5.1252	1.7084	15.99**
Date*Group	18	10.6004	0.5889	5.51**
Error	252	26.9165	0.1068	
Total	279	100.7059		

** = *Highly significant (P<0.01)*

Date x Group interaction mean±SE

Date	Group				Mean
	Control	Group A	Group B	Group C	
24/06/2015	1.550±0.017i	1.550±0.017i	1.550±0.017i	1.550±0.017i	1.550±0.008E
07/07/2015	1.590±0.060hi	1.585±0.045hi	1.570±0.023i	1.800±0.114ghi	1.636±0.037E
24/07/2015	1.630±0.123hi	1.620±0.089hi	1.590±0.035hi	2.050±0.222f-i	1.723±0.072E
08/07/2015	2.000±0.087f-i	1.830±0.032f-i	1.925±0.020f-i	2.115±0.109f-i	1.968±0.039D
24/08/2015	2.370±0.073c-f	2.040±0.040f-i	2.260±0.022d-g	2.180±0.065efg	2.213±0.032C
09/07/2015	2.245±0.067d-g	2.345±0.019c-f	2.825±0.083bc	2.730±0.146cd	2.536±0.059B
22/09/2015	2.120±0.132e-h	2.650±0.027cde	3.390±0.152a	3.280±0.313ab	2.860±0.122A
Mean	1.929±0.049B	1.946±0.050B	2.159±0.083AB	2.244±0.089A	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.



b) Average Total length

Analysis of Variance demonstrated highly significant differences in different fortnights and treatments. The interaction between forth nights and treatments was non significant.

c) Gain in Average Total Length

Analysis of Variance showed highly significant differences in different fortnights and treatments. The interaction between fortnights and treatments was also highly significant.

In the present trial, studies on the growth parameters revealed highly significant difference in the growth parameters i.e. average and gain in total length.

Table 2 : Analysis of variance table for length

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Date	6	80.6200	13.4367	82.68**
Group	3	3.8489	1.2830	7.89**
Date*Group	18	5.4392	0.3022	1.86*
Error	252	40.9522	0.1625	
Total	279	130.8603		

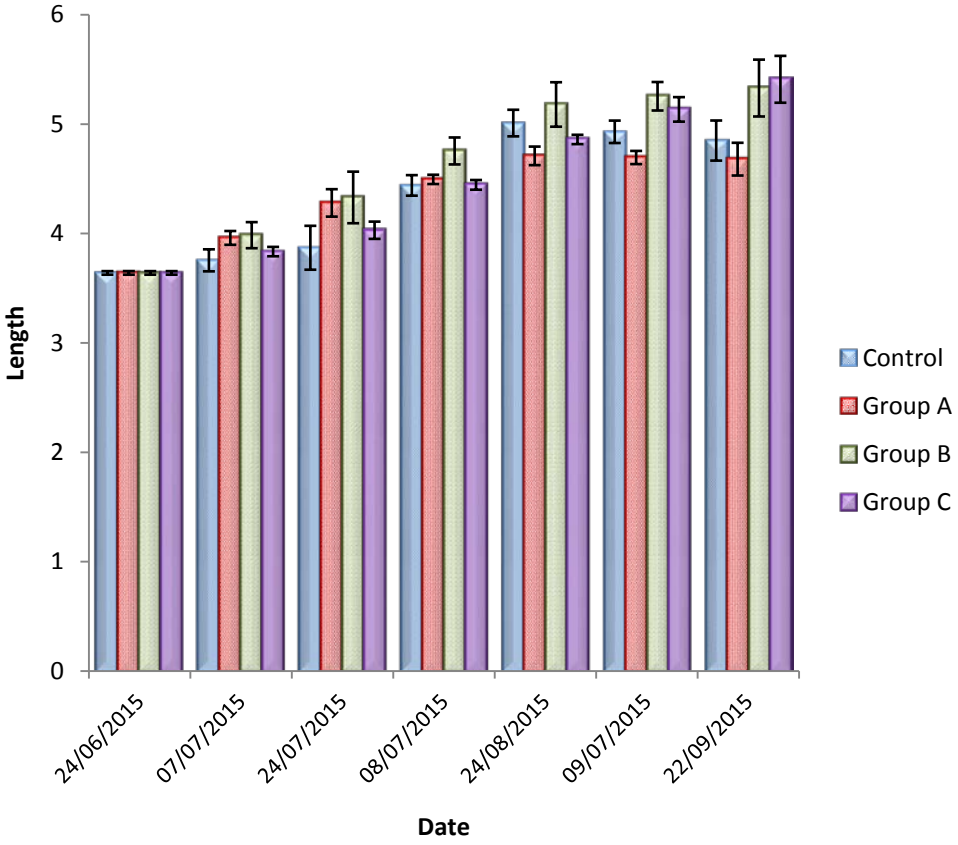
** = Significant (P<0.05); ** = Highly significant (P<0.01)*

Date x Group interaction mean±SE

Date	Group				Mean
	Control	Group A	Group B	Group C	
24/06/2015	3.640±0.016i	3.640±0.016i	3.640±0.016i	3.640±0.016i	3.640±0.008D
07/07/2015	3.755±0.101hi	3.960±0.063ghi	3.835±0.043ghi	3.985±0.119ghi	3.884±0.044CD
24/07/2015	3.870±0.201ghi	4.280±0.125e-i	4.030±0.079f-i	4.330±0.236e-h	4.128±0.088C
08/07/2015	4.440±0.094d-g	4.495±0.042c-g	4.445±0.044d-g	4.755±0.123a-e	4.534±0.045B
24/08/2015	5.010±0.122a-d	4.710±0.085b-e	4.860±0.043a-e	5.180±0.203ab	4.940±0.067A

09/07/2015	4.930±0.103a-e	4.695±0.060b-f	5.135±0.112abc	5.255±0.130ab	5.004±0.061A
22/09/2015	4.850±0.183a-e	4.680±0.150b-f	5.410±0.214a	5.330±0.260ab	5.068±0.111A
Mean	4.356±0.081B	4.351±0.056B	4.479±0.084AB	4.639±0.097A	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.



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Phytochemical Characterization and Insecticidal Property of *Jatropha* Plant

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Abstract- Phytochemical analysis on the pulverized leaf sample of *Jatropha curcas* as well as the insecticidal activity of the ethanolic leaf extract on *Callosobruchus maculatus* was carried out. The study investigated the mortality rate as a result of treatment of grains with the leaf extract at different dilutions of 20, 40, 60, 80 and 100mg/ml. These were tested against *C. maculatus* by treating 20g of bean sample with the extract and then infesting each in a plastic container with 20 adults of the insect and the untreated grains were used as the control. The plant extract resulted in a significant increase ($P<0.001$) in adult mortality at the end of 96 hours but there was no significance difference ($P=0.084$) after 24 hours. The result had a general dose-response characteristic. There was also significant variation in the phytochemical composition of the plant leaf. Compared to other phytochemicals, the concentration of saponin and tannin were higher in the plant leaf.

Keywords: *jatropha curcas, extract, callosobruchus maculatus, phytochemicals.*

GJSFR-C Classification : *FOR Code: 060799*

Strictly as per the compliance and regulations of :

Phytochemical Characterization and Insecticidal Property of *Jatropha* Plant

Mgbemena, I. C [α], Ebe, T. E. [σ], Ezea, C. O. [ρ], Irokanjo, C. E. [ω] & Okechukwu, R. I. [¥]

Abstract- Phytochemical analysis on the pulverized leaf sample of *Jatropha curcas* as well as the insecticidal activity of the ethanolic leaf extract on *Callosobruchus maculatus* was carried out. The study investigated the mortality rate as a result of treatment of grains with the leaf extract at different dilutions of 20, 40, 60, 80 and 100mg/ml. These were tested against *C. maculatus* by treating 20g of bean sample with the extract and then infesting each in a plastic container with 20 adults of the insect and the untreated grains were used as the control. The plant extract resulted in a significant increase ($P<0.001$) in adult mortality at the end of 96 hours but there was no significance difference ($P=0.084$) after 24 hours. The result had a general dose-response characteristic. There was also significant variation in the phytochemical composition of the plant leaf. Compared to other phytochemicals, the concentration of saponin and tannin were higher in the plant leaf.

Keywords: jatropha curcas, extract, callosobruchus maculatus, phytochemicals.

I. Introduction

Jatropha curcas often referred to as 'Jatropha' and is also known as 'physic nut'. The seeds contain between 35 to 40% oil (50-55% oil on kernel basis) (Kaushik *et al*, 2006), which can be processed to produce a high quality biodiesel fuel useable in a standard diesel engine (Kumar *et al*, 2008; Koyejo *et al*, 2010). Besides biodiesel production, *J. curcas* has numerous other uses as it is a multipurpose plant. Among other benefits are health and environmental values. Though extensive work was done on alternative uses of *Jatropha*, there is not much information available on its use as a pesticide (Dowlathabad *et al*, 2010). Within nature, man depends on plants for his food supply. Yet, as a result of population increase, the equilibrium between human beings and their food supply is considered unsound. This results from serious damages caused by insects. Insect and pest control has for long being controlled by majorly chemical method. Other methods such as biological (e.g. natural enemies and predators), cultural and legislative means have been employed as well. However chemical method of insect pest control is the most effective and its result is almost immediate. Control of insect pests is very important especially in agricultural practice as they adversely affect crop yield, as well as in medical practice for most insects act as a vector for most of the life threatening disease like malaria, yellow fever, dengue fever, Chikungunya fever, filariasis, encephalitis, etc (Anupam *et al*, 2011). Hence the control of most pests can indirectly control spread of certain diseases, improve crop yield, longevity of stored crops especially grain, reduce discomfort and so on. With chemical method of insect control being the major means, synthetic insecticides such as organochlorines and organophosphate compounds among others have been employed. But this has not been very successful due to human, technical, operational, ecological and economic factors. Insect pest control by use of chemical pesticides is fraught with various problems like environmental pollution, development of resistance, adverse effect on non-target organisms, residual toxicity, increased cost of chemicals, potential hazards to man and increased demand for hygienic food supplies, clearly demanding the need for alternative approaches (Ghosh *et al*, 2007). Due to the deleterious effect of chemical pesticides, though most effective, a search for natural-product based agrochemicals which are biodegradable, eco-friendly and safe to the environment has intensified (Adebowale and Adedire, 2006), which this study is committed to. However, most previous reports on plants as potential insecticides (eg. mosquitocides) centred on the larvae for *Jatropha sp* (Sakthivadival and Daniel, 2008; Rahuman *et al*, 2007). This approach is usually efficacious for it prevents proliferation by cutting down already developing larvae population in their number, especially in their breeding sites. With this, much population of the insects are targeted before they reach their adult stage. However there are meager reports on insecticidal activity of *J. curcas* leaf extract on adult insects which should actually complement on instances where the breeding sites were hidden or distant or situation where the larvae development was not truncated. Therefore there is need for this study which actually aimed at investigating the phytochemical properties and insecticidal activity of the leaf extract of *J. curcas*. In this work, a null hypothesis was tested which was stated thus: Null hypothesis (H_0): After 96 hours, there was no significant difference in the mortality rate by the various treatments (concentrations). Alternative hypothesis (H_1): There was a significant

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difference in the effect (mean mortality rate) of the various treatments after 96 hours.

II. Materials and Methods

a) Collection and Identification of Plants

Experiments in this study were conducted under laboratory conditions. Fresh healthy leaves of *Jatropha curcas* L. were collected from school of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Imo State in July, 2014 and botanical identification was carried out in the Crop Science Technology Department of the Federal University of Technology, Owerri. The taxonomical identification of the plant was confirmed by Dr. S.O. Ojiako, a plant taxonomist of the Department of Crop Science, Federal University of Technology Owerri, Nigeria. The leaves were washed in tap water and air dried to constant weight at room temperature after which they were ground into powder using an electric blender.

b) Phytochemical analysis of plant material

Phytochemical characterization of the leaves of *J. curcas* was carried out by screening for the presence and percentage concentrations of saponin, flavonoid, alkaloids, tannin, cyanogenic glycoside, oxalate and phytate according to the methods described by Trease and Evans (1989) and AOAC (1990).

c) Saponin

100ml of 20% v/v aqueous solution of ethanol was added to 2.0g of the pulverized sample in an Erlenmeyer flask and heated in a water bath at 55°C for 4 hours with occasional stirring. The mixture was filtered and the residue re-extracted with a fresh 100ml of 20% v/v ethanol solution. Both extracts were combined and volume reduced to about 50ml on a water bath set at 90°C. The mixture was cooled and extracted using 20ml of diethyl ether in a 250ml seperatory funnel. The ethereal layer was discarded followed by addition of n-butanol into the lower layer, then shaken and allowed to separate. The organic layer was shaken twice with 10ml of 5% w/v sodium chloride solution in a seperatory funnel and poured into a pre-weighed beaker. The mixture was evaporated to dryness on a boiling water bath, cooled and reweighed. Percentage saponin content was calculated.

d) Flavonoid

100ml of 80% v/v methanol solution was added to 2.0 g of powdered sample, stirred on a magnetic stirrer for 3 hours and later filtered using a filter paper. The residue was re-extracted and filtered again. Both extracts were combined and put into a pre-weighed beaker and then evaporated to dryness on a boiling water bath. It was cooled and reweighed, and percentage flavonoid computed.

e) Alkaloid

100ml of 20% v/v ethanolic acid solution was added to 2.0g of the powdered sample in a flask, covered and allowed to stand for 3 hours with stirring on a magnetic stirrer. The mixture was filtered while the residue re- extracted as before. Both extracts were combined and concentrated on a water bath to about one-quarter of the original volume. Drops of concentrated ammonium hydroxide were added upon cooling till complete precipitation of the alkaloids had occurred. The resulting mixture was kept in a cool place overnight and filtered using a pre-weighed filter paper which was later dried in an oven set at 70°C for at least 6 hours. Weight of the filter paper and content was reweighed after drying in a desiccator and percentage alkaloids calculated.

f) Tannin

50ml of distilled water was added to 0.5g of powdered sample and stirred for 2 hours on a magnetic stirrer. The mixture was filtered into a 50ml volumetric flask and made up to the mark. Three test tubes (blank, sample and standard) were set up containing 1ml of water, 1ml of sample filtrate and 1ml of 2mg/ml tannic acid solution respectively.

1ml of each of 0.1M hydrochloric acid, ferric chloride and potassium ferrocyanide solution were added to each of the three test tubes. Their absorbances were measured using spectrophotometer set at 720nm within ten (10) minutes and the percentage tannin content was calculated.

g) Cyanogenic glycoside

1.0g of powdered sample was placed in a quick fit round bottom flask. 200ml of deionized distilled water was added to it and allowed to stand for 2 hours. The flask was then connected to complete the distillation setup. 150ml of the mixture was distilled into a 250ml receiver flask containing 20ml of 2.5% w/v sodium hydroxide solution. 100ml of the resulting mixture was measured out into another flask and 8ml of 6.0M ammonium hydroxide solution was added followed by 2ml of 5% potassium iodide solution. The contents were mixed and titrated with 0.02N silver nitrate solution till no further turbidity upon addition of the silver nitrate solution. Percentage cyanogenic glycoside was then calculated.

h) Oxalate

To 5.0g of dry powdered sample, 20ml of 0.3N hydrochloric acid was added in a flask and stirred at 50°C for 1 hour. The mixture was filtered and the process repeated two times while combining the filtrates and making it up to 100ml with distilled water. To 20ml of filtrate, 3 drops of phenolphthalein indicator was added. 5.0N ammonium hydroxide was added in drops till the reaction mixture was alkaline. Glacial ethanoic acid was then added in drops till the pink colouration

disappeaed and then a few more drops just to make the mixture acidic. 5ml of 5% calcium chloride solution was added and mixture allowed to stand for 3 hours, after which it was centrifuged at 300rpm for 15 minutes. The residue was washed three times with hot water using centrifugation method. The residue was then dissolved in 2ml of 3.0N tetraoxosulphate (VI) acid solution with warming at 70°C followed by titrating the resultant solution with freshly prepared 0.01N potassium permanganate solution till permanent pink colouration that lasted for 30 second was obtained. Blank titration was carried out using the same volume of 3.0N tetraoxosulphate (VI) acid as that used in dissolving the oxalate residue. The test was done in duplicates and percentage oxalate content calculated.

i) Phytate

2.0g of dry sample was placed in a flask and 50ml of 0.18M trichloroacetic acid solution added to it. The mixture was stirred for 1 hour and centrifuged at 3000rpm for 10 minutes. 10ml of centrifugate was added to 4ml of 0.036M Iron (III) chloride solution in a boiling tube and placed in boiling water for 45 minutes. Resulting mixture was centrifuged and ferric phytate was collected, washed twice and with 20ml of 0.18M trichloroacetic acid solution and 30ml of water each time. 3ml of 1.5 M sodium hydroxide solution was added to the residue followed by 25ml of water and placed in boiling water till coagulation of ferric hydroxide was complete. The ferric hydroxide was collected by centrifugation and washed twice with water. Ferric hydroxide thus obtained was dissolved in 40ml of 3.2M trioxonitrate (V) acid and made up to 100ml using distilled water. Iron content was determined and content Absorbance was measured in a spectrophotometer at 540nm and percentage phytic acid was then computed.

j) Preparation of plant extract

The powdered plant material (100g) was for ethanolic bulk extraction. Ethanol was added to the sample and shaken intermittently for at least three hours and left standing overnight. The mixture was filtered into a clean dry beaker, obtaining a dark green filtrate. The ethanol was then gently evaporated by placing the beaker in an oven set at 75°C to obtain a thick dark green plant extract. Using an analytical balance, required quantities were weighed into different beakers and diluted appropriately with methanol (Sanis *et al*, 2012) giving rise to five different dilutions of 20, 40, 60, 80 and 100mg/ml.

k) Maintenance of the experimental insect

Bean weevil, *Callosobruchus maculatus* was collected in bulk from bean sellers and identified by Mrs Usenwunne Chinwe, an entomologist in the Animal Science Department of Federal University of Technology, Owerri. They were acclimatized in the laboratory in a clean uninfected jar. The jar was covered with a clean thin cloth and tightly held in place with a rubber band to allow for aeration and prevent entry or exit of insects. It was kept at room temperature 37°C with 12 hour light and dark regimes.

l) Toxicity of Jatropha curcas leaf extract on C. maculatus in grains

About 400g of beans was washed with clean water and then dried in an oven. 20g of the grain was weighed out into different clean petri - dishes. The various dilutions of the leaf extract were applied to them and left to air dry. Each dilution or concentration was applied to three sets of beans, each weighing 20g. The same was done for other treatments while the control had no treatment.

The beans were put into various transparent plastic jars and each container was then infested with 20 *C. maculatus* adults selected at random. The jars were covered with a thin cloth each and held with a rubber band to facilitate aeration and prevent contamination. Each treatment was replicated three times. The adult mortalities were counted and recorded for a period of four days at a 24 hour interval.

m) Statistical analysis

Statistical analysis was performed with GraphPad Prism version 5.0 using one-Way analysis of variance (ANOVA) followed by Dunnet multiple comparison test. Results were expressed as mean ± S.E.M. Groups of data were considered to be significantly different if $P < 0.05$. Schneider-Orelli formula was used to adjust the data where there were deaths in the control treatment while Probit analylsis was employed in the calculation of LC_{50}.

III. Result and Analysis

At the end of 24 hours, after subjection to various concentrations of the plant extract, there was no significant difference ($P=0.084$) when compared to the untreated control. However at the end of 48 hours the treatments resulted in a significant ($P<0.001$) increase in the mean mortality rate, and after 72 hours there was even more significant increase in mortality ($P<0.001$) among the various treatments relative to the control. At the end of three days (72 hours) mortality rate increased significantly ($P<0.001$), approaching a median value. Mortality rate was proportional to the concentration and duration. There was no significant difference in mortality for 20mg/ml over the period of four day which was obviously different in other treatments at some point during the period of 96 hours when compared with the control (Table 1).

Table 1 : Effect of ethanolic extract of *Jatropha curcas* leaves on the mortality rate of *Callosobruchus maculatus*

Treatments (mg/ml)	Mortality count			
	24hours	48hours	72hours	96hours
Control	0.000±0.00	0.000±0.00	0.333±0.3333	0.667±0.3333
20	0.000 ± 0.00^{ns}	1.000 ± 0.5774^{ns}	1.667 ± 0.3333^{ns}	3.000 ± 0.5774^{ns}
40	0.667 ± 0.333^{ns}	1.333 ± 0.3333^{ns}	$2.6\,67\pm0.3333^{a}$	4.667 ± 0.3333^{b}
60	0.667 ± 0.3333^{ns}	1.333 ± 0.3333^{ns}	3.333 ± 0.8819^{b}	5.333 ± 0.8819^{c}
80	1.000 ± 0.5774^{ns}	2.333 ± 0.3333^{b}	4.333 ± 0.3333^{c}	7.000 ± 0.5774^{c}
100	1.333 ± 0.3333^{ns}	3.333 ± 0.3333^{c}	5.667 ± 0.3333^{c}	9.333 ± 0.8819^{c}

Values are expressed as Means ± SEM with n = 3; a, b, c, ns represent $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}p < 0.01$ and no significance respectively compared to control. Means in the same column having the same letter (superscript) are not significantly different at $p < 0.05$.

Mean percentage mortalities were computed (Table 2) and also corrected using the Schneider-Orelli formula (Püntener, 1981) in cases where death was recorded in the control treatments (Table 3), which occurred after 72 and 96 hours as follows:

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treatment plot} - \text{Mortality \% in control plot}}{100 - \text{Mortality \% in control plot}}\right) \times 100$$

Result for the phytochemical analysis carried out is shown below in Table 4. Results were expressed as percentages. The plant *J. curcas* showed a higher content of tannn(7.58%) than other phytochemicals.

Table 2 : Percentage Mortality of *Callosobranchus maculatus* after 96 hours of exposure in ethanolic extract of *J. curcas*

Treatments (mg/ml)	% Mortality			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	1.665	3.335
20	0	5	8.335	15
40	3.335	6.665	13.335	23.335
60	3.335	6.665	16.665	26.665
80	5	11.665	21.665	35
100	6.665	16.665	28.335	46.665

Table 3 : Percentage Mean Mortality Corrected using Scheider – Orelli Formulae

Treatments (mg/ml)	Corrected % Mortality			
	24	48	72	96
Control	0	0	0	0
20	0	5	6.783	12.067
40	3.335	6.665	11.868	20.69
60	3.335	6.665	15.254	24.135
80	5	11.67	20.339	32.757
100	6.665	16.67	27.122	44.825

Table 4 : Phytochemical composition of J. curcas

Phytochemical	Concentration (%)
Saponin	4.89
Flavonoid	3.56
Alkaloid	4.50
Tanin	7.58
Cyanogenic glycoside	4.19
Oxalate	3.62
Phytate	4.10

IV. Discussion

Insecticidal activity of J. curcas

The result from this study showed that the plant, *J. curcas* exhibited an insecticidal action on *C. maculatus* adults with varying susceptibility. After 96 hours there was a significant increase in mortality rate among treatments, hence the null hypothesis was rejected thereby accepting the alternative hypothesis.

From Table 1, there was no significant difference in insecticidal mortality rate by the plant extract compared to the control treatments for all

concentrations (P=0.084). However, by the end of 48, 72 and 96 hours, there were significant differences in mortality rates; P<0.001and P<0.005 respectively. Comparing the various doses after every 24 hours, there is a marked increase showing a positive correlation between concentration and the percentage of insect mortality which confirms the report of Shadia *et al* (2007). Highest mortality rate was 44.83% compared to 12.07% (Corrected %) in 100 and 20mg/ml respectively at the end of 96 hours (Table 3). Death in the control treatment accounted for error as shown in Tables 1 and 2, which is sometimes a part of an experiment. However, the percentage was corrected with that in mind (Table 3).

Tables 1 and 3 showed that the efficacy of the plant extract is both dose and time dependent, for a higher mortality rate is expected from the above trend. Either higher concentration of the extract at a given time and/ or time lapse for a given concentration will both produce better results. This shows that the efficacy of the plant can be maximized by employing higher concentration or allowing a particular treatment to act for a longer period time. However due to the toxic effect of the plant, lower concentration is necessary considering human health especially when used as grain protecting agent. The median lethal concentration (LC_{50}), computed using the Probit analysis (Finney, 1952), gave 149.6mg/ml. This is the concentration that will kill 50% of the insect population after 96 hours. This implies that a higher concentration will be needed to yield a 50% mortality rate within 96 hours, unless allowed for a relatively extended period of time.

Major insecticidal works on *Jatropha* centred on the seed oil (Ebtisam *et al*, 2013; Dowlathabad *et al*, 2010; Adebowale and Adedire, 2006) while meager reports on the leaf are found (Sanis *et al*, 2012). Studies on the seed oil showed higher percentage mortalities for even relatively lower concentrations of the *Jatropha* seed oil extract. Constance *et al* (2013) reported a wide gap between the mortality rates of *Sitophilus zeamais* at the end of 42 days after being treated with leaf, juice and seed oil extracts exclusively. The result from the study showed that the insect mortality increased with the concentration of the extract as expected, but at concentrations more than 10ppm, the seed oil showed significantly (P<0.001) higher insecticidal effect compared to others. In fact 100% mortality was recorded in grains pre-treated with seed oil compared to 58.9% and 55.6% mortality observed for grains pre-treated with leaf extract and juice of *J. curcas* respectively after 42 days.

The phytochemical analysis of the leaf of *J. curcas* in this study revealed the presence of saponin, tannin, flavonoid, alkaloid, oxalate, phytate and cyanogenic glycosides in varying concentrations as shown in Table 4. Some of these secondary metabolites account for the toxicity of the plant extract conferring in it its characteristic insecticidal property, especially phytate, saponin and cyanogenic glycosides. Achten *et al*, (2008) reported high concentration of phorbol esters (phorbol-12-myristate-13-acetate) present in *Jatropha* seed which had been identified as the main toxic agent responsible for *Jatropha* toxicity. Presence of these phytochemicals also confers taxonomic importance on the plant. Among the phytochemicals investigated from the leaf, tannin was found to be the most abundant followed by saponin and alkaloid. flavonoid and oxalate were the lowest in concentration. A similar sequence with tannin being the most abundant phytochemical in plant parts, followed by saponin, alkaloid and so on as seen in this study has been reported by Mallikharjuna *et al* (2007). Other recent investigation showing presence and importance of these phytochemicals similar with the results of this investigation include Ogunkunle and Ladejobi (2006), Ferreira *et al* (2009) and Kumar *et al* (2009).

The presence of phytochemicals also lays credence to the fact that this specie is a potential source of these important phytochemicals. For example, flavonoids are one of the most popular secondary metabolites possessing a variety of biological activities at nontoxic concentrations (Irshad *et al*, 2010). Flavonoids together with other secondary metabolites identified in *J. curcas* has been severally reported in other plants to show curative activity against diverse pathogens, used traditionally as analgesic, antimicrobial (Hassan *et al*, 2004; Singh *et al,* 2009) and insecticidal, as this study has shown. Cardiac glycoside was found to have acaricidal effect against larva and adult stages of the camel tick (Al-Rajhy et al, 2003).

However, *J. curcas* leaf extract have shown adulticidal activity in this study and in fact could serve as an effective replacement for chemical insecticides. The insects used were adults which also explain the relatively low mortality rates recorded. This study has demonstrated that the use of *J. curcas* ethanolic leaf extract can significantly increase mortality rate in *C. maculatus* infesting treated grains and thus reduce the extensive use of synthetic organic chemical insecticides which result in environmental hazards and resistance in major insects, especially insect vectors.

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Review: Distribution and Productivity of Dekoko (*Pisum Sativum* Var. *Abyssinicum* A. Braun) in Ethiopia

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Abstract- The genus pea is originated in Near Eastern and Mediterranean centers of diversity. Since Ethiopia is one of the Vavilov's centers of origin of cultivated flora, distribution of the single *Pisum sativum* subsp. *abyssinicum* A. Braun is in Ethiopia in the Ancient Mediterranean area of origin of cultivated plants. Field pea is one of the oldest crops in Ethiopia with a unique subspecies developed in Ethiopia, *Pisum sativum* subsp. *abyssinicum*. Dekoko (*Pisum sativum* var, *abyssinicum*) an important crop in mixed farming and is widely mixed with the main cereal crops growing in south Tigray such as sorghum, barely and teff and is the potential grain growing or cultivated in the region with chick pea, linseed, grass pea and fenugreek. While varieties of field peas can be found around the world. The existing field pea germplasm endemic to Northern part the Ethiopia particularly the highland areas of South Tigray and the Northern part of the former province of Wollo has a phenotypic diversity and tolerance/resistance to disease. Dekoko is capable of producing seed yield of up to 1.95 t/ha under phosphorus fertilization but not yet record data without fertilizer treatment. Dekoko can be used as landrace in Ethiopia because, land races are the genetic wealth that a crop acquires over many years of its existence and have considerable breeding values as they contain valuable adaptive genes to different circumstances.

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REVIEWDISTRIBUTIONANDPRODUCTIVITYOFDEKOKOPISUMSATIVUMVARABYSSINICUMABRAUNINETHIOPIA

Strictly as per the compliance and regulations of :

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I. Introduction

Legumes (*Fabaceae* Lindl., syn. *Leguminosae* Juss. and *Papilionaceae* Giseke) are one of the plant families comprising the largest number of economically important crops. Among them are some of the first domesticated species in the world, such as common chickpea (*Cicer arietinum* L.), common lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) (Zohary & Hopf, 2000). This has been attested by many archaeobotanical (Tanno & Willcox, 2006) and historical linguistic (Mikić, 2012) analysis. The total world pea grain production fluctuates 10–12 million tons, with Canada as the leading producer, followed by USA, India, Russia, France and China (Smýkal *et al*. 2012). Legumes are facing the bottlenecks caused by environmental factors that emphasize adaptation to deal with productivity and yield quality aspects, raising the questions how to solve the issue of this challenging the productivity basis of cultivated legume species.

Peas and other legumes are desirable in crop rotations because they break up disease and pest's cycles, provide nitrogen, improve soil microbe diversity and activity, improve soil aggregation, conserve soil water, and provide economic diversity (Chen *et al*., 2006). Peas are grown as green manures and cover crops because they grow quickly and contribute nitrogen to the soil (Clark, 2007). Grain legumes, play a crucial function in organic cropping systems (Aslam *et al*., 2010). They possibly will supply nitrogen (N) to the organic cropping system via N_2 fixation and produce grain rich in protein at the same time as improving soil N for the succeeding crop (Corre-Hellou & Crozat, 2005). Like the other legumes crop roots, pea roots have nodules, formed by the bacteria *Rhizobium leguminosarum*, which convert atmospheric nitrogen (N_2) to ammonia (NH_3).

Scarcity of water is a severe environmental constraint to legume productivity (Farooq *et al*., 2009). Early maturing varieties escape terminal drought (Agbicodo *et al*., 2009), but if exposed to intermittent moisture stress during the vegetative growth stage, they perform very poorly. Peas have survival mechanisms when grown in dry regions by reducing loss of water that help them in early and efficient increases in diffusion resistance and reduction of transpiring surface (Agbicodo *et al*., 2009), by having high water conducting capacity through more xylem and dense leaf venation, or peas also can avoid desiccation by storage of water via production of an abundance of root systems that absorb quickly and provide nitrogen (Clark, 2007). Dekoko (*Pisum sativum* var. *abyssinicum* A. Bruan) pea which is this type is the most common type of pea used as a green manure or cover crop in South Tigray and North Wollo because it adapted to cold temperatures and fit in many rotations. Besides to this, grain legumes are good "rupture" crops to pests (Chemining-wa and Vessey, 2006). *Pisum sativum* var. *arvense* A.Bruan, a synonym of Dekoko, produces antifungal proteins help the plant to combat phytopathogenic fungi and thus protect the plant from devastating damage caused by fungal infections and prevent massive economic losses (Wang and Ng, 2007).

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Dekoko (*Pisum sativum* var, *abyssinicum*) an important crop in mixed farming and is widely mixed with the main cereal crops growing in south Tigray such as sorghum, barely and teff and is the potential grain growing or cultivated in the region with chick pea, linseed, grass pea and fenugreek as reported by Girmay *et al*. (2014). While varieties of field peas can be found around the world, this endemic variety is unique to Ethiopia, and particularly the highland areas of South Tigray and the Northern part of the former province of Wollo. Its distribution is limited to Northern Ethiopia.

Ethiopia is the largest producer of cool-season food legumes (CSFLs) in Africa and unique producer of Dekoko in the world. CSFLs including Dekoko are largely produced by subsistence farmers and serve as supplementary protein sources and soil fertility restorers. Yields are very low, mainly limited by soil fertility (Yemane and Skjelvåg, 2002), as CSFLs are cultivated in poor soils, often without fertilization. However, CSFLs require an adequate supply of nutrients for optimum growth and yield (Yemane and Skjelvåg, 2002). Therefore, enhancing the productivity of CSFLs through improved agronomic practice, such as fertilization, expanding in distribution of localized crops like Dekoko to other agro-ecological area contribute to sustainable crop production in Ethiopia.

For expansion, knowledge on the distribution and productivity of native important crops but underutilized is crucial for the discovery of modern productive crops because modern crops were gradually derived from their wild ancestors through continual selection for larger size, improved fruit, and other desired traits (Esquinas-Alcazar, 2005).

Also, despite its domestication thousands of years ago by natives of the North Ethiopian, until this 21th century dekoko is a neglected and less known crop in the whole country where it is commonly cultivated, in and outside the tropical and subtropical belt of Africa. Therefore, information from this study is important in drawing the attention of researchers mainly in the whole country, and hence generally researched for its importance as anti-famine food security source by subsistence farming systems, drought and disease resistant, and export crop that will receive greater attention and interest from many international research and development organizations beyond the owner country.

II. Origin and Distribution of Dekoko in Ethiopia

The genus pea is originated in Near Eastern and Mediterranean centers of diversity. Since Ethiopia is one of the Vavilov's centers of origin of cultivated flora, distribution of the single *Pisum sativum* subsp. *abyssinicum* A. Braun is in Ethiopia in the Ancient Mediterranean area of origin of cultivated plants. Field pea is one of the oldest crops in Ethiopia with a unique subspecies developed in Ethiopia, *Pisum sativum* subsp. *abyssinicum*. The existing field pea germplasm in the country has a phenotypic diversity and tolerance/resistance to disease (IBC, 2007).

The taxon *Pisum sativum* var. *abyssinicum* as its name implies was initially found in the northern region now forming Ethiopia with two ethnolinguistic regions of the country, Tigray and Amhara regions in the northern part of Ethiopia. According to some earlier views, dekoko which was once common is now very infrequently grown in some area of South Tigray and North Wollo. It had not reached other similar agro-ecological areas of the country although the crop was said to have been common in 'the time of Haileselassie'. Yet it is important festival foods and, despite its high price, it is bought from Mekelle market in small quantities, four or five times a year (Wetterstrom, 2006). Dekoko is a unique variety independently developed and cultivated in Ethiopia. Currently, Dekoko is restricted to two provinces of the country particularly of South Tigray and North Wollo in distribution and Southern Yemen and shows a greater affinity to *Pisum sativum* var. *fulvum* (Yemane and Skjelvåg, 2002) and *Pisum sativum* var. *arvense* (Wang and Ng, 2007).

According to Keneni *et al*. (2013), dekoko accessions may show genetic similarities regardless of the differences in places of origin. This may be related to the fact that the Abyssinian field pea exists in confined adjacent regions that could be considered similar in terms of both agro-ecology and crop production system under which distinct pattern of evolution may not be expected. A study previously conducted on *P. sativum* var. *sativum* also suggested that differences in eco-geographic origin might not necessarily suggest presence of genetic divergence for other morpho-agronomic traits (Keneni *et al*., 2013).

III. Taxonomic and Biological Description of Dekoko (*Pisum Sativum* Var. *Abyssinicum*)

The local name of *Pisum sativum* var. *abyssinicum* A.Braun is dekoko, which means "minute seeded." It is also known as yagere ater ("pea of my country") or tinishu Ater ("the smallest pea") in Amharic. *Pisum sativum* var. *abyssinicum* is also locally known as Ye-Ethiopia Ater (Ethiopia pea, Abyssinian pea) in Amharic (Hadiss Yirga and Dargie Tsegay, 2013).

The genus *Pisum* consists of five species: *P. fulvum* (Sibth. *et* Smith.), *P. abyssinicum* ((A.Braun) Govorov), *P. sativum* L., *P. humile* (Boiss. *et* Noë), and *P. elatius* ((M. Bieb.) Asch. *et* Graebn) (Cupic *et al*., 2009). However, Vershinin *et al.* (2003) found close relationships among *P. sativum, P. humile* and *P. elatius* species and divided the genus into three major groups: *P. fulvum*, *P. abyssinicum* and *P. sativum- P. humile - P.*

elatius complex and majority of modern taxonomic classifications recognize the three pea species, namely common pea (*Pisum sativum* L.), red-yellow pea (*Pisum fulvum* Sm.) and dekoko, the Ethiopian pea (*Pisum sativum* var. *abyssinicum* A. Braun).

The exact status of Ethiopian pea has been an object of discussion for decades. *Pisum sativum* var. *abyssinicum,* previously classified as a species, was regarded as an ecotype of *Pisum sativum* var. *arvense;* afterwards, it was considered as synonym of *Pisum sativum* in respect to which *abyssinicum* differentiated into a morphologically distinct form under cultivation and selection in a different geographical area. The results of diverse tests, including ecogeographical (Maxted & Ambrose, 2001), genetic (Baranger *et al*. 2004) and biochemical (Hadačova *et al*. 1980) confirmed the view that Ethiopian pea deserved a status of a separate species within the genus *Pisum* L., together with red yellow (*P. fulvum* Sm.) and common peas. Early hypothesis suggested that Ethiopian pea was simply a geographically isolated variety of common pea, but soon it became clear that it was a separate species domesticated in Ethiopia (Wetterstrom, 2006). An analysis using flow cytometry revealed that, while having the same number of chromosomes of $2n = 14$, Ethiopian pea had between 4% and 8% more DNA than common pea (Baranyi & Greilhuber, 1995). An electrophoretic test with DSD-PAGE showed a clear distinction of Ethiopian pea from common pea (Mishra *et al*., 2012). Various genomic tools also confirmed that Ethiopian pea is characterized by a rather narrow polymorphism in comparison to other *Pisum* taxa and that it was quite independently domesticated from common pea (Vershinin *et al*. 2003). Generally, all these results fully or partially are in accordance with the traditional botanical taxonomy of the genus *Pisum* (Zong *et al*. 2009).

The subspecies appears to be intermediate between *P.* sativum ssp. *sativum* and *P.* sativum ssp. *elatus*. With the former, it shares the traits of indehiscent pod, relatively smooth seeds (Fig.1), and relatively thin testa. However, *Pisum sativum* ssp. *abyssinicum* also is dominant for *A* factor that has a great role for flower colour, and possess an extra set of recessive genes for flowering time, and has relatively small flowers and pods, all characteristics that are fixed or known to occur in *Pisum sativum* ssp. *sativum* and are often lacking in *Pisum sativum* ssp. *abyssinicum* (Weeden and Wolko, 2001).

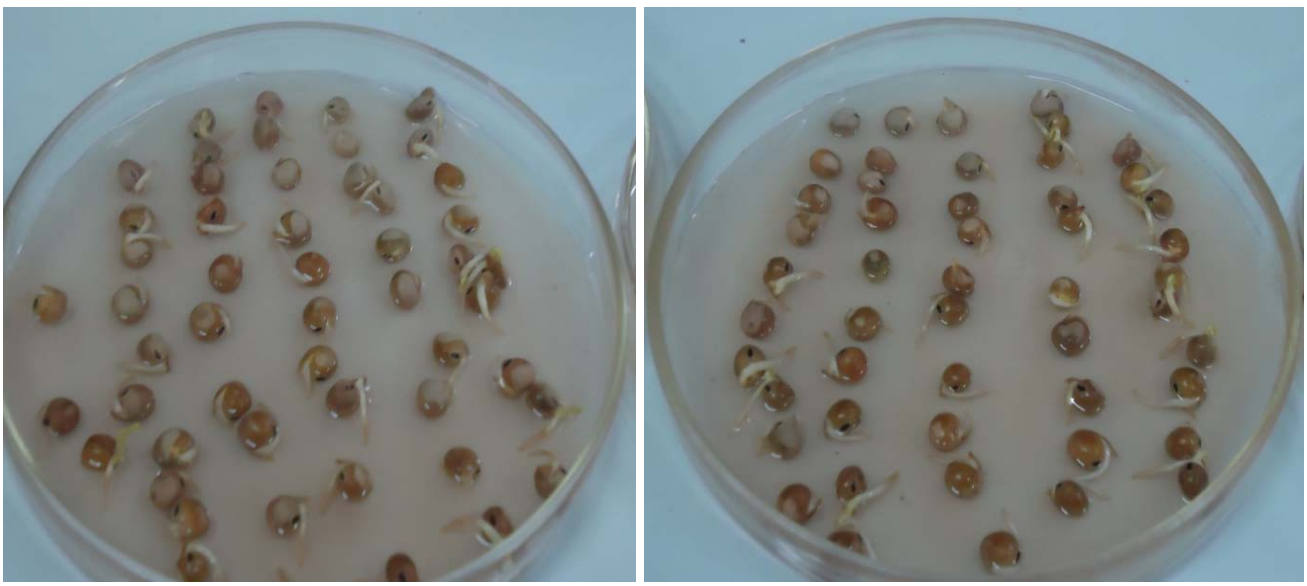

Figure 1 : Seeds of Dekoko (photo: Berhane G. in 2013)

The pea *Pisum sativum* var. *abyssinicum* is a cool-season annual vine that is smooth and has a bluish-green waxy appearance (Fig.2). Pea vines can be up to 9 ft long, however modern cultivars have shorter vines, about 2 ft long. The stem is hollow, and the taller cultivars cannot climb without support (Elzebroek and Wind, 2008), Dekoko (*Pisum sativum* var. *abyssinicum* A.Braun) shares the morphological characteristic of modern cultivars. Leaves are alternate, pinnately compound, and consist of two large leaflike stipules, one to several pairs of oval leaflets, and terminal tendrils in pea (McGee, 2012; Mikić *et al*., 2013c).

Figure 2 : Yellow flower of Ethiopian pea in glasshouse tests at Rimski Šančevi in 2013 (Photo: Sanja Mikić)

The abyssinicum pea, *Pisum sativum* var. *abyssinicum* can be easily recognized from other similar peas strongly by its serrate leaflet margins (Fig. 3) with 5 to 12 nodes which constitute the most obvious morphological character also it has traits such relatively large seeds, lack of dehiscent pods and lack of dormancy mechanism (hard seededness), strongly suggest that *Pisum sativum* ssp. *abyssinicum* has undergone partial domestication (Weeden *et al*., 2001). The serrate character was shown to be controlled by a dominant gene in crosses using the line 808 (= WBH 808). From the three types of leaflet serration present in the *Pisum* germplasm, the *serratus,* or saw-form that controlled by *Ser* distinct loci is common in Dekoko. The *Ser* is typical of *P. sativum* ssp. *abyssinicum* and a line from Afghanistan (WL 1414) or *Ser*: JI 2781 (= WL 1414) (Weeden and Ambrose, 2004).

Figure 3a : Serrate leaflet margins of Dekoko. Photo: Berhane G. in 2013

Figure 3b : Serrate leaflet margins of Dekoko Source: Missouri Botanical Garden

Ethiopian pea is considered examples of wild relative that may be gene pools of desirable traits for introducing into the crops while retaining a high agronomic performance. Ethiopian pea is also regarded as the source of novel genes, such as *er3* (Fondevilla *et al*., 2008), controlling resistance to powdery mildew (*Erysiphe pisi* DC), as well as to *Mycosphaerella pinodes*. On the other hand, there are certain chromosomal differences and partial fertility between Ethiopian peas and common pea. Such complex restrictive behaviour frequently limits a degree of the success of introgressing desirable traits related to enhancing biotic stress resistance from wild into cultivated germplasm. This is also a long process, since numerous back-crosses between Ethiopian and common pea F1 hybrid and common pea cultivar are needed to introgress a trait improving biotic stress tolerance and merge it with high, quality and stable grain yield (Fondevilla *et al*., 2008).

Pisum sativum var. *abyssinicum* is a compact, well delineated taxon with a narrow genetic base and little allozyme variation. It can be clearly defined on the basis of the genotype rather than phenotypically (Weeden and Wolko, 2001). The low genetic diversity in *Pisum sativum* ssp. *abyssinicum* may in part be due to a relatively small number of accessions available with small geographical distribution. In addition, there may be germplasm growing in Ethiopia that is not collected. Based on the data available from different studies, *Pisum sativum* ssp. *abyssinicum* is considered as an isolated and genetically unique taxon that may be important source of genetic diversity for breeding application once the semi sterility problem is overcome. In any event, one and probably only one accession of *Pisum sativum* ssp. *abyssinicum* needed be included in any core collection of *Pisum sativum* germplasm (Weeden and Wolko, 2001).

IV. Productivity of Dekoko (*Pisum Sativum* Var. *Abyssinicum)* in Ethiopia

Agricultural biodiversity, comprising all the elements from gene to agricultural ecosystems, is one of the principal components of natural resources (Atta-Krah, 2004), even though it is often treated separately from other natural resources in many parts of the world (Atta-Krah, 2004), including Ethiopia. A reservoir of irreplaceable genes and gene complexes of a number of crops is currently being lost at a rapid rate through genetic erosion as a result of displacement of landraces by modern varieties, dynamics of agriculture and land uses, destruction of natural habitats, and drought that finally lead to less productivity (Esquinas-Alcazar, 2005). Genetic erosion, the gradual depletion of natural resources in general and crop germplasm in particular with both natural and artificial interferences is, therefore, a current topic all over the world to overcome crop production problems.

Some crops which were common in the past have recently become rare. Faba bean (*Vicia faba* L., *abie*), the common field pea (*Pisum sativum* subspecies *arvense* L., ater), and the Ethiopian variety of the field pea (*Pisum sativum* L. var. *abyssinicum* A.Bruan., dekoko), which were once common, are now very infrequently grown in some parts of Ethiopia as near Adi Ainawalid in south Tigray (Hassan, 2002). They are important festival foods and, despite their high price, they are bought from Mekelle market in small quantities, four or five times a year. All these crops were said to have been common in 'the time of Haile Selassie' (prior to 1973). The main cause for their decline was given as the lack of rainfall, coupled with the small land holdings. Implicated also is the political disruption of traditional farming during the 1970s and 1980s, which caused much loss of germplasm. At a national level, attempts are now being made to conserve and reintroduce the

landraces, which may include varieties better adapted to the current climatic conditions (Hassan, 2002).

Yields of grain legumes are smaller and generally more variable than those of many other crop species. In developed countries, grain yields of legumes have not increased as rapidly as those of cereal crops (Jeuffroy and Ney, 1997). There is thus a need to increase the performance of pulse crops, particularly in developing countries, where most grain legume production is for human consumption and demand is increasing due to population increase. Jeuffroy and Ney (1997) estimated that yields in developing countries were only 45% of those of developed countries for pea, and 75% for faba bean and chickpea.

By the year 2013, Africa ranked fifth in pea crop production (730.4 tons) after North America (Canada and US with 4,669.3 tons), Europe (3,024.0 tons), East Asia (mainly china with 1,567.3 tons) and South Asia (839.1 tons) from the 2013 world major pulse crops producers countries or regions of the world, but the third major chick pea production region (670.4 tonnes/ha) after South Asia (9,895.4 tons) and Oceania (813.3 tons) in the world (CSA, 2011). Overall, from the 2013 world major pulse crops production areas, Africa is second (18,707.1 tons) after Northern America (20,881.5 tons) with the leader producer of cowpea (7,782.1 tons) (CSA, 2011).

In the 2013 world trade of pulse crops, pea held the highest exported (4,978.1 tons) and imported (4,284.7 tons) crop of which Northern America (Canada and US) exported 3,275 tons of the total export and South Asia importing 1,840.9 tons of the total import. There are no clear reasons for the discrepancies between total import and export quantities. According to CSA (2011), perhaps reporting error, duplication of reports, periodicity or a combination may be the reasons.

Field pea (*Pisum sativum* L.) is known to be grown in Ethiopia since antiquity (Keneni *et al*., 2013). Currently, the crop is the fourth most important pulse crop in Ethiopia, preceded only by faba bean, haricot bean and chickpea in terms of both area coverage and total national production (CSA, 2011). There are two botanical cultivars of *Pisum sativum* known to grow in Ethiopia, namely *P. sativum* var. *sativum* and *P. sativum* var. *abyssinicum* (Keneni *et al*., 2013). The botanical cultivar *P. sativum* var. *sativum* dominates the production system in the highlands of Ethiopia (Messiaen *et al*., 2006) while *P. sativum* var. *abyssinicum* is limited to sporadic growth in some pouches, particularly in Wollo and Tigray in the north and Arsi in the southeast (Keneni *et al*., 2013). Like all other cool-season food legumes, the productivity of field pea in general and that of the abyssinian pea in particular was very low in Ethiopia compared to many other continents and countries of the world (Kelley *et al*., 2000), which, among many other factors, may be attributed to poor soil fertility (Getachew *et al*., 2006 in keneni *et al*., 2013). Field pea (*Pisum sativum* L.), the crop that widely grow in mid to high altitude the fourth most important legume crop in Ethiopia after faba bean, haricot bean and chick pea had area coverage over 203,990.64 ha with a total production of 257,031.41 tons which accounts for 13% of the total grain legume production and average yield of 1.26 t/ha until 2011 which is very low (CSA, 2011). However, by 2015 the average yield of field pea in Ethiopia was 12.37 t/ha in area coverage reaching 212,890 ha with an annual production of 2,632,663.87 tons (Cherinet Alem and Tazebachew Asres, 2015) which is 9.8t/ha times more. This may because of increasing number of high yielding and disease resistant varieties, good management practices, supplement of organic and inorganic fertilizers, and reduction in insect and disease problems. Though, both field pea and dekoko are consumed as a protein supplement in the cereal-based diets of Ethiopia there is no national yield record yet for Dekoko (Sentayehu, 2009). The reasons are because of lacking of estimated area coverage per hectare and productions status per tons records of dekoko. This signifies the current review to assess the distribution and productivity of the crucial crop.

There is one scientific field check in strange area on the productivity of dekoko from project TR-31024 of the Ministry of Education, Science and Technological Development of the Republic of Serbia. A field trial with wild populations of Ethiopian pea of diverse geographic origin dealt with the mutual relationship of various seed yield components for the breeding purposes, along with some preliminary crossings with common pea was tested. Seed yield per plant are highly correlated with number of fertile nodes per plant, number of pods per plant (Table 1), while number of fertile nodes per plant was also highly correlated with number of pods per plant (Mikić *et al*. 2013c).

Table 1 : Simple correlation coefficients (*r*) among the seed yield components and seed and straw yields in Ethiopian pea accessions

	2	3	4	5	6	7	8	9
1	-0.263	0.176	-0.406	-0.237	-0.526	0.194	-0.363	-0.023
2		0.156	0.743*	0.706*	0.446	0.367	0.615	0.505
3			0.399	0.303	0.157	-0.006	0.161	0.109
4				0.973**	0.600	0.091	0.638*	0.285
5					0.580	0.233	0.692*	0.285

6						-0.027	0.807**	0.211
7							0.547	0.508
8								0.442

*(1) Main stem length (cm); (2) Number of stems plant^{-1}; (3) Number of total nodes plant^{-1}; (4) Number of fertile nodes plant^{-1}; (5) Number of pods plant^{-1}; (6) Number of seeds plant^{-1}; (7) Thousand seeds weight (g); (8) Seed yield (g plant^{-1}); (9) Straw yield (g plant^{-1}); * = significant at 0.05; ** = significant at 0.01(Mikić et al. 2013c)*

Earliness is one of the prominent traits in Ethiopian pea, giving a basis for a view that earliness may have been selected before Ethiopian pea split from the main track of the domestication within the genus *Pisum* (Mikić and Mihailović, 2014).

V. Soil Requirement

Ethiopian pea is highly suitable for cultivating on the soils with rather low fertility, such as those in northern Ethiopia, where it achieves better results than common pea and other cool season legumes (Yemane & Skjelvag 2003a), as it is resistant to high concentrations of microelements, such as cadmium, in the soil (Manasi *et al*. 2014). In a glasshouse and field trial in Spain, the wild populations of Ethiopian pea with nonspecific resistance genes to pea bacterial blight, proved to be much less affected by all three races of the disease (Elvira-Recuenco *et al.* 2003). The resistance of Ethiopian pea to various pea bacterial blight races was confirmed in several other tests (Martín-Sanz *et al*. 2011, Martín-Sanz *et al*. 2012b). An incomplete resistance to pea powdery mildew was assessed in some wild populations of Ethiopian pea (Fondevilla *et al*. 2007). Ethiopian pea is also much less affected by *Fusarium* root rot in comparison to common pea (Grünwald *et al*. 2003). It was also determined in the case of nematodes, such as *Heterodera goettingiana* Liebscher, where the resistance of Ethiopian pea is controlled by recessive genes.

Dekoko is a neglected and underutilised cool season annual legumes crop in Northern Ethiopia. Concerning the significance of plant genetic resources little has been done recently for dekoko as the concern for significance of other plant genetic resources has been discussed for last several decades. It has always been accurately declared that they are not only a live capital of both one country but also of the whole mankind. However, it has also been stressed that their *ex situ* preservation and *in situ* conservation are very demanding in numerous ways, especially if considering human resources and financial support. It is quite punctually noted that plant genetic resources are in danger to become 'museum items' (Maxted *et al*. 2000 in Mikić *et al*., 2014). But, their *ex situ* preservation is without regard of the ecophysiological nature of the plants in developing countries like Ethiopia.

It is obvious that crop yields can be improved by application of commercial fertilizers but such practices, apart from the environmental concerns, must be repeated each season and hence are expensive particularly for totally neglected crops like the Abyssinian field pea which are grown by the resource-poor farmers under marginal conditions. It is known that the majority of the farmers in Ethiopia did not apply fertilizer to legumes and, out of the total land area under pulse crops; the proportion of the fertilized area was insignificant as compared to cereals (Messiaen *et al*., 2006). Now days, nitrogen and phosphorus fertilizer applications and researches are becoming common in pulse crops. Field pea is known to fix more nitrogen than some of the other legumes like chickpea and lentil but less than the others like faba bean (Schmidt, 1988 as cited in Keneni *et al*., 2013). There are also starting on the effect of nitrogen and phosphorus fertilizers on Dekoko. Biological nitrogen fixation of *P. sativum* var. *abyssinicum* grown in nitisols and vertisols ranged between 38.60 % and 46.10%, the average on the two soils being 42.39 % less than the improved varieties of *P. sativum* var. *sativum,* Holetta-90 with a fixation efficiency of 45.37% and Adi with a fixation of 50.19% which apparently superior (Keneni *et al*., 2013). This may be attributed to: (1) the botanical cultivar *P. sativum* var. *sativum* may be inherently superior for biological nitrogen fixation to *P. sativum* var. *abyssinicum,* (2) the past genetic improvement efforts for other traits may inadvertently improved biological nitrogen fixations in Adi and Holetta-90 (Gemechu Keneni *et al*., 2013).

Nitisols and vertisols are among the dominant soil types in Ethiopia, making 12.5 % and 10.0% of the land area (IFPRI and CSA, 2006 in Gemechu Keneni *et al*., 2013), particularly in the highlands where field pea is among the most dominant pulses (Mussa *et al*., 2006). Dekoko pea has better level of average biological nitrogen fixation under nitisol (45.01 %) than under vertisol (39.29 %) and this could somehow be associated with the variable characteristics of the two soil types particularly the difference in drainage of excess moisture as the impeded drainage in vertisols may reduce the level of nitrogen fixation (Keneni *et al*., 2013).

VI. Effect of N and P Fertilizers on Dekoko (*Pisum Sativum* Var. *Abyssinicum*) Growth

Little green house experimentations are conducted on the physiochemical trait of dekoko seeds based on the application of NP fertilizers although no more work has been done on farm based on native soil characterization. There is an increment of leaf area (LAI) with the application of NP fertilizers. Leaf area

determines the amount of solar radiation intercepted and consequently the amount of dry matter produced (Kiros wolday *et al*., 2015). The increase in LAI was closely related to the number of branches, which increase the total number of leaves. Also it could be in an increase in leaf expansion (Yemane and Skjelvåg, 2003b).

Plant height can be affect by varying nitrogen and phosphorus fertilizer levels (Table 2). From the study of Kiros Wolday *et al*. (2015), there is linear trend between plant height and fertilizer rate as nitrogen is the essential component for growth of the crop. Nevertheless, application of NP beyond 60 kg N ha^{-1} + 60 kg P_2O_5 ha^{-1} has limited increment on plant height that is recommended for further proof in the average values of the higher treatments. Dekoko plant grown without fertilizer application has minimum plant height (40.50cm) compared with plants grown with NP_2O_5 fertilizer application (Kiros Wolday *et al*., 2015). This is common on other pulse crops like chickpea that, increase in plant height with increase in N fertilizer rate and lentil (Togay *et al*., 2005).

Table 2 : Growth parameters of Dekoko (*Pisum sativam* var. *abyssinicum)* as influenced by Nitrogen and Phosphorus fertilizer levels

N + P_2O_5 (Kg ha^{-1})	LAI (m^2m^2)	PHT (cm)	NBBP
0 (Control)	1.04c	40.50[b]	1.68[b]
30 + 30	1.84bc	41.44[b]	1.93b
60 +60	2.13ab	48.98[a]	2.63[a]
90 +90	2.82a	50.83[a]	2.73a
CV (%)	9.4	6.79	13.84
LSD (5)	0.68	4.93	0.48

Where: LAI= Leaf area index, PHT= plant height, NBBP= number of basal branches per plant (Kiros Wolday et al, 2015).

Number of basal branches per plant increased as the NP level increases from the control except treatment T2 (30 kg N ha^{-1} and 30 kg P_2O_5 ha^{-1}) (Table 2). Increasing of N and P fertilizer from 0 to 90 kg ha^{-1} + 90kg P ha^{-1}) enhanced the number of basal branches per plant in a linear fashion. Number of basal branches per plant revealed a similar trend as that of the plant height. EL- Desuki *et al*. (2010) also reported, in field pea where increases in NP level tend to increase basal branches per plant.

The response of cool season grain crops to fertilizer (DAP and Urea) added to vertisol soils is good on the whole because of their high moisture holding capacity, so crops are less likely to be affected by the fluctuation of soil moisture (and low rainfall) as opposed to the other soil types. Crop production is good whenever there is sufficient rainfall and application of fertilizer. Farmers of the dekoko growing area generally use DAP and Urea on vertisol and sandy soils and only DAP on alluvial soils for other crops excluding dekoko. Farmyard manure (FYM) is applied on vertisol soils to improve the workability of the soil. But there is no yet recorded data and clear information about the response of dekoko to composite and its relative impact from DAP and Urea. According Kiros Wolday *et al*. (2015), growth rate of dekoko (*Pisum sativum* var. *abyssinicum*) increases in response to nitrogen and phosphorus fertilizers application increment.

VII. Physical Characteristics of Dekoko Seed

Dekoko seeds have lower seed volume and seed weights than the common pea, Ater by about 30 and 36%, respectively (Table 3). These physical characteristics differences could be attributed to variation in cell number and mean volume of cells in their respective cotyledons, as sizes of matured seeds in peas are determined by the number and mean volume of cells in the cotyledon. The values for percent seed swelling, hydration capacity, and percent husk content are much higher for dekoko than for Ater (Table 3).

Table 3 : Some physicochemical characteristics of the seeds of dekoko and common pea (Ater[a])

Variety	Weight (g 100 $seeds^{-1}$)	Volume (ml 100 $seeds^{-1}$)	Density (g ml^{-1})	Absoption (g 20 g $seeds^{-1}$)	Swelling (%)	Husk (%)
Dekoko	12.5	10.6	1.18	19.54	96	12.2
Ater	17.1	13.8	1.24	13. 9	73	11.0
P value	< 0.001	< 0.001	< 0.022	< 0.001	< 0.001	< 0.001

[a]*Values are mean of four parallel samples. P=probability value from the analysis of variance*
Source: (Yemane and Skjelvåg, 2003b)

Water absorption, in legume seeds, depends on the seed coat thickness, hilum size, and protein content of the cotyledons (Yemane and Skjelvåg, 2003a). The difference in hydration capacity between the varieties could be ascribed to either of these features. As seed volume of legume pea's increase,

their husk content decreases. Dekoko seeds have relatively higher husk percentage (12.2%) than Ater seeds (11%) that could be attributed to dekoko's smaller seeds as husk content were negatively correlated to seed volume.

VIII. Chemical Composition of Dekoko in Relative to Common Pea Seeds

Dekoko seeds contain higher crude protein (CP) than Ater both in the cotyledon (251 vs. 242 g/kg DM) and whole seed flours (235 vs. 229 g/kg DM) (Table 4), also the cotyledon flours contain higher CP than whole seed flours in dekoko seeds. The higher crude protein in dekoko relatively from common pea is due to less incorporation of the seed coat in to the dekoko seeds. In general the CP contents for dekoko seeds are within the ranges reported by a number of workers (Zdunczyk *et al*., 1997 in Yemane and Skjelvåg, 2003b), which are in the range of 207–264 g/kg DM for different pea varieties. Such broad variation in protein content could emanate from genetic differences and variations in growth conditions (soil, fertilizer, etc.). Peas, in general, are known to have low fat contents. It is in the range of 1–2% (Yemane and Skjelvåg, 2003b).

Table 4 : Proximate chemical composition (g/kg DM) for Dekoko and Ater[a]

Seed parts	Variety	CP	Fat	Sugar	Starch	NDF
Cotyledon	Dekoko	251	18.9	31.7	375	13
	Ater	242	16.6	23.7	402	109
Whole seed	Dekoko	235	16.7	26.6	-	-
	Ater	229	15.3	22.4	-	-
Seed coat	Dekoko	71.4	2.60	-	-	-
	Ater	68.0	2.00	-	-	-
	P value	0.024	0.019			

[a]*Values are mean of four parallel samples. P=probability level from the analysis of variance. CP= crude protein, NDF = neutral detergent fiber, - = not determined (Yemane and Skjelva˚g, 2003).*

Fat content of dekoko, in the cotyledon flour (18.9 g/kg DM), is closer to the upper value indicated for peas. Dekoko contain higher fat content than the Ater which contain both in the cotyledon (16.6 g/kg DM) and whole seed (15.3 g/kg DM) flours than those of dekoko cotyledon and whole seeds. Fat is an important source of energy. Its presence also makes food more palatable (Yemane and Skjelvåg, 2003b). The appreciation of the local people for dekoko, in terms of its taste, could be attributed to its relatively higher fat content, although there could be other factors that affect the sensory taste of consumers. Dekoko had a higher NDF content in its cotyledon (131 g/kg DM) than that of Ater (109 g/kg DM) (Yemane and Skjelvåg, 2003b). NDF measures a fraction of the dietary fiber-the insoluble fraction (Yemane and Skjelva˚g, 2003b), which is mainly composed of cellulose, hemicelluloses, and lignin.

Among the 20 building block proteins (standard amino acids) glutamine is found in highest level, followed by asparagines and arginine in dekoko and its relative common pea, Ater (Table 5). These amino acids constituted about 39% of the total amino acid content in both peas. Glutamine is lower in dekoko than in Ater, but they have a difference in relativity only by 4%. Sulfur containing amino acids (methionine and cysteine) exist in smaller quantities (3.01 in dekoko and 2.23 g/16 g N in Ater), like in other legumes reported by a number of workers, which are in the range of 2.2–3.5 g/16 g N. The sulfur containing amino acids (methionine and cysteine) values for Dekoko are also higher than those reported values for a number of pea cultivars, with the maximum value for dekoko about 2.8 g/16 g N even if it is limited to a single accession with a single study report. According to Yemane and Skjelvåg (2003a), the higher total sulfur amino acids content of dekoko compared with common pea might indicate its primitive nature, as primitive and small seeded peas exhibit a higher relative amount of total sulfur amino acids (Yemane and Skjelvåg, 2003b).

Table 5 : Amino acid composition of the cotyledon flour of Dekoko and Ater (g/16 g N), and FAO/WHO (24) reference pattern for children 2-5-year-old[a]

Amino acid	Dekoko	Ater	Dekoko/Ater	Ref
Cysteine[b]	1.97a[c]	1.45b[c]		1.36
Methionine[d]	1.04	0.78		1.33
Asparagine	12.06	11.83	1.02	
Threonine[d]	4.10	3.78	1.08	3.4
Tryptophan[d]	1.14	1.10	1.04	1.1
Serine		5.50	5.36	1.03
Glutamine	16.78a[c]	17.55b[c]	0.96	
Proline[b]	4.29	4.60	0.96	
Glycine[b]	4.31	4.37	0.99	

Alanine	4.22	4.07	1.04	
Valine[d]	4.81	4.73	1.02	3.5
Isoleucine[d]	4.28	4.33	0.99	2.8
Leucine[d]	6.93	6.86	0.99	6.6
Tyrosine[d]	3.52	3.18	1.11	
Phenylanine[d]	4.85	4.74	1.02	
Histidine[d]	2.52	2.46	1.02	1.9
Lysine[d]	6.93	6.86	1.01	5.8
Arginine[b]	10.23	9.57	1.07	
Methionine + Cysteine	3.01a[e]	2.23b[c]	1.35	2.5
Phenylalanine ± Tyrosine	8.37	7.92	1.06	6.3

[a] Values are mean of two parallel samples, [b] conditionally essential amino acids (Reeds, 2000), c a, b significant difference across columns at α = 0.05 and [d] Essentail amino acids.

Lysine, the limiting amino acid in cereals, occurs in fairly high quantity in dekoko seeds. It constitutes about 7% of the total amino acids in dekoko together with Ater variety, the values for comparative contents of the alpha amino acids are (6.93 and 6.86 g/16g N for dekoko and Ater, respectively) and are within the range for common pea (6.19–7.4 g/16 g N) (Yemane and Skjelvåg, 2003b). Therefore, dekoko can be considered as a good protein supplement for a cereal–based diet and an indication of the potential role of dekoko as a good complementary protein source.

In terms of minerals content, the whole seed and cotyledon flours of dekoko contained less calcium (Ca) and magnesium (Mg) than that of the related variety of pea, Ater. Dekoko seeds have much higher phosphorus (P), than Ater (Yemane and Skjelvåg, 2003b). Overall, the Ca and Mg contents of dekoko (34 and 90.7 mg/100 g, respectively) are close to the values reported by Yemane and Skjelvåg (2003b) (34.9 and 87.2 mg/100 g, respectively), while the values for common pea (42.3 and 98.7 mg/100 g) are higher than those of Yemane and Skjelvåg (2003b) for split peas. Ca and Mg for both varieties and P value for dekoko are also higher than the mean values reported for split peas, which were 28, 70, and 278 mg/100g, respectively. Genotypic differences in accumulating these nutrients and/or differences in growing conditions may account for such variations.

With regard to the distribution of minerals in different seed parts of Dekoko, Ca and Mg concentrations are higher in the seed coats than in cotyledon and whole seed flours, while P is highest in the cotyledon flour. Yemane and Skjelvåg (2003a) also reported higher P in cotyledons and higher Ca concentrations in seed coats for a number of legumes. The seed coats are rich in Ca and Mg, and their removal through decortications seems to cause significant losses of minerals. Because of the husk proportion, the losses of Ca and Mg, for example, in dekoko are greater than 55 and 25% of the total, respectively.

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Phytotoxic Efficacy of Rosemary Oil Against Tribolium Castaneum and Callasobruchus Maculatus

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Abstract- The quantitative and qualitative food loses and feed commodities is mainly done by insect pests which are prolific in nature and causes development of hot spots as a result of metabolic heat by developing insect populations, thereby create favorable conditions to various pathogens. Thus they cause two way spoilage of food commodities resulting into economic loss as well as loss to public health. *Tribolium castaneum* and *Callasobruchus maculates* are important pests on household material like wheat flour and Indian chick pea respectively. The essential oil of Rosemary Rosemarinus officinalis L. (Lamiales: Lamiaceae) has been investigated on toxicity of these pests under controlled conditions. The major compounds of the oil were analyzed by GC were as 1, 8 Cineol (20.021%), Borneol (7.17%), Camphor (6.541%), Geraniol (6.281%), Camphene (5.623%), Linalool (4.993%) Alpha fenchyl acetate (4.222%) and Verbenone (4.147%). Efficiency of Rosemary Oil was evaluated by various toxicity assays like Fumigant, Repellency, Contact and Ovicidal on both the pests simultaneously. The oil marked different activity against both of them.

Keywords: essential oil, callasobruchus maculates, tribolium castaneum, GC, fumigant, repellency, contact and ovicidal.

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I. Introduction

Awareness of the environmental health hazards posed by synthetic pesticides, development of resistance to these chemicals leading to recurrent pest outbreaks, danger of misuse and presence of toxic residues in food, has led to a search for safe and environmentally-friendly alternatives (1);(2). Plants are a rich source of natural products. Many species synthesise their own chemicals in defence against attack by herbivores, pests and pathogens. Some are well-known for their insecticidal properties, e.g. Ocimum viridi, Piper mullesua., neem (Azadirachta indica) and tobacco (Nicotiana tabacum L.)(3); (4), (5); (6); (7); (8). Phytochemicals such as rotenone, nicotine and pyrethrum have been used as pesticides by man before the advent of synthetic insecticides (3);(4). Various members of the families Annonaceae, Asteraceae, Meliaceae, Myrtaceae and Piperaceae produce chemical compounds which act as antifeedants, repellents, biocides or growth inhibitors detrimental to many insect species (6).These insecticides of plant origin are commonly used in the form of aqueous/ solvent extracts, powders, slurries, volatiles and oils, or as shredded segments (9) ;(6);(10) . The protection of stored agricultural products using plant materials is an age-old practice in India.

Pest-repelling plants includes plants known for their ability to repel insects, nematodes, and other pests. The essential oils of many plants are also well known for their pest-repellent properties. Oils from families Lamiaceae (mints), Poaceae (true grasses), and Pinaceae (pines) are common insect repellents worldwide. Rosmarinus officinalis L. (Family Lamiaceae) popularly named rosemary, is a common household plant grown around the world. Natural products or eco-friendly pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment.

The currently used fumigants, phosphine and methyl bromide, are still the most effective means for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. However, repeated use of those fumigants for decades has disrupted biological control by natural enemies and led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms (11). Plant essential oils and their components have been shown to possess potential to be developed as new fumigants and they may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (12, 13). The move toward green chemistry processes and the continuing need for developing new crop protection tools with novel modes of action makes discovery and commercialization of natural products as green pesticides, an attractive and profitable pursuit that is commanding attention. The concept of "Green Pesticides" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. Green pesticides are safe, eco-friendly and are more compatible with the environmental components than synthetic pesticide. The advantages of using pesticide oil-in-water micro emulsions for improving the biological efficacy and reducing the

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dosage of pesticides would be a useful strategy in green pesticide technology. Rosemary aerial parts are used as flavoring agent in foods, beverages, and cosmetic preparations and have various traditional uses in ethnomedicine.

II. Materials and Methods

a) Plant material and essential oil extraction

Rosemary Oil was obtained from farm of CIMAP. After weighing 200 gms of raw material the essential oil was obtained by steam distillation. The oil was fractionated in a Clevenger apparatus at 100° C for 3 hrs. Water was kept on through the entire time period to emphasize effective cooling and condensation of the oils. All the oils were analyzed by Gas chromatograph for detection of constituents.

b) Analysis of essential oil

i. *Characterization*

One micro liter of the essential oil was injected to GC-Ms with Varian CP3800 equipped with selective detector (Varian, Mexico) and a DB-5 capillary column (30mx0.25mm,thickness 0.25μm). Column temperature rose from 55 to 65° C at a rate of 1°C/min and held 3 min, then the temperature rose from 60 to 240° C at a rate of 31°C/min and was held at this final temperature for 10 min . Hydrogen was the carrier gas , at a flow at a flow rate of 2 ml/min. Identification of the compounds was based on the comparison of their mass spectra and retention time with the standard spectra data of the GC-MS system. Composition: The chemical composition of Rosemary oil was studied by gas chromatography mass spectrometry (GCMS).About 40 compounds were identified out of which the major compounds were Camphor (29.3%), 1-8 cineol (21.15%), α-Pinene (10.90%), camphene (5.85%), pcymene (4.874%), Berbenone (4.00%), Limonene(3.94%), Caryophyllene Oxide (1.33%), α-Thujene (0.173%).

Table 1 : Chemical constituents of the essential oil of R. officinalis

Compound	Retention Time (Min)	Area/Composition(%)
α-Thujene	5.48	0.173
α-Pinene	5.76	10.904
Camphene	6.13	5.854
p-cymene	8.27	4.874
Limonene	8.43	3.949
1,8 cineol	8.54	21.157
Camphor	12.59	29.339
Berbenone	15.14	4.005
Caryophyllene Oxide	29.80	1.331
Humulus Oxide.	30.76	1.023

c) Insect rearing and evaluation of insecticidal efficiency of essential oil

Parent adults of Chickpea beetle (Callasobruchus *maculates*) and Tribolium *castaneum*, were obtained from laboratory stock cultures. They were reared in climatic chamber at 25 ± 2 °C, 70 ± 10% relative humidity (RH) and a photoperiod of 12: 12 L/D hrs . The food used was whole Chick pea grains and wheat flour respectively. Adults of both Callasobruchus maculates and Tribolium castaneum were placed in separate jars with adequate raw material for culturing.

i. *Fumigant Toxicity Assay*

Exposure time in all the tests was kept the same that is 24 hrs. In order to test the toxicity of essential Oil vapors to the adults of T. castaneum and C. maculatus, gas tight glass bottles of 300ml volume with plastic screw caps were used as exposure chambers(Fig 1). A small piece (6x8) cms filter paper strips were kept inside the glass bottle to serve as an oil diffuser after the appropriate amount of pure essential oil has been applied on it. Doses were calculated based on nominal concentrations and assumed 100% volatilization of the oils in the exposure vessels / glass bottles. In each bottle 5 insects/replication were used and kept inside plastic vials fitted with 40 copper wire net on both the ends. This arrangement with the insects was suspended into the 300 ml glass bottle and then sealed with its cap. This whole set was considered as one replication. 3 such Replns for each concentration of oil was taken. After 24 hrs of exposure to essential oil vapors the dead insects were counted.

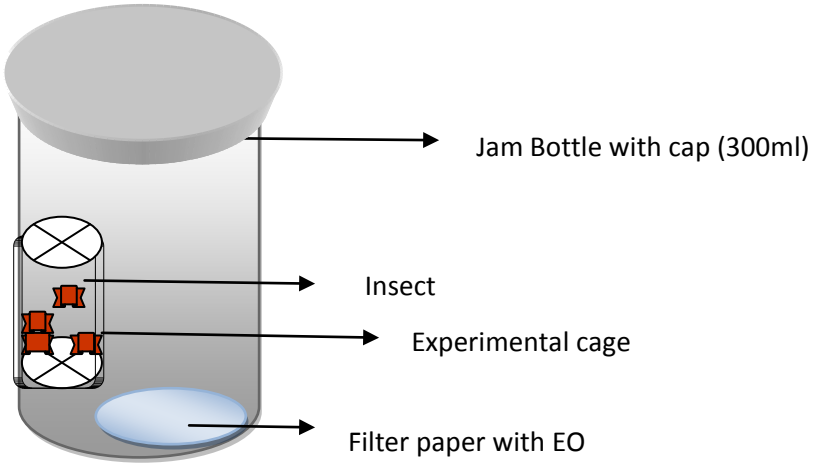


Fig 1

ii. *Repellency Bioassay*

Repellency was arranged in 9 cm test arenas. Whatman filter paper No.1 was cut into half. Test solutions were prepared by dissolving 0.5, 1.6, 2.4, 3.2 and 4 µl (.05,0 .16,0.24,0.32,0.40 % respectively) in 1 ml acetone. The paper disc was cut into 2 equal halves and then joined to a full disc with a sticking tape. Each prepared conc was applied to one half of a filter of the filter paper disc as uniformly as possible with a micropippete. The other half of the filter paper disc was treated with acetone alone and termed as untreated. This dried disc was kept inside the petridishes. Ten adults of mixed sexes of each beetle species were released separately at the centre of the filter disc and the petridish was covered. 10 replicates/conc was prepared. Observation on the no. of insects on the treated and untreated halves was recorded after 3hrs. % repellency was computed using the formula-

$$PR = \{ Nc-Nt\}/ \{Nc+Nt\}X100$$

Where Nc – No. of insects on the control half

Nt – No. of insects on the treated half

iii. *Contact Toxicity assay*

The insecticidal activity of various essential oils against the adults of T.*castaneum* was evaluated by direct contact application assay. (Qui and Burkholder 1981, Broussali's et al 1999). 20 ,40, 60 80 and 100 µl /ml (2,4,6 and 8% solutions) in acetone were prepared). Males and females of T. castaneum were transferred into petridishes and chilled for 2-5 min to reduce their mobility. One µl of the test solution was applied to the dorsal surface of the insect insects with the micropippete. Ten insects were treated /conc of the test solution and this was termed as one replication. Ten such replications for each dose were done. After treatment, insects were transferred into empty 12 cms diameter glass petridishes. Insects were examined after 24hrs of treatment.

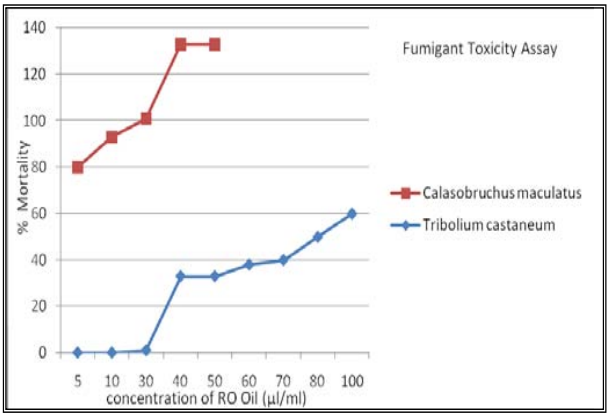
iv. *Ovicidal activity*

Fresh, intact chickpea seeds were palced in plastic jars into which 20 pairs (10Mand 10F) of pulse beetle /CM were released for egg laying. After 7 days the chick pea seeds containing the eggs were sorted. 3,6,9,12 µl essential oil of rosemary officinalis was dissolved in 1ml acetone to make (0.3%, 0.6, 0.9, 1.2%) solutions. Total 50 viable eggs /Repln were mixed thoroughly with the test solution and air dried and considered as one replicate. 5 replicates for each concentration were used . Treated chick pea seeds were placed in 300 ml glass bottles and their mouth covered with muslin cloth and left as it is for 1 month for egg hatching and adult emergence. Data on egg hatching was recorded. Ovicidal assay was not possible to be performed for Tribolium castaneum.

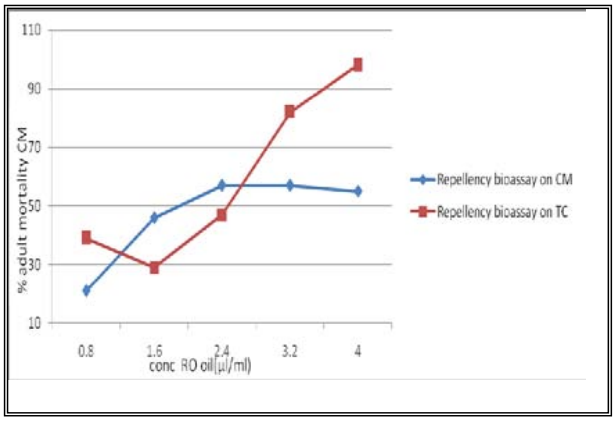
III. Results

In fumigant toxicity assays dose of 10µl/ml caused 100% mortality of *Callasobruchus maculates and no* mortality to Tribolium *castaneum*. LC50 value for *Callasobruchus maculates* was 5µl/ml for fumigant toxicity assays (Fig 2). On the contrary, the oil proved to be very promising for *Tribolium castaneum* in repellency assay with 95% mortality at 4µl/ml (LC50-2.40 µl/ml) and 58% mortality of *Callasobruchus maculates* at the same conc. (Fig 3). In Contact Toxicity assay, dose of 60 µl/ml caused 100% mortality of *Callasobruchus maculates.* LC50 value was 35µl/ml whereas only 50% mortality was achieved with the highest concentration to Tribolium *castaneum* giving 22% mortality to Tribolium castaneum at the LC50 Conc. (Fig 4). In Ovicidal assay for *Callasobruchus maculates,* LC50 value was 6 µl/ml (Fig 5). Rosemary oil was found to be more toxic towards *Callasobruchus maculates* as compared to *Tribolium castaneum.*

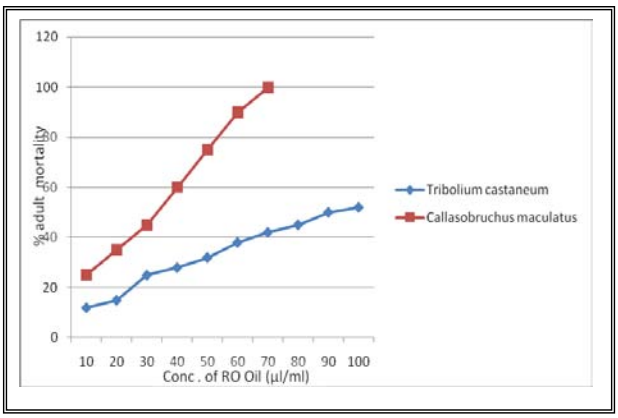
Fumigant Toxicity (Fig2)



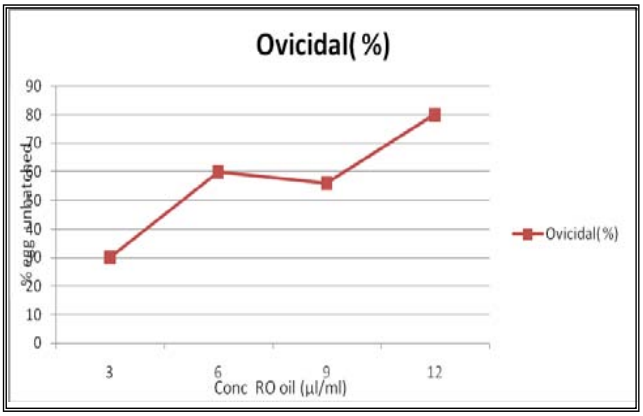
Repellency Bioassay Fig(3)



Contact toxicity Fig(4)



Ovicidal Bioassay Fig(5)



IV. Discussion

This study showed that the essential oil has significant and good toxicity against *Callasobruchus maculatus* as compared to *Tribolium castaneum*. Varying activity of different pests to essential oil of *Rosemary officinalis* indicated that the pest controlling and repelling factors were not uniformly present in this aromatic plant. Essential oil of rosemary was also found to be an effective insecticide against larvae and adults of *sycamore lace bug*. (Helena rojht *et al*, 2009) Therefore, this essential oil may be recommended at farmer level, as it is eco-friendly with low mammalian toxicity and works as a good alternative to synthetic insecticide. It could further reduce the use of synthetic insecticide. According to (Odeyemi et al 2009), Specific protocols could be developed for certain insect groups, which may be modified when required to avoid alteration of results with the same insect species due to variations at morphological and chronological level. Presence of high level of 1,8 cineole may be one of the reasons for differing activity of the oil with different pests.

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Antibacterial Effects of *Phyllanthus discoideus* and *Terminalia avicennioides* on Methicillin Resistant *Staphylococcus aureus* Isolated from Primary School Pupils in Ekiti State

By Ajibade, V. A & Akinruli, F. T
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Abstract- The Antibacterial effects of *Phyllanthus discoideus* and *Terminalia avicennioides* on Methicillin resistant Staphylococcus aureus were studied. Three hundred and twenty four (324) samples were collected from the skin, boil and wound of pupils in twelve (12) selected primary schools in Ekiti State, they were inoculated by streaking on to dried mannitol salt agar plates. *Staph aureus* was identified by agar diffusion method using methicillin disc. Susceptibility testing of the methicillin resistant *Staphylococcus aureus* to the crude extracts of the leaves of *Phyllanthus discoideus and Terminalia avicennioides,* was carried out at various concentrations((10 to100mg/ml).Out of the 324 isolates, 151 (47%) were methicillin resistant and 118 (78%)) were susceptible to *P. discoideus*extract in 151 MRSA isolates, while the remaining 33MRSA isolates were resistant. 121 MRSA isolates were susceptible to *T. avicennioides* extract, 30 were resistant to the extract, the percentage susceptible was 80%. The susceptibility of MRSA isolates to the leave extracts of the two plants was very high,it shows that the crude extracts from the leave of these plants could be used as therapies for the treatment of diseases that are associated with methicillin resistant *staphylococcus aureus*.

Keywords: antibacterial effect, methicillin resistant staphylococcus aureus, phyllanthus discoideus, terminaliaavicennioides.

GJSFR-C Classification: FOR Code: 069999

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I. Introduction

Staphylococcus aureus has been recognized as a very virulent and frequently encountered pathogen in clinical practice (Salgado *et al*., 2003). It is an endogenous microorganism colonizing the nasal cavity, skin, gastrointestinal tract, anus, vaginal and vulvae of healthy persons (Onanuga *et al*., 2005), in this sense, about 20% of human populations are long term carriers of *Staphaureus*. Hence, *Staph aureus* has been considered to be enigmatic due to their existence in a site of infection either as significance or non-significant, significance in this sense is verified by their isolation in deep wound. However, there presence on surfaces is insignificance (Ajibade *et al*, 2010). Methicillin-resistant *S. aureus*(MRSA) was recognized as a nosocomial pathogen in the 1960s and now represents a substantial proportion of *S. aureus* infections in hospitalized (in-patients) and community (out-patient) settings (Diekema *et al*., 2001). MRSA is a specific strain of *Staph aureus* bacterium which is intrinsically resistant to methicillin and all beta lactamase (β-lactamase) antibiotics likedicloxacillin, cloxacillin, nafcillin, penicillin and oxacillin (Diekema*et al*., 2001).

The mechanism of methicillin resistance is an altered penicillin binding protein (PBP2a) in methicillin resistant *Staph aureus* that markedly reduces affinity for all available beta lactamase antibiotics, while maintaining effective cell wall binding activity. The penicillin binding protein (PBP2a) is encoded by the *mecA* gene that is carried on a mobile DNA element, the staphylococcal cassette chromosome (Katayama *et al*., 2000).

Healthy individuals may carry methicillin resistant *Staph. aureus*(MRSA) asymptomatically for periods ranging from a few weeks to many years (Hardy *et al*.,2004). The initial presentation of MRSA is red bumps that resemble pimples, spider bites or boils that may be accompanied by fever and occasionally rashes; within a few days the bumps become larger, more painful and eventually open into deep furuncles (Turnidge and Bell, 2000). Patients with compromised immune systems are at a significantly greater risk of symptomatic secondary infection (Daum *et al*., 2002). Methicillin-resistant *Staphaureus*(MRSA)can have severe public health implications because it can cause a variety of nosocomial- community acquired infections ranging from minor skin infections such as pimples, impetigo, boils (furuncles), cellulitis, osteomyelitis, bacteremia, carbuncle, scalded skin syndrome and abscesses to life threatening diseases such as necrotizing (flesh-eating) pneumonia and toxic shock syndrome (Raygada *et al*., 2009).

Methicillin-resistant *staph aureus* is a strain of *Staphylococus aureus* that is responsible for infections that are difficult to treat(Hardy *et al.*,2004). It has always been identified as one of the banes of many chronic diseases in hospitals and it has also been found to be resistant to most of the antibiotics that are commonly used nowadays. There is a need for antimicrobial agent

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from plants like *Phyllanthus discoideus and Terminalia avicennioides*. Historically, plants have provided antimicrobials that produced successful results in the treatment of notable bacterial infections. Their potency has been ascribed to possession of bioactive agents (phytochemicals) which act either singly or synergistically. *P. discoideus*is aeuphorbiaceae that has antibacterial and antihelmintic properties, extract from the leaves has been used for the treatment of bronchitis, pneumonia and gastrointestinal disorder(Akinyemi *et al*., 2006). The bark is used as a purgative, anthelmintic, toothaches and for kidney and stomach complaints to facilitate parturition. *Terminalia avicennioides* belongs to the family combretaceae and it's root has been commonly used by traditional practitioners in decoction, infusion, maceration or powder, to treat wounds and skin infections including stubborn sores, furuncles, impetigo, athletic foot, burns, bruises, toothache, conjunctivitis, leprosy, venereal diseases and respiratory tract infections including cough, pneumonia and tonsillitis(Mann *et al*.,2008). The powdered bark is taken as a purgative and emetic. Leaves are chewed to treat coughs and pulverized leaves are applied on burns and bruises. Ashes of burnt leaves together with fried bulbs of a *Crinum* species are mixed with butter and applied as ointment to parts affected by articular rheumatism or to swollen joints. This study investigated the antibacterial effects of *Phyllanthus discoideus and Terminalia avicennioides* on methicillin resistant Staphylococcus aureus MRSA isolated from primary school pupils in Ekiti- State.

II. Materials and Methods

a) Collection and treatment of plant materials

Phyllanthus discoideus and Terminalia avicennioides leaves *were* collected from farms around Erinfun in Ado-Ekiti, Southwestern Nigeria. The two plants were identified at the Department of Agriculture, Federal Polytechnic where a voucher specimen no (FPA/Ag/022/2016) was kept. The leaves were air-dried at room temperature of (28°±1°C), pulverized and kept in separate containers.

b) Extraction of Bioactive Compound

The extraction of the crude extracts from the two samples was done by using soxhlet apparatus. Each sample was evaporated to dryness by using rotary evaporator at 20° C.

c) Isolation and identification of the methicillin-resistant Staphylococcus aureus

Staph aureus were obtained from 324 samples of boils, skin and wound from 12 primary schools situated in Ekiti state. Samples collected were inoculated by streaking on to dried mannitol salt agar plates. The isolates were identified using the methods of Sadaka *et al.* (2009)and *S. aureus* was also confirmed by coagulase test(CLSI, 2005). *S. aureus* were collected and subcultured into MaCartney bottles of nutrient agar slant and stored in the refrigerator at 4˚C until required.

The methicillin resistant *Staph. aureus* was identified by agar diffusion method using methicillin disc, those found to be resistant to methicillin were used for the research.

d) Susceptibility testing of the methicillin resistant aureus

The disk diffusion method described by Bradyand Katz (1990) used in Ajibade *et al*.,(2010) was employed. Various Concentrations (10-100mg/ml) of the two extracts were used against the bacterial suspension whose innocular sizes were determined using Mcfarland standard.

III. Results and Discussion

Table 1: Number of MRSA isolates from different schools

Number of Schools	Number of samples	Number(%) positive
1	27	12(44)
2	27	6(22)
3	27	12(44)
4	27	11(41)
5	27	13(48)
6	27	15(56)
7	27	14 (52)
8	27	14(52)
9	27	15 (56)
10	27	12(44)
11	27	14(52)
12	27	13 (48)
Total	324	151(47)

Table 2: Percentage of MRSA Isolates samples susceptible and Resistant to the extract of *Phyllanthus discoideus*

Number of Schools	Number of MRSA	Resistancen(%)	Susceptibility n(%)
1	12	3 (25)	9 (75)
2	6	-(0)	6(100)
3	12	2(17)	10(83)
4	11	2(18)	9 (82)
5	13	1 (8)	12(92)
6	15	4 (27)	11(73)
7	14	3(21)	11 (79)
8	14	4 (29)	10(71)
9	15	3 (20)	12 (80)
10	12	3 (25)	9(75)
11	14	4(29)	10(71)
12	13	4(31)	9(69)
Total	151	33 (22)	118(78)

< 5mm (less than 5mm) : resistant, ≥ 5 mm (greater or equal to 5mm) : Susceptible

Table 3: Percentage of MRSA Isolates samples susceptible and Resistant to the extract of *Terminalia avicennioides*

Number of Schools	Number of MRSA	Resistance n (%)	Susceptibility n (%)
1	12	3 (25)	9 (75)
2	6	1 (17)	5 (83)
3	12	4 (33)	8(67)
4	11	3 (27)	8(73)
5	13	3 (23)	10(80)
6	15	2 (13)	13(87)
7	14	_ (0)	14(100)
8	14	1 (7)	13(93)
9	15	4 (27)	11(73)
10	12	2(17)	10(83)
11	14	4 (29)	10 (71)
12	13	3(23)	10(77)
Total	151	30(20)	121(80)

5mm = resistant; ≥5 mm = susceptible

The number of MRSA isolates from different schools are shown in table 1, 324 samples were collected out of which 151 isolates were methicillin resistant (MRSA),the highest percentage (56%) of MRSA were found in school 6 and 9. The susceptibility of MRSA to the crude extracts of *Phyllanthus discoideus and Terminalia avicennioides* leaves *were* shown in table 2 and table 3. The diameter of the zones of inhibition shown by the MRSA isolates were used to characterize their resistance and susceptibility, the diameter of zones of inhibition less than 5mm indicate resistance while the diameter of zones of inhibition greater than or equal to 5mm indicate susceptibility. In table 2, the total percentage of MRSA susceptible to *P. discoideus* was 78%.In table 3, the total percentage of MRSA susceptible to *Terminalia avicennioides* extract is 80%.

The result from above especially in table1,shows that the high rate of incidence from schools 6 and 9 is predisposed on the composition of the pupils. The pupils in these schools are exposed to environmental hazards. These hazards are poor sanitation, poor waste disposal, water sources sited near dumping site, educational background, location of the schools very close to a dumping site which expose the students to the microbe, body contact with infected pupils while playing or contact with surfaces that are contaminated with MRSA and poor personal hygiene. It could also be as a result of some risk factors of methicillin resistant *Staph. aureus* such as the status of the immune system due to malnutrition, Skin damage from conditions like eczema, insect bites or minor trauma that opens the skin, respiratory illness, burns and surgical wound.

The susceptibility of the bacterium to the crude plant extracts is due to the fact that the plants contain active biochemical that show high potency especially when used in crude form, this is due to one bioactive component potentiating the efficacy of other components. The bioactive components in *P. discoideus* leaves consist of alkaloid, flavonoids, tannins, saponin and trace amount of phenol, tannin promotes healing of wounds and inflamed mucus membranes, it's high flavonoid has antibacterial, antimalarial, anti-oxidant, anti-allergic and antiviral activity.

From the result above, *T. avicennioides* shows the highest potency, viewing the susceptibility pattern of the isolates to the crude extracts, it was shown that the resistance was predominant in *P. discoides* than *T. avicennioides* indicating that efficacy is highest in *T. avicennioides,* this result corroborate the earlier findings of Mann *et al*.,2008. The reason for the efficacy can be attributed to the concentration of bioactive components contained in *T. avicennioides* like glycoside, tannin, phenol and ellagic acid. Even though, when plants extracts are researched to possess the same bioactive component, the level of their concentration in plants and also their dispersing potentials in solvent has significant therapeutic effects.

IV. Conclusion

The susceptibility of MRSA isolates to the leave extracts of the two plants is due to the presence of bioactive components and phytochemicals are utilized for the prevention and treatment of many diseases, therefore this research provides the scientific basis for the use of these two plants as therapies for the treatment of diseases that are associated with methicillin resistant *staphylococcus aureus*.

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The Status of an Ethiopian Endemic Plant *Vepris dainellii* (Pichi-Serm.)Kokwaro, in Arba Minch Natural Forest, Southern Ethiopia

By Mulugeta Kebebew Robi
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Abstract- The study was conducted on The status of an Ethiopian endemic plant *Vepris dainellii*, in Arba Minch Natural forest, Southern Ethiopia, to determine the population status, population structure and regeneration status of the species. Systematic sampling method was used to collect data from 80 quadrats (20m x 20m) established along transects. Analysis on the structure of the species indicated that the species was under good regeneration status. Anthropogenic activities carried out in the area such as cattle overgrazing, cutting of species for fire wood, charcoal and house construction were the major threats to the species. Therefore, it is recommended that timely measures should be taken by all stakeholders to sustain utilization of the species of the study area.

Keywords: *vepris dainellii, population status, population structure, regeneration status.*

GJSFR-C Classification: *FOR Code: 069999*

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I. Introduction

Globally, forests are believed to contain more than 80% of terrestrial biodiversity (FAO 2012) and have consequently been the focus of particular conservation concern in recent years (Newton 2007a). In many areas, native forests are being subjected to intensive human disturbance, through activities such as cutting, burning and browsing by livestock. Such processes can result in forest clearance, degradation and fragmentation, and consequent loss of biodiversity (Newton 2007b; Newton et al. 2009). The nature of forest communities depends on the ecological characteristics in sites, species diversity and regeneration status of species (Habibur et al., 2011).

Natural forests in Ethiopia are declining rapidly due to their conversion to arable lands coupled with unwise and excessive utilization triggered by increasing population growth. This had and continues to have serious consequences on various ecosystems in Ethiopia (Kitessa Hundera, 2010). From the time immemorial people started exploiting the natural environment as the source of their livelihoods. Different wild plant species have been used as source of food, medicine, clothing, firewood, sources of different household utensils (Dereje and Desalegn, 2013). Wild plants continue to play a central role in the livelihood of large proportion of the world's population. This is particularly true in developing countries, where wild collected food and medicine have a long and uninterrupted history of use (Koduru *et al.,* 2007).

Nech Sar National Park is one of the important conservation sites in the country with diverse component of biological resources which are ecologically and economically important. Along with the fast population growth and the development of Arba Minch town there is a high demand for fuel wood and timber production by the urban dwellers and big institutions. For all these institutions the only source of heat and light energy for almost every household in Arba Minch town and for villagers who live near the forest is the Arba Minch forest. It is also used for construction of farm implements, fences, furniture and houses, serve as a source of food, feed and bee fodder, and provide other environmental and social services to the community (Lemlem & Fasil, 2006; Aramde et al., 2012). However, the ongoing consequences of deforestation, cattle grazing, human settlement and over fishing in the park have brought severe stresses and degradation of park ecosystems, positioning the sustainability of the park in question (Svitálek, 2008).

The Arba Minch Natural forest is the best component of Nech Sar National Park and is unique in its vegetation formation from which the miracle forty springs emanate. It included riverine forest, underground water forest, savannah bushland and tree dominated bushland. The PRA survey conducted in the Nech Sar National Park and the woody plant inventory of Arba Minch forest study revealed that there are about 32 tree and 23 shrub species in Arba Minch natural forest (Lemlem & Fasil, 2006). People use these tree and shrub species for several purposes, both for market and household consumption. Currently, this forest is under great threats from the surrounding community particularly from Arba Minch Town. With increasing human population, demand for fuel and other forest products is also progressively increasing (Mateos, 2003; Demeke et al., 2007; Aramde et al., 2012). *Veprls dainellii* is among the gift of nature for the people live in and around the forest. It is used as firewood, timber, local construction, farm implements, handle for implements and food for human and wildlife. The major objective of this study was, therefore, to assess the

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population status, regeneration status and population structure of the plant in its natural habitat.

II. Methodology

a) Description of the Study area

The Arba Minch Natural forest is the best component of Nech Sar National Park, is located in the eastern edge of Arba Minch town, at about 500 kms south of Addis Ababa. The Park lies within the floor of the Ethiopian Great Rift Valley and extends from 5°51'N to 6°50'N and from 37°32'E to 37°48'E with an elevation varying between 1,108-1,650 meters above sea level. It covers an area of 514 km2 of which 85% is land and 15% is water (Israel and Mundanthra, 2016) and is unique in its vegetation formation. The study area comprised the riverine forest, underground water forest, savannah bushland and tree dominated bushland and it has an area of 60 km^2. The temperature of the area ranges between (17-30°C). Rainfall distribution is bimodal mostly occurring in March, April and May and between September and November. Annual rainfall averages around 900 mm. The wet season includes March, April, May, September, October and November and the dry season includes December, January and February.

b) Rational for selecting species

The rationale behind the selection of Ethiopian endemic plant *Vepris dainellii* was basis of their IUCN Red list status (Vivero et al*.,* 2005) and the danger of over collection due to high demand from the study area by local community (NNP annual report, 2015).

c) Data Collection

The data collection was conducted from October to December 2014. A total of 80 quadrats (20 m × 20 m) lying far apart at 100 m was used for plant data based on aspect of the vegetation. The plant species encountered in each quadrate was recorded. In each major plot, subplots (1 m^2) were established at the center and corner for seedlings and saplings data. The plant with DBH ≥ 2.5 cm was recorded as mature plant. In each of these quadrats, the numbers of all seedlings that are less than 1 m in height were recorded. Individuals attaining 1 m and above with DBH less than 2.5 cm were considered as sapling and counted (Gemedo Dalle *et al*., 2006).

d) Data analysis

Density, Diameter at Breast Height (DBH) and frequency were used for description of vegetation structure. Bar graphs were developed using the DBH versus density of individuals for four arbitrary diameter classes (1 ≤ 20 cm, 2 = 20.1–40 cm, 3 = 40.1– 60 cm, and 4≥ 60 cm) of the forest as well as the selected dominant species. The structural parameters were analyzed using the following formula:

1. Diameter at Breast Height (DBH) = (C/π).
2. Density = (number of individuals of species A/area sampled).
3. Frequency = (number of plots in which species A occurs/total number of plots sampled).

Regeneration status of the forest was analyzed by comparing saplings and seedlings with the matured trees according to Dhaulkhandi et al. (2008) and Tiwari et al. (2010); that is, the status was good regeneration, if seedlings > saplings > adults; the status was fair regeneration, if seedlings > or≤ saplings ≤ adults; the status was poor regeneration, if the species survives only in sapling stage (saplings may be≤ or ≥ adults); and if a species is present only in an adult form it is considered as not regenerating.

III. Results and Discussion

a) Natural Distribution and Habitat

Vepris dainellii from *Rutaceae* family is medium-sized, shade tolerant afromontane tree species endemic to Ethiopia. It typically grows as an understory tree mixed with other small tree and shrub species in forests but sometimes seen growing at margin of afromontane forest and open forest areas recently modified by humans (Dereje and Desalegn, 2013). The plant occurs in altitudinal range of 1050-2500m above sea level. It is distributed in Kefa, Ilubabor, Wellega, Bale, Shewa, Sidamo, Gamo Gofa and Gojam in Ethiopia, but not known elsewhere in the world (Gilbert, 1989). In this study *Vepris dainellii* was recorded in all study site. The field observation during data collection clearly confirmed that the species is widely distributed in the riverine forest, underground water forest but less distributed in savannah bushland and tree dominated bushland. The explanation behind the widely distribution of the species is an indication of well adaptation to the ecological condition in riverine forest and groundwater forest.

b) Vegetation Structure of the species

i. *Density and Diameter at Breast Height (DBH)of the species*

The highest density (40.63/ha) of the species was observed for DBH class <20 and the lowest density (2.19/ha) of the species was observed in DBH class >60. The density of the species with DBH class as their contribution of the numbers of species were given in Table 1. The density of the species increases with decreasing number of individual species. So the general pattern of DBH class size distribution forms an inverted J-shape (Figure 2) for the species. Inverted J shaped pattern shows high distribution of individuals of a species in the lower diameter classes and a gradual decrease towards the higher classes. In other words, it shows good reproduction and recruitment potential of the species. This and field observation during data collection clearly confirmed the occurrence of high disturbance in matured tree of the forest by cutting of

trees for charcoal production, firewood, house construction, and fencing.

ii. *Height Class of the species*

The species in study area were divided into five arbitrary height classes. The percentage of the species decrease with increase in height class (that is the highest percentage of the species was found in the height class 1, but, the least percentage of the species was found in height class 3). In other words, the numbers of individuals in height class decrease with increase in height range. The general height class distribution pattern (Figure 3) indicates a normal distribution of the species and maximum values occurred in the first class and reduced gradually up to the third class. This pattern represents the dominance of small sized individuals in the forest which was the attribute of high rate of reproduction status and regeneration potential.

iii. *Regeneration Status of the species*

The effective criteria for successful conservation and management of the forest resources are determining the regeneration status of the forest on the basis of the composition, distribution and density of seedling and sapling (Teketay, 2005; Getachew, 2013). According to Dhaulkhandi et al. (2008), the density values of seedling and saplings are considered as regeneration potential of the species. The analysis of the species reveal that the individual density of seedling, sapling, and matured tree of the species were 82.50, 74.38, and 63.75 ha^{-1} respectively. Density ha^{-1} of the species showed that seedling > sapling > matured tree in Arba Minch natural forest. Based on the criteria of Dhaulkhandi et al. (2008) and Tiwari et al. (2010) the species is categorized under the species with good regeneration. According to Chauhan et al.,(2008) the calculation of the ratio among the mature tree, sapling and seedling can provide information regarding the distribution of mature tree, sapling and seedling and the regeneration status of the species. In line with Chauhan et al., (2008) the ration of seedling to sapling, seedling to mature tree and sapling to mature tree of the species was conducted and the result was 71: 64, 22: 17 and 7: 6 respectively. These reveal that the distribution of seedling density is greater than both sapling and mature tree (i.e. density of seedling > density of sapling > density of mature tree) of the species. According to Dhaulkhadi et al., (2008), a given species had good regeneration if seedling is greater than sapling and mature tree/adult (seedling density > sapling density > mature tree/adults); fair regeneration if seedling > or ≤ sapling ≤ mature tree; poor regeneration if seedling < sapling ≥ or ≤ mature tree; and no regeneration if species are represented only by adult/mature trees. From the three conditions, the species fulfills the first condition and in general, it had good regeneration status. Depending upon the general pattern of frequency distribution, the regeneration of the species within the study area shows the presence of small density of mature species and gradually increases towards the highest density value of sapling and seedling and they formed inverted J-shaped distribution pattern (Figure 4). According to the study of Tesfaye et al.,(2010) and Markos and Simon, 2015), plant species with such distribution pattern had good regeneration and recruitment potential.

c) *Population Structure of the species*

Analysis of population structures for each individual tree and shrub species could provide more realistic and specific information for conservation measures. Based on the assessment of diameter class distributions, the population structure patterns of the species recorded from Arba Minch Natural forest was given in Figure 5. The species exhibited reverse J-shaped distribution. Inverted J-shaped pattern shows high distribution of individuals of a species in the lower diameter classes and a gradual decrease towards the higher classes. In other words, it shows good reproduction and recruitment potential of the species. Population structure of the species indicated the absence of individuals in DBH class 80.1 - 100. This and field observation during data collection clearly confirmed the occurrence of high disturbance in matured tree by cutting of trees for charcoal production, firewood, house construction, and timber.

IV. Conclusion

Vepris dainellii is the most locally useful endemic plant that needs attention for future research. Current over harvesting of the species influenced the population structure, population and regeneration status of this species. The large number of seedling and sapling and small number of mature population of the species is an indication of good regeneration and recruitment potential. If the unsustainable harvesting by local people continues, the capacity of the species to maintain its wild population will be significantly reduced. Therefore, management and conservation strategies are essential to be put in place to save the species.

V. Further Research

Further research is undergoing to determine the conservation status of the species in Arba Minch natural forest, Nech Sar National Park.

VI. Acknowledgements

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Table 1: DBH class and the density of the species in the forest

DBH classes (cm)	Number of species	Density/ha	Percentage
< 20	130	40.63	63.73
20.1-40	53	16.56	25.98
40.1-60	14	4.38	6.86
60.1-80	7	2.19	3.43

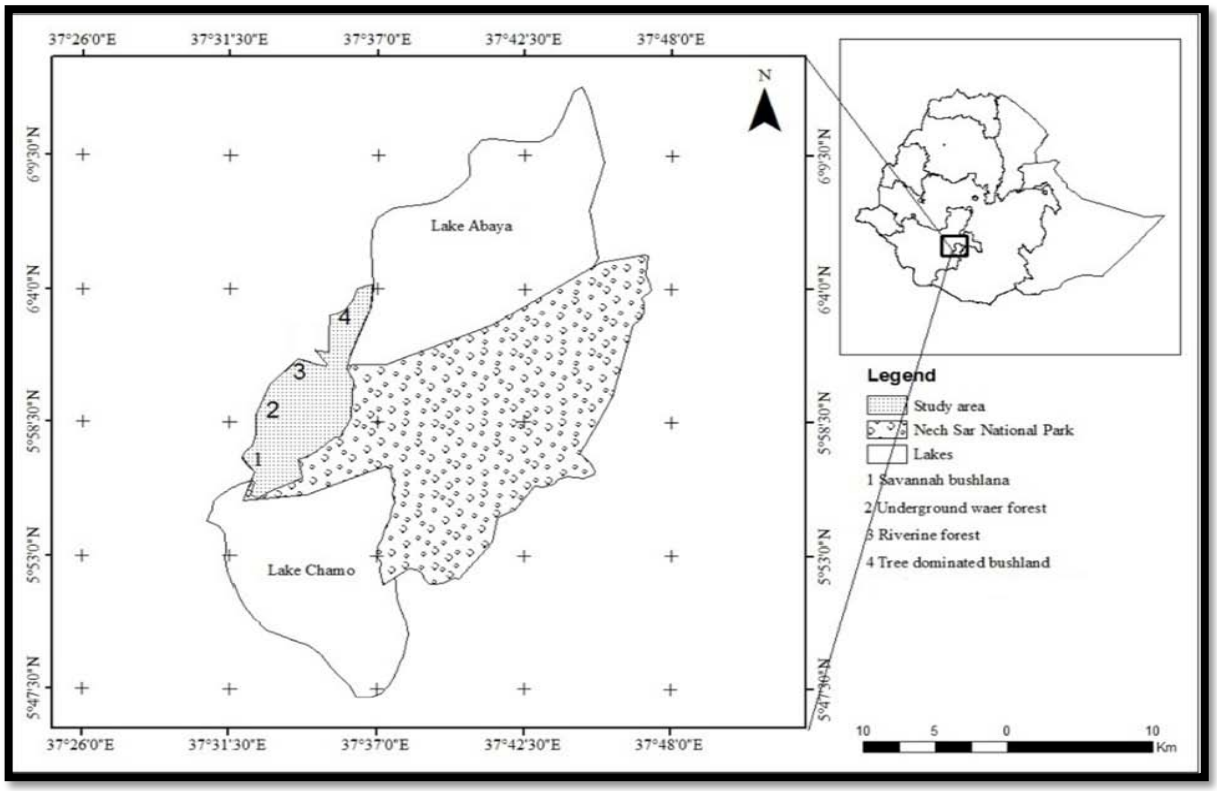

Figure 1: Map of the study area

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Soy Flour as Alternative Culture Media for Yeasts

By Nirmala Ravimannan & Sevvel Pathmanathan
University of Jaffna

Abstract- The high cost of readymade media like Potato Dextrose Agar, Nutrient agar, Peptone yeast Extract agar and media alike has deprived the use of these in laboratories with low facilities. Legume seeds and products have been found to be a very good protein source. The present study deals with the feasibility of using soy flour as an alternative culture media to grow yeasts. Soy flour has several functional properties other than its high protein content which has been reported as 50%. As the starch content is very low it has a higher dissolving property and it solidifies easily due to its gelling ability. Therefore soy flour can serve as a good nutrient source as well as to replace agar to some extent due to its solidifying property. It was found in this study that soy flour had shown to be a simple, cheap source which can replace peptone in the conventional medium.

Keywords: *yeast, soy flour, agar.*

GJSFR-C Classification: *FOR Code: 270499*

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Soy Flour as Alternative Culture Media for Yeasts

Nirmala Ravimannan [α] & Sevvel Pathmanathan [σ]

Abstract- The high cost of readymade media like Potato Dextrose Agar, Nutrient agar, Peptone yeast Extract agar and media alike has deprived the use of these in laboratories with low facilities. Legume seeds and products have been found to be a very good protein source. The present study deals with the feasibility of using soy flour as an alternative culture media to grow yeasts. Soy flour has several functional properties other than its high protein content which has been reported as 50%. As the starch content is very low it has a higher dissolving property and it solidifies easily due to its gelling ability. Therefore soy flour can serve as a good nutrient source as well as to replace agar to some extent due to its solidifying property. It was found in this study that soy flour had shown to be a simple, cheap source which can replace peptone in the conventional medium.

Keywords: yeast, soy flour, agar.

I. Introduction

The preservation and maintenance of stock cultures of yeast is important in laboratories to use in various studies involving identification of yeast strains and protein expression studies etc. A recent study done by Arunava et.al (2014) showed a cost effective technique to maintain stock cultures of yeast in laboratories with less facilities. The pure cultures can be prepared from the stock cultures.

Subculturing in media is necessary to test the viability and structural properties. The use of legume seeds as alternative culture media for fungi and bacteria has been studied (Arulanantham et.al, 2012 and Ravimannan et.al., 2014). Antony et.al. (2014) reported the use of soychunk extract agar as a bacteriological medium. Ojokoh and Ekundayo (2005) used sweet potato agar as a medium to culture yeasts. Peptone yeast extract agar (PYEA) has been used extensively for growing yeasts on regular basis for streak plate and spread plate experiments. This is a commercially available medium which is very costly. The present study is aimed at replacing the nutrient source peptone in the conventional media by soy flour. The medium is a combination of soy flour with a little amount of agar or Soy flour-Agar medium (SA). Recent research papers have been focused on the possibility of using natural plant materials as alternatives to conventional media because of their exorbitant price. Soy flour is a finely ground product processed from full-fat cotyledons or defatted flakes of soybeans. Soybeans and soybean products have been the chief source of protein for millions of people in the Orient (Waggle and Kolar, 1979). Whole soybeans are an excellent source of protein (Nelson et.al., 1978). About 40% of dry matter in soybeans is protein so the quantity is high. Soybean is a profitable crop, grown commercially for human consumption. At present soybean is one of the five major legumes cultivated in Sri Lanka the others being cowpea, mungbean, black gram and groundnut. Soy bean protein is considered as a protein of high quality as it supplies most of the essential amino acids required by the human body. The soybean breeders say that it produces the highest yield of protein per unit area of land. It has been found to be the richest, cheapest and easiest form of protein for a very long time. The protein contents in products made from soybean vary widely due to the processing conditions. Soy flour and TVP (Textured Vegetable Protein) which is a processed product from soy flour has been used as some of the protein sources to formulate alternative culture media to grow bacteria and fungi (Uthayasooriyan et.al., 2016).

II. Materials and Methods

a) Collection of samples

Soybean seeds were purchased from the sales centre of the Soybean Research Institute, Gannoruwa, Central Province, Sri Lanka.

b) Solid media preparation

The soybean seeds were finely powdered using electric blender and sieved. The powder was stored in sterile containers until its use. 3g of soy flour was taken and mixed with 0.5-2.0g agar (HIMEDIA). The solidification times of each media preparation with different amounts of agar were recorded. Finally 1g of agar was added (as the solidification time was more or less equal to that of peptone yeast extract agar – HIMEDIA) and dissolved in 100ml distilled water with soy flour. The pH of the media was adjusted to 6.8. The standard medium PYEA was prepared by dissolving 3g in 100ml of distilled water.

c) Estimation of composition of soy flour

Moisture content was determined using the procedure in AOAC (2000). Crude protein of soy flour was determined using micro-kjeldahl method (AOAC, 2000). Crude fat, crude fiber and ash contents were determined using the standard methods available in AOAC (2000). Carbohydrate content was calculated by

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getting the difference of the contents of the crude fat, fiber and ash.

d) Inoculation of microbial cultures

The yeast cultures (*Saccharomyces* sp and *Schizosaccharomyces* sp) were collected from the microbial culture collection in the Department of Botany, University of Jaffna. Standardized cultures were made by streaking on slants and maintained in the refrigerator. Aqueous suspensions of yeast cells were prepared from pure cultures of the yeast strains. Serial dilutions were prepared with sterile saline water. 0.1ml aliquots of the suitable dilutions were dispensed into triplicate plates. The prepared SA and PYEA were poured on the plates by pour plate technique. The yeast cultures introduced on PYEA served as control. All the plates were incubated at ambient temperature for 5-6 days. After incubation the plates were observed and viable counts were recorded in both media.

III. Results and Discussion

The proximate composition of soy flour is given below.

Constituents	%
Protein (Nx6.25)	50
Fat	6
Fiber	3.5
Ash	6.5
Carbohydrate	32.3
Moisture	8

a) Solidification time

Different solidification times were obtained when 3g of soy flour was mixed with 0.5-2.0g agar which is shown in Table I

Table I: Solidification times of media preparations

Weight of soy flour (g)	Weight of agar (g)	Solidification time (mins)
3	0.5	40
3	1.0	30
3	1.5	22
3	2.0	19
3 (PYEA)	-	30

1g of agar was added to soy flour (3g) as the solidification time was equal to that of PYEA.

This experiment showed results comparable to conventional PYEA and even more. Fig 1 and 2 show the growth curves obtained when *Saccharomyces* sp and *Schizosaccharomyces* sp were plated on different media respectively.

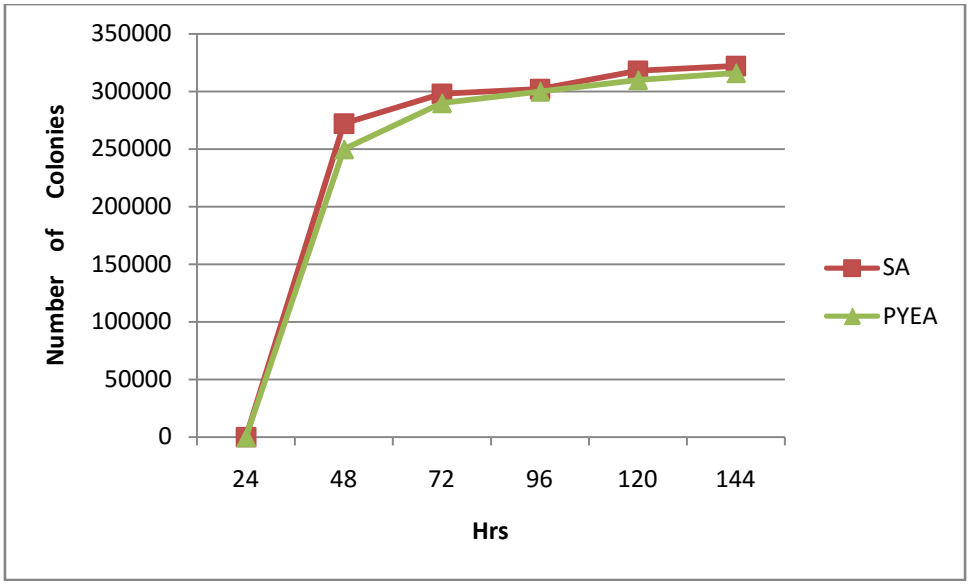

Fig. 1: Growth of *Saccharomyces* sp on SA and PYEA

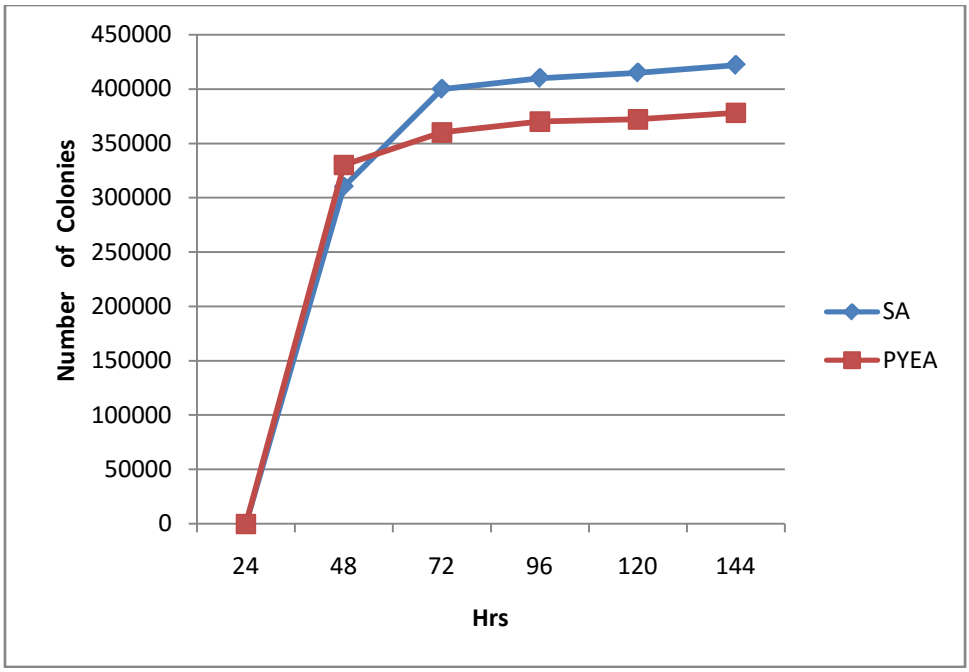

Fig. 2: Growth of *Schizosaccharomyces* sp on SA and PYEA

In both cases, growth of yeasts in terms of No of colonies was higher than the conventional medium PYEA. The medium (SA) in which soy flour was added instead of PYE was very effective on the growth of yeasts. Previous study shows that mature soybean contains little or no starch (Wilson et.al, 1978).

Generally yeasts do not require starch for their growth. But in fact sugars in the form of sucrose or glucose enhance the growth of yeasts. It is interesting to note that due to the gelling property of soy flour it easily solidifies. Further studies are required to confirm its gelling property and future uses in the preparation of media. In this study it was found that SA medium gives a transparent golden brown color which is lighter than the PYEA and the colonies could be observed clearly (Fig 3) though the colony contrast is more clear in the PYEA.

Fig. 3: Growth of *Saccharomyces* sp on SA medium

Due to this easily solidifying property of soy flour it can therefore be used to replace agar which is a common constituent in all the culture media as a solidifying agent. For e.g. In this experiment only 1g agar is used in the preparation of SA medium. The average colony diameter of *Saccharomyces* sp was

higher (2.5mm) in SA than that in PYEA (1.5mm). Similarly the average colony diameter of *Schizosaccharomyces* sp was also higher (2.0mm) in SA than that in PYEA (1.5mm). From the results, it could be seen that the growth of *Saccharomyces* sp in terms of average colony diameter was higher than that of *Schizosaccharomyces* sp on SA. From this study it can be said that soy flour has shown to be a simple efficient and cost-effective medium which can effectively replace the peptone based conventional media. Soy flour which is obtained by grinding soy beans is 50 times cheaper than peptone yeast extract agar. Also it can be stored in dried form in containers for one year. It is readily available and economically feasible which can be used to culture yeasts in laboratories with less facilities. This SA medium can be introduced to developing countries where the conventional medium cannot be purchased due to high cost.

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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.

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

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- • 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 :

"

- 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.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

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30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

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Key points to remember:

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- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the 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 evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
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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