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Highlights

Sensitivity to Biotic Stresses

Factors of Biological Variability

Discovering Thoughts, Inventing Future

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Epigenetic Factors of Biological Variability and Individual Sensitivity to Biotic Stresses

By Kravets A. P. & Sokolova D. A.

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Abstract- The variability of a wide variety of characteristics, including sensitivity to biotic and abiotic environmental factors, is one of the fundamental properties of living things. This study is a continuation of the investigation of the effect of epigenetic differences on the individual resistance of plants to abiotic and biotic stresses. The aim was to investigate the connection between epigenetic polymorphism of two wheat varieties and different sensitivity to fungal infection. Diverse DNA methylation patterns of seed subpopulations with various germination times used as a marker of epigenetic polymorphism. Showed that fast-grown seed subpopulation (Podolyanka variety) which characterized with a higher level of intravariety epigenetic polymorphism, had slow development and lowered final level of fungal infection compared to slow-grown seed subpopulation. The effect observed in plants of this variety for crops of different years. Favoritka variety was characterized with a lower level of intravariety epigenetic polymorphism and shown unstable results in fungal infection of both fast- and slow-grown seed subpopulations. The data obtained indicate the importance of epigenetic mechanisms in determining the individual sensitivity of plants to phytopatogens and the role of epigenetic polymorphism as a factor contributing to the biological diversity of crops.

Keywords: *DNA methylation, epigenetic polymorphism, phytopatogens, plant immunity, biodiversity, crop, mitigation competition factors in mono-crops.*

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The issue about connection intravariety differences in epigenomes with plant individual nonspecific resistance and the role of intravariety epigenetic polymorphism in the biodiversity of monocultures is discussed.

Keywords: DNA methylation, epigenetic polymorphism, phytopatogens, plant immunity, biodiversity, crop, mitigation competition factors in mono-crops.

I. Introduction

Variability of most diverse characteristics – time and dimensional parameters, sensitivity to both biotic and abiotic habitat factors is one of the fundamental properties of living things. A key focus of modern plant breeding is the study of the genetic nature of traits that are important for productivity, their polymorphism, and the use of these data in genetic engineering [2]. A significant limitation of this approach is due to the final result's unpredictability because of environmental factors influence gene expression realized with various epigenetic mechanisms.

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Up-to-date biology, along with the concept of genetic polymorphism, has a concept of epigenetic polymorphism, which implies the existence of a variety of phenotypes while maintaining the unity of the genotype. Epigenetic diversity, like different genetic ensembles expression could be one of the scantily explored biological variability factors. One of the striking phenomena is a phenomenon of asynchronous germination of any seeds sample of the same species, variety, and harvest. The mechanisms defining this phenomenon are still not completely clear. It is significant from a practical point of view since affecting the seeding time and yield of cultivated plants and also complicating weed control, causing significant material costs. Noted for both wild and cultivated plants the level of asynchronous germination is dependent on climatic and environmental conditions. Thus it is controlled not only by genes (genetic factors) but also by environmental factors and points to epigenetic mechanisms participate in the determination of germination time.

A random sample of seeds of the same species, variety and harvest, is a simple and convenient experimental model to investigate the connection of both variabilities of phenotypic characteristic (germination time) and epigenetic polymorphism with sensitiveness to various environmental factors.

The vast majority of researchers explain asynchronous germination via various seeds ripening – differences in the "physiological age" from the same plant up to the time of harvesting due to different pollination terms.

Previously, using DNA methylation patterns as a marker of epigenetic varieties it was shown that seedlings from seeds with other germination time had different DNA methylation pattern. Then was shown that variability of epigenetic patterns is associated not only with maturation and position on some "epigenetic trajectory" but also with diverse "epigenetic trajectories" – in other words, metabolic pathways along which this maturation has taken place [16]. Study of response to UV-C exposure shown that seedlings with different epigenomes and germination time had various sensitivity and formation of adaptive reaction [21].

The work is a continuation of studying plant epigenetic status effect on sensitivity to environmental factors and the formation of protective reactions with sensitivity to phytopatogens.

Crop contamination with phytopatogens is one of key problems both agriculture and food security [20]. Food contamination with spores of pathogenic and conditionally pathogenic fungi is a significant threat as a source of substances with genotoxic effects [8, 20,22,24]. Today grain contamination is studied from different sides because of a lot of various factors while the harvest is formed and stored lead to formation of both qualitative and quantitative characteristics of grain [8, 25]. Up to date, research are focused on several streams. It includes both assessment environmental factors effecting on plant immunity [4,8-11,19,22,25] and mechanisms determining constitutive or inducible way [12,13,17,18,22,] to protect and recognise plant pathogen [12,17,18]. Assessment of epigenetic factors effecting on contamination in one or another degree is a component of listed research areas.

II. Material and Method

The paper provides data obtained on two winter wheat varieties Podolyanka and Favoritka (the originators of both are Institute of plant genetics and physiology NAS of Ukraine and Myronivsky). According to our previous research, the wheat has different ecological plasticity – requirements for sowing, fertilizer application terms, and predecessor [15]. Analyzing different DNA methylation patterns of wheat seedlings with extreme - minimum and maximum germination using the parameter of "epigenetic distance" (D) by Nei. Indicated that plant varieties with increased ecological plasticity characterized with longer epigenetic distance thus wider epigenetic sifference. For example, Podolyanka variety in various experiments shown the parameter 0,1-0,3. The same index for Favoritka one is 0,01-0,056.

DNA methylation patterns from "fast-grown" sample compared with "slow-grown" one. DNA methylation polymorphism of two groups of seedlings was quantified with parameter D – "epigenetic distance" calculated by Nei [15,16].

Experiment repetition is four-time. There were used seeds harvested in 2013-2017 and stored in the refrigerator under +4 -5° C. Experiments were carried out during June-August. Seeds germinated on wet filter paper, ten seeds per Petri dish (to avoid their contact and cross contamination) in thermostat under +23 - +24 °C. As previously studying differences in DNA methylation patterns was carried out on extreme by germination time subpopulations of seedlings.

After 12-hour seed swelling and germination "fast-grown" group was selected, after 24 hours – "slow-grown" one. Then the groups were collected into different Petri dishes. thirty seeds taken per one variant. To determine the type of fungi contamination, we used both microscopy and taking photo of growing seeds with the subsequent zoom the image via a computer. Indicated that the most type of fungi contamination was *Mucor Fresen* – saprophyte belonging to *Zygomycetes*.

The DNA extraction carried out using reagent set NeoPrep DNA (Neogene, Ukraine).

PCR conducted in amplification "Tercik" ("DNA-technologies, Moskow"). Two types of primers used: RAPD-P6 (GAG-CAA-GTT-CAG-CCT-GG) and ISSR 5'-$(AC)_8$C-3'. Oligonucleotides were synthesized by "Metabion" (Germany). To carry out PCR was used reagent set GenPak® PCR Core – ready to use the dry mix for DNA amplification. Reaction mix for ISSR-PCR (20 μl) contained: 1 u Taq-polymerase, 10 μl buffer, 2,5 mM $MgCl_2$, 200 mM each dNTP, 0,1 mM primer, 200 ng total genome DNA, 6,4 μl deionized water. The mix covered with 20 μl vaseline oil. Amplification stages: prior denaturation 5 min under 94°C, then 40 cycles: denaturation under 94°C - 45 sec, annealing temperature 52°C - 45 sec, elongation under 72°C - 90 sec; final elongation 7 min under 72°C [17, 18].

DNA restriction following amplification was conducted in amplification "Tercik" (DNA-technology, Moskow) as well. Two restriction enzymes were used: MspI (5'-CC*CGC-3') and HpaII (5'-C*CGC-3') (Fermentas, Germany). Restriction reaction (25 μl) contained: 0,6 u MspI or 0,2 u HpaII, 2 μl 10xBuffer Tango, 500 ng total genome DNA, 17,1 μl deionized water. The mix covered with 20 μl vaseline oil. Reaction conditions: 16 hours under 37°C, stop reaction - 20 min under 65°C (for HpaII) and 20 min under 80°C (for MspI).

Obtained products of restriction and PCR were separated in 1,0% agarose gel in TBE-buffer with ethidium bromide and visualized in UV-transilluminator. The same volume of PCR products (5 μl) put into gel slots. As a molecular weight marker, GeneRuler 50 bp DNA Ladder (Fermentas, Germany) with fragments length 1000, 750, 500, 250, and 50 bp were used.

III. Results and Discussion

There is diverse initial level, dynamics of development and final level of infection in the subpopulation of seedlings with different germination time from Podolyanka and Favoritka varieties harvested in different years (Fig. 1a-c, 2a-c).

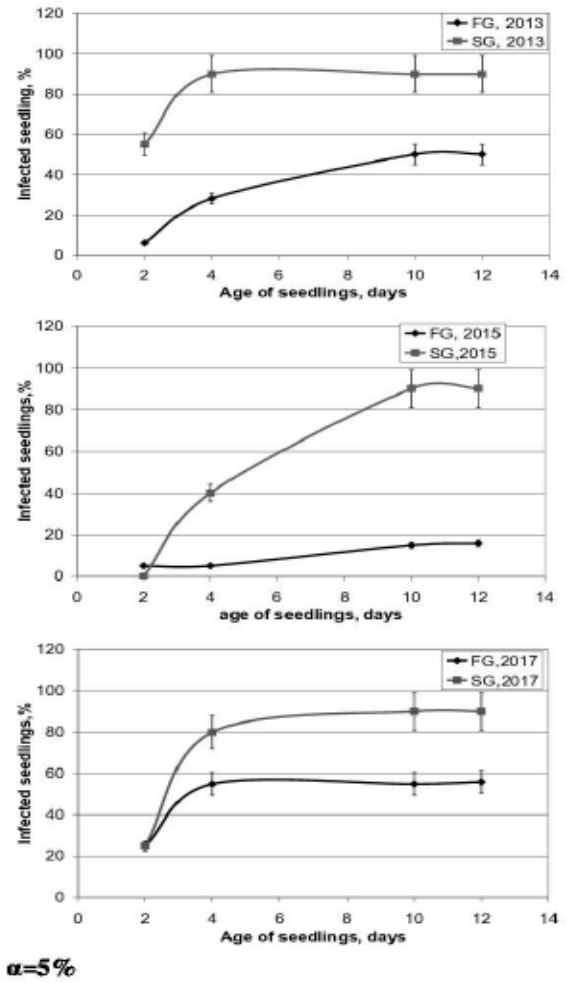

Fig. 1: The dynamics of infection of quickly (FG) and slowly (SG) germinating seedlings of wheat varieties Podolyanka crops of different years.

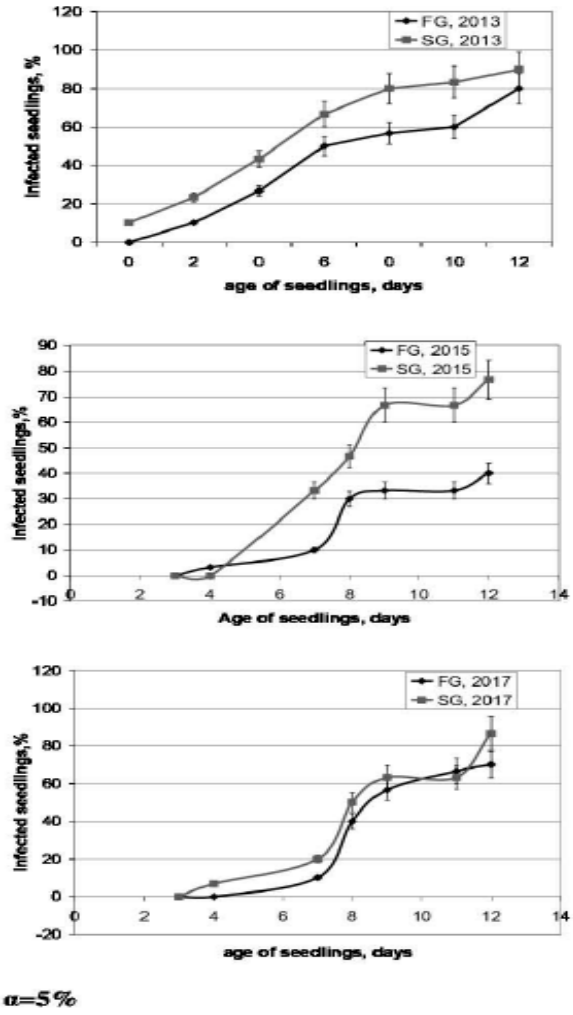

Fig. 2: The dynamics of infection of quickly and slowly germinating seedlings of wheat varieties Favoritka crops of different years.

All the parameters for Podolyanka variety are the same from year to year; in other words, they are stable and therefore less dependent on the weather conditions of the year. Less stable parameters are there for Favoritka variety, but plants from seeds harvested in 2017 have not shown any differences through infection indexes. The phenomena might be considered as the different immune status of plants from other varieties and subpopulations through the same one according to its germination time.

Proceed to the analysis of DNA methylation two wheat varieties belong to subpopulations fast- (FG) and slow-grown (SG) seedlings.

Electroforegram of extracted DNA (Podolyanka variety) shows its nativity that leads carrying out restriction analysis following PCR (Fig. 3 a, b, c). Electroforegrams of P6 and ISSR amplification indicate no polymorphism through these genome elements (Fig.3 b, c).

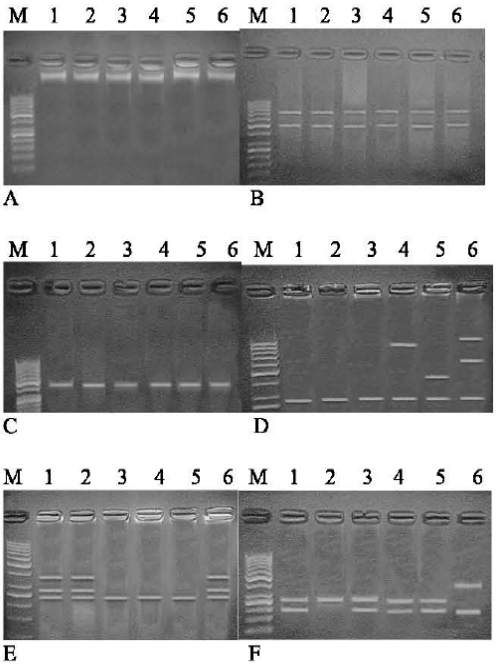

Fig. 3: Variety Podolyanka, harvests of 2013, 2015 and 2017. A. The electroforegram of isolated DNA quality control. B. ISSR-amplification of native DNA. B. P6 amplification of native DNA. G. P6 - amplification of HpaII restricts. D. ISSR - HpaII restriction amplifications. E. ISSR MspI restriction amplifications. **M**- molecular-weight marker GeneRuler 50 bp;1 – FG, 2013; 2 – FG, 2015, 3 – FG, 2017; 4 – SG, 2013; 5 – SG, 2015; 6 – SG, 2017.

Electroforegrams of HpaII restricts P6-amplification show the polymorphism of DNA methylation pattern through FG-SG seedlings (2013 - 2017 years) due to low-molecular-weight amplicons in SG-subpopulation. There is maintaining of DNA methylation pattern through FG seedlings over the years. All variants have low-molecular-weight amplicon 240 bp (Fig. 3 d) that may indicate existing some DNA sequences not conversing into *de novo* mode while forming immune reactions.

Electroforegrams of HpaII restricts ISSR-amplification show polymorphism (2013-2017 years) through both FG-SG seedlings and within each subpopulation with different germination time according to harvest time. As with the variation of methylation patterns in HpaII restricts P6-amplification (Fig. 3 e) there is low-molecular-weight amplicon 250 bp as well.

Electroforegrams of MspI restricts ISSR-amplification also indicate DNA methylation pattern polymorphism between both FG and SG groups of each year and within each subpopulation with different germination time due to the harvest time. There is a match in DNA methylation patterns between SG-subpopulation from 2013 and 2017 and between SG-seedlings from 2013 and 2015 year of harvest. There is observed no mutual amplicon for all variants(Fig. 3 f).

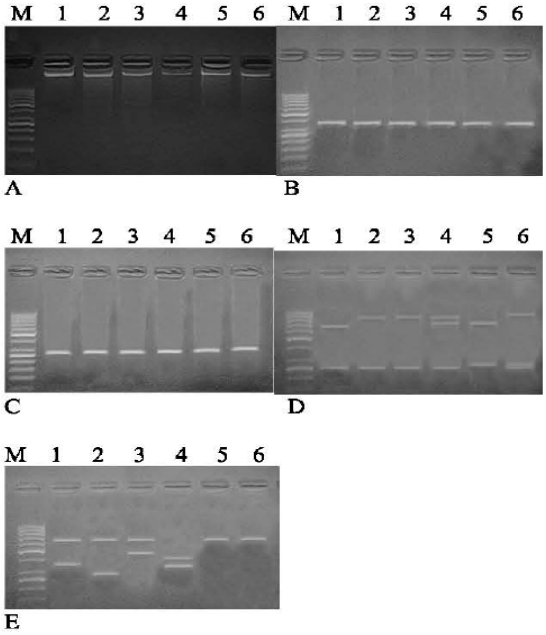

Fig. 4: Variety Favoritka, harvests of 2013, 2015 and 2017. A. Control of the origin of the isolated DNA. B. Electroforegram of native DNA ISSR-amplification. C. Electroforegram of native DNA P6 amplification. D. Electroforegram of HpaII restricts ISSR amplification. E. Electroforegram of MspI restricts ISSR amplification. **M**– molecular-weight marker GeneRuler 50 bp; 1 – FG, 2013; 2 – FG, 2015; 3 – FG, 2017; 4 – SG, 2013; 5 – SG, 2015; 6 – SG, 2017.

Electroforegram of extracted DNA (Favoritka variety) shows its nativity that leads carrying out methylation digest analysis (Fig. 4 a). Electroforegrams of P6 and ISSR amplification indicate no polymorphism through these genome elements (Fig.4 b, c).

Electroforegrams of HpaII restricts ISSR-amplification show polymorphism of DNA methylation pattern both between FG-SG seedlings and within each subpopulation with different germination time due to harvest year. Moreover there is low-molecular-weight amplicon 250 bp in all variants (Fig. 4 d).

Electroforegrams of MspI restricts ISSR-amplification show polymorphism of DNA methylation pattern (Favoritka variety) both between FG-SG seedlings and within with different germination time due to harvest year.

Visualizing infection development for FG- and SG-seedlings, we can conclude that higher steadiness to disease is for FG group. This effect is more stable for Podolyanka variety. At the same time, DNA methylation patterns changed in seedlings FG and SG for both Podolyanka and Favoritka in connection with the year of harvest. Thus it is no connection between phytopatogenes resistance and the specific structure of DNA methylation patterns. However epigenetic distance assessment indicates a general trend for the varieties: while varying DNA methylation pattern by years the

epigenetic distance for Podolyanka variety increasing the same parameter for Favoritka (Table 1).

Problem of individual disease resistance was the subject of research for a long time. I. V. Michurin indicated different stability of cotton seed bolls from the top of the main stem to phytopatogens. Found that the position of cobs on the plant and seeds in the corn cob had immunological significance. These phenomena are associated with varying degrees of seed maturation at the time of harvest [19].

Table 1: Epigenetic distance changing for Podolyanka and Favoritka varieties by years

Variety	D, Epigenetic distance by years		
	2013	2015	2017
Podolyanka	0,23	0,28	0,12
Favoritka	0,02	0,051	0,024

The results suggest a connection between plant individual resistance and DNA methylation characteristics, i.e., some epigenetic features. Assessment of factors determining population epigenetic polymorphism in genetically homogeneous plants [20] indicates its determination not only by other grain maturation degree and factors affecting process but also acting earlier on different growth and development stages of parent organism including gametogenesis. Switching methylation into *de novo* mode is dependent on conditions of plant development. DNA methylation pattern formed to the ripening time of seed from new harvest brings information about the parental organism.

Known that rearrangement of methylated cytosine influence chromatin conformation. Thus the connection between DNA methylation pattern changes and individual plant radiosensitivity admits at least a partial explanation of the fact with different protection of both DNA and chromatin conformation [17]. Correlation between the level of epigenetic polymorphism variety and its unspecific ecological plasticity points into the importance of methylation as a factor of epigenetic regulation affecting various pattern of gene expression and metabolism [18].

Results in the paper also are confirming the role of DNA methylation as an epigenetic regulation factor leading to forming various patterns of gene expression within the genetically homogenous plant material.

The revealed relationship between resistance to phytopatogens and polymorphism of the DNA methylation pattern as a marker of epigenetic and metabolic differences does not indicate possible immune mechanisms that affect the process. Known that plants have both active inducible and passive constitutive (or structural) ways related to morphology and biochemical compound of biological structures [12,17,18] including shell grains. Development just saprophytic infection in grain surface and then in seedling root indirectly indicates epigenetic differences in structure of passive immunity type.

The obtained data indicate the importance of epigenetic mechanisms in determining the individual sensitivity of plants to phytopatogens and the role of epigenetic polymorphism as a factor contributing to the biological diversity of crops.

The issue about factors affecting crops biodiversity is significant. Charles Darwin (Darwin, 1876) was the first to note that the maximum biomass of the annual plant crop observed at their maximum species diversity [6, 23] per unit area. Then the observation formed the main concept of ecological niche. Up to date, it means all biotic and abiotic factors conditioning the existence of different species [1]. There is weakened competition for the resources with possible cooperative interactions among organisms with diverse ecological niches– that could explain the effect revealed by Darwin.

The concepts of life strategies are also widely used in the ecology of populations, one of the biological functions of which is to weak the interspecific competitive relations. At the same time, natural mechanisms reducing intraspecific competition level are not considered. The concept of a variety of life strategies does not apply to the population of cultivated plants that have undergone a lengthy breeding process and grown under artificial conditions of mono sowing. Competitive relationship as a one-species population is partially weakened with exogenous factors, first of all, agrotechnical effects (fertilizing, watering, pests' treatment). However, natural mechanisms decreasing intraspecific competition through organisms taking the same ecological niche are of significant interest.

One of the reasons could be genetic polymorphism appearing in various degrees in any population or planting crops. However, according to recent data, this parameter is relatively low and considerably below the epigenetic polymorphism index [26].

The data suppose that epigenetic polymorphism is efficient and evolutionarily fixed factor decreasing intraspecific competition, including relationship within mono sowing. It confirms with it widespread through various genetically homogeneous biological systems: from populations of wild [28] and cultivated plants [29] to differentiated animal tissues. The prevalence of this phenomenon indicates that the general mechanism increasing the functioning of biological communities any organization level aimed at reducing competitive relations and possibly strengthening cooperative ones.

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No Conflict of Interest

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List of Birds Sigthed on Livestock Farms in Sucre, Colombia

By Sampedro, A. & Camargo, K.

University of Sucre

Abstract- The rural areas of the Department of Sucre, Colombia, have a considerable bird species richness, but there are not many reports on this. In this work, we classify and record the species we sighted in nine cattle farms in the region. We obtained information on the relative abundance of each species (frequency of observation about the total sample), and biotopes where we observe them. We use chi square to know if there was an association between these parameters and also about to the food guild to which the species belong. We obtained the latter in the specialized bibliography. We use the Sorensen Index, to determine the degree of similarity of the communities of species in each biotope. We spot 103 species of birds. The best-represented order was Passeriformes, with eleven families and 36 species. Most of the species were abundant, and the biotope with the highest sighting was the open area, although many species were present in more than one biotope. We found no association between the abundance of species and the biotope in which we observed them, nor about the food guild. Among the species sighted, 21 are migratory, mostly wintering with permanent breeding populations. In this paper we discuss the importance of livestock farms for wildlife conservation.

Keywords: birds, species richness, livestock farms, relative abundance.

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Keywords: birds, species richness, livestock farms, relative abundance.

I. Introduction

One of the causes of decreased biological diversity is habitat loss[1, 2]. Livestock and agriculture are considered a productive sector that causes a high environmental impact due to the necessary clearing, pollution, and soil compaction. According to surveys carried out by the National Administrative Department of Statistics in 2016, in 25 departments of Colombia, 80% of the area is dedicated to livestock, 7.6% to agricultural activity, and only 10.1% to forests. In the department of Sucre, cattle raising is the first line of the economy and 73,204 ha (6.9% of the department) are used for these purposes, while forests cover only 73,204 ha (1.02%). In this case, not only the clearing causes habitat loss, but wrong practices throughout the sector [3], and this does not allow the expected productivity [4]. Environmentalists try to reduce that impact through laws and other mechanisms.

It would be impossible to ensure that forests and other ecosystems transformed for use in livestock, agriculture, and other purposes can recovered for the restoration of biological diversity. Researchers are looking for alternatives that allow a balance between the conservation of biological diversity and agricultural production, both in Colombia [5] and in other countries, such as the Doñana National Park, in southern Spain [6].

Many livestock farms in Sucre have fragments of tropical dry forest, secondary vegetation, wells or jagüeyes, live fences, and pastures with numerous trees, which constitute a silvopastoral system[7]. This could contribute to the increase of biodiversity in these sites.

The investigations carried out on these aspects in the region refer to the birds sighted in artificial water reservoirs that the peasants build, called jagüeyes, to meet the needs of the livestock, small crops, and domestic chores. Several authors have highlighted the importance of jagüeyes for the maintenance of biological diversity[7, 8, 9]. Live fences are also important, especially for birds[10].

This paper aims to expand the information related to the birds present in livestock farms and highlights the possibility that these be considered protected areas, due to their importance for the conservation of biological diversity.

II. Materials and Methods

The places where we do the work are part of the ecosystem that prevailed in ancient times in Sucre. That is, they constituted a typical ecosystem of the Caribbean coastal plain, an Alternate Hydrotropic Zonobioma, also called Dry Tropical Forest (11). In Colombia they appear from the south of La Guajira to Córdoba, San Andres Island, Providencia and Santa Catalina, the canyon of the middle valley of the Cauca River and the high valley of the Magdalena River, as well as in some sectors of Cesar, north of Santander, Antioquia, Valle and Boyacá. They are forests with a prolonged period of drought, which coincides with the astronomical winter of the Northern hemisphere. Plants have water deficits, and most of the canopy woodland loses its foliage.

We carried out the work between November 2017 and the first half of March 2018, the dry season in Sucre.

The livestock farms considered were nine (Table 1), and in all of them the silvopastoral system is developed [4], with the presence of grasses, legumes, jagüeyes (artificial water wells), live fences, trees in the pastures and surroundings the homes of the peasants, as well as fragments of tropical dry forest with a greater or lesser degree of anthropic intervention.

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Table 1: The geographical location of livestock farms in the present work. FA: farm area

Name	Coordinates	FA(ha)
La Camorra	9°29'1.77''N; 75°25'11.67"O	240
Los Charcos	9°29'16.39"N; 75°28'5.72"O	260
La Loma	9°29'1.17"N; 75°27'50.65"O	120
El Vivero	9°28'33.78"N; 75°28'2.71"O	80
Versalles	9°29'50.98"N; 75°26'18.66"O	150
San José	9°27'19.76"N; 75°28'46.32"O	280
Doña Lucy	9°14'31.18"N; 75°23'59.68"O	180
Puerto Alto	9°14'41.48"N; 75°23'57.84"O	280
Villa Carmela	9°14'46.34"N; 75°24'08.26"O	120

The farms were georeferenced with a Garmin GPS (Fig. 1). We visited six farms in the municipality of Sincelejo, near the brick factory El Cinco and the others in the municipality of Toluviejo, near the Argos S. A. plant. There we register birds associated with different types of vegetation and bodies of water.

Source: Google Earth, free version.

Fig. 1: Geographical location of livestock farms where we carry out this investigation. Above, in the vicinity of the Argos S. A. Plant, municipality of Sincelejo; below, municipality of Toluviejo, near the brick factory El Cinco.

Three samplings were carried out on each farm, at times between 0600 and 1000 and between 1600 and 1800. Three professionals participate in the sampling and use the fast ecological method [12, 13]. Species identification was "in situ" and when this was not possible, using specialized bibliography [14, 15, 16] and expert consultations, using photos (Canon EOS Redel XS and a 70 - 300 mm lens) and descriptions. The food guild (FG) to which the birds belong and the migratory species (M), were also consulted in the scientific bibliography [14, 17].

We record the birds sighted, in the following biotopes: open areas (Oa: pastures with trees and live fences), water reservoir (Aca: jagüeyes, puddles, lagoons and vegetation of the riverbank), and forests (F: fragments of the tropical dry forest). The sightings were made using Tasco binoculars (10 x 50 mm).

We perform the data processing, joining the information obtained in the different biotopes of all farms, since they have the same characteristics of the livestock farm.

We constructed the species accumulation curve to establish the sampling efficiency (18), and used the ACE Chao 1, Chao 2 and Bootstrap (19, 20) estimators, based on presence-absence. We combine the results of the different samples, so that an accumulation curve of a single species appears.

We measured the relative abundance (RA) of birds as the frequency of sighting over the total sampling in all farms, and the proportions thus obtained allowed us to establish the following categories for different species: rare (R) (<69%), frequent (C) (70-89%) and abundant (A) (+ 90%).

We made a Contingency Table (X2) to determine if there is an association between the abundance of the species and the biotope in which we see them and also with the type of food they consume. We use the Sorensen Index (21) to make a matrix of similarity between the species present in the different biotopes in which we observe them.

III. Results

The birds we spot on the nine farms belong to 19 orders, 30 families, and 103 species (Table 2 and Fig. 2). The best-represented order is Passeriformes with eleven families (57.9%) and 36 species (34.9%), followed by Pelecaniformes with three families (15.8%) and twelve species (11.6%) and Psittaciformes, one family and eight species. The rest of the orders appear with two or one families and between one and seven species.

The best-represented families are Ardeidae (Pelecaniformes) with nine species, Thraupidae and Tyrannidae (Passeriformes), both with eight species, as well as Psittacidae (Psittaciformes).

Table 2: Bird species observed in nine livestock farms in the department of Sucre between November 2017 and March 2018. RA: relative abundance (R: rare; C: frequent; A: abundant); FG: food guild F: Frugivorous; G: granivore; N: nectarivore; Fi: folivorous; I: insectivore; C: carnivore; P:piscivorous; Sc: scavenger; M: microphage; O: omnivorous); Bi: Biotope: Open areas (Oa: pastures with trees and living fences), water reservoir (Aca: jagüeyes, puddles, lagoons and vegetation of the riverbank) and forests (F: fragments of tropical dry forest)

Order	Family	Species	RA	FG	Bi
ACCIPITRIFORMES	Occipitridae	*Busarellusnigricollis*	A	P, I, M	Aca, Oa, F
		Rupornismagnirostris	A	P, C, I, M	Oa, F
		Elanusleucurus	R	C	Oa
		Buteogallusanthracinus	R	M, C, Sc	F, Aca
		Buteogallusmeridionalis	R	C,I	Oa
		Rostrhamussociabilis	A	M, C	Aca, Oa
	Pandionidae	*Pandionhaliaetus*	R	P	Aca, Oa
CATHARTIFORMES	Cathartidae	*Cathartes aura*	A	Sc, C	F, Oa
		Coragypsatratus	A	Sc, C	Oa
FALCONIFORMES	Falconidae	*Falco rufigularis*	R	C	F, Oa
		Falco sparverius	C	C, I	Oa
		Herpetotherescachinnans	R	C	F, Oa
		Milvago chimachima	A	O	Oa, Aca
		Caracaracheriway	R	Sc, C, M	Oa
ANSERIFORMES	Anatidae	*Anasdiscors*	C	G. M	Aca
		Dendrocygnaautumnalis	R	Fi, G, M	Aca
		Dendrocygna bicolor	C	Fi, G, M	Aca
APODIFORMES	Trochilidae	*Amaziliatzacatl*	A	N, I	Oa
		Anthracothoraxnigricollis	C	I	F, Oa
CAPRIMULGIFORMES	Caprimulgidae	*Nyctidromusalbicollis*	C	I, M	F, Oa
CHARADRIIFORMES	Charadriidae	*Vanelluschilensis*	A	I, M	Oa
	Jacanidae	*Jacana jacana*	A	I,M	Aca
	Scolopacidae	*Actitismacularius*	C	I, M, P	Oa, Aca
	Recurvirostridae	*Himantopusmexicanus*	C	M, P	Aca
COLUMBIFORMES	Columbidae	*Patagioenascayennensis*	A	G	F, Oa
		Columbina minuta	A	G	Oa, F

		Columbina talpacoti	A	G	Oa
		Leptotilaverreauxi	C	G	F, Oa
CORACIIFORMES	Alcedinidae	*Megaceryletorquata*	A	P, M, C	Aca
		Chloroceryle amazona	A	P	Aca
	Momotidae	*Momotussubrufescens*	C	I	Oa, F
CUCULIFORMES	Cuculidae	*Crotophagaani*	A	O	Oa
		Crotophagamajor	A	O	Oa, F
		Tapera naevia	C	I	Oa
GALLIFORMES	Cracidae	*Ortalis garrula*	A	Fi, F	F, Oa
	Odontophoridae	*Colinuscristatus*	C	O	Oa, F
GRUIFORMES	Aramidae	*Aramus guarauna*	R	M, C	Oa, Aca
	Rallidae	*Aramidescajaneus*	R	M	F, Oa
		Porphyriomartinica	C	O	Aca
PASSERIFORMES	Corvidae	*Cyanocoraxaffinis*	A	M	F, Oa
	Dendrocolaptidae	*Dendroplexpicus*	R	I	F, Oa
	Thamnophilidae	*Sakesphoruscanadensis*	R	I, M	Aa
	Furnariidae	*Furnariusleucopus*	A	I, G	Oa, Aca
	Hirundinidae	*Progne tapera*	A	I	Oa, Aca
		Progne chalybea	A	I	Oa, Aca
	Icteridae	*Chrysomusicterocephalus*	C	I, G	Oa, Aca
		Icterusnigrogularis	A	I, F, N	Oa, F
		Molothrusbonariensis	A	I, G	Oa
		Quiscalusmexicanus	C	O	Aca, Oa
		Psarocoliusdecumanus	A	F,N, I	Oa, F
	Mimidae	*Mimusgilvus*	C	O	Oa
	Parulidae	*Mniotilta varia*	C	I, M	Oa, F
		Protonotaria citrea	R	I, M	Oa, Aca
		Oreothlypis peregrina	R	I	Oa
		Setophagaruticilla	C	I	F, Oa
	Thraupidae	*Nemosiapileata*	R	O	Oa
		Ramphocelusdimidiatus	C	I, F	Oa, F
		Thraupisepiscopus	A	F, I, M	Oa
		Thraupispalmarum	A	F, I	F, Oa
		Sicalisflaveola	C	G, I	Oa
		Volatiniajacarina	A	G, I	Oa
		Sporophila intermedia	A	G, M	Oa
		Saltatorcoerulescens	A	F, Fi, G, I	Oa, F
	Troglodytidae	*Campylorhynchusgriseus*	A	I	Oa
		Campylorhynchuszonatus	A	I	Oa
		Donacobiusatricapilla	A	I	Aca, Oa
		Troglodytesaedon	A	I, M	Oa
	Tyrannidae	*Arundinicolaleucocephala*	A	I	Aca
		Elaeniaflavogaster	A	I, F	F, Oa
		Machetornisrixosa	A	I, M	Oa
		Megarynchuspitangua	C	O	F, Oa, Aca
		Pitangussulphuratus	A	O	Oa
		Tyrannusmelancholicus	A	O	Oa
		Tyrannussavana	A	I, F	Oa
		Fluvicola pica	A	I	Aca
PELECANIFORMES	Ardeidae	*Ardeacocoi*	C	P, M	Aca
		Ardeaherodias	R	P, M	Aca
		Ardea alba	C	P, M	Aca
		Bubulcus ibis	A	I, M, C	Oa, Aca
		Butoridesstriata	C	P, M, C	Aca
		Egrettacaerulea	C	P, M, C	Aca
		Egrettathula	C	P,M, C	Aca

		Nycticoraxnycticorax	C	O	Aca
		Tigrisomalineatum	C	P,I,C	Aca, F
	Phalacrocoracidae	*Phalacrocoraxbrasilianus*	A	P, I, C	Aca
	Threskiornithidae	*Phimosusinfuscatus*	A	O	Aca
		Plegadisfalcinellus	R	I, P, M, C	Aca
PICIFORMES	Ramphastidae	*Ramphastossulfuratus*	C	F, I, C	Oa, F
	Picidae	*Campephilusmelanoleucos*	A	I, F	F, Oa
		Colaptespunctigula	A	I	Oa
		Dryocopuslineatus	R	I, G, N. F	F, Oa
		Melanerpesrubricapillus	A	I, F	F, Oa
GALBULIFORMES	Galbulidae	*Galbularuficauda*	A	I	F, Oa
PODICIPEDIFORMES	Podicipedidae	*Podilymbuspodiceps*	C	P, I, C	Aca
PSITTACIFORMES	Psittacidae	*Amazona amazonica*	A	F, G	Oa, F
		Amazona ochrocephala	A	F, I, G	Oa, F
		Ara ararauna	A	G, Fi, F	Oa, F
		Ara macao	A	G, N, Fi, F	F, Oa
		Ara severus	C	G, Fi	F, Oa
		Eupsittulapertinax	C	G, F, Fi	Oa, F
		Brotogerisjugularis	A	F, G, Fi	Oa, F
		Pionusmenstruus	A	F, G, N	F, Oa
STRIGIFORMES	Strigidae	*Bubo virginianus*	R	C, P, M	F, Oa

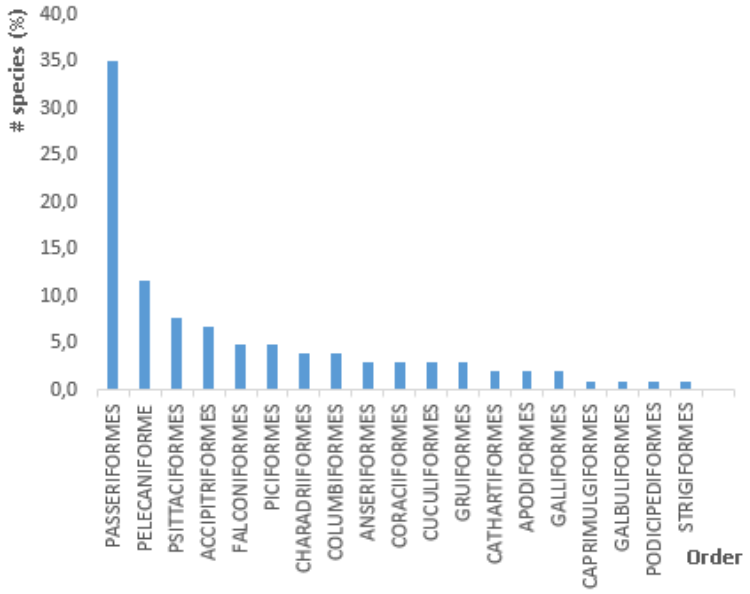

Fig. 2: Bird species (%) distributed in 19 orders, observed in 9 livestock farms in the department of Sucre.

The species accumulation curve showed that the average sampling efficiency was 85%, obtaining representativeness above 83% (Fig. 3).

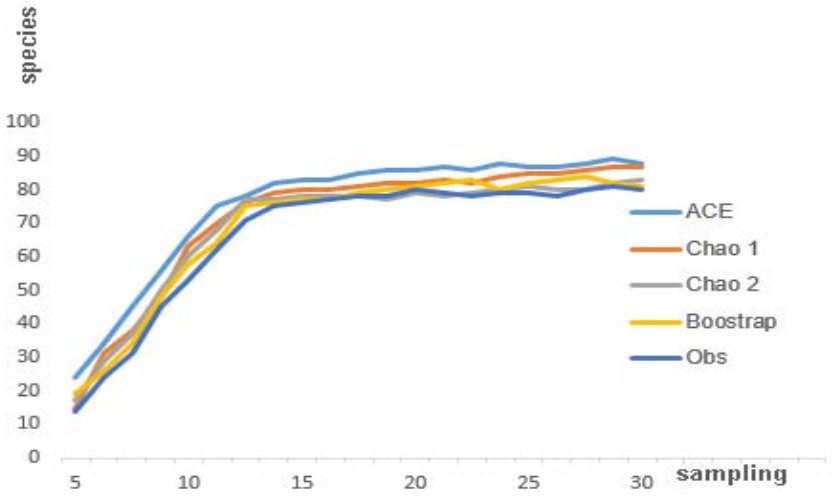

Fig. 3: Accumulation curve of bird species sighted in 27 samples taken in nine cattle farms in Sucre, Colombia

Most of the species observed during the work were abundant (51.4%) and frequent (30.1%), while the rare ones were 18.4%.

In open areas, 80 species (77.7%) were detected, 40 in the forested area (38.8%), and 38 in water reservoir (36.9%). The bird communities sighted in the three biotopes do not show similarity (Table 3).

Table 3: Similarity matrix (Sorensen index) among bird communities sighted in three biotopes of nine farms in the department of Sucre. Oa: open areas; F: forest; Aca: water reservoir.

	Oa	F	Aca
Oa	-	59%	24%
F	-	-	2,5%
Aca	-	-	-

Some species were sighted in only one of those biotopes, and others were detected in more than one. We observe some species in only one of these biotopes, and others detect them in more than one. The highest proportion of species in the different biotopes (Fig. 4) were seen in both open and forested areas (Oa-F) (38.8%), followed by those observed only in open areas (27.2%) and in water reservoirs (20.4%). We did not find species that appeared only in the wooded area.

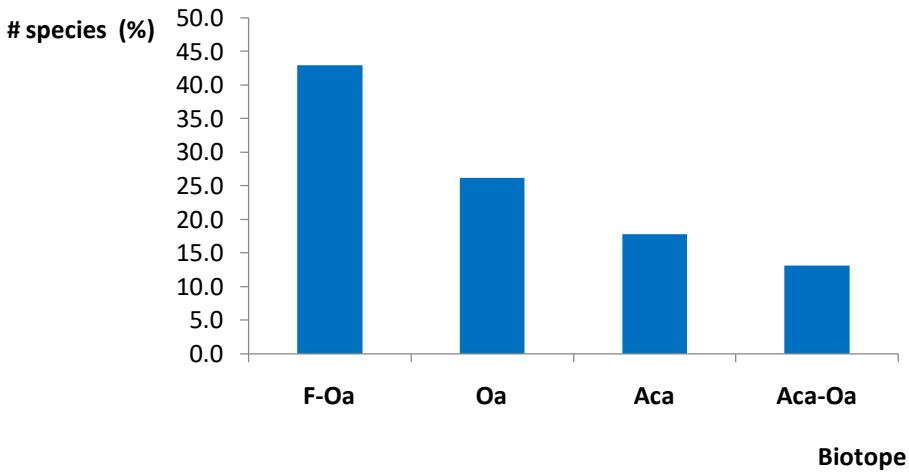

Fig. 4: The proportion of bird species in the different biotopes during the work period, in the nine cattle farms of Sucre, Colombia. Oa: open areas; Aca: water reservoir, F: forest.

We observe the same proportion of abundance (A, C and R) in the different biotopes ($X2 = 5.95$; $p > 0.01$; 8 df).

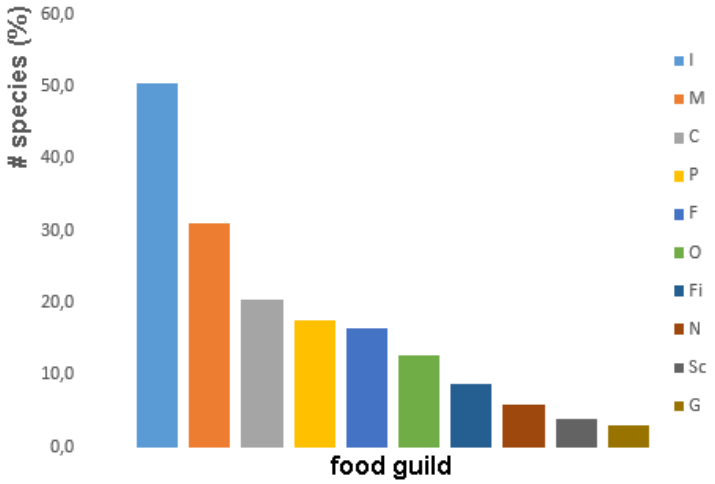

Fig. 5: Type of feeding strategies of birds sighted in 27 samplings in nine farms in Sucre, Colombia. F: Frugivorous; G: granivore; N: nectarivore; Fi: folivorous; I: insectivore; C: carnivore; P: piscivorous; Sc: scavenger; M: microphage; O: omnivorous)

In Fig. 5, we show the type of feeding used by the species observed in the nine farms. Insectivores appeared in greater proportion, followed by the rest of those who consume other types of animal food, while vegetarians generally appear in a smaller quantity. We also found that 30.1% of the species observed in this research could eat both plant and animal foods, although 21% of them are not considered omnivorous.

The type of feeding is not associated with the degree of abundance of the species in the case of those who eat food of animal origin ($X^2 = 15.1$; $p < 0.05$; 8 df), nor for those who eat food from plant origin ($X^2 = 5.1$; $p < 0.05$; 6 df).

As shown in Table 4, the migratory species are 2, and most of them are wintering species with permanent reproductive populations (PRP) (57.2%). In this case, the best-represented family is Ardeidae with six species. Non-reproductive wintering (NR) and local migratory (LM) represent 33.3% and 9.5%, respectively.

Table 4: Migratory species (M) [17] observed in the present investigation in nine farms of the department of Sucre. NR: non-reproductive winters; permanent reproductive wintering; LM: local migratory species.

Order	Family	Species	M
ACCIPITRIFORMES	Pandionidae	*Pandionhaliaetus*	NR
CATHARTIFORMES	Cathartidae	*Cathartes aura*	NR
ANSERIFORMES	Anatidae	*Anasdiscors*	PRP
		Dendrocygnaautumnalis	LM
CHARADRIIFORMES	Scolopacidae	*Actitismacularius*	NR
	Recurvirostridae	*Himantopusmexicanus*	PRP
GRUIFORMES	Rallidae	*Porphyriomartinicus*	LM
PASSERIFORMES	Hirundinidae	*Progne tapera*	NR
	Parulidae	*Mniotilta varia*	NR
		Protonotaria citrea	PRP
		Leiothlypisperegrina	NR
		Setophagaruticilla	NR
	Tyrannidae	*Tyrannusmelancholicus*	PRP
		Tyrannussavana	PRP
PELECANIFORMES	Ardeidae	*Ardeaherodias*	PRP
		Ardea alba	PRP
		Bubulcus ibis	PRP
		Egrettacaerulea	PRP
		Egrettathula	PRP
		Nycticoraxnycticorax	PRP
	Threskiornithidae	*Plegadisfalcinellus*	NR

Almost half of these species (47.6%) are frequent in the locations studied, 28.6% are rare, and 23.8% are abundant. Only the ducks (*Anasdiscors* and *Dendrocygnaautumnalis*) ingest vegetables, which they can complement with small organisms. The rest of the migratory species consume mainly food of animal origin (insects, fish, invertebrates, small vertebrates, and carrion). We observe migratory birds in water reservoirs (71.4%) and open areas (52.4%), but in most cases, we spot them in more than one of the sampled biotopes (Fig. 6).

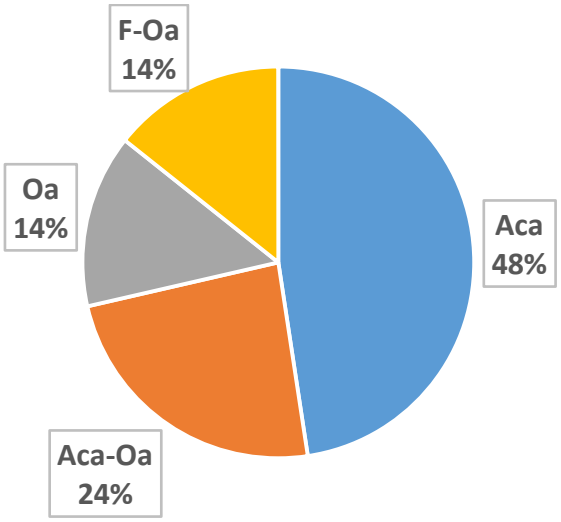

Fig. 6: The number of species (%) of migratory birds observed in different biotopes. (Aca: water reservoir; Aca-Oa: water reservoir and open areas; Oa: open areas; F-Oa: forest and open areas).

IV. Discussion

The species richness found in this work constitutes approximately 43.1%, of that reported for the Caribbean region [14, 15, 16], which gives value to these livestock farms as conservation areas. Sampling did not reach the highest values in number of species, possibly because we don't use fog nets and a significant quantity of species, especially of the Passeriformes order, which presents abundant groups of small size, are difficult to Detected by direct observation and may not have been sighted during sampling. The presence in the work area of migratory species increases the significance of these farms as possible areas for conservation.

The importance of grasslands, living fences, lagoons, jagüeyes, and urban areas for the conservation of biological diversity appears in several investigations [7, 9, 10, 22]. This research confirms that these anthropic ecosystems can serve as buffer zones and complementary to protected or reserve areas for local or migratory wildlife [23].

The little similarity between the different sampled biotopes is logical if one takes into account that birds use different types of resources according to their morphological, physiologica, and behavioral adaptations [24]. Most importantly, the species richness observed constitutes an indicator of the diversity of resources and possibilities of access to them that exist in these localities.

The passerines, which are the most diverse and abundant group[25], are the best represented in this work. Tyranids are the family with the highest number of species, possibly due to the varied food resources they can use and their possibilities to colonize different environments [14, 26, 27].

The absence of moisture in the region during the work period could explain that most of the birds sighted were insectivorous, followed by other consumers of food of animal origin and vegetarians, that in general, appear in a lesser proportion. However, we corroborate the same number of abundant, frequent and rare species, regardless of their preferred type of food resource.

None of the bird species detected are in any category of threat [28]; however, illegal hunting has increased, constituting an important item for the economy of this region, because of the use of wildlife for pet food and trade [29, 30].

Given the degradation suffered by forested areas in the department, a measure that could contribute to the protection of biological diversity could be to establish regional or local protected areas on farms that have the conditions for it, which has been suggested before for wildlife conservation in this region [31, 32].

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Effect of Fluoride on the Reproductive Output of *Drosophila Melanogaster*

By Vanshika Singh, Roushni Chowdhary, Sanjana Shah & Shahla Yasmin

Abstract- *Drosophila melanogaster* is popularly used to study the effect of toxicity of chemicals. Exposure to fluoride may affect the reproductive potential of animals including humans. Therefore, a study was conducted to assess the effect of sub-lethal concentration of Sodium Fluoride (NaF) on the reproductive output of *Drosophila melanogaster*. It was found that 1.0 parts per million (ppm) of NaF was lethal for the adult flies and there was significant fall in the number of 3rd instar larvae, pupae and ecloded flies in different sublethal concentrations of NaF (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm). The study concluded that NaF (commonly used in pesticides), can cause developmental alterations in non-target insects like *Drosophila melanogaster*, thereby suggesting its role in developmental toxicity.

Keywords: *drosophila melanogaster, sodium fluoride, developmental toxicity, reproductive output.*

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Effect of Fluoride on the Reproductive Output of *Drosophila Melanogaster*

Vanshika Singh [α], Roushni Chowdhary [σ], Sanjana Shah [ρ] & Shahla Yasmin [ω]

Abstract- ***Drosophila melanogaster*** **is popularly used to study the effect of toxicity of chemicals. Exposure to fluoride may affect the reproductive potential of animals including humans. Therefore, a study was conducted to assess the effect of sub-lethal concentration of Sodium Fluoride (NaF) on the reproductive output of *Drosophila melanogaster*. It was found that 1.0 parts per million (ppm) of NaF was lethal for the adult flies and there was significant fall in the number of 3rd instar larvae, pupae and ecloded flies in different sublethal concentrations of NaF (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm). The study concluded that NaF (commonly used in pesticides), can cause developmental alterations in non-target insects like *Drosophila melanogaster*, thereby suggesting its role in developmental toxicity.**

Keywords: drosophila melanogaster, sodium fluoride, developmental toxicity, reproductive output.

I. Introduction

Drosophila melanogaster is popularly used as a model to study toxic potential of any chemical (Jatav et al., 2011), due to its easy maintenance in the laboratory and presence of about 50% homology with mammalian proteins (Pomerai et al., 2008). Many studies have been conducted using *Drosophila melanogaster* in laboratory conditions to reveal well defined effects of various insecticides and pesticides on the life cycle, hatchability and emergence of the fly (Nazir et al., 2001; Nazir et al.,2003, Gupta et al., 2005; Das et al., Podder 2010). There are four different stages in the life cycle of *Drosophila melanogaster*, i.e. egg, larva, pupa and adult. The eggs hatch into first instar larvae which moult twice into second and third instar larvae. Third instar larvae pupate and finally metamorphose into adult flies.

It has already been reported that fluoride containing chemicals like cryolite and NaF can cause alterations in the compound eye morphology and developmental stages in *Drosophila melanogaster* (Podder et al., 2012; Dutta et al., 2014). In the recent years, several investigations demonstrated that fluoride can induce oxidative stress and modulate intracellular redox homeostasis, lipid peroxidation and protein carbonyl content, as well as alter gene expression and cause apoptosis (Barbier et al., 2010).

Exposure to fluoride present in pesticides may affect the population of non-target organisms. Adverse effects of fluoride on fertility, fecundity and reproduction has been reported in several insects (Gerdes et al., 1971; Gong and Wu, 1991) and in *Drosophila melanogaster* (Khatun et al 2017). According to Freni (1994), lower birth rate in humans may be linked to intake of fluoride rich groundwater. The present study was undertaken to document the effect of sub lethal dose of NaF on the reproductive output of *Drosophila melanogaster*.

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II. Methods

Drosophila melanogaster were cultured in standard cornmeal medium.

Two sets of cultured bottles were kept in triplicates: 1) Control , and 2) NaF treated.

1) Control set: Flies were cultured in normal cornmeal medium
2) NaF treated set: Flies were cultured in cornmeal medium in which NaF was mixed in different concentration i.e. 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm, and 1.0ppm.

Four adults of the same age group (two males and two females obtained from single line stock culture) were added into each bottle and left undisturbed so that flies of next generation could emerge from the pupae. These flies were counted. This denoted the reproductive output of the initially added flies. 3rd instar larvae and pupae were also counted to find out which developmental stage was most affected.

The statistical analysis of the count data was performed using Analysis of Variance (ANOVA). Because normality of data is a prerequisite for ANOVA, the count data were log transformed to ensure normal distribution.

III. Results and Discussion

In the present study, the life cycle of *Drosophila melanogaster* was completed in nine days (at temperature ~ 28°C). AL-Saffar et al. (1995) found that development time steadily declined for *D. melanogaster* as temperature was raised from 15°C to 30°C. Maximum development rates have historically been observed between 30°C and 28°C (Davidson 1944, Ashburner and Thompson 1978). The generation time is roughly 10 days from fertilized egg to eclosed adult at 25° C (Fernández-Moreno et al., 2007).

Flies could not survive in medium with 1.00 ppm of NaF (Table 1, 2 and 3). Exposure of *Drosophila melanogaster* to sub-lethal doses (0.2, 0.4, 0.6 and 0.8 ppm) of NaF did not affect the duration of life cycle. However, Podder and Roy (2013) observed a distinct delay in emergence of flies in different concentrations of cryolite (sodium aluminum fluoride) as compared to control. Another study also demonstrated developmental delay in *Drosophila melanogaster* after chronic exposure to NaF (Dutta et al., 2014).

There was significant reduction in the number of 3rd instar larvae and pupae in different concentration of NaF as compared to control ($F=36.29$, $P<0.05$) as shown in Tables 1 and 2. There was also significant reduction in the number of flies of the next generation in different concentrations of NaF as compared to control ($F = 42.33$, $P <0.05$). Greatest reduction in reproductive output was seen in fly culture with 0.8 ppm of NaF in the cornmeal medium (Table 3). Decreased fecundity has been reported in *Bombyx mori* and *D. melanogaster* following exposure to environmental fluoride (Gerdes et al., 1971; Chen, 2003a; Chen, 2003b; Khatun et al 2017).

There was no change in number when third instar larvae changed into pupae in the medium containing NaF, suggesting sub-lethal concentration of NaF did not affect the growth phase. This may be due to the reason that ingestion of NaF with food during the larval life might have activated the drug-metabolizing enzymes. Drug-metabolizing enzymes have been reported in *Drosophila melanogaster* by Pai (1983).

Further, fluoride induces oxidative stress in fluoride-intoxicated animals through generation of Reactive Oxygen Species (ROS) and lipid peroxidation (Chlubek, 2003). A significant depression was seen in the number of ecloded flies from the pupae. This might be because NaF could have caused the reduction of oxidative phosphorylation and Adenosine triphosphate (ATP) synthesis. The pupae require energy for morphogenesis and organogenesis. Reduction in the ability of ATP synthesis might have interfered with metamorphosis. NaF might also have interfered with hormones required for metamorphosis.

Table 1: Number of 3rd instar larvae of *Drosophila melanogaster* at the end of 4th day of exposure to different concentrations of NaF

NaF Concentration							
Experimental set number	Initial number of flies	Control	0.2ppm	0.4ppm	0.6ppm	0.8ppm	1.0ppm
1	0.60(4)	1.60(40)	1.30(20)	1.25(18)	1(10)	0.77(6)	All flies died
2	0.60(4)	1.51(33)	1.20(16)	1.11(13)	1(10)	0.69(5)	All flies died
3	0.60(4)	1.41(26)	1.20(16)	1.17(15)	1.11(13)	0.90(8)	All flies died
Mean±SE		1.5±0.05	1.2±0.03	1.17±0.04	1.03±0.03	0.8±0.04	

Values are mean±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses .

Table 2: Number of pupae of *Drosophila melanogaster* at the end of 5th day of exposure to different concentrations of NaF

NaF Concentration							
Experimental set number	Initial number of flies	Control	0.2ppm	0.4ppm	0.6ppm	0.8ppm	1.0ppm
1	0.60(4)	1.60(40)	1.30(20)	1.25(18)	1(10)	0.77(6)	All flies died
2	0.60(4)	1.51(33)	1.20(16)	1.11(13)	1(10)	0.69(5)	All flies died
3	0.60(4)	1.41(26)	1.20(16)	1.17(15)	1.11(13)	0.90(8)	All flies died
Mean±SE		1.5±0.05	1.2±0.03	1.17±0.04	1.03±0.03	0.8±0.04	

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

Table 3: Reproductive output of NaF treated flies after nine days of exposure to different concentration of NaF

NaF Concentration							
Experimental set number	Initial number of flies	Control	0.2ppm	0.4ppm	0.6ppm	0.8ppm	1.0ppm
1	0.60(4)	1.6 (40)	1.25(18)	1.17(15)	1.04(11)	0.69(5)	All flies died
2	0.60(4)	1.49 (31)	1.17(15)	1.07(12)	0.90(08)	0.47(3)	All flies died
3	0.60(4)	1.54(35)	1.20(16)	1.14(14)	1.04(11)	0.77(6)	All flies died
Mean±SE		1.55±0.03	1.2± 0.02	1.13±0.03	0.99±0.05	0.64±0.09	

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

IV. Conclusions

The present study concluded that NaF, which is a regularly used in toothpaste, insecticides and in water fluoridation program, can cause developmental alterations in non-target insects like *Drosophila melanogaster*, hereby suggesting its role in developmental toxicity.

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Antioxidant Activity and Total Phenolic Content of *Eucheuma Cottonii* and *Sargassum sp.* from South Sulawesi Indonesia

By Syaharuddin, Natalia Rombe, Yosefa M, Marianti Manggau, Rosany Tayeb & Sudir Sumarheni

Hasanuddin University

Abstract- Phenolic compounds have been extracted from red algae *(Eucheuma cottonii)* and brown algae (Sargassum sp.) and the antioxidant activity assay using DPPH method has been done. The extractions were carried out on dried and fresh samples by digestion method using ethanol: water (50:50) as solvents and followed by liquid-liquid extraction using hexane and ethyl acetate as solvents. The extraction results were qualitatively analyzed using Folin-ciocalteu and TLC methods, while quantitative analysis was performed by determination of total polyphenol levels and antioxidant activity using UV-Vis spectrophotometer. Identification with Folinciocalteu reagent showed the presence of polyphenol compounds in the ethanol fraction and ethyl acetate fraction. The TLC results showed a spot with a relatively equal Rf value between ethyl acetate fraction and gallic acid standard in both samples. The highest polyphenol content was obtained from the ethyl acetate fraction of fresh samples, 0.59% for red algae and 0.34% for brown algae while the ethanol fraction of dried samples had the lowest polyphenol content of 0.08% for red algae and 0.01% for brown algae.

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Syaharuddin [α], Natalia Rombe [σ], Yosefa M [ρ], Marianti Manggau [Ω], Rosany Tayeb [¥] & Sudir Sumarheni [§]

Abstract- Phenolic compounds have been extracted from red algae *(Eucheuma cottonii)* and brown algae (Sargassum sp.) and the antioxidant activity assay using DPPH method has been done. The extractions were carried out on dried and fresh samples by digestion method using ethanol: water (50:50) as solvents and followed by liquid-liquid extraction using hexane and ethyl acetate as solvents. The extraction results were qualitatively analyzed using Folin-ciocalteu and TLC methods, while quantitative analysis was performed by determination of total polyphenol levels and antioxidant activity using UV-Vis spectrophotometer. Identification with Folinciocalteu reagent showed the presence of polyphenol compounds in the ethanol fraction and ethyl acetate fraction. The TLC results showed a spot with a relatively equal Rf value between ethyl acetate fraction and gallic acid standard in both samples. The highest polyphenol content was obtained from the ethyl acetate fraction of fresh samples, 0.59% for red algae and 0.34% for brown algae while the ethanol fraction of dried samples had the lowest polyphenol content of 0.08% for red algae and 0.01% for brown algae. In the antioxidant activity assay, the best results were obtained from the ethanol fraction of dried red algae sample with IC50 value of 52,36 ppm (strong antioxidant activity) and the ethyl acetate fraction of fresh brown algae sample with IC50 value of 7095 ppm. The lowest antioxidant activity were observed on the ethyl acetate fraction of dried red algae sample with IC50 value of 400,867 (very weak antioxidant activity) and the ethanol fraction of dried brown algae sample with IC50 value of 1918 ppm.

I. Introduction

Excessive free radicals in the body may cause the natural antioxidants produced by the body to be unable to reduce the danger of these free radicals, resulting in cell damages. Therefore, the intake of antioxidants from outside the body that can prevent, overcome and even repair the damage is required. Nowadays, there has been increasing interest in the use of marine natural compounds replacing chemicals either for treatment of specific diseases (Anis M., et al., 2018; Boopathy, N. S., & Kathiresan, K., 2013; Syaharuddin et al., 2018; Sun, Z., et al., 2018) or daily antioxidant intake. There are various resources of natural antioxidants, including the phenolic compounds of the red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.).

South Sulawesi is one of province in Indonesia which has a great potential as a *Eucheuma cottonii* seaweed producer. The total production of *Eucheuma Cottoni* in Indonesia reaches 3,082,113 tons which is about 50% of the world's seaweed product. The red algae (*Eucheuma cottonii*) and brown algae (Sargassum sp.) are also known to contain phenolic compounds that can act as antioxidants (Denny, 2013; Ermina P., et al., 2018). According to Irianti, et al. (2007), polyphenol compounds can be considered as the most important antioxidant component in plants. The antioxidant mechanism of polyphenol compounds is due to the presence of hydroxyl groups which are capable of binding to free radicals, thus forming more stable compounds.

Based on the polarity consideration of the hydroxyl groups, extraction of polyphenol compounds using polar solvents will be more effective than non-polar solvents (Nontji A, 2007). In addition, extraction methods can also affect the value of antioxidant activity. The study by Ermina P. et al., (2018) demonstrated that the n-hexane extract of *Eucheuma cottonii* which was extracted by maceration equipped by sonication method had antioxidant activity with IC50 of 119.208 µg/mL while extract using conventional maceration method had higher value of antioxidant activity with IC50 of 77,62 µg/mL. The extraction of polyphenol compounds from brown algae was also carried out by Indriawati (2015) using ethanol: water (50:50) with the temperature of 40oC by liquid-liquid extraction method, showing the antioxidant activity with IC50 value of 1770 mg/L (Indriawati, 2015). Based on these studies, the extraction of phenolic compounds from red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.) using the method of maceration by digestion using ethanol: water (50:50) as solvents at 40oC. The antioxidant activity assay was carried out by DPPH method. The purpose of this study was to obtain the extract of polyphenolic compounds from red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.) which has antioxidant activity.

II. Experimental

a) Collection and identification of samples

Samples of red algae *Euchema cottoni* and brown algae *Sargassum* sp. were collected from a cultivation area in Takalar Regency, South Sulawesi,

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Indonesia thus identified the taxonomic positions in the Biology Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia. Fresh samples were washed using distilled water and cleaned form salt residue by soaking them in warm water. Washing and soaking were done 3 times. Fresh samples were immediately extracted after the sampling preparation process. While dried algae were obtained after vacuum drying at 70°C in the Laboratory of Biofarmaka, Faculty of Pharmacy, Hasanuddin University.

b) Extraction Process

The fresh and dried samples of *Eucheuma cottonii* and *Sargassum* sp. were blended into a fine powder with a grinder (Sico®) then were weighed (Sartorius®) to 60 g each, and soaked in mixture of ethanol: water (50:50) as a solvent. The maceration method at a temperature of 40°C equipped with constant stirring speed of 800 rpm. After 8 hr., the resulting liquid extract was then centrifuged at 4000 rpm for 5 minutes. The supernatant was evaporated using a rotary evaporator. The liquid extract obtained was then evaporated at dark shade in room temperature. During the evaporation process, ethanol is added to the extract until a thickened extract is obtained.

c) Separation of Compounds Based on Solubility

The obtained extract of dried *Euchema cottonii* was dissolved with 20 ml ethanol and then inserted in separation funnel. 20 ml of n-hexane was added and the mixture was homogenized and allowed to stand for 5 minutes. The n-hexane fraction was taken and then the ethanol fraction is added with 20 ml of ethyl acetate, homogenized and allowed to stand for 5 minutes and then the ethyl acetate fraction was separated from the ethanol fraction. Hexane fraction, ethyl acetate fraction and ethanol fraction were obtained as the final results.

Sargassum sp. extract was dissolved in 50 ml of ethanol and 50 ml of hexane was added and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The upper layer was taken and evaporated. 50 ml of ethyl acetate was added to the lower layer and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The upper layer was taken and evaporated. 50 ml of water was added to the bottom layer and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The top and bottom layers were separated and evaporated at room temperature.

d) Identification of Polyphenol Compounds with the Folin-Ciocalteu Method

The dried fractions were analyzed for their polyphenol content using Folin-Ciocalteu reagents. Each of the fractions was taken sufficiently and dissolved in suitable solvent and then inserted in the test tube. The fractions were added with 7.5% Folin-Ciocalteu reagent and 1% NaOH sufficiently and then homogenized. The change of color to blue indicated the presence of polyphenol compounds in the extract.

e) Identification Using Thin Layer Chromatography

The fractions obtained from liquid-liquid extraction were added into vials separately and dissolved with adequate ethanol and then spotted on TLC plates that had been activated by heating at 110oC for 1 hour. TLC plates were developed using a mixture of methanol: Chloroform (3:1) as eluent. The spots were observed using UV lamps 254 and 366 nm.

f) Determination of Total Phenolic Content

The gallic acid solution was made by dissolving 10 mg of gallic acid in water in a 10 ml-volumetric flask (stock solution of 1000 ppm). For *Eucheuma cottonii,* the standard solution of gallic acid was prepared in the concentration of 0.5; 1.5; 3; 5; and 7 ppm by pipetting: 2.5 µl; 7.5 µl; 15 µl; 25 µl; 35 µl of stock solution respectively and for Sargassum sp. the standard solution of gallic acid was prepared in the concentration of 0.15, 0.5, 1.5, 3, 5, and 7 ppm by pipetting 7.5 µl, 25µl, 75 µl, 150 µl, 250 µl and 350 µl of stock solution respectively. Each of the standard solution was added into 5 ml-volumetric flask followed by 2.5 ml of folin reagent and the the mixture was homogenized. 2 ml of 1% NaOH was added to the mixture and diluted to the mark with water. The absorbances were measured with UV-Vis spectrophotometer.

The sample measurement procedure was performed by preparing a stock solution of 10,000 ppm, each of which 50 mg of ethanol fraction and ethyl acetate fraction were dissolved in 5 ml of ethanol. 3 dilution series were prepared by pipetting 500 µl of stock solution followed by 2.5 ml of 7.5% Folin-Ciocalteau reagent and 2 ml of 1% NaOH. The mixture was allowed to stand for 30 minutes and the absorbances were measured at maximum wavelength using UV-Vis spectrophotometer.

g) Antioxidant Activity Assay Using DPPH Method

8 mg of DPPH was dissolved in 50 ml of ethanol p.a in a volumetric flask (160 ppm). 1 ml of stock solution was pipetted and inserted into a 5 ml-volumetric flask and diluted to mark with ethanol p.a (32 ppm), then homogenized and allowed to stand for a few minutes. The absorbance was then measured with spectrophotometer at the wavelength of 516 nm.

Each 30 mg of the *Eucheuma cottonii* fractions was dissolved with ethanol in a 10 ml-volumetric flask (concentration of 3000 ppm), then a stock solution was prepared by pipetting 160 µl of the solution into a 5 ml-volumetric flask and diluted to mark with ethanol p.a. (concentration of 100 ppm). 1; 1.5; 2; 2.5; and 3 ml of stock solutions were pipetted respectively, followed by 1 ml of DPPH stock solution (160 ppm) and diluted to mark with ethanol p.a. The mixtures were allowed to

stand for 15 minutes at room temperature, then the absorbances were measured using UV-Vis spectrophotometer at the wavelength of 516 nm.

For *Sargassum* sp., 20 mg of the ethyl acetate fraction and ethanol fraction containing polyphenol compound were dissolved with ethanol in a 10 ml-volumetric flask, stock solution was prepared by pipetting 0.05 ml of the solution into a 5 ml-volumetric flask and diluted to mark with ethanol to obtain the concentrations of 20, 40, 60, 80, and 100 ppm and 1 ml of DPPH was added to each concentration and the mixtures were allowed to stand protected from light for 30 minutes. The absorbances were measured at 517 nm.

h) Experimental Analysis

One-way analysis of variance (ANOVA) was used to compare the antioxidant activity of *E. cottonii* and Sargassum sp. extracts, as well as the absorbance value of spectrophotometry results. The SPSS program (version 20.0; SPSS Inc., Chicago, IL, USA) was performed to further analyze the data. All p-values less than 0.05 were considered statistically significant.

III. Results and Discussion

In this study, the extraction of polyphenolic compounds from red algae *Eucheuma cottonii* and brown algae *Sargassum sp.* was carried out using the method of maceration by digestion with low temperature heating (40°C) at 800 rpm for 8 hours. This method was chosen because it had several advantages such as faster extraction time and stirring and slight warming could prevent the thickening of the solvent and precipitation of the extracted compound. The solvents used in the extraction wereethanol: Water (50:50). Previous study by Indriawati (2015) showed that the use of ethanol: Water (50:50) as solvents could provide the highest phenolic content compared to other concentrations. The solvent mixture was used because it is a polar compound which is capable of extracting polar polyphenol compounds.

The results of the extraction of *Eucheuma cottonii* and *Sargassum sp.*are shown in table 1.The results showed that the yield for dried samples was higher compared to fresh samples. The difference was probably caused by lesser water content in the dried samples than the fresh samples thus affecting the extraction process.

Table 1: Percentage yield extracted from dried and fresh of *Eucheuma cottonii* and *Sargassum sp.*

Sample	Sample Weight (g)	Extract weight (g)	Yield (% w/w)
Eucheuma cottonii dried sample	60	1,9	3,17
Eucheuma cottonii fresh sample	60	0,453	0,76
Sargassum sp. dried sample	60	9,1319	15,21
*Sargassumsp.*fresh sample	60	0,2616	0,44

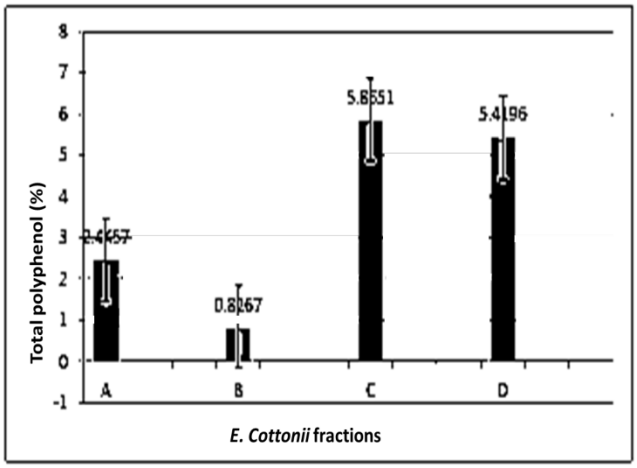
The qualitative identification of ethyl acetate fraction and ethanol fraction using Folin-ciocalteu method obtained a blue solution. The change of color was caused by the reduction of the phosphopolythdate phosphotungstate by the polyphenolic compounds present in Folin Cioucalteu and forminga blue-colored molybdenum. These results indicated that the ethyl acetate fraction and ethanol fraction of *Eucheuma cottonii* contain polyphenol compounds while for the hexane fraction there was no change of color which indicated the absence of polyphenol compounds. Positive results were obtained on ethyl acetate fraction and ethanol fraction of the *Sargassum sp.* samples. While negative results were observed on the hexane fraction. This difference was due to the hexane solvent being nonpolar, hence polyphenol compounds might not be present in the hexane fraction.

Table 2: Identification of and *Sargassum sp* fractions using TLC method

Sample	Fraction	Rf value
Dried *Eucheuma cottonii*	Ethyl acetate	0,61
	Ethanol	0,55
Fresh *Eucheuma cottonii*	Ethyl acetate	0,71
	Ethanol	0,55
Dried *Sargassum sp.*	Ethyl acetate	0,73
	Ethanol	0,68
Fresh *Sargassum sp.*	Ethyl acetate	0,71
	Ethanol	0,65
Gallic acid	-	0,68

The qualitative test by TLC method used silica gel GF 254 as the stationary phase, the mobile phase was a mixture of methanol: Chloroform (1:1) with gallic acid as the standard solution. It could be concluded from the Rf value that the ethyl acetate fraction of both samples might have the same compound with the standard solution.

Total polyphenol content of ethyl acetate fraction and ethanol fraction of *Eucheuma cottonii* were 0.24% and 0.08% respectively, while for the fresh sample the total polyphenol content of ethyl acetate fraction and ethanol fraction were 0.59% and 0.54% respectively. The measurements showed that the highest polyphenol content was found in the ethyl acetate fraction of the fresh sample of *Eucheuma cottonii* and the lowest content was observed in ethanol fraction of dried sample. For *Sargassum sp.*, the highest polyphenol content was obtained on ethyl acetate fraction of fresh sample with the value of 0.34% and the lowest content was obtained in ethanol fraction of dried sample with the value of 0.01%.



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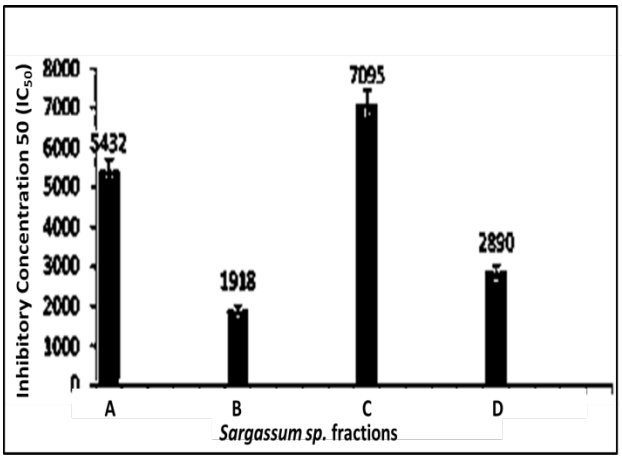

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Figure 1: Comparison of polyphenol content of *E. cottonii* fractions (1) and *Sargassum sp.* fractions (2).

A = ethyl acetate fraction of dried sample; B = ethanol fraction of dried sample; C = ethyl acetate fraction of fresh sample; D = ethanol fraction of fresh sample

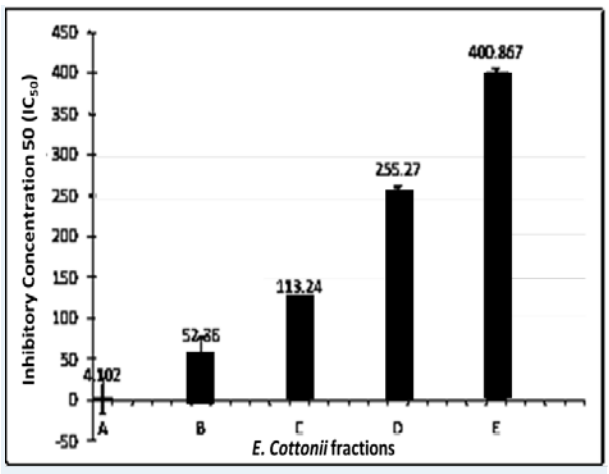
The antioxidant activity assay was performed using DPPH method. This method was chosen because it is a simple, fast and does not require many reagents. Moreover, the results of DPPH measurements show the general antioxidant activity of the sample and does not based on the type of radical inhibited (Juniarti, 2009). Antioxidant activity was measured using UV-Vis spectrophotometer and the percentage of DPPH radical binding will be used to calculate IC50 value. Determination of IC50 value aimed to determine the antioxidant activity of the fraction. IC50 shows the concentration of extracts that can provide DPPH damping by 50%. The smaller the value of IC50, the greater the antioxidant activity.

Table 3: The results of antioxidant activity assay of *Eucheuma cottonii*

Sample	IC_{50}	AAI	Classification
Ethanol fraction Dried sample	52.36 ppm	0.611	IC_{50} (high antioxidant activity) AAI (moderate antioxidant activity)
Ethyl acetate fraction Dried sample	400.867 ppm	0.079	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethanol fraction Fresh sample	113.24 ppm	0.283	IC_{50} (low antioxidant activity) AAI (low antioxidant activity)
Ethyl acetate sample Fresh sample	255.27 ppm	0.125	IC_{50} (moderate antioxidant activity) AAI (low antioxidant activity)
Vitamin C	4.102 ppm	7.801	IC_{50} (very high antioxidant activity) AAI (very high antioxidant activity)

Table 4: The results of antioxidant activity assay of *Sargassum* sp.

Sample	IC_{50}	AAI	Classification
Ethanol fraction Dried sample	1.918 ppm	0.016	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethyl acetate fraction Dried sample	5.432 ppm	0.005	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethanol fraction Fresh sample	2.890 ppm	0.011	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethyl acetate sample Fresh sample	7.095 ppm	0.004	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Vitamin C	4.102 ppm	7.801	IC_{50} (very high antioxidant activity) AAI (very high antioxidant activity)



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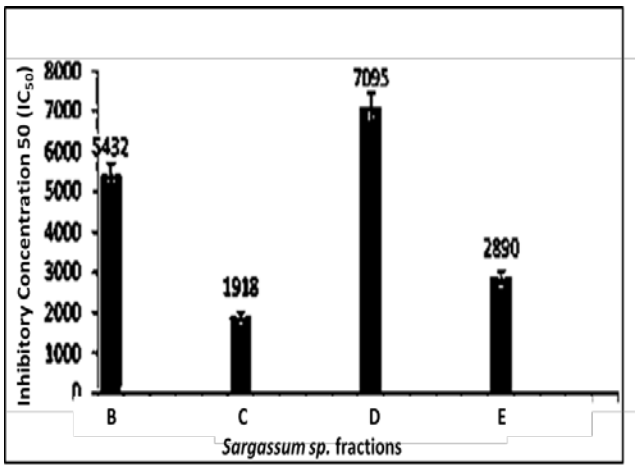

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Figure 2: Comparison of IC_{50}value between vitamin C and samples. 1 = red algae (*Eucheuma cottonii)*; 2 = brown algae(*Sargassum sp*.); A= Vitamin C; B = ethanol fraction of dried sample; C = ethanol fraction of fresh sample; D = ethyl acetate fraction of fresh sample; E = ethyl acetate fraction of dried sample.

The results of the antioxidant activity assay showed the highest IC50 value for ethanol fraction of dried *Eucheuma cottonii* and ethyl acetate fraction of fresh *Sargassum sp.* Meanwhile, the lowest IC50 values was obtained fromethyl acetate fraction of dried *Eucheuma cottonii* and *Sargasum sp*. The low antioxidant activity might result from improper separation processes, as well as other factors that may affect.

IV. Conclusion

Based on this study it can be concluded that the dried sample of red algae (*Eucheuma cottoni*) and brown algae (*Sargassum* sp.) had a higher yield percentage than fresh samples. The highest total polyphenol content was obtained from ethyl acetate fraction of fresh *Eucheuma cottonii* with the value of 0,59%, while the ethanol fraction of dried sample had the lowest polyphenol content of 0,08%. For Sargassum sp., the highest polyphenol content was obtained from ethyl acetate fraction of fresh sample with the value of 0,34% and the lowest was obtained from the ethanol fraction of dried sample with the value of 0,01%. The ethanol fraction of dried *Eucheuma cottonii* had better antioxidant activity than other fraction with IC50 value of 52,36 ppm (classified as high antioxidant activity) and AAI value of 0,611. While the ethyl acetate fraction of the dried sample had the lowest antioxidant activity compared to other fractions with IC50 value of 400.867 (very weak antioxidant activity) and AAI value of 0.079. For *Sargassum* sp., the antioxidant activity of all fractions was very weak.

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Grass Pea Consumption and Present Scenario of Neurolathyrism in the South Central Coastal Area of Bangladesh

By Md. Hasan Rashid, Md. Rabiul Haque, Sujan Kanti Mali, Liton Chandra Sen & Sourav Debnath

Patuakhali Science and Technology University

Abstruct- Grass pea can be consumed as supplementary nutrients in a diet without a neurolathyrism health problem among all classes of people in the south-central coastal area of Bangladesh. The study aimed at assessing the grass pea consumption pattern in diet and detection of neurolathyrism patients over 400 respondents among four villages. An investigation was also done on various paralytic admitted patients at hospital in the south central coastal region of Bangladesh. Among respondents of four villages 63% were male, 38.75% was illiterate, 41.5% was completed primary education and 30% of respondent's monthly income Tk.6000-9500 was maximum. Among respondents 89.6% consumed grass pea was 1/3 or less than cereal at its proportion in a meal and they all consumed meat, fish, egg, vegetables also. In grass pea food items 25.25% respondents prefer Dal barta was maximum and Dobakhesari (snack) 0.75% was lowest. 95.75% respondent was used various spices like onion, garlic, chili, tamarind etc. with grass pea to making food items to increasing its palatability and testes.

Keywords: *grass pea, consumption, neurolathyrism.*

GJSFR-C Classification: *FOR Code: 279999*

Strictly as per the compliance and regulations of:

Grass Pea Consumption and Present Scenario of Neurolathyrism in the South Central Coastal Area of Bangladesh

Md. Hasan Rashid [α], Md. Rabiul Haque [σ], Sujan Kanti Mali [ρ], Liton Chandra Sen [ω] & Sourav Debnath [¥]

Abstract- Grass pea can be consumed as supplementary nutrients in a diet without a neurolathyrism health problem among all classes of people in the south-central coastal area of Bangladesh. The study aimed at assessing the grass pea consumption pattern in diet and detection of neurolathyrism patients over 400 respondents among four villages. An investigation was also done on various paralytic admitted patients at hospital in the south central coastal region of Bangladesh. Among respondents of four villages 63% were male, 38.75% was illiterate, 41.5% was completed primary education and 30% of respondent's monthly income Tk.6000-9500 was maximum. Among respondents 89.6% consumed grass pea was 1/3 or less than cereal at its proportion in a meal and they all consumed meat, fish, egg, vegetables also. In grass pea food items 25.25% respondents prefer Dal barta was maximum and Dobakhesari (snack) 0.75% was lowest. 95.75% respondent was used various spices like onion, garlic, chili, tamarind etc. with grass pea to making food items to increasing its palatability and testes. 90 % respondent was consumed grass pea as various items of Dal in their servings five years or more. Neurolathyrism affected paralysis patients was not detected among the respondents of four villages and admitted patients in hospitals. Among the paralysis admitted patients in hospitals 90.4% were higher suffered from stroke and 0.4% was lowest due to the drug (Isoniazid).

Keywords: grass pea, consumption, neurolathyrism.

I. Introduction

In the south-central coastal region of Bangladesh, most of the respondents have an idea that a chemical contained in grass pea causes a health problem as paralysis and was scared also to consume grass pea. Naturally, grass pea grown in this area contains comparatively low toxin (ODAP) and traditionally used different processing steps like soaking and washing as well as food consumption pattern (blending or mixing with other crops, applying different spices eat with an antioxidant rich foods etc.) also may be decreased toxin level (ODAP) which may favors to decrease risk of neurolathyrism. Grass pea is probably the most drought tolerant legume crop and it is also resistant to moderate salinity. (Yang. Hui-Min and Zhang. Xiao-Yan, 2005). Grass pea is cultivated and consumed in India, Nepal, and Bangladesh and in many parts of Africa that are prone to recurrent droughts (Stodolak B *et al.,* 2008). It is often considered a life saver crop (Lambein F *et al., 2008)*. The protein content of grass pea seeds is higher compared to other legume seeds (Monsoor M A *et al*., 2002). Neurolathyrism is a form of human spastic parapares is related to the over consumption of the legume *Lathyrussativus* or grass pea caused by neurotoxin β-ODAP (Haque A *et al*., 1994). This disease is prevalent in some area of Bangladesh, India, Nepal, Ethiopia and effects more men than women (Spencer *et al*., 1993). Most of the patients develop heavy legs, spasms of the muscles of the legs and spasticity. The risk factors for neurolathyism are heavy physical activity, male gender, young age (15-25years), and micronutrient deficiency like Zn, Cu, Vitamin C and A (Rao SLN; 2001).Sulfur amino acids deficiency caused by grass pea diet plays an important role in the toxicity of l-β-ODAP by increasing the oxidative stress (Eguchi *et al*., 2011). Recent research suggests that sulfur amino acids have a protective effect against the toxicity of β- ODAP (Sriram *et al*., 1998). The mean prevalence of neurolathyrism reaches 6 per 1000 in Ethiopia, 5.3 per 1000 in India and 1.4 per 1000 Bangladesh (IPBO; 2009). At present there is no treatment available for neurolathyrism. Prevention strategies need to be applied in efficiently.

II. Materials & Methodology

A cross-sectional study was performed at the four villages named Shirampur in Dumki, Imamkathi in Bakhergonj, Barobegay in Patuakhalisador and Goaurichanno in Baroguna sador upazila in the south central coastal area of Bangladesh from January to May, 2013. The geographical location of the site of experiment was at 22° 42' 0" north latitude to 90° 22' 0" east longitude. To estimate the consumption pattern of the grass pea in diet and detection of neurolathyrism patients in each village 50 households and 100

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populations were selected using a quota sampling method based on the highest production and consumption of grass pea in that area. The consumption pattern of the grass pea with diet and detection of neurolathyrism patient survey was carried out by the food frequency questionnaire over the 400 respondents in 200 households among four villages. The respondent was mostly senior and responsible person of the household. At the same time another investigation was done on various paralytic patients at the medicine department in Shere-e-Bangla medical college and hospital in where major portion of various paralysis patients was admitted from this area. Identification of neurolathyrism cases by the Snowball sampling method was carried out by observing prescription, register entry book given by a physician and neurological examination of an individual also in the presence of a doctor. The respondents was in separate households. One set of questionnaire for primary data collection were developed with an emphasis on food consumption pattern, specially grass pea consumption and present status of the physical condition of an individual. One set of the questionnaire was pre-tested and necessary improvement was made. The respondents included primarily to represent some of the key characteristics associated were observed to be directly or indirectly related to the consumption grass pea with of various food stuffs, period of consumption of grass pea and the physical condition of respondents. Face to face interview has been carried out following Paper and Pencil (PAPI) method. Database was prepared in Microsoft Excel format separately.

III. Results

a) Socio-economic and demographic profile of the respondents in household

Table 1: Socio-economic and demographic profile of the respondents in household

Characteristics		Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
Sample Size (N)		100	100	100	100	100
Sex (%)						
	male	60	64	62	66	63
	female	40	36	38	34	37
Age (years) (%)						
	<=20	5	7	4	2	4.5
	21-40	28	30	25	22	26.25
	41-60	61	55	66	68	62.5
	>60	6	8	5	8	6.75
Educational status (%)						
	Illiterate	35	38	40	42	38.75
	Primary	34	42	46	44	41.5
	SSC	15	12	10	8	11.25
	HSC	12	6	3	4	6.25
	Degree	4	2	1	2	2.25
Monthly income (in taka) %						
	No income	6	9	10	5	7.5
	1500-2000	5	7	5	10	6.75
	2100-4600	12	14	7	12	11.25
	4600-6000	20	19	18	15	18
	6000-9500	33	24	35	28	30
	Above 9500	24	27	25	30	26.5

b) Food habit of the respondents

Among respondents, 94.5% rice and only 5.5 % was consumed wheat as a stable food in these areas. Grass pea or other food grains was not consumed as stable food in that areas.

Table 2: Stable food consumption rate in the southern central coastal area of Bangladesh

Food grain	Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
Rice	92	94	95	97	94.5
Wheat	8	6	5	3	5.5
Grass pea	0	0	0	0	0
Others	0	0	0	0	0

c) *Nature of grass pea consumption with food items of respondents in a meal*

A majority respondents 89.6% consumed grass pea in these villages were 1/3 or less than at its proportion in a meal. No respondents consumed grass pea as solitary and a majority 92% consumed grass pea with various foodstuffs like meat, milk, fish egg, vegetables in their daily meal.

Table 3: Nature of grass pea consumption with food items of respondents in a meal

Characteristics	Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
How much proportion of grass pea you cosumed in a meal?					
Grass pea at all	0	0	0	0	0
With ≥1/3 cereal	95	90	88	85	89.5
With <1/3 cereal	5	10	12	15	10.5
Did you eat meat, milk, fish, egg, vegetables in your diet with grass pea in your meal generally?					
Yes	100	100	100	100	100
No	0	0	0	0	0

d) *Consumption status of various pulses in diet*

Among respondents, the grass pea consumption 64% was maximum in Goaurichonno and 51% was minimum in Imamkathi. Among pulse grains consumption, lentil 28% was maximum in Shirampur and 15% was minimum in Borobegay. 22% respondents was consumed Mungbean at their servings in Imamkathi hold first position.

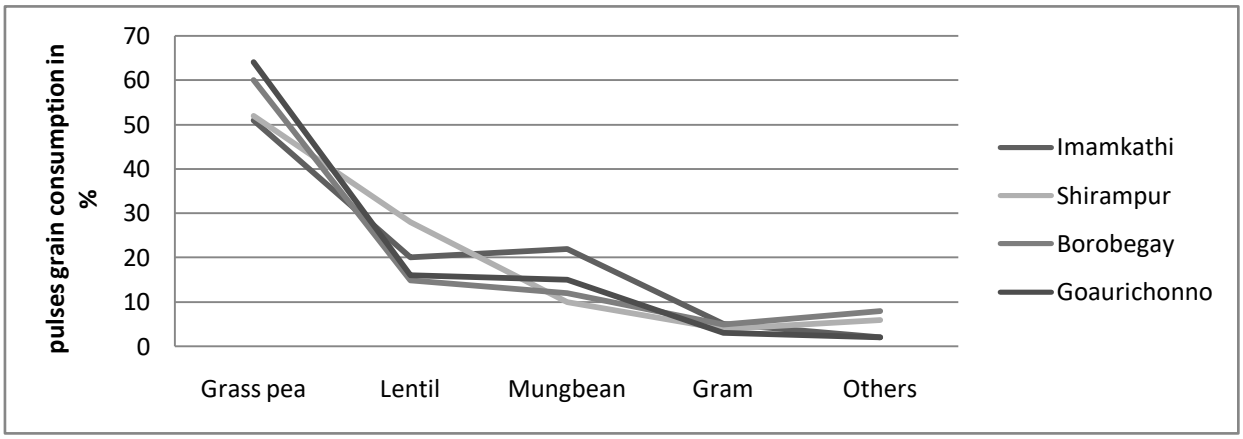

Figure 1: Consumption of various pulses in daily diet in the southern central coastal area of Bangladesh

e) *Processing of grass pea grain at food items preparation*

Now all of the respondents in southern-coastal areas grass pea grains were soaked in clean water for few hours to soften then rinsing by clean water thoroughly in several times at their various food items preparation. 95.75% respondent was used various spices like (onion, garlic, chili, tamarind etc.) with grass pea to making food items to increasing its palatability and testes. Only 4.25 respondents were used grass pea as solitary to making food items in some cases.

f) *Methods of consumption of grass pea in diet*

In the southern-coastal area of Bangladesh People was consumed grass pea as various food items. Among those they prefer Dahl barta was a maximum 25.25% and then as Dahl charchari 19.75%, Dahl bora (piaju) 19.5% respectively in these areas. Comparatively lower amount of grass pea ate as Dahl vaja, Panidahl and Dobakhesari (snack) by the respondents of these villages.

Table 4: Various grass pea food items consumption in (%) at the southern central coastal area of Bangladesh

Forms of khesari meal	Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
Dahl barta	22	26	23	30	25.25
Dahl charchari	23	21	15	20	19.75
Dahl bora (piaju)	24	17	22	15	19.5
Dahl vaja	2	3	5	2	3
Chaldahlkhichuri	5	8	10	5	7
Kaloysak (young shoot)	17	14	15	18	16
Liquid dahl (panidahl)	2	3	2	4	2.75
Sabji/dim chop	5	8	7	4	6
Dobakhesari (snack)	0	0	1	2	0.75

Note: Grass pea food items was given as a local name.

g) *Duration of the consumption of grass pea in the diet of respondents*

90 % respondents was consumed grass pea in their servings for five years or more in the southern-central coastal area of Bangladesh. 93% respondents consumed grass pea was highest in Borobegay and lowest in Imamkathi was 88%. Only 2.50% respondent was consumed Grass pea Six months to one year from in that surveyed areas.

Table 5: Duration of consumption of grass pea in diet on life of respondent

Characteristics	Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
How many months or years do you taken this *dal* (grass pea) in your meal? (%)					
One month to six months	00	00	00	00	00
Six months to one year	04	03	01	02	2.50
One year to five years	08	07	06	09	7.50
Above five years	88	90	93	89	90

h) *Disease occurrence of the respondents among four villages*

In these four villages respondents was not suffered from neurolathyrism paralytic diseases in the south-central coastal area of Bangladesh. About 14.25% respondents of the surveyed areas suffered from skin diseases was highest. Most 61.5% of respondents have no diseases occurrence at surveyed time.

Table 6: Disease occurrence of the respondents among four villages

Characteristics	Imamkathi	Shirampur	Borobegay	Goaurichonno	Mean
Do you have any chronic disease? (%)					
Neurolathyrism	0	0	0	0	0
Diarrhea	10	12	15	19	14
Dysentery	8	11	10	12	10.25
Skin diseases	12	10	16	19	14.25
No diseases	70	67	59	50	61.5

i) *Detection of neurolathyrism among paralytic patients at Shere-e-Bangla Madical College and hospital*

In the southern coastal area of Bangladesh, there were no detected any neurolathyrism affected paralysis patients (caused by continuous consumption of grass pea) among 500 admitted patients at medicince department in Sher-e-Bangla Medical College and hospital. Among these paralysis patients 90.4% were higher suffered from stroke, 0.4% due to drug (Isoniazid) was lower.

Table 7: Prevalence of various types of paralysis patient in (%) at the Shere-e-Bangla medical college and hospital

Common type of paralysis diseases	No. of patient	Mean
Neurolathyrism	0	0
Stroke	452	90.4
Guillain-Barre Syndrome/GBS	13	2.6
Acute transverse myelitis	8	1.6
Nutritional deficiency	7	1.4
Motor neuron disease	6	1.2
Metastasis to spine & spinal cord	5	1
Trauma to spine	4	0.8
Myasthenia gravis	3	0.6
Isoniazide drug	2	0.4

IV. Discussion

The Socio-economic and demographic profile of the respondents was poor in the south-central coastal area of Bangladesh. Among respondents63% were Male and 37% female were less due to cultural restrictions, they were less comfortable to communicate with a male. The literacy rate among the villages found to be 38.75%. Among them 41.5% was completed primary education. About one third 30% of the respondents' monthly income was Tk.6000-9500, 26.5% were above Tk.9500 and 7.50% had no income (Table 01). Most of them depended on agriculture as an occupation. The most commonly cultivated crop in the study area is paddy (*Oryza sativa*) which is sown in July to October season, and it is a major part of diets. Grass pea is mainly cultivated during rabbi season mostly as relay crop in paddy field due to it can withstand low moisture in summer and gives a good yield.

Most of the respondents 94.5% in these areas consumed rice as the stable food and few of them 5.5 % respondent used wheat whose have diabetics' problem in this area. Grass pea or other food grains was not consumed as the stable food in those area. The amount

of grass pea consumed by a majority respondents 89.6% of those villages were 1/3 or less than at its proportion in a meal. Neurolathyrism occurs after prolonged over-consumption of grass pea seed during several months as staple food in anunbalanced diet (Haque A. et al.; 1996) which favors the people of this area can be used grass pea as protein source without scared. Respondents consumed meat, fish, milk, egg, vegetables also in their meal. These types of food items have rich in s- amino acid which may protected from neurolathyrism (Getahun H, Lambein F, Vanhoorne M, Van der Stuyft P, 2005).

Grass pea is probably the most drought tolerant legume crop and it is also resistant to moderate salinity. In this area people has grown plenty of grass pea as relay cropping with rice. Generally grass pea was cheaper than other pulse crops. For poor economic condition of the people they were not able to buy others pulses. Among the pulse grain consumption the grass pea consumption was always higher than others due to its palatability, tested, easy accessible, cheapness etc. in these areas.

Now all of the respondents in southern- coastal areas grass pea grains were soaked in clean water for few hours before they have cooked. They soaked for softening grains to decrease cooking time and also rinsed by water thoroughly in several times to clean on various food items preparation. Although they have not knowledge to the procedure of reduce toxin (ODAP) but their softening and cleaning method decrease the toxin level ultimately well. These ideas have similarity by (Cohn D F, Streifler, 1981).

Several studies have documented that grass pea is consumed in a variety of forms (Roldan *et al.* 1994; Haque *et al.* 1996; Getahun *et al.* 1999) but very few have attempted to associate the type of grass pea preparation with the risk of neurolathyrism. In the south-central coastal areas people consumed grass pea as various food items. Among these they preferred *Dahl varta* (Seeds are boiled, salted and grinding and gave shape as ball with chilly and mustard oil) was maximum 25.25% and then as *Dahl charchari* (Splits seeds cooked as solitary form)19.75% *Dahl bora* / *Piaju* (deep-fried in oil of paste ball with onion and spices)19.5% in those areas. All classes of people ate vegetables of grass pea in those areas. The least amount of people ate grass pea 2.75% as liquid dal (aqueous slurry cooked with spices) due to scared on neurolathyrism. The people of these villages use lentil as liquid form instead of grass pea mostly all servings in their diet and used various spices like (onion, garlic, chili, tamarind etc.) with grass pea to making food items to increasing its palatability and testes. These diet habits grass-pea preparations with cereals may reduce risk of neurolathyrism which have similarity by (Getahun H, Lambein F, Vanhoorne M, Van der Stuyft P, 2003).

Among 400 respondents of four villages and500paralytic patients admitted at medicine department in Sher-e-Bangla Medical College and hospital from those areas neurolathyrism patients not detected caused by continuous consumption of grass pea. Due to poverty (40-60 years) ago, the person of this region was consumed grass pea continuously and a great part at green stage also, so there was a chance to attract by Neurolathyrism (Paralysis).

V. Conclusion

In the present study confirmed that greater amount of grass pea was being consumed as supplementary nutrients in their diet without neurolathyrism health problem among the all classes of people in the south-central coastal area of Bangladesh. It was also evident from this study that people's food habits grass pea consumed mixed with cereals and spices or other food ingredients and soaking and cleaning of grass pea, consumed meat, milk, fish, vegetables etc. as supplementary food did not lead to neurolathyrism. People can be consumed grass pea without scared even marginal sections of people perhaps increased the nutritional status. If the nutritional value of this pulse can be utilized effectively, it may become a good source of protein.

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

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

Keywords

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

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

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

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

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

Numerical Methods

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

Abbreviations

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

Formulas and equations

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

Tables, Figures, and Figure Legends

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

Figures

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

Preparation of Eletronic Figures for Publication

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

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

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

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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

General style:

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

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

Mistakes to avoid:

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

Title page:

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

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

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

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

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

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

Approach:

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

Introduction:

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

The following approach can create a valuable beginning:

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

Approach:

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

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

Procedures (methods and materials):

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

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

Materials:

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

Methods:

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

Approach:

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

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

What to keep away from:

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

Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

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

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

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

Figures and tables:

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

Discussion:

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

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

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

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

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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

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.

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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