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First Global Survey of Evidences the Ichnogenus *Osedacoides* and it Relates to the Rezent Zombie-Worms *Osedax*

By Hans-Volker Karl
Friedrich-Schiller University, Germany

Abstract- In this paper, I present the first global overview of the members of fossil ichnotaxon *Osedacoides* and it recent relatives of the morphogenus *Osedax*. Close on 15 years ago those worms were found on sea floor fallen whalebones that dissolve last recyclers in the food chain to the bone [25]. Before that time it was simply technically not possible to observe a whale carcass over a longer period of time and to study the dynamics of the submarine carcasse- societies. Probably the *Osedacoides* do not specialize in whales, but they did also in past geological ages already been feasting on carcasses, as boreholes show in fossils. The main focus of this work is on the fossil evidences.

Keywords: *ichnofossils, cladistic analysis, osedax like worms, osedacoides, overview.*

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Keywords: ichnofossils, cladistic analysis, osedax like worms, osedacoides, overview.

I. Introduction

The Recent osteophagous marine "Zombie- worm" morphogenus *Osedax* (Polychaeta) was originally discovered in 2002 with two species on the ocean bottom in a depth of approximately, 2.800 m in bones of whales where it produces borings, by now, a dozen are described. Up to now it was believed that it co-evolved with the whales, the source of its preferred diet [8,10]. The cetacean size did not determine the whale-fall communities [25]. In the meantime it could be also experimentally cultivated in cattle bones [13]. This fact as well as the reconstructed molecular clock back to at least the Cretaceous period led to the conclusion that *Osedax* originally might have infested fish or marine "reptiles" long before the marine Mammalia evolved [3,15]. In spite of the fact that the borings most probably were produced by an organism similar to *Osedax* there is no direct evidence that it was the genus *Osedax* itself. There are only ichnofossils known, but not body remains of the animal. A definite assignment of the borings to a particular body-based taxon (morphotaxon) is impossible. In agreement with Bromley [2] the name of the Recent morphospecies *Osedax* should no more be used for fossil borings in vertebrate bones [15], therefore they prefers to establish a new ichnotaxon *Osedacoides* for these fossils, and follows Bromley [2] who proposed not to use the names of the animals but to establish ichnotaxa for borings in hard substrates, even if the producers (like for example Bivalvia or Bryozoa) can be largely ascertained by their typical morphology. Bromley [2] used the ichnogenus *Trypanites* Mägdefrau, [20] as an example, which he defined as simple shaft- or pocket-like borings with a single opening whose producer is not incontestably known. Based upon his revised diagnosis, and calibrated by its type ichnospecies *Trypanites weisei* Mägdefrau, ("Simple, unbranched borings in hard substrate with a single opening to the surface") *Trypanites* can now be generally used in a wider sense for such borings. The principal difference between the two ichnotaxa is: *Trypanites* is mostly in inorganic substrates, *Osedacoides* exclusively in organic substrates (Fig. 1-C). Accorging Muniz et al. [24] the morphology of *Trypanites ionasi* exhibits a proximal, cylindrical, smooth-walled portion of the fossil boring with the deeper, enlarged and more irregular portion may have accommodated the bulbous root-structure. These are features of *Osedacoides* [14]. In compare, Furlong et al. [5] describes a high diversity of biota and low diversity, but high abundance, of borings is present along a modern *Trypanites*-type ichnofacies (Fig. 1-A). Species richness reaches 37 organisms within the study area, and two boring bivalves (*Petricola pholadiformis* and *Zirfaea pilsbryi*), which produce *Gastrochaenolites*-like traces (Fig. 1-B). *Trypanites* isp. seems to descend from cemented strata directly to crocodilian remains also, which occur in massive sandstone bodies [9,28].

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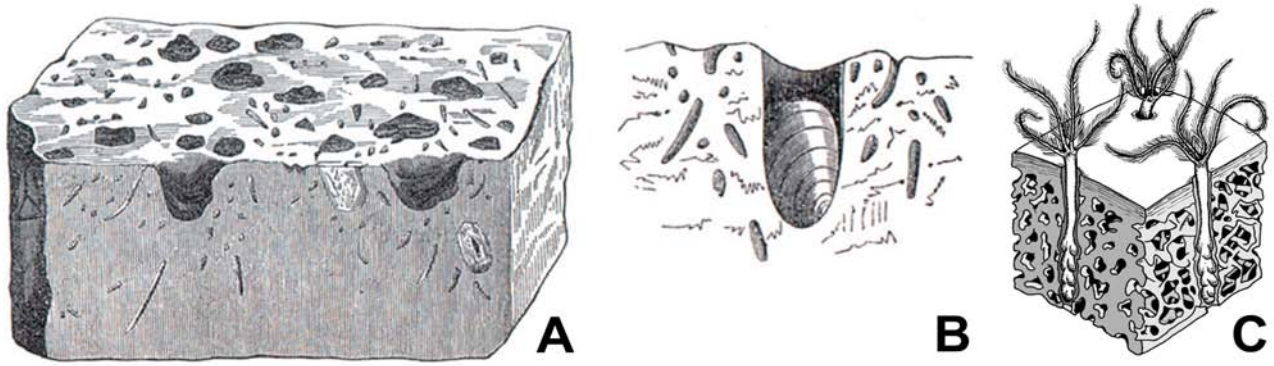

Figure 1 : A - *Trypanites*, B - *Gastrochaenolites*, Jurassic rocky-shore trace fossils from England according De La Beche [5], C - *Osedacoides*, Pliocene whalebone according Muniz et al. [23]

II. Relations between *Osedax* Like Borings and Ichnofossils

Systematics: Kingdom Animalia, Phylum Annelida, Class Polychaeta, Order Sabellida, Family Siboglinidae, Morphogenus *Osedax* Rouse et al., 2004, Type species *Osedax rubiplumus* Rouse, Goffredi & Vrijenhoek, 2004. *Osedax rubiplumus* is a species of bathypelagic Polychaetes that is reported to sustain itself on the bones of falling whales. Their paedomorphic males are 0.4–1.1 millimeters (0.016–0.043 in), and have an incomplete prototroch with a posterior hooked chaete [26]. The species have 16 hooks with 6-8 capitium teeth, which have handles that are 18–23 micrometers (0.00071–0.00091 in). The female ovisac is measured 8 mm by 4 mm by 0.3 mm, with four posterior roots, which have spherical lobes. They also have a trunk, which is 3.8 centimeters (1.5 in) in length and 2 millimeters (0.079 in) wide with the crown plumes, which are 2.1 centimeters (0.83 in) in length. The species is found in East North Pacific where it is abundant. They are used in Calmodulin (CaM, an abbreviation for calcium-modulated protein), a calcium-binding messenger protein expressed in all eukaryotic cells [27]. Higgs et al. [10] published modern *Osedax* traces and also used CT scans to construct 3d models of the borings, and reported borings that were roughly similar to that reported by Kiel et al. [15,18,19,20]. Higgs et al. [10] further found that the borings were mostly restricted to dense cortical bone, generally avoiding lipid-rich cancellous zones. Apparently some isotopic evidence suggests that *Osedax* synthesizes collagen rather than lipids, although other studies have documented *Osedax* in Japanese waters that subsist on blubber in spermaceti [10,11,12]. The hitherto described and named *Osedacoides* ichnofossils are:

a) *Ichnogenus Osedacoides Karl, Brauckmann & Groening, 2012*
LSIDurn:lsid:zoobank.org:act:527977AE-F51F-436D-81AC-A7F75E924913

Type ichnospecies: *Osedacoides jurassicus* Karl, Brauckmann & Groening, 2012

Etymology: Osedacoides = *Osedax*-like.

Diagnosis: Simple, basally thickened to branched borings in marine vertebrate bones with a single opening to the surface.

b) *Osedacoides jurassicus Karl, Groening & Brauckmann 2012*
LSIDurn:lsid:zoobank.org:act:371B736C-4E88-4F88-9CDA-E01C46143FB4

Holotype: Geozentrum Göttingen-GZG.V.773-34: Borings in hyoplastron sin. (Maack [21]: plate 35, fig. 35 = original of *Stylemys lindenensis*, now: *Tropidemys seebachi*, pl. 2 figs. 3-4, pl. 3-4) [19].

Etymology: jurassicus = Jurassic, the type stratum.

Locality: Lindener Berg, Hanover, Lower Saxony, Germany.

Horizon: Middle Kimmeridgian, Late Jurassic.

Dascription: Average diameter of openings 1 - 3, 5 mm, mode of life in groups.

c) *Osedacoides cretaceous Karl & Niehuys 2012*
LSIDurn:lsid:zoobank.org:act:377D8C46-93CF-4451-A57E-1C6D1BF70C0C

Holotype: BGR- Federal Institute for Geology and Minerals- LBEG Hanover, Germany, leg. and coll. Mosbach, 1915, old no. Gr.A.24 no. 8: One boring in peripheral plate of *Ctenochelys stenopurus* described by Karl & Niehuys [14] at their page 174 (4) and illustrated at plate 4, fig. 1- 4).

Etymology: cretaceous = Cretaceous, the type stratum.

Locality: Alsen quarry in Lägerdorf near Itzehoe, 30 m (TK 25: 2123 Lägerdorf), Germany.

Horizon: Lägerdorf-Formation, Chalk Group (Schreibkreide- Gruppe), Untercampanian-Obersantonian, Upper Cretaceous (Lithostratigraphic units of Germany ID: 2008074).

Description: Average diameter of opening = 10, 5x 10, 1 mm, inner diameter = 15, 5 mm and deep = 19, 5 mm (pl. 4 fig. 4), single mode of life.

d) Osedacoides ionasi (Muniz, de Gibert & Esperante 2010)

LSID urn:lsid:zoobank.org:pub:A3A8A74F-4C5F-40DF-B3BD-F2A3B76CD19C (under work)

Original ichnospecies: *Trypanites ionasi* Muniz, de Gibert & Esperante 2010

Holotype: Muniz et al. 2010: 271, figure 2: Arrows in A and B indicate the specimen selected as the holotype.

Etymology: ionasi = from the name Jonas.

Locality: Almería, Spain.

Horizon: Middle platform, lower Pliocene.

Description: Average diameter of opening 0.9 mm, inner diameter 1.9 mm and deep 40 mm, mode of life in groups.

III. Cladistic Analysis

Character coding

1 - life in anorganic substrate= 0, or organic substrate= 1; 2 - single mode of life= 0, or mode of life in groups= 1; 3 - diameter of openings and inner diameter lesser than 10 mm= 0, or more than 10 mm= 1; 4 - inner diameter slightly larger than opening= 0, or significantly larger than opening= 1; 5 - deep equal to or slightly greater than the greatest diameter= 0, or more times greater= 1.

Data matrix

*T.weisei*00001,*O. jurassicus*11001, *O.cretaceous*10110, *O.ionasi*11011

The character differentiation with DOLMOVE (Interactive Dollo and Polymorphism Parsimony by JOSEPH FELSENSTEIN, 1986a) shows a simple tree: (O. ionasi,(O. cretaceous,O. jurassicus,T. weisei))) this calculated with PARS (Discrete character parsimony algorithm, version 3.6a3 by JOSEPH FELSENSTEIN, 1986b) is conform with that. Two most parsimonious trees found: (O. cretaceous:2.50,(O. ionasi:0.50, O. jurassicus:0.50):1.00, T. weisei:1.50)[0.5000];

((O. ionasi:0.50, O. cretaceous:2.50):1.00, O. jurassicus:0.50, T. weisei:1.50)[0.5000]; that are clear specific differentiation in the genus Osedacoides. Two most parsimonious tree found and requires a total of 6.000 in tree A (fig. 2-A)

between	and	length
1	*cretaceous*	2.50
1	2	1.00
2	*ionasi*	0.50
2	*jurassicus*	0.50
1	*weisei*	1.50

Also requires a total of 6.000 in tree B (fig. 2-B)

between	and	length
1	2	1.00
2	*ionasi*	0.50
2	*cretaceous*	2.50
1	*jurassicus*	0.50
1	*weisei*	1.50

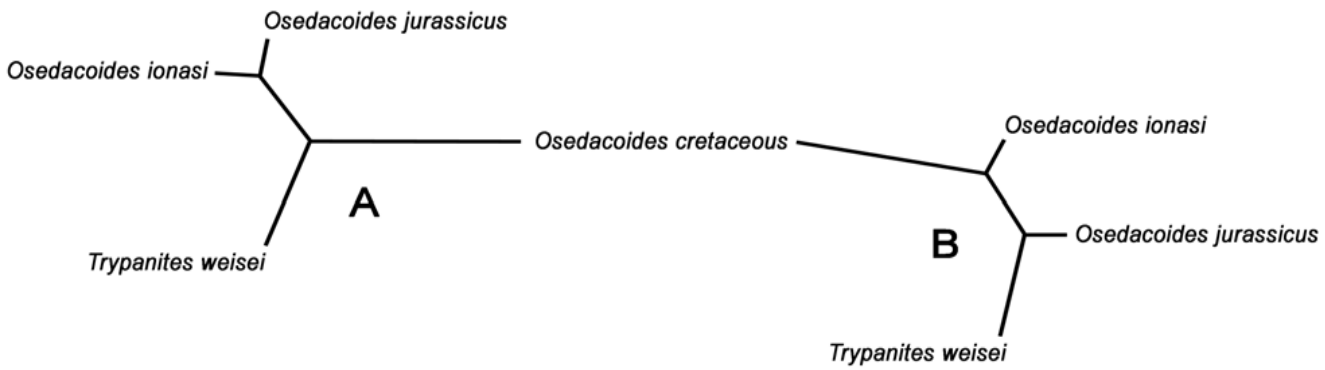

Figure 2 : Cladogram of the hitherto described *Osedacoides* ichnospecies) made with TreeView©Roderic Page. A - Unrooted tree A shows a larger distance of *O. jonasi* and O. *jurassicus* to *O. cretaceous*; B - Tree B construction of the same calculation by PARS shows a larger distance of *O. jonasi* and *O. cretaceous* to *O. jurassicus*.

IV. Results

A shell remain of the quite recently re-discovered type material of the upper Jurassic turtle *Tropidemys seebachi* Portis, 1878 was covered by borings of presumed marine "worms" similar to the Recent *Osedax* for which the new ichnotaxon *Osedacoides jurassicus* was introduced. One another turtle shell remain, a peripheral plate of the upper Cretaceous *Ctenochelys stenoporus* shows a boring of *Osedacoides cretaceous*, which differs to the type species *Osedacoides jurassicus* with the larger dimensions and the single mode of life. An overview of the corresponding fossil ichnofossils hitherto known is given here:

Age	Horizon	Ichnotaxon	Source	Material
Neogene	Pliocene	*Osedacoides ionasi*	[14,23]	whalebones
		undescribed	[12]	whalebones
	Miocene	undescribed	[1]	whalebones
Palaeogene	Oligocene	undescribed	[19]	whalebone and teeth fishbone
		undescribed	[18]	whalebones
		undescribed	[19]	birdbones
Upper Cretaceous	Cenomanian	*Osedacoides cretaceous*	[14,16]	sea turtlebones
		undescribed	[3]	sea turtlebones
Early Cretaceous	Albian	undescribed	[3]	plesiosaurbones
Late Jurassic	Kimmeridgian	*Osedacoides jurassicus*	[14,15]	sea turtlebones
	Oxfordian	undescribed	[3]	ichthyosaurbones

The examination of the Late Jurassic *Osedacoides jurassicus* by Karl et al. [15] shows that *Osedax*-like marine animals lived even much earlier. This would be another strong reference to the fact that the specialization on whalebones evolved secondarily and a long time later. Additionally, the occurrence of *Osedax*-like animals in the Late Jurassic epicontinental sea in North Germany shows that such organisms were originally not restricted to the deep-sea. This is supported by the discovery of a third recent species in 2005, which fed on whalebones in a depth of about 120 m. As osteophagous polychaetes *Osedax* belongs to the decomposing animals among the carcass feeders. As a detritus feeder *Osedax* might not have been closely adapted to particular hosts like a true parasite. *Osedacoides ionasi* from the Pliocene of Spain may represent the first trace-fossil evidence of an *Osedax*-like behavior in whalebones. The first record in bones of birds [19] proves that *Osedax* evidently feed upon penguin-like diving birds. As already mentioned the environmental reconstruction of the Lägerdorf Formation shows pelagic sediments of an open epicontinental sea with 100-150 m water depth. Recent *Osedax*- species fed on whalebones in a depth of about 120 m, but the occurrence of *Osedax*-like animals in the Late Jurassic epicontinental sea in North Germany shows that such organisms were originally not restricted to the deep-sea [15]. The deeper marine areas were not the habitat of the turtles alive. These are only post mortem dropped to the ground. The bones were fragmented before their embedding. The surfaces of all bones showing erosions, which are typical for necrophages animals, such as cancers, echinoderms, worms, snails, mussels or lampreys. One bone fragment shows bite marks. Also fossil traces of *Osedax* like borings from Late Cretaceous plesiosaur and sea turtle bones reports of Danise & Higgs [3], and from early Cretaceous by Danise et al. [6] from ichthyosaur bones.

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VI. Phylogenetic Programs

I. Felsenstein, J. (1986a): PHYLIP/ DOLMOVE-Interactive Dollo and Polymorphism Parsimony © Copyright 1986-2002 by the University of Washington.
II. Felsenstein, J. (1986b): PHYLIP/ PARS-Discrete character parsimony © Copyright 1986-2000 by the University of Washington.
III. TreeView©Roderic Page

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Fungi Associated with Pre-Harvest Deterioration of Egg Plant *Solanummelongenae* L. and their Contorl using Fruit Extract of *Tetrapleuratetraptera*

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Abstract- This study was carried out to investigate the fungi associated with pre-harvest fruit rot of eggplant *(Solanummelongena* L.*)*, their effect on fruit nutritional content and their control using fruit extracts of *Tetrapleuratetrapterain- vitro*. The fungal pathogens isolated as the causative agents of fruit rot in this study were *Phomopsismelongenae* and *Collectotrichummelongenae.* The result of proximate analysis of fungal infected and non-infected eggplant carried out showed that there was an increase in the moisture and protein content of the fungal infected eggplant as compared to healthy ones (control), while there was a decrease in the crude fibre, fat, ash, and carbohydrate contents of the fungal infected eggplant fruits as compared to the healthy ones (control).

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Akwaji, Patrick Ishoro [α], Okon, Ekeng Ita [σ], Markson, Aniedi-Abasi Akpan [ρ] & Iso, Usang Ekong [ω]

Abstract- This study was carried out to investigate the fungi associated with pre-harvest fruit rot of eggplant *(Solanummelongena* L.*)*, their effect on fruit nutritional content and their control using fruit extracts of *Tetrapleuratetrapterain-vitro*. The fungal pathogens isolated as the causative agents of fruit rot in this study were *Phomopsismelongenae* and *Collectotrichummelongenae.* The result of proximate analysis of fungal infected and non-infected eggplant carried out showed that there was an increase in the moisture and protein content of the fungal infected eggplant as compared to healthy ones (control), while there was a decrease in the crude fibre, fat, ash, and carbohydrate contents of the fungal infected eggplant fruits as compared to the healthy ones (control). It was observed that moisture content increased from 42.90±0.12 in the non-infected eggplant fruit to 58.03±0.20 in the eggplant fruit infected with *Phomopsismelongenae* and 42.90±0.12 in the non-infected eggplant to 60.12±0.40 when infected with *Collectotrichummelongenae* while the following parameters viz; protein 5.60±0.10, fat 5.37±0.17, crude fibre5.12±0.09, carbohydrate 3.64±0.19 and ash content 0.50±0.15 of non-infected eggplant were depleted when infected with *Phomopsismelongenae* to 1.40±0.18, 5.60±0.10, 2.98±0.27, 5.37±0.17, and0.25± 0.16 and protein 1.60±1.11, fat 3.0±0.31, crude fibre 3.30±0.7, carbohydrate 1.09±1.21, and ash content 0.30±0.21 of non-infected eggplant were depleted to 5.60±0.10, 5.37±0.17,5.12 ±0.09, 3.64±0.19 respectively when infected with C*ollectotrichum-melongenae.* Results of the *in vitro* antifungal assay carried out showed that the ethanolic extracts of *Tetrapleuratetraptera* had a significant effect ($p<0.05$) in inhibiting the redial growth of the fungal pathogens at the different concentrations (5g/100ml, 10g/100ml and 15g/100ml) tested than the aqueous extracts. Phytochemical test carried out showed that tannin, flavonoid, saponin and cyanogenic glycosides were present in aqueous extract while alkaloids, steroids, triterpens, tannin, flavonoid and saponin were present in the ethanolic extracts.

I. Introduction

Eggplant (*Solanummelongena*L.), family *Solanaceae* is a popular vegetable also known as African eggplant which contains numerous small soft seeds which although edible, taste better because, the plant related to tobacco, and contains nicotinoids and alkaloids and commonly served and eaten as desert mostly with groundnut in this part of the world (Giuliani and Smale, 2000).

It is one of the top ten vegetables in the world and it is grown on more than two million hectare with a production of nearly thirty three million tones (FAO, 2007). The plant had been cultivated in India for the past four thousand years and is one of the most important vegetable of *Solanaceae* family. The global area under eggplant cultivation has been estimated to be at 1.85million hectare with total production of about 32 million metric tons, it is grown on nearly 550,000 hectares in India, making the country as the second largest producers after China. India accounts for about 8.7 million metric tons with an area of about 0.53 million hectares under cultivation (Anon, 1998, Sidhu, 1998).

The northern and southern parts of Nigeria are involved in the cultivation of eggplant which comes in different varieties and they vary in fruit colour, shapes and sizes (Chinedu *et al.,* 2011).

Egg plant is well adapted to high rainfall and high temperatures, and is among the few vegetables capable of high yields in hot- wet environments (Hanson *et al*., 2006). Eggplant contains nutrients such as dietary fiber, folate, ascorbic acids, vitamin K, niacin, vitamin B_6, pantothenic acids, potassium, iron, magnesium, manganese, phosphorus and copper (USDA, 2009); and the nutrients contribute to the diet of the poor and mostly important during time when other vegetables are in short supply.

Eggplant is a delicate tropical perennial, often cultivated as a tender or half-hardy annual in temperate climates (Grubben and Denton, 2004). It grows 40 – 150 cm tall, with large coarsely lobed leaves that are 10 – 20 cm long and 5 – 10 cm broad (Grubben and Denton, 2004.) semi wide types can grow much larger, up to 255 cm with larger leaves, over 30 cm long and 15 cm broad, the stem is often spiny and the flowers are white to purple with a five lobed corolla, yellow stamens and the egg shaped glossy purple fruit has white fleshy with a meaty texture, and the cut surface of the flesh rapidly turns brown when the fruit is cut open (Hanson *et al*., 2006).

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Eggplant fruit and leaves are both eaten as vegetables or used in traditional medicine (Bonsu*et al.,* 2008). Eggplants are highly valued constituents of Nigerian foods and indigenous medicine that are either eaten raw or cooked. Also very popular in rice dishes such as stew and soup of different kind, and also prepared as sauces that are consumed with yam and plantain (Edem*etal*., 2009).

In most advanced countries, such as China, India, Philippines eggplant has been used in their indigenous medicine, the medicinal uses ranges from weight reduction to treatment of several ailments which include asthma, constipation and skin infections, diabetes, leprosy, gonorrhea, dysuria, dysentery, asthenia and hemorrhoids (Gill, 1992).

Diseases and fungal pathogens of eggplant include damping-off caused by (*Pythiumspp*, *Phytophthoraspp*: and *Fusariumspp*) root rot (*Rhizoctoniaspp* and *Sclerotiumspp*). Blight (*Phomopsis-spp*); fruit rot (*Phomopsisvexans* and *Rhizopusstolonifor*) and wilt (*Verticillumspp*; *Fusariumspp*) are noteworthy and take a considerable proportion of the produce annually. Eggplant wilt complex is known to be caused by number of fungi genera such as *Fusarium*, *Verticillum, Rhizoctonia*, Sclerotium and Phytophthora in different parts of the world (Rangas-wami 2000).

In view of the adverse effect of fungi infection on eggplant fruits as observed in the University of Calabar Farm, University of Calabar, Calabar, Cross River State, Nigeria and environs it became necessary to isolate and identify the fungal pathogens associated with the eggplant fruits in the field, determine the effect of the pathogens on the nutritional content of the eggplant fruits through proximate analysis as well as evaluate the phytochemical and antifungal effect of *Tetrapleura-tetraptera* fruit extracts on the isolated fungi *in vitro*.

II. Materials and Methods

a) Collection of Samples

Healthy and infected eggplant fruits were obtained from the University of Calabar Farm, University of Calabar, Calabar Cross Rivers State, Nigeria. Proximate (nutrient) analysis of infected and non-infected eggplant fruits was carried out in the Department of Biochemistry, University of Calabar, Calabar, Nigeria.

b) Source of fungal pathogens and morphological identification

The fungal pathogens used in this research work were isolated from diseased eggplant fruits collected from the University of Calabar Farm, Calabar, Cross River State, Nigeria. Cut sections of the diseased assay fruits were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No. 1filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 5 days, the different isolates were sub-cultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of Barnett and Hunter (1998), Alexopolous and Mins (1989).

c) Pathogenicity Test

Pathogenicity tests were carried out using the techniques of Okigbo and Nmeka,(2005). Healthy eggplant fruits were washed in distilled water and surface sterilized with 1% Sodium hypochlorite solution. A5mm diameter cork borer was used to cut discs from the fruits (three discs per fruit) and cultures of the isolated discs were introduced into holes and replaced with the discs. They were kept for 24-48hours. The inoculated fruits established symptoms on the second day and tissue segments from the infected fruits were cultured.

d) Effect of Fungal Infection on Proximate Composition of Eggplant fruit

To ascertain the effects of fungal infection on nutritional composition of egg plants, matured eggplant fruits showing signs of fungal infection were obtained and the moisture content of the eggplant fruits were determined. The plant fruit were then oven dried at 60°C for 24 hrs and grounded into fine powder using mortar and pestle. The powdered samples were stored in plastic container for laboratory analysis. Most of the methods adopted in this research work were those recommended by Association of Official Analytical Chemist (AOAC 2002).

e) Proximate Analysis of Moisture Content

A clean 100 ml beaker was dried in an oven to constant weight (a). A known amount of the 5 g sample was introduced in the beaker and weighed (b). The samples were then fried in a ventilated electrically heated atmosphere oven at 75°C for about 24hrs and cooled in a desiccators until constant weight was obtained (c). The Percentage Moisture content was calculated from the formula:

$$\% \text{ Moisture content} = \frac{\text{Weight loss of sample} \times 100}{\text{Weigh of the original sample 1}}$$

The experiment was carried out in triplicates.

f) Ash content

5 kg sample were accurately weighed into the crucible. This was ignited at 55°C for about 24 hrs in desiccators and weighted. This step was repeated until a constant weight was obtained. The percentage ash content was calculated from:

$$\% \text{ Ash content} = \frac{\text{Weight of ash x 100}}{\text{Weight of sample 1}}$$

Determination was made in triplicate.

g) Crude fat or ether extract

5 g samples were accurately weighed into a thimble. About 120 ml petroleum ether was poured into a previously dried and weighted round bottom flask. The Soxhlet extractor into which the thimble with content had been introduced was then filled into the round bottom flask and the condenser and extraction apparatus set up with a cramp and stand. Gentle heat had been applied then the heater evaporated and as it condensed, it dropped into the thimble where it extracted ether soluble constituents into the round bottomed flask. The extraction then continued for about 8 hours. The thimble was then removed and air-dried(later far free extract was used for fibre determination). The petroleum ether in the flask was distilled off and collected in the Soxhlet extractor tube. The flask was then fried in an air circulating desiccators for 8 hours. The round bottom flask and the lipid extract were then weighted. The flask and content was again dried and weighed till a constant weight was obtained. The amount of lipid extracted was obtained from the difference between the weight of the flask before and after extraction.

$$\% \text{ Fat} = \frac{\text{Weight loss of sample (extracted fat) x 100}}{\text{Weight of sample 1}}$$

h) Crude fibre

5 g far free material was weighted and quantitatively transferred into 400 ml beaker, which had been previously marked at 200 ml level. 50 ml of 1.25% sulphuric acid were added and the mixture was made up to 200 ml mark with distilled water. The contents of the beaker were heated to boiling point for 30 minutes.

i) Crude Protein (Micro Kjedahl Method)

40% Sodium hydroxide (NaOH) pellets (40 g pellets carbonates free were dissolved in 100 ml distilled water). Concentrated sulphuricacid(H2SO4), Selenium Kjedahl Catalyst (each tablet containing 1g Sodium sulphate, and 0.05 g Copper sulphate(CuSO4)was dissolved in 0.1% hydrochloric acid (HCL). Methyl red-ethylene blue indicator was prepared by mixing the equal volume of 0.2% twice recrystallized methyl red and 0.0% methylene blue made up in absolute ethanol. This sample was then stored in a dark brown bottle in a refrigerator.

j) Digestion (Micro Kjedahl)

1 g sample was weighed out into a 50 ml Kjedahl digestion flask. 20 ml of antidumping chips were added. The mixture was incinerated to gentle boiling on a digestion rack and then heated strongly until the digest became clear. The digest was removed, cooled and quantitatively transferred to a 100 ml volumetric flask and made up to mark. An Erlenmeyer flask containing 10 ml of boric acid indicator solution was placed at the tip of the condenser extended below the surface of the solution. 10 ml of the sample digest was introduced into quick fit micro Kjedahl flask and steam heated. 10 ml of 40% Sodium hydroxide(NaOH) solution was added to the digest and the digested steam distilled into the Erlenmeyer's flask until the contents become more than double of its original volume as the ammonia (NH3) changed to green. A blank determination was carried out in a similar manner as described above except 1 g digestion sample was replaced by 1 ml of distilled water.

k) Titration

The content of the Erlenmeyer flask was titrated with 0.1% hydrochloric acid to a pink end point.

Calculation

1 ml of HCl (Test) – ml of HCl (Blank) × NX × 100 % Protein = 1000 × 10 × 1

N = Normality of the acid

10 = Ml of digest use

I = Gram of sample used

III. Preparation of Plant Materials

Dried fruits used in the study were separately washed thoroughly using distilled water and surface sterilized with 70% ethanol and sun-dried for 3 days. The dried plant fruits were blended separately using a sterile electric blender to obtain 200grams of fine powder of each fruit. Aqueous extracts of fruits were obtained by adding the dried powder (blended) of plant material to distilled water at room temperature 28±1˚C. Three levels of concentrations were obtained by dissolving 5g, 10g and 15g of each sample with 100ml of distilled water. This was vigorously stirred and allowed for 24 hours. The solution was then filtered through four-folds of sterile cheese cloth for all the plant materials. The filtrates obtained were used as aqueous extracts of the test plants and stored in reagent bottles for further use. Ethanolic extracts of plant materials were obtained by adding the powdered sample at different concentrations, 5g, 10g and 15g to 100mls of ethanol. This was stirred vigorously and allowed for 24 hours at room temperature 28±1˚C. The solution was then filtered through four-folds of sterile cheese cloth for all the plant materials. The filtrates obtained were used as ethanolic extracts of the test plants and stored in reagent bottles for further use.

a) Susceptibility test

The extracts percentage concentrations were prepared at 5g/100ml, 10g/100ml and 15g/100ml with ethanol and water as solvent.

b) In vitro antifungal assay

5ml of each concentration of both the aqueous and ethanol extracts was first poured into different Petri-

dishes using sterile syringe. The sterile potato dextrose agar (PDA) was also poured into the plates containing the solvent extracts after which the plates containing the solvent extracts were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized cork borer (5mm in diameter), a disc of the matured culture was punched out from advancing margin of a four- day old pure culture and inoculated at the center of plates and incubated at room temperature (28±10c) for 7 days. The experiment was replicated thrice. Area of inhibition was measured daily for 7days using a meter rule and recorded.

c) Phytochemical Screening

Phychemical screening of the aqueous and ethanolic fruit extract of *Tetrapleuratetraptera* was carried out using the method of (Harborn, 1973). Phytochemical screening was carried out in the Department of Biochemistry, University of Calabar, Calabar, Cross River State, Nigeria.

d) Statistical analysis

Data obtained in this study were analyzed using Student T-test and a one way Analysis of Variance (ANOVA) at 5% probability level ($p<0.05$).

IV. Results

a) Isolated fungal pathogens

The fungal pathogens isolated and identified as the causative agents of pre-harvest eggplant fruit rot from this study were: *Collectotrichummelongenae* and *Phomopsismelongenae.*

b) Pathogenicity Test

Symptoms observed on the fruits inoculated with *Collectotrichummelongenae* and *Phomopsis-melongenae* were similar to those observed on the rotted eggplant fruit obtained from the field. Symptoms such as soft rots and lesions were observed on the fruits.

c) Effect of fungi infection on biochemical composition of the Eggplant

The results of proximate analysis in mg/100 g of *P. melongenae* infected eggplant fruits showed an increase in the moisture content of the fungal infected fruits of eggplant as compared to the healthy ones (control), whereas there was a decrease in the carbohydrate, fat, fibre and ash contents of the fungal infected fruits relative to the healthy ones (control). Moisture content increased from 42.90 ± 0.12 in the non-infected eggplant to 58.03±0.20 in the infected eggplant fruit with *Phomopsismelongenae* and protein content increased from 1.40±0.18 in the Post-infected eggplant to 5.60±0.10 in the non-infected fruits while the following parameters were found to decrease in the infected than in the non-infected fruits viz carbohydrate content 1.02± 0.29, fat 2.98±0.25, crude fibre 3.09±0.06, protein 1.40± 0.10 and ash content 0.25±0.16 as presented in (Table 1).The results of proximate analysis in mg/100 g of *C.melongenae* infected eggplant fruits showed that there was an increase in the moisture content from (42.90 ± 0.12 to 60.12±0.40) when infected with *Collectotrichum-melongenae* and from (1.60±0.11) in the Cs-infected garden egg to (5.60±0.10) in the non-infected eggplant when infected with *Colletotrichummelongenae* while the following parameters were found to decrease when infected with *Colletotrichummelongenae* than in the non-infected fruits viz carbohydrate content (1.09± 0.21), fat (3.0±0.31), crude fibre (3.30±0.7), and ash content (0.30±0.21) as presented in (Table 2).

Table 1 : Proximate composition of *Phomopsismelongenae* infected and non-infected eggplant mg/100g (dry matter)

Sample	Moisture	C/F	Fat	Protein	Ash	CHO
Post-infected	58.03±0.20[a]	3.09±0.06[b]	2.98±0.27 [b]	1.40±0.18[a]	0.25±0.16[b]	1.02±0.29[b]
Non-infected	42.90±0.12[b]	5.12±0.09[b]	5.37±0.17[b]	5.60±0.10[b]	0.50±0.15[b]	3.64±0.19[b]
T-value	3.54					

Note: C/F = Crude fibre, CHO = Carbohydrate, Ps = *Phomopsismelongenae*

Table 2 : Proximate composition of *Colletotrichummelongenae* infected and non-infected eggplant mg/100g (dry matter).

Sample	Moisture	C/F	Fat	Protein	Ash	CHO
Cs-infected	60.12±0.40[a]	3.30±0.7[a]	3.0±0.31[a]	1.60±1.11[a]	0.30±0.21[a]	1.09±1.21[b]
Non-infected	42.90±0.12[b]	5.12±0.09[b]	5.37±0.17[b]	5.60±0.10[b]	0.50±0.15[b]	3.64±0.19[b]
T-value	3.53					

Note: C/F = Crude fibre, CHO = Carbohydrate, Cs = *Colletotrichummelongenae.*

d) Phytochemical screening

Phytochemical screening of the aqueous and ethanolic extract of *Tetrapleuratetraptera* showed that cardiac or cyanogenic glycosides, flavonoid, saponin and tannin were present in the aqueous extract while saponin, flavonoid, alkaloids, steroids, triterpens and tannin were present in the ethanolic extracts as presented in (Table 3).

Table 3 : Phytochemical screening of ethanolic and aqueous extract of *Tetrapleuratetraptera*

Phytochemical constituents	Ethanol extract	Water extract
Flavonoid	+	+
saponin	+	+
Tannin	+	+
Alkaloid	+	+
Triterpens	+	-
Cyanogenic glycosides	-	+
Steroids	+	-

Note: (+) = Present, (-) = Absent

e) In vitro effect of ethanolic extract of Tetrapleuratetraptera on Colletotrichummelongenae and Phomopsismelongenae at the different concentrations.

The *in vitro* effect of the ethanolic plant extract at different concentrations on the radial growth of the fungal pathogens is presented in (Tables 4 and 5). Results from the study showed that extract of *Tetrapleuratetraptera* had a significant effect on the isolated fungal pathogens at all levels of concentration (5g/100ml, 10g/100ml and 15g/100ml) tested as compared with the aqueous extracts. Results (Table 4 and 5) showed that *Tetrapleuratetraptera* extract at 5g/100ml concentration completely inhibited the radial growth of *Colletotrichummelongenae* and *Phomopsismelongenae* in the first to fourth day of incubation and at 10g/100ml concentration on the first to fifth day of incubation respectively while at 15g/100ml concentration, the radial growth of *Colletotrichummelongenae* and *Phomopsismelongenae* was completely inhibited throughout the incubation period as compared with the aqueous extracts.

Table 4 : In vitro effect of ethanolic *Tetrapleuratetraptera* extract on *Colletotrichummelongenae.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.0	0.1	0.2	0.3
10g/100ml	0.0	0.0	0.0	0.0	0.0	0.1	0.2
15g/100ml	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD	0.7*						

Note: Values are means of three replicates

Table 5 : In vitro effect of ethanolic*Tetrapleuratetraptera* extract on *Phomopsismelongenae.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.0	0.1	0.3	0.3
10g/100ml	0.0	0.0	0.0	0.0	0.0	0.1	0.2
15g/100ml	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD	0.8*						

Note: Values are means of three replicates

f) In vitro effect of aqueous extract of Tetrapleuratetraptera on Colletotrichummelongenae and Phomopsismelongenae at the different concentrations

The *invitro* effect of the aqueous plant extracts at the different levels of concentration on the radial growth of the fungal isolates is presented in (Tables 6 and 7). Results from the study showed that aqueous extract of *Tetrapleuratetraptera* had little or no significant inhibitory effect on the isolated fungi (*Colletotrichummelongenae* and *Phomopsismelongenae*) at all levels of concentration (5g/100ml, 10g/100ml and 15g/100ml) tested when compared with the ethanolic extracts.

Table 6 : Invitro effect of aqueous *Tetrapleuratetraptera* extract on *Colletotrichummelongenae*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.4	3.4	4.5	4.5	4.5	4.5	4.5
10g/100ml	2.1	3.6	4.5	4.5	4.5	4.5	4.5
15g/100ml	2.3	3.2	4.0	4.5	4.5	4.5	4.5
LSD	0.96						

Note: Values are means of three replicates

Table 7 : Invitro effect of aqueous *Tetrapleuratetraptera* extract on *Phomopsismelongenae*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.7	3.0	4.5	4.5	4.5	4.5	4.5
10g/100ml	2.4	2.9	4.5	4.5	4.5	4.5	4.5
15g/100ml	1.7	2.9	4.2	4.3	4.4	4.5	4.5
LSD	1.07						

Note: Values are means of three replicates

V. Discussion

In this study, two fungal pathogens were isolated as causative agents of fruit rot of eggplant (*Solanummelongena*), namely *Colletotricummelongenae* and *Phomopsismelongenae* in the infected fruits. Most of the fungal isolates have been found to be associated with the rots of most fruits and vegetables in Kano (Musa and Buashir, 2013), Ibadan (Adio *et al.,* 2013), Port Harcourt (Chuku and Emelike, 2013, Chuku and Barber, 2013), South Eastern States (Iwuagwu *et al.,* 2013) and in Delta state (Taiga and Eyegbagharen, 2013). Pathogenicity test carried out in this study showed that these fungi actually caused the fruit diseases earlier observed on the fruits in the field. The result of proximate analysis carried out showed that moisture content of infected eggplant fruits increase from 42.90 ± 0.12 – 58.03 ± 0.20 when infected with *Phomopsismelongenae* and from 42.90 ± 1.12 – 60.12 ± 0.40 when infected with *Collectrichummelongenae*. The result of proximate analysis (mg/100 g) of non-infected and infected eggplant revealed that moisture content increased in the infected garden egg fruits, to 5.60 ± 0.10 in the infected. This result is in agreement with the findings of Falaye and Fagbohun (2012) who reported an increase in moisture content from 5.09 in the non-infected to 6.13 in the infected and carbohydrate 5.01 to 5.53 of groundnut (*Arachis hypogea*) infected with *Phomospsismelongenae.* Similarly, Nweke and Ibiam (2012) reported an increase in the moisture and protein content of *Anoniamuricata* fruits infected by *Colletotrichumgloeosporoides* and *Rhizopusstolonifer.* Omokolo *et al.,* (1996) have also found that moisture content of non-infected pods (91.0) have decreased to (13.2) in infected cocoa beans. Onifade and Jeff-Agboola (2003) reported that moisture decreased from (36.49 to 10.4) g/100 g in infected samples. The decrease in fat (12.93 ± 0.26), crude fibre (4.67 ± 6.09), ash content (2.30 ± 0.12) and carbohydrate (1.02 0.29) in the infected sample is in agreement with the findings of Shehu and Aliero (2010) have also reported that the infected onion leaf showed a significant decrease in the quantity of the crude protein, fat, fibre and ash content. Opayemi (2012) reported that ash content of non-infected pods (10.7) and beans (8.0) were depleted when infected with *Phytophthora. Palmivora* to (9.3) and (7.8) in cocoa pods and beans, respectively. It could therefore be deduced that the relative increase of moisture in the infected eggplant fruits may be caused by the digestion, degradation and dissolution of the fruit tissue into a mush (water rot) by the pathogens. These degradation activities by pathogens might have also resulted in the relative reduction in the protein, fat, fibre, and ash contents of the infected fruits. The protein, fat, fibre and ash might have been broken down by the fungi into smaller molecules that they absorbed (Nweke and Ibiam 2012). Bonner (1997) reported that complex molecules such as polysaccharide and protein are required by fungi to build the hyphal wall (chitin, glucan and cellulose) and for respiration to obtain energy. Ward and Diener (1991) obtained similar results on groundnut seeds. A decrease in vitamin content of fruits could be due to the increase in moisture content, causing the vitamin to dissolve in it, since it is a water-soluble vitamin. The result of this study shows that the fungi caused deterioration of garden egg fruits and altered the nutritional value of the fruits. In a study conducted by Amer*et al*., (2007), the nutritional contents of garden egg fruits and seeds were greatly affected by the presence of the fungal pathogens *Aspergillusflavus* and *Phomopsis-melongenae,* This suggests that these pathogens might have denied man of these essential nutrients upon consumption through their degradation activities, thereby causing some great damaging effects to human health. Van Duyn and Pivanka (2000) stated that the deficiency of fibre in our diet leads to diverticular diseases and intestinal cancer. However Fanny *et al*., (2000) who also reported a decrease in fat, ash and protein content of maize infected by fungi, stated that the nutrient depletion in entire test plant sample might have been as a result of the internal defense system of the host tissue.

Phytochemical screening of the test plant (*Tetrapleuratetraptera*) extract used in this study was carried to determine its exact phytochemical contents. Results of the phytochemical screening carried out showed thatcardiac or cyanogenic glycosides, flavonoid, saponin and tannin were present in the aqueous extract while saponin, flavonoid, alkaloids, steroids, triterpens and tannin were present in the ethanolic extracts as presented in (Table 3). Phytochemicals, as compounds which occur naturally in plants, form part of plants defense mechanisms against diseases (Eleazu *et al.,* 2012). They are classified into primary and secondary, based on their activity in plant

metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll (Krishnaiah *et al.,* 2007), while secondary ones include the phenolic compounds such as tannins, flavonoids, alkaloids, saponins, anthraquinones, phlobatannins, proanthocyanidins, etc. (Eleazu *et al.,* 2013). These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties (Molan and Faraj, 2010, Zongo*et al.,* 2011). Thus the antimicrobial properties of plants have been attributed to the presence of these secondary metabolites (Prakash and Hosetti, 2010).

In this study, the antifungal activity of *Tetrapleuratetraptera* fruit extract was tested *in vitro* on fungi isolated from infected fruits of *S. melongenae*. Results showed that the ethanolic and aqueous extracts of the fruit of *T. tetraptera* investigated, exhibited various antifungal activities against the species of fungi isolated. The antifungal activity of the ethanolic and aqueous extracts of *T. tetraptera* on the isolated fungal pathogens is presented in (Tables 4- 7) respectively. The results showed that, the ethanolic extract had a significant ($P < 0.05$) effect on the radial growth of the fungal pathogens than the aqueous extracts and the rate of antifungal activity differed from one concentration to the other. The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different plant extracts. This agrees with the results of some workers like Amadioha, (2000) and Okigbo and Nmeka, (2005). Ilondu *et al.,* (2001) reported that some plants contain phenolic substances and essential oils, which are inhibitory to micro-organisms. The presence of these compounds in these extracts has been reported to be responsible for their antifungal properties (Ahmed and Stoll, 1996).It is noteworthy that of all the tested plant extracts(aqueous and ethanolic), ethanolic extracts of *T. tetraptera* had a more significant effect than the aqueous extracts and the level of inhibition increased with a corresponding increase in the concentration of the extracts. Complete inhibition was observed with ethanolic extract of *T. tetraptera* at the highest concentration of 15g/100ml. The inhibitory potency of the plant extracts may be attributed to the phytochemical compounds like tannins, alkaloids, flavonoids and saponins in them as reported by Chiejina and Ukeh (2013). This is also in agreement with the works of Amadioha and Obi, (1999) and Umana *et al.,* 2014, Umana*et al.,*2015) who reported that the high potency of plant extracts containing the same bio-active compounds could be used for the control of fungal pathogens of plants.

VI. Conclusion

The fungal pathogens isolated and identified from this study as the causative agents of fruit rot of eggplant were *Collectotrichummelongenae* and *Phomopsismelongenae*. The results of proximate analysis of fungal infected and non-infected eggplant fruits showed that there was an increase in the moisture content of the fungal infected fruits of eggplant relative to the healthy ones (control), while there was a decrease in the carbohydrate, fat, fibre, protein and ash contents of the fungal infected fruits relative to the healthy ones (control). It is possible that the isolated fungal pathogens might be resident on the leaves and roots of the plant from where they were dispersed into the fruits to initiate infection spore during rainfall. Results of the *in vitro* antifungal assay carried out showed that the ethanolic extracts of *Tetrapleuratetraptera* were effective against the fruit rot fungal pathogens of eggplant at the different concentrations tested. To this effect, use of resistant seed and timely spraying of egg plant crops with extracts of *Tetrapleuratetraptera* prepared at higher concentrations during flowering and fruiting will reduce the damaging activities of the fungal pathogens and contamination with mycotoxins and other related fungal metabolites that might be hazardous to human health.

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Estimation of Liver Glycogen in Normal Control, Diabetic Control and *Tinospora Cordifolia* Extract Treated Albino Rats

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Abstract- Investigations have been carried out to examine the presence of liver glycogen in normal control, diabetic control and extract treated albino rats. Estimation of liver glycogen was carried out by taking liver samples from normal control, diabetic control and extract treated albino rats. The rats weighing 150-190gm were administered intraperitonealy with 180mg/kg body weight dose of alloxan monohydrate for the induction of diabetes, with alcoholic leaf extract of *Tinospora cordifolia* with a oral dose of 20 ml/kg body weight from day 2 to 30 half an hour prior to feeding twice a day. It produces significant decrease in liver glycogen level.

Keywords: liver glycogen, tinospora cordifolia, alloxan, diabetes mellitus.

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Strictly as per the compliance and regulations of :

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Kinkar Shobha Bhanudas [α] & Patil Kishor Gopal [σ]

Abstract- **Investigations have been carried out to examine the presence of liver glycogen in normal control, diabetic control and extract treated albino rats. Estimation of liver glycogen was carried out by taking liver samples from normal control, diabetic control and extract treated albino rats. The rats weighing 150-190gm were administered intraperitonealy with 180mg/kg body weight dose of alloxan monohydrate for the induction of diabetes, with alcoholic leaf extract of *Tinospora cordifolia* with a oral dose of 20 ml/kg body weight from day 2 to 30 half an hour prior to feeding twice a day. It produces significant decrease in liver glycogen level.**

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I. Introduction

In the past 200 years, dramatic advances in our understanding of the regulation of normal glucose metabolism have been made. Beginning in the mid-19th century, Claude Bernard showed that blood glucose levels are regulated not just by the absorption of dietary carbohydrate but also by the liver, which plays a central role in producing glucose from non-glucose precursors [1]. Other investigators built on this discovery to identify the enzymes responsible for the synthesis and breakdown of glycogen [2], the role of anterior pituitary hormones in glucose metabolism and the onset of diabetes [3], the role of reversible protein phosphorylation by a protein kinase [4] and the discovery of cyclic AMP and its role in hormonal action, particularly that of epinephrine and glucagon, both of which elevate the blood glucose concentration and contribute to diabetic hyperglycemia [5].

Number of researches have been accomplished experimental diabetes induce in various mammalian species [6, 7, 8, 9, 10].

It is investigated that the whole plant extract of *Tinospora cordifolia* significantly decreases the blood glucose towards the normal blood [11]. Although several therapies are in use for the treatment, there are certain limitations due to high cost and side effects such as development of hyperglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc.[12]. Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. Present investigations were carried out on albino rat *Rattus norvegicus* due to its metabolic relatedness with human.

Medicinal plants are being looked upon one again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose –lowering agent. Its development was based on the use of *Galiga officinalis* to treat diabetes. *Galiga officinalis* is rich in guanidine, the hypoglycemic component. Because guanidine is too toxic for clinical use, the alkyl biguanides synthalin A and Synthalin B were introduced as oral antidiabetic agents in Europe in the 1920s but these were discontinued after insulin became more widely available. However, experience with guanidine and biguanides prompted the development of metformin. Upto now, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization (WHO) Expert Committee on diabetes has recommended that medicinal herbs be further investigated. Based on this recommendation and need for conducting clinical research in herbal drugs, developing simple bioassays for biological standardization, pharmacological and toxicological evaluation, current study is performed to evaluate the antidiabetic potential of *Tinospora cordifolia*.

Tinospora cordifolia (Guduchi) is an evergreen perennial climber. This deciduous and dioecious plant belong to the family Menispermaceae which consists of about 70 genera and 450 species that are found in tropical lowland regions. They are generally climbing or twining, rarely shrubs. This family is rich source of alkaloid and terpenes [13]. In Hindi the plant is commonly called Giloe [14] which is Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. In Ayurveda, it is designated as Rasayana drug

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recommended to enhance general body resistance, promote longevity and as anti-stress and adaptogen [15,16].

II. Material and Methods

The plant material *Tinospora cordifolia* was collected from hygienic places from in and around the Nagpur city. The plant material was washed with water in order to make it free of dirt and other impurities and was shade dried. Shade dried whole Plant material was grind with mortar and pastel into the fine powder and alcoholic and aqueous extract of *Tinospora cordifolia* was prepared according to the standard procedure.

Healthy albino rats (9 months old) of both the sexes, weighing 150-190gm were used for the experiment. Animals were free to access drinking water and food. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration No.-478/01/a/ CPCSEA).

Four batches of experiment was carried out for standardization of dose. Each batch includes three groups of rats (n=6). Group I included young rats of less than 6 month old, group II included the rat of age group 6-12 month old and group III was included rats of more than 12 month age group. Diabetes was induced in 16hrs fasted albino rats with single intraperitoneal dose of alloxan monohydrate. Alloxan injection was prepared in 0.9%normal saline. Rats with fasting blood glucose more than 220 mg/dl was considered for study. The batch I was injected with 120mg/kg bw, batch II with140mg/kg, bw, batch III with 160mg/kg bw and batch IV with180 mg/kg bw. During dose standardization study it was found that 180mg/kg intraperitoneal dose of alloxan monohydrate was suitable for diabetes induction with the 6-12 month old rats.

For experimental induction of diabetes alloxan monohydrate (A74/3 Sigma Aldrich was used).

For this study the animals were divided into three groups (n=6),

Group-I (NC): Kept as normal control, the animals of this group was free to access drinking water and food; they were neither injected by alloxan nor feed on plant extract.

Group- II (DC): These group of animals were injected with alloxan monohydrate (180 mg/kg bw) and kept as diabetic control. They were not feed on extract.

Group-III (DC+TCE): This group was injected with alloxan monohydrate (180mg/kg bw) and from day 2 to 30 half an hour prior to feeding, orally administrated with TCE (20ml/kg bw) twice a day.

a) Estimation of liver glycogen

Liver sample was dissected out from 16 hrs fasted rat and digested in hot 30% KOH. Liver glycogen was precipitated with alcohol and the precipitate was dissolved in 10% TCA. The sample was processed for centrifugation to sediment the proteins, after centrifugation supernatant was precipitated once again with alcohol. After suitable dilution of the sediment with water, estimation of liver glycogen was carried out with anthrsone reagent [17].

III. Results and Discussion

In the present study we observed the very significantly ($P<0.005$) increased liver glycogen in diabetic control group compared to the normal. This abnormally increased glycogen content is reversed by the *Tinospora cordifolia* whole plant extract. Although almost all the liver glycogen of the normal rats disappeared after only 24 hours of fasting, rather large amount of liver glycogen was reserved in alloxan diabetic rats when they were fasted for 16 hours before testing. The amount of the remaining liver glycogen was affected by the blood glucose level. Several factors which suppress liver glycogen in normal control by 16 hrs fasting may be pointed out; first one of them is the decrease of the blood glucose which may be followed by marked suppression of glucokinase. Since, glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Secondly, the suppression of glycogen synthetase together with the stimulation of phosphorylase may be related to the suppression of liver glycogen. The high liver glycogen level in diabetic rats may be due to either increase in gluconeogenesis or hyperglycemia due to 16 hr fasting before testing. In *Tinospora Cordifolia* whole plant extract treated group the significant ($P<0.005$) reversion of the liver glycogen towards the normal may be due to it's activating effect on the glucokinase and glycogen synthetase.

Table 1 : Liver glycogen Estimation.

Groups	Liver glycogen (mg/g wet wt)
NC	5.2±0.87
DC	11.7±1.2[a]
DC+TCE	6.3±0.45[a]

NC- Normal control

DC- Diabetic control

TCE- *Tinospora cordifolia* extract

(Values are expressed as Mean ± SEM (n=6), paired t-test was performed to compared between groups. [a]P<0.005 when DC compared with NC and DC+TCE compared with DC).

After absorption into a cell, glucose can be used immediately for release of energy to the cell, or it can be stored in the form of glycogen, which is a large

polymer of glucose. All cells of the body are capable of storing at least some glycogen, but certain cells can store large amounts, especially liver cells, which can be stored upto 5 to 8 per cent of their weight as glycogen, and muscle cells, which can be stored up to 1 to 3 per cent glycogen.

The importance of the liver in the regulation of carbohydrate metabolism is recognized by its ability to store carbohydrates in the form of glycogen (glycogenesis) and to release them in the form of glucose (glycogenolysis) when needed. These processes are regulated by 2 key enzymes: glycogen synthase and glycogen phosphorylase.

According to Dewalkar *et al.,* [18] the fasted liver glycogen concentration was found very significantly higher ($P<0.001$) in diabetic control (DC) than normal control (NC). This may be due to the increase rate of glycogen synthase enzyme in alloxan treated group, which in turn account for accumulation of glycogen in diabetic rat liver. Fresh etiolated wheat grass and fruit squash of *Lagerstroemia speciosa* treated groups show statistically significant difference in glycogen concentration like NC. This reversal of glycogen concentration to normal indicates their preventive effect on alloxan induced increase rate of glycogen synthase. They found fresh etiolated wheat grass posses significant ($^{*}P<0.05$) effect on lowering of glycogen concentration than fruit squash of *Lagerstroemia speciosa*.

According to Daisy and Rajathi, [19] the aqueous extracts of *Clitoria ternatea* leaves and flowers decrease in glycogen content of liver and skeletal muscle in diabetic rats is probably due to lack of insulin in the diabetic state. Prevention of glycogen depletion in the liver and muscles, following the administration of the extracts, could therefore have been achieved by stimulation of insulin release. Administration of *Clitoria ternatea* leaves and flowers to the diabetic animals increased the activity of glucokinase in liver. The extract-induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of improved glucose uptake. Similar observations have been made by Singh *et al.* and Shanmugasundaram *et al*. in respect of the extracts of *Catharanthus roseus*, and *Gymnema sylvestre* respectively [20, 21]. The activity of the gluconeogenic enzyme, glucose-6- phosphatase, is usually enhanced during diabetes. following extract administration, blood glucose level falls; while liver glycogen content rose. This may be due to the mobilization of blood glucose into the liver glycogen reserve [22, 23, 24].

IV. Summary and Conclusion

Beside blood glucose, quantitative analysis of liver glycogen content was carried out to interpret the relation between blood glucose and liver glycogen here we observed the significantly increased liver glycogen in diabetic control group compared to the normal. This abnormally increased glycogen content is reversed by the *Tinospora cordifolia* whole plant extract. Although almost all the liver glycogen of the normal rats disappeared after only 24 hours of fasting, rather large amount of liver glycogen was reserved in alloxan diabetic rats when they were fasted for 16 hours before testing. The amount of the remaining liver glycogen was affected by the blood glucose level. Several factors which suppress liver glycogen in normal control by 16 hrs fasting may be pointed out; first one of them is the decrease of the blood glucose which may be followed by marked suppression of glucokinase. Since, glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Secondly, the supression of glycogen synthese together with the stimulation of phosphorylase may be related to the suppression of liver glycogen. The high liver glycogen level in diabetic rats may be due to either increase in gluconeogenesis or hyperglycemia due to 16 hr fasting before testing. In *Tinospora cordifolia* whole plant extract treated group the reversion of the liver glycogen towards the normal may be due to its activating effect on the glucokinase and glycogen synthetase.

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Parkia Biglobosa Jacq (dawa-dawa): The Threatened Giant of the Guinea Savanna of Nigeria (The Cross River State Situation)

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Abstract- *Parkia biglobosa* (Jacq) is a perennial tree of considerable multipurpose importance. In Nigeria, over 60 million people depend on it for food, fuel, wood work and income. This priority tree with enormous economic and social values to the local people of Cross River State (North) is rapidly declining due to rapid human population growth, livestock population pressures, increasing land fragmentation, over exploitation for seed (food) and a high demand for wood fuel especially charcoal. In view of this, a survey was carried out in the Guinea Savanna of Cross River State (North) to determine the level of abundance (A) or rarity (R) of the specie. In each site, ten 20m × 20m randomly selected points were laid along four 1km line transect and accordingly assessed. Seventeen economic important trees were assessed and counted as present with particular emphasis on P. *biglobosa* (taking into consideration the age of the plant). Results showed that the tree was rare (R) and facing extinction with low densities of 2.1/ha for (Ogoja L.G.A), 1.6/ha (Bekwarra), 2/ha (Obudu), 1.7/ha (Obanliku) and 1.5/ha for (Yala).

Keywords: *parkia biglobosa, threatened specie, logistic model, guinea savanna, cross river state.*

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Abstract- ***Parkia biglobosa*** **(Jacq) is a perennial tree of considerable multipurpose importance. In Nigeria, over 60 million people depend on it for food, fuel, wood work and income. This priority tree with enormous economic and social values to the local people of Cross River State (North) is rapidly declining due to rapid human population growth, livestock population pressures, increasing land fragmentation, over exploitation for seed (food) and a high demand for wood fuel especially charcoal. In view of this, a survey was carried out in the Guinea Savanna of Cross River State (North) to determine the level of abundance (A) or rarity (R) of the specie. In each site, ten 20m × 20m randomly selected points were laid along four 1km line transect and accordingly assessed. Seventeen economic important trees were assessed and counted as present with particular emphasis on P. *biglobosa* (taking into consideration the age of the plant). Results showed that the tree was rare (R) and facing extinction with low densities of 2.1/ha for (Ogoja L.G.A), 1.6/ha (Bekwarra), 2/ha (Obudu), 1.7/ha (Obanliku) and 1.5/ha for (Yala). On average, there were 48.34% old trees than juveniles and 20.82% more young trees than juveniles. The Logistic model of population dynamics (with modifications), was used to prove what will be the population size of the specie after ten years (10yrs) at equilibrium critical points of (P=0, P=N and P=M). The study showed that high demand for food, bush burning, farming activities and urbanization within these areas have brought about decimation and near extinction of this valuable tree specie. Domestication and plantation establishment of the specie to enhance its productivity and to curb the threat of extinction is strongly recommended.**

Keywords: parkia biglobosa, threatened specie, logistic model, guinea savanna, cross river state.

I. Introduction

Parkia biglobosa Jacq is a perennial tree of the genus Parkia in the family Fabaceae. In Nigeria, its fruits are fermented into a condiment called "dawa-dawa" (Salim *et al.,* 2002).

Parkia biglobosa is an important economic tree legume of considerable multipurpose importance. The tree attracts bees and is a popular tree among bee keepers. Whole pods are eaten by domestic stock including cattle. The young seedlings are nutritious and heavily browsed by livestock. The seed of the African locust bean when boiled and fermented is known as "dawa-dawa" in Hausa language in Nigeria, a black smelling tasty seasoning, rich in lipid 29%, protein 35%, carbohydrate 16%, good source of protein, fat, calcium for rural dweller. The bark is used as mouth wash, vapour inhalant for toothache, or for ear complaints. It is macerated in baths for leprosy and used for bronchitis, pneumonia, skin infections, sores, ulcers, and washes for fever, malaria, diarrhea, and sterility. Roots are used in a lotion for sore eyes. Pulp is supposedly a water purifier but possibly just sweetens and disguises taste of foul water. The sweet yellow pulp contains 60% sugar when ripe and the seeds contain vitamins as well as minerals (Sacande and Clethero, 2007). The fruit pods are used to produce an insecticide powder for treating crops. Parkia tree is used as timber for making pestles, mortars, bows, hoe handles and seats (Joshi and Joshi, 2009).

Parkia biglobosa (dawa-dawa) is indigenous specie that is economically and socially important for local people in Cross River State (North). The tree serves as source of wood, food, fodder, and medicine for local people. They also provide ecological services including soil fertility and microclimate amelioration. In addition to direct domestic use of the tree products, they are a source of cash for local people (Odebiyi *et al.,* 2004).

According to Odebiyi *et al.,* (2004) the population of this priority tree is rapidly declining due to rapid human population growth, livestock-population pressures, increasing land fragmentation, over exploitation for seed and a high demand for wood fuel especially charcoal. Also, fallowing of farmlands a practice on which the regeneration of this tree relied is no longer being followed. As a result, existing trees are aging.

From the forgoing, the main aim of this research, therefore, was to investigate the population size of *P. biglobosa* in the Guinea savanna of Cross River State (North) as well as build a modified Logistic Model of population dynamics of *Parkia biglobosa* in the next ten years in the face of continuous exploitation without replacement.

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II. Materials and Methods

Cross River State has a total land mass of 20.156km$_2$(7,782squ mi). Of the total land mass, 21,600 hectares is covered by savanna woodland and mostly found in Cross River (North). The state is located in South Eastern Nigeria Latitudes 5˚45˚N' and Longitude 8˚30˚E. It has a humid tropical climate of (1300-3000mm) rainfall and a mean annual temperature of 30˚C.

The study was conducted in January to July, 2013. The study was conducted in the cultivated and fallow land uses in the Guinea Savanna of Cross River (North) making a total of 5 Local Government Areas (5 Locations) Ogoja, Yala, Bekwarra, Obudu, and Obanliku and 3 villages in each of the Local Government Areas (15 study sites) Ishindede, Ndok, Idum (Ogoja), Abuochiche, Afrike, and Gakem (Bekwarra), Okpoma, Ugaga, and Ijegu (Yala), Ukpada, Alege, and Betukwel (Obudu), Sankwala, Busi, and Udeshi (Obanliku). The summary of the agro climatic characteristics of the study sites are given in (Table 1). The study was conducted using line transects. In each site, four 1km line transects were laid, each separated by a distance of 200m.The transects ran through the cultivated and fallow land uses of the Guinea Savanna of Cross River (North). Along each transect, ten 20m × 20m sample plots were laid at randomly selected points with the help of States Forestry Department Officers. In all, a total area of 40,000m^2 was assessed along each transect which gives a gross total area of about 20 hectares. The numbers of stands of P. *biglobosa* and other tree species producing various economically valued products present on the marked plots were counted and recorded (taking into consideration the age of the P. *biglobosa* trees). Data collated was used to determine each species percentage frequency (%), Density, and Relative Abundance using the method of (Sharma, 2009). The (%) frequency for the species in the marked Area was calculated using the formula:

$$\text{Frequency (\%)} = \frac{\text{No. of sampling units in which a particular species occurred}}{\text{Total no. of sampling units studied}} \times 100$$

After determining the percentage frequency of each species, various species were distributed among Sharma's (2009) five frequency classes depending upon their frequency values as presented in (Table 2). A plant species was classified as abundant (A) or rare (R) based on its frequency class in all the assessed segments. A plant species found in 41% and above of all the assessed segments was considered abundant (A) while a species present in less than 41% of all the assessed segments was regarded as rare (R).

However, since frequency does not give correct idea of the distribution of any species unless it is correlated with other characters such as density and abundance. The percentage density of the population was also determined using the formula:

$$\text{Density} = \frac{\text{Total no. of individuals of the species in all the sampling units}}{\text{Total no. of sampling units studied}}$$

While the relative abundance of the species was calculated thus:

$$\text{Abundance} = \frac{\text{Total no. of individuals of the species in all the sampling units'}}{\text{Total no. of sampling units in which the species has occurred}}$$

To predict what will be the population size of P. *biglobosa* after ten years. The Logistic model of population dynamics also called the (Verhulst-pearl) model was used. Modeling was carried out in this study using the methods of (Volterra, 1926, Yodzis, 1978, Law and Blackford, 1992, Cohen, 1995, Mckelvey, 1996, Alexei, 1996, Vandameer, 2010, Arditi and Ginzberg, 2012, Knaust, 2013). The model states that the rate of population increase may be limited i.e., it may depend on population density:

$$r = \frac{r_0 (1- N)}{K}$$

At low densities ($N << K$), the population growth rate is maximal and equals to r_0. Parameter r_0 can be interpreted as population growth rate in the absence of intra-specific competition. Population growth rate declines with population numbers N, and reaches 0 when N=K. Parameter K is the upper limit of population growth and it is called carrying capacity. It is usually interpreted as the amount of resources expressed in the number of organisms that can be supported by these resources. If population numbers exceed K, then population growth rate becomes negative and population numbers decline. The dynamics of the population is described by the differential equation:

$$\frac{dN}{dt} = rN = r_0N(1- \frac{N)}{K}$$

Where N(t) represents number of individuals at time t, r the intrinsic growth rate and K is the carrying capacity, or the maximum number of individuals that the environment can support. Logistic model has two equilibriums N=0 and N=K. The first equilibrium is unstable because any small deviation from this equilibrium will lead to population growth. The second equilibrium is stable because after small disturbances the population returns to this equilibrium state. Logistic

model combines two ecological processes: reproduction and competition. Both processes depend on population numbers or density (Hogeweg and Hesper, 1978, Alexei, 1996, Alder, 1997, May, 2004, Campbell and Reece, 2008).

a) What will be the population size of P. biglobosa after n years (or generations)?

Using the differential equation of the Logistic model which is:

$$\frac{dP}{dt} = kP\left(1 - \frac{P}{M}\right)$$

In order to solve this equation, a non-linear equation which is separable was used. The constant solutions were N=0 and N=K. The non-constant solutions was obtained by separating the variables

$$\frac{dP}{P\left(1 - \frac{P}{M}\right)} = k\,dt$$

And integration

$$\int \frac{dP}{P\left(1 - \frac{P}{M}\right)} = \int k\,dt \ .$$

The partial fraction technique gives

$$\int \frac{dP}{P\left(1 - \frac{P}{M}\right)} = \int \left(\frac{1}{P} + \frac{1/M}{1 - P/M}\right) dP$$

This gives

$$\ln|P| - \ln\left|1 - \frac{P}{M}\right| = kt + c \ .$$

Easy algebraic manipulation gives

$$\frac{P}{1 - P/M} = Ce^{kt} \ ,$$

Where C is a constant solving for P, we get

$$P = \frac{MCe^{kt}}{M + Ce^{kt}} \ .$$

If we consider the initial condition P(0) = P_0 (assuming that P_0 is not equal to both O or M), we get

$$C = \frac{P_0 M}{M - P_0}$$

Which when once substituted into the expression for P(t) and simplified we find

$$P(t) = \frac{MP_0}{P_0 + (M - P_0)e^{-kt}} \ .$$

It is easy to see that

$$\lim_{t \to +\infty} P(t) = M \ .$$

However, this is still not acceptable because this model does not tell us when a population is facing extinction since it never implies that. It can be concluded that extinction never occurs under the logistic model. Even starting with a small population it will always tend to the carrying capacity M. Hence, it became necessary to modify the model using the method of (Mckelvey, 1996) which increased the models realism and usefulness. This was the addition of the ecological idea of a minimum viable population. The idea here is that a population of any species has a minimum level at which the population can thrive. If the population drops below this minimum level, various environmental and genetic factors lead to the elimination of the population. The relevant factors might include inability of trees to reproduce, loss of genetic diversity and increased vulnerability to short and long term environmental changes and disease events. The logistic model was modified to account for the existence of a minimum viable population by using the difference equation:

$$\frac{dP}{dt} = kP\left(1 - \frac{P}{N}\right)\left(\frac{P}{M} - 1\right)$$

Where N is the carrying capacity and indicates when the population is too big and the sparsity parameter M indicates when the population is too small (minimum viable population).

Since P. *biglobosa* is a perennial tree that is confined to a particular habitat (mostly savanna):

- If the population is large, their rate of growth decreases or even becomes negative as a result of intra-specific competition.
- If the population is too small (as with the case in this study), old trees run the risk of not being able to reproduce and as such the rate of growth is also negative. Based on these observations, the modified logistic model was used to find the equilibrium (or critical) points of the P. *biglobosa* population for the next ten years.

b) Application of the Modified Logistic Model of Population Dynamics

$$\frac{dp}{dt} = kp(1 - \frac{p}{N})(\frac{P}{M} - 1).$$

$$<=> \frac{DP}{KP(1 - \frac{P}{N})(\frac{P}{M - 1})} \_\_\_\_\_$$

Integrated both side to get

$$\int \frac{dp}{kp(1 - \frac{p}{N})(\frac{P}{M - 1})} = \int dt + C_o \_\_\_\_\_ *$$

Decompose $\frac{1}{kp(1-\frac{p}{N})(\frac{P}{M}-1)}$. into partial fractions

$$\frac{1}{kp(1-\frac{p}{N})(\frac{P}{M}-1)} = \frac{A}{KP} + \frac{B}{(1-\frac{P}{N})} + \frac{C}{(\frac{P}{M-1})}$$

$$= \frac{[A(1-\frac{P}{N}) + BKP](\frac{P}{M}-1) + CKP(1-\frac{P}{N})}{KP(1-\frac{P}{N})(\frac{P}{M}-1)}$$

$$\frac{1}{KP(1-\frac{P}{N})(\frac{P}{M}-1)} = \frac{(\frac{A}{M} - BK + \frac{A}{N} + CK)P + (-\frac{A}{NM} + \frac{BK}{M} - \frac{CK}{N})P^2 - A}{KP(1-\frac{P}{N})(\frac{P}{M}-1)}$$

A, B, C, are the unknown means

$$\begin{cases} A = -1 \\ -\frac{A}{M} - BK + \frac{A}{N} + CK = 0 \\ -\frac{A}{NM} + \frac{BK}{M} - \frac{CK}{N} = 0 \end{cases} => \begin{cases} A = -1 \\ (-K)B + KC = \frac{1}{M} + \frac{1}{N} \\ (\frac{K}{M})B + (-\frac{K}{N})C = -\frac{I}{NM} \end{cases}$$

$$D = \begin{vmatrix} -K & K \\ \frac{K}{M} & -\frac{K}{N} \end{vmatrix} = \frac{K^2}{N} - \frac{K^2}{M}.Then$$

$$= \left| \frac{1}{M} + \frac{1}{N} K \right|$$

$$B = \frac{-\frac{1}{NM} - \frac{-K}{N}}{D} = \frac{\frac{K}{N}(\frac{1}{M} + \frac{1}{N}) + \frac{K}{NM}}{D}$$

$$= \frac{-\frac{K}{NM} - \frac{K}{N^2} + \frac{K}{NM}}{D}$$

$$= \frac{-K}{DN^2}$$

$$B = \frac{-K}{KN(\frac{N}{M}-1)} = \frac{K}{K^2(-N + \frac{N^2}{M})} = \frac{1}{KN(\frac{N}{M}-1)}$$

$$B = \frac{I}{KN(\frac{N}{M} - 1)}$$

$$C = \frac{-K}{\frac{K}{M} - \frac{I}{\frac{NM}{D}}} \frac{I}{M} + \frac{I}{M} = \frac{\frac{K}{NM - \frac{K}{M}(\frac{I}{M} + \frac{I}{N})}}{D}$$

$$= \frac{\frac{K}{NM} - \frac{K}{M^2} - \frac{K}{NM} = \frac{-K}{DM^2}}{D}$$

$$= . \frac{K}{M2(\frac{I}{N} - \frac{I}{M})K^2} = \frac{I}{K(M - \frac{M^2}{N}}$$

$C = \frac{I}{KM(\frac{I}{-} - \frac{M}{N})}$ With A, B, C, obtained

The left hand side of (*) is

$$\int \frac{dp}{kp(1 - \frac{p}{N})(\frac{P}{M} - 1)}$$

$$= \frac{\wedge}{k}[-\int \frac{dp}{p} + \frac{1}{N(\frac{N}{M} - 1)} \int \frac{dp}{(1 - \frac{p}{N})} + \frac{1}{M(1 - \frac{M}{N})} \int \frac{dp}{(\frac{p}{m} - 1)}$$

$$= \frac{i}{k} Lnp + - \frac{NLn}{N(\frac{N}{M} - 1} (1 - \frac{P}{N}) + \frac{MLn}{m(1 - \frac{m}{N})} (\frac{P}{M} - 1)$$

$$= Lnp - \frac{1}{k\ (1 - \frac{p}{N})} - \frac{1}{(\frac{N}{M} - 1)(\frac{P}{M} - 1)} \frac{1}{(1 - \frac{M}{N})}$$

The right hand side of (*) is

$$\int dt + C_o = t + C_o$$

Hence the differential equation has the general solution

$$Lnp - \frac{1}{k(1-\frac{p}{N})}\ \frac{1}{(1-\frac{N}{M})(\frac{P}{M}-1)}\frac{1}{(1-\frac{M}{N})} = t\_C_o$$

Take the exponential of both sides to finally get:

$$\frac{(1-\frac{p}{N})(1-\frac{N}{M})\ (\frac{P}{N}-1)\frac{1}{(1-\frac{M}{N})}}{P\frac{1}{N}} \quad = C, e^t \ \ where C, = e^{C_0}$$

Since the model is described by the differential equation:

$$\frac{dP}{dt} = kP\left(1-\frac{P}{N}\right)\left(\frac{P}{M}-1\right)$$

Where
P is the population
t is time
N is the carrying capacity which indicates when the population is too big (max).
In this problem N = 2.1
M the sparsity parameter indicates when the population is too small (min).
In this problem M = 1.5

Tentative solution

Assuming the survey took place in 2006 and found that the total population was α = 2.1 + 1.8 + 2.0 + 1.7 + 1.5 = 9.1
So that at t = 0; P = 9.1
Assuming also that at the same time N = 2.1 and M = 1.5

Let t be the extinction period which is 10 years. This means that at time t, P should be very close to zero (0). Mathematically, this is written as:

$$\int_{\alpha}^{o}\frac{dp}{kp(1-\frac{I}{N})(\frac{P}{M}-1)} = \int_{O}^{T} dt$$

$$<=> \int_{a}^{0}\frac{dp}{kp(1-\frac{1}{N})(\frac{P}{M}-1)} = \int_{0}^{10} dt$$

$$\Leftrightarrow \frac{(1-\frac{p}{N})\ (1\frac{N}{M})\ (\frac{P}{M}-1)\ (\frac{1}{1-\frac{M}{N}}\int_{a.1}^{0}}{P\,1/K} \quad = 10$$

With N = 2.1; M =1.5, we have:

$$\left[\frac{(1-\frac{o}{2.1})\ \frac{1}{(1-\frac{2.1}{1.5})(\frac{0}{1.5}-1)}\ \frac{1}{(1-\frac{1.5}{2.1})}}{o\,1/k}\right] - \left[\frac{(1-\frac{9.1}{2.1})\frac{1}{(1-\frac{2.1}{1.r})(\frac{9.1}{1.r}-1)}\ \frac{1}{(1-\frac{1.r}{2.1})}}{(9.1)\,1/k}\right] = 10$$

Note: The 0 in the denominator means the population is close to zero and not exactly equal to zero.

III. Conclusion

Since there is a balance between the left and right hand side of the above equation, then it is proved that the minimum viable population M of *P. biglobosa* decreased and will continue to decrease over time (t) unless viable intervention measures are taken. Based on these findings, It can be concluded that populations less than M (1.5) will decrease to oblivion and will continue to in the face of continuous exploitation without replacement.

Table 1 : Characteristics of the Agro-ecological zone surveyed for P. *biglobosa* in Cross River (North) woodland savanna

Ecozone	Study Locations	Latitude(N)	Longitude(E)	Elevation(ft)	Annual rainfall (mm)	Rainfall pattern
CROSS RIVER STATE Guinea Savanna	Ishindede	6.50˚	8.61˚	403	1250-1300	Unimodal
	Ndok	6.59˚	8.79˚	452	1250-1300	Unimodal
	Idum	6.53˚	8.90˚	597	1250-1300	Unimodal
	Abuochiche	6.69˚	8.97˚	485	1250-1300	Unimodal
	Afrike	6.61˚	8.87˚	436	1250-1300	Unimodal
	Gakem	6.77˚	8.99˚	498	1250-1300	Unimodal
	Okpoma	6.59˚	8.63˚	383	1250-1300	Unimodal
	Ugaga	6.67˚	8.73˚	413	1250-1300	Unimodal
	Ijegu	6.74˚	8.67˚	400	1250-1300	Unimodal
	Ukpada	6.71˚	8.90˚	439	1250-1300	Unimodal
	Alege	6.52˚	8.43˚	393	1250-1300	Unimodal
	Betukwel	6.61˚	9.12˚	646	1250-1300	Unimodal
	Sankwala	6.55˚	9.22˚	1266	1250-1300	Unimodal
	Busi	6.55˚	9.27˚	2057	1250-1300	Unimodal
	Udeshi	6.55˚	9.12˚	1571	1250-1300	Unimodal

Table 2 : Sharma's (2009) five frequency classes

Frequency (%)	Frequency class
0-20	A(R)
21-40	B(R)
41-60	C(A)
61-80	D(A)
81-100	E(A)

Note: (A) Abundant, (R) Rare

IV. Results and Discussion

a) Species Distribution and Density

Cross River state (North) Guinea savanna was assessed for the Abundance (A) or Rarity (R) of *Parkia biglobosa.* In all, seventeen tree species producing various economically important products were encountered. Two of the 17 tree species were observed to be rare (R), while 15 species were abundant (A) as presented in (Table 3). The rarity of *Adansonia digitata* is quite understood, since it is found further up north of the country in abundance (A) mostly in the Sudan savanna. However, P. *biglobosa* which is very common in the Guinea savanna belt of Nigeria was rare (R) in all the assessed areas with low densities of 2.1/ha for Ogoja L.G.A, 1.6/ha (Bekwarra), 2/ha (Obudu), 1.7/ha (Obanliku), and 1.5/ha for Yala respectively (Tables 4, 5, 6, 7 & 8), While *Vitellaria paradoxia* and *Elaeis guinensis* had the highest densities of 3.2/ha for V. *paradoxia*, 10/ha for E. *guinensis* (Ogoja L.G.A), 4/ha and 4.8/ha (Bekwarra), 3.8/ha and 5.9/ha (Obudu), 3.6/ha and 5.2/ha (Obanliku) and 5.3/ha (V. *paradoxia*), 5.7/ha (E. *guinensis*) for Yala L.G.A respectively. On average, there were more old trees (of P. *biglobosa*) than juveniles, and more young trees than juveniles.

The population of P. *biglobosa*, a priority tree with enormous economic and social values to the people of Cross River State (North) was observed to be rare and is rapidly declining. This is due to rapid human population growth, livestock population pressures, increasing land fragmentation, over exploitation for seed and a high demand for wood fuel especially charcoal. In this study, the overall densities for P. *biglobosa* in all the surveyed areas were quite low and facing threat of extinction. The population densities were 2.1stands/ha (Ogoja), 1.6stands/ha (Bekwarra), 2stands/ha (Obudu), 1.7stands/ha (Obanliku) and 1.5stands/ha (Yala) in the cultivated and fallow land uses. However, these findings disagrees with that of Odebiyi *et al* (2004) who reported higher densities 8.2stands/ha, 6.6stands/ha and 5.2 stands/ha respectively in the cultivated and fallow land uses of the Northern Guinea Savanna of Kwara State, Nigeria. In this study we observed that, on average, there were more old trees than juveniles and more young trees than juveniles. Saplings of P. *biglobosa*

were absent in all the surveyed areas, these findings agrees with that of (odebiyi *et al* (2004), Joshi and Joshi (2009). The scarcity of the saplings may be as a result of grazing by livestock, uncontrolled incessant bush burning and over exploitation for seeds which may have hampered their regeneration and aggravated the threat of extinction that the specie is subjected to. More worrisome is the fact that the specie is neither domesticated at present nor planted in organized forestry plantations, and large trees are being over exploited for timber, fuel wood, and production of charcoal and removal of stands to give way for construction works (urbanization).

Table 3 : List of different tree species assessed in the Guinea savanna of Cross River State (North) and their Ecological Status.

S/N	Name of the species	Frequency (%)	Frequency class	Ecological Status
1.	*Parkia biglobosa*	30	B	R
2.	*Elaeis guinensis*	60	C	A
3.	*Hyphaena thebaica*	50	C	A
4.	*Vitellaria paradoxa*	60	C	A
5.	*Isoberlina doka*	70	D	A
6.	*Prosopis africana*	60	C	A
7.	*Adansonia digitata*	20	A	R
8.	*Ceiba pentandra*	60	C	A
9.	*Anarcadium occidentale*	50	C	A
10.	*Gmelina arborea*	60	C	A
11.	*Magnifera indica*	70	D	A
12.	*Azadirachta indica*	50	C	A
13.	*Raffia ruffia*	60	C	A
14.	*Acacia nilotica*	70	D	A
15.	*Moringa oleifera*	50	C	A
16.	*Milicia excelsa*	70	D	A
17.	*Cocos nucifera*	80	D	A

Note: A (abundant), R (rare).

Table 4 : List of different tree species and other data recorded by Transect method in Ogoja L.G.A.

S/N	Name of the species	Segments number 1 2 3 4 5 6 7 8 9 10	Total no. 0f individuals 0f species	Total no. of segments the species occurred	Total no. of segments studied	Frequency (%)	Frequency class	Density	Abundance
1.	*Parkia biglobosa*	- - 3 - 5 - - 7 - -	15	3	10	30	B	1.5	5
2.	*Elaeis guinensis*	4 3 - 12 - 9 -12 -20	60	6	10	60	C	6	10
3.	*Hyphaena thebaica*	5 3- 20 35 - 20 - - -	83	5	10	50	C	8.3	16.6
4.	*Vitellaria paradoxa*	13 - -5 3- - 2 4 5	32	6	10	60	C	3.2	5.3
5.	*Isoberlina doka*	3 21 4 - 6 8 - - 2 9	53	7	10	70	D	5.3	7.6
6.	*Prosopis africana*	- - 10 5 20 - 3 - 6 1	45	6	10	60	C	4.5	7.5
7.	*Adansonia digitata*	5 - - - - 1 - - - 2	8	2	10	20	A	0.8	4
8.	*Ceiba pentandra*	- 9 6 9 2 - - 13 - 3	42	6	10	60	C	4.2	7
9.	*Anarcadium occidentale*	2 10 3 7 - - 15 - - -	37	5	10	50	C	3.7	7.4
10.	*Gmelina arborea*	4 - - 19 - 2 8 - 6 20	59	6	10	60	C	5.9	9.8
11.	*Magnifera indica*	10 - 3 - 7-15- 5 9 8	57	7	10	70	D	5.7	8.1
12.	*Azadirachta indica*	- - 6 13 - 2 -10 - 2	33	5	10	50	C	3.3	6.6
13.	*Raffia ruffia*	- 3 8 2 - 5 20 - 1 -	39	6	10	60	C	3.9	6.5
14.	*Acacia nilotica*	9 2 - - 5 8 1 12 - 3	40	7	10	70	D	4	5.7
15.	*Moringa oleifera*	- - - 10 4 7 2 8 - -	31	5	10	50	C	3.1	6.2
16.	*Milicia excels*	2 8 5 19 1 - - - 6 9	50	7	10	70	D	5	7.1
17.	*Cocos nucifera*	3 12 6 -10 - 9 2 4 7	43	8	10	80	D	4.3	5.3

Table 5 : List of different tree species and other data recorded by Transect method in Bekwarra L.G.A.

S/N	Name of the species	Segments number 1 2 3 4 5 6 7 8 9 10	Total no. 0f individuals 0f species	Total no. of segments the species occurred	Total no. of segments studied	Frequency (%)	Frequency class	Density	Abundance
1.	*Parkia biglobosa*	8 - - 3 - - 6 - -	17	3	10	30	B	1.7	5.6
2.	*Elaeis guinensis*	9 10 6 8 5 3 - - - 7	48	7	10	70	D	4.8	6.8
3.	*Hyphaena thebaica*	11 4 7 9 - - 20 5 - -	56	6	10	60	C	5.6	9.3
4.	*Vitellaria paradoxa*	6 8 5 - 9 - 9 - 2 1	40	6	10	60	C	4	6.6
5.	*Isoberlina doka*	- 4 - 9 12 - 6 3 - 7	41	6	10	60	C	4.1	6.8
6.	*Prosopis africana*	2 9 7 5 8 - - 1 3 -	35	7	10	70	D	3.5	5
7.	*Adansonia digitata*	- - - - - 2 - - - 1	3	2	10	20	A	0.3	1.5
8.	*Ceiba pentandra*	1 4 - - 7 8 10 2 - -	32	6	10	60	C	3.2	5.3
9.	*Anarcadium occidentale*	8 12 6 9 3 2 4 - - 6	50	8	10	80	D	5	6.2
10.	*Gmelina arborea*	- 1 4 20 - 9 2 - - -	36	5	10	50	C	3.6	7.2
11.	*Magnifera indica*	12 4 8 - - 10 2 6 - -	42	5	10	50	C	4.2	8.4
12.	*Azadirachta indica*	- - 10 6 9 11 - - 7 -	43	5	10	50	C	4.3	8.6
13.	*Raffia ruffia*	- - - 14 2 18 3 - - -	37	4	10	40	B	3.7	9.2
14.	*Acacia nilotica*	9 - - 8 6 4 9 - - -	36	5	10	50	C	3.6	7.2
15.	*Moringa oleifera*	- - 10 9 4 7 - 5 - 3	38	6	10	60	C	3.8	6.3
16.	*Milicia excels*	2 8 6 9 - - - 5 - -	30	5	10	50	C	3	6
17.	*Cocos nucifera*	6 10 9 - - - 3 8 4 -	40	6	10	60	C	4	6.6

Table 6 : List of different tree species and other data recorded by Transect method in Obudu L.G.A.

S/N	Name of the species	Segments number 1 2 3 4 5 6 7 8 9 10	Total no. 0f individuals 0f species	Total no. of segments the species occurred	Total no. of segments studied	Frequency (%)	Frequency class	Density	Abundance
1.	*Parkia biglobosa*	- - - 7 - 5 - - - 8	20	3	10	30	B	2	5
2.	*Elaeis guinensis*	18 5 9 8 - 2 4 7- 6	59	8	10	80	D	5.9	7.3
3.	*Hyphaena thebaica*	20 8 6 9 - - 10 - 6 -	59	6	10	60	C	5.9	9.8
4.	*Vitellaria paradoxa*	15 4 3 7 9 - - - - -	38	5	10	50	C	3.8	7.6
5.	*Isoberlina doka*	- - - 9 12 - 7 - 5 7	36	5	10	50	C	3.6	7.2
6.	*Prosopis africana*	2 9 6 4 - - - 7 - 4	32	6	10	60	C	3.2	5.3
7.	*Adansonia digitata*	- - - - - - 1 - - 1	2	2	10	20	A	0.2	1
8.	*Ceiba pentandra*	7 9 - 4 - - 8 3 - -	31	5	10	50	C	3.1	6.2
9.	*Anarcadium occidentale*	16 3 8 4 - 2- 6 9 5	53	8	10	80	D	5.3	6.6
10.	*Gmelina arborea*	- 10 7 4 - 3 9 - - 2	35	6	10	60	C	3.5	5.8
11.	*Magnifera indica*	8 6 3 5 7 - - 3 2 -	34	7	10	70	D	3.4	4.8
12.	*Azadirachta indica*	2 9 3 6 3 - - - 1 -	24	6	10	60	C	2.4	4
13.	*Raffia ruffia*	- - - 9 3 1 8 4 - -	25	5	10	50	C	2.5	5
14.	*Acacia nilotica*	2 8 - 4 - - - 9 1-	24	5	10	50	C	2.4	4.8
15.	*Moringa oleifera*	- - - - 5 9 12 3 2 6	37	6	10	60	C	3.7	6.1
16.	*Milicia excels*	2 9 3 - - - 1 - - 7	22	5	10	50	C	2.2	4.4
17.	*Cocos nucifera*	3 7 4 - 3 - 6 9 - -	32	6	10	60	C	3.2	5.3

Table 7 : List of different tree species and other data recorded by Transect method in Obanliku L.G.A.

S/N	Name of the species	Segments number 1 2 3 4 5 6 7 8 9 10	Total no. 0f individuals 0f species	Total no. of segments the species occurred	Total no. of segments studied	Frequency (%)	Frequency class	Density	Abundance
1.	*Parkia biglobosa*	2 - 8 - - 6 - - - -	16	3	10	30	B	1.6	5.3
2.	*Elaeis guinensis*	8 15 9 5 2 6 3 2 - 2	52	9	10	90	E	5.2	5.7
3.	*Hyphaena thebaica*	6 3 18 26 3 - - - - 2	58	6	10	60	C	5.8	9.6
4.	*Vitellaria paradoxa*	4 7 2 1 9 - - 5 - 8	36	7	10	70	D	3.6	5.1
5.	*Isoberlina doka*	2 6 3 1 6 2 - - - 1	21	7	10	70	D	2.1	3

6.	*Prosopis africana*	5 2 1 - 4 - - 9 7 -	28	6	10	60	C	2.8	4.6
7.	*Adansonia digitata*	- - - - 2 - - - 2 -	4	2	10	20	A	4	2
8.	*Ceiba pentandra*	4 9 6 - - 4 8 - - 2	33	6	10	60	C	3.3	5.5
9.	*Anarcadium occidentale*	8 2 7 - - - - 9 12 -	38	5	10	50	C	3.8	7.6
10.	*Gmelina arborea*	- 9 3 7 8 - - - 2 -	29	5	10	50	C	2.9	5.8
11.	*Magnifera indica*	2 7 9 - 10 3 - - 7 4	42	7	10	70	D	4.2	6
12.	*Azadirachta indica*	- - - 12 3 1 - 4 8 -	28	5	10	50	C	2.8	5.6
13.	*Raffia ruffia*	2 8 4 7 - 5 - 3 - 10	39	7	10	70	D	3.9	5.5
14.	*Acacia nilotica*	2 - - - 6 - - 9 6 4	27	5	10	50	C	2.7	5.4
15.	*Moringa oleifera*	- - 10 4 3 2 1 - - 2	22	6	10	60	C	2.2	3.6
16.	*Milicia excels*	3 6 8 - - - 9 - - -	26	4	10	40	B	0.4	6.5
17.	*Cocos nucifera*	10 4 2 6 - 8 - - 5 8	43	7	10	70	D	4.3	6.1

Table 8 : List of different tree species and other data recorded by Transect method in Yala L.G.A.

S/N	Name of the species	Segments number 1 2 3 4 5 6 7 8 9 10	Total no. 0f individuals 0f species	Total no. of segments the species occurred	Total no. of segments studied	Frequency (%)	Frequency class	Density	Abundance
1.	*Parkia biglobosa*	8 2 - - - - 9 - - 2	21	4	10	40	B	2.1	5.2
2.	*Elaeis guinensis*	9 4 8 9 5 2 7 4 5 8	57	10	10	100	E	5.7	5.7
3.	*Hyphaena thebaica*	9 6 22 - 4 8 7 9 4 -	69	9	10	90	E	6.9	7.6
4.	*Vitellaria paradoxa*	16 8 3 6 2 9 5 - 4 -	53	8	10	80	D	5.3	6.6
5.	*Isoberlina doka*	- - - - 6 10 8 9 - 5	38	5	10	50	C	3.8	7.6
6.	*Prosopis africana*	2 - - 8 5 7 - - - 5	27	5	10	50	C	2.7	5.4
7.	*Adansonia digitata*	- 3 2 4 - - - - - -	9	3	10	30	B	0.9	3
8.	*Ceiba pentandra*	6 3 9 - - - - 8 - 7	32	5	10	50	C	3.2	6.4
9.	*Anarcadium occidentale*	14 3 5 3 7 9 2 1 3 -	47	9	10	90	E	4.7	5.2
10.	*Gmelina arborea*	- - - 6 9 3 5 2 - -	25	5	10	50	C	2.5	5
11.	*Magnifera indica*	7 9 6 - - 3 5 2 - 6	38	7	10	70	D	3.8	5.4
12.	*Azadirachta indica*	- 6 9 2 - - 8 5 - -	30	5	10	50	C	3	6
13.	*Raffia ruffia*	4 9 7 - 6 - - 3 -10	39	6	10	60	C	3.9	6.5
14.	*Acacia nilotica*	- 5 3 - - -9 9 - -	26	4	10	40	B	2.6	6.5
15.	*Moringa oleifera*	7 5 3 2 9 - - - - 2	28	6	10	60	C	2.8	4.6
16.	*Milicia excelsa*	- - - 4 7 3 2 1 2 3 6	28	7	10	70	D	2.8	4
17.	*Cocos nucifera*	7 3 6 9 3 2 1 4 9 -	44	9	10	100	E	4.4	4.4

b) Threat of extinction

Since *P. biglobosa* is a perennial tree that is confined to a particular habitat (mostly savanna):

If the population is large, their rate of growth decreases or even becomes negative as a result of intra-specific competition. If the population is too small (as with the case in this study), old trees run the risk of not being able to reproduce and as such the rate of growth is also negative. Application of the Modified Logistic Model of population dynamics of *Parkia biglobosa* in this study, showed that at P=M (1.5) (population decreased) and will continue to decrease over time (t) unless viable intervention measures are taken. Based on these findings, It can be concluded that populations less than M (minimum viable population) will decrease to oblivion (face extinction) as shown in (figure 1).

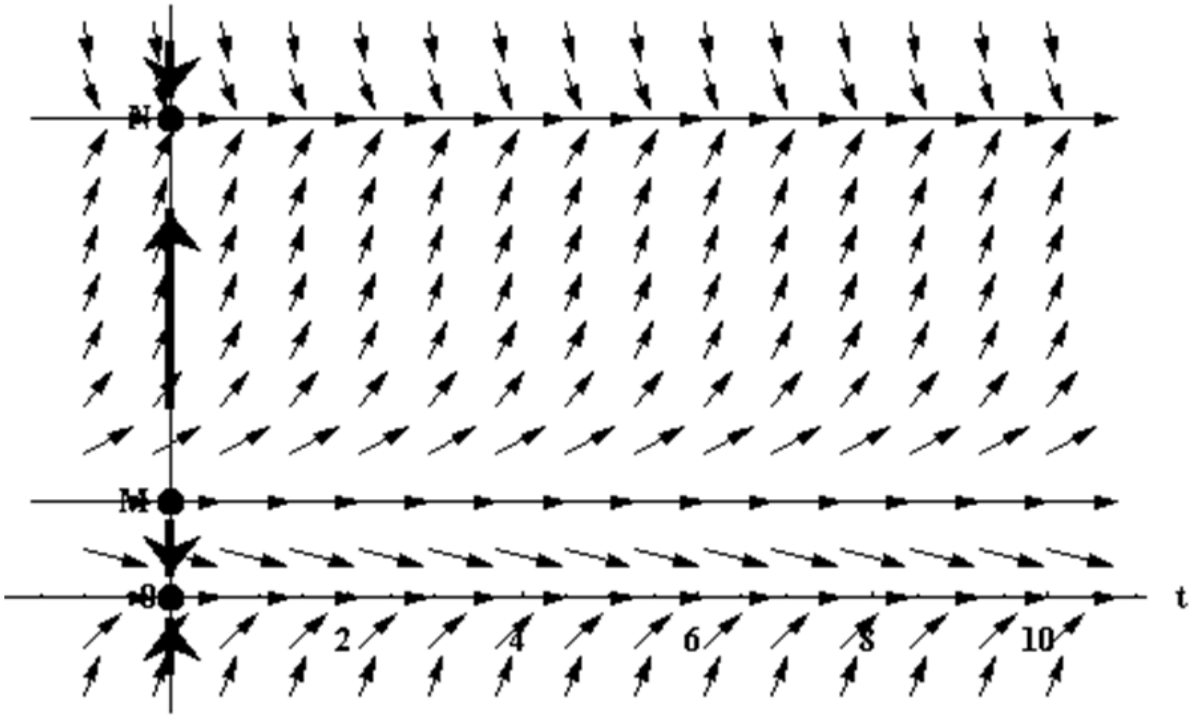

Fig. 1 : Population dynamics of *Parkia biglobosa* in the next ten years based on the modified logistic model

In the face of overexploitation and threat of extinction of the specie, the logistic model of population dynamics (Valhurst-pearl) model was used in this study to predict (model) what will be the population size of P. *biglobosa* after ten years. The model is useful in describing populations which exhibit exponential growth at small populations but who live in environments which enforce an upper limit on population size. In this study, results of the model were not satisfactory because the model does not tell us when a population is facing extinction since it never implies that. Even starting with a small population it will always tend to the carrying capacity. Extinction never occurs under the Logistic Model; this finding agrees with that of Gardner and Ashby (1970), Levins and Culver (1971), Rosenzweig (1971), May (1974), Noy-Meir (1975), Hanksi (1991, 1997,1998), Nee and May (1992),Tilman (1994), Tilman *et al.,* (1997), MaCauley *et al.,* (1999), Scheffer *et al.,* 2001, Scheffer, (2009) on how populations grow using the exponential and logistic equations. Based on this finding, the Logistic Model was modified to increase the models realism and usefulness. This was the addition of the ecological idea of a minimum viable population. The idea here is that a population of any species has a minimum level at which the population can thrive. If the population develops below the minimum level, various environmental and genetic factors lead to the elimination of the population. The relevant factors might include inability to reproduce, loss of genetic diversity and increased vulnerability to short and long term environmental changes and disease events. The use of Modified Logistic Model to predict what will be the population of P. *biglobosa* in the next ten years is similar to that of Mckelvey (1996), Knaust (2013) and Adler (1997) in setting quotas with the modified logistic model and modeling the dynamics of life respectively. In this study, we proved that at critical point of P=M the population of P. *biglobosa* will decrease and will continue to decrease over time in the next ten years into extinction unless viable intervention measures are taken, this agrees with that of Kingsland (1995) in modeling nature and Renshaw (1991) on modeling biological populations in space and time.

V. Conclusion/Recommendation

The study has proven that the population of *P. biglobosa* in the Guinea Savanna of Cross River State (North) is rapidly declining at an alarming rate. In the face of threat of extinction due to continued exploitation without replacement, domestication and plantation establishment of this tree to enhance its productivity and to curb the threat of extinction is strongly recommended.

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Overview of the Biology, Epidemiology and Control Methods Against Hard Ticks: A Review

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Abstract- Ticks are among the obligated ecotparasites that feed blood of vertebrates; particularly mammals; birds; and they are arachnids in the subclass Acari; closely related to mites; surviving for up to several years. This review paper dials about the biology; epidemiology and control methods of hard ticks. Morphologically they are classified into two families known as Ixodidae and Argasidae. All hard ticks feed only on blood of their hosts. They are active during warm periods and easily find their hosts by grapping using their front legs and attach on the suitable site. In their life cycle they have three active stages called larvae; nymphs and adults after eggs is released which is dormant.

Keywords: *control, epidemiology, ixodidae, lifecycle, morphology.*

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Overview of the Biology, Epidemiology and Control Methods Against Hard Ticks: A Review

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Abstract- Ticks are among the obligated ecotparasites that feed blood of vertebrates; particularly mammals; birds; and they are arachnids in the subclass Acari; closely related to mites; surviving for up to several years. This review paper dials about the biology; epidemiology and control methods of hard ticks. Morphologically they are classified into two families known as Ixodidae and Argasidae. All hard ticks feed only on blood of their hosts. They are active during warm periods and easily find their hosts by grapping using their front legs and attach on the suitable site. In their life cycle they have three active stages called larvae; nymphs and adults after eggs is released which is dormant. Three types of Ixodidae are present based on their life cycles called; one; two and three host ticks. Hard ticks life cycles are influenced by factors such as host; climatic; vegetation cover. They widely distributed both in temperate and tropical areas of the world. Hard ticks cause cytolysis; cell reaction; and toxic effects on the host besides anaemia. The commonest tick control methods include chemical; ecological; biological; genetic and immunizations systems.

Keywords: control, epidemiology, ixodidae, lifecycle, morphology.

I. Introduction

Ticks are obligated blood feeding ectoparasite of vertebrates, particularly mammals, birds, and they are arachnids in the subclass Acari, closely related to mites, surviving for up to several years. During this time they feed periodically taking large blood meals, often interspersed with long intervals each meal (Wall and Shearer, 1997).There are at least 884 tick species in two major families, namely the *Ixodidae* comprises approximately 80% and *Argasidae* 20%.There are two well-defined families of ticks, the *Ixodidae* or hard ticks and the *Argasidae* or soft ticks, and the two groups differ from each other markedly in appearance, habits and development(Pegram *et al*.,1987).

It has been estimated that 80% of the world's 1,281 million cattle are infested with ticks and while in Africa about 186 million head of cattle are at risk of ticks and Tick-borne diseases(TBDs)(http://www.fao.org/docrep/U9550T/u9550T04.htm). Besides the direct impact, ticks also have a greatest impact due to their large number and variety of microbial disease that they transmit among domestic animals. Mostly the tick-borne infections of humans, farm and companion animals are essentially associated with wildlife animal reservoirs (Baneth, 2014).

Ticks and the diseases they transmit are widely distributed throughout the world (Nicholson et al., 2010; Dantas-Torres, 2007; Piesman and Eisen, 2008), particularly in tropical and subtropical countries (http://www.mayomedicallaboratories.com/articles /features/tick-borne/).

Ticks impact on animal production is similar in nature and importance; they are responsible for severe losses caused either by the direct effect of the tick through: tick worry, blood loss, damage to hides and udders and the injection of toxins or through mortality or debility caused by the disease transmitted (FAO, 1984, www.nda.agric.za/publications, Rajput *et al*., 2006). DeCastro (1997) estimated that the annual global costs associated with ticks and TBDs in cattle amounted to between US $ 13.9 and 18.7 billion, globally.In Africa, tick-borne diseases are considered the most important animal disease problem (Young *et al.,* 1988). Even though the above estimates are too old reports, yet they reflect the impacts of the problem seriously affecting livestock worldwide. This review paper tried to give an overview of the the Biology, Epidemiology and Control Methods against Hard Ticks.

II. Classification and Morphology

a) *Classification of Ticks*

Ticks are grouped under the phylum Arthropoda;subphylum Chelicerata; ClassArachnida; Order Acarina(Urquhart *et al*., 1996). The present tick species number worldwide estimated to be 889 (702 Ixodid, 186 Argasid and 1 Nuttalliella tick (sub-) species), including their synonyms, (limited) distribution data and common names) which is a searchable taxonomic catalogue of all known ticks (Acari: Ixodida) of the world and is facilitated by the International Consortium on Ticks and Tick-borne Diseases (ICTTD) (Nijhof*et al*., 2016). Family names *Ixodidae* or "hard ticks" and *Argasidae* or "soft ticks" indicatesthe so called by the virtue of their hard dorsal shield and due to their flexible leathery cuticle respectively (Robert *et al*., 1976;http://parasitipedia.net/index.php?option=com_content&view=article&id=2530&Itemid=2804; Latif and Walker, 2004). Recently a new hard tick species, Ixodesariadnaehas been discovered, adding to the two

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known ixodid tick species (*I. vespertilionis* and *I. simplex*) of bats in Europe (Hornok *et al*., 2015). They are dioecious having separate sex (Furman and Loomis,1984).

b) Morphology of Ixodidae

Ixodid ticks are relatively large ,ranging between 2 and 20 mm in length. The body of the unfed tick is flattened dorsoventrally and is being divided into only two sections; the anterior gnathosoma (or capitulum) and posterioridiosoma, which bears the legs. The terminal gnathosoma is always visible when an ixodid tick is viewed from the above. The gnathosoma carries a pair of four segmented palps, which are simple sensory organs, which helps the tick to locate the its host. The fourth segment of each palp is reduced and may articulate from the ventral side of the third ,forming a pincer-like structure. Between the palps lies a pair of heavily sclerotized, two-segmented appendages called chelicerae, housed in cheliceral sheaths. At the end of each chelicera is a rigid, somewhat triangular, plate bearing a number of sclerotized tooth like-like digits. The chelicerae are capable of moving back and fourth and the tooth-like digits are used to cut and pierce the skin of the host animal during feeding (Wall and Shearer, 1997).The enlarged, fused coxae of the palps are known as the basis capituli. The basis capituli varies in shape in the different genera, being rectangular, hexagonal or triangular. The lower wall of the basis capituli is extended anteriorly and ventrally to form an unpaired median hypostome, like an underlip, which lies below the chelicerae. The hypostome does not move but in larvae, nymphs and adult females are armed with rows of backwardly directed ventral teeth. The hypostomeis thrust into the hole cut by the chelicerae and the teeth are used to attach the tick securely to its host. As the hypostome is inserted the palpls are spread flat on to the surface of the host`s skin (Wall and Shearer ,1997; Robert *et al*., 1976).

c) Biological Characteristics of Hard Ticks

i. *Feeding*

Ticks feed only on the blood of their hosts. According to Radostits *et al*.,(1994) all are blood suckers and may cause death from anaemia. All ticks at each stage of the life cycle are parasitic feeding solely on blood, liquefied tissues, and tend to feed at varying depths according to the species. Their bite is relatively painless, but invasions by large number are debilitating (Gray ,1995; Robert *et al*., 1976; Kemp *et al*., 1982).

The salivary gland of ticks produces a secretion, which prevents blood from coagulation. Blood is pumped in by a muscular pharynx and forced backward into the oesophagus and midgut. Diverticula attach to the mid-gut become greatly distended to store and large quantity of blood. The outer body covering (cuticle) is leather-like and capable of great distension during feeding. Un-engorged female ticks are somewhat flattened and tapered anteriorly, but become engorged as much as twenty fold following feeding; male hard ticks take only small blood meals and increase in size only slightly (Bay and Harris, 1988; Robert *et al*., 1976).

ii. *Mechanism of finding their host*

Most ticks are active during warm periods of the year and go into hibernation or become dormant during the cold periods; they hide within fissures in the ground, under rocks or in cracks and in buildings (Galloway, 1974; Robert *et al*., 1976). Ticks find their hosts in several ways. Some ticks live in an open environment and crawl onto vegetation to wait for their hosts to pass by. This is a type of ambush and the behaviour for waiting on vegetation is called questing (Kettle, 1984; Walker *et al*., 2003). Thus in genera such *Rhipicephalus, Haemaphysalis and Ixodes* the larvae, nymphs and adults will quest on vegetation .The ticks grab on to the hosts using their front legs and thus crawl over the skin to find a suitable place attach and feed (Yismashewa, 2005).

Much of the behaviour in ticks is regulated by phermones; this compound greatly enhances inter-species communication, where vision, auditory and tactile means of communication is not believed to play a significant role. Phermones modulate a wide range of behaviour, from mating (sex phermones) to warning other members of the species of impeding communication via phermones affecting assembly, mating and host finding has been demonstrated in ticks (Harwood and James, 1979). Basically tick hormones classified based on their activity profiles tick pheromones are characterized as: (1) assembly pheromones, (2) aggregation/attachment pheromones, (3) pre-attachment or prefeeding pheromones, (4) attractant sex pheromones, (5) contact sex pheromones, (6) fecundity-reducing pheromone and (7) various ungrouped pheromones (Gothe, 1987).

Adult ticks of the genera *Amblyomma* and *Hyalomma* are active hunters; they run across the grounds to seek hosts that are nearby. The general behaves of seeking hosts in an open environment is described as exophilic Ticks such as *Argasids*and many Ixodes species spend their life cycle in their hosts nest and attach to their hosts there. This is endophilic or nidicotous behaviour. A few species of ticks, such as the dog tick *Rhipicephalus* animals there. This is called domestic behaviour (Walker *et al*., 2003).

iii. *Reproduction*

There are three active stages in the life cycle of hard ticks, larvae, nymphs, and adults; each instars takes a blood meal only once and long periods are spent on vegetation between meals. In the hard ticks, mating takes place on the host, except in species of the genus*Ixodes*where it may also occur when the ticks are still on the vegetation. Male ticks remain on the host and

will attempt to mate with many females whilst they are feeding. They transfer a sac of sperm to the female. The females mate only once before they are ready to fully engorge with blood. When they finally engorged, they detach from the host and have enough sperm to fertilize all their eggs (Walker *et al*., 2003). Oviposition usually begins a few days after the female dropped from the host and continues for several days (FAO, 1984). The female dies afterward. Males remain to the host for long period and may mate with other females (Yismashewa, 2005).

In a single batch a female hard tick may lay eggs (2,000to 20,000). The place where all ticks, laid eggs in the physical environment, never on the host (Hoogstral, 1956; Walker *et al*., 2003). The temperature and humidity affects largely the hatching of the eggs and the activity of the larvae. Hot conditions are favourable for hatching (Hoogstraal, 1956 and Gebreab, 1983).

iv. *Life cycle of Ixodidae*

In *Ixodidae,* three types of life cycles can be distinguished based on similarities or differences in tropisms shown by ticks at different instars. These are the monotropic cycle or one-host, the ditropic cycle or two-host and the telotropic cycle or three-host ticks. According to the species, the development is either completed on one, two or three hosts. (Brian, 1997; Walker *et al*., 2003)

In **one-host ticks**, the larvae that emerge from the eggs three to four weeks after deposition at the earliest attaches themselves to host animals where they complete their entire development. On the host they develop to nymph then to adult and then copulate. Afterwards, they drop off and deposit their eggs on the ground. The entire development cycle takes mostly 19-21 days as a rule, with minimum of fifteen and maximum of 40days, each stage taking one week (Seifert, 1996). In these ticks a stricter adaptation eliminates the need to drop to the ground for metamorphosis. All the instars occur on a single vertebrate, attacked by the larva. The larval and nymphal metamorphoses take place on the host, at the point of attachment of the larva and nymph. There is only one parasitic phase (Douglas, 1969).

The **two-host ticks** attach it as a larva to a host, feeds on blood and develops into the nymph stage. After a maximum of 14 days, it drops off on to the ground where it reaches the imago stage in 20-30 days time. Male as well as female ticks then look for another host, feed on blood and copulate. After 6-11 additional days, the female drops to the ground and deposits its eggs. The entire cycle from the time the larva emerges until the engorged female deposits the eggs mainly depends on the time the adult spent on the ground to find a new host. According to the species the nymph may survive on the ground for several weeks (Seifert, 1996). In these ticks the three stages develop on two different individuals that may or may not belong to the same species. In the first phase, the engorged larva molts on the host and the nymph reattaches close by. At the end of the blood meal, the nymph detaches and metamorphoses on the ground. Engorgement of adults occurs during the second parasitic phase. There are only two searches for a host, which eliminates the risks linked with the need for nymphal attachment (Hopla*et al.,* 1994).

The **three-host ticks** looks for a new host during each stage of development in order to feed. The larva emerges from the egg on the ground, looks for a host, feeds on it for three to seven days, drops off and molts after three to four weeks on the ground. The nymph attacks a second host for three to seven days feed on it and drops and develops into an adult on the ground after two to eight weeks. After that, the adult tick looks for a third host to feed on and for copulation that takes one to three weeks. Finally it drops off and completes the cycle with oviposition on the soil. Because of the different time spent in each stage on the ground, the entire development cycle may last up to one year (Seifert, 1996). These ticks require three hosts for development, irrespective of the host species. There are three parasitic phases, separated by two phases on the ground, when metamorphosis occurs (Hopla *et al.,* 1994).

d) Life cycle influencing factors

i. *Host factors*

a. *Character of the hosts*

The adults of many ticks occur on the grass cover and have access to a wide range of host's ungulates or carnivores, wild or domestic. They are not specific but selective towards a group of vertebrates based on their size and origin and mobility. The indigenous wild animals of a given region are important factors regarding the origin and maintenance of population of domestic mammal ticks. The different instars of a given species may or may not have definite microclimatic requirements. These determine their location in the most suitable microhabitat and the choice of hosts according to availability at different levels of the plant cover (Morel, 1980). Specific ticks are associated with hosts of a definite resting site or habitat. The area is small or with a distinct environment nest, burrows, caves, rock piles, thickets, dense specialization in relation to that of the host, rather than phylogenetic specificity of the tick (Samir, 1980; Morel, 1989).

b. *Predilection sites on the hosts*

On initial infestation, ticks were picked up during the day when cattle were grazing, and attached temporarily near the hooves. Subsequently, when cattle rested, especially at night, the ticks detached and then reattached more firmly at the usual predilection sites. Many ticks disappeared during relocation, and other

ticks transferred to different hosts. The tick's location on the host is linked to the possibility of penetration by the hypostome. On ungulates, species with a short hypostome. On usually attach to the head within the ear, nape of the neck, margin of the anus, and under the tail. Long-hypostome. On species attach to the lower part of the body where the skin is thicker, such as the dewlap, groin, udder, testes, perineum, and margin of the anus. Small ticks, all instars of Genus *Rhipicaphalus(Boophilus)*, larvae and nymphs of *Amblyomma*have no marked preference, and can be found all over the body (Strachurski, 2000).

ii. *Climatic factors*

Ticks are found on all continents and are bound to certain climates as far as the requirements for temperature, humidity; sun-radiation and shade of each species are concerned. Furthermore, each species requires specific environmental conditions for its habitat. The respective species only have a chance for survival when these prerequisites are fulfilled (Seifert, 1996). Ticks in a tropical zone the more rapid development pattern are determined during the beginning rainy season. The whole cycle takes one year. Whereas, ticks in an equatorial zone climatic uniformity and the absence of an unfavorable season allow development throughout the year. There is no annual cycle determined by a diapause's, generation follow one another in a pattern depending on the species (Walker and Fletcher, 1982).

a. *Temperature: a dynamic factor*

Each species has its particular threshold temperature below that diapauses occurs in all instars. Egg and larvae development, and egg production in engorged females are inhibited, while immature and unfed adults become quiescent. The average weekly or monthly temperature is useful for predicting the activity threshold and optimum temperature. The tick development and activity periods can be determined from monthly isotherm charts (Morel, 1989).

b. *Relative humidity: a static factor*

Relative humidity is considered at microclimate level. Humid rather than wet conditions are essential for the development and survival of eggs, and the survival of unfed hatched larvae. Each species is adapted to a particular relative humidity range in a biotope and it varies with the instars and its size. Larvae and nymphs have high humidity requirements, whereas the adults can protect themselves better against evaporation because of their larger size and thicker tegument. The requirement ranges from 100 % to very low relative humidity.

Larvae and nymphs adept their humidity requirements by developing in holes in the ground, cracks in rocks, litter, and the base of the vegetation layer and other shelters places. They may alternatively immediately seek a host and not leave it before the engorged female stage. The surface of medium-height (30-150 cm) vegetation and especially bare ground (sand, pebbles, rocks) are less protected. Ticks rarely occur in these sites except under special circumstances in all seasons if adults have thick teguments, or only in the rainy season, or if the site is shaded by a tree to prevent evaporation. Larvae or nymphs found in open spaces are usually active in the rainy or cool seasons (Morel, 1989).

iii. *Climate related factors: seasonal activity*

Climatology includes the particular temperature and humidity conditions prevailing in a country. These parameters are the result of the simultaneous action of several factors, such as latitude, altitude, and their effects sunlight, temperature, rainfall, wind patterns. On a regional scale, these data should be studied for a better understanding of vectors and the diseases they transmit, especially for the application of control measures (Pegram *et al.,*1982). In a region with uniform conditions, a comparison of data on species distribution with isotherm and isohyets maps enables the identification of natural distribution zones in relation to latitude. In mountainous regions, the determining factor is altitude. Various climatic factors condition the presence or absence of a tick species. According to its micro or mesoclimatic requirements, the species will be found in certain similar bioclimatic zones, and not in others. Moreover, seasonal variations within a bioclimatic zone will favor or hinder the development or activity of a tick species during certain periods. In tropical climates, the dominant factor is rainfall. The start and end of the rainy season influence the different phases of the life cycle. Parasitism is reduced during the dry months and increases sharply within days following the first major winter rainfall. The population remains stable for a few weeks, and then slowly diminishes. At the end of the rainy season, there is a marked decrease, with progressive fall to almost zero in the dry season. Tick distribution therefore corresponds to the isohyets. In these cases, the cold and dry seasons impose pattern on tick development that can be observed in parasitism in large mammals. The cycle of seasons determines the alternation of appearance, reduction, and disappearance of ticks. These variations in tick populationsrepresent the frequency or seasonal dynamics of a species, or its phenology (seasonal pattern of appearance) (Fourie *et al.,* 1996).

iv. *Vegetation*

The plant cover as a whole is not an inert intermediate factor between climatic phenomena and the fauna, since it is not in depressant of these factors. It is the result of the adaptation of a particular flora to the temperature, rainfall, and wind patterns prevailing in an area with particular geological and pedological characteristics. In turn, vegetation is also related to temperature and rainfall. Its distribution and feature in

given latitude and altitude represent equilibrium. It is a response to external conditions that creates variety of microclimates at different levels and physical support to the fauna (Glen and Pete, 1969). Vegetation is not only the result of various elements that make up the environment, but it also determines, by its composition, the various microclimates at different levels. It is the best ecological integrator, which influences the biological phenomena seen at a given point. A comparison of the distribution of tick species with the features of the vegetation in a natural zone is very useful. It is of practical use for determining the distribution of a given species, with all the consequences concerning the epidemiology of diseases caused or transmitted by ticks and the possibilities of controlling them (Morel, 1989).

III. Epidemiology and Distribution of Hard Ticks

a) Epidemiology

The distribution of ticks in a temperate climate with frequent and non-seasonal rainfall is closely linked with availability of a micro-environment with a high relative humidity such as occurs in the mat which forms under the surface of rough grazing .In contrast, in tropical grazing areas the grass cover on pasture is discontinuous and often interspersed with bare or eroded patches. Where suitable grass cover does exist it has been generally accepted. Since temperatures are suitable for development throughout a large part of governed by rainfall, and with the exception of *Hyalommaspp*., a mean annual rainfall of more than 60cm is required for survival. However, recent studies in East Africa have shown that the factors underlying the maintenance of the necessary microclimate with a high relative humidity are rather more complex and depend on the transpiration of plant leaves .As long as this continuous adequate humidity is maintained in the microclimate despite the dryness of the ambient temperature. However, when the rate of evaporation increases beyond a certain level, the stomata on the leaves close, transpiration ceases and low humidity created in the microclimate rapidly becomes lethal to the ticks. In the field of course, the stability of the microclimate is dependent on factors such as the quantity of herbage or plant debris and the grass species .The various genera of ticks have different threshold of temperature and humidity within which they are active and feed and their distribution is governed these threshold. Generally, ticks are most active during warm season provided there is sufficient rainfall, but in some species the larval and nymphal stages are also active in milder weather and this affects the duration and timing of control programmes (Urquhart *et al*., 1996).

In recent years, it is common to see increasing of the geographic coverage as well as the incidence and prevalence of TBDs affecting domestic animals and humans has increasing. many important zoonotic TBDs, such as anaplasmosis, babesiosis, ehrlichiosis, and Lyme borreliosis are increasingly gaining more attention from physicians and veterinarians worldwide. With the help and timely instruments developed techniques such as molecular biology, it can be easy to isolate new species, strains, or genetic variants of microorganisms present in ticks worldwide (Pacheco *et al*., 2011; Duh, *et al*., 2010) (Table 1). Some ofthe list of potential tick-borne pathogens continues to increase (Shapiro *et al*., 2010; Pritt et al., 2011; Silva et al., 2011; Subramanian et al., 2012).

Agents, such as *Rickettsia slovaca*, *Rickettsia parkeri*, and *Rickettsia massiliae*, were identified in ticks, decades before these were associated with human diseases (Paddock, 2009). Many other tick-borne pathogens, including *flaviviruses* (e.g., Omsk hemorrhagic fever virus, *Powassan encephalitis virus*, and Kyasanur forest disease virus) have been implicated in human disease in new geographical regions (Dobler,2010; Piesman, and Eisen, 2009) (Table 1).

Table 1 : Distribution of Ticks and TBDs

Diseases	Pathogens	Tick species/vectors	Geographic distribution	Reported in
Mediterranean spotted feve	Rickettsia conorii	Rhipicephalussanguineus,R. turanicus	Africa, Asia, Europe	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008; Matsumoto et al. , 2005)
Lyme borreliosis	Borrelia burgdorferi sensu lato	Ixodes hexagonus,I. pacificus, I. persulcatus, I. ricinus, I. scapularis	Asia, Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Human monocytic ehrlichiosis	Ehrlichia chaffeensis	Amblyomma americanum	North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
African tick bite fever	Rickettsia africae	Amblyomma hebraeum, A. variegatum	Africa, West Indies	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Human granulocytic anaplasmosis	Anaplasma phagocytophilum	Haemaphysalis concinna,H. punctata, Ixodes ricinus, I. pacificus,I. scapularis,Rhipicephalus bursa	Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Q fever	Coxiella burnetii	Many species of differentgenera	Africa, Asia, Australia, Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Relapsing fever	Borrelia spp.	Ornithodoros spp.	Africa, Asia, Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008; Cutler, 2010)
Rocky Mountain spotted fever	Rickettsia rickettsii	Amblyommaamericanum,A. aureolatum,A. cajennense,Dermacentor andersoni,D. variabilis,R. sanguineus	North, South and Central America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008; Labruna, 2009; Breitschwerdt et al.,2011
Tularemia	Francisella tularensis	Many species of differentgenera	Asia, Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008; Milutinovic et al., 2008; Petersen et al., 2009; Gyuranecz et al., 2011)
Babesiosis	Babesia divergens, B. microti	Ixodes ricinus, I. scapularis	Europe, North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Colorado tick fever	Coltivirus	Dermacentor andersoni	Western North America	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Crimean–Congo hemorrhagic fever	Naiovirus	Amblyomma variegatum,H. punctata, Hyalomma anatolicum,H. marginatum,H. truncatum, R. bursa	Africa, Asia, Europe	(Jongejan and Uilenberg, 2004; Piesman and Eisen; 2008)
Kyasanur forest disease [1,5]	Flavivirus	Haemaphysalis spinigera,H. turturis	Indian subcontinent	
Louping ill	Flavivirus	Ixodes ricinus	Western Europe	(Labuda. and Nuttall,2004)
Omsk hemorrhagic fever	Flavivirus	Dermacentor marginatus,D. reticulatus,I. persulcatus	Asia	(Piesman and Eisen; 2008)
Tick-borne encephalitis	Flavivirus	Ixodes persulcatus,I. ricinus, H. concinna,H. punctata	Asia, Europe	(Piesman and Eisen; 2008; Labuda. and Nuttall, 2004)
Powassan encephalitis	Flavivirus	Dermacentor andersoni,Haemaphysalislongicornis, I. cookei,I. scapularis	Asia, North America	Dobler, 2010; Labuda. and Nuttall, 2004)

IV. Pathogenic Effects of Ticks

a) Cytolysis effects

The primary attachment lesion causes cytolysis following production of the hyaline sheath. There is itching accompanied by a tissue and humoral reaction of the host, with hyperemia, eosinophil infiltration and a local edematous reaction. The damaged tissues are pulled by the weight of the feeding tick and this produces a sensation of pain (Morrisan, 1989).

The salivary glands of ticks perform numerous vital functions. They secrete cement that anchors the

mouthparts to the skin soon after the tick attaches to the host. The salivary glands are the major organs of osmoregulation and possess a water vapor uptake mechanism that enables ticks to fast for many months. During feeding the salivary glands of the female secrete excess fluid from the blood meal back in to the host's circulation, thus concentrating the nutrients components of the meal and regulating hemolymph volume and ionic composition. These factors lead to the reaction of the host. The primary challenge or infestation: when ticks attach to a host (primary infestation), they secrete cement and other antigenic materials. In the guinea pig, 40-60 % of the leucocytes infiltrating the lesion are neutrophil, up to the third day of feeding. A day or two later when most of the larvae have engorged the proportions of basophils and eosinophils have increased significantly. Early in tick attachment, the capillaries near the mouthparts become dilated and edema results. This is probably due to vasoactive substances, in tick saliva such as prostaglandins, and to a few deregulated mast cells, which release histamine. Hemorrhage is an obvious characteristic of the feeding lesion. Finally, the langerhans cells of the epidermis trap antigenic material from tick saliva and present it to lymphocytes in the skin and lymph nodes for processing.

(a) Tick attaches to host .Secretes antigens tissue damage Antigens taken up release of mediators Langerhans cell in skin. Antigens presented to lymphocytes inflammation T-lymphocyte activation mild degranulation of mast cells and basophils by slavery B-lymphocyte activation (assumed) gland esterasesIgGIgE Complement erythema and edema activation (primary immune response) (inflammatory response) Host reaction to tick challenge by primary infestation (Morrisan, 1989).

(b) Reactions to a primary infestation. Other than a mild inflammatory response leading to erythema and edema, there are few obvious clinical reactions. The specific immune response involves the activation of T-and B- lymphocytes.

Secondary challenge or infestations: During secondary infestation, the granulocytes in the peripheral blood rise to much higher levels than during the primary infestation and basophils rapidly invade the feeding lesion, degranulate and liberate vasoactive mediators. The later cause edema and might contribute to the formation of blister-like epidermal vesicles underneath the attached ticks. Histamine 5-HT inhibit feeding and salivation, the histamine may induce detachment from the host. Premature detachment and much reduced engorged weights may result and those ticks that do not detach feed very slowly, if at all, and most die *in situ*. As a result of the laceration of blood vessels by the probing actions of tick mouthparts, the host's circulatory homeostatic mechanisms begin to act and in response to these, ticks release salivary secretions to maintain blood flow to the feeding lesion. However the salivary composition of a particular species may be a partial determinate of effective host range. Thus, ticks that posses antihistamine salivary activity probably feed successfully on basophile-rich hosts (cattle, guinea-pigs, rabbits) but may be rejected more effectively by mice, which rely on bradykinin and anaphylatoxins as vasoactive mediators (Bianchi *et al.,* 2003).

After detachment of the tick the necrotic lesion remains indurate, itching, and hot, and can discharge for several months. Bacterial complications may set in with abscess formation. The hypostome may break and remain in the lesion when a tick is extracted. In receptive cattle, the tick remains in place, and lesion is formed with eosinophil infiltration around the necrotic patch near the chelicerae. The allergic reaction is sign of host resistance (Tatchell, 1969).

b) Cell reaction

The lysis cavity opens into internal tip of the sheath, and is formed of amorphous necrotic tissue, with cutaneous and blood cell debris. An edematous zone surrounds the lysis area when the cell structures gradually disappeared collagen remains intact and extravasations and vascular ruptures occur, with hemorrhagic patches. In receptive hosts, the main phenomenon is neutrophil infiltration around the attachment point and capillaries. The resulting inflammation produces vasodilatation, vascular ruptures, and hemorrhages near lysis cavity, while the surrounding dermis becomes fibrous due to the multiplication of fibroblasts. The other cells degenerate at the same time. The dermis and epidermis become edematous. Vesicles and necrotic zones appear at the end of the attachment process, in the presence of eosinophils and basophile. In the receptive host, the main phenomena are vasodilatation and vessels rupture (Allen, 1994). In resistant hosts, tissue reactions are more violent and occur earlier. They are dominated at first by considerable edema of the epidermis and dermis, accompanied by eosinophil and basophile infiltrations from the second day of attachment. Cell decomposition and tissue necrosis are rapid and extensive, but there are not many capillary ruptures. The vesicles appear very early and develop in to pustules. Although the tick saliva has a limited lysis effect, it causes inflammation through degranulation and high antigenic activity. The ingested blood is concentrated by excretion of water and mineral salts starting from the beginning of the meal. Highly engorged species therefore ingest about three times the volume of blood at the end of the meal (Uspenskiy, 1982).

c) Toxic effects (tick toxicosis)

Apart from the mechanical cytolysis effect and blood loss, ticks have a specific pathogenic effect due to the presence of toxins in the saliva. The toxins affect not only the attachment site but also the entire organs of

the host. The toxins act on certain tissues. Neurotropic toxins induce tick paralysis, while dermotropic toxins cause sweating sickness (Tatchell, 1969; Allen, 1979).

V. Control Measures for Ticks

a) Steps of a controlling campaign

The aim of a tick control campaign is not to control all ticks simultaneously, but a definite species because of its particular role. The strategy should therefore be based on the biological characteristics of the target species. Moreover, there is no perfect control method. The efficacy of these methods depends on rational and methodical use (FAO, 1977; Jongejan and Ulenberg, 1994).

Factors to be defined when a campaign is planned against ticks: -

i. *Campaign objectives*

The objectives must be defined on the basis of the biology of the species and epidemiology of the disease caused or transmitted by the particular tick species. Temporary or regular deparasitizing of infested animals is a short-or medium-term measure. It provides relief for the host, but does not affect ticks in pastures. Reduction of tick populations in a pasture has long-term prophylactic effects as it decreases parasitism and frequency of pathogen inoculation to a tolerable level. Premunitionis established or maintained by regular treatments on fixed dates or occasional treatments when the parasitism rate exceeds a certain level. Eradication of ticks has a long-term prophylactic effect and controls transmission of a particularly dangerous pathogen (Kaaya, 2003).

ii. *Target location*

A site is selected for the campaign from the different successive microhabitats of a tick in its free-living phases, and during the parasite phases on the host. The selection is based on ecological data. The control operations are carried out in the field and on the host. In the field, the objective is to attack ticks in their microhabitat during the free-living phases by chemical or ecological measures. On the host, the aim is either to rid it directly of its parasites, or indirectly by means of hosts that collect ticks and serve as bait. This enables long-term deparasitizing of pastures, which is the final goal (Okello*et al*., 2003).

iii. *Campaign schedule*

Information on dynamics is useful for determining the pattern of host treatments and suspension of treatments during seasons of inactivity, on the basis of the life cycle and duration of the parasite phase during seasons of tick activity. In methods requiring removal of livestock, data on survival possibilities of the various instars without feeding will determine how long cattle should be excluded from the pastures. The period required for completion of the cycle should be considered, in the case of species with certain instars that develop on hosts other than ungulates or on wild vertebrates for a part of the tick population (Jongejan and Ulenberg, 1994).

b) Types of tick control

Tick biology data are fundamental factors in chemical control and represent method in biological control. The two control methods differ only in the use of acaricides. Procedures directly affecting the microhabitat and host availability such as using hyperparasites and predators, and immunological control form a part of an integrated biological control program. The practical importance of these methods varies. Some are effective on their own, but it is important to combine them. The use of acaricides is inconceivable without data on the natural environment of ticks and their hosts (Kemp, 1994).

i. *Chemical control*

Arsenic was the first compound to be widely used for tick control but due to problems of toxicity, lack of residual effect and resistance it was largely replaced by the organochlorines in the late 1940s. Increased environmental contamination and consumer resistance to unacceptable levels of organochlorines in meat together with onset of tick resistance to this group of insecticides led to their replacement in the 1960s by several organophosphorus compounds, the carbamates, butocarbonl, more recently the formamidine, amitraz, and some synthetic pyrethroids. Ivermectin or closantel given by the parental route have also been shown to be useful aid in the control against the one-host tick *Rhipicaphalus (Boophilus)*(Urquhart *et al.,* 1996) (Table 2).

Table 2 : Some of the chemicals used for tick control

Group name	Chemical name
Organophosphates	Bromocyclene
	Coumaphos
	Crotoxyphost dichloruos
	Diazinon
	Dichloruos
	Malathion
	Phoxin
	Propoxur
	Stirofos
	Trichlofon (Metrifonot)
Carbamates	Carbaryl
Chlorinated Hydrocarbons	Lindone
	Methoxychlor
Pyrethrins and Pyrethroids	Flumethrin
	Fenualerate
	Permethrin
	Pyrethrin
Avermectin	Ivermectin

Source: Kaufmann (1996).

Avermectins

Avermectins are products of the fungus *Actinomyces*species that are divided into two major components A and B depending on the structures. Avermectins compound is active against intestinal parasites, lungworms, warble flies, lice, midges and different genera of ticks. The active substance inhibits the transmission of stimuli between the interneurons of the ventral nerves and the motor neurons of the parasites. The compound is deposited for a short time in the liver, fat and only in small amounts in muscle and kidney. About50% is excreted unchanged with the faces. Therefore, a withdrawal time of 38 days is required for milk and beef. Because of this, it also does not make sense to utilize the compound in tropical animal production (Seifert, 1996).

ii. *Ecological control*

Information on the ecology of different instars is used for habitat and host linked treatments. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks. Vegetation is periodically removed by burning, but spontaneous or induced fires have little direct effect on ticks since they occur in the season when adults are not active. Annual dry-season fires are widespread in semiarid regions; the value of these fires is very controversial, as they influence not only the availability of an important source of grazing during long harsh dry seasons, but may also diminish the abundance of ticks and vermin such as rats. The influence of burns on tick abundance varies markedly with the time of year, intensity of burn, and the tick species present (Wilkinson, 1979; Baars, 1999).

Replacement of natural vegetation, cropping, and soil cultivation are integrated methods that enable pasture improvement and tick eradication. Using acaricides, careful plastering of walls and ceilings, and installing concrete floors sanitize localized habitats such as stables, sheds, poultry houses, and kennels and the soil of cattle enclosures, markets, areas around wells, etc.

Wild ungulates and carnivores, as well as other possible hosts of the different instars should be eliminated. Since wild ungulates are alternative hosts and can maintain tick populations as effectively as cattle, their removal is the most effective measure. The objective of briefly or periodically withdrawing domestic hosts is to cause the ticks to disappear through inanition since the only available hosts are cattle. Pasture rotation can be used exclusively, or together with the use of acaricides for this purpose. The rotation pattern and pasture area should be determined according to the target species, type to cycle, and hosts of the successive instars, as well as the period of stay and number of cattle. Other important factors are the period required for inanition by each instars, periods of tick activity depending on the seasons and type of climate (McCosker, 1979).

iii. *Biological control*

Entomopathogens are group of organisms that attack ticks and insects. Entomopathogens can be macro- or microorganisms that affect arthropods. In biological tick control the activities of the hyperparasites *chalcid*flies *Hunterellus*are probably important in nature, but they are difficult to evaluate. It is still more difficult to manipulate or reproduce them for practical use. Predators are most effective, especially ants and birds (*Buphagus sp.* Or*oxpeckers*, *Crotophagus*, various magpies, village fowl). Depending on the conditions, these predators can consume a large number of ticks (Samish and Alekseev, 2001).

iv. *Genetic control*

In resistant animals, the blood level of histamine rises considerably within 48 hours of tick attachment. In moderately resistant cattle, there is only a slight increase in the histamine level when larvae attach; it is higher for nymphs, and even higher for females. In receptive animals, there is no change in the histamine level. Antigens extracted from eggs or larvae give the same results in resistant animals. The reaction intensity is not correlated, however, to the degree of resistance (Willadsen, 1999). Resistance to ticks in animals occurs early but is effective mainly in adults. This resistance can be broken by the occurrence of physiological or pathological disorders. The diseases induced or transmitted by ticks can also reduce resistance and are accompanied by an increase in the animal's quantitative tolerance threshold for ticks, which may lead to sever parasitism, with clinical signs (Willadsen and Jongejan, 1999).

The ability is a hereditary character. The ability to resist ticks is acquired, but it develops according to genetic factors. If it is possible to breed cattle for tick resistance, as is done for certain other characteristics, it will provide a solution allowing the costly and dangerous use of acaricides to be abandoned, with the related risk of the emergence of non-sensitive tick strains. The question remains whether breeding for tick resistance can be compatible with breeding for a particular production characteristic (meat, milk)(Brown and Askenas, 1982).

c) *Immunization (anti-tick vaccine)*

Research in to controlling cattle tick *Rhipicaphalusmicroplus(formerly known as Boophilusmicroplus)* has focused on developing vaccines; cattle vaccinated with antigens from the midgut of female ticks were protected from challenge with *Rh. microplus*(Opdebeeck*et al.,* 1988; Willadsen*et al.,* 1988; https://en.wikipedia.org/wiki/Rhipicephalus_microplus).

Ingestion of the blood meal by ticks feeding on vaccinated cattle leads to uptake of antibodies and other components of the host's humoral immune system, resulting in damage to the gut. As a result, the number of ticks engorging, their average weight and their ability to lay eggs may all be adversely affected. Australian tick control project develop a vaccine against *B. microplus.* It was commenced in 1981 with the observation of the cattle, vaccinated with crude or partially purified material from semi engorged adult female ticks, could be effectively protected against tick infestation. The major antigen responsible for this effect, called Bm86, was isolated 4 years later. The antigen is located on the surface of the digest cells, which line the tick's gut. The feeding tick takes in antibody to Bm86. Binding of antibody to the protein on the tick gut leads eventually to lysis of the tick's gut cells. The number of ticks engorging on vaccinated cattle is reduced tick weight and even stronger reduction in the ability of the female ticks to lay eggs. In fact, there is considerable mortality of engorged ticks in the first days following engorgement. The antigen has been sequenced and expressed in *Escherichia coli* or *Aspergillusnidulans* (Willadsen *et al.,* 1989; Tellam *et al.,* 2002).

This recombinant tick vaccine was registered for commercial sale in Australia in 1994, called "Tick Guard ® ". With this vaccine over 18000 cattle had been vaccinated in northern Australia. In a variety of tests of efficacy and safety it was shown that the vaccine was completely safe for use, effective in a variety of different geographical locations and effective against a wide range of ticks that were resistant to the different classes of chemical acaricide. It was found that there were some differences in the vaccine susceptibility of different isolates of tick for reasons, which are not understood. It was further shown that a range of different cattle breeds responded to the vaccine in equivalent ways, as measured by antibody responses following vaccination (Willadsen, 1997).

VI. Conclusions and Recommendations

In Ixodid ticks, there are only two moults following larval hatching: larvae to nymph and nymph to adult, both of which usually occur on the ground. However, in the 2-host cycle, larvae moult to nymphs on the host. Adult Ixodid females usually mate and complete engorgement on the host and then detach and drop to the ground prior to ovipositing. Depending on temperature and relative humidity, oviposition usually begins a few days later and continues for several days .The female dies soon after wards although males remain attached to the host for longer periods and may mate with other females. The number of females attached to the host is thus the most significant indicator of the seasonal activity and population dynamics of these ticks.

Designing an economical, integrated tick control strategy for a particular production system in a specific area are chemical control, ecological control biological control, genetic control, immunization.

Therefore based on the above conclusion the following recommendations are forwarded:

- Strategic tick control based on the biology, ecology aspect should be conducted
- Other safe control methods such as immunization control with other must be implemented
- Identification of tick species must be carried out
- Detailed epidemiology of ticks should be studied
- Movement of animals should be restricted

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FELLOWS

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4. Manuscript's Category,

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TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

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

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

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.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

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.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

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

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the 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):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

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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