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Studies on Morphological Novel Cryptic Ancestral Taxon

Germination in Maize Seeds Pretreatment of Giberellic Acid

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By Gonzalo Guevara-Guerrero, Miguel A. Montalvo Martínez, Víctor M. Gómez Reyes, Zai-Wei Ge, Michael A. Castellano, Gregory Bonito, Matthew E. Smith & James M. Trappe
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Keywords: *fungi evolution, truffle, angiocarpic fungi, hypogeous fungi.*

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New Molecular Data in the Truffle-Like Fungus, *Aroramyces Herrerae*, Reveal a Novel Cryptic Ancestral Taxon, *Pterosporomyces Herrerae Gen. Nov. & Comb. Nov.* (Trappeacea, Phallales)

Gonzalo Guevara-Guerrero [α], Miguel A. Montalvo Martínez [σ], Víctor M. Gómez Reyes [ρ], Zai-Wei Ge [ω], Michael A. Castellano [¥], Gregory Bonito [§], Matthew E. Smith [χ] & James M. Trappe [ν]

Abstract- Recently, *Aroramyces herrerae* was described based on morphology, ecology, and taxonomically placed in the family Hysterangiaceae (Hysterangiales). However, a DNA analysis revealed that *A. herrerae* belongs to a new sequestrate cryptic genus, *Pterosporomyces*, in the family Trappeaceae (Phallales), nested with *Restingomyces reticulatus* and *Phalobata alba* and jointly forming a basal clade of Phallales with strong statistical support. *Pterosporomyces herrerae gen. nov & comb. nov.* is characterized by an olive green to brown gleba and wing spores (utriculum) up to 6 µm broad and its ITS and ATP6 variation. *Pterosporomyces* is similar in morphology to *Restingomyces* but differs by the spore ornamentation been utriculate in the former and alveolate in the latter. A taxonomic amendment is presented for the family Trappeaceae to include the new genus and recombine *Aroramyces herrerae* into *Pterosporomyces herrerae gen. nov.* & *comb. nov.* and a redescription with new morphological, rDNA, and mtDNA data with illustrations are provided.

Keywords: fungi evolution, truffle, angiocarpic fungi, hypogeous fungi.

I. Introduction

Aroramyces herrerae Guevara, Gómez & Castellano, and *A. balanosporus* Guevara & Castellano were described for the family Hysterangiaceae based mainly on morphology and ecology without DNA support (Guevara et al. 2016). However, a recent ATP6 and ITS genes analysis was performed on these two species to corroborate their novel status. The phylogenetic analysis showed that, in fact, *A. balanosporus* belongs to the family Hysterangiaceae as expected; however, in *A. herrerae*, the ITS and ATP6 analysis indicated that this taxon belongs to the family Trappeaceae into the order Phallales. Based on this, the recombination of *A. herrerae* to the new genus *Pterosporomyces herrerae* is proposed here. This new genus, among other hypogeous and stinkhorn fungi, belongs to the monophyletic subclass Phallomycetidae which is one of the most amazing fungi in recent mycological research due to the great diversity and evolutionary forms present in this group (Hosaka et al. 2006; Trappe et al. 2009). The family Trappeaceae, which includes *Trappea darkeri* (Zeller) Castellano, *Restingomyces reticulatus* Sulzbacher, B.T. Goto & Baseia, *Phallobata alba* G. Cunn., and the new taxon *Pterosporomyces herrerae* is ancestral in the Order Phallales (Hosaka et al. 2006; Sulzbacher et al. 2016). This research will increase our taxonomic and systematic understanding of the Gomphales and Phallales in North America.

II. Material and Methods

Basidiomata sampling, macro and microscopic analysis were performed following the recommendations of Castellano et al. (1989) and Pegler et al. (1993). The colors of fresh fruiting bodies are in general terms of the authors. For microscopic observations herborized specimens were hand-cut and mounted in 5% KOH, Melzer's reagent, or water. Dried and herborized specimens are deposited at José Castillo Tovar herbarium, ITCV. Thirty measurements including means were obtained from mature basidiospores with a compound microscope at 1000 × under oil immersion.

DNA sequencing and phylogenetic analyses.—Molecular protocols follow those of Guevara et al. (2008). First, DNA was obtained from basidiomata by the chloroform extraction technique using CTAB buffer.

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The internal transcribed spacer (ITS) region was amplified with the primer set ITS1f- ITS4 (White et al. 1990, Gardes and Bruns 1993). Then, the ATP6 gene was amplified with the primer set atp6-1 and atp6-2 (Kretzer and Bruns. 1999). Amplicons were cleaned enzymatically with antarctic phosphatase and endonuclease (New England Biolabs, Ipswitch, Massachu- setts). Posteriorly, Sanger sequencing was performed by Big Dye chemistry 3.1 (Applied Biosystems, Foster City, California) with the forward primer ITS or atp6-1 and reverse primers ITS4 or atp6-2. In addition, the DNA sequences were determined on an ABI 3700 capillary sequencer (Applied Biosystems, Foster City, California), viewed and manually edited in Sequencher 4.0 (Gene Codes Corp., Ann Arbor, Michigan). Later, sequences were queried against GenBank with the BLASTN algorithm to verify that sequences belonged to *Aroramyces*. MUSCLE (Edgar 2004) was used to align sequences and manually checked, and ambiguities were excluded in Mesquite 2.5 (Maddison and Maddison 2009).

Furthermore, the phylogenetic analyses were performed with maximum likelihood (ML) in PAUP* (Swofford 2002), and Bayesian inference (BI) with MrBayes (Huelsenbeck and Ronquist 2001). The Akaike criterion model (best-fit nucleotide substitution) information was selected and executed in PAUP* 4d106 (Swofford 2002). ML bootstrap support based on 1000 replicates was assessed with RAxML (Stamatakis et al. 2008). BI analyses and posterior probability were run through the CIPRES Web portal (http://www.phylo.org/). BI was based on parallel runs of 20 million generations sampling every 1000 generations for the phylogenetic tree.

Finally, sequences of this study were uploaded in GenBank under accession numbers MZ343611, MZ343612, and MK811032,

III. Results

a) Molecular analyses

A total of 49 sequences published or reported in the NCBI were selected for the ITS and ATP6 phylogenetic analysis (Table 1& 2). As previous studies have shown, the family Trappeaceae is a basal clade in the Phallales according to ML and Bayesian Inference analyses (Fig. 1 & 2). The Bayesian inference analysis recovered *Pterosporomyces herrerae* as a monophyletic group. The amendment to accommodate *Aroramyces herrerae* to *Pterosporomyces herrerae* in the basal family Trappeaceae is proposed as a new combination supported by ITS and ATP6 analysis with strong statistical support (PP=1), the internal nodes of *Pterosporomyces* were well supported too, in addition to new morphological characters.

IV. Taxonomy

Trappeaceae P.M. Kirk *In*: Kirk PM. Cannon PF. Minter DW, Stalpers JA, eds. 2008. Dictionary of the Fungi. 10th ed.

a) *Taxonomic amendment of the family Trappeaceae to include Pterosporomyces*

Basidiomata hypogeous, sequestrate, gregarious, scattered, globose, subglobose or irregular, white pale tan to brownish, mottled dark brown with pale areas when handling and when dried, smooth to slightly tomentose, with mycelial strands, odor acetone/ether solvent-like. Peridium two layers (separable from the gleba when dried). Gleba green, brown or blackish, locules ellipsoid to elongate, columella dendroid, gelatinized, grayish, rhizomorphs few, attached at the base. Basidiospores small, smooth, reticulate or utriculate, (wing-like ornamentation), fusoid, ellipsoids, up to (5) 6 μm broad laterally, numerous fine spines within the "utriculum," the utriculum encompassing hilar appendage to give a truncated appearance, utriculum often no evenly inflated to protrude from one side or another, occasionally surrounding entire spore originating from the spore base, pale brown in mass in KOH, inamyloid, nondextrinoid.

Type genus: Trappea Castellano

Other genera: Pterosporomyces Guevara, Gomez & Z.W. Ge, *Restingomyces* Sulzbacher, T. Grebenc & Baseia and *Phallobata* G. Cunn.

Pterosporomyces Guevara, Gómez, & Z.W. Ge gen. nov.

Myco Bank: 834809 GenBank: MZ343611, MZ343612 & MK811032

Typification: *Pterosporomyces herrerae* (Guevara, Gómez, Castellano) Guevara, Gómez & Z.W. Ge

Etymology: "in reference to a fungus with wing (utricle) spore " the genus is dedicated to the pioneer mycologist from northern Mexico José Castillo Tovar.

Diagnosis: The genus differs from all other known Phallales by the spores with distinct inflated utricle laterally, and *ITS* and *ATP6* analysis placed this taxon into the family Trappeaceae.

Pterosporomyces herrerae (Guevara, Gómez, Castellano) Guevara, Gómez & Z.W. Ge, *gen. nov. & comb. nov.*

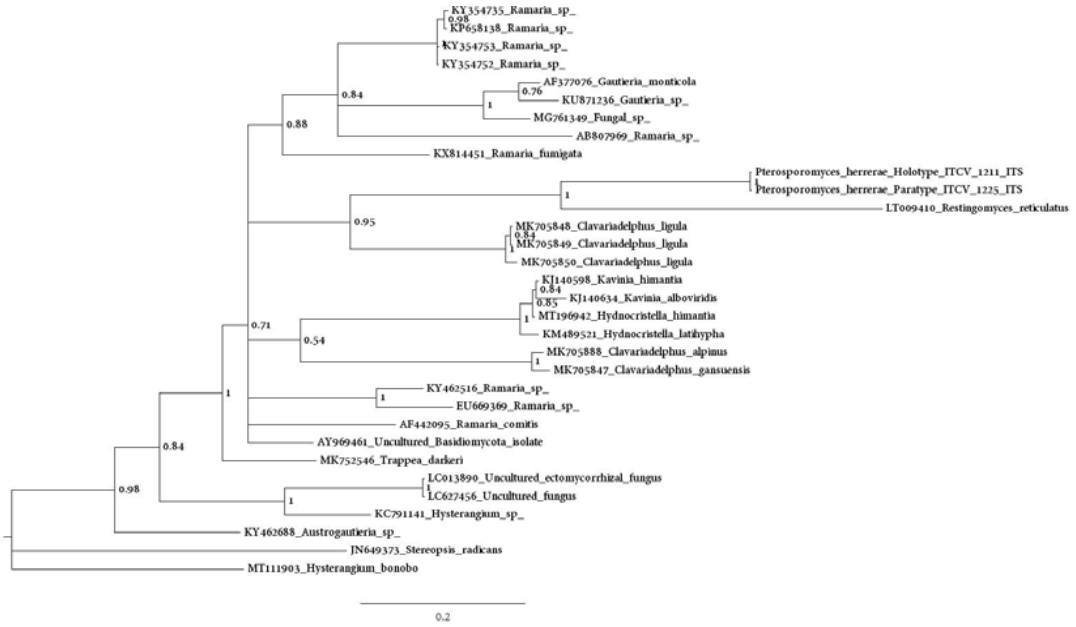

Figure 1: *Pterosporomyces herrerae*, phylogenetic tree inferred under the maximum-likelihood (ML) criterium for ITS gene with Mr. Bayes . The posterior probabilities for each clade are shown on the branches. The accession numbers in the sequence labels indicate the GenBank accession numbers

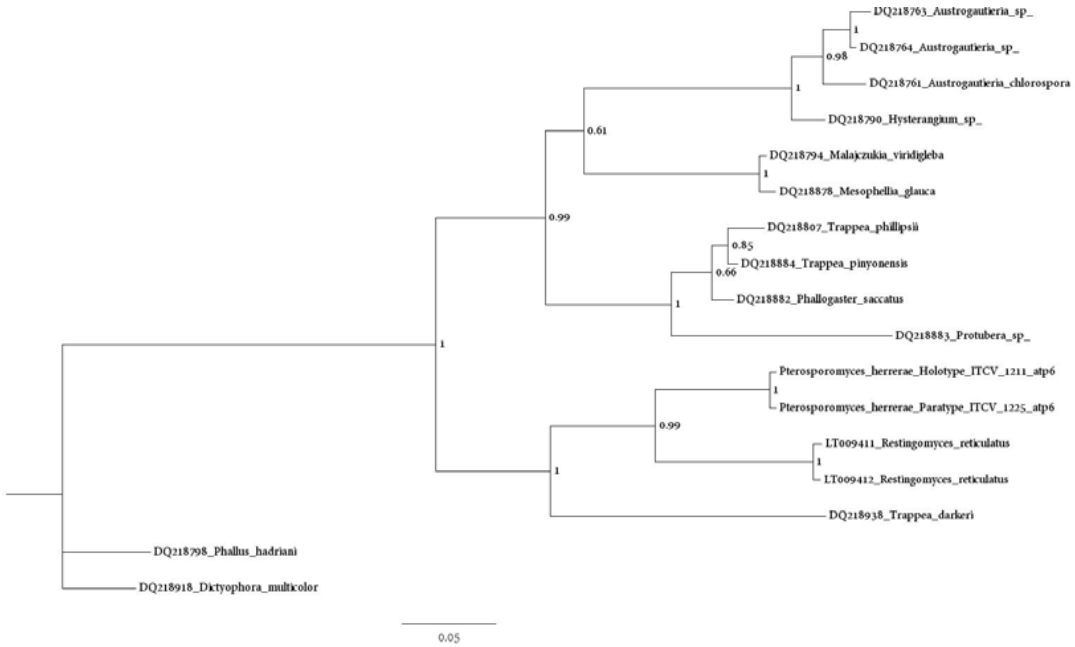

Figure 2: *Pterosporomyces herrerae*, phylogenetic tree inferred under the maximum-likelihood (ML) criterium for ATP6 gene with Mr. Bayes . The posterior probabilities for each clade are shown on the branches. The accession numbers in the sequence labels indicate the GenBank accession numbers

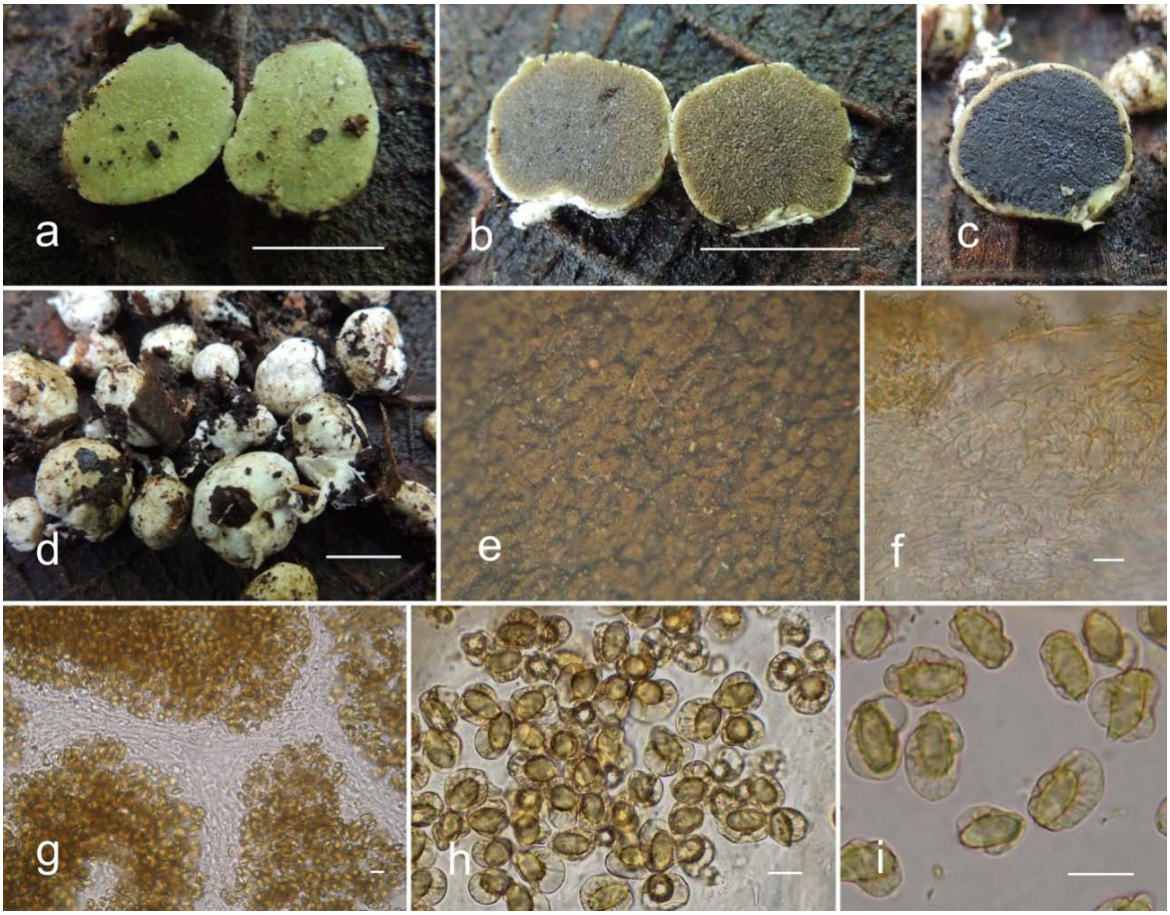

Figure 3: a-i, *Pterosporomyces herrerae comb. nov*.(ITCV 1211Typus), a, b, & c: cross-sections of basidiome (bar = 1 cm), d: basidiomata (bar = 1 cm), e: close up of gleba, f: epicutis with pseudoparenchyma cells, (bar = 10 μm), g: Trama (bar = 10 μm), h & i: basidiospores with inflated utricle (bar 10 = μm)

= *Aroramyces herrerae* Guevara, Gómez & Castellano, IMA Fungus 7(2) 235-238, 2016 (basionimo)

MB 812928, GenBank: MZ343611 (Type), MZ343612 (paratype) & MK811032

Holotype Guevara 1211 (ITCV1211), paratype Guevara 1225 (ITCV 1225)

Macromorphology. Fruiting bodies 5-18 x 6-15 x 5-10 mm, irregular, globose or subglobose. Basidiomes surface white, pale tan to brownish, mottled dark brown with pale areas when handled and dried, smooth when fresh, much wrinkled when dried, with some white mycelial strands, soil, and organic matter adherent to surface. Peridium <0.5 mm wide, somewhat separable. Hymeneal gleba olive green when young, green-brown to dark brown when mature, nearly black when dried, locules ellipsoid to elongate, stuffed with spores, columella absent when young, thin dendroid in mature, gelatinized, grayish. Rhizomorphs few, small, white attached at base, brownish when handled or when herborized. Odor organic solvent-like. Taste not recorded. *Macrochemical characters.* Positive reaction with KOH (5%), brown to blackish on the surface of dried specimens.

Micromorphology. Peridium 70-400 μm wide, two-layered. Epicutis 45-175 μm wide, usually on the thinner side with some areas with wart-like protrusions, of septate hyphae, thin-walled, pale yellow-brown to yellow-brown in KOH, repent hyphae 4.5-6.5 μm broad, occasionally inflated cells up to 18 μm broad, with interspersed small crystalline particles scattered across the layer, subcutis 110-135 μm wide, of septate, thin-walled, hyaline in KOH, interwoven to subparallel or cross-weaved hyphae, 6.5-11.0 (-15.0) μm wide. Mycelial strands on peridium of dark brown, filiform, branched hyphae, 2-3 μm broad, encrusted with small crystalline particles, clamp connection present. Trama 37-112 μm wide, hyaline in KOH, thin-walled, compactly interwoven to parallel hyphae, 2-5 μm wide, in a gelatinized matrix, clamp connections present. Basidia not observed. Spores without utricle and hilar appendage 10.5-12.3 x 5.3-7.0 μm, mean = 11.3 x 6.0 μm, with utricle and hilar appendage (12.3-) 13.2-14.0 x 7.9-9.7 (-10.5) μm, walls up to 1 μm thick, oblong fusoid, ellipsoid, symmetrical, smooth when young, spinny within the utricle when mature and not encompassing hilar appendage, often not equally inflated, rarely encompassing entire spore, commonly laterally inflated

up to 5 (-6) μm broad, hyaline to yellow-orange singly, pale brown in mass in KOH, inamyloid, non-dextrinoid in Meltzer reagent.

b) Distribution, habit, habitat, and ecology

México, Michoacán, in the Trans-Mexican Volcanic belt, hypogeous, solitary to groups, under *Quercus castanea* Muhl. *Q. obtusata* Bonpl., *Q. magnoliifolia* Née, *Q. rugosa* Née, *Pinus leiophyla*, *Pinus pseudostrobus* Schl. & Cham., and *Pinus michoacana* Mtz. at approximately 2160 m elevation, September and October.

c) Specimens examined

State of Michoacán, MÉXICO, locality Puerto Madroño; ejido Atécuaro, Municipality of Morelia, 19° 32' 113", 101° 12' 5", 18 Oct. 2011, *G Guevara 1211* (*ITCV 1211* holotype), *G. Guevara 1225* (*ITCV 1225* paratype), *G. Guevara 1218* (*ITCV 1218*). Ichaqueo, 20 Sept. 2014, *V Gomez-Reyes 863, 877*, (EBUM paratype).

Observations. *Pterosporomyces herrerae* is recognized morphologically by its olive-green to the brown-green color of the gleba and spores with a distinctive inflated, wing-like appearance to the utricle (inflated up to 6 μm), associated with *Quercus* spp. and ITS, ATP6 gene variation. *Restingomyces reticulatus* is similar to *P. herrerae*, but the former has reticulated basidiospores. This novel taxon also resembles to *Hysterangium inflatum* Roadway, a member in the family Hysterangiaceae with olive-green, brown to black gleba but differs by presenting smaller spores of 9-12 x 8-9 μm including ornamentation with utricle up to 2.5 μm on the side, ectomycorrhizal associated with *Eucalyptus* spp. and very distant genetically in ITS and ATP genes. Similarly, *Hysterangium stoloniferum* Tul. & C. Tul. resembles *Pterosporomyces herrerae*, but the former presents bigger spores of 17-21 x 6-8 μm and a narrow adnate rugose utricle.

Discussion. The study of hypogeous fungi has been very limited in Mexico (Cázares et al. 1992; Trappe & Guzman 1971). However, in 2016 *Aroramyces balanosporus* and *A. herrerae* were proposed as new taxa based only in macro (brown to blackish gleba) and microscopic (fine spines within the utricle) morphology without molecular support (Guevara et al. 2016; Castellano et al. 2000). Posteriorly, an ATP6 and ITS gene analysis were performed on these species to confirmed their novel status. The analysis confirmed the novel status of *A. balanosporus* (Genbank MK811031) as expected (Peña-Ramirez et al. 2019). However, the phylogenetic study surprisingly revealed that *A. herrerae* belongs to the evolutive basal family Trappeaceae and *Trappea darkeri*, *Restingomyces reticulatus*, and *Phallobata alba*. Thus, *Aroramyces herrerae* was transferred from the Hysterangiaceae to *Pterosporomyces* within the family Trappeaceae, an ancestral taxon in Phallales. The basidiomata of *Pterosporomyces herrerae* in cross-section are similar to those in *Restingomyces reticulatus* having both brown jelly dendroid gleba as seen in *Aroramyces*, but differs in the reticulate spore ornamentation present in *Restingomyces*; in contrast, *Pterosporomyces herrerae* shows utricle (wing) spores up to 5(6) μm tall. It seems to be that the utricle is a convergent evolutive feature similarly observed in other hypogeous species such as *Austrogautieria* in the family Gallaceaceae, which possesses longitudinally ridged spores alike to those in *Gautieria* (Hosaka et al. 2006). Similarly, the ITS and ATP6 genes analysis showed that *Pterosporomyces herrerae* along with *Restingomyces reticulatus* is ancestral of epigeous related genera such as *Clathrus*, *Phallus*, *Dictyophora*, *Mutinus*, among other stinkhorn taxa that evolved from hypogeous gasteroid forms in the Phallales clade in agreement with Hosaka et al. (2006) and Sulzbacher et al. (2016). Fig. 1 & 2.

With these results, the members of the family Trappeaceae increase to five, *Trappea darkeri, T. phillipsii, T. pinyonensis, Restingomyces reticulatus*, and *Pterosporomyces herrerae*, hoping to answer the question, "how many more taxa await to be described from the Neotropical Forest ?"

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Current Approach to Solving the Problem of the Mind-Body

By Vahram R. Sargsyan & Maia E. Hovsepyan
International Academy of Neuroscience

Abstract- The article presents a new and effective way to solve a psychophysical problem or the problem of the mind-body relationship. The psychophysical problem is a scientific task of a universal human scale, which requires a revision of the fundamental foundations of modern science and creating a new scientific and philosophical concept. The scientific article presents some of the results of our previous work: the place and function of viruses in nature, a new classification of the genome, and a nano-model theory of the functioning of the genome. These made it possible to revise the cell theory, to understand the mechanisms of the formation of human higher nervous activity, and to formulate a new scientific and philosophical concept of the universe. Understanding the mechanisms of interaction between mind and body will contribute to the intensive development of the health care system, education system, psychology, neuro-linguistics, sociology, and many other practical areas.

Keywords: *psychophysical problem, mind, body, viral theories, cell theory, nano-model genetic theory, the concept of the unity of the universe.*

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Vahram R. Sargsyan [α] & Maia E. Hovsepyan [σ]

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Keywords: psychophysical problem, mind, body, viral theories, cell theory, nano-model genetic theory, the concept of the unity of the universe.

I. Introduction

A psychophysical problem ("mind-body problem") is an issue of mental- physical phenomena. There are no convincing hypotheses in science explaining how objectively recorded brain processes generate a subjective psyche devoid of the attributes of materiality.

Until now, science has not been able to formulate a sufficiently substantiated working theory explaining the occurrence of mental phenomena; therefore, in this review scientific article, based on the results of our previous works, we will try to solve the problem of the mind-body relationship (psychophysical problem).

Our scientific approach to solving a psychophysical problem involves interdisciplinary and theoretical research using scientific meta-analysis. It is necessary to revise some fundamental knowledge in biology, and to formulate a new and effective scientific and philosophical concept of the universe. Since 2018, we have already taken the first steps to solve this problem. We started by defining the functions and place of viruses in nature and rehabilitating the classical cell theory [19]. Further, a nano-model theory of the functioning of the genome, a new classification of the genome, and the classification of viruses according to V.Sargsyan were formulated[18]. Thanks to the above achievements in theoretical and fundamental biology, we have 14 viral theories and a new genetic theory.

Based on this, we can understand and substantiate the mechanisms of the formation of human higher nervous activity [12]. As a result, this achievement became the scientific basis for creating our scientific and philosophical concept of the unity of the universe [17].

Several more scientific theories have already been created and logically substantiated. For example, the theories of the big biological explosion, integration, hierarchical universe functioning and, knowledge about the biocommunication system of humans (and other multicellular biological species) [20], [15]. However, we will not consider these theories since they are practically not needed to solve a psychophysical problem within the framework of this scientific article.

So, let's start by defining the true place and role of viruses in nature. Next, we present our rehabilitated classical cell theory [16], new genome classification, and nano-model theory of genome functioning [14]. The presentation of this scientific knowledge is very important for a clear understanding of the mechanisms of the formation of human higher nervous activity. It is impossible to solve the problem of the mind-body relationship. Let us also dwell in detail on our scientific and philosophical concept of the unity of the universe.

II. Scientific Meta-Analysis in Virusology and Viral Theories

a) Scientific meta-analysis in virology

Viruses are one of the biggest mysteries in modern biology. A virus (Latin virus – poison) is a non-cellular infectious agent that can reproduce only inside living cells. Viruses infect all types of organisms, and we have already described about 5-6 thousand types of viruses. However, we believe that there are more than one hundred million of them. Viruses are the most abundant biological form and are found in almost every ecosystem on planet Earth.

However, given the current level of knowledge in various fields of science, there is a need to revise some of the fundamental ideas about the true place and

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functions of viruses in nature. For this, a meta-analysis of various reliable scientific data has been perfected.

For the first time, the existence of a virus (as a new type of pathogen) was proved in 1892 by the Russian scientist D.I. Ivanovsky.

Five years later, in the study of diseases in cattle, asimilar filterable microorganism was isolated. And in 1898, when D. Ivanovsky's experiments were reproduced by the Dutch botanist M. Beijerinck, such microorganisms were called "filterable viruses."In an abbreviated form, this name began to denote this group of microorganisms.

In 1901 they discovered the first human viral disease – yellow fever. The American military surgeon W. Read and his colleagues made this discovery. In 1911, Francis Routh proved the viral nature of cancer – Rous sarcoma.

Based on the knowledge of viruses in the nature, it will be possible to study the fundamentals of life and its manifestations. According to our opinion, that is the answers lie to many scientific and practical problems of modern humanity.

So, we have formulated 14 new viral theories, each of which reveals one of the functions of viruses in nature (Table 1).

Table 1: Viral theories

1.	The Viral Theory of the Electromagnetic Reception
2.	The Viral Theory of Biocommunication
3.	The Viral Theory of Signal Transduction
4.	The Viral Theory of The Functioning of the Energy system of Cell
5.	The Viral Theory of The Functioning of The Immune System
6.	The Viral Theory of Information Perception
7.	The Viral Theory of Memory Formation
8.	The Viral Theory of The Functioning of The Somatic Nervous System
9.	The Viral Theory of The Functioning of The Autonomic Nervous System
10.	The Viral Theory of The Functioning of The Endocrine System
11.	The Viral Theory of the Functioning of The Cardiovascular System
12.	The Viral Theory of The Functioning of The Reproductive System
13.	The Viral Theory of The Evolution of The Organic World and Homo Sapiens
14.	The Viral Theory of Aging

But first, here are some data that confirm the consistency of viral theories suggested by us.

1. To date, we have identified and investigated approximately 5-6 thousand types of viruses, although we assume that there are more than one hundred million of them. Why is such huge biodiversity necessary? The answer to this question lies in our theories. Here we will only note that nature does not create anything in vain.
2. Viruses differ in a special way of reproduction: in the cell, the nucleic acids of viruses and their proteins synthesize separately, they are assembled into viral particles.
3. The genetic apparatus of viruses can easily mutate and thus change their "behavior."
4. Viruses are widespread, capable of infecting almost all representatives of Flora and Fauna, even many microorganisms. Many viruses can infect one or many cell types of various cellular life forms.
5. There are 5×10^{7} bacterio phages per milliliter of ocean water.
6. According to geneticists, 1/3 of the human genomeconsists of so-called "junk genes" ("non-coding DNA"). It is also known to be a space where one can find viruses.
7. We can get the biological information required for the growth, development, and maintenance of the organism's functions in the genome. It is a known fact that human genes contain 100,000 DNA fragments of endogenous retroviruses, which make up 5–8% of the human genome.
8. Viruses, their derivatives, and closely related structures make up at least 43% of the human genome[4].
9. According to recent data, half of the human genome is made up of the DNA of viruses. In fact, a person is a product of symbiosis, that is, the relatively peaceful coexistence of a person and a virus", says

Frank Ryan. - "If it weren't for them, there would be no us, or we would be completely different"[3], [4].

10. We know that even in a healthy body, numerous viruses live without causing any particular harm.
11. Viruses promote the fertilization process and the formation of the placenta in humans successfully, in fact, we owe our existence as a biological species to the functioning of viruses.
12. Why do children under a certain age have almost no developed long-term memory function? The fact is that only by 1-2 years of age a person has formed that necessary arsenal of viruses, the viral composition that makes it possible for a person to exercise this unique opportunity – to remember, archive information for a long time. Human Virom is unique for each person, and this can explain the individuality of the cognitive abilities of each person.

Thus, we concluded that viruses are migrating organelles of eukaryotic cells. They are part of us – cellular life forms and have multiple functions. Viruses are not independent forms of life, and the cellular theory evidences it.

We describe three principles of classical cell theory below:

1. All living organisms consist of one or many cells.
2. The cell is a structural and functional unit in organisms.
3. A cell arises from the division of the pre-existing cell.

Many scholars dispute the first of these principles. Because non-cellular objects such as viruses are considered life forms.

However, according to our viral theories, the cell theory is scientifically completely sound[16].

In 1898, when reproducing the experiments of D. Ivanovsky, the Dutch botanist M. Beijerinck first used the term "virus," as he called such microorganisms "filterable viruses." One hundred twenty years later, relying on the above, in 2018, we proposed to replace the term "virus" with the term bio communicator, which is more consistent with the functions they perform.

If we consider the place and role of various living organisms at the level of the planet Earth (biocenosis), then only animals (including Man),plants, fungi, archaea, and bacteria are living biological organisms. And viruses are not living biological species, since, for their vital activity (development and reproduction), the presence of a cell is necessary. In fact, at this stage of the evolutionary development of the organic world of the planet Earth, this is the case. Viruses are not non-cellular life forms; they are only components of cells (migrating organelles of eukaryotic cells) but very important. In other words, it is due to the functional activity of viruses (bio communicators) that the cell is "revived." Without viruses and/or entero viruses, a cell is an almost "dead" conglomerate of organic matter.

Thus, the smallest unit of life is a cell with viruses, enteroviruses, and other mobile genetic elements.

The following are only those viral theories necessary for understanding the functioning of the higher nervous activity of man, and solving the psychophysical problem. For the full acceptance of viral theories, it is necessary to consider the new genome classification, which we present below.

b) The viral theory of information perception

Viruses of humans, animals, and other organisms play a leading role in the process of information perception. The information we receive from the sensory organs (receptors) goes to the central nervous system, where they transfer into electrical impulses. And the process of electrical activity in the central nervous system leads to the formation of a sequence of nucleotides of DNA/RNA-containing viruses (biocommunicators) and also changes their configuration (3D) and motor activity (it turns out 4 D). Microtubules of the cells play an important role in this process, which also form an antenna on the cell surface. Microtubules are the transport infrastructure for DNA and RNA-containing biocommunicators (viruses). Thus, biological nano-models of various objects "noticed" by the body's receptors are created in neurons and, consequently, in the brain. A person can also think figuratively. Each thought can correspond to one specific "virus", and the emotion is already a whole group of "viruses". Often, a "ready-made virus" or a group of the moutside (a thought or emotion of another organism) can enter the brain and thus carry out communication. This canconfirm the fact known to science that viruses can control the consciousness of various species of animals and humans. This, in turn, creates the prerequisites for the formation of long-term memory.

Since the perception process begins with the receptor, we should note that the functional activity of a single receptor also depends from the activity of biocommunicators.

c) The viral theory of memory formation

The process of constant electrical activity in the central nervous system during reverberation leads to structural changes in DNA/RNA-containing viruses (biocommunicators) of humans and animals. All these changes in neural responses are called consolidation, and biocommunicators are the material carriers of information that enters long-termmemory. The formation and further storage of biological nano-models take place. Further, the expression of these genes leads to the extraction of information from long-term memory. In the human body herpes viruses perform the function of the information carrier in long-term memory.

Herpes viruses (lat. Herpesviridae) – a large family of DNA-containing viruses that infect most of the population of our planet.

As of May 2016, the International Committee on Virus Taxonomy (ICTV) has registered 86 species. A distinctive feature of viruses of this family is the presence of the virus in the cells latently, persisting, indefinitely long time, without clinical manifestations. Therefore, according to our theories, at this time, they perform the functions of the higher nervous activity of man described by us.

Memory is localized not only in certain areas of the brain but also distributed throughout the body. However, the brain of the body plays a key role and the place of storage of memory. Brain structures are responsible both for the formation of memory in the DNA (possibly also in RNA and in some proteins) of biocommunicators, as well as for the processes of implementing the information contained in these molecular memory carriers.

d) The viral theory of the somatic nervous system functioning

Viruses of the human and animal bodies play a leading role in transforming the will and intentions of the body into movements. They store all the acquired skills of the body during life in the form of changes in the structural and spatial organization of the genetic material of bio communicators in the long-term memory of humans or animals, in the future, if necessary, the expression of these genes occurs. It is thanks to the above-described molecular mechanisms that the body has the opportunity to exercise motor and speech activity and subordinate the functioning of the somatic nervous system to its will. This can explain the formation of linguistic abilities in humans. And, therefore, we must search for the genes responsible for speech in the acquired genome (biocommunicator genes). For more information, see our nano-model theory of genome functioning, presented below.

e) The viral theory of the functioning of the autonomic nervous system

Human and animal viruses also play a leading role in the functioning of the autonomic nervous system. Many innate and acquired skills of the organism during life are represented in the form of changes in the structural and spatial organization of the genetic material of bio communicators in the genetic/long-term memory of the organism and in future, if necessary, the expression of these genes occurs. Thus, the autonomous functions of the human and animal nervous systems, which are vital for the body, are provided. It is thanks to the above-described molecular mechanisms that the body has the opportunity to better adapt to changing environmental conditions. However, we should take into account that the genes of the main genome also carry a significant burden in ensuring the functioning of the autonomous nervous system.

III. Modern Genome Classification and New Genetic Theory

a) The main and acquired genome

Genome is a set of hereditary material contained in the cell of the body. The genome contains the biological information needed to build and maintain the body. They are built from DNA. There is also another definition of the term "genome", according to which the genome is a set of genetic material haploid set of chromosomes of this species. According to classical data, in humans (Homo sapiens), 23 pairs of chromosomes represent the hereditary material of the somatic cell (22 pairs of autosomes and a pair of sex chromosomes) located in the nucleus, and the cell also has many copies of mitochondrial DNA. Autosomal chromosomes, sex chromosomes: X and Y, and human mitochondrial DNA contain approximately 3.1 billion base pairs.

In many species, only a small fraction of the total genome sequence encodes proteins [21]. So, only about 1.5 % of the human genome consists of protein-coding sequences of DNA-exons (DNA fragments, copies of which make up mature RNA – mRNA). The reasons for the presence of such a large amount of non-coding DNA in eukaryotic genomes and the difference in genome size (C-value) is one of the unsolved scientific mysteries; research in this area also points to a large number of fragments of relict viruses in this part of the DNA.

We were reading the sequence of letters in the human genome – the sequence of four types of nucleotides – does not show how the genome works. They are not a decoding of the genome, but, on the contrary, an encrypted text, the meaning of which we do not yet understand. According to modern classical concepts the "main intrigue" is that all the body cells have the same DNA, which contains information about the encoding of proteins. But the cells of different tissues: muscle, nerve, or blood cells are not similar to each other, although they arise from the same cell – the zygote. In the process of development, each organism goes from a fertilized egg (zygote) to an adult and at the same time changes all the time, but the genome does not. The work of genes is not the same at different stages of ontogenesis. How all this is regulated is "the main mystery of life."

Below is the scientific position proposed by us, which allows us to explain" the basic puzzle of life."It became possible by understanding the formation and functioning of the acquired genome in ontogenesis. In other words, the plasticity of the genome acts in nature[18].

Below is the classification of the genome according to Vahram Sargsyan.

The main genome is a set of all the genes received by the body from the egg and sperm due to fertilization (nuclear, mitochondrial, plastid). It is the *vertical transfer of genes.*

The acquired genome is a set of all the genes received by the body during the embryonic and postembryonic periods from the migrating organelles of the cells –biocommunicators (viruses) in the form of DNA and RNA molecules. It is important to note that the formation of the acquired genome occurs based on existing genes (biocommunicators) under the influence of, for example, electrical processes occurring in the nervous system of the body (see viral theories of information perception, the formation of long-term memory and the functioning of the somatic nervous system)[19]. They occur as a result of the activity of the body's sensory systems. Electromagnetic radiation influences on the formation of the acquired genome (for example, the ultraviolet spectrum of radiation) of natural and artificial origin. For more information, see the viral theory of electro-magneto reception. It turns out that all changes occurring in the external and internal environment of the body are fixed (cause changes) in the acquired genome. Those that are important we can find in the body's long-term memory reserves. This is *horizontal gene transfer.* This genome is individual for each somatic cell. If the process takes place in gametes, then endo-viruses genes can be formed, which, as is known, are already inherited from generation to generation.

Plasmids play the role of biocommunicatorsin single-celled prokaryotic organisms (for example, in bacteria). However, they are not able to perform all the functions inherent in biocommunicators. Below we will draw your attention that plasmids carry out active horizontal gene transfer in prokaryotes. The analogs of plasmids for eukaryotes are viruses. Bacteriophages (bacterial viruses) are not biocommunicators (migrating organelles) of bacterial cells. The fact that they forcibly introduce their genetic material into the bacterial cell speaks about it. Thus, bacteriophages are biocommunicators of various eukaryotic cells (their migrating organelles), which carry out and ensure the regulation of biochemical processes in bacterial cells and their number (on the part of the host of this biocommunicator).

It is interesting to imagine the following analogy: *plasmids are derivatives of the bacterial chromosome, and viruses are derivatives of the cell nucleus.* Although from an evolutionary point of view, the cell nucleus arose from a virus.

According to the above information about the genomes, we can give a new definition of the term "phenotype". *A phenotype* manifestsaset of genes obtained by vertical and horizontal gene transfer and the result of their interaction. Therefore, the phenotype is the expression of the genotype. Naturally, recombination and mutation variations have their contribution.

Throughout its life – from the moment of fertilization of an egg (formation of a zygote) to death the organism has the opportunity to enrich its genotype by increasing the proportion of the acquired genome. It takes place by horizontal gene transfer. The information received by the sensory systems (receptors) of the body about the external and internal environment actively affects the change (enrichment or impoverishment) of the acquired genome of the body. As a result, the phenotype changes. However, these changes affect only the genes of cells and tissues of the body. For example, the cells of the central nervous system of humans or animals, the immune system, or the liver cells change. If the changes affect the germ cells, then new signs and properties will be inherited, from generation to generation.

According to the "additional" position of the cell theory, some cells of multicellular organisms, such as a zygote, are totipotent. That is, they can give rise to the entire organism, having the genetic strength of all cells of this organism, that is, equivalent in genetic information, but differing from each other in different expressions of different genes, which leads to morphological and functional diversity - to differentiation.

Our opinion is radically different from the above "additional" position of the cell theory. Bearing in mind that there is an acquired genome(other than the main one) – cells in the process of ontogenesis of the organism no longer become equivalent in genetic information and therefore differ from each other not only by different expression of different genes but also by a different gene set of the acquired genome. This is of crucial importance in the morphological and functional diversity (differentiation) of cells. It is a necessary condition for the appearance of highly specialized cells of multicellular organisms (in humans, for example, during the perinatal and postnatal periods of ontogenesis). This feature is not taken into account by many bio-engineers when obtaining tissues and organs *in vitro* for their further use for medical purposes (transplantation of tissues and organs) and therefore cannot get many types of human tissues and organs that are fully functioning and suitable for transplantation to the recipient. To date, no scientist in the world has managed to obtain a human brain *in vitro*, and it will never work if you do not take into account the presence of the acquired genome of the cell, because in complex functioning organs (for example, the brain), horizontal gene transfer plays a key role.

Thus, considering our classification of the genome into main and acquired, it is possible to achieve a complete understanding of the various biological processes occurring at the genetic, cellular

(biochemistry, biophysics), and organizational (physiology) levels of the organization in norm and pathologies.

b) Brain plasticity and genome plasticity

According to the above, the genome of an organism is an actively and dynamically developing system throughout the entire period of ontogenesis. Still, for the greater credibility of this thesis, we will give an analogy with the plasticity of the human brain below.

Neuro plasticity is a property of the human brain, which consists of the ability to change under the influence of experience, as well as to restore lost connections after damage. We start to speak about this property relatively recently. Previously, we generally accepted that the brain remains unchanged after its formation in childhood.

The discovery that thoughts can change the structure and function of the brain, even in old age, is the most important achievement in the field of neurology over the past four centuries. Norman Doge offers a revolutionary view of the Human Brain [6].

The brain consists of interconnected nerve cells(neurons) and glial cells. The process of learning can occur through changes in the strength, the emergence or destruction of connections between neurons, as well as the process of neuro genesis. This is due to neuro plasticity.

During the 20th century, we generally accepted that the structure of the brain stem and neocortex remained unchanged after the completion of formation in childhood. It meant that learning processes there can only proceed by changing the strength of connections, while the areas responsible for memory processes (the hippocampus and the dentate gyrus) and preserving the ability to neurogenesis throughout life are highly plastic. This opinion is changing due to new research, which claims that the brain retains its plasticity even after the period of childhood.

Neuroplasticity can manifest at different levels, from cellular changes in the brain, up to large-scale changes with reassigning roles in the cerebral cortex as a response to damage to specific parts. Modern medicine widely recognizes the role of neuroplasticity and is also used as a phenomenon in memory development, learning, and repair of the damaged brain. William James was the first to propose the idea of "plasticity" of the brain in 1890, but everybody ignored it for the next fifty years. The Polish neurophysiologist Jerzy Konarski was the first to coin the term "neuroplasticity".

One of the fundamental principles of neuro plasticity is the phenomenon of synaptic pruning: in the brain, there is a constant process of destruction and the creation of connections between neurons. Recall that *synaptic pruning*– "neuronal pruning"- reducing the number of synapses or neurons to increase the efficiency of the neural network, removing redundant connections. Pruning involves both axon pruning and dendrites pruning.

Thus, scientists accepted *the fact of neuro plasticity.* Why not accept *the fact of the plasticity of the genome* (the processes of the origin/destruction of genes in the process of ontogenesis of the organism) and use this understanding of the fundamental biological processes to explain the numerous processes that occur in nature and are "riddles" of science. I suggest that geneticists do not make the "mistakes" of neuroscientists and timely review and determine the question of the plasticity of the genome of the organism, which will have an impact on the development of biological sciences and numerous practical areas of knowledge.

IV. Nano-Model Theory of Genome Functioning

According to our nano-model theory of genome functioning, a DNA molecule stores biological information not only in the form of a genetic code, consisting of a sequence of nucleotides but also in the form of a spatial-structural organization. It means that the information component is hidden not only in the primary structure of the organization of DNA molecules but also in II and III structures. These are special kinds of biological nano-models.

RNA molecules can perform a similar function in nature, as well as, to some extent, the protein molecules.

DNA contains information about the structure of various types of RNA and proteins[1]. But this does not mean at all that the DNA molecule cannot carry out numerous independent biological functions that ensure the vital activity of living systems.

Almost all genes function like nano-models. However, proceeding from the fact that many genes of the genome are localized in the cell nucleus and must function in the cytoplasm or outside the cell, nature has created the processes of transcription and translation known to modern biology. The protein has a bulky structure (definite shape) due to its II, III, and sometimes also IV structure. It is known, for example, that an enzyme protein has an active center that functions according to the principle of a key to a lock. Depending on its form, it has had a functional activity. The DNA molecule (it's specific part – the gene) also has II and III structures; that is, it is not just a linear molecule consisting of nucleotides.

The whole point of the processes of transcription and translation comes down to creating a copy of the nano-model (DNA gene) in the form of ribosomal RNA (rRNA), transport RNA (t-RNA), or messenger RNA (m-RMK). In mRNA, the process of biosynthesis of the polypeptide chain (primary structure

of the protein) follows – translation on polyribosomes in the cytoplasm of the cell. Ready-made copies of DNA nano-modelscan function outside the cell nucleus of a eukaryotic cell. As is known, protein biosynthesis takes place based on mRNA information; rRNAs are part of ribosomes that are actively involved in the biosynthesis of proteins (primary structure). The delivery of amino acids to the site of protein synthesis requires a t-RNA. Exons – codingregions and introns –non-coding regions make up many genes. When transcribed from a gene, RNA carries both exons and introns. In the process of splicing, introns excise, and exons stitch together to form a mature mRNA. Further, the polypeptide chain of the protein synthesized during translation will acquire a spatial-structural organization and become a functional product of full value.

Thus, here we have presented in a simplified form the process of forming copies of nano-models based on biological information embedded in the DNA of genes.

And some of the genes (mainly "junk genes" and some genes of the acquired genome) do not need such intermediary processes. Therefore, there is no need for transcription and translation. The genes can leave the cell nucleus and cells. This applies to biocommunicators(containing DNA and RNA). For efficient functioning and transportation a protein shell – a caps id covers them. Along with biocommunicators, there are transposons. *Transposons* are DNA regions of organisms capable of movement (transposition) and reproduction within the genome. Transposons, also known as jumping genes, and are examples of mobile genetic elements. Transposons are by no means "genetic parasites."

That is why the overwhelming part of the human genome is non-coding protein.

This is essentially the "language of the genes."

Below we will show the consistency of the nano-model theory of genome functioning in case of DNA molecules using the example of the spatial-structural organization of RNA molecules, which clearly shows the importance of their volumetric (3 D) organization for performing biological functions.

The sequence of nucleotides (primary structures) determines the secondary structure of RNA, which determines the tertiary structure of loops consisting of unpaired bases and open sections of the chain, held in some kind of fixed state, about each other. Such bare areas are potential points through which t- RNA can specifically interact with other nucleic acids (for example, the interaction of t-RNA with rRNA or m-RNA), and they contain new possibilities used in the processes of encoding or transferring information in living systems that are not inherent in destructured single-stranded strands or ideal double helices. The same is true for the three-dimensional structure (3D) of DNA molecules. Like t-RNA, its function largely depends on the three-dimensional structure.

Scientists have discovered an unusual form of DNA in human cells. Its shape is not classical but in the form of a knot. It became known that the previously discovered spiral (more precisely, in the form of a double screw) DNA structure is not the only one in our body. This type of DNA could only have artificial origin. The structure in its structure resembles a knot of four threads, connected in a very intricate manner. In addition, the knotty structure of DNA is capable of forming and decaying during a person's life.

Let's discuss the issues of epigenetics, which have become an excellent confirmation of our genetic theory. Arthur D. Riggs introduced the most commonly used definition of epigenetics in the 90s of the XX century and formulated it as "the study of mitotically and /ormeiotically inherited changes in gene function that the changes in the sequenced DNA cannot explain.

The molecular basis of epigenetics is quite complex even though it does not affect the primary structure of DNA but changes the activity of genes. This explains the expressiveness of the genes necessary for their activity in differentiated cells of a multicellular organism. A feature of epigenetic changes is that they don't disappear during cell division. We know that most epigenetic changes are manifested only within the life of one organism. At the same time, if a change in DNA occurs in a sperm or egg, then some epigenetic manifestations can be transmitted from one generation to the next.

Our nano-model theory of the functioning of the genome perfectly reflects the numerous processes occurring at both the cellular and organismal levels. Genes functioning according to the principle of nano-models are, in fact, a kind of copy of the macrocosm. Depending on the adequacy of its reflection at the cellular level, it is possible to judge the level of quality of information perception from the body. The well-known expression, "The Brain is in the World, and the World is in the Brain", becomes fully explainable thanks to the above scientific data.

a) Functioning of protein-coding genes in the light of the modern genetic theory of genome functioning

We know that in the human genome, a small part of genes (according to some sources, 1.5%) are protein-coding. But even the functional activity of these genes cannot be logically and substantiated scientifically within the framework of only the concept of the genetic code.

For protein biosynthesis, the following processes are necessary:

1. *Transcription* (from Latin transcription – rewriting) – the process of RNA synthesis using DNA as a matrix, occurring in all living cells. In other words, it

is the transfer of genetic information from DNA to RNA.

2. *Formationofmature mRNA.*
3. *Translation* (from Latin translation – transfer, movement) – the process of protein synthesis from amino acids on the matrix of informational (matrix) RNA (i-RNA, m-RNA), carried out by the ribosome.

At the stage of mature mRNA formation, the principles of the nano-model theory of genome functioning operate.

In addition, polyribosomes synthesize a protein that is not ready in a functional aspect (with a secondary, tertiary, or even a quaternary structure), but only its primary structure (a polypeptide chain consisting of the corresponding amino acid residues). After leaving the endoplasmic reticulum, the production of ready-made and functionally complete proteins is carried out in the Golgi apparatus of the cell. This process also takes place due to the nano-model organization of the genome.

Thus, we can conclude that information is not completely embedded in the gene responsible for its synthesis; moreover, mRNA does not carry all the information. The rRNA, included in the protein synthesizing ribosome, can carry part of the decisions about the biological activity of the produced protein. The genes responsible for the synthesis of rRNA in the cell nucleus (in eukaryotic cells) in fact are regulatory in the production of a protein, and the protein that synthesizes the gene carries "raw" information about the sequence of amino acid residues in the produced protein.

According to our viral theories, biocommunicators contained in large quantities in the Golgi apparatus control the processes of formation of already functionally active proteins (4D, a bulky macromolecule in motion).

V. Differentiation of Cells in Multicellular Organisms: Formation of Higher Nervous Activity and Immune System in Human

An organism (cell) has a main and acquired genome. This fact sheds light on many currently unsolved scientific issues and, first of all, on aspects of the genetic level of the organism's development .In turn, it becomes clear how and what molecular mechanisms carry out the differentiation of cells in multicellular organisms and individual development (ontogenesis). Scientifically fully substantiated, for example, the emergence of highly specialized functions in neurons of the human brain and the manifestation of various functions of higher nervous activity at the organismal level.

Therefore, it is not surprising that geneticists studying the human genome struggle to find the genetic traits that led to the increase in the brain and, possibly, its more efficient work. We pin particular hopes on the comparison of the human genome with the chimpanzee genome, which allows us to immediately exclude from consideration that 98%of the genome that are identical in our species. Somewhere out there, in the remaining two percent, the secret of human uniqueness is encrypted. It remains to understand where and how.

Nowadays, the biological theories we have proposed are capable of explaining all this scientifically. The behavior and mental abilities of humans are at a qualitatively new level compared to those of the monkeys. It is reasonable to assume that these differences are genetic.

As a result of serious research, scientists have proven that during the origin of man, there was no universal and large-scale accumulation of amino acid changes in the genes involved in the work of the nervous tissue.

Yet, after all, people are still smarter than chimpanzees, and our relative brain size is larger! "As cattering of genes encode the development of our mental abilities(changes in their sequence or level of expression), and these changes do not affect the average characteristics of all genes of the nervous system".

And according to our proposed classification of the genome(based on our viral theories) and the nano-model theory of the functioning of the genome, we can explain all this very logically and scientifically. The thing is that modern classical genetics study only the main genome of the organism, that is, the genes obtained from the parental germ cells (egg and sperm). However, for the functioning of highly specialized cells (such as, for example, brain neurons), those genes that were received from parents by vertical transmission (from germ cells as a result of the formation of a zygote)are not enough. According to our viral theories, for a full-fledged perception of information, of long-term memory and, the functioning of the somatic nervous system, the body in the process of ontogenesis must additionally receive a set of genes through horizontal gene transfer. Which occurs in the perinatal and postnatal periods of the individual development of the organism. For most of the highly specialized cells in the human body (or other multicellular organisms) to begin to perform their intended functions fully, it is not enough just to "switch on" (express) groups of genes and "switch off" other groups of genes of the genome. If everything were so simple, the geneticists would have long ago found many genes from the main human genome, which are inherent only in us (humans) and distinguish us, for example, from monkeys. The fact that a person in terms of his level of development issuperior to other species of animals is beyond doubt. And these differences are due precisely to the receipt of additional genes already in the process of human ontogenesis. The human genome creates the prerequisites (favorable conditions) for the

implementation of this process, and this requires a small number of genes. By the way, according to modern genetic research, this is what distinguishes us, for example, from chimpanzees in terms of the genome.

a) *Formation of the immune system as evidence of genome plasticity*

We know that during the formation of acquired immunity, the cells can acquire new genes that are not characteristic of the human genome. This process happens depending on the influence of the environment on the body – what viruses and foreign agents will inflect on the body during ontogenesis. After all, all sane scientists understand that at the moment of fertilization, it is not known yet in what conditions the individual development of the organism is proceeded. We inherit only a part of the immune system, and therefore the immune system of humans and many species is a dynamically changing system. And this is another confirmation of the inconsistency of the concept of totipotency of all cells or even some cells of a multicellular organism and indicates the validity of the genome plasticity.

Conclusions to this part of the scientific article

1. Along with the main genome, a cell has an acquired genome. In other words, the genome is plastic.
2. The functioning of the genome in the overwhelming majority of cases is based on the activity of biological nano-models.
3. The processes of cell differentiation in multicellular organisms, the formation of the functions of higher nervous activity in humans, and many other biological phenomena are closely related to horizontal gene transfer during the ontogenesis of the organism.
4. Taking into account our classification of the genome into main and acquired, as well as our nano-model theory of the functioning of the genome, it is possible to achieve a complete understanding of various biological processes occurring at the genetic, cellular (biochemistry, biophysics) and organismal (physiology) levels of the organization, inhealth and pathologies. Therefore, we can talk about another revolution in biology, which will affect such practical areas as medicine[9], [10], agriculture, bioengineering, ecology, psychology, sociology and the like[7], [8].

VI. Scientific and Philosophical Concept of the Unity of the World

Philosophy is a form of discovering of the world, which develops a system of knowledge about the most general characteristics, generalizing concepts and fundamental principles of reality (being) and cognition, human being, about the relationship between man and the world[5]. The tasks of philosophy throughout its history included both the study of the universal laws of the development of the world and society and the study of the very process of cognition and thinking. Among the ultimate philosophical questions are, for example, the questions "Is the world cognizable?", "Does God exist?","What is truth?", "What is Man?", "What is primary – matter or consciousness?" and others[2]. Here we discuss and try to give a scientifically substantiated answer to the question - "What is a primary matter or consciousness?" and to find out how appropriate this question is, if it is not artificial and unnecessary for the development of philosophy and science in general. It is very important for the further intensive and correct development of neurobiology.

For many centuries the materialists and idealists have been trying to find approaches to the structure of the universe. According to the materialistic approach, the matter is primary. According to the idealists the idea is primary.

Idealism is a mode of explanation that considers the spiritual to be before the material, while the materialists say the material to be before the spiritual. Idealism believes that everything material supposedly depends on something spiritual, while materialism claims that everything spiritual depends on the material.

Materialism seeks to explain these issues in terms of the material world, with the help of factors that can be checked, understood, and controlled.

For idealism, there is always a higher, supposedly more real immaterial world, which precedes the material world, is its ultimate source and cause, and to which the material world is subordinate. For materialism, on the contrary, there is only one world – the material world, the one in which we live.

Below are the presented main provisions of idealism and materialism, as well as their opposite.

We can formulate the main points put forward by any form of idealism as follows:

1. Idealism claims that the material world depends on the spiritual.
2. Idealism asserts that spirit, mind, or idea can and does exist separately from matter.
3. Idealism asserts that there is a realm of the mysterious and unknowable, "above" or "beyond" or "behind" that which can be established and known through perception, experience, and science.

a) *In turn, we can state the main provisions of materialism as follows*

1. Materialism teaches that the world is material by its very nature, that everything that exists appears based on material causes, arises, and develops by the laws of motion of matter.
2. Materialism teaches that matter is an objective reality that exists outside and independently of

consciousness and that the spiritual does not exist separately from the material. Still, everything spiritual or conscious is a product of material processes.

3. Materialism teaches that the world and its laws are fully cognizable, and although much may be unknown, there is nothing that we cannot cognize.

As you can see, all the basic tenets of materialism are opposite to the tenets of idealism. The opposition of materialism to idealism, expressed in its most general form, is not the opposition of abstract theories about the nature of the world but between different ways of understanding and interpreting any question. So, it is so important.

Based on the latest advances in cell biology and neuroscience, we propose a new scientific and philosophical concept of the unity of the universe.

Because new biological theories (viral and genetic theories) serve as a base for our scientific and philosophical concept, then the method we use to create new biological theories is the scientific method. Biological theories published in 2018 – 2019. To do this, we applied scientific meta-analysis. We analyzed the reliable scientific information, and based on the synthesis of this scientific information, we have new progressive and innovative scientific theories, and in the future, a new scientific and philosophical concept of the unity of the universe.

Everything is known to humanity and at the same time that a person can perceive with his senses (receptors) and through modern equipment can be material. And all the dreams, ideas of people that today the human senses(receptors) or modern technical means do not perceive are ideal. However, with the development of science, more and more concepts and phenomena pass into the material world. For example, with the discovery of electromagnetic radiation, it became clear that many previously mysterious-sphenomena have a material basis. With the development of genetics, it became known that the material carriers of genetic information are nucleic acids (DNA, RNA). With the development of microbiology and virology, the material causes (pathogens) of various infectious diseases, that we previously interpreted as the action of "evil spirits" on the body, became known.

Our works on neurogenetics and neurophysiology have shown that the DNA of viruses (biocommunicators) is responsible for the long-term memory and the formation of higher nervous activity in humans and animals, evidenced by our biological theories (the viral theory of information perception, the viral theory of memory formation, the viral theory of the functioning of the somatic nervous system, the nano-model theory of the functioning of the genome, and others). This discovery, together with our other biological theories, pushed us to create a new philosophical concept of the unity of the universe. In other words, what we previously considered ideal (thoughts, emotions, mind, and consciousness of a person) today we already begin to explain at the level of DNA/RNA macromolecules and proteins.

Thus, over time, everything, the ideal, is transformed into a material (Figure 1). However, if we hypothetically imagine that everything that exists in nature is material, then it, as a separate category, will automatically cease to exist for the simple reason that it will lose its opponent. Everything that we will have will become one. Therefore, the division into material and ideal will not make sense. And the question - "what is primary, matter or idea?" will lose its significance.

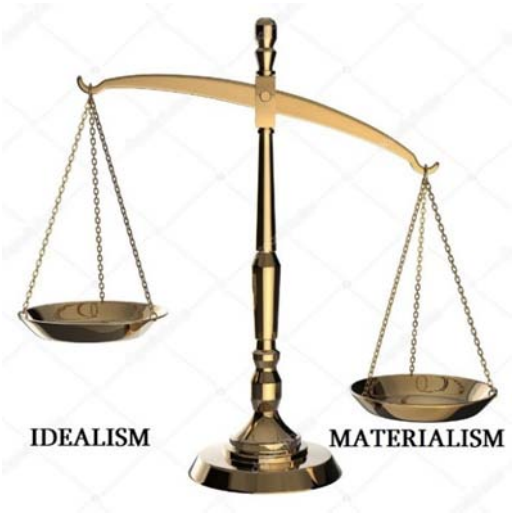

Figure 1: Philosophical balance of Vahram Sargsyan With the development of science, many "mysteries of nature" or "mystical" find their materialistic confirmation

It turns out that in NATURE, everything is ONE[13]. It indicates that the division into material and ideal was artificial and meaningless. In addition, the need of a person, to study all phenomena from the position of his feelings, resulted in such a division of the world, or in other words, the perception of the world from the side of a person. A person is the center of the Universe and the Judge, who determines what is considered material and what is ideal. After all, as noted above, "what a person can perceive with his senses (receptors) and through modern equipment is considered to be material." But any sane scientist and philosopher will agree that such an understanding of the universe cannot be objective. It is subjective because the main criterion is a person's feelings or his instrumental methods of registration, the data (indicators) of which are ultimately again evaluated and interpreted by a person.

The dualistic approach has also exhausted itself and is scientifically unsound.

VII. Conclusion

The struggle and contradictions between the materialistic and idealistic approaches with the emergence of new biological theories and the philosophical concept of the unity of the universe are over.

We can only talk about the existence of different hierarchical levels in a single universe. If the smallest unit is conventionally considered the electron, and the largest – the Universe, then these hierarchical levels (taking into account biological systems) can be conventionally represented as follows:

electron – atom – molecule – cell organelle – cell – tissue – organ – organ system – organism (for example, man) – society – biocenosis –biogeocenosis– planet Earth – Solar system – Milky Way Galaxy – Universe.

As noted at the beginning of the scientific article, the "mind-body problem" is an issue of the relationship of mental phenomena to physical ones. Thanks to the results of our many years of work and the creation of new biological theories and the scientific and philosophical concept of the unity of the universe, it became clear that the mind (mental phenomena) and the body (physical) are only different hierarchical levels in the ONE WORLD. It explains how objectively recorded brain processes generate a psyche, supposedly devoid of the attributes of materiality. After we have clarified the numerous functions of biocommunicators in Nature, learned about the new classification of the genome and the nano-model genetic theory of the functioning of the genome, the mechanisms of the formation of the "inner world" or the human psyche are small copies of society. Viruses (biocommunicators) are migratory organelles of eukaryotic cells, and they perform many vital functions. Biocommunicators are essentially the foundation of life. Now it is clear what forms the "inner world" of a person, and this is very important for understanding the processes in the formation of society[11]. However, we should not forget about the influence of the external environment in the forming the human psyche. *The sensorimotor activity of a person provides the connection between the mind and the body.* It shows how the human psyche and the human impact on the world around us. The" inner world "of each person should normally be a small copy of the general "big world".

Thus, at this stage of the historical development of humanity, it is BIOLOGY that has become the driving force of scientific and philosophical progress. At an earlier stage in the development of mankind, this role was played many times by Philosophy and Physics. Today we can even talk about another revolution in biology, which will have a very positive effect on the development of such practical areas as medicine, pedagogy, agriculture, ecology, sociology, psychology, bioengineering, and the like.

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Assessment of Corticolous Lichen Diversity in Romblon State University, Main Campus, Odiongan, Romblon

By Alwin F. Maulion
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Abstract- Corticolous lichens grow on barks of living host trees. Their ubiquitous distribution and sensitivity to pollutants makes them ideal model organisms to biomonitor air pollution and assess air quality. But before these lichens can be used as bioindicators, it is imperative that baseline information on their diversity in a given area must be established for comparison. In this study, the diversity of the corticolous lichens was evaluated in two different sites of Romblon State University Main Campus, Odiongan, Romblon. Sampling ladders with 10 x 10 cm contiguous quadrats were used to assess the diversity of corticolous lichens from different tree species within 10 x 10 m plots set-up in the study sites. Results showed that site 2(LDV= 170.2) showed the highest lichen diversity values than site 1 (LDV= 117.9). At least nine different species of corticolous lichens were reported in this research study. Our research is one of few studies in the Philippines looking at the application of corticolous lichens in biomonitoring environmental quality.

Keywords: *corticolous lichens, species diversity, bioindicator, biomonitoring.*

GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of:

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I. Introduction

Lichens are mutualistic associations of a fungus and an alga or cyanobacterium and occur as crusty patches or bushy growths on trees, rocks and bare ground. The names given to lichens strictly refer to the fungal partner; the algae have separate names. Lichens are very sensitive to sulphur dioxide pollution in the air. Since industrialisation, many lichen species have become extinct in large areas of lowland Britain, one example being the beard moss *Usnea articulata*. This is mainly due to sulphur dioxide pollution, but the loss of habitat, particularly ancient woodland, has also led to reductions in some species. Lichens are sensitive to sulphur dioxide because their efficient absorption systems result in rapid accumulation of sulphur when exposed to high levels of sulphur dioxide pollution. The algal partner seems to be most affected by the sulphur dioxide; chlorophyll is destroyed and photosynthesis is inhibited. Lichens also absorb sulphur dioxide dissolved in water. Lichens are nature's pioneers especially in plants. They are not a single organism the way most other living things are, but rather it is a combination of two organisms which live together intimately. Most of the lichens are composed of fungal filaments, but living among the filaments are algal cells, usually from a green alga or a cyanobacterium and they are poikilohydric, meaning they are capable of surviving extremely low levels of water content.

According to Kershaw (1985) environmental conditions such as climate, substrate, light and moisture play important roles in the distribution of lichen. Lichen species with similar distribution models tend to have similar ecological requirements. Boundreaultet al., (2008) found that the dominance of bryophytes at trunk base and the dominance of lichens at breast height are related to different humidity levels along a tree. So, lichens bark structure influences epiphyte colonization and growth. Lichens in plants are not considered plant pathogens. Only a few cases of parasitic activity by lichens have been reported. The fungal partner of lichen was suspected of killing twigs and small branches of elm by infecting the cork cambium, which is found just below the bark. But this suspected pathogenic activity was never proven.

Instead, lichens are an important part of the ecosystem providing substrate or later succession species, microhabitats and food for herbivores. More importantly for recovering ecosystems, many lichen species have cyanobacteria photobionts or cyanobacteria that closely associated with them are therefore important in nitrogen cycling in which the natural circulation of nitrogen by living organism (Romagni and Gries, 2000). They have several important functional roles in forest ecosystems and they may constitute an important component of the total biodiversity (Dettki and Esseen, 2003). They increase structural complexity, modify canopy water regimes, influence nutrient cycling and provide habitat, food and nest material for many animals (Galloway, 1992; Rhoades, 1995) and are amongst the most significant indicators of air pollution (Richardson, 1992, Wolseley et al 1995, Upreti1995) because lichens are very sensitive to pollution in the air. One indication is when there are too many harmful things in the air, lichens die. If there are many lichens it probably means the air is clean. But, if there are only a few lichens in the neighborhood, the air is probably clogged with automobile fumes or industrial wastes. The bioindicator features of lichens are suitable for determining special ecological conditions such as substrate and air pollution. In recent

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years, studies done on these issues have indicated that community structure and diversity of epiphytic lichens vary due to the differences in the environmental conditions and the preferences to substrate of lichens (Pirintsos et al., 1993; Burgaz et al., 1994; Pirintsos et al., 1995; Loppi et al., 1997).

They are also used as a food in many parts of the world. Some species of lichens such as *Cetrariaislandica* was an important human food in northern Europe, and was cooked as bread, porridge, pudding, soup, or salad and *Bryoriafremontii* was an important food in parts of North America, where it was usually pit cooked. Northern peoples in North America and Siberia traditionally eat the partially digested reindeer lichen (*Cladina spp.*) after they remove it from the rumen of caribou or reindeer that have been killed. Rock tripe(*Umbilicaria spp.* and *Lasalia spp.*) is lichen that has frequently been used as an emergency food in North America, and one species, *Umbilicariaesculenta*, is used in a variety of traditional Korean and Japanese foods.

In sense of biological activity, lichens have attracted much attention in investigations because of their antiviral, antibiotic, antioxidant, antitumor, allergenic and plant growth inhibitory activities (Boustie and Grube, 2005; Muller, 2001) and they produce secondary compounds, including pigments that reduce harmful amounts of sunlight and powerful toxins that reduce herbivory or kill bacteria. There are reports dating almost 2000 years old of lichens being used to extract purple and red colors. The pHindicatorlitmus is a dye extracted from the lichen genus *Rocellatinctoria* which was used in dyeing silken and woollen goods by boiling. Extracts from many Usnea species were used to treat wounds in Russia in the mid-twentieth century. The substance olivetol is found to be naturally present in certain species of lichens. This is a property it shares with the cannabis plant, which internally produces the related substance olivetolic acid (before using it to biosynthesis tetrahydrocannabinol (THC).

However, lichens have been essentially ignored by the modern pharmaceutical industry, despite the fact that lichen produce a large number of secondary metabolites with diverse structures and that studies have provided evidence of biological activity extracts from whole native lichens.

Some species of lichens are one of the most threatened organisms. The main threats that apply to biodiversity in general are also true for lichens, e.g. habitat degradation and loss (Groomet al., 2006), habitat fragmentation (Bergamini et al., 2005), overexploitation (Upreti et al., 2005), species invasions (LaGreca and Stutzman, 2006), and climate change. For instance, climate change is likely to have dramatic effects on distribution and abundance of lichen populations (Ellis and Coppins, 2007; Ellis et al., 2007).

Overexploitation of lichen populations for human uses is a serious problem, even if the demand is not increasing, but the size or quality of the habitat is declining. Habitat degradation and loss is the most serious threat to biodiversity in general (Groom et al., 2006) and in lichens in particular (Wirth, 1976, 1999). Loss of habitat leads to a reduction of local population sizes, and saxicolous, terricolous and epiphytic species are all similarly affected. Habitat loss has been identified as the most widespread threat to lichens, clear-cuts of old or natural forests accounting for 63 % of lost sites (Wolseley, 1995). Deforestation and degradation of lichen habitats by the replacement of natural forests with plantation forests have both a drastic effect on species richness and composition of lichen communities (Rose, 1992).

Monitoring programs and more specific concerns about environmental monitoring are required to ensure that lichens ecosystem are conserved and manage sustainably to maintain their environmental benefits in the ecosystem.

Unfortunately, in the case of Romblon State University there are no studies regarding lichens diversity and distribution that are made to catch the attention of the public agency in the government. Assessment of these organisms was the effective tool in giving the information about environment monitoring.

II. Statement of the Problem

This study was conducted to assess the diversity and of corticolous lichens found in the Romblon State University, Main Campus.

Specifically it aims to find answers to the following questions;

1. What is the diversity of corticolous lichens in Romblon State University (Main Campus), Odiongan, Romblon?
2. What are the different lichens species found in the study area?
3. Is there a presence of lichen indicator species in the study area?

III. Significance of the Study

The primary concern of this study was to assess the diversity and the different identification of lichens found in Romblon State University, Main Campus.

Findings of the study would help to determine the different kinds of lichens species present inside the Romblon State University, Main campus. With this, we could provide a basis for identification of lichens found in RSU, Main Campus.

The study would enable the researchers to be familiarized with the lichen species and to become aware of the environmental conditions that lichens contribute such as bioindicator of air pollution.

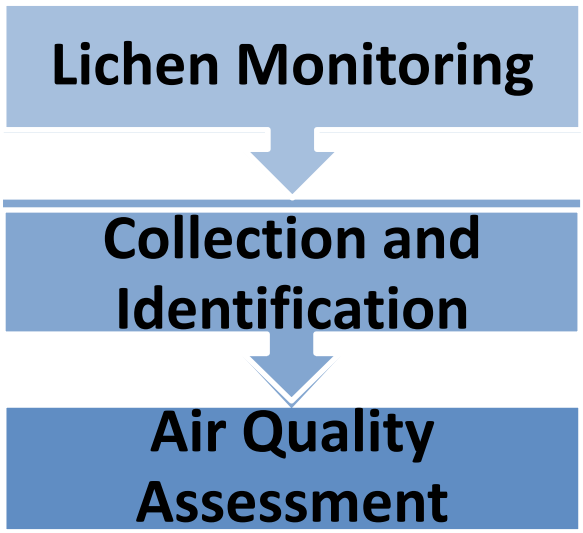
The result of this study would be a source of reference and guidance for replication of those who are interested to conduct other studies regarding lichens.

IV. Scope and Delimitation of the Study

The study was conducted to assess the diversity and distribution of lichen species found in RSU, Main Campus. The study focused entirely on lichens growing on trees. Making of quadrats in different aspect (N, E, W, S) of trees are determined in the study.

The study was limited to assessment of corticolous lichen species diversity found in RSU, Main Campus. The duration of the study lasted for three (3) months.

Conceptual Framework



a) Related Literature

Lichen was introduced into Greek literature about 300 B.C by Theophrastus primarily to describe outgrowths from the bark of olive trees, and this is the first written record on lichens (Hawksworth and Hill, 1984). Up to end of the 16thcentury, descriptions of lichens were entirely based on their physical appearance and were often incorrectly described as type of mosses or seaweeds.

Lichens were alluded to by only a few writers during the next 2000 years. This reflected not only the small amount of study in natural history, but also the relative lack of economic worth of lichens. However, the advent of microscope in the beginning of 18thcentury enabled detailed anatomical studies of lichens, which revealed their special dual character consisting of algal and fungal partners. This led to a series of more refined definitions.

Schneider (1897) wrote a history of lichenology, recognizing the following periods:

1. From the earliest times to the end of the seventeenth century.
2. From 1694, when Tournefort, the first to separate lichens taxonomically from the bryophytes, arranged plants into classes called genera, to 1729.
3. From 1729, when Micheli divided lichens into different orders, to 1779.
4. From 1779, when Weber established definite and reasoned lichen genera based on thestructure of thallus and fruits, to 1825.
5. From 1825, when Wallroth and Meyer each published works dealing with detailed morphological, ecological, and biochemical observations, to 1868.
6. From 1868, when Schwendener discovered the dual nature of lichens, to 1894.

The difficulty in finding a universal definition for 'lichen' results from the variability of fungal-algal associations and the range of symbiosis. A number of definitions of lichens are provided in contemporary literature (Hawksworth and Hill 1984, Orange 1994, Purvis 2000, Ulloa and Hanlin 2002, Wolseley et al. 2002, Allaby 2004, Gilbert 2004, Lawrence, 2005). However, the interpretation of lichen as an association of two organisms living in symbiotic relationship seems to be the most common dimension of these definitions. Indeed, in the Ainsworth and Bisby's Dictionary of the Fungi (Kirk etal. (eds.) 2001) lichen is defined as a stable self-supporting association of a fungus (mycobiont) and an alga or cyanobacterium (photobiont). More precisely, lichen is described as an ecologically obligate means able to exist under only one set of environment conditions, stable mutualism between an exhabitant fungal partner and an inhabitant population of extracellularly located unicellular or filamentous algal or cyanobacterial cells.

Perhaps a more widely accepted idea was given by Imshaug (1951)who defined a lichen as "an entity capable of reproducing itself, and consisting of two organisms, an alga and a fungus, living together in a state of symbiosis, as is manifested by some change in the anatomy, morphology, or physiology of at least one of its components."

Lichens do not have independent scientific names; the fungal and photosynthetic partners each have separate names, and names given to lichens are considered as referring to the fungal partner alone. The classification of lichens is therefore integrated into the system of Fungi. Current nomenclature is consistent with the recognition of lichens as a nutritional rather than a taxonomic group. The nomenclature of fungi including lichen–forming fungi is governed by the international code of botanical nomenclature (Kirk et al. (eds.) 2001).

b) Use as Bio-indicators

Lichens are widely used as environmental indicators or bio-indicators. If air is very badly polluted with sulphur dioxide there may be no lichens present, just green algae may be found. If the air is clean,

shrubby, hairy and leafy lichens become abundant. A few lichen species can tolerate quite high levels of pollution and are commonly found on pavements, walls and tree bark in urban areas. The most sensitive lichens are shrubby and leafy while the most tolerant lichens are all crusty in appearance. Since industrialisation many of the shrubby and leafy lichens such as *Ramalina, Usnea* and *Lobaria* species have very limited ranges, often being confined to the parts of Britain with the purest air such as northern and western Scotland and Devon and Cornwall.

c) Zonation of Lichens

A lichen zone pattern may be observed in large towns and cities or around industrial complexes which corresponds to the mean levels of sulphur dioxide experienced. The table shows the lichen zone scale of Hawks worth & Rose (1970). Particular species of lichen present on tree bark can indicate the typical sulphur dioxide levels experienced in that area. For example if there are no lichens present, the air quality is very poor (zone 1), whilst generally only crusty lichens such as *Lecanora conizaeoides* or *Lepraria incana* can tolerate poor air quality (zone 3). In moderate to good air, leafy lichens such as *Parmelia caperata* or *Evernia prunastri* can survive (zone 6) and in areas where the air is very clean, rare species such as 'the string of sausages' *Usnea articulata* or the golden wiry lichen *Teloschistes flavicans* may grow (zone 10).

It is important to note that the zone chart in Table 1 applies to areas where sulphur dioxide levels are increasing. If sulphur dioxide conditions are falling, lichens rarely colonise in exactly the same sequence; lichens are slow growing and may take a year or two to recolonise bark or other substrates following a reduction in air pollution levels, and tiny recolonising specimens can be difficult to spot and identify.

During the early and mid-twentieth century, air pollution levels were much greater than they are today in towns and cities of the UK. Sulphur dioxide levels were highest in the inner city areas becoming less polluted out towards the edges of the urban areas. At such times, the lichen zone scale would often highlight zone 1 as the inner city area, moving through the zones to the cleaner air at the edge of the city. From the 1970s onwards, sulphur dioxide levels have been falling markedly in the central and outer areas of cities, such that there may be no differentiation between levels in central and outer areas of many cities. The fall in sulphur dioxide levels between the 1970s and the 1990s has led to a number of lichens recolonising in areas from which they had previously been eliminated.

d) Ecology of Lichens

In general, three major life forms of lichen thallus are recognized, crustose (crust-like biofilm), foliose (leaf-like), and fruticose (branched tree-like, shrubby, pendulous; thalli (Hawksworth et al. 1995; Büdel and Scheidegger, 1996). The fourth type, gelatinous thallus, is restricted to some cyanobacterial lichens (Büdel and Scheidegger, 1996). Even without roots, lichens can efficiently extract nutrients (phosphorus, magnesium, calcium, potassium, sulfur, and iron) from recalcitrant surfaces (Richardson, 1975). Rhizinae on lichen thalli may have a function in the uptake of nutrients. Lichens often grow in habitats with extreme light, dryness, or temperature, which are less favorable or unsuitable for higher plants (Kershaw, 1985; Vrablikova et al. 2006).

Lichen thalli are poikilohydrous, which means that their water status passively follows the atmospheric humidity (Nash, 1996; Kappen, 2000). The presence of water rapidly activates lichen metabolism (Nash 1996, Schlensog et al. 2004). Recovery of the photosynthetic apparatus after the dark winter takes only minutes in Antarctic lichens, whereas in mosses it is a longer process (Schlensog et al.2004). Incredible adaptations enable some cold-adaptedgreen algal lichens to activate their photosynthesis at −20°Cwith water vapor obtained from snow. Photosyntheticactivity can be high by at 0°C (Kappen et al. 1996; Kappen, 2000; Richardson, 2002). Certain strategies increase the fitness of some lichen over others in dry habitats. The right choice of the photobiont, the water holding structures, and a tolerance to osmotic stressare some of the survival strategies. While green algae in lichens are able to activate their photosynthesis with water vapor, cyanobacteriain lichens need liquid water (Rundel 1988; Richardson 2002). This explains why algal lichenssurvive in dryer habitats than cyanobacterial lichens green; which in humid tropics represent nearly half of the known lichen species. Some cyanobacteriallichen species with gelatinous polysaccharides-containingthalli and green algal lichens with cushions' water-storing thalli are able to extend their daily metabolism compared to thin, easily drying lichen species (Richardson, 2002).

e) Lichen Symbiosis

Lichens are the symbiotic phenotype of nutritionally specialized fungi that acquire; in an ecologically obligate symbiosis, fixed carbon from a population of green algal or cyanobacterial cells (Dembitsky, 2003; Honegger, 1998; Yuan et al., 2006).

According to Hawksworth et al. 1995, lichen is an ecologically obligate, stable mutualism between an exhabitant fungal partner and an inhabitant population of extracellularly located unicellular or filamentous algal or cyanobacterial cells.

Scholler (1997) described how in the 18thcentury lichens on the bark of trees and rocks were recognized as physically joined algae and filaments of fungi. Indeed, this dual character of lichens was recorded as comprising algae and fungi living in a symbiotic relationship. This symbiotic description provided a more

specific explanation of the living arrangement between both partners.

Fink (1913) gave his own idea in the following statement:

> "The lichen is a fungus which lives all or a part of its life in parasitic relation with an algal host and also sustains a relation with an organic or an inorganic substratum."

The lichen symbiosis probably evolved around 400– 600 million years ago (Yuan et al. 2005). Lichens can be considered as ecosystems where the interaction of partners results in behavior and life forms that are not found in the isolated partners (Nash 1996).

Lichens are not regarded as a taxonomic group, butlichen taxonomy is based on the taxonomy of the fungal partner, the mycobiont (Tehler, 1996). In a course ofevolution, about 13,000 extant fungal species (Hawksworth, 2001) have specialized in gaining their carbon and about1, 500 species also in gaining their nitrogen from a photosynthesizingpartner (Hawksworth et al. 1995).Nearly 19% of all fungi are lichenized (Lutzoni et al. 2001; Hawks worth et al. 1995). The fungal diversity alone offers a great metabolic potential for new ecological and biotechnological discovery.

More than 98% of lichenized fungal species belong to phylum Ascomycota, a few to orders of phylum Basidiomycota and some to Mitosporic fungi (Hawks worth et al. 1995; Tehler 1996). Most of the lichenized fungi (mycobionts) form lichen symbiosis with green alga (Chlorophyta; Lewis and McCourt 2004), only about 10% with cyanobacteria, and 3% with both green alga and cyanobacteria (by Scheider et al. 1987 as cited by Woess 1988). Most of the tripartite lichen thalli consist of lichen fungi and green alga while the cyanobacteria are spatiallyseparated from alga in internally or externally occurringfungal compartments called cephalodia (Büdel and Scheidegger 1996). Some mycobionts can also changetheir photosynthesizing partner from green alga tocyanobacterium and vice versa and this leads to changesin thallus morphology. This behavior was suggested to be due to an environmental adaptation and related to ecological compatibility of the photobiont (Honegger1996; Stenroos et al. 2003).

Future studies with careful evaluation of cyanobacterial taxonomy (Oren, 2004) and carefully chosen DNA markers should result in a clearerpicture of the taxonomic diversity of lichen photobionts (Oksanen, 2006). There are challenges in finding appropriate DNA markers that have descended directly from a common ancestor that provide sufficiently but not too much nucleotide variation and have conserved sites for primer design (Oksanen et al.2004; Sánchez-Baracaldo et al. 2005).

f) Reproduction of Lichens

Lichens reveal various reproductive strategies where the mycobiont and its photobionts either disperse separately, in the case of sexual reproduction (horizontal transmission ofphotobionts) or where the lichen symbionts are co-dispersed with clonal, symbiotic propagules (vertical transmission of photobionts; Yahr et al., 2004). Lichens reproduce either with fungal spores (Büdel andScheidegger1996; Murtagh et al. 2000) that have to find a suitable photobiont or by vegetative propagules including both partners (Büdel and Scheidegger 1996). Crustoselichens grow slowly, ≤0.87 mm/year (Karlen and Black, 2002); other growth forms from0.06 to 36.5 mm/year Richardson, 1975). With a few exceptions, where photobionts grow betweesn the meiosporangia of the mycobiont and are co-dispersed with the ascospores (Ahmadjian, 1993), sexual reproduction is always associated with horizontal transfer of photobionts. A high number of species develop symbiotic propagule types such as is idia or soredia that facilitate clonal reproduction of the symbiosis (Budel and Scheidegger, 2008). In many species, these diaspores are multifunctional and can develop intoregeneration structures (Ott et al., 1993). Species with a predominantly clonal reproductive mode can exhibit extensiveclonal genetic structure. Some predominantly sexually reproducing lichen fungi may lack any structure at the local scale (Werth and Sork, 2008).

Zoller et al. (1999) were the first to recognize that lack of ascomata in strongly fragmented and geographically isolated populations of *Lobaria-pulmonaria* (''lungwort'') might be due to missing mating partners.

Sexual reproduction in lichens refers specifically to the sexuality of the lichen-forming fungus. During fungal sexual reproduction, ascospores are formed in ascomata (Ascomycetes) or, in basidiolichens, basidiospores are formed. Some lichen-forming fungi are capable of both selfing and outcrossing (homothallism), while others are obligatory outcrossers (heterothallism) (Zoller et al., 1999).

Some lichen-forming fungal species exhibit contrastingre productive strategies in different parts of their ranges (Poelt, 1970, 1972; Tehler, 1982; Mattsson and Lumbsch, 1989; Lohtander et al., 1998; Kroken and Taylor, 2001, Cornejo et al., 2009). Often, due to their different reproductive mode, these were described as separate species, when in fact they are conspecific. One example of these so-called ''species pairs'' is the sexual lichen *Porpidiaflavocoerulescens* and its clonal counterpart, *P. melinodes* (Buschbom and Mueller, 2006).

g) Ecological Diversity and Distribution

i. *World Status*

Lichens are widespread in many forests ecosystem (Dettki and Esseen, 2003).Lichens are the most successful symbiotic organisms in nature, dominating 8% or more of the earth's terrestrialarea (Ahmadjian,1995). According to Upreti (1998) India is a rich centre of lichendiversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world and its share of just 2×4% of global land surface. Since then, they have colonized almost all habitats and extreme conditions, from epiphytic (growing on trees) to end olithic (growing under the surface of rocks), and from Antarctica to the highest mountains and sea shores (Nash III, 1996).

Lichens occur commonly as epiphytes on trees and other plants, and in some ecosystems epiphytic lichen biomass may exceed several hundred kg ha^{-1}(Coxson, 1995). In addition, they frequently colonize bare soil, where they are an important component of cryptogrammic soil crusts in arid and semi-arid landscapes(Belnap and Lange, 2003). Furthermore, lichensoccur almost ubiquitously on rocks with the most obvious ones occurring asepiliths, either growing over the surface or embedded within the upper fewmillimeters. A few lichens even occur to endolithically within the upper fewmillimeters of the rock, such as occurs in Antarctica (Friedmann, 1982).

Lichens occur in most terrestrial ecosystems of the world, but their biomasscontribution varies from insignificant to being a major component ofthe whole ecosystem (Kershaw, 1985).

In the study of Giao (2009) Eighty three (83) species of macrolichens are reported from Langbian Mountain and Ngoclinh Mountain, located in the Western Highlands of central Vietnam, including 61 new records for Vietnam (Aptroot and Sparrius,2006) estimated at least 1000 species lichen is in Vietnam.

New microlichen species for Thailand are described by Sparrius and Saipunkaew (2005).

Dodge (1973) reported 86 genera including 424 species of lichens from Antarctica and its adjacent islands.

According to Negi (2000) India is a rich center of lichen diversity, contributing nearly15% of the 13,500 species of lichens so far recorded in the world.

ii. *Philippine Diversity*

In Philippines, the total number of lichen species credited now is 790 (DENR1999; Tacio 2004).

Recently, Dulnuan (2006) reported 3 species of lichens with fruticose type of growth from a total of 52 lichen genera collected in Ifugao, Mountain Province.

Earlier study by Herre (1957) reported only 68lichen species from 26 provinces in Luzon (12), Visayas (6), and Mindanao (8). Majority of the species were foliose (45 species) and crustose (36 species) type of lichen growth. Eleven species were recorded as fruticose, while only one species was noted as squamulose. Among the fruticose type of lichens reported were species of Cladonia(2), Stereocaulon(2), and Usnea(7). Herre(1963) also reported from Bataan, Ilocos Sur, and Misamis, Negros Oriental, and Rizal five species ofUsnea: *U. hossei, U.longissima, U. marivelensis, U. misamisensis, and U. squarrosa.*

iii. *Biological Activities*

The challenge for today's pharmaceutical industry lies in the discovery and development of new pharmacological active molecules due to resistance to available antibiotics (Bahera et al., 2005). Similar to higher plants, lichens were used since antiquity as natural drugs, together with some marine organism and frog venom, are important sources of biologically active compounds (Barner, 2000). Their efficacy is due to the synthesis of unique secondary compounds, a number of which have important biological roles (Perry et al., 1999).

According to Elix (1996) lichens produce a wide range of organic compounds that can be divided into two groups calledprimary metabolites and secondary metabolites. Primary metabolites are proteins, lipids, carbohydrates, and other organic compounds that are essential to the lichen's metabolism and structure. Some of these metabolites are produced by the lichen's fungal partner and others by the lichen's algal or cyanobacterial partners. Secondary metabolites are produced by the fungus alone and secreted onto the surface of lichen's hyphae either in amorphous forms or as crystals. If these substances are only found in lichens, then they are called lichen substances (Ozturket al., 1999). Burkholder et al. (1944) reported for the first time the presence of antibiotic substances in lichens.

The chemistry of about one third of all lichen species has been studied up to now and about 350 secondary metabolites are known from lichens. The chemical structures of approximately 200 of them have been established. They are extracellular products of relatively low molecular weight crystallized on the hyphal cell walls. Also they are usually insoluble in water and can be extracted into organic solvents. They amount to between 0.1 and 10% of the dry weight of the thallus, sometimes upto 30% (Galun, 1988).

After the discovery of penicillin from a fungus, numbers of lichens were screened for antibacterial activity in the 1940s and 1950s . For example, usnic acid has been used as atopical antibacterial agent and also it showed antimicrobialactivity against Gram-positive organisms 'in vitro (Lauterwein et al., 1995). Protoli-chesterinic acid exhibited in vitro activity against Helicobacter pylori (Ingolfsdottiret al., 1997). Lauterwein et al. (1995) investigated in vitroactivities of vulpinic acid and usnic acid against some aerobic and anaerobic microorganisms. Fournet et al. (1997) studied the activity of the lichen compounds usnic acid, pannarine

and 1_-chloropannarine against promastigotes forms of three strains of *Leishmaniaspp*. In addition lichens have been used for medicinal purposes throughout centuries. For example, *Lobariapulmonaria*, *Cetrariaislandica*, and *Cladonia*species were reputed to be effective in the treatment of pulmonary tuberculosis. Both enantiomeric forms of usnic acid inhibited the growth of *Mycobacterium tuberculosis* and *Mycobacterium tufu* in vitro at a relatively low concentration (Krishna and Venkataramana, 1992). In vitro activities of five common lichen compounds were screened for *Mycobacterium aurum* by Ingolfsdottir et al. (1998).

Two recent reviews summarize its antimicrobial, antiprotozoal, antiviral, antiproliferative, antiinflammatory, analgesic, antipyretic, and antitumoractivities as well as some other propertiessuch as UV protection, allergenic potential, toxicity (Cocchiettoet al., 2002; in Ingolfsdottir, 2002). Ingolfsdottir's review presents a comprehensive listfor the antimicrobial activity of (+)-usnic acid and(D)-usnic acid against gram positive and gramnegative, anaerobic bacteria, mycobacteria, andyeast/fungi with the relevant references.

Ghioneet al. (1988) reported the antibacterial activity ofusnic acid against *Streptococcus mutans*, *Streptococcuspyogenes*, and *Staphylococcus aureus*.

Lauterwein et al. (1995) determined in vitro activitiesof (+)-usnic acid, (D)-usnic acid, and vulpinic acid against aerobic and anaerobic microorganisms. They found that these lichen compounds did not inhibit gram negative rods or fungi at concentrations lower than32 μg/ml but were active against clinical isolates of *Enterococcus faecalis*, *Enterococcusfaecium*, *Staphylococcus aureus*, and someanaerobic bacteria.

The usnic acids (Ingolfsdottir, 2002) have also been found to exhibit antihistamine, spasmolytic, and antiviral properties as well as being active against gram-positive bacteria and streptomycetes. Indeed they are used in commercially available antiseptic creams including ''Usno'' and ''Evosin.'' Usnic acid is reported to be more effective than penicillin salves in the treatment of external wounds and burns and is also used to combat tuberculosis. The active centers of the usnic acid molecule seem to be the benzofuran or dihydrodibenzofurannucleus, the phenolic hydroxy groups and the 4,4a-double bond in the dihydroaromaticring. The antibiotic action of usnic acidis due to the inhibition of oxidative phosphorylation, an effect similar to that shown by dinitrophenol. More recently Shibuya et al. (1983) showed that4-O-methylcryptochlorophaeic acid was a powerful inhibitor of prostaglandinbiosynthesis and a potentially useful anti-inflammatory drug.

Lichen substances are also known to exhibit anti-tumor activity. Usnic acid has low level activity against lung carcinoma. Pannarin inhibited cell growth and induces cell death in human prostate carcinoma DU-145 cells (Maier et al. 1999).The orcinol derivatives tenuiorin and methyl orsellinate present in extract of *Peltigera leucophlebia* (Nyl.) Gyeln. (Peltigeraceae) exhibited in vitro inhibitory activity against 15-lipoxygenase from soybeans. A correlation has been observed between 5-lipoxygenase inhibition and anti-proliferative effects for related lichen metabolites. On this account, tenuiorin and methyl orsellinate were further tested for anti-proliferative activity on cultured human breast, pancreatic and colon cancer cell lines. Methyl orsellinate lacked anti-proliferative activity but tenuiorindepicted moderate activity (Ingolfsdottir et al. 2002). Bianthraquinone glycosides, colleflaccinosides isolated from *Collemaflaccidum* (Ach.) Ach. (Collemataceae) collected in Israel and Russia, were reported to have antitumor activity (Rezanka and Dembitsky, 2006).

However, the most active anti-tumor lichen substances are water soluble polysaccharides which appear to bepartially O-acetyled homo-D-glucans (Nash, 1996).

Various plant-derived and lichen-derived compounds that are known to have antimicrobial activity against "normal" microbes constitute one noteworthy group of candidates that might have activity also against MRSA or VRE or against resistant bacteria in general. One such compound is (+)-usnic acid, an old lichen-derived drug with antimicrobial and many other interesting biological activities (Ingolfsdottir et al. 1998; Huneck1999). Topical formulations of this drug, either as such or in salt form, have been subject to pilot clinical studies(Cocchietto et al. 2002), and the drug is usedin antifeedant products (Durazo et al. 2004), mouth rinses,and dentifrices (Grasso et al. 1989) as well as in cosmetics(Najdenova et al. 2001).

According to Crockett et al. (2003) and Rankovic et al. (2007) used lichens as medicine in treatingwounds, stomach diseases, and whooping cough in America andin Europe.

Quisumbing (1951) earlier reported the medicinal properties of fruticose lichen *Usneaphilippina*. Santos et al. (1964) tested the biological activities of these lichens and other fruticose lichens, e.g., *Usneasp*., *Ramalina sp*. and *Stereocaulon sp.*, and reported their inhibitory activitiesagainst Gram-positive bacteria such as *Micrococcus pyogenesvaraureus* 209 P (syn=Streptococcuspyogenes), penicillin-resistant *Micrococcus pyogenesvaraureus*, *Bacillus subtilis*, and the acid-fast bacilli, *Mycobacterium tuberculosis* 607.

Santos and Mondragon (1969) also conducted thin layer chromatographic analysis ofthese lichens and detected the following lichen acids: salazinicacid, stictic acid, usnic acid, barbatic acid, protocetraric acid, zeorin, atranorin, lecanorin, and homosekikaic acid. However, itwas not reported whether any of these metabolites is responsible for its antibacterial activities.

Antifungal activity of lichen extracts and lichen acids against plant pathogenic fungi was reported

(Gulluce et al., 2006; Halama and VanHaluwin, 2004; Oh et al., 2006).

Lichens have a large variety of uses and for some of them, ethnopharmacological properties are reported as for *Cetrariaislandica* still indicated as a cough remedy (Van Haluwyn and Lerond, 1993).Studies reporteda variety of very interesting properties e.g. antibiotic (Ogmundsdottir et al. 1998), antioxidant (Hidalgo et al.1994), anti-HIV (Neamati et al. 1997).

Caperatic acid and extracts of the lichens *Flavoparmelia baltimorensis* and *Xanthoparmelia cumberlandia have* antiherbivore activities against the snail *Palliferavaria* (Lawrey 1983, 1989). Methyl b-orcinolcarboxylate, ethyl hematommate and 5-chlorohematommate show nematocidic activity on larvae of Toxocaracanis (Ahad et al. 1991). Giez et al. (1994) and Emmerich et al. (1993) studied the effect of lichen substances on the growth and development of the polyphageous insect Spodopteralittoralis: atranorin, pulvinic acid dilactone, calycin, parietin, evernic, psoromic, physodic, 3-hydroxyphysodic, fumarprotocetraric, stictic, norstictic, salazinic, vulpinic, rhizocarpic, and usnic acids.

Heteroglycans and a beta-glucan isolated from *Thamnolia vermicularis* var. *subuliformis* were tested for in vitro immune modulation activity and reported to have various influences on the immune system (Omarsdottir,, et al, 2007).

Previous phytochemical studies on *Usnea longissima Linn*. also known as Old Man's Beard, resulted in the isolation of several lichen acids, with anti-inflammatory, analgesic, antipyretic, anti-tumor, anti-cholesterol and nematocidal properties (Yamamoto et al., 1995; Nishitobaet al., 1987).

According to Nash (1996), lichen substances have a harmful property. In northern Europe the lichen *Letharia vulpine* was used traditionally as a poison for foxes and wolves. The toxic principle is the pulvinic acid derivative vulpinic acid, which is not only poisonous to all meat eaters but also to insects and molluscs. Surprisingly this compound is ineffective against rabbits and mice. The secalonic acid derivatives are also highly poisonous. These substances are mycotoxins and, like vulpinic acid, may have evolved to serve a twofold ecological role. Thus, in addition to screening incoming light, they are highly poisonous to grazing herbivores (Nash, 1996).

Contact dermatitis, a severe skin rash, is well known among forestry and horticultural workers in North America, forming part of a syndrome known as' woodcutter's eczema'' or ''cedar poisoning.'' These complaints are an allergic response resulting from exposure to various lichen substances. Among the lichen substances responsible are usnic acid, evernic acid, fumarprotocetraric acid, stictic acid, and atranorin. Usnic acid, for instance, is a common lichen substance in the corticolous species of Alectoria, Evernia, and Usnea, which are widespread in the forests of North America. A dusting of soredia on clothing causes allergic reactions in the wives of lumbermen not directly exposed in the forests. Atranorin and stictic acid are also capable of photosensitizing human skin as well as being contact allergens. This can lead to photo contact dermatitis, where the allergic reactions become much more acute when the persons are exposed to the lichen substances in combination with light (Hale, 1983; Richardson, 1988).

Periodically hundreds of elks die in western North America when these largeruminants are forced out of their normal winter habitats by excessive snows and at lower elevations primarily find *Xanthoparmelia chlorochroa* to eat. Although the toxin is fully resolved, the abundance of salazinic acid is suspected. In contrast, these animals eat other epiphytic lichens without apparent ill effects (Nash, 1996).

iv. *Lichens in Perfume*

Large amounts of two lichen species are being processed in the perfume industry (mainly at Grasse, France): 1900 tons/year of *Pseudevernia furfuracea* ("tree moss"; 1997 level) and about 700 tons/year of *Evernia prunastri*. The lichen extracts have a certain "green" aspect caused by esters of substituted aromatic acids and act as fixatives. The combined lichen material and tree bark is subsequently extracted with an organic solvent and treated with ethanol. The concentrate of this solution contains a mixture of essential oils and depside derivatives (degradation products). The final extract with its sweet ''mossy'' smell is used in some perfumes to ensure persistence on the skin, as the major ingredients do not evaporate readily. The lichen extract may amount to 1–12%of the finished perfume. The precise identity of the scented component remains a trade secret but comprises a very small proportion (c. 0.04%) of the total extract, the majority of which comprises borneol, cineole, geraniol, citronellol, camphor, naphthalene, orcinol, orsellinate esters and their homologues (Moxham 1980; Richardson 1988; Hiserodt et al. 2000).Usnic acid is used as a preservative in cosmetic creams (Seifert and Bertram 1995), and atranorin, pannarin, gyrophoric, acid and usnic acid are applied in suntan preparations (Fernandez et al. 1996).

v. *Lichens in Dyeing*

Lichens were used as a source of dyestuff from the time of the ancient Greeks and probably earlier (Henderson 1999), but are of little economic importance today. Historically *Roccellamontagnei*, common fruticose lichen on rocks,provided valuable red or purple dyes in the Mediterranean region. These dyes were produced by ''fermenting'' the Roccella or chemically equivalent species (*Ochrolechiatartarea*, *O. androgyna*, or *Parmotrematinctorum*) with dilute ammonia solution. The macerated lichen and dilute ammonia were sealed in a container containing twice the volume of air. The

purple color developed after a week and was used as a direct dye (orchil) for protein fibres (wool and silk). The simple para-depsideserythrin (*Roccella*) and lecanoric acid (*Ochrolechia* and *Parmotrematinctorum*) present in these lichens are responsible for these colors. Rapid basehydrolysis of the lecanoric acid or erythrin by ammonia gives ammoniumorsellinate and then orcinol (by decarboxylation). Subsequent oxidative coupling in the presence of ammonia gives rise to the dyestuff, orcein, which comprises a mixture of three major chromophores, 7-hydroxyphenoxazone, 7-amino-phenoxazone and 7-aminophenoxazine (Hale, 1983). The common acid-base indicator litmus, formerly widely used in chemistry laboratories, is closely related to orcein but represents a more complex mixture of polymeric compounds with the 7-hydroxyphenoxazone chromophore and its anion being responsible for the sensitivity of the color to pH (Nash, 1996).

vi. *Lichens as Environmental Indicators*

Lichens have been recognized as being very sensitive to air pollution for many years (Nimis et al. 2002).

According to Garty, 2001, lichens adsorb and are sensitive to heavy metals. Coccomyxa photobiont species were more sensitive to metals than Trebouxia species and this may affect the habitat preference of lichens containing these green algae.

Lichens are used in environmental monitoring of industrial pollution (Garty, 2001). Monitoring methods include quantification of lichen populations, examination of lichen morphology, and heavy metal analyses of natural or transplanted thalli (Garty, 2001). The emission of ethylene is one of the measures of air pollution stress even though ethylene biosynthesis and its control in lichen are not fully understood (Ott et al. 2000; Oksanen, 2004). A new method for environmental monitoring involves the reduction of triphenyltetrazolium chloride to colored triphenylformazan in lichen (Backor and Fahselt, 2004).This measure of lichen dehydrogenase activityindicates environmental stress in lichens and their isolated bionts.

The strongest case for using lichens as bioindicators of air pollution involves sulfur dioxide (Grace et al. 1985a; Seaward 1993; Hawksworth, 2002; Nash and Gries, 2002). Some forms of coal (and other fuel products) have particularly high levels of sulfur, and its oxidation leads to the formation of sulfur dioxide, one of the major gases associated with acid rain. In fact sulfur dioxide has only an average atmospheric residency time of about 12 hours, because its high solubility in water leads to its trapping in water vapor aerosols and rapid conversion to sulfuric acid, one of the stronger acids (Nash, 1996).

h) Related Studies

Macrolichens cover and their distribution pattern on two common *Quercus semecarpifolia* and *Rhododendron arboreum* trees from the moist temperate forest (Chopta) of Garhwal Himalaya. Out of three d. b. h. classes trees (diameter at breast height), d. b. h. between 0.1-0.30 m, has found maximum cover of macro-lichens at southeast aspect (Nature and Science, 2009).

Recently, on 26 April 2012, scientists reported that lichen survived and showed remarkable results on the adaptation capacity of photosynthetic activity within the simulation time of 34 days under Martian conditions in the Mars Simulation Laboratory (MSL) maintained by the German Aerospace Centre (DLR).

In a pioneering study, Culberson et al. (1988) attempted to elucidate the sexual cycle in *Cladonia chlorophaea* using chemical markers. Earlier investigations on North American populations of *C. chlorophaea* had distinguished 14 distinct chemotypes, which were interpreted as sibling species. Culberson et al. (1988) analyzed secondary products in progeny of individuals of *C. chlorophaea* taken from populations of mixed chemotypes. According to Vartia (1973) lichens prevent to decay wood by fungi. He reported that characteristic secondary metabolites of lichens, such as usnic, divaricatic and lichesterinic acids, inhibit the growth of some filamentous fungi. It was therefore expected that lichen mycobiont cultures would yield growth inhibitors of wood decaying fungi (Yamamoto et al. 2002a).

In 2008, scientists from the European Space Agency (ESA) sent a suitcase-sized Expose-Experiment package to the International Space Station (ISS) filled with organic compounds and living organisms to test their reaction to outer space. The samples returned to Earth in 2009. Lichen has proven to be tough cookies – back on Earth, some species continue to grow normally. ESAs Rene Demets explains: “These organisms go into a dormant state waiting for better conditions to arrive.”

According to the Bergquist of Journal Sentinel, 2011, a laboratory study has found that lichens on Wisconsin's landscape break down the infectious proteins that are responsible for causing chronic wasting disease, or CWD - the devastating neurological disorder that was discovered in Wisconsin's wild deer population in 2002. The study by researchers at a federal government animal health laboratory in Wisconsin showed that certain lichen organisms contain an enzyme that is capable of degrading prions.

V. Research Methodology

This study utilizes an experimental research design. Corticolous lichens were collected from different species of trees from two study sites. The collected specimen will be identified by observing its characteristics using “Consortium of North American

Lichen Herbaria and "A Guide to the Study of Lichens by Schneider".

a) Research Locale and Time

This study was conducted at Romblon State University, Main Campus from the month of August to October, 2013. The location map of the study is presented in Figure 3.

Figure 1: MAP OF ROMBLON STATE UNIVERSITY Main Campus

b) Selecting tree species

Tree species are selected according to circumference of trunks must not be less than 110 cm, and injured trees are not suitable for the survey.

c) Surveying lichen diversity on tree trunks

Lichen diversity (LD) was surveyed on the selected trees, using a surveying quadrat. This quadrat consisted of four independent quadrat segments; each 50cm in height and 10cm in width. Quadrat segments were placed on the North, East, South and West side of thetrunk 100cm above the ground. Each quadrat segment was subdivided into five quadrat squares 10 x 10cm (Figure 2) and the presence of lichen species was recorded in each quadrat square.

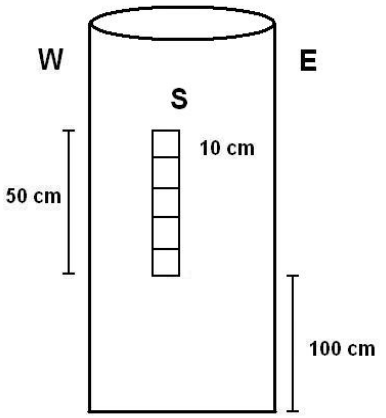


Figure 2: Surveying quadrat segment with five quadrat squares (adapted from Asta et al. 2002).

d) Collection and Identification

All samples of lichens collected in the RSU, main campus were processed for identification in the laboratory.

Thalline Spot tests was applied for identifying lichen species that have a color reaction using 10% solution of Potassium hydroxide (K), 4%solution of Sodium hypochlorite or chlorine (C) and the Lugol' s iodine.

e) Thalline Spot test procedures

1. Remove a piece of lichen from the specimen.
2. Place the sample on a white filter paper.
3. Add a minute amount of spot test reagent with a dropper.
4. Observe colour changes quickly.

f) Species Identification

The lichen species were identified by comparing them with the characteristics of lichens published in the book of Schneider entitled "A Guide to the Study of Lichens" and the documented samples of "Consortium of North American Lichen Herbaria".

g) Data Analysis

i. *Calculation of lichen diversity values*

Following the procedures of Asta et al. (2002 a, b) LD values for each sample plot were calculated.

Within each sample plot a sum of frequencies of all lichen species for each aspect on each tree was calculated. For each tree there were four Sums of Frequencies (SFi) on the North (SFiN), East (SFiE) South (SFiS) and West (SFiW) side of the trunk. Then the arithmetic Mean of the Sums of Frequencies (MSF) for each aspect (North, East, South, and West) in sample plot j was calculated following the formula:

$$MSF_{Nj} = (SF_{1Nj} + SF_{2Nj} + SF_{3Nj} + SF_{4Nj})$$

where;

MSFN: is the mean of the sums of frequencies of all trees of plot j for each aspect(e.g. North)

SF: is sum of frequencies of all species recorded for each aspect (e.g. North) of tree i
N: is the number of surveyed trees with a given aspect in unitj

The LD value of sample plot j (LDVj) was then calculated as the sum of the MSFs ofall aspects:

$$LDV_j = (MSF_{Nj} + MSF_{Ej} + MSF_{Sj} + MSF_{Wj})$$

The comparison of lichen species in trees were taken and analyzed by getting the overall population of lichens per site. Results from the study were presented in graphical form by showing the standard form.

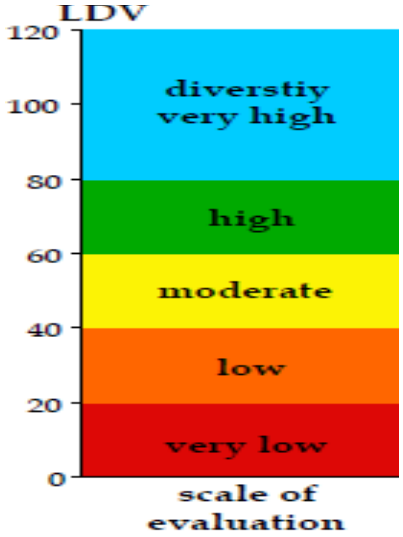
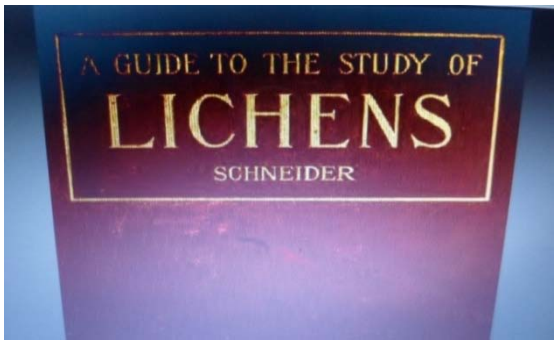


Figure 3: Assessment of Lichen Diversity (Asta et. al.)

VI. Results & Discussions

a) The Identification of Lichen Species

The lichen species were identified by comparing it with the characteristics of lichens published in the book of Schneider entitled "A Guide to the Study of Lichens" and the documented samples of "Consortium of North American Lichen Herbaria".



Collected from Consurtium of North American
Study site Lichen Herbaria
Common name: Blemish lichens
Scientific name: Phlyctis argena (white)

Description:

Thallus: crustose, typically continuous, even to tuberculate, but rarely coarsely tuberculate or tuberculate-plicate, sometimes scurfy, usually distinctly rimose in thick specimens, thin to thick, forming extensive patches up to several dm in diam.; prothallus: often conspicuous, white, felty, composed of radiating hyphae forming a marginal border to about 1 cm wide. *Surface:* white, sometimes with a brownish tinge sorediate.

Collected from Study site

Consurtium of North American Lichen Herbaria

Common name: Common green shield lichen
Scientific name: Parmelia caperata

Description:

Thallus: adnate to loosely adnate, foliose, 5-20 cm in diam., sometimes forming extensive patches, irregularly lobate. *Lobes:* subirregular, elongate, plane to subconvex, separate, 5-13 mm wide, contiguous to somewhat imbricate; apices rotund, crenate, eciliate. *Upper surface:* yellow green to pale yellow, occasionally green-gray (in shade), smooth but becoming rugose and folded with age, dull to somewhat shiny; epruinose and emaculate. *Soredia:* laminal, granular to wart-like, initially in circular soralia but becoming diffuse and confluent; *Isidia:* absent. *Medulla:* white with continuous algal layer. *Lower surface:* black centrally, brown and naked peripherally; ***Rhizines:*** dense to sparse centrally to edge of brown zone, black, simple, sometime brown or white tipped.

Collected from Study site

Consurtium of N. American Lichen Herbaria

Common name: Drinaria lichen
Scientific name: Drinaria applanata

Description:

Thallus: foliose, appressed to agglutinated, loosely appressed at the lobe tips, up to 6 cm in diam., pinnately or subpinnately lobate. *Lobes:* radiating, confluent, flat or convex, but sometimes concave towards the lobe tips, 0.5-2 mm wide, distinctly flabellate towards the lobe tips. *Upper surface:* gray, bluish gray or almost white, with a punctiform, rarely patchy white pruina or epruinose, sorediate. *Soredia:* farinose, in laminal, globose or elongated soralia. *Pseudocyphellae:* distinct, marginal, rarely also laminal, usually restricted to the peripheral parts of the lobes, sometimes reticulately confluent. *Medulla:* white, the lowest part sometimes orange, especially towards the lobe tips. *Lower surface:* black in center, paler towards lobe tips, erhizinate. *Apothecia:* very rarely present, laminal on thallus, 0.5-1.5 mm wide.

Collected from Study site

Consurtium of American Lichen Herbaria

Common name: Gold dust lichen

Scientific name: Chrysothrix xanthina

Description:

Thallus: crustose-leprose, bright yellow, unstratified, adnate, diffuse, irregularly spreading, sometimes forming scattered granules, but usually ±continuous. Soredia fine, with individual granules minutely convex to spherical, 20–80 µm wide, not agglomerated. *Medulla:* not apparent. *Apothecia:* reported to be rare, to 0.5 mm wide, sessile, rounded; disc orange, plane to convex, heavily yellow-pruinose; margin very thin, ecorticate, soon becoming excluded; hymenium colourless, to 50 µm thick; epihymenium colourless, composed of a reticulate layer of richly branched paraphyses; hypothecium colourless, poorly developed; *Ascospores;* (2–) 3-septate, obovoid to ellipsoidal, straight or curved, often constricted in the middle, 9–14 × 3 µm.

Collected from American Study site

Consurtium of N. Lichen Herbaria

Common name: Blemished lichen
Scientific name: Phlyctis argena (gray)

Description:

Thallus: crustose, typically continuous, even to tuberculate, but rarely coarsely tuberculate or tuberculate-plicate, sometimes scurfy, usually distinctly rimose in thick specimens, thin to thick, forming extensive patches up to several dm in diam.; prothallus: often conspicuous, white, felty, composed of radiating hyphae forming a marginal border to about 1 cm wide. *Surface:* gray sometimes with a brownish tinge (in herbarium specimens only?), sorediate. *Soredia:* forming coarse consoredia, up to 90-125 μm in diam., often mixed with eroding cortex fragments, in pale yellow to greenish white (rarely pure white and sometimes becoming pink in the herbarium) irregular soralia often somewhat elongate and angular and usually delimited by a ±raised rim formed by the cortex and often to a cm or more wide or sometimes becoming confluent and accounting for most of the thallus.

Collected from Study site

Consurtium of North American Lichen Herbaria

Common name: pencil mark lichen

Scientific name: Graphis scripta

Description:

Thallus: crustose, continuous to slightly rugose. *Surface:* cream-colored, white or pale gray or grayish green, dull. *Apothecia:* raised from the thallus, lirellate. *Lirellae:* oblong, ±flexuous and branched, 1-3 x 0.2-0.4 mm. *Disc:* narrow to wide and open, dark gray to brown with whitish pruina. *Margin:* well developed, covering the lateral part of the ascocarps; excipular lips: black, entire, sometimes narrow. *Exciple:* poorly developed and not carbonized at the base, carbonized laterally, with entire excipular lips whose basal part is sometimes less developed and less carbonized. *Epihymenium:* brown, 5-10 μm thick. *Hymenium:* not inspersed, 90-100 μm tall; paraphyses: 1.5-2 μm thick, dense, tips distinctly brown or yellowish brown; subhymenium: hyaline, 10-20 μm thick.

Collected from Study site

Consurtium of North American Lichen Herbaria

Common name: none

Scientific name: Miriquidica atrofulva

Description:

Life habit: lichenized; Thallus: crustose or squamulose, usually composed of contiguous to scattered areoles, sometimes rimose; prothallus: sometimes present. Areoles: angular to roundish in outline or irregularly shaped. Surface: white, gray, brownish yellow or brown, lacking secondary reproductive structures. Cortex: eucortex or phenocortex, often with a distinct epinecral layer. Medulla: white to spotted brown, I-. Photobiont: primary one a chlorococcoid green alga, secondary one absent. Ascomata: apothecial, black or dark brown, immersed to sessile, lacking a thalline margin;

Collected from American Study site

Consurtium of North Lichen Herbaria

Common name: none

Scientific name: Parmelia soredians

Description:

Thallus: adnate to tightly adnate, to 4–8 cm wide. Lobes imbricate, 1–3 mm wide; margins shallowly incised; apices rotund. Upper surface: yellow-green, smooth, dull to slightly shiny, without dactyls and isidia; marginal lobes developing laminal rugae, the surfaces of which disintegrate to form orbicular soralia with granular soredia; rugae coalesce to form sorediate ridges which eventually develop into large, pulvinate soralia in thallus centre. Medulla: white. Lower surface: with moderately dense, simple rhizines. Apothecia and pycnidia: not seen.

Collected from Study site

Consurtium of North American Lichen Herbaria

Common name: Jelly lichen

Scientific name: Collema furfuraceum

Description:

Thallus: foliose, medium-sized to large, (1-)3-6 (-10) cm across, membrane-like, closely adnate, conspicuously, deeply and broadly lobate. Lobes: (0.2-) 0.5(-1) cm broad, thin, (50-)60-105 μm thick, apically rotund or extended, +overlapping. Upper surface: dark olive-green to brownish black, paler and +transparent when moist, strongly but broadly ridged (wrinkled); ridges: radiate, sometimes postulate in young parts of thallus, becoming long, narrow and flexuous, 0.1-0.3 mm wide, up to 1.5 mm tall, simple or branched.

SITE 1

Elevation: 36 masl

Location: N 12^0 23.792' E 121^0 59.037'

10 Trees/species were assessed

Table 2: Tree No.1

Polyalthia longifolia (Indian tree)	N	E	S	W
Phlyctis argena (white)	106	118	90	86
Parmelia caperata	110	136	107	103
Drinaria applanata	20	35	15	21
Chrysotrix xanthina	36	22	26	28
Phlyctis argena (gray)	12	9	10	12
Graphis scripta	31	26	22	20
Miriquidica atrofulva	4	4	2	4

SF: Sum of frequencies of all lichen species found at one aspect of tree
N=319, E= 350, S=272, W= 274
MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 31.9, E= 35, S= 27.2, W= 27.4
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(31.9+35+27.2+27.4)
LDV = 121.5

Table 3: Tree No. 2

Swietenia mahogani (Mahogany)	N	E	S	W
Phlyctis argena (white)	100	92	88	86
Parmelia caperata	104	98	96	94
Drinaria applanata	12	26	11	18
Chrysotrix xanthina	32	21	22	26
Phlyctis argena (gray)	10	12	12	11
Graphis scripta	36	22	24	24
Miriquidica atrofulva	2	2	2	4

SF: Sum of frequencies of all lichen species found at one aspect of tree
N=296, E= 273, S=255, W= 263
MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 29.6, E= 27.3, S= 25.5, W= 26.3
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(29.6+27.3+25.5+26.3)
LDV = 108.7

Table 4: Tree No.3

Areca catechu (Betel nut)	N	E	S	W
Phlyctis argena (white)	112	106	100	111
Parmelia caperata	120	112	116	115
Drinaria applanata	10	21	26	20
Chrysotrix xanthina	36	28	26	20
Phlyctis argena (gray)	11	11	14	10
Graphis scripta	32	30	26	22
Miriquidica atrofulva	2	0	2	1

SF: Sum of frequencies of all lichen species found at one aspect of tree N= 323, E= 308, S=310, W= 299
MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 32.3, E= 30.8, S= 31, W= 29.9
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(32.3+30.8+31+29.9)
LDV = 124
Total LDV of SITE 1
LDV (T1) + LDV (T2) + LDV (T3)/3
(121.5+108.7+124)/3
SITE 1 Total LDV = 117.9
SITE 2
Elevation: 59 meters above sea level
Location: N 12^0 23.736' E 121^0 59.187'
10 trees/species were assessed

Table 5: Tree No.1

Polycias nodosa (Malapapaya Tree)	N	E	S	W
Phlyctis argena (white)	101	90	96	108
Parmelia caperata	106	98	102	90
Drinaria applanata	10	9	11	10
Chrysotrix xanthina	12	8	10	12
Phlyctis argena (gray)	90	88	96	100
Graphis scripta	102	97	99	90
Miriquidica atrofulva	2	6	6	8
Parmelia soredians	22	26	26	20
Collema furfuraceum	4	3	6	4

SF: Sum of frequencies of all lichen species found at one aspect of tree
N= 449, E= 425, S= 452, W= 442
MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 44.9, E= 42.5, S= 45.2, W= 44.2
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(44.9+42.5+45.2+44.2)
LDV = 181.8

Table 6: Tree No.2

Swietenia mahogani (Mahogany Tree)	N	E	S	W
Phlyctis argena (white)	96	100	90	96
Parmelia caperata	102	98	88	90
Drinaria applanata	8	12	8	6
Chrysotrix xanthina	10	10	8	4
Phlyctis argena (gray)	80	86	86	90
Graphis scripta	96	99	97	97
Miriquidica atrofulva	2	0	2	4
Parmelia soredians	30	32	22	28
Collema furfuraceum	2	2	4	2

SF: Sum of frequencies of all lichen species found at one aspect of tree
N= 426, E= 439, S= 405, W= 417

MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 42.6, E= 43.9, S= 40.5, W= 41.7
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(42.6+43.9+40.5+41.7
LDV = 168.7

Table 7: Tree No.3

Polyalthia longifolia (Indian tree)	N	E	S	W
Phlyctis argena (white)	96	90	104	100
Parmelia caperata	90	82	90	92
Drinaria applanata	8	8	6	8
Chrysotrix xanthina	10	6	6	4
Phlyctis argena (gray)	92	86	86	82
Graphis scripta	86	90	90	94
Miriquidica atrofulva	6	6	2	4
Parmelia soredians	20	22	18	24
Collema furfuraceum	2	0	1	1

SF: Sum of frequencies of all lichen species found at one aspect of tree
N= 408, E= 390, S= 403, W= 409
MSF: Mean of the sums of frequencies of all the sampled trees of unit
N= 40.8, E= 39, S= 40.3, W= 40.9
LICHEN DIVERSITY VALUE(LDV)
MSF(N)+MSF(E)+MSF(S)+MSF(W)
(40.8+39+40.3+40.9)
LDV = 161
Total LDV of SITE 1
LDV (T1) + LDV (T2) + LDV (T3)/3
(181.8+168.7+161)/3
SITE 2Total LDV = 170.2

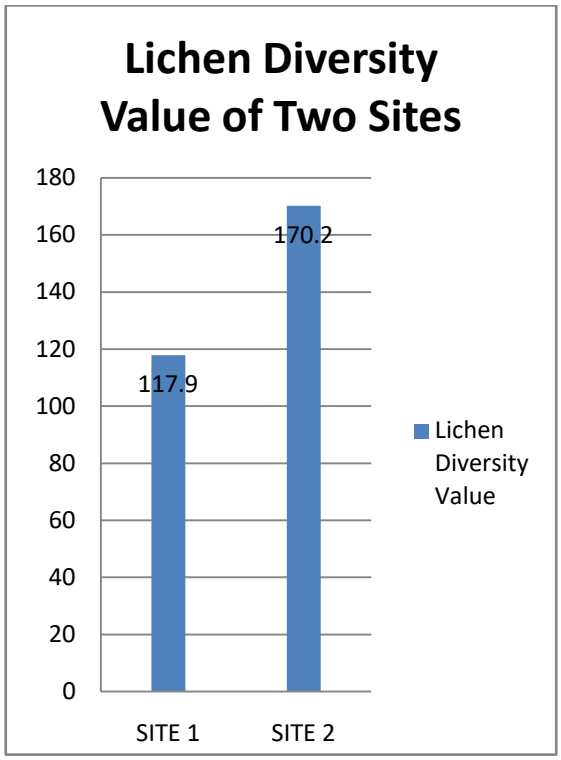


Figure 4: Shows that Site 2 has the higher Lichen Diversity Value than Site 1. Both sites have very high lichen diversity based on the scale of evaluation by Asta et. al.

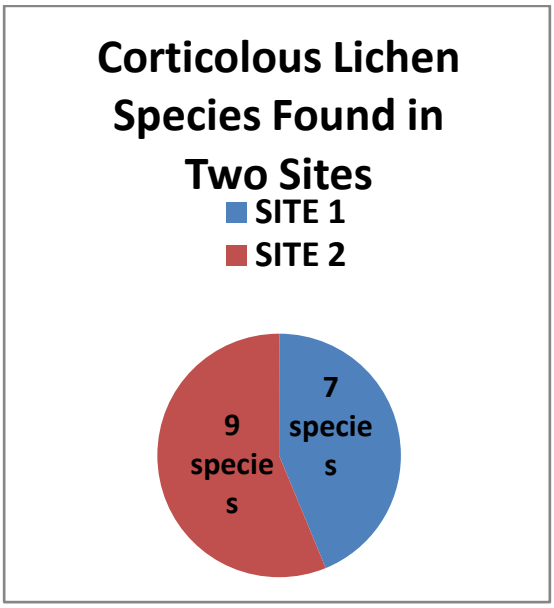


Figure 5: Show that there were 7 species of corticolous lichens in found Site 1 and 9 species in Site 2

Table 8: Thalline Spot Test

SPECIES	TYPE OF THALLUS	SPOT TEST REACTION		
		KOH Sol.	Chlorine Sol.	Lugol' s Iodine
Phylctis argena	Crustose	K+ (positive) black	C- (negative)	I+ (positive) gray
Parmelia caperata	Foliose	K+ (positive) dirty yellow	C+ (positive) copper green	I- (negative)
Drinaria applanata	Foliose	K+ (positive) yellow	C- (negative)	I+ (positive) reddish
Chrysothrix xanthina	Crustose	K+ (positive) yellow	C+ deep yellow	I- (negative)
Phlyctis argena	Crustose	K+ (positive) yellow	C- (negative)	I+ (positive) reddish
Graphis scripta	Crustose	K+(positive) black	C- (negative)	I- (negative)
Miriquidica atrofulva	Crustose	K+ (positive) pink	C+ (positive) orange	I- (negative)
Flavoparmelia soredians	Foliose	K+ (positive) yellow	C+ (positive) white	I- (negative)
Collema furfuraceum	Foliose	K-(negative)	C- (negative)	I- (negative)

As shown in this table, thallus of each species were identified and spot test reactions are noted that a +denotes color reaction and a – indicates that there is no color change.

Table 9: Common Lichen Indicator of Air Pollution (Hawks worth & Rose)

Polluted Areas	Moderately Polluted	Slightly Polluted	Clean Air
Buellia punctata	Evernia prunastri	Anaptychia ciliaris	Degelia plumbea
Cladonia coniocraea	Foraminella ambigua	Graphis elegans	Lobaria pulmonaria
Cladonia macilenta	Hypogymnia	Graphis scripta	Lobaria scrobiculata
Diploicia Canescens	physodes	Opegrapha varia	Pannaria rubiginosa
Lecanora conizaeoides	Lecanora chlarotera	Parmelia acetabulum	Permelia perlata
Lecanora dispersa	Lecidella elaeochroma	Parmelia caperata	Ramalina calicaris
Lecanora expallens	Parmelia glabratula	Phaeophyscia orbicularis	Ramalina fastigiata
Lepraria incana	Parmelia saxatilis	Physcia aipolia	Ramalina fraxinea
Xantoria parietina	Parmelia sulcata	Physconia distorta	Teloschistes flavicans
	Physcia adscendens	Physconia enteroxantha	Usnea species
	Physcia tenella	Pseudevernia furfuracea	
	Plastismatia glauca		
	Ramalina farinacea		

This table show that there were two common lichen indicator of air pollution present in the two study sites, Graphis Scripta and Parmelia caperata.

VII. Findings

1. Site 2 (LDV = 170.2) has the higher Lichen Diversity value than Site 1 (LDV = 117.9).
2. Both sites have Very High Lichen Diversity based on the scale of evaluation by Asta et. al.
3. Seven (7) corticolous lichen species found in Site 1 and nine (9) corticolous lichen species in Site 2.
4. Two (2) types of lichens were found in the study sites, crustose and foliose.
5. Two (2) species of lichen indicator found in the study sites, *Graphis scripta and Parmelia caperata.*

VIII. Conclusions

1. The diversity of corticolous lichens in the Romblon State University, Main Campus is Very High.
2. There were nine (9) species of corticolous lichens present in the Romblon State University, Main Campus.
3. Crustose and Foliose types of lichens were present in the study area.
4. There were two (2) lichen air pollution indicators present in the study area.
5. The air status of the study area is slightly polluted.

Recommendations

1. Other studies should be conducted by assessing all the factors in the growth of lichens including humidity, temperature, ph bark, light intensity and climate conditions.
2. Additional references must be provided to other researchers for their guidance.

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The Effects of Pretreatment of Giberellic Acid, Alpha Tocopherol and Ascorbic Acid on Germination in Maize Seeds

By Hatice Cetinkaya, Mehmet Akgün & Burcu Seçkin Dinler
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Abstract- The present study was conducted to determine the effects of gibberellic acid (GA), alpha-tocopherol (Vitamin E) and ascorbic acid (Vitamin C) on germination in maize seeds. The seeds were subjected to priming with 250 ppm GA, 300 ppm alpha-tocopherol, 100 ppm ascorbic acid for 24 h. The seeds which has grown at 25°C and 60% mousture, germination rate, germination duration, germination speed, germination index, length of radicle and plumula, fresh and dry weight, relative water content, protein content and catalase enzyme activity were determined after 7 days. The results clearly revealed that GA application has an positive effects on the physiological and biochemical parameters in maize seeds. Otherwise, Vitamin E has a positive effect on germination whereas Vitamin C has a negative effects depending on the application dose. The results of GA and E applications were significiant but others were not.

Keywords: *ascorbic asid, alpha-tocopherol, catalase, gibberellic acid, maize.*

GJSFR-C Classification: *FOR Code: 060799*

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Hatice Cetinkaya [α], Mehmet Akgün [σ] & Burcu Seçkin Dinler [ρ]

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Keywords: ascorbic asid, alpha-tocopherol, catalase, gibberellic acid, maize.

I. Introduction

Corn is one of the most common grains grown worldwide, used both as food and forage. Corn seeds are rich in protein, sucrose, and various vitamins, minerals [1]. It ranks third in total world production after wheat and rice and is considered a staple food in many countries, especially in tropical and subtropical regions [2]. Seed sowing and germination of the seed under suitable conditions constitute the first and most important stage of plant production [3, 4]. Seeds and seedlings are very sensitive to physiological and environmental stress factors during germination, emergence, and early seedling.

Various seed applications have been developed to improve the quality of seeds and to minimize the risk of environmental pressure [5]. The effectiveness of the pretreatment method is determined by the osmotic processes, water potential, pretreatment agent and time, temperature, presence or absence of light, oxygen availability, initial seed quality and post-pretreatment drying factors [6]. Pre-sowing soaking, acid etching, growth regulators, vitamins, sowing as a gel after germination, holding in nutrients or osmotic solutions, coating and banding are some of the preliminary applications [7, 8, 9, 10, 11, 3]. Similarly, plant growth regulators and hormones increase the performance of crops with various seed pre-applications [12].

Gibberellins are widely used as a pre-treatment method because they are effective in eliminating seed and bud dormancy, controlling and stimulating seed germination [13]. Gibberellins are engaged in the stimulation of enzymes involved in the seed germination phase. However, in the next stage of germination, gibberellins are transported from the embryo to the endosperm and play a role in converting starch to sugar to provide the necessary energy by stimulating the α-amylase enzyme [7, 8].

Tocopherols are chemically lipophilic antioxidants belonging to the vitamin E family. They are naturally produced in green photosynthetic organisms [14]. Alpha-tocopherol is the compound with the highest antioxidant activity because it contains three methyl groups in its molecular structure. This antioxidant deactivates photosynthetic reactive oxygen species (O_2, H_2O_2, OH) and prevents the propagation of lipid peroxidation in thylakoid membranes [15]. Vitamin E (alpha-tocopherol) penetrates between cellular and organelle membranes and protects the membranes against lipid peroxidation by converting free radicals to less reactive compounds.

Ascorbic acid (AsA) is a cofactor for certain enzymes by protecting various physiological processes in a plan; Barth et al. work [16] on its help with the creation of signal generation and Farooq et al. [17] work on it as a phytohormone. AsA plays a role in photosynthesis, cell division, cell expansion, increase of antioxidants and hormone biosynthesis. As A affects cell division in plants and causes cell elongation, development and ageing, as well as vegetative reproduction [16].

This study comparatively demonstrated the effects of gibberellic acid, alpha-tocopherol and ascorbic acid on the germination of corn seeds. Upon reviewing the literature, numerous studies in which gibberellic acid, alpha-tocopherol and ascorbic acid were applied alone in plants have been found. However, there are no studies on gibberellic acid, alpha-tocopherol and ascorbic acid as a pre-application in the

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corn plant. For this reason, we think that the study preserves its original value.

II. Materials and Methods

In the research, the seeds of 71MAY82 varieties of maize (*Zea mays* L.) were used, and the seeds were obtained the Bursa MAY Agro seed company. Seed pre-applications in germination trials are:

1. C: Control
2. GA: GA, 250ppm
3. E: Alpha-Tocopherol, 300 ppm (Vitamin E)
4. C: Ascorbic acid, 100 ppm (vitamin C)
5. G+E: GA+ Vitamin E
6. G+C: GA+Vitamin C
7. E+C: Vitamin E + Vitamin C
8. G+E+C: GA+Vitamin E +Vitamin C

Corn seeds were kept in de-ionized water (H_2O), gibberellic acid, tocopherol and ascorbic acid solutions at 20±1°C in dark conditions for 24 hours. After the waiting period, ten seeds were placed in each sterile petri dish, after which the water of the seeds was filtered, and two layers of blotting paper were placed. Germination tests were carried out in Petri dishes with a diameter of 10x2, between double-layered blotting papers, at 60% humidity and 25°C, on 3x10 seeds, with three replications, for seven days [18]. In pre-treated seeds, the initial viability of seeds was determined according to ISTA [18] rules and then taken to germination and emergence test.

a) Analyses

Physiological analysis of germinating plants: The fresh and dry weights and lengths of the radicle and plumula were calculated [19].

Relative water content: Fresh weights of radicle and plumula samples were measured in 6 [20]. They were kept in Petri dishes in dI-H_20 for 6 hours to become turgorized. Then, their turgorous state was measured. After drying at 70°C for 72 hours, their dry weights were determined. Relative water contents are calculated as % (WW)/(TW-DW)×100 connection.

Germination times: Average germination time (AGT) was calculated with the formula (AGT) = ∑Ti Ni/∑Ni. Ti: refers to the number of days after planting, Ni: refers to the number of seeds germinated on the day of observation [19].

Germination rate (%): 3x10 seeds were placed in Petri dishes and germinated in the air-conditioning room. The germination rate was calculated at the end of 7 days when germination was fixed, by proportioning sown and germinated seeds [21].

Germination index: The formula (GI) = ∑(Gt/Tt) was used to calculate the germination index (GI). Gt: the number of seeds germinated on day t after sowing, Tt: the number of days after sowing [19].

Germination speed: The germination speed was calculated according to Ellis and Roberts [22]. Σn (Seeds germinated on the day of counting) x d (Day of counting) germination speed = Σn (Total number of germinated seeds).

Protein content: Protein determination in radicle and plumula samples of germinated seeds is made according to Bradford [23] method. 5 µL of samples homogenized with phosphate buffer is taken, 250 µL of Bradford's reagent is added to it, and it is mixed and kept at room temperature for 15 minutes. At the end of this period, the samples were recorded by reading their absorbance at 595 nm in the spectrophotometer.

Catalase enzyme activity: It was carried out according to the method of Bergmeyer [24]. The decrease in the content of H_2O_2 was determined by the decrease in maximum absorbance at 240 nm. The reaction mixture in quartz cuvettes with a final volume of 1 ml consists of 0.1 mM EDTA, 50 mM Na-phosphate buffer (pH: 7), dI-H_2O and 0.3% H_2O_2. The decrease in absorbance during the reaction was followed for 180 seconds. CAT activity was expressed as µmol H_2O_2 consumed per minute.

Results were evaluated according to the analysis of variance in the statistical program, SPSS (Statistical Package for Social Sciences, Version 22.0). Complementary statistics and significance statuses of the analysis results were presented in tables.

III. Results

a) Germination Rate, Germination Time, Germination Index and Germination speed

The effects of pre-applications (GA, vitamin E, vitamin C) on maize seeds (71MAY82) on germination rate, germination time, germination index and germination speed were found to be statistically significant at $P < 0.01$ according to the results of analysis of variance (Table 1).

Table 1: The effects of preliminary applications (GA, vitamin E, vitamin C) on germination time, germination rate and germination index and speed in maize seeds (71MAY82)

	Aplication	Germination time (day)	Germination rate (%)	Germination index	Germination speed
Control	1	3.17 C	100 A	62.06 B	83.33 C
GA	2	3.07 G	96.67 B	63.14 A	90 A
E	3	3.10 F	96.67 B	62.03 C	86.67 B
C	4	3.11 E	93.33 C	59.78 D	83.33 C
GA+E	5	3.44 B	90 D	48.41 E	56.67 E
GA+C	6	3.13 D	53.33 G	33.84 H	46.67 F
E+C	7	3.05 H	66.67 F	43.97 F	63.33 D
GA+E+C	8	3.58 A	80 E	38.54 G	33.33 G
	F value	<0,0001	<0,0001	<0,0001	<0,0001
	LSD	4,8334	0,0071	4,367	7,0805

In terms of germination time, it was determined that corn seeds decreased in GA (3.07), E (3.10) and C (3.11) groups compared to control (3.17) groups. In addition, an increase in germination time was observed in GA+E (3.44), GA+C (3.13), GA+E+C (3.58) groups compared to GA application alone. Germination time in the C treated group increased compared to the E+C application. Based on these results, it can be said that E and C applications act in opposition to each other in terms of germination time.

The germination rate increased in the GA and E (96.67) groups compared to the C (93.33) group. While the germination rate decreased in GA+E+C (80) groups compared to GA+E (90) application, it increased compared to the groups treated with GA+C (53.33) and E+C (66.67). The lowest germination rate; was achieved with GA+C (53.33) application and C (93.3) application.

Compared to the GA (63.14) application, it was determined that there was a decrease in germination index in E (62.03) and C (59.78) applications. It was observed that there was a decrease in the GA+E(48.41), GA+C (33.84), GA+E+C (33.54) application group according to the GA application. The lowest germination rate and germination index were seen in GA+C and GA+E+C groups (Table 1). Pre-application of vitamin C showed a negative effect on the germination index.

The greatest decrease in germination speed was in the GA+E+C (33.33) group compared to the GA (90) and E (86.67) groups. A decrease was observed in the group treated with E+C (63.33) compared to vitamin E application.

b) Radicle and Plumula fresh, Dry Weight and Length

The effect of pre-applications (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on the fresh, dry weights and lengths of the radicle and plumula was found to be statistically significant at P <0.01 according to the analysis of variance results (Table 2).

Table 2: The effects of preliminary applications (GA, vitamin E, vitamin C) on radicle and plumula lenght, dry and fresh weight in maize seeds (71 may 82).

Aplications		Radicle length (cm)	Plumula length (cm)	Fresh weight Radicle (mg)	Dry weight Radicle (mg)	Fresh weigth Plumula (mg)	Dry weight Plumula (mg)
Control	1	7.0 A	2.17 ab	76.24 A	8.49 A	84.60 A	10.22 A
GA	2	5.17 B	2.17 ab	48.27 D	5.56 B	70.30 D	6.17 E
E	3	3.83 B	2.17 ab	42.37 E	5.53 B	58.73 F	7.51 C
C	4	5.33 B	2.33 ab	61.44 B	5.17 C	62.33 E	8.15 B
GA+E	5	4.67 B	2.83 a	50.64 C	3.60 D	80.33 B	6.82 D
GA+C	6	1.83 C	2.00 ab	23.53 F	1.17 E	52.17 G	3.30 F
E+C	7	1.67 C	1.83 b	22.33 G	0.83 F	49.19 H	3.15 G
GA+E+C	8	1.83 C	2.33 ab	21.53 H	1.32 E	75.17 C	3.17 G
	F VALUE	<0,0001	0,4663	<0,0001	<0,0001	<0,0001	<0,0001
	LSD	1,5997	0,8833	0,7592	0,2024	0,7956	0,1179

A statistically significant decrease in radicular length was detected in the co-administration groups compared to the C treatment (Table 2). In addition, GA (5.17), E(3.83), C (5.33) according to application groups GA+E(4.67), GA+C(1.83) and GA+E+C(1.83). It was determined that there was a decrease in the application groups. On the other hand, E(2.17) application increased compared to GA+E (2.83), GA+E+C(2.33) and E+C(1.87) groups. Length measurements in the plumula were among the values in the GA+E(2.83) and E+C(1,83) application groups but were not found to be statistically significant.

The fresh weight of the radicle was decreased in the treatment groups compared to the control (76,24) group. In addition, a decrease was observed in the GA+C(23.53) and E+C(22.33), GA+E+C(21.53) application groups compared to the C(61.44) application (Table 2). In the dry weight of the radicle, compared to the E(5.53) and C(5.17) application groups, the greatest decrease was in the E+C (0.83) group.

If the plumula is on fresh weight, compared to the control (84.60) group, the highest decrease was observed with the E(58.73) application. If it is on dry weight, the highest decrease was observed with the application of GA (8.15 mg) compared to the control (10.22) group (Table 2). According to the GA application, an increase in GA+E fresh (80.33) and dry (6.82) weights was observed. It was determined that there was an increase in fresh weights in GA+E+C(75,17) application compared to GA+C(52,17) and E+C(49,19) application groups.

c) Relative Water Content (RWC)

The effect of pre-applications (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on the relative water content (RWC) was statistically significant at $P < 0.01$ according to the results of the analysis of variance (Figure 1).

The relative water content of the radicle increased by 7.75% in group C compared to the control treatment. Compared to the applications performed alone, RWC increased by 26.14% in the GA+C group (Figure 1).

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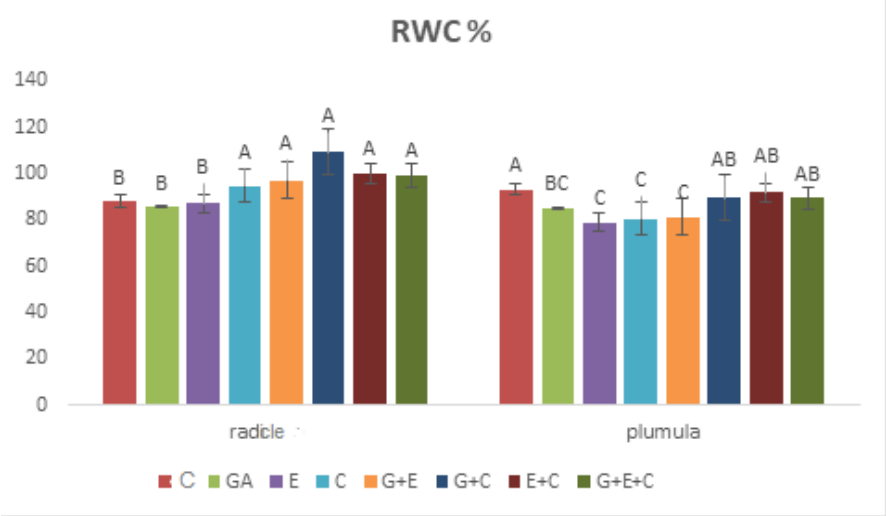

Figure 1: The effects of preliminary applications (GA, vitamin E, vitamin C) on relative water content (RWC) (%) in maize seeds (71may82)

In the relative water content of the plumula, a decrease of 7.225% was observed in GA+E applications compared to GA+C and GA+E+C applications. However, it was determined that there was a 3.13% increase in the GA applied groups compared to the G+E application (Figure 1). Compared to the E+C application, a decrease of 16.15% and C 14.14% was observed in the E applied groups, respectively.

d) Protein content

The effect of pre-applications (GA, vitamin E, vitamin C) on maize seeds (71May82) on protein content (mg) was found to be statistically significant at $P < 0.01$ according to the results of the analysis of variance (Figure 2).

The content of protein increased in all treatments in the radicle compared to the control group. On the other hand, it was determined that there was an increase of approximately 1.36 times in the GA+C application compared to the GA and C application in the compass (Figure 2).

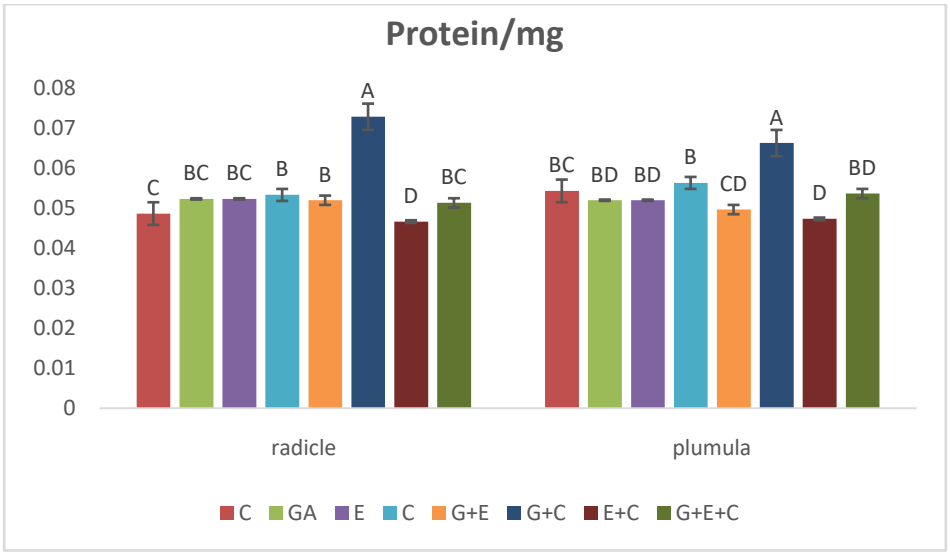

Figure 2: The effects of preliminary applications (GA, vitamin E, vitamin C) on protein content in maize seeds (71MAY82)

e) Catalase Enzyme Activity

The effect of pre-treatments (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on CAT enzyme activity (unit/mg-¹ protein) was found to be statistically significant at $P < 0.01$ according to the results of analysis of variance (Figure 3).

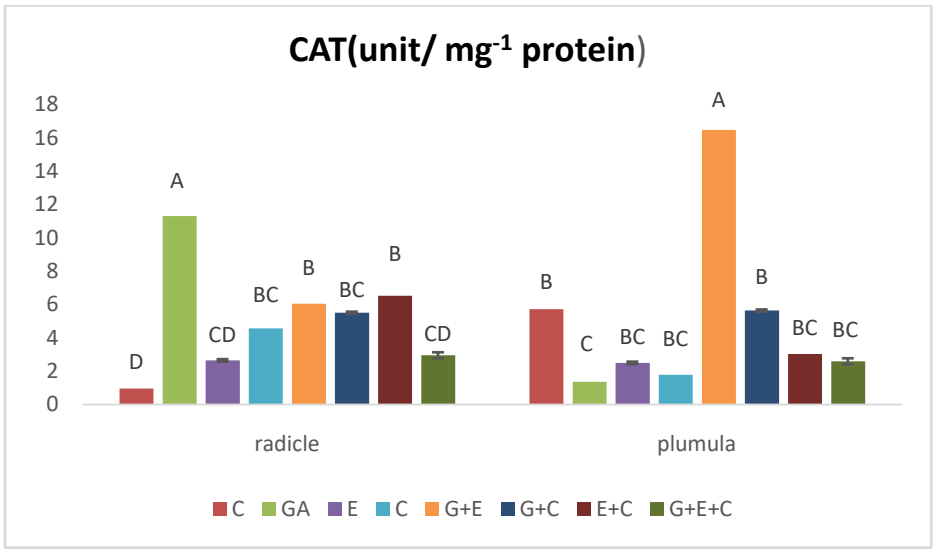

Figure 3: The effects of preliminary applications (GA, vitamin E, vitamin C) on CAT enzyme activity (unit mg-¹ protein) in maize seeds (71MAY82)

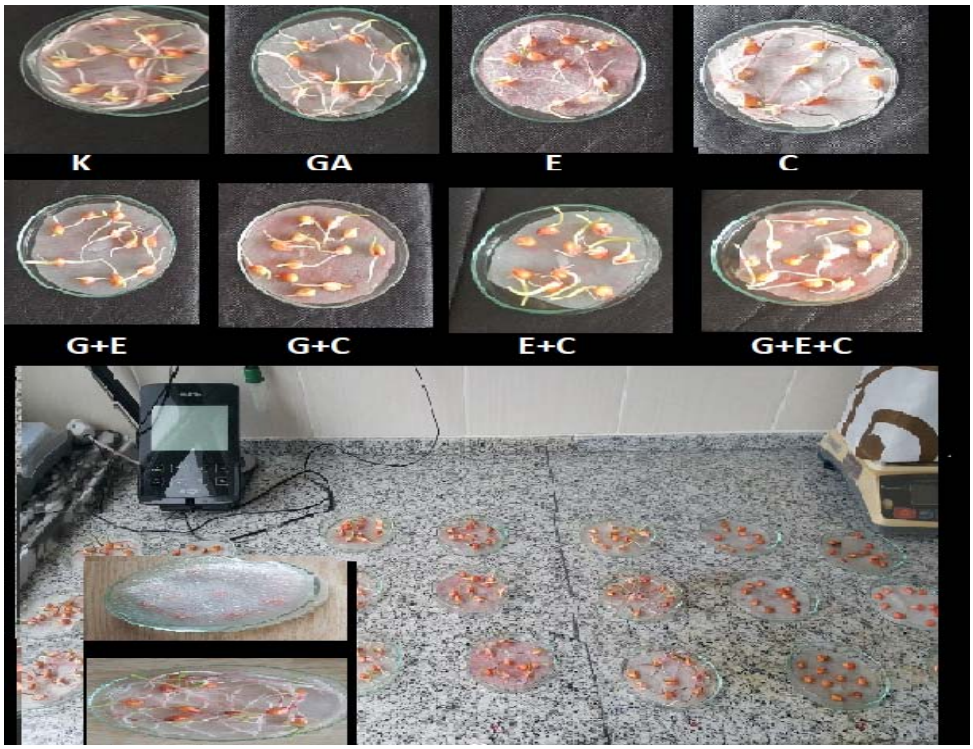

Photo 1: Maize seed spretreated with gibberellic acid, alphatocopherol and ascorbic acid SU Plant Physiology Laboratory

In the radicle, while CAT enzyme activity increased with the application of GA, it caused a decrease in plumula. It was determined that a 3.32-fold decrease in the radicle with the GA+E+C application compared to the GA application. In the plumula, a 12-fold increase was observed with the GA+E application compared to the GA application (Figure 3). E+C application increased CAT enzyme activity in the radicle compared to the E application.

IV. Discussion

The germination time of seeds varies depending on the amount of air and water in the germination medium, its temperature and the water absorption capacity of the seeds [21]. In this study, it was observed that there was a decrease in the GA treatment group compared to GA+E, GA+C, GA+E+C and control groups in corn seeds in terms of germination time. Similarly, Oral et al. [25] and Topcu et al. [26] revealed in their study that GA pre-application reduced the germination time. Accordingly, we think that the increase in germination rate observed in GA application compared to the co-treatment groups is related to the decrease in germination time. Depending on the results obtained in the study, GA and E application groups alone decreased the germination time compared to the combined application groups. The C application decreased it compared to the control group and increased it compared to the E+C application.

It has been demonstrated that seed pretreatments improve germination rate, speed and homogeneity [27]. The germination rate of corn seeds increased with GA and E application compared to C application (Table 1). It has been reported that the increase in germination rate in Arabidopsis mutant seeds treated with vitamin E is associated with a decrease in lipid peroxidation [28]. This result is in good agreement with our data. The greatest decrease in germination rate was with GA+C application compared to C application and co-treatments. Based on these results, we think that the applied vitamin C concentration (100ppm) negatively affects the germination rate.

In terms of germination index, GA application in corn seeds increased compared to GA+E, GA+C, GA+E+C application. In a study conducted by Yuonesi and Moradi [29], on wheat, it was stated that seeds subjected to GA pretreatment had a positive effect on plant growth. Similarly, it has been emphasized that GA applications increase productivity in plants [30]. This situation can be associated with an increase in germination rate and a decrease in germination time. The decrease observed in the groups where vitamin C was administered alone or together is associated with the decrease in germination rate. Similarly, the application of vitamin C to wheat seeds in high concentrations has been reduced germination [31].

In maize seeds, GA application increased fresh and dry weights and radicle length compared to the co-administered groups. Supporting this result, Kaya et al.

[32] found that pre-GA application to sugarcane seeds increased the length and weight values. In our study, radicular and plumula lengths increased with GA+E application compared to E application. This increase is in parallel with the increase in fresh and dry weight observed in the same groups.

Furthermore, while it causes a decrease in radicular lengths and weight values in GA+C and GA+E+C application groups compared to vitamin C application, an increase in RWC content was observed. In contrast, pre-application of vitamin C an increased root length and dry weight [17]. However, it can be said that an increase in plumula fresh weight observed in the groups treated together compared to the C application is due to the increase in RWC. This may be related to the fact that vitamin C has a positive effect on RWC, increasing the germination speed and rate. E+C application caused a decrease in all physiological parameters compared to the applications performed alone. We believe that this situation caused the negative effect of E+C application on seed germination by affecting the germination index and rate.

The content of protein constitutes the main nutritional source important during the development and maturation of seeds [33]. In our study, the protein content in the radicle and plumula increased with GA+C application than the control groups (Figure 2). We think that this result is related to the stimulation of enzyme activities. Mohsen et al. [34] showed that pre-treatment of *Viciafaba* seeds with vitamin C increased their protein content. Similarly, it has been determined that the application of 100 and 200 ppm vitamin C in oilseeds increases the activity of CAT and protein [35]. Accordingly, the increase in weight values of the radicle and plumula is associated with the increase in protein content.

In maize seeds, catalase enzyme activity increased with GA application in the radicle, decreasing in the plumula. In addition, we think that the increase observed in catalase enzyme activity with GA+E application in the radicle is due to GA application (Figure 2). Similarly, an increase in CAT enzyme activity is associated with limiting hydrogen peroxide production by preventing dehydration-related oxidative damage [36] and preserving lipid mobilization [33]. Moreover, Younesi and Moradi [29] observed an increase in catalase activity with pre-GA application to seeds of the Medicago sativa plant.

In our results, C application in the radicle increased catalase activity compared to control and co-administration decreasing it in plumula compared to control, causing no change compared to co-administrations. Thus, Dolatabadian and Sanavy Modarres [34] found that the application of vitamin C to sunflower seeds reduced catalase activity.

V. Conclusion

While it was observed that gibberellic acid and vitamin E applications on corn seeds had positive effects, it was determined that vitamin C caused negative effects depending on the applied concentration. Additionally, it was observed that the combined applications had a negative effect on the physiological and biochemical parameters of the radicle and plumula. Furthermore, we believe that the data obtained from this study will shed light on the applications to be made to increase the yield of maize seeds under stress.

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Studies on Morphological, Anatomical and Phytochemical Characteristics of *Costus lucanusianus* J. Braun & K. Schum. of Costaceae.

By Wahua, C., Agogbua, J., Ugiomoh I. & Awogbayila, O. D.
University of Port Harcourt

Abstract- The research was set to investigate the morphological, anatomical and phytochemical characteristics of *Costus lucanusianus* J. Braun & Schum. of pan tropical family of monocots, Costaceae in the Order Zingiberales. It is amedicinal perennial herb which grows up to 3m in height without branches. The glabrous stem is cylindrical, greenish, masked with brown leaf sheaths and hairs observed around the petiole base. The petiolate leaves are lanceolate and large in spiral phyllotaxy measuring up to 25±10cm long and 7±5cm wide. Region of leaf blade is pubescent and leaf apex is acute. Flowers are in terminal clusters with cymose inflorescence. The corolla is whitish with pinkish lips and deep yellowish throat. Creeping rhizomes are present with fibrous root system. The micro-morphological study revealed polygonal epidermal cells with tetracytic stomata and amphistomatic. Anatomical study showed circular sections of spiral leaves with numerous scattered vascular bundles in the main stem. The stem cortex is dominated with sclerenchyma while parenchyma occupied the ground meristem. Pith is present in the root section, which is surrounded with large vessels in ring form. Phytochemical investigation revealed presence of flavonoids, terpenoids, steroids, cardenolide and saponins.

Keywords: *costus lucansianus, anatomy, morphology, phytochemistry, stomata.*

GJSFR-C Classification: *FOR Code: 060799*

STUDIESONMORPHOLOGICALANATOMICALANDPHYTOCHEMICALCHARACTERISTICSOFCOSTUSLUCANUSIANUSJBRAUNANDKSCHUMOFCOSTACEAE

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Keywords: costus lucansianus, anatomy, morphology, phytochemistry, stomata.

I. Introduction

The Order Zingiberales consists of 8 family members which include Costaceae which has distinct aerial shoot that have characteristic monostichous phyllotaxy (1). The non-aromatic vegetative body, spirally arranged leaves and anther appendages to separate the Costaceae from Zingiberaceae. *Costus lucanusianus* is commonly called Bush cane, in Ijaw and Nembe it is referred to as Ogbodoin and Ogbodain, in Ikwerre it is called Opete and in Efik it is known as Mbritem (2). *Costus* is the largest genus in the family Costaceae with about 150 species which are mainly tropical in terms of distribution (3 and 4). There are about 4 genera in Costaceae (5).*Costus lucanusianus* has the calyx-tube longer than the bracts and often densely puberulous, leaf sheaths have long bristly rim below the apex and inflorescence globose as markly differentiated from other species of *Costus* (6).The sap is used to relieve malaria attack by the Ijaw people and occasionally chewed to quench thirst (2). In Ikwerre ethnicity, it is administered to reduce the severity of snake bites. It is used in Southern Ivory Coast for anti-abortive activities(7). A decoction of the stem sap is used as eye wash and for treatment of early eye diseases.

Considering The justification focuses on the fact that *Costus lucanusianus* is used variously in treating different diseases, It is observed that the herb contains sodium chloride (NaCl), Sodium hydroxide (NaOH) and lots more which are very important industrial and domestic compounds, it is therefore necessary to consider Taxonomic lines of evidence as concerns this plant, hence the objectives focus on morphological, anatomical and phytochemical characteristics on *Costus lucanusianus* J. Braun & K. Schum. belonging to the family Costaceae.

II. Materals and Methods

a) Geographic Location

The location of the parent plant studied was University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

b) Morphological Studies

The meter rule was used to measure the plant height, starting from the root-collar to the terminal bud while leaf length from the leaf tip to the petiole base. The leaf width is measured across the leaf lamina, from one margin to another at the widest point on it.

c) Micro-morphological Studies

Harvested leaves and young stem for this study were peeled and subjected to alcohol solutions in the ratio of 50%, 75% and absolute alcohol respectively following the method of (8). The cleared epidermal layers were stained with safranin for 5 minutes, washed and counter stained with Alcian blue for the same time interval, rinsed and temporarily mounted in aqueous glycerol solution. Photomicrographs were taken from good preparations.

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d) Anatomical Study

The plant grew in the wild. The harvested stems, leaves, petioles, flowers, fruits and roots were dehydrated in alcohol solutions of 50%, 75%, absolute alcohol and thereafter subjected to alcohol chloroform series in the ratio of 3:1 of alcohol chloroform series, 1:1, 1:3 and pure chloroform following the method of (9) modified. Free hand section was done using a systematic arrangement of 5 razor blades as described by (10) was adopted. Microphotographs were taken from good preparations using Sony camera of 7.2 Mega pixels having 2.411 LCD monitor and High sensitivity ISO 1250.

e) Phytochemical Study

The leaves of *Costus lucanusianus* studied were sun dried for 72 hours (3 days) and weighed. Fifty grammes (50g) of the dried leaves were macerated in 96% ethanol with the aid of a pestle and mortar. The extract was thereafter filtered and evaporated to dryness (constant weight) using a rotary evaporator set at 45^0C. Residue yields were noted and used for the phytochemical screening.

f) Test for alkaloids

This was done using 0.5g of the plant extract stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. The third 1ml was treated with Wagner's reagent. Turbidity or precipitation with any of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (11 and 12). A modified form of the thin-layer chromatography (TLC) method as described by (13) was used. One gramme (1g) of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. The extracts were combined and concentrated to 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvent systems were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatograms (indicated by an orange or darker colored spot against a pale yellow background) was confirmatory evidence that the plant extract contained alkaloid.

g) Test for tannins

i. *Ferric chloride test ($FeCl_3$)*

5g of the pulverized sample was boiled in 5ml of distilled water for 5minutes on water bath. This was filtered while hot. 1ml of 5% $FeCl_3$ was added to the filtrate and observed. Blue-black, green or blue-green precipitate was taken as the presence of tannins (14)

h) Test for anthraquinones

Born trager's test was used. Five grammes (5g) of each plant extract were shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones.

i) Test for combined anthraquinones

Five grammes (5g) of each plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammonia phase (lower layer) indicated presence of anthraquinone derivatives in the extract (12).

j) Test for phlobatannins

The deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins(12).

k) Test for cardiac glycosides

Lieberman's test was used in which 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled in ice. One milliliter (1ml) of Sulphuric acid was carefully added in drops until a color change from violet to blue to green indicated the presence of a steroidal aglycone portion of the cardiac glycoside (14).

l) Test for Saponins

Frothing tests was done following the method described by (15). The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. 0.5g of the plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. The disc was then washed in ether, dried and placed on a 7% blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was taken as further evidence of presence of saponins. (15)

III. Results

a) The Geographic location

The geographic location of the parent plant is University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

b) Morphological Study

Costus lucanusianus J. Braun & Schum. is a perennial herb which grows up to 3 m in height with no branches hence growth is monopodia in nature. Figure 1a, b, c and d. The glabrous stem is cylindrical, greenish, masked with brown leaf sheaths and hairs observed around the petiole base. The petiolate leaves are lanceolate and large in spiral phyllotaxy measuring

up to 25 ± 35 cm long and 7 ± 12 cm wide. Region of leaf blade is hairy and leaf apex is acute. Flowers are in terminal clusters with cymose inflorescence. The corolla is whitish with pinkish lips and deep yellowish tube or throat. Creeping rhizomes are present with fibrous root system.

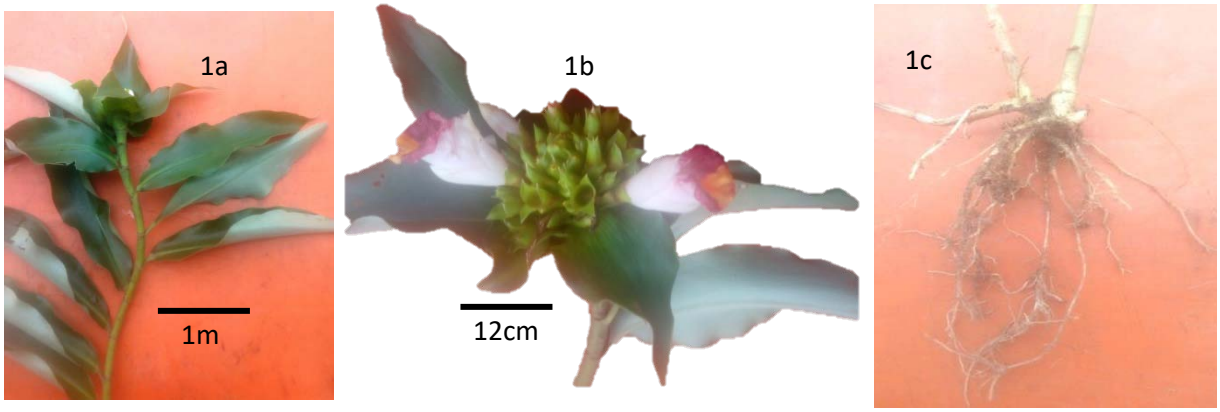

Figure 1a: *Costus lucanusianus*; 1b: Flower head of *C. lucanusianus*; 1c:*C. lucanusianus* fibrous root system

Table 1: Summary of morphological Properties of *Costus lucanusianus* J. Braun & K. Schum.

Characters	
Habit	Evergreen herb.
Duration	perennial
Root	Fibrous Root system from rhizomes
Stem Description	Greenish to brownish, fleshy edible herbs
Leaf type	Simple lanceolate with acute apex
Leaf organization	Simple and petiolate
Phyllotaxy	Spiral arrangement
Leaf outline or shape	About three times as long as wide, lanceolate with acute apex and cuneate base.
Leaf margin	Even or smooth
Length of leaf (cm)	about 25 cm long
Range	15 to 35 cm long
Mean	25 ± 10 cm long
Breadth of leaf (cm)	8 cm wide
Range	3 cm to 12 cm wide
Mean	7 ± 4cm wide
Flower description	Flowers are terminal clusters with cymose in florescenceon capitulum

c) *Epidermal Study*

Epidermal studies revealed tetracyticstomata which are amphistomatics and epidermal cells are in polygonal in shape. Lower epidermis has more stomata than the upper one. See plates 2a and2b.

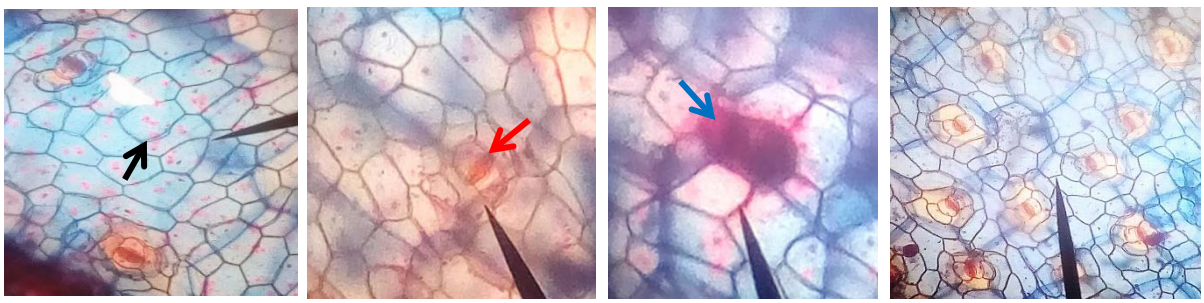

Plate 2a, 2b and 2c: *Costus lucanusianus Adaxial* Foliar Epidermis; 2d: *C. lucanusianus*Abaxial Epidermis.Black arrow in 2a revealed nucleated epidermal cells; Red arrow in 2b showed tetracytic stoma; Blue arrow in 2c showcased uniseriatetrichome; and 2d revealed more stomata in abaxial surface than as observed in the adaxial region

d) Anatomical Study

Anatomical study showed circular sections of spiral leaves with numerous scattered vascular bundles in the main stem, mid-ribs and root, but absence of pith in the stem while the root anatomy has large central pith. Plates 3a, 3b, 3c, 3d, 3e and 3f. The hypodermis is preoccupied with sclerenchyma and vasculation is closed type. Parenchyma occupied the ground meristem. Pith observed in the root section surrounded with large vessels in ring form at pith region.

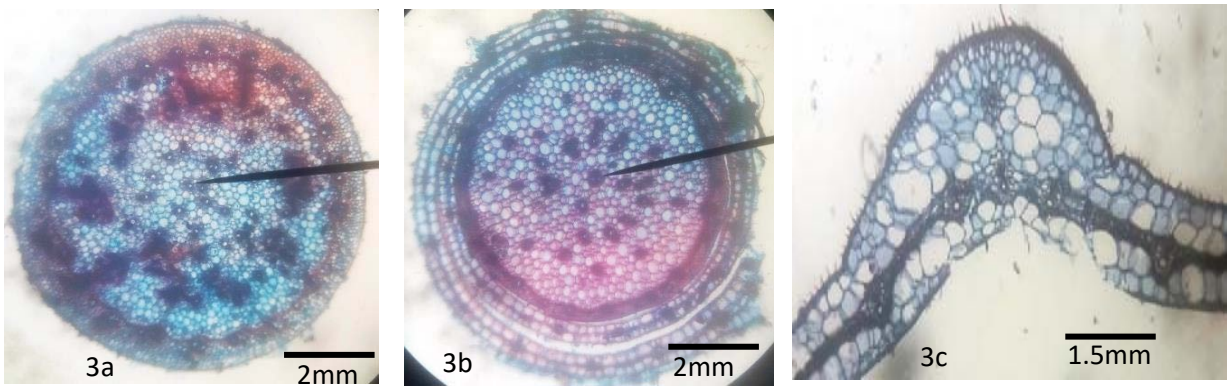

Plate 3a and 3b: *Costus lucanusianus* stem anatomical sections (T.S.); 3c: *C. lucanusianus* Mid-rib (T.S.)

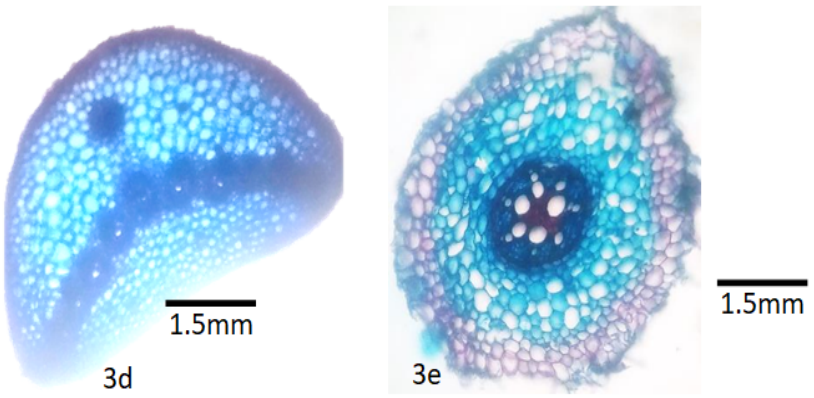

Plate 3d and 3e: *Costus lucanusianus* stem anatomical sections (T.S.)

IV. Phytochemical Study

Phytochemical investigation revealed presence of flavonoids, terpenoids, steroids, fixed oil, cardenolide and saponins. See table 1.

Table 1: Qualitative Phytochemical Test on *Costus lucanusianus*

Phytochemicals	Test methods	*Costus lucansianus*
Flavonoids	ACIB	+
Triterpenoids/Steroids	Lisbermann-Buchard's Test	-
	Salwoski	+
Cardenolide	Keller Killani	++
	Kedde	++
Saponins	Frothing Test	+
	Emulsion Test	++
Alkaloids	Drangerdoffs	-
	Mayer's	-
	Hager's	-
Tannins	$FeCl_3$	-
Cyanogenic glycosides		-

Key: ++ represents more abundant while – showed absence.

V. Discussion

The micro-morphological study revealed tetracytic stomata on both sides of the leaf surfaces. Unlike most members of the Order Zingiberales, *Costus lucanusianus* has non-aromatic characteristics. The description accorded to *Costus lucanusianus* in this investigation tallied with those of (6) who stated that *Costus lucanusianus* has the calyx-tube longer than the bracts and often densely puberulous, leaf sheaths have long bristly rim below the apex and inflorescence globose and as differentiated from other species of *Costus*, The sap is used as remedies to eye infections, anti-snake poisons and used to quench thirst where drinkable water is far-fetched. The absence of tannins and presence of saponins in the stem sap is an indicator of no dyes or staining properties but could be used for soap making, unlike most other members of Zingiberales such as *Musa* species which have lots of dyes.

VI. Conclusion

More research using the rhizomes, roots and fiber content should be encouraged for proper exploitation of the species and extended to other members of the genus Costaceae. The fiber could be good source for paper and thread making.

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ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

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Financial

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Organize seminar/conference

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Career | Credibility | Financial

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Associate	Fellow	Research Group	Basic
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Preferred Author Guidelines

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

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

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

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

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

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2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
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7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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

Acknowledgments

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

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

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

Manuscript Style Instruction (Optional)

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

Structure and Format of Manuscript

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

A research paper must include:

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

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

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

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

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

Format Structure

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

All manuscripts submitted to Global Journals should include:

Title

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

Author details

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

Abstract

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

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

Keywords

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

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

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

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

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

Numerical Methods

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

Abbreviations

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

Formulas and equations

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

Tables, Figures, and Figure Legends

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

Figures

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

Preparation of Eletronic Figures for Publication

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

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

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

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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

General style:

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

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

Mistakes to avoid:

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

Title page:

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

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

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

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

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

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

Approach:

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

Introduction:

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

The following approach can create a valuable beginning:

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

Approach:

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

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

Procedures (methods and materials):

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

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

Materials:

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

Methods:

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

Approach:

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

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

What to keep away from:

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

Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

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

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

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

Figures and tables:

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

Discussion:

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

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

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

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

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

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	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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