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Editorial

Greetings to all our contributors and readers.

The year 2016 marks the 12th year of publication of Scientia (ISSN: 0976-8289), an annual peer reviewed national science journal from Mercy college, Palakkad. We are happy to bring out this issue of Scientia which features 16 articles from various areas of science. As Scientia enters the 12th year of publication, we are delighted to present this issue with an interesting contribution on Digital Signature, Virtual Reality and a review on Doped Zinc Oxide Thin Films. In this issue we bring to introduce an article on Moonlighting proteins in the opportunistic fungal pathogen *C.albicans*-A computational study. Also including an interesting work on butterflies of MGM college campus, Udupi District, Karnataka. Intensive Seed Germination Studies reported in Rauvolfia hookeri ,A rare and Endemic Plant of Southern Western Ghats. Scientia is interested in publishing a wide range of manuscripts presenting original research and reviews in all areas of science. The arena of original research brings articles from Chemistry, Physics, Biotechnology, Botany and Zoology. Two article on oxide thin films. Effect of NaCl, KCl, epinephrine and vitamin A, B and C on melanophore indexes of *Anabas testudinues* were studied. A Study on the Expression of Some Selected Human Morphogenetic Traits in Thrissur District was conducted and explained. Scientia congratulates all our contributors and readers for your achievement in 2016.

We invite your continued support and contribute articles/news items of interest/research papers etc on science.

With warm personal regards

Dr. Jayasree S.
Chief Editor

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Digital Signature: A digital version of conventional signature

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Abstract

Electronic transactions are gaining importance in our day to day life. Be it in the field of banking, stock trading, and the sale and purchase of merchandise. Their main aim is to minimize operational costs and provide enhanced services. This has led to a phenomenal increase in the amounts of electronic documents that are generated, processed, and stored in computers and transmitted over networks. Since all of these kinds of data are sensitive they need to be safe guarded against malicious parties who could tamper with it. Traditionally, paper documents are validated and certified by written signatures, which provides authenticity. For electronic documents, a similar mechanism is necessary, which is, digital signatures.

Keywords: Cryptosystem, Decryption, Encryption.

Introduction

Digital Signature[1] is a method to encrypt a message (such as documents, contracts, notifications) which will be transferred, adopting data-exchanging protocol and data-encrypting algorithm. An abstract is produced in this procession. The abstract is like a signature or seal which can be used by receiver to verify the identity of the sender. Digital signatures, are nothing but a string of ones and zeroes generated by using a digital signature algorithm, it serves the purpose of validation and authentication of electronic documents. Validation refers to the process of certifying the contents of the document, while authentication refers to the process of certifying the sender of the document.

Digital signature technology

Since a digital signature is a sequence of zeroes and ones, it is desirable for it to have the following properties: the signature must be a bit pattern that depends on the message being signed (thus, for the same originator, the digital signature is different for different documents); the signature must use some information that is unique to the sender to prevent both forgery and denial; it must be relatively easy to produce; it must be relatively easy to recognize and verify the authenticity of digital signature; it must be computationally infeasible to forge a digital signature either by constructing a new message for an existing digital signature or constructing a fraudulent digital signature for a given message; and it must be practical to retain copies of the digital signatures in storage for arbitrating possible disputes later.

The functions of digital signature include: (1) Assuring data integrity - once the message changes a little, the abstract will change a lot for hash function's peculiarity, so that avoids the message being distorted. (2) Anti-deniability - using public key cryptography algorithm, the sender can't deny that he/she has sent the message for he/she has the private key. (3) Avoiding receivers forging message that is claimed to be from the sender.

Digital signatures are computed based on the documents (message/ information) that need to be signed and on some private information held only by the sender. In practice, instead of using the whole message, a hash

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function is applied to the message to obtain the message digest. A hash function, in this context, takes an arbitrary- sized message as input and produces a fixed-size message digest as output. Among the commonly used hash functions in practice are MD-5 (message digest 5) and SHA (secure hash algorithm). These algorithms are fairly sophisticated and ensure that it is highly improbable for two different messages to be mapped to the same hash value.

There are two broad techniques used in digital signature computation, symmetric key cryptosystem and public-key cryptosystem (cryptosystem broadly refers to an encryption technique) [2]. In the symmetric key system, a secret key is known only to the sender and the legitimate receiver is used. However, there must be a unique key between any two pairs of users. Thus, as the number of user pairs increases, it becomes extremely difficult to generate, distribute, and keep track of the secret keys.

Public key cryptosystem

A public key cryptosystem, on the other hand, uses a pair of keys: a private key, known only to its owner, and a public key, known to everyone who wishes to communicate with the owner. For confidentiality of the message to be sent to the owner, it would be encrypted with the owner's public key, which now could only be decrypted by the owner, the person with the corresponding private key. For purposes of authentication, a message would be encrypted with the private key of the originator or sender, who we will refer to as A. This message could be decrypted by anyone using the public key of A. If this yields the proper message, then it is evident that the message was indeed encrypted by the private key of A, and thus only A could have sent it.

In "public key cryptosystem" each user places in a public file an encryption procedure E. That is, the public file is a directory giving the encryption procedure of each user. The user keeps secret the details of his corresponding decryption procedure D. These procedures have the following four properties:

(a) Deciphering the enciphered form of a message M yields M. Formally:D (E (M)) = M.

(b) Both E and D are easy to compute.

(c) By publicly revealing E the user does not reveal an easy way to compute D. This means that in practice only he can decrypt messages encrypted with E, or compute D efficiently.

(d) If a message M is first deciphered and then enciphered, M is the result. Formally:E (D (M)) = M.

An encryption (or decryption) procedure typically consists of a general method and an encryption key. The general method, under control of the key, enciphers a message M to obtain the enciphered form of the message, called the cipher text C. Everyone can use the same general method; the security of a given procedure will rest on the security of the key. Revealing an encryption algorithm then means revealing the key.

When the user reveals E he reveals a very inefficient method of computing D(C): testing all possible messages M until one such that E (M) = C is found. If property

(c) Is satisfied the number of such messages to test will be so large that this approach is impractical.

A function E satisfying (a)-(c) is a "trap-door one-way function;" if it also satisfies

(d) It is a "trap-door one-way permutation."

Diffie and Hellman introduced the concept of trap-door one-way functions. These functions are called "one-way" because they are easy to compute in one direction but (apparently) very difficult to compute in the other direction. They are called "trapdoor" functions since the inverse functions are in fact easy to compute once certain private "trap-door" information is known. A trap-door one-way function which also satisfies (d) must be a permutation: every message is the cipher text for some other message and every cipher text is itself a permissible message. (The mapping is "one-to- one" and "onto"). Property (d) is needed only to implement "signatures."

Creating and verifying a digital signature

A hash function is applied to the message that yields a fixed-size message digest. The signature function uses the message digest and the sender's private key to generate the digital signature. A very simple form of the digital signature is obtained by encrypting the message digest using the sender's private key. The message and the signature can now be sent to the recipient. The message is decrypted and can be read by anyone. However, the signature ensures authenticity of the sender. At the receiver end, the inverse signature function is applied to the digital signature to recover the original message digest. The received message is subjected to the same hash function to which the original message was subjected. The resulting message digest is compared with the one recovered from the signature. If they match, then it ensures that the message has indeed been sent by the (claimed) sender and that it has not been altered.

Creating and opening a digital envelope

A digital envelope is the equivalent of a sealed envelope containing an unsigned letter. The message is encrypted by the sender using a randomly generated symmetric key. The symmetric key itself is encrypted using the intended recipient's public key. The combination of the encrypted message and the encrypted symmetric key is the digital envelope. First, the encrypted symmetric key is recovered by a decryption using the recipient's private key. Subsequently, the encrypted message is decrypted using the symmetric key.

Creating and opening digital envelopes carrying signed messages

A digital signature is created by the signature function using the message digest of the message and the sender's private key. The original message and the digital signature are then encrypted by the sender using a randomly generated key and a symmetric-key algorithm. The symmetric key itself is encrypted using the recipient's public key. The combination of encrypted message and signature, together with the encrypted symmetric key, form the digital envelope containing the signed message. First, the symmetric key is recovered using the recipient's private key. This is then used to decrypt and recover the message and the digital signature. The digital signature is then verified as mentioned earlier.

A public versus a private approach to digital signatures

One way of classifying digital signature schemes is based on whether a private-key cryptosystem or a public-key cryptosystem is use①incorporated into the Digital Signature Standard (DSS), which was published by the National Institute of Standards and Technology as the Federal Information Processing Standard.

(1) RSA is a commonly used scheme for digital signatures. Plain text is encrypted by group. The length of every group is less than or is equal to log(n), n is a integer.

Algorithm description:

The sender chooses two prime integer p, q which is possessed by him.

$n = p*q$ is public.

e is public and is chosen freely. e is a co-prime with

$(p-1)(q-1)$.

$d = e^{-1} \bmod (p-1)(q-1)$

The private key is $\{d, n\}$,public key is $\{e, n\}$, M is plain text, C is cipher text.

Encrypting: $C = M^{c} \bmod n$,

Decrypting: $M = C^{d} \bmod n = M^{ed} \bmod n$.

Due to number theory, it is impossible to compute e from d while it is easy to compute d from e. The data which will be signed is transformed into a hash value with fixed length. The hash code is M which is encrypted into digital signature (DS). DS along with the data is sent to receiver. The receiver computes the hash value of the received data as M ' with the same hash algorithm, decrypting the DS1 (perhaps different from DS).The validity of the signature will be known by comparing M and M '.

In a broad outline of the RSA approach, the message to be signed is input to a hash function that produces a secure hash code of fixed length. This hash code is then encrypted using the sender's private key to form the signature. Both the signature and the message are then concatenated and transmitted.

The recipient takes the message and produces a hash code. The recipient also decrypts the signature using the sender's public key. If the calculated hash code matches the decrypted signature, the signature is accepted as valid. This is because only the sender knows the private key, and thus only the sender could have produced a valid signature.

(2) The DSA approach also makes use of a hash function. The hash code is provided as input to a signature function together with a random number generated for this particular signature. The signature function also uses the sender's private key and a set of parameters known to a group of communicating parties, referred to as global public key. DSA is based on the difficulty of computing logarithm. DSA is proposed by ElGamal and Schnorr. Description of the DSA:

①p is prime and $2^{L-1} < p < 2^{L}$, $512 < L < 1024$, L is multiple of 64, and it is public.

②q is the prime factor of $(p - 1)$ which can be divided exactly by q, and satisfies $2^{159} < q < 2^{160}$, could be public.

③ $g = h^{(p-1)/q} \bmod p$, of which h is a integer, and satisfies$1 < h < (p - 1)$, can be computed and be public.

④ The user's private key X, random integer or false random integer q which satisfies $0 < x < q$, is private.

⑤The user's public key y, satisfying: $y = g^k \bmod p$.

⑥ k is random integer or false random integer, satisfying: $0 < k < q$.

When signing, computation is:

$r = (g^k \bmod p) \bmod q$;

$s = [k^{-1} (H (M) + xr)] \bmod q$, getting the signature: (r , s) .

When testing, computation is:

$w = (s')^{-1} \bmod q$;

$u_1 = [H (M ') w] \bmod q$;

$u_2 = (r ') w \bmod q$;

$v = [(g^{u_1} y^{u_2}) \bmod p] \bmod q$;

Need to verify: whether v is equal to r', if then, the signature is valid.

M is the data which is to be signed: $H (M)$ produces the hash code of M using SHA-1,and M ', r ', s ' is the actual data which receiver gets as M , r , s.

The output signature consists of two components. At the receiving end, the hash code of the incoming message is generated and input to a verification function, together with the two components of the signature. The verification function uses the global public key as well as sender's public key and recreates (one of the two components of) the original digital signature. A match between the recreated and the original signature indicates the authenticity of the signature. The signature function is such that it assures the recipient that only the sender, with the knowledge of the private key, could have produced the valid signature[4].

The basis of the RSA scheme is the difficulty of factoring of large prime numbers. That of the DSA scheme is the difficulty of computing discrete logarithms. The DSA provides only the signature function where as the RSA scheme could additionally provide encryption and key exchange. The signature verification using the RSA scheme is about 100 times faster than a DSA scheme. The signature generation is slightly faster in the DSA scheme. It is as follows:

①The file sent is encrypted into abstract of 128bit;

②The sender encrypts the abstract S1 with his private key into signature F;

③A random key K is produced, with which the message will be encrypted into P1;

④The symmetric key K and digital signature F is encrypted with the receiver's pubic key into P2;

⑤P1 and P2 are sent to the receiver;

⑥The receiver decrypts P2 with his private key into symmetric key K and digital signature F;

⑦ F is decrypted with the sender's public key into abstract S1;

⑧ P1 is decrypted into original text with the symmetric key K;

⑨ The recovered original text is encrypted into another abstract S2 using SHA;

⑩ Comparing S1 to S2, if they are equal, the transferred message isn't damaged and distorted, vice versa.

Digital signatures in real applications

In the present time, digital signatures are being used in secure e-mail and credit card transactions over the Internet. The two most common secure e-mail systems using digital signatures are Pretty Good Privacy and Secure/Multipurpose Internet Mail Extension. Both of these systems support the RSA as well as the DSS-based signatures. The most widely used system for the credit card transactions over the Internet is Secure Electronic Transaction (SET). It consists of a set of security protocols and formats to enable prior existing credit card payment infrastructure to work on the Internet. The digital signature scheme used in SET is similar to the one used in the RSA scheme.

Conclusion

Many traditional and newer businesses and applications have recently been carrying out enormous amounts of electronic transactions, which have led to a critical need for protecting the information from being maliciously altered, for ensuring the authenticity, and for supporting no repudiation. Just as signatures facilitate validation and verification of the authenticity of paper documents, digital signatures serve the purpose of validation and authentication of electronic documents. It has been widely used in many areas of technology and will continue to grow with more added features that will ensure safer transactions and information exchange.

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Virtual Reality, the Illusion, now in Education!

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Abstract

Education is the best area which has adopted virtual reality for teaching and learning. The advantage of this is that it enables large groups of students to interact with each other as well as within a three dimensional environment. It is able to present complex data in an accessible way to students which is both fun and easy to learn. Plus these students can interact with the objects in that environment in order to discover more about them.

Key Words: Virtual reality, Virtual Artifact, CAVE, Heptic Interaction, Quasi VR

Introduction

Virtual reality or virtual realities (VR), also known as immersive multimedia or computer-simulated reality, is a computer technology that replicates an environment, real or imagined, and simulates a user's physical presence and environment to allow for user interaction. Virtual realities artificially create sensory experience, which can include sight, touch, hearing, and smell. Most up-to-date virtual realities are displayed either on a computer screen or with a special virtual reality headset (also called head-mounted display), and some simulations include additional sensory information and focus on real sound through speakers or headphones targeted towards VR users. Some advanced haptic systems now include tactile information, generally known as force feedback in medical, gaming and military applications. Furthermore, virtual reality covers remote communication environments which provide virtual presence of users with the concepts of telepresence and telexistence or a virtual artifact (VA) either through the use of standard input devices such as a keyboard and mouse, or through multimodal devices such as a wired glove or omnidirectional treadmills. The immersive environment can be similar to the real world in order to create a lifelike experience for example, in simulations for pilot or combat training or it can differ significantly from reality, such as in VR games.

The reasons to use virtual reality in education and training relate particularly to its capabilities. Immersive VR furnishes first-person non-symbolic experiences that are specifically designed to help students learn material. These experiences cannot be obtained in any other way in formal education.This kind of experience makes up the bulk of our daily interaction with the world, though schools tend to promote third-person symbolic experiences. Constructivism provides the best theory on which to develop educational applications of VR. The convergence of theories of knowledge construction with VR technology permits learning to be boosted by the manipulation of the relative size of objects in virtual worlds, by the transduction of otherwise imperceptible sources of information, and by the reification of abstract ideas that have so far defied representation.

Methodology

A panel discussion has been conducted among college students about the variations in formal education and the advanced education involving new technology. In the new Information Technology world, students were having suggestions for a new method that experience all subject to the students as they were experience in real even for

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science students or literature students. Both the stream will have greater opening for each students. This opens a window to this paper "Virtual reality, the illusion now in Education".

About the Virtual Reality Concept

The definition of virtual reality comes, naturally, from the definitions for both 'virtual' and 'reality'. The definition of 'virtual' is near and reality is what we experience as human beings. So the term 'virtual reality' basically means 'near-reality[1]. This could, of course, mean anything but it usually refers to a specific type of reality emulation.

We know the world through our senses and perception systems. In school we all learned that we have five senses: taste, touch, smell, sight and hearing. These are however only our most obvious sense organs. The truth is that humans have many more senses than this, such as a sense of balance for example. These other sensory inputs, plus some special processing of sensory information by our brains ensures that we have a rich flow of information from the environment to our minds.

Everything that we know about our reality comes by way of our senses. In other words, our entire experience of reality is simply a combination of sensory information and our brains sense-making mechanisms for that information. It stands to reason then, that if you can present your senses with made-up information, your perception of reality would also change in response to it. You would be presented with a version of reality that isn't really there, but from your perspective it would be perceived as real. Something we would refer to as a virtual reality.

It is an emerging technology for learning, virtual reality (VR) dates back four decades, to early work by Ivan Sutherland in the late 1960s. At long last, interactive media are emerging that offer the promise of VR in everyday settings. Quasi-VR[2] already is commonplace in 2-1/2-Dvirtual environments like Second Life and in massively multiplayer online role-playing games(e.g., World of Warcraft). Realizing the potential of VR for education, however, is much more complex than simply making its interface practical and affordable. Learning applications are not like fire, a wonderful technology that provides a benefit from merely standing in its vicinity. In education, technologies achieve their power indirectly, as catalysts for deeper content, more engaging activities, more active forms of learning and instruction, and richer types of assessment.

Virtual reality Astronomy

Astronomy students can learn about the solar system and how it works by physical engagement with the objects within. They can move planets, see around stars and track the progress of a comet. This also enables them to see how abstract concepts work in a three dimensional environment which makes them easier to understand and retain.

This is useful for students who have a particular learning style, e.g. creative or those who find it easier to learn using symbols, colours and textures. One ideal learning scenario is medicine: virtual reality can be used to develop surgery simulations or three dimensional images of the human body which the students can explore. This has been used in medical schools both in the UK and abroad.

Virtual reality and tech-savvy children

There is the fact that children today are familiar with all forms of technology and use these at school as well as at home. They have grown up with technology from a very early age and unlike adults, do not have any fear or hesitation in using it. And we live in a technological society. So it makes sense to implement virtual reality as one of several forms of technology in order to educate tomorrow's technological elite. Education has moved on from books, pencils and pens to the use of interactive technologies to help impart knowledge and understanding.

There are two ways of using virtual reality in the classroom: the first involves a traditional desktop set up in which the student explores a virtual environment using

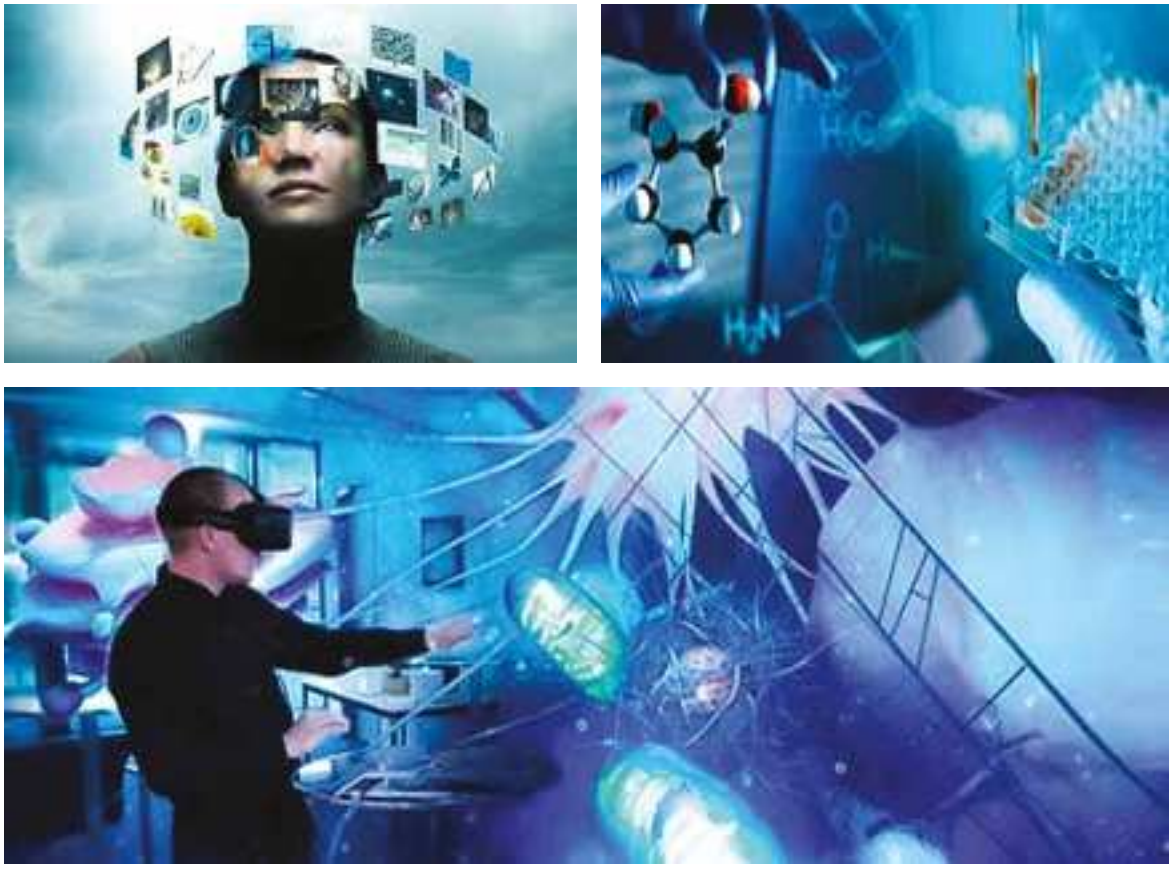

Fig.1 a, b, c focus on the imaginary world created by Virtual Reality

a computer, keyboard and mouse. Or use some other input device, e.g. controller (similar to the WiFi remote controller).The second set up is fully immersive and requires the student to wear a head mounted display[3] (HMD) and data glove for interaction within a virtual environment. This environment may take the form of a series of large screens or a complete CAVE virtual reality system.

The fully immersive set up will include a tracking system which is included in the HMD that records and analyses the student's movements in a virtual space. This also has an effect on what they see as any movement of their head will cause a shift in perception due to the tracking device. They will see images which show the illusion of depth that only adds to the experience.

Example of VR in the classroom: For example: if you are a history teacher then your students may have the opportunity to explore a historic building or era in time such as Ancient Greece. They will be able to walk around a Greek city, e.g. Athens, and explore various aspects, often by using touch via the data glove. This is a great way of learning about day to day life in Ancient Greece which brings it to life in a way that books or online media are unable to.So what we are saying is that virtual reality can be used in many areas of the curriculum. This includes Maths, English, Science, History, Geography, Languages and newer subjects such as design technology.

Also there are students who respond to computer generated learning than traditional methods of teaching. In these cases virtual reality learning is an ideal way of engaging these students with a particular subject in a manner they are comfortable with. Students can touch and manipulate objects within a virtual environment in order to generate a greater understanding of them. But this doesn't only apply to objects, students are able to interact with data sets, complex formulae and abstract concepts

that they may have previously found inaccessible. For some students, learning by doing is easier than learning by listening.

CAVE Fully Immersive Virtual Reality

This is the name given to a virtual environment in which the person is fully immersed within it. CAVE stands for CAVE Automatic Virtual Environment and takes the form of a cube-like space in which images are displayed by a series of projectors.A typical CAVE set up includes the following: Rear projection walls, Down projection floor, Speakers at different angles, Tracking sensors in the walls, Sound/music, Video.There may be a few differences between different CAVE systems but generally, this is the standard set up.

The person wears a pair of virtual reality glasses or a head mounted display (HMD) which displays a three dimensional image via a process known as stereoscopy. This is where someone is shown two images one per eye which the brain combines into a single image. These images are generated by powerful computers and a motion capture system which records the person's actions inside the CAVE. These actions are converted into a series of images which are then displayed to the person via their glasses.

CAVE interaction

A main feature of the CAVE[5] system is interaction. The combination of interaction and total immersion is known as telepresence in which a person can literally lose themselves within the virtual environment.

Interaction takes place using a variety of input devices, for example a joystick, wand or more commonly, a haptics device, i.e. data glove. This enables the person to interact with objects, for example, pulling, twisting or gripping by means of touch. The ability to do this is known as haptics.

Haptic interaction

A haptics system can also enable the person to experience a reaction also known as force feedback. These reactions take the form of vibrations or other forms of movement and contribute to the experience. They are often accompanied by sound, video and/or images.Force feedback is often seen in computer games, for example racing car games where the person feels the car losing control or fighting to stay on the road when cornering. But it is becoming increasingly popular in other areas such as touch screen in-car navigation systems and surgery simulation.

Virtual Reality Programming Languages

For virtual reality to be truly effective, it must have a good sense of realism. Just on its own this is a technical challenge and, as such, virtual reality is highly demanding on many resources. From hardware performance to the intellectual ability of the implementer of the system, how these are managed are massive issues. As mentioned above, the realism involved in virtual reality requires a large amount of hardware resources. The most obvious requirement is processing speed, which will become more of a problem as Moore's law becomes less effective. A convincing virtual environment must have extremely realistic visuals so good usage of the GPU, for graphics rendering, is also a definite requirement. Sound is also another factor and the quality of audio output must be extremely high, which requires good use of the sound card. This barely scratches the surface of the technical requirements involved.

Not only is the use of hardware an issue, the person using these resources must also be highly skilled. A good knowledge of in-depth computer science topics is a must have, usually requiring post-graduate education.

Much like software development today, as time progresses and the field expands, these requirements must be dealt with effectively and the barrier of entry much be lowered. One such method is to employ a domain-specific programming language geared especially towards virtual reality.

Programming Languages Specifically for DSL (domain-specific language) can be carefully tailored to a problem domain in many critical ways. This is applies to virtual reality too. Carefully crafting the correct

language(s) to virtual reality will allow developers to write less code which is optimised especially for the creation of a virtual environment.

Visual Programming Language: This programming language was invented by Jaron Lanier of VPL[5] Research to aid the building of virtual reality experiences. VPL is described as a "post-symbolic" programming language, which is taken to mean programs are 'written' by means other than letters, numbers and other written characters. For reference, most computer programs are written using the letters of the alphabet, numbers and punctuation characters.

Microsoft have developed a language with the same name which fits a "post-symbolic" description using drawing as a method of writing programs.

There is Unimersiv to help VR developers working on educational content find an audience. Unimersiv is a platform dedicated to virtual reality educational content that aims to help developers get their experience noticed and earn money through those. Educational experiences on Unimersiv can be free or paid according to the choice of the developers (*https://www.eonreality.com*).

Future enhancement

- Collaboration in virtual reality classroom fosters social integration of learners
- Not possible in reality is possible in virtual reality
- Virtual game-based experience increases students' motivation
- Virtual reality introduces new approach to rewards
- Virtual platforms and headsets are the new tools for inspiring creative learning

Conclusion

According to the cone of learning from Edgar Dale, after two weeks, the human brain tends to remember 10% of what it reads, 20% of what it hear but 90% of what it does or simulate. As we say, a picture is worth a thousand words, well we believe that a 15 minutes virtual reality experience worth a 1H30 documentary. We now have the ability to take back people in 1942 to relive some of the worst period of humanity or in 1969 to become Neil Armstrong on what is the 20th century's most enduring achievement. So let the education be in-cooperated with virtual reality and our education system become useful for all students. Let the education become an experience.

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A Review on Doped Zinc Oxide Thin Films

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Abstract

ZnO is an attractive material for application in electronics, photonics, acoustics and sensing. In optical emitters, its high exciton binding energy (60 meV) gives ZnO an edge over other semiconductor such as GaN.Reproducible and reliable p type doping in ZnO is to be achieved, which is currently a main obstacle for realization of bipolar devices.Hence we are going to review study of doped zinc oxide thin films. Recent improvement in the control of back ground conductivity of ZnO and demonstration of p type doping have intensified interest in this material for applications in light emitting diodes (LEDs). In this paper, we summarize growth and doping effect on various properties of zinc oxide thin films prepared by various methods. It is an innovation if it is made reproducible and reliable.

Key words- ZnO, doping, co-doping

Introduction

Much attention has been paid on zinc oxide (ZnO) as a promising material for various optoelectronic and photonic devices due to its large exciton binding energy of 60meV and wide band gap of 3.37eV at room temperature[1]. Due to its non-Centro symmetric crystallographic phase, ZnO shows the piezoelectric property which is highly useful for the fabrication of devices, such as electromagnetic coupled sensors and actuators[2]. Zinc oxide has also attracted attention for electrical and optical applications such as light emitting diodes, photocatalysts, photodetectors[3], piezoelectric devices and solar cells. In addition, ZnO films with high transmittance in the visible region and low resistivity are suitable for use as transparent electrodes in electronic displays.

To develop a light emission device, such as light emitting diodes and laser diodes which are having high efficiency &low energy loss is an important topic to be dealt with in the 21stcentury . It is reported thatGaN device technology is commercialized due its high performance in electronic and optical properties. However, thereare still some problems of using GaN, such as the high processing temperature required for them. ZnO has a lot of advantages over GaN as a good light emitting diode. ZnO has a high exciton binding energy, same structure (wurtzite) as that of GaN, lower toxicity, lower cost and high abundance. Furthermore, the potential worldwide shortage of indium in face of growing demand for indium tin oxide (ITO) seems to be stimulating the exploration of zinc oxide based transparent oxides, which if successful, could become a huge application area.

In order to use zinc oxide thin films for different applications,it is very important to obtain films with desired and reproducible properties. Taking into account that the deposition parameters of each deposition techniques can be varied, the properties of thin films obtained may differ significantly. Therefore researchers are still conducting studies to establish the most convenient deposition parameters for processing ZnO thin films with excellent optical and electrical properties. Many techniques have been employed by different authors in order to prepare ZnO thin films. They include Metal Organic Chemical Vapor Deposition (MOCVD)[1,4,5], Molecular Beam Epitaxy(MBE)[6,7], Pulsed Laser Deposition (PLD)[8,9,10], Aqueous Solution Method[11,12], Sputtering[13,14,15,16], SILAR[17,18,19] and Sol Gel methods[20,21]. In

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this paper we summarize effect of doping on electrical and optical properties of zinc oxide thin films prepared by various methods. The organization of this review is given as follows. ZnO material properties such as physical and electrical properties are described in the first section. This is followed by doping of ZnO thin film in the next section .In the last section the effect of different dopant (p-type and n-type) on electrical and optical properties of ZnO thin films are also described. Final section deals with conclusion arrived from the reviews.

ZnO Material Properties

Recently Zinc Oxide has attracted much attention for its applicationsin LEDs, solar cell, sensors and transparent electronics. In this section, we focus on the material properties of ZnOthin films such as physical and electrical properties.

Physical Properties

Zinc oxide is a II-VI compound semiconductor with a hexagonal wurtzite structure whose lattice constant is 3.25Å. Zinc oxide is characterized by wide band gap (3.37eV) and a large exciton binding energy (60meV)[22,23], when compared with GaN (3.4eV, Direct) which in principle enables optoelectronicapplications in the blue and UV region of the spectrum.The prospect of such applications has been filled by impressive progress in bulk crystal as well as thinfilm growth over the past few years. ZnO has a number of advantages over GaN, the wide band gap semiconductor currently utilized in optoelectronic industry. Some of these advantages include a large exciton binding energy , a higher radiation hardness, simple processing due to amenability to conventional chemical wet etching, and the availability of large area substrate at relatively low material costs. Table 1 shows some of basic physical properties of ZnO at room temperature[3].

Electrical Properties

The electrical behavior of undoped ZnO film has found to be n type and it is widely believed that is due to native defect such as oxygen vacancies and zinc interstitials.

Table 1: Basic physical properties of ZnO at room temperature

Lattice parameters at 300K	
a_0	0.32495 nm
c_0	0.52069 nm
a_0/c_0	1.602
Density	5.606g/cm^3
Melting point	1975^{0}C
Static dielectric constant	8.656
Refractive index	2.008,2.029
Energy gap	3.37eV direct

The electrical conductivity measurements were performed by A.P.Rambuetet.al[24] and it was observed that values ranged between $1.15x10^{-2}(\Omega\text{-cm})^{-1}$ and $5.43x10^{-3}(\Omega\text{-cm})^{-1}$.ZnO film annealed at 400^{0}C,was found to have the highest electron concentration of $7.247x10^{18}$/cm^{-3} with carrier mobility of 6.53cm^2/Vs at room temperature. Free electron concentration was found to have a minimum value of $1.426x10^{17}$cm^{-3} for ZnO film annealed at 600^{o}C[22].In general the carrier concentration is typically 10^{16}-10^{17}cm^{-3}. The electron mobility and hole mobility of undoped ZnO films varies depending on the growth method but is usually 120-440cm^2/Vs and 5.53cm^2/Vs at room temperature respectively[25,26].The hole mobility is much lower than electron mobility as it is evident from the difference in effective mass (effective mass of electron is $0.24m_o$ and that of hole is $0.59m_o$) and carrier scattering mechanism. Undoped ZnO has an electrical conductivity ranging to small extent, which could be changed by doping as seen in later section.

Doping Of ZnO

ZnO is an important material as is seen from its use in electronic and optoelectronic devices due to its varied properties such as semiconducting, magnetic, piezoelectric etc. One of the key requirements for many of these applications is the doping of ZnO with various elements for enhancing

and controlling its electrical and optical properties.

n-type Doping

The as grown ZnO films is unintentionally n-type and it is widely believed that intrinsic defects such as the zinc interstitials and oxygen vacancy are sources of donors in ZnO. Having established that native defects cannot explain the n type conductivity in ZnO, it is relevant to investigate possible impurities that act as shallow donors. For the sake of controlling the n-type conductivity in ZnO,it is also relevant to investigate other possible impurities that act as shallow donors and can be used to make n-type ZnO in a stable manner. The properties of dopant impurities in ZnO are summarized in table 2.[15,16,27,28]

Table 2: Properties of dopant impurities in ZnO

Impurity	Character	Carrier concentration experimentally cm^{-3}
B	Donor	10^{20}
Al	Donor	$4.1x10^{21}$
Ga	Donor	$6.9x10^{20}$
In	Donor	$4.75x10^{20}$
F	Donor	-

Intentional n-type doping has been accomplished using group III elements such as B, Al, Ga and In, which can easily substitute Zn ions. Many groups have attempted doping with B, Al, Ga and In for application to n-type layers in light emitting devices as well as transparent ohmic contacts. Doping method with B[28,29], Al[6,27,30], Ga[14,16,31,32], and In[15,33] can produce highly conducting n-type ZnO films with a carrier concentration of $>10^{20}cm^{-3}$ range. The group VII elements F and Cl which substitute oxygen ions have also been suggested for n type dopant. Dwight reported that indium, gallium and fluorine can be doped on ZnO grown by spray pyrolysis with a resistivity of $4.1x10^{-3}$ Ωcm, $3.6x10^{-2}$ Ωcm and $6.7x10^{-3}$ Ωcm respectively[7]. ZnO based dilute magnetic semiconductors doped with transition metals(Co, Mn, Fe.etc.) have been predicted theoretically to be good candidates for room temperatureferromagnetism and large magnetization.[8,57,58] . Besides these dopants, by doping with luminescent centers the emission properties of ZnO can be tuned towards selected wavelengths in the visible region, which is of interest for a variety of application including multicolor emission in light emitting devices.[59,60]

A.P.Rambu et al[61]reported that the electrical conductivity of ZnO:In thin films is increased with few orders of magnitude by comparing with the conductivity of undoped ZnO. It is due to the incorporation of indium atoms in ZnO lattice, which increases the donor concentration. Min Chul Jan et al[45] and A.Alkahlout[30] doped the aluminum and gallium on ZnO thinfilms by spin coating on glass substrate. They observed that GZO films showed better properties that AZO ones when used as transparent conducting coatings and also aluminum and gallium dopants acted as electrical dopants at the initial doping concentration but as impurities at grater doping concentration. Adding indium impurity to ZnO Indium substitutes Zn and it will act as donor impurity and creates n-type conductivity. Increasing impurity (higher that 6%) leads to change n typeto p type. The reason for this change might be a decrease in donor property of indium following an increase of impurity. So indium act as acceptor impurity and is substituted for oxygen. Therefore change in carrier type resulted in change of n-type to p-type semiconductor[33,34,47,62]. The photoluminescence studies on GZO films shows that the broad visible emission is originated from the intrinsic shallow traps(Vzn) and deep level Vacancies (Zn_i,Ozn and Vo) in ZnO films[36,37]. Table 3 is a survey of dopants, growth techniques, substrates and electrical properties of n type ZnO.

Table 3: Survey of dopants, growth techniques, substrate, transmittance and electrical properties of n type ZnO

Growth techniques	Electrical Properties			Dopant	Substrate	Trans-mittance	Refer-ences
	Carrier concentration cm^{-3}	Mobility cm^2/Vs	Resistivity Ω/cm				
Ion beam assisted MBE	$4.11x10^{19}$	13.09	$5x10^{-3}$	Al	Glass	< 80%	6
Spray pyrolysis	-	-	$3.42x10^{-3}$	In	Sodocalcic glass	50-70%	34
	-	-	$5.63x10^{-1}$	In	Glass	90%	35
	$6.58x10^{19}$	18.99	$5x10^{-3}$	Ga	Glass	80%	36
	-	-	$9.37x10^{-3}$	Ga	Glass	85-95%	37
	$9.03x10^{19}$	2.99	$2x10^{-2}$	Al	Glass	80%	38
	-	-	$10.17x10^{3}$	In	Soda-lime glass	82%	39
	-	-	10^{-1}	In	glass	80%	40
	-	-	$4.1x10^{-3}$ $3.6x10^{-2}$ $8x10^{-3}$	In Ga F	Sodocalcic glass	97%	7
Spin coating	-	-	1.45 $7.4x10^{-2}$	Al Ga	Glass	>80%	30
	-	-	-	Ga	Silica	85%	41
	-	-	10.03-0.503	In	Glass	-	42
Sol Gel	$8.72x10^{17}$	234	422	In	Glass	>90%	33
	$1.25x10^{19}$	0.328	1.52	Sn	Silica	94%	43
	-	0.06	-	In	Silica	-	44
	-	-	$4.3x10^{-3}$ $3.3x10^{-3}$	Al Ga	Glass	>85%	45
Thermal oxidation	-	-	-	In	Glass	88-95%	46
Atmospheric pressure plasma processing	$2.69X10^{20}$	12.86	$1.8X10^{-3}$	In	Glass	80%	47
Reactive thermal evaporation	-	-	36.24	Al	Glass	>70%	48
PLD	-	-	-	Ga Fe	Glass	94% 94.94%	8
	-	-	$1.44x10^{-4}$	Al	Quartz corning	84-92%	10
SILAR	10^{14}	20	-	Al	Glass	-	49
	-	-	10	Sn	Corning glass	-	50

Growth techniques	Electrical Properties			Dopant	Substrate	Trans-mittance	Refer-ences
	Carrier concentration cm^{-3}	Mobility cm^2/Vs	Resistivity Ω/cm				
Magnetron sputtering	$6.99x10^{20}$	29.49	$3.03x10^{-4}$	Ga	Glass	80%	16
	-	-	$1.31x10^{-3}$	Ga	Glass	89%	14
	$4.75x10^{20}$	-	$9.13x10^{-4}$	In	Non alkali glass	85%	15
	-	-	$3.9x10^{-3}$ $1.41x10^{-3}$	Ga	Glass	>80%	32
	-	-	$1.3x10^{-3}$	Al	Glass	>90%	51
	$6.1x10^{20}$	28.4	$0.36x10^{-3}$	Ga	Glass	-	52
	$4.1x10^{21}$	8.93	$3.9x10^{-4}$	Al	Glass	90%	27
PECVD	$5.4X10^{20}$	-	$3x10^{-2}$	Al	Corning glass	95%	53
MIST CVD	-	-	$1.1x10^{-3}$	Ga	Glass	-	54
Spray CVD	10^{20}	-	$0.39x10^{-3}$	B	Glass	90%	28
MOCVD	-	-	$1x10^{-3}$	Al	GaN wafers	80%	55
	$3.16x10^{20}$	4.85	$4.07x10^{-3}$	Ga	Sapphire	96%	56

p-type Doping

In order to develop zinc oxide based opto-electronic devices the most important issue is the fabrication of p type ZnO. One reason is that ZnO has affinity to make n type conductivity. Another reason is that there are native defects, such as zinc vacancies, oxygen vacancies or back ground impurities such as hydrogen in the pure ZnO. A third reason is the fact that there are very few elements allowacceptors in ZnO. To grow p type ZnO, the acceptor concentration should be higher than unintentional donor concentration. Column IA elements (Li, Na, K..) on the Zn site are either deep acceptors or are also stable as interstitial donors that compensate p type conductivity. Column IB elements (Cu, Ag, Au) are deep acceptors and donot contribute p-type conductivity, because O is highly electronegative. The other column V elements (P, As, Sb) substituting on sites are all deep acceptors. P type doping in ZnO may be possible by substituting either Group I elements Li[25,64], Na[65,66], and K[67] for Zn sites or group V elements, N[59], P[68,69,70], As, Sb[65,72] for O sites. It has been believed that the most promising dopant for p- type ZnO is the group V elements, though theory suggests some difficulty in achieving a shallow acceptor level. Recently another p type doping mechanism was proposed for group V elements. It suggested that these impurities would substitute for Zn and form complexes with two Zn vacancies[59]. However, the choice of p-type dopant and growth techniques remains controversial and the reliability of p-type ZnO and the doping mechanism are still subjects for debate. Nitrogen has been used as p-type dopant due to similar ionic radius compared to oxygen and availability of gas sources such as N_2, NO_2 and NH_3.

M.Ardyanianet *et. al*[64] reported heavy lithium doped p type ZnO films by spray pyrolysis method. The Hall effect results

describe that Li doping leads to change in the conduction type from n-type to p-type, and again to n-type (high concentration) and is attributed to self-compensation effect. The reported carrier density was in the order of $10^{13}cm^{-3}$. Bing wang et al[25] prepared Li doped ZnO thin film on quartz substrate by radio frequency magnetron sputtering, getting resistivity 78.61Ωcm and mobility $1.02cm^2/Vs$ respectively when annealing ambient is O_2. ZnO films annealed under Ar ambient presents n type conduction with low resistivity (0.51Ωcm) due Li incorporation at interstitials sites, on other hand annealed under O_2 ambient showed p type conduction due to Li incorporation at substitutional sites.

Nitrogen doping was considered to be a promising approach to achieve p-type conductivity of ZnO. The N ion is expected to enter the lattice in two distinct configurations, either as substituent on the oxygen site or as defects complex in combination with Zn or O vacancies . It will induce a deep acceptor level in ZnO band gap, giving rise to a broad luminescence peak at-730 nm.[59]A.Saaediet. al[72] investigated possible p-type with the group I elements in ZnO. They obtained that Li and Na are better acceptor dopants with shallow acceptor levels than N, while they are mostly self-compensated by Li and Na interstitials respectively. They also found that Li doped ZnO have higher optical quality than Na, K doped and undoped ZnO nanowires. Compared with the undoped ZnO nanowires, doped ZnO shows a red shift in the UV emission.Thisshows a step to obtain p-type nanowires. The p type conductivity of Sb doped ZnO materials have been reported by group of researchers[65,71],getting the lowest resistivity 0.185Ωcm with a Hall mobility of 54.05 cm^2/Vs, and a hole concentration of 6.25x1017 cm^{-3}. Sodium doped ZnO thin films were prepared by many researchers by different methods such as sol gel[73] and hydrothermal[74]. The p-type carrier concentration and mobility of Na doped ZnO nanorod arrays range from $3.1x10^{16}cm^{-3}$ to $1.7x10^{17}cm^{-3}$ and $41.9cm^2/Vs$ to 106 cm^2/Vs respectively. In addition to that there is a change of green emission peak in PL, and it may be connected with competition between oxygen vacancy(Vo^+) and interstitials oxygen (O_1^-). The violet emission is attributed to both Zn vacancy (Vzn) and defect level in the green boundaries of ZnO crystals.[73]

The origin of green luminescence in ZnO is shown to be related to Zn interstitials formed due to the escape of oxygen when ZnO is fired in reducing atmospheres. The population of oxygen vacancies and hence, the luminescence intensity could be enhanced with alkali metal (K+ at substitutional sites of Zn^{2+} form oxygen vacancies) doping in ZnO[75,76]. Bousmahal *et al*[77] reported the electronic properties of pure and K doped ZnO using first principle calculations based on Density Functional Theory (DFT) to describe the possibility of achieving p type ZnO by K doping. Both the electronic band structures and density states showed an appearance of a new narrow band in the valence band and the Fermi level shifts towards the valence band which indicates that p type ZnO can be achieved by K doping. The microscopic defects in phosphorus doped ZnO films epitaxially grown on the (0001) sapphire and the (0001) ZnO substrate and their effects on the electrical properties were studied by Allenic *et al*[68]. They reported that there are edge type dislocations associated with the antisite defect. This result in the formation and stabilization of Pzn-2Vzn shallow acceptors that can lead to p type conductivity due to the anhilation of native donors by getting and out diffusion from the dislocation cores. Kyoung-kook kim *et al*[70] report on the production of p-ZnO:P thin films on a sapphire substrate using phosphorus doping and a thermal annealing process. As grown n-ZnO:P thin films is converted in to p-ZnO: P by an RTA process under a N_2 ambient. The hole concentration,-carrier mobility and resistivity of p-ZnO:P films were found to be $1.0x10^{17}$-$1.7x10^{19}cm^{-3}$, 0.53-3.51 cm^2/Vs and 0.59-4.4Ωcm respectively. Table 4 is a survey of dopants, growth techniques, substrates and electrical properties of p type ZnO.

Table 4: Survey of dopants, growth techniques, substrate transmittance and electrical properties of p type ZnO

Growth techniques	Electrical Properties			Dopant	Substrate	Trans-mittance	Refer-ences
	Carrier concentration cm^{-3}	Mobility cm^2/ Vs	Resistivity Ω/cm				
Spray pyrolysis	10^{13}	-	40	Li	Glass	97%	64
	$6.25x10^{17}$	54.05	0.185	Sb	Glass	75%	65
Chemical Method	$2.36x10^{19}$	-	-	K	-	-	67
	-	1.84	-	Na	-	-	72
PLD	$4.78x10^{17}$ -$4.68x10^{18}$	0.12-1.42	13.8-19	Na	Silicon	-	65
	$1.3x10^{17}$	1	$4.9x10^{1}$	P	Saphire	-	66
Hydro-thermal	$3.1x10^{16}$-$1.7x10^{17}$	41.9-106	-	Na	Si	-	74
CVD	10^{15}	-	-	Na	Si	-	66
MOCVD	$1.8X10^{18}$	0.227	14.9	P	Quartz	-	69
R.F Magnetron sputtering	$6.48x10^{16}$	1.02	78.61	Li	Quartz	80%	25
	$1x10^{17}$-$1.7x10^{19}$	0.53-3.51	0.59-4.4	P	Saphire	-	70
	$13-41x10^{21}$	$11-16x10^{-3}$	.012-.025	P	Zinc wafer	-	71
	$5-28x10^{21}$	$2-20x10^{-3}$	0.042-0.146	Sb			

Co-doping

Co-doping has been suggested to be an effective method of achieving p-type conductivity in ZnO. The term co-doping means that, along with the acceptors that are incorporated to produce holes, donors are also incorporated during the growth. At first sight, this would lead merely to compensation. In fact, compensation during the growth is actually quite desirable, since, it shifts the Fermi level away from the VBM toward the middle of the gap. This results in a lowering of the formation energy of acceptors, as well as an increase in the formation energy due to compensating donor type native defects [such as Vo]. However, the compensation by the intentionally introduced donor well persist after growth, and the material will not exhibit p type conductivity. One potential strategy for overcoming this limitation is to remove the donor after growth by annealing at modest temperature.

Difference of radii between doping ions and Zn^{2+} ions orO^{2-} ions will result in variation of lattice and degeneration of crystallinity in zinc oxide. This issue could be partially resolved by co-doping, which has led to an upsurge study in recent years. Theory predicted that co-doping of ZnO simultaneously with acceptors (N,P,As or Sb) and donors (Al,Ga or In), enhances incorporation of the shallow acceptors and supports

the formation of shallow acceptors levels. Hence, co-doping provides better perspectives for obtaining of a p-type ZnO material than mono-doping.

Karmvirsinghet al[9]studied the effect of (aluminum and indium) co doped in ZnO thin films prepared by PLD technique. They reported high transmittance in the range of 81% in the visible region. The surface morphology of the AIZO thin films were affected due to the presence of aluminum and indium. Experimentally, Jinhyun shin et al[78] hasreportedGalium and Aluminium co-doping in ZnO(GAZO). They reported high concentration ($9.4x10^{20}cm^{-3}$), low resistivity of $2.18x10^{-4}\Omega cm$ and carrier mobility of $28.4cm^2/Vs$ and also found average transmittance of 85%. As the doping concentration was increased, the optical absorption edge shifted toward high photon energy.Another type of co- doping has been proposed by G.shanmuganathanet al[79]. This proposal was based on simultaneous doping of Fe and K in ZnO films using chemical bath deposition. The Fe incorporation in Zn lattice site results in red shift. Table 5 is a survey of dopants, growth techniques, substrates and electrical properties of co doped ZnO.

Conclusions

ZnO offers some advantages in providing electronic, photonic and spin based devices as is evident from the encouraging progress made in the research phase enlightened above . Despite this progress, there are still a number of important issues that are in need of further investigations before this material can be transitioned to commercial use for the stated applications. The difference between the current surge to turn ZnO in to a functional semiconductor and past attempts is that better thin film growth techniques are available, ZnO substrates have higher quality and experience has been gained from research on GaN, which posed similar difficulties with respect to controlling conductivity.

A key conclusion from the recent studies is that native defects cannot explain the often observed n type conductivity, but the latter is likely to be caused by the incorporation of impurities during the growth or annealing. ZnO with controlled n type conductivity has many important applications, such as transparent contacts and high electron mobility transistors (HEMTs). If reliable and reproducible p type doping can be achieved, it would hugely boost the applications of ZnO, for instance in LEDs s and lasers. An important factor in these developments is that the quality and availability of ZnO substrate have dramatically improved in recent years, and that high quality epitaxial layers are now being controllably produced. Still, there are a large number of areas that require more investigations: in particular, we note the possible presence of an electron accumulation layer on the surface,

Table 5. Survey of dopants, growth techniques, substrate transmittance and electrical properties of co-doped ZnO.

Growth techniques	Electrical Properties			Dopant	Substrate	Transmittance	References
	Carrier concentration cm^{-3}	Mobility cm^2/Vs	Resistivity Ω/cm				
Spray pyrolysis	-	-	-	Li &Sn	Glass	-	9
CBD	-	-	-	Fe & K	Glass	<85%	78
PLD	$9.4x10^{20}$	28.4	$2.1x10^{-4}$	Al &Ga	Glass	85%	79
	-	-	-	Al & In	Glass	81%	80
Sol-gel	-	-	10^2	Na& Mg	Glass	-	66

and its effect on measurements of conductivity of the underlying layers.

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Preparation and characterisation of nickel oxide thin films using sol gel spin coating technique

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Abstract

Nickel oxide(NiO) thin films were deposited on glass substrates using a facile sol-gel method. The effects of annealing temperature on the structural and optical properties of NiO films were examined. These films have been characterized with regard to structure, morphology, elemental nature, optical absorption and electrical behaviour using X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), UV-Visible double beam spectrophotometer and Hall effect measurements. From the structural studies, we have obtained diffraction peaks which matched with the standard JCPDS data, thus confirming polycrystalline nature and fcc (NaCl-type) structure of NiO. SEM analysis shows that the film consists of nano crystalline grains with uniform coverage on substrate surface with randomly oriented morphology. The elemental analysis showed the presence of nickel and oxygen in the sample. NiO oxide thin films showed high optical transmittance (80-90%) in the wavelength range of 300-800 nm. Optical study revealed that band gap energy of NiO thin films decreases progressively with increasing annealing temperature. These results suggest that NiO thin films prepared by spin coating technique should be useful as transparent electrodes for optoelectronic devices.

Key Words: Sol-gel, NiO, Spin coating, Structural properties, Optoelectronic properties.

Introduction

Transparent electronics is a promising industrial science involving the accomplishment of invisible electronic devices and circuits. Transparent conducting oxides (TCO) have a wide utilisation range in solid state devices such as display devices, solar cells, gas sensors and electro chromic devices. Transparent p and n-type semiconductors are necessary for a variety of applications in optoelectronics and transparent electronics. For p-type TCOs, hole mobilities are low due to the localised nature of hole transport path. In almost all TCOs, localized O 2p orbital form the valence band maxima and hence the hopping conduction is responsible for charge transport. NiO is a wide band gap p-type semiconductor with energy band gap ranging from 3.47 eV-3.86 eV. It is reported that nickel oxide is an anti ferromagnetic, electro chromic and catalytic material with excellent chemical stability, high transparency and electrical conductivity[1]. Various techniques have been adopted for the preparation of nickel oxide thin films. It includes thermal evaporation[2], spray pyrolysis[3], vapour deposition[4], electrochemicaldeposition[5], sol gel[6,7], sputtering[8-10], chemical bath deposition[11-15]etc. Of the above deposition techniques, sol-gel process is a cost effective technique that can be used for large area film deposition.

In the present work, nickel oxide films of different thickness were coated on glass substrate by sol-gel coating technique. The effect of annealing temperature on their structural, morphological, and optical properties has been studied.

Materials and method

Microscopic glass slides (2.5 cm x 2.5 cm x1.45 mm) were used as substrates for thin film deposition. Substrates were first

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cleaned using laboratory detergent followed by distilled water. Then they were cleaned ultrasonically with acetone and methanol each for 10 minutes using an ultrasonic cleaner. Finally the slides were dried in oven. The precursor solution was prepared by dissolving 0.5M nickel acetate tetra hydrate [$Ni(CH_3COO)_2 \cdot 4H_2O$] as source of Ni in 60ml of 2-methoxyethanol (solvent) and Mono Ethanol Amine (C_2H_7NO, MEA) was added to the solution as stabilizer. The molar ratio of mono ethanolamine to nickel acetate was kept at unity. The solution was stirred at 60°C for two hours and then aged for 24 h at room temperature to obtain green coloured homogeneous and clear solution. Using static dispense spin coating method, the films were casted by dropping 2ml of sol using an accupippet variable volume at a

Results and Discussions

Thin film fabrication technologies are in high demand and have given the widespread use of coatings in all engineering and science fields. Mechanical, functional and geometrical properties of thin films can vary dramatically and this fact makes it difficult to find a general purpose characterization technique. After the production of thin films, they were characterized using different techniques concerning their electrical, optical, structural and morphological properties.

Structural Studies

Structural study for the NiO films prepared on glass substrate with 5 coatings of sol of molarity 0.5 at a spinning speed of 3000rpm for 30s, annealed at 400 – 600˚C

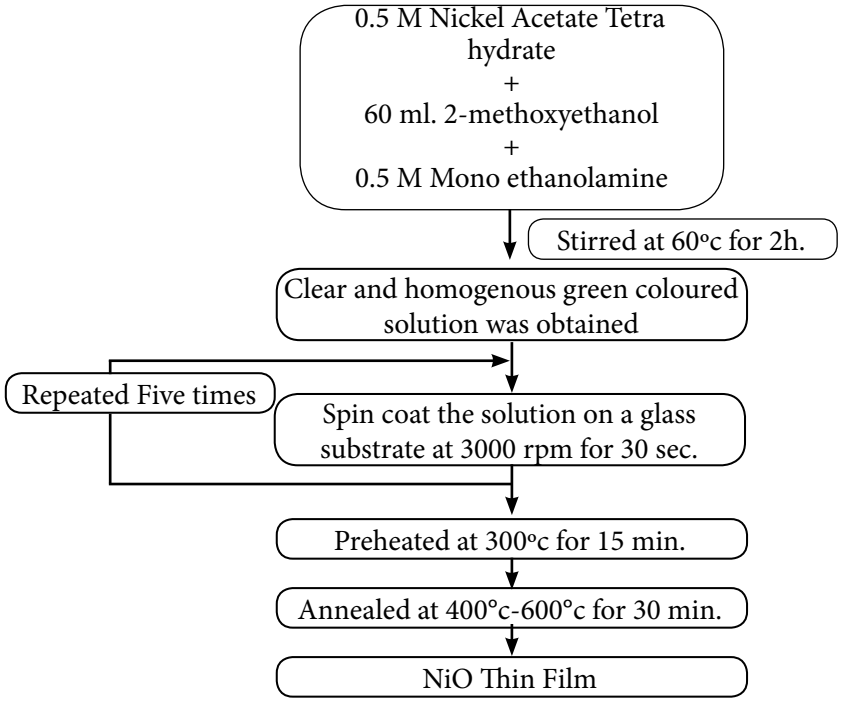

Fig.1. The flow diagram for NiO films prepared from sol gel process using spin coating technique

revolution of 3000 rpm for 30 seconds. After the deposition, the films were preheated at 300°C for 15 minutes in a muffle furnace to remove solvent and organic residuals. The procedures from coating to drying were repeated five times in order to obtain films with desired thickness. Finally the films were annealed at different temperatures from 400°C-600°C for 30 minutes.

was carried out by using CuK_α radiation (wavelength λ=1.54056A^0). Figure 2shows the XRD patterns for NiO films annealed at 400 – 600˚C. The observed peaks in this XRD pattern are fully matched with pure cubic-structured crystalline NiO. Several peaks are observed in the pattern at 2θ = 37.38˚, 43.46˚, 63.03˚and 75.67˚assigned to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) crystal

planes, respectively. The indexed peaks are fully consistent with the cubic-structured crystalline NiO (JCPDS)No 47-1049[16].

In addition to this, no peaks for any impurities or other phases were observed in the pattern which further confirms the crystalline and pure phase of the cubic NiO. It may be noted that the crystallinity of the films improve progressively with increase in the annealing temperature. The crystallite

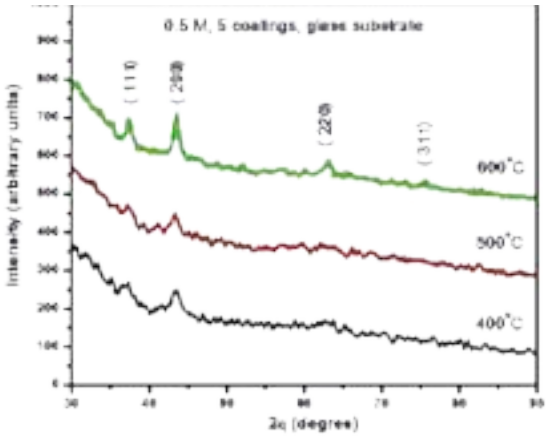

Fig.2: XRD pattern of NiO films annealed at different temperatures

size (D) is calculated using the Scherrer formula[18], $D = 0.9\ \lambda/\beta\ cos\ \theta$. Where β is the full width half maximum of diffraction peak measured in radians, D is the grain size, λ is the wave length of x-ray, θ is the angle of diffraction. The average crystallite size increases with increase in the annealing temperature and lies in the range 7 – 11 nm for 400–600°C. The lattice parameter decreased slightly from 4.18 A° to 4.16 A° with increase in the annealing temperature (Table 1).These values are in agreement with other reports on NiO thin films[17].

Table 1: Effect of annealing on crystallite size and lattice parameter

Annealing temperature (°C)	Crystallite size (nm)	Lattice parameter (Ao)
400	7.0	4.18
500	9.0	4.17
600	11.0	4.16

Surface Morphology

Two-dimensional high magnification surface images of NiO thin film annealed at 600°Care shown in Fig.3. From the micrographs, it is seen that the film consists of nanocrystalline grains. However, some cracks were observed on film surface which may be considered as a drawback of spin coating method for NiO film deposition.When the number of coating layers increases, cracks appears on the film. This is due to removal of organic solvents during pre heating process after each layer coating,

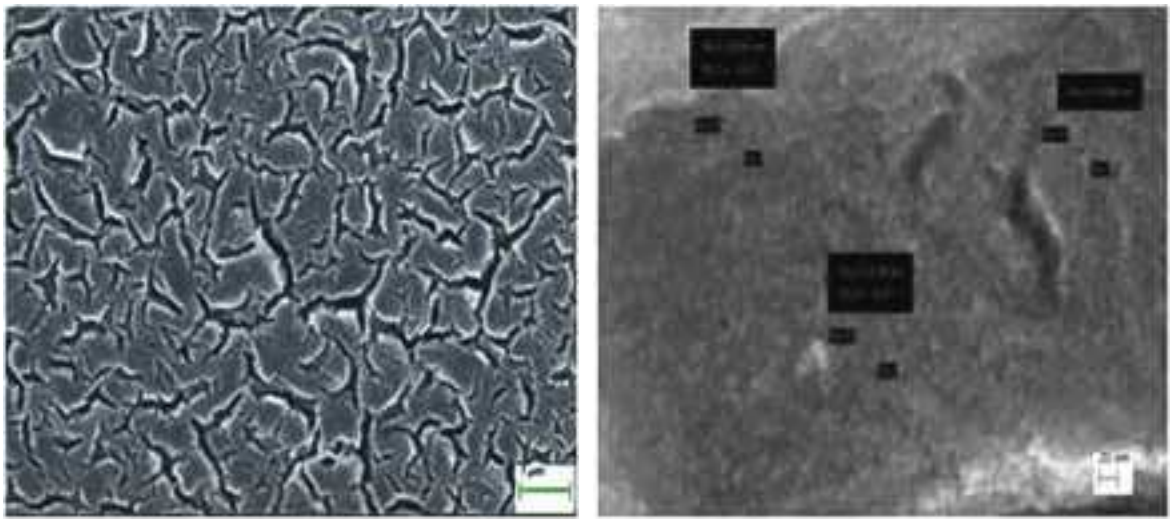

Fig. 3. SEM of NiO thin film annealed at 600° C

which is an inevitable procedure in spin coating method. The grain size at 600°C is found to be 5.84 nm.

Energy Dispersive Spectroscopy (EDS)

The EDS provides the composition of nickel oxide thin films. The EDS spectrum in fig.4 shows the presence of nickel and oxygen in the sample. The presence of Si, other elements and extended peak of oxygen may have come from the substrate used. Also some excess oxygen can also be expected in p-type TCOs

Optical studies

The optical characterisation of the films was done using Intech Model 2700 Double Beam UV –Visible spectrophotometer.

The optical band gap (E_g) of NiO thin films annealed at 400-600°C is calculated from the optical absorption spectra using the following equation[19]. $\alpha h\nu = A(h\nu - E_g)^n$

Where, A is a constant, E_g is the semiconductor band gap and n is a number equal to ½ for direct gap and 2 for indirect gap compound. Since NiO is a direct band gap compound, n=½.

The above equation can be employed to plot against to determine the energy band gap (E_g). The linear portion of the curve when extrapolated intersects the *x*-axis and gives the value of E_g. E_g value decreases from 3.47 eV to 3.41 eV with increase in the annealing temperature from400 °C to

600°C.The reduction in *Eg* with annealing temperature is due to increase in crystalline size and decrease of defect sites. This is in good agreement with the experimental results of XRD analysis. It is reported that there is reduction in the optical band gap energy with the annealing temperature in direct-transition-type semiconductor films[1]. The change in optical band gap energy, E_g, reveals the impact of annealing on optical properties of the NiO films.

The room temperature optical transmittance verses wavelength plots are shown in Fig.5. The transparency of the films

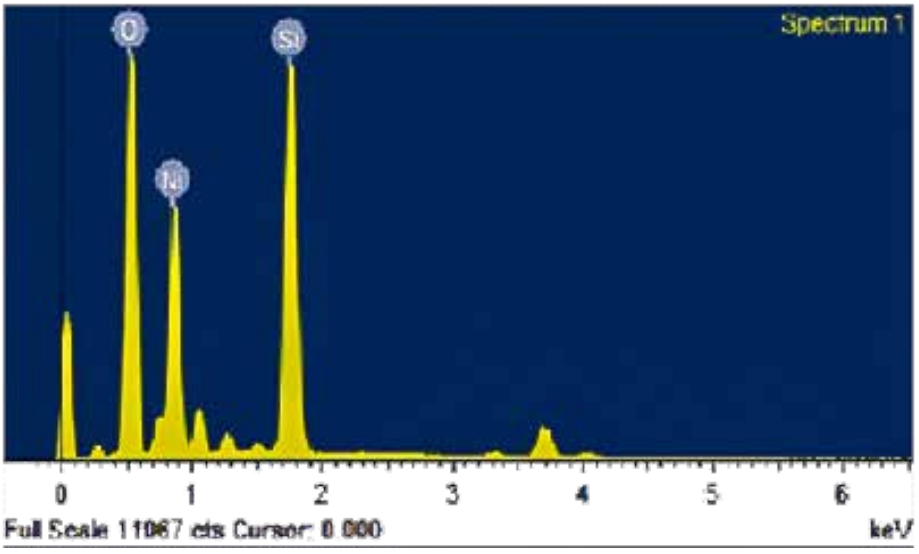

Fig.4. EDS spectrum of NiO thin film

Table 2. Elemental composition of NiO thin films obtained from EDS analysis

Element	Atomic%	Weight%
Ni	17.35	43.53
O	82.64	56.46

is about 80-90% in the visible region but decreases gradually with increasing annealing temperature.

Conclusions

Nano-crystalline nickel oxide thin films were prepared by cost effective sol gel spin coating

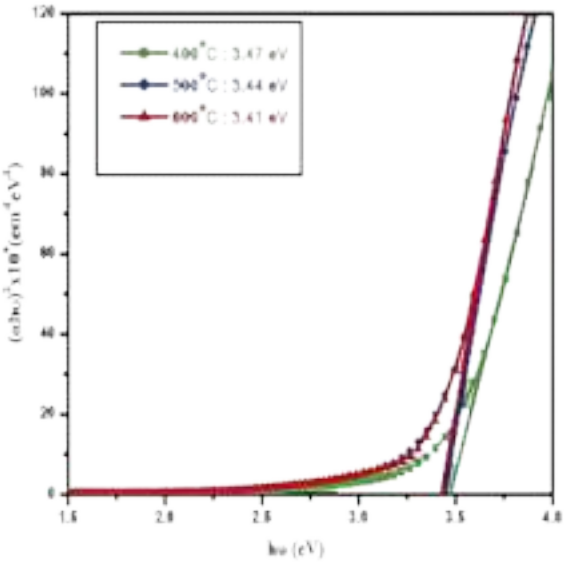

Fig.5. Plot of $(\alpha h\nu)^2$ versus $(h\nu)$of NiO thin film for different annealing temperatures for different annealing temperatures

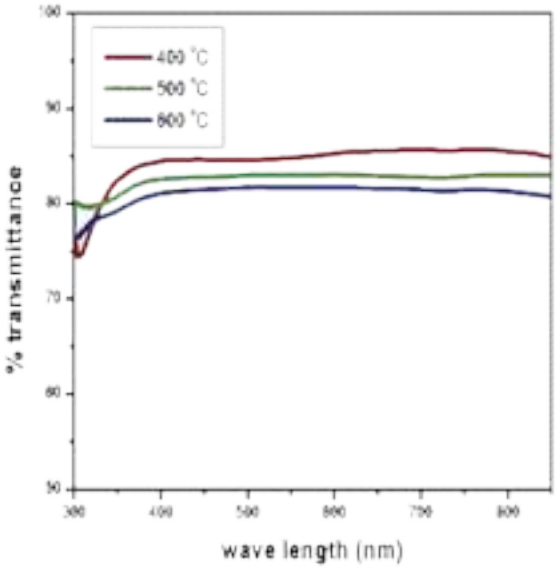

Fig. 6. Plot of optical transmittance verses wavelength of NiO thin film

technique. The NiO films were annealed for various temperatures between 400°C to 600˚C. The XRD results revealed that the NiO thin film has nano-crystalline cubic structure. The SEM results depicted a uniform surface morphology and fine nano-particles with an average grain size of about 5.8 nm. Optical absorption studies showed high transmittance with band gap 3.47eV (at 400˚C) which was decreased to 3.41 eV (at 600˚C). The p-type electrical conductivity is confirmed from Hall Effect measurement. These results suggest that NiO thin films prepared by spin coating technique can turn out to be a promising transparent electrode for optoelectronic display devices.

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Effect of annealing on the structural, morphological and optical properties of Sb_2S_3 thin films

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Abstract

Antimony sulphide is a metal chalgogenide that finds its application in variety of optoelectronic devices. This paper reports the preparation of Sb_2S_3 thin film by the cost effective method of CBD. The effect of annealing on these as-prepared thin films is analyzed in detail. The preparation is carried out at 25°C and the annealing temperature is varied from 150°C to 300°C. XRD analysis reveals that the material transformed from amorphous to polycrystalline on annealing. SEM analysis reveals homogenous and well distributed spherical grains indicating the formation of the antimony sulphide thin film. With increase in annealing temperature the band gap of the material is found to decrease.

Key Words: Antimony sulphide, Chemical bath deposition, XRD, EDAX, SEM, UV-Visible Spectroscopy

Introduction

Metal chalcogenides (sulfides, selenides, telluride's) serves important applications as photo conducting cells, photovoltaic cells and other optoelectrical devices 1-5. Antimony sulphide belongs to the group V-VI of periodic table. It exists in nature in crystalline form with band gap in the range 1.7-2.3 eV. They serves as potential absorber for photovoltaic applications. Antimony sulphide (Sb2S3) thin films are known for their high refractive index and well-defined quantum size effects6. Of the various methods for the preparation of antimony sulphide, chemical vapour deposition has proved to be a cost effective one. Chemical bath deposition technique is yet other method that can be carried out easily at low temperature. This technique is the least expensive and non-pollutant method that can be used for preparing thin films of large surface area.

In the present work antimony sulphide is prepared from a bath containing antimony trichloride and sodiumthiosulphate. These samples are annealed at three different temperatures and the effect of annealing on the structural, morphological and optical properties is studied.

Materials and methods

0.5 M solution of antimony chloride was prepared in 10 ml acetone. 25 ml of 1 M solution of sodium thiosulfate is added to this under constant stirring. This solution was made up to 100 ml by adding 65 ml of distilled water. Both water and thiosulfate solution were precooled to 10°C[7]. The glass substrate is cleaned well with laboratory detergent and distilled water. It is further cleaned using ultrasonic cleaner before being dried in oven. Clean microscope glass slides were placed vertically on the walls of the beaker. The deposition temperature was maintained at 25°C for two hours using a cryostat. The orange-yellow thin films formed on the substrate, is removed from the bath and washed well with distilled water. Films deposited on the lower side of the substrates was found to be more uniform as it avoided gravity settling. The films were uniformly reflective, smooth and adherent.

The formation of antimony sulfide films can be explained as follows:

The dissociation of thiosulfate ions in acidic solution occurs according to the reaction:

$$S_2O_3 + H^+ \rightarrow S + HSO_{3-}$$

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Thiosulfate being a reducing agent, act as an electron donor and reduce the S to S^{2-}.

The thiosulfate forms a very strong complex $Sb_2(S_2O_3)_3$ with antimony ion (Sb^{2+}) , which hydrolyses to form Sb_2S_3 as

$Sb_2(S_2O_3)_3+3H^++6e-\rightarrow Sb_2S_3+3HSO_{3-}$ Or

$2Sb_3^++3S_2^-\rightarrow Sb_2S_3$

The Sb_2S_3 film starts depositing at the substrate, when the ionic product (IP) of Sb^{3+} and S^{2-} ions produced through reactions in the bath is greater than the solubility product (Ksp) of Sb_2S [8,9]. Similarly 0.3 M, 0.8 M and 1 M solutions of antimony chloride in acetone were prepared and the experiment was repeated. These films were annealed at 150°C, 200°C and 300°C for two hours. The films were smooth and reflective when observed through naked eyes.

Results and Discussions

Surface Morphology

Micrograph images were taken using SEM machine JOEL ModeL JSM-6390LV. Image shows spherical shape of grains that are homogeneous and well distributed indicating the formation of uniform thin film. Though the presence of agglomerated surface particles is reported as a common morphological characteristic in films grown by CBD method, this was not pronounced in our film surface.

The grain size was evaluated from SEM images and the value is about 0.646 µm in the case of as prepared sample and is about 1.25 µm in the case of sample annealed at 150˚C. This indicates that particle size increases when annealed. Well defined spherical shapes and grain boundaries were observed in the case of films deposited at 25˚C and annealed at 200˚C for 2 hours.

Chemical Composition

Elemental analysis for a selected area by EDAX analysis is illustrated in figure 2. The result shows the presence of Sb and S, while the impurity elements O and C are

Figure 1

Figure 2

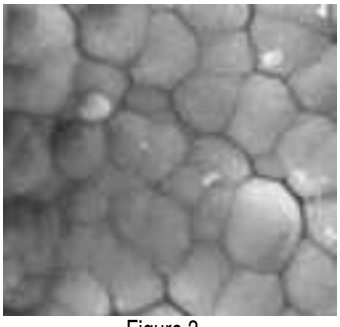

Figure 3

Fig. 1, 2 & 3: Sem images of sample deposited at 25˚C- as prepared samples, samples annealed at 150˚C and at 200°C respectively

Table 1 : atomic percentage of Sb and S content in the film

Element	Atomic%
S	56.0926
Sb	43.9074
Totals	100.00

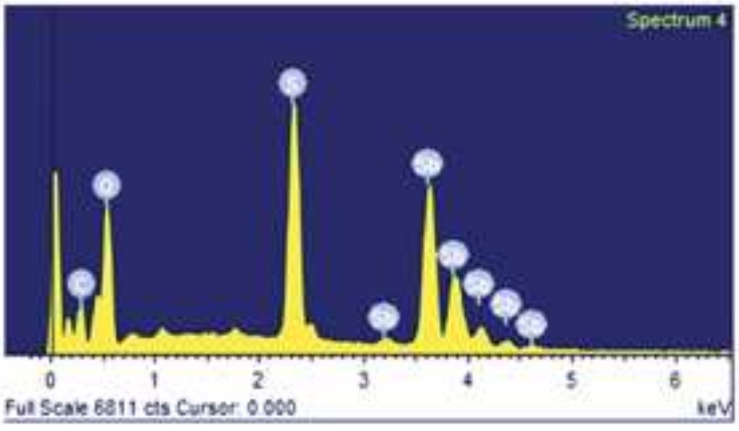

Fig. 4. EDAX analysis of the sample deposited at 25˚C

the contributions from the glass substrate. The stoichiometry of the prepared sample is found to be close to that of Sb_2S_3.

Table 1 shows atomic percentage of Sb and S content in the film. While the expected stoichiometry of S/Sb in antimony sulphide film is 1.5 the obtained ratio is 1.28. The slight decrease in the ratio of S/Sb may be due to the process of annealing in air which results in the formation of Sb_2O_3 with the release of sulphur[7].

Structural Studies

XRD patterns were recorded using Cu Kα source of Rigaku D Max X-ray diffractometer. Figures3-5 shows the XRD spectrum of films annealed at different temperature. The pattern shows that as- prepared sample and films annealed at 150˚C are amorphous. The crystallinity of the sample increase with increase in annealing temperature. It appears that at an annealing temperature of 300˚C, the film formed is polycrystalline with an orthorhombic crystal structure with narrow peaks. The peak positions obtained experimentally are in close agreement with the theoretical value. The films prepared at 25˚C and annealed at 300˚C are found to be polycrystalline.

Optical Studies

Optical studies were done using INTECH model2700 UV –Visible spectrophotometer. The optical band gap of the Sb_2S_3 thin films due to band–band transition is determined using the empirical relation $(\alpha h\upsilon)^n = A(h\upsilon - Eg)$, where Eg is the optical band gap and n = 2, 1/2, 2/3 respectively for allowed direct, allowed indirect and forbidden direct transitions and A is a constant[(7)].

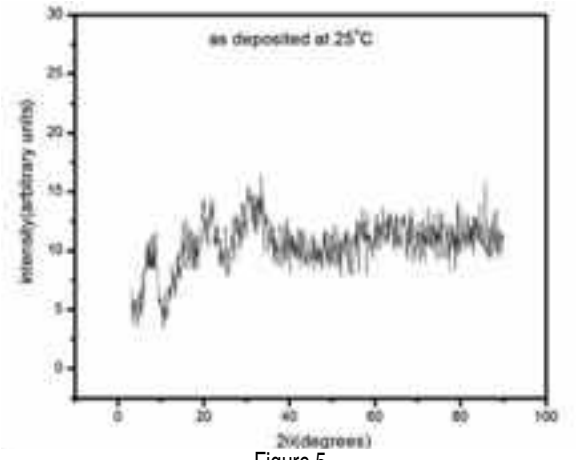

Figure 5

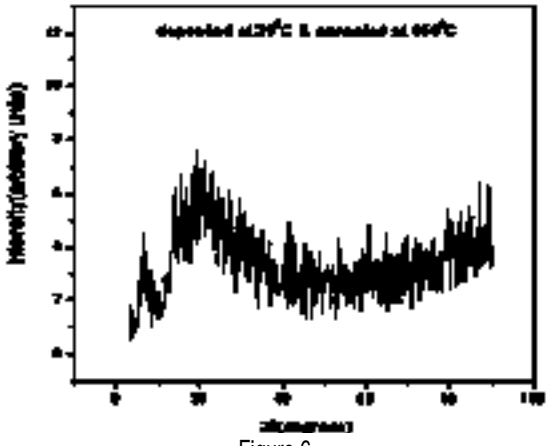

Figure 6

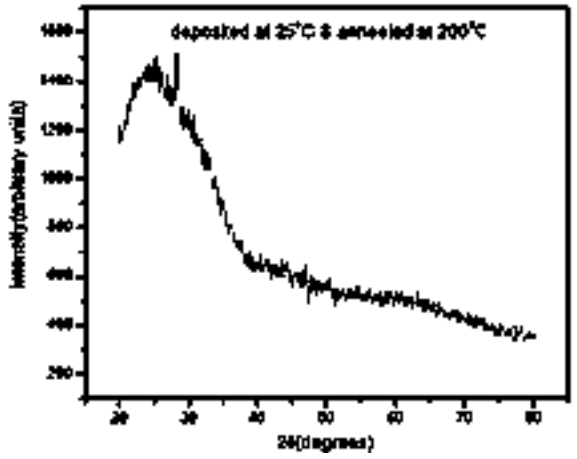

Figure 7

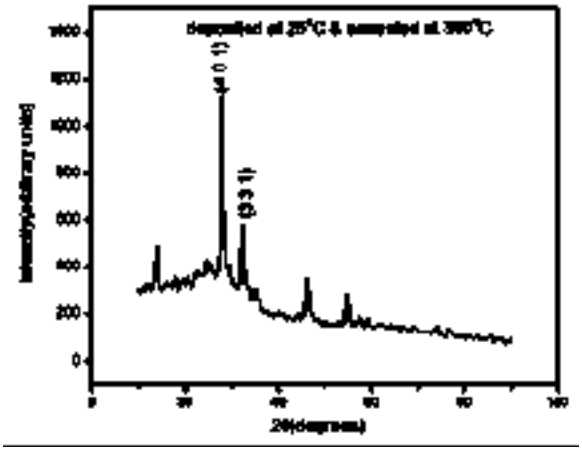

Figure 8

Fig.5, 6, 7 &8 show the XRD spectrum of films prepared by varying the annealing temperature.

The value of $(\alpha h\nu)^2$ for Sb_2S_3 is plotted against $h\nu$ (shown in figures 9-12). The as-prepared sample shows a direct band gap of 2.17 eV. With the increase in annealing temperature, band gap decreased from 2.17 eV to 1.71 eV (Table 2). This is in accordance

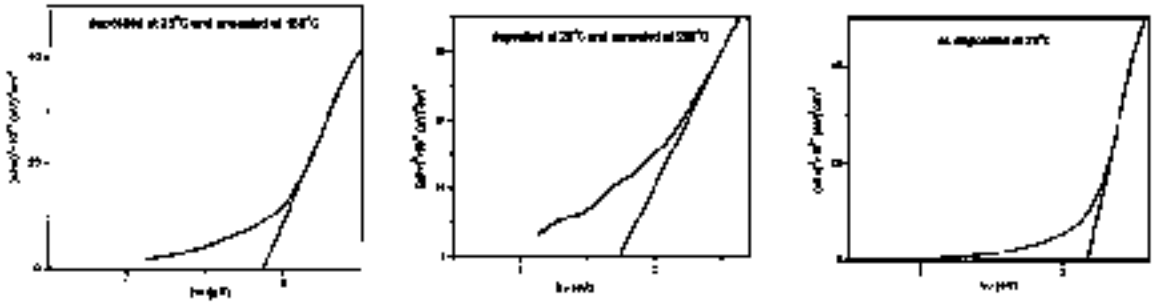

Fig. 9, 10& 11 The plot of $(\alpha h\nu)^2$ against $h\nu$ for Sb_2S_3 film deposited at25°C and at various annealing temperatures

Table 2. shows the variation of band gap with annealing temperatures

Concentration	Deposition temp.	Annealing temp.	Band gap
0.5	25˚C	As prepared	2.173
0.5	25˚C	150˚C	1.76
0.5	25˚C	200˚C	1.738
0.5	25˚C	300˚C	1.71

with the variation of band gap as reported in paper [7, 10]. This can be attributed to quantum confinement effect due to increase in particle size with increase in annealing temperature.

Conclusion

The deposition of crystalline thin films by an easy and cheap technique has always presented a challenge for researchers. Through our work we were successful in developing Sb_2S_3 thin films by the very simple technique of Chemical Bath Deposition.

XRD studies show that the films deposited at 25˚C and annealed at 300˚C were polycrystalline. SEM shows homogeneous and well distributed spherical grains indicating the formation of uniform thin films. Optical measurement showed as prepared samples have a well-defined band gap of 2.175 eV. Further work is required to improve the stoichiometry of these Sb_2S_3 thin films for better device applications.

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Moonlighting proteins in the opportunistic fungal pathogen *C.albicans*—A computational study

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Abstract

Moonlighting proteins are multifunctional proteins that carry out independent, unrelated functions and are found in all kingdoms of life. They play important roles in many biological processes like establishment of infections by pathogens in their hosts. But, they are poorly described in *Candida sp*., the opportunistic fungal pathogens which are in the limelight due to high mortality rates in patients. This study focuses on the identified moonlighting proteins in C.albicans, the most prevalent species causing candidemia. The moonlighting proteins are linked to plasminogen binding, complement inhibition and ferritin utilization in C.albicans. Gene Ontology analysis of the consolidated protein list was performed and the domains of the proteins were studied. The protein disorder was predicted, which was the highest in ALS3. The interaction network of the proteins in S.*cerevisiae* was obtained which can serve as a starting point to predict other moonlighting proteins in C.*albicans*.

Keywords: Moonlighting proteins (MPs), C.*albicans*, Virulence, Protein disorder, Gene ontology.

Introduction

The term moonlighting proteins is used to refer to the multifunctional proteins, excluding the products of gene fusion and homologs, which carry out unrelated and independent functions without partitioning them into different domains. The process has been described in many species of plants, animals, prokaryotes as well as yeasts. Moonlighting is found in highly conserved proteins, usually enzymes, which are relatively abundant in the cell. It can be an evolutionary mechanism to improve the functions of the cell without expanding its genome. It has been found that a novel acquired function of a protein that benefits the cell is selected for during evolution. This novel function may be the result of mutations altering a few amino acid residues, as observed[1].

Protein moonlighting is evident in yeasts. For instance, aconitase which catalyses the conversion of citrate to aconitate during Krebs cycle is also found to be essential for mitochondrial DNA maintenance in *Saccharomyces cerevisiae*[2]. But, it is less documented in Candida sp, the opportunistic fungal pathogens. They cause mucosal as well as deep tissue infections especially in hospitalized individuals and immunocompromised patients. *C. albicans* is the most prevalent species causing invasive fungal infections. These nosocomial infections pose a serious threat due to high mortality, worsened by the increasing drug resistance of Candida sp[3].

Moonlighting proteins have important roles to play in the host-pathogen interactions and can be advantageous to the pathogen by aiding establishment of infection. Such functions have been observed in many pathogens like *Listeria monocytogenes* [4]. This study aims at consolidating the available data on the moonlighting proteins of *C.albicans* and analysing their characteristics, especially their link to infections.

Materials and Methods

The moonlighting proteins in *C.albicans* were chosen from literature and databases like MoonProt (http://www.moonlightingproteins.org/)[5]. Gene Ontology analysis

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was done using Gene Ontology Slim Mapper available at Candida Genome Database (CGD- http://www.candidagenome.org/). The protein sequences were retrieved from Candida genome Database. The protein disorder prediction was performed using DisEMBL (http://dis.embl.de/)[6]. The domains were detected using CD-search tool at Conserved Domains Database (CDD-https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml)[7]. The interaction network was obtained using GeneMANIA (http://genemania.org/)[8].

Results and Discussion

The moonlighting proteins in *C.albicans* were identified through literature survey and using databases. They are listed in Table 1. Most of the moonlighting functions are associated with plasminogen, which is involved in the host-pathogen interaction. The MPs bind plasminogen which is then activated to plasmin by the host plasminogen activators. Thus, the plasminogen-binding is a major step in establishing infections. It is interesting to note that majority of the reported moonlighting proteins bind plasminogen[9]. The site of this function is the cell surface while the canonical functions (Function 1) are carried out in the cytoplasm.

Gene ontology analysis shows that each protein is associated with multiple GO terms, the prominent being the ones linked to their canonical function. But, 9 of them are also associated with the GO term 'Interspecies interaction' which reflects the moonlighting function. Biofilm formation and filamentous growth are important virulence features of *C.albicans* as it is linked to increase in drug resistance [3]. Many of the moonlighting proteins are linked to biofilms and filamentation, as is evident from GO Biological process (Table 2). This emphasizes the medical importance of moonlighting proteins. GO Function, on the other hand, provides information primarily of the canonical function (Table 3).

Table 1: Moonlighting proteins in *C.albicans*

Protein	Function 1	Function 2	Reference
ADH1	Oxidoreductase activity	Plasminogen binding	9
ENO1	Phosphopyruvate hydratase activity in carbohydrate degradation, glycolysis	Plasminogen binding	10
FBA1	fructose-bisphosphate aldolase activity in carbohydrate degradation, glycolysis, gluconeogenesis	Plasminogen binding	9
TSA1	Oxidative/ reductive stress	Plasminogen binding	9
CTA1	Catalase activity	Plasminogen binding	9
TDH3	Dehydrogenase activity in carbohydrate degradation, glycolysis	plasminogen binding fibronectin and laminin binding	9, 11
PGK1	Kinase in carbohydrate degradation, glycolysis	Plasminogen binding	9
GPM1	Phosphoglyceromutase in carbohydrate degradation, glycolysis	Plasminogen binding	9
TEF1	Transcription elongation factor in translation	Plasminogen binding	9
ALS3	Cell wall adhesin	Endothelial invasion, utilization of host cell ferritin	12
HGT1	High affinity glucose transporter	Complement inhibitor and HIV receptor	13

Domain analysis of moonlighting proteins usually detects the domains responsible for the canonical functions, not the domains related to moonlighting. The reported MPs of *C.albicans* were analysed using CD-search tool in Conserved Domain Database. The metabolic enzymes had the common domains- the ones for substrate/ co-factor binding, multimer interfaces or active sites. Adh1 has the necessary domains for its dehydrogenase activity which includes NAD, substrate and Zinc binding sites along with the tetramer interface site. Als3 has the cell wall agglutinin N-terminal ligand-sugar binding site. Metal binding site, dimer interface and substrate binding pocket were identified in Eno1. Active site, zinc binding site, Na+ binding site, intersubunit interface are the main domains present in Fba1. Catalytic core was predicted in GPM1. NAD binding domain and C-terminal domain were identified in Tdh3.

GTP/Mg^{2+} binding site, EF1B alpha binding site, pelota interface, heterodimer interface, 18S rRNA binding site,putative GEF interaction site, EF1 beta interface, G1-G2-G3 box, switch I region, switch II region are present in Tef1. Tsa1 has catalytic triad, dimer interface, decamer interface, peroxidatic and resolving cysteines. Snf7 superfamily domain was the only one predicted in Cta1. Hgt1, which belongs to the Major Facilitator Superfamily has a putative substrate translocation pore.

Moonlighting proteins were suggested to be intrinsically disordered, though some reports claim that the percentage is same

Table 2: Gene Ontology- Biological Process analysis of MPs in *C.albicans*

GO-ID	GO term	Frequency	Gene(s)
44419	Interspecies interaction between organisms	9 out of 11 genes, 81.8%	*TSA1 ADH1 TDH3 ALS3 TEF1 PGK1 ENO1 GPM1 FBA1*
50789	Regulation of biological process	8 out of 11 genes, 72.7%	*TSA1 ADH1 TDH3 CTA1 PGK1 HGT1 ENO1 FBA1*
6810	Transport	6 out of 11 genes, 54.5%	*TDH3 ALS3 CTA1 TEF1 HGT1 FBA1*
5975	Carbohydrate metabolic process	5 out of 11 genes, 45.5%	*TDH3 PGK1 ENO1 GPM1 FBA1*
6091	Generation of precursor metabolites and energy	5 out of 11 genes, 45.5%	*TDH3 PGK1 ENO1 GPM1 FBA1*
30447	Filamentous growth	5 out of 11 genes, 45.5%	*TSA1 ALS3 CTA1 HGT1 ENO1*
42221	Response to chemical	3 out of 11 genes, 27.3%	*TSA1 CTA1 HGT1*
7155	Cell adhesion	2 out of 11 genes, 18.2%	*TDH3 ALS3*
42493	Response to drug	2 out of 11 genes, 18.2%	*CTA1 HGT1*
6950	Response to stress	2 out of 11 genes, 18.2%	*TSA1 HGT1*
19725	Cellular homeostasis	2 out of 11 genes, 18.2%	*TSA1 ALS3*
42710	Biofilm formation	2 out of 11 genes, 18.2%	*ADH1 ALS3*

Table 3: Gene Ontology- Biological function analysis of MPs in *C.albicans*

GO ID	GO term	Frequency	Gene(s)
5515	Protein binding	9 out of 11 genes, 81.8%	*TSA1 ADH1 TDH3 ALS3 TEF1 PGK1 ENO1 GPM1 FBA1*
16491	Oxidoreductase activity	3 out of 11 genes, 27.3%	*TSA1 ADH1 TDH3*
16829	Lyase activity	2 out of 11 genes, 18.2%	*ENO1 FBA1*
3723	RNA binding	2 out of 11 genes, 18.2%	*TDH3 TEF1*
16740	Transferase activity	2 out of 11 genes, 18.2%	*ADH1 PGK1*
16853	Isomerase activity	1 out of 11 genes, 9.1%	*GPM1*
5215	Transporter activity	1 out of 11 genes, 9.1%	*HGT1*
30234	Enzyme regulator activity	1 out of 11 genes, 9.1%	*TSA1*
16787	Hydrolase activity	1 out of 11 genes, 9.1%	*TEF1*
3674	Molecular_function unknown	1 out of 11 genes, 9.1%	*CTA1*

as in other proteins [14, 15]. This was analysed using DisEMBL. The disorder was restricted to the C-terminal and N-terminal regions in most of the proteins, just like any other protein in general. But, Crowe et al. had attributed the moonlighting functions of five of the proteins, namely, Adh1, Tsa1, Cta1, Gpm1 and Tef1 to the lysine residues at the C-termini[9]. It is possible that the disorder can facilitate the function. Short

Fig.1. Protein disorder in Als3 as predicted by DisEMBL. The regions of disorder (hot loops) are marked in bold.

Table 4: Gene Ontology- Cellular component analysis of MPs in *C.albicans*

GO ID	GO term	Frequency	Gene(s)
5737	Cytoplasm	9 out of 11 genes, 81.8%	*TSA1 ADH1 TDH3 CTA1 TEF1 PGK1 ENO1 GPM1 FBA1*
5618	Cell wall	9 out of 11 genes, 81.8%	*TSA1 ADH1 TDH3 ALS3 TEF1 PGK1 ENO1 GPM1 FBA1*
16020	Membrane	8 out of 11 genes, 72.7%	*ADH1 TDH3 CTA1 TEF1 PGK1 HGT1 ENO1 FBA1*
5739	Mitochondrion	7 out of 11 genes, 63.6%	*ADH1 TDH3 TEF1 PGK1 ENO1 GPM1 FBA1*
5634	Nucleus	7 out of 11 genes, 63.6%	*TSA1 TDH3 TEF1 PGK1 ENO1 GPM1 FBA1*
5576	Extracellular region	7 out of 11 genes, 63.6%	*ADH1 TDH3 ALS3 TEF1 PGK1 ENO1 GPM1*
5886	Plasma membrane	6 out of 11 genes, 54.5%	*ADH1 TDH3 PGK1 HGT1 ENO1 FBA1*
5773	Vacuole	3 out of 11 genes, 27.3%	*CTA1 TEF1 ENO1*
5740	Mitochondrial envelope	1 out of 11 genes, 9.1%	*GPM1*
5777	Peroxisome	1 out of 11 genes, 9.1%	*TDH3*

stretches of disorder were also found, but the disorder is not high enough to classify the proteins under Intrinsically Disordered Proteins (IDP). Als3, on the contrary, has larger stretches of disorder, denoted as 'hot loops' by the tool (Figure 1).

MPs may have different cellular localizations or they may be found in exceeding amounts[16]. The GO cellular compartment provides information on this aspect (Table 4). It can be seen that all the proteins in the list are associated with more than one

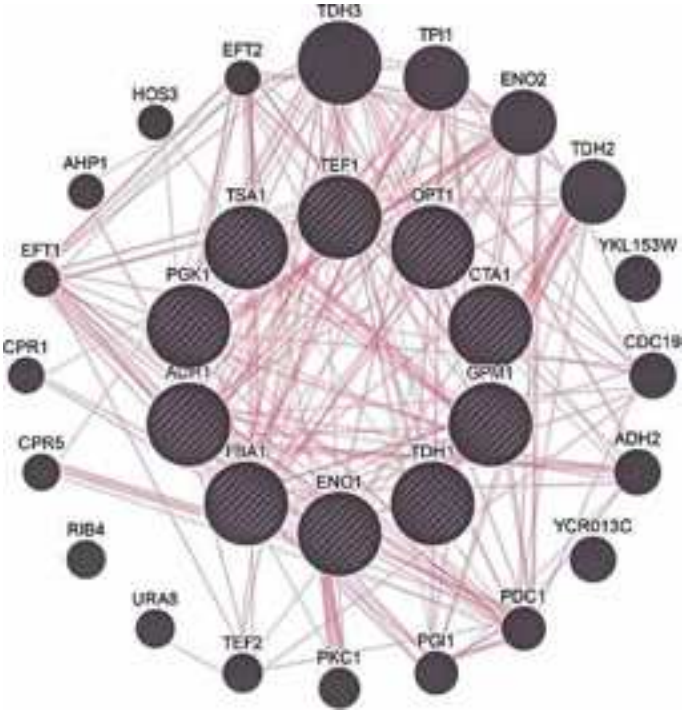

Fig.2. The protein-protein interactions of the homologs of MPs in *S.cerevisiae* (using GeneMANIA)

GO-cellular component term. Interestingly, the terms cytoplasm and cell wall appear in the same frequency, in accordance with the locations of the two functions listed.

Another approach to study moonlighting proteins is to analyse the neighborhoods using protein interaction databases [17]. Unfortunately, the protein-protein interactions in Candida sp. are poorly documented. So, the interactions of the proteins were checked in *S.cerevisiae*, the most similar, well-characterised model organism (Figure 2). In the extended interactome of the moonlighting proteins, Eno2 and Adh2 are reported to exhibit moonlighting functions in *S.cerevisiae* and *Entamoeba histolytica*, respectively. It is probable that more proteins in the neighbourhood may show moonlighting activities, as the biochemical pathways may be common to these proteins. This approach can be used to predict moonlighting proteins which can be confirmed experimentally.

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The impact of *Aspergillus* species on the quantitative and qualitative changes in the total proteins of Coriander *(Coriadrum sativum)* seeds

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Abstract

Role of seed borne fungi on the quantitative and qualitative changes in the total protein of Coriander seeds (*Coriadrum sativum L*) was analyzed. The seeds were infested with different species of *Aspergillus* namely *A. niger* Van Tieghem*, A.flavus* Link, *and A. terreus* Thom. The seeds were kept in different containers such as earthen pot, polythene bags and metallic bin. Qualitative variation in mycoflora was observed in relative to the moisture contents of seeds, humidity and temperature of the atmosphere. The results revealed that the fungal activity is higher at higher relative humidity and there severity is maximum in blotter method than the Agar plate method. There was a marked reduction in the percentage of total protein level in the seeds infested with *A.niger, A. flavus and A. terreus.*

Keywords: *Coriandrum sativum,* Aspergillus, Total Protein.

Introduction

Coriander (*Coriandrum sativum* L.) which belongs to the family Apiaceae (Umbelliferae) is mainly cultivated from its seeds throughout the year.[1] India is the biggest producer, consumer and exporter of coriander in the world with an annual production of around three lakh tonnes. It is an annual, herbaceous plant which originated from the Mediterranean and Middle Eastern regions and known as medicinal plants. It contains an essential oil (0.03 to 2.6%)[2]. All the parts are edible, used for cooking and use as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations [3].

Seeds has been recognized as one of the vital input in modern agricultural production. About 90% of all the food crops are grown today, are propagated by seeds. Increasing crop productivity through the use of high yielding cultivars and avoiding crop failures are the two ways of boosting food production. Benefits of using high yielding cultivars, however, may get nullified by dangerous seed borne disease as seed is just not a germplasm but a microhabitat as well.

Plant diseases have been found to affect the growth and productivity of crop plants. The causative agents of diseases in plants are the same to those causing diseases in humans and animals. These include pathogenic micro-organism, such as viruses, bacteria, fungi, protozoa and nematodes. More over seeds relatively more susceptible to fungi infestations, in stored conditions, in comparison to other microorganisms. Seed borne diseases have been affect the growth and productivity of crop plants[4]. The pathogenic organisms can utilize and exploit nutrients according to their utilization efficiencies, thereby, lowering germ inability and nutritional values of the seeds. Observations have been recorded for the presence of mycoflora on seed surface with qualitative and quantitative incidence. The objective of this study was to determine the presence of mycoflora on seed surface and its impact

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on the nutritional status(total protein) of seeds of Coriander on stored condition.

Material and Methods

Coriander seeds were collected from different distantly located fields of Durg – Bhilai region of Chhattisgarh and stored lots from farmers. The seed lots right from its harvest were stored in different types of containers as earthen pots, polythene bags and GI sheets containers etc. for 12 months. Monthly isolation of mycoflora was done for 12 months. Qualitative variation in mycoflora was observed in relative to the moisture contents of seeds, humidity and temperature of the atmosphere.In all three samples collected from seed lots of coriander was tested by Thom and Church[5] Czapek'sDox Agar media, and Anonymous[6] blotter technique, simultaneously for the presence fungal organisms. 1000 seeds of coriander– 500 seeds each for Czapek'sDox Agar media and blotter plated in five replicates of 100 each. After leaving them for 48 hours to ascertain sterility they were incubated at 28± 1°C for the growth of seed mycoflora. All the operations were done under aseptic conditions observations of the plates were started from fifth day for microscopic examination by stereoscopic binocular microscope. For the protein estimation, the 5 gram of seeds were treated with 0.01 % aqueous $HgCl_2$ solution for 1-2 minutes and washed with a several changes of sterilized distilled water. This surface sterilized seeds were inoculated with respective test organisms and incubated for seven days. On the other hand for protein estimation the test organisms were cultured in Richard's Broth media. Surface sterilized, healthy seeds were also kept as control. The total protein was estimated by the method of Lowry *et al* [7].

Results and Discussion

Fungal activities were observed at different temperature and moisture content of the seeds at regular intervals of two months for one complete year, Fungal infestation were maximum in the seeds kept in earthen pots than those of metallic bins and polythene bags, The blotter method showed maximum frequency of fungi than the Czapek'sDox Agar media(Table 1). The observation also confirm the superiority of 'Blotter method' over 'Agar plate method' for determining the seed born fungi [8].

Coriander seeds were infected mainly by *Aspergillus niger, Aspergillus flavus,*and *A. terreus.* As the moisture content of the seeds increased in the months of July August, the severity of *Aspergillus niger,* Aspergillus flavus, and A. terreus also increased. The maximum severity of test organisms was observed in month of August possibly due to higher level of moisture content. At high humidity level, spores of the pathogen germinated well within four hours in vitro as well as on the ragi leaves of both the varieties highly susceptible and moderately resistant[9]. The fungal activity is higher relative humidity's at lower relative humidity's (33 and 55 %) they are unable to act upon the seed substrate to bring about any remarkable change in its chemical properties [10].

The cultures of three test fungus in Richard's Broth media showed the following amount of protein content respectively: *A.niger* – 0.402 mg/gram mycelium, *A.flavus – 0.50 mg*/gram mycelium and *A.terreus* – 0.056 mg/gram mycelium. (Table-2). Coriander seeds infested with *A niger, A.flavus and A.terreus* showed the following amount of protein content respectively: 0.682mg, 0.582mg, 0.146mg. (Table.3 & Fig.1). The healthy Coriander seeds which are kept as control is estimated to be 2.51mg/gram seeds. The percentage loss in protein content of healthy seeds after they are infested with *A niger, A.flavus and A.terreus* respectively are 72.8%, 76.8% and 94.18%. When healthy coriander seeds infested with *A.terreus* the percentage loss in protein is found to be much greater when compare to the others two. According to the Cherry *et.al*[11], Protein content of seeds decreases due to the activity of the hydrolytic enzymes produced by fungus. It is also observed that the decreases in protein contents during early phase of incubation can be attributed to its hydrolysis to simpler components which ultimately results in an increases in the soluble nitrogen[12, 13]. Protein in Ground nut seeds infested with *A. parasiticus* were

Table.1. Periodic screening for the qualitative variation in mycoflora of seed surface to Environmental Changes

Coriander seeds				Czapekdox media						Blotter methods		
				Earthen Pot		Metallic bin		Polythin bag		Earthen Pot	Metallic bin	Polythin bag
Sl.No.	Fungal Isolates	Months	Room Temp.	% Moisture content of seeds	Incidence	% Moisture content of seeds	Incidence	% Moisture content of seeds	Incidence	Incidence	Incidence	Incidence
1	*A. niger*	April	26.5°C±3°C	29.1	++++	26.4	+++	25.0	++	+++++	++++	+++
		June	40°C±5°C	23.9	+++	21.2	++	18.7	+	++++	++++	+
		August	25.5°C±1°C	33.4	+++++	27.5	++++	25.6	+++	++++++	+++++	++++
		Oct.	29.8°C±3°C	27.2	++++	25.0	+++	24.3	++	+++++	++++	+++
		Dec.	28.1°C±2°C	30.0	++++	26.8	+++	25.2	++	+++++	++++	+++
		Feb	26°C±2°C	30.3	++++	27.0	+++	25.3	++	+++++	++++	+++
2	*A. flavus*	April	26.5°C±3°C	29.1	++++	26.4	+++	25.0	++	++++	+++	++
		June	40°C±5°C	23.9	+++	21.2	++	18.7	+	++	+	+
		August	25.5°C±1°C	33.4	+++++	27.5	++++	25.6	+++	+++++	++++	+++
		Oct.	29.8°C±3°C	27.2	++++	25.0	+++	24.3	++	++++	+++	++
		Dec.	28.1°C±2°C	30.0	++++	26.8	+++	25.2	++	++++	+++	++
		Feb	26°C±2°C	30.3	++++	27.0	+++	25.3	++	++++	+++	++
3	*A.*terreus	April	26.5°C±3°C	29.1	++++	26.4	+++	25.0	++	+++++	++++	+++
		June	40°C±5°C	23.9	+++	21.2	++	18.7	+	++++	++++	++
		August	25.5°C±1°C	33.4	+++++	27.5	++++	25.6	+++	++++++	+++++	++++
		Oct.	29.8°C±3°C	27.2	++++	25.0	+++	24.3	++	+++++	++++	+++
		Dec.	28.1°C±2°C	30.0	++++	26.8	+++	25.2	++	+++++	++++	+++
		Feb	26°C±2°C	30.3	++++	27.0	+++	25.3	++	+++++	++++	+++

Note: +++++/ ++++- Maximum, +++- Moderate, ++- Minimum

Table. 2: Total protein in healthy and infested seeds of coriander grown in Richard's Broth media

Host	Category of sample	Total protein in (mg/gm mycelium)
Coriander	*A. niger*	0.40 2 ±0.07
	A. flavus	0.50 ±0.086
	*A.*terreus	0.056 ±0.010

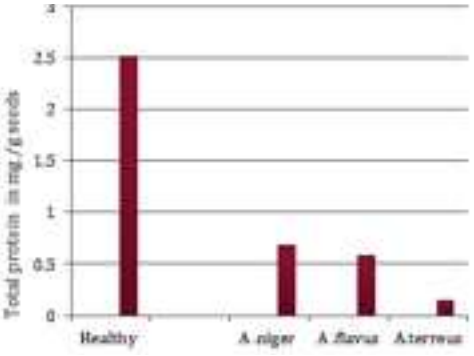

Fig.1. Representation of total protein in the isolates of coriander seeds

Table 3: Total protein in healthy and infested seeds of coriander with % loss of protein due to infestation

Host	Category of sample	Total proteinin (mg/gm seed)	% loss of protein due to infestation
Coriander	Healthy seeds	2.51 ±0.313	-
	Seed infested with		
	A. niger	0.682 ±0.073	72.8 %
	A. flavus	0.582 ±0.089	76.8 %
	*A.*terreus	0.146 ±0.073	94.18 %

hydrolyzed primarily to small polypeptides and finally to simple free amino acids. When stored seeds are infected with fungi they take control of physiological activities of the host and their by degrade the host completely.

Conclusion

In the present work results showed that storing of seeds in earthen pots provide more chances of fungal infestations than storing them in polythene bags and metallic bins. The reason may be the moisture content of the seeds, as high moisture content of the seeds provided more chance of infestation as were recorded in the month of July and August. Chances of moisture absorption is always more for the seeds stored in earthen pots because they provide comparatively better aeration, that help the fungus to thrive well. It was observed that the protein content of *A. terreus* in Richard's Broth media is very low compared to *A niger, A.flavus.* Similarly the protein content of coriander seeds infested with *A. terreus* showed maximum loss of protein when compared to other samples infested by *A niger, A. flavus.* Thus it is quite evident that *A. teereus is* most efficient an secreting proteolytic enzymes during pathogenesis but it stores a less quantity of protein (enzymes) in it mycelia. Overall results indicate that various spp. of Aspergillus are quite harmful for the stored seeds in terms of lowering protein of the coriander seeds.

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Evaluation of relative toxicity (LT_{50} and LC_{50}) of some organophosphate insecticides against the hairy caterpillar, *Pericallia ricini* Fab. (Lepidoptera: Arctiidae)

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Abstract

The effectiveness and relative toxicity of four different insecticides *viz,* triazophos, dimethoate, profenofos and chlorpyrifos were evaluated against the fourth instar larvae of the hairy caterpillar*, Pericallia ricini* Fab. in laboratory conditions by Probit analysis. *P. ricini* is one of the major pests of several crops. Based on LC_{50} values, the toxicity of triazophos was found to be the highest, followed by chlorpyrifos, profenofos and dimethoate. Mean LT_{50} value was found to be least for triazophos followed by profenofos, chlorpyrifos and dimethoate. In this study, lowest LC_{50} and LT_{50} values were recorded for triazophos indicating that it is the most toxic organophosphate insecticide against the *P. ricini*. The LT_{50} and LC_{50} of triazophos were recorded as 7.08 hr and 0.008% respectively; profenofos 8.65 hr and 0.029 % respectively and chlorpyrifos 8.85 hr and 0.027 % respectively. Dimethoate recorded 10.63 hr and 0.099 % respectively indicating that it is least toxic to *P.ricini*. The toxicity of insecticides tested in their decreasing order of magnitude is triazophos>chlorpyrifos>profenofos >dimethoate. At the current market price, the insecticides could be ranked as chlorpyrifos (Hilban, Rs. 203/l) < dimethoate (Rogor, Rs. 342/l) < profenofos (Cypro, Rs. 380/l) < triazophos (Taran, Rs. 432/l). Although the cost of triazophos is higher, the lethal time and dose required is low, and hence could be more cost effective than other organophosphate insecticides tested.

Key words: *Pericallia ricini,* Indian bean, LT_{50}, LC_{50}, relative toxicity, Kerala, India

Introduction

The substantial increase in crop losses due to insect damage despite increased insecticide use can be accounted for by some of the major changes that have taken place in agriculture since the1940s. Through the centuries, many cultural and physical control practices were developed by man for the protection of the crop even before the biology and life cycle of various insect pests were understood. Crop production suffers greatly due to the attack of various pests. The agricultural revolution of 20th century has further disrupted and complicated the stability of pest species in our agro-ecosystems. Indiscriminate use of various insecticides leads to ecological imbalance, biomagnification and develop resistant strains of pest population. In Kerala, very few studies were conducted to find out the relative toxicity of different commonly used insecticides against major pests. The relative toxicity of different insecticides on the Rice bug, *Leptocorisa acuta* were studied[1] while LT_{50} and LC_{50} of organophosphate insecticides against the same pest revealed that dimethoate was the most effective insecticide[2].

Pericallia ricini Fab. is one of the major pests of castor, gingelly, cotton, Indian bean, brinjal, drumstick, chilly, cotton, pumpkin, tea, oleander, sweat potato[3] and vanilla[4]. Caterpillars are active feeders and damage the plant by eating the photosynthetic machinery, leading to the wilting of plant. Hence effective measures are required to control this pest and several insecticides are in use[5]. In Kerala, *P. ricini* is a major pest

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of vegetables and is causing considerable damage, but still, very few studies were conducted to evaluate the extent of the damage caused and the possible control measures. The relative efficacy of the commonly used organophosphate insecticides against *P. ricini* is lacking in Kerala and hence a laboratory evaluation was done. A comparative evaluation of the cost of the insecticides was also attempted to find out the most cost effective insecticide.

Materials and Methods

Egg masses and newly hatched caterpillars were collected from various unsprayed fields of *Dolichos lablab* (Indian bean). Emerged caterpillars were allowed to grow in clean glass bottle covered with cotton cloth. They were fed with fresh leaves of Indian bean and were transferred to fresh bottle daily. When the larvae reach the fourth instar stage (2.76 ± 0.13cm and weight 410 ± 12.83mg) they were used as test organism.

Four organophosphate insecticides were compared, namely triazophos, profenofos, dimethoate and chlorpyrifos. Graded concentrations (0.01%, 0.025%, 0.05%, 0.075%, 0.1%) of the emulsifiable concentrate of the insecticides were prepared and fresh leaves of Indian bean collected from unsprayed fields were treated with each dilution using 5ml per leaf and placed in a glass tube containing water which was further kept in a cylindrical glass jar and closed with muslin cloth. After 20 min, 20 fourth instar caterpillars were released into the jar and mortality was recorded every hour up to 48 hours after treatment. Three replications were set up for each treatment and control. The data were subjected to Probit analysis[6,7] to obtain LC_{50} and LT_{50} values.

Results and Discussion

Based on LC_{50} values, the toxicity of triazophos was found to be the highest, followed by chlorpyrifos, profenofos and dimethoate (Table1). Mean LT_{50} value also was found to be least for triazophos followed by profenofos, chlorpyrifos and dimethoate (Table 2). The results of insecticidal properties of four organophosphate insecticides at different concentration had shown that different concentration of triazophos was more effective in reducing the population of *P. ricini.* The LT_{50} and LC_{50} of triazophos were recorded as 7.08 hr and 0.008% respectively. The LT_{50} and LC_{50} value of profenofos were recorded as 8.65 hr and 0.029 % respectively and that of chlorpyrifos was 8.85 hr and 0.027 % respectively. LT_{50} and LC_{50} values of dimethoate were 10.63 hr and 0.099 % respectively. The relative toxicity of various insecticides against the larvae of *P. ricini* was reported[8] on the basis of LC 50 of compounds with monocrotophos as unity, determined that Fenthion was 20-93 times toxic (highest toxicity reported) and Chlorpyrifos has 7-30 times toxic. The ovicidal effect of Neem derivatives on the eggs of *P. ricini* was also reported[9]. In a previous study, it was reported that dimethoate was the most effective organophosphate insecticide against the rice bug, *Leptocorisa acuta*[2]. This indicates that efficacy of different organophosphate insecticides against

Table 1. Relative toxicity of insecticides commonly used against *Pericallia ricini* Fab.

Insecticide	Heterogeneity	Regression equation	LC_{50}	Fiducial limits	Relative toxicity	Order of relative efficacy
Triazophos	χ^2 (13) 0.978	Y = -0.512+ 66.523 χ	0.008	-0.008 - 0.013	12.37	1
Chlorpyrifos	χ^2 (13) 5.105	Y = -0.595+ 22.267 χ	0.027	0.012 - 0.037	3.66	2
Profenofos	χ^2 (13) 6.008	Y = -0.551 + 19.084 χ	0.029	0.012 – 0.041	3.41	3
Dimethoate	χ^2 (13) 1.907	Y = -1.290 + 13.008 χ	0.099	0.079- 0.151	1	4

Table 2. LT_{50} of insecticides commonly used against *Pericallia ricini* Fab.

Insecticide	Heterogeneity	Regression equation	LT_{50}	Fiducial limits	Order of relative efficacy
Triazophos	χ^2 (13) 9.915	Y =1.827+ -0.258 χ	7.088	6.195- 8.467	1
Chlorpyrifos	χ^2 (13) 5.011	Y = 2.808+ -0.317 χ	8.85	8.122 - 9.902	3
Profenofos	χ^2 (13) 11.792	Y = 1.823+ -O.211 χ	8.646	7.554– 10.402	2
Dimethoate	χ^2 (13) 7.171	Y = 2.020+ -0.190 χ	10.632	9.419- 12.435	4

different pest species are varied. This may due to the changes in the genetic constitution and the physiological aspect of different pest species.

Present study recorded dimethoate with highest LC_{50} value (0.099 %) indicating that it is less toxic than others. Lowest LC_{50} and LT_{50} values were recorded for triazophos indicating that it is more toxic than all the other three organophosphate insecticides tested. The toxicity of insecticides against *P. ricini* in their decreasing order of magnitude is triazophos> chlorpyrifos> profenofos>dimethoate. At the current market price, the insecticides could be ranked as chlorpyrifos (Hilban, Rs. 203/l) < dimethoate (Rogor, Rs. 342/l) < profenofos (Cypro, Rs. 380/l) < triazophos (Taran, Rs. 432/l). Although the cost of triazophos is higher, the lethal time and dose required is low, and hence it is more cost effective than other organophosphate insecticides tested for crop protection.

The results from the study proved that in almost all insecticides Normally Recommended Concentration (NRC) is much higher than their actual required concentration and that might lead to produce many serious health hazards. Recent reports showed that pesticides cause about 10,000 deaths world wide every year. The high dose application of pesticide has serious impact on ecosystem. They affect the whole biota of the ecosystem and upset the equilibrium between insect pests and their natural enemies, increased disease susceptibility, bioamplification, disturbance in reproductive physiology of birds, humans and contamination of food. Sub lethal concentration (Concentration below their LC_{50} value) spraying is also dangerous; it will make genetic changes in the genome of the insect pest, as they become resistant and develops insecticide tolerance. Thus the insecticides must be applied in correct concentration in order to control the target pest insect. The effective use of insecticides in correct concentration is the need of the day, for the continued existence of life in the biosphere.

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Beetle (Insecta: Coleoptera) diversity in an agroecosystem: A study in the Thrissur District, Kerala, India

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Abstract

The impact of agricultural practices on beetle diversity was studied in Kariyannur village, Thrissur District, Kerala. Altogether 175 beetles were recorded which belonged to 20 species and 16 families. The overall Shannon's diversity index for the study area was 2.80 and Fisher's alpha diversity index was 5.82. Changes in the insect diversity in the study area between different habitats were evident and this might be due to changes in the floral composition and floral structure. The dominant families recorded were Coccinellidae, Chrysomelidae, Carabidae and Scarabaeidae. The species abundance model was close to log normal which indicated that most beetles recorded are of moderate abundance. Rarefaction curve showed that almost all the species present in the study area were recorded. The study indicated that anthropogenic activities have adversely affected the beetles in the study area. This might be due to the loss of habitats for their survival.

Keywords: beetles, insect diversity, agro ecosystem, Western Ghats, Kerala, India

Introduction

Beetles are by far the largest order in the Animal kingdom, with about 350,000–400,000 species[1]. They make up about 40% of all insect species described, and about 30% of all animals. More than 358,000 species of beetles have been described and are considered valid of which about 62% are in six extremely diverse families; *Curculionidae, Staphylinidae, Chrysomelidae, Carabidae, Scarabaeidae,* and *Cerambycidae*. Coleoptera are found in nearly all natural habitats, including freshwater and marine habitats, trees, bark, flowers, leaves, roots and even inside plants as in galls, and in dead or decaying organic matter. Their role as herbivores, predators and pollinators is well recognized. Destruction of these insects may in the long run affect the habitat structure. They are also economically important as many of them are pests of a variety of crops. Information on their host range, habitat preferences and distribution is very important in forest and agriculture systems. They form important links in the food webs and have also been looked upon as important tools for monitoring changes taking place in the terrestrial habitats[2].

Kerala, with its variety of ecosystems ranging from the high mountains supporting thick tropical evergreen forests, coastal plains, riverine and mangrove vegetation is known for its rich diversity. The publications of earlier workers like Sir George Hampson (Lepidoptera), Guy Marshall (Coleoptera), Maulik (Coleoptera), De niceville (Butterflies) and Bingham (Hymenoptera) contained references to species found in Kerala. More recent works on insect diversity in the Kerala part of Western Ghats include a study of insect fauna in the Malayattoor forests[3], a study on the butterflies and moths of Silent Valley[4,5], a study on the hymenopteran fauna of Silent Valley[6] and a detailed study on the insect diversity of the Silent Valley National Park in which about 800 species were recorded[7]. Recently several studies are carried out on the diversity of different insect groups in different areas of Thrissur District[8,9,10]. In the present study,

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the beetle diversity of an agro ecosystem in the Kariyannur village, Thrissur District, Kerala, has been carried out.

Materials and Methods

Study area

The present study was carried out in the Kariyannur village, Thrissur District from April 2013 to June 2013. To study the effect of agricultural practices on beetles, three different habitat types were selected.

Site 1: It represents an area exclusively with flowering and leafy plants. The flora included trees such as *Cassia fistula, Artocarpus hirsutus* and *Murraya koenigii* which dominate this habitat. The understorey is a combination of shrubs like Sida sp., Hibiscus sp., *Helicteres isora, Ixora* sp. and herbs like *Smithia geminiflora, Centella asiatica* and *Globba marantiana.*

Site 2: This site has limited flowering plants but lot of herbs and shrubs. The main trees were *Mangifera indica, Cocos nucifera, Michelia champaca, Artocarpus sp., etc.* The main shrubs were *Lantana camara, Chromolaena odoratum* etc. and the herbs included *Tridax procumbens, Centella asiatica,* etc.

Site 3: The site was characterized by tall trees, mainly Coconut trees and Banana plants which are well watered and frequently fertilized. Herbs and shrubs are very few.

Materials and Methods

The insects were collected using forceps, by handpicking and by hand nets. The insects were then transferred to the killing jars containing chloroform. After numbering, the individual specimens were preserved as dry specimens. They were properly pinned and were later stored in entomological boxes. Identification up to family level with common name was done with the help of standard reference books.

Insect diversity studies

Representative plots in three study sites were established for systematic collection of the insects. At each location, two plots of 25m x 25m were laid out. The plots were separated by a distance of 25m. The insects collected were sorted out to species and the number of individuals for each species was recorded on data sheets for estimating the diversity. As spot identification could not be made in all cases, code numbers were assigned to the various species, which were later labeled after establishing their identity. Data on insects from each habitat were computed and from these indices of insect diversity, dominance, evenness, species richness, etc., were computed using PAST. The values for different habitats were pooled for deriving the overall values for the study area.

Diversity indices

The quantification of diversity must address two statistical properties common to any mixture of different objects. The first property is the number of different classes or types of objects *i.e.,* species, genera, families, different habitats and so on. The second property is the distribution of objects among classes such as the relative abundance of individuals of different taxa or the relative area of the habitat that falls into different habitat types[11]. In this study, only species diversity was studied.

1. Shannon-Weiner diversity index

(H/): $H/=-\Sigma P_i \log_e (P_i)$

where 'H/' is the Shannon's index of species diversity and P_i is the proportion of individuals in the 'i' th species

2. Fisher's alpha: a diversity index, defined implicitly by the formula $S = \alpha(1 + n/\alpha)$ where S is number of species, n is number of individuals and α is the Fisher's alpha.

The standard deviation of α was estimated as $X = N/(N + \alpha)$

Where $SD(\alpha) = \alpha/-\log(1 - X)$

Dominance index

Patterns of relative abundance of species determine the dominance component

of diversity. In this study, the relative dominance of each insect order in a locality was determined by calculating the dominance index using the following formula:

Dominance = 1-Simpson index. Ranges from 0 (all species are equally present) to 1 (one species dominates the community completely).

$$D = \sum \left(\frac{n_i}{n} \right)^2$$

where n_i is the number of individuals for the ith species.

3. Simpson index = 1-dominance. This index measures 'evenness' of the community from 0 to 1.

Shannon's evenness or equitability index

This index which measures the evenness of species abundance is complimentary to the diversity index concept and it indicates how the individuals of various species are distributed in the community.

The Shannon's evenness index of the community (E) was calculated using the formula

$E = H/\log_e(s)$

where 's' is the number of species recorded and ' H' is the Shannon-Weiner index of diversity.

Species richness

In the ecological literature the number of species at a site, in a region or in a collection is called species richness, which is the simplest and most useful measure of species diversity. In this study, the total number of insect species collected in each month from each locality was considered as species richness.

Results and Discussion

Coleopteran richness

20 species of beetles belonging to 16 families were collected from the study area. Of the species collected 19 species were from Site 2, 14 species from Site 1 and only 8 species from Site 3 (Table 1). A total of 175 individuals were also recorded during the study. The most abundant species was Coccinellidae (32 individuals) followed by Chrysomelidae (27 individuals) and Carabidae (22). Species richness was higher for Scarabaeidae (Fig.1).

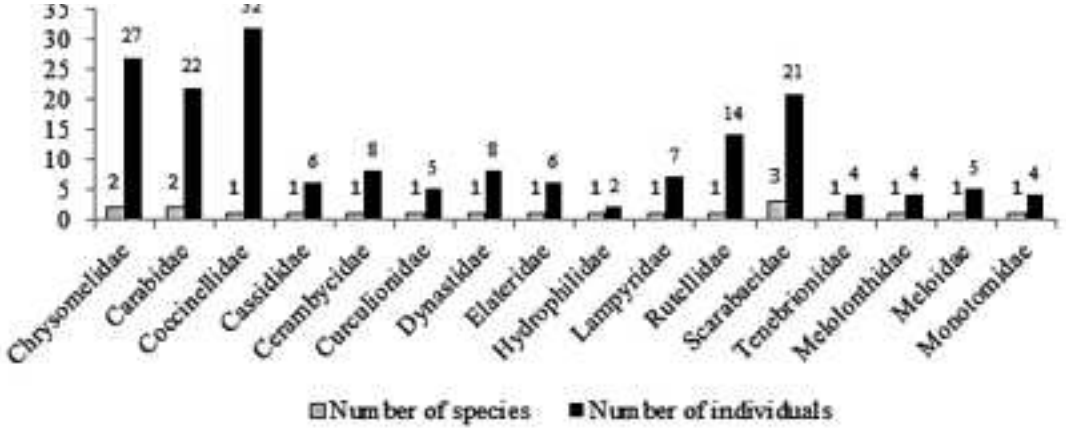

Fig.1. Family wise distribution of beetles in Kariyannur, Thrissur District

The total number of species recorded was more in site 2 (19 species) than in site 1 (14 species) and site 3 (9 species). The difference in the beetle species richness between different sites might be due to the effect of vegetation. The structure of Coleopteran community in the study area is provided in Table 1.

Species abundance

A total of 68 beetles were recorded from Site 2 as against 60 from site 1 and 47 from site 3. In site 2 a total of 19 families were recorded of which Coccinellidae contained the maximum number of individuals (15) followed by Chrysomelidae (11) and Carabidae (11) (Table 1).

Table 1. Structure of Coleopteran community in Kariyannur, Thrissur District

Family	Species No.	Beetle Population		
		Plot 1	Plot 2	Plot 3
Chrysomelidae	1	8	7	0
Chrysomelidae	2	8	4	0
Carabidae	3	7	5	0
Carabidae	4	4	6	0
Coccinellidae	5	9	15	8
Cassididae	6	3	3	0
Cerambycidae	7	3	2	3
Curculionidae	8	1	3	1
Dynastidae	9	0	0	8
Elateridae	10	3	3	0
Hydrophilidae	11	1	1	0
Lampyridae	12	6	1	0
Rutellidae	13	0	5	9
Scarabaeidae	14	0	1	7
Scarabaeidae	15	0	1	6
Scarabaeidae	16	0	1	5
Tenebrionidae	17	1	3	0
Melolonthidae	18	3	1	0
Meloidae	19	3	2	0
Monotomidae	20	0	4	0
Total		60	68	47

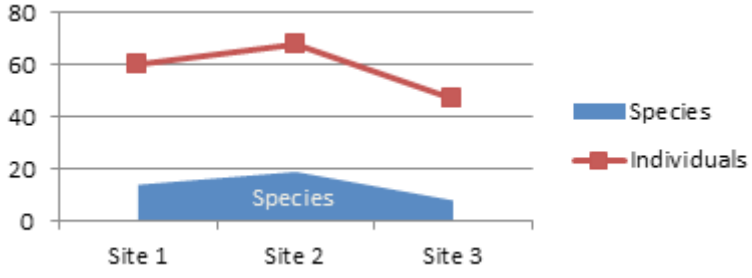

Fig.2. Species abundance of beetles at Kariyannur, Thrissur District

Effect of vegetation on beetle diversity

Species diversity index

Shannon's index of diversity was calculated for various sites. The diversity of beetles in site 2 (2.63) was more than that in site 1 (2.44). The least diversity was obtained at site 3 (1.96). Fisher's alpha index showed that beetle diversity was more in site 2 (8.75) than that in site 1 (5.74) and site 3 (2.77). Similar trend was also recorded for other diversity indices (Table 2). Changes in the beetle diversity between different habitats might be due to changes in the floral composition, floral structure, and micro climatic conditions.

Table 2. Diversity of Coleoptera at different sites in Kariyannur, Thrissur District

Diversity	Site 1	Site 2	Site 3	Total
No. Species	14	19	8	20
Individuals	60	68	47	175
Dominance_D	0.099	0.095	0.148	0.076
Shannon_H	2.437	2.629	1.962	2.795
Fisher_alpha	5.743	8.749	2.769	5.821
Simpson_1-D	0.901	0.904	0.851	0.924
Margalef's	3.175	4.266	1.818	0.8184
Evenness	0.82	0.73	0.89	3.679

Dominance index

The dominance indices for Coleoptera are given in Table 2. The dominance (D) of a single species was more in Site 3. The Simpsons index revealed that site 1 and 2 have a more diverse community.

Rarefaction

The data collected was analysed and rarefaction curves were plotted (Fig.3). The graph line has reached an asymptote with x axis which indicates that almost all the species in this area has been sampled. More samplings in the area are less likely to unearth more number of species.

Evenness or equitability index

Based on the data collected, the Shannon's evenness indices for site 1, site 2 and site 3 were 0.82, 0.73 and 0.89 respectively.

Species abundance model

Species abundance model is the most complete depiction of diversity data. Species abundance model was fitted for the data collected on beetles. The model obtained was close to log normal model. There was no significant difference between the hypothetical model (polygon curve) and the original data (bars). It indicates that species with intermediate abundances were more abundant than the very dominant and very rare species.

The coleopteran richness as well as diversity was more in areas with high occurrence of herbs and shrubs as well as flowering plants. This might be due to the phytophagous nature of the beetles. The overall Shannon diversity index for the study area was 2.80 which were more or less similar to previous studies conducted in Thrissur District[8]. Beetles were prone to erosion in diversity due to habitat modification and degradation[2].

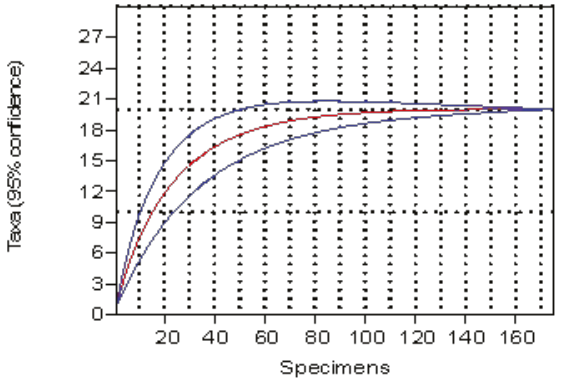

Fig.3. Rarefaction curve showing the observed (blue) and expected (red) beetle abundance in the study area

A total of 175 individuals were recorded during the study which belonged to 20 species and 16 families of which Coccinellidae, Chrysomelidae and Carabidae are more abundant. Coccinellids are mostly predators while chrysomelids and carabids are mostly leaf eaters. Presence of high number of herbivorous insects might have resulted in the appearance of large number of predators in the study area. Earlier studies have reported that the species composition of insects has indicative value in monitoring changes taking place in the environment[7,8,12,13]. Although insect diversity indices are available for very few localities in Kerala, it is interesting to find that the overall beetle diversity of Kariyannur (2.80) was similar to beetle diversity reported in similar conditions[2]. But it is significantly lesser than insect diversity reported for protected

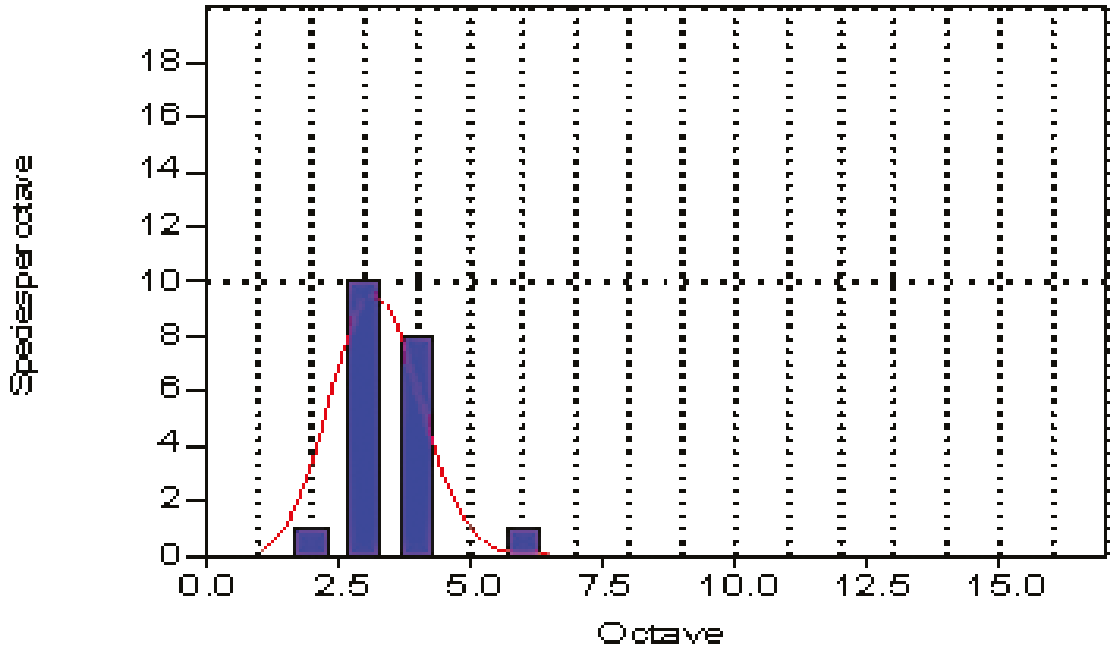

Fig. 4. Species abundance model for the data collected on beetles

areas like Parambikulam (4.76), Sholayar (4.74), Silent Valley (4.83) and Nelliampathy (5.13) indicating that anthropogenic activities have adversely affected the insect populations. Therefore conserving the remaining habitats is very crucial for the survival of the insects in general and human race at large.

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Checklist of butterflies of MGM college campus, Udupi District, Karnataka

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Abstract

Butterflies, the flying jewels of nature, are one of the matchless, colourful and conspicuous of insects. Among insects, lepidopteron (Butterflies and Moths) are the only ones having scaly wings which provide them their amazing colours and patterns. Butterfly communities form a valuable part of the ecosystem because most butterfly larvae have strong associations with host plants, and adults require a specific range of nectar plants. A preliminary study was carried out from June to Decembers in 2015 at MGM College campus, Udupi district, Karnataka state, to find the diversity of butterfly species and a total of 28 species belonging to 23 genera and 5 families were identified and documented.

Key words: Butterflies, Lepidoptera, scaly wings.

Introduction

Arthropods are invertebrates, constituting nearly a million of species, conquering most of land, sea and air in a cosmopolitan range. The body is bilaterally symmetrical each segment equipped with jointed appendages; chitin constituting exoskeleton; simple or compound eyes. Phylum Arthropoda is classified into five classes among which class insecta includes all insects. The unique feature of class insecta is the presence of three pairs of legs, which gives them the name Hexapoda; two pairs of wings; and body division into head, thorax and abdomen. Butterflies, the flying jewels of nature, are one of the matchless, colourful and conspicuous of insects. Among insects, lepidopteron (Butterflies and Moths) are the only ones having scaly wings which provide them their amazing colours and patterns. This intricate pattern and bright colours of the wings of butterflies are made up of a mass of tiny scales which overlap each other almost like the roof tiles of a house[1]. Butterfly communities form a valuable part of the ecosystem because most butterfly larvae have strong associations with host plants, and adults require a specific range of nectar plants. Usually a female shows host specificity while laying its eggs on the plants i.e., specificity towards appropriate plant on which caterpillars feed, after hatching out of the eggs. Based on the flora of an area, one can easily predict the existing butterfly fauna of that area[2]. They are valuable pollinators when they move from plant to plant gathering nectar[3]. As butterflies are highly sensitive to any environmental changes and are delicate creatures, they act as good bio-indicators of the health of a habitat[4]. Thus increased butterfly populations may indicate an increase in plant diversity. Butterflies being the small insect in the food web become an important element of the food chain. Apart of being an important component in food web, butterflies still manage to survive because of its survival strategies such as camouflage, unpalatable nature by storing toxic substances in body or wings, mimicry with other unpalatable species and larval association with ants[5]. Butterflies are seasonal in their occurrence. They are common for only a few months and rare or absent in others. The seasons when they are rare or not active as adults are usually spent either as caterpillars or as pupae. The months when the adults are active are called as the "flight period"[4]. There are about 18,000 species of butterflies in the world.

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India alone has 1501 species, of which 321 are Skippers, 107 Swallowtails, 109 Whites and Yellows, 521 Brush footed butterflies and 443 Blues[1]. Butterflies are classified under 2 super families, Hesperioidea (Skippers- link between true butterflies and moths) and Papilionoidea (all other butterflies). Earlier butterflies were classified into smaller families and India alone had 9 families. However, many of the old families are merged into family Nymphalidae, finally maintaining only 5 families- Hesperiidae, Papilionidea, Pieridae, Lycaenidae and Nymphalidae across the world, all of which are represented in India[5].

Area of Study

Udupi district topographically lays 13°20.0802 N latitude and 74°44.7702 E Longitude, bound by Arabian Sea in west and Western Ghats (world heritage site) in the east making it a beautiful part of coastal belt of South Kanara. Land nearer to sea is plain with small hills and paddy fields, coconut gardens etc. Land bordering the Western Ghats in the east is covered with forests and hilly terrain. The study area Mahatma Gandhi Memorial College (MGMC) lies 3 kilometers away from Udupi municipal town in Udupi district, South Kanara, Karnataka state. MGMC is one of the premier college in Udupi located in the sylvan terrain of a small town Sagri, close to Manipal-International centre for education, covering a total area of 47acres of land accommodating two botanical gardens and one herbal garden with number of flowering plants and rare species of plants and trees. The vegetation is of urban type.

Materials and Methods

The study was conducted in a period of approximately seven months from the beginning of June 2015 to December 2015. Butterflies were observed during active morning hours (8am to 10am) afternoons (2pm to 3pm) and evenings (after 4 o'clock) in alternate days. However some butterflies spotted were identified and recorded, out of observation hours in the college campus. Butterflies were observed in the campus; photographs were taken with Sony cyber shot- point and shoot camera and identified using the available literature. No specimen was collected for this study. Morphological features were taken into consideration and species were identified. To this account 3 species of Hesperiidae, 2 species of Papilionidae, 2 species of Pieridae, 14 species of Nymphalidae and 7 species of Lycaenidae were observed and recorded (Table 1).

Result and Discussion

A total of 28 species belonging to 23 genera and 5 families recorded during the study period is listed in table 1 and depicted in figure 1. Among the 5 families, Nymphalidae dominated with 14 species(50% of the population), followed by Lycaenidae with 7 species (25%). In remaining 3 families Hesperiidae records 3 species (11%), Papilionidae and Pieridae records 2 (7%) species each. Based on the status of distribution, butterflies were classified as common, very common, less common and rare. Accordingly 9 species are classified as common, 5 species as very common, 7 species as less common, and 7 species as rare in occurrence. The dominance of Nymphalidae from Western Ghats has been reported by earlier workers [2,3,4,6,8,9,11] this species richness may be due to the rich distribution of food plants of the Nymphalidae members, favorable climatic conditions which serves as an best habitat for butterflies.

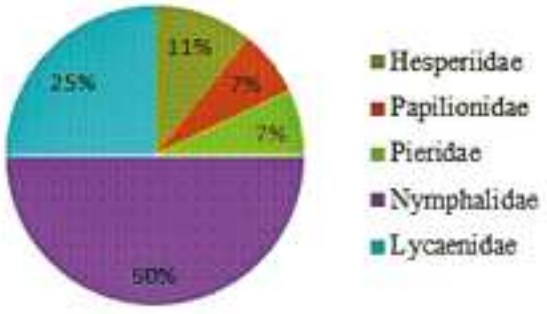

Fig.1. Family wise composition of Butterfly species at MGM college campus, Udupi

Table 1. Checklist of butterflies of MGM College campus, Udupi

Sl. No	Common name	Scientific name	Family	Status
1	Chestnut Bob	*Iambrix salsala* M	Hesperiidae	Rare
2	Suffused Snow Flat	*Tagiades gana* M		Rare
3	Pigmy Scrub Hopper	*Aeromachus pygmaeu* F		Rare
4	Common Mormon(male)	*Papilio polytes* L	Papilionidae	Common
5	Lime Butterfly	*Papilio demoleus* L		Rare
6	Mottled Emigrant	*Catopsilia pyranthe* L	Pieridae	Common
7	Common Grass Yellow	*Eurema hecabe* L		Very common
8	Grey Pansy	*Junonia atlites* L	Nymphalidae	Common
9	Lemon Pansy	*Junonia lemonias* L		Common
10	Chocolate Pansy	*Junonia iphita* C		Very common
11	Grey Count	*Tanaecia lepidea* Butler		Less common
12	Common Sailer	*Neptis hylas* Moore		Rare
13	Common Bush Brown	*Mycalesis perseus* F		Common
14	Common Evening Brown	*Melanitis leda* L		Very common
15	Great Evening Brown	*Melanitis zitenius* Herbst		Less common
16	Nigger	*Orsotrioena medus* F		Less common
17	Tawny Coster	*Acraea violae* F		Common
18	Common Five- ring	*Ypthima baldus* F		Less common
19	Common Four- ring	*Ypthima huebneri* Kirby		Less common
20	Common Castor	*Ariadne merione* C		Rare
21	Common Hedge Blue	*Acytolepis puspa* H		Less common
22	Common Lascar	*Pantoporia hordonia* Stoll	Lycaenidae	Less common
23	Common Pierrot	*Castalius rosimon* F		Very common
24	Red Pierrot	*Talicada nyseus* Guerin Meneville		Common
25	Banded Blue Pierrot	*Discolampa ethion* Hewitson		Rare
26	Tiny Grass Blue	*Zizula hylax* F		Common
27	Dark Grass Blue	*Zizeeria karsandra* M		Common
28	Plains Cupid	*Chilades pandava* H		Very common

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Seed Germination Studies in *Rauvolfia hookeri* Srinivas. & Chithra,

A rare and Endemic Plant of Southern Western Ghats

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Abstract

Rauvolfia hookeri is a shrub found in the evergreen forests of southern Western Ghats. Conventional propagation in this species is problematic due to various reasons including reproductive failure. The mode of propagation in the wild habitat is mainly through root suckers. The objective of the present study was to check the germination capacity of seeds and develop methods to improve the rate of germination through thermal and chemical treatments. Poor seed set and delayed germination is witnessed in *R. hookeri.* Seeds without any pre-treatment when transferred to soil will take nearly 45 days to germinate and only 10% of seedlings are emerged from them. Seed viability test carried out in freshly harvested seeds revealed high viability which goes on decreasing with storage. Hot water and acid treatments did not give any considerable increase in germination percentage. Diffuse light along with GA3 treatment enhanced percentage of seed germination. GA3 treatment significantly increased the germination percentage and the mean time for germination (MTG) under *ex vitro* conditions. A significant increase in the rate of germination (98.3%) and MTG (17days) was achieved by 100ppm GA3 treatment. From the results it can be evident that seed dormancy existing in *R. hookeri* is due to multiple factors excluding physical parameters.

Key words: Endemic, seed dormancy, seed viability, GA3, mean time for germination

Introduction

The genus *Rauvolfia* comprises about 60 species with a pantropical distribution[1,2]. *Rauvolfia hookeri* Srinivas & Chithra is a shrub, endemic to southern Western Ghats and found rare in evergreen forests of Thiruvananthapuram and Kollam districts of Kerala[3]. It undergoes flowering and fruiting throughout the year with a peak flowering period during September to November. Inflorescence is a dichotomously branched axillary cyme. Flowers small with pinkish white corolla tube and shows expansion below the throat where the stamens are attached[4]. This plant exhibits poor seed-set, low germination percentage and delayed rooting of seedlings and vegetative cuttings which made its propagation difficult.

The reproductive success of a plant to a certain extent can be evaluated by germination percentage of its seeds. Germination is a complex process which involves the finely co-ordinated changes in the embryo and the surrounding tissues along with the participation of several genes. Dormancy is a condition in which the seeds do not germinate even when the environmental conditions are suitable for germination[5]. Seeds of a number of medicinal plants express different types of dormancy that makes its survival a little bit difficult under natural conditions. Major categories of dormancy include – exogenous, endogenous, double and secondary dormancies. It can be overcome by mechanical, thermal and chemical treatments.

Most of the *Rauvolfia* species are characterized by poor seed viability and low germination percentage[6,7&8] and this could be the

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possible reason for their restricted distribution in the wild habitat. Seed germination studies conducted in *Rauvolfia* species are scarce. The present study deals with the seed germination under *ex vitro* conditions, assessment of seed viability and effect of thermal and chemical treatments on germination of *R. hookeri*. Propagation through seeds was not found in wild populations of the plant under the present study. Hence, it is advisable to assess the germinating ability of the seeds and introduce methods to improve the germination percentage for further propagation.

Materials and Methods

Mature fruits were collected from wild populations of R. hookeri (Fig.1.a) distributed in the Braimore forest region of southern Western Ghats. Fruits were depulped and thoroughly washed under running tap water and excess water content was removed by blotting paper (Fig. 1.b).

Seed viability test

Two sets of experiments to assess seed viability were carried out, one with freshly harvested seeds and another with seeds after 7 days of storage. Seed coat was partially removed with the help of scalpel and the seeds were immersed in 10 ml of 0.1% 2, 3, 5 – triphenyl tetrazolium chloride solution taken in a culture tube. A total of 25 seeds were taken and put into five tubes, each containing five seeds. Observations were made after 3 h of incubation.

Hot water and acid treatments

Dried seeds (10 seeds) were put into boiling water taken in a 100 ml beaker, immediately removed from the heat source and rinsed in distilled water. The seeds were taken into laminar air flow hood (Medi tech, Mumbai) and treated with 0.1% mercuric chloride for 10 min. After decanting the sterilant, the seeds were thoroughly washed with sterile distilled water. To remove the excess water, they were transferred to Petri dish containing sterile Whatman No.1 filter paper. Finally, the seeds were inoculated onto Murashige and Skoog (MS) basal medium with the help of forceps and the cultures were incubated at 25±2oC under 8 h photoperiod. Seeds without treatment served as the control.

For acid treatment, ten dried seeds were taken in 50 ml beaker containing 20 ml of conc. H2SO4. The beaker containing the seeds was gently stirred and incubated for 5 min. After that the seeds were taken out, washed several times and kept overnight in a beaker containing sterile distilled water. Next day, they were taken out and rinsed 3 - 4 times with distilled water. Surface sterilization was done with 0.1% HgCl2 for 5 min followed by a thorough washing to remove the traces of sterilant, inoculated onto MS basal medium and incubated under standard conditions.

GA_3 treatment

To study the effect of Gibberellic acid (GA3) on seed germination, dried seeds were soaked in varied concentrations of GA3 (25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm) and incubated in room temperature for 24 h. GA3 treated seeds were subjected to germination trials against water soaked, untreated seeds. After 24 h of incubation, the treated seeds were taken out and washed with distilled water. Finally, they were transferred to clay pots (15 cm) filled with sand and garden soil in 1:1 ratio to know ex vitro germination percentage. To study germination under in vitro conditions, seeds after GA3 treatment were surface sterilized with 0.1% HgCl2 according to the method described above and cultured in MS basal medium.

Statistical analysis

All experiments were conducted using a randomized block design (RBD). For each experiment, 10 seeds were used with three replications. Analysis of variance was performed using data on germination percentage and mean number of days taken for germination. Mean values were compared with DMRT.

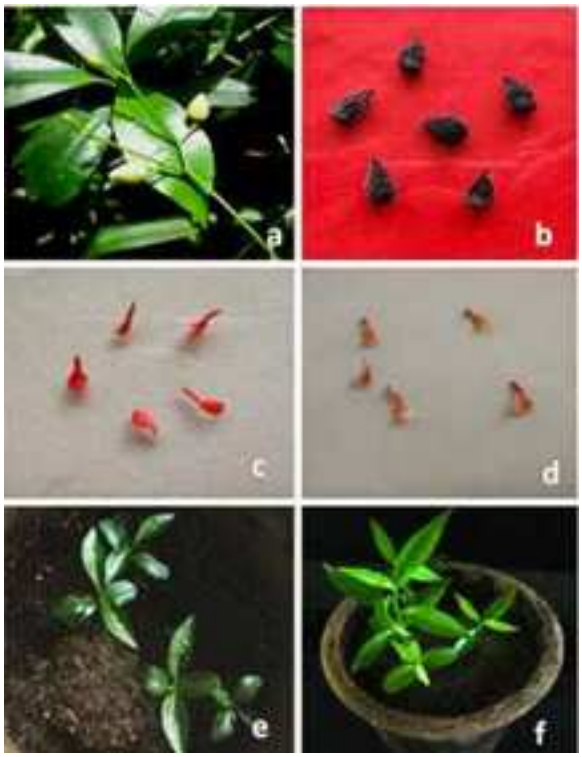

Fig.1 a. Habit of *Rauvolfia hookeri,* **b**. Dried seeds used for germination studies, **c.** TTC tested embryos of freshly harvested viable seeds, **d**. Less viable embryos of stored seeds, **f**. Seedlings emerged from GA_3 treated seeds under *ex vitro* condition, **g**. Control seedlings.

Results

Seed viability

The TTC test conducted in freshly harvested and stored (7 days after collection) seeds expressed marked differences in their viability. Embryos isolated from fresh seeds treated with TTC for 3 h turned completely red i.e., both the radicle and plumule portion stained red and no necrotic areas were observed in the entire embryo (Fig.1.c). The cotyledons also turned reddish which indicated high viability of seeds. Embryos isolated from stored seeds after TTC treatment exhibited a gradual decrease in the staining intensity. Radicle portion of some embryos appeared colourless whereas in others plumule portion remained unstained, an indication of declining viability (Fig.1.d). Completely unstained embryos were also encountered in some stored seeds.(fig.1)

Hot water and acid treatments

Hot water and acid treated seeds under in vitro conditions didn't show any sign of germination even after 45 days. Sub culturing on to the same medium also had no response.

GA_3 treatment

The effect of various concentrations of GA3 treatment on ex vitro and in vitro seed germination was determined (Table.1). GA3 treatment significantly (P<0.001) increased the germination percentage and the mean time to germination (MTG) under ex vitro conditions. The germination was poor in control (without GA3), i.e., only 21.6% of seeds undergone germination with 36.5 MTG. Treatment with GA3 (25 ppm) improved the germination up to 26.6% and resulted in the significant reduction of MTG (30.5). A significant (P<0.05) increase in the rate of germination (98.3%) and MTG (17) was achieved by 100 ppm GA3 treatment. All the treatments significantly (P<0.05) improved the germination percentage and MTG of R. hookeri seeds. Seedlings emerged from GA3 treated seeds were healthy with dark green leaves (Fig.1.e) and exhibited rapid growth compared to control (Fig.1.f).

GA_3 treatments (25 ppm, 50 ppm, 100 ppm, 150 ppm) under *in vitro* conditions

Table 1. Effect of different concentrations of GA_3 treatment on ex vitro seed germination of R. hookeri

GA_3 Conc. (ppm)	Germination (%)	MTG (days)
0.0	21.66^{c}	36.5^{a}
25	26.66^{c}	30.5^{b}
50	61.66^{b}	25.1^{c}
100	98.33^{a}	17.0^{d}
150	56.66^{b}	26.6^{c}
Main effect F df (n-1) 4	45.949***	65.878***

Means within a column followed by same letters are not significantly different as determined by DMRT (P<0.05). **Significant at P<0.01 level; *** Significant at (P<0.001) level.

evoked poor response in germination percentage even after 30 days of culture. Among all the treatments, germination (10%) was observed only in 100 ppm GA_3 treated seeds.

Discussion

In *R. hookeri,* conventional propagation is problematic due to various reasons including reproductive failure. The mode of propagation in the wild habitat is mainly through root suckers, so the present study was attempted to check the germination capacity of seeds and develop methods to improve the rate of germination through thermal or chemical treatments. There are no previous reports regarding the viability and seed germination studies on *R. hookeri* except one i.e., a comparative study on the morphology of fruits and seeds of *R. hookeri*, *R. serpentina* and *R. micrantha*[9].

Low flower-fruit percentage, poor seed set and delayed germination is witnessed in *R. hookeri*. Seeds without any pre-treatment when transferred to soil will take nearly 45 days to germinate and only 10% of seedlings are emerged from them. The dormancy may be imposed either by seed coat or by the embryo. Covering structures interfere with the water uptake and gaseous exchange of seeds, sometimes it acts as a barrier for the release of inhibitors from the embryo or as a chemical restraint. One or combined action of these effects may be responsible for the maintenance of dormancy in seeds[10].

In the present study, seed viability was assessed by TTC test. It is based on the activity of dehydrogenase enzymes that reduce tetrazolium compounds to insoluble red formozans. Activity of these enzymes may be low or absent in deeply dormant seeds[5&10.] From the TTC test it is evident that the embryo of *R. hookeri* seeds are fully viable immediately after harvest and the viability goes on decreasing with the increase in storage period. Loss in embryo viability appears at the extremity of the radicle, epicotyls and cotyledon tips[11]. Seeds stored for one week, when subjected to this test revealed gradual loss in viability that is evidenced by the appearance of necrotic regions in radicle tip and may give reduced germination under unfavourable soil conditions. TTC test was adopted to test the viability of *Stevia* seeds at two different stages to select suitable one for further germination experiments[12]. It distinguishes living and dead tissues within a single seed and can indicate impaired weakness before germination[13]. Thus highly viable freshly harvested seeds are selected for further germination experiments.

Seed dormancy may be an evolutionary adaptation that delays the germination after the seed has been shed from the parent plant. Physical dormancy (seed coat dormancy) in which the seed coat is impermeable to water can be relieved by hot water and acid treatments. Physical dormancy in *Corchorus* imposed by hard seed coat was released by the treatment with hot water and acid treatments[14]. In order to know the type of dormancy in *R. hookeri*, hot water and acid treatments were carried out. These treatments had no effect on the germination of *R. hookeri* seeds. The results of the treatments in the present investigation corroborate the reports in *R. serpentina*[15]. Thus it is clear that the dormancy prevailing in *R. hookeri* is not due to the hard seed coat but due to some endogenous factors it possesses.

Gibberellins are a class of hormones directly involved in the control and promotion of seed germination. They act at two different stages of germination either in the initial enzyme induction or in the activation of reserve food mobilizing systems[5]. Treatment of *R. hookeri* seeds with various concentrations of GA_3 for 24 h remarkably improved the germination percentage and MTG under *ex vitro* conditions. A significant increase in the germination percentage through pre-soaking of seeds in GA_3 was achieved in *Penstemon digitalis*[16]. In another study, GA_3 treatment along with short time chilling increased the germination of three endemic species of Asteraceae[17]. Pre- soaking of seeds initiates a number of metabolic processes before radicle protrusion whereas GA_3 stimulates germination by the

production of amylase enzyme, especially in cereals[18]. The efficiency of GA_3 in stimulating seed germination was recorded in a number of plant species by various workers[19, 20&21].

Poor and delayed germination of *R.hookeri* seeds may be attributed to the action of physiological dormancy. Generally, in mature dormant seeds GA_3 concentration drop to a lower level. In such cases an exogenous GA_3 supply will increase the imbibition process and stimulate germination. It is evident from the study that pre-soaking of seeds in water significantly increased the germination. Light also plays a pivotal role in germination influencing the phytochrome action which can either inhibit or stimulate the process. In the present study, experimental pots containing GA_3 treated seeds were kept away from direct sunlight. From the results it was evident that diffuse light along with GA_3 treatment enhanced seed germination percentage. GA_3 treated seeds under *in vitro* conditions did not respond well and it may be attributed to 8 h photo period provided in the culture conditions. Short time dim light treatment along with 250 ppm GA_3 treatment enhanced germination percentage and reduced the mean time to germination in *Cirsium leucopsis*[17]. Dormancy is regulated by relative levels of growth promoters (GA_3) and inhibitors (ABA). When GA_3 level exceeds that of ABA, germination will take place. Thus an exogenous GA_3 supply can overcome dormancy of *R. hookeri* seeds. Role of GA_3 in endogenous dormancy breaking was reported earlier by several authors[22, 23&24].

From these results it can be concluded that the seed dormancy existing in *R. hookeri* is due to multiple factors excluding physical parameters and GA_3 application is the most beneficial treatment to enhance maximum germination under *ex vitro* conditions.

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Effect of NaCl, KCl, epinephrine and vitamin A, B and C on melanophore indexes of *Anabas testudinues* (Bloch) and *Channa striata* (Bloch)

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Abstract

Chromatophore responses of selected fish species to known melanophore dispersal and aggregation agents (KCl, NaCl and Epinephrine) and (agents of unknown effect) Vitamin-A, Vitamin-B and Vitamin-C. Scales from live specimens of *Anabas testudineus and Channa striata* of either sex ranging from 7-11cm were treated with 3.5% of NaCl solution, 1% KCl, Epinephrine solution, and vitamin solutions and chromatophore responses were studied calculating melanophore index after Hogben and Slome. The Melanophore Index of *Anabas testudineus* for showed that the NaCl, Vitamin A, Vitamin B and Vitamin C causes melanophore dispersal. KCl and epinephrine induces aggregation of melanophores. In *Channa striata* NaCl, Vitamin B and Vitamin C causes melanophore dispersal whereas KCl, epinephrine and Vitamin A causes aggregation of melanophores. Vitamin A caused dispersal in *Anabas testudineus* but aggregation in *Channa striata.* Vitamin B showed strongest dispersing effect in *Anabas testudineus* while in *Channa striata* Vitamin C showed strongest dispersing effect. Epinephrine showed maximum aggregating response in both species. The punctate, punctostellate and stellate stages did not show significant difference between responses of the two fish species during the application of different solutions; but reticulostellate and reticulate stages show significant difference in the response of the two fishes. It can be concluded that the application of Vitamin A, Vitamin B and Vitamin C elicits significant melanophore responses from the selected fish species. Inter specific responses showed maximum variation in the reticulostellate and reticulate stages.

Key Words: Melanophore Index, Chromatophore responses, *Anabas testudineus, Channa striata*

Introduction

Fishes change their colour in response to background colouration and also display colour responses during excitement and courtship. These spectacular changes are mediated through integumentary pigment containing cells called '"chromatophores". With some exceptions, these chromatophores are capable of changing their appearance; and thus the aspect of the fish as a whole is altered. Either the number of chromatophores or the amount of pigment which they contain may increase or decrease.

The pigment within most chromatophores varies in its position. It may appear as a solid, rounded mass in the centre of the cell or may be distributed throughout the cell, revealing its finest branches. Occasionally clusters of pigment granules move with a certain degree of independence, and concentration and dispersion of pigment has been observed within a process detached by micro dissection. Chromatophores in isolated tissues respond to treatment with hormones or drugs more promptly than they do while still controlled by the animal.

According to the colour of pigments they contain, the chromatophores of fishes have been commonly classified as melanophores (brown or black), erythrophores (red), xanthophores (yellow), leucophores (white), and

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iridophores (reflecting). Sometimes more than one type of pigment can be found in the same chromatophore. The degree of intensity of darkness of these cells depends on the amount of dispersion of melanin pigment within the cell. When the pigment is dispersed widely throughout cytoplasm, skin macroscopically appears darker (black or brown). When the pigment granules aggregate or contract, the cell loses its darkness.

Spaeth[1] first observed that the K^+ and other alkaline ions except Na^+ cause aggregation of melanosomes on the scale melanophores of *Fundulus*. Falk and Rhodin[2] presented their electron microscope observations of *Lebistes reticulatus* melanophore. Kinosita[3, 4], working with the scale melanophores of *Oryzias latipes*, reported that melanosomes are negatively charged and that under influence of K^+ or epinephrine causes the aggregation of pigment in cell. The reverse changes are recorded following application of physiological saline or atropine solution in which melanin dispersion takes place. Identical action of alkaline earth ions was also reported[5, 6]. Quantitative descriptions of the effect of K^+ ions on the scale melanophores of some fresh water forms has been studied[6, 7, 8]. Among alkaline ions and alkaline earths, K, Rb, Cs, NH_4, Ba, and Sr ions are generally effective enough to induce melanin aggregation[1, 5,6,9]. No response was observed in response to these ions in Chasmichthys[9]. The effects of drugs and ions on melanophore pigment movements and transmembrane potentials of stoneloach (Noemacheilus) have also been studied[10].

Many researchers have conveniently used the isotonic NaCl or proper physiological saline solutions for the purpose of obtaining the dispersed state of melanophores in various species of fish.

Most of the chromatophore activities results from the pigment movements. Chromatophore responses can be studied by finding out the Melanophore Index (MI) and the Mean Melanophore Index (MMI). In present investigations effect of different solutions such as KCl, NaCl, Epinephrine and Vitamin-A, Vitamin-B, Vitamin-C etc. on fish chromatophore was studied.

Materials and methods

Live specimens of *Anabas testudineus and Channa striata* (both were selected because they are hardy) of either sex ranging between 7-11cm were collected from natural habitats and stocked in transparent glass aquaria under natural photoperiodic conditions in the laboratory. 3.5% of NaCl solution, 1% KCl, Epinephrine solution, and vitamin solutions (1 capsule/1litre) were prepared. A scale was plucked from the antero dorsal region of the trunk from the live fish using forceps. The scale was mounted on a microslide with a drop of fresh water and kept under the microscope for observation. The number of melanophore belonging to each stage was counted. This is taken as normal (control). The same procedure was repeated using NaCl, KCl, epinephrine, vitamin A, vitamin B and vitamin C. Experiments were carried at room temperature ranging 25-30^0 C.

Chromatophore responses were studied by finding out the melanophore index as originally used by Hogben and Slome. In the melanophore index system, the range from the stage1 means maximal pigment aggregation and each increase in number tending the increased pigment dispersal. Stage 5 corresponds to the fully dispersed stage. Stages Of Chromatophore are 1-Punctate, 2-Punctostellate, 3-Stellate, 4-Reticulostellate, 5-Reticulate. The Melanophore index (MI) permits the estimation of the degree of dispersion of melanin in a melanophore according to scale ranging from stage1 (full aggregation) to stage 5 (full dispersion). The Mean Melanophore Index (MMI) is derived by selecting 10 melanophores and studying these according to the MI. The number of melanophore in each stage of dispersion is multiplied by the index number of the stage and the sum of the product is divided by 10 to get the MMI.

Results and discussion

Anabas testudineus

By observing the melanophore indexes in *A. testudineus*, the lowest value was observed for the first stage *ie*; Punctate

(9.18) and the highest value was observed for the final stage *ie*; Reticulate (47.58). Punctostellate, Stellate and Reticulostellate stages have the values 12.83, 26.75, 35.93 respectively. The normal values for melanophore indexes in *A. testudineus* increased from the first stage (Punctate) to the final stage (Reticulate).

On application of NaCl, there was change in the melanophore index of *A. testudineus*. For NaCl, the lowest value was obtained for the Punctate stage (4.68) and the highest value for the Reticulate stage (51.83). Punctostellate, Stellate, Reticulostellate stages showed the values 10.63, 34.05, 46.47 respectively. When compared to normal (control); it was observed that in the first 2 stages, ie. Punctate and Punctostellate the MMI values on application of NaCl becomes lower and the values for the other 3 stages increase. Therefore it can be concluded that NaCl has dispersing effect on melanophores in *A. testudineus*.

On application of KCl, the lowest value was obtained in the Punctate stage (9.55) and the highest value was obtained in the stellate stage (31.75). Punctostellate, reticulostellate, reticulate stages have the values 20.80, 20.13, 23.33 respectively. When compared to normal (control) it was observed that the first 3 stages such as punctate, punctostellate, and stellate have higher values than the normal but the values of the last 2 stages (reticulostellate and reticulate) decreases. Therefore one may be concluded that KCl shows aggregating effect on the scale melanophores of *A. testudineus*.

On application of epinephrine, the lowest value was obtained in the punctate stage (11.77) and the highest in the stellate stage (29.50). Punctostellate, reticulostellate, reticulate stages have the values 21.87, 18.73, 19.00 respectively. When compared to normal (control) it was observed that the punctate, punctostellate, and stellate values are higher during the application of epinephrine and the other 2 stages - reticulostellate and reticulate have lower values. From this it can be concluded that the epinephrine shows much aggregating response on the scale melanophores of *A. testudineus*. When compared with the other aggregating agent KCl, epinephrine was observed to have more aggregating effect than KCl in *A. testudineus*.

On application of vitamin A in *A. testudineus*, the lowest MI value was obtained in the punctate stage (8.13) and highest value of MI obtained in the reticulate stage (70.42). When compared to normal (control) it was observed that, except for the punctate stage, all other stages had higher values. From this it can be concluded that vitamin A also plays an important role in pigment dispersal in *A. testudineus* which is higher than the response to NaCl.

On application of vitamin B to the scale melanophores of *A. testudineus*, the lowest value was obtained in the punctate stage (2.93) and highest value was obtained in the reticulate stage (80.33). Punctostellate, stellate, and reticulostellate stages have the values 8.63, 40.35, 61.07 respectively. When compared to normal (control), the first 2 stages of vitamin B shows lower value and in the other 3 stages very high values were observed. From this it can be concluded that vitamin B also enhance pigment dispersal like vitamin A and NaCl.

On application of vitamin C the lowest value was obtained in the punctate stage (6.55) and highest value was obtained in the reticulate stage (78.58). Punctostellate, stellate, reticulostellate stages have the values 16.33, 37.20, 55.53 respectively. On comparison with the normal values, it was observed that the values for punctate is decreased and that of other four stages increased; from which one can conclude that vitamin C also initiates pigment dispersal like other vitamins.

Channa striata

In the normal MI for *C. striata* (Fig. 2), the lowest value was obtained for the punctate stage (4.02) and the highest value was obtained for the reticulate stage (95.58). Punctostellate, stellate, and reticulostellate stages have the values 9.30, 17.50, 44.40 respectively.

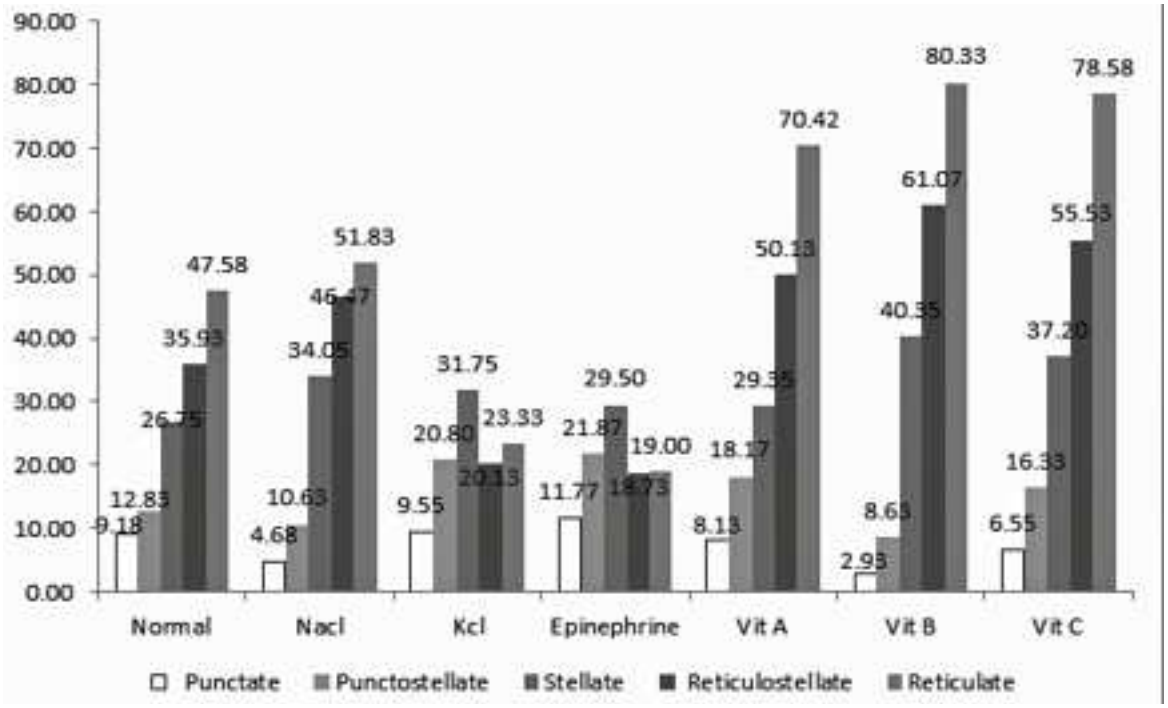

Fig 1. Melanophore Index of *A. testudineus* with responses to Vitamin treatment

On application of NaCl, the lowest value was obtained for the punctate stage (2.88) and highest value for the reticulate stage (96.83). Punctostellate, stellate, reticulostellate stages have the values 8.17, 22.90, 53.93 respectively. On comparison with the normal (control), it was observed that the punctate and punctostellate values was less than the normal values in *C. striata* ; but in the other 3 stages such as stellate, reticulostellate and reticulate higher values were obtained by the application of NaCl. Hence it is concluded that NaCl shows dispersing effect for both *A. testudineus* and *C. striata.*

On application of KCl, the lowest value was obtained for the punctate stage (14.55) and the highest value was obtained for the

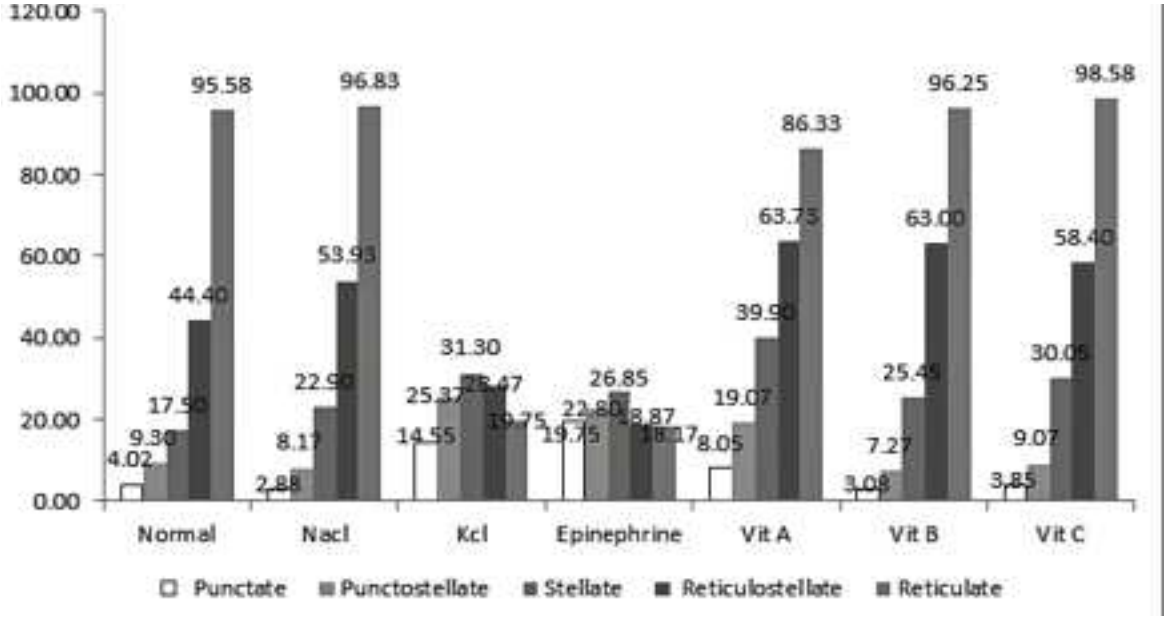

Fig 2. Melanophore Index of *C. striata* with responses to Vitamin treatment

stellate stage (31.30). Punctostellate, reticulostellate, reticulate stages have the values 25.37, 28.47, 19.75 respectively. On comparison with the normal (control), it was observed that the first 3 stages - punctate, punctostellate, and stellate had higher values than the normal and reticulostellate, reticulate stages had lower values than the normal; indicating the KCl's aggregating effect on the scale melanophores of *C. striata*

On application of epinephrine, the lowest value was obtained for the punctate stage (19.75) and the highest value was obtained for the stellate stage (26.85). Punctostellate, reticulostellate and reticulate stages have the values 22.80, 18.87, 18.17 respectively. On comparison with the normal (control), it was observed that in the first 3 stages - punctate, punctostellate and stellate the MI values were higher than the normal and the reticulostellate and reticulate values was lower. From this it can be concluded that the epinephrine shows aggregating effect on the scale melanophores in *C. striata*. In *A. testudineus* also the same effect is noticed.

On application of vitamin A in *C. striata*, the lowest value is obtained in the punctate stage (8.05) and the highest value is obtained in the reticulate stage (86.33). Punctostellate, stellate, reticulostellate stages have the values 19.07, 39.90, 63.73 respectively. On comparison with the normal (control), all stages except for the reticulate stage had higher values. This was different from the observation seen in the *A. testudineus*. *C. striata* shows a aggregational response to vitamin A application while *A. testudineus* showed visible dispersion. From this observation, it can be concluded that the response of scale melanophores to different chemicals may be species specific to some extent.

On application of vitamin B to the scale melanophores of *C. striata*, the lowest value was obtained in the punctate stage (3.08) and the highest value was obtained for the reticulate stage (96.25). Punctostellate, stellate, and reticulostellate stages have the values 7.27, 25.45, 63.00 respectively. On comparison with the normal, the punctate and punctostellate values for vitamin B was lower than the normal and the stellate, reticulostellate and reticulate stages showed higher values. From this it can be concluded that, vitamin B does not show the same effect of vitamin A in *C. striata*. Vitamin B induces dispersion of melanophores in *C. striata* species as in *A. testudineus*.

On application of vitamin C in *C. striata*, the lowest value was obtained for punctate stage (3.85) and highest value was obtained for reticulate stage (98.58). Punctostellate, stellate and reticulostellate stages have the values 9.07, 30.05, 58.40 respectively. On comparision with normal (control) it was observed that, in the punctate and punctostellate stages the values decreases and in stellate, reticulostellate and reticulate stages the values increases.This indicates the maximum dispersion of melanophores during the application of vitamin C. In *A. testudineus* also vitamin C showed complete dispersion.

When one compares the response of *C. striata* to different treatments, it was

Table 1. Student T test (two tailed) results for inter-specific response comparison

	DF	ALPHAVALUE	P VALUE	T VALUE OBSERVED	CRITICAL VALUE	INFERENCE
Punctate	6	0.05	0.788	-0.281	2.448	No Significant Difference
Punctostellate	6	0.05	0.443	0.822	2.448	No Significant Difference
Stellate	6	0.05	0.168	1.569	2.448	No Significant Difference
Reticulo stellate	6	0.05	0.014	-3.447	2.448	Significant Difference
Reticulate	6	0.05	0.039	-2.636	2.448	Significant Difference

observed that vitamin C causes maximum dispersal of melanophores.

Two tailed Students T- test was done for the purpose of inter species comparison. *ie*; response of melanophores to treatment with different solutions which are tabulated in Table 1.

While here was no significant difference between the two selected fish species in the punctuate, the punctostellate, and stellate stages the reticulostellate stage and reticulate stage showed a significant difference in the response of melanophores to different treatments.

It was concluded that in *Anabas testudineus* NaCl, Vitamin A, Vitamin B and Vitamin C causes melanophore dispersal and KCl and epinephrine induces aggregation of melanophores. In *Channa striata* NaCl, Vitamin B and Vitamin C causes melanophore dispersal. KCl, epinephrine and Vitamin A causes aggregation of melanophores. The response of the two species to Vitamin A was different-dispersal in *Anabas testudineus* but aggregation in *Channa striata*

Vitamin B showed strongest dispersing effect in *Anabas testudineus* while in *Channa striata* Vitamin C showed strongest dispersing effect. Epinephrine showed maximum aggregating response in both species. It can be concluded that application of Vitamin A, Vitamin B and Vitamin C elicits significant melanophore responses from the selected fish species. Inter specific responses showed maximum variation in the reticulostellate and reticulate stages. Since only two species were studied and effect on live fishes were not checked one cannot arrive at fool proof conclusions. It is suggested that more studies involving other species, live fishes, different concentrations of Vitamins in the water and different diet need be undertaken. Similar applications on live aquarium fishes would be helpful in maintaining or manipulating the attractiveness of fishes without using harmful additives.

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Efficacy of different extracts of Glycosmis *pentaphylla* on the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

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Abstract

Experiments were conducted to study the bioefficacies of plant derivatives that affect the development of rice weevil, *Sitophilus oryzae* (L.) Effect of aqueous, ethanol and acetone extracts of the leaves of the plant *Glycosmis pentaphylla* was tested against the rice weevil. After 7 days of extract exposure of the plant, total body protein, glycogen and total free amino acids of the insects were checked. The efficacy of the extracts on the insects was dose-dependent. Different doses were checked and the results showed that high doses of the extracts were significantly more toxic to *Sitophilus oryzae* compared to lower doses. LD50 value was assessed The result showed that the acetone extracts were effective in checking insect infestation. Total body protein and glycogen were found to be depleted compared to control, while total free aminoacid was incresed. Extracts of the plant is proved to have strong insecticidal activity against *Sitophilus oryzae* .

Key words: *Sitophilus oryzae* ,*Glycosmis pentaphylla*, insecticidal activity, mortality rate, bio molecules.

Introduction

Insects cause a lot of damage to stored seeds, grains and their products. There is a continuous need to protect the stored products against deterioration, especially loss of quality and weight during storage. Quantitative and qualitative losses of stored grains may result from the feeding and waste production by insects, mites, rodents and birds or from the growth of microorganisms all of which are influenced by environmental condition[9].In India, unscientific storage, rodents, insect pests, mites, microorganisms account for 10 per cent wastage of food grains. In comparison to other regions of the country, storage of food grains in North East India is very difficult due to high humidity, encouraging infestation of stored grains by insect pests. In storage rice is damaged by a number of insect pests, particularly by rice weevil,Sitophillus oryzae (L.) (Coleoptera: Curculionidae) ;angoumois grain moth, Sitotroga cerealellea (Olivier); lesser grain borer, Rhizopertha dominica (Fabricius); rust red flour beetle, Tribolium castenium (Herbst) and khapra beetle, Togoderma granarium (Everts) [2] while S. oryzae caused heavy damage to wheat, rice, maize and sorghum grains [4] To overcome the problem of storage insect pests, plant products have the potentiality to play an important role.

Botanical pesticides are emerging as a possible alternative to chemical pesticides and a viable component of Integrated Pest Management strategies on all crops in view of their efficacy to pests, easy biodegradability,and photo stability etc. Various botanicals have been found to be effective against different pests. Research reveals that extracts prepared from plants have a variety of properties including insecticidal activity, repellency to pests, anti feedant effects, insect

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growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens [12].This study is an attempt to analyze the pesticidal properties of selected plant against the stored product pest *Sitophilus oryza*. The test plant would yield environmentally sound chemicals having no harmful effects on the non target organisms. Keeping this in view, the present study was carried out to test the efficacy of the plant leaf extracts of the plant *Glyosmis pentaphylla.*

Materials and methods

Culturing of test insects

The pest, *Sitophilus oryzae,* was collected from stored rice from a local shop. Fresh rice was washed and dried in sunlight. This rice was taken in containers and the insects were transferred to it. Thus stock cultures were prepared. Holes were drilled on the container lid for permitting the passage of air. The culture was maintained at room temperature. For getting newly emerged adults, 100 insects, including both male and female, from the stock were transferred to fresh rice. They were allowed to lay eggs on fresh rice. Then after 2 weeks, they were removed, and the rice containers were kept undisturbed. On the sixth week, new insects began to emerge in the containers. These insects were used for further studies. The stock culture was cleaned by sieving once in five days. This helps to remove the food waste and faecal matter of the insects to avoid fungal attack.

Preparation of aqueous extract of plants

The plant leaves were collected and washed well with distilled water. The leaves were ground without adding water. The ground mass was then transferred into a beaker containing 100 ml of distilled water. Then it was mixed well and kept for three days. After three days the mixture was filtered. Then this mixture was kept in a water bath at 60-70^{0}C. After drying, this residue is dissolved in water and made up to different concentrations.

Preparation of acetone and ethanol extracts of plants

For the extraction, soxhlet apparatus was used. About 25g powder of each plant leaves were extracted with 250ml ethanol and acetone. The extraction of each plant sample was done in about 12 hrs. After soxhlet extraction; the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept in a water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Treatments

The extracts were applied at different doses on Whatmann No. 1 filter paper and air-dried for an hour. The controls were treated with acetone or ethanol or distilled water only. The treated and control filter paper discs were placed singly at the bottom of plastic jars and 20gm of rice were placed on the papers. Ten insects *were* released in each plastic container. There were five replicates for each treatment and control. Observations were recorded on the seventh day of treatment.

Biochemical and enzyme assay

Bioassay of protein[8],total free amino acids and glycogen[6] were conducted on both control and insects, treated with sub lethal doses of *Glycosmis pentaphylla*.

Statistical analysis of data

The data obtained are recorded as mean± standard deviation. For testing the significance of the data obtained, statistical analysis were carried out using ANOVA ($p \leq 0.05$) using SPSS software[5].LD 50 was calculated using probit analysis.

Results and Discussion

Effect of plant extracts on mortality of insects

The total number of adult insects surviving after the treatment was recorded for seven days consecutively. The percent

mortality was then calculated. Acetone and Ethanol extracts of the plant showed significant mortality compared to the aqueous extract. (Table 1) No mortality was seen in the case of control.LD 50 was calculated using probit analysis

Table 1. Effect of plant extracts on mortality of insects

Dose (%)	Mortality(%)		
	Glycomis pentaphylla		
	Acetone extract	Ethanol extract	Aqueous extract
1%	40±0.44	40±0.44	30±0.40
5%	48±0.20	46±0.09	40±0.20
10%	75±0.45	74±0.14	50±0.45
15%	80±0.54	75±0.14	60±0.44
25%	84±0.45	78±0.45	70±0.40

Table 2. LD_{50} of different extracts of *Glycosmis pentaphylla* on *Sitophilus oryzae*

Plant	Extracts	LD_{50}(%)
Glycomis pentaphylla	Aqueous	10.14
	Ethanol	4.38
	Acetone	4.06

Table 3. Sub lethal doses of adult insects

Plant	Sub lethal dose(%)		
Glycomis pentaphylla	Aqueous	ethanol	acetone
	10	4	3.8

Effect of plant extracts on bio molecules of insects

After extract exposure ,total body protein and glycogen were found to be depleted compared to control, while total free aminoacid was incresed. (Table 4).

The use of plant extracts to control stored products insects is an ancient practice. Insecticidal properties of a number of plant extract have been evaluated against stored product insects [1] Essential oils from some medicinal and aromatic plants are known to possess bioactive compounds that are either toxic to a number of insects at various stages of life or elicit anti-feedant properties [7]

Results obtained in the present investigation clearly demonstrate that both solvent and aqueous extracts of *Glycosmis pentaphylla* is toxic to *Sitophilus oryzae*. Maximum mortality was obtained in acetone extract of the plant.

Extract from *Glycosmis pentaphylla* has potentially reduced the body content of glycogen highest in acetone extract of the plant. This may be due to depletion of glycogen indicates more and more utilization of food reserves to cope up the insecticide induced stress [11] .This decrease in glycogen level may be due to high release of glucagon, corticosteroids catecholamines which stimulate glucose production to combat energy demand. Normally in the body free glycogen floats in the haemolymph/ blood that after breakdown help to maintain glucose level in hemolymph. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment[3].Protein may block at cellular level and catabolism get increased which results into low availability of proteins. Total free aminoacid level in the treated was high compared to control.The high level of total free aminoacids indicates a disturbance in the metabolism of adult insects.

Similar results were reported in Pimpla turionella wasp when its larvae, pupae and adult females were treated with cypermethrin Cypermethrin affected the level of glycogen, protein and lipid[10]. Similarly cypermethirn decrease the protein level in Spodoptera litrua larvae in comparison to control [14] Few organophosphorus insecticides such as chloropyrifos, thiamethoxam, fipronil and malathion caused significant depletion in total protein in haemolymph and fat body of silk worm Bombyx mori [13]

Table 4.Effect of sub-lethal doses of three extracts of *Glycosmis pentaphylla* on total body protein, glycogen and total free amino acid of adult *Sitophilus oryzae*

Parameters	*Glycosmis pentaphylla*			
	Control	Aqueous	Ethanol	Acetone
Total body protein	9.27±0.03	7.46±0.01	6.86±0.01	5.89±0.04
Glycogen (mg/gm)	10.26±0.01	8.46±0.01	7.88±0.01	6.89±0.03
Total free aminoacid	10.37±0.03	12.24.±0.01	14.32±0.01	16.89±0.01

Values are mean ±SE; all values are significant at p≤0.05 level of significance

However, it can be concluded that *Glycosmis pentaphylla* possess few active ingredients that might be highly effective against stored grain insects. It is proved by the results that these ingredients cause high lethality in *Sitophilus oryzae* at a low dose and caused significant inhibition of metabolic enzymes. Therefore, it is recommended that active ingredients of *Glycosmis pentaphylla* could be used for preparation of herbal insecticidal formulation to control stored grain insects, so it could be integrated into pest management system.

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Resource utilisation in mutualists: Fig (Moraceae) and fig wasp (Chalcidoidea)

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Abstract

In the relationship between fig and their pollinator wasp, the survival and reproductive success are based on certain strategies adopted to facilitate mutualism. In the mutualism benefit is for both the partners. The pollination system of *Ficus* is highly specific with its pollinating wasps. Any disruption is a serious matter because the plant must balance with seed production with maintenance of the pollinator population. The investigation made on two monoecious fig species has shown that distribution of style length exhibit single mode. There is a strong correlation between ovipositor lengths of the pollinating wasps and the average style length of the respective *Ficus*. Increased length of ovipositor is thus capable of infesting nearly 100% of the flowers of host plants by the pollinator wasps. This antagonistic trait may lead to the collapse of mutualism. However, such an event is not met with as they sustain mutualism by ensuring equity in sharing of resources.

Key words: Mutualism, *Ficus,* pollination, wasp, co evolution

Introduction

The unique association between *Ficus* plants and their pollinator wasps is evidently a very good example of co evolution of plants and insects. Generally each *Ficus* species is pollinated by specific pollinating wasp which can only breed in female flowers of its host fig plant. The female pollen transporting wasps enter, pollinate, lay eggs in the ovary of some female flowers and usually die within the inflorescence. Each larva develops within single fig ovary. Some times after pollination the female flowers reach maturity, male flowers produce pollen and new generation of wasps attains adult stage. After mating, female wasps get loaded with pollen of their natal fig. They eclose out and fly in search of a new receptive fig to lay egg. After the wasps' exit, the fig ripens and the seeds are dispersed by various frugivore species. The figs, when they reach receptive stage emit volatile compound to attract their specific pollinating wasps. Almost half of all fig species are monoecious, produces seeds, pollen and pollinators. The other half are functionally dioecious female trees producing only seeds, while the male trees produce pollen grains and the pollinating wasps. The fig- wasp relationship probably cannot be understood without considering certain non-pollinating wasps that develop within the fig. Little is known about the impact up on mutualism caused by these insects. Figs and their pollinating wasps are highly co evolved mutualists that depend completely on each other for continued reproduction[8].

To maintain the obligate mutualism, the pollinating wasps and host figs exhibit a range of peculiar structural features and specialized behavioural patterns favou8ring their strong co evolution. One such feature of the fig plant is the production of two distinct types of female flowers in the fig, as short styled and long styled. The style length variation has been generally assumed to be the mechanism by which the figs generate the retention of at least some uneaten seeds, whereas short styled flowers contain seed

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eating wasps. In monoecious figs, the evolutionary conflict is aimed at leaving as many offspring as possible, an individual tree producing not only pollen carrying wasp offspring, but some undestroyed seeds as well. In this paper we examine the degree of bilateral exploitation of resources through relative production of pollinating wasps and seeds without affecting their reciprocal benefits.

Materials and Methods

The studies were performed on *Ficusbenghalensis* and *F.drupacea*, the two monoecious fig species on the campus of University of Calicut, Kerala and their active pollinator wasps *Eupristinamasoni* and *E.belgaumensis*. For measuring the style length, 30 flowers were randomly collected from each B-phase fig according to the method followed by[6]. The tepals were removed and stigma to the point of attach to the ovary, to the nearest 0.01 mm, under a microscope. The figs of D-phase collected from the field were kept in jars covered with muslin cloth. A minimum of 30 pollinator wasps were randomly selected from each species to measure the length of ovipositor. The ovipositor was separated from its enveloping sheaths and measured from the point of attachment to the gaster till the tip, to the nearest 0.01 mm. Kolmogrow-Smirnow test was employed to test the fitness of the style length. To estimate the allocation of flowers in the fig, the near ripe figs *Ficusbenghalensis* and *F. drupacea*were collected at different times and the contents in the figs were scored as wasp containing ovary, seed and gall ovary. The male flowers were also recorded.

Result and Discussion

The distribution of style lengths observed in two monoecious fig species showed single mode. There was no indication of bimodality or existence of discrete classes of short and long styled flowers. Therefore there is normal distribution. However, it is possible to identify short and long styled flowers relative to the length of the ovipositor of the respective pollinating wasp. It indicates the fact that in *Ficus drupacea* 100% of the styles in the fig were shorter than mean ovipositor length of their respective wasps, *Eupristina belgaumensis*. In the case of *F. benghalensis*, pollinated by *E. masoni* ; the percentage of short styled flowers in the fig was 97.62 (Table 1). The coefficient of variation in the distribution of style length of these species were more that in the ovipositor length of their pollinating wasps. The average seed and wasp production in two studied species of *Ficus* were almost identical i.e. in *Ficus drupaca* 40% seeds; 50% insects; whereas in *F. beghalensis* 45% seeds, 43% insects. The percentage of male flowers within two species was identical, which was 8%. On the other hand the percentage of gall flowers showed much variation with 2% and 4% respectively (Fig.1&2).

In fig/fig pollinator relationship, the dependence on the pollinator wasp for the pollination of the flowers and the production of viable seeds is highly profound. It is true that, of the potential seeds, roughly 50% may remain seeds and the rest may be eaten by wasps. However, the figs also dependent on the female offspring of the pollinator wasp to carry pollen off to pollinate other figs. The two monoecious fig species studied have shown a normal distribution of

Table 1: Mean style length of two monoecious *Ficus* species and ovipositor length of their pollinator wasps

Sl. No.	Ficus species with the wasp species in brackets	Style length and ovipositor length (in mm)				
		n	Mean	SD	CV %	Percentage ofshort styled flowers
1	*Ficus drupacea* *(Eupristinabelgaumensis)*	91 54	0.6012 1.364	0.169 0.182	28.11 13.34	100
2	*Ficusbenghalensis* *(E. masoni)*	126 51	1.122 1.841	0.400 0.132	35.65 7.17	97.62

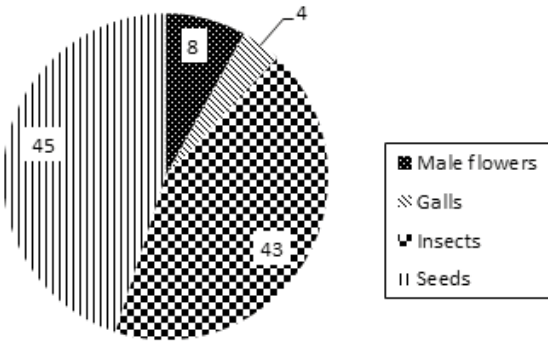

Fig.1.Pie diagram to show the allocation of flowers of Ficus benghalensis fig sampled

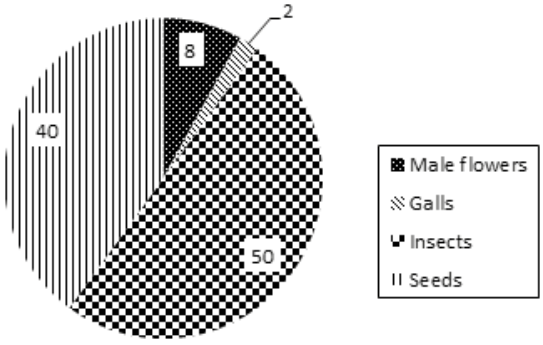

Fig. 2. Pie diagram to show the allocation of flowers of *Ficus drupacea* fig sampled

style length with no indication of bimodality and hence no direct sign from the side of fig plants to employ dimorphism in style length as a strategy to regulate the extent of over exploitation by wasp. As the ovipositor of *Eupristina belgaumensis,* the pollinator wasp of *Ficus drupacea* and *E.masoni,* the pollinator wasp of *Ficus benghalensis* are long, can potentially oviposit in all the flowers with the style length shorter than their ovipositors. Several independent studies [10, 11 and 14] reveal that style length shows normal distribution. Several workers have suggested that the fig allocate about equal proportions of flowers to the pollinating wasps and for seed production. These were either on the basis of preliminary observation or on the assumption that in style length exists a perfect bimodal distribution. For two monoecious *Ficus* species, the proportion in *Ficus benghalensis* was 100% and in *Ficus drupacea* was 97.62%. This will result in a linear increase in the benefit, which means that the selected ovipositor would be equal to the longest style; this could not leave any flower for seed production and hence the mutualistic relation collapses. On other hand the results of the study contain allocation of resources such as male flowers, seeds, gall flowers and pollination offspring. There are implications of variation from species over a longer period of time. The phenomenon of gall ovaries deserve attention in studies of fig/ pollination wasp interaction since it appears to be intimately involved with the process that limit fig and wasp reproductive success in nature. There are more vacant ovaries in figs which were entered by fewer pollinators[1].The well recognized conflict of interest exists between figs and their pollinator mutualists [2, 12 and13], but the expected negative correlation has never been found in seed and pollinator number in the wake of nearly 100% of the styles in the fig being shorter than mean ovipositor length of their respective wasps so that complete flowers in the fig could be usurped by the wasps. Instead, pollinated figs show that there is positive association between seeds and pollinator wasps themselves. Natural variation in resources available to different species *Fcius*and at different times may have independently affected both seeds and wasp numbers maturing, leading them often to be strongly positively correlated [4]. In mutualism, the resource relationships within and among figs are crucial in controlling the reproductive success of both partners [3, 8].Certain features have been evolved among figs and pollinating wasps to maintain their strange association. It has been suggested that the wasp can lay its eggs in the ovules of the short styled flowers, but not in the long styled flowers as its ovipositor does not reach their ovules [5, 15]. It was believed that by this strategy the fig plants can regulate the proportion of the flowers allocated to the seed production and pollinator wasps [9, 13]. The investigations on the other species of *Ficus* would further reveal the intriguing aspects in the fig/fig pollinator interaction.

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Fecundity Analysis: *Amblypharyngodon melettinus muriyadensis* —A Freshwater Fish

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Abstract

The fishes surpass all other groups of vertebrates in their diversity in mode of reproduction. Most of the teleosts are seasonal breeders with definable reproductive and breeding phases. Even though the fishes exhibit immense prodigality in reproduction, due to environmental resistance, the population may not increase to an explosive level. Analysis of fecundity is a tool to study the reproductive phases of fish. In this study analysed the fecundity of *Amblypharyngodon melettinus muriyadensis*. They were collected from three different regions of Muriyad wetland namely, Thommana canal, Konthipulam fields and Nandhi canal. 30 fish of advanced stage of maturity were selected and ovaries were preserved in 4% neutral formalin. 0.1 gm of the middle part of the ovary was taken and the number of eggs in these samples was determined. The total number of eggs in the ovaries was estimated. The mean values were recorded as the absolute Fecundity. Relationship between the fecundity and fish weight, fish length, ovary weight were established by applying the method of least square. The number of ripe ova was found to vary from 2031 to 9962 in fish ranging in total lengths 77.5mm to 120mm, body weight between 4.10g and 14.73g and total ovary weight ranging from 0.26g to 1.12g. Fecundity was related to the total length of fish, body weight and total gonad weight using regression equations. The fecundity in fishes is often correlated with length, weight and age of fish and also with the length, weight and volume of the ovary. In *A. melettinus muriyadensis* it was found that the fecundity increased at a rate of 1.2 times of the fish length. In *A. melettinus muriyadensis*, a straight line relationship was obtained between the weight, length, ovary weight and fecundity.

Key words: Fecundity, *Amblypharyngodon melettinus muriyadensis*,least square method, fish weight, fish length, ovary weight

Introduction

The fishes surpass all other groups of vertebrates in their diversity in mode of reproduction. Most of the teleosts are seasonal breeders with definable reproductive and breeding phases. Even though the fishes exhibit immense prodigality in reproduction, due to environmental resistance, the population may not increase to an explosive level. From the beginning of gametogenesis to the attainment of maturity, there are a number of factors responsible of ultimate growth of fish. If any fish species is to be managed and exploited scientifically, the fundamental knowledge of various aspects of reproduction and breeding behavior are important.

Reproductive processes of fish are governed by a number of biotic and abiotic factors of the environment[1]. Any alterations in the environmental conditions may affect the process of gametogenesis. The environmental stimuli are believed to be mediated through the central nervous system affecting secretary changes in the hypophysis of the fish. Of all the environmental factors, photoperiod and temperature appear to be of crucial importance. By photo-thermal manipulations, maturation in many fishes can be accelerated or retarded and it is possible to extend the spawning season in seasonal breeders[2]. The effect of photoperiod is more pronounced in salmanoid group of fishes. The effect of temperature also exerts

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influence on most of the physiological functions in fishes. Rainfall triggers courtship behaviour leading to spawning in commercially important culture species including Indian and Chinese carps. Rainfall associated with flooded condition is one of the major environmental factors responsible for the spawning of fishes.

The endocrine system acts as a "major link" in the chain of events leading from the perception of environmental stimuli to the release of gametes[3]. Knowledge on the reproductive biology of a fish is of great importance for its rational exploitation through proper management of resources, development of selective brood stock, domestication and genetic improvement[4]. Habit destruction and rampant aquatic pollution resulting from anthropogenic intervention decimates the fish population to a great extent. Over exploitation is another major factor that contributes to the decline of the rich aquatic wealth. As fishes provide cheap and delicious food for our consumption, development of aquaculture techniques for indigenous species seems to be the only solution to meet the challenges of our times. The spawning season is May to July during which gonads enlarge and attain maturity. The males and females expel their gametes to the surrounding water bodies where fertilization takes place. The eggs hatch to larvae and the larvae attain sexual maturity around one year.

Recently, there is a renewed interest in diversifying aquaculture from the traditional exotic carps to indigenous and locally available fish species. Considering the indigenous status of the fish, together with its appeal in the domestic market as a food fish as well as ornamental variety, we thought of assessing the potential of the species for aquaculture. The present study focuses on the assessment of fecundity of *A. melettinus muriyadensis.*

Materials and methods

Muriyad carplet *A. melettinus muriyadensis* (Plate I) were collected from three different regions of Muriyad Wetland namely, Thommana canal, Konthipulam fields and Nandhi canal. Monthly collections from these 3 sites were done and the Fish were brought to the laboratory and grouped according to size. Each fish was then weighed up to the nearest gram using electronic balance (Shimadzu AY series). They were then measured up to the nearest centimetre (cm) and their total length (TL: from the tip of the snout to the tip of the caudal fin) and standard length, (SL: from the tip of snout to the tip of the caudal peduncle) were determined. Then the fish were dissected and the gonads were removed, weighed and thoroughly examined for the determination of sex and maturity stages. The gonads were categorized to 5 different maturity stages as described by El-Greisy[5].

Fecundity

Fish of advanced stage of maturity were selected and ovaries were preserved in 4%

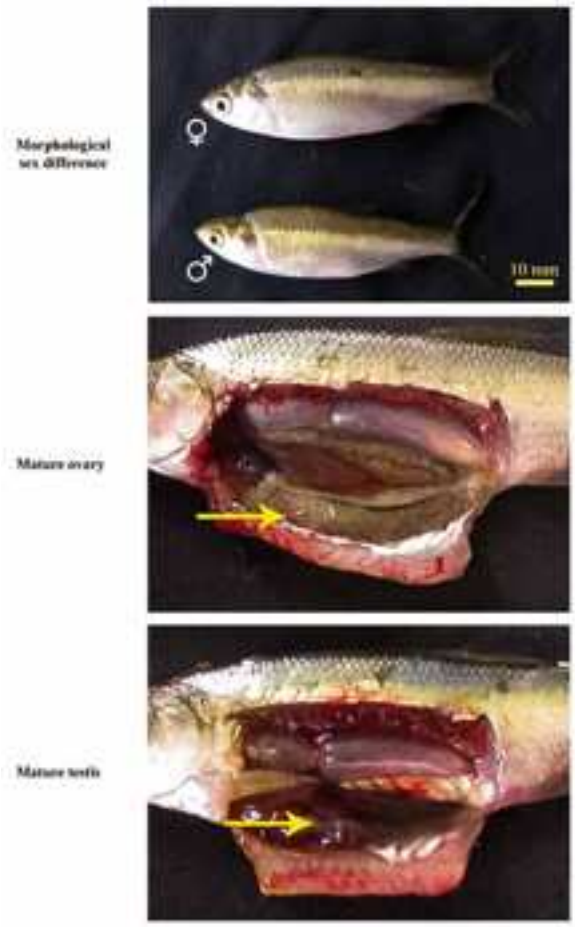

Plate1. Sexual dimorphism in *Amblypharyngodon melettinus muriyadens*

neutral formalin. 0.1 gm of the middle part of the ovary was taken and the number of eggs in these samples was determined. The total number of eggs in the ovaries was estimated using the following formula:

Total number of eggs =

$$\frac{\text{Weight of ovary} \times \text{Number of eggs in the sample}}{\text{Weight of sample}}$$

The mean values were recorded as the absolute Fecundity.

Relationship between the fecundity and fish weight, fish length, ovary weight were established by applying the method of least square, i.e.

$$Y = a + b x$$

Or in logarithmic form

$$\text{Log } Y = \text{Log } a + b \text{ Log } X$$

Where Y = Fecundity, X = Body measurements such as body length / body weight / ovary weight a (intercept) and b (slope) are the constant.

Results

Fecundity was estimated in 30 specimens of *A. melettinus muriyadensis* with ripe ovaries. The number of ripe ova was found to vary from 2031 to 9962 in fish ranging in total lengths 77.5mm to 120mm, body weight between 4.10g and 14.73g and total ovary weight ranging from 0.26g to 1.12g. Fecundity was related to the total length of fish, body weight and total gonad weight using regression equations.

The relationship between fecundity (F) and total length of fish (L) was found to be linear as follows (Fig. 1).

$$\text{Log } F = 2.8606 + 0.7433 \text{ log } L$$

$$F = 735.4\ (L)^{0.7433},\ r=0.233$$

F was found to be linearly related to the total body weight (W) as follows (Fig. 2).

$$\text{Log } F = 3.2722 + 0.3656 \text{ log } W$$

$$F = 1872(w)^{0.3656},\ r=0.3531$$

Fecundity was fond to be linearly related to ovary weight (OW) as follows (Fig. 3).

$$\text{Log } F = 3.7338 + 0.49375 \text{log } OW$$

$$F = 5418(OW)^{0.49375},\ r = 0.784965$$

Discussion

The onset of maturity varies considerably among different species of fishes, within different populations of the same

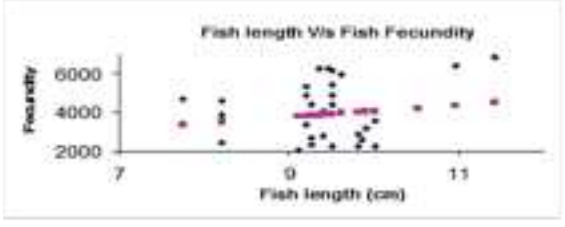

Fig. 1 Relation between fecundity and total length of A. melettinus muriyadensis

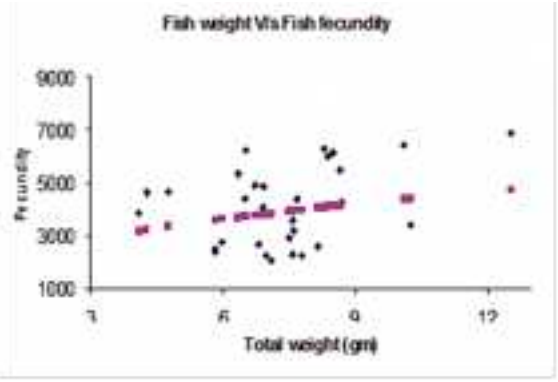

Fig. 2 Relation between fecundity and total weight of A. melettinus muriyadensis

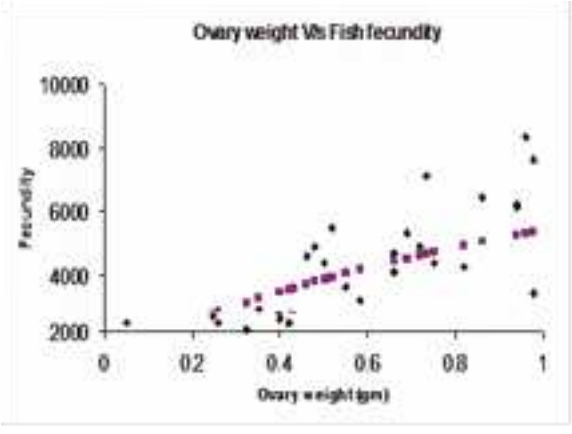

Fig. 3 Relation between fecundity and ovary weight of A. melettinus muriyadensis

species, and also within the limits of a single population[6]. Teleosts exhibit different spawning periodicity and many fishes are seasonal breeders. In Indian subcontinent most of the freshwater fishes breed during monsoon[7]. Many cyprinid fishes as *Cirrhinus mrigala* (July-August); *Labeo rohita* (July-August); *Labeo calbasu* (July-August); *Puntius sarana* (Late June to early September); *Puntius amphibius*, *Puntius parrah*, *Puntius chola* (May-July) show its peak spawning during monsoon months[8]. *A. melettinus muriyadensis* gonad shows five different stages of development. The fish collected from 3 different sites showed almost same developmental stages. In several other teleosts, left and right gonads are generally of equal length (e. g. *Ompok malabaricus*), but sometimes one may be larger (e. g. *Channa striatus*, *Puntius* sps.) In *A. melettinus muriyadensis*, the right ovary and testes are longer than that the left one. The fecundity in fishes is often correlated with length, weight and age of fish and also with the length, weight and volume of the ovary. In *A. melettinus muriyadensis* it was found that the fecundity increased at a rate of 1.2 times of the fish length. In *Labeo gonius* it increases at the rate of 2.27 times of the fish length[9]. In addition to these, many have described different relationships between length and fecundity of different species[9, 10,11,12,13]. In teleosts, fecundity estimations fluctuate from a few hundred to several lakhs. In *A. melettinus muriyadensis*, a straight line relationship was obtained between the weight, length, ovary weight and fecundity.

Several workers reported a straight line relationship between the fish weight and fecundity[14 ,15]. A close correlation is usually expected between ovarian weight and fecundity. The number of egg production more closely depends upon weight of the ovary. In this fish also a higher correlation could be seen between fecundity and ovary weight[16]. Various factors like food availability, rain fall and salinity of water and genetic difference of the stock affect fecundity of fishes causing variation among population of different water bodies and even in the same habitat in different years[17]. The quality and quantity of food consumed by the parent population determine not only the fecundity but also the quality of the sexual products and the viability of the offsprings[18]. Lowering of fecundity is said to be due to poor food intake in Stickleback [19, 20].

Conclusion

At present there is a high demand for edible fresh water fishes in domestic and international markets. As *A. melettinus muriyadensis* is a delicious indigenous food fish which is well adapted for backyard farming. *A. melettinus muriyadensis* being an ornamental fish, induced breeding of it seems to be economically valuable. We hope the present study will contribute for the conservation and sustainable management of the fish population in our state.

The scientific management of inland fishery resources essentially needs to consider the community ownership of the resource. Owing to the degraded nature of the aquatic environments, the thrust of the management strategy should be on conservation of stocks and biodiversity. While in the lacustrine system, the focus should be on optimization of production by various bio-manipulation practices, in the open water fisheries, the focus may be on enhancement for breeding and management strategies.

Successful resource management undoubtedly depends on the increased participation of stake holders, groups and communities. Such an effective partnership and linkage by close involvement of the local interest will go a long way to ensure protection of the resources and environment.

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A Study on the Expression of Some Selected Human Morphogenetic Traits in Thrissur District

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Abstract

Morphogenetic characters are physical characters of an individual and the pattern of inheritance of these traits is autosomal dominant as well as autosomal recessive. The present study was conducted to screen random population for ten different morphogenetic traits *i.e.* ear lobe attachment, dimples, crossing of thumb, crossing of arms, widow's peak, tongue rolling, chin cleft, arched foot, Hitchhiker's thumb, handedness to find out the frequency of expression in individuals, which is dominant or recessive. A total of 1130 individuals were subjected to this study. The prevalence of these morphogenetic traits was observed as; dominant traits such as free ear lobe (65.5%), able to tongue rolling (53%), arched foot (67%), straight thumb (63.6%), right handedness (97.6%), etc. are expressed more frequently in the population. But, same time the recessive characters like absence of dimples (79.5%), crossing of thumb right over left (56.9%), absence of widow's peak (70%), crossing of arm left over right (53.8%), smooth chin (76.2%), *etc,.* are expressed more frequently in the population. We analysed whether a trait is dominant or recessive. The survey result shows a variation of this argument, it shows that some typical dominant character was not expressed but the expression of recessive character was prevalent as in the case of widow's peak (absent), smooth chin, crossing of thumb; right over left, Hitchhiker's thumb (straight), absence of dimple, *etc,* all of which are recessive traits.

Keywords: Morphogenetic traits, Human genetics. Hitchhiker's Thumb. Widow's peak. Dimples

Introduction

Genetic variability is the characteristics of living things, especially in human beings. They show varieties of morphogenetic characters among one population itself[1]. Morphogenetic characters are physical characters of an individual and the pattern of inheritance of these traits is autosomal dominant as well as autosomal recessive [2]. Human population provides an exclusive opportunity to study the morphogenetic variation among the endogamous populations living in different geographical and ecological circumstances [3]. The presence of genetic variation in man is controlled by many factors including assortment, migration, and genetic drift[4]. Human genetics deals with the study of inheritance as it occurs in human beings. The advancement and research in the field of human genetics have made great socio economic contribution for human welfare. The principle of genetics concern largely with an explanation of the differences existing among individual [5]. It helps in analysing the potentialities of individuals already leaving as well assign predicting the trait of future offspring from a given mating[5].

In this study, we discuss some human traits which are more prevalent in our community. When one learns about dominant and recessive alleles, there is often a misconception that dominant alleles are the most common and they will tend to crowd out the recessive alleles in course of time. The frequency of a character in a population is related to whether its phenotypic effect is favourable or unfavourable. It was only a

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preliminary attempt to study the expression of some traits in a population of Thrissur .The main objectives of this study were to assess the frequently expressed morphogenetic traits among the population of selected areas of Thrissur district and also to check which trait is more dominantly or recessively expressed in the population and to make a comparison of male and female percentage difference in the expression of the traits.

Materials and Methods

Study area

i) The survey was conducted in randomly selected areas of Thrissur district (Fig. 1).

ii) Data collection and tabulation

The Survey method was chosen for data collection. We had prepared a data collection table, which include the individual's name, sex, age, and trait, whether it as a dominant or recessive trait based on the phenotypic expression in that individual. We preferred the subjects within the age range of 10 to 70 years. A total of 1130 individuals were observed for 10 different morphogenetic traits such as Ear lobe, Dimples, Crossing of thumb, Crossing of arms, Widow's peak, Tongue rolling, Chin cleft, Arched foot, Curved thumb, Handedness, from the random population. Out of 1130 subjects, 565 were females and 565 were males.

Following are the description of selected morphogenetic traits, we have observed for dominance and recessiveness that can be easily observed in people around us.

1. Earlobe attachment

If earlobes hang free, they are detached. If they attach directly to the side of the head, they have attached earlobes [6]. This is due to a gene that is dominant for unattached ear lobes and recessive in the case of attached ear lobes. If more people have attached earlobes then that is the most prevalent [7].

2. Dimples

Dimples are round indentations in the cheeks when smiling, not lines or clefts. They are reportedly due to a single gene with dimples dominant and a lack of dimples recessive [8].

Fig.1 Study area (source: http://www.thehindu.com/news/national/kerala/)

3. Crossing of Thumbs

In a relaxed interlocking of fingers, left thumb over right results from having one or two copies of the dominant version of the gene [9]. People with two recessive place right thumb over left.

4. Crossing of arms

When the arms are folded across the chest, having the left arm positioned on top is the dominant characteristic whereas having the right arm positioned on the top is the recessive characteristic [9].

5. Widow's Peak

A widow's peak or the mid-digital the hairline is due to expression of the gene for hairline. This gene has two alleles, one for widow's peak and one for the straight hairline. The widow's peak allele is dominant and the straight allele is recessive [10].

6. Rolling of Tongue.

Tongue rolling is the ability to roll the lateral edges of the tongue upwards into a tube. If you can roll the lateral edges of your tongue together, then this means you have inherited a dominant trait. Those who are unable to do so are expressing inheritance of recessive gene for tongue rolling [11].

7.Chin

People can have a cleft chin or smooth chin. Cleft chin or dimple chin refers to a dimple on the chin. It is a y- shaped fissure on the chin with an underlying bony peculiarity. This is an inherited trait in humans, where the dominant gene causes the cleft chin, while the recessive genotype presents without a cleft [12].

8. Foot

In humans, the allele for having a foot with normal arches is dominant. The allele for flat foot is recessive. People with arched foot have a distinct curve along the inside of the foot with a band slightly less than half the width of the foot connecting the heel & toe. Flat foot refers to a change in foot shape in which the foot does not have a normal arch when standing.

9. Hitchhiker's Thumb

Thumb can be straight or curved (Hitchhiker's thumb). Straight thumbs can be seen as nearly a straight line and may contain a slight arch when viewed from the side. A Curved thumb is dominant over a straight thumb.

10.Handedness

The gene for right-handedness is dominant and the gene for left hand is recessive. Thus, majority of the people have inherited the dominant gene resulting in right-handedness.

iii) Statistical analysis

After data collection a primary consolidation of data was done. Appropriate graphical representation and statistical analysis (Student's paired T test (two tailed), Spearman's correlation-significance level 5%) was also done.

Result and Discussion

A total of 1130 individual were observed for morphogenetic traits - Ear lobe, Dimples, Crossing of thumb, crossing of arms, Widow's peak, Tongue rolling, Chin cleft, arched foot, Hitchhiker's thumb and Handedness from the random population.

The results show the clear cut view of the expression of traits in each individual in the population. A graph (Fig.2) were plotted for dominant and recessive traits classified based on their expression. Out of 1130 individuals, 599 individuals were capable to roll their tongue and others were not able of rolling their tongue. About 741 individuals of the population have free ear lobes and 44.9% has attached ear lobes. The survey showed that 231 of the population had facial dimples and 899 subjects are without this trait. About 339 subjects are having widow's peak in their head and 791 individuals are not having this trait. About 719 individuals have curved thumb and 411 subjects have straight thumb. Out of 1130 individuals, 758 individuals are having arched foot and 372 are having flat foot.

The results showed that 268 individuals are having cleft chin and 862 individuals are

Table 1: The percentage wise difference of dominant and recessive traits between male and female

Trait	Number of male dominant	Number of male recessive	% of dominant males	% of recessive males	Number of female dominant	Number of female recessive	% of dominant female	% of recessive female
Ear lobe	351	214	62.12	37.87	390	175	69.02	30.97
Dimples	116	449	20.53	79.46	115	450	20.35	79.64
Crossing of thumb	331	234	58.58	41.41	323	242	57.16	42.83
Crossing of arms	289	276	51.15	48.84	232	333	41.06	58.93
Widow's peak	182	383	32.21	67.78	157	408	27.78	72.21
Tongue rolling	339	226	60	40	260	305	46.01	53.98
Chin	153	412	27.07	72.92	115	450	20.35	79.64
Foot	404	161	71.50	28.49	354	211	62.65	37.34
Thumb	365	200	64.60	35.39	354	211	62.65	37.34
Handedness	549	16	97.16	2.83	555	10	98.23	1.76

having smooth chin. The pattern of crossing of arms and thumbs were also surveyed, here results showed that 644 individuals are cross their thumb in a pattern of right over left, and others are cross their thumb left over right.521 individuals cross their arms right over left and 609 individuals cross their arms left over right. Right Handedness were showed as one of the most dominant trait in the survey, this shows a clear differentiation between dominant and recessive characters in handedness trait, only 26 individuals are have left handedness.

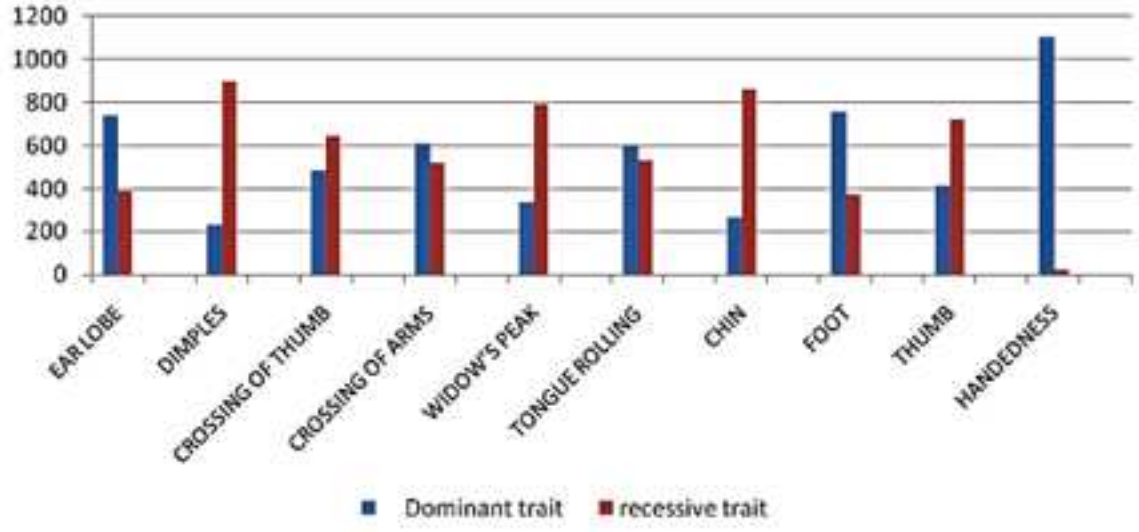

Fig. 2. Difference between dominance and recessiveness of the trait among total subjects.

Table.1 shows the percentage wise difference between male and female subjects. About at 46% female and 60% males were able to roll their tongue. About 53.8% of the population cross their arms right over left. Fifty nine percent of female and 48.8% male had crossed their arms right over left. Fifty seven percentage of the sampled individuals cross their thumb right over left; 58.8% male and 57.1% females sampled showed this trait. The survey showed that 20.4% of the population had facial dimples. Only 20.3% of females and 20.5% of males showed this trait. About 23.7% subjects have chin cleft; 20.3% of females and 27% of male subjects had this trait. About 30% of individuals possess widow's peak. Gender wise, 27.7% of females and 32.2% males had widow's peak. About 67% subjects have arched foot; 62.6% of females and 71.5% of males had arched foot. About 65.5% individuals have free ear lobes. Free ear lobes were seen in 62.1% males and 69% females. Posture of thumb was curved in 63.6% individuals; 64.6% males and 62.6% were females. Right Handedness was one of the prominent distinguishable characters in this survey; it shows about 97.6% of expression in individuals. Right handedness was expressed in 97.1% of males and 98.2%of females.

Fig. 2 shows that traits like ear lobe, crossing of thumb, arched foot, Hitchhiker's thumb, and handedness are expressed as dominant in both males and females. Whereas traits like dimples, widow's peak and cleft chin are expressed as recessive in both male and females. Some traits like crossing of arms and tongue rolling showed difference in the expression of alleles in both sexes with more males showing the dominant character and more females showing the recessive character.

The study found that there was no significant difference between the number of dominant and recessive characters expressed either in male and females. There was also no significant gender difference in the expression of dominant or recessive characters with most of the traits showing the same pattern of expression in either sex.

i) Male dominant and recessive

There is no significant difference between the expression of dominant and recessive characters of selected traits in males [(t

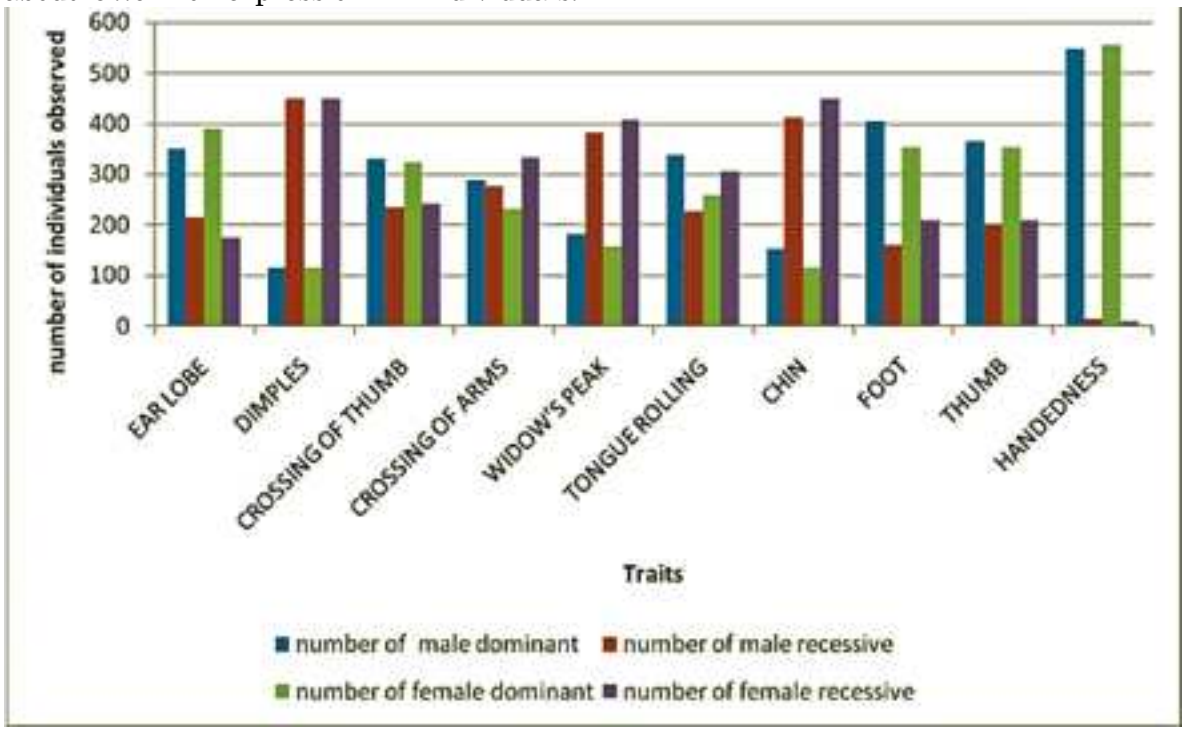

Fig.3. Percentage difference between dominant and recessiveness in male and females

(observed value) = 0.620, t (critical value) = 2.262) two tailed paired t-test at 5% significance for N (traits) =10. The risk to reject the null hypothesis H0 while it is true is 55.06%.

ii) Female dominant and recessive

There is no significant difference between the expression of dominant and recessive characters of selected traits in females [(t (observed value) = 0.069, t (critical value) = 2.262) two tailed paired t-test at 5% significance for N (traits) =10. The risk to reject the null hypothesis H0 while it is true is 94.69%.

iii) Male dominant and Female dominant

There is no significant difference in the expression of selected dominant characters in both males and females [(t (observed value) = 2.046, t (critical value) = 2.262) two tailed paired t-test at 5% significance for N (traits) =10. The risk to reject the null hypothesis H0 while it is true is 7.10%.

iv) Male recessive and Female recessive

There is no significant difference in the expression of selected recessive characters in both males and females [(t (observed value) = - 2.046, t (critical value) = 2.262) two tailed paired t-test at 5% significance for N (traits) =10. The risk to reject the null hypothesis H0 while it is true is 7.10%.

We analysed whether a trait is dominant or recessive, and if the expression happens based on dominance. The survey result shows a variation of this argument; it shows that some typical dominant characters were not expressed but the expression of recessive character was prevalent as in the case of widow's peak. Presence of widow's peak is a dominant character in population but, as per our study the absence of widow's peak (regarded as a recessive character) was more prominent in the population. The same was in the case of cleft chin, crossing of thumb; right over left, Hitchhiker's thumb (curved), absence of dimple, etc, all of which are recessive traits. In one study conducted in Nigeria, on Morphogenetic traits combination pattern amongst the population shows some significant results like morphogenetic combinations might be rare in that population[13].

Study of the inheritance pattern of human traits is one of the significant methods for analysing the genetic history of a population [14]. It has an important role in the determination of the chance of occurrence of genetic diseases in the family otherwise in the society, pedigree analysis is one of the basic and important methods of genetic analysis of human families. It will study and trace their ancestral inheritance patterns and reaches at accurate understandings about the family history [15].

We conclude from our study that unlike the preconceived notion that dominant alleles are always expressed more, the expression of dominant and recessive characters may vary and may depend on various factors.

Acknowledgement

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