



INTERNATIONAL INSTITUTE OF EDUCATIONAL RESEARCH & DEVELOPMENT

International Conference on Life Sciences  
(ICLS-20)

Mashhad, Iran

15<sup>th</sup>-16<sup>th</sup> September 2020

International Institute of Education, Research and  
Development

[www.iierd.org](http://www.iierd.org)

Publisher: IIERD Explore

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IIERD-Explore

## **Editorial:**

We cordially invite you to attend the International Conference on Life Sciences (ICLS-20), which will be held in Mashhad, Iran on September 15<sup>th</sup>-16<sup>th</sup>, 2020. The main objective of ICLS-20 is to provide a platform for researchers, students, academicians as well as industrial professionals from all over the world to present their research results and development activities in Micro Biology, Life Science, Bio Medical, Bio-Technology. This conference provides opportunities for the delegates to exchange new ideas and experience face to face, to establish business or research relations and to find global partners for future collaboration.

These proceedings collect the up-to-date, comprehensive and worldwide state-of-art knowledge on software engineering, computational sciences and computational science application. All accepted papers were subjected to strict peer-reviewing by 2-4 expert referees. The papers have been selected for these proceedings because of their quality and the relevance to the conference. We hope these proceedings will not only provide the readers a broad overview of the latest research results on Micro Biology, Life Science, Bio Medical, and Bio-Technology but also provide the readers a valuable summary and reference in these fields.

The conference is supported by many universities and research institutes. Many professors played an important role in the successful holding of the conference, so we would like to take this opportunity to express our sincere gratitude and highest respects to them. They have worked very hard in reviewing papers and making valuable suggestions for the authors to improve their work. We also would like to express our gratitude to the external reviewers, for providing extra help in the review process, and to the authors for contributing their research result to the conference.

Since July 2020, the Organizing Committees have received more than 40 manuscript papers, and the papers cover all the aspects in Micro Biology, Life Science, Bio Medical and Bio-Technology. Finally, after review, about 12 papers were included to the proceedings of ICLS-2020.

We would like to extend our appreciation to all participants in the conference for their great contribution to the success of International Conference 2020. We would like to thank the keynote and individual speakers and all participating authors for their hard work and time. We also sincerely appreciate the work by the technical program committee and all reviewers, whose contributions make this conference possible. We would like to extend our thanks to all the referees for their constructive comments on all papers; especially, we would like to thank to organizing committee for their hard work.

## **Acknowledgement**

IIERD is hosting the International Conference on Life Sciences this year in month of September. International Conference on Life Science will provide a forum for students, professional engineers, academician, and scientist engaged in research and development to convene and present their latest scholarly work and application in the industry. The primary goal of the conference is to promote research and developmental activities in Micro Biology, Life Science, Bio Medical and Bio-Technology and to promote scientific information interchange between researchers, developers, engineers, students, and practitioners working in and around the world. The aim of the Conference is to provide a platform to the researchers and practitioners from both academia as well as industry to meet the share cutting-edge development in the field.

I express my hearty gratitude to all my Colleagues, Staffs, Professors, Reviewers and Members of organizing committee for their hearty and dedicated support to make this conference successful. I am also thankful to all our delegates for their pain staking effort to travel such a long distance to attain this conference.



**Dr. Simpson Rodricks**

**President**

**International Institute of Education, Research and Development (IIERD)**

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# Is Plastics Bad?

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**Abstract:** -- We use plastics in a number of objects and it brings up the question, how much damage or cost does it create and where? This study maps harmful effects of plastic waste on environment, animals, humans and marine environment. It also lists few countries playing a major role to combat plastic waste pollution. The paper concludes with few suggestions on the ways to decrease its waste, junk or trash.

**Index Terms**— Plastics, cost, waste pollution

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## I. INTRODUCTION

The early use of Plastic dates back to 1600 B.C. with its exploitation that started in 1839 followed by its mass production in the year 1940s and is still continuing to expand. The plastics produced globally since the 1950s exploded from 2 million tons to 440 million tons in 2015. If production continues at a current rate, plastic trash could top 13 billion tons by 2050. Most of the things, we use today is made up of plastics. “Take out all the stuff in your house that’s made of plastic and probably your space will end up with virtually nothing”. The reason being, Plastics are cost efficient in terms of lightweight that matters in industries and makes it easier for issues like storing and shipping. Second, it is cheap to manufacture, inexpensive and economical (E.g. plastic spoons and forks). Third, in terms of hygiene quality, it helps prevent the spread of diseases caused due to improperly cleaned metal cutlery.

Today, we are dependent on plastic to a great extent as it makes our life so much easier. They are found in the form of different products that we use every day and are present in a range of applications close to a user like bed mattresses, fibres in most clothes, food package, PVC tubes in medical devices, children’s toys and much more. Unfortunately, many materials used in making plastics include chemicals that are actually very harmful. These days, most plastics are made up of fossil oil or gas and using more of it adds to global warming.

There are two distinct groups of plastics namely Thermoplastics and Thermosets:

- Thermoplastics can be heated and reformed repeatedly (E.g. Celluloid). Its property allows easy processing and recycling.
- Thermosets cannot be remelted and once formed, reheating causes material to decompose rather than melt (E.g. Bakelite, poly phenol formaldehyde).

## II. NEGATIVE IMPACTS OF PLASTIC

- Plastic causes dangerous risk on health, environment, animal and on the marine environment. Packing of hot food in plastic bags causes harmful chemicals to dissolve in the food which flows into the bloodstream on ingestion may induce several diseases including liver and lung damage. On disposing plastic with other waste, it pollutes land, air and water and takes ages to degrade naturally. The disposed plastic bags normally end up being swallowed by animals and block the wind pipe and digestive tract which in could lead to death in most cases. Plastics are also a hazard for marine life as they block the gills of fish and also are known to be causing harm to the coral reef by wrapping around them and blocking the flow of water and sunlight thus cutting out their source of nutrition.
- Disintegration of plastics leads to release of harmful chemicals that percolate into the soil and water thus making it toxic, this is consumed by humans and animals in various forms which lead to serious diseases and in many cases death. The production of plastics releases various toxins including various greenhouse gases which are effective in fast-forwarding climate change and polluting the air.
- As plastics are unable to pile in landfills they block storm drains, litter streets, stick to trees and further contaminates oceans where all marine animals eat or get tangled in them.
- The animals and sea creatures are hurt and killed every day due to the high amount of discarded plastic waste in the sea.
- A new investigation by Orb Media and researchers discovered plastic fibres are found in tap waters over the globe where 83% of samples were contaminated with plastic fibres. The micro

plastic contamination in water that we consume every day is now a huge problem.

- Studies show plastics and micro plastics are ingested by fish becoming part of a fish diet and unfortunately, we are eating plastic eating fish.

A study by researchers published in Science Journal quantified the amount of plastic entering oceans from coastlines of 192 countries. The findings show China ranks first followed by countries in Southeast Asia, Srilanka, Egypt, Nigeria, Bangladesh and South Africa. India ranks 12th in the list of top 20 countries discharging maximum junk of plastic waste into high seas. In addition, the study calculated 275 million metric tons of plastic waste generated by 192 countries in 2010, an average of 8.8 million plastic waste entering oceans and globally 620% increase in production of plastics since 1975.

As per Central Pollution Control Board, India generated 15,342 tonnes of plastic waste (the year 2016), of which 9,205 tonnes reported to be recycled and 6,137 tonnes left uncollected and littered. These plastic bags have become an indispensable part of Indian shopping and the waste elements generated by it stays for a very long time in the environment which is very harmful. It can remain underground for 500 years, contaminating the soil and polluting the environment. The table shows Plastic waste consumption (Tones) in India for the years 1996, 2000, 2001 and 2007 in an increasing trend

S. No.	Year	Consumption (Tones)
1.	1996	61,000
2.	2000	3,00,000
3.	2001	4,00,000
4.	2007	8,500,000

*Plastics Consumption In India*

*Source: Central Pollution Control Board, as cited in Plastic Consumption in India (Atulesh).*

### BAN ON PLASTIC BAGS

A number of countries around the world has taken move on different ways towards ban on plastic bags and few of which are listed below.

- In Kenya producing, selling or using plastic bag will cost four years jail or \$ 40,000 fine, one of the toughest plastic bag ban.
- Norway recycles 60% of plastic packaging.
- Costa Rica taking stand against plastic waste flooding and planning to ban all single use plastics.

- Seattle to ban plastic straws, utensils at restaurants from 1st July 2018.

- Today Rwanda ban on plastic bags in year 2008 is seen by most as a success.

- Initiatives taken by many states in India against the use of plastic. Few to be mentioned are Sikkim, Himachal Pradesh, Karnataka, Goa, Rajasthan and Mumbai.

### AN EXAMPLE OF PLASTIC WASTE USED IN CONSTRUCTING ROADS

The benefits of using plastic waste on roads are plenty. It makes roads stronger, gives better resistance towards rain water and water stagnation, helps in reduction of pores, no effect of UV radiation, load withstanding benefits, cost in road construction reduces with nil maintenance cost. Countries like United Kingdom, Netherlands, Ghana, US, Bhutan and India are paving way forward using plastic waste to construct roads as an alternative that are greener, stronger and requires less maintenance.

In India context, cities are playing a major role by using plastic waste in construction of roads. Few examples are as follows:

- Indore is recycling half of its plastic waste daily with 30% used for roads and more than 500 km roads are constructed.
- Chennai claims to construct 1,035.23 km length of roads using 1,600 tonnes of plastic.
- Pune has given a contract to Rudra Environmental Solution (India Ltd) for building 12 trial plastic roads across the city.

#### Suggestions

So, is it a problem if we burn or bury plastics? And the answer is definitely YES.

Burning plastics releases harmful chemicals that pollutes the air, damages our atmosphere and as we breathe in the polluted air it causes a range of health issues including cancer. By burying it, we won't see but ends up in number of problems. Example, the rubbish dumped in environment can lead to breeding ground for disease carrying pests like mosquitoes and rats. Hence recycling, reusing as something valuable, and reducing its use is a better solution.

Simple ways one can take in decreasing large junk of plastic waste we generate in 16 ways (Moss Laura), are as follows:

- Saying no to plastic straws at restaurants and bringing reusable stainless steel or glass drinking straw.
- Using reusable bags from plastic produce bags.
- Giving up gum made of plastic that may also be toxic plastic (polyvinyl acetate manufactured using vinyl acetate a chemical to cause tumour's in lab rats).
- Making use of boxes that is easily recycled and made into more products than plastic bottles.



- Buying food items from bulk bins that opt to fill a reusable bags or container and saves unnecessary packaging.
- Buying variety of prepared foods in glass jars and instead of throwing or recycling, better to reuse jar while buying bulk food.
- Using reusable cup to coffee shops instead of using plastic, paper or Styrofoam cups preventing lot of unnecessary waste (e.g. office).
- Making use of our own containers whether pickup or bringing home leftovers from restaurants.
- Opting for using matches over disposable plastic lighters for various purposes for starting fire.
- Skipping frozen foods, even those eco-friendly packaged items that are actually coated in thin layer of plastic. This helps in consumption of fewer processed foods and avoiding chemicals in plastic packaging.
- Saying goodbye to plastic ware (disposable chopsticks, knives, spoons, forks, sporks) and keeping a set of utensils that reduces carbon footprint.
- While buying things at farmers market, bring plastic containers, if need a refill, or ask the grocer to take back the containers and reuse.
- Use cloth diapers than disposable diapers that consume a huge amount of plastic and trees a year while manufacturing.
- Prepare own fresh squeezed juice or eat fresh fruits that is good for the body rather than plastic bottled juices.
- Replace use of multiple plastic bottles of (tile cleaner, toilet cleaner, window cleaner) with own cleaning products (baking soda and vinegar).
- Pack lunch in reusable containers or reusable snack bags instead of packing in disposable plastic containers and bags.

Urgent things we can do today calls for four R's of plastic use as follows:

- Refusing all sorts of plastic use
- Reducing the use of plastic bags each week
- Reusing the plastic bag for number of purposes at home or outside, and
- Recycling of plastics by taking our own used bags, returning unwanted plastic for recycling if shopping is delivered, checking food scraps before recycling to avoid contamination causing problems in production preventing recycled plastics from being used, approach local council to provide plastic bag recycling.

Although plastics are seen disposed everywhere, it is simply ignored. There is an urgent need to act now and take action towards saying no to plastic bags, cleaning the trash around, using eco-friendly products, reducing plastic waste, using reusable cloth bags, following healthy environmental habits, raising awareness on impacts of plastic pollution. Additionally, we need to encourage eco-friendly industries,

push government to charge fine on ones who uses plastics or manufactures it with a similar kind of law what Kenya and many others has adopted. In conclusion, plastic pollution will become a big problem for future generations and significant effort is needed to tackle the problem now, if not us then who? Therefore, big focus in the coming years is everyone should make a little effort in incorporating the above mentioned good ways that contributes in reducing plastic waste towards clean and healthy environment. Success in combating various problems of plastic pollution we face today is in our hands and the need of the hour is to make changes in our lifestyles.

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# Angling: An Emerging Fishery in Lake Naivasha?

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**Abstract**— Large carnivores are experiencing massive decline in their population and abundance due to loss of habitat and loss of prey species. The present study was carried out in Mundanthurai Plateau of Kalakad-Mundanthurai Tiger Reserve, Tamil Nadu, India. In Mundanthurai Plateau, Tiger *Panthera tigris* has been absent in past two decades which is linked with low density of large ungulate prey species such as gaur and sambar. In this study we examined the status of large carnivore and prey species after the removal of cattle grazing in the plateau. The study reveals that the overall density of ungulate prey species was  $12.4 \pm 1.5/\text{Km}^2$  and gaur density found to be  $3.37 \pm 1.40/\text{Km}^2$ . The available prey biomass of 3282.02 kg can support around 11 tigers/100 Km<sup>2</sup> and it may be lower due to biomass which is shared by other sympatric carnivores. The present estimated leopard density in the plateau is  $24.32 \pm 4.38$  using camera traps spatially explicit capture-recapture method. Overabundance of leopard may be due to the absence of tiger in the plateau and we have confirmed the presence of one male tiger in the plateau so far. The present study may provide baseline information on monitoring tigers and co-predators in the Mundanthurai Plateau of Kalakad Mundanthurai Tiger Reserve..

**Index Terms**— Prey species; Biomass; Western Ghats; Mundanthurai plateau

## I. INTRODUCTION

Lake Naivasha is an endorheic lake lying in the eastern part of Kenyan Rift Valley. The Lake is found south of the equator ( $0^{\circ}45'S$ ,  $36^{\circ}20'E$ ) at an altitude of about 1890 m above sea level and about 80 km north west of Nairobi, Kenya's capital city [1]. The lake is shallow with a mean depth of 3.4 m, deepening towards its south-western part to a maximum of 8 m in depth, though the deepest part of the lake is at 16 m off Crescent Island [1,2]. The areas surrounding the lake are semi-arid with the average annual rainfall of 1350 mm in the mountains to 600 mm on the shores of the lake. The rainy season is divided into the long rains from April to May and short rains from October to November [3]. The average temperature is  $25^{\circ}\text{C}$  with minimal annual variability and the lowest yearly temperatures are recorded in May-August [4]. The Lake covers a surface area varying between 120 km<sup>2</sup> and 150 km<sup>2</sup> depending on the dry and wet spells respectively [2].

Lake Naivasha is the only freshwater Lake along the chain of East African Rift Valley saline lakes. The freshness of the lake is attributed to inflow from rivers Malewa, Gilgil, and Karati and underground outflow through seepage [5,6]. The Lake Naivasha catchment is approximately 3400 km<sup>2</sup> and contains a large amount of small-scale agriculture. River Malewa with a catchment of approximately 1730 km<sup>2</sup> is the largest and provides about 80% of the Lake's inflow, while River Gilgil drains an area of about 500 km<sup>2</sup> and contributes about 20% of the lake's inflow (Figure 1) [7]. Threats of the lake revolve around unsustainable resource exploitation both within the lake and its catchments [8].

These include exotic species' introductions and accidental arrivals, pollution from agricultural activities, sewage waste, siltation, habitat degradation, illegal fishing, climatic change, fluctuations in Lake Level and water abstraction. The lake became a Ramsar site in April 1995 [9], but this does not seem to have slowed down pressures on the lake's ecosystem and the fishery [10]. This paper looks at angling as an emerging fishery in Lake Naivasha, its challenges and suggests management strategies that may enhance sustainable exploitation of these resources. The study used a combination of literature review, interviews with the locals and personal observations by the Kenya Marine and Fisheries Research Institute and Fisheries Department.

### Illegal Fishing on Lake Naivasha

Currently we have observed there are various techniques of illegal fishing being practiced on Lake Naivasha. For example fishers using passive gillnets as active gears (used as seines), seine nets and monofilament nets by both legal and illegal fishers. There is also use of

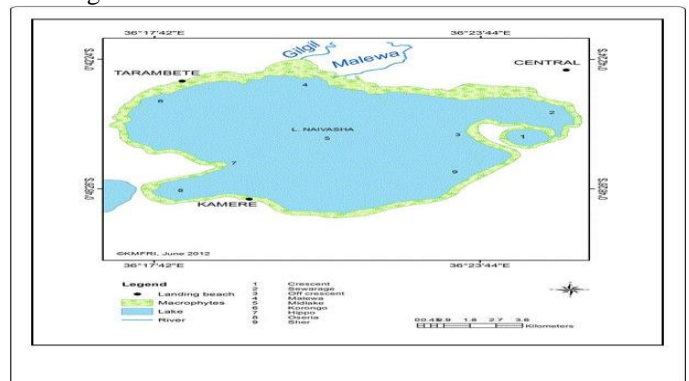


Figure 1: Map of Lake Naivasha

gillnets of 3.5'' and below to target the smaller sized *Oreochromis niloticus*, *Oreochromis leucosticus* and *Tilapia zillii*. This has rendered common carp as a by-catch of the tilapia fishery and capture of immature fish. Experimental gill netting studies shows that gears below 4'' mainly capture fish below size at first maturity. The fishers usually use illegal nets/mesh size to fish in shallow, protected areas which act as breeding and nursery grounds for most fishes. We also observed fishers targeting the bigger brooder specimen of the *Cyprinus carpio* using gillnets of 8'' to 10''. The use of undersize nets that catch juvenile fishes and large sized nets increases fishing effort than may be permitted and seining by illegal fishers has the potential to affect the performance and sustainability of the fishery (Figure 2) [11,12].



**Figure 2: Active fishing using illegal gears on fish breeding areas**

Illegal Angling has also been observed to be an emerging fishery in Lake Naivasha being practiced by both legal and illegal fishers. It is a selective fishing method in nature targeting a particular species and had been previously introduced in Lake Naivasha strictly for sport fishing with black bass or *Micropterus salmoides* being the target species. However with the dwindling catches of the common carp which since 2002 constituted up to 95% of the total annual fish landed from Lake Naivasha, the fishers have resulted to engaging in intensive fishing using the hook and line. The fishing lines are tied on to the papyrus vegetation along the lake shore or on the boats. (Figure 2). Illegal fishers practicing this fishery use ugali or boiled maize as bait for the carp species (Figures 3 and 4).



**Figure 3: Carp is the target species of angling**



**Figure 4: An illegal fisher displaying a fishing hook baited with ugali .**

The legal fishers set gillnets overnight and lift them at dusk and then proceed to sell them to the traders/middlemen at the fish landing beaches. By 8.00 am they return to the lake to start fish angling. It has been observed that fishers using this technique can harvest approximately 15 Kg or (20 pieces) of fish within three hours which is three times more than what they harvest from gillnetting (Figure 5). It was also observed that the fishers use hooks of different sizes ranging between 8 and 12 inches targeting mainly brooders



Angling in Lake Naivasha has attracted many people due to its high yields and low investment level; it's predominantly being carried out by male youths who are school dropouts, former workers of the collapsed floriculture and horticulture farms and individuals who are looking for a source of livelihood. Boat crew members have also abandoned their regular fishing activities to participate in this illegal activity due to its quick and high returns. This has led to huge losses for boat owners who now lack boat crews to harvest for them fish from the lake [13-15]. From an environmental perspective, illegal angling has totally changed the landscape of Lake Naivasha due to the large deposits of plastic waste that are strewn all over the riparian zones and particularly in areas with papyrus fringes and macrophyte population which act as refugee for fish and other water birds have been degraded and in some cases completely destroyed as a result of their fishing activity (Figure 6).



*Figure 6: Environmental degradation along the papyrus fringes.*

This therefore raises a big environmental concern on the impact of this fishery on the sustainability of the fish populations, the impact on the ecological integrity of the lake and the social economic impact. Further, the two fold increase of illegal fishers, fishing using hook and line pose a serious problem to the Lake's fishery negating the efforts by the State Department of Fisheries of controlling the fishing effort. This is mainly because illegal angling is mostly done in far off areas where enforcement of the fisheries regulations is difficult and without licenses.

## II. RECOMMENDATIONS

Illegal fishing is mostly undertaken in far off 'hidden' areas where enforcement of the fisheries regulations is almost impossible or difficult. Fisheries Managers need to devise an effective Monitoring Control and Surveillance Program to stem this menace which is increasing fishing pressure. The government through the ministry of fisheries, agriculture and livestock development should strive to sensitize and educate the fishing communities on the dangers associated with illegal fishing and in particular destruction of the fish refuge which are critical for feeding and breeding. They should also address issues concerning waste management and pollution of the ecosystem.

## III. CONCLUSION

Angling should be undertaken in an environment that aims to promote collection of good catch statistics and encourages clean and safe management practices to avoid polluting the lake with plastics. Increased and uncontrolled fishing pressure has been heavily blamed for the decline of Lake Naivasha fisheries. Such vital information as good fish catch statistics, biological parameters, and indices of the distribution and abundances of commercial fish species, which are needed for defining management policies, should be sought regularly.

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# Diversity, Distribution, Indigenous Uses and Conservation of Orchids in Parvati Valley of Kullu District, Himachal Pradesh, Northwestern Himalaya

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**Abstract**— The Indian Himalayan Region (IHR) is known as the repository of biodiversity. It supports 8,000 species of angiosperms. The orchidaceae is among the dominant family of angiospecies and highly evolved family among the monocotyledon. The species of this family are facing tremendous pressure due to habitat degradation, overexploitation and changing environmental conditions. Therefore, while exploring the floristic diversity of Parvati Valley, attempts have also been made to explore the orchid diversity and analyze for diversity, distribution patterns, nativity, endemism, threat categories and indigenous uses. Total 19 species of the orchids representing 13 genera were recorded between 1100-3600 m amsl. Of these, 13 species were natives, 6 species near endemic and 5 species non-natives. These species represented in grassland, shady moist, forests, alpine meadows, moist rocks and bouldary habitats. These species were used for curing various diseases/ailments by the inhabitants of the valley. Due to habitat degradation the populations of these species are decreasing fast. The over exploitation, habitat degradation and changing environmental conditions of these species has led rapid population depletion. Therefore, appropriate strategy has been suggested for the conservation and management of these orchids

**Index Terms**— Diversity; Distribution; Native; Threat categories; Indigenous uses; Orchids

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## I. INTRODUCTION

The Indian Himalayan Region (IHR) comprises of three bio-geographic zones and eight bio-geographic provinces and extends from Jammu & Kashmir in the North-West to the Arunachal Pradesh in the East [1]. Indian Himalayan Region one of the mega hot spot of biological diversity [2], is a source of a great diversity of food, fuel, fodder, timber, dye and medicinal plants. It comprises about 18% of India and is more than 2,800 km long and 220 to 300 km wide, with altitudes from 200-8000 m [3]. The vegetation comprises of tropical, sub-tropical, temperate, sub-alpine and alpine types [4].

The IHR supports about 8,000 flowering plants and family orchidaceae is one of the species rich families of angiosperms [5,6]. Orchids are worldwide famous for their charming and long lasting flowers. They form a unique group of plants and represent a peak in the evolution of monocots. They are terrestrial (including lithophytes, epiphytes and saprophytes) in nature. The diversity of orchids decreases from North East to North West Himalaya [6-8].

In general, a large number of studies have been carried out on the orchids of IHR [5,6,9-15]. In particular in Himachal Pradesh a very few studies are available on orchids [15-18]. However, studies at watershed/ valley level for the exploration of orchids have not been carried out, which is most important for the conservation and management of orchids. Therefore, the present attempt has been made to:

- (1) Assess and identify the orchids diversity,
- (2) Assess the status and distribution pattern of native and endemic orchids,
- (3) Assess the economically important orchid diversity,
- (4) Assess orchid diversity for threat categories, and
- (5) Suggest strategy plans for the conservation of orchid diversity.

## II. MATERIALS AND METHODS

### Study area

The Parvati Valley (31° 58' 41'' to 32° 05' 51''N Latitudes and 77° 14' 23'' E to 77° 27' 08'' E Longitudes), ranging

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from 1,100-4,800 m, is remarkably beautiful and just a little sinister (Figure 1). The valley is very narrow with the mountains rising steeply on both sides, allowing a couple less hours of light than in erstwhile areas. The Parvati River is a main drainage of the watershed and supported by its tributaries such as Malana nallah, Tosh nallah, Grahan nallah, etc. The unique topography, diverse habitats and climatic conditions support rich biodiversity. The village Malana nestled between Jari and Khiksa thatches is the oldest democracy in the world. The area receives heavy snowfall during winter and rainfall in rainy season. The soil comprises laterite, red, sandy, loamy and alluvial types and composition of the soil depends upon the underlying rocks and effect of various agencies from time to time. The vegetation mainly comprises of sub-tropical, temperate, sub-alpine and alpine types. The inhabitants are largely dependent on biodiversity for their sustenance. Due to over exploitation and habitat degradation the economically important biodiversity is under tremendous pressure.

### Surveys, sampling, identification and data analysis

The extensive field surveys were conducted to explore the orchid diversity of the Parvati Valley between 1,100-3,600 m during 2009-

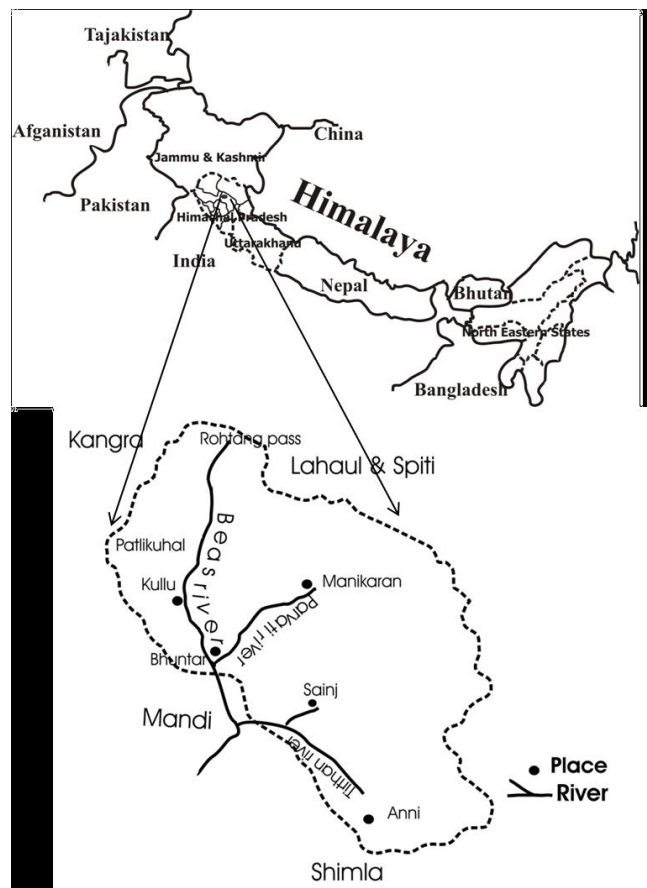


Figure 1: Location map of study area.

2013. The rapid sampling of species was done and the samples of each species were collected for proper identification. For each species, information on habit, habitat, altitudinal range, population size, indigenous uses, etc. was collected. The species were identified with the help of flora [8,14,19,20]. Species were analyzed for nativity, endemism and rarity. Nativity of the species was identified [4,5,21]. Endemism of the species was identified based on distribution range [4,22]. Species confined to the IHR were considered as endemic, and those with a distribution extending to neighboring countries i.e., Himalayan region of Afghanistan, Pakistan, Tibet, Nepal, Bhutan and adjacent states of the IHR were considered as near endemics. Threat categories were identified [23]. Information on the indigenous uses of the species is based on the available literature and interviews of the inhabitants [5,24].

### III. RESULTS

#### Diversity and distribution

A total of 19 species of the orchids representing 13 genera were recorded between 1100-3600 m amsl. Of these, 7 species of orchids were recorded from <1800 m altitudinal zone, followed by the 1801-2800 m zone (15 spp.) and >2800 m (16 spp.), respectively (Figure 2). The diversity of orchid increases with the increase in altitude and vice-versa. These species were found in grasslands, shady moist places, forests, alpine meadows, moist rocks and boulders.

#### Nativity and endemism

Thirteen species (i.e., *Calanthe tricarinata*, *Dactylorhiza hatagirea*, *Epipactis helleborine*, *Galeola lindleyana*, *Goodyera biflora*, *G. fusca*, *Gymnadenia orchidis*, *Habenaria edgeworthii*, *H. elisabethae*, *Herminium lanceum*, *H. monophyllum*, *Neottia listeroides* and *Satyrium nepalense*) were natives, 6 species (i.e., *Calanthe tricarinata*, *Epipactis helleborine*, *Galeola lindleyana*, *Goodyera biflora*, *Habenaria edgeworthii* and *Herminium lanceum*) near endemic and 5 species (i.e., *Cephalanthera longifolia*, *Epipactis gigantea*, *Herminium monorchis*, *Malaxis muscifera* and *Spiranthes sinensis*) non-natives (Appendix).

#### Threat categorization

Of the total species, 3 spp. have been identified as Critically Endangered, 1 species as endangered, 2 species as vulnerable and 9 species as near-threatened. According to the International Union for Conservation of Nature (IUCN), 2 species have been categorized as Critically Endangered globally, 4 species as rare and 1 species near-threatened (Figures 3-5; Appendix) [23].



### Indigenous Uses

Leaves (6 spp.), bulb (2 spp.), stems (1 sp.), tubers (12 spp.) and aerial parts (3 spp.) were used by the inhabitants for their therapeutic use (Figure 3). These species are used for curing various ailments such as sores, eczema, paralysis, healing wounds, bone fracture, cough, cold, cuts, sexual disability, rheumatism, fever, blood purification, cold, kidney disorder, female disorder, dysentery, sterility, leucorrhoea, diabetes, malaria, etc., and also used as aphrodisiac, antispasmodic, sedative, febrifuge, appetizer and tonic (Appendix). Due to habitat degradation the populations of these species are decreasing fast. *Dactylorhiza hatagirea* and *Malaxis muscifera* are commercially exploited by the inhabitants.

### IV. DISCUSSION AND CONCLUSION

The state Himachal Pradesh forms the parts of Trans and North Western Himalaya, supports relatively very less number of orchids compared to Western, Central and Eastern Himalaya [5,8]. Mostly terrestrial orchids are found in the state except *Gastrochilus calceolaris*, *Aerides multiflora* and *Rhynchostylis retusa*, which are epiphytic in nature [8,18]. The occurrence of representative, natural, unique and socio-economically important orchids in the area indicates high conservation and socio-economic values and merit priority attention for conservation of these species [5]. In Parvati Valley, the inhabitants are largely dependent on forests for grazing, fuel, fodder, timber, medicinal and wild edible plants, making agricultural tools, etc. Due to continuous use of economically important species, their populations are depleting rapidly and habitat degradation has increased many folds [24]. Besides these, orchids are extensively used in the traditional systems of medicines [18]. *Dactylorhiza hatagirea* and *Malaxis muscifera* are commercially exploited in the area. Due to high commercial values of *Dactylorhiza hatagirea* and *Malaxis muscifera* as medicine and food, these species are facing tremendous pressure and these species have been identified as Critically Endangered, globally [23-26]. If over exploitation and habitat degradation continues, these species may become extinct from the area. The other species are also exploited for curing various diseases and other purposes. Therefore, study on habitat ecology, mass multiplication using convention and in-vitro, propagation methods, establishment and maintenance in ex-situ and in-situ conditions, promotion of orchids, educational and awareness programmes on status, conservation and management of the orchids, promotion of orchid species with high aesthetic value in floriculture; and involvement of inhabitants in the conservation management have been suggested. So that the gene pool of this unique group of plants could be maintained posterity.

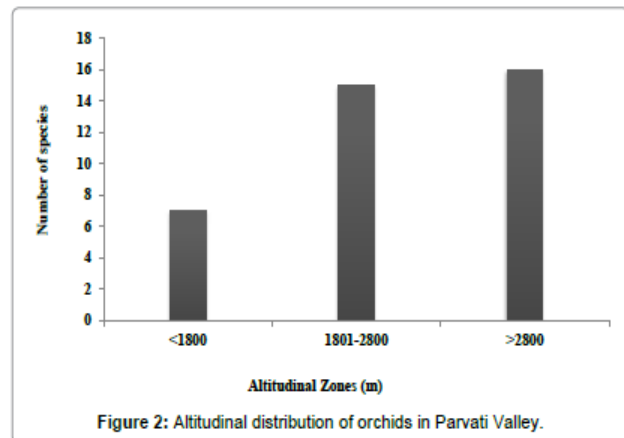


Figure 2: Altitudinal distribution of orchids in Parvati Valley.

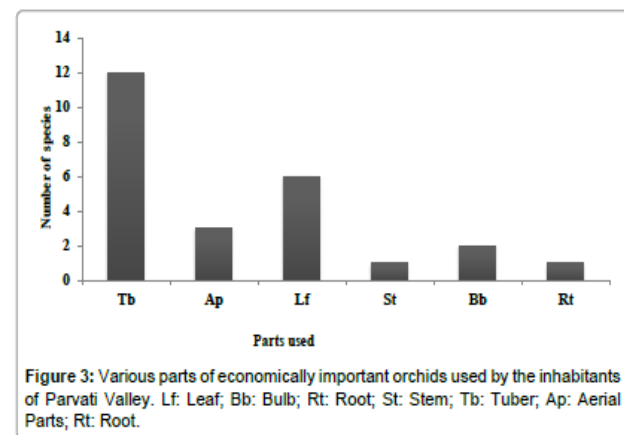


Figure 3: Various parts of economically important orchids used by the inhabitants of Parvati Valley. Lf: Leaf; Bb: Bulb; Rt: Root; St: Stem; Tb: Tuber; Ap: Aerial Parts; Rt: Root.

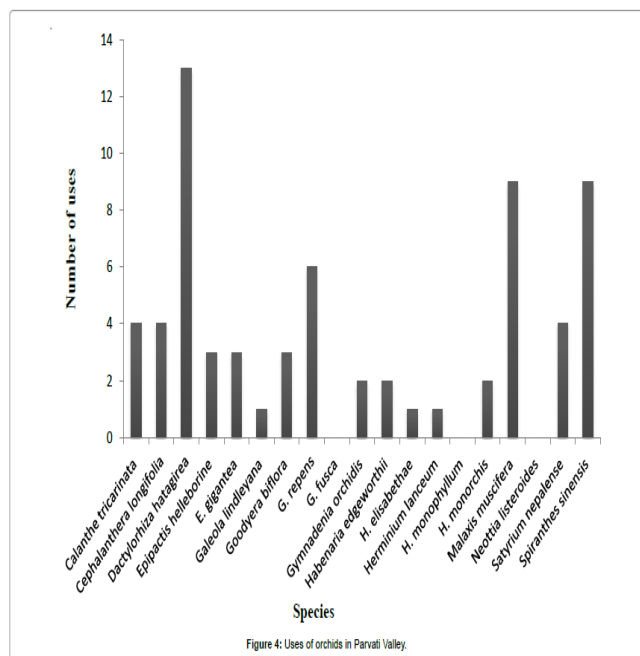
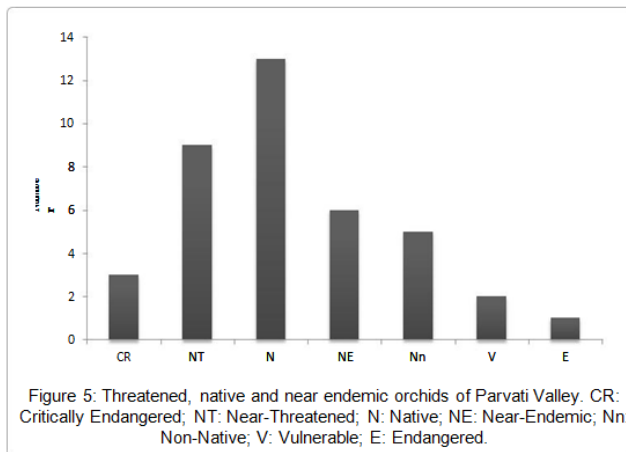


Figure 4: Uses of orchids in Parvati Valley.

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### V. ACKNOWLEDGEMENT

The authors are thankful to the Director, G.B. Pant National Institute of Himalayan Environment & Sustainable Development (GBPNIHESD), Kosi-Katarmal, Almora for facilities and encouragement. The inhabitants of the area are acknowledged for providing valuable information during the field surveys.

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# New Records of Bloodsucking Flies Associated with Wild Birds of Haftad-Gholleh Protected Area, Iran (Diptera: Hippoboscidae, Calliphoridae)

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**Abstract**— We have studied the parasitic flies of wildlife of Haftad-Gholleh Protected Area, Iran, for the first time and report here the three hematophagous fly species of birds: the louse fly *Ornithophila metallica* (Schiner) (Hippoboscidae), and bird nest flies *Trypocalliphora braueri* (Hendel) and *Protocalliphora azurea* (Fallen) (Calliphoridae). The genera and species *O. metallica* and *T. braueri* are new to Iran

**Keywords:** Avian myiasis; Louse flies; *Ornithophila metallica*; *Trypocalliphora braueri*; *Protocalliphora azurea*; Blow flies

## I. INTRODUCTION

Avian myiasis-causing flies and bird's blood-feeding ectoparasite flies mainly belong in the family's Calliphoridae and Hippoboscidae. The family Hippoboscidae, commonly known as louse flies, consists of 213 hematophagous species of birds and mammals worldwide [1]. This family is known in Iran only by the single species *Pseudolynchia canariensis* (Macquart), pigeon fly that has been repeatedly recorded from various cities across the country [2-5]. Aside from being a nuisance to their hosts, hippoboscids are capable transmitters of pathogenic and parasitic agents, including avian trypanosomes and mammals' bacteria, causing serious diseases in wild birds [6] and ruminant animals [7,8]. They are also the only known vectors of apicomplexan parasites of the genus *Haemoproteus* to birds and transmitters of filarial nematodes to domestic and wild mammals [9,10].

The majority of myiasis-inducing species belong to the family Calliphoridae, esp. subfamily Chrysomyinae, whose members are known as important facultative and obligatory parasites. Bird myiasis records are not as frequent as mammals' most likely due to the inaccessibility of the hosts. With respect to Iran, the reports of avian myiasis have been poorly documented [11,12], mainly because of difficulties in larval identification. Although the said technical problem often necessitates the rearing of the maggots for a reliable identification at adult stage, in a recent case of avian wound myiasis in southwestern Iran the myiatic agent was successfully identified at larval stage [13]. The genera *Protocalliphora* Hough and *Trypocalliphora* Peus contain

specialist bird nest parasites whose larvae feed on the blood of nestling birds through tunneling under their skin, causing a type of myiasis called subcutaneous myiasis, and eventually leading to heavy damages to the tissues or death of young birds [14]. The species *P. azurea* (Fallen) is widely spread in the Palaearctic region and remains the only species of birds' subcutaneous myiasis agents that has been recorded from Iran so far [15].

## II. MATERIAL AND METHODS

Haftad-Gholleh Protected Area covers an estimated area of 97,400 hectares (240,680 acres) and is home to a large number of vulnerable mammal and bird species (Figure 1). Using Malaise traps, the specimens were collected in 75% ethyl alcohol and preserved at the Hayk Mirzayans Insect Museum (HMIM), Tehran, Iran. In case of the examination of male genitalia, we detached the whole abdomen to clear it in hot 10% KOH and then washed it lightly in glacial acetic acid



**Figure 1:** A general view of Chekab valley, Haftad-Gholleh protected area, Iran.

to remove the base. After dissecting the male genitalia, the abdomen was glued back to its original place and the genitalia transferred to a microvial and pinned below the associate specimen. Specimen data: 1♂ 1♀ *Ornithophila metallica*; 2♂♂ 3♀♀ *Protocalliphora azurea*; 1♀ *Trypocalliphora braueri*; Iran: Markazi province, Amr-abad village, Haftad-Gholleh Protected Area, Chekab valley, 2219 m, 34°07'05.3"N 050°16'25.3"E, 28 May-15 June, 2016, Malaise trap near pool, E. Gilasian & M. Parchami-Araghi. Birds of haftad-gholleh protected area Haftad-Gholleh is home to an estimated 71 species within 26 families of wild birds and serves as a sanctuary for a number of migrating birds as well. We have listed the following common avian taxa of this area to underline the impact of hematophagous flies on the bird fauna: *Monticola solitarius* (L.) (blue rock thrush), *Accipiter* spp. (hawks), *Falco* spp. (falcons and kestrels), *Coturnix* spp. (quails), *Columba* spp. (pigeons), *Cuculus* spp. (cuckoos), *Coracias* spp. (rollers), *Merops* spp. (bee-eaters), *Upupa* spp. (hoopoes), *Galerida* spp. (larks), *Hirundo* spp. (passerines), *Muscicapa striata* (Pallas) (spotted flycatcher), *Emberiza melanocephala* Scopoli (black-headed bunting), *E. cia* (L.) (rock bunting), *E. citrinella* L. (yellowhammer), *Turdus* spp. (true thrushes), *Motacilla* spp. (wagtails), *Lanius* spp. (typical shrikes), *Parus* spp. (tits), *Passer* spp. (sparrows.), *Sturnus* spp. (starlings), *Corvus* spp. (crows), *Pica pica* (L.) (Eurasian magpie), *Ammoperdix* spp. (partridges), *Gypaetus* spp. (vultures), *Aquila* spp. (eagles), *Athene noctua* (Scopoli) (little owl), swifts (Apodidae) and woodpeckers (Picidae) [16].

### III. RESULTS AND DISCUSSION

We, for the first time, collected three species of bloodsucking flies from Haftad-Gholleh Protected Area where strictly feed on wild birds. The recorded fly species are as follows: *Ornithophila metallica* (Schiner), *Protocalliphora azurea* (Fallen) and *Trypocalliphora braueri* (Hendel). Both *O. metallica* and *T. braueri* are new genus and species records for the Iranian fauna.

#### *Ornithophila metallica* (Schiner)

Both sexes of the hippoboscid *O. metallica* are hematophagous ectoparasites and ingest blood from a wide variety of birds (Figures 2 and 3). Maa [17] listed the host birds for the two Palearctic members of *Ornithophila* Rondani, *O. metallica* and *O. gestroi* Rondani, and categorized the former as a species with “having high population density and very wide host and distributional ranges” and found the latter to be a species with “low population density and much more restricted host/ or

distributional ranges.” *O. metallica* is widely distributed in the Old World including Iran’s neighboring countries of Pakistan, Afghanistan and Turkey (Figures 4-10) [17]. These species are commonly known as bird blow flies or bird nest flies. *Trypocalliphora* is a monotypic genus, with a single Holarctic



Figure 2: *Ornithophila metallica* (Schiner): Dorsal view.



Figure 3: *Ornithophila metallica* (Schiner): Lateral view.

species *T. braueri* which differs from its closest related genus *Protocalliphora* in having additional notopleural setae. Although some Dipterists consider *Trypocalliphora* a subgenus within *Protocalliphora* [18], other calliphorid taxonomists argued that *Trypocalliphora* is to be considered as a valid genus [19-21]. These species display different types of parasitic strategies as the larvae of *P. azurea* feed on the blood of young birds of the order Coraciiformes and remain on the surface of the birds, but the hematophagous larvae of *T. braueri* infest nestlings of the order Falconiformes and burrow beneath the skin of their hosts, causing a form of parasitism called subcutaneous myiasis [18].



Figure 4: *Protocalliphora azurea* (Fallen): Dorsal view



Figure 5: *Protocalliphora azurea* (Fallen): Lateral view.



Figure 6: *Protocalliphora azurea* (Fallen): Male genitalia, lateral view



Figure 7: *Protocalliphora azurea* (Fallen): Male genitalia, posterior view.



Figure 8: *Protocalliphora azurea* (Fallen): Male sternite



Figure 10: *Trypocalliphora braueri* (Hendel): Lateral view

#### IV. CONCLUSION

Haftad-Gholleh Protected Area, like most of Iranian natural habitats, has been experiencing destructive interventions from illegal human activities, including poaching, that aggravating the vulnerability of its wildlife to epidemics and parasites as an ovine rinderpest epidemic heavily emaciated the population of wild goats of this area in 2015. In terms of birds, the sprawling build-up areas, power lines and transmission towers pose significant threats to migrating birds of the area and nearby parks. In a framework of a faunistic project, we are working to document the insect diversity of Haftad-Gholleh Protected Area to underscore the need for improving the conservation measures and policies towards a standard protection of the area and its fauna and flora.

#### V. ACKNOWLEDGEMENTS

We wish to thank Dr Nil Rahola (Université de Montpellier, Montpellier, France) for confirming the identity of *Ornithophila metallica*. We are also grateful to the Department of Environment of Markazi province and the rangers at Haftad-Gholleh Protected Area for facilitating our research through the area.

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# Socio-Economic Determinants of Sweet Melon Production in Balanga Local Government Area of Gombe State, Nigeria

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**Abstract**— The potentials of the horticulture sub-sector in solving the prevailing food crisis in Nigeria remain largely untapped because of inefficient use of production resources. The study examined socio-economic determinant of sweet melon production in Balanga local government area of Gombe state. A two stage sampling procedure was used in drawing a sample size of sixty sweet melon farmers from three communities. Data collected were analyzed using both descriptive and multiple regression models. The result revealed that the majority of the farmers were male, married, and literate, with small holding. A coefficient of multiple determinants,  $R^2$  of 0.765 indicated a high relevance of the input in explaining the observed variation in melon production. The regression co-efficient of experiences, farm size and house hold size were significant at 5% level of probability, therefore, making the three factors important determinant of output from sweet melon production. Based on findings from the study, it is recommended that government should provide credit facilities with less bureaucracy and low interest rate to producers; this will enable farmers to increase their farm size and in turn increase output

**Keywords:** Socio-economic; Determinant; Sweet melon; Production; Balanga

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## I. INTRODUCTION

Sweet Melon (*Cucumis melon* L.) is a warm, long season horticultural crop that is adapted to all climatic zones. Annual world production of melon has increased from 9 million (700,000 ha) in 1992 to 22 million (1.2 million ha) in 2002. Major producing countries are China with 400,000 ha, West Asia (Turkey, Iran, Iraq) 200,000 ha, the America (United State, Mexico, Central and South American countries) 165,000 ha, Northern African (Egypt, Morocco, Tunisia 110,000 ha. Southern Asia (India, Pakistan, Bangladesh 100, 000 ha). European Union (Spain, Italy, France Greece, Portugal) 95,000 ha, Romania 50,000 ha Japan 13,000 ha and Korean republic 11,000 ha FAO [1]. Each country has its own specific melon cultivars of the crop which are sold in local markets. In Africa, it is an economic crop for urban markets, grown in drier region and non-high lands. Statistics on production and marketing in Africa are not available for most countries except Cameroon (3500 ha) and Sudan (1200 ha), Senegal and surrounding countries are exporting the melon during the winter to Europe FAO [1]. Mature fruits of sweet melon cultivars are usually consumed fresh for the sweet and juicy pulp. The pulp is also mixed with water and sugar or sometimes with milk, and served as a refreshing drink or made into ice cream. Immature fruits of non-sweet types, including snake melon are used as a fresh cooked or pickled vegetable. They

can also be stuffed with meat, rice and spice, and fried in oil. Sweet melon is often confused with cucumber and often used as such. The seeds are eaten after roasting they contain edible oil. The Hausa people in Nigeria grind the kernels to a paste and make it into fermented cakes. The young leaves are occasionally consumed as a pot herb and in soups. The leafy stem and also the fruit provide good forage for all livestock. In reunion and Mauritius a decoction of seeds and roots is used as a diuretic and vermifuge [2]. Sugar content and aroma are important factors determining the quality of sweet melon. Esters derived from amino acids are important components of the characteristics flavour, sulphur containing compound also play a role. Several C-9 alcohol and aldehydes, including Z-non 6-Enal, are characteristics of the melon aroma. To get the best aroma fruits should be harvested only 2-3 days before they are fully ripe. The edible seed kernel contains approximately 46% of yellow oil and 36% protein [3]. Given the increasing popularity and importance of sweet melon as a desert to many households in Nigeria, it is imperative to understand the problems facing the producers of sweet melon. Olukosi and Isitor[4] identified several possible factors that are constraints to production of fruit and vegetables. These include low farm gate price, high cost of labor input, inadequate supply of improve inputs and inefficient marketing system Due to the increasing demand and importance of sweet melon, venturing in to its enterprise holds promising potentials. However, there is little or no attention given to sweet melon production technology while only a few is done on its



marketing as well. Thus, there is need for further investigation into socio-economic determinants of sweet melon production in Balanga local government area of Gombe state and also determine the factors influence sweet melon production in the study area.

### **Problems of sweet melon production**

According to Adamu et al. [5], in his studies of profitability of sweet melon production and marketing in Kirfi Local Government Area; Bauchi State shows that majority (87.5%) of the producers and marketers face the problem of transportation due to poor feeder roads, similarly (50%) and (62.5%) of the producers and marketers experienced inadequate capital to improve their productivity and farming business respectively. Moreover, 75% and 37.5% of the producers and marketers complained of glut (on-season problem) respectively. It is noteworthy that about 31.3%, 56.3% and 81.3% of all the producers complained of inadequate improved seeds, labor, disease attack as well as low farm gate price respectively. This indicated that water melon producers in the study area undergo the water melon business under unpredictable situation as was also reported by Singh [6], for vegetable and tomato producers in the semi-arid regions and Yamaltu Deba Local Government Areas of Gombe State, Nigeria. Similarly, other authors reported many problems that are limiting the fruit production as, Dieter [7], shows that in his report, fluctuation in the price of fruit also contributes a major problem in its business. Agricultural production has been increasing at (2%) two percent per year while demand has been increasing at slightly less rapid rates. This means that agricultural prices income have to be low. Similarly Adegeye and Dittoh [8], reported that prices of fruits and other agricultural produce are often manipulated by speculators with adverse effects on the producers and the consumers there is too much seasonal variation in price due mainly to lack of storage facilities and insufficient supply. Also according to the Abbott [9], shows that most fruit do not have adequate storage or ware housing facilities. The existing infrastructural facilities such as access roads, transport, market storage and processing are far from being adequate Singh [6]. Food processing plants are virtually none existing. These pose a serious problem for effectively processing of agricultural producers. Hence, affect the effective production of fruit and other agricultural produce. In the same studies carried by Adegeye and Dittoh [8], also reported that some marketing problems can be traced to lack of information about production, for example sellers may not be able to identify source of supply of commodities, while producers may curtail their production as a result of poor sales. Therefore, there must be an information system where buyer and seller can be aware of each other problems. Also according to them, the problem of transport in

marketing of fruit and vegetables has many dimension, in some cases there are insufficient vehicles to carry goods from farm (purchase place) to markets (serving places) and from rural market to the towns. In other cases, transport accounts for a large proportion of production costs. In some instances there are no roads where they exist they might be seasonal. Feeder roads are usually few and in most cases have to be construction and maintained by communal efforts. Adegeye and Dittoh [8] reported that all effort has geared towards producing more without thinking about how to market them. There is need to know about new technologies in food storage preservation and marketing. Thus, there is need for research on consumers demand and preferences, handling and packaging to reduce lose in fruit and vegetable as well as in other agricultural produce marketing.

Some of these problems reported by Singh [6] include:

- Problems of price variability.
- Inadequate processing and storage facilities.
- Lack of information about production and marketing.
- Lack of transport facilities.
- Lack of uniform weight and measures.
- Inadequate research on fruit market, etc.

## **II. METHODOLOGY**

### **Area of the study**

Gombe state was created on 1st October, 1996 by the military Government headed by General Sani Abacha, the commander-in-chief of Armed forces of the federation. It was formally under Bauchi state. The state has eleven local government councils with its administrative headquarters in Gombe. Gombe state shares common boundary with Borno state in the east, Bauchi state by the west, Yobe state by the north and Adamawa state by the south. It is located in latitude 10°15' north and longitude 110 east. Her population is estimated of 1.5 million covering the area land mass of about 20, 265 square kilometer [10]. The area of the study was Balanga Local Government and it has covered three distinct areas in the local government namely, Maidara, Daban Magarya and Bakasi. The study area is located in co-ordinates 9°58'N 11°41'E. Balanga is a local government area in the south east of Gombe State, Nigeria bordering Adamawa State. It's headquarters Talasse. It has an area of 1,626 Km<sup>2</sup> and a population of 212,549 at the 2006 census. The climate condition of the local government area is characterized by two distinct seasons, dry and wet. The hottest months are March and April which recorded up to a temperature of about 40-42°C while the coldest months are December to February with a minimum temperature of about 20-22°C and the area received the

mean annual rainfall of 321.4 mm/annum [11]. Sample procedure and sample size The data for this study were generated through the use of structured questionnaire complemented with data were collected from sixty sweet melon producers in Balanga local government area of Gombe state. Purposive sampling technique was used to select three villages and proportional sampling was used in selecting twenty respondents from each village. Twenty questionnaires each were administered to sweet melon producers in Maidara, Daban Magarya and Bakasi. The data were collected by the researcher with help of two well trained personnel's within the period of eight weeks, beginning from June-July 2015.

#### Method of data analysis

The statistical tools employed in this study include descriptive statistics analysis, such as frequency distribution, percentage and mean were used for the analysis of socio-economic characteristics of sweet melon producers. The relationship between the socio-economic characteristics and production of sweet melon was determined using multiple regressions model. The model was specified as:

$$Y=f(X1,X2,X3,X4,X5,X6,X7,X8,u)$$

Where,

Y=Output in Pyramid/Bill (Kg)

X1=Age (Years)

X2=Years of Experience (Years)

X3=Farm Size (Ha)

X4=Household Size (No. of Person)

X5=Level of Education (Years)

X6=Marital Status (1=Married, others=0)

X7=Extension Contact (Yes=1, No=0)

X8=Membership of Cooperative (Yes=1, No=0)

U=Constant term

The above model was specified and estimated in four functional forms. The functional forms tried include the linear, exponential, semi-log and double-log. The functional form which gives the best fit in term of R<sup>2</sup> value was selected because it agree with a priori expectation.

### III. RESULTS AND DISCUSSION

#### Socio-economic characteristics of sweet melon producers

Table 1 shows that majority (98.3) of the sweet melon producers in the study area were male. This implies that the

participation of female in sweet melon production in the study area is very low. This agrees with the finding of Adamu et al., [5] who reported that males dominated the farming aspect of water melon in Kirfi Local Government Area of Bauchi State Nigeria. This is because most of the people in the study area are Muslims and "Purdah" is practice for female and house wives (not allowed in to farming) as enshrined in the culture of northern Nigeria. On marital status of the respondents, the results revealed that 95% of the sweet melon producers in the study area were married while only 5% were single in the study area. This is in line with study made by Atman et al., [12] which revealed that (98.99%) of vegetable marketers in Yamaltu Deba Local Government of Gombe State, Nigeria were married. This is because majority of the producers in the area were Muslims and their religion permits them to marry at early ages. It is clear from the table that majority of the respondents were in the age category of 31-45 years representing 61.7% followed by those within the range of 16-30 years with 20%, those within the range of 46-60 years represents 18.3%, none of the respondent fall within the age of 61-75 years. The result further shows that a minimum of 19 and maximum of 53 years was recorded with a mean ages of the producers as 39 years, standard error of the mean was found to be 0.88% and 54.3% co-efficient of variability, implying that there is variability in the age of the respondents. The result depict that production of sweet melon in the study area are mostly carried out by relatively middle aged people. This category of people is believed to be more flexible in their decision making and adopt new ideas more readily, and the aged are risk-aversive. The results also indicated that 50% of the respondents attended Qur'anic schools, 23.3% had primary education, 16.7% had secondary education, while 8.3% had no formal education and 1.7% had attained tertiary education. The literacy level among the respondents was relatively high. It is expected that the sweet melon producers in the study area could readily adopt new ideas of agricultural production and can make accurate use of production decision. This is in line with the study made by Atman et al., [12] which reported that literacy status of respondents is necessary to explain the strength or weakness observed in their management ability and adoption of innovation. Educations are an important tool in increasing adoption of improved farm practices and ultimately improve in farm production and productivity. On occupation of the respondents, the result indicated that 43.3% of the sweet melon producers in the study area were engaged in farming alone; 33.3% engaged in farming and trading/business. 8.3% were into farming and livestock rearing while, 8.3% were sweet melon producers and civil service, 6.7% of the respondents were engaged in farming and others artisan activities such as driving, mechanic and car washing. The result means that majority of the

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respondents were full time farmers in the study area. The results revealed that majority had between 11-15 and 16-20 persons in their

	Frequency	Percentage
<b>Sex</b>		
Male	59	98.3
Female	1	1.7
<b>Marital Status</b>		
Married	57	95
Single	3	5
<b>Age</b>		
16-30	12	20
31-45	37	61.7
46-60	11	18.3
<b>Educational Level</b>		
No formal Education	5	8.3
Quaranic Education	30	50
Primary Education	14	23.3
Secondary Education	10	16.7
Tertiary Education	1	1.7
<b>Occupation</b>		
Farming alone	26	43.3
Farming and Trading	20	33.3
Farming and Livestock	5	8.3
Farming and Civil Services	45	8.3
Farming and Artisanship	4	6.7
<b>Household Size</b>		
1-5	12	20
6-10	12	20
11-15	13	21.7
16-20	13	21.7
21-26	9	15
27-30	1	1.6
<b>Years of Experience</b>		
1-5	56	93.3
6-10	4	6.7
<b>Farm Size</b>		
0.5-1	29	48.3
1.5-3	31	51.7
<b>Source of Finance</b>		
Family and Friend	53	88.3
Bank Loan	4	6.7
Cooperative Societies	3	5
<b>Cropping Pattern</b>		
Intercropping	26	48.3
Sole Cropping	20	33.3
Both	14	23.4

**Table 1: Socioeconomic characteristics of respondents (Source: Field Survey, 2015).**

households, representing 21.7% each, Household size of 1-5 and 6-10 persons also had 20%. The mean households' size of the respondents was found to be 13 persons with standard error of the mean of 0.25, and co-efficient of variability of 23.7%. The result clearly indicated that, the minimum number of persons in a household was found to be one with a maximum of 27 persons in the study area. The low variability among the household size is most likely

attributed to polygamous nature in Northern Nigeria, similar to what was reported by Umoh [13] in Bauchi metropolis. The result also revealed that minimum year of experience was found to be 1 year with the maximum of 6 years. The mean years of experience of respondents was found to be 3.1 years, standard error of the mean value of 0.12 and the co-efficient of the variability of 28.7%. This means that majority of the producers in the study area had not stayed long in the business because the fruit production was newly introduced to the area. The result indicated that majority (51.7%) of the respondents had a farm size category of 1.5-3.0 hectares following by those in the category of 0.5-1.0 hectare representing 48.3%. The result further shows that a minimum of 0.5 and maximum of 3 hectares was recorded with mean hectares of 1.5, standard error (SEX) of 0.008 and co-efficient of variability (CV) was found to be 1.19%. This implies that the farmers have small-scale managed farms. Majority (88.3%) of the respondents sourced their initial capital through family and friends, 6.7% obtained their capital from bank loan while, only 5% obtained financial support from co-operative societies. This indicated, the only means of financing their business was through family and friends. This agrees with the findings of Atman et al. [12] who reported that, tomato producers and marketers were only financing their business through informal means that is through own savings, money lenders, family and friends), as none of the respondent's claimed to have obtained money for financing his business from government. The result indicated that majority (43.33%) of the sweet melon producers engaged in intercropping pattern while 33.33% of the respondents were engaged in sole cropping and only 23.33% were engaged in both sole and inter cropping. This is in line with the study by Yusuf et al., [14] which stated that the higher the number of crops in the mixture the less the profitability. Also Yusuf [15], discovered in his research on Egusi melon that the more the number of crops in the mixture the less the yield and the less the profitability, which he attributed to the competitive effects of the various crop in the mixture (Table 1).

### Regression results for the socio-economic determinants of sweet melon production

Multiple regression analysis was used to determine the socio-economic factors influencing the sweet melon production in the study area. In order to compare and assess in detail the necessary parameters, four functional forms viz: linear, double-log, semi log, and exponential function were fitted to the data. The result presented in Table 2 shows the estimated impacts of socio-economic factor of respondent on production output (age, experience, farm size, house hold size, education attainment, married status). Double-log function was found to have the best fit and therefore chosen

as the best lead equation. The R2 of the double-log function was found to be 0.768. This implies that about 76.8% of the variation in output of the respondent was accounted for by joint action of the six independents factor while the rest 23.2% of the variation was due to error. The overall regression result was significant with F-statistic value of 35.717 at 5% level of probability. The regression co-efficient of experiences, farm size and house hold size were significant at 5% level of probability, therefore, making the three factors important determinant of output from sweet melon production. The other three factors age, education attainment and marital status were not significant and therefore, constituted weak determinants of production output. Farmers with high experience are more likely to produce more sweet melon than their counterparts with low experience, and also farmers with large farm size are more likely to produce more melon with their counterparts with small farm sizes and the farmers with large house hold size are more likely to produce more sweet melon than their counterparts with small household size which are similar to Ugwumba [16].

#### IV. CONCLUSION

The study was conducted in Balanga Local Government Area, Gombe State. The main objective of the study is to obtain information

Factors	Linear Function	Semi-Log Function	Double-Log Function	Exponential Function
Constant	338.245 (0.269)	670.601 (1.223)	3.617 (10.479)**	3.071 (35.889)**
Age	-0.110 (-0.780)	-0.186 (-1.073)	-0.168 (-1.409)	-0.86 (-0.825)
Experience	0.519 (4.780)**	0.463 (3.846)**	0.583 (7.065)**	0.598 (7.419)**
Farm Size	0.312 (2.752)**	0.362 (2.839)**	0.317 (3.625)**	0.245 (2.909)**
House Hold Size	0.158 (1.249)	0.155 (0.869)	0.268 (2.191)**	0.256 (2.734)**
Education Attainment	0.009 (0.097)	0.030 (0.312)	0.025 (0.382)	0.013 (0.185)
Marital Status	-0.005 (-0.045)	-	-	0.68 (0.831)
F-Statistic	11.070**	11.109**	35.717**	27.361**
R <sub>2</sub>	0.556	0.507	0.768	0.756
R <sub>2</sub> Adjusted	0.506	0.461	0.746	0.728

**Table 2: Regression results for the socio-economic determinants of sweet melon production**

on socio-economic determinant of the respondents, in achieving these objectives; three villages (Maidara, Daban Magarya and Bakasi Areas) were purposively selected. Sixty respondents were randomly selected from the list frame of the sweet melon farmers. The respondents were issued with questionnaires, which were filled with the help of well trained enumerators and the researcher. Statistical tools such as descriptive statistics and multiple regression

analysis were used in data analysis. The major findings of this study revealed that the majority of the sweet melon producers were male, married and were within the age bracket of 31-45 years with mean age of 39 years. The result further showed that the respondent had one form of education or the other with Qur'anic education as the highest up to 50%, and had 1-5 years experience with the mean 3.1 years of experience. Moreover, the result also showed that 43.3% of the respondents engage in farming alone and mainly sources their initial capital for the business through family and friends been (88.3%). The regression analysis of the socio-economic characteristics show that double-log regression was chosen as the lead equation based on the values of R2 of 0.768 with a standardized co-efficient of 0.256. The regression co-efficient of experiences, farm size and the house hold size were significant at 5% level of probability, therefore, making the three factors important determinant of output from sweet melon production.

#### V. RECOMMENDATION

Based on the findings the following recommendations were made:

1. Socio-economic characteristic of sweet melon farmers should be taken into consideration when formulating policies and also when introducing new technologies to rural farmers.
2. Provision of credit facilities with less bureaucracy and low interest rate to producers. This will enable farmers to increase their farm size and in turn increase their output.
3. Extension agent should be mobilized in the area to enhance the level of agronomic practices of melon farmers. Access to extension agents enhances the chances of having access to better crop production techniques, improved inputs as well as other production incentives and in turn leads to increase in output.

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# Interrogator for FBG Strain Sensor

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**Abstract:**-- A structural health monitoring system (SHM) is the need of the hour because continuous monitoring of large structures is a cumbersome task. In this paper a fiber Bragg grating (FBG) sensing system for strain measurements is being described. Economically feasible and simple grating based FBG has been used to produce strain and induced voltage corresponding to wave length shift. The fiber optic grating sensors have been used in this research work in static operating conditions. The feasibility of using a FBG sensor system in real time monitoring of strain in an optic fiber has been demonstrated experimentally. Experimental and theoretical results showing capability of the proposed system to perform strain measurement and giving an approximate linear response are presented.

**Index Terms:**-- fiber Bragg grating, optical fiber, strain sensor, wave-length shift

## I. INTRODUCTION

Civil infrastructures such as long span bridges, offshore structures, large dams and other hydraulic engineering, nuclear power stations, tall buildings, large space structures and geo technical engineering etc. often have a long service period, maybe several decades or over one hundred years, during which they are inevitable to suffer from environmental corrosion, long term loading or fatigue effects, material aging or their coupling effects with extreme loading, and then the damage accumulates, performance degenerates or capacity resisting from disaster actions reduces and even disaster occurs since their failure under the extreme loading. Therefore an intelligent Structural Health Monitoring system (SHM) becomes more and more important technology to study the damage or even to predict disaster. This SHM with FBG strain sensors will be very useful to reduce the maintenance costs with increased levels of safety.

## II. FIBER SENSOR MODULE

In a typical optical fiber sensor, light from a source such as a laser diode or LED is guided by an optical fiber to the sensing region as shown in the fig.1. Some property of the propagating light beam such as intensity, phase, state of polarization or wavelength gets modulated due to change in pressure, temperature, strain, magnetic field and so forth. The modulated light is then sent via another (or the same) optical fiber for detection and processing. Optical-to-electrical conversion is obtained using photo detectors and thus enables any measurements to be performed through the information of optics.

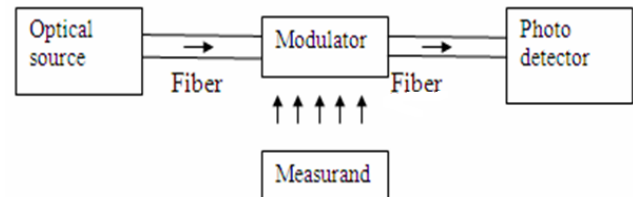


Fig.1 Block Diagram of Fiber Sensor

## III. DIFFERENT FIBER OPTICAL SENSING TECHNIQUES

Techniques of implementation of optical fiber sensors are very wide and broad. Some of the major sensing techniques of optical fiber sensors based on modulation are discussed here.

### a. Intensity Modulation Sensors

The basic concept of intensity based sensors is very simple, in this intensity of either reflected or transmitted light is modulated by the measurand. The major limitation of any intensity based sensor is the lack of any suitable reference intensity signal. Any intensity fluctuations in the output which may not be associated with the measurand may produce erroneous results [2].

### b. Phase Modulation Sensors

The total phase of light path along an optical fiber depends on three properties of the fiber guide namely-

- i. Total physical length.
- ii. The refractive index and index profile.
- iii. The geometrical transverse dimension of the guide.

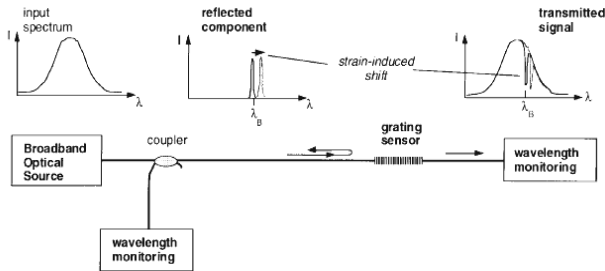
In this type of sensors transmitted and reflected light are compared using various techniques to detect any change in phase caused by the measurand.

### c. Wavelength or Frequency Modulation Sensors

In this type of the sensor the wavelength of the transmitted and reflected light in the fiber is modulated by the measurand. The commonly used sensor is Fiber grating sensor which is discussed as follows:

#### Fiber grating sensor:

Bragg gratings are periodic refractive index variations written into the core of an optical fiber by exposure to an intense UV interference pattern. For a Fiber Bragg grating (FBG) sensor [7], changes are enclosed as changes in the periodicity or refractive index of the grating thereby causing shift in the wavelength of the reflected wave or transmitted wave. The measurements of the measurand are achieved by detecting the wavelength of the reflected wave or transmitted wave.



**Fig.2 Basic grating-based sensor system with transmissive or reflective detection options**

When broad band light propagates through the FBG the wavelength of light satisfying the Bragg condition is given as

$$\lambda_B = 2 n_{\text{eff}} \Lambda$$

where,  $\lambda_B$  is the reflected Bragg wavelength,

$n_{\text{eff}}$  is the average refractive index of the fiber core,

$\Lambda$  is the grating spacing..

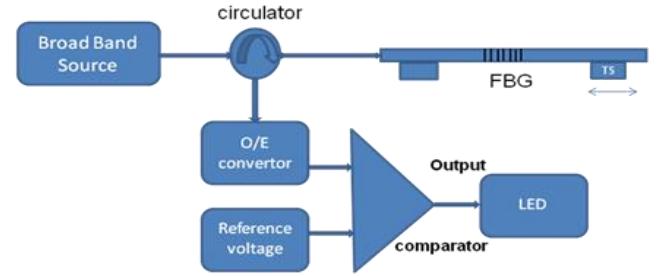
A part of light will get reflected and remaining will be transmitted. The spacing of the periodic variation of the refractive index will change because of external stress on the fiber. Also as a result of the strain-optic effect the average refractive index will be changed. Thus a shift in the Bragg wavelength is observed. This shift can be expressed as [1],

$$\frac{\Delta \lambda_B}{\lambda_B} = K \epsilon$$

where  $K$  is a constant,  $\Delta \lambda_B$  is the wavelength shift and  $\epsilon$  is the applied strain.

## IV. EXPERIMENTAL TECHNIQUE

We are proposing a method for real time monitoring of the strain. Fig.3 shows the experimental setup of the proposed FBG interrogation system. Light from the Broad Band source (ASC) passes through the 3-port circulator to the FBG that is instrumented. The part of input light satisfying the Bragg condition is reflected and rest is transmitted. A physical elongation has been produced creating a tensile in the FBG.



**Fig.3 Experimental set-up**

As the wavelength  $\lambda_B$  is shifted corresponding to the applied strain, therefore, calibration is mandatory to achieve a precise relation between the wavelength shift and the physical signal being measured. For that the shifted wavelength is given to the Optical to Electrical converter (here it is OSA itself) where the wavelength shift is converted into the voltage which is given to the one input of comparator and second input of comparator is Reference voltage. The reference voltage is set on the lowest possible value of the voltage corresponding to the maximum value of the strain that a FBG can bear. If the sample voltage is greater than the reference voltage than the output of comparator will be negative and whenever the sample voltage is less than the reference voltage output will be positive, and indicator in the output of the comparator will glow showing maximum limit of strain is crossed.

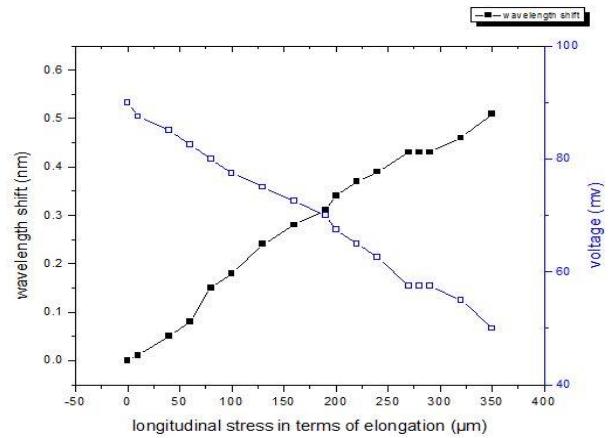
A relationship exists between the wave-length shift i.e. caused due to strain and analog output voltage. We are using this relationship in our program. The experimental values of wavelength shift for applied longitudinal stress in terms of elongation are obtained from OSA and corresponding analog output voltage of the OSA is observed on digital storage oscilloscope (DSO) and tabulated in the table.1

**Table 1. Output voltage for wavelength shift corresponding to longitudinal stress**

Longitudinal Stress( $\mu\text{m}$ ) (elongation)	Wavelength Shift(nm)	Voltage (mv)
00	00	90
10	0.01	87.5
20	0.02	85
30	0.03	82.5
40	0.05	82.5
50	0.09	80
60	0.12	80
70	0.14	77.50
100	0.21	77.50
110	0.23	80
120	0.24	75
130	0.26	75
140	0.27	75
150	0.27	75
160	0.28	72.5
170	0.29	72.5
180	0.31	72.5
190	0.34	70
200	0.35	67.5
210	0.37	67.5
220	0.38	65
230	0.39	65
240	0.39	62.5
250	0.42	60
260	0.43	57.5
270	0.43	57.5
280	0.44	57.5
290	0.45	57.5
300	0.46	55
310	0.47	55
320	0.48	55
330	0.50	50

**V. RESULT AND DISCUSSION**

The experimental values of the Bragg wavelength shifts were obtained from the reflected signal and corresponding value of voltage is measured on Digital Oscilloscope (DSO). The plot were drawn between the wavelength shift and voltage as shown in the fig.4 The alert system which glows when the applied strain is beyond the maximum limit is successfully demonstrated.



**Fig.4 Voltage Vs Wavelength Shift.**

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# Acoustoelectric Effect In Solid State Materials and Devices

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**Abstract:** Acoustoelectric effect is the appearance of a dc electric current, when an acoustic wave passes through a conducting medium. This paper reviews the study of Ultrasonic stress on the semiconductor materials and devices used in various scientific and other measurements. A historical review of earlier findings is also reported with special reference to associated mechanisms. Ultrasonic stress studies in solid state devices require further attention and the work done in this area is also discussed. Different kinds of the mechanism, interpretation responsible for the change in the characteristics of the solid state devices and materials have been discussed. Ultrasonic stress produces a pressure effect on a target object placed in the ultrasonic field. Ultrasonic wave may be regarded as a coherent beam of phonons, absorbed in front of the material. In terms of the charge carriers, an electron hole pair is created due to the Ultrasonic field.

**Keywords:** Intervalley Scattering, Free electrons of the materials, Resistance of the material, Ultrasonic stress, Radiation Pressure, Ultrasonic Amplifier, Bunching of electrons and Holes in a Material, Carrier Mobility.

## I. INTRODUCTION

When a sinusoidal Ultrasonic wave propagates through a semiconductor, it gives rise to an electric field travelling through the semiconductor material with the velocity of the Ultrasonic wave. A dc field, under the action of the travelling ac field is generated across the semiconductor material while the wave traverses the semiconductor material. Ultrasonic energy (signals) containing phonons shower down on unexcited atoms. The phonons are absorbed by the atoms of the said solid state materials and the devices. The energy of the phonon is converted into internal energy of the atom. The atom is then raised to an excited quantum state. Later on, the excited atom radiates this energy. In this way, there is a travelling wave amplification through semiconductor materials and solid state devices. Under the assumption of the conservation of momentum, Weinreich has derived a relationship between the absorption coefficient of ultrasonic waves and the ultrasonic field:[1 – 14]

$$\alpha_i = \delta_{ne} V_s E_{ae} / Q \text{ ----- (1)}$$

$\alpha$  is the attenuation per unit length due to conduction electrons in nepers –  $\text{cm}^{-1}$ ,  
 $n$  the density of conduction electrons in  $\text{cm}^{-3}$ ,  
 $V_s$  the ultrasonic wave velocity in  $\text{cm} - \text{sec}^{-1}$ ,  
 $Q$ , the ultrasonic – power density in watt –  $\text{cm}^2$ ,  
 $E_{ae}$ , the ultrasonic electric field in volt –  $\text{cm}^{-1}$ ,  
 $\delta$ , the numerical factor depending on the scattering process of conduction electrons. The field created in the solid state

devices and materials due to the ultrasonic stress effect is defined as the electric field equivalent to the dc forces acting upon the electrons due to the ultrasonic wave. Weinreich pointed out, the rate of loss of momentum from the ultrasonic wave (which is equal to the rate of energy loss divided by the velocity of sound) is a dc force in the direction of propagation of sound and is equivalent to an electric field.

The concept of a deformation potential is useful in discussing the motion of electrons and holes in a semiconductor material and devices in the presence of ultrasonic deformation of the semiconductor material. In the simplest form, deformation potential is applicable to low energy electrons and holes whose bands have a simple structure, the assumption is that such a particle has a potential energy  $V_1$  proportional to the dilatation of the semiconductor material:

$$V_1 = -\epsilon_1 \Delta \text{ ----- (2)}$$

$\epsilon_1$  is a constant and  $\Delta$  the dilatation. This interaction leads to forces exerted on particles by ultrasonic wave and in certain cases to radiation of ultrasonic wave by particles.

Treating the ultrasonic stress effect on the semiconductor materials and devices classically, ultrasonic wave lengths are much larger than carrier mean free paths (and the periods much larger than mean free times), describing the net particle current  $j$  in the usual macroscopic way as being composed of a drift term and a diffusion term

$$j = D \left( \frac{F}{KT} - \nabla \right) \eta \quad \text{----- (3)}$$

$\eta$  is the particle density, F the force applied to the particles, KT the thermal energy, D the diffusion constant.

To avoid space charge difficulties, in addition to the deformation potential force  $-\nabla V_i$ , the force exerted by electric fields resulting from the redistribution of charges is also considered. For small sinusoidal disturbances, induced electrostatic potential is proportional to the deformation potential of the applied ultrasonic wave. The change in the ultrasonic propagation properties of the medium can be thought of as the continual addition to the original wave of a wave which is radiated by the redistributed carriers. The ultrasonic stress effect on the semiconductor materials and devices produce simultaneous bunching of electrons and holes in a semiconductor under the action of the deformation potential of a travelling ultrasonic wave.

## II. DISCUSSION

Propagation of an ultrasonic wave causes a change in the resistance due to the heating of the carrier caused by absorption of energy from the ultrasonic wave and the corresponding change in carrier mobility.

Ultrasonic wave propagation through a semiconductor material and solid state devices lead to thermal motion and lattice vibration analogous to the photons in the electromagnetic waves. The mode of ultrasonic wave propagation through a semiconductor and solid state material gives information about the lattice vibration. The acoustical branch of lattice spectrum is described by the dispersion relation.

$$W^2 = C \left( \frac{1}{M} + \frac{1}{m} \right) - C \left[ \left( \frac{1}{M} + \frac{1}{m} \right)^2 - \frac{4 \sin^2 ka}{Mm} \right]^{1/2} \quad \text{--- (4)}$$

C is elastic force constant.

m is the linear array of identical atoms with interatomic spacing a

K is the wave vector

W is the angular frequency

One dimensional crystal formed by the alternate placing of two different atoms of masses M and m.

$m < M$

The maximum possible angular frequency for the acoustic mode is  $W_1 = (2c/M)^{1/2}$

which is independent of the mass of the lighter atom. The acoustic mode minimises changes in the second nearest neighbour distance by maximising changes in the nearest neighbour separation. The nearest neighbour

stiffness constant is considerably larger than any other, each harmonic oscillator of the acoustic mode will store a considerable amount of energy.

The range of frequency for which K must be complex extends from  $W_1 = (2c/M)^{1/2}$  to  $W_2 = (2c/m)^{1/2}$

The width of the forbidden band is  $(W_2 - W_1)$ .

The mass ratio (m/M) determines width of the forbidden band.

Both electromagnetic and acoustic waves exert forces of radiation upon an obstacle placed in the path of the wave, the forces being proportional to the mean energy density of the wave motion.

The energy of a lattice vibration is quantized. The quantum of the energy is called a phonon. Elastic wave in crystals are made up of phonons. Thermal vibrations in crystals are thermally excited phonons.

The energy of an elastic mode of angular frequency W is  $E = (n + 1/2) \hbar w$ .

when the mode is excited to quantum number n and the mode is occupied by n phonons. The term  $1/2 \hbar w$  is the zero point energy of the mode.

A simple model of phonons in a crystal is defined as the vibrations of a linear lattice of particles connected by springs. Quantizing the particle motion for a harmonic oscillator. Transforming from particle coordinate to phonon coordinate known as wave coordinate as it represent a travelling wave.

Let N particles of mass M be connected by springs of force constant C and length a. For fixing the boundary conditions, the particle form a circular ring. Considering the transverse displacements of the particles out of the plane of the ring. The displacement of the particle s is  $q_s$  and its momentum is  $p_s$ . The Hamiltonian of the system is

$$H = \sum_{s=1}^n \left\{ \frac{1}{2M} p_s^2 + \frac{1}{2} C (q_{s+1} - q_s)^2 \right\} \quad \text{(5)}$$

The Hamiltonian of a Harmonic oscillator is

$$H = \frac{1}{2M} p^2 + \frac{1}{2} CX^2 \quad \text{----- (6)}$$

And the energy eigen values are, for  $n = 0, 1, 2, 3, \dots$

$$E_n = (n + 1/2) \hbar w. \quad \text{----- (7)}$$

An ultrasonic wave exerts a static pressure on any interface or medium across which there is a decrease in ultrasonic intensity in the direction of wave propagation. This static pressure is distinct from the oscillating particle pressure of the wave. The force is proportional to the mean energy density of the wave motion. In case of complete absorption of a finite beam of plane waves,

$$F = W/C \quad \text{-----} \quad (8)$$

F is the force due to radiation pressure.

W is the ultrasonic power.

C is the ultrasonic velocity in the medium.

The amount of energy carried by sound vibration in 1 sec through an area of  $1\text{cm}^2$  perpendicular to the direction of propagation, determines the strength, or intensity, of the sound.

For a plane progressive Sine wave, the sound intensity I is expressed in Watt/  $\text{Cm}^2$  or erg/ sec –  $\text{cm}^2$  as

$$I = P^2 / 2\rho C \\ = (\frac{1}{2}) V^2 \rho C = PV/2 \quad \text{---} \quad (9)$$

P is the pressure of the ultrasonic wave at any point in the medium.

$\rho C$  is specific acoustic impedance of the medium,

$\rho$  is the density of medium,

C is ultrasonic velocity in the medium,

V is the velocity of the vibrational movements of the particles of the medium through which an ultrasonic wave is propagated.

The acoustic pressure in water irradiated with ultrasonic waves of intensity I is given as

$$P = \sqrt{2\rho C I} \quad \text{-----} \quad (10)$$

The intensity I is proportional to the square of the displacement. If  $I_0$  is the initial intensity, in watts per square centimetre of a progressive ultrasonic wave, the intensity at a distance X is

$$I = I_0 e^{-2\alpha X} \quad \text{-----} \quad (11)$$

where  $\alpha$  is the loss coefficient in nepers per cm of the wave through a medium.

The time averaged force per unit area on the surface on an obstacle termed as radiation pressure is related to the time averaged momentum flux of the ultrasonic wave.

The presence of an ultrasonic field, the carriers of the device acquire energy from the field and lose it to phonons, by emitting more phonons than those absorbed. The carriers on the average, acquire more energy than they have at thermal equilibrium. The average energy of the carriers also increases, and they acquire an effective temperature  $T_e$ , which is higher than the lattice temperature T.

The presence of a sinusoidal travelling acoustic wave gives rise to a sinusoidal electric field, this field travelling through the material with the same velocity as that of the acoustic wave. For most of the conduction electrons, this component of velocity will be much larger in magnitude than the speed of acoustic wave, so that these electrons are out of phase with respect to the travelling electric field. Thus the time average of this field over their trajectories is zero, and these electrons are essentially unaffected by the

presence of the acoustic wave. There are a few electrons, having components of velocity parallel to the wave which are comparable to the speed of the wave. These electrons are capable of being trapped by the moving electric field so that their time – averaged velocity in the direction of the field is exactly that of the field. Among these electrons, those having a maximum energy will be found to give rise to a net electric current.

In an n – type semiconductor, these electrons are in the conduction band, such a generation of an electric current by a travelling acoustic wave is defined as the acoustoelectric current. These are the consequences of the ultrasonic stress effect on the material.

The acoustoelectric effect has been explained by wave mechanics using one electron approximation and Schroedinger's equation. The acoustoelectric effect is the production of a dc electric field under the action of a travelling acoustic wave in a medium containing free carriers. The term acoustoelectric effect refers to the appearance of a dc electric field along the direction of propagation of a travelling acoustic wave in a medium containing mobile charges and known as wave particle drag.

Ultrasonic wave changes the resistance of the device which is due to the compression and tension by the ultrasonic wave (stress).

### III. CONCLUDING REMARKS.

The present study would help the manufacturer's and users to add a proper necessary correction factor while using the solid state devices in the ultrasonic (stress) field.

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# Effect of Ultrasonic stress in Semiconductor Materials and Devices

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**Abstract:**-- Effect of Ultrasonic Stress in solid state devices and materials have been discussed with the help of electromagnetic wave theory. Ultrasonic stress (radiation pressure) changes the characteristics of solid state devices and materials. Analytical treatment of changed characteristics of solid state devices and materials have been discussed.

**Index Terms**— Maxwell's wave equation, Poisson's equation, Space Charge density, Attenuation and Dispersion of Stress Wave, Ultrasonic Radiation Pressure.

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## I. INTRODUCTION

Effect of ultrasonic stress is known in pure materials. Further, a systematic study has been made in solid state devices made from pure materials defined as Acoustoelectric effect. [1-13] due to ultrasonic radiation pressure effect when a sound wave propagates through a material containing free electrons, its momentum, as well as energy is attenuated. The momentum attenuation acts a dc force, causing the electrons to drift in the direction of force. When there is a closed circuit in this direction, a direct current is produced called "Acoustoelectric current" which is proportional to the sound intensity, as the momentum attenuation itself is. If on the other hand, the circuit is open, the drifting electrons produce a space charge whose electric field cancels the dc force due to the sound wave momentum attenuation. This back electric field is the "Acoustoelectric Effect" due to ultrasonic radiation pressure effect. The resistance of the material is changed due to acountic stress. There is a simultaneous bunching of electrons and holes in the solid state devices under the action of deformation potential of the travelling ultrasonic wave.

The phenomenon of phonon drag contributes to the thermometric power due to the momentum transfer to electrons from thermal phonons streaming down temperature gradient. It is qualitatively equivalent to the acoustoelectric effect, while quantitatively, it is different, since the ralations between typical wave length, mean free times and frequencies are entirely changed. For the propagation of acontic wave in piezoelectric semiconductor, there is a possibility of achieving acoustic gain by applying a dc electric field which causes the interacting charge carriers to drift in the direction of wave

propagation faster than the sound. Ultrasonic wave carries a flux of momentum. A loss in energy from wave is equivalent to a proportional loss in momentum. This loss in momentum constitute a constant force acting on the object absorbing the energy (radiation pressure). The absorbers are the free charged carriers, and the ultrasonic radiation pressure is the acoustoelectric effect.

There is a simultaneous bunching of electrons and holes in a solid state devices under the action of deformation potential of the travelling acoustic wave. A sound wave in a solid gives rise to electric fields which accelerate electrons in much the same way as an electromagnetic wave. An analytical treatment of ultrasonic radiation pressure effect has been discussed.

## II. ANALYTICAL TREATMENT: DESCRIPTION *Stress waves and Electrical Phenomena in Piezoelectric Semiconductors*

For a one dimensional approximation, Electric field  $E$  produces a stress in the  $x_1$  direction as follows:

$$\sigma = c\varepsilon - eE \quad (1)$$

$$D = eE + pE \quad (2)$$

$c$  = elastic constant,  $E$  = electric field

$D$  = electric displacement

$\varepsilon$  = permitivity of a medium

$\sigma$  = surface charge density

$e$  = piezoelectric constants relating electric field to stress

$p$  = magnitude of the elastic displacement component

Expressing  $E$  in terms of  $\varepsilon$  and differentiating (1) with respect to  $x$  leads to a wave equation. If  $D$  is assumed constant, this equation takes the form

$$\rho \frac{\partial^2 S}{\partial t^2} = c \left( 1 + \frac{e^2}{cp} \right) \frac{\partial^2 S}{\partial x^2} \quad (3)$$

S is the displacement. The change in c due to the presence of the electric fields is thus obvious. The condition of constant D further leads to a zero space charge density through Poisson's equation

$$\frac{\partial D}{\partial x} = Q \quad (4)$$

where as the continuity relation

$$\frac{\partial J}{\partial x} = - \left( \frac{\partial Q}{\partial t} \right) \quad (5)$$

For an extrinsic semiconductor in thermal equilibrium, the total space charge density Q may be expressed in terms of the energy levels and densities of the impurity states in the forbidden band, and the concentration of electrons in the conduction band. The condition of electrical neutrality corresponds to Q=0, and the acoustically produced space charge is the periodic variation of Q about zero.

J is the current density, indicates that in this case the varying current density due to the piezoelectric fields is zero, which corresponds to a very low conductivity in the medium.

In the case of very high conductivity, the field E accompanying the wave will be zero, and the elastic constant C will remain unaffected (Equation 1), whereas the stress wave will be accompanied by D fields, currents and varying space charge.

The case of specific interest here is that corresponding to intermediate values of conductivity, in the range encountered in semiconductors. In this range, equation (4) and (5) are used, together with an appropriate expression for J, to obtain values of D and E. These, in turn permit one to eliminate E from the wave equation.

For an extrinsic semiconductor (assumed to be n type), the current density may be expressed by

$$J = q (n + fn_s) \mu E + (\mu KT) f \left( \frac{\partial n_s}{\partial x} \right) \quad (6)$$

where the first term is due to drift and the second term is due to diffusion, q is the electronic charge, K is Boltzman's constant, T is the temperature, n is the mean density of electrons in the conduction band, and f is the fraction of acoustically produced space charge density  $n_s$  which is mobile.

Thus,  $(n + fn_s)$  is the instantaneous local density of electrons in the conduction band. Equation (1) to (4) combined with plane wave representations of D and E

$$D = \frac{-i(nq \mu/\omega)E}{1+i\omega\left(\frac{k}{\omega}\right)^2(\mu fKT/q)} \quad (7)$$

In the case of small conductivity modulation ( $fn_s \ll n$ ), equation (7) may be further simplified and written in the form

$$D = \frac{-i(b/\omega)E}{1+i\omega\left(\frac{k}{\omega}\right)^2(\mu fKT/q)} \quad (8)$$

$b = nq\mu$  represents the average conductivity.

The condition of small conductivity modulation ( $fn_s \ll n$ ) is satisfied when the effective drift velocity of the carriers in the piezoelectric field  $f\mu E$  is much less than the velocity of the stress wave v. This imposes a limitation on the strain value:

$$\varepsilon \ll pv/f\mu e \quad (9)$$

In order to determine the attenuation and dispersion of stress waves, use is made of the conductivity frequency, defined by  $\omega_c = \frac{b}{p}$ , and the diffusion frequency, defined by,

$$\omega_D = \frac{q}{f\mu KT} \left( \frac{\omega}{K} \right)^2 \approx \left( \frac{q}{f\mu KT} \right) v^2$$

From equation (1), (2) and (8), one obtains

$$E = -\frac{e\varepsilon}{p} \left[ \frac{1+i(\omega/\omega_D)}{1+i(\omega/\omega_D)+i(\omega_c/\omega)} \right]$$

In the case of negligible charge carrier diffusion ( $\omega_D \gg \omega_c$ ) equation (9) may be simplified to:

$$E = -\frac{e\varepsilon}{p} \left[ \frac{1-i(\omega_c/\omega)}{1+(\frac{\omega_c}{\omega})^2} \right] \quad (10)$$

and the effective elastic constant is obtained by substitution into (1)

$$\sigma = C \left[ 1 + \frac{e^2}{cp} \frac{1-i(\frac{\omega_c}{\omega})}{1+(\frac{\omega_c}{\omega})^2} \right] \varepsilon \quad (11)$$

The velocity and attenuation are obtained in terms of the real and imaginary parts of the complex elastic constant

$$V = V_0 \left[ 1 + \frac{\frac{e^2}{2cp}}{1+(\frac{\omega_c}{\omega})^2} \right] \quad (12)$$

$$\alpha = \frac{\omega}{V_0} \frac{e^2}{2cp} \left[ \frac{\frac{\omega_c}{\omega}}{1+(\frac{\omega_c}{\omega})^2} \right] \quad (13)$$

This expression show that at very low frequency  $V$  tends to  $V_0$  and  $\alpha$  tends to zero, whereas in the high frequency limit the become.

$$V = V_\alpha = V_0 \left[ 1 + \frac{e^2}{2Cp} \right] \quad (14)$$

$$\alpha = \alpha_\alpha = \frac{\omega_c e^2}{V_0 2Cp} \quad (15)$$

$\omega_D$  is the frequency above which the wave length is sufficiently short for diffusion to smooth out carrier density fluctuations associated with the periodicity of the stress wave.

Expression (11) and (12) are obtained on the assumption that  $\frac{e^2}{Cp}$  is small.

In the vicinity of  $\omega = \omega_c$ , a simple relaxation –type dispersion occurs. It should be emphasized that the relaxation frequency is given by the conductivity of the material.

When carrier diffusion is taken into account, the complete expression for velocity and attenuation become

$$V = V_0 \left[ 1 + \frac{e^2}{2Cp} \frac{1+(\omega_c/\omega_D)+\left(\frac{\omega}{\omega_D}\right)^2}{1+2\left(\frac{\omega_c}{\omega_D}\right)+\left(\frac{\omega}{\omega_D}\right)^2+\left(\frac{\omega_c}{\omega}\right)^2} \right] \quad (16)$$

$$\alpha = \frac{\omega}{V_0} \frac{e^2}{2Cp} \left[ \frac{\omega_c/\omega}{1+2(\omega_c/\omega_D)+\left(\frac{\omega}{\omega_D}\right)^2+\left(\frac{\omega_c}{\omega}\right)^2} \right] \quad (17)$$

In this case, for  $\omega_D \gg \omega_c$ , expression (12) and (13) retain their validity for all frequencies, except that  $\alpha$  approaches a constant value  $\left(\frac{\omega_c}{V_0}\right) \left(\frac{e^2}{2Cp}\right)$  in the frequency range between  $\omega_c$  and  $\omega_D$ , and drops to zero as  $\omega$  becomes larger than  $\omega_D$ . For  $\omega_c \gg \omega_D$ , the velocity and attenuation may be expressed by:

$$V = V_0 \left[ 1 + \frac{e^2}{2Cp} \frac{(1+\omega^2/\omega_D\omega_c)}{2+(\omega^2/\omega_D\omega_c)+(\omega_D\omega_c/\omega^2)} \right] \quad (18)$$

And

$$\alpha = \frac{\omega}{V_0} \frac{e^2}{2Cp} \left( \frac{\omega_D/\omega}{2+(\omega^2/\omega_D\omega_c)+(\omega_D\omega_c/\omega^2)} \right) \quad (19)$$

The maximum velocity change occurs at the frequency  $\omega = \left(\frac{\omega_D}{\omega_c}\right)^{1/2}$ , whereas the frequency corresponds to maximum attenuation is

$$\omega = \left(\frac{\omega_D\omega_c}{3}\right)^{\frac{1}{2}}$$

Ultrasonic radiation pressure has been discussed analytically [13].

### III. CONCLUSION

Discussion of the interaction of ultrasonic waves with lattice vibrations or with defects in a solid follows a pattern very close to that of thermal conductivity theory. The interaction or coupling between ultrasonic waves and conduction electrons proceeds through the absorption and emission of phonons. At sufficiently low temperature there is also energy transfer from ultrasonic waves to conduction electrons.

Electrons are coupled to the lattice and interact with stress waves in at least two distinct ways. Lattice waves interact with ultrasonic stress waves, and nuclear quadrupole coupling link the nucleus with the lattice and hence with ultrasonic stress waves.

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# Effect of Inorganic and Organic Fertilisers on Walnut Quality and Leaf Macro Nutrient Status

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## I. INTRODUCTION

Supply of all the essential plant nutrients in sufficient quantity and appropriate proportion is one of the major factors controlling the nut quality and leaf nutrient status. Production of fruit crops has undergone enormous change due to continuous use of inorganic fertilizer over a long period causing serious damage to soil fertility, environment and health. Sustainability in horticulture with respect to maintenance of soil fertility and stabilized fruit production is the main concern in the present situation. Hence, there is a need to think of alternate source of safe fertilizers which may improve quality and leaf nutrient status without having adverse effects on soil properties. The high nutrient requirement of fruit crops can be met through an integrated use of organic manures and chemical fertilizer since organic manures in INM generally improve the physical, chemical and biological properties of the soil along with its moisture holdings capacity which results in enhanced crop productivity and the quality of crop produced. Therefore, present investigation will be carried out to standardize integrated nutrient management programme for sustainable walnut production as no systemic work has been done in relation to walnut nutrient management.

## II. MATERIAL AND METHODS

The experiment consisted of four selections [SKAU/002 (S1), SKAU/008 (S2), SKAU/024 (S2) and SKAU/040 (S2)] and six treatments [T1 (NPK recommended as per package of practices through inorganic fertilizers), T2 {100 % through manure (FYM 50% + vermicompost 25% + poultry manure 25%)}, T3 (75% NPK through inorganic fertilizers + 25 % through FYM), T4 (75 % NPK through inorganic fertilizers + 25 % through vermicompost), T5 (75 % NPK through inorganic fertilizers + 25 % through poultry manure) and T6 {75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3

vermicompost + 1/3 poultry manure)} replicated thrice in Factorial Randomised Block Design during 2011 and 2012. The observations were recorded on kernel weight, Kernel percentage, Kernel fill, kernel protein content and kernel oil content by following standard procedures. Leaf samples from walnut trees were collected and analysed for macronutrient status.

## III. RESULTS AND DISCUSSION

The results obtained in present study indicate that kernel weight, kernel protein and kernel fill were significantly affected by different fertilizer treatments. Maximum kernel protein content was found in treatment T4 which differ significantly from treatments T1, T2, T3 and T6 but is statistically at par with treatment T5. The improvement in nut quality might be due to improvement in physical properties of soil and increase growth of micro-organisms. The maximum kernel protein content in treatment T4 might be due to the fact that protein is made up of amino acid which is mostly constituent of nitrogen. Treatment T4 enhanced the uptake of nitrogen which must have assimilated in amino acid and finally into protein. The increase in oil content under combined fertiliser application may be due to increased availability of micronutrients and K that help in converting primary fatty acids to their end products by increased activity of acetyl CO-A. This increase in nut parameter with combined application of vermicompost and inorganic fertilisers might be due to the fact that vermicompost would have improved soil texture and provided micronutrients such as zinc, iron, copper, manganese etc. and better microbial establishment in the soil. The biological activity of the micro-organism would have helped the soil to become ready to serve zone for essential nutrients to plant root system. Zinc is involved in the biochemical synthesis of the most important phytohormone IAA through the pathways of conversion of tryptophan to IAA. Iron is involved in the chlorophyll synthesis besides being part of co-enzymes of respiratory chain reaction. Copper and manganese are important activators of co-enzymes. Organic manures in combination

with inorganic fertilisers must have helped in metabolic changes through the supply of such important micro-nutrients and enzyme activation which ultimately must have improved nut parameters.

Among selections S1 showed highest kernel weight, which differed significantly from S3 and S4 but is at par with S2. Kernel percentage was maximum in S1 followed by S3 and S2. This difference in nut parameters among different selections might be due to their genetic make-up. Different selections showed marked differences with regard to, kernel protein content and maximum protein content was observed in selection S2 which differed significantly from S1, S2 and S3. Kernel fat content was highest in selection S2 followed by S1, S4 and S3. This difference in quality parameters of nut may be due to genetic constitution of individual selections.

Maximum leaf nitrogen content was observed in treatment T4 followed by T5, T1 and T3 while lowest was recorded in treatment T2 and T6. Phosphorus content of leaves differed significantly with different fertiliser treatments and recorded higher P content in treatment T2 which showed marked difference with all other treatments but is statistically at par with treatment T4. Potassium content also showed marked difference among different treatments. The highest K content was observed in treatment T4 followed by T1 and T5, while as lowest was found in treatment T6. The highest leaf N content in treatment T4 might be due to the fact that application of vermicompost alongwith NPK must have enhanced mineralization of organic nitrogen thus making more nitrogen available to the plants. Higher nitrogen can also be attributed to the improvement in soil aeration, better soil moisture retention in root zone, increased microbial nitrogen fixation due to the conjoint application and improved its availability to the plants. The addition of vermicompost improves physical properties of soil, moisture retention in soil rhizosphere, improved root development by mycelial network of arbuscular mycorrhizal fungi, thus increased the water absorption and hence improved nutrient contents of leaf Phosphorus applied to the soil in inorganic form get fixed but addition alongwith organic manure release P slowly due to microbial culture present in the soil, solubilised the fixed phosphorus and make it easily and readily available to plants.

It is evident from the data that maximum leaf calcium was observed in treatment T4 followed by T5 and T3, while as lowest was found in treatment T1. Highest magnesium content was recorded in treatment T4 and minimum in T1. This increased calcium and magnesium content in leaves might be due to the fact that vermicompost is a rich source of calcium and with the application of higher quantity of vermicompost, availability of Ca would have increased hence more leaf Ca.

#### IV. CONCLUSION

Thus it may conclude that conjoint application of organic and inorganic fertilizers showed substantial improvement nut quality and leaf macro nutrient status. Application of 75% RDF through inorganic coupled with 25% vermicompost was the best treatment for optimum nut quality and leaf nutrient status. Among the selections, S2 showed better performance with respect to yield and quality followed by selection S1 in walnut under Kashmir conditions.

##### *Effect of integrated nutrient management on kernel weight (g) in walnut*

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	6.40	6.73	5.70	6.37	<b>6.30</b>	6.53	6.73	5.84	6.43	<b>6.38</b>	6.47	6.73	5.77	6.40	<b>6.34</b>
T <sub>2</sub>	6.15	6.07	5.53	5.71	<b>5.87</b>	6.61	6.13	5.70	5.89	<b>6.08</b>	6.38	6.10	5.62	5.80	<b>5.97</b>
T <sub>3</sub>	6.07	6.55	5.78	5.99	<b>6.10</b>	6.67	6.68	5.78	6.46	<b>6.40</b>	6.37	6.62	5.78	6.23	<b>6.25</b>
T <sub>4</sub>	7.46	7.40	6.87	6.66	<b>7.10</b>	7.53	7.50	6.97	7.00	<b>7.25</b>	7.50	7.45	6.92	6.83	<b>7.17</b>
T <sub>5</sub>	7.21	6.52	6.57	5.90	<b>6.55</b>	7.21	6.76	6.63	6.07	<b>6.67</b>	7.21	6.64	6.60	5.99	<b>6.61</b>
T <sub>6</sub>	6.47	6.15	5.86	5.67	<b>6.04</b>	6.70	6.39	5.89	6.01	<b>6.25</b>	6.58	6.27	5.88	5.84	<b>6.14</b>
Mean	<b>6.63</b>	<b>6.57</b>	<b>6.05</b>	<b>6.05</b>		<b>6.88</b>	<b>6.70</b>	<b>6.14</b>	<b>6.31</b>		<b>6.75</b>	<b>6.63</b>	<b>6.09</b>	<b>6.18</b>	
C.D.S0.05 (S)	0.27					0.37					0.21				
C.D.S0.05 (T)	NS					0.48					0.45				
C.D.S0.05 (S×T)	NS					NS					NS				

T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002

T<sub>2</sub> = 100% through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008

T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25% through manure (FYM) S<sub>3</sub> = SKAU/024

T<sub>4</sub> = 75% NPK through inorganic fertilizers + 25% through manure (vermicompost) S<sub>4</sub> = SKAU/040

T<sub>5</sub> = 75% NPK through inorganic fertilizers + 25% through manure (poultry manure)

T<sub>6</sub> = 75% NPK through inorganic fertilizers + 25% through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

##### *Effect of integrated nutrient management on kernel percentage in walnut*

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	56.99	52.18	53.57	52.22	<b>53.74</b>	56.76	52.45	53.71	52.31	<b>53.81</b>	56.88	52.32	53.64	52.27	<b>53.78</b>
T <sub>2</sub>	57.01	54.20	53.53	50.71	<b>53.86</b>	57.01	54.20	53.53	52.47	<b>54.30</b>	57.01	54.20	53.53	51.59	<b>54.08</b>
T <sub>3</sub>	54.05	52.69	52.59	52.20	<b>52.88</b>	54.80	53.13	52.73	52.40	<b>53.27</b>	54.43	52.91	52.66	52.30	<b>53.07</b>
T <sub>4</sub>	58.01	55.43	55.05	53.32	<b>55.45</b>	58.14	55.62	55.37	53.55	<b>55.67</b>	58.08	55.53	55.21	53.43	<b>55.56</b>
T <sub>5</sub>	56.77	54.33	54.16	52.40	<b>54.42</b>	56.85	54.22	54.44	52.60	<b>54.53</b>	56.81	54.28	54.30	52.50	<b>54.47</b>
T <sub>6</sub>	53.96	53.35	53.71	52.35	<b>53.34</b>	55.46	54.01	53.81	52.57	<b>53.96</b>	54.71	53.68	53.76	52.46	<b>53.65</b>
Mean	<b>56.13</b>	<b>53.70</b>	<b>53.77</b>	<b>52.20</b>		<b>56.50</b>	<b>53.94</b>	<b>53.93</b>	<b>52.65</b>		<b>56.32</b>	<b>53.82</b>	<b>53.85</b>	<b>52.43</b>	
C.D.S0.05 (S)	1.76					1.56					1.18				
C.D.S0.05 (T)	NS					NS					NS				
C.D.S0.05 (S×T)	NS					NS					NS				

## Effect of Inorganic and Organic Fertilisers on Walnut Quality and Leaf Macro Nutrient Status

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
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 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### Effect of integrated nutrient management on kernel protein content (%) in walnut

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	16.26	16.84	15.41	16.21	<b>16.18</b>	16.19	16.8	15.52	15.28	<b>15.95</b>	16.23	16.82	15.47	15.74	<b>16.06</b>
T <sub>2</sub>	14.41	15.48	15.07	14.33	<b>14.82</b>	15.15	15.48	15.23	15.33	<b>15.30</b>	14.78	15.48	15.15	14.83	<b>15.06</b>
T <sub>3</sub>	15.26	16.35	16.57	16.49	<b>16.17</b>	15.17	16.55	16.75	16.29	<b>16.19</b>	15.21	16.45	16.66	16.39	<b>16.18</b>
T <sub>4</sub>	18.79	18.81	17.58	17.8	<b>18.25</b>	18.85	19.07	17.63	17.97	<b>18.38</b>	18.82	18.94	17.61	17.88	<b>18.31</b>
T <sub>5</sub>	16.92	17.95	17.14	17.13	<b>17.29</b>	17.21	17.85	17.14	17.23	<b>17.36</b>	17.06	17.9	17.14	17.18	<b>17.32</b>
T <sub>6</sub>	15.32	16.74	15.29	16.1	<b>15.86</b>	15.52	16.75	15.39	16.27	<b>15.98</b>	15.42	16.75	15.34	16.18	<b>15.92</b>
Mean	<b>16.16</b>	<b>17.03</b>	<b>16.18</b>	<b>16.34</b>		<b>16.35</b>	<b>17.08</b>	<b>16.28</b>	<b>16.39</b>		<b>16.25</b>	<b>17.05</b>	<b>16.23</b>	<b>16.37</b>	
C.D <sub>50.05</sub> (S)	0.70					0.62					0.58				
C.D <sub>50.05</sub> (T)	1.45					1.85					1.22				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### Effect of integrated nutrient management on kernel fat content (%) in walnut

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	57.34	58.12	55.93	56.57	<b>56.99</b>	57.6	58.15	56.09	56.67	<b>57.13</b>	57.47	58.13	56.01	56.62	<b>57.06</b>
T <sub>2</sub>	54.97	56.23	51.73	55.9	<b>54.71</b>	57.26	58.16	56.31	56.16	<b>56.97</b>	56.11	57.19	54.02	56.03	<b>55.84</b>
T <sub>3</sub>	59.63	59.03	56.89	57.47	<b>58.26</b>	59.13	59.17	57.3	57.57	<b>58.29</b>	59.38	59.1	57.1	57.52	<b>58.27</b>
T <sub>4</sub>	61.4	62.76	57.47	59.07	<b>60.18</b>	61.83	63.22	57.67	59.66	<b>60.60</b>	61.62	62.99	57.57	59.37	<b>60.39</b>
T <sub>5</sub>	59.01	60.27	57.27	58.21	<b>58.69</b>	59.01	60.58	57.27	58.35	<b>58.80</b>	59.01	60.43	57.27	58.28	<b>58.75</b>
T <sub>6</sub>	58.21	58.2	52.78	56.36	<b>56.39</b>	58.34	58.34	54.52	56.61	<b>56.95</b>	58.28	58.27	53.65	56.49	<b>56.67</b>
Mean	<b>58.43</b>	<b>59.1</b>	<b>55.35</b>	<b>57.26</b>		<b>58.86</b>	<b>59.6</b>	<b>56.33</b>	<b>57.5</b>		<b>58.64</b>	<b>59.35</b>	<b>55.94</b>	<b>57.38</b>	
C.D <sub>50.05</sub> (S)	2.55					2.21					1.65				
C.D <sub>50.05</sub> (T)	NS					1.98					1.24				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

### Effect of integrated nutrient management on leaf nitrogen (%)

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	2.73	2.75	2.75	2.73	<b>2.74</b>	2.74	2.76	2.75	2.75	<b>2.75</b>	2.73	2.75	2.75	2.74	<b>2.74</b>
T <sub>2</sub>	2.66	2.64	2.68	2.64	<b>2.66</b>	2.74	2.76	2.78	2.76	<b>2.76</b>	2.70	2.70	2.73	2.70	<b>2.71</b>
T <sub>3</sub>	2.69	2.73	2.68	2.68	<b>2.70</b>	2.73	2.75	2.74	2.75	<b>2.74</b>	2.71	2.74	2.71	2.72	<b>2.72</b>
T <sub>4</sub>	2.77	2.78	2.75	2.78	<b>2.77</b>	2.78	2.80	2.83	2.83	<b>2.81</b>	2.78	2.79	2.79	2.81	<b>2.79</b>
T <sub>5</sub>	2.71	2.75	2.74	2.75	<b>2.74</b>	2.73	2.76	2.76	2.78	<b>2.76</b>	2.72	2.76	2.75	2.77	<b>2.75</b>
T <sub>6</sub>	2.68	2.69	2.68	2.72	<b>2.69</b>	2.70	2.73	2.76	2.75	<b>2.74</b>	2.69	2.71	2.72	2.74	<b>2.71</b>
Mean	<b>2.71</b>	<b>2.72</b>	<b>2.71</b>	<b>2.72</b>		<b>2.74</b>	<b>2.76</b>	<b>2.77</b>	<b>2.77</b>		<b>2.72</b>	<b>2.74</b>	<b>2.74</b>	<b>2.75</b>	
C.D <sub>50.05</sub> (S)	NS					NS					NS				
C.D <sub>50.05</sub> (T)	0.06					0.04					0.04				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### Effect of integrated nutrient management on leaf phosphorus (%)

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	0.25	0.24	0.25	0.24	<b>0.25</b>	0.26	0.25	0.26	0.25	<b>0.26</b>	0.25	0.26	0.24	0.25	<b>0.25</b>
T <sub>2</sub>	0.29	0.29	0.26	0.26	<b>0.28</b>	0.3	0.3	0.31	0.3	<b>0.30</b>	0.3	0.29	0.29	0.28	<b>0.29</b>
T <sub>3</sub>	0.24	0.23	0.25	0.25	<b>0.24</b>	0.25	0.25	0.25	0.26	<b>0.25</b>	0.25	0.24	0.25	0.25	<b>0.25</b>
T <sub>4</sub>	0.21	0.80	0.22	0.23	<b>0.36</b>	0.27	0.26	0.27	0.28	<b>0.27</b>	0.24	0.53	0.25	0.25	<b>0.32</b>
T <sub>5</sub>	0.27	0.26	0.25	0.25	<b>0.26</b>	0.28	0.27	0.26	0.28	<b>0.27</b>	0.27	0.27	0.26	0.27	<b>0.27</b>
T <sub>6</sub>	0.25	0.25	0.25	0.22	<b>0.24</b>	0.26	0.26	0.26	0.27	<b>0.26</b>	0.26	0.25	0.25	0.25	<b>0.25</b>
Mean	<b>0.25</b>	<b>0.34</b>	<b>0.25</b>	<b>0.24</b>		<b>0.27</b>	<b>0.28</b>	<b>0.27</b>	<b>0.26</b>		<b>0.26</b>	<b>0.31</b>	<b>0.26</b>	<b>0.25</b>	
C.D <sub>50.05</sub> (S)	NS					NS					NS				
C.D <sub>50.05</sub> (T)	0.07					0.02					0.02				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

## Effect of Inorganic and Organic Fertilisers on Walnut Quality and Leaf Macro Nutrient Status

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### *Effect of integrated nutrient management on leaf potassium (%)*

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	1.63	1.66	1.66	1.68	<b>1.66</b>	1.64	1.66	1.67	1.69	<b>1.67</b>	1.63	1.66	1.67	1.69	<b>1.66</b>
T <sub>2</sub>	1.42	1.43	1.36	1.44	<b>1.41</b>	1.64	1.69	1.68	1.71	<b>1.68</b>	1.53	1.56	1.52	1.57	<b>1.55</b>
T <sub>3</sub>	1.55	1.49	1.47	1.51	<b>1.51</b>	1.59	1.51	1.57	1.60	<b>1.57</b>	1.57	1.50	1.52	1.56	<b>1.54</b>
T <sub>4</sub>	1.70	1.69	1.69	1.68	<b>1.69</b>	1.71	1.74	1.74	1.71	<b>1.73</b>	1.70	1.71	1.72	1.70	<b>1.71</b>
T <sub>5</sub>	1.56	1.64	1.65	1.64	<b>1.62</b>	1.61	1.67	1.69	1.68	<b>1.66</b>	1.59	1.66	1.67	1.66	<b>1.64</b>
T <sub>6</sub>	1.52	1.51	1.47	1.55	<b>1.51</b>	1.59	1.53	1.57	1.58	<b>1.57</b>	1.55	1.52	1.52	1.56	<b>1.54</b>
Mean	<b>1.56</b>	<b>1.57</b>	<b>1.55</b>	<b>1.58</b>		<b>1.63</b>	<b>1.62</b>	<b>1.66</b>	<b>1.67</b>		<b>1.59</b>	<b>1.60</b>	<b>1.61</b>	<b>1.62</b>	
C.D <sub>50.05</sub> (S)	NS					NS					NS				
C.D <sub>50.05</sub> (T)	<b>0.16</b>					<b>0.04</b>					<b>0.05</b>				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### *Effect of integrated nutrient management on leaf Magnesium (%)*

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	0.5	0.49	0.51	0.5	<b>0.50</b>	0.51	0.5	0.52	0.51	<b>0.51</b>	0.51	0.5	0.51	0.51	<b>0.51</b>
T <sub>2</sub>	0.52	0.52	0.52	0.52	<b>0.52</b>	0.53	0.53	0.53	0.53	<b>0.53</b>	0.53	0.53	0.52	0.53	<b>0.53</b>
T <sub>3</sub>	0.53	0.54	0.53	0.54	<b>0.54</b>	0.54	0.55	0.54	0.54	<b>0.54</b>	0.54	0.55	0.54	0.54	<b>0.54</b>
T <sub>4</sub>	0.55	0.55	0.54	0.56	<b>0.56</b>	0.57	0.57	0.56	0.57	<b>0.57</b>	0.56	0.56	0.55	0.57	<b>0.56</b>
T <sub>5</sub>	0.57	0.55	0.54	0.55	<b>0.55</b>	0.57	0.55	0.55	0.56	<b>0.56</b>	0.57	0.55	0.55	0.56	<b>0.55</b>
T <sub>6</sub>	0.52	0.51	0.52	0.51	<b>0.52</b>	0.53	0.52	0.53	0.52	<b>0.53</b>	0.53	0.52	0.52	0.52	<b>0.52</b>
Mean	<b>0.54</b>	<b>0.52</b>	<b>0.53</b>	<b>0.53</b>		<b>0.56</b>	<b>0.53</b>	<b>0.54</b>	<b>0.54</b>		<b>0.55</b>	<b>0.53</b>	<b>0.53</b>	<b>0.54</b>	
C.D <sub>50.05</sub> (S)	NS					NS					NS				
C.D <sub>50.05</sub> (T)	NS					<b>0.05</b>					NS				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

- T<sub>1</sub> = NPK (recommended as per package of practices) through inorganic fertilizers S<sub>1</sub> = SKAU/002  
 T<sub>2</sub> = 100 % through manure (FYM 50%+ vermicompost 25%+ poultry manure 25%) S<sub>2</sub> = SKAU/008  
 T<sub>3</sub> = 75% NPK through inorganic fertilizers + 25 % through manure (FYM) S<sub>3</sub> = SKAU/024  
 T<sub>4</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (vermicompost) S<sub>4</sub> = SKAU/040  
 T<sub>5</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (poultry manure)  
 T<sub>6</sub> = 75 % NPK through inorganic fertilizers + 25 % through manure (1/3 FYM + 1/3 vermicompost + 1/3 poultry manure)

### *Effect of integrated nutrient management on leaf calcium (%)*

Treatment	2011					2012					Pooled				
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
T <sub>1</sub>	2.26	2.24	2.25	2.24	<b>2.25</b>	2.27	2.25	2.26	2.25	<b>2.26</b>	2.27	2.25	2.26	2.24	<b>2.25</b>
T <sub>2</sub>	2.31	2.31	2.31	2.32	<b>2.31</b>	2.37	2.38	2.35	2.34	<b>2.36</b>	2.34	2.35	2.33	2.33	<b>2.34</b>
T <sub>3</sub>	2.43	2.42	2.42	2.44	<b>2.43</b>	2.44	2.42	2.43	2.45	<b>2.44</b>	2.44	2.42	2.43	2.45	<b>2.43</b>
T <sub>4</sub>	2.57	2.58	2.59	2.57	<b>2.58</b>	2.58	2.59	2.61	2.59	<b>2.59</b>	2.58	2.58	2.6	2.58	<b>2.58</b>
T <sub>5</sub>	2.54	2.56	2.54	2.57	<b>2.55</b>	2.55	2.56	2.55	2.58	<b>2.56</b>	2.55	2.56	2.55	2.58	<b>2.56</b>
T <sub>6</sub>	2.45	2.43	2.44	2.44	<b>2.44</b>	2.46	2.44	2.45	2.45	<b>2.45</b>	2.46	2.44	2.45	2.44	<b>2.45</b>
Mean	<b>2.43</b>	<b>2.42</b>	<b>2.43</b>	<b>2.43</b>		<b>2.45</b>	<b>2.43</b>	<b>2.44</b>	<b>2.44</b>		<b>2.45</b>	<b>2.42</b>	<b>2.43</b>	<b>2.44</b>	
C.D <sub>50.05</sub> (S)	NS					NS					NS				
C.D <sub>50.05</sub> (T)	NS					<b>0.24</b>					NS				
C.D <sub>50.05</sub> (S×T)	NS					NS					NS				

# Quantitative Analysis of Methyl-Coenzyme M Reductase (MCRA) Gene in a Biogas Producing Reactor Treating Brewery Wastewater

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**Abstract:**— Among the organisms that are involved in digestion of organic matter degradation methanogens are the major microbial group responsible for methane production. This study quantify the concentration of total bacteria and methyl-coenzyme M reductase  $\alpha$ -subunit (*mcrA*) gene, specific functional gene for methane-producing Archaeal in an anaerobic reactor treating brewery wastewater using quantitative real-time polymerase chain reaction (Q-PCR). Primer sets targeting *mcrA* gene and total bacteria were used to detect and quantify the concentration present in the sludge samples. Q-PCR results showed that a high amount of *mcrA* gene that codes for the functional enzyme in methane producing Archaea are present in the reactor. However, the ratio of Archaea to bacteria concentration is lower and this revealed that the quantity of methane producing communities need to be enhanced in this reactor, in order to increase biofuel production. The results further increased our understanding on the ability of methanogens to grow in high concentration at an optimum reactor performance in anaerobic condition to transform organic substrate present in industrial wastes into biogas as source of renewable energy.

**Keywords** — Archaea, brewery wastewater, methanogens, quantitative PCR

## I. INTRODUCTION

Anaerobic treatment of wastewater have been widely adopted for the treatment of high strength wastewater using different anaerobic technology and for a proper functioning of any bioreactor, the biodiversity of the microbial community is very important [1]. Brewery wastewater consist of complex organic compounds and the breakdown of these organic matters during anaerobic digestion process using UASB reactor involves the ultimate action of several groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic bacteria) through a variety of intermediates to biogas production [2,3,16,17]. Due to the huge structural complexity of the granular sludge, it is hard to assess the diversity, colonization and topological distribution of these groups of microorganisms using normal conventional methods (isolation, plate-counting, etc). Advances in understanding the microbial ecology of anaerobic systems are needed for an efficient and better effluent quality as well as to enhance bioenergy production. Knowledge on the quantification of microbial communities using culture-independent molecular tools to determine the impact of shallow reactor on microbial concentration Granules from a full-scale UASB reactor treating brewery wastewater in Durban, South Africa was investigated in this study. Sample collection and treatment was earlier

described in [6]. The direct isolation of total genomic DNA from granular sludge samples was carried out according to phenol-chloroform extraction method described by [7]. The concentration of the DNA was checked by Nanodrop ND-1000 Spectrophotometer. The purified DNA

Target group	Target microorganisms	Primer names	Sequences(5'–3')	Reference
<i>McrA</i>	Functional gene for	MLf	GGTGGGTGTMGGATTCACARTAYGCWAC	[15]
	methanogenic	MLr	AGC	
	Archaea		TTCATTGCRTAGTTWGGRTAGTT	
16S rDNA	Bacterial	27f	AGA GTT TGA TCM TGG CTC AG	[9]
		1492r	TAC GGY TAC CTT GTT ACG ACT T	

molecular techniques, such as the rRNA-approach: FISH, cloning and sequencing of 16S-rRNA genes [2]; DGGE: denaturing gradient gel electrophoresis and pyrosequencing [18,20] are been used to know the interaction of bacterial populations of anaerobic sludge granules [5,11,19,20]. Little work has been done on the distribution and quantity of microbial community across shallow reactors. The aim of this study was to quantify the spatial distribution of microbial communities especially methyl coenzyme M reductase (*mcrA*) genes. The functional genes that are responsible for methanogenic activity during fermentation and anaerobic digestion of wastes using Q-PCR as compared to the total bacteria.

## II. MATERIALS AND METHODS

A. Sample Collection and Genomic DNA Extraction from Granular Sludge Sample

B. Amplification of Genomic DNA Using Quantitative PCR Purified PCR amplicons for methyl coenzyme M reductase (*mcrA*) genes and total bacteria were used as a template for the standard curve. DNA from each amplicon was diluted a 10-fold and bacteria series concentration using PCR grade water. Range of 10<sup>1</sup> to 10<sup>8</sup> target DNA copies/ $\mu$ l was generated and analyzed in duplicate by qPCR with its corresponding primer set. Quantitative real-time PCR (qPCR) to quantify 16S rRNA copies in DNA extracted from the samples were performed using a thermal Cycler instrument (C-1000 Touch, CFX 96, Biorad Laboratories Pty Ltd, USA) using two primer sets targeting domain bacteria and *mcrA* gene (Table 1). QPCR reaction mixture for the amplification was carried out in a final volume of 20 $\mu$ l containing PCR-grade water, 1  $\mu$ l of each primer (final concentration, 10  $\mu$ M), 10  $\mu$ l of the Sso fast Eva green Master Mix (Biorad Laboratories Pty Ltd, USA) and 4  $\mu$ l of template DNA. Two-step amplification of the target DNA was carried out using the protocol described by [6] as follows: initial denaturation for 3.5 min at 94°C followed by 40 cycles at 95°C for 30 s and annealing at 55°C for 30 s and final extension with image capturing at 72°C for 30s. The temperature was increased at 0.5°C every 10 s from 40 to 95°C for melting curve analysis. Each QPCR assay was conducted in duplicates. For all experiments, appropriate negative controls containing no genomic DNA were subjected to the same procedure to exclude any possible contamination or carry-over. For each qPCR assay, the value of the logarithmic starting quality for the different 16S rDNA gene were plotted against the threshold cycle (C<sub>q</sub>) numbers and the linear ranges of the standard curves were selected based on the R<sup>2</sup> of the slope greater than 0.990. For quantification of 16S rDNA gene concentration that were present in the DNA obtained from the different compartment, the C<sub>q</sub> values for each sample were compared with the corresponding standard curves. The 16S rDNA gene copy was calculated with the average molecular weight of 660 Da and avogadro's numbers (6.02  $\times$  10<sup>23</sup>) per base pair was of double-stranded DNA [8].

## III. RESULTS AND DISCUSSION

A. Quantification of 16S rDNA gene concentration of microbial community using real-time PCR

The validation and accuracy of quantified the 16S rDNA gene copy numbers were determined using coefficients (R<sup>2</sup>) values of 0.991 and 1.000 respectively for *mcrA* gene

and total bacteria in the reactor. The 16S rDNA gene copies of *mcrA* in the samples were calculated against the total bacterial 16S rDNA gene copies. The compartment showed a noticeable disparity in terms of the composition of bacteria and methanogenic population using real-time PCR (Fig. 1). It is observed that the concentration of *mcrA* gene decreases with an increase in the total bacteria concentration along the compartments of the full-scale reactor. Real-time PCR revealed high percentage of *mcrA* gene in Compartment 1 as compared to bacteria concentration. The percentage of 16S rDNA gene copies of *mcrA* gene per nanogram of sample in Compartment 1 was much higher (34.90%) than the total bacterial (2.62%). Similar variation in spatial distribution of this gene colonizing the lower and middle parts in the anaerobic reactor was reported by Kubota et al. [12]. However, the bacterial concentration increased to 95.80% in the last compartment with a decrease in the amount of *mcrA* 16S rDNA gene copies. Fluctuation in the quantity of bacterial in the different compartments was noticed and this might be as a result of production of metabolites by some group of bacteria to inhibit or suppress the growth of other bacterial in the reactor [14]. The abundance of *mcrA* genes at the lower compartment of the UASB reactor as determined by real-time PCR suggest the presence of lower toxic substances that supports the growth of methanogens in the reactor. The reduction in *mcrA* gene concentration or cell number indicates the production of intermediates metabolites that do not favour or inhibit the growth of methanogens [10,13]. The study further confirms that there is variation in the microbial population in each compartment. It can further be deduced that different compartment in the reactor might be involved in different phases of anaerobic degradation of organic matter in brewery wastewater with different concentration of metabolic products been produced as confirmed by the qPCR assays.

## IV. CONCLUSIONS

Quantitative PCR-based technique produced a better concentration of the microbial consortium present in the UASB reactor treating brewery wastewater. This technique helps us to quantify the microbial population in each compartment and possibly the phases at which anaerobic fermentation takes place in the investigated UASB reactor.

## Quantitative Analysis of Methyl-Coenzyme M Reductase (MCRA) Gene in a Biogas Producing Reactor Treating Brewery Wastewater

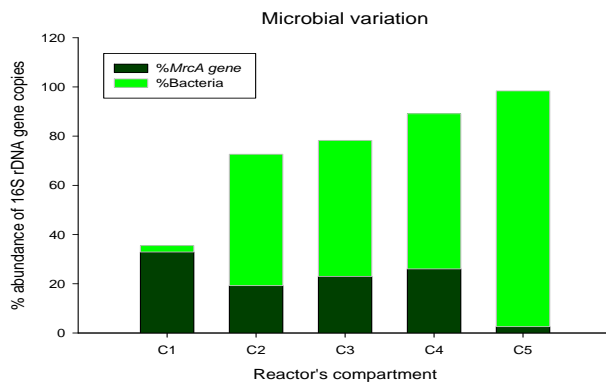


Fig. 1. Comparison of microbial population in samples taken from different compartment of UASB reactor treating brewery wastewater as determined by domain-specific and methyl-coenzyme M reductase 16S rDNA-targeted oligonucleotides primer sets

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# A Review of Lung Cancer in Never Smoking Chinese Women

Shihan Zhang

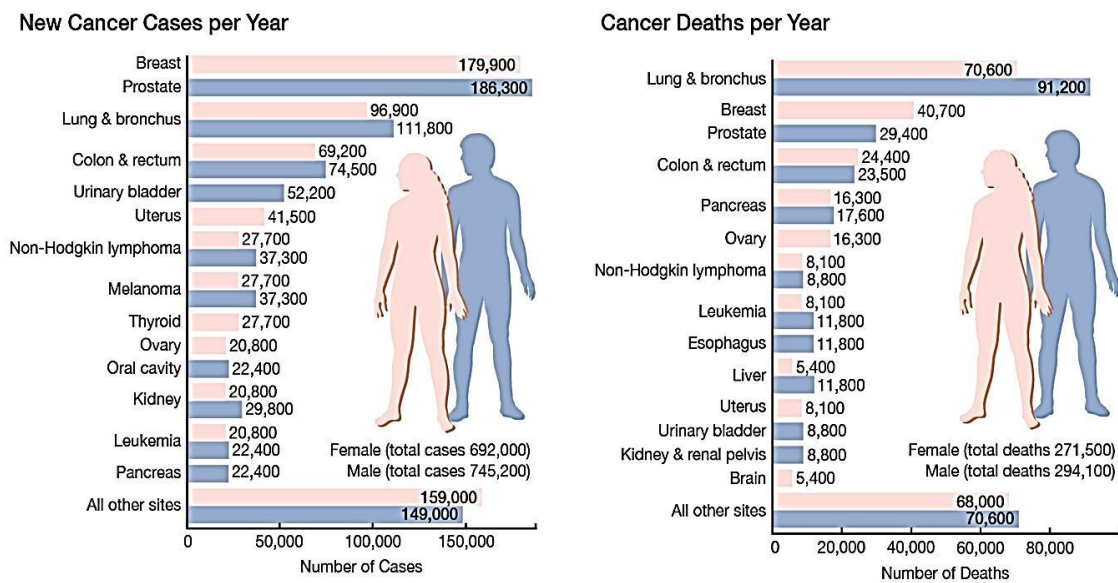
**Abstract:**-- Lung cancer has been a leading cause of cancer deaths among women worldwide, causing about 1.6 million annual deaths. Of all cancer deaths, lung cancer deaths among never smokers have been estimated to be the 7th leading cause of cancer mortality. Chinese women are recognised to have a high incidence of lung cancer despite a low smoking prevalence. Several studies have investigated the risk factors associated with lung cancer. This paper outlines the biology of cancer, discusses the possible causes including carcinogens as well as summarising the current research status and suggesting possible measures of prevention, diagnosis and treatment.

## I. BIOLOGY OF CANCER

Cancer is a genetic disease derived from a single cell with changes in DNA sequences of the critical genes such as tumour suppressor genes and oncogenes by mutagens, such as UV radiation, chemicals and viruses (Hanahan and Weinberg, 2011). A group of mutagens that can cause cancer are called carcinogens, which implies that not all mutations result in cancer. Mutations are also introduced naturally during DNA replication and accumulate over time, resulting in a progressive and subtle divergence of the DNA sequence from the original copy from the

fertilized egg to cancerous mutations causing cancer cells to grow independently and continually in an unrestrained manner (Hanahan and Weinberg, 2011). A collection of cancer cells forms a tumour, which are able to detach from their primary site, change from benign to malignant tumours and invade the surrounding tissues by metastasis. Metastatic cells are able to survive in the blood circulation and lymphatic system after they break the basement membrane in order to be transported by the circulatory system throughout the body and form secondary tumour in other parts of the body. Cancer cells are categorised by their primary sites (Fig 1) which can affect different tissues and organs in the body such as lung (Fig 2).

### Cancer Incidence and Mortality in the United States\*



\*Estimated numbers from the American Cancer Society, Inc., 2008.

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**Figure 1. Incidence and mortality of different types of cancer in both men and women in the United States. The figure is adopted from <https://www.britannica.com/science/cancer-disease>**



Figure 2. A comparison of lung tissue in healthy (left) and cancerous (right) individuals. The figure is adopted from <http://www.lungcure.in/lung-cancer-treatment-in-delhi-gurgaon.php>

Lung cancer has been a leading cause of cancer deaths among women worldwide over the past decades (Siegel et al., 2014), causing about 1.6 million annual deaths (Brambilla, 2014). Most cancers initiating in the lung, known as primary lung cancers, are carcinomas. They can be categorised as either small-cell lung carcinoma (SCLC) or non-small-cell lung carcinoma (NSCLC) (Neal et al., 2019). Signs and symptoms which may suggest lung cancer include: (Horn and Lovly, 2018) (Fig 2)

- Respiratory symptoms: coughing, coughing up blood, wheezing, or shortness of breath
- Systemic symptoms: weight loss, weakness, fever, or clubbing of the fingernails
- Symptoms due to the cancer mass pressing on adjacent structures: chest pain, bone pain, superior *vena cava* obstruction, or difficulty swallowing.

In addition to the symptoms of lung cancer mentioned above, cancer growth in the airways may obstruct airflow and cause breathing difficulties which can also lead to accumulation of secretions behind the blockage and predispose to pneumonia (Horn and Lovly, 2018). Many of the general cancer symptoms such as poor appetite, weight loss, fever, fatigue are not specific for lung cancer (Lu et al., 2010). In many people, the cancer has already spread beyond the original site to other sites like brain, bone, adrenal glands, opposite lung, liver, pericardium and kidneys by the time they present symptoms such as weight loss, bone pain, and neurological symptoms (headaches, fainting, convulsions, or limb weakness) and seek medical attention (Frederick, 2002; Horn and Lovly, 2018).

## II. FORMATION AND PREVENTION OF LUNG CANCER

Inhalation of tobacco during active cigarettes smoking has been the oldest (Doll and Hill, 1950) and most well-established (Sadik et al., 2001) predominant risk factor for lung cancer (Adeline et al., 2000). It accounts for the vast

majority of cases (85%) of lung cancer (Alberg et al., 2016) and about 10–15% of cases occur in people who have never smoked (Thun et al., 2008). Of all cancer deaths, lung cancer deaths among never smokers have been estimated to be the 7th leading cause of cancer mortality (Siegel et al., 2014). It is estimated that 15% of male patients and 53% female patients of lung cancer are non-smokers worldwide (Shi et al., 2019).

### Lung Cancer Among Non-Smokers

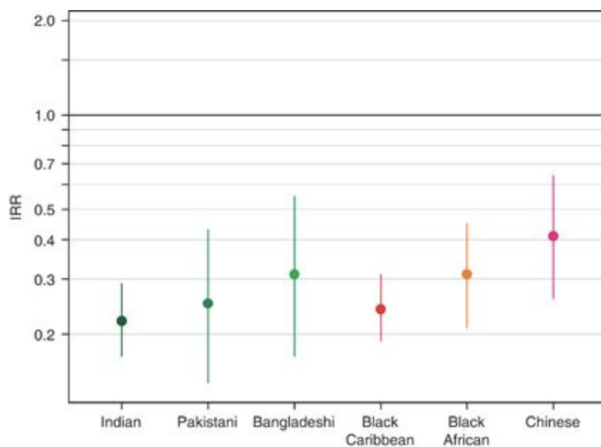
Lung cancer in non-smokers has been rising, showing a different carcinogenic pathway, clinicopathological characteristics, epidemiology and natural history (Ziebarth, 2018). Lung cancer is often caused by a combination of genetic factors and exposure to substances such as radon gas where it takes the leading cause (Darby et al., 2005), second-hand smoke exposures (i.e. passive smoking), asbestos exposure, outdoor air pollution, family history of lung cancer as well as other forms of air pollution such as cooking fumes. (Table 1) (de Groot et al., 2018). Of all risk factors, cooking oil fumes takes the highest estimated risk (odds ratio=2.12, 95% confidence interval) compared with other factors.

Risk Factors of Lung Cancer in Never Smokers Summary	
Non-Modifiable	Modifiable
Family history	Domestic radon exposure
Use of menopausal hormone replacement therapy	Environment tobacco smoke
Patient history of lung cancer include tuberculosis/COPD/emphysema /chronic bronchitis/parenchymal infection	Environmental air pollution
	Occupational exposure
Low socioeconomic status	Cooking oil fumes

	Smoke from domestic combustion for heating and cooking
	Low intake of fruit

**Table 1. A summary of non-modifiable and modifiable risk factors of lung cancer in never-smokers. COPD, Chronic Obstructive Pulmonary Disease.**

However, compared with women of other countries, Chinese women worldwide are known to have a high risk of lung cancer, despite having a low smoking prevalence (Hinds et al., 2006) (Fig 3).



**Figure 3. A graph demonstrating the prevalence of lung cancer among Chinese women compared to women from different ethnicities. The figure is adopted from <http://anthropology.msu.edu/anp204-us18/2018/07/10/week-2-reflection-lung-cancer-among-chinese-women/>**

The incidence of lung cancer among women living in China has been one of the highest in the world corresponding to about 20 per 100000 persons per year. High rates have also been found among Chinese women living in different countries such as Singapore, Hong Kong, Taiwan, United States and Australia (Hinds et al., 2006). Therefore, cigarette smoking solely cannot explain the epidemiologic characteristics of higher lung cancer prevalence in Chinese women and other factors play a significant role (Yin et al., 2015).

### III. LUNG CANCER AND INDOOR POLLUTION

One of the main causes of lung cancer is exposure to cooking oil fumes (de Groot et al., 2018). Stir-frying, which involves heating of oil in a wok to high temperatures before ingredients are added is a common culinary practice among Chinese women. This process involves volatilisation of oils which potentially exposing people to

more fumes than cooking methods from other regions of the world (Zhong et al., 1999). International Agency for Research on Cancer has classified emissions from burning wood or from high-temperature frying as ‘probably carcinogenic to humans (group 2A) (Straif et al., 2006). The risks were strengthened when the fumes were not reduced by an extractor (Ko et al., 2000) or with a poor ventilation.

Research has shown that other sources of indoor air pollution (IAP) besides cooking oil fume exposures is coal combustion, in particular, IAP from coal use in houses is a lung cancer risk factor and is an International Agency for Research on Cancer class 1 carcinogen (Hosgood et al., 2011). This is especially popular in rural and developing populations with high exposure (Armstrong et al., 2004; Barone-Adesi et al., 2012) and poor home ventilation which can increase exposure to carcinogenic particulates thus elevates lung cancer risk (Lan et al., 2002). IAP is a major concern in less developed countries where biomass fuels are used for cooking and heating (Smith et al., 1983). Indoor coal burning increases particulate matter (PM) (Ezzati et al., 2011), polycyclic aromatic hydrocarbons (PAHs), and heterocyclic aromatic compounds (Zhang et al., 2007) in the air, which are associated with lung toxicity and cancer risk. This is an important public health issue in Chinese cities where people may have lower socioeconomic status and live in houses with inadequate kitchen ventilation and using coal for cooking (Kim et al., 2014).

Cooking fumes (CFs) are mainly produced during cooking fuels burning, cooking oil volatilisation and reaction during heating process (Sun et al., 2007). CFs, which have been recognised as the main source of indoor air pollution, are mixtures of many toxic components such as aldehydes, heterocyclic amines, polycyclic PAHs (known carcinogens) (Sun et al., 2007), fat aerosols, trans, trans-2, 4-decadienal (t,t-2,4-DDE) (high-fat frying) and PM, where the composition of them may depend on the types of food, cooking oil and the cooking method (Chunyan et al., 2017). Studies have shown that indoor air pollution from Chinese-style cooking when the cooking oil fumes emitted at high temperatures and smoke from domestic combustion of coal for heating and cooking may cause lung cancer in Chinese women by causing DNA damage or blocking the function of related protein (Chunyan et al., 2017; Zhao et al., 2006).

Lung cancer takes the leading place among diseases being reported caused by CFs exposure, especially adenocarcinoma (Ren et al., 2015). Adenocarcinoma is a type of non-small cell lung cancer which starts in the glands that line the inside of the organ, making up about 40% of lung cancers and the majority of lung cancer in non-smokers (Ren et al., 2015). It is most often found in the outer part of the lungs and grows more slowly than other types of lung cancer. Molecular and biochemical

studies have found that CFs exposure may lead to lung cancer by gene damage, formation of reactive oxygen species, blockage of related proteins' function, and even cell death. (Chunyan et al., 2017). t,t-2,4-DDE in cooking fumes, can induce cell proliferation and cytokine production from oxidative stress (Chang et al., 2005; Wang et al., 2010; Young et al., 2010).

#### **IV. DIAGNOSIS AND TREATMENT OF LUNG CANCER**

Approximately, 50% of never-smoker patients present molecular mutations that may be treatable currently or in the near future via targeted therapies compared to potentially 10% of ever smokers (Sébastien et al., 2012). On the other hand, about 10% of people with lung cancer do not have symptoms at diagnosis; these cancers are incidentally found on routine chest radiography (Collins et al., 2007).

#### **V. NOVEL METHODS TO TREAT LUNG CANCER**

Traditionally, lung cancer was treated in the following three methods (Zappa and Mousa, 2016):

- Removal of cancerous tissue by surgery,
- Treating cancer cells by chemotherapy drugs, and
- Using radiation therapy to treat cancer cells.

Recently, immunotherapy and targeted therapy have been introduced to treat cancer patients (Rybarczyk-Kasiuchnicz and Ramlau, 2018). Immunotherapy fights against cancer cells which escape the immune system by using body's own immune cells. Targeted therapy delivers medications specifically to the target tissue to minimise the side effects and increase the efficiency of the treatment. All the therapies can be performed individually or in combination. For example, surgery can be followed by chemotherapy to ensure all the cancer cells are removed from the site of operation.

#### **VI. SUGGESTED SOLUTIONS**

One of the major problems with IAP is that it has remained under-studied over the past decade. With the advances in urbanisation and increased migration from rural areas to cities, it is assumed that IAP does not pose a threat to the population health. However, sufficient statistical data is not available to support this argument. Therefore, to establish a thorough study of the current status of the lung cancer among non-smoking Chinese women, I suggest the following measures

1- A population level investigation on the epidemiology of indoor pollution among Chinese women - Statistical data should be gathered on the number of people who cook Chinese style foods (including those who cook Chinese food in industrial scales such as restaurant

workers) and use indoor combustion of coal for heating. This can be done by sampling and conducting survey of different population cohort.

2- An investigation for assessing the efficacy of measures to reduce indoor pollution. Data should be collected from people who use effective measures to alleviate indoor air pollution such as using extractors. Surveys could focus on access and utilisation of resources to reduce IAP such as possessing and using the extractors as well as the effectiveness of these devices to minimise IAP.

In addition to the measures to establish the current status of lung cancer among non-smoking Chinese women, this paper suggests that authorities and responsible healthcare organisations can take the following actions:

1- Application and monitoring construction regulations for building houses with sufficient ventilation systems, as well as regulation on cooking industry to optimise the oil ingredients to minimise potential carcinogens.

2- Setting a minimum time duration for advertisements on TV programs and social media about health and measures to prevent lung cancer.

Many people assume the higher lung cancer rate in never-smoking Chinese women can be explained by many reasons. Other than indoor air pollution from domestic practices, outdoor air pollution from factories is a major social issue in China where many people blame the authorities for its under-developed environmental policies.

#### **CONCLUSION**

As discussed above, lung cancer in never smoking Chinese women is particularly prevalent in rural and under-developed areas. Diagnosis can be very difficult in those areas due to a scarce of healthcare access such as professional doctors and equipments. Cultural difference between women from different countries also play a role because some people have different standards when it comes to presenting illness to seek medical attention. Some people choose to not have any treatments even when diagnosed because they cannot afford the high health and travelling expenses and they are not very used to live far from their familiar environment. Therefore, an interdisciplinary approach composed of cultural and medical solutions is required to reduce the incidence and prevalence of lung cancer among non-smoking Chinese women.

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# Effect of Ethanol Extract of Capsicum sp. On The Protoscolices of Sheep Hydatid Cysts and Pathogenic Bacteria

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**Abstract— Background:** Hydatidosis is a zoonotic disease that requires surgery as one of the best methods for treatment, in conjunction with scolicidal agents to prevent the formation of new cystic echinococcosis. Bacterial infection is another risk in hydatidosis surgery. Due to the side effects of chemical scolicidal agents, this study aimed to evaluate the effect of ethanol extract of *Capsicum* sp. on the protoscolices of hydatid cysts and some pathogenic bacteria species *in vitro*.

**Methods:** The hydatid cysts of *Echinococcus* sp. were collected from the livers of infected sheep. The scolicidal effect was determined at different exposure times (5, 10, 15 mins.) and different concentrations (50, 100, 500 mg/ml), and the viability of the protoscolices was detected by 0.1 % eosin staining. The antibacterial activity of the Capsicum ethanol extract was estimated using the agar well diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The secondary metabolites of ethanol extract were assessed using several qualitative tests.

**Results:** The results showed the highest scolicidal efficacy after 15 mins of exposure at all used concentrations with a 22.4%, 19.8% and 0% survival rate respectively. The highest antibacterial activity was recorded against *Staphylococcus aureus* with MIC 125 mg/ml.

**Conclusion:** Capsicum ethanol extract has a good protoscolicidal effect in a short exposure time and could be used as a scolicidal agent. However, *in vivo* studies are necessary to confirm the effect.

**Index Terms— Antibacterial: Capsicum: Ethanol extract: Hydatid cyst: scolicidal agents**

## I. INTRODUCTION

Hydatidosis is a zoonotic parasitic disease in people and domestic animals that causes huge economic damage. Hydatidosis is caused by the larval or adult stages of the *Echinococcus* species, Taeniidae family.<sup>1</sup> Dogs are the final host for the mature stage of *Echinococcus* spp., while humans and sheep are intermediate hosts infected by the ingestion of eggs.<sup>2</sup> The embryophore releases the oncosphere in the stomach, where the enzymatic action and bile assist the oncosphere to penetrate the wall and move to the liver, lungs and sometimes other internal organs to stay and cause Cyst Echinococcus (CE).<sup>3</sup>

The best method for treating CE is surgery with the injection of protoscolicidal agents into the hydatid cyst to avoid the risk of spillage of the protoscolices and formation of a secondary cyst.<sup>4</sup> In addition, bacterial infection of the hydatid cyst can occur and cause destruction of the endocyst and sterilization of the cyst which leads to many hazardous consequences.<sup>5-7</sup>

Many protoscolicidal agents have been used such as silver nitrate, formalin and hypertonic saline to sterilize cysts. However, the use of each agent involves risks of complication such as cholangitis and necrosis. Therefore, it has been suggested that further studies be conducted to find more effective and safer protoscolicidal agents.<sup>8-9</sup>

Medicinal plants have been widely used for over a thousand years to prevent or cure diseases.<sup>10</sup> Recently data have demonstrated that about third of medicines are derived from natural products including plants. Natural products from plants have played an important role in drug discovery as a direct source of medicine, or provide the raw material for the development of semi-synthetic new drugs or as lead molecules. Therefore, evaluation of medicinal plants is an aim in different countries.<sup>10-11</sup>

Capsicum or pepper (red, green, bell and chili) is a part of the Solanaceae family, *Capsicum* genus. In total, 5 out of 40 Capsicum species are consumed by humans as a flavoring, spice or vegetable added to food as a raw or cooked ingredient throughout the world.<sup>12</sup> Capsicum fruit is also used in folk medicine to reduce blood pressure, increase the efficacy of the circulatory system, break down cholesterol, and aid digestion.<sup>13</sup> In addition, it has been used to treat dysentery, diarrhea and stomach ache, toothache, asthma, ulcers, arthritis and for wound healing.<sup>12-14</sup> Many species of Capsicum have been documented as possessing antimicrobial properties against fungi and Gram positive and negative bacteria.<sup>15-16</sup> Dogan *et al.* determined that the methanol extract of *Capsicum annum* was effective on *Helicobacter pylori* and *Campylobacter jejuni*, while its water extract was highly effective on *Arcobacter cryaerophilus*.<sup>17</sup> Moreover, red



Capsicum has displayed effects on metabolic syndrome including obesity, diabetes and lipid mass, and revealed anticarcinogenic, antioxidant and antigenotoxic effects.<sup>18-19</sup> A few pieces of research in the literature have reported the anti-parasitic activity of the Capsicum species. Extracts of *C. frutescens* showed a potential effect against the fish ectoparasite *Ichthyophthirius multifiliis* and anthelmintic activity.<sup>18, 20</sup> Therefore, the aim of this study was to assay the protoscolicidal effects of ethanol extracts of the fruit of *Capsicum sp.* on the protoscolices of sheep hydatid cysts, which has not been studied before, and to investigate its antibacterial activities against two types of pathogenic bacteria.

## II. MATERIALS AND METHODS

### 2.1 Plant Materials:

Fruits of the hot red pepper *Capsicum sp.* were purchased from a local market, dried, ground and extracted using 70% ethanol and a magnetic stirrer for 48 hours each. The extracts were filtered and the solvent was then removed under vacuum using a rotary evaporator.

### 2.2 Phytochemical Analysis:

Ethanol extract of Capsicum was subjected to further analysis to detect the secondary metabolites. 1 g of each plant extract was dissolved in 20 ml of distilled water and filtrated; 1 ml of each filtrate extract was used for the phytochemical tests. The presence of alkaloids was detected using Dragendorff's reagent, flavonoids using alcoholic potassium hydroxide reagent, free amino groups using Ninhydrin, glycosides using Benedict reagent, phenols using ferric chloride and Folin reagent, saponins using mercury chloride reagent and tannins using lead acetate 1% reagent.<sup>21-24</sup>

### 2.3 Protoscolices Collection:

*Hydatid cysts* were sourced from the livers of infected sheep. The cysts were transferred to the laboratories and their *outer surfaces were sterilized with* 70% ethanol before being dissected. The cyst fluid was collected *with a 1 ml syringe and transferred* into a sterile container, The supernatant was *removed and the sediment protoscolices were washed* three times with PBS.<sup>25</sup>

### 2.4 Viability of the Protoscolices:

This test was accomplished by adding 0.1% aqueous eosin to the protoscolices *clean glass slide in a* which was examined under a light microscope. The green *protoscolices were* considered to be *alive* and there *ones were* considered to be dead.<sup>26</sup>

### 2.5 Scolicidal Assay

In this study, 1 ml of three concentrations (50, 100, 500 mg/ml) of ethanol extract of Capsicum, *was placed in a test* tubes. *Protoscolices* sediment was added ( $\approx$  500

protoscolices/ml) and gently *mixed*. The test tubes were incubated for 5, 10 and 15 min at 37 °C for each concentration. Then the supernatant of the solution was *carefully removed*, and one millilitre of 0.1% eosin stain was added *and mixed*. The yielded *protoscolices* were then washed and *examined under light microscope*. For each experiment, minimum of *500 protoscolices* were counted, and *ntreated protoscolices-on* were designed as control groups. The *experiments were* repeated three times. Statistical analysis was carried out using SPSS software version 24. A two-way ANOVA test was used to compare each experiment. The least significant difference (LSD) was used to compare the mean survival rate of protoscolices for all used concentrations, exposure times and the control. A *P value* of less than 0.05 was considered significant.

### 2.6 Bacterial Isolates

Bacterial isolates were obtained from burned patients and identified in the microbiological lab at the Department of Biology, College of Science, University of Basrah, using traditional biochemical tests which were then confirmed by automated vitek<sub>2</sub> compact system.

### 2.7 Antibacterial Activity and Minimal Inhibitory Concentration Assay

This assay was performed using the agar well diffusion method as described in<sup>27</sup>. Wells were formed with a sterilized Pasteur pipette in a nutrient agar plate inoculated with tested bacteria. Bacterial concentrations were adjusted according to McFarland 0.5 standard tube and the final bacterial concentrations were streaked on a solid culture medium using sterilized cotton swabs. After the plates had been dried for 15-20 mins, wells were filled with 100 µl of *ethanol* extract of Capsicum that was dissolved in dimethyl sulphoxide (DMSO), whereas the extract was prepared with serial dilutions (250, 125, 62.5 mg/ml) to determine MIC. The plates were incubated at 37 °C for 24 hours to form zones of inhibition through the agar media. The diameters of the zone of inhibition for plant extract against tested bacteria were measured in millimeters.

## III. RESULTS

### 3.1 Phytochemical Analysis Result:

The phytochemical analysis of ethanol extract of Capsicum fruits showed the presence of alkaloids, flavonoids, free amino groups, phenols, saponins and tannins as shown in

Plant	Compounds					
	Alkaloids	Flavonoids	Free amino group	Glycosides	Phenols	Saponin
<i>Capsicum sp.</i>	+	+	+	-	+	+

Table 1.

### 3.2 The Scolicidal Assay Result:

The percentage of live protoscolices in the *sample was calculated by dividing the number of live* protoscolices

in the sample with the total number of calculated **headings 100**×. **The process was repeated three times and the survival rate was determined.** The viability of the control group of protoscolices was 100% as shown in Table 2.

The results revealed that there were significant differences between the ethanol extract of Capsicum groups and the control group at all concentrations and exposure times ( $p < 0.05$ ).

The highest reduction in protoscolices was at 500 mg/ml with a 23%, 9% and 0% survival rate after 5, 10 and 15

mins of exposure time respectively, with significant differences between the means for survival rates of used concentrations ( $p < 0.05$ ). After 15 mins of exposure time there was high scolicial activity at all concentrations 50, 100, 500 mg/ml with a 22.4%, 19.8% and 0% survival rate respectively with significant differences when compared 5 and 10 mins of exposure time ( $p < 0.05$ ).

The percentage survival rate of the protoscolices decreased with increasing concentrations and exposure times Figure 1.

Plant	Compounds						
	Alkaloids	Flavonoids	Free amino group	Glycosides	Phenols	Saponins	Tannins
<i>Capsicum sp.</i>	+	+	+	-	+	+	+

Table 1: Phytochemical compounds present in the ethanol extracts of Capsicum

Table 2: Protoscolicidal effects of the Capsicum ethanol extract at different concentrations according to the time of exposure

Concentrations mg/ml	% Survival rate after			Mean of concentration	Sig.
	5 min ± SD	10 min ± SD	15 min ± SD		
<b>50</b>	69.60 ± 7.54	56.30 ± 3.67	22.40 ± 3.51	<b>100 ± 0.00</b>	<b>a</b>
<b>100</b>	35.60 ± 3.80	34.40 ± 4.81	19.80 ± 1.65	<b>49.34 ± 21.56</b>	<b>b</b>
<b>500</b>	23.00 ± 2.61	9.00 ± 1.53	0.00 ± 0.00	<b>29.95 ± 8.27</b>	<b>c</b>
<b>Control</b>	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	<b>10.67 ± 10.16</b>	<b>d</b>
<b>Mean of survival rate</b>	<b>57.05 ± 31.63</b>	<b>49.92 ± 34.99</b>	<b>35.55 ± 39.93</b>		
<b>Sig.</b>	<b>a</b>	<b>b</b>	<b>c</b>		

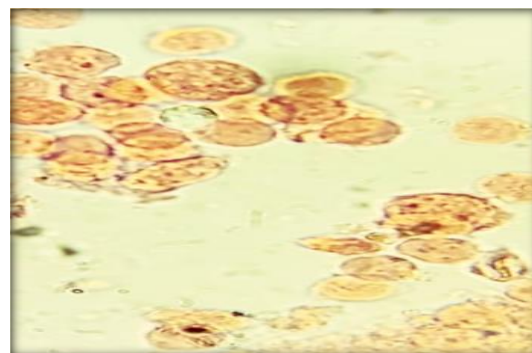
<sup>a, b, c, d</sup> The values are statistically significant at  $P < 0.05$



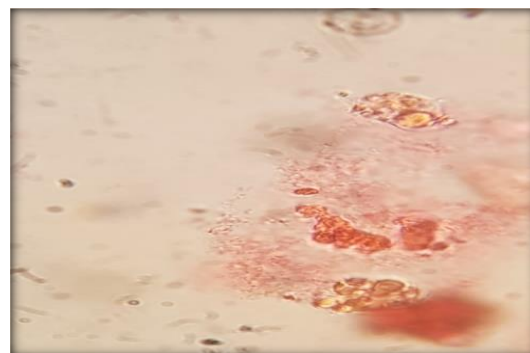
(A)



(B)



(C)



(D)

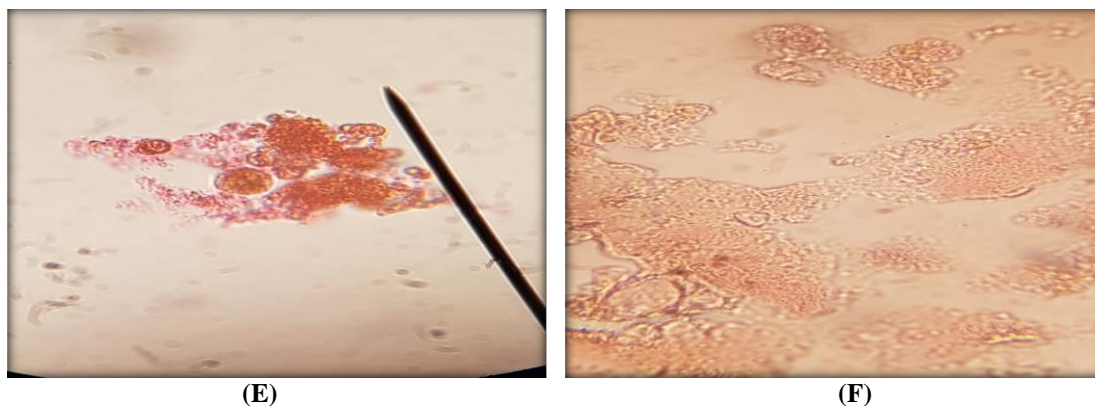


Figure 1: A: hydatid cyst from sheep liver. B: protoscolices of hydatid cyst collected from naturally infected livers of sheep. C: live protoscolices after staining with 0.1% of eosin. D: dead protoscolices treated with 50 mg/ml of ethanol extract of Capsicum Capsicum and stained with 0.1% of eosin. E: treatment with 100 mg/ml of ethanol extract of Capsicum and stained with 0.1% of eosin. F: treatment with 500 mg/ml of ethanol extract of Capsicum and stained with 0.1% of eosin.

### 3.3 Antibacterial Activity Assay and MIC Result:

The current study showed that the ethanol extract of Capsicum had different inhibitory activities against tested pathogens. Whereas it exhibited the highest inhibitory activity against Gram positive bacteria *Staphylococcus aureus* with an MIC value of 125 mg/ml and an inhibition zone of 17 mm, the results did not observe any effect of the plant extract on Gram negative bacteria *Pseudomonas aeruginosa* as shown in Table 3 , Figure 2.

Table 3: Zones of inhibition of ethanol extract of Capsicum against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Bacterial species	Zone of inhibition in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Staphylococcus aureus</i>	17	17	0
<i>Pseudomonas aeruginosa</i>	0	0	0

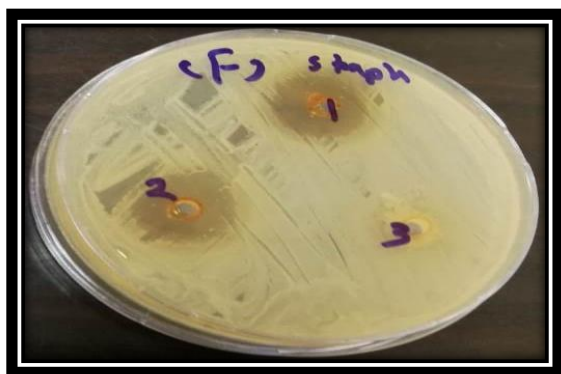


Figure 2: Zone of inhibition of ethanol extract of Capsicum against *Staphylococcus aureus* with

concentrations: 1=250 mg/ml, 2=125mg/ml, 3=62.5 mg/ml.

## IV. DISCUSSION

in spite of the risk of spillage of hydatid fluid and formation of secondary cysts, surgery remains the best method for removing a hydatid cyst. During surgery, protoscolicidal agents are injected into the cyst to prevent its reformation.<sup>3, 9</sup> The WHO has referred to the urgent need for new protoscolicidal agents with fewer side effects and more efficacy.<sup>3,8</sup> Various studies have referred to the protoscolicidal effects of some plant extracts, for example *Allium sativum*, *Nigella sativa*, *Punica granatum* and *Salvadora persica*.<sup>28-30</sup> However, there is still a need for more effective and available agents requiring only a short exposure time. In another direction, hydatid cysts can be infected by bacteria and fungi, such as *Escherichia coli*, *klebsiella*, Streptococci, Staphylococci, *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.<sup>7</sup> Therefore, this study has attempted to find an agent which combines antibacterial and anti-protoscolices activity.

Both traditional medicine and academic researchers have discussed the importance of Capsicum fruits to treat many disorders, syndrome and pathogenic diseases.<sup>12</sup> The bioactive compounds of the plants which include alkaloids, flavonoids, glycoside, phenols, saponins and tannins have been documented for their antimicrobial and antiparasitic activity. The ethanol extract of Capsicum fruits was found to possess most of these compounds. This result is in line with Tasdemir *et al.* and Koffi-Nevry *et al.*<sup>15, 31</sup>

The activity of Capsicum extract against protoscolices has not been previously reported. However, the methanol extract of *C. frutescens* leaves has demonstrated significant antihelmintic activity in different concentrations by causing paralysis then mortality of worms.<sup>18</sup> Aqueous

extracts of *C. frutescens* also demonstrated 70% death of *Ichthyophthirius multifiliis* during *in vitro* experiments.<sup>20</sup> Our results indicate that the Capsicum extract worked as a good scolicidal agent in a short exposure period. It was shown to have a destructive effect on *E. granulosus*. This activity, considering its low cost and high safety make it a good antihelmintic substance that can be used during surgery to prevent the recurrence of hydatid disease. Generally, different concentrations were proved to have significant scolicidal effects at  $p \leq 0.05$  in the present work. It has been observed that 70% ethanol extract of Capsicum showed the best antifungal activity compared with water and acetate extracts. Moreover, Capsicum extracts displayed high diffusion, when using the well method to evaluate the antimicrobial activity, due to direct contact between the extract and the agar.<sup>16</sup> Therefore, ethanol extract was chosen in this study to evaluate its activity using the well method.

In this study, ethanol extract obtained from hot Capsicum exhibited antibacterial properties against Gram positive pathogenic bacteria *S. aureus*, with no activity against Gram-negative bacteria *Pseudomonas aeruginosa*. These results disagreed with previous studies,<sup>32-33</sup> which showed that Capsicum extracts had antibacterial properties against both Gram-positive and Gram-negative bacteria. While our findings agreed with those of Koffi-Nevry *et al.*,<sup>15</sup> which showed activity of Capsicum extract against some bacterial species (*Staphylococcus aureus*, *Salmonella typhimurium*, and *Vibrio cholerae*) and no efficacy against *Shigella dysenteriae* and *Pseudomonas aeruginosa* and *Escherichia coli*, this difference could be attributed to the low permeability of Gram-negative bacteria membrane. This membrane consist of uneven and inflexible bilayer of lipopolysaccharides (LPS) and phospholipids. This led to specific uptake channels and nonspecific porins are implanted, which caused reducing passive diffusion of hydrophobic compounds to selective size of hydrophilic solute.<sup>34</sup>

In spite of the fact that the mechanism of action for the extract was not studied, the activity of phytochemical compounds can be belong to blockage in the cell wall synthesis by formatting complexes between protein and tannin. Flavonoids can be work as same as tannin by inhibiting cell wall of bacteria through formatting complex with different types of proteins. The saponins are responsible on outflow of important constituents from the cell. Terpenoids can cause dissolution of the bacteria cell wall.<sup>35</sup> Phytochemical analysis of Capsicum ethanol extract detected these compounds (flavonoids, saponins and tannins). Many studies have revealed that Capsaicin alkaloids are the most active compound in the fruit extract of Capsicum. It has been demonstrated to have different biological thermogenic influence effects, anti-inflammatory, antilithogenic and beneficial effects on the gastrointestinal system.<sup>36</sup> It has been suggested that

capsaicin and dihydro-capsaicin prevent cariogenic processes, and decrease the level of acid secretion and biofilm formation by *Streptococcus mutans*.<sup>37</sup> Yeast DNA micro-array methods were used to understand the antimicrobial mechanism of capsaicin. It was found that capsaicin induced 39 genes from about 6,000 genes; these genes are responsible for membrane biosynthesis genes, multi-drug resistance transporter genes and genes encoding stress proteins.<sup>38</sup>

## V. CONCLUSION

The Capsicum extract is an ideal scolicidal agent which is defined by its potency at lower concentrations, high efficacy after only short exposure times, high availability and ability to be prepared rapidly. No studies have previously used it as a scolicidal agent. This is the first report on the scolicidal activity of Capsicum extract, in conjunction with its antibacterial activity. Nevertheless, there is a need to explore *in vivo* scolicidal activity of the ethanol extract of Capsicum.

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