Proceedings of the International Conference on
Creating and Enabling Future through
Science, Technology and Innovation: Dynamics
and Challenges for Development Endeavors

Date: 18th and 19th May 2017; Venue: Nekemte, Ethiopia.

Editors
Dr. Eba Mijena
Dr. Hirpa Legesse
Dr. Diriba Diba
Dr. Raghavendra HL
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Date: 18-19 May 2017; Venue: Wollega University, Nekemte, Ethiopia.

Actors and Linkages in the Innovation System

Editors
Dr. Eba Mijena
Dr. Hirpa Legesse
Dr. Diriba Diba
Dr. Raghavendra HL

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A International Conference on
Creating and Enabling Future through Science, Technology and Innovation: Dynamics and Challenges for Development Endeavors

Date: 18-19 May 2017

Thematic Areas

THEME 1: SCIENCE RESEARCH FOR DEVELOPMENT ENDEAVORS
- Health (Human and Animal) Research
- Biodiversity, Biotechnology, Nanotechnology and Material Science Research
- Research and Development in Applied Sciences
- Computational (Physics, Chemistry and Biology) Research
- Mathematical Modeling for Multidisciplinary Applications
- Environmental and Earth Sciences Research

THEME 2: ENGINEERING AND TECHNOLOGY RESEARCH FOR DEVELOPMENT
- Construction Technology and Management Research
- Industrial Process Improvement and Product Design and Development
- Communication and Information Technology Research
- Water, Irrigation and Energy Technology Research
- Highway and Transportation Engineering and Management
- Electrical, Electronics and Telecommunication Engineering
- Automation and Industrial Mechanization
- Architecture and Urban Development

THEME 3: POLICIES FOR INNOVATION, TECHNOLOGY TRANSFER AND COMMERCIALIZATION
- Innovation and Entrepreneurship Development in the Tropics
- Technology Transfer and Commercialization Strategies for Development
- Ethiopian and International Research Policy for Development Endeavors

THEME 4: SCIENCE, TECHNOLOGY AND INNOVATION FOR SUSTAINABLE DEVELOPMENT
- Environment, Pollution and Strategies to Cope up with Climate Change
- Re-cycling and Re-engineering: Dynamics and Challenges
- Economic, Social and Environmental Analysis of Green Technology

THEME 5: DYNAMICS & CHALLENGES OF SCIENCES, TECHNOLOGY AND INNOVATION IN THE 21ST CENTURY
- Dynamics of Science, Technology and Innovation in the 21st Century
- Challenges of Science, Technology and Innovation in the 21st Century
Preface

Welcome you to this volume of the proceedings of a International Conference on “Creating and Enabling Future through Science, Technology and Innovation: Dynamics and Challenges for Development Endeavors”, which was held on 18th and 19th May 2017 at Wollega University, Nekemte, Ethiopia. In this proceeding, the opening and welcome addresses, the keynote addresses and key technical papers presented on the conference have been compiled. Conferences traditionally take a broad approach to thinking and cognition, in all their various aspects and manifestations, and this is broadly reflected in the content of the various papers submitted for publication in this proceedings. The papers are from researchers working in academia and research institutes. All the papers are compatible with the core thematic areas requested for the conference. The publication of the papers aimed at importance of Science, Technology and Innovation towards development endeavors and avail it to the wider audience.

Science, Technology and Innovation (STI) has globally emerged as one of the major drivers of national development. Science and technology influence society as never before. Scientific achievements continue to push back the frontier of knowledge and increasingly contribute to the technological progress that affects the way we live and work. Scientific advances and technological change are important drivers of recent economic performance and is a key to long-term growth and higher standards of living. In the unfolding scientific revolution and industrial changes, science and technology has become an increasingly important contributor to socioeconomic progress. Moving upwards not only staying top on the global economy necessitates that a country excels in science, technology, and innovation. The ability to create, distribute and exploit knowledge has become a major source of competitive advantage, wealth creation and improvements in the quality of life. The stages of development of a country may vary depending on whether it is factor, efficiency, or innovation driven.

The heart of the long run economic growth in the all economic growth models is technological change and innovation. On the other hand the heart of technological change and innovations is scientific developments. In this context, countries must design economy policies in order to develop science-technology-innovation environment in the society and economy, leading sustainable economic growth and global competitiveness. In order to achieve science-technology-innovation based global competitiveness level, it is required the transformation of the knowledge-based
economy for the countries. The knowledge-based economy is an expression coined to describe trends in advanced economies towards greater dependence on knowledge, information and high skill levels, and the increasing need for ready access to all of these by the business and public sectors. Knowledge and technology have become increasingly complex, raising the importance of links between firms and other organisations as a way to acquire specialised knowledge. A parallel economic development has been the growth of innovation in services in advanced economies. One must design and develop science-technology innovation based competitiveness, economic growth and development strategies by improving the conditions for research and development, qualified human capital, infrastructure, higher education, cooperation between the state, industry and university, information and communication infrastructure, accessing the internet, patent protection laws, royalty fees, financial, institutional and structural deficiencies, government policies and externalities.

It is found that the countries that have science-technology-innovation based economic policies and strategies have great superiority and sustainable competitive advantage in not only global competitiveness but also economic growth and development leading to wealth and welfare of the country. Advances in science-technology-innovation are main driving engine of global competitiveness, economic growth and development in both in economic theory and country practices. Therefore, countries can direct global competitiveness, economic growth and development in the long run by applying appropriate economic policies stimulating developments in science-technology-innovations.

In Ethiopia research is needed to address the resolution of major social and economical problems; contribute to the achievement of national development objectives; and to meet technology demand. However, it is learnt that there is a gap between the research activities and focuses in higher education and research institutions and the national development need. Hence, the national research system should be strengthened and orientated to focus on the national technological demands for searching for, learning about, adapting and utilizing effective foreign technologies.

Universities, research institutes, TVET institutions and industry can be demonstrated to be core actors in the national innovation system. The strength as well as effectiveness of the established linkages among these institutions largely depends on their tendency and capability to be involved in activities dealing with technology transfer. As far as
technology learning is concerned, the current situation of our country confirms that universities are not taking the leading role and are lagging behind the industries. Therefore, the linkages that exist among these actors should focus on contributing to capacitating the productivity of manufacturing and service providing enterprises. The shared effort should also focus on identifying appropriate technologies and their sources, understanding the technologies through learning-by-doing and adaptation as well as effective utilization.

The Conference Purpose and Thematic Areas
The purpose of this conference is to provide platform for stakeholders from different areas related to Science, Technology and Innovation in order to present and discuss on the practical problems of STI towards development of Ethiopia and prospects based on research outputs, ideas, development and applications in all areas of STI in Ethiopia. Researchers, Scholars, Policy Makers and professionals working in the Ministries, Universities, Research Institutes, Non-government Organizations, Investors, TVET's and different offices are invited to exchange ideas and experiences, and to showcase methods and innovations relevant for agricultural development in Ethiopia. The main thematic areas of the conference are as follows,

Theme 1: Science Research for Development Endeavors
Theme 2: Engineering and Technology Research for Development
Theme 3: Policies for Innovation, Technology Transfer and Commercialization
Theme 4: Science, Technology And Innovation for Sustainable Development
Theme 5: Dynamics and Challenges of Sciences, Technology and Innovation in the 21st Century

Organization of the Proceedings
This publication is arranged into three main sections. The first section is comprises the opening addresses given on the formal commencement of the conference. The conference had formal welcome addresses from Dr. Eba Mijena, President, Wollega University, Nekemte, Ethiopia and opening speech from Ato Mr. Moges Edae, East Wollega Zone Administrator and WU Board Member. The second section contains keynote addresses made by Dr. Abera Deressa, Former State Minister of Ministry of Agriculture, and WU Board Member, Prof. Endashaw Bekele, Addis Ababa University, Dr. Adugna Woyessa, Ethiopian Public Health Institute, Dr. Dawit Tesfaye Institute of Biotechnology Research and Mr. Wasihun Tamirat, Ministry of Science and Technology, Addis Ababa. Third section comprises those plenary addresses for which presenters made detailed papers available. It is unfortunate not to include all papers presented in the two days conference because of lack of space.
Papers published in here were submitted as formal research papers by authors, and were subject to a peer review and editing process conducted by a panel of academics from Wollega University, Nekemte, Ethiopia. These papers were also proof-read and edited for English style, grammar and syntax. The editors of these papers trust that the editing of certain English expressions, grammar, and so on, have not changed the central meaning and content of the papers, and that these remain true to the authors’ intent. Therefore, the views expressed therein are entirely those of the authors. We would like to thank all those who sent their papers in time.

Editors

Dr. Eba Mijena  
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## Abbreviations

<table>
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<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>AAU</td>
<td>Addis Ababa University</td>
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<tr>
<td>AFLP</td>
<td>Amplified Fragment Length Polymorphism</td>
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<tr>
<td>AMH</td>
<td>Anatomically Modern Humans</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ATA</td>
<td>The Agriculture Transformation Agency</td>
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<tr>
<td>CBD</td>
<td>Convention on Biological Diversity</td>
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<td>cm</td>
<td>Centimeters</td>
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<td>CSA</td>
<td>Central Statistical Agency of Ethiopia</td>
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<td>CV</td>
<td>Coefficient of Variation</td>
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<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>EBF</td>
<td>Exclusive Breast Feeding</td>
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<td>EBTi</td>
<td>Ethiopian Biotechnology Institute</td>
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<td>EBW</td>
<td>Electron Beam Welding</td>
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<td>EC</td>
<td>Ethiopian Calendar</td>
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<td>EIB</td>
<td>The Ethiopian Institute of Biodiversity</td>
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<td>ENACTS</td>
<td>Enhanced National Climate Services</td>
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<td>ENEA</td>
<td>Ethiopian National Examination Agency</td>
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<td>FAO</td>
<td>The Food and Agriculture Organization</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FAU</td>
<td>Forensic Acquisition Utilities</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FDRE</td>
<td>The Federal Democratic Republic of Ethiopia</td>
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<td>FNS</td>
<td>Food and Nutrition Security</td>
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<td>GC</td>
<td>Gregorian Calendar</td>
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<td>GCMS</td>
<td>Gas Chromatography-Mass Spectrometry</td>
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<td>GDP</td>
<td>Gross Domestic Product</td>
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<td>GMCs</td>
<td>Genetically Modified Crops</td>
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<td>Description</td>
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<tr>
<td>GTP</td>
<td>Growth and Transformation Plans</td>
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<td>HEIs</td>
<td>Higher Education Institutions</td>
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<td>ICT</td>
<td>Information and Communications Technology</td>
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<td>IFPRI</td>
<td>International Food Policy Research Institute</td>
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<td>ISAAA</td>
<td>International Service for the Acquisition of Agri-biotech Applications</td>
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<td>ISSR</td>
<td>Inter Simple Sequence Repeats</td>
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<td>IT</td>
<td>Information Technology</td>
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<td>JU</td>
<td>Jimma University</td>
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<tr>
<td>m.a.s.l</td>
<td>Metres above sea level</td>
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<td>MDGs</td>
<td>Millennium Development Goals</td>
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<td>mm</td>
<td>Millimetre</td>
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<td>MoA</td>
<td>The Ministry of Agriculture</td>
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<td>MoE</td>
<td>Ministry of Education</td>
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<td>MoST</td>
<td>Ministry of Science and Technology</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
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<td>NVI</td>
<td>National Veterinary Institute</td>
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<tr>
<td>OD</td>
<td>Optical Density</td>
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<td>ONRS</td>
<td>The Oromia National Regional State</td>
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<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
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<tr>
<td>RAPD</td>
<td>Random Amplification of Polymorphic DNA</td>
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<tr>
<td>SAW</td>
<td>Submerged Arc Welding</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<tr>
<td>SS</td>
<td>Stainless Steel</td>
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<td>STI</td>
<td>Science, Technology and Innovation</td>
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<tr>
<td>UNICEF</td>
<td>United Nations Children's Fund</td>
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<tr>
<td>WB</td>
<td>World Bank</td>
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<td>WHA</td>
<td>World Health Assembly</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>WU</td>
<td>Wollega University</td>
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Welcome Address

By

Dr. Eba Mijena
President, Wollega University, P.O.Box 335, Nekemte, Ethiopia

Your Excellency Mr. Moges Edae, East Wollega Zone Administrator & WU Board Member
Your Excellency Dr. Abera Deressa Former State Minister, Ministry of Agriculture and WU Board Member
Your Excellency Prof. Endashaw Bekele, Professor of Genetics and Member of the World Academy of Sciences

Distinguished Guests, Paper Presenters, Ladies and Gentlemen and Dear Participants,

It is my pleasure and honor to welcome you all to this international conference on “Creating and Enabling Future through Science, Technology and Innovation: Dynamics and Challenges for Development Endeavors” organized by our University. I would like to thank keynote speakers, invited guests, paper presenters, media personnel and participants taking their valuable time, and came over here in order to share their valuable experiences, ideas and to contribute their best for the grand success of the conference. Honorable guest, researchers, scientists and scholars, please accept my warm greetings this morning on this special event!

Excellencies and Ladies and Gentlemen,

The title of the conference is timely and very appropriate, as science and technology are continuously evolving, influenced by structural shifts in the world economy, the steady globalization of innovative activity, the rise in new actors and new ways of innovating. It is hoped that it fosters a better learning space in the area of STI for all of us. It is clear that the world is shaped by STI. We need technological innovations in education, in agriculture, in medicine or health care, construction, and all activities for better and quality of life. Science and technology unlocked the keys to mankind’s mastery over nature. Let me mention what President Barack Obama stated once in his talk regarding the role of science in life. He said “Science is more essential for our prosperity, our security, our health, our environment, and our quality of life than it has ever been before.” STI is a key to long-term growth and higher standards of living. In the unfolding scientific revolution
and industrial changes, science and technology has become an increasingly important contributor to socioeconomic progress. Moving upwards not only staying top on the global economy necessitates that a country excels in science, technology, and innovation. The stages of development of a country may vary depending on whether it is factor, efficiency, or innovation driven.

Dear Participants,

The scientific revolution began about 400 years ago with the work of Galileo, and the technology spawned from this ongoing revolution transformed the world and it will continue to do so. Communications were primitive, only crude telephones existed and there was no radio or television, no computers, no internet. The average person knew little of the rest of the world. Transportation was slow and there were no vehicles or airplanes. There was no knowledge of the subatomic world, no computers, etc. Indeed, most of the work that people do today is in areas that did not exist back then and is based on the technologies derived from the scientific revolution begun by Galileo 400 years back. Science as the search for truth is always there as far as the world exists and so does innovation as far as we live in a competitive world.

Today the world is under various global challenges. To address these, the STI plays paramount roles. For example, climate change, loss of biodiversity, loss of topsoil, disease threats and the consequences of population ageing are some of the major complex challenges facing the globe today. Ethiopia is not exceptional and faces similar challenges. So, what would the solution be? Breakthroughs in science and technology are needed to address such global challenges in cost effective ways.

Ladies and Gentlemen,

If we agree on the importance of STI, I believe it is important to answer how the STI positively affects development. Many agree that for the STI to be a driver for sustainable development, it is important that Development Agenda are people-centered, creating an enabling environment for the power of the STI to be a cause for development. This implies that countries in the globe have to develop, implement and monitor their STI policies and programs that promote knowledge production, dissemination and utilization as well as the development and appropriation of technologies that spur innovation not only at large production facilities but also at grassroots level as part of a broader development agenda. These frameworks require that special attention is given to human capital development, a fundamental block of any sustainable development agenda, and to governance.
mechanisms that promote broader participation in decision making in the STI related issues.

We obviously observe that technological developments influence society and vice versa. For example, the genetic modification of crops has already made it possible to increase their yield, protect them from insects and pests. Using such technologies, human beings are able to modify plants and possibly animals and other living organisms. Technological innovations shaped the world and will continue to shape even in the future. Among the technologies which shaped this world is the information and communication technology. Information and communication technologies developed rapidly and will continue to rapidly develop. It seems certain that people everywhere will be in more or less constant communication and interaction with each other through mobile phone, Internet, teleconferencing, and GPS technology today. The Internet and its successors will enable unprecedented exchange of information and knowledge across the globe. The Internet already is bringing together like-minded people from different cultural and economic environments.

What are the driving forces for technological innovations? Technologies change with the existing situations. The technological innovations which existed in the past are completely different from those which we have today. The ancient technologies used to solve the then societal problems. Technologies of today however serve to solve these days’ challenges. This means that the forces driving the technologies may also change in the future. Such knowledge will help man to be aware of the dynamisms and challenges of STI in development endeavours and accordingly prepare himself.

Ladies and Gentlemen and Dear Participants,

Is it possible to create enabling future through science, technology and innovation? If ‘Yes” how? Enabling future cannot occur without complementary investments to create a supportive environment. The enabling future for the STI and through the STI encompasses factors that influence innovation positively but are controlled by policy domains. Given the resource limitations and numerous choices, investments in an enabling environment must be prioritized and sequenced with great care. This happens through the proper preparation of the STI policy. Managing existing technology and non-technological innovation also counts. National and international policies, including intellectual property systems, need to adapt to this evolving environment and address the special needs of different countries, especially for Ethiopia.
Distinguish Guests and Dear Participants,
It is also important to be aware of the risks, which might science, and new technologies create and how should policy makers prepare. While there is a consensus that science is playing significant role,” there is a danger that a lack of understanding of how science leads to the development of new technologies and applications will end up short-changing the long term and thus damaging the prospects for succeeding at what the policymakers are trying to do. There is the effect of science on technology and that of technology on science. It is not too difficult to observe that today’s technology is based on yesterday’s science; and today’s science is based on today’s technology.

Future technologies will have diverse positive economic and social implications. But other effects are potentially destructive. It is argued that human survival is in greater danger from the potential consequences of new technologies than is widely realized. Existential risks range from accidental release of destructive technology, to the unforeseeable consequences of scientific experiments gone wrong. Therefore, there must be serious attention and care in this regard. This caution should focus not only on today’s capabilities, or likely near-term advances, but also on developments that might take place decades ahead. Questions need to be examined concerning the demands that future technology combinations might place on national and international governance institutions as well as other actors with risk-management responsibilities. For instance, in different scientific-technological domains, assessments could consider which practical forward-looking risk reduction and pre-emptive measures need to be planned for and prioritised. Undertaking such work through national and international forum is particularly appropriate, given the global nature of many of the risks in question.

Distinguished Guests and Dear Participants,
We aspire to be the middle income country by 2025. How does this happen? Do the strategies and policies which we have at hand strong enough to transform our country? How do we solve the pertaining challenges we have today? I believe this shall happen by rigorously working on STI. Yet, there are lots of challenges in all our systems; we are still using backward technologies in agriculture, education, investments, etc. We must work hard to reverse this condition. We need to focus on major deliverables in all sectors focusing on STI which I hope, will be the outcome of this particular conference. It is expected to have a larger impact on the future intervention policies. We also hope that we would be able to provide for a wider dissemination of the existing knowledge and present experiences in the thematic areas indicated.
Excellencies, Ladies and Gentlemen,

The 176 Abstracts were submitted based on the call for paper for this conference. Out of these, only 82 (nearly 46.6% of the total papers submitted) papers were provisionally accepted of which 48 (nearly 27.3% of the total papers submitted) papers have been selected for today’s presentation based on their relevance and quality. More than 300 participants are expected from different universities, institutes, Wollega zones and Woredas. Sharing experiences on existing international trends and views becomes paramount important whereby conferences of this kind give opportunity for better understanding of the issues. It is hoped that the following questions will be answered during this conference:

- What roles does the STI play in creating enabling future?
- How does the STI play these significant roles?
- What challenges does the STI have in the development endeavours?
- How might the STI affect the future development endeavours?
- What measures must be taken as intervention mechanisms?
- What dynamics and challenges does the STI have on future development endeavours?

I believe that lots of valuable initiatives and policy issues will come out of it. Having said all this, finally, I would like to thank you all for your participation and friends and colleagues of Wollega University who have contributed a lot for conducting this conference.

So far we organized three national conferences. Yet, this forth conference is unique for some reasons. It is the exact time when we:

1. won our first mega research project under the industry theme form the Ministry of Science and Technology on the research topic entitled “Method Development, Optimization and Production of Shelf Stable Liquid Coffee Concentrate and Its Waste Management” proposed by Dr. Raghavendra HL, Dr. Hipa Legesse, Dr. Temesgen Garoma and Mr. Girmaye Kannasa. I take this opportunity to congratulate the team, and call other researchers to write proposals for possible grants from different agencies and conduct research in the broad area of STI.

2. Acquired our own teaching hospital, which we have been awaiting for the last ten years. Launch three different new journals in the areas of Health Sciences, Agriculture and Afan Oromo.
3. Celebrate our 10th anniversary which shall take place by this Saturday (May 20, 2017 here in this auditorium) which I take this opportunity to invite you all to join us.

Lastly, I wish you all a fruitful discussion and I look forward to welcoming you again to the conference and wish you all have the most pleasant time with us.

Thank you for your attention!
Opening Speech

By

His Excellency Mr. Moges Edae
East Wollega Zone Administrator

Honorable Guests!
Dear Presenters and participants!
Ladies and gentlemen!
Good Morning!

It gives me great pleasure and I am really honored to attend this International Conference.!!

First, Let me take this great opportunity to congratulate Wollega University on its 10th Anniversary celebration. It is really an important milestone in the educational and development history of Ethiopia.

In my opinion, this is not a celebration for its simple existence during the last 10 years but it is a celebration for giving a meaning for your presence in this region.

I am really proud to tell you this: It is a celebration for making yourself visible through your excellent contributions for the nation in general and wollega region in particular in difficult conditions.

Allow me to extend my best wishes to you all to continue your valuable services for the betterment of our citizens in the coming decades.

Let me to appreciate the organizers of this International Conference for - focusing on one of the critical aspects of our nation’s developmental journey, effective integration of science, technology and innovation for our nation’s development, which in fact has far-reaching consequences on our sustainable development and the future of our nation.

This International Conference is a major academic event which is, in fact, in line with our government’s committed efforts to incorporate science, technology and innovation for the accelerate economic growth and socio-economic transformation.
Equally, the role of science, technology and innovation to translate our developmental objectives is clearly outlined in each and every policy framework of our government. Allocation of 70% of student intake for science and technology shows the kind of importance that government attaches on this.

No doubt, Ethiopia is maintaining a double digit growth rate for more than a decade. Sustaining this rapid economic growth and ensuring structural transformation will not be possible without establishing strong national technological capability in our country. In other words, accelerated economic growth would not be sustainable without effective utilization of appropriate technologies.

Again, achieving the mission of bringing ‘middle income’ status for our country in the future needs a systematic integration of science, technology and innovation with our core national development agendas.

Today, we are in a globalized world, of course in a competitive world. Our global economic system is fully knowledge-based. We cannot integrate our economy with international economy if our products are not as per the global standards, we need to produce quality products to survive in the global competition. We need to add value.

This, of course, needs innovation, technological upgradation and high standard equipments, most importantly, well-trained skilled manpower.

Also, Let us not to forget our society at large.!
Today our society is facing numerous developmental challenges that need effective solutions. This can be possible only if we rightly apply science, technology and innovation.

In line with this, our National Science, Technology and Innovation Policy encourages transfer and adaptation of technologies from abroad while building the essential technological capabilities at home.

Similarly, the Ethiopian Higher Education Proclamation clearly calls upon the academic institutions to ensure that research and technology transfer should be in consistent with our country’s priority needs.

With significant expansion in the number of universities and research institutes in the country, there is an urgent need to develop our technological capabilities and to
effectively support our economic growth. Also applying right scientific tools in solving community problems is mainly emphasized.

However, today, there is a majority opinion that says "Universities are not taking the leading role and are lagging behind the industries in innovation and technology transfer."

This serious trend needs our special attention. I will appreciate if the organizer of this conference encourages discussion about it.

Basically, the accumulation of technical capabilities is not an easy process, and it will never happen in a short time. But continuous and sincere efforts must be made. Our higher educational institutions and specialized research centres need first to strengthen their capacities to fulfill what is expected from them.

To achieve this, the higher educational institutions should focus in effectively training our graduates in science- and technology and equip them well to address the pressing development challenges that are related to poverty, food security, climate change, urbanization and health. Of course, the selection of thematic areas for this conference truly highlights these issues. Let me to appreciate the organizers!!

We have to encourage our students to come up with creative and innovative ideas. We need to provide forum for discussing and sharing their innovation. Higher educational institutions should develop appropriate mechanism to identify and explore the true scientific potentials of our students. Efforts must be made to create interest among students in science, technology and innovation. Very importantly, we should have adequate infrastructure- like labs, training centres etc. with international standard.

I am sure, task like this kind of nature, involves collaborations and partnerships among different actors.

Therefore, the linkage between University-Industry and the relationship between University-community should be developed that must be based on mutual interest. A constant and regular interaction will serve the purpose well.

Also it is the right time for our educational institutions to think of not only imparting scientific knowledge among our students but encourage them to develop certain
values and attitudes which will help them in applying scientific knowledge to address developmental problems facing our society.

I am sure, the scientific knowledge shared during this conference and innovative ideas exchanged here will serve the nation in its dynamic journey towards development.

Again, I congratulate Wollega University for making sincere efforts in bringing a number of well-known scholars, researchers, academicians and practitioners to share their experiences, best practices, innovative ideas and research outputs on this critical issue of our national development!

I wish all the presenters and participants a good time.

With these words, I officially open this International Conference.

Thank you!
 Boost your Memory with Food

**Human nutrition** refers to the provision of essential nutrients necessary to support human life and health. Generally, people can survive up to 40 days without food, a period largely depending on: The amount of water consume stored body fat, muscle mass and genetic factors.

**Major Classes of Nutrients**

The seven major classes of nutrients are: carbohydrates, fats, fiber, minerals, proteins, vitamins, and water. These nutrient classes are categorized as either macronutrients (needed in relatively large amounts) or micronutrients (needed in smaller quantities). The micronutrients are minerals and vitamins and the rest listed are macronutrients.

**Food Security**

- The concept of food Security and insecurity is complex
- Governs the essence of future of humanity and human well being

Food security requires a transdisciplinary approach. Transdisciplinary approaches offer innovative methodologies for high-impact science through understanding and taking action on complex societal problems that cannot be approached and solved by single disciplinary...
approaches. Promotes the cognitive age that followed the physical, chemical and biological age.

The essence of future of humanity and human wellbeing Depends on:

- The enhancement of basic biological capacities to effectively develop S & T and utilize the benefits from this to sustain Food and Nutrition Security.
- Capacity to overcome conditions of profound uncertainties through potentially transformative technologies such as advances in genomics, nanotechnology, material science, artificial intelligence and capacity of not selling the future.
- Realization of the Supply side of the food equation’s difficulties to expand beyond food production and acting as civilized society with the capacity to care about others.

Examples of collapses of civilization from archeological sites due to selling the future & shrinkage in harvest:

- Sumerians irrigation system with rising salt level soil depleted the productivity of wheat and barley.
- Series of intense drought and soil erosion undermined Mayans civilization.
- Sustenance of our current civilization cannot be solved with business as usual since business as usual results in a collapse of several past civilizations including the Hidden but becoming vivid historical Cushitic civilization.

Trends of Current situation towards collapses of civilization:

- Tripled grain prices from 2006-2008 due to soil erosion, falling water tables and rising carbon emission.
- A monsoon impact in India.
- Severe drought including Soviet Union and other countries.
- Crop-shrinkage due to heat waves in US mid-west.
- Diversion of grain to produce fuel for cars.
• Spreading shortage of irrigation water
• Increase in the number of hungry people
• Emerging new diseases and erosion of important indigenous social values
• Supply side of the food equation difficulties to expand food production
• Soil erosion egg. Lesotho & Mongolia with 50% reduction of harvest for the last three decades and vast dust storms with the loss of top soil in Sub-Sahara, China, Western Mongolia and Central Asia
• Aquifer depletion due to over pumping of water egg. A nearly Phasing out of wheat production in Saudi Arabia,
• Crop-shrinkage heat waves
• Melting ice sheet and mountain glaciers that feed major rivers and irrigation systems
• Rising sea level with flooding
• Loss of cropland to non-farm uses due to soil erosion egg. Sugar industry for ethanol production
• The diversion of irrigation water from farm land to the growing cities
• The coming reduction in oil supplies
• Climate Change where for each 1 degree Celsius increase 10% of decline in wheat, rice and corn with expected rise of 6 degree Celsius in this century with serious consequence.
• Advancing desert as a result of overgrazing, over plowing and deforestation egg. Sahara Africa expanding both south and north ward, the middle East, Central Asia and China

New techniques to expand production
• Unused agricultural technology is decreasing/ shrinking
• In developed countries farmers are using all available technology to raise land productivity
• Not many new ways to raise yield are coming out as anticipated
• In China, Egypt, France and Japan the yield rise is now becoming history
• For the world as a whole, the rise in grain land productivity dropped from 21% a year from 1950 to 1990 to 1.3% from 1990 to 2008
• No genetically modified grains have dramatically raised yields, nor are they likely to do so
• The inherent limit of photosynthetic efficiency reflects the upper bounds of the Earths biological productivity determining the human carrier capacity
The Emerging Politics of Food Scarcity

Food security will deteriorate: Unless Mobilization by all countries to stabilize

- population,
- climate,
- aquifers,
- conserve soil,
- protect croplands and
- restrict the use of grain to produce fuel for cars

- Banning export of food commodity to counter domestic food price rises and the inability to negotiate long term trade agreement
- Unprecedented buying of land by rich countries and or acquisition of land to grow food in other countries e.g. Saudi Arabia, South Korea, Kuwait, Libya, India, Egypt, Jordan, the united Arab emeritus and Katar from World food program supported poor countries
- One characteristics of land acquisition is that they are also water acquisition whether the land is irrigation water or rain fed
- Due to land acquisition public hostility builds by the native holders and the natives employment undermined due to mechanized farming
- Competition for land and water resources is crossing national boundaries increasing hunger and political instability
- Global Ponzi-economy is created resulting consumption of the asset base itself like overfishing, over pumping, over grasing etc.

An Integrated Plan to save Civilization

- Cutting net carbon dioxide emission by 80% by 2020 and raising energy efficiency and expanding renewable energy
- Stabilizing population at eight billion or lower
- Eradicating poverty and
- Restoring the Earth’s natural system including its soils, aquifers, forest, grassland and fisheries

The Present Situation in Africa

- low investment in S & T;
- declining quality of S&T education;
- brain drain and brain wastage;
- decaying and acquisition of outdated infrastructure;
• disorientation from local and continental priorities

Not allowing the rightful delivery of S&T to FNS quality products from S&T and its linkages
Need for the national and continental innovation system for FNS is clearly defined and get optimized.

The FNS Depends on
  ❖ the existence of different S&T actors and its policy should address issues related
to access to knowledge and technology, finance,
  ❖ human resource,
  ❖ Appropriate and sustaining physical infrastructure, and
  ❖ efficiently built organizational structure and enabling framework conditions

African Agriculture is Challenged by:
Climate change is calling for novel S&T approaches in agricultural and livestock production. Malnutrition is greatest in countries with underdeveloped agricultural productivity processing, and storage technologies.

The Key Factors Influencing African Food Insecurity Need is:
To adapt multidisciplinary science-based technological innovations to address the FNS issue.

Food and nutrition security and eradication of hunger on a continental scale can only be within reach provided that Appropriate technological innovations are accepted, promoted and implemented at all levels.

Some of the Missing and Underdeveloped S&T Based Methods Include
  • green and blue water S&T;
  • Hydroponics and drip irrigation;
  • technologies for postharvest conservation, processing, and storage;
  • technologies to overcome a biotic stresses like drought for FNS with promising solutions from transgenic and genomics based sciences;
  • agro forestry as biodiversity based technology for carbon sequestration;
  • x-ray based technologies for soil mineralogy analysis;
• technologies based on electromagnetic fields controlled food borne pathogen like electric field processing technologies;
• Recent development in novel shelf life extensions technologies like preserved inert gases, electron beam irradiation, pulsed light, cold plasma and chemical technologies;
• Bio preservation with bacterio-phages and bacteriocine; and nanotechnology.

**Figure 1:** Major Migration Routes during the inhuman slave trade as an input to the site specific genetic diversity 18th and 19th century
Genetic Diversity in World-wide Population

African samples reveal more genetic diversity than samples outside of Africa.
• Ethiopia is a key region in human evolution.
• It is now widely accepted that Anatomically Modern Humans (AMH), which are the major ancestors of all humans alive today, arose in Africa sometime ~200 thousand years ago (Kya).
• From Africa, AMH travelled to the rest of the world at some point between 60-120 Kya, and
• it has been suggested that these first migrants from Africa left through Ethiopia.
• Supporting this theory, some of the earliest known AMH remains were found in Ethiopia, including Omo I from Kibish first discovered by Richard Leakey in 1967 dated to 190-200 Kya, and the Herto fossils dated to between 160-154 Kya.
• Ethiopia has some of the highest levels of linguistic, cultural and ethnic diversity in the world, being home to over 70 different ethnic groups and over 80 living languages.
• One can argue that the nutritional need in terms of type and specification could vary as a burden of genetic diversity egg lactase persistence and tolerance

Stunting Target of Various Countries (IFPRI, 2016)
• Only 4 countries are in good progress
• 10 countries are off course on some progress Ethiopia included
• 5 countries with no progress
• 4 countries with insufficient data to make any progress

Countries Requiring External Food Assistance, December 2016 (FAO, 2016a)
Status of Food and Nutrition Security

- About 793 million people are undernourished globally, down 167 million over the last decade, and 216 million less than in 1990–92.
- For the developing regions as a whole, the share of undernourished people in the total population has decreased from 23.3 percent in 1990–92 to 12.9 per cent.
- Some regions, such as Latin America, the east and south-eastern regions of Asia, the Caucasus and Central Asia, and the northern and western regions of Africa have made fast progress.
- Progress was also recorded in southern Asia, Oceania, the Caribbean and southern and eastern Africa, but at too slow a pace to reach the MDG 1c target of halving the proportion of the chronically undernourished.
- For the developing regions as a whole, the two indicators of MDG 1c – the prevalence of undernourishment and the proportion of underweight children under 5 years of age – have both declined.
- Economic growth is a key success factor for reducing undernourishment, but it has to be inclusive and provide opportunities for improving the livelihoods of the poor. Enhancing the productivity and incomes of smallholder family farmers is key to progress.
Water Availability of Different Countries for Food Self-sufficiency

Percent of Green Water use of Total (Green Plus Blue) Agricultural Water use on Cropland and Pasture, 1995–2005 Average
Contemporary water availability for cropland and/or pasture for (a) blue water only and (b) total amount of blue plus green water (after extraction of consumed water in upstream locations).

Status of Bio-economy Policy around the World

Bioeconomy Policies around the World
Food Loss from Food Supply Chain in Developed and Developing Countries. Source: Ennart. Illustration: Britt-Louise Andersson

The numbers and shares of undernourished people by region, 1990–92 and 2014–16: Region C is of concern to us.
Undernourishment trends: progress made in almost all regions, but at very different rates

Constraints ranked according to stunting (%), lowest to highest prevalence, and assessment of progress towards WHA 2025 target (IFPRI, 2016)

- 8 countries are in good progress
- 33 countries are off course on some progress Ethiopia included
- 5 countries with no progress
- 3 countries with insufficient data to make any progress

Countries ranked according to anemia (%), lowest to highest prevalence and assessment of progress towards WHA target (IFPRI, 2016)

- 1 country on course
- 53 countries off the course Ethiopia included
- 1 country with insufficient data

Countries ranked according to exclusive breast feeding (EBF) of infants less than 6 months (%) highest to lowest prevalence and assessment of progress towards WHA target (IFPRI, 2016)

- 23 countries on course
- 3 countries off course with some progress Ethiopia included
- 12 countries off course with no progress
- 17 countries with insufficient data

Countries ranked according to wasting (%), lowest to highest prevalence, and assessment of progress towards WHA target (IFPRI, 2016)
• 17 countries with good progress
• 34 Countries with no progress Ethiopia included
• 3 countries with insufficient data

Wasted fruit and vegetables near a market in due to a lack of appropriate postharvest handling facilities.

Current Rates of Malnutrition in Africa
• 58 million children under age five are too short for their age (stunted), 13.9 million weigh too little for their height (wasted), and 10.3 million are overweight. None of these children are growing healthily (UNICEF-WHO-WB, 2015)
• 163.6 million Children and women of reproductive age are anaemic (WHO, 2015a)
• 220 million people are estimated to be calorie deficient (FAO, 2015a)
• 8 per cent of adults over 20 are obese (WHO, 2015b)
• Adult obesity is on the rise in all 54 African countries (2010-2014) (WHO, 2015c)
• 13 countries in Africa have to manage serious levels of stunting in children under 5 or anaemia in women of reproductive age and adult overweight
• The central lesson from my 35 years in AAU and other Sister Scientific Institutions
• It is critically important but not yet realized that modern science, and scientists, achieve a much higher degree of integration with indigenous knowledge, throughout both their teaching and research.

The creativity, rationality, openness, and tolerance that are inherent to science --- a "scientific temper" – can only be achieved through appropriate understanding of indigenous knowledge.

Some Examples of IK
• Medicinal plants
• Farmers indigenous soil knowledge
• The rotational cropping, organic manuring
• IK and plant breeding in terms of seed selection and extensive subsistence farmers knowledge in agro botany egg Ethiopian high lysine sorghum
• Diverse home garden plants, pastoralist herbal traditional knowledge
• Astrological, metrological and calendar setting knowledge system

Power of Science-Education Partnerships for FNS
• Scientists are urgently needed to be exposed to indigenous knowledge domains to support teachers in FNS
• And scientists have a great deal to learn from outstanding teachers and indigenous knowledge custodians that will improve not only appropriate teaching but also research.
World Population since 11000 Years and Innovations

The 21st Century Frontiers

- Genomics and agriculture
- Genetic medicine and Biotechnology
- Energy and water resource
- Femto and nano technology
- IT and others like
- Quantum theory, relativity, new dimensions in time and space (FEMTO & NANO),
- black holes and the expanding universe, and the deciphering of the genetic code are examples that have transformed human thoughts and are the bases of new quest for new frontiers.
Integrating FNS with the Three Major Functions of Schooling: Social, Innovative and Liberative

Integrating FNS with what is spelt out as objectives in the Ethiopian Educational & training policy

The development of abilities to think independently and constructively (to solve problem)

Enable children (Learners) to acquire an understanding of self and the environment

Develop of desirable social and civic and vocational skills and attitudes

Upbring citizens who respect human rights and stand for the wellbeing of peoples, etc

What university represents has to accommodate FNS

Universities represent many important values and functions in a society:

research,

Education and knowledge production

and the role of universities in democratic development.
What is on the ground for FNS?

- Not conducive for analytical and critical thinking.
- Does not encourage dialogue and questioning for science learning.
- The environment of learners and their success has not been motivated to a degree it deserves due partly to:
  - Inefficient school academic leadership
  - Paucity of well trained and committed teachers, and
  - Material shortage.

The role of universities under the circumstances prevailing is obvious: require working university and inputs and processes is needed

Percent of Gross Expenditure on R&D in Ethiopia by Main fields of S&T as reported:

- Natural Science 7%
- Engineering and Technology 5%
- Medical and Health Sciences 16%
- Agricultural Sciences 46%
- Social Sciences 7%
- Humanities 3%
- Others unspecified 16%

NB: Gross Expenditure on R&D amounted 931.4 million birr of which 84% expenditure goes to Gov sector with a lion share to HEI.

**Capacity differences in research and their implications in development and regional integration**

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28
National R&D Personnel by Occupation Of The Total that equals 8282 in full time equivalent:

- 45% are researchers
- 17% are Technicians
- 38% are support staff

The three critical capacities needed for any R&D including FNS include:

Population growth and urbanisation

- Rapid population growth in Africa will have important consequences for agriculture and food systems:
- Rapid population growth will put pressure on African food systems to feed its fast-growing cities
- Land values may

Urban Vegetable Production in Cotonou (Benin) in 2006)
Priorities to be set

- NUTRITION for sustainable and healthy diets
- CLIMATE smart and environmentally sustainable food systems
- CIRCULARITY and resource efficiency of food systems
- INNOVATION and empowerment of communities
- Research breakthroughs
- Innovation and Investment
- Bio-fertilizers for Plants
- Less Food Waste for Food Systems
- Alternatives to Meat
- Aquaponics for Agricultural Circularity
- Personalized Nutrition
- Fighting obesity with proper pre- and post-natal nutrition
- Photosynthesis for Food & Energy Towards a healthy and sustainable African Diet through basic and applied research on African crops Open Science
- International collaboration

Eu-Africa High Level Policy Dialogue (HLDP) On Science Technology & Innovation

1. Health, demographic change and wellbeing
2. Food security, sustainable agriculture and forestry, marine and maritime and inland water research and the bio-economy (€3.8 billion 2014-2020)
3. Secure, clean and efficient energy
4. Smart, green and integrated transport
5. Climate action, resource efficiency and raw materials
6. Inclusive, innovative and reflective societies
7. Secure societies
Conceptual framework for the links between diet quality and food systems (Global Panel on Agriculture and Food Systems for Nutrition, 2016)

The need to urgently take the new biosciences to mitigate the problems of FNS

- Advances in computing power
- Genomic sequencing
- Crystal structures of proteins
- High through-put technologies
- Biological databases
- Diverse biological sampling/collection
To set deserving direction in S&T for FNS and related issues

Rooted problems among our researchers in the universities

- Interested group of self promoting “intellectual” trade mark
- Repeated copying and reiterating of past problems with out meaningful suggestion
- Being the results of copied educational systems and researchers from various systems and various persuasions.
- The loss as analogous to what is called Semantic Satiation
- Our learning institutions and professional societies being a subsidiary and pretentious of the western academic system under a situation of intellectual imitation have to free themselves.

FNS demands emancipation from Negative effects of Intellectual imitation

- deprive local ability and collegiality enhancing frustration
- Denudes a foundation for democratic society since when knowledge is made a public good and service, it advances not only learning but defends civil rights.
- result in creating ‘pretending intellectuals’ that are not cohesive group, that are heterogeneous with no single class position
- Promote self interest and cannot create a deep world to reflect justice and thus lacks great faith in humanity.
- Lack attitudes for pluralism, tolerance and broad mindedness with-out which there are no democratic society
- Lack the understanding of the heterogeneous internal dynamics of the society and the country at large

The foundation on which the strength of professional S&T rest to enhance FNS:

- Any S&T group, if it is to achieve excellence must have moral in ethical sense of the word and moral in its intellectual sense.
- Devoid of multiple opportunistic mode of personalities
• Modest man power development,
• Enabling environment initiated for S&T.
• On ability to overcome the human selfish values with a performance of social values and intellectual.
• The university with universal mind needs encouragement for:
  • diversity of thought,
  • dialogues that we believe further create intellectual forum to each other
• and break the intellectual alienation forum that have disintegrated the miniature of S&T group in the country.
• Should side innovators or what one may call the proletariat of knowledge industry.

Research in the FNS is fundamental to a 21st century economy
• Should the FNS sciences be defined as ‘the sum of all knowledge’?
• The public doesn’t own FNS Science and Technology but has a right to criticize if researchers do not deliver what the public wants.
• Citizens need to feel some ownership of FNS science to be comfortable in making informed decisions
• It is important to address trust issues in FNS science
• Future challenges for society will require a change in behavior by the whole population rather than just a technical fix.

An example of a national food security and nutrition plan (Hendriks 2016).
Summary of the presentation

- The presentation of the title starts with the definition of nutrition, classes of nutrients and their importance to human civilization, memory and health. It addresses the complexity of the food and nutrition security and the need for trans boundary approach to mitigate the food security problems and promote the essence of future of humanity and human wellbeing in order to avoid collapses of civilization due to selling the future & shrinkage in harvest as was witnessed from various archeological sites. The presentation gives example for the current trends towards collapse of civilization due to problems of food and nutrition security with the supply side of the food equation and difficulties to expand food production.

- The paper addresses the emerging politics of food scarcity with the need of an integrated plan to save civilization with an effort to overcome the S&T situation in Africa. The presentation lists some of the missing and underdeveloped S&T based methods and calls for addressing them. The paper also touches on the status of Ethiopia as a cradle of man and the burden and opportunity of genetic diversity in the country with a concerted effort on resource mapping, identification, characterization, conservation, utilization and bio prospecting on available conventional and alternative food and feedstuffs for man and livestock in Africa.

- The presentation reviews target of various countries, situation of food insecurity, the African Union and the World Food Programme Cost of Hunger estimates, Status of Food and Nutrition Security, water availability of different countries for food self-sufficiency with reference to World population 2050 (from 7 to 9 billion) and demographic transition.

- S&T and status of Bio economy Policy around the World is presented and this is followed with Food loss from food supply chain in developed and developing countries with emphasis on numbers and shares of undernourished people by region and undernourishment trends due to poor capacity in S&T in food and nutrition security.

- The author underlines the central lesson from his 35 years in AAU and other Sister Scientific Institutions and recommends modern science, and scientists, to integrate with indigenous knowledge where Power of Science-Education Partnerships for FNS has to be spelled out. The author uses the various technological development and Innovations since the origin of agriculture 11000 Years ago and links this with the 21st century frontiers where FNS with the major functions of schooling, the Ethiopian Educational & training policy, and realization of university to accommodate FNS R&D for economic and social development need to be perceived and integrated. The presentation focuses also on urban agriculture and the experiences of some countries priorities to be set.
the need to urgently take the new biosciences to mitigate the problems of FNS to set deserving direction in S&T for FNS and related issues.

• As a form of advice the presentation touches on the rooted problems among our researchers in the universities with a necessity of emancipation from negative effects of intellectual imitation and promotion of university with universal mind. Towards the end the presentations lists S & T advantages for FNS Research where this should be considered as fundamental to a 21st century economy. An example of a national food security and nutrition plan from quoted sources is reported.
Keynote Address on
Climate Change and Health: Research Experience, Opportunities and Challenges

By

Adugna Woyessa Gemeda (PhD)

Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

Who is at risk?
• ALL POPULATIONS will be affected by climate change, but some are more vulnerable than others.
• Children – in particular, children living in poor countries – are among the most vulnerable to the resulting health risks and will be exposed longer to the health consequences.
• The health effects are also expected to be more severe for elderly people and people with infirmities or pre-existing medical conditions.
• Areas with weak health infrastructure – mostly in developing countries – will be the least able to cope without assistance to prepare and respond.

WHO Response?
• In 2015, the WHO Executive Board endorsed a new work plan on climate change and health. This includes:
  • Partnerships: to coordinate with partner agencies within the UN system, and ensure that health is properly represented in the climate change agenda.
  • Awareness raising: to provide and disseminate information on the threats that climate change presents to human health, and opportunities to promote health while cutting carbon emissions.
  • Science and evidence: to coordinate reviews of the scientific evidence on the links between climate change and health, and develop a global research agenda.
  • Support for implementation of the public health response to climate change: to assist countries to build capacity to reduce health vulnerability to climate change, and promote health while reducing carbon emissions.
Malaria and Climate in Ethiopia

- Weather variability is a key driving force for malaria transmission (Siraj et al., 2014; Midekisa et al., 2015)
- Anomalies in both Rainfall & Temp. are precipitating factors for malaria epidemics (Abeku et al., 2003)
- Malaria Control & Elimination program is both a public health & national development priority (MoH, 2014)
- Malaria transmission varies & depends on local climatic & environmental conditions
- detailed knowledge about each locality as well as global drivers, for designing focused interventions
- Malaria and Climate Research Experience
- Highland malaria in Ethiopia (Addis Ababa University, Faculty of Science, Department of Biology as Postgraduate study

Aim: elucidation of malaria transmission and determine prevalence in Akaki Town and its environs (1998/9)

- Prospective parasitological, entomological and climate information
- Record review (21 Health Centers in Addis Ababa City Administration) of malaria cases, 1993-1999ma
- Compare climate data at different part of the City
  - Highland malaria in Ethiopia (Addis Ababa University, College of Health Sciences, School of Publichealth postgraduate study
  - Epidemiology of highland malaria in Butajira area (2008-2010)
  - Longitudinal parasitological data
  - Climate and intervention data
  - Relationship of El Nino and historical malaria epidemics
  - Record review (1950-2015)
  - Health and Settlement: Ethiopian Panel on Climate Change/ Ethiopian Academy of Sciences
  - Climate sensitive diseases
  - Vulnerability and Adaptation Assessment of Health to Climate Change in Ethiopia
  - Health vulnerability index for each region
A Case Study: El Niño and Malaria in Ethiopia (1950-2010)

- However, available information on the association of malaria epidemics and climate drivers in the peer reviewed and grey literature were seen as inconsistently presented, patchy in geographic distribution and often limited to small area studies
- However, available information on the association of malaria epidemics & climate drivers in the peer reviewed and grey literature were seen as inconsistently presented, patchy in geographic distribution and often limited to small area studies
- **Purpose:** Investigate the historic association of ENSO events and recorded malaria epidemics across Ethiopia with an emphasis to its future implication on malaria control and elimination
- Enhanced National Climate Services (ENACTS) initiative emerged in Ethiopia in direct response to perceived user needs
- NMA provide quality-assessed spatially and temporally complete data services
- In Ethiopia, half of the populations live in cool highland areas where malaria is restricted by low temp. (Lindsay and Martens, 1998).
- Malaria in highland areas is highly sensitive to climate anomalies and trends (Tulu, 1996; Abeku et al., 2002; Abeku et al., 2003; Woyessa, 2003; Kiszewski and Teklehaimanot, 2004; Teklehaimanot et al., 2004; Alemu et al., 2011; Woyessa et al., 2012).
- To investigate the relationship of ENSO indices to historical malaria epidemics in Ethiopia obtained from the peer-reviewed and grey literature for the period 1953-2010
- Hypothesis: widespread malaria epidemics are related to ENSO and therefore potentially predictable

A Case Study: Aim and Methods of a Case Study

- Search of peer reviewed publications and ‘grey’ literature (reports)
- Web of Science/Google search: “Ethiopia” and “malaria” and “epidemic” or “outbreak” and year (and tracing of referenced articles therein) to identify records of malaria epidemics in Ethiopia for the period 1953-2010
A Case Study: Findings

- Total Years of ENSO and Widespread/Local Epidemics with Corresponding ENSO Phase (1951-2015)

<table>
<thead>
<tr>
<th>ENSO Phase</th>
<th>Total years</th>
<th>Widespread epidemic years</th>
<th>Local Epidemic Years</th>
<th>No of spatial info provided</th>
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<td>1</td>
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<tr>
<td>La Niña Phase (La Niña + Neutral/La Niña)</td>
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<tr>
<td>Neutral Phase</td>
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<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
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<td>12</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

A Case Study: The relationship of ENSO and malaria epidemics between 1951 and 2011 in Ethiopia

http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml

A Case Study: Discussion

- The relationship of historical malaria epidemics in Ethiopia documented between 1953 and 2010 to ENSO events
- Epidemics may occur during any ENSO state, widespread epidemics appear to be more common during El Niño phases
- While local epidemics are more frequently identified with La Niña or Neutral periods
• However, inconsistencies in epidemic reporting make it impossible to quantity the precise relationship.
• Still, not all malaria epidemics were preceded by ENSO events.

Summary
• The relationship of historical malaria epidemics in Ethiopia documented between 1953 and 2010 to ENSO events
• Epidemics may occur during any ENSO state, widespread epidemics appear to be more common during El Niño phases
• While local epidemics are more frequently identified with La Niña or Neutral periods
• The current analysis and the recent ENSO Map Rooms provides an opportunity to assess the association of climate variability with malaria epidemics at multiple temporal and spatial scales
• The ENACTS products, enriched by the national observational archive and monitoring data, and associated web-based Map pages provide basic information that can be used for detailed research and local decision-making

Why Malaria Surveillance Suite?
• Malaria elimination strategies
• Operational research and developing new tools
• Surveillance is a key tool in malaria elimination
• Environmental information such as temperature, rainfall and topography and altitude
• Districts
• District is an important unit in terms of:
  • decision point
  • Political administrative unit
  • Planning
• Malaria elimination focus at lowest level
• Surveillance
• Climate information recommended to be part of surveillance for malaria elimination

Malaria Surveillance Suite
• Climate and topography ready as part of surveillance
• 80 districts selected for malaria elimination
• Piloted for developing the surveillance suite
Ethiopia Proxy Surveillance Suite for Malaria

Are there significant trends in the seasons that affect this region/zone/district (Monthly-Yearly)?
How is the current and recent rainfall in area (region/zone/district) - (past 3 dekads and cumulative)?
Is the climate suitable of the for malaria transmission?
Is ENSO important in my area?
(1) Are there significant trends in the seasons that affect this region/zone/district (Monthly-Yearly)?

- [Graphs showing seasonal trends]

(2) How is the current and recent rainfall in area (region/zone/district) - (past 3 dekads and cumulative)?

- [Maps and graphs showing rainfall patterns]

---

(3) Is the climate suitable for malaria transmission?

- Malaria transmission in XX is not limited by rainfall.
- Malaria transmission in XX is not limited by temperature.
- Malaria transmission in XX is not limited by humidity.
Summary
The surveillance tool helps to simplify our understanding about local climate parameters and use for decision

Recommendations
• Incorporate climate variables into the malaria surveillance system at local level
• Strengthening human resource and technology capacity to use ENACTS at district level

Opportunities
Global Matters
• Climate change an agenda for the last decade
• Previous US Government considered climate change as part of all its program
• Open access tools and technology
• WHO and WMO collaboration: a unit established

Local Matters
• National Meteorological Agency and Ministry of Health strong collaboration since 2001
• Malaria and climate issues
• Global Fund application and resource sharing
• Monthly Health Bulletin & data sharing experience
• Climate and Health Working Group established February 2008

Challenges
Global Matters
• Commitment of the current US Government under question in accomplishing agreements of the recent years
• Resources are limited for Health and climate

Local Matters
• Institutional arrangement for health and climate issues
• Evidence-based action
• Resource for health research and developing tools as well as using available ones poor
• Collaboration and resource mapping limited
• Access and availability of data sharing system requires improvement
Keynote Address on
Biotechnology Research and Education in Ethiopia: Progress and Prospect

By

Kassahun Tesfaye (PhD), Hailu Dadi (PhD) and Dawit Tesfaye (PhD)
Ethiopian Biotechnology Institute (EBTi), Addis Ababa, Ethiopia.

Background

- The word "biotechnology" was first used in 1917 to describe processes using living organisms to make a product or run a process, such as industrial fermentations.
- Biotechnology began when humans began to plant their own crops, domesticate animals, ferment juice into wine, make cheese, and leaven bread.
- Traditional biotechnology has been used for thousands of years to produce improved food & health care products.
- Today, modern biotechnology enables us to develop improved products more safely and more rapidly than ever before.

Biotechnology

- “Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.” (UN CBD, 1992)
- Using scientific methods with organisms to produce new products or new forms of organisms.
The Potential Applications ....

- As in theory any gene from any organism could be transferred into a plant/animal/microbes, the potential applications of genetic engineering to food and agriculture are virtually unlimited.

- Not all the possibilities presented here have been developed yet:
  - many are still out of reach for the moment,
  - some are subject to fundamental research,
  - Some are at experimental stage and some have already been commercialized.

1. **Fight against** crop protection traits such as pests, pathogens and weeds deployed in soybean
   - Maize, canola, cotton, sugar beet, etc
     - Resistance to insects, nematodes, virus, fungi, Herbicide tolerance
     - Environmental benefits: crops can facilitate no-tillage or low-tillage weed management

2. **Agronomic traits**
   - Crops with higher yield
   - Drought and salt tolerance
   - Tolerance to cold or high temperature
3. Quality traits
   - Delayed softening (Flavr Savr tomato)
   - Nutritional value (fatty acids, vitamins, etc.)
   - Specific substance production (antibiotics, vaccines, biodegradable plastics, fibres, etc.)

4. Environmental traits
   - The decontamination of industrial sites polluted by heavy metals
   - Use of transgenic plants, fish, or microbes as bio-monitors to detect pollutants which is more effective and/or less expensive than current methods

What are the stages of biotechnology?
   - **Ancient Biotechnology**
     - Early history as related to food and shelter, including crop and animal domestication
   - **Classical Biotechnology**
     - Built on ancient biotechnology
     - Fermentation promoted food production
     - Traditional Medicine
   - **Modern Biotechnology**
     - Manipulates genetic information in organism
     - Genetic engineering
     - Gene Editing

Agricultural Biotechnology (A Lot More than Just GM Crops)
The biotechnology tools that are important for agricultural biotechnology include:-
   - Conventional plant breeding
   - Tissue culture and micro-propagation
   - Molecular breeding or marker assisted selection
   - Genetic engineering and GM crops
   - Molecular Diagnostic Tools

Case study on Bt technology
Bt Proteins to Control Insect Pests
   - Bt protein products are sold across the globe to control these agricultural pests
     - Lepidoptera: corn borers, armyworms, bollworms
     - Coleoptera: corn rootworm
     - Dipterans: flies and mosquitoes
The Facts

- **Bt = *Bacillus thuringiensis***
  - A spore-forming soil-inhabiting gram positive bacterium
  - Is found in >70% of the soils on the earth
  - Insecticidal activity of Bt protein(s) discovered in 1901

- **Bt first sold for agricultural use in 1958**
  - Product: a crude formulation of dehydrated bacterial cells that contains the Bt insecticidal protein
  - Popular with small scale farmers and the organic farming community
    - Environmentally safe and non-toxic except to the target insect pests

How do *Bt* Genes work to protect plants against insect pests?

![Diagram showing the mechanism of action of *B. thuringiensis*](image)
Insect Resistant Crops

Bt Cotton
- Traits Conferred (1995) resistance to cotton bollworm and budworm
- Developers: Monsanto
- Gene source: Bacillus thuringiensis; NATURALLY OCURRING Soil borne bacterium

Bt Corn
- Trait: resistance to insect pests

European corn borer; Armyworm; Earworm; Rootworm
- Developers: Dow, Monsanto, Pioneer, Syngenta
- Gene source: Bacillus thuringiensis; NATURALLY OCURRING Soil borne bacterium
Basis of Specificity in Bt Activity

The basis of the specificity lies in the binding domain which recognizes specific receptors in the gut lining of the insect:

- Cry1 Bt: only binds to receptors present in some Lep species
- CryII Bt: only binds to receptors present in some Lep and Dip species
- CryIII Bt: only binds to receptors present in some Coleop species
- CryIV Bt: only binds to receptors present in some Dipteran species

Bt Corn Protects from Corn Borer

GM Cotton Resistant to Bollworm
Global Controversy has had a Significant Effect on Biotechnology Development and Adoption in Africa

High Dependency on Food Aid
- In 2007, sub-Saharan Africa accounted for 67% of global emergency food aid deliveries
- SSA - 2.5 Million tons
- Asia - 0.9 Million tons
- M. East and N. Africa - 0.2M tons
- Eastern Europe - 53, 000

Africa Union: Africa Biosciences Initiative
Important Biotech Crops in the Pipeline for Africa

- **Banana**: enhanced nutrition and bacterial wilt
- **Cowpea**: insect resistant
- **Cassava**: virus resistant; enhanced nutrition
- **Maize**: drought tolerant
- **Potato**: disease resistant
- **Rice**: salt and drought tolerant; enhanced nutrition
- **Sorghum**: enhanced nutrition
- **Sugarcane**: drought tolerant

GM Crop Trend in Africa

Why is Africa Lagging Behind?

- Technical (R&D) capacity; not at critical mass
- Science and Technology still not a priority; underfunded by national governments
- Conflicting “donor” attitudes
- Regulatory
- Trade concerns with Europe
- Misinformation
- Lack of political will

Biotechnology R & D and Education in Ethiopia: Current Status

**Ethiopian Agricultural System**

- It accounts for half of the GDP & 90% of the exports with 85% work force employment;
- The smallholder farmers cultivate 95% of the cropped area;
- This farming practice generally characterized by low yield per unit area:-
  - Caused by the use of local landraces along with traditional management practice and effect from biotic and a biotic stress.
Agricultural Constraints

- Biotic stresses
  - Diseases (fungi; bacteria; virus, ...)
  - Pest
  - Weed

- A biotic stresses
  - Drought
  - Soil acidity
  - Soil salinity

- Nutrition; Quality & Storability

Agriculture in Ethiopia

- Conventional research has significantly increased the country's agricultural productivity in the past.
- However, the performance of agriculture is poor in terms of feeding the country's population (ca. 2.9%)
The realistic option is to increase the food productivity per unit of land; Hence, the science based technological options including biotechnological tools/methods and genomics the only way to realize this; Biotechnology would complement these efforts & speed up the processes to identify the target traits of agronomic importance.

Main ingredients for biotechnology R & D

- Main ingredients for biotechnology development
  - Biodiversity (plant, animal, microbial)
  - Skilled manpower, and
  - Biotech R&D infrastructure

Ethiopia’s Potential for Biotechnology

- Biodiversity
  - Key to Biotechnology
  - Ethiopia is endowed with huge biodiversity centre of origin & diversity for many crops
    - Has several unique habitats
  - The flora of Ethiopia is estimated ca. 7,000 species, of which 12% is considered to be endemic.
  - One of the 8 Vavilovian gene center.
Current Biotech Reality in Ethiopia

- Current reality in relation to biotechnology
  - Lack of a critical mass of trained personnel in biotechnology;
  - Poor biotech R&D infrastructure;
  - Lack of nationally coordinated biotechnology research effort;
  - Limited biosafety monitoring capacity at national level;
  - Lack of public awareness.

Biotech R & D Attempts in Ethiopia

- It is lagging behind on activities of “Modern Biotechnology” involving recombinant DNA technology;
- Most of the works revolve around “Conventional Biotechnology”

Current activity focused on

- Plant tissue culture;
- Disease free planting materials production & dissemination;
- QTL mapping;
- PCR based genetic analysis

Plant Tissue Culture

- Plant tissue culture has been given the highest priority in biotechnology R & D in Ethiopia;
- Tissue culture activities first started in Ethiopia in 1980’s at AAU with focus on micropropagation of indigenous forest species and local crops;
- EIAR (Holetta, DZ, Jimma, Melkassa), AARI, SARI, OARI & some higher learning institutions (JU, MIT….)
- Emphasis on protocol optimization for mass propagation, low cost protocols, disease cleansing…;
Prunus africana

Sweet potato
Meristem culture

- More than 10 varieties
- 100% virus free sweet potatoes were obtained

Micropropagation of Apple (AAU-Holetta Lab)

Rooting response of MM106 on half MS medium containing different concns. of IBA & IAA
Genomic Resources & Molecular Markers

- RFLPs, RAPD, AFLP, ISSR and Microsatellites (SSR) & Sequence data derived from nuclear, and cp DNA have been used.
- Not geared towards more important practical applications MAS via Association study, QTL mapping & candidate gene identification.
- Lack of close integration of the research activities with the existing conventional breeding research programs.
- Moreover, most of the studies were conducted as part of MSc & PhD.
## Application of Molecular Markers to Study the Genetic Diversity and/or Phylogenetics of Crops from Ethiopia

<table>
<thead>
<tr>
<th>Crops/plants</th>
<th>Marker type used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>AFLP</td>
<td>Demisse et al., 1998</td>
</tr>
<tr>
<td><em>Brassica carinata</em></td>
<td>RAPD</td>
<td>Teklewold and Becker, 2006</td>
</tr>
<tr>
<td>Coffee (cultivated, forest)</td>
<td>RAPD, ISSR, AFLP, SSR</td>
<td>Aga et al., 2003, Aga et al., 2005, Tesfaye 2014</td>
</tr>
<tr>
<td>Coffee</td>
<td>Sequence of part of cpDNA</td>
<td>Tesfaye et al., 2007</td>
</tr>
<tr>
<td>Enset</td>
<td>AFLP, RAPD, cpDNA</td>
<td>Negash et al., 2002, Birmeta et al., 2002, Bekele 2010</td>
</tr>
<tr>
<td><em>Guizotia</em> spp.</td>
<td>ITS sequence</td>
<td>Bekele et al., 2007</td>
</tr>
<tr>
<td><em>Guizotia</em> spp. (weedy &amp; wild)</td>
<td>AFLP; RAPD</td>
<td>Geleta et al., 2007</td>
</tr>
<tr>
<td><em>Hagenia abyssinica</em></td>
<td>ISSR</td>
<td>Feyissa et al., 2007</td>
</tr>
<tr>
<td>Highland maize</td>
<td>AFLP</td>
<td>Beyene et al., 2006</td>
</tr>
<tr>
<td>Linseed</td>
<td>AFLP</td>
<td>Wajkira et al., 2005</td>
</tr>
<tr>
<td>Potato</td>
<td>SSR</td>
<td>Abebe et al., 2004</td>
</tr>
<tr>
<td>Sorghum</td>
<td>AFLP, SSR, RAPD</td>
<td>Geleta et al., 2006, Ayana et al., 2000a</td>
</tr>
<tr>
<td>Sorghum, wild</td>
<td>RAPD</td>
<td>Ayana et al., 2000b</td>
</tr>
<tr>
<td>Finger millet</td>
<td>SSR</td>
<td>Yifru et al., 2006, Wang et al., 2007</td>
</tr>
<tr>
<td>Wheat (tetraploid)</td>
<td>SSR, EST-SSR</td>
<td>Tamiru et al., 2007</td>
</tr>
<tr>
<td>Yam</td>
<td>AFLP</td>
<td>Genet et al., 2005</td>
</tr>
<tr>
<td>Mustard</td>
<td>AFLP</td>
<td></td>
</tr>
</tbody>
</table>
Molecular Characterization of Ethiopian Indigenous Breeds

- Characterize five Ethiopian indigenous cattle breeds using Microsatellite markers and Restriction Fragment analysis of milk protein genes;
- Molecular Characterization of local Goat Breeds

Potentials of Modern Biotechnology (GE) to Solve Crop Production Constraints

- GE offers several benefits when used responsibly by addressing the environmental and food safety concerns.
- Crop production problems that are either difficult or impossible to address using conventional research techniques are likely to be a target;
The potential of genetic engineering in solving crop production constraints in Ethiopia (Adane Abreham 2009)

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Constraints</th>
<th>Candidate Transgenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>Bollworm</td>
<td>Insect resistance (Bt) gene</td>
</tr>
<tr>
<td>Wheat, barley, tef</td>
<td>Grass weeds, striga, abiotic stress (drought, frost and salinity tolerance)</td>
<td>Herbicide resistant gene</td>
</tr>
<tr>
<td>Maize/sorghum</td>
<td>Stem borer, striga, abiotic stress (drought, frost and salinity tolerance)</td>
<td>Bt gene, striga resistance gene, stress genes and promoters</td>
</tr>
<tr>
<td>Tef</td>
<td>Lodging resistance</td>
<td>Dwarfing gene from wheat or rice</td>
</tr>
<tr>
<td>Tomato</td>
<td>Tomato yellow leaf curl virus</td>
<td>Viral rep protein C1 gene</td>
</tr>
<tr>
<td>Potato</td>
<td>Late blight</td>
<td>Resistance genes from wild potato</td>
</tr>
<tr>
<td>Enset/banana</td>
<td>Bacterial wilt</td>
<td>Hrap gene or bacteria from pepper</td>
</tr>
<tr>
<td>Enset</td>
<td>Protein deficiency</td>
<td>Protein enhancement via amino acid modification</td>
</tr>
<tr>
<td>Pepper</td>
<td>Potyviruses (Potato virus Y and others)</td>
<td>Viral replicase or protease gene</td>
</tr>
<tr>
<td>Sesame</td>
<td>Sesame seed bug</td>
<td>Bt gene</td>
</tr>
<tr>
<td>Faba bean</td>
<td>Chocolate spot</td>
<td>Chitinase or glucanase gene</td>
</tr>
<tr>
<td>Chickpea</td>
<td>Pod borer, stunt virus</td>
<td>Bt gene, Viral coat protein gene</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>weevil</td>
<td>Bt</td>
</tr>
</tbody>
</table>

Biotechnology Education in Ethiopia
- As oldest and largest University, AAU was the first to start Training in Biotechnology
- Collaborative research projects with Sweden
  - Mainly at the then of Faculty Natural Sciences
  - Started in the 1980s and 1990s
  - Few staff trained and modest facility acquired
  - Helped to lay the foundation

Biotechnology Education in Ethiopia
- Currently a total 10 HLIs are offering biotechnology undergraduate training;
- MSc training: a total of 7 Universities offers
Other Institutes

- AHRI (Health)
- Akililu Lema Institute of Path-biology (Health)
- EPHI (Health)
- National Veterinary Institute (NVI),
- The Ethiopian Institute of Biodiversity (EIB)
- EIAR
- Private Companies (Tissue culture)
  - MIT established in 2006 as largest tissue culture facility
  - VCI Ethiopia Tissue Culture Laboratory Based in Bishoftu
  - Olij Roses Ethiopia based in Bishoftu
  - Waginos Biotech (Plant Micropropgation laboratory based in Addis)
  - NARUS Biotechnology at Modjo

Ethiopia’s Conducive Environment for Biotech R&D

- Biotechnology has been identified as a key driver of growth for Ethiopia to fulfils its vision to becoming middle income by the year 2025
- Biotechnology
  - In the government’s priority area as part of GTP I & II (Conducive Policy Env’t)
  - Education Sector Development Program IV ESDP IV) (2011-2015) with special attention to Science & Technology

Biotechnology National Road Map

- Ministerial Steering Committee, Establish Biotech National Taskforce
- Developed Biotechnology Road Map for the next 10 years
  1. Ethiopian Biotech Research & Development Roadmap (135 pages)
  2. Ethiopian Human Resource Development Roadmap (93 pages)
  3. Ethiopian Agricultural Biotechnology Roadmap (114 pages)
  4. Ethiopian Industrial Biotechnology Roadmap (108 pages)
  5. Ethiopian Environmental Biotechnology Roadmap (89 pages)
  6. Ethiopian Health Biotechnology Roadmap (104 pages)

Biotechnology Council and Institute

- The Government recently established the Ethiopian Biotechnology Council and Institute-EBTi (June 2016) with Ministerial Council Regulation No. 388/2016
Members of the Ethiopian Biotech Council
The council shall have the following members:

1. The Prime Minister .................................................Chairperson
2. Minister of Education ............................................Member
3. Minister of Agriculture & Natural Resources ...............Member
4. Minister of Health ..................................................Member
5. Minister of Environment, Forestry & Climate Change ..Member
6. Minister of Industry .................................................Member
7. Minister of Livestock and Fisheries ............................Member
8. Minister of Science and Technology ..........................Member
9. Heads of Sectors (as required) .................................Member
10. Senior Researchers ................................................Member
11. Director General of the Institute ..............................Member and Secretary
Ethiopian Biotechnology Institute (EBTi)

- National Policy
- National Strategy
- Short & Long term R&D plan

- R&D Activities
- Technology Transfer
- Technical Support

- Training & capacity building
- System development
- Infrastructure

- Coordination
- Biosafety & Moral Issues
- Develop manual, guideline
- Partnership

EBTi Organogram

Director General Office

Policy and Strategy Directorate
- Law Directorate
- Ethics Directorate

Public and International Relations Directorate
- Gender Directorate
- Audit Inspection Directorate

Emerging Technology Centre
- Nanotechnology Directorate
- Material Science Directorate
- Artificial Intelligence Directorate
- Reverse Engineering Directorate

Biotechnology Centre
- Agricultural Biotechnology Directorate
- Health Biotechnology Directorate
- Environmental Biotechnology Directorate
- Industrial Biotechnology Directorate
- Bioinformatics and Genomics Biotechnology Directorate

Corporate Administration Directorate
- Human Resource Development Directorate
- Finance and Purchasing Directorate
- Planning Monitoring and Evaluation Directorate
- General Service Directorate

EBti

Ethiopian Biotechnology Institute
Risks Associated with Transgenic Crops

Summary of environmental risks associated with transgenic crops

1. Detrimental effects on non-target organisms
2. Gene flow to wild relatives or non-transgenic verities
3. Development of weediness
4. Development of resistance or tolerance
5. Production of novel toxins
6. Recombination of bacteria or viruses to produce new pathogens
7. Impacts of changes in agricultural management practices on biodiversity
8. Loss of crop genetic diversity
9. Unanticipated consequence

Hence, proper regulatory mechanism should put in place to promote safe biotech R & D.

Challenges for future Agricultural Biotech R & D

Challenges

- Lack of Biotech infrastructure & manpower for modern Biotech;
- Lack of incentive and/or low remuneration to compete & maintain biotechnologist locally;
- Procurement procedural limitation for Biotech equipments & supplies;
- Absence of local private company interested to import biotech items;
- Weak linkage among national biotech institutes;

Materials Used in this presentation

- AccesExcellence.org
- Few slides from ISAAA
- Biotechnology and Biosafety in Africa by Judith A. Chambers, Ph.D.
Mechanical Behaviour of Laser Welded 316L (N) Stainless Steel

D Harish Kumar

Department of Wollega University, Wollega University, P.O. Box: 395, Nekemte, Ethiopia

Email: harishkumardr1@gmail.com

Abstract

Laser Welding is a specialized welding process recommended for welding materials used in the aerospace, surgical and power plant industries. Laser welding is a non contact, low heat input, widely accepted welding process for welding a wide variety of materials due to its advantages like deep narrow welds, minimum distortion, narrow heat-affected zone, excellent metallurgical quality, ability to weld smaller size, thinner and thicker components and increased travel speeds compared to other welding processes. Wide variety of welding processes has been used in the past in the construction of Fast Breeder Reactors. The important welding processes which included were Multi – Pass Tungsten Inert Gas Welding (MP-TIG), Activated Tungsten Inert Gas Welding (A-TIG) and Submerged Arc Welding (SAW). Recent studies on 316L (N) stainless steel have recommended the use of Laser welding and Electron Beam Welding processes. The 316L (N) Stainless Steel is an austenitic grade recommended for the construction of Fast Breeder Reactor components. The present investigation was conducted on welding aspects of 316L (N) Stainless steels with carbon content 0.03-0.05Wt% and fixing the nitrogen content approximately at 0.07Wt %. The welded joints have been fabricated using 5.5mm thick plates at 3KW and 3.5KW laser powers by protecting the environment by argon and nitrogen shielding. The welded joints were subjected to micro-hardness measurements across the fusion zone. Transverse tensile tests both at room temperature and 650°C and creep-rupture tests at stresses in the range 180MPa-220MPa keeping the temperature constant at (650+273) °K have been conducted. Creep-ruptured tests carried out at stress levels in the range 180MPa-220MPa have been thoroughly investigated to characterize the creep behaviour of 316L (N) SS and the contributive creep failure mechanisms. The strengths of the welded joints when tested under tension were observed to be equivalent to those of the base metal. Micro hardness test results across the welded joints are also in agreement with the tension test results. Creep-rupture times at 180MPa are observed to be higher than those of the base material. The rupture lives at all the stress levels tested are higher as compared to those of MP-TIG, A-TIG and closer to the values of 316L (N) base material. In the present work, laser welding process has proved to be effective in producing satisfactory welded joints.

Keywords: Laser welding, 316LN stainless, Creep, Tension test, Temperature
INTRODUCTION

Laser welding is a non-contact, fusion welding process that melts and joins metals or non-metals at high travel speeds with a very low heat input using a laser beam. Laser beam can be produced either by a solid state laser or by a gas laser, in either case; the beam has to be focused to a small area. (Harish Kumar et al., 2009). The power density in laser welding can be defined as the ratio of laser power by the area of the spot. For example if a 1KW laser beam is focused to a spot of 200µm in diameter then the power density or the irradiance is 1000/area of the spot (3.14x10^-8 m^2) or 3.2x10^6 W/cm^2. (Leonard Migliore, 1998). In general, laser welding usually employs a power density of 10^5-10^7 W/cm^2. It means laser welding requires tremendous concentration of power for welding to occur. Laser beams are just light-beams, and metals are very good reflectors of light. This problem is compounded by the fact that the major industrial laser types emit infrared light, which metals reflect even better than visible light. As a result, most of the incoming power bounces right back at the source. (This, by the way, can damage lasers, lenses or whatever else is back there). Since metals are also good conductors of heat, the power that does get in to the work is rapidly dissipated away from the spot being heated. The only way lasers can weld metal is by applying so much power that the small fraction absorbed in the work is enough to meet it. That’s why laser welding requires 10^6 W/cm^2 for welding to occur.

The focusing of the laser beam to a small area is achieved by optical means. In solid state laser, a single crystal is doped with small concentrations of the transition elements or rare earth elements. For instance, in Nd-YAG laser, the crystal of Yttrium-Aluminum-Garnet (YAG) is doped with Neodymium. The electrons of the dopant can be selectively excited to higher energy levels upon exposure to high intensity flash lamp. Lasing occur when these excited electrons return to their normal energy state by emitting energy in the form of light. As mentioned earlier, laser welding popularly uses two types of lasers namely CO_2 lasers and Nd: YAG lasers with different laser power. Nd: YAG lasers are used to weld materials of different thicknesses involving powers up to 5KW. Whereas, CO_2 lasers are used for the applications which involve higher powers upto 20KW.

In laser welding the gap between the plates to be butt welded is one of the most important issues to be solved because the gap results in the formation of various weld defects such as under filling or non bonded joints, lack of fusion, porosity and under cuts (The codigo et al., 2009). The gap tolerance in butt joints is dependent on the material thickness, welding speed, and beam diameter and beam quality. It may be noted that the gap tolerance
increases with material thickness. However, as the gap increases, their reinforcement associated with the line-on-line fit up of laser welds normally decreases. The effects of zero gaps on the weld metal quality have been reported variously in the literature. (Wouters et al., 2006) Wouter reported that maintaining a zero gap prior to welding gives a weak weld. This effect they have attributed to the weld geometry within which they have observed a sharp crack where the unweld parts met each other. Codigo Do Trabalho has studied systematically the effects of the gap tolerances on the weld metal quality of 316 SS Nd: YAG pulsed laser welded joints by varying the gap in the range 0-350 microns. Pores were observed in specimens with zero gaps. The presence of spatter has also been reported in wider gaps.

Low carbon grade type 316 austenitic stainless steel, alloyed with nitrogen designated as 316LN SS exhibit superior strength at ambient and high temperatures, excellent corrosion resistance to replace other expensive materials. Austenitic stainless steel gets sensitized during welding. The problem of sensitization can be overcome by decreasing the carbon content. Reducing the carbon content would cause drastic reduction in mechanical properties. Replacing much of the carbon with nitrogen can offset this deterioration in mechanical properties. Nitrogen in solid solution is the most beneficial alloying element in promoting high strength in austenitic SS without sacrificing their good ductility and toughness. The upper limit of nitrogen is set on considerations of minimizing scatter in mechanical properties and improving weldability. Phosphorous, sulphur and silicon are treated as impurities having adverse effects on weldability. The selection of the material is based on its good combination of tensile, creep strength, ductility and its high resistance to stress corrosion cracking and sensitization. Nickel content has been known as an element that stabilizes austenite and increases toughness. Nitrogen has diverse effects on the microstructure and solidification cracking behavior of type 316 stainless steel.

The 316L(N) Stainless Steel (SS) is used as a structural material in the construction of Fast Breeder Reactor (FBR) components due to its compatibility with liquid sodium coolant, excellent high temperature mechanical properties, good weldability, freedom from sensitization during welding and the associated stress corrosion cracking that may occur during storage of these components in chloride environments in coastal construction site prior to installation and commissioning of the nuclear plants, availability of design data, indigenous availability, good service life under FBR operating conditions, vast experience and enhanced pitting resistance in the presence of molybdenum (Narayana Rao, 2010; Baldev Raj et al., 2010; Srinivas et al., 2011; Girish Shastry et al., 2004).
In the fabrication of FBTR, thickness in the range 5-40mm is usually used. For joining thick plates above 10mm, welding processes like shielded metal arc welding (manual metal arc welding), MP-TIG welding and submerged arc welding used these processes requires 10-20 number of passes for completion of the joint. This is time consuming and also leads to distortion of the welded joints. To overcome these difficulties welding processes like A-TIG welding, electron beam welding and laser beam welding are used.

A-TIG welding is an activated TIG process using multi-component flux. The flux in form of paste is applied on surface of joint before welding penetration up to 300% achieved in Austenitic Stainless Steel reduce the magnitude of tensile residual stress and distortion in welds MULTI-PASS (TIG) uses 1.V-groove for plates of 6mm thickness 2. Filler wire of 316LSS of 1.6mm diameter.

Using A-TIG welding it is possible to weld 12mm thick in a single pass .Laser welding and electron beam density welding are high energy high power welding processes. As far as electron beam welding there are no thickness limitations.40mm thick Plates can be joined in a single pass.

Laser welding can be used to join 316LN SS in a single pass for thickness up to 12mm or more. Laser welding aspects of 316LN SS has not been understood. In view of this laser welding process has been selected in the present work.

The effects of welding processes like Shielded Metal Arc Welding (SMAW), Multi–Pass Tungsten Inert Gas Welding (MP-TIG), Submerged Arc Welding (SAW) (Woo Gon Kim et al., 2007) and Activated flux Tungsten Inert Gas Welding (A-TIG) (Vasudevan et al., 2007; Chandrasekhar et al., 2010; Vasantharaja et al., 2012; Vasudevan et al., 2009) on the mechanical properties of different grades of austenitic stainless steels have been investigated in detail by numerous workers in the past. However the effects of other welding processes like Electron Beam Welding (EBW), Activated flux Electron Beam welding (A-EBW) and Laser Beam Welding (LBW) on the mechanical properties of 316L (N) stainless steel have not been studied in detail. From the reviewed literature on the aspects related to weldability of 316L (N) stainless steel it is clear that there is exists considerable scope on the laser welding aspects of 316L (N) stainless steel.
Weldability of 316 LN SS:
The austenitic stainless steels are considered the most weldable of the stainless steels. They are routinely joined by all fusion and resistance welding processes. Two important considerations for weld joints in these alloys are (1) avoidance of solidification cracking, and (2) preservation of corrosion resistance of the weld and heat-affected zones. Type 316LN stainless steel often is welded autogenously. If filler metal must be used for welding Type 316LN, it is advisable to utilize the low carbon Types 316L or E318 filler metals. Contamination of the weld region with copper or zinc should be avoided, since these elements can form low melting point compounds, which in turn can create weld cracking. Fusion welding is widely used to fabricate the reactor vessel and piping due to its complexity and size involved. Service experience has shown that the cracking in weld joints is the life limiting factor (Laha et al., 2007 and Manning et al., 1980). Major welded component often have to be taken out of service at a point in time where, little damage would have occurred in the parent material. In general, Tungsten Inert Gas welding (TIG) process with and without filler metal are used to join this class of materials. However, the most constraints of TIG welding of stainless steel lie in the limited thickness of the material which can be welded in a single pass, poor tolerance to chemical composition of the deposited weld metal leading to the formation of undesirable phases and the low productivity of joining. In this process, weld penetration achievable in single pass welding of stainless steel (SS) is limited to 3 mm when using argon as shielding gas. The penetration capability of the arc in TIG welding can be significantly increased by application of a flux coating containing certain inorganic compounds on the joint surface prior to welding (Mathew et al., 2007 and Matsuda et al., 1982). A-TIG welding is nothing but an activated TIG process using multi-component flux. The flux in the form of paste is generally applied on the surfaces of the joint before welding. Using A-TIG welding, penetration up to 300% has been achieved in austenitic stainless steel. Use of activated fluxes during TIG welding has been found to reduce the magnitude of tensile residual stresses and distortion in welds. Multi-pass (TIG) welding process generally uses a V-groove for plates of 6mm thickness and a filler wire of 316L SS of 1.6mm diameter. To reveal the δ-ferrite in the weld metal immersion etching in boiling murakkami etchant is generally used. This etchant consists of (10gm potassium ferric cyanide, 10gm potassium hydroxide and 100ml water). The presence of σ –phase can be detected by etching with modified murakkami etchant (30gm potassium ferric cyanide, 30gm potassium hydroxide and 150 ml water). Wealth of information exist in the literature on the welding process effects of 316LN material on the low temperature and high temperature mechanical properties. A-TIG and multipass TIG welding processes have been used to join 316LN
material and evaluated and compared the mechanical properties, microstructures of these weldments. The chemical compositions 316L(N)SS, 316L(N) weld metal of MP(TIG), and 316L(N) weld metal of(A-TIG) have been given in Table-1. The welding parameters of A-TIG and multi-pass TIG welding of 316LN have been given in Table-2.

Table 1: The chemical compositions 316L(N)SS, 316L(N) weld metal of MP(TIG), and 316L(N) weld metal of(A-TIG)

<table>
<thead>
<tr>
<th>Material</th>
<th>C</th>
<th>Cr</th>
<th>Ni</th>
<th>Mo</th>
<th>Mn</th>
<th>Si</th>
<th>S</th>
<th>P</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>316L(N)SS Base metal</td>
<td>0.028</td>
<td>17.5</td>
<td>12.2</td>
<td>2.3</td>
<td>1.65</td>
<td>0.44</td>
<td>0.01</td>
<td>0.024</td>
<td>0.085</td>
</tr>
<tr>
<td>316L(N)Weld Metal(MPTIG)</td>
<td>0.026</td>
<td>19</td>
<td>11</td>
<td>2.3</td>
<td>1.5</td>
<td>0.53</td>
<td>0.01</td>
<td>0.025</td>
<td>0.087</td>
</tr>
<tr>
<td>316L(N)Weld Metal(ATIG)</td>
<td>0.03</td>
<td>17.5</td>
<td>12.0</td>
<td>204</td>
<td>1.45</td>
<td>1.45</td>
<td>0.5</td>
<td>0.005</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Table 2: The welding parameters of A-TIG and multi-pass TIG welding of 316LN

<table>
<thead>
<tr>
<th>Welding Parameters</th>
<th>A-TIG</th>
<th>Multi-Pass TIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current (A)</td>
<td>200</td>
<td>110</td>
</tr>
<tr>
<td>Voltage (V)</td>
<td>12.5</td>
<td>12</td>
</tr>
<tr>
<td>Travel Speed(mm/min)</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>Number of layers</td>
<td>Single pass</td>
<td>7 passes</td>
</tr>
<tr>
<td>Heat input (KJ/mm)</td>
<td>1250 J/mm</td>
<td>990 J/mm</td>
</tr>
<tr>
<td>Plate thickness</td>
<td>6mm</td>
<td>6mm</td>
</tr>
</tbody>
</table>

The weldability aspects of 316L(N)SS has been documented in the recent literature (Mathew et al., 2010, 2012; Saktivel et al., 2012 and Ganesan et al., 2008 and 2010) with the intention that the service life of FBR can be extended from the present 40 years service life to 60 years service life. Investigations (Ramazan-Yimaz et al., 2002) on the mechanical properties of 304L and 316L stainless steel welded by Gas Tungsten Arc Welding (GTAW) and Gas Metal Arc Welding (GMAW) had shown that the mechanical properties such as yield strength, tensile strength, impact strength and micro-hardness values of GTAW were higher as compared to those of GMAW. Studies (Kim et al., 2007) on the mechanical properties of Submerged Arc Welded (SAW) 316L(N) SS revealed that the ultimate tensile strength values for the base and weld metal were almost similar at all the stress temperatures tested in the range 25-700 MPa. However, in the case of yield stress values the weld metal values were increased above about 50 MPa at all temperatures to the base one. Especially, the elongation value of the weld metal was decreased by about 50% to that of the base one. Investigations have also been carried out on the tensile properties of A-TIG welded and Multi-pass TIG welded 304LN SS and 316L (N) SS (Vasudevan, 2007; Vasudevan et al., 2008). It has been noted that transverse strength properties of the 316 and 316L (N) SS welds produced by A-TIG.
welding exceeded the minimum strength values of the base metal. Improvement in toughness values were observed in the 316L (N) SS produced by A-TIG welding (Vasudevan, 2007; Vasudevan et al., 2008).

The effects of welding processes like Shielded Metal Arc Welding (SMAW), Multi-Pass Tungsten Inert Gas Welding (MP-TIG), Submerged Arc Welding (SAW) (Woo Gon Kim et al., 2007) and Activated flux Tungsten Inert Gas Welding (A-TIG) (Vasudevan et al., 2007; Chandrasekhar et al., 2010; Vasantharaja et al., 2012; Vasudevan et al., 2009) on the mechanical properties of different grades of austenitic stainless steels have been investigated in detail by numerous workers in the past. However the effects of other welding processes like Electron Beam Welding (EBW), Activated flux Electron Beam welding (A-EBW) and Laser Beam Welding (LBW) on the mechanical properties of at least 316L (N) stainless steel have not been studied in detail. In the present investigation constant load creep tests have been carried out on laser welded 316L (N) stainless steel joints and 316LN SS base material. The 316LN base material is used as a high temperature structural material for FBTR applications (Reddy et al., 1980; Girish Shastry et al., 2004; Baldev Raj et al., 2010; Ganesan et al., 2010; Sakthivel et al., 2011; Mishra et al., 1997; Mathew et al., 2012; Tjong et al., 1995). The selection of this material is based on the considerations of its compatibility with liquid sodium coolant, superior creep strength at the FBTR operating temperatures, weldability availability of design data, and resistance to oxidation and resistance to corrosion and free from sensitization (Girish Shastry et al., 2004; Sakthivel et al., 2011; Tjong et al., 1995; Sakthivel et al., 2011).

MATERIAL AND METHODS
Fabrication of 316L (N) SS weldments
Preparation of the 316L (N) plates prior to welding
Three weldments have been fabricated using 6 plates of dimensions 250mm x 125mm x 5.5mm (length x width x thickness). These plates were milled in the thickness direction by keeping them in the vice of the milling machine. The plates were machined using a milling cutter and ensured that there will be zero clearance between the two plates when the plates were end-end fitted. The end-end fitted machined plates prior to running the laser beam along the length wise direction is shown in Figure 1.
Laser Welding Process Details

Equipment Details

The laser machine supplied by Messers Rofin, Germany Model was used for the fabrication of three 316LN SS weldments. The manufacturer details of the CO₂ laser used in the fabrication of the weldments are listed in Table 3.

Welding Conditions

Two weldments have been prepared using two different laser powers such as 3.0KW and 3.5KW. During the preparation of these two weldments, nitrogen shielding gas was employed to protect the fusion zone against the atmospheric contamination. One more weldment has been fabricated with 3KW laser power using Argon shielding gas. The details of the laser welding process employed during welding and parameters of laser welded joints have been listed in Table 4 and Table 5.

![Figure 1: Butt joint with (a) zero gap and (b) variable gap](image)

**Table 3: CO₂ laser welding conditions**

<table>
<thead>
<tr>
<th>Laser Type / Model</th>
<th>CO₂ Slab Laser (Model # DC-035, Rofin, Germany)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year Of Installation</td>
<td>2005</td>
</tr>
<tr>
<td>Wavelength</td>
<td>10.6µm</td>
</tr>
<tr>
<td>Output Power Peak Power</td>
<td>100-3500 W(CW)  8KW (pulsed)</td>
</tr>
<tr>
<td>Pulse Duration</td>
<td>5 ms – CW</td>
</tr>
<tr>
<td>Repetition Rate</td>
<td>5000 HZ</td>
</tr>
<tr>
<td>Workstation</td>
<td>4 – CNC (1500 mm x 3000 mm with 300 mm Z- stroke and 350 mm diameter rotary table)</td>
</tr>
<tr>
<td>Optics Available</td>
<td>360° rotating welding head with 150,200 and 300 mm focal length mirrors</td>
</tr>
<tr>
<td>Processes Possible</td>
<td>Welding, Cutting, Surface Modification</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4: Welding conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion-Cooled</td>
</tr>
<tr>
<td>Output Power</td>
</tr>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Wavelength</td>
</tr>
<tr>
<td>Beam Diameter</td>
</tr>
<tr>
<td>Excitation</td>
</tr>
<tr>
<td>Beam Quality</td>
</tr>
<tr>
<td>Power Stability</td>
</tr>
<tr>
<td>Focal point</td>
</tr>
</tbody>
</table>
Table 5: Parameters of laser welded joints

<table>
<thead>
<tr>
<th>Welding joint</th>
<th>shielding gas</th>
<th>flow rate</th>
<th>power</th>
<th>welding speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I joint</td>
<td>Argon</td>
<td>30lt/min</td>
<td>3KW</td>
<td>1m/min</td>
</tr>
<tr>
<td>II joint</td>
<td>Nitrogen</td>
<td>30lt/min</td>
<td>3.5KW</td>
<td>1m/min</td>
</tr>
<tr>
<td>III joint</td>
<td>Nitrogen</td>
<td>30lt/min</td>
<td>3KW</td>
<td>1m/min</td>
</tr>
</tbody>
</table>

X – Ray Radiography of weldments

All the laser fabricated weldments have been subjected to X-ray radiographic Non Destructive Test (NDT) to ensure soundness. Figure 2, 3 and 4 give the NDT results of the fabricated weldments it may be seen from Figure 2 that the argon shielding gas weldment has not confirmed to the radiographic quality and hence the weldment was not used for carrying out Tension test and Creep Tests. It may be noted that the weldment which fabricated with the argon as the shielding gas revealed the presence of under cuts, lack of fusion and spatter.

Machining of Tension Test, Creep Test, and Impact Test Samples

Machining of samples

Prior to machining of the tensile, creep and impact samples all the fabricated weldments have been dressed to the original base metal thickness. Tension test, creep test and impact test samples were machined in the transverse direction from the dressed weldments. Figure 5 depicts the configuration diagram of the weldment showing machining details of the tension and creep test samples. Fig 6 gives the design details of the tension test samples used for carrying out the tension test. Figure 7 gives the design details of the creep test samples used for carrying out the creep test. The details of the impact test sample machined from the dressed weldments are given in Figure 8. The high temperature tension tests have been carried out as per the ASTM standards.

Figure 2: The 3.0 KW Laser Power (Argon)
Figure 3: The 3.5 KW Laser Power (Nitrogen)

Figure 4: The 3.0 KW Laser Power (Nitrogen)

Figure 5: Schematic diagram of the weldment showing machining details of the tension and creep test samples
**Tensile Test Samples**

The Universal Testing machine with the capacity 100KN (10 Tonne) manufactured by Hung Ta (model HT 242) supplied by Blue Star India was used for carrying out all the room temperature as well as high temperature (650°C) tension tests. All the tensile tests were carried out at a nominal strain rate of $3 \times 10^{-4}$/sec. Room temperature tension tests have also been carried out from the 316L (N) SS base metal. The design details of the tension test samples used for carrying out the room temperature and high temperature tension tests are given in Figure 6.

**Creep Test Samples**

Creep tests have been conducted in air using constant load creep testing machines. Single creep test was carried out on each material in air under the prescribed test conditions 650°C (923K) at 180MPa, 200MPa, 220MPa and was repeated whenever found necessary. Elongation of the creep specimens was monitored by an extensometer and digital dial gauge attachment.

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**Figure 6:** Tension test sample design.

**Figure 7:** Creep test sample design.
Impact Test Samples
Impact tests have been conducted on the impact samples machined in the transverse direction providing the notch in the weldmetal. Impact tests have also been carried out on the basemetal. All the charpy impact test samples have been carried out using charpy impact testing machine (Figure 8).

Chemical Composition
Analysis and the chemical composition of the same (Wt %) has been provided in Table 6 along with the base metal chemical composition.

Metallography
Sample Preparation for Optical Microscope
Metallographic samples for macro and micro structures were machined from the weldments. The machined metallographic samples were subjected to usual emery polishing procedures followed by cloth polishing procedures, ultrasonically cleaned, and etched in an electrolytical bath containing 60% HNO₃ and 40% water for 15sec at 3V to reveal the fusion zone macro structures, microstructures, Heat Affected Zone (HAZ) and base metal microstructures.

Optical microscope was used to observe the macro as well as the micro structures. The observed macro and micro structures were photographed at a suitable magnification.

![Figure 8: Impact test sample design](image)

Table 6: Chemical composition (Wt %) of the 316LN Base metal and weld deposit

<table>
<thead>
<tr>
<th>Material</th>
<th>C</th>
<th>Cr</th>
<th>Ni</th>
<th>Mo</th>
<th>Mn</th>
<th>Si</th>
<th>N</th>
<th>S</th>
<th>P</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>316L(N)SS Base plate</td>
<td>0.028</td>
<td>17.5</td>
<td>12.2</td>
<td>2.3</td>
<td>1.65</td>
<td>0.44</td>
<td>0.085</td>
<td>0.01</td>
<td>0.024</td>
<td>Balance</td>
</tr>
<tr>
<td>316L(N)SS Weld Metal</td>
<td>0.025</td>
<td>17.6</td>
<td>12.2</td>
<td>2.41</td>
<td>1.7</td>
<td>0.36</td>
<td>0.082</td>
<td>0.006</td>
<td>0.021</td>
<td>Balance</td>
</tr>
</tbody>
</table>
Delta - Ferrite Measurements
Immersion etching in boiling murakkami’s etchant (10gr potassium ferric cyanide, 10g potassium hydroxide and 100ml water) was used to reveal the delta ferrite. Ferrite scope was also used to know the ferrite numbers in the base metal and as well as in the fusion zone. The ferrite scope had a measuring accuracy of 0.001%. In the as welded condition the fusion zone has not revealed any delta ferrite when immersion boiling murakkami’s etchant was used and observed under optical microscope. The ferrite scope results given as Ferrite Numbers (FN) are compared to those of the other welding processes. The ferrite number results reveal that the weld deposit FNs are considered to be insignificant as compared to those of the other welding processes.

RESULTS AND DISCUSSIONS
Weldzone Microstructures
Optical Micro Structures
The macrostructure of the 3KW and 3.5KW laser welded 316L (N) SS weldment reveals, weldzone, heat affected zone and the base metal shown in (Figure 9 (a) and 9(b)). The microstructure of the interface between weld bead and parent metal 3KW and 3.5KW laser welded 316L (N) SS shown in (Figure 10(a) and 10(b) The as welded microstructure of the laser welded 316L (N)SS using 3.5KW laser power is shown in Figure 11(a), 11(b) and 11(c). It shows columnar dendritic and equiaxed dendritic microstructures. The microstructure shown in Fig.12 is the base metal microstructure of 316L (N) SS. It shows equiaxed grain structure.

![Figure 9(a): 3Kw 50X](image1)
![Figure 9(b): 3.5KW 50x](image2)

The macrostructure of the 3KW and 3.5KW laser welded 316L (N) SS weldment reveals, weldzone, heat affected zone and the base metal shown in (Figure 9(a) and 9(b)).
The microstructure of the interface between weld bead and parent metal 3KW and 3.5KW laser welded 316L (N) SS shown in (Figure 10 (a) and 10(b)).

Figure 10(a): 3KW 200x  
Figure 10(b): 3.5KW 200x

Figure 11: (a) Optical microstructure of the 3.5KW laser welded fusion zone; (b) Optical microstructure of the 3.5KW higher magnification laser welded fusion zone and Figure 11(c) Optical microstructure of the 3 KW laser welded fusion zone.

Figure 12: Optical microstructure of the 316L (N) basemetal
The melted base metal insitu solidified due to the conduction of heat through the base metal perpendicular to the welding direction. As a result, the base metal acted as a nucleation site causing the dendritic grains to grow towards the centre of the fusion zone. On other hand the equiaxed grains might have developed by homogenous nucleation. The growing dendrites might have broken and there by acted as nucleation site.

**Mechanical properties, Tensile testing, Stress Strain Curves**

Engineering stress strain curves of the material 316L(N) Base Metal and laser welded tensile tested at a strain rate of 3x10^{-4} s^{-1}over the temperature range of room temperature and high temperature 650°C (923K) shown in figs 13- 18.

![Stress strain curve of tension tested 316 L(N) base metal at Room Temperature](image1)

**Figure 13:** Stress strain curve of tension tested 316 L(N) base metal at 650°C (923K)

![Stress strain curve of tension tested 316 L(N) base metal at Room Temperature](image2)

**Figure 14:** Stress strain curve of tension tested 316 L(N) base metal at Room Temperature
Figure 15: Stress strain curve of tension tested 3KW laser welded 316 L(N) at 650°C (923K)

Figure 16: Stress strain curve of tension tested 3KW laser welded 316 L(N) at Room Temperature

Figure 17: Stress strain curve of tension tested 3.5KW laser welded 316 L(N) at 650°C (923K)
Comparison of the stress-strain curves of room temperature tension tested 316L (N) base metal, 3.0KW laser welded and 3.5Kw laser 316L (N) weldments given in table. The high temperature deformation of the 316L (N) SS is seen in the form of serrated yielding. This may be due to the fact that the interstitial elements carbon and nitrogen diffuse and pin the dislocation structure. The serrated yielding is variously known as dynamic strain ageing, negative strain rate sensitivity and changes in the internal friction behavior.

**Mechanical Properties**

The results of the tension tests conducted on the transverse samples machined from the 3.0 KW and 3.50 KW laser welded joints are given in Table 7, along with the base metal.

**Table 7:** Room temperature and high temperature mechanical properties of 316L(N)SS basemetal and the weld deposits

<table>
<thead>
<tr>
<th>No</th>
<th>Power KW</th>
<th>Room Temperature</th>
<th>650°C</th>
<th>Impact Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 Y.S.</td>
<td>U.T</td>
<td>%E</td>
<td>%R.A.</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>329</td>
<td>344.</td>
<td>593</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>326</td>
<td>344.3</td>
<td>602</td>
</tr>
<tr>
<td>3</td>
<td>Basel</td>
<td>325</td>
<td>345.4</td>
<td>598.6</td>
</tr>
</tbody>
</table>
DISCUSSION

On comparison, it could be seen that the properties of the welded joints are not significantly different from those of the base metal. The yield strength, 0.2% proof stress and ultimate tensile strength properties of the welded joints fabricated at 3.0KW and 3.50KW laser power are more or less remain unchanged from the base metal.

Impact Tests

The charpy impact test results of the base metal are compared with those of the welded joints fabricated at 3.0KW and 3.50KW laser power in Table 7. It may be noted that the impact strength properties of the both the welded joints had shown same values to those of the base metal.

Mechanical properties and Micro structure

The creep rupture properties of laser welded 316L(N) SS are given in Table 8, where rupture lives of weld deposits are compared with those of basemetal it may be noted that the 3.0KW laser power and 180Mpa rupture samples showed weak strength under creep at 650°C.At other stresses, the weld deposits displayed superior strength then the basemetal. Upon comparing laser welded joints with the electron beam welded joints, S.C.Tjong et al, have observed that both have almost similar creep ruptured properties. The near fracture regions of the creep ruptured laser welded 316L (N) SS samples at stresses in the range 180–220MPa have been studied under optical microscope to know the mechanisms of creep failures. Fig 19(a) shows the extent of creep damage in the near fracture region of the 3.5KW laser welded 200MPa stress creep tested 316L (N) SS. The damage could be seen in the form of voids, micro cracks and wedge cracks. The extent of damage at 220MPa stress on the near fracture region of the laser welded 316L(N)SS is given in Fig 19(b). Fig 19(b) shows damage in the form of wedge cracks and full length boundary cracks formed due to void coalescence. At the regions where the slip bands intersected the grainboundary nucleation of voids were also observed. Fig 19(c) shows voids, micro cracks and the formation of micro cracks due to void coalescence in the 3.5KW, laser welded 200 MPa creep tested 316L(N)SS. Fig 19(d) shows wedge cracks at triple boundary junctions and full length boundary crack due to linkage of micro cracks. Fig 19(e) shows voids along the grain boundaries and the formation of major crack due to the linkage of full length boundary cracks in the 3.5KW laser welded and 180MPa creep tested 316L(N)SS. Overall, it could be seen that at 200 and 220 MPa stress wedge crack population at the triple boundary junctions was higher. At 180MPa stress cavities nucleated along the grain boundaries, at triple boundary junctions and at the regions where slip bands intersected the grain boundary. The results
are well in agreement with the others who reported that wedge cracks nucleate at higher stresses.

**Figure 19(a):** The creep damage in the near fracture region of the 3.5KW laser welded 200MPa stress creep tested 316L(N) SS.

**Figure 19(b):** The creep damage at 220MPa stress on the near fracture region of the laser welded 316L(N)SS.

**Figure 19(c):** Voids, micro cracks and the formation of micro cracks due to void coalescence in the 3.5KW, laser welded 200 MPa creep tested 316L(N)SS.

**Figure 19(d):** Wedge cracks at triple boundary junctions and full length boundary crack due to linkage of micro cracks.

**Figure 19(e):** Voids along the grain boundaries and the formation of major crack due to the linkage of full length boundary cracks in the 3.5KW laser welded and 180MPa creep tested 316L(N)SS.
Table 8: Creep – Rupture Properties of 316L (N) SS welded joints at different stresses and at 650°C.

<table>
<thead>
<tr>
<th>No</th>
<th>Power KW</th>
<th>Creep Properties @ 650°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stress, MPa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Rupture Life (hrs)</td>
<td>% E</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1328.3</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>3048.8</td>
</tr>
<tr>
<td>3</td>
<td>Base Metal</td>
<td>1837</td>
</tr>
</tbody>
</table>

CONCLUSIONS

- 316L (N) SS can be satisfactorily welded using laser welding with weld metal properties at least equivalent to those of the base metal.
- Laser welded 316L (N) SS weld metal has not revealed any delta-ferrite in the weld metal, when etched and observed metallographically. However, ferrite scope had shown ferrite numbers in the weld metal which are not significantly different from those of the base metal.
- Creep failures occurred in an intergranular fashion at all the stresses studied. Cavities and wedge cracks nucleated during creep deformation. Voids linked up together during the course of the creep deformation forming micro-cracks. Micro-cracks linked up together forming full length boundary cracks.
- Full length boundary cracks constituted the fracture forming a major crack along the grain boundaries.
- Creep rupture times of the weld deposits are significantly higher than those of the base metal except the 3.0KW laser power and 180Mpa creep tested.
- At higher stresses creep rupture samples revealed higher percentage population of wedge cracks as compared to that of at lower stress levels.
- The welded microstructure of the laser welded 316L (N) SS shows columnar dendritic and equiaxed dendritic microstructures. The base metal microstructure of 316L(N)SS shows equiaxed grain structure.
- The room temperature and high temperature yield strength and ultimate tensile strength values of both the weld deposits are more or less similar to those of the base metal.
- Both the weld deposits failed in the base metal.
- High temperature tension tested welded samples of 316L(N) SS showed mixed mode of fracture in the case of 3KW laser power and inter granular fracture in the weld deposits of 3.5KW laser power.
The laser deposited weldments were considerably stronger, ductile and the mechanical property values were equivalent to those of the base metal.

Laser welding can be used with confidence for the joining of the 316L(N) SS.

This work can be extended to study the effects of Argon or helium shielding on the laser welding of 316LN stainless steel.

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Cyber Forensic Tools and its Exploration

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Abstract
As the usage of internet has become a major portion of daily human life of general public, thus increasing the growth of cyber crime and so the scope of cyber crime investigation has also been extended. Cyber forensics is a new and fast growing field that involves carefully collecting and examining electronic evidence of cyber crime. As criminals aggressively expand the use of technology in their enterprise of illegal activities (cyber crimes) cyber forensics is very important and that not only estimates the damage to a computer as a result of cyber crime, but also to recover lost information from such a system to prosecute a criminal. This paper aims to demonstrate the importance of cyber forensics by describing investigation procedures, tools and differences in the use for individuals/small groups vs. large groups. The investigation procedures described in this paper deal with how to collect evidence and the laws that need to be followed for admission of evidence into a court room. The cyber forensic tool (Encase) demonstrated in this paper is the basis for all tools that are available. The role of cyber forensic tools include, backing up data, authentication, decryption, file auditing, IP tracking, and data recovery and document examination. This Paper also explores emerging cyber crimes and its forensic investigation procedures to create logical evidences for prosecuting the criminals using Encase forensic tool and its techniques.

Keywords: Cyber Forensic Investigation; Cyber forensic tools; Cyber crime; Criminals and Encase

INTRODUCTION
Now a day’s our digital world is becoming targets of crime activities, such as web defacement, vandalism, espionage and even cyber war. Different types of cyber attacks from various sources may adversely affect computers, a network, an agency’s operation or the Internet itself. So the banks and various financial organizations and products aim to take assistance of legal and cyber forensics. Cyber forensics is involved in analyzing, investigating and presenting digital evidence which encompasses the areas of computer, network and internet forensics.

Computers Forensics—is forensics performed on storage media, hard drives, thumb drives, etc.

Network Forensics – is forensics performed on the communication traffic data between networked devices; such as computers, network devices, printers, etc.
Internet Forensics – is forensics performed on global Internet communications from multiple networks.

The information, which is in the form of digital, can be easily modified, duplicated, restored or destroyed. And it should not be modified without proper authorization. To achieve this, the forensic investigator should use different forensic tools. Forensic tools are playing vital role in criminal investigation process for gathering and preserving evidence, reconstructing events, and assessing the present state of an attack or event.

This paper first section explains introduction and second section describes phases in the cyber forensic investigation and third part explains about the types of cyber crimes fourth section explains the investigation procedure of different cyber crimes including complete investigation of storage media. Any crime committed in the computer or cyber world starts from the basic investigation procedure of storage system. So our paper helps an investigation become easier and faster.

Cyber Forensics Phases

There are four phases in cyber forensics: Identification phase, Acquisition phase, Analysis phase and Reporting Phase. Below figure shows various phases of cyber forensics process and each phase responsibility. The identification phase mainly deals with incident identification, evidence collection and checking of the evidence. The acquisition phase saves the state of a computer system that can be further analyzed. The analysis phase collects the acquired data and examines it to find the pieces of evidences. The reporting phase comprises of documentation and evidence retention.

![Cyber Forensics Phases Diagram]

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Identification Phase

The identification phase is the process of identifying evidence material and its probable location. This phase is unlike a traditional crime scene it processes the incident scene and documents every step of the way. Evidence should be handled properly. Basic requirement in evidence collection is evidence must be presented without alteration. This requirement applies to all phases of forensics analysis. At the time of evidence collection, there is a need of thorough check of system logs, time stamps and security monitors.

Once evidence collected, it is necessary to account for its whereabouts. Investigators would need detailed forensics to establish a chain of custody, the documentation of the possession of evidence. Chain of custody is a vital part of computer forensics and the legal system and the goal is to protect the integrity of evidence, so evidence should be physically secured in a safe place along with a detailed log. Below figure shows the evidence and chain of custody which is useful during incident investigation. Handling specific type of incidents like Denial of Service, Malicious Code, Unauthorized access etc are described in computer security incident handling guide.

Acquisition Phase

The acquisition phase saves the state of evidence that can be further analyzed. The goal of this phase is to save all digital values. Here, a copy of hard disk is created, which is commonly called as an image. As per law enforcement community, there are three types of commonly accepted forensics acquisition: Mirror image, Forensics duplication and live acquisition (RAM).
Mirror images, bit-for-bit copy, involve the backups of entire hard disk. Creation of mirror image is simple in theory, but its accuracy must meet evidence standards. The purpose of having mirror image is evidence available in the case of the original system need to be restarted for further analysis.

A forensic duplicate, sector-by-sector, is an advanced method that makes a copy of every bit without leaving any single bit of evidence. The resultant may be single large file and must be an exact representation of the original drive at bit-stream level.

It is often desirable to capture volatile information, which is stored in RAM; it cannot be collected after the system has been powered down. This information may not be recorded in a file system or image backup, and it may hold clues related to attacker. All currently running processes, open sockets, currently logged users, recent connections etc., are available in volatile information.

**Analysis Phase**
Forensic analysis is the process of understanding, re-creating, and analyzing arbitrary events that have gathered from digital. The analysis phase collects the acquired data and examines it to find the pieces of evidences. This phase also identify that the system was tampered or not to avoid identification. Analysis phase examines all the evidence collected during collection and acquisition phases. There are three types of examinations can be applied for the forensics analysis; limited, partial or full examination.

Limited examination covers the data areas that are specified by legal documents or based on interviews. This examination process is the least time consuming and most common type. Partial examination deals with prominent areas. Key areas like log files, registry, cookies, e-mail folders and user directories etc., are examined in this case of partial examination. This partial examination is based on general search criteria which are developed by forensic experts. Most time consuming and less frequent examination process are full examination. This requires the examiner to look each, and every possible bit of data to find the root causes of the incident. File slack inspection is done in this examination. EnCase forensic tool has the ability to process larger amounts and allow the user to use predefined scripts to pull information from the data being processed.

**Reporting Phase**
The reporting phase comprises of documentation and evidence retention. The scientific method used in this phase is to draw conclusions based on the gathered evidence. This
phase is mainly based on the Cyber laws and presents the conclusions for corresponding evidence from the investigation. There is a need of good policy for how long evidence from an incident should be retention. Factors to be considered in this process are prosecution, data retention and cost. To meet the retention requirements there is a need of maintaining log archival. The archived logs must be protected to maintain confidentiality and integrity of logs.

**Types and Examples of Cyber Crimes**

Cyber crime is an unlawful act wherein the computer is either a tool or a target or both. Crime committed over Internet is also treated as cyber crime. The first recorded cyber crime took place in 1820. Cyber Crime refers to all activities done with criminal intent in cyberspace. These fall into three slots.

- Those against persons.
- Against Business and Non-business organizations.
- Crime targeting the government.

Let us examine the acts wherein the computer is a tool for an unlawful act. This kind of activity usually involves a modification of a conventional crime by using computer. Some examples are:

1. **Financial Crimes** - Financial crimes are crime against property, involving the unlawful conversion of the ownership of property (belonging to one person) to one’s own personal use and benefit.
2. **Sale of illegal articles** – This would include sale of narcotics, weapons and wildlife etc., by posting information on websites, bulletin boards or simply by using e-mail communications.
3. **Online gambling** - There are millions of websites; all hosted on servers abroad, that offer online gambling. In fact, it is believed that many of these websites are actually fronts for money laundering.
4. **Intellectual property crimes** - These include software piracy, copyright infringement, trademarks violations etc.
5. **Email spoofing** - A spoofed email is one that appears to originate from one source but actually has been sent from another source. This can also be termed as E-Mail forging.
6. ** Forgery** - Counterfeit currency notes, postage and revenue stamps, mark sheets etc., can be forged using sophisticated computers, printers and scanners.
7. **Cyber-pornography** - This would include pornographic websites; pornographic magazines produced using computer and the Internet (to down load and transmit pornographic pictures, photos, writings etc.)

8. **Cyber defamation** - This occurs when defamation takes place with the help of computers and or the Internet e.g. someone published defamatory matter about someone on a websites or sends e-mail containing defamatory information to all of that person’s friends.

9. **Cyber stalking** - Cyber stalking involves following a person’s movements across the Internet by posting messages on the bulletin boards frequented by the victim, entering the chat-rooms frequented by the victim.

10. **Web defacement** - The changing or defacing of a web page or web site by an unauthorized individual or process, usually a hacker.

11. **Email bombing** - Email bombing refers to sending a large amount of e-mails to the victim resulting in the victims’ e-mail account or mail servers.

12. **Data hiding** - This kind of an attack involves altering the raw data just before it is processed by a computer and then changing it back after the processing is completed.

13. **Salami attacks** - Those attacks are used for the commission of financial crimes. The key here is to make the alteration so insignificant that in a single case it would go completely unnoticed e.g. a bank employee inserts a program into bank’s servers that deducts a small amount from the account of every customer.

14. **Denial of Service attack** - This involves flooding computer resources with more requests than it can handle. This causes the resources to crash thereby denying authorized users the service offered by the resources.

15. **Virus/worms attack** - Viruses are programs that attach themselves to a computer or a file and then circulate themselves to other files and to other computers on a network. They usually affect the data on a computer, either by altering or deleting it. Worms, unlike viruses don not need the host to attach themselves to.

16. **Trojan horse** - A Trojan as this program is aptly called is an unauthorized program which functions from inside what seems to be an authorized program, thereby concealing what it is actually doing.

17. **Internet time theft** - This connotes the usage by unauthorized persons of the Internet hours paid for by another person.

18. **Web jacking** - This term is derived from the term hi jacking. In these kinds of offences the hacker gains access and control over the web site of another. He may even change the information on the site. This may be done for fulfilling political
objectives or for money. E.g. recently the site of MIT (Ministry of Information Technology) was hacked by the Pakistani hackers and some obscene matter was placed therein.

19. Email frauds - Almost as soon as e-mail became widely used, it began to be used to defraud people via e-mail fraud. E-mail fraud can take the form of a "con game" or scam. Confidence tricks tend to exploit the inherent greed and dishonesty of their victims: the prospect of a 'bargain' or 'something for nothing' can be very tempting.

20. Cyber terrorism - Cyber terrorism is the use of Internet based attacks in terrorist activities, including acts of deliberate, large-scale disruption of computer networks, especially of personal computers attached to the Internet, by the means of tools such as computer viruses.

Cyber Forensic Tools (Encase Forensic Imager Tool)

There are many cyber forensic tools are used during a digital investigation process. Data collection / Acquisition Tools: are help to collect needed evidence for the investigation. For collecting the evidence during the investigation process following concepts are playing vital role. Let us have a look on them.

A. Hidden data analysis in storage media

Suspects can hide their sensitive data in various areas of the file system such as Volume slack; file slack, bad clusters, deleted file spaces [8].

Hard disk: The maintenance track / Protected Area on ATA disks are used to hide information. The evidence collection tools can copy the above contents.

File System Tables: A file allocation table in FAT and Master File Table in NTFS are used to keep track of files. These entries are manipulated to hide vital and sensitive information [8].
**File Deletion:** When a file is deleted, the record of the file is removed from the table, thereby making it appear that it does not exist anymore. The clusters used by the deleted file are marked as being free and can now be used to store other data. However, although the record is gone, the data may still reside in the clusters of the hard disk. That data we can recover by calculating starting and end of the file in Hex format and copy it into a text file and save with corresponding extension.

**Recover a JPEG file:**
- Open file in the hex format
- Check the file signature
- Copy from starting signature to ending signature.

For example: JPEG/JPG/JPE/JFIF file starting signature is FF D8 FF E1 XX XX 45 78 69 66 00 (EXIF in ASCII Exchangeable image file format trailer is FF D9.
- Open the file with corresponding application.

**Partition Tables:** Information about how partitions are set up on a machine is stored in a partition table, which is a part of the Master Boot Record (MBR). When the computer is booted, the partition table allows the computer to understand how the hard disk is organized and then passes this information to the operating system. When a partition is deleted, the entry in the partition table is removed, making the data inaccessible. However, even though the partition entry has been removed, the data still resides on the hard disk.

**Slack space:** A file system may not use an entire partition. The space after the end of the volume called volume slack that can be used to hide data. The space between Partitions is also vulnerable for hiding data. file slack space is another hidden storage. When a file does not end on a sector boundary, operating systems fill the rest of the sector with data from RAM, giving it the name RAM slack. When a file is deleted, its entry in the file system is updated to indicate its deleted status and the clusters that were previously allocated to storing are unallocated and can be reused to store a new file. However, the data are left on the disk and it is often possible to retrieve a file immediately after it has been deleted. The data will remain on the disk until a new file overwrites them however, if the new file does not take up the entire cluster, a portion of the old file might remain in the slack space. In this case, a portion of a file can be retrieved long after it has been deleted and partially overwritten.
**Free space:** However, when a file is moved from one hard disk or partition to another, it is actually a multistep process of copying and deleting the file. First, a new copy of the file is created on the target partition. After the file has been copied, the original file is then deleted. This process also requires some housekeeping in the FAT or MFT tables. A new entry is created in the table on the partition where it has been copied, whereas the record for the deleted file is removed from the table on its partition. When a file gets deleted, that space considered as free space, there also criminal can hide sensitive information.

**EnCase Forensic Software Suite:**
EnCase is another popular multi-purpose forensic platform with many nice tools for several areas of the cyber forensic process. It is a Windows-based comprehensive and complete forensic application. This tool can rapidly gather data from various devices and find potential evidence. It also produces a report based on the evidence. EnCase is recognized as a court-validated standard in computer forensics software. This tool does not come for free. The license costs $995.

Encase can have the following functionalities.

1. File signature analysis
2. Filter conditions and queries
3. View deleted files and file fragments in unallocated or slack space
4. Folder recovery
5. Log file and event log analysis
6. File type search
7. Registry viewer, external file viewer
Types of Evidence Files

EnCase Evidence Files:
Legacy EnCase evidence files (.E01) are a byte-for-byte representation of a physical device or logical volume. Current EnCase evidence files (.Ex01) can be encrypted; however, .Ex01 files are not backward compatible with legacy versions of EnCase.

EnCase evidence files provide forensic level metadata, the device level hash value, and the content of an acquired device. Dragging and dropping an .E01 or .Ex01 file anywhere on the EnCase Forensic Imager interface adds it to the currently opened case.

Logical Evidence Files
Logical evidence files (.L01) are created from previews, existing evidence files, or Smartphone acquisitions. These are typically created after an analysis locates some files of interest, and for forensic reasons, they are kept in a forensic container. Current logical evidence files (.Lx01) provide encryption and hashing options, but they are not backward compatible with legacy versions of EnCase.

When an .L01 or .Lx01 file is verified, the stored hash value is compared to the entry’s current hash value.

- If the hash of the current content does not match the stored hash value, the hash is followed by an asterisk (*).
- If no content for the entry was stored upon file creation, but a hash was stored, the hash is not compared to the empty file hash.
- If no hash value was stored for the entry upon file creation, no comparison is done, and a new hash value is not populated.

Raw Image Files
Raw image files are a dump of the device or volume. There are no hash comparisons or CRC checks. Therefore, raw image files are not as forensically sound as EnCase evidence files. Although the files are not in EnCase evidence file format, EnCase Forensic Imager supports a number of popular formats.

Before you can acquire raw image files, they must be added to a case. Raw image files are converted to EnCase Forensic Imager evidence files during the acquisition process, adding CRC checks and hash values if selected.

Creating an Encrypted Logical Evidence File in EnCase:
Step 1: In the Evidence tab, select one or more entries in the left pane. Right click, then click Acquire > Create Logical Evidence File from the dropdown menu.

Note: The folder highlighted when you click Create Logical Evidence File is treated as the root folder for including entries in the logical evidence file. Only blue checked child entries inside that folder are included. To include files from more than one folder, you must highlight a folder that is a common parent.

Step 2: The Create Logical Evidence File dialog displays. It opens to the Location tab by default.
Step 3: In the **Location** tab:
- a. Enter the evidence file name.
- b. Enter the evidence number.
- c. Enter the case number.
- d. Enter the examiner name.
- e. Add notes, if desired.
- f. Check the **Add to existing evidence file** checkbox if you want to add this file to an existing logical evidence file. You must specify the output path to an existing logical evidence file that is not locked.
- g. Specify the output path for the logical evidence file.

Step 4: In the **Logical** tab:

![Create Logical Evidence File dialog box](image)

**Source** is the root level folder or device containing blue checked items to include in the logical evidence file.

**Files** contains the number of files and the total size of the file or files to include in the logical evidence file.

**Target folder within Evidence File** is an optional user-specified folder that is created inside the logical evidence file. Any selected files in the source location are placed inside this folder. This is useful for organizing multiple additions to a single logical evidence file.
Include contents of files checkbox: If checked, file content data displays in the View pane when you open the logical evidence file.

File in use checkbox: If checked, the hash is computed when the file is read from evidence. This is valuable when previewing live data that may have changed since initially calculating the hash value.

Include original extents checkbox: If checked, original extent information is added to the logical evidence file. Physical Location, Physical sector, and File Extents columns in the logical evidence file will match the original entries.

Include contents of folder objects checkbox: If checked, folder content data displays in the View pane when you open the logical evidence file.

Lock file when completed checkbox: If checked, the logical evidence file is locked after creation.

Step 5: In the Format tab:

1. For the Evidence File Format, select Current (Lx01). This is the default.
2. From the Entry Hash dropdown menu, select a hashing algorithm: MD5 (default)
3. Specify Compression as Enabled (default) or Disabled.
Step 6: Click the Encryption button to open the Encryption Details dialog.

Note: By default, Encase Forensic Imager saves encryption keys to the My Documents folder of the current user profile. To save the encryption keys to a different location, right-click in the Encryption Details dialog, then click Change Root Path from the dropdown menu.

Step 7: Click the key icon in the upper pane to open the New Encryption Key dialog.

Step 8: Click Next to generate a new encryption key.
**Step 9:** after the key is generated, the Password dialog displays.

![Password dialog](image)

**Step 10:** Enter a name for the encryption key, then enter a password and enter the password again to confirm it. The Password Quality bar indicates if the password you entered is acceptable.

**Step 11:** When you have entered an acceptable password, confirm the password, and then click **Finish**.

**Step 12:** EnCase Forensic Imager prompts you to save the public key file you just created.
Step 13: Back in the Encryption Details dialog; click **Update** to display a checkbox for the key you just created.

![Encryption Details dialog](image)

Step 14: Click the checkbox for the new key, and then click **OK**.

![Encryption Details with new key](image)

Some Other Cyber Forensic Tools

Cyber forensic primarily is used in the investigation of cyber crimes (i.e., crimes that occur over and on the technology front). However this need not be the case, since most forensic techniques and tools are also used for scientific purposes and research. With serious issues like terrorism that threaten the national integrity of a country it is only wise to learn and know the tools of the trade that terrorists use against the state. Cyber forensic tools aid not only in investigating crime cases but also for drafting and creating hard evidences for the same. Let us see some of these tools that have been used since long by forensic investigators and scientists.
**WinHex** - WinHex is used as a universal hexadecimal editor and is primarily useful in low-level data processing, file inspection, digital camera card recovery, recovery of files even from corrupt files systems, etc. This is one heck of a powerful tool and can especially be used in gathering digital evidence. It is an open source tool.

**First On Scene (FOS)** - FOS is the only one tool of its kind. It is rather a visual basic script code than a executable binary file. First On Scene works with other tools such as PSTools, LogonSessions, FPort, NTLast, PromiscDetect, FileHasher, etc. to gather an evidence log report. This log report can further be analyzed by forensic experts to extract important information.

**Rifiuti** - Rifiuti is a unique tool that aids investigators in finding the very last details of your system’s recycle bin folders. Rifiuti is useful to gather critical information on your entire delete and undelete activities.

**Pasco** - Pasco is a Latin word for "browse". Pasco helps in the analysis of the contents of internet explorer’s cache. So in short it can be particularly useful to gather internet activity records from a target computer.

**Galleta** - Galleta is a Spanish word that means "cookie". Galleta is useful in examining the contents of cookie files on your machine. Cookie files are basically temporary internet files used by websites to maintain their indigenous logs for tracking and other such purposes.

**Forensic Acquisition Utilities (FAU)** - Forensic Acquisition Utilities is a set of forensic tools such as md5 checker, file wiper, etc. used for assorted purposes in research and investigation.

**NMap** - NMap is particularly associated with network security. NMap is a port scanner tool that helps find open ports on a remote machine. What separates NMap from other tools is its ability to evade source machine identity and to work without causing any Intrusion Detection System (IDS) alarms to go off.

**Ethereal** - Ethereal is another network security tool which is not a port scanner but rather a network packet sniffer. Ethereal sniffs data packets over the network and can provide investigators with incoming/outgoing data that is sent over a network. However, ethereal it cannot be useful in cases where strong encryption algorithms are in place at the source and destination computers.
BinText - BinText does not directly investigate but can be useful to browse through gathered evidence files such as that of log files generated by other forensic tools. BinText can be used for pattern matching and filtering these log files.

PyFlag Tools - PyFlag are a couple of tools used for log analysis and can be a very effective tool for investigators if coupled and used with other forensic tools.

Current Research in Cyber Forensics
Cyber Forensics is sizzling topic of the current trends. Many researchers started doing intensive research in this current area. New directions in this field include authorship analysis, digital evidence collection and forensics investigation process, proactive forensics, intrusion detection systems with the help of honey pots, building evidence graphs, identifying usage of mobile phones in cybercrimes and hash function for preserving the integrity of evidence. The complete picture of Cyber Forensics in the form of Cyber forensics ontology which can be helpful for studying cyber forensics is given in. Proactive forensics helps in the creation of preventive intelligence and threat monitoring besides post incident investigations.

Currently in Ethiopia INSA (Information & Network Security Agency), a government organization is providing the technical intelligence pertaining to national interest. The main aim of INSA is to build national cyber power capable of protecting the national interest. The INSA is taking care of all the cyber security related issues happened in Ethiopia.

In India RCCF (Resource Center for Cyber Forensics) a government organization is providing the technical services like Cyber Crime Analysis, Cyber Investigation, Expert witness and creating awareness about cyber crimes. Currently RCCF is developing various forensic tools in the areas of Disk Forensics, Network Forensics, Mobile Device Forensics and Live Forensics.

CONCLUSIONS
Cyber forensics is a promising field in the current research scenario. Detailed study of the field of cyber forensics is given in this paper. When analyzing cyber forensics, the process of doing so is different from the traditional forensics. In this paper, we described various cyber forensics related definitions, phases of cyber forensics and forensics methodology. Various phases of the Cyber forensics have been discussed and each phase explored clearly. We also described about the types and examples of various cyber crimes. Moreover, we explained about Encase forensic imager tool with the practical example of
creation of logical evidence. EnCase software comes in several products designed for forensic, cyber security, security analytics, and e-discovery use. The company also offers EnCase training and certification. Finally, we had shown the current research trends in this new era of cyber forensics; it still evolves and will remain a hot topic as long as attackers have the methods to violate the data security.

Our intention in writing this paper is to bring together the different views of cyber forensics in one place, but it is not meant to be a complete description of the field. We hope earnestly that, this paper will cater the needs of novice researchers and students who are interested in the development of cyber forensic tool software’s.

Acknowledgment
We are very thankful to the management of Wollega University for hosting this esteemed International Conference to share our knowledge and to interact with the eminent professor’s of the country and worldwide.

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Production and Characterization of Bacterial Alkaline Protease for Skin Dehairing

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Abstract

Enzymes are highly efficient environmental-friendly protein catalysts, synthesized by living systems. Alkaline proteases are one of the most important groups of microbial enzymes that find varied uses in various industrial sectors. The study focused on production and characterization of bacterial alkaline protease that has potential application in dehairing of skin and the diversity study of microorganisms. It also to identify the best protease producing isolate and to optimize the feasible production conditions of the enzyme from different substrates. Many strains of alkaliphilic proteolytic bacteria were isolated from samples collected from different habitats around Arba Minch and their diversity studied. The screening of these isolates took place depending on clear zone formation, dehairing application of goat skins and keratinase activity by plate assay method. The selected isolate was identified as Bacillus badius by morphological and biochemical characterizations. The dehairing test was done on fresh goat skins with alkaline protease by incubating the skin pieces with crude enzyme and incubating at 40°C for 6h and hair successfully removed with improved skin quality. Alkaline protease characterized with different conditions and found that it is more active at 40°C, pH10, 0.5% casein as a substrate and stable at temperature 30-60°C and pH 7-11. Culture conditions were characterized for the optimum growth and enzyme production of selected isolate. Accordingly, temperature 40°C, pH 9-10, 1% inoculum size and incubation time of 36 h was identified as optimum condition for growth and enzyme production by Bacillus badius. The maximum production of enzyme 997.36 U/ml was found at these conditions. Production optimization of alkaline protease was done with different cheap substrates, carbon and nitrogen sources. Cow dung was found to be the best substrate for enzyme production by Bacillus badius. Also in this study chicken manure was found as the best cheap nitrogen source for alkaline protease production. Alkaline protease produced by Bacillus badius in the present study has high potential in skin dehairing with improved skin quality. Its feasible production from cheap substrates and industrial application for dehairing will have implications on environment protection, product quality and shortened processing time.

Keywords: Bacillus badius, Dehauling, Keratinase, Protease
INTRODUCTION
Enzymes are highly efficient environmental-friendly protein catalysts, synthesized by living systems. They have significant advantages over chemical catalysts, of which the most important is specificity, high catalytic activity, ability to work at moderate as well as extreme temperatures, and the ability to be produced in large qualities. The current demand for better utilization of renewable resources and pressure on industry to operate within environmentally compatible limits stimulated development of new enzyme-catalyze industrial process (Barredo, 2005).

Recent developments in industrial biotechnology has offered an alternative approach for the reduction (or in some cases total elimination) of pollution from many industrial sectors without affecting production efficiency and product quality. At present enzymes find increasing application in many industrial processes. As a result the global industrial market is growing very fast with a current estimated value of US$7 billion. Although enzymes are found in all living organisms, most industrial enzymes currently in use are obtained from microorganisms (Gessesse et al., 2011). Ethiopia is endowed with unique microbial diversity which could serve as a source of novel enzymes for industrial application. Despite its huge potential for biotechnology innovation, to date the country make no use of this resource (Gessesse et al., 2003).

Bacterial proteases are the most preferred group of industrial enzymes as compared to animal and fungal proteases, because of their ability to grow in simple culture medium with minimum space requirement, faster growth rate, higher productivity and low production cost. Alkaline proteases have extensive applications in industries like laundry detergents, pharmaceutical, food industry, leather processing and proteinaceous waste bioremediation (Bayoudh et al., 2000). Recently, bacterial alkaline proteases have received attention as a viable alternative for bioremediation of protein rich tannery waste and their use in treatment of raw hide by replacing the hazardous chemicals to produce quality leather without causing environmental pollution (Ahmad and Ansari, 2013).

Leather processing involves a complex set of steps, from skin to finished product, including soaking, dehairing, bating, and tanning. These operations involve the application of materials that are capable of degrading proteinaceous matter present in the hides and skins. The conventional methods of leather processing involve the application of various hazardous chemicals, notably sodium sulfide, which generates several environmental and waste disposal problems. In order to overcome the hazards caused by these effluents, enzymes have often been proposed as viable alternatives (Gupta et al., 2002).
Keratinases are a group of metallo or serine proteinases that can degrade the insoluble structure forming keratin substrates. In enzymatic catalysis, the disulfide bonds of keratin are reduced by disulfide reductase followed by the action of keratinases, which simultaneously degrade the keratin into oligo and monomeric products (Gupta and Ramnani, 2006).

Currently, microbial alkaline proteases are used to ensure a faster absorption of water and reduce the time required for soaking (Pillai and Archana, 2012). The conventional lime-sulfide process is, however, known to generate large amounts of sulfide, which poses serious health and waste disposal problems (Pandeeti et al., 2011). Bio treatment of leather using an enzymatic approach is preferable as it offers several advantages such as easy control, speed and waste reduction, thus being eco-friendly (Verma et al., 2011).

In the process of enzyme production up to 40% of the production cost is always accounted for by the growth substrate. The enzymes considered in this study grow using very cheap substrates such as cow dung and wheat bran. For example if glucose, peptone, and yeast extract are to be used for our 300 l fermenter the cost of the substrate (based on current local price) is estimated to be US$ 350 per batch. If cow dung or wheat bran is used the cost of the growth substrate will be less than US$50 per batch. However, cost effectiveness a certain enzyme does also depend on the level of enzyme production. Therefore, due attention has been given in selecting high yielding strains and in the optimization of the fermentation condition (Gessesse et al., 2011).

Even though some microorganisms found from certain habitats, until now no proteolytic microorganisms with high potential for dehairing applications of alkaline protease have been isolated in Ethiopia. Therefore, this study focuses on isolation and diversity study of best alkaline protease producers of bacterial isolates for dehairing application in Ethiopian leather industry.

MATERIALS AND METHODS

Materials

Goat skins were purchased from Arba Minch town. Chicken feathers, animal blood and other cheap substrates were supplied from local sources. Agricultural byproducts of carbon and nitrogen sources were found from local agricultural farm. Other materials and reagents were supplied from Arba Minch University research laboratories and also purchased from chemical suppliers in Addis Ababa.
Sample Collection and Isolation of Bacterial Strains

Strains of alkaliphilic proteolytic bacteria were isolated from samples collected from different habitats around Arba Minch such as: Chamo Lake, Abaya Lake, fish processing area, Arba Minch town abattoir, Compost site, Arba Minch University abattoir, Arba Minch University garden soil and Arba Minch University effluents. These samples were suspended in water by vigorous vortexing and serial dilutions were made up to $10^{-6}$ in sterile water. From the appropriate dilution, 0.1 ml was added to petri plate on milk agar at pH 10 and incubated at 40°C for 24h. A clear zone of milk agar hydrolysis around the colonies indicated alkaline protease production by an organism. Isolates with good zone formation were further analyzed. The colonies were picked and purified by streaking on milk agar. The purified proteolytic isolates were stored and maintained for further study.

Screening of Best Alkaline Protease Producers

Many bacterial isolates with special ability of protease enzyme production (proteolytic activity) were screened depending on different growth parameters such active growth on alkaline medium, active growth at high temperature, clear zone formation, etc. They were screened by sub culturing repeatedly on alkaline media at pH 10 and 40°C. These isolates were screened for further enzyme production and biodiversity studies. The screening took place depending on size of clear zone formed.

Biodiversity Study of Selected Isolates

Bacterial isolates with prominent zone of clearance and showing efficient enzyme production were processed for the determination of colony morphology, Gram staining, motility, biochemical tests and identified in accordance with the Bergey’s Manual of Determinative Bacteriology.

Protease Production Media and Culture Conditions

The culture was grown in 250 ml of Erlenmeyer flasks containing 100 ml medium consisting of glucose 1.5%, $K_2HPO_4$ 0.2%, MgSO$_4$.7H$_2$O 0.5%, CaCl$_2$ 0.1%, peptone 0.75% for 48 h on an orbital shaker at 150 rpm at 40°C inoculated with a loop full of 24 h old colony from milk agar plate. The pH of the medium was adjusted to 10 and the supernatant was collected after centrifugation at 5000 rpm for 20 min as the crude enzyme source.

Testing Dehairing Activity of an Enzyme

Dehairing activity was performed by some modification of the method described by Pravin et al. (2014). Fresh goat skin was cut into 5cm$^2$ pieces and it was washed gently with tap
water and rinsed with distilled water to remove chemicals from the skin, which may hinder enzyme activity during dehairing activity. Then it was incubated with 10 ml of crude enzyme for 6 h at 40°C. Goat skin treated with only buffer was taken as a control. The skin pieces were virtually analyzed for dehairing activity. The quality of dehaired goat skin was checked with high quality indicators such as clear hair pore, clear grain structure and no collagen damage.

Further Screening of Isolates for Alkaline Protease Production

Screening Depending on Dehairing Potential of Isolates

The screening of an isolates was depending on their ability in dehairing of hide or skin. Different characteristics of an organism were considered during the screening. The morphology and anatomy of dehaired skin was studied by looking up the physical structure of the skin with naked eye and also observing under microscope. The completely dehaired skins with white to grey spots, clear hair pore and clear grain structure were selected and the isolates were further analyzed.

Screening of Keratinase Production by Plate Assay

The isolates were screened for keratinase activity. This was by inoculating an organisms according to method described by Raju and Divakar (2013), on the feather powder agar plates containing 0.4% feather powder (washed feathers was dried at 50°C in a forced draught oven (ISO 9001 Glass ware drying cabinet). The dried feathers were ground into fine fractions (<90, 90, 150, 300, 425 and 850µM) with test sieves of appropriate diameters) incubated at 40°C for 48 h. A clear zone formation by an isolate indicated keratinase activity. Keratinase positive isolate was selected for further study.

Further Identification of the Selected Bacterium

Further identification of the selected bacterium was carried out by doing biochemical tests such as starch hydrolysis test, indole production test, citrate utilization test, round spore test and Voges Proskauer test (Prabhavathy et al., 2012).

Characterization of Growth Conditions of Selected Isolate

Effect of pH, Temperature and Incubation Period on Bacteria Cell Growth

To evaluate the effect of pH on bacteria cell growth, production media of different pH (7, 8, 9, 10, 11, 12 and13) was taken in each flask and inoculated with bacteria. After 36 h incubation at 40°C, the cell growth was measured. To find out the effect of different temperature (30, 40, 50, 60 and 70°C) on growth of bacteria, Production media was prepared in 5 different flasks and inoculated with bacteria. Then they were incubated at
these temperatures for 36 h and cell growth measured by spectrophotometer. To test the
effect of different incubation period on the growth of selected isolate, culture media was
incubated at different time intervals (12, 24, 36, 48, 60, 72, 84, and 96 h) and cell growth
measured.

**Enzyme Characterization**

**Effect of pH and Temperature on Alkaline Protease Activity and Stability**

The protease activity was evaluated using the standard assay method in the following
buffer systems at 0.1 mol l\(^{-1}\) concentrations in the reaction mixture: sodium phosphate
buffer, pH 6 to 7; tris-buffer, pH 8; NaHCO\(_3\)–NaOH buffer, pH 9 to 11; NaHPO\(_4\)–NaOH
buffer, pH 12 and KCl-NaOH buffer, pH 13. To check the pH stability, 1 ml of the enzyme
solution was mixed with 2 ml of the buffer solutions (pH 7–13) and aliquots of the mixture
was taken to measure the protease activity under standard assay conditions after
incubation for 1 h. The effect of temperature on enzyme activity was studied according to
method described by Chaudhari *et al.* (2013) by conducting the reactions at various
temperatures (30, 40, 50, 60, and 70\(^0\)C) using the standard assay method. To evaluate
heat stability of the protease, enzyme was denatured at various temperatures ranging from
30 to 70\(^0\)C for 1 h.

**Effect of Metal Ions, NaCl and Methanol on Enzyme Stability and substrate
Concentration on Enzyme Property**

To evaluate the effect of ions (0.01 mol l\(^{-1}\)) on enzyme activity, the enzyme sample was
pre-incubated with various divalent ions (Ca\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), Hg\(^{2+}\), and Zn\(^{2+}\)) at 40\(^\circ\)C
for 1 h and the residual activity was measured (Vijayaraghavan *et al.*, 2014). The effect of
NaCl on enzyme stability was studied by following the method described by Suganthy *et al.*
(2014) with some modifications. The crude enzyme was mixed with 0.5 M NaHCO\(_3\)-
NaOH buffer pH 10 contained different NaCl concentrations ranging from 0M to 3M with
0.5 unit intervals. The reaction mixture was pre-incubated at room temperature for 1 h.
Todetermine the residual activity, 1ml of the enzyme solution was taken from pre-
incubated solutions for standard protease assay.

Effect of methanol on enzyme stability was tested by following the slight modification of the
method described by Pravin *et al.* (2014). The concentration range used in this experiment
was in the range of 0-20%. Aliquots of the crude enzyme were mixed with methanol in
equal proportion and incubated for about 1 h at 40\(^\circ\)C. Then by taking 1ml of pre-incubated
enzyme from each concentration, the standard protease assay was carried out at 40\(^\circ\)C for
1 h and compared with control considered as 100% activity.
Different concentrations of casein according to Lakshmi et al. (2014) (0.5%, 1%, 2%, 3%, 4% and 5%) in Carbonate–Bicarbonate buffer pH 10.0 was used as enzyme substrate with the above mentioned parameters to determine optimum concentration of substrate.

**Optimization of Culture Conditions for Alkaline Protease Production**

**Effect of Cheap Substrates on Alkaline Protease Production**

Banana peels, cow dung, chicken feather and wheat bran were used as the substrate for the production of alkaline protease in the production medium (glucose 1.5%, peptone 0.75%, KH$_2$PO$_4$ 0.2%, MgSO$_4$.7H$_2$O 0.1%, CaCl$_2$ 0.1%, substrates 2% (w/v). All substrates were sun dried for several days and further dried at 60°C for 1 h. Two g of substrate was taken in separate flasks and autoclaved at 121°C and 15 lb pressure for 20 min. After cooling, the flasks were inoculated with equal quantity of inocula. After 36 h of incubation on an orbital shaker at 150 rpm and 40°C, the enzyme was extracted by centrifuging at 5000 rpm for 20 min and enzyme activity was assayed according to Roja et al. (2012). The best substrate which allowed the secretion of higher protease was selected for further process.

**Effect of Different Carbon and Nitrogen Sources on Alkaline Protease Production**

The production medium was separately amended with 2g of glucose, fructose, sucrose, maltose, lactose, mannitole and starch in flasks inoculated with equal quantity of inoculum and incubated for 36 h on an orbital shaker at 150 rpm and 40°C. Nitrogen sources such as ammonium nitrate ((NH$_4$)$_2$NO$_3$), ammonium carbonate (NH$_4$)$_2$CO$_3$, yeast extract, peptone and urea were amended in the production medium separately and incubated on an orbital shaker at 150 rpm at 40°C for 36h.

Cheap carbon sources such as banana peel, wheat bran, corn cob and sugar cane bagasse were prepared in each flask and inoculated with equal size of inoculum. It was incubated for 36 h on an orbital shaker at 150 rpm at 40°C. Cheap nitrogen sources such as chicken manure, ground nut pod, dried blood and chicken feather were studied by following the same production mentioned above.

**Effect of pH, Temperature, Incubation Time and Inoculum Sizes on Alkaline Protease Production**

To observe the effect of initial pH on enzyme production, 7 production medias of different pH (7, 8, 9, 10, 11, 12 and 13) was taken in each flask and inoculated with equal size of inoculums and incubated for 36 h at 40°C. The culture media with pH10 and inoculated with equal inoculums was incubated to find out the effect of different temperatures (30, 40,
50, 60 and 70°C) on protease production and incubated for 36 h at these temperatures. The effect of incubation periods on alkaline protease activity by the test isolate was studied. For protease production, culture media was incubated at different time intervals namely 12, 24, 36, 48, 60, 72, 84 and 96 h. The effect of different inoculum size on alkaline protease production was done by following method described by Smita et al., 2012. Inoculum concentrations of 0.1ml, 0.5 ml, 1ml, 1.5 ml & 2ml of a 24h old culture was inoculated into the assay medium for protease production at 40°C, pH 10, 150 rpm for 36h.

**Alkaline Protease Assay**

According to Roja, et al. (2012), the enzyme was assayed in the reaction mixture containing 2.0 ml of 0.5% casein solution in 0.1M Carbonate–Bicarbonate buffer pH10 and1ml enzyme solution in a total volume of 3.0ml. Reaction mixture was incubated for 5min at 40°C. The reaction was terminated by adding 3ml of 10% trichloro-acetic acid. The tubes were incubated for 1h at room temperature. Precipitate was filtered through whatman no.1 filter paper and the filtrate collected. For the color development for the assay of tyrosine in the filtrate, 5ml of 0.4 M Sodium carbonate and 0.5 ml of Folin phenol reagent was added to 1ml of filtrate, vortexed immediately, incubated for 20 min at room temperature and optical density (OD) was taken at 660 nm. Concentration of tyrosine in the filtrate was read from a standard curve for tyrosine already prepared.

One unit enzyme activity was taken as the amount of enzyme producing 1 g of tyrosine under standard assay conditions and expressed as units per ml enzyme.

**Method of Data Analysis**

For statistical analysis of data, a standard deviation for each experimental result was calculated using the Excel Spreadsheets available in the Microsoft Excel. Graphs, tables and photographs were prepared and used to present the data.

**RESULTS**

**Isolation of bacterial strains**

From samples collected from different habitats around Arba Minch University 197 strains of proteolytic bacteria were isolated. These isolates were screened from total strains of bacteria grown on each petri plate depending on proteolytic activities. Pre-screening took place according to clear zone of an organism (table 1).

**Screening of Best Alkaline Protease Producers**

From total 197 proteolytic isolates, 53 bacteria with special ability of protease enzyme production (proteolytic activity) were screened depending on different growth parameters.
such active growth on alkaline medium, active growth at high temperature, proteolytic activities, etc. They were screened by sub-culturing repeatedly on alkaline media with pH10 and incubated at 40°C. These isolates were screened for further enzyme production and biodiversity studies. The first selection was depending on size of zone formed by an organism. Therefore, in this situation high clear zone formers were suggested as high alkaline protease producers.

**Table 1: Morphological characterization of selected isolates**

<table>
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<th>C.No</th>
<th>Morph</th>
<th>Color</th>
<th>Motility</th>
<th>G.stain</th>
<th>C.Z.Size (mm)</th>
<th>C.No</th>
<th>Morph</th>
<th>Color</th>
<th>Motility</th>
<th>G.Stain</th>
<th>C.Z.Size* (mm)</th>
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<td>15</td>
</tr>
<tr>
<td>S28</td>
<td>Rod</td>
<td>White</td>
<td>&quot;</td>
<td>+</td>
<td>25</td>
<td>S6</td>
<td>Rod</td>
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<tr>
<td>S31</td>
<td>Rod</td>
<td>White</td>
<td>&quot;</td>
<td>+</td>
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<td>S22</td>
<td>Cocci</td>
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<tr>
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<td>Yellow</td>
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</table>

**Biodiversity study of selected isolates**

Bacterial isolates with prominent zone of clearance and showing efficient enzyme production were processed for the determination of colony morphology, colony color, Gram staining, endospore staining, motility, biochemical tests and identified in accordance
with the Bergey’s Manual of Determinative Bacteriology. The morphologies of these 53 isolates were observed as rod and cocci and colony colors white and yellow. Gram reaction indicated that 49 bacteria were gram positive and the others 4 were gram negative. From these isolates, 25 were spore formers and the others non formers.

Table 2: Biochemical characterization of selected isolates

<table>
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<th>No</th>
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<td>-</td>
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<td>3</td>
<td>Citrate utilization</td>
<td>-</td>
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<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Voges Proskauer</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Indole production</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Starch hydrolysis</td>
<td>-</td>
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<td>1</td>
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<td>Roundspore test</td>
<td>-</td>
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<tr>
<td>8</td>
<td>Strict anaerobes</td>
<td>-</td>
<td>25</td>
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</tr>
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</table>

From 41 isolates identified by different biochemical tests, 25 Bacillus sp., 7 Staphylococcus sp., 5 Micrococcus sp., 2 Corynebacterium sp. and 2 Neissaria veillonilla were found from samples collected from different habitats.

![Graph of Sites of isolation](Image)

*Sites of isolation: AB = Abaya, FP = Fish processing, AMA = Arba Minch Abattoir, AMUE = Arba Minch University Effluent, AMUS = Arba Minch University Soil, AMUA = Arba Minch University Abattoir, RLA = Research Laboratory Air

Figure 1: Distribution and taxonomic characteristics of micro-organisms isolated from 7 different habitats around Arba Minch.

Testing Dehairing Activity of an Enzyme

Dehairing of skin tested for each 53 isolates and 51 isolates were observed as good to excellent in dehairing potential. Only 2 isolates were not successful in hair removal from goat skin pieces. More than 56.6% of isolates successfully removed hairs completely (100%) from the skin (figure 2 and 3). About 37.7% isolates removed hair partially (70-90%) and the skin was not completely dehaired (figure 4). The result of this study did not
only focus on complete removal of hair from the skin (dehairing), but also on skin quality improvement and reduction of the duration of incubation time for dehairing. The complete removal of hair from the skin took place within 6 h incubation time.

Figure 2: Dehairing test of goat skin using alkaline protease produced by selected (S35) isolate without use of any lime, sodium sulphide and buffer in reaction mixture at 40°C for 6 h: A) Skin treated with only buffer (control), B) Enzymatically dehaired skin (experiment).

Figure 3: Enzymatically dehaired goat skins by alkaline proteases of 21 different isolates in reaction at 40°C for 6 h with only enzymes.
Figure 4: Partial to complete enzymatic dehairing of goat skins by alkaline proteases of 16 isolates at 40°C for 6 h.

Further Screening of Isolates for Alkaline Protease Production

Screening Depending on Dehairing Potential of Isolates

The dehaired skin with high quality showed clear hair pore, clear grain structure and no collagen damage. Depending on these parameters 5 isolates with best dehairing characteristics were selected from all 53 bacteria checked for dehairing. These isolates did not only completely dehair hides, but also improved the skin quality. The structure of dehaired skins observed under microscope and the isolate S35 found without damaged part and showed the highest quality of skin.
Screening of Keratinase Production by Plate Assay

The isolates were screened for Keratinase activity. Depending on this screening technique isolate S35 was selected for further study. This isolate formed large clear zone on feather powder agar plates (figure 7).

Figure 6: Plate assay for keratinase production by isolate S35 at pH 10, 40°C for 36 h in milk agar plate amended with 0.4% powdered feather.

Further Identification of the Selected Bacterium

Further identification of the selected bacterium was carried out by doing biochemical tests such as citrate utilization test, Voges Proskauer test, indole production test, Starch hydrolysis and round spore test (table 2). According to Bergey’s Manual of Determinative Bacteriology, the isolate was identified as Bacillus badius.

Characterization of Growth Conditions of Selected Isolate

Effect of pH on Growth

The maximum cell growth of selected (S35) isolate was found at pH 8 with OD value of 0.369 (figure 8).

Figure 8: Effect of pH on growth of Bacillus badius at 40°C and 150 rpm for 36 h
Effect of Temperature on Growth
The highest cell growth of the selected isolate was observed at 40°C. At this temperature the maximum OD value of 0.355 was found. But gradually the cell growth decreased at temperature above 40°C (figure 9). At 70°C, the growth was reduced by 26.5 % from the maximum.

![Figure 9: Effect of temperature on growth of Bacillus badius at pH 10 & 150 rpm for 36 h](image)

Effect of Incubation Period on Bacteria Cell Growth
The optimum cell growth of Bacillus badius observed at 24 h incubation time. As it can be observed from figure 10 below, the growth of cell was slowly decreasing after 24 h incubation time. At 96 h incubation, the cell growth decreased by 42.9% of the optimum.

![Figure 10: Effect of incubation period on cell growth of Bacillus badius at 40°C, pH 10 & 150 rpm.](image)
Enzyme Characterization

Effect of pH on Alkaline Protease Activity and Stability

The optimum protease activity (459.24 U/ml) was found at pH 10. The enzyme activity increased from pH 7 to 10 (Figure 11). As indicated on figure 11, the activity of the enzyme was found to be 78.78% at pH 12 and 65.9% at pH 13.

The protease produced by *Bacillus badius* was stable in pH 7-11 (Figure 11). But the stability decreased above pH 11.

![Figure 11: Effect of pH on activity and stability of alkaline protease from *Bacillus badius* incubated for 1 h in different buffers](image1)

Effect of Temperature on Alkaline Protease Activity and Stability

The enzyme produced by *Bacillus badius* was found optimally active at 40ºC. The maximum activity of 775.85 U/ml (100%) was found at this temperature after which the activity slightly decreased. The alkaline protease by *Bacillus badius* was stable at moderate temperature range of 30ºC to 60ºC (Figure 12).

![Figure 12: Effect of temperature on alkaline protease activity and stability by incubating enzyme at different temperatures for 1 h.](image2)
Effect of Metal Ions on Enzyme Activity
All of the cations tested inhibited the enzyme activity (Figure 13). Ions such as Hg$^{2+}$, Fe$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$ strongly inhibited the enzyme activity, and the enzyme activities were 32.34%, 44.88%, 47.85% and 51.15%, respectively. Ca$^{2+}$ and Cu$^{2+}$ resulted 70.63%, and 64.36% activities respectively.

Figure 13: Effect of different metal ions on alkaline protease activity by incubating an enzyme for 1 h in the solution of metal ions.

Effect of NaCl on Enzyme Stability
The maximum enzyme activity as compared with others concentrations was recorded at 0.5M NaCl (Figure 14).

Figure 14: Effect different concentrations of NaCl on alkaline protease activity by incubating crude enzyme in 0 to 3M NaCl for 1 h.
Effect Different Concentrations of Methanol on Enzyme Stability

Methanol inhibited the protease activity of *Bacillus badius*. As it can be observed from figure 15, the activity of the enzyme at 1% concentration methanol was 76.13% of the control. The enzyme was more repressed at 20% concentration of methanol (62.25%).

![Figure 15: Effect of different concentrations of methanol on stability of protease enzyme pre-incubated with methanol for 1 h.](image)

Effect of Substrate Concentration on Activity of Protease

The enzyme activity was negatively affected by higher concentrations of casein (figure 16). Maximum activity of protease was found at 0.5% casein concentration (463.88 U/ml) and decreased as concentration increased.

![Figure 16: Effect of different concentrations of casein on activity of protease produced by *Bacillus badius*.](image)
Optimization of Culture Conditions for Alkaline Protease Production

Effect of Cheap Substrates on Alkaline Protease Production

Among cheap substrates evaluated for alkaline protease production by *Bacillus badius*, cow dung showed the highest protease activity (429.09 U/ml) followed by banana peel with enzyme activity of 390.83 U/ml (Figure 17).

![Figure 17: Effect of cheap substrates on alkaline protease production](image)

**Figure 17:** Effect of cheap substrates on alkaline protease production by inoculating the isolate in production media containing different substrates and incubating at 40°C, pH 10 and 150 rpm for 36 h. Control was production medium with no added substrate.

Effect of different carbon sources on alkaline protease production

Among different carbon sources evaluated for alkaline protease production by *Bacillus badius*, fructose indicated the highest protease activity of 198.31 U/ml (Figure 18). Glucose and lactose showed equal enzyme activity of 141.48 U/ml while sucrose, mannitol, maltose and starch resulted in 138 U/ml, 129.89 U/ml, 128.73 U/ml and 117.13 U/ml activities respectively.

![Figure 18: Effect of different carbon sources on alkaline protease production](image)

**Figure 18:** Effect of different carbon sources on alkaline protease production by *Bacillus badius* by incubating the inoculated media at 40°C & 150 rpm for 36h.
Effect of Different Nitrogen Sources on Alkaline Protease Production
Among different nitrogen sources amended for protease production, yeast extract indicated the maximum protease activity of 322.40 U/ml. Peptone indicated protease activity of 115.97 U/ml (Figure 19). But urea, (NH₄)₂CO₃ and ((NH₄)₂NO₃ showed almost similar lower protease activities of 40.59 U/ml, 35.95 U/ml and 33.63 U/g respectively.

![Figure 19: Effect of different nitrogen sources on alkaline protease production by Bacillus badius by incubating the inoculated media at 40°C and 150 rpm for 36 h.](image)

Effect of Cheap Carbon Sources on Protease Production
Among cheap carbon sources evaluated for protease production, banana peel showed the highest protease activity of 346.76 U/ml followed by corncob and wheat bran with equal enzyme activity of 323.56 U/ml (Figure 20). Also sugar cane bagasse indicated 216.87 U/ml activity of alkaline protease by Bacillus badius.

![Figure 20: Effect of cheap carbon sources on alkaline protease production by Bacillus badius by incubating the inoculated media at 40°C and 150 rpm for 36 h 150 rpm.](image)
Effect of Cheap Nitrogen Sources on Protease Production

Among cheap nitrogen sources evaluated for production alkaline protease by *Bacillus badius*, chicken manure showed the maximum protease activity of 245.86 U/ml (Figure 21). Chicken feather, ground nut pod and dried blood indicated enzyme activities of 106.69 U/ml, 70.74 U/ml and 38.27 U/ml respectively.

![Figure 21: Effect different cheap nitrogen sources on alkaline protease production by *Bacillus badius* by incubating the inoculated media at 40°C & 150 rpm for 36 h.]

Effect of pH on Alkaline Protease Production

The highest alkaline protease production by *Bacillus badius* was found at pH 9-10 (Figure 22). At pH 7 and 13 the relative activities of the enzyme were 69 and 60.53% respectively.

![Figure 22: Effect of pH on alkaline protease production by *Bacillus badius* by incubating inoculated media with different pH at 40°C and 150 rpm for 36 h.]

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Effect of Temperature on Alkaline Protease Production

The maximum alkaline protease production by Bacillus badius was observed at 40°C (Figure 23). The enzyme production declined after 40°C.

![Figure 23: Effect of temperature on alkaline protease production by Bacillus badius incubating inoculated media at different temperatures and 150 rpm for 36 h.]

Effect of Incubation time on Alkaline Protease Production

The maximum enzyme activity of 997.36 U/ml was found at 36 h incubation by Bacillus badius (Figure 24). After this optimum production time, the enzyme activity was slowly decreased up to 48 h after which it sharply decreased.

![Figure 24: Effect of different incubation times on alkaline protease production by Bacillus badius at 40°C and 150 rpm.]

Effect of Inoculum Size on Alkaline Protease Production

Results from the present study showed that optimum inoculum size of Bacillus badius for protease production was 1ml from 24 h old culture broth. The enzyme activity at this
inoculum size was 303.85 U/ml. As indicated on figure 25, the activities at 0.1 and 2 ml inoculum sizes were 256.29 U/ml and 247.02 U/ml respectively.

![Figure 25: Effect of inoculum size on production of alkaline protease by *Bacillus badius* inoculated with different sizes at 40°C and 150 rpm for 36 h.](image)

**DISCUSSION**

A total of 197 proteolytic bacteria were isolated from different habitats around Arba Minch such as Chamo Lake, Abaya Lake, and fish processing area, Arba Minch town abattoir, Arba Minch University abattoir, and Arba Minch University garden soil and Arba Minch University effluents. Depending on clear zone formation 53 bacteria were then screened as potential alkaline protease producers. Clear zones were formed because of the hydrolysis of casein by protease produced from the isolates. In fact these bacteria are known for their abilities to secrete large amounts of alkaline proteases having significant proteolytic activity and stability at considerably high pH and temperatures.

As shown on figure 1, 29.3% of these isolates were found from Arba Minch town abattoir, 17% from Lake Abaya and 17% from Arba Minch University effluents. This indicates that these three habitats are rich in proteolytic bacteria for alkaline protease production. It might be due to the fact that, these three sites have more alkaline condition and proteolytic in nature that suits for distribution of proteolytic bacteria in the area. About 36.7% were isolated from Arba Minch University garden soil, Arba Minch University abattoir, fish processing area and also from air around research laboratory. Fish processing area was reach in most proteolytic organisms. This is because the area has much protein residues which were accumulated from fish byproducts. The strain with highest proteolytic activity (selected for this study) was found from the area.
Dehairing test done on goat skin indicated that about 96.23% of tested isolates were with potential ability of dehairing skin and only 3.77% were not at all. This dehairing test is in addition for completely removal of hairs from the skin, it also focused on improvement of the quality of the skin dehaired and the duration of incubation time. In this case, the successfully dehaired skins were soaked in the enzyme for 6 h. So that, the time required for unhairing in the present study was the minimum. This is because when it is compared with previous works, the incubation period is the shortest. For example, Pravin et al. (2014) reported complete dehairing of goat skin after 8 h incubation time by *Bacillus licheniformis* U1. Prabhavathy et al., (2013) has shown complete dehairing of cow skin after 24 h incubation time by isolate *Bacillus subtilis*. Nadeem et al., 2009 reported complete dehaired goat skin after 12 h incubation with alkaline protease from *B. licheniformis UV-9*.

Depending on their dehairing potential, 5 different bacteria with optimum proteolytic activities were screened for further enzyme production and characterization. The morphology and anatomy of dehaired skins were studied by observing the physical structure of the skin with naked eye and under microscope during screening. This was because some strains of bacteria have negative effect on quality of dehaired skin and in this study these were checked by observing the physical structure of the skin to identify the damaged collagen. These isolates were identified as 3 *Bacillus* sp., 1 *Micrococcus* sp. and 1 *Staphylococcus* sp. When bacterial isolates were identified which yielded prominent zones of clearance on the skim milk agar medium, it was found that about 80% were represented by the genus *Bacillus* (Nadeem et al., 2009).

These 5 selected isolates were further screened based on plate assay techniques to evaluate keratinase activities of organisms. Based on this technique, isolate S35 was with highest clear zone formation on feather agar medium and selected as best producer of alkaline protease (Figure 6).

The selected S35 isolate was characterized morphologically and biochemically and identified as *Bacillus badius* (table 1 & 2). The strain was isolated from fish processing area which is rich in protein sources. Due to the fact that, this isolate was found with highest proteolytic activity and stability at considerably high pH and temperatures.

The effect of pH on bacteria cell growth was evaluated and maximum growth found at pH 8 and the growth decreasing above that point. Likewise, Najafi et al. (2005) reported similar result from *P. aeruginosa* PD100 grown on culture media of different pH. Microbial
cells are significantly affected by the pH of their immediate environment because they apparently have no mechanism for adjusting their internal pH. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth (Bhattacharya et al., 2011).

From the evaluated effect of different temperatures on cell growth of bacteria, maximum growth was found at 40°C. This is because most *Bacillus sps* were thermophilic in nature and have optimum temperature requirement at 40°C and above. Likewise, similar result was reported by Josephine et al. (2012) from study done on alkaline protease from *Bacillus sp*.

The maximum cell growth of bacteria was observed at 24 h incubation time. But after this optimum period the cell growth starts slowly decreasing, because decline phase started at the end stationary phase. At 96 h incubation time the relative cell growth was 57%. Josephine et al. (2012) reported comparable result from study done on alkaline protease production by *Bacillus* SRN01. Suganithi et al. (2013) reported the same result from the study done on *Bacillus licheniformis*. Moreover, Johnvesly et al. (2002) found that a high level of extracellular thermostable protease activity was observed after 24 h incubation and hence this result is in complete agreement with earlier reports.

The optimum protease activity of 459.24 U/ml was determined at pH 10 (100%). This was suggested that it was an alkaline protease and potential candidate for industrial application. The result was similar with that of Gururaj et al., 2012. He reported the highest protease activity of 184.8 U/ml at pH 10. Generally, commercial proteases from microorganisms have maximum activity in the alkaline pH range of 8-12 (Gupta et al., 2002). Optimum pH of 10 for alkaline proteases from various *Bacillus* species has been reported by some workers (Adinarayana et al., 2003; Gupta et al., 2005).

PH stability of alkaline protease produced by *Bacillus badius* was evaluated and the result indicated that the enzyme was stable under various pH ranges 7 to 11 (figure 11). The activity of the protease was 78.78% at pH 12 and 65.9% at pH 13. It shows that as an enzyme has more potential for application in industries at harsh conditions. Similar result was recorded by Smita et al. (2012) from study done on alkaline protease of *Serratia liquefaciens*.

As it indicated on figure 12, the optimum activity of alkaline protease was found at 40°C. At 50°C the enzyme activity was 98.5% and at 60°C it was 95.66%. Also at 70°C the activity
of the enzyme was 64.4%. This indicates that the enzyme was thermostable because it has good activity even at higher temperature. Ravindran et al., 2011 and Ahmad, 2011 reported similar results from study done on alkaline protease produced by *Bacillus cereus* and *Streptomyces aurantiogriseus* EGS-5 respectively.

The enzyme was thermally stable through different temperatures from 30°C to 60°C (residual activity 100%) and gradually decreasing at temperature higher than 60°C. Also at 70°C the activity of the enzyme was 77.7%. This indicates the thermostability of the selected isolate and the enzyme. The result seems to be very interesting as the broad optimal temperature range of the isolate is a very suitable characteristic for its industrial acceptability including tanneries and a common feature for getting the bacterial alkaline protease commercialized. The result suggests elevated temperature required for optimum catalytic activity. The result is in agreement with earlier reports (Pravin et al., 2014).

The influence of various divalent ions on protease activity of *Bacillus badius* was investigated and a significant inhibitory effect was observed with all ions (Hg²⁺, Fe²⁺, Mg²⁺,Ca²⁺, Cu²⁺ and Zn²⁺) (figure 13). This demonstrated that the alkaline protease produced by *Bacillus badius* is metal independent and does not require any metal ion for its activity. Likewise, Najafi et al. (2005) and Vijayaraghavan et al. (2014) reported a metal-independent protease from *Pseudomonas aeruginosa* PD100 and *Pseudomonas putida* strain AT respectively.

The effect of NaCl on enzyme stability was evaluated and the maximum enzyme activity as compared with others concentrations was recorded at 0.5M NaCl. The residual activity of the enzyme was 79.49% at this concentration. As shown on Figure 14, the enzyme showed good activity (57.62%) even after incubation for 1 h at 3M NaCl. This indicates that NaCl has a minimum effect on the activity of alkaline protease produced by *Bacillus badius* in concentration less than 3M. Similar result was reported by Pravin et al. (2014) from study done on thermostable and solvent tolerant serine protease from hot spring isolated thermophilic *Bacilluslicheniformis* U1.

The effect of different concentrations of methanol on protease activity was tested and the result indicates that methanol inhibits the alkaline protease produced by *Bacillus badius*. Specifically, the activity of the enzyme was more repressed at 20% methanol concentration. Pravin et al. (2014) reported similar result from study done on thermostable and solvent tolerant serine protease from hot spring isolated thermophilic *Bacilluslicheniformis* U1. The level of stability towards solvents is the unique properties of
enzymes. Biocatalysis in organic media offers several advantages including the higher solubility of hydrophobic substrate enabling their active reactions, reduced microbial contamination and reusability leads to the development of novel application prospects (Gupta and Khare, 2006).

The effect of substrates on the activity of alkaline protease was tested and the result indicates that the activity of the enzyme was negatively affected at higher concentration of substrate. As it can be observed from figure 16, the activity of the enzyme was 416.33 U/ml at 5% concentration of casein. Likewise, Lakshmi et al., 2014 reported similar result from alkaline protease produced by *Halo alkaliphilic Bacillus sp.*

From cheap substrates amended for alkaline protease production by *Bacillus badius*, cow dung showed the highest enzyme activity of 429.09 U/ml. Cow dung was able to provide all the necessary nutrients for the growth of the bacterium and for the synthesis of the enzyme. Therefore, the result indicated that cow dung is the best substrate for alkaline protease production by *Bacillus badius*. Similar result was reported by Vijayaraghavan et al. (2014) from the investigation on alkaline protease produced by *Pseudomonas putida* Strain AT. Likewise, Vijayaraghavan et al. (2013) reported the same result from the study done on alkaline protease by *Bacillus cereus* strain AT.

Among various carbon sources evaluated fructose indicated as the best carbon source for alkaline protease production by *Bacillus badius*. Similar result was reported by Kumar et al. (2012) from study done on protease produced by *Bacillus subtilis*. Likewise, Joshi et al. (2007) reported comparable result from alkaline protease produced by *Bacillus cereus* MTCC 6840.

Among the various nitrogen sources tested, yeast extract exhibited the maximum production of protease (322.40 U/ml) (Figure 19). Likewise, Nayera et al. (2014) reported similar result from alkaline protease produced by *Streptomyciesambofaciens* in free and immobilized form. Also the same result was reported by Raj et al. (2012) from protease produced by *Pseudomonas aeruginosa*. Vijayaraghavan et al., 2014 reported similar result from alkaline protease produced by *Pseudomonas putida* strain AT. Nadeem, (2009) reported the same result from alkaline protease produced by *Bacillulslicheniformis* N-2.

Among various complex nitrogen sources, yeast extract and casamino acid have been reported as suitable sources for alkaline protease production (Rahman et al., 2005; Prakasham et al., 2006).
Ammonium nitrate \((\text{NH}_4\text{H}_2\text{NO}_3)\), ammonium carbonate \((\text{NH}_4\text{H}_2\text{CO}_3)\) and urea repressed the growth and yield of alkaline protease by *Bacillus badius*. The repression of growth and protease biosynthesis might be attributed to the fast release of ammonia from these inorganic nitrogen sources. Rahman *et al.* (2003) reported comparable results for thermostable alkaline protease production by *B. stearothermophilus* F1 in the presence of organic and inorganic nitrogen sources. Many other researchers have also reported that organic nitrogen sources are better for enzyme production than inorganic ones (Shikha *et al.*, 2007; Ravishankar *et al.*, 2012 and Vijayaraghavan *et al.*, 2014).

Among different cheap carbon sources evaluated for protease production banana peel showed the highest protease activity of 346.76 U/ml (Figure 20). It indicated that banana peel is the best source of carbon for alkaline protease production by *Bacillus badius*. Among different cheap nitrogen sources evaluated for alkaline protease production, chicken manure showed the maximum protease activity of 245.86 U/ml by *Bacillus badius* (Figure 21). The study result indicates that chicken manure is the best nitrogen source for alkaline protease production by *Bacillus badius*. This result is new and not reported by other researchers.

Among various pH evaluated for alkaline protease production by the isolate, the highest enzyme activity was found at pH 9 (100%) followed by pH 10 (98.68%) (Figure 22). At higher pH, the metabolic action of the bacterium could have been suppressed, thus decreasing the enzyme production. Similar trends have been observed in protease production by *Bacillus sp.* (Prakasham *et al.*, 2006; Okafor and Anosike, 2012). Likewise, Vijayaraghavan *et al.* (2013) reported the comparable result from the study done on alkaline protease production by *Bacillus cereus strain AT*.

The optimum alkaline protease production was found at 40°C (100%). At 50°C the activity was 79%. At 60 and 70°C the protease retained 58.4% and 41.58% activities respectively (figure 23). This is because the metabolic reaction and biosynthesis of enzyme highly takes place at temperature 40°C and slightly decreased after that. Similar result was reported by Josephine *et al.* (2012) from a study done on alkaline protease production by *Bacillus SNR01*. This indicates that the result of the present study is similar with previous work by other researchers. The growth and enzyme activity of microorganisms is greatly influenced by different incubation temperatures. Temperature significantly regulates the synthesis and secretion of bacterial extracellular proteinase by changing the physical properties of the cell membrane (Balaji *et al.*, 2012).
The effect of incubation periods on the for alkaline protease production by the test isolate was studied and maximum enzyme activity of 997.36 U/ml found at 36 h incubation time (Figure 24). This indicates that *Bacillus badius* is fast grower and produce the highest protease enzyme during last log phase at 36 h incubation period. The enzyme activity was 494.04 U/ml at 96 h. The growth of the organism is essential for the production of enzyme. Most extra cellular enzymes are produced during log phase of the organisms. Maximum enzyme activity of 410 U/ml at 36 h incubation period was reported by Verma and Baiswar, (2013) from study done on thermo alkaline protease producing *Bacillus cereus* isolated from tannery effluent. Many workers have reported a broad incubation period ranging from 36 to 96 h for the maximum yield of protease enzyme by *Bacillus* strains (Shafee *et al*., 2005; Genekal and Tari, 2006; Jaswal and Kocher, 2007; El-Enshasy, *et al*., 2008; Khosravi-Darani *et al*., 2008; Raj *et al*., 2012 and Vijayaraghavan *et al*., 2014).

Results from the present study showed that optimum inoculum size of *Bacillus badius* for protease production was 1 ml from 24 h old culture broth. The less protease production in small inoculum sizes of 0.1ml and 0.5ml may be due to insufficient number of bacteria, which would have led to reduced amount of secreted protease and the decrease even though luxurious growth was observed in higher inoculum size of 2.0ml, may have resulted due to reduced dissolved oxygen and increased competition towards nutrients. Similar result was reported by Sai Smita *et al*. (2012) from investigation on quantification and optimization of bacterial isolates for production of alkaline protease.

**CONCLUSION AND RECOMMENDATIONS**

About 197 proteolytic bacteria were isolated from different habitats around Arba Minch. From these isolates 53 with enough proteolytic properties were screened for alkaline protease production. Among these one isolate was selected by its enzyme titer, keratinase production and quality of skin after dehairing. The bacterium was identified as *Bacillus badius*. It grew well at pH 10, 40$^0$C for 36 h. The protease produced was maximally active at pH 10 and 40$^0$C. The optimum enzyme production conditions were pH 9-10, 40$^0$C, 1% inoculum and 36 h incubation. The optimum activity was expressed at pH 10, 40$^0$C and 0.5% casein as substrate. It was stable in pH 7-11 and 30-60$^0$C after pre-incubation for one h in respective buffers and temperatures. All metal ions tested (Ca$^{2+}$, Mg$^{2+}$, Fe$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ and Cu$^{2+}$) inhibited the enzyme at 0.01M and 1 h pre-incubation. Methanol also inhibited the enzyme. However, NaCl had minimal effect on the enzyme stability. Among carbon and nitrogen sources tested for the enzyme production fructose and yeast extract resulted in maximum yield. Cow dung, banana peel and chicken manure were identified as
the best cheap substrates for potential feasible production of the enzyme. Alkaline protease produced by *Bacillus badius* in the present study has high potential in skin dehairing with improved skin quality. Therefore, its feasible production from cheap substrates and industrial application for dehairing will have implications on environment protection, product quality and shortened processing time.

Based on the result of the study the following recommendations are given.

1. Isolation of industrially important enzymes from different habitats has to continue so as to enable the country use its microbial diversity and save foreign currency.
2. Purification and determination of properties of the alkaline protease needs to be studied further.
3. The alkaline protease production needs to be optimized with statistical methods that could show interactions of various parameters.
4. Scale up study recommended for further use
5. The enzyme recommended for dehairing application at leather industry.

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Abstract

The issue that this study addresses is the need for secondary students to work on relevant non-routine statistical problems to develop their modeling abilities in multidisciplinary areas. A Modeling Approach in the study integrates Contextual and Socio-critical Modeling Perspectives for teaching a unit of descriptive statistics through problem solving and, therefore; justifies the statistics rather than treating it as a means to an end. The modeling lessons had four Model-Eliciting Activities (MEAs) so as to allow students to 'express, test, and revise' their models iteratively in an engineering way. Model-Eliciting Activities are non-routine problems which require students to make interpretation and conclusion of meaningful real life situations by making models using system of representations. The purpose of this study is to explain secondary students’ modeling experiences on a unit of descriptive statistics in mathematics education. The research question set forth is, how do students gain modeling experiences working collaboratively in a team on MEAs?. Two Grade Nine Sections with a total of 80 students were selected by using purposive sampling techniques at two high schools in urban district in West Shewa Zone, in Ethiopia. Probing worksheet questions based on a modeling cycle are prepared to analyze students’ team work on the MEAs. Quality Assurance Guide instrument was used to assess students’ models on MEAs (Lesh et.al. 2000). Descriptive statistics and content analysis were used to analyze the data on students’ reports on MEAs. Content analysis of Grade 9 text book on a unit of descriptive statistics also made to investigate the extent in which relevant non-routine problems exists. Though students found MEAs cognitively challenging tasks, they constructed different models working in a team collaboratively. Teams of students were able to write meaningful reports on MEAs working on multidisciplinary problems which involve matrix of real data sets and several variables. Finally, the successes and challenges of the Modeling Approach to teach statistics in Secondary Education were discussed. The findings of this study are remainders that Modeling Approach likely help to enhance students modeling experiences with similar school setting, but needs further efficacy study at different school settings and populations. If relevant non-routine
problem solving tasks are important starting from lower grades to nurture the scientist and engineers of the future, they need to be integrated in the curriculum and instruction in an education system.

**Keywords:** Non-routine problems, Modeling Approach, Model-Eliciting Activities, Statistics Education

**INTRODUCTION**

In an education system, stakeholders agree students need to solve problems; however, there is little consensus on what entails problem-solving curriculum and instruction in mathematics education (Chamberlin, 2008 and 2010). Teaching approaches that have some nature of problem solving in mathematics generally could be classified as teaching for, teaching about, and teaching through problem solving (Bostic, 2012; Cai, 2003; Schroeder, Thomas, and Lester, 1989). Teaching through problem solving considers problem solving as integral to the development of an understanding of any given mathematical content and process (Doerr and Lesh, 2003; English, 2013a; Hamilton, 2007). It is different from the practice of teaching for problem solving which deals with solving problems using the already taught concepts. It also differs from teaching about problem solving which deals with solving problems using heuristics and strategies. A Modeling Approach in the study integrates Contextual and Socio-critical Modeling Perspectives to teach through problem solving.

The nature of problems beyond classroom demands educators to give students interdisciplinary trans-disciplinary and multidisciplinary problem solving experiences (English, 2016). Modeling needs to be integrated within all topic areas across the mathematics curriculum, and, there is a need, across disciplines (English, 2013a; Gouvea, Sawtelle, Geller and Turpen, 2013). There is, however, a limited research on how to integrate other disciplines within mathematics curriculum which can be done through modeling instruction (English, 2013a; Mousoulides and English, 2012). A model in this article refers to “a system for describing (or explaining or designing) another system(s) for some clearly specified purpose” (Lesh & Fennewald, 2010, p.7).

More importantly, mathematics curricula must extend their goals to take account of key concepts and processes like statistical reasoning and mathematical modeling which optimize students’ chance of being successful in the 21st century (English, 2002; Kuntze and Engel, 2011). According to Sriraman and English (2010), the issue of enhancing students’ statistical reasoning through data modeling requires ‘substantial research’.
Modeling would be appropriate for the nature of statistics which emphasize problem solving “in the wild” (Lesh and English, 2005; Roth, 2007) to describe and interpret meaningful socio-cultural real life situations. This kind of problem solving is needed in Ethiopian educational policy and curriculum (TGE, 1994). Descriptive statistics is part of Ethiopian high school mathematics curriculum with the aim of teaching students for developing foundational concepts of statistics to solve problems related with ‘every bit of students’ everyday life’ (MoE, 2010a).

Although Ethiopian education policy and documents on mathematical curriculum recommend that there should be relevant problem solving activities on socio-cultural issues, researchers have indicated that covering the text book is the most common teaching practice in Ethiopia instead of engaging students with rich problem-solving tasks (Asgedom, 2009; Micheal and O’Connell, 2014). This may lead teachers to use simple textbooks problems, and as a result students couldn’t develop cognitive abilities to solve problems in novel situations.

The best possible levels of cognitive tasks are required to prepare students for nature of understanding and abilities needed beyond schools (Lesh, 2000, 2012). Significantly, students need to solve non-routine statistical problems like MEAs which are sometimes identified as ‘rich tasks’ (Bostic, 2012) so as to enhance students’ foundational and critical understanding of statistics now and then (Chamberlin, 2012; Lesh and Doerr, 2003).

MEAs possess specific qualities that ask students to engage in multiple iterations to solve the problem similar to the engineering design process (Chamberlin and Coxbill, 2012). The process of creating and refining multiple iterations of the models has been referred to as the process of, ‘express, test, and revise’ (Hamilton, Lesh, Lester and Yoon, 2007) in an attempt to seek a highly refined mathematical model. Magiera (2013) argued that MEAs have provided problem-solving experiences that help a wide range of mathematical expertise that creates problem solvers, innovators, inventors, self motivation and self reliance, logical thinkers, technological literacy which supports the goal of STEM. Researchers had found dramatic and positive results using MEAs in STEM education at different school levels (Diefes-Dux, Whittenberg and McKee, 2013; English, 2010, 2013a; English and Mousoulides, 2011; Eric, 2008; Moore, 2007; Mousoulides and English, 2012).

Statistics is at the intersection of all these disciplines in order to provide “a coherent set of ideas and tools for dealing with data” (Cobb & Moore, 1997, p.801). English (2013b) had

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investigated that how first grade students learn to competently generate, test, revise and represent data through data modeling before formally being taught to do so. She had showed that through scaffolding using rich and motivating contexts, students at young age could construct their own representation, ways of understanding, and they learned to think about their data. A similar study conducted by Kinnear (2013) showed young students at age of 5 had competence and capacity to develop statistical reasoning. However, the literature on teaching statistics using a modeling approach still shallow and needs further studies (Garfield and Ben-Zvi, 2008; Sriraman and English, 2010).

Significance of the Study
A study of the Modeling Approach to teach statistics is important for several reasons. First, it helps to lay the ground for further similar empirical studies across different school settings and populations on how to enhance students’ modeling experiences in multidisciplinary areas in Statistics Education. Second, it serves as a basis for further effectiveness and large scale studies for teaching statistics using Modeling Approach. Third, the study will show research directions on how to design curriculum using Modeling Approach. Fourth, it will help teachers as a heuristic devise on how to prepare and implement relevant non-routine problem solving tasks like MEAs. Fifth, the study will provide directions on aspects of professional development that have to be given for teachers for teaching statistics in secondary schools. Finally, it will serve as an available asset for policy decision makers, curriculum developer, and assessment builders on how to include relevant non-routine problem solving tasks in an education system.

MATERIAL AND METHODS
Instructional Design
Four MEAs were designed for this study as described in Table 1 based on the six principles of constructing MEAs (Lesh and Doerr, 2003) with other statistical activities using representations systems. The MEAs had four main components: newspaper article, readiness or warm up questions, data table, and a problem statement. Each component serves a valid purpose and used to engage problem solvers in the task (Chamberlin & Moon, 2005). The purpose of the newspaper was to familiarize students with the context of the problem and to develop their statistical literacy. This article required 15 minutes or so to read and provide further information for the second part of MEAs. The second part of an MEA was readiness questions or warm-up questions. These questions were designed to evaluate their understanding of their reading and basic statistical literacy ability of the media article. The third part of MEA was a problem statement which required students
pose and solve problems on the socio-cultural problem situations. The fourth part of an MEA was usually a data table that may be used to solve the problem.

<table>
<thead>
<tr>
<th>Title of MEA</th>
<th>Problem Context</th>
<th>Statistical Concept</th>
<th>Objectives with Social Agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe-Water</td>
<td>Students are asked to write a report based on 20 households data to give an awareness program for rural people how to drink safe water and keep their environment sanitation and hygiene.</td>
<td>Different graphs, distribution, average, little or a lot variability</td>
<td>To provide awareness on health and sanitation problems</td>
</tr>
<tr>
<td>Millennium Dam</td>
<td>Students are asked to write a report based on three grade 9 sections of students’ data on the contribution of money to Ethiopian Millennium dam which is going to be sent by the director for the news agency.</td>
<td>Outliers, measure of centers (mean, median and mode), Range</td>
<td>Empower students on their contribution for developing their identity</td>
</tr>
<tr>
<td>Football</td>
<td>Students are asked to write a report to present on school min media to create awareness program and for consulting the Ethiopian football coach based on data on two football teams who plays against two other African football teams.</td>
<td>Center(mean, mode, and median), Distribution, Graphs</td>
<td>Helping others to control emotions and for creating awareness program about Ethiopian Football</td>
</tr>
<tr>
<td>Tourist</td>
<td>Students are asked to give reliable information based on the weather data of five tourist sites on what to eat, cloth and shoes to wear.</td>
<td>Distribution, measure of center, measure of variation</td>
<td>Use variability in everyday life like knowing variability of weather conditions</td>
</tr>
</tbody>
</table>

A typical modeling approach lesson sequence was shown in Figure 1. After finishing the MEA, there would be other follow up activities that used system of representations and MEA extension problems. For example, in Safe-Water MEA students were introduce other graphs from simple graph type dot plot to histogram and how to interpret by comparing distribution of various graphs. Then, as assignment students wrote a report for Ministry of Water and Energy on Safe-Water MEA based on their own data and got feedback on the structural similarity of the pervious client report on Safe-Water MEA. Students were expected to use the newly introduced representation systems in the MEA extension problem.
Quality Assurance Guide

The quality assurance Guide was designed to help teachers, researcher and students evaluate the products that were developed in response to the MEAs with the following characteristics: (a) the goal is to develop conceptual tools, (b) the client purposes are known and met, and (c) the tool must be sharable with other people and must be useful in situations where the data are different than those specified in the problem as shown in Table 2.

The Quality Assurance Guide was used to quantitatively assess students’ models. The levels were designed to categorize how well students’ solution artifacts or reports satisfy the needs of the client and how well they explained their reports in general way (Lesh et al., 2000). The range of response went from level 5, where the response satisfied the needs of the client for the current situation and for other similar situations as well, 1, where the response were going in the wrong direction and the team would need to rethink their reports completely.
Table 2: Quality Assurance Guide

<table>
<thead>
<tr>
<th>Performance Level</th>
<th>How useful is the product?</th>
<th>What might the client say?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level one: Require Redirection</td>
<td>The product is on the wrong track. Working longer or harder won’t work. The students may require some additional feedback from the teacher.</td>
<td>“Start over. This won’t work. Think about it differently. Use different ideas or procedures.”</td>
</tr>
<tr>
<td>Level two: Require Major Extensions or Refinements</td>
<td>The product is a good start toward meeting the client’s needs, but a lot more work is needed to respond to all of the issues.</td>
<td>“You’re on the right track, but this still needs a lot more work before it’ll be a form that’s useful.”</td>
</tr>
<tr>
<td>Level three: Requires only Minor Editing</td>
<td>The product is nearly ready to be used. It still needs a few small modifications, additions, or refinements.</td>
<td>“Hmmm, this is close to what I need. You just need to add or change a few small things.”</td>
</tr>
<tr>
<td>Level four: Useful for this Specific Data Given</td>
<td>No changes will be needed to meets the immediate needs of the client.</td>
<td>“Ahhh, this will work well as it is, I won’t even need to do anything”.</td>
</tr>
<tr>
<td>Level five: Sharable or Reusable</td>
<td>The tool not only works for the immediate situation, but it also would be easy for others to modify and use in similar situations</td>
<td>“Excellent, this tool will be easy for me to modify or use in other similar situations when the data are slightly different.”</td>
</tr>
</tbody>
</table>

Site Selection and its Rationale - The Schools

This study was conducted at West Shewa, Oromia Region schools (School A and School B; pseudonyms) in Ethiopia based on grade 9 secondary mathematics school curriculum which had one unit of descriptive statistics with 22 period allotments (Micheal & O’Connell, 2014). There were 9 and 6 sections of grade students with average class size of 45 and 42 students per class in School A and B respectively. The dominant languages spoken by students were Amharic and Oromigna.

The first researcher chose this research site for a number of reasons. First, the researcher had familiarity of the study site living at the place for more than 15 years to investigate the nature of socio-cultural problems that could arise from their everyday life. Second, Ethiopian National Examination Agency (ENEA), (2010; 2013) had revealed that in secondary school 15.5% of the observed variation in students academic achievement attributed to differences among schools. The study selected the two schools to show direction how it was possible to work jointly to improve the quality of education. Third, the distance between the two schools is 0.5 km which could make the data collection process convenient.
Participants in the Intervention

From the two high schools, 80 students participated in this study from two grade 9 sections. The sampling method used to select the two classes was purposive sampling because the method would allow selecting classes that teachers can implement Modeling Approach. The demographics of the combined sections consisted of 49 % females (39 out of 80). The students’ ages were within the range of 14-17 years old. Background information on the intervention participants based on their sex and mathematics achievements levels in the last semester were described as shown in Table 3. Students score were transformed using standard scores and students whose mathematics score were below 50 taken as low achievers, if students score between 50 and 70 assumed to be medium achievers and lastly if a students’ score were above 70 the students were considered as high achievers as set by the schools district officers. Two teachers were selected one from school A, and the other from school B based on their willingness to participate in the research.

<table>
<thead>
<tr>
<th>School</th>
<th>School A</th>
<th>School B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>*Levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Medium</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Low</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

Piloting the Intervention

As part of the design and development cycle of the research, the instructional design was piloted tested in two schools to see the feasibility of the design. First, the researcher designed the instructional design for the intervention based on MEA design principles and gave to experts for review and validation purpose. Second, the researcher selected six teachers for seminar on the Modeling Approach and there was discussion on the instructional design in a workshop and teachers’ comments were taken. Third, the instructional design was pilot tested by two of the teacher from the seminar groups in their own classrooms. The researcher recorded some of the lesson videos and took observational notes. Then, after implementing the instructional design from the pilot test, the instructional design was revised and implemented in two Grade 9 classes in the selected school for the study. The school for a pilot test was assumed similar to the schools for the major study. There were major decisions made by the researcher in
revising and improving the intervention in the pilot test. Students were not accustomed to modeling and the researcher, based on modeling processes cycle, developed a worksheet for each MEA that could help teacher to scaffold students’ modeling of real world problems as shown in Figure 2.

Then, piloting the intervention helped to observe the feasibility of the study and to modify and improve some parts of the instructional design materials. Training was given on the Modeling Approach for a week on goals, orientation and resources of Modeling Approach to implement it in class. The design was pilot tested one year (in 2012/2013) before the final intervention (in 2014).

![Figure 2: Modeling cycle used for scaffolding purpose](image)

Based on the modeling cycle as shown in Figure 10 a worksheet with the following probing questions were given for the students

1. **To understand the real problem**: What is the thing that the client wants you to do for him/her?

2. **To set up a statistical model based on reality**: How could you use the data using your model so that it is meaningful for the client purpose?

3. **To solve statistical questions using the statistical models**: Could you show how to use the statistical models to provide some solution for the problem?

4. **To interpret statistical results in a real situation**: What are your interpretations on the models you made in question 3?
5. **To validate the obtained solution and see the limitations of the model**: Do you think your statistical models enough for the client purpose and have limitations?


**Data Collection Methods and Analysis**

To answer the research question, appropriate mixture of quantitative and qualitative data collection methods were used. Both quantitative and qualitative data collection methods administered during and after the implementation of the Modeling instruction. The source of data for the research drew on content analysis of textbook and students reports on the four MEAs. Content analysis of teams of students’ models on the four MEAs was made using Quality Assurance Guide. Descriptive statistics such as means and percentage were used.

**Ethical Considerations**

Prior to conducting the study the proposal was approved by the Science and Mathematics Education Department in Addis Ababa University; and then, the study got local permission from the school district officers and school directors. Ethical issues in the research were considered starting from topic selection of the research problem, to carrying research goals, to the interpretation and reporting of the research findings beyond informed consent.

**RESULTS**

**Content Analysis of Grade 9 Text Book**

As shown in Table 4 content analysis is made on the current Ethiopian Grade Nine mathematics textbook on statistics unit which is published by Ethiopian Ministry of Education in 2010. The percentages of exercises and word problems tasks in statistics unit in Ethiopian Grade Nine mathematics textbook are 68% and 27% respectively. Although Ethiopian education policy and documents on mathematical curriculum recommend that there should be relevant problem solving activities on socio-cultural issues, the percentage of statistical problem solving tasks (4%) in Grade Nine textbook is very low and non-routine statistical problem solving tasks are almost non-existent.
### Table 4: Levels for types of tasks

<table>
<thead>
<tr>
<th>Level</th>
<th>Tasks name</th>
<th>Example as task statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Exercises</td>
<td>*Find the mean of the following data 5, 7, 8, 9, 11</td>
</tr>
<tr>
<td>Level 2</td>
<td>Word or story problems</td>
<td>*In mathematics test the scores for boys were 6, 7, 8, 7, 5 and the scores for girls were 6, 3, 9, 8, 2, 2, 5, 7, 3. Find the mean and median for the boys and girls.</td>
</tr>
<tr>
<td>Level 3</td>
<td>Statistical problem solving tasks</td>
<td>*Mamo’s brothers are 174cm, 180cm, 179cm, and 172cm tall. If Mamo and his brothers have an average height of 176.50 cm, then how tall is Mamo?</td>
</tr>
<tr>
<td>Level 4</td>
<td>Non-routine statistical problem solving tasks (like Model-Eliciting Activities)</td>
<td>In your team, write a report that could be given to the news broadcast center by the director of the school using the provided students’ data of three Grade Nine sections.</td>
</tr>
</tbody>
</table>

*The three examples are taken from Grade Nine mathematics textbook (MoE, 2010a)*

**Students' Models for MEAs**

In this study out of the 4 MEAs in the Modeling Approach lessons we consider students’ reports on the first MEA (Safe-water) and the last MEA (Tourist) for illustrative purpose by selecting samples of students’ reports from each level based on Quality Assurance Guide.

**Students' Models for Safe-Water MEA**

**Students’ Models at Level 1: Safe-Water MEA**

Two teams of students were at level 1 on Safe-Water MEA. The reports were unrelated to the clients’ needs and did not fit the purpose of the task. For example, one team of students drew the bar graph as shown in Figure 3. First, they drew the bar graph putting on the x-axis the name of the representative of the 20 householders. Then, they drew the annual income of householders in thousands on the y-axis.

![Figure 3: Team of students’ model on Safe-Water MEA at Level 1.](image-url)
Report: Dear Ladies and Gentlemen, today we will give you an awareness program on keeping the sanitation of latrine houses. We have drawn a bar graph with the income of family in thousand birr and the number of children whose age is greater than or equal to the age of 5 years old. From the graph, we see that a family with 22 thousand birr income has 4 children, a family with 15 thousand birr income has again 4 children and a family who has 40 thousand birr income has 5 children and so on. Each family has better income and at least has 2 children at home. Thus, a family should keep the latrine clean together and should not use open defecation and wash hands after going to latrine house. We thank you for your attention!

They tried to modify the graph putting the income of householders on the x-axis and the number of children on the y-axis. But, they couldn’t justify why they drew the graph. In addition, the report merely included little data from the provided data table and partial data was used for the bar graph. The report needs a complete redirection.

Students’ Models at Level 2: Safe-Water MEA

Seven teams’ of students were at this level. One team of students’ drew pie chart on drinking water sources and histogram on the number of families whose age was greater or equal to 5 as shown in Figure 4. The team drew a histogram with the bars overlapping, but the variable on the x-axis was a discrete variable. The team showed meaningful interpretation, though they selected only two variables. The work was on the right direction, but it needs adjustment to satisfy the clients’ needs. The team had to redraw the histogram by labeling the axis and making the bars non-overlapping.

Figure 4: Team of students’ model on Safe-Water MEA at Level 2.

Report: Good morning! Today, we will see how to treat drinking water in a family. Based on our data as shown in the pie chart, for 20 householder families 40% of drinking water source is protected well, 35% -borehole, 15% -spring and 10% comes from rain water. The histogram has also showed the number of family members whose age is greater or equal to 5. There are 3 householders who have 2 family members, 6 householders who have 3 family members, 5 house holders have 4 family members and another 5 householders have 5 members and one householder have the maximum number of family members which is 6. Dear families, the water you drink
is not well treated which causes many water born diseases like typhoid and typhus. We should first boil the water and then cool it for drinking purpose. Every family member should feel responsible for keeping the water safe. We thank you for listening!

Students’ Models at Level 3: Safe-Water MEA
Nine teams of students were at level 3. Teams of students’ reports at this level were closely ready to give an awareness program on safe drinking water, but the reports needs some modifications. Among these teams of students, one team of students prepared a report on safe drinking water on how to wash hands after using a latrine as shown in Figure 5. The two bar graphs and the pie charts were appropriate and visible except they need titles. The interpretations on the graphs were correct and used more than three variables unlike the students’ models at Level 2.

![Figure 5: Team of students’ model on Safe-Water MEA at Level 3](image)

Report: Welcome! How are you? We are going to discuss with you on keeping our environment sanitation and on how to give our children safe drinking water based on data and graphs. As the graph shows, out of 20 householders’ in our rural community, there are 8 householders who have used protected well for drinking water source, 2 householders use rain water, 3 from spring and 7 get from Borehole water source. There are family members who do not wash their hands. For example, out of 9 people 6 people do not wash hands taking 9 householders sample from the 20 householders. This is a serious matter because 50% of householders in the community use open defecation. Hence based on the data we need a solution to keep our children safe because every family have at least one child except two householders. Children are most affected by unsafe water. We recommend the following solution: (1) All people have to wash their hands, and (2) All people have to save the children. Next time we will see how to filter and treat the water.

Students’ Models at Level 4: Safe-Water MEA
One team of students’ was at level 4. The team wrote the report to give an awareness program for the society. The team drew histogram for the income of the householders for 20 families and the income ranges from 13 thousand birr to 90 thousand birr. They also drew bar graphs and pie charts. Then, they made association with the area each
householders had in its compounds. They could see the paradox in that the area the 20 householders own was large and they had high incomes (Figure 6).

Report: Dear Ladies and Gentlemen, Good morning! We have called you to solve the problems the community have for building the latrine and keeping our environment safe and wash our hands. Based on our data we found that 20 householders had better income and enough area. The people income ranges from 13 thousand birr to 90 thousand birr yearly. It surprises us because half of them (50%) practice open defecation and lack no latrine houses. Most of the people use water sources like protected well (40%), borehole (35%), spring water (15%), and rain water (10%). All of this water sources may contain impurities and should be treated. But, we need also to build pump water or ask the government to get tap water service. So we need to solve the problem together. How could we create awareness for every member of our family young and old to wash hands? How we build latrines in our compound? And how do we get safe drinking water? Please, give us your comments if you have any better options!

Figure 6: Team of students’ model on Safe-Water MEA at Level 4

But, half of the householders with their family practiced open defecation. This was interesting, because they had created relations among the variables looking at some data pattern. But, the report might not be sharable or reusable if the data provided or the context was different.

Students’ Models for Tourist MEA
Students’ Models at Level 1: Tourist MEA
Two teams of students were at this level. For example, a team of students gave general information on what clothes and shoes to wear, what food to eat and what drinks to drink, but they barely used data in their report to meet the client needs as shown in Figure 7.

Dear Tourist, it is advisable that a tourist visit a country in September because the weather condition is conducive. When the tourist wants to visit a country, he should not bring clothes for cold weather, that is, he/she should bring light clothes. The shoes should be Sandals as there is no snow in the tourist sites. The tourist needs to bring an umbrella in case it rains. Besides, the tourist should bring fast foods and cold drinks. M.D = 28+23+35+23/4 =23 M.D= /-5/+/-7/+/-5/ +/0/ divided by 4 equals 7 And M.D= 1.25

Figure 7: Team of students’ model on Tourist MEA at Level 1.

Students’ Models at Level 2: Tourist MEA
Eight teams of students were at level 2. They used only one model which was range to describe the average annual temperature of two tourist attraction sites out of the four sites. They used partial data of the provided data and they tried to give information on two tourist
attractions sites. The reports needed further refinements using more variables and data to furnish the tourist with good information for the four tourist sites as shown in Figure 8.

Dear Tourist, Welcome to the attractive tourist sites of Ethiopia! We would like you to introduce two historic tourist attraction sites in Ethiopia. The two sites are known as Harar and Aksum. Harer is a city protected by stone wall and it is recognized by UNESCO. The people of Harer are well known for their hospitality and love. Both Christian and Muslims have lived in Harmony for centuries. Harer is found at an altitude of 55m above sea level. The range of average temperature and rain days for a year in Harer is 3 degree centigrade and 9 respectively. Axum is a city well known for its obelisks for example one obelisk has a height of 33 meters. Aksum is found at an altitude of 2355 m which is at higher altitude than Harar. The range of average temperature and rain days for a year in Harer is 3 degree centigrade and 9 respectively. Dear tourist, Harar is hotter than Axum. You need to visit both places, because both of them are historical places and their social life and culture are interesting. Good Luck!

Eight teams of students were at level 3 on Tourist MEA. For example, a team of students at this level used two models (range and bar graphs) to give information to the tourists using the provided data. They tried to present the data using bar graphs and range on average annual temperature on the two tourist attraction sites (Figure 9).

![Figure 9: Team of students’ Model on Tourist MEA at Level 3.](image)

Dear Tourist, We would like to give you reliable information about health requirement, customs, transport, time, currency, topography, etc. We would like to introduce two tourist attraction sites which are known as Axum and Lalibla. The range of the annual average temperature of Axum is 3°c and the range of the annual temperature of Lalibla is 15°c. So the Lalibla temperature is hotter than the Axum temperature. So if you go to Lalibla you must wear white or light clothes, since it will be hot there. You can see and compare the temperature difference using the pair of bar graphs as shown for the two sites. Come and visit us we will give you further information!
Students’ Models at Level 4: Tourist MEA

One team of students was at level 4. Similar to team of students at level 3, the team had used range and bar graphs. But the team of students gave description on the rainfall amount of the four places in addition to using the average annual temperature.

Figure 10: Team of students’ model on Tourist MEA at Level 4.

Things to do for Tourist! There are many things that we do for tourists; they may come from a country far from Ethiopia. Thus, they may not know our local languages that we have to translate the local language for them. We can also help them by carrying their goods, food, clothes and other necessary materials. We can also use range to show the tourist sites climate variations to give information for tourists.

Axum- has low range b/c 18-15= 3°c and rain-high =11mm
Lalibela-has high variation b/c 28-13=15°c and rain-low=1mm
Gonder-has high variation b/c 35-23= 12°c and rain-low=1mm
Harar-low variation b/c 28-23=5°c and rain high=9mm

Dear tourists, welcome to the attractive sites of Ethiopia. Ethiopia is a country abundant with varied tourist sites which are attractive and you will have memories of these sites in your mind. We will say, welcome again! Now I am going to tell you about Ethiopian tourist sites. Ethiopia has many innumerable tourist sites that it is difficult to count in short period of time. Among the well known sites by tourism sector, we take today Axum, Lalibla, Gonder and Harar. We can see different amazing things at these places. Our dear tourists, if you want to come to Axum, you have to wear sweater, normal trousers and you need to have tea because there will be rain days. Again if you want to come to Lalibla, you have to wear t-shirt and need to have cold water and you have to have vegetable food because this place is very hot. As we notice from the graphs most months have high variation by temperature. At the end, please try to come to visit Ethiopian tourism.

Students’ Models at Level 5: Tourist MEA

One team of students was at level 5. The team’s report assumed to be sharable and reusable as the students used all the variables from the provided data and different models to describe the four tourist sites. They interpreted the data correctly within cultural
contexts of the tourist sites like wearing style, social life of the people as shown in Figure 11. They presented the information as if it was given in FM radio transmission.

This is Ethiopian FM RADIO!
Dear tourists, first welcome to Ethiopia! We are happy to announce you that you will be happy for visiting Ethiopia, the country which has several historic, cultural and wildlife tourist attraction sites. Among the cities for tourist attractions I will give you important information on Harer and Gonder. And Helen will give you information about the attractive tourist sites of Lalibla and Axum. Please be with us!
Based on data, Harer is located at an altitude of 55m above sea levels. It is known for its people kindness and the city is called a ‘love country’. And thus, this culture is closer to Brazilian culture and that many Brazilian come to visit Harar. The range of the average temperature for Harar for a year is 5 degree centigrade with medium temperature. Thus, we need to wear light clothes like traditional Harar clothes called ‘dereya’. When we look at the rainfall amount it has a standard deviation of $\sqrt{6.24}$. When we go to Gonder, it is located at an altitude of 380m above sea levels and it is a city that we found several historic and cultural places to visit. The range of the average temperature for Gonder is 12 degree centigrade for a year with hot temperature. The standard deviation of the rainfall amount of Gonder for the year is $\sqrt{239}$. Now, Helen will present you information on other two cities.

Thank you Hanan! I will present you information on the great Ethiopian cultural Heritage placed called Aksum and Lalibla. Axum is a place where it attracts many tourists in the world and located at an altitude of 2355m. The range of average temperature for Axum is 3 degree centigrade which means it is not a hot place. The standard deviation of the rainfall amount is $\sqrt{14.35}$ and you can wear whatever cloths you like. When we see Lalibla, it has range of average temperature of 15 degree centigrade. The people who lived in Lalibla often wear white clothes to reflect the sun light radiation. You could also stay there wearing light clothes suitable for the weather condition. Lalibla is located at an altitude of 74 m from sea level and the standard deviation of the rainfall amount is $\sqrt{1.34}$. Thank you for staying with us! We will meet in another program.

**Figure 11:** Team of students’ model on Tourist MEA at Level 5.

Agreement was reached among the two teachers and the researcher using Quality Assurance Guide for the four MEAs that the majority of teams of students were at level 2 and 3 as shown in Table 5. The percentage of number of teams of students’ solution at level 1 and 2 were 10% and 38.8% respectively. The percentage of number of teams of students’ solution at level 3 and 4 were 45% and 5% respectively. Only one team of students’ solution was considered at level 5 across the four MEAs.
Table 2: Number of teams’ at the five performance levels for the four MEAs

<table>
<thead>
<tr>
<th>Performance Level</th>
<th>MEA-1</th>
<th>MEA-2</th>
<th>MEA-3</th>
<th>MEA-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level one: Requires Redirection</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Level two: Requires major extension or revision</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Level three: Requires editing and revision</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Level four: Useful for the specific data given</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Level five: Shareable and reusable</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

DISCUSSION

Successes

Students’ in a team worked on multi data sets and on multi variables to write reports and letters. Learning statistics is not only doing, but also involves describing and interpreting situations statistically (Lesh, 2000; 2012). The finding of the study suggests students can draw conclusion informally from data in a context on MEAs as contents of students’ reports showed. Students can construct, test and revise their models by expressing their thinking through representation systems (Lesh and Doerr, 2003; Lesh and Fennewald, 2010). The multiplicity of students’ solution on MEAs illustrated students can develop authorships of ideas than relaying simply on text books and teachers.

The finding also suggested students found the MEAs relevant to their life and they were ‘hard fun’ activities. Students were able to go through the modeling cycle as shown in Figure 2 and they were able to think ‘outside of the subject box’ in multidisciplinary areas. Statistics is at an intersection of many subjects, since all subjects will use some data to work with concepts. This is an opportunity to all allow students to work with a team in multidisciplinary area which is the ability and understanding needed beyond school in an age of information (English, 2002, 2013; Lesh, 2000). Students get used to the statistical inquiry cycle doing MEAs (Wild & Pfannkuch, 1999). By working on real world problem, a set of data and the need for a solution, students would experience the statistical inquiry cycle.

Much emphasis is given to Science, Technology, Engineering and Mathematics Education in Ethiopia Education System (MoE, 2010b). Hence, Modeling Approach would come into spotlight to bring together different disciplines. Working on relevant non-routine problems like MEAs starting from lower grades would create opportunities to students to prepare them for their future careers like engineering and other sciences. The finding of the study among others indicated it is possible to develop students' modeling experiences from early grades (English, 2013b; Eric, 2008; Kinner, 2013; Lesh, 2012).
Challenges
Defenders claim that if teachers teach statistics using Modeling Approach, students may not get the basic building blocks of knowledge. They assume that students need to learn first the basics, then apply what they learned later on in another statistics lessons or in application problems. Of course students need to learn a healthy dose of basic concepts and a focus only on problem solving process without skills will deter their procedural fluency; however it is possible to use Modeling Approach to enhance basic skills as well as the cognitive thinking and reasoning at the same time, since Modeling Approach enhance students’ basic knowledge while they solve modeling problems (Lesh, 2012). A decade of mathematics education research showed that students could enhance high cognitive thinking on ‘rich tasks’ without compromising the basic skills and knowledge (Schoenfeld, 2004). The findings of the study suggest that it is possible to enhance students’ conceptual understanding and modeling abilities without affecting their procedural understanding.

Defenders also may claim that modeling problems are challenging that students could not attempt to solve the problems. Neither a teacher wants to teach statistics by giving students challenging problems which frustrate them to attempt, nor does he/she want to teach students by spoon feeding. However, teachers need to strike a balance to teach students with some sense of challenges so that students get a chance to enhance their cognitive thinking. Even a child from the early age naturally requires some challenge; the challenge for six month baby may be walking.

Teachers need not avoid modeling problems in the pretext of students are young that they do not need to work on modeling problems for the reason that they are challenging. But, learning theory supports that students learn well through perturbations, since human minds disallow the disequilibrium created by the challenge. Through scaffolding, students could solve the modeling problems through iterative cycles of assimilation and accommodation. The findings of the study suggest, students could undergo data modeling processes; even if, they were not experts at modeling. Social interactions in teams of students were the cause of accommodations as observed in MEAs lessons.

Many defenders raises practical issues by claiming that it is not possible to teach statistics using Modeling Approach due to time constraints and content coverage in the school programs. Of course, some problem-driven curriculum may take longer period of time than the allotted time in schools (Chamberlin and Coxbill, 2008). However, as this study among others showed, MEAs did not take longer more than one or two periods that time constraints may not be a problem (Chamberlin and Coxbill, 2008, Lesh, 2012). Students
need to develop both content and process objectives for learning descriptive statistics. Most importantly, the best possible ranges of problem types that involve routine and non-routine problems need to be used. Students could learn on big ideas of descriptive statistics using MEAs based on few design principles as this study suggests. Further, students could benefited more if they do open-ended project to experience statistical investigative processes that could save enough time to meet the process objectives as this study findings suggested.

Other defenders simply leave the matter of using non-routine problem solving tasks like MEAs for teachers who teach statistics in schools. Even though the teacher is the ultimate decision maker in the classroom, it is not honest recommendation since using non-routine needs a coordinated effort from all stakeholders. First the teacher may have little time to design MEAs, because MEAs requires selecting topical themes and need to follow design principles to construct them. Second, using Modeling Approach the teacher needs to use diagnostic teaching method to attend students’ thinking (Schoenfeld, 2010).

It is not just the lesson goes according the plan way of teaching is used for Modeling Approach. But, among other studies, the study showed MEAs primary purpose is to elicit students thinking by externalizing their thinking using external representation systems or models (Doerr and English, 2003). Thus, students’ models serve as windows so that teacher made interpretation on their models to build the instruction (Lesh and Fennewald, 2010). In the Modeling Approach, teaching is not only doing; it also involves observation and interpretation and making inference based on cognitive tasks as this study suggests. Hence, to use modeling problems in an education system needs serious attention beyond leaving the matter simply to teachers on what they liked to do with non-routine problems.

Finally, many defenders of the modeling practice claim that it is simply a tradition to teach statistics using a formal approach and teachers ought to continue with the usual practice. However, following traditions may have negative side to enhance the kinds of understanding students’ need in the 21st century in an age of information. Statistics being in mathematics curriculum may be taught in traditional formal approach, despite statistics as discipline originates to solve practical problems.

In fact, to reveal a particular practice as having a status of a tradition couldn’t shed light on whether it is a good one or a bad one. Statistics is at the intersection of many disciplines that stakeholders need to take part for enhancing students’ understanding for the benefit of all not just for mathematicians or mathematics educator. If Modeling Approach
enhances students’ understanding of descriptive statistics within the interest of all stakeholders, then it needs to be considered seriously to incorporate modeling problems in the statistics curriculum, instruction and assessment in an education system.

CONCLUSIONS

High school students worked on relevant non-routine problem solving tasks called MEAs. Students elicit their models by externalizing their thinking through representation systems working on MEAs and can develop their own models by testing, revising and refining iteratively in an engineering way. The findings of this study are remainders that Modeling Approach likely help to enhance students modeling experiences with similar school setting, but needs further efficacy study at different school settings and populations; it also needs large scale effectiveness study to meet the interest of stakeholders in an education system.

REFERENCES


Characterization and Ranking of Vermicompost Obtained from Different Plant Materials and Animal Wastes in Terms of their Major Nutrient Contents and Manurial Value

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Abstract
Composting of organic materials and returning it back to the soil is a common technique in maintaining soil fertility through natural nutrient cycle. To examine this, a study was conducted at Bako Agricultural Research Centre (BARC) during 2015 – 2016 E.C. The experiment was designed to characterize vermicomposts prepared through organic waste recycling from residue of main crops in the area and animal wastes in terms of the major plant nutrient composition in order to identify the best quality compost. Laboratory analysis of the vermicompost for its chemical property and nutrient composition revealed that unlike the pH value of conventional compost which falls in alkaline array detrimental to growth of plants, the pH values of all type of vermicompost were found in suitable range for plant growth. In terms of organic carbon, CN ratio and total nitrogen content, all types of vermicompost has outsmarted the conventional compost significantly. The vermicompost obtained from the combination of Maize Stover, Niger seed residue and sheep manure has shown higher value of total nitrogen (2.42%). However, these types of vermicomposts were found to be very poor in other primary and secondary plant nutrient elements. With regard to other plant growth-limiting nutrients, the vermicompost produced from soybean residue and cattle manure scored a higher value in total phosphorus, total potassium, and total magnesium. The result of this study indicated that in spite of supplying other macro and micro nutrients needed for plant growth, 4.64 tons of this type of vermicompost can replace the recommended amount of urea (200kg) in terms of nitrogen which at the same time supply 139 kg of DAP (64.49 kg P₂O₅), an amount which exceeds the blanket recommended dose of phosphorus for maize. Thus, by virtue of the accessibility of raw materials, simplicity of its production and better availability of the nutrient contained in it to the plant, utilizing the vermicompost of soybean straw and cattle manure has a paramount importance in enhancing crop productivity and improving soil fertility.

Keywords: Soil fertility; Vermicompost; Feedstock; Eisenia fetida
INTRODUCTION

Tropical soils are deficient in all necessary plant nutrients on the one hand and large quantities of such nutrients contained in domestic wastes and agricultural byproducts are wasted on the other hand. It is estimated that in cities and rural areas of developing countries million organic wastes are generated annually which is either burned or land filled (Gandhi et al., 1997)

The extensive use of chemical fertilizers leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and causes soil degradation. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection (Aveyard, 1988; Wani and Lee, 1992 and Wani et al., 1995).

In maintaining soil fertility through natural nutrient cycle, composting of organic materials and returning it back to the soil is a common activity in developed nations (Peter et al., 2000)). Composting is a technology for recycling organic materials in order to achieve enhanced agricultural production.

Vermicomposting appears to be the most promising as high value bio fertilizer which not only increases the plant growth and productivity by nutrient supply but also is cost effective and pollution free. Vermicompost can be described as a complex mixture of earthworm faeces, humified organic matter and microorganisms, which promotes soil aggregation and stabilizes soil structure improving the air-water relationship of soil, when added to the soil or plant growing media, increases germination, growth, flowering, fruit production and accelerates the development of a wide range of plant species (Ndegwa et al., 2001).

Vermicomposting is faster and less labor intensive than traditional composting methods, requires less space, and creates little odor. It is a promising biotechnology for many waste management applications and is an easy way to make a positive environmental impact by reducing the amount of green-waste that finds its way into landfills, incinerators, and sometimes the ocean. The resulting nutrient-rich compost end product is an environmentally sound amendment to enrich soil for plant growth that contributes in counteracting the deterioration of the environment due to rampant use of chemical fertilizers (Inbar et al., 1993)

Composting worms are small mesophillic, red purple worms that prefer an environment of decaying organic matter rather than soil (Piper, 2005). They reproduce quickly, consume
large amounts of organic material, and tolerate the environment of a worm bin. Earthworms consume various organic wastes and reduce the volume by 40–60 (Dominguez, 2004). Earthworms and its excreta (vermicast) promises to usher in the ‘Second Green Revolution’ by completely replacing the destructive agro chemicals which did more harm than good to both the farmers and their farmland. Earthworms excreta (vermicast) is a nutritive ‘organic fertilizer’ rich in humus, NKP, micronutrients, beneficial soil microbes—‘nitrogen fixing& phosphate solubilizing bacteria’ and ‘actinomycets’ and growth hormones ‘auxins’, ‘gibberlins’ and ‘cytokinins’. Both earthworms and its vermicast& body liquid (vermiwash) are scientifically proving as both ‘growth promoters and protectors’ for crop plants (Rajiv et al., 2010).

Extensive research on inorganic fertilization and plant breeding, carried out within the framework of conventional agriculture, has allowed agricultural producers to fine-tune nutrient inputs and plant needs in order to maximize yields. However, such detailed knowledge has not yet been attained as regards the nutrient composition of organic fertilizers as vermicompost in sustainable agriculture. Given the complex and variable composition of vermicompost in comparison with inorganic fertilizers and the myriad of effects that it can have on soil functioning, a clear and objective concept of vermicompost is required, and the complex interactions between vermicompost-soil-plant must be unraveled in order to maintain consumer confidence in this type of organic fertilizer (Cristina and Domínguez, 2010).

In Ethiopian context, vermicomposting is a recently adopted biotechnology in which the effort of on farm verification and demonstrating its utilization was made by Haramaya University, Ambo Plant Protection Research Center and Holeta Agricultural Research Center. However, there were very limited attempt of characterizing vermicompost and identifying it by the nutrient content and other quality parameters considered in enhancement of crop productivity and soil fertility due to lack of experience in analyzing this fertilizer by domestic laboratories. Among few individual efforts domestically made, Gezahgn et al. (2012) have vermicomposted coffee husk, enset waste, khat waste and vegetable waste using the epigeic earthworm Eiseniafoetida and found to be as a good option for improving solid waste management in Ethiopia and production of excellent bio fertilizers for agronomic purposes.

Among the wettest parts of Ethiopia western Oromia receives rainfall from April to December, that allow the growth of considerable amounts of decomposable materials needed to prepare compost. However, due to lack of awareness and technical know-how,
these materials are usually wasted without proper use despite the fact that soil fertility in
the region is declining rapidly from time to time. The sub optimal level of NP fertilizers
currently being used for crop production under farmers’ conditions has aggravated the
situation of soil fertility degradation and reduction of crop productivity (Heluf et al., 2004).
These and other facts have sparked the idea of looking for alternative sources of fertilizers
other than the commercial one. To this effect, vermiculture station was established and
Vermicompost preparation was launched at Bako Agricultural Research Center in the last
cropping season. Therefore, this study was conducted subsequently to characterize
vermicomposts prepared from residue of main crops in the area and animal wastes in
terms of major plant nutrient composition in order to identify the best quality vermicompost.

MATERIALS AND METHODS

Description of the Study Area
The study was conducted at Bako Agricultural Research Centre (BARC) during 2015 -
2016. The centre is located in the western part of Ethiopia at a distance of 250 km away
from Addis Ababa. It lies at latitude of 9° 6’ 00”N and longitude 37° 9’ 00”E and at an
altitude of 1650 m above sea level. It has a warm humid climate with annual mean
minimum and maximum temperature of 13.5 and 23.7°C respectively. The area receives
an annual rainfall of 1237 mm from May to October with maximum precipitation in the
month of June to August (Metrological station of the centre).

Establishment of Vermiculture Station
Vermiculture station which comprises three rooms namely raw material preparation
vermicomposting and drying and storage rooms was constructed in shady and ventilated
area in the compound of the center. The station is a simple prototype of un elevated barn
like housing with corrugated iron roof and netted strips of bamboo walls with meshed wire
extension on its upper part designed to ventilate the rooms and to avoid the entrance of
flying predators. A protective structure was also laid out at the basement and around the
walls of the room to prevent the composting worms from the attacks of ants and other
crawling enemies of the worms.

Experimental Materials and Procedure

Vermicompost Preparation

Feed Stock
The materials used in this experiment were crop residue; maize Stover, Soybean straw
and Niger seed refuse that are obtained from experimental fields of the center as a
bedding material and animal wastes; cattle manure sheep dropping and poultry manure collected from animal farms of the center as a feedstock for composting worms.

**Composting Worms**
The earth worms employed in the study were the red non burrowing type of specious known as Esinea Fatida, which are 10 to 15 cm /3-4 inch long with life span of only 28 months, collected from Ambo Crop Protection Research Center.

**Treatment and experimental setup**
In this study, fourteen types of bedding and feed materials combination were used as a treatment which undergone partial fermentation for 20 days using a cylindrical plastic container as a worm bin with the combination ratio of crop residue (DOW) to animal manure 1: 2 on weight basis. All the basic things needed by the worms that are referred to as ‘five essentials’: an hospitable living environment, usually called “bedding”, a food source; adequate moisture (greater than 50% water content by weight); adequate aeration; protection from temperature extremes were fulfilled.

**Treatments (Feedstock combination)**
1. Maize Stover + cattle manure
2. Soybean straw + cattle manure
3. Niger seed straw + cattle manure
4. Maize Stover + soybean straw + cattle manure
5. Maize Stover + Niger seed straw + cattle manure
6. Maize Stover + soybean straw + sheep waste
7. Maize Stover + Niger seed straw + sheep waste
8. Maize Stover + soybean straw + poultry manure
9. Maize Stover + Niger seed straw + poultry manure
10. Soybean straw + Niger seed straw + cattle manure
11. Soybean straw + Niger seed straw + sheep waste
12. Soybean straw + Niger seed straw + poultry manure
14. Cattle manure only

The vermicomposting process was started by releasing worms in to the partially decomposed medium in condition where the three most important environmental factors (Temperature, Adequate moisture and Ventilation) were maintained (Glenn, 2009). However, during the composting process it was observed that worms in the three
treatments which poultry manure was used as major feed material couldn’t survive much longer than a day to sustain the composting activity. This was probably due to toxic effect of the poultry waste which was possibly contaminated with chemicals used as disinfectant in the farm that paralyzed and finally killed the compost worms. The materials in the other eleven combination was safely transformed in to vermicompost after 3 months to give a uniform humus like loamy material in which no food scraps and residue materials are identifiable. It is light and black or dark brown in color. The compost was collected by manual harvesting which involved hand-sorting, or picking the worms directly from the compost by hand. The vermicomposts were dried, heaped, and stored while their representative samples were taken and prepared for laboratory test along with a sample of conventional compost prepared simultaneously from similar materials to determine their nutrient level.

Vermicompost Laboratory Analysis
The prepared vermicompost samples were analyzed in JIJE Analytical Testing Service Laboratory for their major plant nutrient composition and some chemical properties worth considering in characterizing the material to an extent.

Major Parameters and Test Methods
1. Organic carbon (OC) - FAO-Loss on Ignition at 450°C
2. Total Potassium (K) - FAO - Aqua regia digestion extract – Flame photometer
3. Total Nitrogen (TN) - FAO- Kjeldahl
4. pH water FAO-Potentiometric-Water extract
5. Total Phosphorous (TP) - FAO - Aqua regia Digestion extract – Flame photometer
6. Total Calcium (Ca) - FAO - Aqua regia Digestion extract – EDTA Titration
7. Total Magnesium (Mg) - FAO - Aqua regia Digestion extract – EDTA Titration

RESULT AND DISCUSSION
Chemical Property and Nutrient Level of the Vermicomposts
pH, Organic Matter and Total Nitrogen
According to the result of laboratory analysis, the vermicompost obtained from the combination of Maize Stover, Niger seed residue and sheep manure as well as the compost from the combination of Soybean residue, Niger seed straw and Sheep Manure has shown higher value in total nitrogen content. The compost formed from the combination of corn pulp, Soybean Straw and Sheep Manure and that of cattle manure only hold second and third position with the value of 2.17 and 2.1% respectively (table 1).
Table 1: Laboratory analytical result of the vermicomposts

<table>
<thead>
<tr>
<th>Feed material Combinations</th>
<th>pH (H₂O 1:5 ratio)</th>
<th>% OC</th>
<th>% OM</th>
<th>% T.N</th>
<th>CN ratio</th>
<th>% T.P</th>
<th>% T.K</th>
<th>% T.Ca</th>
<th>% T.Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize. St + Cattle Manure.</td>
<td>8.29</td>
<td>32.11</td>
<td>55.3</td>
<td>1.53</td>
<td>20.99</td>
<td>1.22</td>
<td>2.42</td>
<td>5.32</td>
<td>2.1</td>
</tr>
<tr>
<td>Soybean St + Cattle Manure</td>
<td>8.20</td>
<td>32.22</td>
<td>55.5</td>
<td>1.98</td>
<td>16.27</td>
<td>1.39</td>
<td>3.94</td>
<td>7.91</td>
<td>8.7</td>
</tr>
<tr>
<td>Niger St + Cattle Manure</td>
<td>8.51</td>
<td>35.21</td>
<td>60.7</td>
<td>1.98</td>
<td>17.78</td>
<td>0.69</td>
<td>2.29</td>
<td>3.08</td>
<td>1.85</td>
</tr>
<tr>
<td>MaizeSt.+Soybean st.+Cattle Manure</td>
<td>8.4</td>
<td>35.38</td>
<td>61.0</td>
<td>1.37</td>
<td>25.82</td>
<td>0.68</td>
<td>1.8</td>
<td>5.27</td>
<td>3.8</td>
</tr>
<tr>
<td>MaizeSt.+Niger. St. +Cattle Manure</td>
<td>8.12</td>
<td>34.43</td>
<td>59.3</td>
<td>1.75</td>
<td>19.67</td>
<td>0.69</td>
<td>1.75</td>
<td>8.39</td>
<td>3.78</td>
</tr>
<tr>
<td>MaizeSt.+Soybean St.+Sheep Manure</td>
<td>8.74</td>
<td>33.00</td>
<td>56.8</td>
<td>2.17</td>
<td>15.21</td>
<td>0.80</td>
<td>2.7</td>
<td>6.26</td>
<td>6.89</td>
</tr>
<tr>
<td>Maize St.+ Niger St. +Sheep manure</td>
<td>8.88</td>
<td>35.09</td>
<td>60.5</td>
<td>2.42</td>
<td>14.50</td>
<td>0.90</td>
<td>2.32</td>
<td>3.08</td>
<td>3.33</td>
</tr>
<tr>
<td>Soybean St.+ Niger st.+Cattle Manure</td>
<td>8.12</td>
<td>37.24</td>
<td>64.2</td>
<td>1.98</td>
<td>18.81</td>
<td>0.72</td>
<td>1.92</td>
<td>5.29</td>
<td>3.18</td>
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<tr>
<td>Soybean St.+ Niger St.+Sheep Manure</td>
<td>8.56</td>
<td>36.02</td>
<td>62.1</td>
<td>2.42</td>
<td>14.88</td>
<td>0.83</td>
<td>2.53</td>
<td>5.29</td>
<td>5.71</td>
</tr>
<tr>
<td>Crop Residue (Ms. Sbs. NSS)+Fym(cattle+sheep manure)</td>
<td>8.05</td>
<td>35.50</td>
<td>61.2</td>
<td>1.98</td>
<td>17.93</td>
<td>0.83</td>
<td>2.22</td>
<td>3.17</td>
<td>8.24</td>
</tr>
<tr>
<td>Cattle Manure only</td>
<td>8.16</td>
<td>42.87</td>
<td>73.9</td>
<td>2.1</td>
<td>20.41</td>
<td>0.69</td>
<td>1.7</td>
<td>5.31</td>
<td>1.91</td>
</tr>
<tr>
<td>Check.Conven. Compost</td>
<td>9.25</td>
<td>19.32</td>
<td>33.3</td>
<td>0.87</td>
<td>22.21</td>
<td>0.47</td>
<td>1.53</td>
<td>8.39</td>
<td>4.40</td>
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</tbody>
</table>

The conventional compost is superior in its pH value which falls in the very alkaline range of pH scale. Unlike the pH value of conventional compost which is detrimental to growth of plants, the pH values of all type of vermicompost are found in moderately alkaline range suitable range for plant growth (table 1). Considering the organic carbon, CN ratio and total nitrogen content, all types of vermicompost has outsmarted the conventional compost significantly. The modification of acidity was possibly due to nitrogenous waste excreted by the earth worms and the vermin wash released in the process which increased the moisture content thus neutralizing the pH of the vermicompost. This is in conformity with the study of Nagavalleemma et al. (2004) who found that the worm castings (vermicompost) contain higher percentage of organic carbon (13.8%) and total nitrogen (1.61%) compared to the conventional compost that contained 12% organic carbon and 0.8% total nitrogen. The same trend was obtained by Musaida et al. (2012) who stated that earthworms play an important role in the recycling of N in different agro ecosystems evident in vermicomposting which converts household and agricultural waste into compost within 8 weeks, reduces the C:N ratio and retains more N than the traditional methods of preparing composts.
Total phosphorus, Total potassium, Total calcium, and Total magnesium

With regard to other plant growth limiting nutrients the vermicompost produced from soybean residue and cattle manure scored a higher value in total phosphorus, total potassium and total magnesium while the vermicompost produced from Maize Stover, Niger seed Straw and Cattle Manure has scored higher value in total Calcium (table 1).

The higher total phosphorus content in the vermicompost is attributed to the mineralization and mobilization of phosphorous contained in feedstock due to earthworm activity as earthworms play an important role in the release of phosphates on organic matter. The increase in potassium and magnesium is boosted in similar way by the earthworm activity on the feed material. The result of this study is in line with the finding of Amir and Fouzia (2011) which indicated that vermicomposts have rich source of nutrient content, a higher Base Exchange capacity and more exchangeable sodium, magnesium and potassium than pit compost and garden soil. The analytical result of this experiment is also concordant with the observation of Pius and Thompson (2000) which showed vermicomposting resulted in a significant increase in total and available P, exchangeable K, exchangeable Ca and total Mg, emphasizing that the higher concentrations of plant nutrients in end product of vermicomposting indicate a potential for using agriculture wastes in sustainable crop production.

SUMMARY

In terms of the nitrogen economy of the vermicompost, the material that is obtained from Maize Stover, Niger seed residue and sheep manure as well as the compost from the combination of Soybean residue, Niger seed residue and Sheep Manure out smarts the others. However, these type of vermicomposts are found to be very poor in other primary and secondary plant nutrient elements as it is shown in the table. With respect to other major plant nutrients such as Phosphorus, Potassium and Magnesium, the vermicompost prepared from Soybean Straw and Cattle Manure has out ranked the other types of compost.

The manuring value of later type of vermicompost can be illustrated by taking maize, which is one of the major crops in the experimental area and other parts of western Oromia as an example. The blanket fertilizer recommended for this crop which is being used nowadays is 200kg urea and 100kg DAP. According to the result of this study, the recommended amount of urea can be replaced by 4.64 tons of vermicompost prepared from soybean straw and cattle manure in terms of nitrogen which at the same time supply
139 kg of DAP (64.49kg P$_2$O$_5$), an amount which exceeds the recommended dose of phosphorus for the crop.

CONCLUSIONS AND RECOMMENDATION
According to the results of this study, integrated effect of all the nutrients present in vermicompost helps to avoid plant nutrient imbalance in the soil in general. And by virtue of the accessibility of raw materials, simplicity of its production and better availability of the nutrient contained in it to the plant, utilizing the vermicompost obtained of soybean straw and cattle manure has a paramount importance in enhancing crop productivity and improving soil fertility.

Acknowledgements
The authors are highly thankful to the Natural Resource Research staff of Ambo Plant Protection Research Center for providing the composting worms to launch this study and for sharing their experiences in this regard. We are also grateful to JJJE Analytical Testing service Laboratory for handling the compost analysis task. The Soil Fertility Improvement Research case team members of BARC also deserve appreciation for their commitment to put the brief of this experiment to effect.

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Chemical Constituents and Larvicidal Effects of Dichloromethane Extract of Oreosyce africana on Anopheles arabiensis

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Abstract

Malaria continues to be one of the most deadly diseases in tropical countries. The efficacy of synthetic inorganic insecticides to control malaria vector mosquitoes is compromised by increased mosquito resistance to insecticides. Furthermore, use of inorganic insecticides raises environmental toxicity concerns. Therefore, plant-based biodegradable insecticides would be ideal alternatives for the control of malaria vector, which need to evaluate its efficacy against mosquito and to promote traditional methods of mosquitocidal activity. The selected plant, Oreosyce africana is collected mainly on the basis of ethnobotanical information combined with literature search. The aim of the study was to purify and characterize the chemical constituents from the extracts of O. africana and evaluate their larvicidal effects against Anopheles arabiensis. The selected plant is exhaustively extracted by 80% aqueous methanol and crude extracts were partitioned between different solvent systems by increasing polarities (dichloromethane, ethyl acetate and water). Purification of the dichloromethane fractions of O. africana were performed on flash column chromatography (eluted by n-hexane and chloroform solvent systems) in a step-wise gradient. The identity of the active fractions of O. africana was analyzed by a combination of TLC, HPLC, UV-Vis, NMR and GC-MS methods. The fractions of O. africana were tested using test cups against An. arabiensis larvae and analyzed by PoloPlus software. The active purified fractions of O. africana designated as Fraction IV had the most potent larvicidally active components among the fractions tested (LC50 of 2.507 and LC90 of 7.721 ppm) at 24 hr post-exposure and showed significant differences at p<0.05. The structural elucidation of the active ingredients in fraction IV of O. africana was determined using a combination of 1H-13C-NMR, DEPT-135 and GC-MS measurements. From fraction IV of O. africana which was eluted with hexane-chloroform.

(80:20) by flash column chromatography revealed the presence of methyl 9,12-heptadecadienoate (molecular ion peak 280 m/z). The results concluded the promising effect of purified fraction IV of *O. africana* isolated by using flash column chromatography for the control of mosquito larvae as they pronouncedly enhanced the mortality of mosquito larvae with the treatment.

**Keywords:** *Oreosyce africana*, Flash Column chromatography, TLC, HPLC, NMR, GC-MS, Larvicidal

**INTRODUCTION**

The approach to combat malaria largely relies on interruption of the disease transmission cycle by targeting the adult mosquitoes using indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs), and by killing mosquito larvae through treatment of stagnant waters with larvicides, as well as through environmental modification to prevent mosquito breeding. However, malaria control is a big challenge due to various factors. Of which the complexity of disease control process, and the complexity of the vectors and expensiveness of the control program. For example, *Anopheles arabiensis* is strongly resistant to DDT and pyrethroids (Abose *et al.*, 1998; Yewhalaw *et al.*, 2010).

Vector control measures targeting the larval and adult stages have been established to control the transmission of malaria. Among these, larviciding in mosquito control involves the killing of immature stages of mosquitoes by application of various methods including water management, using insecticides and biological control agents to the breeding sites (WHO, 2006). These control methods are used to stop mosquito larvae from maturing into biting adults that can transmit the disease. According to World Health Organization report more than 80% of the population in developing countries relies on traditional medicine, and it is now widely accepted that traditional medicines are more affordable, less toxic, and have a wide acceptance around the world (WHO, 2002).

Although the application of plant derivatives as larvicides and adulticides to malaria vector control is not common, some reports show that many plant species are traditionally used as repellents and insecticides (Abebe *et al.*, 2003; Berhanu *et al.*, 2006). It is plausible to assume that it is the phytochemicals contained in such plants that act as insecticides against both the larvae and adult stages of mosquitoes. Plant species including *Olea europaea* ssp. *cuspidata*, *Ostostegia integrifolia*, *Azadirachta indica*, *Silene macrosalen* and *Echinops* sp. were shown to be the most commonly used traditional mosquito repellent plants in Addis Zemen Town, South Gonder, Ethiopia (Karunamoorthi *et al.*,...
2009). In a study by Bekele et al. (2012) showed the people in Akaki District (east-central Ethiopia) used *O. africana*, *B. nigra* and *Aloe* spp. in traditionally for mosquito management and for the control of cattle ticks and other arthropod pests. Mullai et al. (2008) showed the benzene crude extract of *Citrullus vulgaris* leaves (Cucurbitaceae) to have an LC$_{50}$ value of 18.56 ppm and LC$_{90}$ value of 39.08 ppm against *An. stephensi* larvae.

*Oreosyce africana* Hook.f (Cucurbitaceae) is a slender climber or trailer growing to 3 m and its habitat is in wet or moist *Pouteria (=Aningeria) adolji-friederici-Syzygium guineense* forest margins, grassland and in plantations at an altitude between 1650-2000 m asl (Jeffrey, 1995). The leaf part of *O. africana* is used as an anthelmintic for intestinal worms and for healing burned skin (Yamada, 1999). The water homogenate obtained from *O. africana* was also reported to be given through hypodermal injection to treat gonorrhea (Yineger and Yewhalaw, 2007). The present paper deals with the purification of *O. africana* extract and reveals its bioactive properties against the malaria vector, *An. arabiensis*.

**MATERIALS AND METHODS**

**Collection of Plant Materials**

Plant sample of *Oreosyce africana* Hook.f was collected from Yerei Lencho kebele, Akaki District in November 2005. The plant sample was identified by taxonomist and a voucher specimen was given (voucher specimen no; DB.18) which was kept at National herbarium of Addis Ababa University for further references.

**Preparation and application of test extracts**

The concentrated extracts of *O. africana* fractionated with flash column chromatography fractions were first dissolved in 0.02% Tween-80 (Sigma, USA), which was used as an emulsifier (surface-active material) to ensure complete solubility of the extract and then diluted with deionized water. Stock solutions of the extracts were prepared and then the test concentrations ranging from 4 to 16 ppm of fractions (I-V) of *O. africana* were serially diluted with deionized water and applied to test cups. The bioassay tests for the larvicidal effects of *O. africana* fractions against *An. arabiensis* fourth instars larvae were carried out in accordance with the WHO standard procedure (WHO, 2005). The mixtures was gently stirred to ensure a homogeneous test solution and kept at ambient temperature. Fourth instars larvae of *An. arabiensis* were transferred by means of dropper to separate test cups (200 ml) with respective concentrations of the test solution. Determination of the desired concentration was based on the formula $C_1V_1 = C_2V_2$ (Kudom et al., 2011).
Test Mosquitoes and Experimental Design
Larvicidal activity of purified fractions of *O. africana* was carried out on *An. arabiensis* using cup bioassay. Three replicates per concentration per test were used to determine the bioactivity of the extracts on the larvae. Tests were carried out simultaneously using four batches of 20 larvae from which one batch was used for control with a total of 80 larvae for each extract concentration. The set up of the test was assigned in a completely randomized design (Gomez and Gomez, 1984). In the test controls the larvae were exposed to 0.02% Tween-80 in deionized water. The larvae in each treatment solution were held for 24 hr, after which they were transferred into 0.02% Tween-80 in deionized water for another 24 hr, as a test for recovery (Edriss et al., 2013; WHO, 2013). Larvae were counted as dead when they were not coming to the surface for respiration and were probe-insensitive (Sivagnaname and Kalyanasundaram, 2004). To prevent decomposition, which may cause rapid death of the remaining larvae, dead larvae were removed as soon as possible when they failed to move after probing with a needle in the siphon or cervical region. The bioassay tests were performed under 25-27°C and 70-80% relative humidity.

Purification of Dichloromethane Fraction of *Oreosyce africana* using Flash Column Chromatography
To concentrated 80% aqueous methanol extract of *O. africana* was added dichloromethane and deionized water and placed on a flash column chromatography (a column of silica gel, Merck with 25-100 mesh size) and a glass column (5 x 40 cm). The components were successively eluted with n-hexane-chloroform in a step-wise gradient (100:0, 95:5, 90:10, 80:20, and 70:30, v/v), fractionated (20 ml/tube), and examined by TLC. Fractions were pooled together according to their TLC profile and stored at –20°C until tested for larvicidal activity. The active components were monitored by an assay of the larvicidal effects on *An. arabiensis*, and the eluate from hexane-chloroform (80:20) showed activity. After separating the eluate by silica gel flash column chromatography with the hexane-chloroform (80:20) solvent, active fraction IV was obtained.

Thin layer chromatography
Isolation of the secondary metabolite from *O. africana* was monitored during the separation stages by thin layer chromatography on Kiesel gel 60 F_{254} plates (Merck) which has a 0.5 mm thickness with an eluent of methanol-chloroform (1:9, v/v). The components of TLC plate were visualized by spraying with 3% sulfuric vanillin in ethanol and then heating the plates at 110°C, which was developed twice.
High Performance Liquid Chromatography Analysis
The active fractions of *O. africana* were further purified by high performance liquid chromatography (HPLC) using Waters LC-2000 model equipped with Waters 600 pump controller (Milford, MA, USA). 10 μl of each sample fraction - fraction IV of *O. africana* was injected manually into a column of Tracer Extrasil ODS2, TR416059 (particle diameter, 5 μm; pore size, 30 nm; column size, 25 x 0.46 cm (Teknokroma, Barcelona, Spain) and eluted by use of solvent mixture of methanol and 0.1 % trifluoroacetic acid (20:80, v/v) with running time of 0-30 min. Column temperature of 20°C and flow rate of 1.0 ml/min were maintained and the eluate detected at 271 and 254 nm using Waters 2487 dual absorbance UV detector operated by Millennium 32 software from WATERS (Milford, MA, USA).

UV-Visible Spectroscopy Analysis
The UV-Visible spectra were recorded on UV-1800, ENG 240 V. SOFT, and SHIMADZU (Japan) spectrophotometer using a 10 mm light path cuvette in a Beckman Du 64 spectrophotometer (Waldwick, NJ) at Ethiopian Public Health Institute.

Characterization of Pure Constituents
The identity of fraction IV from *O. africana* was analyzed using nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS).

Nuclear Magnetic Resonance Spectroscopy
$^1$H and $^{13}$C-Nuclear Magnetic Resonance (NMR) spectra were recorded using Brucker Avance 400 MHz NMR spectrometer at the Department of Chemistry, Addis Ababa University. The NMR analysis for the isolated fraction of *O. africana* was made following the method described by Silverstein *et al.* (1991). The spectra were recorded at room temperature in deuterated chloroform (CDCl$_3$). The chemical shifts (δ) are reported in parts per million (ppm). For the $^{13}$C-NMR spectra, multiplicities were determined by Distortionless Enhancement by Polarization Transfer (DEPT) method. Multiplicities of $^1$H-NMR signals were indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet) and m (multiplate). The chemical structures were proposed based on the interpretation of the combined spectra.

Gas Chromatography-Mass Spectrometry (GC-MS)
Identification of the compounds was carried out by GC-MS analysis at Governors state University, USA. Gas chromatography-mass spectrometry spectra were recorded with
Agilent Technologies 7890B GC system, which was combined with Agilent Technologies 6977A MS system.

**Data Analysis**

The 95% confidence intervals of upper confidence limit (UCL) and lower confidence limit (LCL) for the lethal concentration in ppm (LC$_{50}$ and LC$_{90}$), chi-square ($\chi^2$) values, the slope for larvicidal effects were used to measure differences between test samples using the statistical package PoloPlus (version 2.0, LeOra Software, Petaluma, California; 2007). PoloPlus was also used to fit the data in probit and logit regression analysis and to estimate natural response. Statistically significant differences between the compared LC$_{50}$ concentrations, the 95% confidence intervals for lethal concentration ratios were calculated. In this pairwise comparison, lethal concentrations were considered significantly different if the value ‘1’ did not fall within the confidence interval for the ratio (Robertson *et al*., 2007). Standardized residual plots were generated to show how results fit the log-probit model using PoloPlus (LeOra Software, 2007). Standardized residuals were calculated by taking the difference of the observed value and the expected value and dividing the result by their standard errors. These results were plotted against the lethal concentration estimate for the expected values. Goodness of fit was considered as residuals scattered randomly within a horizontal band around zero and mostly between -2 and 2.

**RESULTS**

**Purification of Dichloromethane Fraction of *Oreosyce africana***

Thin layer chromatographic analysis confirmed that the major components were in fractions No. 11-14 (Figure 1). This fraction was also found to have pronounceable larvicidal effects on *An. Arabiensis*.

![Figure 1: Thin-layer chromatograms showing the products eluted from *O. africana* leaf fractions collected from flash column chromatography. 5 μl of each fraction were spotted on TLC plate from left to right: O$_{1-8}$ was labeled as plate A, O$_{8-14}$ was labelled as plate B, O$_{15-20}$ was labeled as plate C developed with methanol-chloroform (1:9) solvent.](image)

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The yields of the five fractions (Fr I to Fr V) obtained from a flash column chromatographic fractionation of the dichloromethane soluble portion of the extract of *O. africana* is shown in Table 1. The active fraction IV (Fr IV) was used in the following tests and its identity was characterized employing NMR and GC-MS.

**Table 1:** Flash column chromatographic fractionation of the dichloromethane extract of *Oreosyce africana*.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Mobile phase</th>
<th>Fractions combined</th>
<th>Yield (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr I</td>
<td>Hexane (100:0)</td>
<td>O₁-₅</td>
<td>17</td>
</tr>
<tr>
<td>Fr II</td>
<td>Hexane-chloroform (95:5)</td>
<td>O₆&amp;₇</td>
<td>23</td>
</tr>
<tr>
<td>Fr III</td>
<td>Hexane-chloroform (90:10)</td>
<td>O₈-₁₀</td>
<td>27</td>
</tr>
<tr>
<td>Fr IV</td>
<td>Hexane-chloroform (80:20)</td>
<td>O₁₁-₁₄</td>
<td>30</td>
</tr>
<tr>
<td>Fr V</td>
<td>Hexane-chloroform (70:30)</td>
<td>O₁₅-₂₀</td>
<td>21</td>
</tr>
</tbody>
</table>

Dichloromethane fraction of *O. africana* was eluted successively with hexane and chloroform represented by pooled fractions Fr I, Fr II, Fr III, Fr IV, and Fr V (Table 1) from a flash column chromatography. The eluate with hexane-chloroform (80:20) (Fr IV) had high yield (30 mg) followed by hexane-chloroform (90:10) (Fr III) with yield of 27 mg.

**Larvicidal activity of Isolated Fractions against *Anopheles arabiensis***

Results of testing different concentrations of *O. africana* isolated fractions against larvae of *An. Arabiensis* are tabulated in Table 2. The results show that as the concentration increase percent mortality increase.

**Table 2:** Larvicidal activity of different fractions of *O. africana* (Fraction I-V by using flash column chromatography (Flash CC) against *An. arbiensis* larvae 24 hr post-exposure in test cups (n = 60 in each test)

<table>
<thead>
<tr>
<th><em>O. africana</em> Fractions*</th>
<th>LC₅₀ ppm (95% CL)</th>
<th>LC₉₀ ppm (95% CL)</th>
<th>Slope±SE</th>
<th>χ² (P&lt;sup&gt;c&lt;/sup&gt;)</th>
<th>LC₅₀ ratio (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr I</td>
<td>8.890 (7.063-12.073)</td>
<td>83.975 (44.390-266.239)</td>
<td>1.314±0.200</td>
<td>0.552(0.48)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Fr II</td>
<td>6.906 (6.047-7.950)</td>
<td>23.731 (18.580-33.420)</td>
<td>2.391±0.229</td>
<td>1.666(0.56)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.287(0.961-1.725)</td>
</tr>
<tr>
<td>Fr III</td>
<td>7.388 (6.329-8.776)</td>
<td>32.366 (23.443-52.165)</td>
<td>1.997±0.215</td>
<td>1.687(0.53)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.203(0.887-1.633)</td>
</tr>
<tr>
<td>Fr IV</td>
<td>2.507 (1.734-2.632)</td>
<td>7.721 (6.470-10.173)</td>
<td>2.334±0.273</td>
<td>0.571(0.53)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.030(2.898-5.603)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fr V</td>
<td>13.136 (10.175-19.369)</td>
<td>107.348 (55.200-354.087)</td>
<td>1.405±0.210</td>
<td>1.465(0.56)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.677(0.453-1.011)</td>
</tr>
<tr>
<td>Negative control**</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The codes used for the fractions; ** Tween-80(0.02%) in deionized water. <sup>a</sup> Good fit of the data to the probit model (P>0.05). <sup>b</sup> LC₅₀ ratio significant at P<0.05; 95% confidence interval did not comprise the value 1.0. <sup>c</sup> Probability of good fit of the probit model.

The eluate with hexane-chloroform (80:20) had detectable larvicidal activity.
Plot of residuals against predicted values were between -2 and 2, which indicated its fitted plot (Figure 2).

Figure 2: PoloPlus plot showing standardized residual of fraction IV of Oreosyce africana at different concentrations on Anopheles arabinesis larvae (n = 60 in each test).

Chemical Analysis of Oreosyce africana Fraction
To purify the active fraction, fraction IV of O. africana was injected into analytical HPLC. The HPLC profile of fraction IV of O. africana is shown in Figure 3 and 4.

Figure 3: HPLC separation of fraction IV of O. africana in Octadecylsilane column eluted with water-methanol (20:80) at a flow rate of 1 ml/min for 35 min at 271 nm.
HPLC analysis of fraction IV of *O. africana* showed that the retention time of the first peak is 6.329 minute with an area percent of 55, while the retention time of the second peak is 9.921 minute with area % of 45 at absorption of 271 nm.

![HPLC separation of fraction IV of *O. africana* in Octadecylsilane column eluted with water-methanol (20:80) at a flow rate of 1 ml/min for 35 min at 254 nm.](image)

Quantitation of the fraction is based on the area percentage of absorption at 254 nm. The area percentages of the first and second peak, respectively, were 68.51 and 31.46 at retention time of 3.57 min and 5.21 min (Figure 4).

**UV-Vis Spectral Characteristics**

The UV-Vis spectrum of fraction IV of *O. africana* exhibited maximum absorption at 267 nm indicating the presence of chromophore.

Identification of fraction IV of Oreosyce africana

The structure elucidation of fraction IV of the dichloromethane extract of the leaves of *O. africana* was achieved using UV-Vis, NMR and GC-MS. The UV-Vis spectrum revealed an absorption band at 267 nm which is characteristics of fatty acids containing two double bonds. The GC-MS spectral analysis showed peak at m/z 280 establishing the molecular formula as C\textsubscript{18}H\textsubscript{32}O\textsubscript{2} (Figure 5). The $^{13}$C-NMR spectrum of fraction IV demonstrated carbon resonances of 18 carbon atoms which agreed well with the GC-MS analysis.
Oreosyce africana earance indicated that fraction IV is in agreement with linoleic acid whose structure is depicted in Figure 6. The proton NMR spectrum of fraction IV showed proton signals belonging to olefinic and alkanic protons. The signal at δ 5.4 (4H, multiplet) is ascribed to the presence of four olefinic protons in the structure of the compound. This was supported by the appearance of four methine carbon resonances at δ 130.2, 130.0, 128.0 and 127.9 in the carbon NMR spectrum of the compound. The presence of multiplet at δ 2.8 which integrates for two protons is due to protons alylic to two double bonds. The presence of signal adjacent to carbonyl carbon is ascertained by the presence of signal at δ 2.3 in its proton NMR spectrum. The presence many methylene proton signals is evident from the broad signal appearing at δ 1.3. To the end the triplet signal observed δ 0.89 justifies the presence of terminal methyl group.

The proton decoupled $^{13}$C-NMR spectrum in conjunction with DEPT-135 demonstrated the presence of four methane, one methyl and twelve methylene carbon atoms. The remaining one carbon atom is ascribed to quaternary carbon. The carbon signal at δ 31 is distinctive of methylene carbon adjacent to carboxyl group. This was supported by the appearance of triplet signal at δ 2.30 (2H) in the proton NMR spectrum of the compound. The most up field carbon signal at δ 14.0 in the $^{13}$C-NMR spectrum accounts for the presence of terminal methyl group. The remaining eleven methylene carbon signals were observed in the range between δ 30.0 to 22.6. The NMR spectral analysis in combination with the UV-Vis and GC-MS mentioned above indicated that fraction IV is in agreement with linoleic acid whose structure is depicted in Figure 6.

Counts (%) Vs. Mass-to-Charge (m/z)

**Figure 5:** The GC-MS profile for the fraction IV of *Oreosyce africana.*
**DISCUSSION**

The uses of plant materials for insecticides compared with many synthetic insecticides have a relatively low mammalian toxicity, a short period of activity, and a fairly broad spectrum of control (Ahmed *et al*., 1984). Bekele *et al* (2014) reported that the 80% methanol crude leaf extract of *O. africana* exhibited potent larvicidal activities against *An. arabiensis*.

On the TLC plate showed in plate B of Figure 1 of the active compound present was identified and carried out in order to isolate the active compound in large enough amounts for further confirmation and quantification of bioactivity, as well as structural elucidation. Flash column chromatography method is economically feasible and suitable for purification of bioactive plant materials.

The eluate with hexane-chloroform (80:20) had detectable larvicidal activity. Therefore, it is to be expected that since different phytochemical constituents dissolve in specific solvents (Sukumar *et al*., 1991). After 18th/19th hour of treatment most of the treated larvae were become immobile and unable to reach the water surface but ultimately die.

The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various economically important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control program (Alkofahi *et al*., 1989), and they could lead to development of new classes of possible safer insect control agents. In view of the potential of this compound as a promising anti-mosquito agent, it should further be refined and used for insecticide residual spray and insecticidal treated nets or even formulated as ointment repellents for effective malaria control.

For malaria vector control it is worthwhile to consider exploiting the plant material to incorporate it into integrated vector control. Therefore, the use of the products of *O.*

\[ \text{Figure 6: Chemical structure of linoleic acid} \]
*O. africana* an indigenous plant to Ethiopia for the larvicides must be seriously considered in malaria control and elimination effort currently in place.

Furthermore, the advantage of organic insecticides in reducing the problem of insecticide resistance among mosquitoes to synthetic insecticides, as demonstrated by the use of Azadiracthin (Shivakumar *et al.*, 2010), a plant product is good evidence to consider the methyl 9,12-heptadecadienoate containing products identified from *O. africana* in the present study.

According to Kihampa *et al.* (2010), the phenylpropenoids compounds of *Uvariodendron pycnophyllum* showed long term mortality effects to adults of *An. gambiae* on impregnated mosquito nets. In the use of mosquito nets for protection against malaria, physical barrier provided by the nets, usually needs to be supplemented by a chemical barrier consisting of a long-lasting deposit of insecticides on the netting. However, as the most commonly used insecticides are inorganic chemicals that cause concern as environmental hazards, the need for their replacement with organic insecticides, such as fraction IV isolated in the present study from *O. africana* must be encouraged.

The use of dichloromethane for isolating linoleic acid from the leaves of *Helichrysum pedunculatum* (Asteraceae) (Dilika *et al.*, 2000) for its antibacterial activity, is good evidence that bioactive substances with a broad activity can be extracted with dichloromethane. The larvicidal effect of dichloromethane fraction of *O. africana*, of which linoleic acid is the major component, is proof to its broad spectrum of bioactivity. Furthermore, the broad spectrum of bioactivity of linoleic acid is evident from its inhibition of parasitemia in mice infected with *Plasmodium vinckei* and *Plasmodium yoelii* in a 4-day suppressive test (Krugliak *et al.*, 1995). Ramos-López *et al.* (2012) had reported that linoleic acid isolated from *Ricinus communis* has insecticidal activity against *Spodoptera frugiperda* (Noctuidae). The extracts of *Annona squamosa* and *Annona muricata* (Annonaceae) contained linoleic acid against adults of *Aedes albopictus* and *Culex quinquefasciatus* had significant insecticidal effects compared to mortality induced by deltamethrin (Ravaomanarivo *et al.*, 2014).

Specific evidence for linoleic acid, the chemical compound identified as larvicide in this study, as a larvicide exists from *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) with an LC$_{50}$ value of 9.79 ppm and LC$_{90}$ value of 37.42 ppm against fourth instar larvae of *An. stephensii* Liston (Rahuman *et al.*, 2008). In addition, a study by Ramsewak *et al.* (2001) showed that linoleic acid isolated from *Dirca palustris* (Fabaceae), exhibited activity
against fourth instar larvae of *Ae. Aegypti* with LC$_{50}$ values of 100 μg/ml after 24 hr exposure. Edriss et al. (2013) and Gutierrez et al. (2014) also showed the presence of linoleic acid in the seed extracts of *Jatropha curcas*, a plant that has been shown to posses larvicidal effects against *An. arabiensis* (Tomass et al., 2011).

**CONCLUSIONS**

The results of this study indicated that the plant-based compounds such as methyl 9, 12-heptadecadienoate have a potential to be developed as an effective alternative to conventional synthetic insecticides for the control of *An. arabiensis*. Reports on high active compounds of plant origin used for insecticides are less. Further research undoubtedly will lead the improved formulations with enhanced activity, which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control.

**Acknowledgements**

Authors wish to acknowledge the Department of Molecular, Cellular and Molecular Biology and Thematic Research of Malaria and other Parasitic Diseases for their financial support. Our thanks forwarded to Prof. Ensermu Kelbessa, Department of plant biology and biodiversity management for identifying the investigated plant species and Department of Chemistry, Addis Ababa University, for NMR measurements. We are grateful to the staff of Ethiopian Public Health Institute: Dr. Asfaw Debeella, who allowed us to use various instruments in their laboratory, Mr. Yehualashet Belete for skilled technical assistance in processing plant materials and Mr. Fitsum Tesfaye for technical assistance in mosquito rearing and providing eggs of *Anopheles arabiensis*.

**REFERENCES**


Assessment of Pesticidal Activity of Marine Bacterial Extracts using Brine Shrimp (Artemia salina) Lethality Bioassay

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Abstract

Pests affect agriculture and transmit diseases to human and plants. The increase in number of resistant varieties of pests, ineffectiveness of chemical insecticides, necessitates the development of vector control strategies. Thus microbial insecticides can be considered as alternatives to chemical insecticides. In Present study marine sources such as Seaweed (Enteromorpha compressa) and jelly fish samples were collected and used for screening of bacteria. Secondary metabolites from the bacterial isolates were extracted and tested for its pesticidal activity against Brine shrimp (Artemia naupli). The secondary metabolites were evaluated for its toxicity and the percentage mortality against the larvae Artemia naupli was determined. The LC50 of the extracted secondary metabolites of the isolates were found to be very high in Micrococcus sp., (JF2) about 10µg/ml, medium activity in Steptomyces sp.,(Em5) about 100µg/ml and low activity expressed by Vibrio sp., (Em1 and JF1) about 1000µg/ml. Therefore, the secondary metabolites of Micrococcus sp., isolated from Jelly fish and Steptomyces sp., from Seaweed Enteromorpha commpressa can control the pests which transmitting diseases to human and plants.

Keywords: Pesticide, Marine bacteria, Artemia toxicity, Brine shrimp and insecticide.

INTRODUCTION

The Marine environment is a rich source of biologically active natural products mant of which have not been found in terrestrial sources. Natural products are secondary metabolites produced by microorganisms, plants and animals and the chemical novelty associated with such natural products are higher than that of any other sources. Natural products, the secondary or non primary metabolites produced by living organisms, have been exploited by people for a variety of purposes excluding use of food, fragarances,
pigments, insecticides and medicines. The marine environment is an exceptional reservoir of bioactive natural products (Ireland et al., 1988).

Marine microbes are producing high potential compounds than terrestrial microbes. The symbiotic bacteria can express various bioactivities such as Antimicrobial, Pharmaceutical, Cytotoxic and pesticidal activities.

Pests include Mosquitoes which are known to harbour plethora of viruses viz. West Nile virus, Saint Louis encephalitis virus, Eastern equine encephalomyelitis virus, Everglades virus, Highlands J virus, Dengue fever, Yellow fever, Ilheus virus, Rift Valley fever and Japanese Encephalitis. There are over 2500 different species of mosquitoes throughout the world. In India, three vector-borne diseases namely Malaria, Dengue, and Chikungunya are more prevalent and thus measures to control such mosquito vectors are habitat control, use of insecticides, introduction of sterile male mosquitoes, reduction of the breeding rates and larvicides (Bela et al., 2015).

Development of insecticidal resistance in mosquito populations, inhibition of non-target beneficial insects and contamination of food and drinking water sources leads to damage to the biodiversity and are major drawbacks of overuse of chemical insecticides. At the same time genetic alterations are also contributing to increase in the number of resistant variety of mosquitoes and reducing the effectiveness of insecticides (Chevillon et al., 1999).

Microbial insecticides on the other hand; due to their selective toxicity and ready decomposability in the ecosystem, are being considered as alternatives to chemical insecticides. Unlike the inherent dangers associated with the process of production of synthetic insecticides, the process for the manufacture of microbial products is safe, well-contained and less polluted (Syed and Leal, 2008). Microbial metabolites and antimicrobial substances exhibit specific insecticidal activity, bacteria pathogenic to insects such as Wolbachia are found to reduce the susceptibility of Aedes mosquito towards the Dengue virus (Frentiu et al., 2010). Similarly Bacillus thuringiensis, Serratia sp., through their metabolites inhibit Aedes, Anopheles and Culex mosquito larvae (Patil Chandrashekhar, 2012).

Artemia salina the brine shrimp is an invertebrate component of the fauna of salina aquatic and marine ecosystem. It plays an important role in the energy flow of the food chain (Lewan et al., 1992). And it can be used in a laboratory bioassay in order to determine the
toxicity by the estimation of the medium lethality concentration LC$_{50}$ (Meyer et al., 1982). Which have been reported for a series of toxins and plant extracts (Lagadic and Caquet, 1998).

This study to assess the pesticidal activity of marine bacterial metabolites using Brine shrimp lethality bioassay to prevent various diseases transmitted to human and plant by pests and mosquitos.

MATERIALS AND METHODS

Collection of Samples
Marine samples such as seaweeds and jelly fish were collected from Tuticorin, Mahapallipuram and Kovalam coastal waters and transferred to the laboratory aseptically for the isolation of bacteria.

Isolation and Screening of Microorganisms
For isolation of endosymbionts the samples seaweeds and jelly fish were homogenised with sterile seawater separately. The bacteria were isolated in 3 different types of medium using pour plate technique. Triplicates were maintained and incubated at 270 C for 7 days. The individual bacterial strain was isolated by repeated streaking (Vijayalakshmi et al., 2008). Thus, Colonies were obtained, and allowed for antimicrobial activity using cross streak method.

Out of 21 isolates only 11 strains were exhibited anti microbial activity against five human pathogens (E. coli, Streptococcus aurogens, Klebsiella pneumoniae, Enterobacter aerogens and Candida albicans). Active strains only preserved and were utilized to study their pesticidal activity.

Identification of Microorganisms
Bacterial isolates with pesticidal activity were identified on the basis of Bergey’s manual of determinative bacteriology 9th edition (Holt, 1994). Their Gram’s character, sugar fermentation tests and specific biochemical tests were carried out and identified upto Genus level.

Extraction of Secondary Metabolite from the Bacterial Isolates
The secondary metabolites were extracted from bacterial isolates and were inoculated in nutrient broth and incubated at their respective temperatures for 2 weeks (Ahmed, 2012). Broth was then filtered, centrifuged at 15000 rpm, for 20 min to obtain cell free supernatant. Equal volume of ethyl acetate was added to the cell free supernatant and
kept under shaker conditions for 1 hour. Secondary metabolite was extracted as middle layer after allowing the mixture to settle in separating funnel. The layer of secondary metabolite was collected in a petri dish and dried to evaporation. The dried secondary metabolite was dissolved in dimethyl sulfoxide (DMSO) and used for testing the *Artemia nauplii* larvicidal activity.

**LC$_{50}$ determination:**
The LC$_{50}$ of the secondary metabolite was determined by using the WHO guidelines 2005. The concentrations of secondary metabolites were prepared in the range of 10, 100 and 1000 µg/ml. Tests were carried out in 3 batches of 10 larvae in 1ml. Test was conducted in a closed vessel having air space of its 2/3rd volume. This vessel was monitored for next 24 hrs. The minimum concentration inhibiting 50% of the larva population was considered as LC50. The mortalities of treated groups were calculated according to the formula:

\[
\text{Inhibition \%} = \frac{\text{Total no. of shrimps died}}{\text{Total no. of shrimp inoculated}} \times 100
\]

**Statistical Analysis**
Ten larvae were introduced in each of the test solution as well as the control. The LC$_{50}$ value was calculated after 24 hrs by probit analysis (Finney *et al*., 1971).

**RESULTS AND DISCUSSION**
In past few years resistance to pesticides and chemical agents have been increasing rapidly. Hence there is a persistent demand of developing and searching of new pesticidal agents from natural environments. Extreme natural environments have been consistently generating microbial species which contributes to the control of diseases and their transmission. In the present study 21 samples were collected from two different marine sources such as Seaweed *Enteromorpha compresa* and Jelly fish. 11 isolates were expressed broad spectral activity against five human pathogens (*E. coli*, *Streptococcus aurogens*, *Klebsiella pneumoniae*, *Enterobacter aerogens* and *candida albicans*). These isolates were carefully screened for their potential toxicity against *Artemia nauplii*. Preliminary study revealed that the 11 bacterial isolates showed Pesticidal activity against Brine shrimp.

Secondary metabolites were extracted from the 11 effective isolates and the bioassay was conducted to estimate the sub lethal pesticidal concentrations of secondary metabolites. **Bioassay was carried out using secondary metabolite in the range of 10 µg/ml, 100 µg/ml**
and 1000 µg/ml. Out of 11 isolates one strain (Em2) showed 100 percent mortality at 10 µg/ml concentration.

**Tables 1-11:** Pesticidal activity of Marine bacterial extracts (Brine shrimp lethality bioassay)

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<th>Concentration of Extract mg/ml</th>
<th>No. of shrimps surviving after 24 hours in Em1</th>
<th>Total Number of Shrimps Alive</th>
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### Table: 6

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<th>No.of shrimps surviving after 24 hours in Em6</th>
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<th>% of Inhibition</th>
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<th>No.of shrimps surviving after 24 hours in Em7</th>
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### Table: 8

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<th>Total Number of Shrimps Alive</th>
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The LC$_{50}$ of the extracted secondary metabolites of the isolates were found to be very high in JF2 about 10µg/ml, medium activity in Em5 about 100µg/ml and low activity expressed by Em1 and JF1 about 1000µg/ml. The results of which are presented in Table 1-11, which represents the distribution of effective isolates collected from the marine sources as well as the identification of the isolate upto the Genus level using Bergey’s manual of determinative bacteriology (Table-12). It was observed that these 11 isolates were belonging to *Vibrio, Bacillus, Alteromonas, Micrococcus, Steptomyces, Pseudomonas* and *Alcaligenes*. Therefore, *Micrococcus sp.*, was predominant followed by *Steptomyces sp.*, and *Vibrio sp.* The secondary metabolites of *Micrococcus sp.*, (JF2) isolated from Jelly fish and *Steptomyces sp.*, (Em5) from Seaweed *Enteromorpha compressa* can act against various pests.

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**Table 12: Identification of Bacteria**

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<td>Micrococcus sp.,</td>
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The brine shrimp lethality bioassay represents a rapid, inexpensive and simple bioassay for testing Marine bacterial extract’s bioactivity which in most cases correlates reasonably well with pesticidal properties (McLaughlin et al., 1991). The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials (Harwig and Scott, 1971; McLaughlin et al., 1991 and Pelca et al., 2000).

The hatchability test detected toxicity in a number of species similar to the lethality test, but seemed less sensitive to detect toxicity of macro algae extracts. In general, the groups with the highest percentage of toxic species, and with the most toxic extracts, were the invertebrates. Some species such as echinoderms, the sponges *Mycale parishii*, *Dysidea sp.*, and the gorgonians *Muricea sp.*, etc., significantly lowered hatching in the hatchability test, interfering with normal development of the nauplii. The echinoderms *Toxopneustes roseus*, *Isostichopus fuscus*, etc. presented a high lethality (almost 100%). The high incidence of toxicity in Sponges and echinoderms seems to be an effective defense mechanism against many predation fishes, which increases closer to the tropics (almost 100% of all species tested) (Bakus and Green, 1974).

This bioassay show that standard test employing organisms such as brine shrimp are useful in identifying metabolites with a high potential for activity against marine organisms. In this study the result show that the shrimp lethality test could be an easy bioassay to screen marine natural products and it is used to detect the pesticidal activity of marine bacterial metabolites.
CONCLUSIONS AND RECOMMENDATION
The Brine shrimp lethality bioassay is rather inadequate regarding the elucidation of the mechanism of action. It is useful to assess the bioactivity of the marine bacterial extracts. Secondary metabolites from marine microorganisms have immense potential to counter the threat associated to human health and agriculture crop from pest vectors. The study provided secondary metabolites which are effective in control of agricultural pests and mosquitos which transmit diseases to human and plants. 100% mortality was obtained by the secondary metabolites isolated from Vibrio at 10 µg/ml concentrations. Thus the metabolites can be a potential substitute for the pesticides against which the resistance have already been developed. The pure compound may be active against yellow fever transmitting mosquito and we can prevent yellow fever in Ethiopia.

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Community’s Knowledge, Attitudes and Practices on Rabies in Gimbi District, Western Wollega, Ethiopia

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Abstract

Rabies is encephalitis, almost inevitably fatal zoonotic disease responsible for death of people mainly in developing countries. A cross-sectional study was carried out from March 2014 to October 2014 to assess the community’s knowledge, attitude and practice about rabies in Gimbi district West Wollega, Ethiopia. The data were collected from randomly selected 384 households through face to face interview using pretested and structured questionnaires. The participants for this study were all individual more than 18 years from four Kebele of the district. Of the 384 respondents interviewed 132 (34.4%) of males and 39 (10.2%) females were had good KAP level and 50 (13.0%) were above 45 years old people had good KAP level. Only 28.6% had mentioned on the relative cause of rabies and minority (22.7%) of the respondents wash wound with water and/or soap, as first aid if they are bitten by suspected rabid animals. All of the respondents indicated that they had previously heard about rabies. The finding show that overall KAP of the communities were 44.5%, 48.4%, 70.2% and 49.2% of the respondents had good KAP, good knowledge, positive attitude and acceptable practice scores, respectively with mean of 18.16 out of all items. There was statistical significance association between KAP scores and middle (31-45) years old (P=0.043; OR=1.65; 95%CI= 0.97-2.79). Generally these findings indicate that the Gimbi community has poor KAP about rabies transmission, disease outcome, and prevention and control in humans and animal populations. So a need for educational outreach in Gimbi district to raise accurate KAP on mode of transmission, appropriate prevention and treatment measures.

Keywords: Attitude, Community, Gimbi, Knowledge, Practice, Rabies

INTRODUCTION

Rabies is zoonotic diseases it affects all warm blooded animals including human beings and caused by rhabdovirus and transmitted to humans by animal bites. As a result, it is also one of global disease causing major public health and economic problems in the most part of the world (Rupprecht et al., 2002).
The number of human deaths due to the disease is estimated to be 40,000 to 70,000 people die every year around the world, while more than 10 million people are exposed to rabies annually. Yet, the disease does not attract as much attention as it should either from the government or from the public and remains a major socio-economic and public health problem in developing countries (Knobel et al., 2005 and WHO, 2008).

It is a persistent problem throughout the developing world where it spreads primarily by domestic dogs (Katie et al., 2007). It is reported that at least 87 countries are at risk of rabies covering a 2.4 billion populations, mainly in the developing countries (WHO, 2008). Canine rabies is geographically widespread and continues to represent a significant public health threat, particularly in developing countries (WHO, 1998) and dog-mediated rabies contributes to more than 99% of all human rabies cases (Thomas and Mettenleiter, 2011).

In Africa, where the incidence of as well as the range of species involved is increasing, it needs attention to develop public health awareness (Radostits et al., 2007). The disease is a prime example of a neglected tropical disease that mostly affects poor communities suffering from inequitable health care, children and elderly people (Agarvval and Reddaiah, 2003).

In Ethiopian context, it is an important disease that has been recognized for many centuries (Pankhrust, 1990) and domestic dog being the most important vector of human exposure (Wandeler et al., 1993). The incidence of human post exposure treatments and human rabies cases per million population of Ethiopia were 73.6 and 12.6, respectively (Bogel, et al., 1986).

The virus is transmitted by bites of infected animal and following bite, the uptake of virus by neuro-muscular spindles provides an important for virus entry into the nervous system. In all animals, rabies is characterized by typical signs of central nervous system disturbance. In endemic areas, suspect domestic carnivores or others should be isolated and observed for up to 14 days (Quinn et al., 2002).

Where the urban dog population is increasing at the same rate as the human population, the problem of dog bite from possibly rabid dogs was found to rise with alarming rate (Yimer et al., 2002). Consequently, the contributing factors include failure to seek treatment at healthcare facilities, failure to make a laboratory diagnosis and failure to report the status of the disease (Radostits et al., 2007).
However, the disease has the highest case fatality rate; it is preventable (Ertl, 2009). Some studies indicated as the control and prevention of rabies requires the engagement and collaboration of many stakeholders (Jonson et al., 2004). In many countries including Ethiopia many people receive anti rabies post-exposure treatments annually due to the widespread nature of dog rabies. There is, however, lack of sufficient information to public health and economic importance of rabies in developing countries. Poor public awareness towards rabies is considered as one of the bottle necks for the prevention and control of the disease in Ethiopia. Rabies was reported to be one of the public health concerns that need formulation of intervention strategy in Ethiopia.

Similarly, according to the information obtained from different source, there was no vaccination of dogs against rabies, while several cases of peoples bitten by dog were thought to visit the different health center of western Wollega (personnel communication). These all indicate the presence of significant public health risk of rabies in the areas. Information on knowledge, attitudes and practices (KAP) of the community a given community is crucial to plan and implement appropriate control measures. Though, rabies was reported to be common in Gimbi district, there is no study conducted on occurrence of the disease, associated risks and community’s knowledge, attitudes and practices towards the rabies in Gimbi district. Thus, the objective of this research was to assess the knowledge, attitudes and practices of the community in Gimbi district of West Wollega zone on public health risk and prevention strategies of rabies.

MATERIAL AND METHODS

Description of the Study Area
The study was conducted in purposively selected district Gimbi of west Wollega zone. Gimbi: was located between 90°10'- 9°17' North latitude and 35°44'- 36°09' East longitudes. The mean minimum and maximum annual temperature ranges between 10°C and 30°C. The mean annual rainfall is 1400-1800ml. It lies at altitudinal range of 1200m-2222m above sea level. As reported by Ghimbi District Finance and Economic Development office (2001), the district has high livestock potential with 107,334 cattle, 13,476 Ovine, 5124 Caprine, 5211 Equine, and Poultry 44144, 25600 Bee Colonies and unknown over populated of canine population (Ghimbi District Finance and Economic Development Office, 2001).

Study Design and Population
Cross-sectional study was conducted on randomly selected kebeles of Gimbi district, from March, 2014 to October, 2014.
Inclusion and Exclusion Criteria
Volunteer individuals with age of greater than 18 years of study subject’s householders were included in the assessment of KAP of the community.

Sample Size Determination and Sampling Procedure
Sample Size Calculation
Number of householders for assessment of knowledge, attitudes and practices (KAP) was calculated on the basis of the 50% expected prevalence by using a 95% confidence interval (CI) and 5% level of precision as follows: 

\[ N > \frac{(1.96^2 \times P_{exp} \times (1 - P_{exp}))}{d^2} \]

Where, 
- \( N \) = minimum sample size required
- \( P_{exp} \) = expected prevalence
- \( d \) = desired absolute precision (5%) 

(Threshold, 2005) Accordingly, a total of 384 householders were considered for data collection in the area.

Sampling Technique
Zone and district were purposively selected to conduct this study and eight (8) kebeles were randomly selected using lottery method from list of kebeles from the district, followed by selection of households from each kebele by using simple random sampling method.

Data Collection Procedure
To gather relevant information pertinent for the assessment of KAP of the study participants on rabies, face to face interview was made by using structured questionnaire specifically developed for this purpose. The head of the householders or the next responsible persons (in the absence of household head), was interviewed. Interview was made by the assigned data collector and supervisor in the respective Kebele after giving sufficient awareness for data collectors about the objectives of the study and use of the questionnaire for data collection. Demographic details (age, sex, residential place, educational status and religion) of the respondent were covered by the questionnaire. Each respondent was asked if she/he is keeping dog (s) and others questions which explore their knowledge concerning the disease, and its means of transmission to humans, their attitudes on the public health risk of the virus and its managements and their practices on activities used for prevention and control of the disease.

Operational Definition
Knowledge: Information concerning the respondent’s awareness on disease, virus and means of transmission was assessed by asking knowledge question. Appropriate proportions for correct and incorrect answers were determined.
**Attitude:** "a relatively enduring organization of beliefs, feelings, and behavioral tendencies towards socially significant objects, groups, events".

**Practice:** A numbers of questions were presented on practice of individuals on prevention and control measure of the disease and appropriate proportion for incorrect and correct practices were determined.

**Data Analysis**
The collected data was entered into excel Microsoft word and analyzed using SPSS version 20.0 statistical software package. Descriptive statistics was computed as appropriate. Outcome variables (awareness on rabies and associated factors, perceptions towards the public health risk and its prevention strategies and their practices on the major prevention and control activities of the disease and occurrence of rabies) were analyzed as appropriate. Socio-demographic characteristics of the study participants were major independent variables. The associations of the proportions on knowledge, attitudes and practices of the study participants were assessed based on logistic regression. For each respondents who score greater than or equal to the mean value (Mean ≥) grouped to good KAP and knowledge; positive attitude and acceptable practice while less than the mean value (<) Poor KAP and knowledge; negative attitude and unacceptable practice. Univariable and multivariable logistic regression models were fitted containing the appropriate independent variable (s) with 95% confidence interval and less than 0.05 level of precision.

**RESULTS**

**Socio-Demographic Characteristics of the Respondents**
All of the respondents (100%) were from the rural kebeles of the district.

**Table 1:** Address of study participants in Gimbi district, Western wollega, 2014/15

<table>
<thead>
<tr>
<th>Kebeles of respondents</th>
<th>Numbers of respondents</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bikiltu Tokuma</td>
<td>54</td>
<td>14.1</td>
</tr>
<tr>
<td>Chuta Gochi</td>
<td>40</td>
<td>10.4</td>
</tr>
<tr>
<td>Choli Mikael</td>
<td>35</td>
<td>9.1</td>
</tr>
<tr>
<td>Dalo Sewa</td>
<td>52</td>
<td>13.5</td>
</tr>
<tr>
<td>Waligala Dalo</td>
<td>59</td>
<td>15.4</td>
</tr>
<tr>
<td>Loya Gafare</td>
<td>51</td>
<td>13.3</td>
</tr>
<tr>
<td>Ware Sayo</td>
<td>37</td>
<td>9.6</td>
</tr>
<tr>
<td>Lalisa Eyasus</td>
<td>56</td>
<td>14.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>384</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
A total of 384 respondents were interviewed during the study period. The age of the respondents ranged from 18 to 91 years, 38.8% were between the age of 31-45 years and the majority of the respondents were male (74.2 %).

Table 2: Socio-demographic characteristics of study participants in Gimbi district, 2014

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number (%) of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups in years</td>
<td></td>
</tr>
<tr>
<td>18-30 (young)</td>
<td>143 (37.2)</td>
</tr>
<tr>
<td>31-45 (middle age)</td>
<td>149 (38.8)</td>
</tr>
<tr>
<td>greater than 45</td>
<td>92 (24.0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>285 (74.2)</td>
</tr>
<tr>
<td>Female</td>
<td>99 (25.8)</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
</tr>
<tr>
<td>Informal education</td>
<td>52 (13.5)</td>
</tr>
<tr>
<td>Primary school (1-8)</td>
<td>225 (58.6)</td>
</tr>
<tr>
<td>Secondary School (9-12)</td>
<td>80 (20.8)</td>
</tr>
<tr>
<td>College/University Graduate</td>
<td>27 (7.0)</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
</tr>
<tr>
<td>Orthodox</td>
<td>89 (23.2)</td>
</tr>
<tr>
<td>Protestants</td>
<td>269 (70.1)</td>
</tr>
<tr>
<td>Muslim</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Catholic</td>
<td>18 (4.7)</td>
</tr>
<tr>
<td>Total of each variables</td>
<td>384 (100)</td>
</tr>
</tbody>
</table>

Communities Knowledge, Attitude and Practice in Gimbi Districts West Wollega

Knowledge of the Respondents

From the total of 384 respondents, 100% had previously heard about rabies. Only 28.6% respondents were able to mention germ as causative agent of rabies. Majority 368(95.8%) of the respondents knew that dogs are the common source of rabies even though rabies could affect all animals in West Wollega (Table 3).
Table 3: Selected variables on knowledge of the respondents on rabies Gimbi district, 2014

<table>
<thead>
<tr>
<th>Selected Knowledge Question</th>
<th>Numbers</th>
<th>% of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you know the disease called rabies?</td>
<td>384</td>
<td>100</td>
</tr>
<tr>
<td>From where you heard of rabies?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>From friends /family</td>
<td>375</td>
<td>97.7</td>
</tr>
<tr>
<td>From mass media/others</td>
<td>9</td>
<td>2.3</td>
</tr>
<tr>
<td>Germ causes rabies?</td>
<td>109</td>
<td>28.4</td>
</tr>
<tr>
<td>Clinical signs of rabid animals in generally?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal behavior</td>
<td>221</td>
<td>57.6</td>
</tr>
<tr>
<td>Lack of fear, aggressive, attempt to biting nothing (air)</td>
<td>297</td>
<td>77.3</td>
</tr>
<tr>
<td>Hydrophobia</td>
<td>124</td>
<td>32.3</td>
</tr>
<tr>
<td>Excessive salivation</td>
<td>256</td>
<td>66.7</td>
</tr>
<tr>
<td>Others (tail dropping)</td>
<td>18</td>
<td>4.7</td>
</tr>
<tr>
<td>Clinical signs of rabid humans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>141</td>
<td>36.7</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>65</td>
<td>16.9</td>
</tr>
<tr>
<td>Hydrophobia</td>
<td>33</td>
<td>8.6</td>
</tr>
<tr>
<td>Photophobia</td>
<td>16</td>
<td>4.2</td>
</tr>
<tr>
<td>Altered mental status (nervous)</td>
<td>150</td>
<td>39.1</td>
</tr>
<tr>
<td>Others (sound of puppy heard from stomach)</td>
<td>81</td>
<td>21.1</td>
</tr>
<tr>
<td>Don't know</td>
<td>137</td>
<td>35.7</td>
</tr>
<tr>
<td>Humans and animals acquire rabies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Due to bite (lick) by rabid dogs</td>
<td>368</td>
<td>95.8</td>
</tr>
<tr>
<td>Due to bite (lick) by rabid cats</td>
<td>52</td>
<td>13.5</td>
</tr>
<tr>
<td>Due to bite (lick) by rabid farm animals</td>
<td>147</td>
<td>38.1</td>
</tr>
<tr>
<td>Due to bite (lick) by rabid wildlife</td>
<td>221</td>
<td>57.6</td>
</tr>
<tr>
<td>How could you prevent yourself from acquiring rabies?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoiding stray dogs</td>
<td>134</td>
<td>34.9</td>
</tr>
<tr>
<td>Avoiding being bitten by animals</td>
<td>345</td>
<td>89.8</td>
</tr>
<tr>
<td>Confining dogs</td>
<td>36</td>
<td>9.4</td>
</tr>
<tr>
<td>Vaccinating dogs and cats</td>
<td>35</td>
<td>9.1</td>
</tr>
<tr>
<td>Don’t know</td>
<td>8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Fifty-seven percent were able to describe rabies as a change of behavior in animals, and 66.7% were able to describe hydrophobia as clinical sign of rabies.

The mean knowledge score of the respondents was 9.53 with standard deviation 2.585 out of the 21 items scored. There was strong association between knowledge scores and middle (31-45) age (OR= 1.91, p = 0.016). The majority of respondents of more than 45years groups had moderate level of knowledge while the age category 18-30years had poor knowledge. The good scores were higher in males (38.0%) than females (10.4%).
From educational level (Primary school (1-8)) was significantly associated with knowledge scores (OR= 2.33, P= 0.05). That means primary school attendant respondent 2.3 more likely had good knowledge about rabies than college graduate.

**Table 4:** Knowledge scores about rabies and some key independent variables among study respondents of Gimbi district (N=384), 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Knowledge score</th>
<th></th>
<th>P-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor (%)</td>
<td>Good (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>139(36.2)</td>
<td>146(38.0)</td>
<td>0.06</td>
<td>1.55(0.97-2.46)</td>
</tr>
<tr>
<td>Female</td>
<td>59(15.4)</td>
<td>40(10.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30(young)</td>
<td>72(18.8)</td>
<td>71(18.5)</td>
<td>0.23</td>
<td>1.38(0.81-2.33)</td>
</tr>
<tr>
<td>31-45(middle age)</td>
<td>87(22.7)</td>
<td>62(16.1)</td>
<td>0.02</td>
<td>1.91(1.12-3.22)</td>
</tr>
<tr>
<td>Greater than 45</td>
<td>39(10.2)</td>
<td>53(13.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Education status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informal education</td>
<td>24(6.2)</td>
<td>28(7.3)</td>
<td>0.44</td>
<td>1.46(0.56-3.77)</td>
</tr>
<tr>
<td>Primary school(1-8)</td>
<td>130(33.9)</td>
<td>95(24.7)</td>
<td>0.05</td>
<td>2.33(1.02-5.30)</td>
</tr>
<tr>
<td>Secondary School(9-12)</td>
<td>34(8.9)</td>
<td>46(12.0)</td>
<td>0.62</td>
<td>1.26(0.51-3.08)</td>
</tr>
<tr>
<td>College/University Graduate</td>
<td>10(2.6)</td>
<td>17(4.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Respondents with secondary school (9-12) levels (12.0%) had higher percentages of good rabies knowledge compared with those who had informal education (7.3%) and high education (4.4%). Statistically no significant difference was observed among different dependent variable in the level of knowledge of respondents in table 4 above.

**Attitude of the Respondents**

All (99.5%) of the respondents believe that rabies is an important health risk to them. Almost all respondents (94.8%) agreed to immediately consult health professionals if they were bitten by dogs.

**Table 5:** Attitude of the respondents on rabies and associated risks in Gimbi district, West Wollega, 2014.

<table>
<thead>
<tr>
<th>Attitude Questions</th>
<th>Number of respondent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (99.5%)</td>
</tr>
<tr>
<td>Rabies is health risk to you?</td>
<td>382</td>
</tr>
<tr>
<td>Exposed individuals immediately seek medical evaluation</td>
<td>364 (94.8)</td>
</tr>
<tr>
<td>It impossible to live without keeping pets</td>
<td>201 (52.3)</td>
</tr>
<tr>
<td>Keeping free roaming dogs is no harm?</td>
<td>266(69.3)</td>
</tr>
<tr>
<td>Rabies preventable?</td>
<td>330 (85.9)</td>
</tr>
<tr>
<td>Traditional healers or herbal med. cure rabies?</td>
<td>366 (95.3)</td>
</tr>
</tbody>
</table>
The mean attitude score was 4.97 with standard deviation of 1.025 out of 6 items indicating that the respondents had moderately negative attitude towards the disease. Middle ages respondents were slightly more positive attitudes than those aged more than 45 years. This study found that no statistical association exists between attitude scores and independent variables (Table 6).

Table 6: Attitude scores about rabies and some key independent variables among study respondents of Gimbi district west Wollega, 2014

<table>
<thead>
<tr>
<th>Variables</th>
<th>Attitude score</th>
<th>P-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (%)</td>
<td>Positive (%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>79(20.6)</td>
<td>206(53.6)</td>
<td>0.290</td>
</tr>
<tr>
<td>Female</td>
<td>33(8.6)</td>
<td>66(17.2)</td>
<td></td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30(young)</td>
<td>39(10.2)</td>
<td>104(27.1)</td>
<td>0.381</td>
</tr>
<tr>
<td>31-45(middle age)</td>
<td>43(11.2)</td>
<td>106(27.6)</td>
<td>0.538</td>
</tr>
<tr>
<td>Greater than 45</td>
<td>30(7.8)</td>
<td>62(16.1)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informal education</td>
<td>18(4.7)</td>
<td>34(8.9)</td>
<td>0.655</td>
</tr>
<tr>
<td>Primary school(1-8)</td>
<td>64(16.7)</td>
<td>161(41.9)</td>
<td>0.898</td>
</tr>
<tr>
<td>Secondary School(9-12)</td>
<td>22(5.7)</td>
<td>58(15.1)</td>
<td>0.831</td>
</tr>
<tr>
<td>College/University Graduate</td>
<td>8(2.1)</td>
<td>19(4.9)</td>
<td></td>
</tr>
</tbody>
</table>

Practice of the Respondents Towards Rabies
The majority (75.7%) of the respondents do nothing on wound as first aid if bitten by suspected rabid animals while (24.3%) wash wound with water and/or soap. Majority of dog owners 172(45.1%) keep non-restricted dogs and cats while, 25(6.5%) secured their pets by tied in the compound (Table 7).

Table 7: Practice of the respondents on rabies and associated risks in Gimbi district, 2014

<table>
<thead>
<tr>
<th>Practices questions</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>What measure is given if one individual suffer dog bite?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Will be wash wound with soap and ethanol?</td>
<td>93</td>
<td>24.3</td>
</tr>
<tr>
<td>Do nothing on bitten wound?</td>
<td>291</td>
<td>75.7</td>
</tr>
<tr>
<td>Did you keep pets?</td>
<td>198</td>
<td>51.6</td>
</tr>
<tr>
<td>Did you vaccinate for your pets?</td>
<td>6</td>
<td>1.6</td>
</tr>
<tr>
<td>How do you keep your pets?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you keep in totally restriction/confined your dog (s)</td>
<td>25</td>
<td>6.5</td>
</tr>
<tr>
<td>Do you Keep your dogs in cohabiting with family or free roaming dog(s)?</td>
<td>172</td>
<td>45.1</td>
</tr>
</tbody>
</table>
The results show that about (49.2%) of the respondents had acceptable practice scores with mean of 4.02 out of 7 items, while 190(50.8%) were found to have unacceptable practice. College graduated almost 3times (P=0.03, B= 0.34, 95%CI on (0.13-0.88) more likely favor acceptable practice than informal educated individuals. However, having acceptable practice score increase with age to some extent from young (14.8%) to middle age (22.9%) but decreased from young to older age. There was no significantly association between Practice scores and different independent variables.

Table 8: Relationships between Practice scores about rabies and some key independent variables among study respondents of Gimbi district West Wollag, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Practice score</th>
<th>P-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unacceptable</td>
<td>Acceptable</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>135(35.2)</td>
<td>150(39.1)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>55(14.3)</td>
<td>44(11.5)</td>
</tr>
<tr>
<td>Age in years</td>
<td>18-30(young)</td>
<td>86(22.4)</td>
<td>57(14.8)</td>
</tr>
<tr>
<td></td>
<td>31-45(middle age)</td>
<td>61(15.9)</td>
<td>88(22.9)</td>
</tr>
<tr>
<td></td>
<td>Greater than 45</td>
<td>43(11.2)</td>
<td>49(12.8)</td>
</tr>
<tr>
<td>Education status</td>
<td>Informal education</td>
<td>19(4.9)</td>
<td>33(8.6)</td>
</tr>
<tr>
<td></td>
<td>Primary school(1-8)</td>
<td>110(28.6)</td>
<td>115(29.9)</td>
</tr>
<tr>
<td></td>
<td>Secondary School(9-12)</td>
<td>44(11.5)</td>
<td>36(9.4)</td>
</tr>
<tr>
<td></td>
<td>College/University Graduate</td>
<td>17(4.4)</td>
<td>10(2.6)</td>
</tr>
</tbody>
</table>

Over all of community KAP about Rabies in Gimbi district
The overall KAP results show that about 44.5%, 48.4%, 70.2% and 49.2% of the respondents had good KAP, good knowledge, positive attitude and acceptable practice scores, respectively with mean of 18.16 out of all items.

Table 9: KAP of participants related to summery on rabies in Gimbi areas west Wollega, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Numbers</th>
<th>% (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor knowledge (Less than mean)</td>
<td>198</td>
<td>51.6</td>
</tr>
<tr>
<td>Good knowledge (Greater than mean)</td>
<td>186</td>
<td>48.4</td>
</tr>
<tr>
<td>Attitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative attitude (Less than mean)</td>
<td>198</td>
<td>29.2</td>
</tr>
<tr>
<td>Positive Attitude (Greater than mean)</td>
<td>186</td>
<td>70.8</td>
</tr>
<tr>
<td>Practice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unacceptable Practice (Less than mean)</td>
<td>190</td>
<td>50.8</td>
</tr>
<tr>
<td>Acceptable Practice(Greater than mean)</td>
<td>194</td>
<td>49.2</td>
</tr>
<tr>
<td>KAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor KAP(Less than mean)</td>
<td>213</td>
<td>55.5</td>
</tr>
<tr>
<td>Good KAP(Greater than mean)</td>
<td>171</td>
<td>44.5</td>
</tr>
</tbody>
</table>
The data show that about 213 (55.5%) of the study participants were found to have poor KAP about rabies and 171 (44.5%) were found to have good KAP level. Respondents who score greater than or equal to the mean value (Mean=18.1615, SD=2.585) grouped to good KAP and less than the mean value Poor KAP level.

Table 10: Relationships between KAP scores about rabies and some key independent variables among study respondents of Gimbi district West Wollega, 2014.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Poor</th>
<th>Good</th>
<th>P-value</th>
<th>Odd ratio (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>153(39.8)</td>
<td>132(34.4)</td>
<td>0.23</td>
<td>0.33(0.83-2.11)</td>
</tr>
<tr>
<td>Female</td>
<td>60(15.6)</td>
<td>39(10.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30 (young)</td>
<td>83(21.6)</td>
<td>60(15.6)</td>
<td>0.064</td>
<td>1.65(0.97-2.79)</td>
</tr>
<tr>
<td>31-45 (middle age)</td>
<td>88(22.9)</td>
<td>61(15.9)</td>
<td>0.043</td>
<td>1.72(1.02-2.90)</td>
</tr>
<tr>
<td>Greater than 45</td>
<td>42(10.9)</td>
<td>50(13.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-educated</td>
<td>23(6.0)</td>
<td>29(7.6)</td>
<td>0.986</td>
<td>0.99(0.39-2.53)</td>
</tr>
<tr>
<td>[1-8] (Primary school)</td>
<td>135(35.2)</td>
<td>90(23.4)</td>
<td>0.126</td>
<td>1.88(0.84-4.19)</td>
</tr>
<tr>
<td>[9-12] (Secondary School)</td>
<td>43(11.2)</td>
<td>37(9.6)</td>
<td>0.404</td>
<td>1.45(0.60-3.49)</td>
</tr>
<tr>
<td>&gt;12 (College Graduate)</td>
<td>12(3.1)</td>
<td>15(3.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Rabies is well established in Ethiopia and it is still continue to be major public health problems. Almost 95.6% respondents had heard about rabies from different sources. About 97.7 respondents have heard about rabies from Friends /parents (table 3). It is agreement with Kabeta et al, (2015) who report majority (83.9%) of the victims had heard about rabies from their family/friends in Jimma town. Additional Sambo and his colleagues who reported the most common source of information on rabies in Tanzania was from personal contacts (neighbors, parents and friends) (sambo et al., 2012). On other hand, this finding was higher when compared with that reported lower proportion (68.7%) in a survey of knowledge, attitudes and practices about animal bite and rabies in general community in India and in Zimbabwe (Ichhupujani et al., 2006). This is mainly because of the fact associated with the source of information determining the appropriateness of the knowledge transferred. This is supported by the result obtained in this study that only 2.3% of respondents receive information about rabies from mass media (formal source) (Table 3). However, such information tended to be superficial and it did not adequately enable public to acquire appropriate level of knowledge on rabies. Asabe et al. (2012) also report that the mainly source of information about rabies, for children from talking with their friends i.e. information is passed best from friend to friend, orally.
The overall KAP analysis of rabies in Gimbi areas revealed that about 44.5%, 48.4%, 70.2% and 49.2% of the respondents had good KAP, good knowledge, positive attitude and acceptable practice scores respectively (Table 9). A study conducted in the city of New York, USA reported that 94.1% of the study participants know rabies as a killer disease (Eidson et al., 2004). The study carried out in Bahir Dar Town indicated that, about 64.1% of the study participants were found to have good KAP about rabies (Tadesse et al., 2014). As the study reported by Abraham et al., (2013) indicated, majority of the respondents (71.9%) know that rabies can affect all warm blooded animals and 73.5% of the respondents identified that dogs are major sources for the spread of rabies in human population. The score of level of knowledge in this study was lowest when compared with that of New York City and Bahir Dar Town due to the poor awareness creation conducted in the area.

Of total respondents, only 28.6% had understanding on the relative cause of rabies (Table 3). This result is agree when compared with the result obtained from study conducted in Gondar and Dabat, Ethiopia indicated that most of respondents believe that the disease in dogs is caused by starvation; thirst and prolonged exposure to sun heat (Wudu et al., 2013).

In this study the knowledge of the respondents was assessed, and therefore from the total interviewed peoples on the clinical signs of rabid animals, abnormal behavior, (lack of fear, aggressive, disoriented and attempt of biting nothing (air)), excessive salivation, tail dropping, and hydrophobia, were aware of common clinical signs of rabies in animals which were mentioned by (57.6%, 77.3%, 66.7%, 4.7%, and 32.3% respondents, respectively (Table 3)). This finding is supported by study done in Nigeria Asabe et al. (2012).

Association between independent variables and knowledge scores on rabies was calculated using logistic regression. The majority of respondents of all educational levels had moderate level of knowledge. However, educational level was significantly associated with knowledge scores on young ranged between 18-39 years (P<0.05). That means respondents with lower (informal) education (P=0.03; OR=0.34(0.13-0.88) less (0.34) likely had awareness of good rabies knowledge when compared with those who had complete higher education. The good scores were higher in males (38.0%) than females (10.4%) (Table 4) The finding is agreement with Abrium et al, (2013) who report the same ideas.
The majority of respondent of middle age group had poor knowledge levels scores while young and the age group greater than 45 years old communities had good knowledge levels. The good knowledge levels scores were observed in young (P=0.04; OR= 1.72 (1.01-2.92) than adult more than 45years old (Table 4).

Majority (95.3%) of the respondents had strong believed on traditional medicine for rabies prevention and treatment (Table 5). Majority of male (53.6%) had positive attitude than female (17.2%). All age group and educational level had positive attitude to words different attitude variables. However, this study found that no statistical association exists between attitude scores (Table 6). This finding agreement with the finding of Abraim et al, (2013) who report there is no statistical association exists between attitude scores. Additional agreement with Kabeta et al. (2015) who report that the communities had good attitude and WHO, (2013) also report that the communities have favorable attitude. In agreement with India’s surveyed Agarwal and Reddaiah, (2003), 49.6% participants had strong belief on traditional medicine.

On other hand, in contrast in Sri Lanka almost all respondents agreed to consult health professional in case of animal bite (Gino et al., 2009). This may be due to lack information and unavailability of health centers in immediate vicinity. Statistically no significant difference was observed between attitude scores and different attitude variables.

The current study found that, only 22.7% of the respondents wash wound with water and/or soap, while 75.8% do nothing on wound as first aid if they are bitten by suspected rabid animals (Table 7). It’s lower than the report of Tenzina and Bir (2013) in Bhutan. Additional, Tadesse et al (2014) report that, 70.8% of the respondents following dog bite know that wound washing is immediate action after dog. This difference might be due to respondents believed that the infection could be treated with herbs.

In this study about 51.6% of the respondents had pets (dogs and/or cats) of which only 1.6% report to give vaccination for their pets. From those who owned pets 45.1% of them keep non-restricted dogs and cats (Table 7). The majority of the respondents indicated depopulation of stray dogs is effective measure for controlling the disease in the study area (Table 3). This finding was consistence with results recorded in Sir Lanka in which the majority of the participants were in favor of rabies control programs that mainly focused on stray dog population control (Gino et al., 2009). The current finding also consistence with results recorded in Bahir Dar Tadesse et al. (2014) and Kabetta et al.
who report majority of the owner not vaccinate their pets. This could be due to study area and community awareness difference.

CONCLUSIONS AND RECOMMENDATIONS

Rabies is a highly fatally virus disease that responsible for many million people death in developing countries especially in Africa and Asia. The assessed communities' knowledge on rabies in Gimbi district was indicated that, there is low communities awareness on knowledge question such as on etiology (causes), clinical signs, transmissions method, and prevention and control measures on the disease. On other hand, the communities had positive attitude toward public health importance and preventable measures of the disease. However, on the practice of the communities there is unacceptable practice favorable in the areas even though all interviewed respondent knew the presence of the disease in the areas. Regarding dogs and rabies such as vaccination of dogs is not adequate. Furthermore, the mass media are not exploited for rabies education. Therefore the following recommendation is forwarded:

- Accurate rabies education with emphasis on transmission and potential exposure as well as first aid treatment should target communities.
- Awareness creation emphasis on all age group and all level of education they can take appropriate and prompt actions like immediate washing of bite wounds and visit nearby health, where dog bites have occurred.
- Enforcement of the laws regarding dog ownership is also vital to control rabies in Ethiopia.
- The government should be creating awareness on the impact of a disease through one health principle with cooperation of ministries of health, education and livestock to control rabies effectively.
- Awareness should be give for the local healers not to restrict the victim to herbal medicine

Acknowledgments

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REFERENCES


