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DISCOVERING THOUGHTS AND INVENTING FUTURE

HIGHLIGHTS

Automobile Radiators

Saprolegnia Species

Date Palm and Carob Plants

Floristic Composition

Yellow Jellyfish

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On the Origin of Sex

By A.I. Ibraimov
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Abstract - The problem of sex origin of eukaryotes in the process of evolution still has not settled. Existing theories and hypothesizes mainly concern the maintenance and biological reasonability of sexual mode of replication. Their theoretic foundation is based on Darwin's and Mendel's ideas that sex was originated due to natural selection and genes. Another model is proposed – sex of eukaryotes was originated as a result of long-term evolution of non-coding DNAs in a genome at one of the branches of prokaryotes. Non-coding DNAs accumulation and evolution in prokaryotes' ring chromosomes eventually led to emergence of mitotic chromosomes and mitotic way of cell division. Sex and sexual replication became possible since that time when modified variant of mitosis – meiosis – have originated. Separate stages of the proposed model may be exposed to experimental check.

Keywords : *origin of sex, origin of chromosomes, noncoding DNAs, in vitro meiosis.*

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On the Origin of Sex

A.I. Ibraimov

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Keywords : origin of sex, origin of chromosomes, non-coding DNAs, in vitro meiosis.

I. Introduction

The evolution and maintenance of sexual replication is one of the central questions in modern evolutionary biology (Bell 1982; Williams 1975; Maynard-Smith 1978; Michod 1995; Hurst and Peck 1996). The evolution of sex contains two related, yet distinct, themes: its origin and its maintenance. Reasons for origins of sex are not necessarily the same as for the maintenance of sex (Birdsell and Wills 2003). Since the hypotheses for the origins of sex are difficult to test experimentally, most current work has been focused on the maintenance of sexual reproduction.

There are many contested theories for what made organisms evolve in a pattern which is less efficient for population growth, but no sure-fire answers (Maynard Smith and Szathmáry 1999). Several explanations have been suggested to explain how sexual reproduction is maintained in a vast array of different living organisms (Bell 2001; 2006; Bernstein et al. 1989; Hamilton et al. 1990; Michod 1995; Agrawal and Chasnov 2001; Cavalier-Smith 2002; Otto 2003; Dolgin and Otto 2003; Hörandl 2009). Yet, as these theories valiantly attempt to explain why sex exists now, they do not explain the origin of sex (Harrub and Thompson 2003). We have no intention to make comprehensive analysis of works devoted to maintenance of sexual reproduction.

Most theories of the origin of sex include either facultative or obligate sexual cycles (Crow 1994; Dacks and Roger 1999). Simply stated, organisms that have facultative sexual cycles can reproduce sexually or asexually, whereas organisms that reproduce in an obligate sexual cycle are forced to reproduce sexually or not at all. Dacks and Roger (1999), for instance, suppose that facultative sex was most likely the sexual cycle which developed first, at the origin of sex and the arrival of obligate sex may have something to do with the evolution of increasingly complex multi-cellular organisms. Nevertheless, this article dodges the question of why mammals don't reproduce through a facultative sexual cycle.

The predominant theory for the origin of sex has always been the benefits of DNA repair. However using DNA repair as the strongest argument for sex is problematic as it gives asexual diploids an equal footing with sexual organisms in that regard. DNA repair in and of itself does not provide a satisfactory explanation for the necessity of sex, only a strong argument for diploidy (Harrub and Thompson 2003).

According to Rothschild (1999) the origin of sex is that UV radiation stirred evolution in such a way as to make sex advantageous. UV radiation is a mutagen, and exposure thereto causes genes to mutate in ways that may or may not be good for the organism. Sex splits the homologous pairs of chromosomes and allows them to recombine in haploidy before they are passed on, so that daughter cells receive different combinations of beneficial and harmful mutations.

Another theory is that sex originated as a way to protect cells from infection by plasmids and other parasitic bodies (Sterrer 2002). According to this theory, cells that come to contain parasites through phagocytosis or another method of ingestion will co-evolve with the parasites, allowing the primary cell protection against further infection and the secondary body assurance of reproduction. Sex must emerge to keep this relationship stable, preventing the parasite from taking over the host by breaking up the symbiont genomes and asserting host control over replication.

It is one thing to develop a theory or hypothesis to explain something that already exists, but it is entirely another to develop a theory or hypothesis to explain why that something (in this case, sex) does exist. In his book, The Masterpiece of Nature: The Evolution of Genetics and Sexuality, G. Bell (1982) described: 'Sex is the queen of problems in evolutionary biology. Perhaps no other natural phenomenon has aroused so much interest; certainly none has sowed as much confusion. The insights of Darwin and Mendel, which have illuminated so many mysteries, have so far failed to

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shed more than a dim and wavering light on the central mystery of sexuality, emphasizing its obscurity by its very isolation'.

Indeed it is hard to believe that having impressive breakthrough in modern genetics and molecular biology the reasons and mechanisms of sex origin are still unknown. This probably has to do with the fact that in the basis of all hypothesizes and theories on sex biology lies idea on all-powered role of natural selection and genes in eukaryotic organisms' evolution. Although they help to explain reasonably and justify such widespread propagation of sexual reproduction in the world of eukaryotes; nevertheless, these approaches had little help in the development of theories and hypothesizes explaining sex origin. More over they were not able to show ways of their experimental check. As Ridley (2010) begrudgingly admitted: 'Sex is not used simply for want of an alternative. Nothing, in an evolutionary sense, forces organisms to reproduce sexually'.

II. Theoretic Modeling

Our approach is relies on the non-coding DNAs (ncDNAs) evolution. Hypothesis is based on suppositions that: a) sex was originated at one of the branches of prokaryotes, in genome of which ncDNAs were accumulated and then evolved; b) the beginning of sex emergence is related to linear chromosomes emergence from a "bare" ring chromosome which due to ncDNAs presence in its composition has acquired nucleosomal structure (mitotic chromosome); c) on the basis of various types of ncDNAs centromeres, telomeres and kinetochores originated; they help chromatids of replicated mitotic chromosomes keep together or on the contrary split on two daughter cells in the process of cells division (mitosis); d) sexual replication became possible due to emergence of modified mitosis – meiosis (Ibraimov 2003; 2004; 2008; 2009; 2010).

It is commonly known that eukaryotic organisms have meiotic sex. Since meiosis is modified mitosis it is obvious that sex origin directly connected with mitotic chromosomes emergence. Unfortunately we were not succeeded in finding in literature any hypothesis explaining origin of mitotic chromosomes (Ibraimov 2009). Perhaps this is due to the fact that it is impossible to explain the origin of mitotic chromosomes by simple increase of genes number in prokaryotes chromosomes and by their further multiplication. Note that centromeres, telomeres and kinetochores consist of high repetitive DNA sequences and do not have structural genes which testify that they were originated from ncDNAs. As for the amount of coding DNAs in human's chromosomes they constitute less than 2% of total DNA of his genome.

Earlier, we presented data that probably sex and sexual reproduction of eukaryotic organisms are the result of the long evolution of ncDNAs, which step by step led to the origin of mitotic chromosome, mitosis, meiosis, sex determination and differentiation mechanisms (Ibraimov 2008; 2009; 2010). As we suppose, so complicated evolutionary changes were the consequence of an amazing ability of ncDNAs to provide the very different forms of DNA organization: from nucleosomes to mitotic chromosome body. Apparently, the basis of the ncDNAs' potential for different forms of self-organization is formed by their common capability of mutual nonspecific attraction – "stickiness", – which is connected to the presence of short repeated sequences of nucleotides in them.

Though, the modes of DNA packaging into interphase cells do not influence on the contents of the genetic information of a nuclear genome, nevertheless, they are essential factors in a vital activity of not only single cells (Ibraimov 2003), but of the whole organism (Ibraimov 2004; 2007; 2011). Hereby, we do not assert that ncDNAs are capable of specific reactions. Their nonspecific molecular composition does not allow this. We just want to say that nonspecific reactions can serve as the basis for the creation of specific forms of response to different environmental changes, and this circumstance can be related to the sex origin of eukaryotic organisms.

Formation of nucleosomes is the first step in DNA packaging into a minor metaphase structure. We believe that it is connected with the availability in eukaryotic genomes intervening sequences of ncDNAs, which has the ability to attach to histones (by DNA-protein recognition mechanisms). Lack of nucleosomes in prokaryotes in spite of the availability in the cells the histone-like proteins is possibly attributed to this important reason. In other words for formation of nucleosomes, chromomeres, centromeres, telomeres, kinetochores and chromosome bands it is necessary that in the DNAs should be nucleotide sequences with anchorage dependence features, due to which they will be inside the nucleus (in more detail see Ibraimov 2003, 2004; 2009; 2011).

Since meiosis represents a special type of mitosis, and mitosis is not possible without mitotic chromosome, then the solving of the sex origin problem is probably to be started with the mitotic chromosome origin examination. The following model of the origin of mitotic chromosomes and mitosis seems highly probable (Fig. 1).

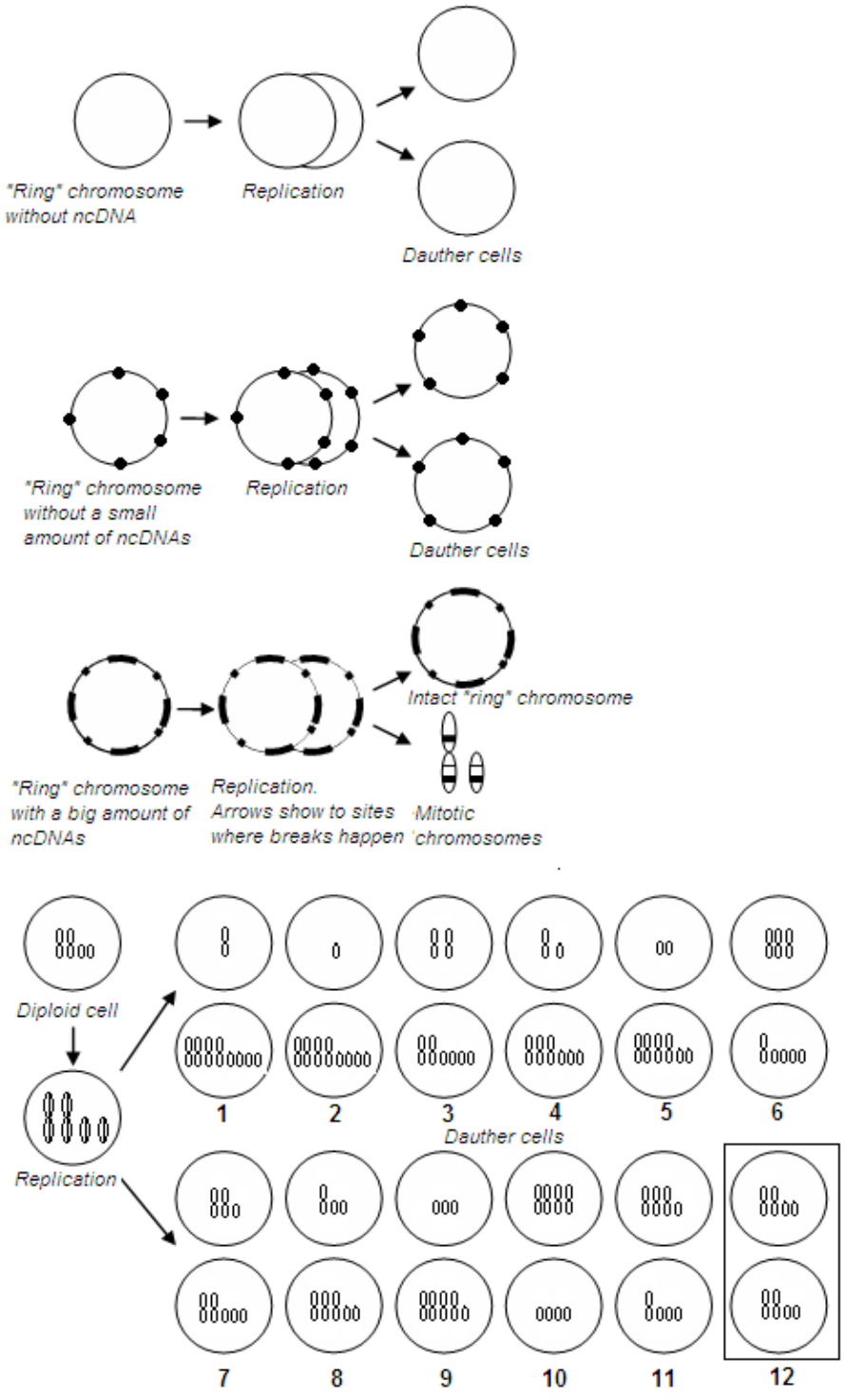

Figure 1 : Origin of mitotic chromosomes and mitosis.

At a certain stage of "bare" ring chromosome evolution of some lines of prokaryotes the sites with ncDNAs started to emerge (Ibraimov 2003, 2004). This has led to: a) the increase of the length of such chromosomes; b) the delay of separation of already replicated DNAs because of the mutual attraction of chromosome sections with ncDNAs. To divide such ring chromosomes, at the least they need to be shortened to

the maximum. This can happen only owing to ncDNAs according to the principle, which has a place at mitotic prophase stage. When the thickness of such cylinder reaches the certain limit, "sister ring chromosomes" will start to repulse from each other and finally will divide in two.

In the cases when this division mode becomes difficult, ring chromosomes break. Perhaps, more favorable outcomes expected those ring chromosomes where breaks happened in the sections with the considerable amount of ncDNAs. In due course, these ends could be transformed into centromeres, telomeres and kinetochores (Lima-de-Faria 1983). Thus, could be originated the eukaryote genetic linkage groups in the form of meta-, acro- or telocentric chromosomes. There appeared possibilities for the endless combination of genes in the population of eukaryote organisms through meiosis, having opened yet unknown prospects for their further development.

Major problems happen at attempt to explain transformation of mitosis into meiosis. As it is known, meiotic division having much in common with mitosis, nevertheless has a number of peculiar properties: at mitosis centromeres divide and sister chromosomes, connected to them, move towards the opposite poles. At meiotic division paired centromeres do not divide, but each one moves separately from others, carrying one chromosome from each pair to the opposite poles.

It ought to be admitted that we know almost nothing about the mechanism of chromosome pairing in meiosis, due to which homologues appear to be so tightly brought together that there can start synapsis with the formation of synaptonemal complex. We assume that during temperature reduction the slow down of heterochromatin (one of the types of ncDNAs) compactization takes place, and as a consequence formation of metaphase chromosome body detains, thus giving time to prophase homologous chromosomes to "know" each other (Ibraimov 2009).

As we assume, ncDNAs in chromosome bands play the important role in both mutual attraction and repulsion of chromosomes. In mitosis, for instance, sister chromatids separate without the help of mitotic spindles having the dividing cells treated with colchicine ("C-mitosis"). Apparently, the separation of sister chromatids in "C-mitosis" is also caused by a complete fusion of chromomeres and chromosome bands along the chromosome into one homogeneous body at the end of metaphase. When chromatids turn into short "thick" cylindrical bodies, the contact area between sister chromatids becomes so small that they are not in the position to remain tied together in the "boiling" cytoplasm. Here, the attractive forces between chromatids, even if remained, depend mainly on the quantity and the quality of repeated DNAs.

Hence, at the stage of the cell division, ncDNAs derivatives participate: 1) in shortening and dense packaging of the chromatin fibres for formation of the body of the metaphase chromosomes; 2) in keeping the sister chromatids up to the end of anaphase together; 3) in repulsion of sister chromatids from each other at the stage of anaphase; and 4) it gives chromosomes the necessary strength and flexibility so that they can pass the mitotic cycle.

Could certain stages of sex origin be experimentally checked? Fortunately, the certain stages of the supposed sex origin account can be checked experimentally. In principle the proposed model of mitotic chromosome origin can be considered to have been already checked. Hereof testify the experiments on generation of artificial chromosomes for use in gene therapy. For example, it is demonstrated that the short arm of human acrocentric chromosomes, which contains tandemly repeated ribosomal DNA genes and different satDNA sequences, is an optimal chromosomal region for inducing *de novo* chromosome formation (Hadlaczky 2001).

Evidently, with sophistication of cell cultivation, cloning and *in vitro* fertilization methods there has come the time for experiments on carrying out meiotic division of somatic cells – *in vitro* meiosis (IVM). The main point of IVM is to expose somatic cells to meiotic division in order to receive haploid cells ("gametes") for *in vitro* fertilization (IVF). As we believe there already exist methodical and theoretical prerequisites for realization of IVM:

a) Availability of culture of Sertoli cells;
b) Techniques of germ cell transplantation;
c) The demonstration that spermatogenesis can be successfully carried out in a testis of different species;
d) Fertilization has been achieved even when sperm motility and morphology is poor. Sperm recovered from the epididymis or from the testis can also be used in introcytoplasmatic sperm injection. For men whose ejaculates contain even a few sperm, in which a single sperm is injected into the cytoplasm of the egg, has proved unexpectedly successful, giving pregnancy rate equaling that normal IFV;
e) There have been no published reports of primordial germ cells entering meiosis *in vitro*, when maintained as isolated cells. However, if mouse germ cells do indeed have a cell-autonomous tendency to enter meiosis irrespective of the urogenital ridge, then theoretically somatic cells can also enter meiosis in an environment of a tissue or an organ culture system;
f) It has managed to show that the diploid spermatogonia progressing *in vitro* to haploid spermatidis involves coculture with an immortalized

Sertoli cell line (in more detail see Rassoulzadegan et al. 1993; McLaren 1998; McLaren and Southee 1997).

Hence, we believe that inasmuch as all somatic cells are pluripotent, then at least some of them (e.g. less specialized cells, like fibroblasts) can experience the meiotic division, if the respective *in vitro* conditions to be created. Schematically, for realization of such experiments it is required to:

a) Prepare a culture of fibroblast cells from a donor as a source material for receiving haploid cells – "gametes";
b) Have a culture of Sertoli cells as supporting tissue to nourish and regulate the development of somatic cells from diploid to haploid stages;
c) Prolong the mitotic prophase stage as much as it is required to make prophase homologous chromosomes conjugate as they do during the ordinary spermatogenesis. For that, carry out IVM at a temperature 2-3 °C lower than the core temperature of a corresponding type of mammals;
d) Separate donor cells with haploid sets of chromosomes;
e) Use nuclei of such "gametes" for further IVF or intracytoplasmic injection.

III. Discussion

Our hypothesis on possible mechanisms of sex origin is close to well-known works of Margulis and Sagan (1984; 1986) as we also believe that organization of the genome in chromosomes and the evolution of mitosis are important. These authors developed a comprehensive hypothesis for the evolution of sexual reproduction in the context of the endosymbiotic origin of eukaryotic cells. Organization of the nuclear genome in chromosomes is coupled with the evolution of mitosis. Starvation and cannibalism are seen as the main triggers for merging of cells and the evolution of outcrossing. Meiosis evolved out of mitosis through tardy kinetochores, consequently segregating chromosomes rather than chromatids. Meiosis was maintained as a mechanism to sort better the genetic diversity that has resulted from the merging of genomes. These two evolutionists have admitted that meiosis is critical for sexual reproduction. However our point of view on cell nucleus and mitotic chromosome origin substantially differ from the hypothesis on endosymbiotic origin of eukaryotic cells (see Ibraimov 2003; 2004).

As it seen from Fig.1 at the process of disjunction only two pairs of mitotic chromosomes into two daughter cells out of twelve possible combinations only one is compatible with life. For three pairs of mitotic chromosomes only one out of 44 possible combinations is viable, etc. If the situation was similar to what we think than it is not hard to imagine why in the process of evolution sex and sexual reproduction originated so late. Margulis and Sagan (1997), for instance, suppose that meiotic sex evolved '520 million years ago'. At that major problems perhaps were connected with the emergence of mitotic way of division than with emergence of mitotic chromosomes.

We certainly do not say that we were able to unravel the mystery of the origin of sex. Another hypothesizes will occur and they may be exposed to experimental check. We only would like to say that it is too early to despair and give up by quoting such lines of biologists: 'But we would suggest that there is no naturalistic explanation at all for the origin or maintenance of sex. The highly complex and intricate manner in which the human body reproduces offspring is not a matter of mere chance or a "lucky role of the dice." Rather, it is the product of an intelligent Creator' (Harrub and Thompson 2003).

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A. Prokaryotic cell with "bare" ring chromosome without ncDNAs disjunction into two parts after replication.
B. Small amount of ncDNAs in ring chromosomes might not affect on their disjunction into two daughter cells.
C. In some cases replicated ring chromosomes might have difficulties with disjunction into daughter cells due to "sticking" of parts with a big amount of ncDNAs. For successful disjunction of such ring a chromosomes breaking on separate pieces is demanded. As is known parts of chromosomes with ncDNA are inclined to ruptures and complicated structural transformations that probably, eventually transformed into centromeres, telomeres and kinetochores (Lima-de-Faria 1983).
D. In the absence of due mechanism of mitotic division, replicated chromosomes to divert into daughter cells in different number and combinations. Cells, for instance, containing only two pairs of mitotic chromosomes are able to produce daughter cells with twelve different combinations of chromosomes where only one of which is viable.

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Identification of Appropriate Sample and Culture Method for the Isolation of Thermophilic Bacteria from Automobile Radiators

By Adetunji C.O, Makanjuola O.R , Lateef. A , Oloke J. K , Arowora K.A, Adetunji J.B , Ajani A.O & Umanah J.T.
Ladoke Akintola University of Technology

Abstract - The purpose of this study was to identify appropriate samples and culture techniques on the isolation of thermophiles from automobile radiators. Water samples from these artificial environments were used for the isolation of thermophilic bacteria at 60°C and pH 7.65. Samples from Honda, Jetta and Passat (automobile vehicles) were screened for the growth of thermophilic organisms using nutrient broth medium and their turbidity were measured using spectrophotometer at 600nm. Results of this study suggest that optimal isolation rates of thermophiles from automobile radiator samples were achieved by culturing the sample on nutrient broth and agar at high temperature. Extremophilic microorganisms, especially thermophilic bacteria can facilitate the enzymatic degradation of polymeric substrates such as starch, cellulose, xylan, pectin and chitin.

GJSFR-C Classification : FOR Code: 060501, 060503

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Identification of Appropriate Sample and Culture Method for the Isolation of Thermophilic Bacteria from Automobile Radiators

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Abstract - The purpose of this study was to identify appropriate samples and culture techniques on the isolation of thermophiles from automobile radiators. Water samples from these artificial environments were used for the isolation of thermophilic bacteria at 60°C and pH 7.65. Samples from Honda, Jetta and Passat (automobile vehicles) were screened for the growth of thermophilic organisms using nutrient broth medium and their turbidity were measured using spectrophotometer at 600nm. Results of this study suggest that optimal isolation rates of thermophiles from automobile radiator samples were achieved by culturing the sample on nutrient broth and agar at high temperature. Extremophilic microorganisms, especially thermophilic bacteria can facilitate the enzymatic degradation of polymeric substrates such as starch, cellulose, xylan, pectin and chitin.

I. Introduction

Microorganisms can be grouped into broad categories according to their temperature ranges for growth; psychrophiles, mesophiles, thermophiles and hyperthermophiles. Thermophiles are microorganisms that thrive at relatively high temperatures, between 45 °C and 80 °C. During the past few years, most research on the microbes of hot springs has concentrated on the cultivating and isolating of extreme thermophilic and acidophilic strains (Belkova *et al*., 2007). It is generally believed that at high temperature, biomolecules such as enzymes denature thereby losing their function and hence, stopping the metabolism. Also, the fluidity of membranes increases significantly, disrupting the cell.

The molecular basis for adaptations of thermophilic organisms to extreme environments is to prevent denaturation and degradation. Their membrane lipids contain more saturated and straight chain fatty acids than do mesophiles, which grow typically between 15 °C and 40 °C (Ulrih *et al*, 2009). This allows thermophiles to grow at higher temperatures by providing the right degree of fluidity needed for membrane function. The presence of chaperones which refold denatured proteins increase the stability of thermophilic proteins (Jaenicke *et al*, 1996). Also, thermophilic proteins appear to be smaller and in some cases more basic, which may also result in increased stability (Kumar *et al*, 2001).

The study of extreme environments has considerable biotechnological potential. For example, the two thermophilic species *Thermus aquaticus* and *Thermococcus litoralis* are used as sources of the enzyme DNA polymerase, for the polymerase chain reaction (PCR) in DNA fingerprinting, etc. The enzymes from these organisms are stable at relatively high temperatures, which is necessary for the PCR process which involves cycles of heating to break the hydrogen bonds in DNA and leave single strands that can be copied repeatedly. Another thermophile, *Bacillus stearothermophilus* (temperature maximum 75°C) has been grown commercially to obtain the enzymes used in 'biological' washing powders. Extremophilic microorganisms, especially thermophilic bacteria, can facilitate the enzymatic degradation of polymeric substrates such as starch, cellulose, xylan, pectin and chitin (Stetter *KO*, 1996; Kristjansson *JK* and Hreggvidsson *GO*, 1995; Bertoldo *C* and Antranikian *G*, 2001).

Hence, the current study was carrying out with the objective to isolate and identify thermophilic organisms from automobile radiator.

II. Materials and Methods

a) Collection of Sample

Water sample from three different automobile radiators; Honda, Jetta and Passat were collected using sterile syringe, one for each and labeled as sample A, B and C accordingly. The samples were immediately taken to the laboratory for subsequent sampling to reduce the proliferation of mesophilic organisms.

b) Sampling of Thermophilic Microorganisms from Automobile Radiators

0.5ml of each sample recovered by sterile syringe was transferred to each of the bottles, consisting of 10 ml sterilized nutrient broth (pH 7.65) in a

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McCartney bottles which were labeled accordingly. The bottles were incubated in a water bath at 60°C for 7 days. A control medium consisted of sterilized nutrient broth only and was incubated in the water bath alongside with the samples. Growth was followed by measuring the turbidity at 600nm at the 7th day of incubation.

c) Isolation of Thermophilic Organisms

After the 7th day of incubation in water bath, the cultures were introduced into a prepared plate containing nutrient agar using streak method and incubated for 2 days. Isolation of pure culture was done using spread plate method and streak plate method recommended by Rath and Subramanyam (1998).

d) Characterization and identification of microorganisms

Colonial characteristics of the bacterial isolates were determined using parameters such as size, elevation, pigment, surface, opacity, edge and shape. Cellular characteristics of the isolates were determined through the following experiments:

e) Gram's staining

Smear of each bacterial isolate was prepared on a clean slide. In preparing the smear a drop of sterile distilled water was placed in the middle of the slide. A sterilized inoculating needle was used to pick from the bacterial colony and rubbed on the slide containing a drop of sterile distilled water. The bacterial cells were spread into a thin smear, air dried and heat fixed (Fawole and Oso, 2001).

The heat fix smear was stained with crystal violet for 1 to 2 minutes after which the stain was poured off. The smear was rinsed off with Gram's iodine and the iodine was allowed to react for 1 minute with the smear. The slide was then washed with 95% alcohol until the violet was seen to stop running from the slide. The slide was rinsed with gentle running tap water and counterstained with safranin for 1 to 2 minutes.

The slide was rinsed with water, blotted dry and examined under microscope with oil immersion (Olutiola *et al.,* 1991). Gram positive cells appeared purple while gram negative cells appeared pink.

f) Motility Test

The hanging-drop method was used to determine the motility of the bacterial isolates. A little Vaseline was placed around the edge of the hallow of a clean cavity slide. A loopful of each isolate was transferred to the center of a clean coverslip laid on the bench. The cavity slide was carefully inverted over the coverslip and the slide was pressed down gently in order to seal the coverslip with the slide.

The unit was then inverted in such a way that the loopful of the bacterial colony was in hanging position. The preparation was examined immediately under the X40 objective lens. The microscopy was done quickly in order to avoid excessive illumination, which could quickly cause the organism under study to lose motility (Olutiola *et al.,* 1991).

Motile cells came in view and were seen moving rapidly in the field while non-motile were not moving.

g) Spore Staining

Heat-fixed smear of each isolate was prepared in a slide. Malachite green solution was added to the smear and steamed for 10 minutes. The stain was not allowed to dry out. The stain was then washed off with cold water.

The smear was counterstained with safranin solution for 15 seconds. It was wash with water, blotted dry and examined under the microscope with the oil-immersion objective (Olutiola *el al.,* 1991). Spores appeared green and bacterial cells appeared red.

h) Capsule Staining

Air-dried smear of each isolate was prepared and fixed on a slide. Crystal violet was applied on the slide for 2 minutes and the slide was then steamed for 40 minutes. The crystal violet was rinsed off with copper sulphate solution. Each slide was blotted carefully, dried in the air and examined under the microscope using oil immersion lens (Olutiola *el al.,* 1991). Bacterial cells appeared deep violet while the capsules appeared pale violet.

i) Catalase Test

A thick emulsion of each test organism was prepared on a clean slide. Several drops of 3% hydrogen peroxide were added on each of the slides. A positive result was indicated by effervescence which was caused by the liberation of oxygen gas as a result of catalase production by the bacterium. There were no gas bubbles in the bacteria that do not produce catalase (Fawole and Oso, 2001).

j) Oxidase Test

A filter paper was soaked in 1% sodium oxalate solution. A portion of each bacterial colony was picked and rubbed on the filter paper. A blue colour change within 10 seconds indicated the production of the enzyme oxidase.

k) Methyl Red Test

10 ml of glucose phosphate broth was prepared into different test tubes. The test tubes were then inoculated with different bacterial isolates. The test tubes were incubated for 3 days at 37^{0}C. After 3 days, 5 drops of methyl red indicator was added to 5 ml of each cultured broth. Acid production was indicated by a yellow colouration.

l) Indole Test

One of the end products of the amino acid tryptophan hydrolysis is indole. Some microorganisms are capable of hydrolyzing tryptophan to give indole. Production of indole revealed the possession of the enzyme tryptophalase by the test organism.

1% tryptone broth was prepared in different test tubes. The test tubes were inoculated with each

bacterial isolate. The tubes were then incubated for 48 hours at 35^{0}C. After incubation, 2 ml of chloroform was added to each broth culture and was shook gently.2ml of Kovac's reagent was added to the broth culture and shook gently. The tubes were allowed to stand for 20 minutes in order to permit the reagent to rise to the top. A red colour at the reagent layer indicated indole production (Fawole and Oso, 2001).

m) Starch Hydrolysis

Starch agar was prepared following manufacturer's instructions. The agar was poured in sterile Petri dishes and allowed to set. The plates were inoculated with each test organism using streaking method. The plates were incubated for 48 hours at 37^{0}C. After incubation, each of the plates was flooded with iodine solution. There was no bluish black colour in the plates of the bacteria that utilized starch while bluish black colour was observed in the plates of the bacteria that did not utilized starch. Starch utilization indicated the possession of the enzyme amylase by the test organism (Fawole and Oso, 2001).

n) Citrate Utilization

Some bacteria are capable of utilizing citrate as the sole carbon source. In the test, citrate agar plates were inoculated with the bacterial isolates using streaking method. The plates were incubated at 37^{0}C for 24 hours. Colour change from green to blue on the plates indicated citrate utilization by the test organisms.

o) Sugar Fermentation

The sugar tested for fermentation included fructose, maltose, lactose, sucrose and glucose. Nutrient broth containing 0.5% of each of the sugar was prepared. Two drops of 0.01% phenol red indicator was added to each of the broth media. 10ml of each of the broth media was dispensed into test tubes containing inverted Durham tubes. The media setup was sterilized by steaming for 30minutes on three successive days.

Each indicator-sugar-broth was inoculated with a loopful of each of the bacterial isolates. One test tube of each medium was left uninoculated as a control. The test tubes were incubated at 35^{0}C for 4 days.

After incubated it was seen that growth occurred in the inoculated tubes while growth did not occur in the uninoculated tubes. The change in colour from red to yellow indicated acid production and the presence of air space in the Durham tubes indicated gas production (Fawole and Oso, 2001).

p) Oxygen Relationship

MacCarney bottles containing sterile nutrient agar were used. The bottles were inoculated while at semi-solid state with each of the bacterial isolate using stabling technique. The agar in the MacCartney bottles were allowed to solidify and incubated at 37^{0}C for 48 hours. Anaerobes grew at the bottom of the bottles, aerobes grew on the surface and facultative anaerobes grew from the bottom through the bottles to the top.

III. Result and Discussion

Out of 3 samples cultured, all samples grew on nutrient broth and nutrient agar and they showed a clear distinction compared to the control. The growth rate (Turbidity) of the thermophilic organisms in the nutrient broth at the 7th day of culturing were determined spectrophotometrically and the results of the optical density (OD) were 0.937, 0.897, 0.140 and 0.050 for sample A, B,C and Control respectively as shown in Fig.1.

The result of the OD showed that sample A has the highest growth rate follow by sample B and C. The increasing order of the growth rate is; Control < Sample C < Sample B < Sample A .

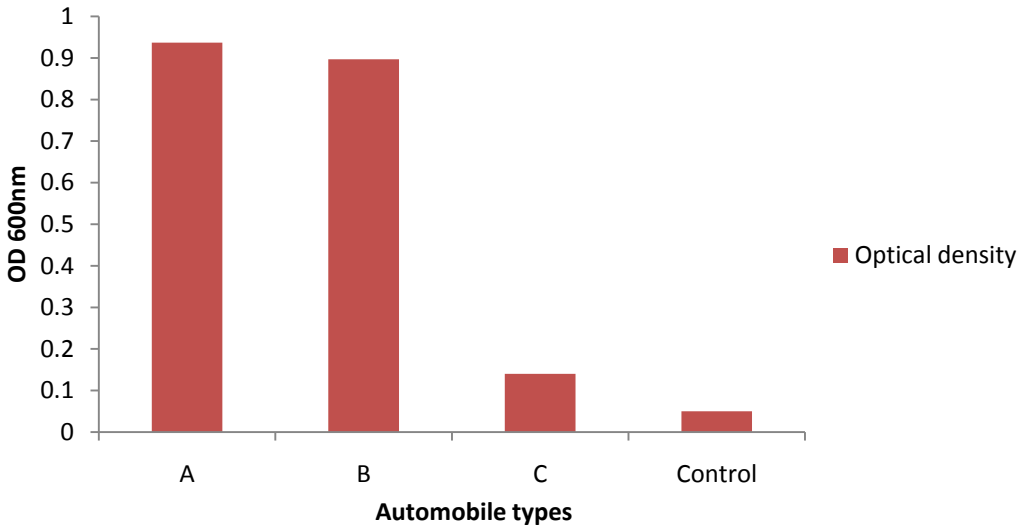

Figure 1 : Growth rate of bacteria from water samples of automobile radiators after 7days.

The bacteria showed some morphological and biochemical characteristics which were summarized in the Tab.1 below. Pure culture isolates were observed and identified from each of the sample cultured on nutrient agar while no growth was observed in the control. *Thermoanaerobacter* spp was isolated from sample A while *Clostridium* spp was isolated from sample B and C.

Table 1 : Colonial morphology, cellular morphology and biochemical characteristics of the bacterial isolates.

Isolate	Cellular shape	Colonial elevation	Colonial edge	Colonial opacity	Colonial surface	Colonial pigmentation	Cellular arrangement	Gram's staining	Motility test	Spore staining	Capsule staining	Catalase test	Methyl red test	Starch hydrolysis	Citrate utilization	Oxygen reaction	Action on simple carbohydrates					probable microorganism
																	Lactose	Glucose	Sucrose	Maltose	Fructose	
A	Rod	Raised	Lobate	Opaque	Smooth	Creamy White	Clusters	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	FAN	A	A	A	A	AG	*Thermoanaerobacter* species
B	Rod	Raised	Entire	Opaque	Rough	Cream	Chain	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	AN	A	AG	A	AG	A	*Clostridium* species
C	Rod	Raised	Entire	Opaque	Rough	Cream	Chain	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	AN	A	AG	A	AG	A	*Clostridium* species

IV. Conclusion

From our experiment, we were able to isolate rod shaped, obligately anaerobic and facultatively thermophilic bacteria belonging to the genera *Thermoanaerobacter* and *Clostridium* from automobile radiators. These organisms can be used in the production of various enzymes for industrial and biotechnological importance.

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Ectomycorrhization of Date Palm and Carob Plants

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Abstract - The effectiveness of plastic pots inoculation technique to infect Ceratonia siliqua and phoenix dactylifera L. seedling roots with ectomycorrhizal fungal Pisolithus tinctorius [(Pers) Coker & Couch] was established. Spores powders inoculum units were prepared from crushed carpophores of Pisolithus tinctorius. Six months after inoculation, ectomycorrhizas were established in date palm and carob seedlings roots. The ectomycorrhization of Ceratonia siliqua and Phoenix dactylifera using spores powders inoculum was performed for the first time in our experiment conditions. These ectomycorrhizas results can provide an enormous potential for the development of large scale inoculation procedures of these species seedlings in commercial nurseries. The ectomycorrhization of date palm can be exploited to fight against Fusarium wilt using Pisolithus tinctorius as biocontrol.

Keywords : *pisolithus tinctorius, ceratonia siliqua, phoenix dactylifera, ectomycorrhization.*

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Ectomycorrhization of Date Palm and Carob Plants

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Abstract - The effectiveness of plastic pots inoculation technique to infect *Ceratonia siliqua* and *phoenix dactylifera* L. seedling roots with ectomycorrhizal fungal *Pisolithus tinctorius* [(Pers) Coker & Couch] was established. Spores powders inoculum units were prepared from crushed carpophores of *Pisolithus tinctorius*. Six months after inoculation, ectomycorrhizas were established in date palm and carob seedlings roots. The ectomycorrhization of *Ceratonia siliqua* and *Phoenix dactylifera* using spores powders inoculum was performed for the first time in our experiment conditions. These ectomycorrhizas results can provide an enormous potential for the development of large scale inoculation procedures of these species seedlings in commercial nurseries. The ectomycorrhization of date palm can be exploited to fight against *Fusarium* wilt using *Pisolithus tinctorius* as biocontrol.

Keywords : pisolithus tinctorius, ceratonia siliqua, phoenix dactylifera, ectomycorrhization.

Résumé - L'efficacité de la technique d'inoculation des racines de *Ceratonia siliqua* et *Phoenix dactylifera* L. par le champignon ectomycorhizien *Pisolithus tinctorius* [(Pers) Coker & Couch], dans des pots en plastique, a été démontrée. L'inoculum en poudre a été obtenu par broyage des carpophores du *Pisolithus tinctorius*. Six mois après l'inoculation, les racines des plantules du palmier dattier et du caroubier ont formés des mycorhizes. L'ectomycorhization du caroubier et du palmier dattier en utilisant un inoculum naturel sous forme de poudre est ainsi, démontré pour la première fois dans nos conditions de travail. Ces résultats sur les ectomycorhizes peuvent fournir un énorme potentiel pour le développement à grande échelle des procédures d'inoculation de ces deux espèces dans les pépinières commerciales. En outre, l'ectomycorhization du palmier dattier pourrait être exploitée pour lutter contre la fusariose vasculaire du palmier en utilisant le *Pisolithus tinctorius* comme agent de lutte biologique.

Motsclés : pisolithus tinctorius, ceratonia siliqua, phoenix dactylifera, ectomycorhization.

I. Introduction

Ectomycorrhizal (ECM) fungi like *Pisolithus* (Alb. & Schwein) are known to enhance tree growth by increasing the uptake of nitrogen (N) (Martin 1985; Chalot and Brun 1998) and phosphorus (P) by roots in P deficient soils (Grove and al. 1994; Marc and al. 2004). In addition ECM fungi are able to mobilize nutrients from organic substrates (proteins, amino acids, chitin, phosphomonoesters and phosphodiesters) or nutrients linked to organic residues by secreting extra-cellular enzymes (Dighton 1983; Abuzinadah and Read 1986 a,b; Leake and Read 1990; Guttenberger and al. 1994). The ability to secrete extra-cellular enzymes differs with ECM fungal species and with season (Buée and al. 2005). Some authors have reported that ectomycorrhizae can also protect seedling roots against pathogens and since then, several studies have noted this protective capability of mycorrhizas (Davis and al. 1942; Chakravarty and Hwang 1991; Chakravarty et al. 1991; Duchesne 1994; Hwang and al. 1995; Morin et al. 1999). It is well known that mycorrhizal fungi create a physical barrier between roots and pathogens, exude antimicrobial metabolites and use surplus carbohydrates to reducing roots pathogenic organisms attractiveness (Machon and al. 2009).

For these reasons, research on ectomycorrhizas has evolved greatly over the last 40 years (De Roman et al. 2005).

Pisolithus tinctorius is an ectomycorrhizal fungus frequently used for inoculation in controlled mycorrhization programs (Marx and al., 1982; Burgess and al., 1995). Isolates of this fungus are some of the most commonly used in forestry, with growth stimulation reported for several tree species including Eucalypts, Pines and Acacias (Marx et al. 1977; Garbaye et al. 1988; Duponnois et Ba 1999). The common occurrence of *Pisolithus* fruiting bodies, the ability of this fungus to form ectomycorrhizae and its wide host range makes it a very interesting organism for artificial inoculation of nursery plants.

In this paper we report for the first time the mycorrhization of *Phoenix dactylifera* and *Ceratonia siliqua* by *Pisolithus* inoculum. The development of these ectomycorrhizal associations can provide an enormous potential for the development of large scale inoculation procedures of these species seedlings in commercial nursery. In view of limited studies on antifungal action of endomycorrhizal fungi to defy *Phoenix dactylifera* wilt (Bayoud) caused by *Fusarium oxysporum albedinis fsp.* (F.O.A) (Jaiti and al. 2007; Oihabi 1991), and no studies using ectomycorrhizal fungus, the present study try to explore *Pisolithus tinctorius* as biocontrol agent against F.O.A

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II. Materiel and Methods

a) Fungus inoculum

Pisolithus tinctorius is the cosmopolitan basidiocarps in warm temperate regions (Martin et al. 2002) and forms ectomycorrhizal associations with a broad variety of forested plants including: Myrtaceae, Mimosaceae, Pinaceae, Fagaceae, Cistaceae, Diptero-carpaceae and Caesalpiniaceae (Moyersoen et al. 2004).

Pisolithus tinctorius basidiocarps (fig1.a) used in this study were collected during the spring season (April 2009) in *Eucalyptus gomphocephala* plantation (fig1.b) in eastern region of Morocco. *Pisolithus* basidiocarps harvested were dried at 35°C for 72 hours (Rincon et al. 2005), and crushed to produce spores powder. Initial fungal spores concentration was measured with a haematocytometer and mixed with sterile peat before being sown by the germinated seeds. One gram of *Pisolithus tinctorius* powder spores contained $1x10^8$ spores.

b) Host plants and seeds germination

Before use, all seeds were first surface sterilized with 10% sodium hypochlorite for 5 min and rinsed four times in sterile distilled water.

i. *Date palm seeds*

Phoenix dactylifera L. seedlings were obtained from seeds produced by 'Boufaggous' during 2009 date palm production season in Figuig (South East of Morocco). Boufeggous is a high quality variety grown in Morocco. It is mainly attacked by *Fusarium oxysporum fsp. Albedinis* (F.A.O) responsible for date palm wilt (Bayoud) disease (Hakkou and Bouakka 2002) and Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), causing extensive losses in storage (Azelmat et al. 2006).

Boufaggous seeds were first soaked in boiling water and then allowed to cool in tap water for 48 hours before being transferred to germination in autoclaved peat. Seeds were incubated in darkness at 30 °C for 16 days. The germinated seeds were sown in plastic pots (V: 500 ml. 14cm diameter; 10cm height) containing 300g of autoclaved peat.

ii. *Carob seeds*

Ceratonia siliqua seeds used in this study were collected manually from street trees in Oujda city (Eastern Morocco). Only, intact seeds of *Ceratonia siliqua* were selected to dip with boiling water. After that, seeds were allowed to cool in tap water for 72 hours and were then allowed to germinate in sterile peat in darkness. After 11 days, seeds rate germination was 95%.

All germinated seeds were then transferred in plastic pots (V: 500ml. 14cm diameter; 10cm height) containing a sterilized peat (300g) and inoculated by 7g powder spores in a growth chamber maintained at 28 ± 2°C with 16 h photoperiod. The pots open at the bottom were placed in containers support, filled with water which level was adjusted to ensure substrate a constant humidity.

c) Ectomycorrhizal synthesis

i. *Using natural inoculum*

These systems are routinely used for synthesis of ectomycorrhizae (Mulette 1976). It consists of a 14 cm diameter plastic pot filled with autoclaved peat mixed to fruiting bodies powder ($70x10^8$ spores/g of autoclaved peat). Sterile date palm and Carob seedlings destined to inoculation were then, inserted into the substrate. Plastic pots were set in a saucer of water to ensure substrate humidity.

ii. *Using mycelia inoculum*

We have previously tested the compatibility of *Pisolithus tinctorius* isolate, *in vitro* with *Eucalyptus turquata.* Plugs of fungus cultures were taken aseptically with a sterile cork borer (10mm in diameter) from the actively growing mycelium front and subcultured in modified Melin Norkrans (MNM) agar slant surface (Marx 1969) in glass test tubes. *Eucalyptus turquata* seedlings were placed then with fungus inoculum of *Pisolithus tinctorius*. After six weeks incubation, roots seedlings were harvested, washed gently with tap water, colored (Philips and Hayman 1970) and observed under microscope (fig 2).

d) Microscopic studies

Six months after inoculation, date palm and carob seedlings roots were rinsed under water, incised and colored before being examined using an Olympus optical microscope. A randomly samples of short roots was cleared in 10% KOH for 30min at 90°C and stained for 15 min with Trypan Blue (0.1 % in lactoglycerol). Tinted short roots were mounted on microscope to check ectomycorrhizas (ECM) presence and mycelia structure. Observations of control (fig.3) mycelium structure collected in margin of a rapid growth culture on MNM agar medium have been made.

e) Data collection and mycorrhizal measurement

The experimental protocol used in this study is completely random using five repetitions. And the percentage of mycorrhized plants was determined according to the method established by Maroneck and al. (1982).

III. Results and Discussion

a) Sampling of plants and evaluation of root ectomycorrhizas

Six months after inoculation, seedling roots were washed free of substrate and ectomycorrhizas were identified according to coloration methods (trypan blue). Each seedling root was cut into 2-3 cm segments

(Rincon et al. 2005) to evaluate the possible presence of ectomycorrhizas and frequency of ectomycorrhized plants. Twenty four weeks post inoculation, *Pisolithus tinctorius* mycelia characteristics were revealed in Carob (fig.4) and date Palme (fig.5) roots systems. Microscopic observations revealed the presence of *Pisolithus tinctorius* mycelia derived from germinated spores (fig.6). The ectomycorrhizae interface cell was composed by both *Pisolithus* mycelia and host cells wall. We have also noted that the mycelium grew only between cells cortex showing a typical Hartig net hyphae (fig.5b, c, d) characterized by labyrinthine branching. A fungal mantel around roots and emerged hyphae outwards roots were also observed (fig.7). This complex hyphal is considered to increase the fungal surface area in contact with the cells roots and explored soil area.

In this study ectomycorrhizal plants frequency after inoculation by *Pisolithus tinctorius* in this study was 6.66% for date palm and 10% for *Ceratonia Siliqua*. Thus, *Phoenix dactylifera* and *Ceratonia siliqua* were ectomycorrhized for the first time in our experience conditions.

Examination of control plants root systems was shown that they are free from mycelia contamination (fig.8).

If there are several works on endomycorrhization of date palm (Jaiti et al. 2007) and carob plants (Cruz et al. 2004) in controlled conditions, there are no results using the ectomycorrhizal fungi as inoculum. In the date palm *Fusarium Oxysporum Albedinis* interaction, little is known concerning the contribution of mycorrhizas to Bayoud disease control. Oihabi (1991) has shown that the inoculation of date palm seedlings with *Glomus mosseae* reduces the disease severity. In this context, antagonism between F.A.O and other micro-organisms was studied. El Hassni and al. (2005) have shown some isolates of *Bacillus spp., Pseudomonas spp.,* and *Rahnella aquatilis* are able to enhance defence reactions of date palm without causing any seedlings mortality. In other study, Machón and al. (2009) have demonstrated that inoculation of *Pinus pinea* seedlings by the ectomycorrhizal fungi *Laccaria laccata* in nurseries reduces both damage intensity and seedlings mortality caused by *Fusarium* damping-off. In the present study, ectomycorrhized roots are characterised by the presence of fungal sheath. This mantle adheres to the root surface and consists of aggregated hyphae (Ammarellou et al. 2007). Barker and al. (1998) have reported that these hyphae are responsible for the mineral nutrition and water uptake of the symbiotic tissues. Cruz and al. (2004) have demonstrated that *Ceratonia siliqua* colonized roots by *Glomus intradices* improves the nitrogen nutrition of plants, generally when growing at low levels of nutrients.

In the perspectives of using controlled ectomycorrhization to induce date palm plants resistance against the *Fusarium* wilt, the antagonism between *fusarium oxysporum albedinis fsp.* (F.A.O) and our *Pisolithus tinctorius* isolate, in vitro, were already begun in our laboratory (data not shown). In addition, the controlled ectomycorrhization of carob plants was performed for the first time.

Our results on ectomycorrhization allowing biologists a very useful tool for studies on resistance to wilt date Palm and a rigorous way to defy biotic and abiotic constraints.

Figure 1 : Pisolithus tinctorius basidiocarps (a) associated with Eucalypts plant (arrow) (b). Scale bar = 33000 μm.

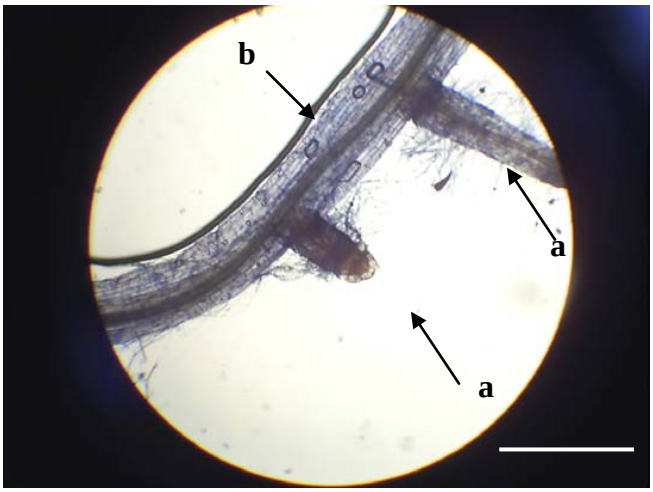
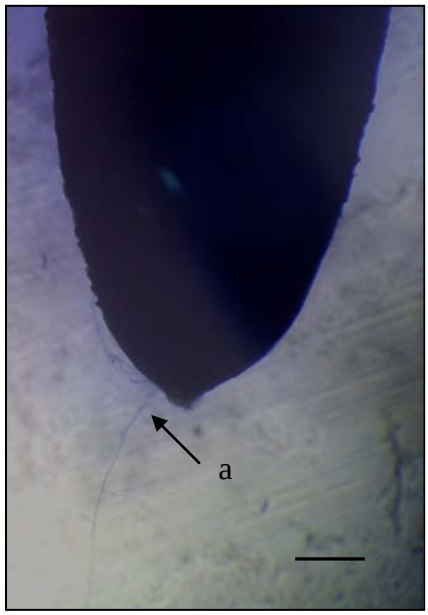


Figure 2 : Eucalypts root ectomycorrhized by *Pisolithus tinctorius* mycelia obtained in glass test tube (X 100). Arrow a: Lateral root; Arrow b: Tap root .Scale bar = 500 μm.



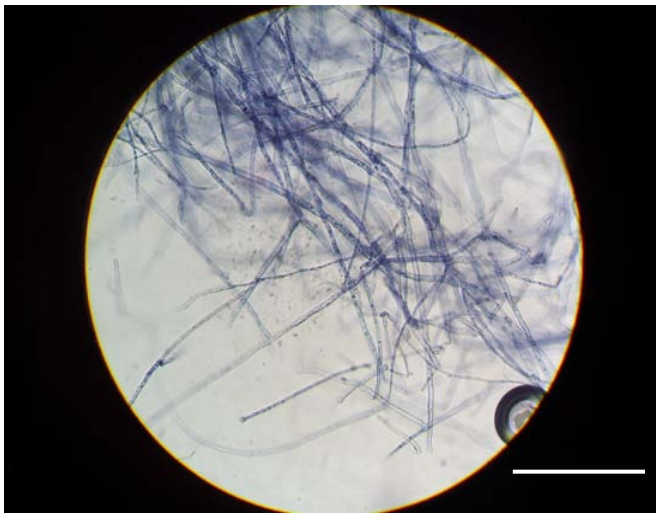


Figure 3 : Microscopic observation of the *Pisolithus tinctorius* mycelia developed in MNM medium (X100). Scale bar = 500 μm.

Figure 4 : *Ceratonia siliqua* lateral root forming a mycorrhizas with *Pisolithus tinctorius*. Fungus hyphae (a) (arrow) grow around the root to form a fungus mantle (b) (arrows) (X100). Scale bar = 200 μm

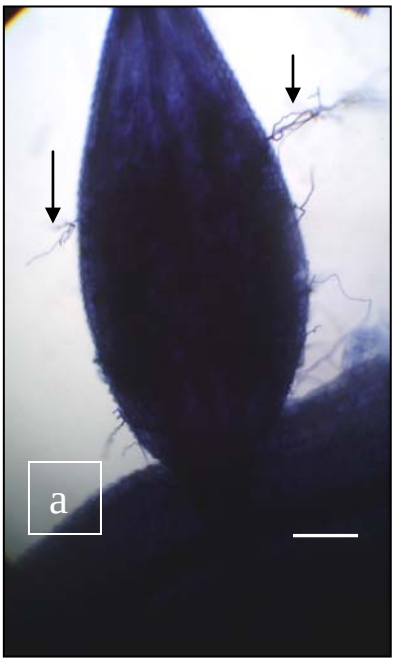
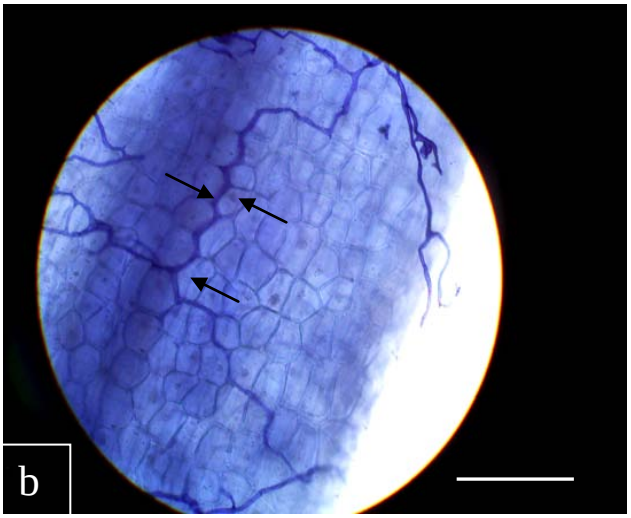
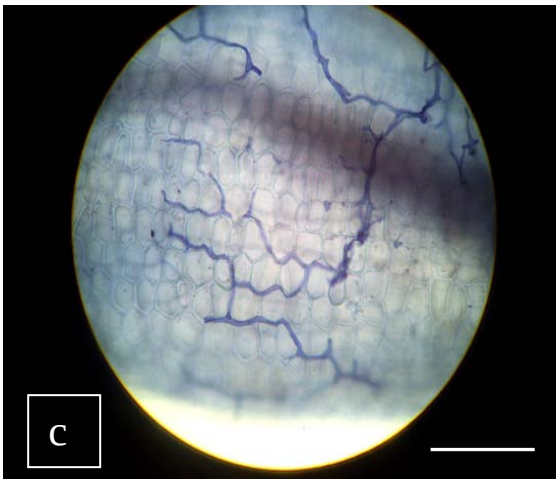
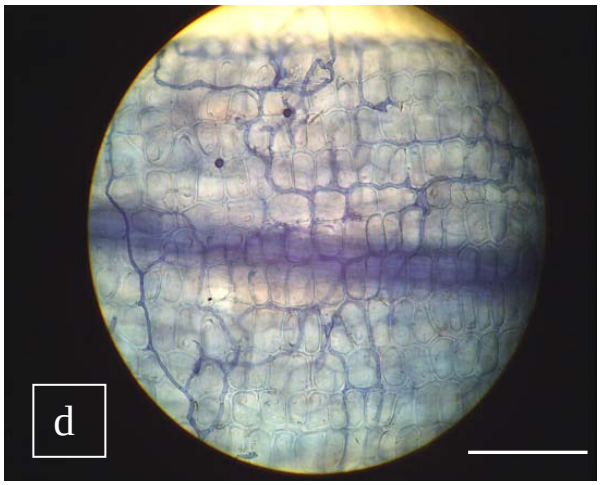


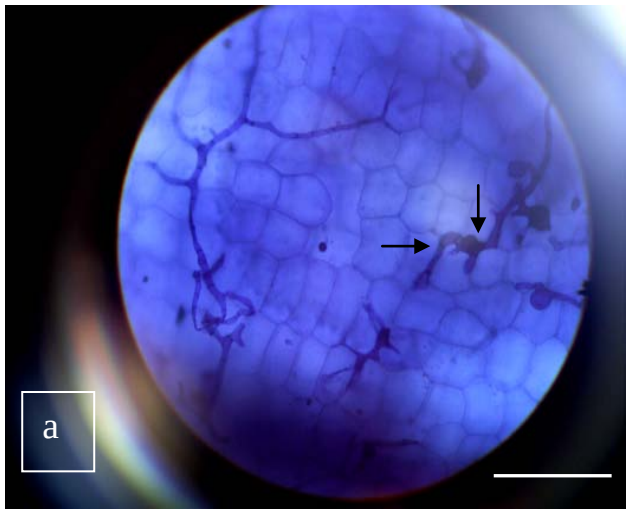
Figure 5 : Lateral root of *Phoenix dactylifera* colonised bay *Pisolithus tinctorius* hyphae (a) (arrows) (X100). Scale bar = 200 μm.

The mycorrhizal fungus grows by branching hyphae and continues to elongate only between cortical cells interfaces (arrows) (b, c and d) (X400). Scale bar = 100 μm.









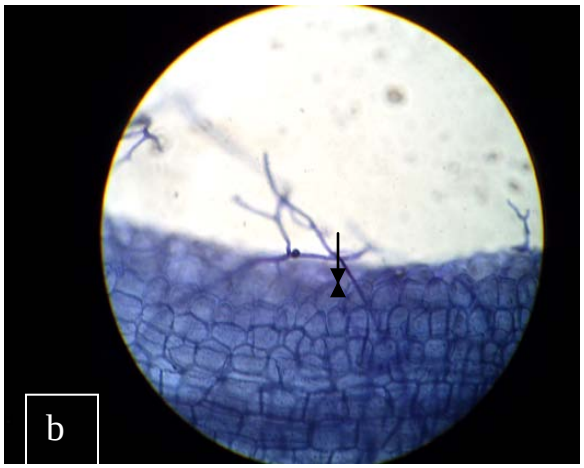
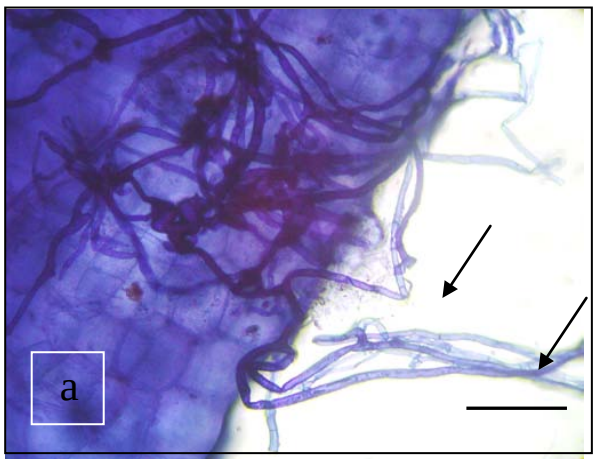


Figure 6 : Pisolithus tinctorius mycelia derived from germinated spores (arrow), in root, grew only between cortical cells wall (a). Scale bar = 100 μm. A *Pisolithus tinctorius* spore can also germinate on the exterior of root and emits mycelia which penetrate between cortical cells (arrow) (b). Scale bar = 2000 μm.



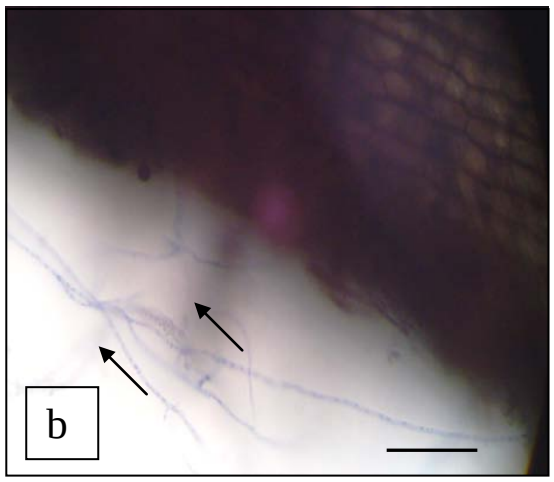
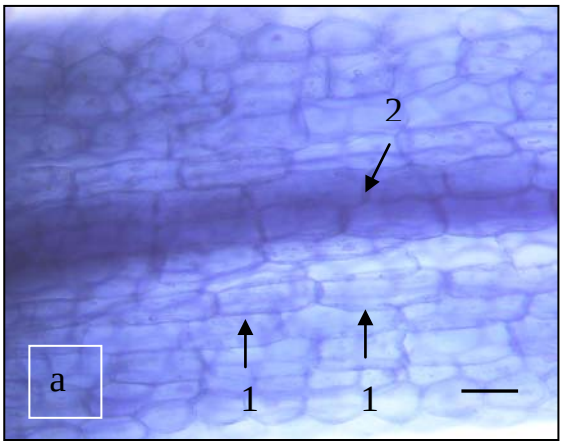


Figure 7 : Pisolithus tinctorius hyphal emergence to outside host roots. a: Date palm root (arrows) (X400). Scale bar = 50 μm; b: Carob root (arrows) (X100). Scale bar = 200μm.



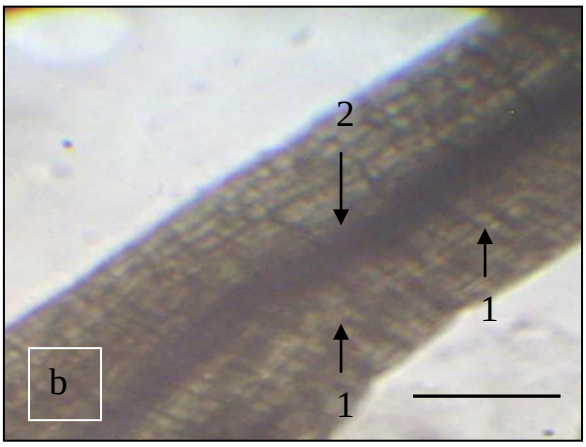


Figure 8 : Control plants root free from mycelia contamination. a: Date palm root. Scale bar = 50 μm; b: Carob root. Scale bar = 500 μm. Arrows 1: Cortical cells; Arrows 2: Central cylinder.

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Use of *Euphorbia Kamerunica* (Spurge) Extract in the Control of *Saprolegnia Species* Growth in Incubated Eggs of *Clarias Gariepinus*

By Agbebi O. T., Oyeleke G.O. & Agbon O.A.
Fisheries University of Agriculture

Abstract - *Saprolenia* is a major aquatic fungus affecting the smooth successful operation of fish hatcheries because it causes mortality during incubation. A number of cheap synthetic antifungal products have been prohibited because of human health risks associated with them. A study was conducted to investigate the antifungal potential of *Euphorbia kamerunica* (Spurge) using latex from fresh extract. Fertilized catfish (*Clarias gariepinus*) eggs were incubated in three different treatment concentrations of 100ml, 50ml and 25ml extract with a control.This study on the effectiveness of *Euphorbia kamerunica* (Spurge), a perennial woody shrubs whose caustic milky sap (Latex) is readily soluble in water was extracted as anti fungi agent on the growth of *Saprolegnia species* on incubated eggs of *Clarias gariepinus species*.

Keywords : *spurge, saprolegnia, incubation, fungi extract, concentration.*

GJSFR-C Classification : *FOR Code: 830501, 070405*

Strictly as per the compliance and regulations of :

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Abstract - *Saprolenia* is a major aquatic fungus affecting the smooth successful operation of fish hatcheries because it causes mortality during incubation. A number of cheap synthetic antifungal products have been prohibited because of human health risks associated with them. A study was conducted to investigate the antifungal potential of *Euphorbia kamerunica* (Spurge) using latex from fresh extract. Fertilized catfish (*Clarias gariepinus*) eggs were incubated in three different treatment concentrations of 100ml, 50ml and 25ml extract with a control.This study on the effectiveness of *Euphorbia kamerunica* (Spurge), a perennial woody shrubs whose caustic milky sap (Latex) is readily soluble in water was extracted as anti fungi agent on the growth of *Saprolegnia species* on incubated eggs of *Clarias gariepinus* species.The study revealed that was a significant difference ($P<0.05$) in the number of eggs infected at different concentration of 100ml (38 ± 10), 50ml (109 ± 8), 25ml (140 ± 14) and 0ml (197 ± 2.186) and the number of eggs hatched 100ml (648 ± 45.863), 50ml (942 ± 21.032), 25ml (1088 ± 52), and 0ml (1950 ± 63). Some of the physico-chemical parameters of the water monitored are pH, Electrical conductivity, Total dissolved solids and Temperature which were within the tolerable range/limit of the fish species. From the results, it was inferred that 25ml concentration of the spurge extract could be used to control the growth of the fungi *Saprolegnia species* in fish hatchery operations as a result of high hatchability rate (1088 ± 52.699) and reduced level of infection.

Keywords : spurge, saprolegnia, incubation, fungi extract, concentration.

I. Introduction

Saprolegnia is ubiquitous in freshwater ecosystem and is the main genus of water moulds responsible for fungi infection of freshwater fish and eggs. *Saprolegnia* has a fairly wide range of temperature tolerance from 3°C to 33°C, this appears to reflect the thermal preferences of the host (Pickering and Willoughby, 1982). Almost every freshwater fish is exposed to at least one species of fungi during its lifetime (Neish, 1991; Noga, 1996), especially from the egg stage through smoltification (Bruno and Wood, 1994). *Saprolegnia* is characterized by an external cotton-like appearance that radiates out in a circular crescent shaped or whorled pattern. It infects eggs by adhesion to and penetration of the egg membrane (Willoughby, 1994) and can spread from dead eggs to live eggs via positive chemotaxis (Bruno and Wood, 1994). The physiological state of fish generally determines if fungi will be successfully established (Neish, 1977). *Saprolegnia* generally invades dead eggs in hatchery condition and fish that have been stressed or otherwise have a weakened immune system (Pickering, 1994). Sudden changes in temperature can also make fish vulnerable to *Saprolegniasis* due to increased physiological stress.

Euphorbia *kamerunica* (Family: *Euphorbiaceae*) is found primarily in the tropical and subtropical region of Africa and America as well as in the temperate zones of the world. Succulent species originate mostly from Africa, the Americas and Madagascar (Bruyns, 2006). Several spurges are grown as garden plants, and the succulent species are used in traditional medicine in China. The latex (milky sap) of *E. kamerunica* is usually white, but in rare cases yellow. The latex exudates congeal within a few minutes on contact with air. Partially or completely congealed latex is often not soluble in water. Several *Euphorbia species* serve as food for the larvae of some Lepidoptera (butterflies and moths) like the spurge Hawk-moth and the Giant Leopard Moth (Carter and Smith, 1988).

The use of some commonly available chemicals such as Hydrogen Peroxide, Formalin and Malachite green etc. in the control of *Saprolegnia species* has been reported to have teratogenic and carcinogenic effects on both fish and man (Doerge *et al*., 1998) and their use has been prohibited. The search for alternative anti-fungal agents for use in fish hatchery has intensified in recent years. The search has been extended to plants that possess fungicidal properties. Mori *et al*. (2002) reported that some plant extracts possesses anti-fungal properties which inhibits the growth of aquatic fungi such as *Saprolegnia species*.

This study was designed to provide baseline information on the potential of *Euphorbia kamerunica* in the control of *Saprolegnia* in catfish hatchery by determining the effect of various concentrations of the extract on the growth of *Saprolegnia* during incubation of the catfish eggs with a view to ascertain the most convenient concentration of the extract that will be most effective in controlling the growth of *Saprolenia*.

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II. Materials and Methods

Gravid brooders of *Clarias gariepinus* were selected and kept in plastic bowls prior to inducement for breeding. Single doses of 0.5ml/kg body weight of ovaprim (Syndel) was injected into the female broodfish intramuscularly below the dorsal fin above the lateral line and were monitored during latency period of that lasted from 10-12 hours before stripping. Thirty minutes prior to stripping, the testes of male brooder fish were surgically excised and upon stripping of the females of their eggs into a bowl, the testes were lacerated and the milt squeezed on to the eggs. The eggs were fertilized by gently mixing them with the milt and freshwater added and the bowl swirled for about 3 minutes at 26^{0}C to allow for proper fertilization. Fertilized eggs were incubated in standard hatching jars.

a) Preparation of Euphorbia kamerunica extract

100g of fresh spurge *Euphorbia kamerunica* was crushed in a porcelain mortar and dissolved in one litre of distilled water. The mixture was sieved through a whatman filter paper and the filtrate stored in a refrigerator at 4°C inside a clean sterile plastic contain and labeled as the stock solution until ready for use. The following volumes: 100ml, 50ml and 25ml were measured out with the aid of a glass measuring cylinder into glass aquaria (hatching trough) containing 10 litres of water each for incubation of the eggs. There was also a control (0ml extract).

There were three replicates for the treatments (100ml, 50ml, 25ml and 0ml) which were distributed in twelve hatching trough of 0.49m×0.49m×0.2m (L×B×D:) size containing 5g of fertilized eggs each for incubation using static renewal method with aerators. They were monitored for three days to observe the growth of *Saprolegnia species* in the different treatment concentrations and their respective replicates.

b) Determination of physico-chemical parameters

The following physico-chemical parameters: pH, Temperature, Electrical conductivity and the Total Dissolved Solid (TDS), of the water in the incubation troughs were monitored with the aid of Hanna HI 9810 meter and their values were recorded.

c) Statistical analysis

The results obtained were subjected to subjected to Analysis of Variance while Duncan Multiple Range test was used to separate means that showed significant level of variance at 5% with the aid of SPSS version 15.

III. Results

The mean number of eggs infected with *Saprolegnia* at different concentration level of plant extract, number of eggs hatched and number of eggs not hatched are shown in Table1.

Table 1 : Mean number of eggs infected, hatched eggs and not hatched eggs.

Parameters	100ml	50ml	25ml	0ml (Control)
Number of eggs infected	38 ± 10^{a}	109 ± 8^{b}	140 ± 14^{b}	197 ± 2^{c}
Number of eggs hatched	648 ± 45^{a}	942 ± 21^{b}	1088 ± 52^{b}	1950 ± 63^{c}
Number eggs not hatched	2352 ± 45^{a}	2058 ± 21^{b}	1912 ± 52^{b}	1050 ± 63^{c}

Values with the same super-script on the same row are not significantly different at P<0.05.

The control (0ml) had the highest mean number of eggs infected (197±2) followed by 25ml (140±14) with 100ml (38±10) being the least infected with *Saprolegnia*. The number of hatched eggs ranged from 648±45 (in 100ml) to 1950±63 (in 0ml). The highest treatment with eggs not hatched was recorded in 100ml concentration (2352±45) followed by 50ml (2058±21 and the least in 0ml (1050±63). Anova revealed that there was significant difference (P<0.05) in the number of eggs infected, number of eggs hatched, and number of unhatched eggs.

The highest mean number of percentage hatchability was recorded in 0ml (In Table 2) concentration (65±2) followed by 25ml concentration (36±1) and the least in 100ml concentration (21.60±1.525). The highest value of percentage unhatched eggs was (78±1) in 100ml concentration followed by 50ml and the least in the control 0ml concentration (34±2). Percentage infected within unhatched eggs ranges from (18±1) to (1 ±0.41).

Table 2 : Percentage hatchability, percentage infection within unhatched eggs and percentage unhatched eggs.

Parameter	100ml	50ml	25ml	0ml
% Hatchability	21 ± 1^{a}	31.40 ± 0.685^{b}	36.27 ± 1.752^{b}	65.00 ± 2.868^{c}
%infection unhatched eggs	1 ± 0.41^{a}	5.31 ± 0.469^{b}	7.30 ± 0.525^{b}	18.98 ± 1.019^{c}
% unhatched eggs	78.41 ± 1.528^{a}	68.60 ± 0.699^{a}	63.72 ± 1.756^{a}	34.99 ± 2.132^{a}

Values with the same super-script on the same row are not significantly different at P<0-05.

There was a significant difference (P<0.05) in the percentage hatchability and percentage infection of unhatched eggs but there is no significant difference in the percentage of unhatched eggs.

In Table 3, the value of temperature in all the concentration is the same (31.3±0.000) and the electrical conductivity increase as the concentration level increases with value ranging from 225±0.471 to 274±0.471. Also the Total dissolved solids value range from 113±0.816 to 141±0.471 (0ml to 100ml) while the highest pH value (7.11±.0.005) was associated with 0ml and least value (6.83±0.005) with 100ml.

Table 3 : Mean values of physicochemical parameters of the culture media.

Parameter	100ml	50ml	25ml	0ml
Temperature (°C)	31.3	31.3	31.3	31.3
Electrical conductivity (us/cm)	274±0.471[a]	236±0.471[b]	226±0.471[c]	225±0.471[c]
Total Dissolved Solids (ppm)	141±0.471[a]	119±0.943[b]	114±0.471[b]	113±0.816[b]
pH	6.83±0.005	6.87±0.009	7.02±0.005	7.11±0.005

Values with the same super-script on the same row are not significantly different at P<0.05.

There were significant differences (P<0.05) only in the Electrical conductivity and Total Dissolved Solids. Temperature and pH had no significant difference (P>0.05).

IV. Discussion

The physico-chemical parameters of the incubation media were adequate. Boyd (1990) and Adeniji (1986) reported that to maintain a good population of Fish, it was necessary to keep the pH between 6.5 and 9.0. The pH values obtained during this study (6.83-7.11) were suitable for fish eggs to develop. The conductivity of the water was observed to decrease as the concentration decreased. The conductivity values in all the treatments were high than that of the control (0ml). Boyd (1990) stated that natural water conductivity ranges from 20 to 1500us/cm. The Electrical Conductivity value obtained ranged between 225-274μs/cm, which was within the range for development and survival of the eggs. The mean temperature obtained from the result showed that it was constant (31.30°C) in all the treatments during the study. Boyd (1990) reported that eggs hatched at temperature between 25°C to 32°C and between 24 to 48 hours. The values of the Total Dissolved Solids obtained were within the range 113-141 ppm. These values agrees with that of Boyd (1990).

The study revealed that there was an inverse relationship between the plant extract and eggs hatchability. There was an increase in the number of infected eggs as the concentration of plant extract decreased. The study further showed that there was a significant difference (P<0.05) between the concentrations of the *E. kamerunica extract* used in the experiment. On the infection with *Saproglenia*, the mean number of infected eggs in the control (0ml) was greater when compare to the treatment with the 100ml concentration treatment. Similarly the infection was visibly higher in 0ml concentration. This may probably due to the absence of spurge. This agrees with the report by Khomvilai *et al* (2006) who used Horseradish Extract on *Saprolegnia parasitica.* Moreso, there was a significant difference (P<0.05) in the number of eggs hatched at different concentration level. The result indicated that the number of eggs hatched increases as the concentration level decreases. The control (0ml) recorded highest mean value of hatchlings compare with the 100ml treatment concentration.

There was a significant difference (P<0.05) in the number of eggs unhatched at different treatment concentration levels. The highest mean number of unhatched eggs was recorded in 100ml treatment concentration while the lowest was in control thus indicating that the extract of *E. kamerunica* had aborticidal effect on the fertilized eggs of *C. gariepinus.* There was a direct relationship for as the concentration level the extract of *E. kamerunica* increased, the percentage of unhatched eggs also increased.

On the percentage infection of unhatched eggs, there was a significant difference in the percentage infection of unhatched eggs (P<0.05). The highest treatment concentration of the extract of *E. kamerunica* had the lowest mean number of infected unhatched eggs while the control had the highest mean number of infected unhatched eggs. This shows that the spurge (*E. kamerunica*) inhibited the development of *Saprolegnia species* on the eggs of catfish. This finding agrees with Isshiki *et al* (1992) and Mori *et al* (2002) who reported that allyl isothiocyanate (AIT) present in *E. kamerunica* had strong antifungal activity against aquatic fungi including *S. parasitica.*

V. Conclusion

From the result obtained in the present study, it can be concluded that 25ml and 50ml concentration of spurge (*Euphorbia kamerunica*) could be used to control the growth of *Saprolegnia species* in fish

hatchery operations since the level of hatchability was high and degree of infection reduced thus making it a good candidate to be considered as an antifungal agent.

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Floristic Composition and Ecological Characteristics of Olea-Acacia Forest of Shamshokii District Karak

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Abstract - Floristic study of Olea-Acacia semi evergreen arid montane forest conducted during summer, 2011 indicated that there were 110 species belonging to 51 families. The families include 45 dicots and 5 monocots, four Bryophytes, one Peridophytes, algae and lichens. The flora is divisible into 92 dicots, 18 monocots, four bryophytes,some algae and lichens. Poaceae had the maximum species.

Keywords : *montane forest, olia-acacia modesta, karak, shamshooki.*

GJSFR-C Classification : *FOR Code: 820199*

Strictly as per the compliance and regulations of :

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Shahida Naveed [α], Furrukh Hussain [σ], Inayatullah Khattak [ρ] & Lal Badsha [ω]

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I. Introduction

Ecologically Forest is a plant community of diverse plant life in a certain unit area having similarity in their requirements. Forest vegetation is the most precious resourse that predicts need of the people in the form of food, fodder, fuel, medicine, timber, resins, and oil, etc. (Gaur, 1999). Vegetation plays a pivotal role in sustainable management by maintaining biodiversity and conserving the environment (Farooquee & Saxena, 1996). Floristic composition of any area is a prerequisitefor understanding the overall structure and function of any ecosystem. Fundamental botanical research revolves around floristic composition. A flora enumerates plants of a particular geographical area (Venu, 2002).Floristic study is prerequisite for any advanced work. Local flora is easy to handle and understand. Some floristic listing of various parts of Khyber Pakhtoonkwawa has been made (Badsha, 2011, Sha, 2011, Sher, 2011, Badshah *et al.* (1996), Wali (1966), Tareen & Qadir (1993) and Ayaz *et al.* (1993). Rashid et al. (1988), Qadir & Tareen (1987), Hussain *et al.* (1985).Literature survey reveal that no such work has been done on the vegetation of study area, the present study was undertaken to report the flora of Shamshokii Valley and its ecological characteristics. The findings might be of help to ecologists, ethno botanists and conservationists.

a) Study area

Karak is situated between 32° 47 to 33° 28 N and 70° 30 to 71° 30 E. Shamshaki Masti Khel Olea – Acacia forest area (Naveed et al.,2012) is 5763.9375 Hectares with an elevation ranging from 800 m to 1400m above sea level, Annual rainfall is frequent in summer. Mean summer temperatures varies from 23C^0 - 35C^0 and temperature may go down below freezing point in winter.

II. Materials and Methods

Plants were collected from November 2010 to 2011July .Plants were dried, preserved identified through available literature (Nasir & Ali 1971; 1995; and Ali & Qaisar 195;-2006). Leaf size and life forms were determined after Raunkier (1934) and Hussain (1989).

III. Result and Discussion

There were 110 plant species belonging to 57 families (Table.1).*Pteridophytes* were represented by only 1 family(*Adiantaceae*), Bryophytes by4 (*Marchantiaceae, Rebouliaceae, Funariacea* and *Politricaceae*) Monocots by 5 families (*Alliaceae, Asparigaceae, Cyperaceae, Liliaceae* and *Poaceae*) while the remaining 45 families were represented by the Dicots. The well represented families were *Poaceae* (11 spp.), *Asteraceae* (8 spp.) *Papilionaceae* (6 spp.), *Lamiaceae* (7 spp.), *Solanaceae* by 5 spp, *Euphorbiaceae* by 4, *Moraceae, Amaranthaceae, Brassicaceae, Alliaceae, Liliaceae, Chenopodiaceae, Mimosaceae* and *Malvaceae* by 3 spp. each. *Asparagaceae, Polygonaceae, Cucurbitaceae, Caryophyllaceae, Apocynaceae* by and *Rhamnaceae* by 2 spp.While remaining 28 Families (*Asclepiadaceae, Bignoniaceae, Boraginaceae, Buxaceae, Nyctaginacea, Urticaceae, Cascutaceae, Canabanaceae, Primulaceae, Papaveraceae, Oxaliceae, Meliacea, Oleaceae, Punicaceae, Celesteraceae, Convolvulaceae, Fumariaceae, Menispermaceae, Portulacaceae, Ranunculaceae, Rosaceae, Rubiaceae, Sapotaceae, Sapendaceae, Schrupholariacea and Salicaceae, Telliaceae, Verbinaceae and Zygophylaceae*) were represented by one spp. each.

The biological spectrum (Table 1) showed that Therophytes (47%) were the dominant followed by Nanophanerophytes (12.7%), Chamaephytes (12.7%), Geophytes (6.3%), Hemicryptophytes (10.9%), Magaphanerophytes(4.54%), Microphanerophytes (0.9%). Phytoplanktons and Parasitic plants were 0.9% each. Leaf spectra showed that Microphylls was dominant

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(52.7%), followed by Nanophylls (14.54%), Leptophylls (14.54%), Mesophylls (11.81%), and Megaphylls (2.7%).

The dominance of Therophytes (44.6%) indicated that the investigated area was under heavy biotic pressure due to deforestation and over grazing. Many plant species were decreasing in the area. It would be the moral and ethical duty of the local people to protect the plant resources. Most of the medicinal plants were uprooted for burning purposes and grazed by the livestock. It therefore, seemed appropriate to manage the grazing system. Most of the fuel wood and timber wood was extracted from these forests. Even fruiting trees were also grazed by animals and used for burning. The forests were refuge for valuable and endangered animals. Further study is needed to quantify the data and suggest plans for the conservation of the area.

Table 1 : Floristic list, Life form and Leaf size classification of some plants of Olea-Sanatha Forest Shamshaki ,District Karak.

No	Phylum	Family	Botanical Name	Local name	Life form	Leaf size	Habit	Habitate	Occurence	Economic Uses
1	Algea	Chlorophyceae	Spirogyra spp	Ghorhbaiy	Phytoplankton		fillamentous	Stream beds	common	Md
2	Lichens	Crustose Lichens	White, gray ,yellow and orange colored crustose lichens		LCh		Crust like	Rock/tree barks	common	-----
3	Bryophytes	Liverworts	Marchantia spp		BrCh		Flate	Open places	rare	------
4		Mosses	*Funaria spp & polytricum spp*		Br Ch	Le		Moistplaces	abundent	-------
5	*Ptridophytes*	*adianthaceae*	*Adianthem incisum Forsk.H*		*H*	*Na*	*Herb*	*Shady places*	*Rare*	-------
6	*Monocots*	*alliaceae*	*Allium griffithium Bioss*	*Paharhi piyaz*	*G*	*Mic*	*herb*	*Open places*	*Rare*	*Md*
7			*Asphodilus tenoifolius Cav*	*piyazakai*	*G*	*Na*	*herb*	*Open places*	*common*	*Fd*
8		*Asparagaceae*	*Asparagus officinalis Wall.*	*laychagha*	*Ch*	*Le*	*shrub*	*Shady places*	*common*	*Fd*
9		*Poaceae*	*Cymbopogon jawarancus*	*Sargarhi*	*H*	*Na*	*herb*	*Open land*	*common*	*Fd*
10			*Aristida*	*washa*	*T*	*Mic*	*herb*	*Open land*	*common*	*Fd*
11			*Avena sativa*	*karyanrha*	*T*	*Mic*	*herb*	*Cultivated field side*	*common*	*Fd*
12			*Bromus japonicas Thumb exMurr.*	*washa*	*H*	*Mic*	*Herb*	*Hill side*	*common*	*Fd*
13			*Cenchrus ciliaris*	*washa*	*H*	*Mic*	*Herb*	*Open land*	*common*	*Fd/FW*
14			*Cynodon dictylon L.pers.*	*bayrawa*	*H*	*Mic*	*Herb*	*Cultivated Field Side*	*commen*	*Fd*
15			*Saccharum spontaneum*	*kana*	*Ch*	*Na*	*Herb*	*Hill side*	*common*	*RT/FW*
16			*S. bengalense Ritz*	*kaee*	*Ch*	*Na*	*Herb*	*Hill side*	*common*	*RT/FW*
17			*Desmostachya bipinnata*	*drab*	*H*	*Mic*	*Herb*	*Hill side*	*common*	*Fd*
18			*Dictylatum annulatumStafl.*	*wakha*	*H*	*Mic*	*Herb*		*common*	*Fd*
19			*Achnatherun thurberianum*	*wakhan*	*H*	*Mic*	*Herb*	*Cultivated Field side*	*common*	
20		*Cypiraceae*	*Cypirus rotendus*		*G*	*Mic*	*Herb*	*Cultivated n field side*	*common*	*RT*
21			*Cypirus spp*	*Wakhan*	*G*	*Mic*	*Herb*	*Hill side*	*common*	*RT*
22		*Liliaceae*	*Polygonatum verticilatumAll*	*Noo e alm*	*G*	*Mic*	*Herb*	*Stream bed*	*V. rare*	-------
23			*Unidentified sp*	*Shandai*	*G*	*Mic*	*Herb*	*Shady places*	*Rare*	-------
24	*Dicotyledon*	*Amaranthaceae*	Amaranthus viridis	*Ranzaqa*	*T*	*Mic*	*Herb*	*Cultivated field side*	*common*	*Fd*
25			Achyranthus aspera L.	*Kurashkay*	*T*	*Mes*	*Herb*	*Cultivated field side*	*common*	*Fd*
26			Digera muricata	*Sur golai*	*T*	*Mic*	*Herb*	*Cultivated field side*	*common*	*Fd*
27		*Anacardiaceae*	*Pistacia integerrima J.L.Stewart ex* Brandis	*Sroon*	*Ch*	*Mic*	*Tree*	*Hill side*	*Rare*	*FW*
28		*Apocynaceae*	*Caralluma edulisEdgew.*	*Pawana*	*T*	*Le*	*Herb*	*Hill side*	*Rare*	*Fd*
29			*Cynanchum auriculatum*	*Lewanai perwata*	*T*	*Mic*	*Herbaceous Climber*	*On trees*	*Rare*	*P*
30		*Asclepiadiaceae*	*Calotropis procera (wild) R.Br.*	*Spelmakai*	*Ch*	*Mes*	*shrub*	*Open field*	*common*	*FW*

31		Asteraceae	Launea procumbens (Roxb.) Amin	Tarezsha	T	Mes	herb	Hill side	common	Fd
32			Calendula arvensis L.	Zairh gwall	T	Mic	herb	Open places	common	Fd
33			Carthamus oxycantha M.B.	Azghi boota	T	Mic	herb	Open places	common	FW
34			Conyza canadensis L.	Harhsaasi	T	Le	herb	Open fields	v. common	FW
35			Cichorium intybus L.	boota	Mēs	Mes	herb	Cultivated field side	common	Fd
36			Sonchus asper L.	Kandiari	T	Na	herb	Cultivated field side	v. common	Fd
37			Taraxacum officinale Weber.	Zairh gullai	T	Mic	herb	Cultivated Field side/open fields	v. common	HN/Md
38			Xanthium strumarium L.		Ch	Mes	herb	Open fields	common	Fd
39		Berberidaceae	Berberis lyceum	Zerh larga	Np	Mic	Small Tree	Hill side	common	Md/RT
40		Brassicaceae	Brassica compestris L.	Wree, sharhsham	T	Mes	Herb	Cultivated field side	v. common	Fd
41			Capsella bursa-pestoris medic	Akhso bota	T	Mic	Herb	Cultivated field side	common	Fd
42			Lepidium sativumL.	boota	T	Mic	Herb	Cultivated field side	common	Fd
43		Bignoniacea	Tecomella undolata	Raddoon	T	Mic	Tree	hill Side	Rare	RT
44		Boraginaceae	Onosma hipida	bota	NP	Na	Herb	Open fields	common	Fd
45		Buxusaceae	Buxus papilosa	shamshood	NP	Mic	Tree	Hill lside	common	Md
46		Canabanaceae	Cannabis sativa L.	bhaang	T	Mic	Herb	open fields	common	Md/FW
47		Caryophyllaceae	Stellaria media (L.) Cry.	wakha	T	Na	Herb	Cultivatd field side	common	Fd
48			Silene conoidea	bota	T	Mic	Herb	Cultivated field side	common	Fd
49		Celesteraceae	Gymnosporia royleana(Wall.)Lawson.	soorAghza	NP	Mic	Shrub	Hill Side	common	
50		Chenopodiaceae	Chenopodium album L.	Harh sooba	T	Mic	Herb	Open fields	common	Fd
51			Chenopodium murale L.	Toorasaray	T	Le	Herb	Cultivated fieldside	common	fd
52			Arva javanica (Burm. f.) Juss. ex Schultes	sperai	T	Na	Herb	Open places	common	Fd
53		Convolvulaceae	Convolvululus arvensis	perwata	T	Mic	Herb	Cultivated field side	common	Fd
54		Cuscutaceae	Cuscuta reflexa Roxb.	chambarh	Par	Le	Parasitic climber	Epiphyte	common	Md
55		Cucurbitaceae	Citrullus colocynthis	Tharkha maran	T	Le	Herb	Open fields	common	Md
56			Luffa cylindrica (L.) Roem.	thoray	T	Mac	Herb	Open field	common	Fd
57		Euphorbiacea	Riccinis communis L.	arand	Np	Mac	Tree	Open field	Rare	Md/FW
58			Euphorbia helioscopia Mewski	bota	T	Na	Herb	Open places	common	FW
59			Euphorbia prostrata L.	bota	H	Le	Herb	Open places	common	FW
60			Euphorbia hirta	bota	H	Le	Herb	Open places	common	FW
61		Fumariaceae	Fumaria indica (Hsskn) H.N.	bota	T	Le	Herb	Cultivated field side	common	Fd
62		Lamiaceae	Ajuga parviflora Benth.		T	Mic	Herb	Hill side/field side	common	Md
63			Leucas aspera	bota	T	Na	Herb	Under trees	v .common	Hn
64			Otostegia limbata Bth.	Boota	Np	Mic	Shrub	hillside	v. common	Md/FW

65			Salvia aegyptiaca	bota	H	Mic	Herb	Open places	common	Fd
66			Ocimum basilicum L.	boobrai	Ch	Mic	herb	Cultivated field sides	Rare	Md
67			Salvia argenta	Drashaal	T	Mac	herb	Openplaces	common	Fd
68			Isodon roguses (Wall. ex D. Don)	Boi bota	Ch	Mic	herb	Hill side	v. common	HN
69		Malvaceae	Malva officinalis	shastari	T	Mic	Herb	Cultivated field side	common	Fd
70			Malva neglecta Waller.	tiklay	T	Mic	Herb	Cultivated field side	common	Fd
71			Malvastrum coromandelianum(L) Grub.	boti	H	Mic	Herb	Cultivated field side	common	Fd
72		Meliaceae	Melia azedarach L.	bakarhan	Mp	Mic	Tree	Open places	common	FW
73		Menispermaceae	Cocculus pendulus	Kamar perwata	Np	Mic	Shrubby Climber	On rocky cliffs	common	FW
74		Mimosaceae	Acacia modesta Wall.	Paloosa, wanna	Mp	Le	Tree	hillside	v. common	HN/FW
75			Acacia nilotica (L.) Delile.		Mp	Le	Tree	Open places	common	HN/Fd
76		Moraceae	Morus nigra	Toor toot	Mp	Mes	Tree	Open places	common	Fd/Md
77			M. alba	Speen toot	Mp	Mes	Tree	Open places	common	Fd/Md
78			Ficus palmata	Inzar	P	Mes	Tree	Hillside	common	Fd/Md
79		Nyctaginaceae	Boerhaavia diffusa L.	bota	H	Mic	Herb	Open places	common	FW
80		Papilionaceae	Astragallus oblongifolia	Gull bota	T	Le	Shrub	Hillside	common	HN
81			Lathyrus aphaca L.	Zanglimatar	T	Mic	Shrub	Cultivated field side	common	Fd
82			Medicago minima (L.) Grub.	Sooba	T	Na	Herb	Cultivated field side	common	Fd
83			Medicago denticolata L.	shpathlaray	T	Na	Herb	Cultivated field side	common	Fd
84			Vicia sativa L.	boota	T	Na	Herb	Cultivated field side	common	Fd
85			Sophora millis subsp. griffthii	Gajarail	NP	Le	Huge Shrub	Hill side	common	FW
86		Primulaceae	Anagalis arvensis	Gul boti	T	Le	Herb	Cultivated field side	common	Fd
87		Oleaceae	Olea ferruginea	Shoona,Zaitoon	P	Mic	Tree	Hillside	v. common	Md/FW
88		Oxaliceae	Oxalis corniculata	Tarwaka	G	Mic	Herb	Cultivated field side	common	HN
89		polygonaceae	Rumex hastatus	Leewanai sooba	T	Mic	Herb	Cultivated field side	common	Fd
90			Polygonum aviculare	bota	T	Mic	Herb	Open places	common	Fd
91		Portulacaceae	Portulaca olearaceae L.	waarkhorha	H	Na	Herb	Cultivated field side	common	Fd
92		Punicac eae	Punica granatum L.	Anar	P	Na	Shrub	Hill side	common	Md
93		Ranunculaceae	Clematis montana	Perwata	Np	Mic	Weedy climber	On tree	Rare	FW
94		Rosaceae	Cotoneaster nummularia Fish & Mey.	Mamorha	Np	Mic	Shrub	Hill side	common	Fd
95		Rhemnaceae	Ziziphus numularia	Karkarhana	Np	Mic	Shrub	Hillside	common	HN/Fd
96			Z. mouritiana	Bera	mp	Mic	Tree	Hillside	common	HN/Fd
97		Rubiaceae	Gallium aparine L.	Shin boota	T	Le	herb	Cultivated field side	Rare	Fd
98		Sapindaceae	Dodonaea viscosa	Zerha wanny,Sanatha	Np	Mic	shrub	Hill side	Dominant	FW

99		Sapotaceae	Monotheca buxifolia	Gurgura	P	Mic	Shrub/ Tree	Hillside	V. common	Fd
100		Salicaceae	Salix alba	Waley	P	Mic	Tree	Along stream	rare	FW
101		Scrophulariaae	Verbascum thapsus L.	Drashal	T	Mes	Herb	Hillside	rare	FW
102		Solanaceae	Solanum surratense Burm.f	Marghonaiy	T	Mic	Herb	Open places	common	HN
103			Datura innoxia Mill.	Barbaka	Ch	Mes	Shrub	Open places	common	Poisonous
104			Solanum nigrum L.	Kachmacho	T	Mic	Herb	Shady places	common	Fd
105			Withania somnifera (L.) Dunal.	Payshanga	Ch	Mes	Herb	Open places	common	Md
106			Withania cogulanse	Shapyaanga	Ch	Mic	shrub	Open places	v. common	Md
107		Tiliaceae	Grewia oppositifolia	Pastawana	NP	Mes	Shrub	Open places	common	FW
108		Verbenaceae	Vitex negundo L	Varmandi	NP	Mic	Shrub	Along stream	v. common	FW
109		Zygophyllaceae	Tribulus terrestris L.	Maarkondy	T	Mic	Herb	Open places	common	Fd

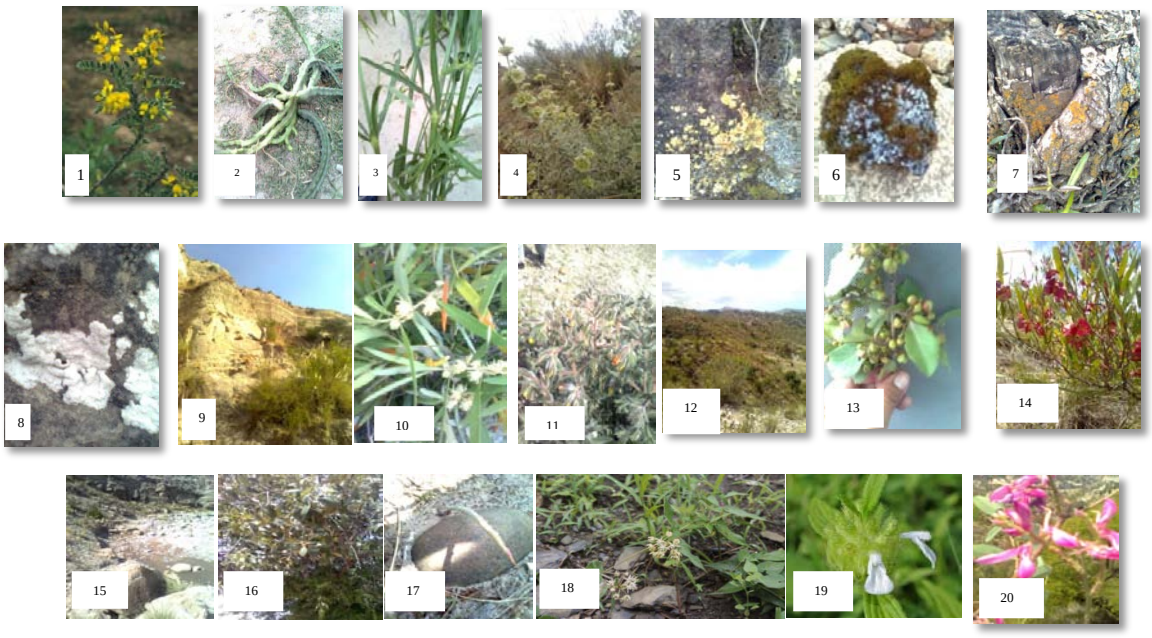
Table 2 : Summary of life form and leaf size classes and Economic uses of some plants of Olea-Sanatha forest Shamshooki, Karak.

Economic Uses	Perentage
Fodder	50.9%
Medicinal	16.36%
Fuel wood	22.72%
Roof Thaching	5.45%
Honey bee Nector	9.09%
Poisonous plants	0.90%

Life Form Classes	Percentage
Therophytes (T)	47 %
Chamaephytes (Ch)	12.7 %
Hemicryptophytes (H)	12.7 %
Geophytes (G)	6.36 %
Parasite (Par)	0.9 %
Phanarophytes(Ph)	5.45%
Nanophanerophytes(NP)	12.7%
Mega-Phanerophytes (MP)	4.54 %
Microphanerophytes (mp)	0.90%
Phytoplankton (Phpk)	0.9%

Leaf Size Classes	Percentage
Nanophylls (Na)	14.54 %
Leptophylls (L)	14.54 %
Microphylls (Mic)	52.7 %
Mesophylls (Mes)	11 .81 %
Megaphylls (Mac)	2.7 %

PLATES



1. *Sophora millis,*
2. *Caralluma edulis,*
3. *Polygonatum verticillatum,*
4. *Otostegia lambata,*
5/6/7/8. *Colorful Crustose lichens*
9. *Sedimentary Rocks*
10. *Buxus papilosa*
11. *Onosma hispida*
12. *Area showing Vegetation*
13. *Mytenus roylena*
14. *Dodonaea viscose*
15. *Algea,*
16. *Olea ferruginea*
17. *Unidentified Liliacea Plant*
18. *Cynanchum auriculatum*
19. *Leucas aspera*
20. *Astragalis oblongifolia*

IV. Acknowledgement

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final Points:

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

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

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

General style:

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

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

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

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

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

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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

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

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

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

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

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

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

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

Methods:

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

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

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

Results:

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

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

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

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

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

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

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

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

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

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

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

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

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

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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