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Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-7, July 2021, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development,* and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

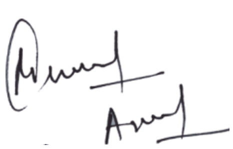
Environmental Research:

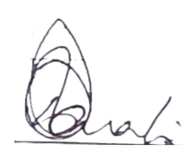
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.





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Role of Urban Agriculture in Achieving Food Security and other Social Missions – A Global Study

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***Abstract*—** *There is an increasing importance of addressing food scarcity and food safety issues as the population is increasing at a very fast rate. With the urban population increasing even more rapidly, there is a need for a radical change in the perception of food production and agriculture. Urban Agriculture has become significant in recent times as it provides a way for urban people to practice farming and easing the pressure on food production, in a way that can satisfy other important goals in a society. This paper analyses the different ways in which urban agriculture can benefit society through qualitative research. The paper finds that urban agriculture is practiced with the primary intent of food security and with other socio-economic goals in mind, through a review of various forms of qualitative data. The paper concludes by advocating for policy implications that will focus on bringing urban agriculture from the purview of the informal and unorganized sector and promoting it to urban dwellers as a viable economic and social activity.*

***Keywords*— Food Security, Local Economies, Social Missions, Urban Agriculture, Urbanization.**

I. INTRODUCTION

Urban Agriculture has been defined as "the growing of plants and the raising of animals for food and other uses within and around cities and towns, and related activities such as the production and delivery of inputs, processing, and marketing of products"(FAO, 2007). In simple words, urban agriculture encompasses the agricultural activities conducted in urban areas. Urban agriculture and peri-urban agriculture almost go hand in hand, peri-urban agriculture being practiced in peri-urban areas. This form of agriculture comprises a variety of tasks such as horticulture, animal husbandry, and aquaculture. Urban agriculture can take up many forms depending on the economic and social status of the person(s) practicing it; it can be a person having a garden, a community coming together to cultivate plants in a vacant area of the city, or a company taking up an urban farming project. The goal behind practicing urban agriculture may also vary, such as for income, food security, recreation, better climate, community building, etc.

As urbanization is taking place rapidly, estimates show that the urban population would increase by more than 10% by 2050 (United Nations, 2018). With such rapid growth, there is immense pressure on rural farmers for the production of food, and given the increasing conversion of vacant lands into commercial buildings and other constructions, the issue of food production and food security is indeed quite alarming. Urban poor spend around 60 to 85 % of their income just on food (Mougeot, 2005) (Redwood 2008). This makes them extremely susceptible to an increase in food prices. As the urban population increases, the number of urban poor increases, leading to issues of food security and food safety. The issues of food insufficiency and food insecurity must be addressed at the earliest, sustainably. One way in which this problem can be dealt with is with urban agriculture complementing rural agriculture. Through urban farming, food security can be reached as locally produced food is more accessible to urban dwellers. In fact, people growing food in their houses can have immediate access to nutritious food. Apart from food security, urban farming can also help achieve several socio-economic goals (income, employment, community, social inclusion) of a society.

With increasing concerns of food shortages and lack of accessibility to nutritious food, especially in the aftermath of the COVID-19 pandemic, it is time that urban dwellers too start contributing to the production of food and not just rely on rural farmers and put them under an excessive burden. Urban agriculture is gaining more importance and attention by the day as it provides a flexible mechanism for even regular urban dwellers with no prior knowledge of agriculture to cultivate food and contribute to food supply and food security, along with contributing to a better climate, biodiversity, a close-knit community, and a better local economy. Urban farmers throughout the world and urban farming projects are testimony to the fact that urban agriculture can really benefit people in a society in numerous ways.

While this form of agriculture can be crucial for dealing with a society's food security issues, it must be noted that effective quantitative data and quantitative analysis of urban agricultural activities is extremely low. It may be due to the fact that various benefits of urban farming can't be quantified, but even for the quantitative aspects of urban agriculture such as the yield from urban farming, income derived from urban farming, etc., there is no record for urban agriculture data. Hence many papers about urban agriculture usually carry out a qualitative analysis rather than a quantitative one.

This paper too, through qualitative analysis, seeks to analyze and find out the urban farming activities throughout the world, major interventions made through this practice, and the impact it has on the urban communities. The paper seeks to emphasize the increasing relevance of urban farming and why urban dwellers should definitely involve themselves in urban farming.

1.1 Objectives

- To analyze the role of urban agriculture in ensuring food security for the urban poor in the wake of rapid urbanization
- To find out the different social missions undertaken by various urban farming projects worldwide and study the ways in which urban agriculture has helped achieve these missions.

1.2 Importance

Urban dwellers, especially youngsters lead a very busy life with little connection with nature and farming. However, one can never isolate themselves from the matters concerning food as one needs food to survive. It is extremely crucial that an individual participates in the process of making one's food as the quality and even the mere existence of food is a matter of deep concern. This study is significant in the sense that it provides an enhanced insight into the capabilities of urban dwellers in being involved in the process of food production and ensuring food security without having to live in rural areas, and emphasizes the goals that urban dwellers can achieve through their participation in urban farming, regardless of its scale.

1.3 Methodology

The methodology adopted is of a qualitative nature. Qualitative data in both textual and visual forms such as case studies, documentaries, interviews, theses, articles, reports, etc. have been studied and analyzed. Literature about urban and peri-urban across various countries has been reviewed for a broad understanding. The first objective, food security has been analyzed concerning two parameters: i) food production with minimal use of pesticides and chemicals (to retain nutrients in food) and ii) accessibility to nutritious food. The second objective of achievement of social missions is studied through social, economic and environmental factors.

1.4 Limitations

- The scope of this paper is limited to urban agriculture and its implications for urban dwellers, mostly the urban poor. Thus, this paper does not shed light on the rural farmers and rural poor who form an equally important segment.
- As pointed by many studies about urban agriculture, there is an absence of a reliable database for urban agriculture. Thus, the impact of urban agriculture has not been statistically proven in this paper.

II. REVIEW OF LITERATURE

(Lintelo, D.T.H., et. al, 2001) assesses the nature, extent and significance of Urban and Peri-urban Agriculture in the context of Delhi, the rapidly growing city in India. The research was based in Delhi and Varanasi. Land use patterns were obtained through digitized satellite imagery from the National Remote Sensing Agency in Hyderabad. Six villages (peri-urban areas) were taken as the case study and all the households were categorized into different landholding and non-landholding groups. The data indicated that agriculture was a major land use in and around Delhi, and the research showed that Urban and Peri-urban agriculture was not only a dominant and dynamic land use but also an important livelihood strategy for poor people in

India, providing families with employment, income, and food. The paper emphasizes policy implications of spreading awareness about its significance and creating effective linkages with research and policy measures.

(Thornton, 2008) presents results from case studies exploring the nature and geographical extent of Urban and Peri-urban Agriculture in one of South Africa's poorest provinces, the Eastern Cape. The study collects baseline socio-economic data using household questionnaire surveys and to identify formal and informal sources of income and expenditure of households engaged in urban and peri-urban agriculture activity. 251 people from parts of Eastern Cape formed the sample size. Results showed that youngsters had a general stigma around urban agriculture as most of them had migrated from rural to urban areas and looked at subsistence food production negatively. Due to the lack of promotion of the idea that urban and peri-urban agriculture creates some social welfare, its impact among these people is low, and there is little incentive for the urban poor, particularly young unemployed people, to strive for subsistence or commercial benefits from urban agriculture and to supplement household incomes.

(Zezza, A., et. al, 2010) investigates the potential of urban agriculture in playing a substantial role in urban poverty and food insecurity. The paper looks into the household data of 15 developing countries from different continents (Africa, Asia, Eastern Europe and Latin America). The analysis is based on the Rural Income Generating Activities (RIGA) database, which is constructed from a pool of several Living Standards Measurement Study (LSMS) and other multi-purpose household surveys prepared by the World Bank and other national and international institutions. The paper studies the relationship between various variables such as the impact of participation in urban agriculture in each of these 15 countries on their share of income from agriculture and on their calorie consumption. The results (derived from simple data analysis) pertaining to income show that urban agriculture doesn't play a huge role in the alleviation of poverty as the income derived is very minimal. Pertaining to calorie consumption, regression analysis shows that there is a statistically significant relationship between engagement in urban agriculture and nutritional intake. While it recommends the reader not to overemphasize its role since its overall contribution to income and output is limited, it also advises not to underestimate its role as it does play a significant role in the livelihoods of people in developing countries as it promotes an increase in calorie consumption and nutrition intake.

(Badami, M., et. al, 2015) reviews various perspectives about the contribution of Urban Agriculture to food security and poverty alleviation, and assess its potential to contribute to urban food security in different regions based on the magnitude of urban land required to grow the daily vegetable intake for the urban poor. The target population of the study is the urban poor from various countries; the food items included in the analysis are vegetables, and the intake is considered to be 400g, as per recommendations from WHO and FAO. The population is taken from the United Nations 'World Urbanization Prospects' database. Urban poverty data is drawn from or calculated based on data from OECD and World Bank. An expression is derived to measure the percentage of the total urban area needed to grow 300 g of vegetables per capita per day to feed the urban poor in each of the selected countries. Calculations through this expression show that urban agriculture may be beneficial for high-income countries in terms of urban land availability, but low potential and feasibility in lower-income countries in terms of land availability in urban areas.

(Dimitri, C., et.al, 2016) examines the increasing concerns regarding the implementation of social goals during the process of farming with the increasing importance of urban agriculture in recent years, and analyzes the various social missions to be undertaken during farming. The paper examines various social goals of urban farmers in the USA. The paper makes use of primary data obtained from a national survey of urban farmers and questions farmers on various matters such as their production strategies and practices, farm size, location, and farm characteristics. The data so obtained was analyzed by a multinomial logistic model. The results showed that urban farms were relatively small, and urban farmers primarily concentrated on the production of food as an essential mission in their agricultural practice. These farms, especially those located with lower median income were more likely to have social goals such as increasing food security, education, and building community.

(Nicholls, E., et. al, 2020) reviews the potential of urban and peri-urban agriculture to contribute to sustainable food production using the 17 sustainable development goals set by the United Nations General Assembly as an agenda for evaluation, SDG2 (towards more productive, sustainable food production systems and resilient agricultural practices) being particularly relevant to the study. 185 allotment holders and home growers were recruited to participate in the study, wherein they self-reported yields and agrochemical use. They were also made to record any pests or diseases encountered in their plots and the control method they used and were asked to weigh each crop in their allotment. The results showed that there was a significant yield (an average of 1kg, with some ranging even to 9kgs) with minimal use of pesticides (adherence to SDG15), there was also considerable savings for the home growers (£550 per year). The case study showed that urban farming not only contributes to food security in the city (SDG2), but that it may also be more beneficial to environmental (SDG15) and human (SDG3) health as compared to current industrial practices.

III. ANALYSIS

3.1 Urban Agriculture and Food Security

Over half of the world's population lives in urban areas, and this proportion is expected to increase to 60% by 2050 (United Nations, 2018). With the rapid increase in population by the day and urbanization too taking place at a very fast rate, estimates show that the amount of land required to feed the people in cities would be half of South America (Despommier, 2015). With such a huge burden on both land and food production, there arises a need to rethink the existing agricultural system and reimagine the concept of food production. There is a need to pave the means for newer ways of practicing agriculture, giving emphasis on food security and nutrition-sensitive agriculture rather than the cultivation of commodity and cash crops.

As defined by (FAO, 2000), 'Food Security' means that 'all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life'. Urban agriculture, comprising of agriculture in both urban and peri-urban areas is significant in the sense that the primary goals of urban farmers and people involved in urban farming projects across various countries like the USA (Chicago, Dallas, Philadelphia, California), India (Bengaluru, Chennai), Singapore, Kenya (Nairobi), Malawi (Lilongwe) and many more is food security and ensuring adequate nutrition intake for the community, especially the socio-economically deprived ones. Urban agriculture produces 15 to 20% of the world's food supply and could play a major role in achieving global food security, since urban poor are more vulnerable to food insecurity because they rely on external sources for their food needs and are thus exposed to greater supply risks (Corbould, C., 2013). Studies on urban agriculture and its impact on nutrition focus on dietary diversity and kilocalorie consumption as two main aspects which influence the outcome of improved nutrition (Alberto Zezza et. al, 2010). Also, urban agriculture seems to have a positive influence on maternal care, as mothers engaged in urban agriculture are able to positively impact child nutrition rather than mothers who are not (Maxwell DG, 1998). It is notable to mention that role of women in urban agriculture is quite significant and a woman's involvement in urban farming can also bring down food insecurity among women, which tends to be on the higher side.

TABLE 1
FOOD INSECURITY LEVELS BY REGION AND GENDER (2019)

Countries	Severe food insecurity (%)		Moderate food insecurity (%)	
	Men	Women	Men	Women
Africa	26.2	27.6	28.6	29.4
Asia	8.6	9.5	13.6	13.6
Latin America	8.6	11.2	17.2	21.4
Northern America and Europe	3	3	8.1	9.2
Oceania	3.4	3.3	7.4	9.8
World	10	11	15	15.7

Source: FAO, IFAD, UNICEF, WFP and WHO, (2020)
Note: *The Food Insecurity Experience Scale (FIES) data collected by FAO in more than 140 countries at the individual level provide a unique opportunity to produce gender-disaggregated estimates for the prevalence of food insecurity.*

As mentioned by an entrepreneur conducting urban farming projects (Sriram Gopal), urban farming has the potential to grow things at smaller places with higher intensity and technologies that help produce more from less. Many urban areas are characterized by tall buildings and high-story constructions. The rooftops of these buildings can be used to practice urban farming. The entrepreneur has confirmed that buildings with rooftop farms have claimed to be cooler 20% against regular buildings. Urban farming can bring together 2 different worlds, minimum area and maximum food production. This is especially an advantage for countries like Singapore which have scarce amounts of land and are importing 90% of their food (Chandran, R., 2020).

Case Study: Future Farms

Future Farms is a start-up based in Chennai using hydroponics technology to grow vegetables and other plants. Founder and CEO Sriram Gopal wanted people to gain access to healthy food free from pesticides and also without excessive use of water. Initially on his own, he was later joined by like-minded people who shared the same goals as him. The company gets clients for various medicinal and other plants and is growing at 300% per year. Sriram is pinning hopes to an urban farming revolution where there would be perfect harmony between agriculture and technology in a way that can sustain food production and with minimum inputs.

While food production and nutrition-sensitive agriculture is important, the way in which it is produced also plays a major role in food security. The use of pesticides is over 4.1 million tons worldwide (FAO, 2020). With the increasing use of pesticides to tackle the increase in population, the toxicity level of food increases which results in health issues. A report from 1FCA showed that the highest toxicity and poison levels were found in mint and coriander. If food produced from excessive chemicals is consumed, it may not satisfy the minimum amount of nutritional intake which must be consumed and also may create health hazards. These pesticides also deplete the amount of water in the groundwater table. Water is the most important input in agriculture and scarcity in water will be an alarming issue. A recent intervention in light of both of these issues is a technology called Hydroponics. Hydroponics is a technology wherein plants don't require soil to grow; they grow from a solution of water and nutrients. Since there is no need for soil and improving the fertility of the soil in the first place, hydroponics does not need pesticides, and no issue of groundwater table deterioration arises as well. Hydroponics is estimated to use almost 10 times less water than conventional farming practices (National Park Service, 2018). Hydroponics thus aids vertical farming, a type of farming where plants are grown vertically (Used in the production of micro greens and herbs, and other plants). Newer practices such as rooftop farming, hydroponics, and vertical farming are key features of urban farming which are practiced in various parts of the world, including Singapore and India. A study in the UK has also shown the significant yield obtained from urban agriculture with minimal use of pesticides and with the use of organic pesticides (Nicholls, E., et. al, 2020).

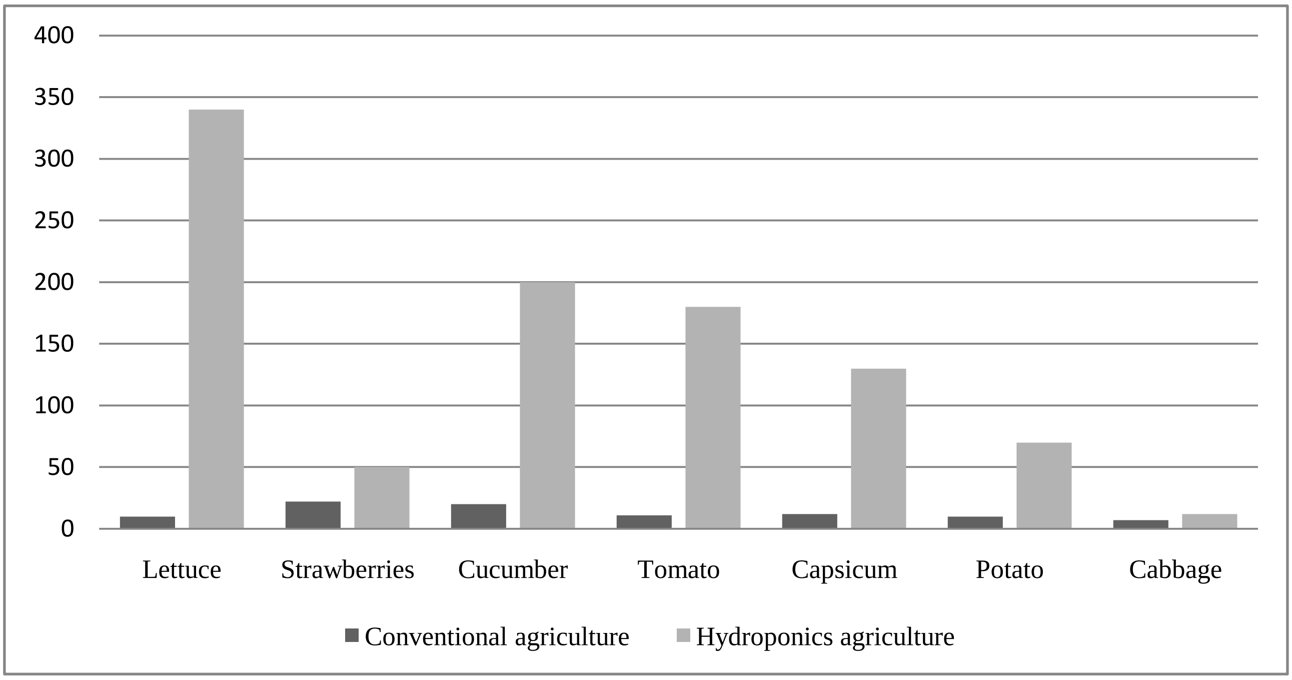

FIGURE 1: Output per Acre (in tons)

Source: DataM Intelligence Analysis (2021)

Note: *In spite of the output using hydroponics is high, lack of knowledge and awareness among farmers, initial investment and maintenance costs and more is still a barrier towards the implementation of hydroponics*

Another key aspect in the matter of food security is its accessibility for all, most importantly the socio-economically backward communities. People from peri-urban areas and outskirts of cities have claimed to face the issue of the lack of a supermarket in their vicinity or the lack of availability of required food items. The recent COVID-19 pandemic has seen immense food shortages, caused due to panic buying and an overall blow to production activities. Urban farmers themselves admit the lack of availability of food in markets during the outbreak of the virus. Urban farming is a solution for many such people as it makes food readily available locally for self-subsistence and for the community. Many people have taken up urban farming even in the most basic levels such as rooftop gardens, etc. during the pandemic to ease the access to nutritious food. Urban farming projects such as Urban Creators were formed in order to make nutritious food accessible to the local community since there was only one supermarket in their area which was too far away. Entrepreneur Sriram Gopal has initiated a project targeted at anemic women and kids suffering from iron deficiency, by setting up urban rooftop farms on commercial buildings which will grow only spinach and employ women to cultivate the plants. Several other projects like these have targeted to achieve accessibility of food across all communities and trying their best to ensure all get adequate nutritious food.

Case Study: Urban Creators

Urban Creators is a collective based in Philadelphia formed to take up the responsibility of feeding the people of the community and to create a space where people learn to grow their own food. According to co-CEO and co-founder Jeanine Kayembe, everyone should be provided fresh food, and the fact that such food is inaccessible to so many communities is a problem, a problem she among many is trying to solve. Young people are educated and employed to be urban farmers, and are also taught basic carpentry. In addition, it intends to provide a space for young people, giving artistic and cultural experiences. The collective has provided job opportunities to more than 136 local youths since 2012 and has hosted several events and engaged a large number of new people in the practices of urban farming.

3.2 Urban Agriculture and Social Missions

Social missions refer to the end goals that benefit a community, a society or an economy. Urban Agriculture is usually practiced by people as a way of giving back to the world. Hence, many urban farmers have strong social missions which they wish to accomplish through urban farming. Looking at the goals of many urban farmers and urban farming communities worldwide, food security seems to be the first and foremost goal, but it is coupled with other goals such as community development, strengthening local economies, providing jobs to socio-economically backward people, a feeling of social inclusion and bonding, environment and biodiversity conservation, effective use of vacant land and so on. Apart from health-related goals such as food security and food safety, there are economic goals, environmental goals, and social goals which urban farmers try to achieve through urban farming projects.

A case study of an urban farming project has been undertaken to deal with climate change is given below. Urban areas are way more polluted and prone to climate change conditions because of excessive vehicles, etc. Urban farming is a way of promoting greenery and improve climate, other than increasing biodiversity and beautifying the place. There is also a wide vegetative (fruits, vegetables, herbs, medicinal and ornamental plants) and insect diversity (various species of bees and spiders) achieved through urban agriculture (Brenda B. L., et al., 2015). Thus, biodiversity is also a significant aspect of urban farming.

Case Study: Asia's largest Rooftop Farm

Bangkok's Thammasat University is home to Asia's largest urban rooftop farm. Built to achieve urban food security and very importantly to tackle climate change, the founder Kotchakorn decided to implement this project as a climate resilient green space, since Thailand is subject to intense flooding and monsoons and World Bank estimates show that nearly 40% of the Thai Capital may be flooded by 2030. (Chandran, R., 2019). Kotchakorn considers urban rooftop farms to be an easy and effective climate solution, and with increasing climate risks, rooftop farms may become a viable and effective solution.

Community building, educational and cultural activities become significant in urban community gardens as well. Many a time, participants of a community garden follow a joint agenda based on solidarity and horizontal decision-making (Mudu & Marini, 2016). It is also a space to meet like-minded people with a common mission. A study has also proven that urban farmers tend to have social goals such as education and community building apart from the primary goal of food security (Dimitri, C., et.al, 2016).

Patrick, an employee at Bonton Farms, has admitted that urban farming has changed his life and given it a new perspective. Having admitted to the usage of narcotics for seeking pleasure and passion previously and seeking help, Patrick has turned over a new leaf through his involvement in the urban farming practices, which he confesses gave him peace of mind, new meaning, and purpose. The Executive Director of Bonton Farms, Daron Babcock, emphasizes how the community can normalize in a span of 20 years through urban farming and how better it is instead of having a prison or a homeless shelter in the same place.

Case Study: Bonton Farms

Bonton Farms, situated in Dallas, is one of the largest urban farms in America. The Bonton community based in Dallas suffered from poverty and various diseases. Half of the men in the community have been to prison too. This prompted Daron Babcock to do something for this downtrodden community. He wanted to provide these people with some opportunity, and so he set up a garden (now what is called Bonton Farms) for providing the community with jobs and also produce their own food. To sell the produce, a market and a café have been set up. Employees are given accommodation and transportation facilities. Today, they have over42 acres of land under cultivation, and getting more than 18,000 people who visit their market, café and coffee house.

Other than providing scope for environmental and social benefits, urban farming is also able to generate employment and income for the urban poor. On an individual level, it gives scope for entrepreneurship through selling organic food and produce from the urban garden, and on a community level, urban poor are provided employment working on these farms. Urban farming thus helps urban people (especially the poor) earn some income and sustain their livelihood and has important implications on economic goals.

Case Study: City Farms

City Farms is yet another urban farming project founded by Ken Dunn based in Chicago. City Farms have taken up the project of composting vacant land area in Chicago (almost 12,000 acres) and providing employment to the local neighborhood that farm the land and cultivate produce. Compost is prepared from food waste produced by nearby restaurants and grocery stores (around 2 tons a day). Ken Dunn, the founder of City Farms emphasizes on the cultivation of food crops rather than commodity crops as health of the local community comes first.

There are many more case studies and reports of urban farmers trying to overcome issues surrounding climate change, unemployment, building community, and so on who have caused a difference and brought each of their communities a step closer to a better lifestyle through the accomplishment of their social, economic and environmental goals.

IV. FINDINGS

The following findings have been derived from the above analysis:

- Urban agriculture has a positive impact on food production as it offers scope for the production of food with minimal inputs. Urban farming techniques such as vertical farming, rooftop farming and hydroponics use minimal land, water and chemical inputs compared to conventional farming.
- Urban agriculture increases the access to food for the urban poor. Communities involved in urban farming are able to secure nutritious food, and individuals with urban gardens or farms get immediate access to healthy food.
- Urban agriculture tries to achieve various social missions. It tries to improve social (social inclusion, community building and development), economic (employment and income generation) and environmental (dealing with climate change and enhancing biodiversity) factors of urban people, especially the poor communities.
- Urban farmers undertaking projects across the world give serious importance to several of these perspectives (health, environmental, economic and social). Food security is given utmost importance and the achievement of social missions too is a crucial aim.

V. SUGGESTIONS

Urban agriculture is mostly practiced by private individuals and communities. It is important that the government step up and invest in urban agricultural activities in a way that people undertake it as a viable activity. There should be incentives provided to those who practice urban farming at all levels and any resources or assistance needed should be readily available. Databases should be made available for people to understand the trends and viability of urban agriculture. Young people too need to participate in agriculture in the urban areas they live in.

VI. CONCLUSION

This paper looks into the impact of urban agriculture on food security and the extent of achievement of various social missions through the practice of urban farming. FAO (Food and Agriculture Organization of the UN) defines Urban Agriculture as "the growing of plants and the raising of animals for food and other uses within and around cities and towns, and related activities such as the production and delivery of inputs, processing, and marketing of products". The relevance of urban agriculture has increased as estimates show that there will be a huge increase in the urban population which would achieve adequate food supply and food security of the urban poor difficult (United Nations, 2018). This is coupled with the decrease in the amount of land available for agriculture as land is increasingly used for commercial and industrial purposes. Such issues call the need for a slightly different way of practicing agriculture, with minimum inputs and maximum output whilst promoting social missions; and urban agriculture is a way of farming that can handle several of these issues.

The paper analyses qualitative data regarding urban agriculture based on two aspects: urban agriculture's influence on food security and its role in achieving social missions. Food security has been analyzed based on the parameters of food production and food accessibility. Achievement of social missions is analyzed through the positive contribution of urban farming to various social, economic, and environmental factors in urban areas.

There is an increasing need for agriculture to be food and nutrition-sensitive. The focus of agriculture should be on the production of nutritious food rather than commodity crops or cash crops. Moreover, what is as important as the scale of food production is the production of food without excessive use of pesticides and chemical fertilizers. Excessive use of chemicals in the production of food can negatively impact the nutrient content in food. Urban agriculture offers scope for various interventions that can be made in order to reduce the amount of inputs and harmful chemicals used in food. One of the interventions is hydroponics which refers to growing plants with only water and no soil at all. Plants grown in this method use 10 times less water than plants grown in the conventional way. In this way, food production can be greatly improved in urban areas due to lesser requirement of water and no requirement of soil or pesticides. For countries with scarce amount of land, vertical farming and rooftop farming can be a great alternative because these techniques take up extremely less space. Moreover, analysis of literature and projects undertaken with respect to urban agriculture show that food security is a primary goal of all urban farmers. Urban farming projects are carried out in localities where there is limited accessibility to a supermarket or a grocery store. Urban gardens provide immediate access to nutritious food for communities.

With respect to the contribution of urban farming to the fulfillment of social missions, case studies of urban farming projects have been analyzed to find out the goals behind them and their importance in the project. Urban farming has been conducted to achieve various goals. Urban agriculture is conducted in urban and peri-urban areas; hence it encourages the growth of a variety of crops, plants, and trees in these urban areas, and thus reduces the amount of toxic gases and pollution in the atmosphere and helps deal with climate change. It also promotes biodiversity as urban gardens are home to a variety of species of plants and insects. Urban agriculture also offers economic benefits as urban farming employs the local communities and helps them generate an income. Individuals with urban farms can either use the produce of their urban farms or gardens for self-consumption which may reduce the income spent on its purchase, or sell the produce and obtain income from it. Apart from economic and environmental benefits, urban farming provides an opportunity for various purposes like social inclusion and community development where people can address various issues together and deal with them in the best way possible.

Studies regarding urban agriculture usually highlight the fact that support must be provided from authorities for urban agriculture to tackle various issues (Anderson, M., 2014) (Thornton, 2008) and policies should establish urban farming as a legitimate and viable economic activity in many cities (Nugent, R., 2000). Indeed promotion and implementation of urban agriculture should be encouraged by governments throughout the world so that more people involve themselves in urban agriculture and reap the benefits.

Today, there are several issues in the context of agriculture. Need for modification of agricultural policies, farming seen as one of the least viable and desirable professions especially among young people, less access to land, credit, water, inputs, etc., impact of climate change on agriculture and agricultural produce and so on are just a few of the many issues surrounding agriculture. These issues face each and everyone in the world and it is not directed only at a few communities, because at the end of the day everyone needs food to survive. These issues should be fought radically, and urban agriculture gives the opportunity to do so. It helps bring people together, regardless of several differences and paves the way to address these problems whilst addressing several other non-agricultural issues as well. Whether practiced at an individual level, a community level or a company level, urban farmers at all levels confirm that urban farming is extremely capable of solving many problems and giving way to a better future.

REFERENCES

[1] Zezza, Alberto & Tasciotti, Luca. (2010). Urban agriculture, poverty, and food security: Empirical evidence from a sample of developing countries. Food Policy. 35. 265-273. 10.1016/j.foodpol.2010.04.007.

[2] Dimitri, Carolyn & Oberholtzer, Lydia. (2016). Urban agriculture: connecting producers with consumers. British Food Journal, 118(3). British Food Journal. 118. 10.1108/BFJ-06-2015-0200.

[3] Nicholls, Elizabeth & Ely, Adrian & Birkin, Linda & Basu, Parthiba & Goulson, Dave. (2020). The contribution of small-scale food production in urban areas to the sustainable development goals: a review and case study. Sustainability Science. 15. 10.1007/s11625-020-00792-z.

[4] Badami, Madhav & Ramankutty, Navin. (2015). Urban agriculture and food security: A critique based on an assessment of urban land constraints. Global Food Security. 4. 8-15. 10.1016/j.gfs.2014.10.003.

[5] Lintelo, D.T.H. & Marshall, Fiona & Bhupal, D.S.. (2002). Urban food: The role of urban and peri urban agriculture in India: A case study from Delhi. Food, Nutrition and Agriculture. 29. 4-13.

[6] Thornton, Alec. (2008). Beyond the Metropolis: Small Town Case Studies of Urban and Peri-urban Agriculture in South Africa. Urban Forum. 19. 243-262. 10.1007/s12132-008-9036-7.

[7] Nugent, Rachel. (2000). The impact of urban agriculture on the household and local economies. Growing Cities, Growing Food: Urban Agriculture on the Policy Agenda.

[8] Orsini, Francesco & Kahane, Remi & Nono Womdim, Remi & Gianquinto, Giorgio. (2013). Urban agriculture in the developing world: A review. Agronomy for Sustainable Development. 33. 695-720. 10.1007/s13593-013-0143-z.

[9] Monica, R., & Gupta, V. (2018). Transforming Urban Farming Approaches to achieve the SDGs.

[10] Agri Committee, European Parliament (2018). Research for AGRI Committee-Urban and Peri-urbanAgriculture in the EU

[11] Anderson, Mallory (2014). Building Community Capacity through Urban Agriculture

[12] B.B.Lin, S. M. Philpott, S. Jha (2015). The future of urban agriculture and biodiversity-ecosystem services: Challenges and next steps. Basic and Applied Ecology. 16(2015)189–201

[13] FAO, Urban Food Agenda

[14] Chandran, R. (2019). With few green spaces, Bangkok plants Asia's biggest rooftop farm. Thomson Reuters Foundation News. Retrieved from <https://news.trust.org/item/20191210054949-uczwr>

[15] Game, I. & Primus, R. (2015) Urban Agriculture. GSDR 2015 Brief

[16] National Park Service. Hydroponics: A Better Way to Grow Food

[17] Evans, D. & Davies. J. (2020). 4 reasons why the world needs more urban farming post-pandemic. World Economic Forum. Retrieved from <https://www.weforum.org/agenda/2020/09/urban-farming-flourish-post-pandemic>

[18] DataM Intelligence (2021). India Hydroponics Market, Size, Share, Opportunities and Forecast, 2020-2027

[19] FAO. 2020. World Food and Agriculture - Statistical Yearbook 2020. Rome.

Biogeochemical aspects of selected elemental content in *Ilex paraguayensis* S.H from Eastern Paraguay

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***Abstract*—** *Yerba mate, Ilex Paraguayensis, is a plant of Paraguayan origin used in infusions/macerations by the ancient inhabitants of Paraguay as a "reviver"/energy beverage and mineral supplier which consumption is lasting up today; furthermore, it is extended almost worldwide. In regard to its mineral content very few studies are known; moreover, none has been published related to the occurrence of REE (rare earth) and other refractory elements in the leaves. In this work, minor and trace elements composition have been investigated by XRF techniques to determine their correlation as well as provenance. The analysis of complex spectra was performed by the AXIL software and the quantitative analysis by the QAES software. Analyzed trace elements were the refractory Rb , Sr, Y, Zr, Nb, Ba, La, Ce, Nd , 3d as Ti, Cr, Ni, Cu, Zn. Minor elements were Mn, Fe which are often related to the above refractory together with S and K.*

***Keywords*— *Ilex Paraguayensis, uptake of minerals, elementary content, refractory and rare earth elements*.**

I. INTRODUCTION

The role of mineral constituents and nutrients is of remarkable importance for *Illex paraguayensis*, according to its generally mentioned/accepted properties. *Ilex paraguayensis* or *Ilex paraguariensis* (Aquifoliaceae), is a small tree of Paraguayan origin called *yerba mate* in Spanish and *Ka'a* in Guaraní *(Paraguay is a bilingual country. Official languages are Spanish and Guarani),* and is used as infusions in hot water (*mate*) or as a maceration in cold water (*terere*) by the ancient inhabitants of Paraguay as a "reviver"/energy beverage *(According the Paraguayan Codex Alimentarious the minimum caffeine content shall be no less than 1%)* and mineral supplier whose consumption continues and is expanded to these days especially in Paraguay, Argentina, Brazil and Uruguay; furthermore, it is used almost worldwide as an infusion. When people, including those who are undernourished, drink in appropriate amounts any of these beverages, they gain/recover strength and their working yield improves. *(Usually workers are entitled to a "mate break" or "tereré break" of about 30 min)*. In the *Codex Alimentarius* of Paraguay and elsewhere yerba mate is considered as a true food stuff [1-2].

Mate tea, as it is cited in [3] has, due to some of its components, important pharmacological properties. Chlorogenic acid and caffeoyl derivatives, among others polyphenols such as tannins, rutin etc, contribute prominently for its antioxidant capacity. Xanthines, as theophilline, theobromine and caffeine (the latter present at higher concentration), account for diuretic, CNS stimulant, hepatoprotective as well as other biological/ pharmacological properties. Saponins (so called matesaponins) in addition to their role in the flavor, have hypocholesterolemic and antiinflamatory properties; some of them have antiparasitic effects, *inter allia* anti-trypanosomal, due to its content in tri-terpenoids (IC_{50} around 4μM for *Trypanosome brucei*). An excellent and comprehensive review in this regard is presented in [4*&* references therein].

The traditional way of manufacturing the yerba mate or yerba *(as the product is called)* is as follows: after raking leaves, twigs, and petioles, one proceeds to make bundles which are quickly passed over the flames of a bonfire, for achieving by fast evaporation a desiccation that prevents the decomposition of the *yerba;* the fire must be strong but without smoke; (a treatment called *sapecado*). Then comes the roasting, made over a framework of canes and sticks, done very carefully to avoid the loss of the green of the leaves. Afterwards, the material is submitted to milling [5]. The product consists of a powder-like mixture of tiny crushed leaves and small sticks.

Despite its wide use in Paraguay few papers & reports were produced in the country, such as those from the middle of the 20th century [6-8]; more recently, two interesting and recommended papers can be cited [9,10] & references therein, mainly related to minerals content, as well as others more recent in similar line [11,12]. Also some more can be cited from the neighboring countries of Brazil and Argentina, *inter alia* at the State do Parana [13,14] in the former at the Provinces of Misiones and Corrientes in the later, [15,16]) all of them bordering the East of Paraguay; just for expand the comparison, some other *data*[17] are also included.

They are mainly related to their multi-elemental contents; many of them, such as the essential microelements, are of utmost importance for living organisms.

An aspect very little considered is the geochemical approach, which represents a significant perspective on the ecology of plant nutrition [18-20], particularly in relation to their content of incompatible and refractory elements and their relationship in the *Ilex Paraguayensis.*

In this work, the composition of selected minor and trace elements have been investigated by EDXRF (Energy Dispersive X-Ray Fluorescence) techniques to determine their correlation as well as *provenance*. Analyzed elements were: K, Ti, Cr, Mn. Fe, Ni, Cu, Zn. Rb, Sr, Y, Zr, Nb, Ba, La, Ce, Nd .

II. MATERIALS AND METHODS

2.1 Materials

The materials analyzed were from the north and the south of the Eastern Region of Paraguay main production areas of Ilex. In this way were analyzed:

2.1.1 Specimens of different commercial brands of yerba mate

For the analysis, packages of samples were selected from major production areas, that is, in the north (Areas of San Pedro and of Pedro J Caballero and in the south, Itapúa,(Area of Bella Vista) in the Eastern Region of Paraguay. Thus, were analyzed samples from different brands taken at random in several shops four kg of 0,5 and 1kg packages , ea of the same brand. They are constituted by ground leaves and shoots mixed with small fragments of petioles and twigs, called usually sticks, whose presence in the product is admitted up to no more than 35% according to the art 1193 of the Código Alimentario Argentino. Moisture ranges from 8.3 to 12.5%.

2.1.2 Specimens of leaves from fresh plants

Leaves, shoots, petioles and twigs were collected at localities of Azote'y in the North and of Capitán Miranda in the South (Itapua). Samples were taken at random from at least 12 plants in each cropping site. At the laboratory, materials were dried over night under a fan at room temperature, crushed, dry again and ground.

Composite samples from each pkg were prepared by quartering, being then dried at 105ªC in an oven, ground and sieved. Samples from fresh plants were prepared in a similar way. For XRF measurements, the powdered samples were pressed into pellets of area weight of ~ 0.1 to 0.3 g.cm^{-2}.Samples from commercial products were analyzed in Asunción while those from fresh materials in Ljubliana.

2.1.3 Ashes samples

In order to check the results, aliquots of A and B materials were reduced to ashes at 550ºC in an oven. Ashes content range from ~ 4.0 up to 6.5 %.

The distribution of sampling stations appears in **table 1,** including the soil typology.

TABLE 1
ILEX SAMPLING SITES

North: Departments of S. Pedro – Amambay		
	Coordinates	**Soil tipology**
Nueva Germania (NG)	23° 54' 41.712" S, 56° 41' 56.734" W	Ultisol – Sandy from sandstones
Azotey (AZ)	23° 19' 7.855" S, 56° 29' 17.147" W	Ultisol – Sandy from sandstones
P.J.Caballero (PJC)	22° 32' 45.586" S, 55° 43' 55.622" W	Inseptisol– Sandy from sandstones
South: Departments of Itapua		
	Coordinates	**Soil typology**
Cap. Miranda (CM)	27° 12' 53.592" S, 55° 47' 48.306" W	Oxisol– Sandy clay from basalt
Bella Vista (BV)	27° 3' 0.000" S, 55° 33' 0.000" W	Oxisol –Sandy clay from basal

2.2 XRF measurements and analysis

The XRF measurements and quantification were performed utilizing the facilities of the XRF laboratories at the Jožef Stefan Institute in Ljubljana and at the Atomic Energy Commission in Asunción. For the excitation of the fluorescence radiation the radioisotope sources of Cd109 (30 mCi) and Am-241 (100 mCi) were utilized as well as a X-Ray tube with the Mo anode &Mo secondary target. The energy dispersive X-ray spectrometer was based on Si (Li) semiconductor detectors. The analyses of complex spectra were performed by the AXIL software [21] which is based on iterative nonlinear least square fit of the spectra by the Gaussian shaped spectral lines. The resulting intensities of pure K_α and L_α lines of measured elements were then utilized in quantitative analysis, employing the quantification software of QAES (quantitative analysis of environmental samples) designed by P. Kump [22,23]. This software utilizes the i.e. transmission-emission (TE method) method for determination of the absorption in the sample and then iteratively finds the solution of the system of basic XRF equations (for each measured element there is one equation). The basic XRF equation namely relates the measured intensity to the respective concentration of the element in the sample. Since this relation is nonlinear and the intensities depend also on concentrations of all unmeasured elements, the information of the absorption in the total sample at single energy (Mo K_α line) obtained by the mentioned TE method is crucial in solving such a system of equations. On the other hand the quantification would be possible only if a set of standards very much resembling the unknown samples would be at hand to perform the necessary calibrations. The absorption measurement on the sample was in principle equivalent to additional measurements on a set of standards.

The uncertainties of elemental concentrations obtained by the QAES software were assessed to be between 5 % and 15 % which has been confirmed by the analysis of some standard reference materials (RM Soil-7 and Sediments SL-1, SL-3, V8Flour rye from International Atomic Energy Agency, and Orchard leaves1571 from NIST.

The preparation of the samples, were carried out at the Laboratory of Hydroconsult in Asunción.

III. RESULTS AND DISCUSSION

The primary uptake of metals from soils is through the root to the plant system [24]. In the rhizosphere the ability of the plant to absorb elements is often limited by their availability at the root surface; the concentration and oxidation state of them are significant in such an availability. In the dissolution of metallic compounds of the soil, pH and redox potentials, as well as the formation of complexes, play an important role in releasing the element, incorporating it into the soil pool [25,26]. Then, assisted by microorganisms activities as well as by the interactions of the root surface, which has the ability to release compounds that alter the solubility and bioavailability of nutrients, they can be absorbed and transported.

Rock minerals, silicates, oxides, sulfides, carbonates, sulfates, etc., due to weathering, undergo alterations, modifications, originating various compounds that precipitate or co-precipitate or become solubilized, being incorporated into the soil solution. In regard to 3d series, from Sc to Fe most of their minerals are oxides, while from Co to Zn, sulfides. In the soil solution they can be present as aquo-complexes according their most stable/important oxidation state Ti^{4+}, VO^{2+}, Cr^{3+},

Mn^{2+}, Fe^{2+}, Co^{3+}, Ni^{2+} , Cu^{2+}[27], That compares, in broad sense, the relative stability in solution of 3d metals complexes, which order is Mn<Fe<Co<Ni<<Cu> Zn according to Irving Williams Rule (IWR) [28].

Generally, the "*3d"* elements do not maintain the original geochemical composition of the precursor materials. The elements are considered mobile and this mobility is related to their oxidation states: is very low for states+3 and +4 and fairly high for the +2 state. As they usually do not precipitate directly (but Fe and Mn), their incorporation into sediments and eventually in the soil solution, are mainly due to co-precipitation processes. [29].

In general, their lower valence states are the most convenient, in this case M^{2+}, on the availability of the *3d series* elements.

At pH < 5.5, which is the case for most of the soils at the *yerba* cropping area in Eastern Paraguay, (pH ranging from 4.50 to 5.50), in reducing ambience metals oxides are reduced to M^{2+}, increasing their availability. On the other hand these soils are low in organic matter (OM) with negative effect on such availability. Besides the humic complex that strongly affects the biogeochemical fate of micronutrients, a variety of other organic molecules can reduce and dissolve metallic oxides [30].

The humic complex are constituted basically by fulvic and humic acids (FA, HA) fractions. *HA* with various metals, forms much more stable complex than those formed with *FA*. Thus, the former are only partially soluble while FA metal complexes are more soluble: therefore more at hand to for the roots.

Recently, studies carried out in complexes of some transition metals, with ligands of carboxylic and phenol functional groups in humic substances (O-donor humic-like ligands) 1:1, indicate the following stability order Co (II) <Ni (II) << Cu (II)> Zn (II), ie, in accord with IWR; however, if Pb (not transition) is included, its complexes with such ligands are comparatively more stable and the order results Pb >> Co <Ni <Cu >Zn [31]. On the other hand in a study of complexes of several metals with HA isolated from soils, the order of their stabilities results: Cu >Fe >Pb > Ni > Co > Ca > Cd > Zn > Mn >Mg. not following IWR [32] . This suggests that the complicated structure / composition of the humic substance and the concentration of the different functional groups present play an important role in the stability of the metal complexes. According, *inter alia*, another recent paper [33] the order of the binding capacity of these metals with *HA* is: Pb>Ni>Cr>Cu>Cd while with *FA* is Cr>Pb>Ni>Cu>Cd.

The average absolute values of K, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr occurrence in three different commercial brand materials from three Departments are shown in Table 2. The results for the fresh leaves analysis of Rb, Sr, Y, Zr, Nb, Ba, La, Ce, Pr, Nd are breaking down in Table 3.

TABLE 2
MINERAL CONTENT OF COMMERCIAL PRODUCTS

	Ti	Cr*	Mn	Fe	Ni*
San Pedro (whole)	18.6±3.7	1.1± 0.13	510.25 ± 42.7	844.75± 57.02	1.7 ±0.5
San Pedro (leaves)	17.25±1.33	1.1±0.13	776.3± 22.2	1433.8±126.3	1.7±0.4
PJC (whole)	18±1.54	1.1±0.14	609.8± 60.3	620±110.12	1.6±0.7
PJC (leaves)	17.6 ± 2.1	1.16±0.11	624.95± 63.8	1034.48±111.74	1.74±0.98
Itapua (whole)	38.2±3.8	4.2±0.7	213.36± 29.2	213.36±29.171	1.248±0.524
ItapuA (leaves)	37±4.0	4.4±0.7	1036± 150	263.2 ±19	1.6 ±0.38
	Cu*	**Zn**	**K**	**Rb**	**Sr**
San Pedro (whole)	6.57 ±1.76	38.17 ±6.02	16653± 1824.	20.36 ± 0.93	34.47 ±1.66
San Pedro (leaves)	7.34±1.26	43.92±3.65	19100.± 614.	29.82±1.95	45.12±0.88
PJC (whole)	9.2±1.5	36.7±6.4	16170.± 2398	23.4±2.4	25.72±2.51
PJC (leaves)	8.4±1.5	38.1±3.4	16507 ± 3295	27.8±3.1	38.4±2.67
Itapua (whole)	13.8±1.9	100.2±13.0	22132 ± 2445.	21.3±2.5	26.5±20.25
ItapuA (leaves)	15.4±2.1	114.5 9.46	22107 ± 1080	21±2.5	36.5 ±3.2

recalculated from ashes

TABLE 3
ANALYSIS OF FRESH LEAVES

	K	Ti	Cr*	Mn	Fe	Ni*	Cu*	Zn
S1 (Azotey	7948 ± 916	21±2.6	2.0±3.1	625±64.9	295±24.9	5.4±0.8	11.9±1.2	19.7±1.8
S2 (Cap.Miranda)	25300 ± 2130	45.0±4.5	5.5±1.0	1680±142	318±26.9	9.8±21.5	18.1 ±1.9	188±24.1
	Rb	**Sr**	**Zr***	**Nb***	**Ba**	**La***	**Ce***	**Nd***
S1 (Azotey	16.6±1.8	46.5±4.13	1.0±0.11	0.06±0,008	25.7±2.1	1.3±LOD	1.4± LOD	1.99±0.6
S2 (Cap.Miranda)	44.9±4.1	42.7±3.9	1.1±0.15	0.06±0.008	56.1±4.4	2.0±0.5	1.6±0,20	2.2±0,25
	Y*							
S1 (Azotey)	0.33±0.06							
S2(Cap.Miranda)	0.7±0.09							

recalculated from ashes

For comparison, correlation and *provenance* studies, as per the method widely used in geochemistry, the content of mineral components (specially at trace level) in soils and plants must be standardized in relation to their concentration / recommend values in primordial materials like, as chondrite, primordial mantle (PM), upper crust (UC), that is, by building the arachnograms or multi-element diagrams; the unit value obviously corresponds to those of the analyzed component layer [34,35].

Here we will refer to PM and to UC in relation to processes/products resulting from the *major* and the *minor* geochemical cycles [36,37].

Thus, in figures 1a, 2a and 3a are presented the spidergrams of the results of the samples from San Pedro, PJ Caballero and Itapúa respectively, normalized to UC recommended values. The higher values indicate that the element is concentrated at the point of sampling.

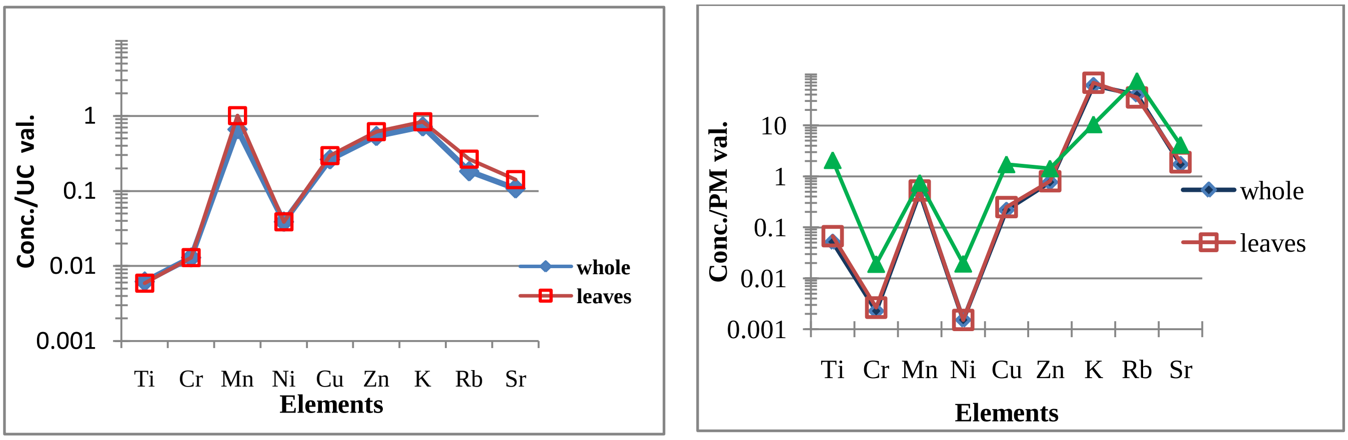


FIGURE 1 a: Spidergrams of whole & leaves of *I. paraguayensis* from San Pedro standarized to UC values. b: Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standarized to PM values

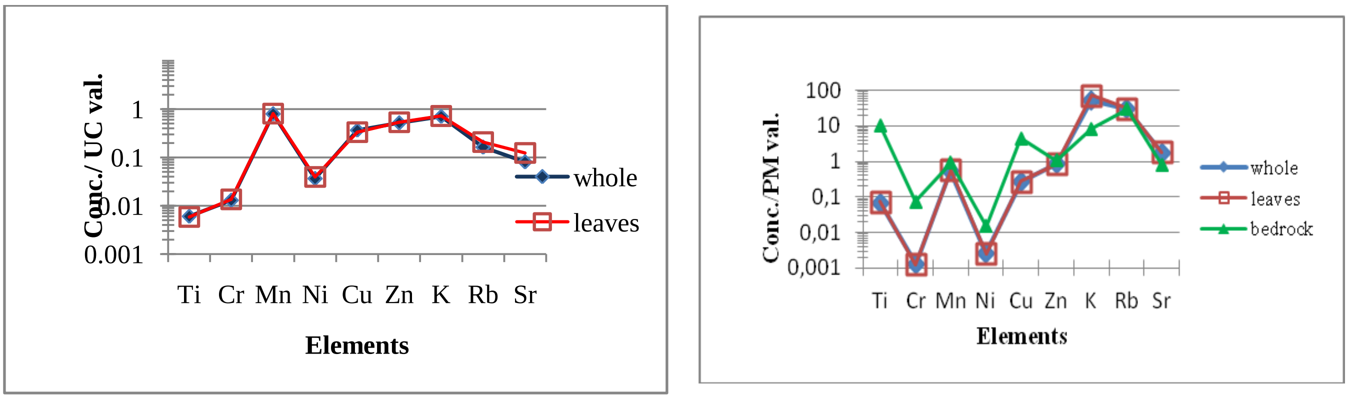
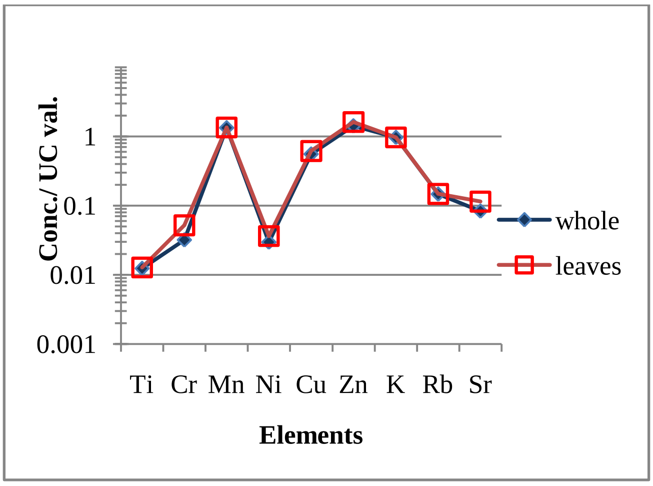


FIGURE 2 a: Spidergrams of whole & leaves of *I. paraguayensis* from P.J. Caballero standardized to UC values. b Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standarized to PM values



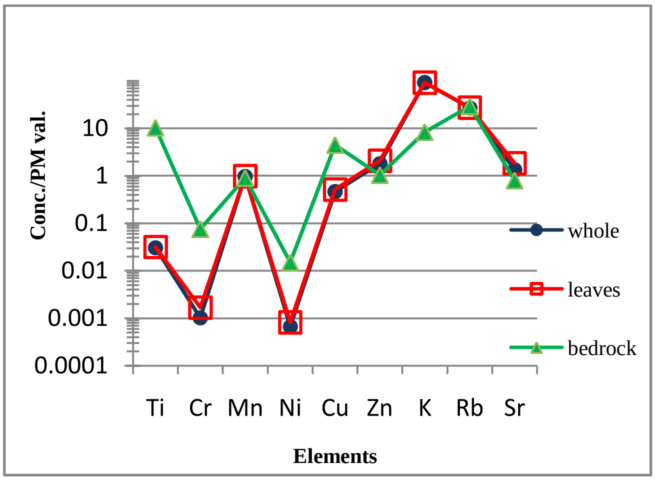


FIGURE 3 a: Spidergrams of whole & leaves of *I. paraguayensis* from Itapua standarized to UC values. b: Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standarized to PM values

The absorption of transition elements in yerba mate is little known [3]*; e*fforts have been although made on the" complex paleo history" of the genus Ilex (~600species) and besides, some species from Sud America such as *I dumosa* and *I guayusa* which infusions are also used as the *mate* [38-40]. In other plants it has been found the action of different NRAMPs in their absorption, transport and homeostasis. (NRAMP or natural resistance associated macrophage protein refers for an integrated membrane protein) [24].

3.1 Elements in the Ilex commercial samples

Uptake and distribution of K^+ in plant cells is carried out by a variety of transporter proteins categorized into several families with varied structures and transport mechanisms that comprise the channel families *Shaker*-like voltage-dependent [**41]**. The K^{1+} is strongly incompatible in the mantle melting and in basalt differentiation. During weathering and by hydrolysis, feldspar originated clay minerals rich in K that remains as a residue and thus the concentration of dissolved K^{1+} in inland waters is low. [36]. It is an essential element for plant development and is considered as a macronutrient, taken in account the amounts absorbed by plants such as the yerba mate. The element promotes esteric changes in a great number of enzymes exposing reactive centres for chemical attack. It is essential for the synthesis and function of chlorophyll. It must be mentioned that potassium (K_2CO_3?) also neutralizes various anions and other compounds within the plant [25].

In the case of *yerba mate,* the plant concentrates K (See fig) and its recovery in dissolution is high [7-11]; its alkalinity (methyl orange) according to references 7 &8 is around 22mg in K_2CO_3 $*L^{-1}$. This supports the explanation given in [6,7] on the effect of .K of the neutralization into the lactic acid/lactate/ [H^+] system, which promotes muscular fatigue. This should explain why people drinking the beverage, gain/recover strength and their working yield improves.

Essential microelements as well as of the other analytes found here, are also within the range of those from several studies on yerba mate and other vascular plants made elsewhere [12-17 & ref. therein].

It has been mentioned the existence of several large families of metal transporter genes. Members of the ZIP a metal transporter family first identified in plants, are capable of transporting cations, of the *transition d series*. [42]. Another important transporter of divalent metals are members of the Cation Diffusion Facilitator (CDF) super family and NRAMP homologues. In addition, members of the vacuolar cation proton exchange (CAX) and of the ABC transporter family are involved in metal homeostasis in plant cells [43].

Cations of the *"d"* elements like Cr, Cu, Fe, Mn have unpaired electrons that allow their participation in redox reactions. Several of the biological toxic effects of these elements can be explained by their capacity to catalyze the initiation of free radical reactions. On the other hand, Zn without unpaired electron however, functions as antioxidant when it replaces metals that are capable to catalyze free radical reactions; also, *inter allia*, Zn acts as a structural or regulatory co-factor for a certain number of enzymes.

Mn usually occurs in *Ilex paraguayensis* at relatively high concentration (see Table 2) perhaps also as a normal constituent of oxidizing enzymes. Small amounts of Mn^{+2} in the oxidases and peroxidases accelerate their oxygen carrying power. The major part of Mn in soils is in the higher oxidation states and only in very small quantities as Mn^{+2}. Fig 1a-3a show, in comparison with the upper crust values, that the element is concentrated in the *yerba mate*; note in addition, that samples from the south present higher concentration [3].

Cu is a component of proteins found in enzymes that regulate the role of many reactions in plants, which would not grow without their specific presence; therefore, Cu plays essential roles in a wide range of physiological process (an example: chlorophyll formation) and promotes seed production and contributes to plant formation. A lower threshold (Cu deficiency) is not known but like several other plants, *Ilex* could be very sensitive to a low level concentration of Cu [20].

Ti is generally present in plants in low concentrations but is very dependent on the soils (6.12 to 85.1 $mg.Kg^{-1}$ in Chong Qing; with a wider range in different regions of China. [44] In Paraguay Ti has been studied in sandstones and its content ranges from 0.39 to 4.62mg/g in rocks from the Misiones and Eusebio Ayala Formations respectively [45]. The element is an inherent constituent of the ash from all plants and might participate in their metabolism as a redox catalyst, although its role in plant development is not well known. Some beneficial effects (and a few adverse though) of Ti application have been described. Recent efforts have been made to look for an explanation for the fact that a harmful substance at low doses can induce counter effects ("*hormesis*") that neutralize those toxic [46].

Cr^{+3} and Ni^{+2}, both with unpaired electrons, are essential elements, recently recognized as such. At low concentrations, it was reported that Cr^{+3} promotes plant growth, stimulates chlorophyll synthesis and photosynthetic activity. In the human diet Cr deficiency causes lowered tolerance of glucose, which is the consequence of changes in insulin affinity of its receptors on the cells. In addition Cr also increases the functional activity of immune system of organism. Also, it has metabolic and cardiovascular benefits. When present in excess, Cr causes oxidative stress in cells**.** Ni has antioxidative effects on enzyme proteins; in addition, it is a unique component of urease, which is responsible for the hydrolysis of urea which nitrogen atoms are available for plants only if the compound is hydrolyzed. It has been called an "ubiquitous trace element" occurring in soil, water, air and the biosphere; however it has no known biological role in mammal metabolism. When present in excess Ni is genotoxic and causes oxidative stress. In this work, Cr could be quantified in the ashes; Ni also in some whole samples [20 & therein]. Other elements quantified by means of their ashes were Y, Zr, Nb, La, Ce, Nd. Their recalculated values are those included in Tables 2& 3.

An interesting aspect of the *"d"* elements in sediments/sedimentary rocks is that referring to the primordial mantle values [36], they present a W distribution (Allegre *"in W"* distribution); resulting from the crystal field stabilization energy (CFSE) and the electronic distribution. During the crystalization processes of the magma, those elements with high stabilization energy stay more stabilized in the solid but in the magma they become depleted. One can see this in the normalized multi-element diagrams of Fig 1b-3b of *Ilex* and bedrocks, with chromium and nickel at the bottoms of the troughs, whereas manganese and zinc are at the higher spikes; this could be an indicator that *Ilex* follows in some way the cations content/distribution of the sedimentary environment. In addition it should noted that it is apparent the enrichment of the Ilex fractions in Mn, K and perhaps Zn in relation with the UC values (Fig 1a-3a); enrichment that is higher in the southern samples in agreement with the nature of latheritic soils.

In those spidergrams were also included rubidium and stroncium. Rb in soils is closely connected to K. Its occurrence in soil is strongly related to the bed rocks. This element is taken up easily by plants; it substitutes K sites in them but does not substitute K metabolic function. At high concentration is toxic to the plants. Rb uptake and transportation in plants seems too different from that of K. Most of higher plants as the Ilex show concentrations of rubidium ~ 20-70ppm.

Geochemical and biochemical properties of Sr are similar to those of Ca and very often are associated with it in the terrestrial environment. Interactions between both are complex; Sr usually cannot replace Ca in biochemical function. Its content in soils is mainly controlled by bedrock and climate: its concentration ranges from 750 to 1000ppm; in Paraguayan sandstones from ~15 to ~130ppm [45].

3.2 Elements in the fresh Ilex samples

Several of the elements analyzed K, Rb, Nb, Ba, La, Ce, Nd, Zr, Ti, Y like, are often used as provenance indicators in geochemistry. They are refractory as well as their compounds. Very little is known about their role in plants despite recent efforts made to its understanding [18,19] especially in relation to the REE (Rare Earth Elements). Table 3 brakes down the average of their absolute values which show to be very low; in fact some of these values were recalculated from measurements of the ashes.

Comparison among light rare earth elements and other refractoru is doing by normalizing the analyses results to primordial mantle (PM)) [36] as well as upper crust (UC) reference values [37], the latter in order to look for enrichment/contamination of the upper crust materials from other sources.

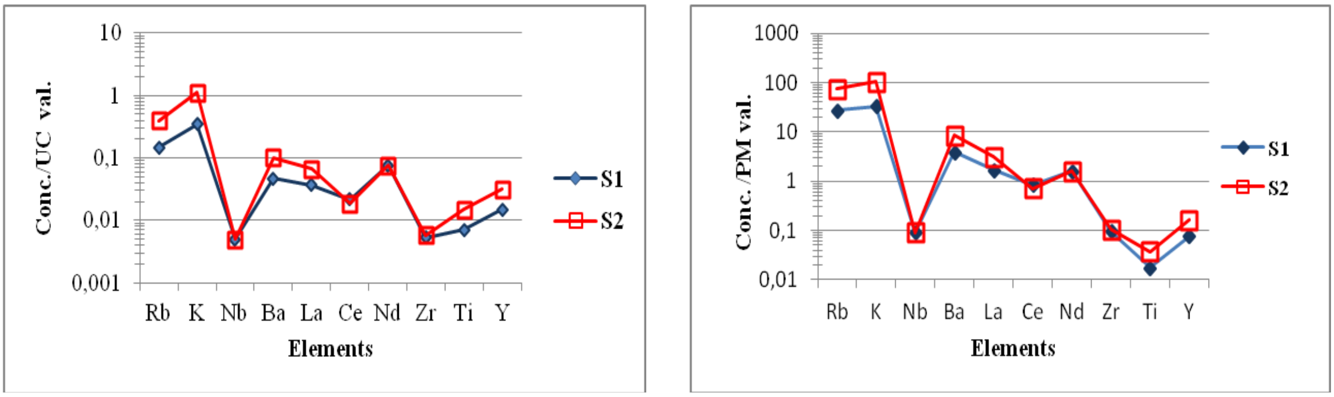


FIGURE 4 a: Spidegrams of fresh samples leaves of *I. paraguayensis* from Azotey (S1) & Cap. Miranda (S2) standardized to UC values.
b: Spidegrams of fresh samples leaves of *I. paraguayensis* from Azotey (S1) & Cap. Miranda (S2) standardized to PM values.

The spidergrams of incompatible elements results from yerba mate, are assembled and compare in Figures 4a & 4b normalized to UC and PM values respectively; the first does not show any enrichment/contamination of the upper crust materials from other sources; they follow closely the uptake referred to PM of the second one. In both cases, results of these refractory are low, and except by monovalent captions (K & Rb), well below the line of reference when normalized to UC (fig 4a).

Their availability to the plant, both, below and above the ground, that is, roots and aerial parts are the key factor; in regard to the soil, it has been shown that the system Mn / Fe oxyhydroxides/pH in soil fractions are the major contributors to the release and availability of REE compounds [47,29].

Although REEs have not known biological role in plants, some toxic effects have been mentioned, although there is not much data [25]. They can compete with other cations and interfere their bioprocesses. Such is the important case of Ca^{2+}, which ionic radius (0.99 Â) is close to those of REEs, whose their higher oxidation state motivates besides, a higher density of charges compared to Ca bivalent with a lower density. Thus it can be supplanted in its bonding site, generating therefore undesirable effects on the plant, especially when referring to the shoots/leaves, taken into account inter alia, the role of Ca channels on photosystem II [48 & ref therein].

It has be found some negative effects of REE on biomass development and also in the biogermination; among REE, Ce is of the more concern due its radius (1.0 &1.1 Â for IV & III valences) and its capacity to change its oxidation state. [48]. Lanthanides are toxic to cell metabolism, although there is not much data about. On the other hand, it was reported that La, Pr, Nd to, specifically and competitively, inhibit calcium uptake / accumulation by the mitochondria of microorganism cells [25].

Although the uptake of LREE is low in *Ilex* and other plants, the sustained increase in their use in modern / contemporary technology has turned them into "emerging" pollutants / toxics, which should be under permanent consideration as indicated by several articles among which can be cited [49 **& ref herein]**.

As above mentioned, the multi-element diagrams show that aerial materials of both, S1 & S2 have almost the same behavior: a deep through in Nb, Ce, Zr,Ti and bumps at K, Ba and Nd; it is apparent a negative anomaly for Ce as well as a *W distribution* of the LREE first tretad in the spidergrams normalized either to UC as to PM values. According to the well documented study on *Vitis vinifera* **[50 & ref herein]**, these *W distribution* of the first tetrad associated with the negative Ce anomaly in the aerial parts of the plants, found in this effort also, suggest the oxidation of Ce^{3+} to Ce^{4+} insoluble as CeO_2 which, comes out of the solution and therefore originates the negative Ce anomaly. On the other hand, a M distribution was observed in the root of grapes associated with positive Ce anomaly being apparent the redox processes $Ce^{3+} \leftrightarrow Ce^{4+}$ in the rhizosphere and its uptake as organic complexes of Ce^{4+} but changes to Ce^{3+} in the plant metabolic processes [50,51].

IV. CONCLUSION

The samples indicate that *Ilex paraguayensis* present in the aerial parts an important content of *3d* elements, several of them considered essentials; besides K plays a prominent rol in the "reviver" property of mate beverages. On the other hand, results of the refractory are low, and except monovalent cations (K & Rb), well below the line of reference when normalized to UC. When compared to bed rocks PM normalized values, *Ilex* follows in some way the captions content/distribution of the sedimentary environment. In addition, spidergrams show the enrichment of the Ilex fractions in Mn, K and perhaps Zn in relation with the UC values, enrichment that is higher in the southern samples: this can be used as provenance indicator of *yerba mate* in Eastern Paraguay.

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REFERENCES

[1] Código Bromatológico del Paraguay (1932). XI art 110 Edic.America. Asunción.
[2] Código Alimentario Argentino (2000) XV art.193 Edic. La Roca Buenos Aires
[3] J.Cabello, P. Kump, A. Dávalos, J.F. Facetti- Masulli, "Biogeochemical aspects of manganese content in Ilex paraguayensis SH from Paraguay by EDXRF and INAA" IJOEAR-vol.5(9) pp.22-28,set 2019.
[4] C.I. Heck , E.G De Mejia,"Yerba Mate Tea (*Ilex paraguariensis*)": A Comprehensive Review on Chemistry, Health Implications, and Technological Considerations, J of Food Science.,vol. 72 (9), pp 138-15, 2000. doi.org/10.1016/j.foodchem.2016.04.059
[5] H. Schmidt Hebel, "Química y Tecnología de Alimentos".Editorial Salesiana. Santiago de Chile.1966.
[6] P.B.Guggiari , G. Crovato et al, "Código Sudamericano de Alimentos". Draft-Report of the Oficina Química Municipal de Asunción to the First South American Congress of Chemistry .Buenos Aires,1927.
[7] F.S. Facetti-Villasanti, "Alcalinidad, potasio calcio y manganeso en yerba mate".Report prepared for the book "La nutrición en el Paraguay",1953.
[8] F. Montalto, "La nutrición en el Paraguay". Ediciones Oeste. Buenos Aires,1956
[9] R.Vera-Garcia, I. Basualdo, I.Peralta, M.Herebia, S.Caballero, "Mineral Content of Paraguayan Yerba Mate",Arch. Latinoam. Nutr., vol 47, pp.77–80,1991.
[10] R. Vera- Garcia, I. Peralta, S. Caballero, "Fraction of minerals extracted from Paraguayan yerba mate (*Ilex paraguariensis, S.H.*) by cold tea(maceration) and hot tea(infusion) as consumed in Paraguay". Rojasiana, vol. 7(1), pp.21-25,1992.
[11] A.Dávalos, P. Kump, J. F. Facetti -Masulli , "Biogeochemical aspects of mineral content in *Ilex paraguayensis* S.H", 17RAD Conference, Booklet of Abstracts, vol.28, 132. Marianske Lazne, June 2014.
[12] M.C.A Marcello, C.A. Martins, D. Pozebon, V. Dressler, M.F. Ferrao." Classification of Yerba mate (Ilex paraguayensis) according of the country of origin based on elemental concentration". Microchemical Journal, vol. 117, pp.164-171,Oct 2014.
[13] G. Ducat , S.P.Quinaia ,"Avaliacao do teor de minerais da *Ilex paraguarienses* da regiao Centro-Oeste do Estado do Parana". *Rev. Ciencias Exatas e Naturais*, vol.6(1), 31-42,2004.
[14] C.B. Reissmann, M.I. Radomski , R.M.Bianchini de Quadros "Chemical composition of *Ilex paraguariensis* St. Hill. under different management conditions in seven localities of Paraná State". *Braz. Arch. Biol. Technol*., vol. 42(2), pp.187-193,1999.
[15] M.G. Maiocchi , L.DelVito , M. Peteneti ,E. Marchewsky, M.Avanza , R.G. Palle , G.Petenati, "Multi-elemental composition and nutritional values of dumosa (*Ilex dumosa*), yerba mate (*I. paraguayensis*) and their commercial mixture in different forms of use". *Rev. Fac. Cienc. Agrar. Univ. Nac. Cuyo*., vol. 48(1), 145-159,2016
[16] C.Carducci , P.C.Dabas , J.O. Muse, "Determination of Inorganic Cations by Capillary Ion Electrophoresis in *Ilex paraguariensis* (St. H.)", a Plant Used to Prepare Tea in South America . *J. AOAC Int.*, vol.83 (5), pp.1167-1173,2000.
[17] K. Wróbel, E.M. Colunga-Urbina, "Determination of Total Aluminum, Chromium, Copper, Iron, Manganese, and Nickel and Their Fractions Leached to the Infusions of Black Tea, Green Tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (Mate) by ETA-AAS". *Biol. Trace Elem. Research.*, vol. 78, pp.271-280,2000.
[18] T.Liang, S. Zhang, L. Wang, HT.Kung, Y. Wang, A. Hu, S. Ding, "Environmental biogeochemical behavior of rare earth elements in soil–plant Systems" Environmental Geochemistry and Health, vol. 27: pp.301–311.2005. doi: 10.1007/s10653-004-5734-9
[19] W. X. Han, J. Y. Fang, P. B. Reich, F. I. Woodward, Z. H. Wang, " Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China". Ecology Letters, vol.27, 301-311, 2011 doi: 10.1111/j.1461-0248.2011.01641.x
[20] V. Romero, A. De Lorenzi , P. Kump, J.F. Facetti-Masulli, "Selected Mineral contents in wheat from Paraguay by XRay Fluorescence". J. Chem. Chem Eng., vol. 6:pp.1114-1120, 2012.
[21] P.Van Espen , H. Nullens, F.Adams, "A computer analysis of XRF spectra Nucl Instr Meth", vol. 142: pp.243, 250, 1997.
[22] P.Kump, QAES Instruction Manual.Jožef Stefan Institute, Ljubljana, 20 Slovenia,1988.

[23] P.Kump. M. Necemer, P. Kupnic, “Development of the Quantification procedures for *in situ* XRF analysis. In situ applications of X Ray Fluorescence Techniques”. IA E A-TECDOC, 1456: pp.217-229, 2005

[24] S .Thomine , J.I. Schroeder, “Plant Metal Transporters with Homology to Proteins of the NRAMP Family” Madame Curie Bioscience Data Base [Internet] Landes Bioscience Austin (TX), 2001.

[25] A. Kabata Pendias , H. Pendias, “Trace elements in soils and plants” 3d edit – CRC Boca Raton, 2003.

[26] M. L. Guerinot, D. E. Salt, “Fortified Foods and Phytoremediation. Two Sides of the Same Coin”. Plant Physiol., vol. 125, pp.164-167, 2001

[27] F.A. Cotton , G.Wilkinson, “Advanced Inorganic Chemistry”, J. Wiley & Sons. New York.1988.

[28] R. B. Martin, “A stability ruler for metal ions complexes”. J. Chem. Educ., vol. 64, 402, May 1,1987,doi.org/10.1021/ed064p402

[29] J.F. Facetti-Masulli, P. Kump, “Selected Minor and Trace Elements from Water Bodies of Western Paraguay” - J. Radioanal. Nucl. Chem., vol.286,pp. 441-448,2010

doi: 10.1007/s10967-010-0775-2.

[30] M. Millaleo, M. Reyes-Diaz, A.G. Ivanov, M. Mora, M. Alberdi, “Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms”, J. Soil Sci. Plant Nutr., vol. 10 (4), 470 – 481. 2010.

doi.org/10.4067/S0718-5162010000200008

[31] I. Kostić, T. Anđelković, D. Anđelković, R. Nikolić, A.Bojić, T.Cvetković, G. Nnikolić, “Interaction of cobalt(II), nickel(II) and zinc(II) with humic-like ligands studied by the ESI-MS and ion-exchange methods” J. Serb. Chem. Soc., vol.81 (3)pp. 255–270.March 2016. 1947, available

[32] S. Lalas, V. Athanasiadis, V.G. Dourtoglou , “Humic and Fulvic acids as potencially toxic metal reducing agents in water”. Clean Soil Air Water 1700608. 2018
doi 10.1002/clen.201700608

[33] F. Flores, M .Delgado, J.F.Facetti –Masulli, “Bottom Sediments from Tapiracuai and Cuarepotí Brooks.” Rev Soc Cient Paraguay, vol. 23 (2): pp.251-262. 2018. (in Spanish).

[34] J. F. Facetti- Masulli, P. Kump , M. Delgado, “ Provenance, Guideline Values and Trace and Minor Elements in Bottom Sediments of Tebicuary River and Tapiracuai Stream from Eastern Paraguay by X-Ray Fluorescence”. J. Chem. Chem. Eng., vol.15, pp.5-13 2021. Doi: 10.17265/1934-7375/2021.01.002.

[35] F .Albarède Geochemistry- Cambridge Universty Press. Cambridg 2003.

[36] R. Rudnick, S. Gao ,2003, “Composition of the Continental Crust.” In *Treatise Geochem*, edited by Turekian, K., and Holland, D., vol. 3. New York: Elsevier, pp.1-64.

[37] F. Manen,M. Boulter,Y .Naciri, “The complex history of the genus Ilex L. (Aquifoliaceae): Evidence from the comparison of plastid and nuclear DNA sequences and from fossil data” Plant Syst. and Evol. , vol.235:79-98. 2002.

doi:10.1007/s00606-002-0225-x

[38] P. Cuenoud, M. A. Del Pero -Martinez , P. A.Loizeau , R. Spichiger , S. Andrews , J. F. Manen, “Molecular phylogeny and biogeographyof the genus Ilex L. (Aquifoliaceae)”. Ann. Bot., vol.85: pp.111–112, 2000.

[39] J.F. Dueñas, C. Jarrett, I. Cummins, E . Logan–Hines. “Amazonian guayusa (*Ilex guayusa* loes.)”: a historical and Ethnobotanical Overview; Economic Botany, vol. 70(1), pp. 85–91. The New York Botanical Garden Press; New York, 2016.

[40] P. Ragel, N. Raddatz, E. O. Leidi, F. J. Quintero, J. M. Pardo ¨Regulation of K^+ Nutrition in Plants Front Plant” Sci., vol. 10: pp.1-21, 2019 .Doi: 10.3389/fpls.2019.00281.

[41] M.L. Guerinot “The ZIP family of metal transporters” Biochim Biophys Acta, vol. 1465, pp.190-198, May 2000.
doi/10.1016/S0005-2736(00)00138-3.

[42] V. Lanquar, M. Schnell- Ramos, F. Lelièvre, A. Krieger-Liszkay, U. Krämer, S. Thomine, “Export of Vacuolar Manganese by AtNRAMP3 and AtNRAMP4 is Required for Optimal Photosynthesis and Growth under Manganese Deficiency “. Plant Physiol., vol. 152(4) pp. 1986–1999, Feb 2010.

doi.org /10.1104/pp.109.150946

[43] J.J. Hao, “Study on the Titanium Distribution Characteristics in Chong Qing Typical Soil Profiles and Ti Biology Validity “. M.Sc. Thesis, Chong Qing SW University, November 2009.

[44] J.F.Facetti-Masulli; P. Kump; E. Gonzalez-Erico- “Selected Trace and minor elements in sandstones from Paraguay “– Radiochim. Acta, vol. 98, pp.441-446, 2010

doi:10.1524/ract.2010.1734.

[45] M. Hrubý, P. Cígler, S. Kužel, “Titanium in Plant Nutrition, A Contribution to Understanding the Mechanism of Titanium Action in Plants”, J. Plant Nutrition, vol. 25, pp.577-598, 1991.

[46] [47] X.Cao, Y. Chen, X. Wang, X. Deng, “ Effects of redox potential and pH value on the release of rare earth elements from soil”. Chemosphere , vol.44, pp.655–661, 2001.

[47] P.J .Thomas, T.E .Carpenter, C. Boutin, J. Allison, “Rare Earth elements : effects on germination and growth of selected crop and native plants species “ , Chemophere , vol. 96, pp.57-76 ,February 2014.

doi: 10.1016/j.chemosphere.2013.07.020

[48] W. Gwenzi , L. Mangori, C. Danha , Ch.Nhamo , D.Nothando , E. Sanganyado, “Sources, behaviour, and environmental and human health risks of 1 high-technology rare earth elements as emerging contaminants " Sci Total Environ., vol. 15, pp.299-313, sep,2018. doi: 10.1016/j.scitotenv.2018.04.235.

[49] P.Censi, F. Saiano, A. Pisciotta , N. Tuzzolino , "Geochemical behaviour of rare earths in Vitis V*inifera* " – Science of the Total Environment. Vol. 473-474 , pp.597-608, 2014

doi.org/10.1016/j.scitotenv.2013.12.073

[50] A.Pisciotta, L.Tutone, F. Saiano,"Distribution of YLOID in soil-grapevine system (Vitis vinifera L.) as tool for geographical characterization of agro-food products. A two years case study on different grafting combinations, Food Chemistry, vol. 221 , pp.1214-1220, 2017.

doi:10.1016/j.foodchem.2016.11.037.

Screening of Sponge-associated Actinobacteria against Human Pathogenic *Candida albicans* in Kien Giang Sea, Vietnam

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Abstract— *From 198 actinomycetes isolated from sponges at the Kien Giang Sea in Vietnam, 42 actinomycete isolates were selected with the ability to resist Candida albicans, a species of yeast causes human diseases. There were eight isolates having strong resistance, 31 moderate, and three weak resistances. Eight best isolates were selected to identify by 16S rDNA gene analysis and sequencing. The result showed that six strains were characterized as Streptomyces spp., one strain belonged to genus Microbacterium, and one strain was genus Rhodococcus*.

***Keywords— Antimicrobial activity, Candida albicans, Kien Giang Sea, sponge, Streptomyces*.**

I. INTRODUCTION

Candida albicans is an opportunistic pathogenic yeast [1] that is a standard member of the human gut flora. It can also survive outside the human body [2]. It is detected in the gastrointestinal tract and mouth in 40–60% of healthy adults [3]. It is usually a commensal organism, but it can become human pathogenic in immunocompromised individuals under a variety of conditions [4]. It is one of the few species of the genus Candida that causes the human infection candidiasis, which results from an overgrowth of the fungus [5]. Candidiasis is, for example, often observed in HIV-infected patients [6]. Candidiasis is understood to cause gastrointestinal (GI) symptoms particularly in immunocompromised patients or those receiving steroids (e.g. to treat asthma) or antibiotics. Recently, there is an emerging literature that an overgrowth of fungus within the intestine of non-immunocompromised subjects may cause unexplained GI symptoms. *Candida albicans* is a species of yeast - a single-celled fungus, in fact – that lives naturally in the body. This yeast may be a normal part of the microbes that survive your skin and in your alimentary canal, but under some circumstances, it can multiply out of control. Small amounts of *Candida albicans* also sleep in various warm, moist areas throughout the body, including on the skin, within the mouth and gut, and also the rectum and vagina.

Elbendary [7] using ethyl acetate extraction method, the isolates culture's supernatants were tested by diffusion method against indicator microorganisms. These results indicated that actinobacteria isolated from Egypt farms could be sources of antimicrobial bioactive substances.

These identified isolates showed antimicrobial activity against the test organism, and the range of inhibition zones was between 2.5 and 10.2 mm. Out of 287 actinomycetes166 isolates were found antagonistic to *Candida albicans* isolated and selected a *Streptomyces* sp. [8]. Al-Dhabi [8] used the chromatogram of GC-MS analysis of this ethyl acetate extract (EA) had diverse chemical compounds namely benzene acetic acid (7.81%), acetic acid, methoxy-, and 2-phenylethyl ester (6.01%) were the major compounds. Minimum inhibitory concentrations (MIC) values were observed against Candida albicans and *Aspergillus niger* by (312 μg/ml).

This study aimed to selected and identify the Actinobacteria and discover potential sources of antimicrobial secondary metabolites to human pathogenic yeast, especially *Candida albicans*.

II. Materials and Methods

2.1 Materials

One hundred and ninety-eight actinobacteria were isolated from sponges in the Kien Giang Sea, Vietnam, and *Candida albicans* (ATCC 10231) used for testing the agent of antibacterial isolates.

2.2 Screening assays for antibacterial activity

The liquid cultures were grown with shaking at 150 rpm for one day at 30°C. The broth was centrifuged at 5,000 rpm, 15 minutes. The supernatant was stored at 4°C. The *Candida albicans* test organisms were plated in the LB medium. The antimicrobial extract was added to the wells, the plates were incubated at 4°C for 2 hours for the diffusion of antimicrobial extract and observed for the zones of inhibition at 28°C for 48 hours.

2.3 The agar well diffusion method

The active isolates were cultured by the method given in the previous step. The supernatants were used for testing extracellular antimicrobial activity by the agar well diffusion method. By using a sterile cork borer, wells were punctured in the appropriate agar medium previously seeded with *Candida albicans*. One hundred microliters of the culture supernatants were added to each well. The plates were then incubated at 4°C for at least 2 hours to allow the diffusion of crude extracts followed by incubation for 48 h at 28°C for yeast. The diameters of inhibition zones were monitored and measured [9], and the positive control was nystatin.

Screening of isolated microorganisms had for inhibitory activity. The isolates screened for antibacterial metabolite production using the agar well diffusion method that inocula were prepared by growing the varied test organisms on separate agar plates. The colonies from plates were transferred with inoculating loop into 3 mL of normal saline in a test tube. The density of these suspensions adjusted to 0.5 McFarland standards.

By means of a sterile cork borer wells (8 mm in diameter) were made in the agar and filled with 0.2 ml of 72 hours culture of the isolated microorganism. Two replicates of the experiment were done, and the plates were incubated at 37°C for 18 hours. The diameters of the zone of growth-inhibition produced were measured, and the mean values calculated.

2.4 Genomic DNA extraction and 16S rDNA gene amplification and sequencing

Actinobacteria cells from these cultures were collected by centrifugation, and genomic DNA was extracted [10]. The PCR was performed in a final volume of 25 µl which was composed of about 50ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 200 ρM of Actinomycetes specific primers S-C-Act-0235-a-S-20 (5'-CGCGGCCTATCAGCTTGTTG-3') and S-C-Act-0878-a-A-19 (5'-CCGTACTCCCCAGGCGGGG-3') [11] and 1U of Taq polymerase with the appropriate reaction buffer under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 50s, annealing at 52°C for 50s, and 72°C for 90s. The amplified products were separated by gel electrophoresis in 1.2% agarose gels which were stained with Safeview dye.

2.5 Sequence analysis

The 16S rRNA gene sequences are compared with those from the type strains available in NCBI (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST).

For phylogenetic analysis, multiple sequence alignment performed using CLUSTALX, version 1.81. The Phylogenetic tree constructed using Mega 7.0. The consistency of the trees was verified by bootstrapping (1000 replicates) for the Neighbor-joining method.

2.6 Statistical analysis

The experimental results were analyzed as ANOVA with the isolates and with levels of diameters of inhibition zones. All analyses were conducted using the program MSTATC, Minitab 16. The data were considered significantly different at $P<0.01$. Duncan's test at $P = 0.01$ was used to differentiate between statistically.

III. RESULTS AND DISCUSSION

3.1 Screening assays for antibacterial activity

Total 198 isolates of endophytic actinomycetes were obtained from sponges collected from the Kien Giang Sea. However, there were 42/198 isolates against *Candida albicans* among 8 isolates strong resistance (7.1%), 31 moderate resistance (73.8%), and 3 weak resistance (19.1%) (Table 1, Figure 1).

TABLE 1
MICROBIAL ACTIVITY OF 42 ACTINOBACTERIAL ISOLATES ON *CANDIDA ALBICANS*

No	Actino-bacterial isolate	Diameter of Sterile ring (mm)	Evaluated of Galindo (2004)	No	Actino-bacterial isolate	Diameter of Sterile ring (mm)	Evaluated of Galindo (2004)
01	ND1.1a	23.0 c	+++	22	HD1.5c	6.0 t	++
02	ND1.3b	16.0 i	++	23	HD1.6a	18.0 g	++
03	ND1.5a	12.0 n	++	24	HD2.1a	18.0 g	++
04	ND1.5c	14.0 l	++	25	HD2.3a	16.0 i	++
05	ND1.7a	23.7 b	+++	26	HD2.3b	6.0 t	++
06	ND1.7b	25.7 a	+++	27	HD2.3c	26.0 a	+++
07	ND2.4	5.0 u	+	28	HD2.3d	3.0 v	+
08	ND2.6c	21.7 cd	+++	29	HD2.3e	11.7 o	++
09	ND2.7b	6.0 t	++	30	HD2.4a	17.0 h	++
10	ND2.7c	22.0 d	+++	31	HD2.5a	14.0 l	++
11	RL1c	6.0 t	++	32	HD2.5b	8.0 r	++
12	RN3c	6.0 t	++	33	HD2.5d	12.0 n	++
13	HD1.2a	5.0 u	+	34	HD2.6c	10.0 p	++
14	HD1.2c	26.0 a	+++	35	HD2.7d	15.0 k	++
15	HD1.3c	16.0 i	++	36	HD2.8l	14.0 l	++
16	HD1.3d	14.0 l	++	37	HD2.8p	14.0 l	++
17	HD1.3e	19.0 f	++	38	HD2.9a	14.0 l	++
18	HD1.3f	9.0 q	++	39	N1a	21.0 e	+++
19	HD1.4b	9.0 q	++	40	N4a	7.0 s	++
20	HD1.4d	6.0 t	++	41	N5c	13.7 m	++
21	HD1.5a	8.0 r	++	42	N10b	8.0 r	++
	CV (%) = 2.46			Positive control (tetracycline)		7.0 p	

Means within a column followed by the same letter/s are not significantly different at p < 0.01

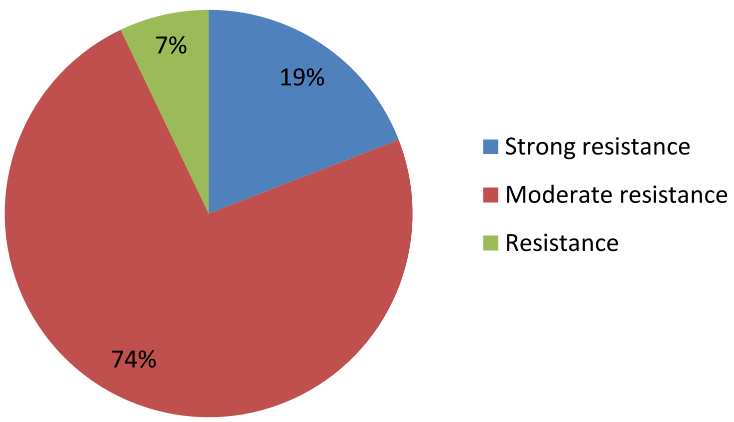

FIGURE 1: Microbial activity of 42 actinobacterial isolates to *Candida albicans* [9]

Based on evaluated of Galindo [9], the isolates as HD2.3c, HD1.2c, ND1.7b, ND1.7a, ND1.1a, ND2.7c, ND2.6c, and N1a were the best isolates with diameter >21 mm differed from the others statistically. They were chosen to identify by 16S-rDNA gene PCR technique and sequencing (Table 2).

3.2 Identify actinobacterial isolates

TABLE 2
PHYLOGENETIC AFFILIATION OF ISOLATES SUPPORTED 16S RRNA GENE SEQUENCES BY USING BLAST PROGRAM WITHIN THE GENBANK DATABASE SUPPORTED SEQUENCES SIMILARITY

No	Actinobacterial isolates	Cloest species relative	Similarity (%)
	Actinomycetaceae		
1	ND1.1a	*Streptomyces coelicolor* strain DSM 40233 (KY820720.1)	100
		Streptomyces sampsonii strain NRRL B12325 (KY820696.1)	100
2	ND1.7a	*Streptomyces tateyamensis* strain 18I (MG009024.1)	100
		Streptomyces chumphonensis strain HQA999 (MH041238.1)	100
3	ND1.7b	*Streptomyces ambofaciens* strain I (MK929479.1)	100
		Streptomyces olivaceus strain HQA933 (MH044533.1)	100
4	HD2.1c	*Streptomyces recifensis* strain WZS121 (MH497607.1)	100
		Brevibacterium sediminis strain YIM102079 (MN099340.1)	100
5	HD2.3c	*Streptomyces coelicolor* strain DSM 40233 (KY820720.1)	100
		Streptomyces sampsonii strain NRRL B12325 (KY820696.1)	100
6	N1a	*Streptomyces coelicolor* strain DSM 40233 (KY820720.1)	99.67
		Streptomyces sampsonii strain NRRL B12325 (KY820696.1)	99.67
	Microbacteriaceae		
7	ND2.7c	*Microbacterium tumbae* strain C3 (MG958700)	100
		Microbacterium kyungheense strain MK (MF373498)	100
	Nocardiaceae		
8	ND2.6c	*Rhodococcus hoagii* strain AL01 (MF928189)	100
		Rhodococcus equi strain TRB132 (KX981343)	100

A Neighbor-joining phylogenetic tree (Figure 2) of these isolates described the two clusters. Cluster A had five strains including *Streptomyces coelicolor* N1.1a, *Streptomyces coelicolor* HD2.3c with high similarity, both strains related with *Microbacterium tumbae* ND2.7c, and three had a high relationship closely with *Streptomyces coelicolor* N1a. All four had a relationship with strain *Rhodococcus hoagie* ND2.6 while cluster B had three strains: *Streptomyces recifensis* H1.2c, *Streptomyces tateyamensis* N1.7a, and *Streptomyces ambofaciens* N1.7b had a close relationship.

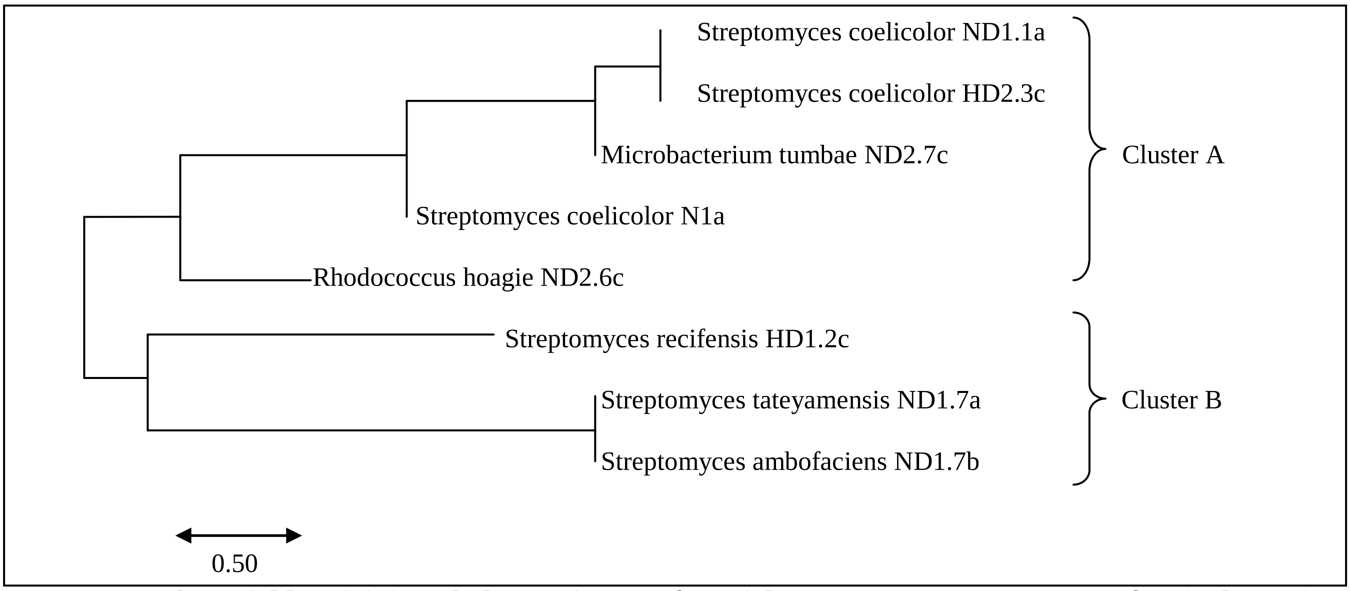

FIGURE 2: The Neighbor-joining phylogenetic tree of partial 16S rRNA gene sequences of actinobacteria isolated from sponges that closely related type strains. Numbers in the figure refer to percentage bootstrap values calculated for 1000 replicates. Bar, 0.02 was per nucleotide position.

Candida albicans is the most important fungal opportunistic pathogen of humans [12]. *Candida* species and other microorganisms are involved in this complicated fungal infection, but *Candida albicans* continue to be the most prevalent. In the past two decades, it has been observed that abnormal overgrowth in the gastrointestinal, urinary, and respiratory tracts, not only in immunocompromised patients but also related to nosocomial infections and even in healthy individuals. There is a wide variety of causal factors that contribute to yeast infection which means that candidiasis is a good example of a multifactorial syndrome [4]. The antibiotics for the treatment of infections are derived either directly from natural sources, semi synthesized from a natural product parent, or completely synthesized but modeled after a natural product lead compound [13]. It has been estimated that about two-thirds of the natural antibiotics have been isolated from actinobacteria, especially from the genus *Streptomyces* [14]. Belghit [15] found a strain of actinobacteria, designated G61, was isolated from Saharan soil and tested for its activity against these microorganisms. New antifungal antibiotics were active against *Candida albicans* and other pathogenic fungi. The analysis of G61 by PCR 16S rDNA gene and sequencing showed a similarity level of 100% with *Streptomyces mutabilis* NBRC 12800T and was determined NMR to be 2,4-Di-tert-butylphenol (2,4,DTBP). Palla [16] isolated the actinomycetes from Koringa mangrove soil samples near Kakinada, Andhra Pradesh, India. The scientists found the potent strain KMFA-1 having activity against dermatophytes *Candida albicans* and *Pectinotrichum llanense.* Based on physiological, morphological characteristics and 16s rRNA gene sequencing the isolated strain was identified as *Streptomyces hydrogenans*. The crude antifungal metabolite produced by the *Streptomyces* spp. isolate KMFA-1 is found to thermostable and the antifungal activity was not lost over a wide range of pH (2–10) indicating that it is active at various physiological pH. The antifungal activity of crude supernatant was not lost even after 24 months at 4°C, suggesting that the bioactive metabolite possess longer shelf life at refrigerated temperature and the specific nature of this bioactive metabolite produced by the selected isolate could be a diversified molecule and exerts unique mode of action to inhibit the growth of *C. albicans*.

Our results also discovered at least five actinobacterial strains (*Streptomyces* sp.) having the high antimicrobial activity to *Candida albicans* from originating from sponges in the Kien Giang Sea. Further investigations are necessary for isolation and chemical characterization of the compound by chromatographic and other spectral analysis as GC-MS.

IV. CONCLUSION

From 198 isolated actinomycetes, 42 isolates had the ability against *Candida albicans*, a species of yeast that causes human diseases. The six best isolates were chosen to identify by PCR 16S rRNA technique. They belonged to four *Streptomyces*, one *Microbacterium*, and one *Rhodococcus*. The present study showed that the potential bioactive compound from sponge-associated actinobacteria has not been exploited yet in the Kien Giang Sea, Vietnam. The other invertebrates as coral with endophytes containing many novel secondary metabolites especially, antibiotics, antimicrobial, antifungal, anti-cancer, and so on, need to study in the future.

REFERENCES

[1] N. A. R. Gow, "Microbe Profile: Candida albicans: a shape-changing, opportunistic pathogenic fungus of humans", Microbiology. Vol. 163(8), pp. 1145–1147, 2017.

[2] D. Bensasson, J. Dicks, J. M. Ludwig, C. J. Bond, A. Elliston, I. N. Roberts and S. A. James, "Diverse Lineages of Candida albicans Live on Old Oaks", Genetics, Vol. 211(1), pp. 277–288, 2019.

[3] C. Kerawala, C. Newlands, "Oral and maxillofacial surgery". Oxford University Press, pp. 446, 447, 2010.

[4] N. Martins, I. C. Ferreira, L. Barros, S. Silva and M. Henriques, "Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment", Mycopathologia, Vol. 177(5–6), pp. 223–240, 2014.

[5] A. Erdogan and S. S. C. Rao, "Small intestinal fungal overgrowth", Current Gastroenterology Reports, 17(16), 2015.

[6] R. Calderone and C. J. Clancy, "*Candida and candidiasis",* 2nd, ASM Press, 2012.

[7] A. A. Elbendary, A. M. Hessain, M. D. El-Hariri, A. AdelSeida, I. M. Moussa, A. S. Mubarak, S. A. Kabli, H. A. Hemeg and J. K. El-Jakeea, "Isolation of antimicrobial producing Actinobacteria from soil samples", Saudi Journal of Biological Sciences, Vol. 25(1), pp. 44-46, 2018.

[8] N. A. Al-Dhabi, G. A. Esmail, V. Duraipandiyan and M. V. Arasu, "Chemical profiling of *Streptomyces* sp. Al-Dhabi-2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications", Saudi Journal of Biological Sciences, Vol. 26(4), pp. 758-766, 2019.

[9] A. B. Galindo, "*Lactobacillus plantarum* 44A as a live feed supperlement for freshwater fish", Ph.D Thesis, 2004.

[10] J. Sambrook, E. P. Fritsch and T. Manlatis, "In vitro amplification of DNA by the polymerase chain reaction", In: Molecular cloning. A Laboratory Manual 2nd, New York: Cold Spring Habor Laboratory Press, 1989.

[11] J. E. M. Stach, L. A. Maldonado, A. C. Ward, M. Goodfellow and A. T. Bull, "New primers for the class Actinobacteria: application to marine and terrestrial environments", Environ. Microbiol, Vol. 5, pp. 828-841, 2003.

[12] C. Spampinato and D. Leonardi, "*Candida* infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents*",* BioMed Research International, Vol. 2013, pp. 1-13, 2013.

[13] C. C. Cain, D. Lee, R. H. Waldo III, A. T. Henry, J. E. Jr. Casida, M. C. Wani, M. E. Wall, N. H. Oberlies and J. O. Falkinham III, "Synergistic antimicrobial activity of metabolites produced by a nonobligate bacterial predator". Antimicrob, Agents Chemother, Vol. 47(7), pp. 2113 – 2117, 2003.

[14] J. Berdi, "Bioactive microbial metabolites: a personal view", The Journal of Antibiotics, vol. 58(1), pp. 1-26, 2005.

[15] S. Belghit, E. H. Driche, C. Bijani, A. Zitouni, N. Sabaou, B. Badji and F. Mathieu, "Activity of 2,4-Di-tert-butylphenol produced by a strain of *Streptomyces mutabilis* isolated from a Saharan soil against *Candida albicans* and other pathogenic fungi", Journal de Mycologie Médicale, Vol. 26(2), pp. 160-169, 2016.

[16] M. S. Palla, G. S. Guntuku, M. K. K. Muthyala, S. Pingali and P. K. Sahu, "Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil", Beni-Suef University Journal of Basic and Applied Sciences, Vol. 7(2), pp. 250–256, 2018.

Optimization of a Filter Medium Suitable for Direct Irrigation with Seawater through a Water Table

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Abstract— *The method that humanity has adopted to moisturize and thus bring to life the plants, imitating the model that was most visible, is rain. However, the great secret of irrigation lies in the land, in the water table and aquifers that treasure and manage water, sending away every drop of rain and spreading water through the underground basins of rivers, indirectly watering from the mountain to the Sea. The key is in the different circulation rates of groundwater because of the nature of the substrates. However, agriculture has adopted irrigation from above as we know it and has focused especially on drainage capacity. From this point of view, saline water is not beneficial for irrigated agriculture, but it may be the only source of irrigation water in large arid regions, especially in developing countries, where extreme freshwater scarcity and rapid population growth require more water.*

When it is raised the possibility of watering with seawater without desalination, always through capillary systems, it is essential to take into account the different soil strata, the distance to the water table, the composition of the seawater, the capacity soil chemical reactions to salts, etc. Modification of any of these parameters may produce salinization effects, moisture loss or desertification among others.

Keywords— ***Desertification, Desalination, Reuse, Marine Water Table.***

I. INTRODUCTION

Soil salinization is the process of accumulation in the soil of water-soluble salts. It can occur naturally, when it comes to low and flat soils, which are periodically flooded by rivers or streams; or if the groundwater level is shallow and the water rising by capillary contains dissolved salts (Chhabra, 1996).

When this process has an anthropogenic origin, it is usually associated with irrigation systems. It is called saline soil to a soil with excess soluble salts. Typically, the dominant salt is sodium chloride (NaCl). One consequence of soil salinization is the loss of fertility, which harms or makes agricultural cultivation impossible. Usually, the process is slowed or reversed by expensive washing of the soil to leach the salts or, alternatively, to grow plants that better tolerate salinity (Hoorn & Alphen, 2006).

On the other hand, it is known that the Inca culture developed a technology based on modifying the surface of the soil to facilitate the movement and storage of water. The main feature of this system is the construction of a network of embankments and channels, currently in use, as shown in Figure 1.

The water channels serve two very important functions, the first is to provide the water necessary for the growth of plants, since the proximity to water allows the area to remain moist and the plants can absorb the nutrients they need. The second function is to achieve a temperature more suitable for plants than the environment. The body of water allows to absorb excess cold in the nights and winter periods, preventing frosts from affecting the plants. Likewise the water absorbs the midday heat and radiates it at night where it is necessary to balance the cold of the night (Carolina Sparavigna, 2010).

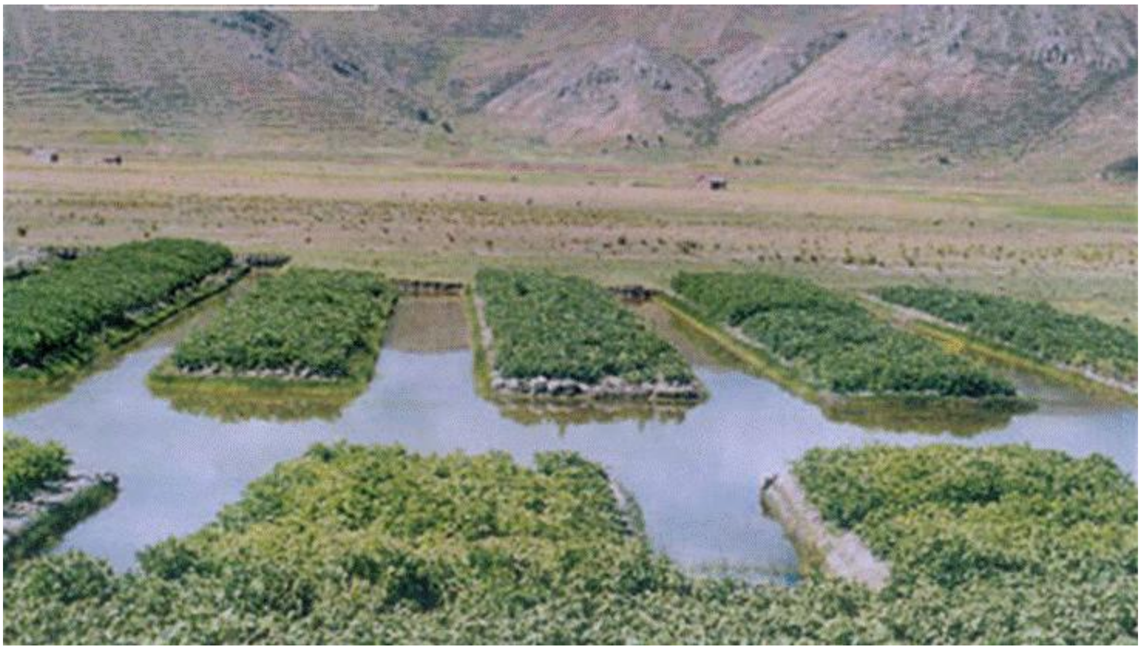

FIGURE 1: Set of embankments known as waru in the highlands of Peru.

Terraced agriculture is a phenomenon developed since time immemorial and distributed throughout the globe. In Asia: Sumatra, Philippines, Yemen, Nepal, China, Turkey, Pakistan and elsewhere. In Africa: Ethiopia, Sudan, Uganda, Tunisia, Algiers and others. In Europe: Spain, Portugal, Italy, Romania, France, Switzerland and other places. In America: from the southern United States to northern Argentina (Donkin, 1979).

Almost two thirds of the water used by man goes to agriculture. In Asia, the proportion increases to four fifths. Agriculture also impacts the basis of its own future through land degradation, salinization, excess water extraction and a reduction in agricultural genetic diversity. To date, issues related to water resources have not been adequately addressed in climate change analyzes or in climate policy-making (Bates, et al., 2008).

Globally, a water volume of more than 1,000 m3 per inhabitant per year is considered more than necessary for domestic, industrial and agricultural uses. As a result, a basin is estimated to suffer from water stress when its water availability per capita is less than 1,000 m3 /year (based on the historical average runoff) or when the ratio between water extraction and the annual historical average of runoff is greater than 0.4. There are such basins in North Africa, the Mediterranean region, the Middle East and the Middle East, South Asia, Northern China, the United States of America, Mexico, northeastern Brazil, and the western coast of South America. The population living in these basins amounts to an estimated total of between 1.4 billion and 2.1 billion people (V.R.Smarty et al., 2000; Alcamo et al., 2003a, b; Oki et al., 2003; Arnell, 2004).

Currently, agriculture is one of the main contributors of greenhouse gases, with 13.5% of the world's emissions. At the same time, climate change increases the risks and uncertainty of farmers, by warming and consequent aridity, by changes in rainfall regimes and by the increasing incidence of extreme weather events (FAO & Earthscan, 2011).

Irrigation accounts for about 70% of the water extracted worldwide and accounts for approximately 40% of agricultural production (Fischer et al., 2006). In fact, irrigated lands, which account for only 18% of the world's agricultural land, produce 1 billion tonnes of cereals per year, accounting for about half of the total world supply; this is because irrigated crops produce, on average, between 2 and 3 times more than rain-dependent crops (Alexandratos, N., 2005).

Overall, global warming appears likely to benefit agriculture in developed countries in temperate areas and have adverse effects on the production of many developing countries in tropical and subtropical areas. Climate change could therefore increase developing countries' dependence on imports and accentuate the differences between north and south in food security (Canadell et al.,2007).

Water management aims to improve the quantity and quality of available water. The ways to achieve this are: regulate the use of surface and groundwater, develop alternative sources of water, rationalize their consumption, control the supply of pollutants and recover initial conditions through purification processes. The objective of good water conditions should be pursued in each watershed, so that measures relating to surface water and groundwater belonging to the same ecological, hydrological and hydrogeological system are coordinated (Directive 2000/60/EC). From this perspective, the reuse of purified waters is an essential element of the natural water cycle and is, in fact, seen as a measure to solve the problems of water scarcity.

Given the high pollution rates of rivers, reservoirs and groundwater, an important option is presented: the desalination of seawater to obtain consumable water (Lechuga et al., 2007). Their demand has increased considerably in recent years. This is mainly due to the serious water resource shortage suffered in various parts of the planet.

In recent years, the idea that water management should be understood as an instrument in the service of an explicit territorial policy has been reinforced and that it will also be supported by the growing demand for integration between water management and sectoral policies (Moral 2009). From this perspective, this study proposes the direct use of seawater, without going through a process of prior desalination, as a fluid to be used in the irrigation of various types of cultivation.

II. MATERIAL AND METHOD

All the experiences that relate in this study have in common that seawater was circulated underground. In this way, by capillary, the seawater was dispersed through the corresponding solid substrate.

2.1 Material in contact with seawater

Preliminary studies (García et al., 2019) showed that the direct use of seawater as an irrigation fluid, as long as it is administered phreatically, is viable. However, this necessary condition is not enough. It is necessary to have a substrate as a filter with certain characteristics (composition and granulometry) that allow reducing the saline content and keeping the humidity at a sufficient height so that the roots of the cultivated vegetables can absorb enough water and nutrients without reaching to toxicity limits.

Based on the climatic classification, proposed by Kóppen and modified by Geiger (Kottek et al., 2006), it was estimated that a first use of seawater as an irrigation fluid would be appropriate in semi-arid climates, both warm (BSh) with an average temperature annual above 18°C and an average annual rainfall between 300 and 700 mm (tropical and subtropical semi-deserts) as cold (BSk) with an average annual temperature below 18°C and an average annual rainfall between 250 to 500 mm (temperate and cold semi-deserts). Consequently, as substrates materials were chosen that I can find in arid areas such as beach sand and fine grain aggregate, in this case from quarries and subjected to a crushing and sorting process. The particle size of both substrates was between 0.06 and 4 mm.

The initial objective was to determine the height that, by capillary, seawater would reach in the presence of these substrates. For this purpose, some specimens made of plastic tubes (PVC), 60 cm high and 18 cm in diameter were prepared. At the lower end, in contact with seawater, a net was incorporated thick enough to maintain the substrate. Once carefully refilled to prevent the formation of air chambers, these tubes were inserted in trays 2 m long, 1 m wide and 7 cm high through which seawater circulated and that allowed to maintain a constant water height of 3 cm. It worked on open circuit, so that seawater was continuously renewed.

Two working groups were established. The first group of tubes was filled only with the fine grain arid, while the filling of the second group consisted of a 10 cm high layer of beach sand at the lower end and the rest of the tube was filled with the fine grain arid, as shown in Figure 2.

In order to determine the evaporation rate of water on the surface of each tube, both groups were subdivided into three subgroups: a) tubes without surface protection, b) tubes with full surface protection, c) tubes with partial surface protection, as shown in Figure 3, and an experience was scheduled to subject all the tubes to a 24-hour seawater flow over a variable time period of 1, 2, 4, and 8 weeks, as shown in the diagram in Figure 4.

(a) **(b)**

FIGURE 2: Appearance of the two types of tube used a) without filter b) with beach sand filter.

(a) **(b)** **(c)**

FIGURE 3: a) tube without surface protection b) tube with partial surface protection c) tube with full surface protection.

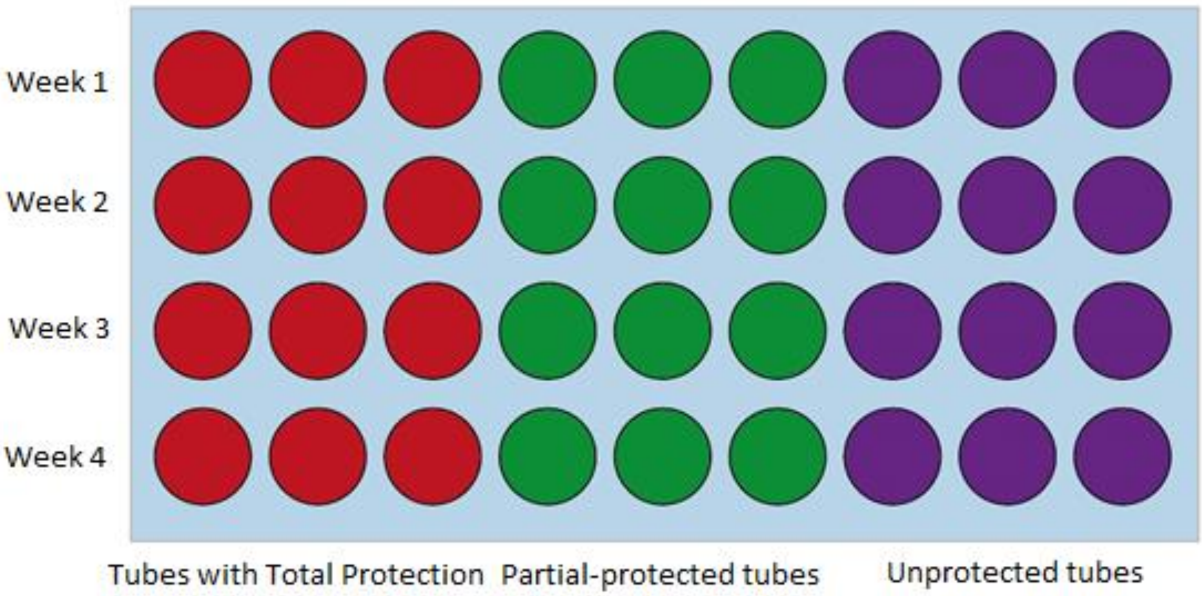

FIGURE 4: Temporal and spatial distribution of all tubes (with filter and without filter)

2.2 Sampling and determined parameters

The height of the tubes that did not contain a beach sand filter was divided into 5 sections, while the tubes that contained this type of filter their height was divided into 6 sections, so that one of the samples corresponded to the sand filter of beach and the rest to the fine grain aggregate used in the experience.

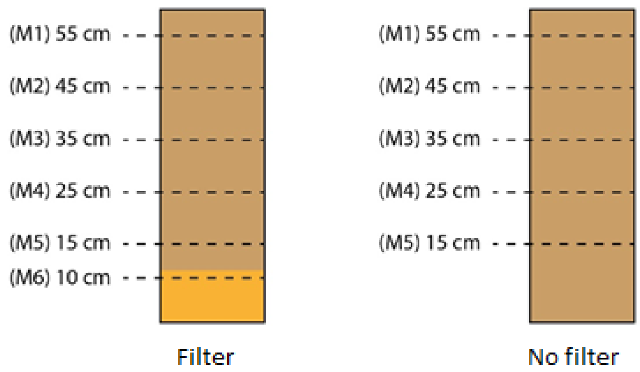

FIGURE 5: Dimensions where samples were taken in filter and no filter tubes

Once the samples had been collected and closed in airtight containers, moisture, conductivity and pH were determined.

Experimentally, the first parameter determined was humidity at the different dimensions set (see Table 1). To do this, different samples, of known weight, were subjected to 110oC inside a laboratory stove over a period of 48 hours. Then, they were introduced into a desiccator until room temperature was reached and it was weighed. The weight difference determined the moisture value of each sample. The determination of the weights was carried out with a SCALTEC SBA 52 precision balance and heat treatment with a Nahita 631/4 laboratory stove.

TABLE 1
EVOLUTION OF MOISTURE IN THE DIFFERENT SETS OF TUBES.

Humidity (%)		With filter (B1)				Without filter (B2)			
		Week 1	Week 2	Week 4	Week 8	Week 1	Week 2	Week 4	Week 8
With lid (T)	M1	4,56±1,44	6,35±1,52	7,80±0,05	6,55±0,22	2,87±0,36	6,48±0,34	7,54±0,23	6,57±0,34
	M2	5,18±1,57	5,78±0,61	6,84±0,41	7,33±0,29	3,24±0,05	6,76±0,79	7,16±0,09	6,83±0,56
	M3	6,14±2,02	6,91±0,26	7,96±0,66	7,88±0,34	3,56±0,21	7,09±0,29	8,73±1,11	7,35±0,47
	M4	7,45±2,05	8,88±0,34	9,18±0,71	8,99±0,86	9,11±0,43	8,63±0,43	9,22±0,75	8,60±0,36
	M5	11,93±0,46	9,76±0,73	10,56±0,65	10,99±1,88	11,34±0,47	11,08±0,91	11,57±1,04	10,31±0,57
	M6	7,76±0,20	10,11±1,14	8,00±0,09	5,63±1,55				
With hole (A)	M1	2,56±0,09	2,29±0,76	6,64±0,08	6,31±0,39	4,21±1,61	5,46±1,11	7,43±2,34	6,18±0,40
	M2	4,04±0,79	4,89±1,17	6,65±0,87	6,27±0,23	5,24±1,52	6,38±0,84	6,68±1,12	7,00±0,47
	M3	5,18±1,44	6,20±0,96	7,57±0,86	7,02±0,10	6,51±1,53	7,16±0,68	7,47±1,08	7,77±0,60
	M4	6,91±1,37	7,76±1,31	9,97±1,09	8,37±0,67	9,18±0,09	9,09±0,47	8,44±0,06	9,32±0,65
	M5	8,61±2,24	8,94±1,67	10,45±0,50	9,73±0,30	12,67±0,44	10,65±0,25	10,35±2,71	10,58±0,23
	M6	7,17±0,20	12,19±1,41	9,18±1,02	8,32±0,23				
Without lid (N)	M1	1,26±0,08	0,91±0,04	4,38±2,50	7,31±0,90	1,06±0,03	5,52±0,22	6,36±0,22	8,13±0,01
	M2	2,97±0,03	2,61±0,32	6,09±1,99	6,97±1,07	2,93±0,10	6,44±0,27	7,27±0,06	10,24±1,41
	M3	3,18±0,06	3,59±0,23	7,27±1,69	7,15±1,61	4,07±0,54	8,08±0,15	8,06±0,32	10,01±0,21
	M4	3,43±0,10	5,10±0,33	9,36±2,63	8,16±1,23	9,08±0,21	8,62±0,05	8,85±0,30	10,52±0,63
	M5	3,53±0,14	5,31±0,48	8,23±2,60	9,21±0,88	12,43±0,75	9,80±0,13	11,10±0,32	11,93±0,49
	M6	5,07±0,28	8,66±1,76	8,19±1,48	6,78±0,31				

In unfiltered tubes, a clear trend was observed: the decrease in humidity as they were taken show further away from dimension 0, especially in those tubes that were not protected. In which they were protected, in whole or in part, the values at the upper end of the tube were influenced by the effect of evaporated water condensation on the protective surface.

Once the humidity present in the samples was determined, the content of salts that had been retained in the substrate as a result of direct contact with seawater was determined.

It was preceded as follows: 10 grams of each dry sample from the previous test were inserted into a screw cap container. Then 200 mL were introduced distilled water. To achieve a total dissolution of the salts contained in the sample, a magnetic stirrer (fly) was inserted into the beaker and kept in agitation at 800 rpm for 10 minutes. The contents of the container were left at rest for 24 hours in an airtight manner, decanted and filtered.

The volume of filtered liquid was sufficient to determine conductivity and pH. The conductivity was determined at a temperature of 25°C using a Jeulin JLC20 conductivity meter and the pH using a Thermo Scientic Orion 2 Star pHmeter.

It was found that the pH value remained virtually constant in all experiences, with a value of approximately 8 units. On the contrary, the values of humidity and conductivity evolved differently, observing a progressive decrease of both parameters, as they are determined in samples increasingly far from the base of the tubes, where seawater was constantly circulated with a conductivity value of 55 mS/cm.

As far as conductivity is concerned, both in the experiences carried out in fully covered tubes and in partially covered tubes, the value of this parameter, at eight weeks was maintained, at surface level, at values below 5 mS/cm. Only in those experiences made with tubes without any protection were achieved values of 15 mS/cm, in the case of using the beach sand filter and 19 mS/cm in the absence of this filter.

TABLE 2
EVOLUTION OF CONDUCTIVITY IN THE DIFFERENT SETS OF TUBES.

Conductivity (mS/cm)		With filter (B1)				Without filter (B2)			
		Week 1	Week 2	Week 4	Week 8	Week 1	Week 2	Week 4	Week 8
With lid (T)	M1	2,91±0,57	2,84±0,77	3,51±0,96	3,15±0,25	4,08±0,33	3,68±0,76	3,39±0,22	5,25±0,47
	M2	3,57±0,50	3,69±0,47	3,98±0,43	4,19±0,29	2,58±0,84	4,15±0,07	3,76±0,50	4,39±0,25
	M3	5,71±2,55	5,19±0,26	7,26±1,16	7,23±0,51	2,92±0,95	6,02±1,41	6,42±0,10	7,79±0,39
	M4	7,22±3,45	7,96±0,53	10,28±0,85	8,57±1,53	5,68±1,86	10,36±2,28	9,29±0,50	12,57±0,84
	M5	8,97±2,42	10,49±1,34	11,34±0,67	12,48±3,95	13,60±3,33	12,50±3,06	11,50±0,25	14,89±2,32
	M6	6,71±0,46	8,80±2,06	8,80±0,96	8,39±3,22				
With hole (A)	M1	3,48±0,22	3,65±0,53	3,67±0,44	4,78±0,16	2,18±0,25	3,29±0,17	4,83±1,06	5,27±1,51
	M2	3,81±0,49	3,57±0,67	4,18±0,24	5,50±0,78	2,97±0,84	4,01±0,36	4,29±1,23	4,84±0,90
	M3	3,34±0,78	4,89±0,68	6,29±1,69	7,59±0,64	4,39±0,54	5,48±1,17	6,88±1,99	7,31±1,24
	M4	6,67±2,82	8,77±1,62	9,33±0,93	9,81±1,23	6,15±1,01	9,43±0,74	10,14±1,79	10,52±3,21
	M5	11,85±4,21	9,83±0,59	9,90±0,15	10,95±0,76	11,87±1,88	11,02±0,15	11,78±2,08	15,42±2,32
	M6	9,27±1,42	10,90±2,83	7,94±1,68	8,36±0,29				
Without lid (N)	M1	2,93±0,30	3,03±0,66	7,44±2,90	15,65±1,65	4,23±0,61	3,56±0,75	6,53±0,97	18,65±5,16
	M2	3,08±0,78	2,82±0,82	6,33±2,13	7,23±2,55	4,31±0,16	4,17±0,04	5,98±0,22	8,02±1,80
	M3	2,98±0,98	2,83±1,18	7,08±2,56	8,14±1,06	3,47±0,11	6,68±0,06	7,13±0,43	11,37±1,43
	M4	2,71±0,88	5,17±0,68	12,50±4,07	9,30±1,20	5,24±1,08	9,08±0,36	8,40±0,42	12,73±1,66
	M5	7,31±2,84	5,75±1,52	8,51±3,14	9,76±0,64	12,61±2,41	10,84±0,26	10,29±0,37	27,11±0,52
	M6	7,52±1,55	6,92±2,08	7,96±0,92	9,05±0,92				

2.3 Optimizing the filter media

In view of a reduction in the order of 90% achieved for surface values of conductivity with a height of 60 cm, it was proposed to optimize the behavior of the filter medium.

Two objectives were set:

a) Minimize conductivity and maximize moisture.
b) The filter media had to be recoverable on site, since in a large-scale capillary irrigation system it is unfeasible to have to replace the materials used often.

The filter media component used in this study was beach sand screened and separated according to particle size. The sand composition used based on the particle size is shown in the following table.

TABLE 3
COMPOSITION OF THE SAND ACCORDING TO THE PARTICLE SIZE

Diameter (mm)	Composition (%)
d > 1,5	6,04
1,5 > d > 1	34,45
1 > d > 0,5	58,55
0,5 > d > 0,3	0,35
0,3 > d	0,61
TOTAL	100,00

Separately used 1 mm top granulometry sand, with a granulometry of less than 1 mm and unscrat sand. In this way, the potential for ascension by capillary of seawater could be compared in the same medium with variable granulometry.

In a first experience a PVC tube of 57 mm diameter, filled with beach sand of granulometry less than 1 mm to a height of 30 cm, was introduced in a container containing a layer of seawater 3 cm thick and that was renewed each day to prevent the formation of brine due to evaporation.

At four weeks, the electrical conductivity was determined at different levels, taking as a reference the interface between beach sand and seawater (0 cm). The dimensions chosen were: 0; 2,5; 5; 7,5; 15 and 30 cm. It was found that seawater had not exceeded the 15 cm height. Conductivity values are shown in Table 4.

TABLE 4
EXPERIMENTAL VALUES OF CONDUCTIVITY AND ITS EQUIVALENCE IN NaCl (g/L)

Dimension (cm)	CE (mS/cm)	[NaCl] g/L
0	1,017 ± 0,004	4,60
2,5	0,785 ± 0,002	3,48
5	0,558 ± 0,006	2,39
7,5	0,224 ± 0,001	0,78
15	0,123 ± 0,001	0,30
30	0,104 ± 0,001	0,21

In view of this result, a waterproofed canal with drainage system was built to control the maximum seawater height of 80 cm long, 34.7 cm wide and 20 cm high. A pump was used for water circulation with a flow rate of 4 L/min and worked in open circuit, i.e. with seawater renewal. Periodic renewal of the canal water was scheduled every 12 hours, to maintain an artificial water table 3 cm thick by renewing the water, avoiding the generation of brines by progressive accumulation of salts.

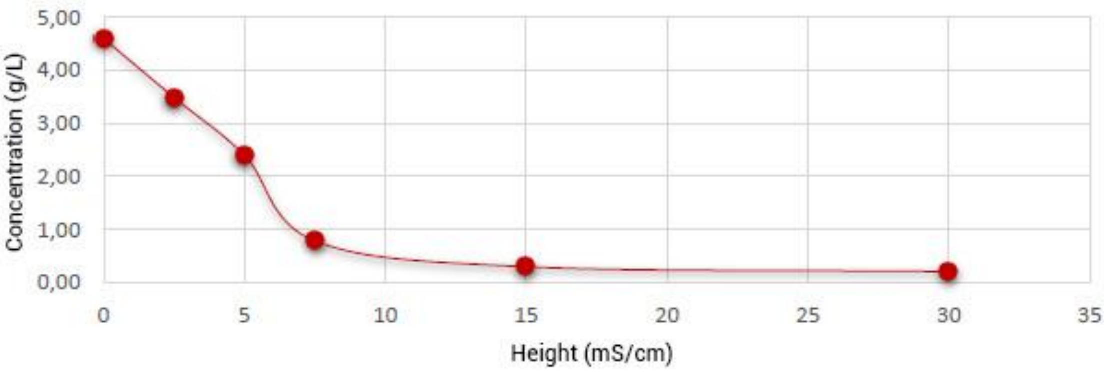

FIGURE 6: Graphical representation of electrical conductivity relative to the sampled dimension at four weeks

Thirty-six tubes 57 mm in diameter were prepared and available as shown in Figure 7. The different tubes were differentiated as follows: "P" corresponds to particles less than 1 mm; "G" with particles greater than 1 mm and "M" with unscreld beach sand.

P.4.1	P.4.2	P.4.3	G.4.1	G.4.2	G.4.3	M.4.1	M.4.2	M.4.3
P.3.1	P.3.2	P.3.3	G.3.1	G.3.2	G.3.3	M.3.1	M.3.2	M.3.3
P.2.1	P.2.2	P.2.3	G.2.1	G.2.2	G.2.3	M.2.1	M.2.2	M.2.3
P.1.1	P.1.2	P.1.3	G.1.1	G.1.2	G.1.3	M.1.1	M.1.2	M.1.3

FIGURE 7: Arrangement of PCV pipes in the seawater channel

Three tubes were allocated for each type of composition and sampling time, and three repetitions of each test can be made. Samples were taken at 4 and 8 weeks of different dimensions.

The pipes were divided into two groups: those that were not subjected to freshwater irrigation (rows 1 and 3) and those that were subjected to irrigation with 2 L of distilled water from its top (rows 2 and 4) at three weeks after the experiment began. Figure 8 shows the mean values of electrical conductivity within four weeks of starting the experiment.

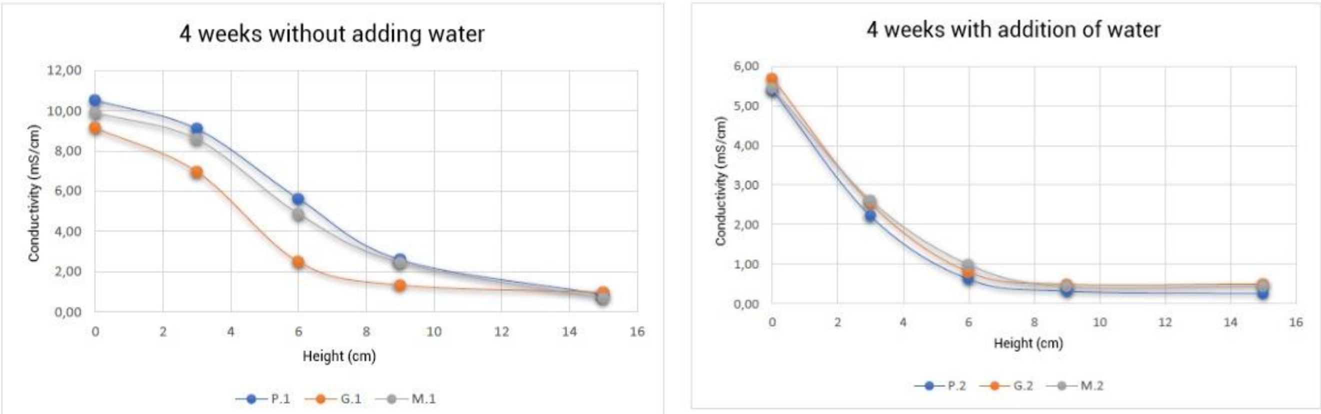

FIGURE 8. Evolution of conductivity at four weeks a) samples without irrigation b) samples with irrigation.

The EC value at level 0 in samples that were not washed with distilled water was significantly higher than the EC value at level 0 in samples subjected to distilled water irrigation, a fact that confirmed the reversibility of the filter. Leaching to reduce salt concentration was found to be a highly effective method. It is achieved by accumulating fresh water on the surface of the soil and allowing it to infiltrate. Leaching is effective when salt drainage water is discharged through underground drains that carry leached salts, as was the case.

The behavior observed in the samples analyzed after a four-week period was corroborated by the one observed at eight weeks (Figure 9).

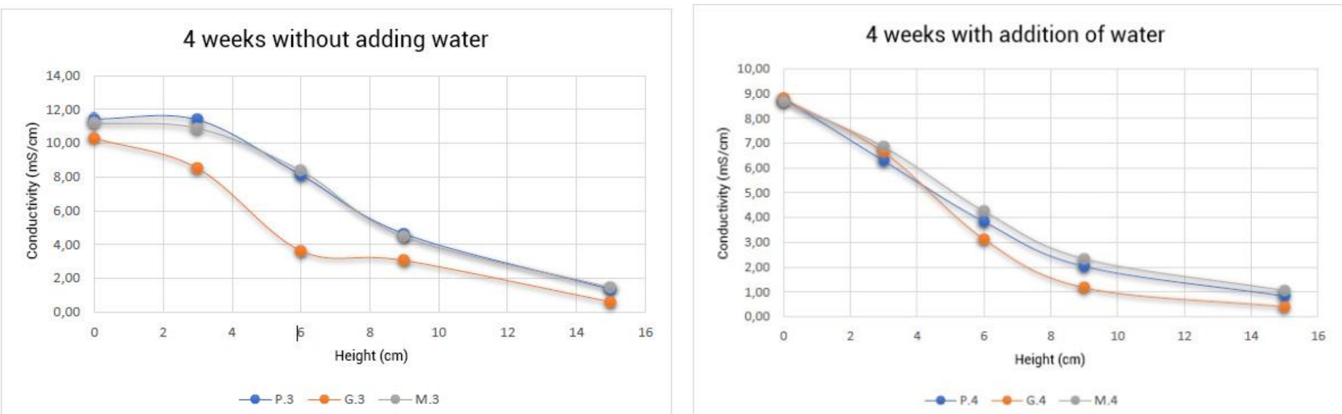

FIGURE 9: Evolution of conductivity at eight weeks a) samples without irrigation b) samples with irrigation.

From all the experiences, the different behaviour of the unscreened samples is inferred in experiences without surface irrigation with distilled water, at heights increasingly far from the reference point (0 cm), with lower CE values.

Based on the classification of soil types according to electrical conductivity (Abrol et al., 1988), unscrel dying provided a non-salt floor 9 cm from the water table (dimension 0) within four weeks of the experiment. By eight weeks, the EC had increased and slightly saline ground was available (see Table 5).

TABLE 5
CLASSIFICATION OF SOILS ACCORDING TO ELECTRICAL CONDUCTIVITY

Soil type	CE (dS/m)	Effect on crops
Non-salt	0 - 2	Insignificant salinity effects
Slightly saline	2- 4	Yields of sensitive crops may be affected
Moderately saline	4 - 8	Yields of many crops are restricted
Strongly salted	8 – 16	Only tolerant crops yield satisfactorily
Very strongly salty	> 16	Only a few very tolerant crops yield satisfactorily

Moreover, when EC values were considered in freshwater leaching experiences, the results corresponded to non-salt terrain on both occasions.

III. DISCUSSION

The capillary height that reaches the water in the soil is determined by considering a mass of soil as a large network of capillary tubes formed by the voids existing in its mass. Unlike capillary tubes, soil vacuums have variable width and communicate with each other in a latain. If this latat is communicated below with the water, its bottom becomes completely saturated. Higher the water only occupies the small voids and the larger ones are left with air. In a thin soil, the saturation level is above the water table by capillary ascension. The height reached is bounded by the weight of the water in the canals, when equalising the surface tension force (Atkinson, 1978).

Salinization is one of the major threats to agriculture worldwide, and it is a growing problem. In general, crops produce lower yields at higher salinity levels, and in the worst case, farmers have to leave their fields and clean up new land that increases pressure on natural ecosystems and associated biodiversity. In addition, salinity is expected to increase further, according to current predictions of climate change. Therefore, with a growing global population and climate change on a global scale, salinity is a problem that will only grow in importance and urgently requires a solution (Qadir et al., 2014).

A practical salinity index is electrical conductivity (EC), expressed in deciSiemen units per meter (dS/m), equivalent to mS/cm. Electrical conductivity values are always expressed at a standard temperature of 25oC to allow comparison of readings taken under different climatic conditions. Water with a CE less than 0.7 dS/m is considered non-salt or sweet water, while 2 dS/m is considered the maximum value for irrigation water (Rhoades et al, 1992).

High levels of salinity in the soil affect plants in several ways. First, it decreases the osmotic potential of soil pore water. This makes water absorption more difficult for plants as the plant has to decrease the osmotic potential of the roots to levels below the osmotic potential of soil moisture.

Second, NaCl molecules that are incorporated into the plant with water can cause physiological damage. Na+ ions, especially, can quickly reach toxic levels within the plant. Finally, due to the high concentrations of Na+ in soil pore water, competition occurs with K+ potassium ions, essential for plant growth, and plants may have difficulty absorbing enough K+ (Vos et al, 2016).Arid lands are among the most fragile ecosystems in the world, and their fragility is accentuated by periodic droughts and the increasing overexploitation of meager resources. Arid and semi-arid lands cover about a third of the area of emerging land, and there is a population of approximately one billion people who are mostly among the poorest in the world (Belaz, 2003).

The United Nations Conference on Environment and Development (UNCMAD, 1992) defined the concept of desertification as "the degradation of lands in dry, semi-arid and sub-humid areas as a result of various factors, including climatic variations and human activities". Desertification does not consist of the advance of existing deserts, but is a consequence of the effect of localized land degradation, and occurs rapidly after deforestation and soil depletion.

Desertification is a global problem that directly affects 250 million people; desertification particularly affects Africa, as three-quarters of the continent is drylands and deserts. However, more than 30 percent of the lands of the United States of America are also affected by desertification. A quarter of the surface of Latin America and the Caribbean are deserts and drylands. In Spain, 20 percent of the land is at risk of becoming desert. In China, since the 1950s, sand displacement and degradation have affected nearly 700,000 hectares of cultivated land, 2.35 million hectares of grasslands and 6.4 million hectares of forest, clear mountain and shrubland.Worldwide, about 70 percent of the 5.2 billion hectares of dry land used for agricultural activities are degraded and endangered desertification (FAO, 2007).

Currently, the main limitation of irrigated agriculture is the availability of water, both in terms of quality and quantity (García-Vila & Fereres, 2012). This scarcity can be due to several causes: aridity, drought, desertification or water stress (Pereira, et al., 2002).

With the increasing competition for non-renewable water resources around the world and the growing demand for agricultural products, the need to improve the efficiency and productivity of water use for crop production has never before been so pressing to ensure future food security and to address the uncertainties associated with climate change (Steduto, et al.2012).

The incorporation of desalinated seawater in irrigated areas is another alternative that has been on the rise in recent decades. Globally, major agricultural irrigation experiences with desalinated water show that, in many countries with arid or semi-arid climate, and which also have highly technical agriculture, brackish water desalination represents an additional source of (Anzecc & Armcanz, 2000). There are many countries (Israel, Spain, Malta, Australia, southern US states, Middle Eastern countries, North African countries, etc.) that have made use of different desalination technologies to ensure the domestic supply of water to their populations (Duranceau et al., 2011).

The Reverse Osmosis (OI) technique has become the reference technology for seawater desalination, as it has reduced energy consumption and production costs compared to other large-scale applicable technologies (Shaffer et 2012). Despite the competitive advantages of the (OI) over other desalination techniques, it still involves very high-energy consumption (Melgarejo & Montano, 2011).

Desalinated seawater is presented as a guaranteed resource of water, as it is part of an inexhaustible source. However, it presents a number of special features that may limit its direct use for agricultural irrigation, which must be considered and analyzed appropriately to be corrected if necessary with appropriate after treatments (Martinez-Beltran, 2006).

Desalinated water, especially if it has not undergone remineralization processes (ROSW), is characterized by low salinity and a very different salt composition than natural waters. The concentration of Na^{+} and Cl^{-} is usually high in relation to natural waters, while the presence of Ca^{2+}, $Mg^{2+}$and SO_4^{2-} is minimal (Yermiyahu et al., 2007).

If only irrigation water quality is taken into account, regardless of the other factors, you can ensure that, beyond thresholds characteristic of each crop or variety, as the electrical conductivity of the water (EC) increases the crop yields. However, there is a history that seawater has been used without desalination as irrigation water for crops (Iyengar, et.al, 1968).

Given the forecasts accepted by the main international agencies, the use of filter media that allows the development of crops directly with seawater (without desalination) will provide a number of advantages that will allow alleviating the short-, medium- and long-term needs. These advantages include:

- The release of fresh water for irrigation (redistribution for other uses)
- The use of arid areas for cultivation
- The provision of an unlimited resource at the global level
- The promotion of sustainable agriculture with job creation
- The contribution to satisfying the nutritional demand of a significant part of the world's population, which is constantly growing, in the near future.

The use of affordable materials will allow a wide development of this proposed irrigation model.

IV. CONCLUSIONS AND PERSPECTIVE OF THE FUTURE

The results obtained experimentally allow to state that the direct use of seawater as irrigation fluid, provided that it is administered in a phreatic way is feasible. However, this necessary condition is not sufficient. A filter substrate with certain characteristics (composition and granulometry) that reduce saline content and maintain moisture at a sufficient height is required so that the roots of cultivated vegetables can be reduced absorb enough water and nutrients without reaching toxicity limits.

In this study, it has been shown that the beach sand meets these requirements and that it can be regenerated by leaching with fresh water. An economic and abundant filter environment is therefore available. Conductivity levels need to be controlled. In this sense, in addition to the method of regeneration of the filter media, it is advisable to look for alternatives that allow controlling this balance. A very promising prospect is the incorporation of bioadsorbents, mainly from waste from the agri-food industry.

In addition, it is worth taking into bear in mind the climatic influence (rain, wind, temperature...) as long as this experience is not carried out inside a greenhouse where environmental conditions can be regulated. This is especially important for areas with a very low or no rainfall regime where the phenomenon of evaporation at surface level can cause a concentration of salts at undesired levels.

REFERENCES

[1] Abrol IP, Yadav JSP, Massoud FI. (1988). Salt affected soils and their management. FAO soil bulltin 39. FAO, Rome.

[2] Alcamo, J., P. Döll, T. Henrichs, F. Kaspar, B. Lehner, T. Rösch and S. Siebert. (2003a). Development and testing of the WaterGAP 2 global model of water use and availability. Hydrol. Sci. J., 48, 317–338. DOI: org/10.1623/hysj.48.3.317.45290

[3] Alcamo, J., P. Döll, T. Henrichs, F. Kaspar, B. Lehner, T. Rösch and S. Siebert (2003b) Global estimates of water withdrawals and availability under current and future “business-as-usual” conditions. Hydrol. Sci. J., 48, 339–348. DOI: 10.1623/hysj.48.3.339.45278

[4] Alexandratos, N., (2005). Countries with Rapid Population Growth and Resource Constraints: Issues of Food, Agriculture, and Development. Population and development review. 31(2): 237-258. DOI: org/10.1111/j.1728-4457.2005.00064.x

[5] Anzecc & Armcanz. (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality. The Guidelines Australian and New Zealand Environment and Conservation Council Agriculture and Resource Management Council of Australia and New Zealand.

[6] Arnell, N.W., (2004). Climate change and global water resources: SRES emissions and socio economic scenarios. Global Environmen. Chang., 14, 31–52. DOI: 10.1016/j.gloenvcha.2003.10.006

[7] Atkinson, J.H. y Bransby, P.L. (1978). The mechanics of soils. An introduction to critical state soil mechanics. McGraw-Hill, Londres.

[8] Bates, B.C., Z.W. Kundzewicz, S. Wu y J.P. Palutikof, Eds., (2008). El Cambio Climático y el Agua. Documento técnico del Grupo Intergubernamental de Expertos sobre el Cambio Climático, Secretaría del IPCC, Ginebra, 224 págs.

[9] Belaaz, M. (2003). Le barrage vert en tant que patrimoine naturel national et moyen de lutte contre la désertification. En *Proceedings of the XII World Forestry Congress*, Quebec, Canadá, 21-28 de septiembre de 2003.

[10] Canadell, J. G., C. Le Quere, M. R. Raupach, C. B. Field, E. T. Buitenhuis, P. Ciais, T. J. Conway, N. P. Gillett, R. A. Houghton y G. Marland. (2007). Contributions to Accelerating Atmospheric CO_2 Growth from Economic Activity, Carbon Intensity, and Efficiency of Natural Sinks. Proceedings of the National Academy of Sciences 104(47):18866–70. DOI: 10.1073/pnas.0702737104

[11] Chhabra, R. (1996). Soil salinity and water quality. Brookfield, USA A. A. Balkema Publishers. 284 p. ISBN 90 5410 727 8

[12] CNUMAD, Conferencia de las Naciones Unidas sobre el Medio Ambiente y el Desarrollo (1992). Capítulo 12: Ordenación de los ecosistemas frágiles: lucha contra la desertificación y la sequía. En Programa 21.

[13] Carolina Sparavigna, A. (2010) Landforms of Titicaca Near Sillustani. Editore: Lulu.com

[14] Directiva 2000/60/CE del Parlamento Europeo y del Consejo de 23 de octubre de 2000 por la que se establece un marco comunitario de actuación en el ámbito de la política de aguas.

[15] Donkin, R. A. (1979). Agricultural Terracing in the Aboriginal New World. Viking Fund Publications in Anthropology Nº 56, Wenner-Gren Foundation for Anthropological Research. Tucson. University of Arizona Press.196 pp

[16] Duranceau SJ, Pfeiffer-Wilder RJ, Douglas SA, Peña-Holt N, Watson IC. (2011). Posttreatment stabilization of desalinated water. Water Research Foundation, Denver, USA, 194 pp.

[17] FAO (2007). Situación de los bosques del mundo 2007. Roma.

[18] FAO & Earthscan (2011). The State of the World’s Land and Water Resources for Food and Agriculture. Managing systems at risk.

[19] Fischer, G., Tubiello, F.N., van Velthuizen, H., Wiberg, D. (2006). Climate change impacts on irrigation water requirements: global and regional effects of mitigation, 1990–2080. Tech. Forecasting Soc. Ch., 74, DOI: 10.1016/j.techfore.2006.05.021.

[20] García-Vila, M., & Fereres, E. (2012). Combining the simulation crop model AquaCrop with an economic model for the optimization of irrigation management at farm level. *European Journal of Agronomy*, *36*(1), 21–31. https://doi.org/10.1016/j.eja.2011.08.003

[21] García, J., Monagas, P., Martos, N., Gálvez, D., Arnal, O., Pujolà, M., Carol, J. (2019). Viability, method and device for horticultural crops with brackish and marine water. International Journal of Environmental & Agriculture Research (IJOEAR) 5(6): 5-25. DOI: 10.5281/zenodo.3264041

[22] Hoorn van J.W.; Alphen van J.G. (2006), Salinity control. In: H.P. Ritzema (Ed.), Drainage Principles and Applications, p. 533-600, Publication 16, International Institute for Land Reclamation and Improvement (ILRI), Wageningen, The Netherlands. ISBN 90-70754-33-9.

[23] Iyengar, E. R. R., Kurian, T., & Tewari, A. (1968). Utilization of Sea-water and Coastal Sandy Belts for GrowinB Crops in India. In H. Boyko (Ed.), Saline Irrigation for Agriculture and Forestry (pp. 24–40). https://doi.org/10.1007/978-94-017-6016-4

[24] Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F. (2006). World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift 15(3): 259-263. DOI: org/10.1127/0941-2948/2006/0130

[25] Lechuga J.; Rodríguez M.; LLoveras J. (2007). Análisis de los procesos para desalinización de agua de mar aplicando la inteligencia competitiva y tecnológica. Ingeniería, Revista Académica de la FI-UADY, 11-3, pp. 5-14, ISSN: 1665-529X

[26] Martínez-Beltrán J, Koo-Oshima S. (2006). Water Desalination for agricultural applications. Rome (Italy). FAO Land and water discussion paper, No 5, 60 pp.

[27] Melgarejo J, Montano B. (2011). Power efficiency of the Tajo-Segura transfer and desalination. Water Science & Technology 63:536–541. DOI: 10.2166 / wst.2011.254

[28] Moral, Leandro del (2009). «New trends in water management, spatial planning and integration of sectorial policies». Revista Electrónica de Geografía y Ciencias Sociales, XIII, 285.

[29] Oki, T., Agata, Y., Kanae, S., Saruhashi, T.,Musiake, K. (2003). Global water resources assessment under climatic change in 2050 using TRIP. Water Resources: Systems Water Availability and Global Change, S.W. Franks, G. Böschl, M. Kumagai, K. Musiake and D. Rosbjerg, Eds., IAHS Publication, 124–133

[30] Pereira, L. S., Oweis, T., Zairi, A. (2002). Irrigation management under water scarcity. Agricultural Water Management. DOI: org/10.1016/S0378-3774(02)00075-6

[31] Qadir M, Quillerou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Crechsel P, Noble AD 2014. Economics of salt-induced land degradation and restoration. National Resources Forum, United Nations. DOI: 10.1111/1477-8947.12054

[32] Rhoades JD, Kandiah A, Mashali AM. (1992). The use of saline water for crop production. FAO irrigation and drainage paper 48. FAO, Rome.

[33] Shaffer DL, Yip NY, Gilron J, Mebachem E. (2012). Seawater desalination for agriculture by integrated forward and reverse osmosis: Improved product water quality for potentially less energy. Journal of Membrane Science 415-416:1–8.

DOI: 10.1016/j.memsci.2012.05.016

[34] Steduto, P., Hsiao, T.C., Fereres, E., Raes, D. (2012) Respuesta del rendimiento de los cultivos al agua. Estudio FAO: riego y drenaje 66. Organización de las naciones unidas para la alimentación y la agricultura Roma

[35] Vörösmarty, C.J., P.J. Green, J. Salisbury and R.B. Lammers, 2000: Global water resources: vulnerability from climate change and population growth. Science, 289, 284–288. DOI: 10.1126/science.289.5477.284

[36] Vos, Arjen de; Bruning, Bas; Straten, Gerrit van; Oosterbaan, Roland; Rozema, Jelte; Bodegom, Peter van. (2016). Crop salt tolerance under controlled field conditions in The Netherlands, based on trials conducted at Salt Farm Texel. Den Burg: Salt Farm Texel - 39

[37] Yermiyahu U, Tal A, Ben-Gal A, Bar-Tal A, Tarchitzky J, Lahav O. (2007). Rethinking desalinated water quality and agriculture. Science 318:920–921.

Real Time Nitrogen Management in Rice using Leaf Colour Chart under Rainfed Condition of Western Hills of Nepal

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Abstract— *A field experimentation to determine the response of real time nitrogen management in rice using leaf colour chart on yield of rice under rainfed condition of western hills of Baitadi district of Nepal during rainy season of 2015. The variety for the field experimentation was carried in the variety "Rato Basmati". This variety was tested at five LCC based nitrogen management practices (N omission + recommended dose of P and K, recommended dose 100:30:30 kg NPK ha^{-1}, 30 kg N ha^{-1} + LCC < 4 @ 30 kg N ha^{-1}, No basal + LCC<4 @30 kg N ha^{-1} and 30 kg N ha^{-1} 15 DAT + LCC<4 @ 30 kg N ha^{-1}) in simple RCBD with three replication. The soil of experimental site was sandy loam in texture with pH 5.93. The data showed that there was saving of 10 Kg N ha^{-1} as compared to recommended N practice (100 Kg N ha^{-1}) in that LCC management practices where the basal application is omitted. Real time nitrogen management in rice using leaf colour chart significantly influenced the growth, yield attributes and yield over control. The highest plant height (122.90 cm), grain/panicle (103), grain yield (3890.83 kg/ha), harvest index (57.58) was obtained with no basal nitrogen plus LCC based nitrogen application. Maximum effective tiller (283.75) obtained from N2 application at 15 DAT+LCC based N application. The application of nitrogen only using the LCC or omitting the basal application further improve the efficiency of applied nitrogen and increase the yield by 75.53% and 25% respectively over the control and recommended practice.*

Keywords— Real Time Nitrogen Management, Leaf Colour, Western Hills of Nepal, rice management.

I. INTRODUCTION

Rice is the most important staple food crops in Nepal occupying hectares of land producing 5045045 tomes of grains with national productivity of 3.39 t ha^{-1} (ABPSD, 2014). Rice is the major cereal crop of the terai and inner terai occupying 67.87% of total area (ABPSD, 2013) but can be grown throughout all agro-ecological regions from terai plains to the high hills up to 3000 m above sea level (NARC, 2007) including valleys and foothills (Dhital *et al.*, 1995). Rice provides 50% of total calories requirement to Nepalese population and contributes 20% to the agriculture gross development product (NARC, 2007).

The national yields (ABPSD, 2014) of rice (3.39 t ha^{-1}) is far below the attainable yield of 5.00 t ha^{-1} (Dey and Hossain, 1995) and yield obtained in other major rice growing countries in the world. Furthermore this present yield of rice is not sufficient to meet the national demand. It is estimated that about 1259 thousand tons of additional rice need to be produced by 2030, which is equivalent to an overall increase of 27.96% in the next 17 years (Prasad *et al*., 2011; ABPSD, 2014). But the possibility of expanding the area in future is very limited. Therefore, the required extra production has to come only through increase in productivity with less water, labor, and chemicals and ensuring long-term sustainability. Agronomic management and technological innovations are needed to address these issues at present.

Among various reasons for this low productivity, inefficient utilization of nitrogen in considered to be the most critical one especially in south Asia including Nepal (Shukla *et al*., 2004; Witt *et al*., 2005). Nitrogen fertilizer must be applied only when necessary and must be based on the crops' nitrogen status. However, most farmers rely on the age (days after transplanting) of rice and not on nitrogen status of crop (Alam *et al*., 2005). They generally apply nitrogen fertilizer in fixed time recommended nitrogen split schedule and ratio at basal, maximum tillering and panicle initiation stages, without taking into account whether the plant really requires nitrogen at that time. The number of splits, amount of nitrogen applied per split and the time of applications vary greatly among the farmers (Witt *et al*., 2005; Regmi, 2003). This, consequently, caused inefficiency levels of nitrogen in terms of growth, development, and yield. Moreover, there are several farmers applying nitrogen fertilizer even if the crop does not need. As a result, there is an insignificant addition to the production cost and undesired effects on the growth of rice.

On the recent world-wide evaluation of fertilizer, its recovery efficiency has been found to be around 30% in rice (Krupnik *et al*., 2004). It has been observed that more than 60% of applied nitrogen is lost from the soil plant system due to lack of synchronization between the nitrogen demand and nitrogen supply (Yadav *et al*., 2004). The optimum use of nitrogen can be achieved by matching nitrogen supply with crop demand (Bijay *et al*., 2002) and caused greater yield responses to nitrogen application compared to farmer practice (Witt *et al*., 2005).

Viewing the scenario of low yield of rice in the hills of western Nepal, the experiment was conducted for sustainable improvement of the yield through the most economic and ecological prospective by effective nutrient management. The main objective of this study was to find out the effective nitrogen management practices through the use of LCC on rice production and to access the effect of different nitrogen management on growth, yield and yield attributes of rice under western mid hills of Nepal.

II. MATERIALS AND METHODS

The details of methods adopted and materials used during the course of study have been described in this chapter under following headings.

2.1 Description of the field experiment

2.1.1 Location

The experiment was carried out from June 2015 to October 2015 in the Agronomy farm of GAASC, Baitadi, Nepal which is located at Gokuleshwor, VDC of Baitadi District. The elevation of the sites, i.e. GAASC is at 700 masl with $24^{0}75'$ N latitude and $80^{0}50'$ E longitude. The research was conducted in the GAASC agronomy farm during rainy season of 2015.

FIGURE 1: Map showing research station (Gokuleshwor) of the Baitadi district

2.2 Physico-chemical characteristics of experimental soil

Composite soil sample were randomly taken from different spots from 0 – 15 cm depth using auger to record the initial soil physico-chemical properties of the experimental site. Soil sample was air dried, grounded and sieved through 2 mm sieve and tested for their properties.

The total nitrogen was determined by Kjeldhal distillation unit (Jackwson, 1967), available phosphorus by spectrophotometer (Olsen *et al.*, 1954) and available potassium by Ammonium acetate method (Black, 1965). Organic matter was determined by Walkey and Black method (1934), pH (1:2 soil: water suspensions) by Beckman Glass Electrode pH meter (Wright, 1939) and soil texture by hydrometer method. Physico-chemical properties of the soil of the experimental site are presented in Table 2. From the analysis, sand was found to be dominated in the physical properties of soil than silt and clay, possessing the sandy loam texture (Table 2).

On the chemical properties of soil, organic matter, total nitrogen, available phosphorus and potassium were observed. The average soil pH was found slightly acidic (pH 5.93) in the experimental field. The available nitrogen in the field represented medium (Khatri Chhetri, 1991), phosphorus in medium and high potassium in lower range whereas organic matter of soil (Jaishy, 2000) indicated the low soil fertility status (Appendix 2).

TABLE 1
PHYSICO-CHEMICAL PROPERTIES OF THE SOIL OF THE EXPERIMENTAL SITE AT GAASC, BAITADI

S.N.	Properties	Average content	Scale
1.	**Physical properties**		
	Sand (%)	30	
	Silt (%)	40	
	Clay (%)	20	
2.	**Chemical properties**		
	Soil pH	5.93	Slightly acidic
	Soil organic matter (%)	3.2	Medium
	Total nitrogen (%)	0.17	Medium
	Available phosphorus (Kg ha^{-1})	74.23	Medium
	Available potassium (Kg ha^{-1})	79.2	High
3.	**Texture/Rating**	Silt loam	

Source: NARC (Soil testing lab), Khajura, Banke district

2.3 Climatic condition during experimentation

The experimental site lies in the subtropical humid climate belt of Nepal. The area has sub-humid type of weather condition with cool winters, hot summers and distinct rainy season with annual rainfall of about 1919.5 mm. The metrological data for cropping season was recorded from NASA Power and presented Figure 2.

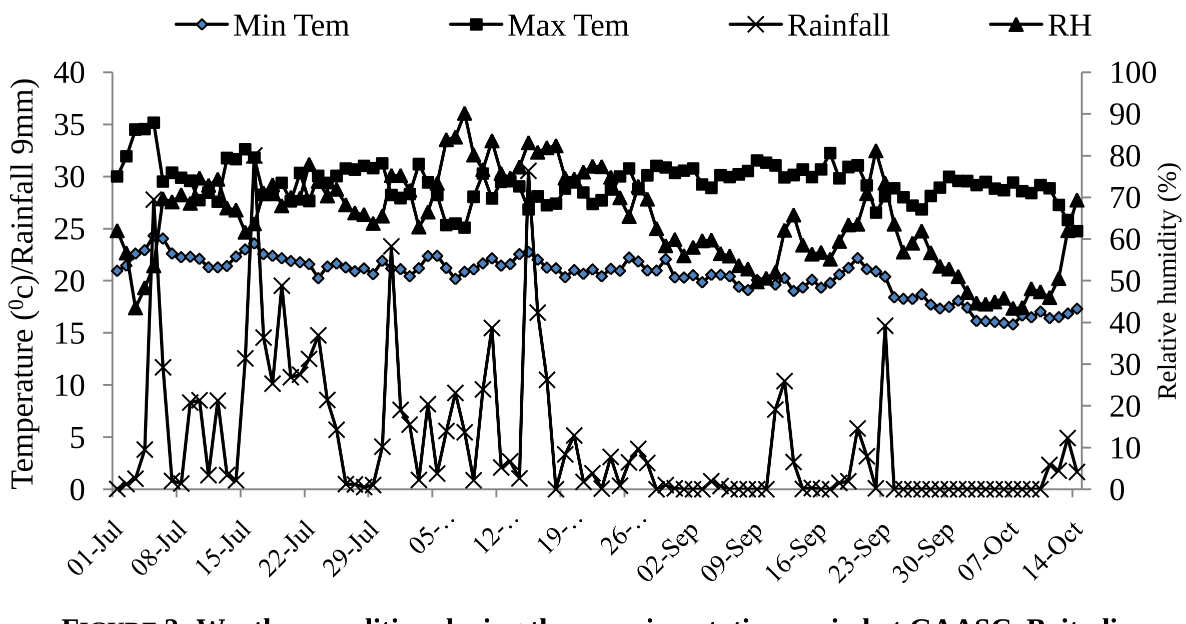

FIGURE 2: Weather condition during the experimentation period at GAASC, Baitadi
(Source: NASA power 2015)

The total rainfall of 472.8 mm was received during the entire period of experimentation whereas 300.00 mm of rainfall was recorded in the rice transplanting. The highest rainfall was recorded during July (256.05 mm) and lowest in October (10.67 mm).

The temperature regime in the experimental field was 15.81^0C -35.15^0C with average of 25.48^0C. The average relative humidity during the experimentation period was 64.70%.

2.4 Experimental details

2.4.1 Field layout

The experiment was laid out in one factors randomized complete block design with three replications having five treatments (Figure 2). The treatments consisted of combination of different levels and methods of nitrogen management namely control (nitrogen omission and recommended P and K), recommended NPK (100:30:30 kg NPK ha^{-1}), 30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}, No basal N + LCC at <4 @ 30 kg N ha^{-1} and 30 kg N ha^{-1} at 15 DAS+ LCC at < 4 @ 30 kg N ha^{-1} detailing in Table 3. The variety used in experiment is Rato Basmati, a long duration variety. The size of individual plot was 3 m × 2 m (6.0 m^2). There was a bund of 0.5 m width between two experimental plots and each replication was separated by bund of 1 m width. The crop geometry of rice was maintained at 20 cm × 20 cm (hill to hill and row to row spacing) with two-three to four seeding per hill with 10 rows in each plot. The five central rows were treated as net plot rows for harvesting and remaining five rows in each side were used for biometrical observation.

2.4.2 Treatment details

TABLE 2
TREATMENT DETAIL OF THE EXPERIMENT REAL TIME NITROGEN MANAGEMENT IN RICE USING LEAF COLOUR CHART ONRICE DURING RAINY SEASON OF 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

S.N.	Treatment combination	Treatment code
1	Nitrogen omission + Recommended P and K	T1
2	Recommended 100:30:30 kg NPK ha^{-1}	T2
3	30 kg N/ha basal+ LCC at <4 @ 30 kg N ha^{-1}	T3
4	No basal N + LCC at <4 @ 30 kg N ha^{-1}	T4
5	30 kg N/ha at 15 DAT+ LCC at < 4 @ 30 kg N ha^{-1}	T5

2.4.3 Cultivation practices

Rice was transplanted on 20 July by using 24 day old seedling in main field. Fertilizer was applied according to treatment combination, no basal nitrogen were applied in basal dose skip plot. Gap filling was done one week after rice transplantation. Two manual weeding were carried at 24 and 45 days after transplanting. Crop form net plot area was harvested and dried in-situ for 3 days then threshed and yield measurements were recorded.

2.5 Observation recorded in rice

2.5.1 Leaf color chart reading

For N top dressing under LCC based treatments, LCC reading was taken at an interval of 10 days on randomly selected 10 plants from 14 days after transplanting (DAT) to the beginning of flowering. Readings were taken on the top most fully expanded leaf of randomly selected fixed 10 disease free plants by placing its middle part on top of the color strips in the chart. If six or more leaves read bellow a set critical value (4), predetermined rate of N was applied immediately. N top dressing through LCC was 30 Kg N ha^{-1}.

- **Plant height**

Randomly selected entire two rows' 10 hills were used for the measurement of plant height at an interval of around 15 days from 15^{th} day, after transplanting and ending with 48 DAT. It was measured from base to tip of the upper leaves of the main stem.

- **Yield and yield attributing characters**:

Effective tiller/m^2 was counted from net harvested area. No of grain/panicle from 20 randomly selected panicle, thousand grain weight form threshed seed, adjusted straw yield and grain yield and harvest index were recorded in kg /ha.

2.6 Statistical analysis

Dependent variables were subjected to analysis of variance using GenStat for simple RCBD. Repeated measure analysis was performed to account for the measurements over time on plant height. All the recorded data were subjected to analysis of variance and Duncan's multiple range test (DMRT) for mean separations from the reference of Gomez and Gomez (1984). Treatments differences were considered statistically significant at 0.05 levels of significance. A simple correlation and regression analysis was run between selected parameters. And SPSS was used for the regression analysis was used for the graphical analysis.

III. RESULTS AND DISCUSSION

The results obtained during the experiment are analyzed and presented in this chapter with the help of the tables and figures wherever necessary. The results obtained are discussed with possible reasons and literature support.

3.1 Effect of LCC readings on N fertilization in rice

Nitrogen fertilizer, while essential for high yield and profit in rice farming, is often managed inefficiently by farmers (Alam *et al.*, 2005). To avoid the underutilization and over utilization of N to the rice crop, IRRI introduced the leaf color chart (LCC) to help farmers measure leaf color intensity, an indicator of the crop's need for N (Budhar and Tamilselvan, 2003). Thus LCC is a simple tool for improving the time and rate of N fertilizer in rice.

TABLE 3
TOTAL AMOUNT OF NITROGEN FERTILIZER APPLIED IN DIFFERENT TREATMENTS COMBINATIONS DURING 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

S.N.	Treatment detail	Nitrogen applied (kg ha^{-1})
1	T1 = N omission + Recommended dose of P and K	0
2	T2 = Recommended 100:30:30 kg NPK ha^{-1}	100
3	T3 = 30 kg N/ha basal+ LCC at <4 @ 30 kg N ha^{-1}	120
4	T4 = No basal N + LCC at <4 @ 30 kg N ha^{-1}	90
5	T5 = 30 kg N/ha at 15 DAT+ LCC at < 4 @ 30 kg N ha^{-1}	90

Note: LCC, Leaf color chart, DAT days after transplanting

The data on the amount of nitrogen applied either through the use of LCC or through other ways of nitrogen management is presented in Table 5. The data showed that there was saving of 10 Kg N ha^{-1} as compared to recommended N practice (100 Kg N ha^{-1}) in that LCC management practices where the basal application is omitted. Saving might be due to application of N fertilizer when crop demands, thus enhancing the efficiency of applied nitrogen by reducing losses.

3.2 Effect of nutrient management on plant height

The plant height of rice was increased up to 48 DAT and recorded maximum (117.07 cm). The different nutrient management practices significantly influenced the plant height at all dates of observation (Table 2).

TABLE 4
EFFECT OF REAL TIME NITROGEN MANAGEMENT IN RICE USING LEAF COLOUR CHART ON PLANT HEIGHT (CM) OF RICE DURING RAINY SEASON OF 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

Treatments	Plant height (cm)		
	15 DAT	33 DAT	48 DAT
T1 = N omission + recommended P and K	61.10^{c}	80.70^{c}	100.90^{b}
T2 = Recommended 100:30:30 kg NPK ha^{-1}	70.60^{b}	81.55^{c}	119.05^{a}
T3 = 30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}	72.05^{ab}	101.35^{a}	119.90^{a}
T4 = No basal N + LCC at <4 @ 30 kg N ha^{-1}	74.10^{a}	96.15^{b}	122.90^{a}
T5 = 30 kg N ha^{-1} at 15 DAT+ LCC at < 4 @ 30 kg N ha^{-1}	70.80^{b}	101.35^{a}	122.60^{a}
SEm (±)	0.82	1.45	1.52
LSD (=0.05)	2.78**	4.74**	4.95*
CV, %	2.10	2.70	2.20
Grand mean	69.73	92.22	117.07

Note: DAT, Days after transplanting; LCC, leaf color chart. Same letter(s) with in the column are not significant, **highly significant * significant

At 15 DAT, The average plant height was 69.73 cm and significantly varied among the treatments. The maximum plant height (74.10 cm) was recorded under treatment 4 (No basal N + LCC <4 @ 30 kg N ha^{-1}) and it was statistically similar to T3 (30 kg N ha^{-1} basal + LCC <4 @ 30 kg N ha^{-1}) but higher than other treatments. There was no significant difference among T3 (30 kg N ha^{-1}basal+ LCC at <4 @ 30 kg N ha^{-1}), T2 (Recommended 100:30:30 kg NPK ha^{-1}), and T5 (30 kg N ha^{-}

^{1} at 15 DAS+ LCC at < 4 @ 30 kg N ha^{-1}). The shortest plant height (61.10 cm) was recorded for treatment (N omission + recommended dose of P & K) and it was statistically lower as compared to rest treatments.

The average plant height at 33 DAT was recorded 92.22 cm and significantly varied among the treatments. The maximum plant height (101.35 cm) was recorded under T3 (30 kg N/ha basal+ LCC at <4 @ 30 kg N ha^{-1}) and T5 (30 kg N ha^{-1} at 15 DAS + LCC at < 4 @ 30 kg N ha^{-1}) and they were significantly taller than other treatments. The lowest plant height (80.70 cm) was recorded on T1 (N omission + recommended dose of P and K) which is statistically at par with T2 (Recommended 100:30:30 kg NPK ha^{-1}). The treatment 4 (No basal N + LCC <4 @ 30 kg N ha^{-1}) was intermediate in height (96.15 cm).

At 48 DAT, the average plant height was recorded 117.07 cm and which is significantly varied among other treatments. The maximum plant height (122.9 cm) was recorded under treatment 4 (No basal N + LCC <4 @ 30 kg N ha-1) and it was statistically similar to T2 (Recommended 100:30:30 kg NPK ha^{-1}), T 3 (30 kg N/ha basal+ LCC at <4 @ 30 kg N ha^{-1}) and T5 (30 kg N ha-1 at 15 DAS+ LCC at < 4 @ 30 kg N ha^{-1}). The lowest plant height (100.90 cm) was recorded for T1 (N omission + recommended dose of P & K) and it was statistically lower as compared to treatments.

3.3 Effect of nutrient management on yield attributes

The average effective tiller was (260.75) per square meter and significantly varied among the treatments. The highest effective tiller (283.75) was recorded in T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}) and it was significantly different from rest of treatments. T2 (Recommended 100:30:30 kg NPK ha^{-1}) and T3 (30 kg N ha^{-1}basal+ LCC at <4 @ 30 kg N ha^{-1}) had statistically similar effective tiller per square meter and the lowest effect number of tiller per square meter (240.00 was recorded in T1 (N omission + recommended dose of P and K) and statistically lower as compared to rest treatments.

TABLE 5
EFFECT OF REAL TIME NITROGEN MANAGEMENT IN RICE USING LEAF COLOUR CHART ON YIELD ATTRIBUTES OF RICE DURING RAINY SEASON OF 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

Treatments	Yield attributes		
	Effective tillers per square meter	Grains per panicle	Thousand grain weight (g)
T1 = N omission + recommended P and K	240.00^{d}	58.03^{c}	17.00^{a}
T2 = Recommended 100:30:30 kg NPK ha^{-1}	258.75^{c}	82.12^{b}	15.50^{a}
T3 = 30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}	267.50^{b}	76.50^{bc}	17.00^{a}
T4 = No basal N + LCC at <4 @ 30 kg N ha^{-1}	253.75^{c}	103.08^{a}	16.50^{a}
T5 = 30 kg N ha^{-1} at 15 DAT+ LCC at < 4 @ 30 kg N ha^{-1}	283.75^{a}	78.08^{bc}	16.50^{a}
SEm (±)	1.69	5.95	0.63
LSD (=0.05)	5.52	19.41	Ns
CV, %	1.10	13.00	6.60
Grand mean	260.75	79.56	16.50

LCC, leaf color chart, Common letter(s) with in the column are not significantly different at 0.05 level of significance by DMRT, Same letter (s) with in the column are not significant, **highly significant * significant

The average grain per panicle was 79.56 and maximum grain per panicle (103.08) recorded in T4 (No basal + LCC< 4 @30 kg N ha^{-1}) and it was statistically different rest of treatments. The treatment T2 (Recommended 100:30:30 kg NPK ha^{-1}) was statistically similar to T3 (30 kg N ha^{-1}basal+ LCC at <4 @ 30 kg N ha^{-1}) and T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}). The minimum grain per panicle (58.03) was recorded in T1 (N omission + recommended dose of P and K) and it was statically similar to T3 (30 kg N ha^{-1}basal+ LCC at <4 @ 30 kg N ha^{-1}) and T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}).

The average value of TGW was 16.50 g and highest TGW (17 g) was recorded in T1 (N omission + recommended dose of P and K) and T3 (30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}). All treatment had not significantly influenced the TGW.

3.4 Effect of LCC based nitrogen management yield and harvest index

The average grain yield was 3114.83 kg ha^{-1} and was significantly varied among the treatments. The maximum grain yield 3890.83 kg ha^{-1} was recorded in T4 (No basal + LCC< 4 @30 kg N ha^{-1}) and it was statistically similar to T2 (Recommended

100:30:30 kg NPK ha^{-1}), T3 (30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}) and T5(30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}). The minimum grain yield 2216.67 kg ha^{-1} was recorded in T1 (N omission + recommended dose of P & K) and it was statistically differ among rest treatments. It showed that grain yield was 28.78 % more in T2 (Recommended 100:30:30 kg NPK ha^{-1}) over T1 (N omission + recommended dose of P and K). The grain yield of T3 (30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}), T4 (No basal + LCC< 4 @30 kg N ha^{-1}) and T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}) was 44.36%, 75.52% and 42.29% respectively more than that of T1 (N omission + recommended dose of P and K). Also, the grain yield of T3 (30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}), T4 (No basal + LCC< 4 @30 kg N ha^{-1}) and T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}) was increased by 2.734%, 20.004% and 1.33% respectively as compared to T2(Recommended 100:30:30 kg NPK ha^{-1}). The per kg of nitrogen applied in recommended NPK resulted in 31.13 kg grain, while T3 (30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}), T4 (No basal + LCC< 4 @30 kg N ha^{-1}), T5 (30 kg N ha^{-1} @ 15 DAT + LCC < 4 @ 30 kg N ha^{-1}) resulted in 26.67 kg, 43.23 kg and 35.05 kg.

TABLE 6
EFFECT OF REAL TIME NITROGEN MANAGEMENT IN RICE USING LEAF COLOR CHART ON GRAIN YIELD (kg ha^{-1}), STRAW YIELD (kg ha^{-1}) AND HARVEST INDEX (%) OF RICE DURING RAINY SEASON OF 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
T1 = N omission + recommended P & K	2216.67^{b}	2104.17^{a}	50.97^{a}
T2 = Recommended 100:30:30 kg NPK ha^{-1}	3112.50^{a}	3091.67^{a}	50.72^{a}
T3 = 30 kg N ha^{-1} basal+ LCC at <4 @ 30 kg N ha^{-1}	3200.00^{a}	2487.50^{a}	56.17^{a}
T4 = No basal N + LCC at <4 @ 30 kg N ha^{-1}	3890.83^{a}	2850.00^{a}	57.58^{a}
T5 = 30 kg N ha^{-1} at 15 DAT+ LCC at < 4 @ 30 kg N ha^{-1}	3154.17^{a}	2579.17^{a}	54.90^{a}
SEm (±)	238.20	2.76.70	3.46
LSD (=0.05)	776.90	Ns	Ns
CV, %	13.20	18.20	11.10
Grand mean	3114.83	2622.50	54.07

LCC, leaf color chart, Common letter(s) with in the column are not significantly different at 0.05 level of significance by DMRT, Same letter (s) with in the column are not significant, **highly significant * significant

The average straw yield was 2622.50 kg ha^{-1} and maximum straw yield (3091.67 kg ha^{-1}) was recorded in T2 (Recommended 100:30:30 kg NPK ha^{-1}) and all the treatment had not significantly influenced the straw yield.

The average harvest index was 54.07 %. The maximum harvest index 57.58% was recorded in T4 (No basal + LCC< 4 @30 kg N ha^{-1}) and all the treatment had not significantly influenced the harvest index.

3.5 Correlation among yield attributes and yield parameters

Correlation among the yield attributes and yield was presented in Table 5. The grain yield was positively correlated with effective tillers per square meter (0.343) and number of grains per panicle (0.943**) and non-significant association with the thousand grain weight. The increase or decrease in the grain yield was greatly affected by the changed in effective tillers per square meter and grains per panicle. Similarly correlation among the harvest index and number of grains per panicle was also positive and highly significant.

TABLE 7
CORRELATION COEFFICIENTS FOR RELATIONSHIPS BETWEEN YIELD ATTRIBUTING CHARACTERS WITH YIELD OF RICE DURING RAINY SEASON OF 2015 AT GOKULESHWOR, VDC OF BAITADI DISTRICT, NEPAL

	GPP	TGW	GY	SY	HI
ET	0.177	-0.052	0.343	0.184	0.208
GPP		-0.229	0.943**	0.255	0.664**
TGW			-0.002	-0.038	0.044
GY				0.281	0.711**
SY					-0.466

Note: GPP, grains per panicle, ET, effective tillers per square meter, TGW, thousand grain weight, GY, grain yield; SY, straw yield and HI, harvest index; * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed)

IV. CONCLUSIONS

The data showed that there was saving of 10 Kg N ha^{-1} as compared to recommended N practice (100 Kg N ha^{-1}) in that LCC management practices where the basal application is omitted. Real time nitrogen management in rice using leaf colour chart significantly influenced the growth, yield attributes and yield over control and at par with the recommended nitrogen application. The application of nitrogen only using the LCC or omitting the basal application further improve the efficiency of applied nitrogen and increase the yield by 75.53% and 25% respectively over the control and recommended practice.

REFERENCES

[1] ABPSD. 2013. Statistical information on Nepalese agriculture 2012/2013 (2069/070). Government of Nepal Ministry of Agricultural Development, Agri-Business Promotion and Statistics Division, Agri-statistics Section, Singha Durbar, Kathmandu Nepal

[2] ABPSD. 2014. Statistical information on Nepalese agriculture 2013/2014 (2070/071). Government of Nepal Ministry of Agricultural Development, Agri-Business Promotion and Statistics Division, Agri-statistics Section, Singha Durbar, Kathmandu Nepal.

[3] Alam, M. M., J. K. Ladha, S. R. Khan, Foyjunnessa, Harun-ur-Rashid, A. H. Khan and R. J. Buresh. 2005. Leaf color chart for managing nitrogen fertilizer in lowland rice in Bangladesh. Agron. J., 97: 949-959.

[4] Bijay, S., S. Yadvinder, J. K. Ladha, K. F. Bronson, V. Balasubramanian, S. Jagdeep and C. S. Khind. 2002. Chlorophyll meter and leaf color chart-based nitrogen management for rice and wheat in northwestern India. Agronomy J., 94(4): 821-829.

[5] Black, C. A. 1965. Method of soil analysis. Part II. American Society of Agronomy. INC publisher, Madison, Wisconsin, USA. pp 1372-1376.

[6] Budhar, M. N. 2005. Leaf colour chart with nitrogen management in direct seeded puddled rice (*Oryza sativa*L). Fertilizer News, 50(3): 41-44.

[7] Dey, M.M. and M. Hossain, M. 1995. Yield potentials of modern rice varieties: an assessment of technological constraints to increase rice production. In: Proceedings of the Final Workshop of the Projections and Policy Implications of Medium and Long Term Rice Supply and Demand Project. Beijing, China, April 23-26, 1995.

[8] Dhital, B. K., T. B. Gurung, R. P. Bhari and K. D. Subedi. 1995. Agronomic research in rice. LARC working paper 1995, Lumle Kashi, No. 96:51.

[9] Gomez, K. and A. Gomez. 1984. Statistical Procedures for Agricultural Reserch, 2nd edition. Two factor experiments. A wiley interscience publication. John Wiley and Sons, New York. p 108.

[10] Jakson, M. L. 1967. Soil chemical analysis. Prentices Hall of India. Pvt. Ltd., New Delhi.

[11] Joshi, K.D., R.B. Rana, and A. Subedi. 2001. Farmers and researchers contribution to the selection of landraces of Gaiya (Upland Rice) for Tar areas of Nepal. LIBIRD/SANFEC: Kathmandu / Dhaka.13:1-32.

[12] Khatri-Chhetri, T. B. 1991. Introduction to soils and soil fertility. Tribhuvan University, Institute of Agriculture and Animal Science, Rampur Chitwan, Nepal. pp. 164-198.

[13] Krupnik, T. J., J. Six, J. K. Ladha, M. J. Paine and C. van Kessel. 2004. An assessment of fertilizer nitrogen recovery efficiency by grain crops. *In*: Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Production and the Environment, Scientific Committee on Problems of the Environment (SCOPE), A. R. Mosier, J. K. Syers and J. R. Freney (Eds.), Paris.

[14] NARC. 2007. Research highlights: 2002/03-2006/07. Communication, Publication and Documentation Division, Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur. 17 p.

[15] Olsen, S. R., C. V. Cole, F. S. Watanable and L. Dean. 1954. Estimation of available phosphorus in soil by extraction with Sodium carbonate. US Dept of Agric.

[16] Prasad, S. K., H. Pullabhotla and A. G. Kumar. 2011. Supply and Demand for Cereals in Nepal 2010-2030, In: IFPRI Discussion Paper 01120, Environment and Production Technology Division, New Delhi.

[17] Regmi, A. P. 2003. Improving the productivity of rice-wheat system through field specific nutrient management in Nepal. Thesis (Ph.D.) University of the Philippines at Los Banos (UPLB), Los Banos, Laguna, Philippines.

[18] Shukla, A. K., J. K. Ladha, V. K. Singh, B. S. Dwivedi, V. Balasubramanian, R. K. Gupta, S. K. Sharma, Y. Singh, H. Pathak, P. S. Pandey, A. T. Padre and R. L. Yadav. 2004. Calibrating the leaf color chart for nitrogen management in different genotypes of rice and wheat in a systems perspective. Agronomy Journal, 96: 1606–1621.

[19] Witt, C., J. M. C. A. Pasuquin, R. Mutters and R. J. Buresh. 2005. New leaf color chart for effective nitrogen management in rice. Better Crops, Vol. 89 (1): 36-39

[20] Wright, C. H. 1939. Soil analysis: A hand book of physical and chemicals. Thomas Murty and Co., London.

[21] Yadav, R. L., A. T. Padre, P. S. Pandey and S. K. Sharma. 2004. Calibrating the leaf color chart for nitrogen management in different genotypes of rice and wheat in a system. Agronomy J., 98: 1606-1621.

An Evaluation of different Forms of Granulated Compound Fertilisers and Micronutrients on Solanum lycopersicum var. Swaraksha

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Abstract— *An open field experiment was conducted at the University of Mauritius Farm to evaluate the effects of different forms of compound fertilisers and the response of incorporating micronutrients along with the compound fertilisers on the vegetative growth and yield of Solanum lycopersicum, var. 'Swaraksha'. Nitrogen, phosphorus and potassium being three essential plant nutrient components, play important roles in the production of Solanum lycopersicum. Besides, micronutrients in minute amounts are also indispensable for proper plant development. The treatments were randomly arranged in block design (RBD) with four replicates. These were: control (T1), 13-13-20-2 complex (T2), 13-13-20-2 complex with micronutrients (T3), 13-13-20-2 blended form with micronutrient (T4) and 13-13-20-2 in compacted form (T5). The results showed that treatment T4 had the most significant upsurge in growth and yield of Solanum lycopersicum with respect to the control block and compared to the other forms of fertilisers. The yields were found to be 47.83 t/ha under T4 followed by 35.52 t/ha under T3. The highest number of flowers and fruits per plant were also observed in T4. Hence, it is essential to employ these nutritive components for satisfactory growth and yield of Solanum lycopersicum while taking into consideration the costs of inputs.*

Keywords— blended, compacted, complex, costs, growth, yield.

I. INTRODUCTION

Tomato (*Solanum lycopersicum)* belonging to the Solanaceae family, is one of the utmost importance crop cultivated in Mauritius. It is a highly consumed crop both locally and around the World making a global production of up to 182,256,458 tonnes for the year 2018 (FAO, 2018). With regards to its nutritive value, 95% of S.lycopersicum content comprises of water and the other 5 % is carbohydrates and fibre (Bjarnadottir, 2015). The production of crop is the outcome of how well resources such as soil, water and nutrients are being used. A rise in the world's population has led to an increase in the food production to be able to cater for the demands of the population which clearly comes from the amplification of agricultural production. The amount of fertilisers consumed is estimated to rise by 40 % between 2002 and 2030 (FAO, 2000).

In the current experiment, the method of conventional farming is being put into practice which makes use of chemically manufactured fertilisers to monitor the health, growth and development of the plant for optimum yield. Fertilisers are crucial for the plant growth and development at their initial stage (Loks *et al*., 2014). In conventional agriculture, large amount of inorganic fertilisers is added to the soil to increase the quantity and the quality of the plant.

Agro-climatic conditions such as temperature, light and wind speed are also determining factors for a good plant performance. A day temperature of 25 ºC to 30 ºC and a night air temperature of 15 ºC to 20 ºC and a root zone temperature of 25.4 ºC to 26.3 ºC (Diaz-Perez & Batal, 2002). Below 10 ºC, the plants suffer from chilling injury and can cause fruit suppression (Naika *et al*., 2005). Light intensity has a powerful impact on the quality of the product and at the same time on the nutritional quality (Savvas and Passam, 2002). For photosynthesis, visible light is the source of energy (Xiong and Bauer, 2002). As the concentration of carbon dioxide increases in the atmosphere, the net photosynthesis also increases (Thongbai *et al*., 2010) and also when the stomata are opened and the plants are not in severe water stress (Kitaya *et al*., 2004).

Fertilisers are categorised mainly under macronutrients and micronutrients. In the farming context, nitrogen is the first primary macronutrient for plant growth and is needed in great amounts. It is absorbed as nitrate and ammonium ions (Forde and Clarkson, 1999). Nitrogen is important for the formation of amino acids, proteins and vitamins. The deficiency of nitrogen in plants causes poor growth and development of and causes young leaf blades to turn yellow or light green, die back on older leaves (Bianco *et al*., 2015).

De Groot *et al*., (2002) proclaimed that the rate of growth of S.lycopersicum expand rapidly with a high level of phosphorus. It promotes early root formation, growth and increase water-use efficiency (Grant *et al*., 2011). Deficiency of phosphorus include purple and red discoloration of leaf tips and margins, slender leaves, thin stalks, poor or no tillering and short internodes and finally poor growth (Kuo and Chiou, 2011). Potassium is the third macronutrient which is useful in carbohydrate breakdown and protein synthesis (Kadam *et al*., 2011) and also increases rate of photosynthesis (Battie-Laclau *et al*., 2014). A need in potassium can result in leaf wilt, distorted spindles, slim and short shoots. Gap analysis in the historical use of fertilisers revealed that no studies were conducted on the possible effects of different forms of compound fertilisers on a specific plant and the interest of incorporating microelements in the granules. Hence, it was hypothesized that the different forms of granulated compound fertiliers (13-13-20-2) would have no differences in plant development and yield of S.lycopersicum. The objectives of the experiment were to assess soil chemical parameters before and after experiment and to evaluate the effects of the different forms of compound fertilisers including micronutrients on S.lycopersicum

II. MATERIALS AND METHODS

2.1 Site Description

The field experiment was conducted on the University of Mauritius Farm (20°14'08.1"S, 57°29'26.6"E) located at Reduit during the summer season from October to January (2018-2019). The soil was prepared mechanically at a depth of 30 cm. Water was fed through sprinkler irrigation on a uniform basis.

2.2 Experimental design and data analysis

The trial was carried out to evaluate the different forms of granulated compound fertilisers and micronutrients on *Solanum lycopersicum* var. *Swaraksha* on 20 sub-plots of land giving an area of 500 m^2. The experiment was laid out on a completely randomised block design (RBD) with 5 replicates for each treatment and 4 blocks. The unit plot was 5.0 m by 2.8 m and would contain 20 *S.lycopersicum* plants. Statistical analysis was completed using Minitab 18® Statistical Software for Windows at 5% significance level by means of Tukey's Pairwise Comparisons. Plant height, leaf area and root dry matter content was modeled by using Microsoft Excel ® 2016.

2.3 Planting material

A spacing of 1.0 m was maintained between two plants horizontally and 0.6 m vertically between seedling to seedling. About 4 weeks of age of the '*Swaraksha*' variety from Maxiplex Ltd were collected and were transplanted to the field at a planting density of 33,333 plants/ha followed by sprinkler irrigation. Cultural practices for weeding, fertiliser and pesticide application were followed as stated in Le Guide Agricole (2010) of the Food and Agricultural Research and Extension Institute (FAREI).

2.4 Treatments

There were 20 plots (4 blocks and 5 treatments) comprising of NPK fertilisers as follows:

TABLE 1
TREATMENT ALLOCATIONS

T1 = Control (No fertiliser)
T2 = 13-13-20-2 (complex)
T3 = 13-13-20-2(complex + micronutrients)
T4 = 13-13-20-2(blended + micronutrients)
T5 = 13-13-20-2(compacted)

The fertilisers were applied at 3 stages throughout the crop cycle namely at the date of transplantation, 3 weeks after transplantation and at the flowering stage. Composted cow manure was applied only at the transplanting stage.

TABLE 2
AMOUNT OF FERTILIZER AND MANURE USED AT DIFFERENT STAGES

Fertiliser used (13-13-20-2)	Amount (g) - per hole	Amount (kg) - total
At transplantation	24	67.2
3 weeks after transplantation	9	3.6
At flowering	9	3.6
Manure	900	360

TABLE 3
RATE OF APPLICATION OF 13-13-20-2 AT DIFFERENT STAGES OF THE GROWTH

Stages	Nutrients (Kg/ha)		
	N	P_2O_5	K_20
At transplantation	52	52	80
3 weeks after transplantation	39	-	-
At flowering	20	20	30

2.5 Soil parameters analysis

Soil sample at a depth of 30 cm were collected before conducting the experiment from the experimental field using W-method as illustrated by Mungla and Chooneea (2016). Soil pH, electrical conductivity, total nitrogen, total potassium and total phosphorus before and after the experiment were analysed.

2.6 Plant parameters analysis

Physical plant analysis such as plant height, shoot and root dry matter content, root length, leaf area, harvestable and non-harvestable yield were recorded. Plant chemical analysis was also done by tagging the plants. Plant height was being monitored each week throughout the vegetative, flowering and fruiting stage to assess plant growth. The fruits were harvested when matured and ripened. The number of fruits per plant, weight per plant and yield were registered during harvest. Root length and leaf area were measured at the same time. Shoot and root dry matter content, root length and leaf area were also being supervised throughout the 3 stages upon uprooting, washed thoroughly with tap water first to remove the adhering soil and dust. They were dried first, oven dried at 70ºC to constant weight.

III. RESULTS AND DISCUSSION

3.1 NPK concentrations in soil

After the experiment, an increase in total nitrogen, total phosphorus, total potassium and electrical conductivity was noted.

The nitrogen content heightened from 3.55 ± 0.127 mg/kg before plantation to 8.56 ± 0.173 mg/kg after plantation. This increment can be justified by the administration of inorganic fertilisers as well as manure which was rich in organic matter; that is nitrogen. As the plant grows in size from the juvenile phase till the ageing phase, nitrogen in form of inorganic fertiliser is added at different phases namely at the transplantation day, three (3) weeks after transplantation week and lastly at the flowering stage in recommended doses which adds to the level of nitrogen in the soil. Nitrogen promotes rapid development, enhances fruit quality, and improves the foliage for photosynthesis. When the plant is mature enough, its roots' is far down the soil which boots up the uptake of Nitrogen (Leghari *et al*., 2016). This was similar to Afghan *et al*., (2005) who indicated that by making use of blended fertilisers has caused NPK levels to rise in sugarcane.

The amount of phosphorus level increased from 74.98 mg/kg at transplanting stage to 94.01 mg/kg post-transplanting. Phosphorus exhibits a noteworthy impact on the growth of the plant as a primary macronutrient (Radin and Eidenbock, 1984). To explain this increase in phosphorus level, it was related to its mobility in the soil, that is, phosphorus is relatively immobile in the soil. Very little amount inorganic phosphate travel through mass flow to the roots of plants and it provides in minute quantity as 1 to 5 % of the plant phosphorus requirement (Oliveira *et al*., 2010), hence more phosphorus remains in the soil for a longer time compared to nitrogen.

Total potassium level in the soil improved by 4.86 mg/kg. In an experiment conducted by Hariprakash and Subramanian in 1991, higher amounts of potassium increased the yield of tomatoes and it was also noted that the quality of fruits was affected by potassium supply (Lester *et al.*, 2005). By comparing soil-applied fetiliser with foliar potassium application, the foliar application is a better point of view according to Jifon and Lester (2009) who claimed that productivity and quality of fruits can be upgraded. It is similar to the actual field experiment in a sense that blended, compacted and NPK granular coated with micronutrients fertilisers were used and the potassium level was augmented. It was somewhat contradictory to Fufa (2018) stating that high amount of potassium inorganic fertilisers did not show any changes to it's' growth and changes in fruit weight in chillies.

3.2 Plant height

Plant height is one of the most important characteristics of tomato plant. The plant height of the tomato was measured 30 and 45 days after transplantation (DAT) of the tomato seedlings throughout the crop cycle and the results are presented in the histogram below

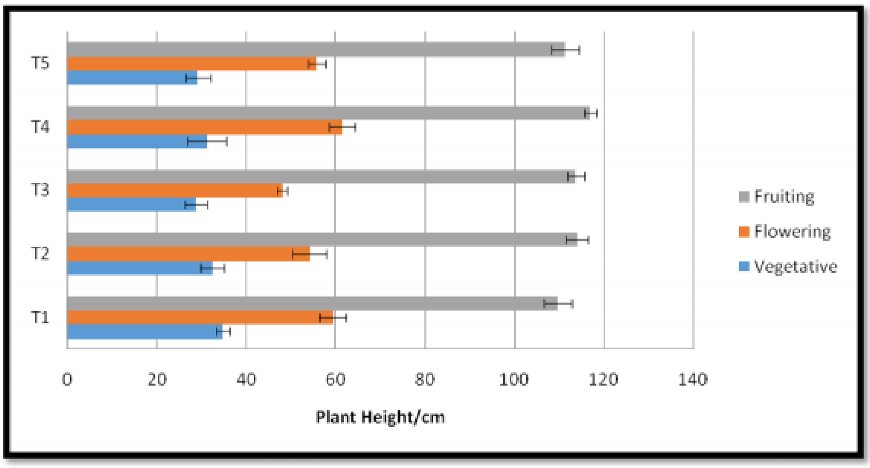

FIGURE 1: Plant height at the different stages of growth.

The mean plant height at the vegetative stage for T1, T2, T3, T4 and T5 was 34.69, 32.35, 28.68, 31.06 and 29.18 cm.

The mean plant height at the flowering stage for T1, T2, T3, T4 and T5 was 59.31, 54.14, 47.95, 61.48 and 55.71 cm.

The mean plant height for the fruiting stage for T1, T2, T3, T4 and T5 was 95.21, 86.70, 91.93, 97.15 AND 103.1 cm respectively.

Regarding the effect of different complex fertilisers on tomato plant height, the results of this experiment is in line with those obtained by Majumdar *et al.*, (2000) stating that potassium in the complex fertiliser strengthened the plant canopy and widened photosynthetic activity and therefore led to the growth of the plant.

3.3 Shoot and root dry matter content

Shoot dry matter content of tomato plants at fruiting stage revealed that plants treated with the blended, compacted and with micronutrients form of fertiliser had the highest shoot and root dry matter content. For the shoot biomass; at the vegetative stage it was optimum, 23.65 cm for the compacted form (T5), at the flowering stage, it was the highest for the control (T1), 35.53 cm and at the fruiting stage, it was the maximum for the blended form (T4), 50.83 cm. The increase in shoot biomass content from vegetative to the fruiting stage can be explained by Isitekhale *et al.*, (2013) stating that manure can be considered as a reservoir of the main macro elements and trace minerals. The General Linear Model revealed that the blocks did not differed statistically ($p>0.05$); while there was significant difference among the treatments ($p<0.05$) for the shoot biomass.

For the root biomass; at the vegetative stage and the flowering stage, it was optimum with the compacted form (T5), and at the fruiting stage, it was maximum with the control (T1). This increase can be related to the level of phosphorus in the soil. The movement of phosphorus to the root system occurs by diffusion with soil particles (Rahmatullah *et al.*, 1994). The root morphology of plants is such that it is crucial for absorbing dormant minerals such as Phosphorus (Zhu *et al.*, 2005). The diameter of the roots, root hairs affect the movement of Phosphorus in the soil (Aziz *et al.*, 2011). Root hairs improve the potential of the roots to look for rhizosphere (small zones found in soil) for Phosphorus because of its high surface area of absorption (Hill *et al.*, 2010). There is statistically no difference between root dry matter content and treatment ($p>0.05$) and block ($p>0.05$).

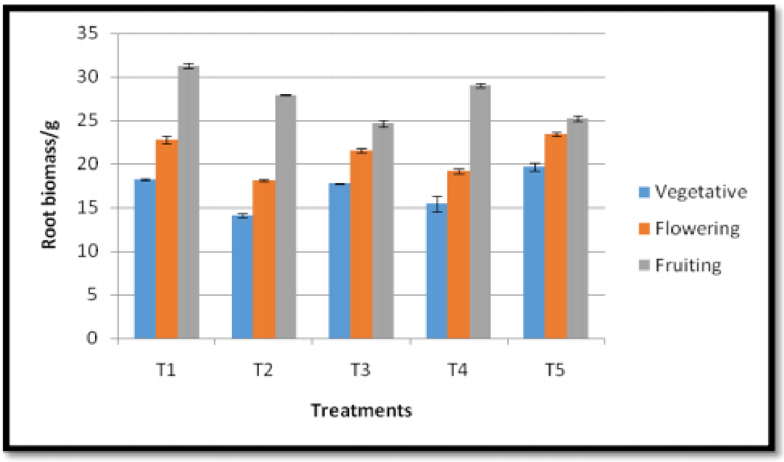

FIGURE 2: Root biomass at different stage of crop cycle

3.4 Root length

At the vegetative stage, the compacted form (T5) showed an increase in the root length with 24.25 cm. At the flowering and fruiting stage, the root length was the highest with the blended form of fertiliser (T4). There was no statistical difference in the length of the root for the five (5) treatments at the three consecutive phases of the growth cycle ($p>0.05$); in the same way there was no statistically significant differences between the blocks ($p>0.05$).

T3 contained micronutrients. It demonstrated that in the vegetative stage, the trace element Zinc favored the production of the auxin precisely Indole Acetic Acid (IAA) (Marschner, 1995) to achieve its maximum length. Indole Acetic Acid is the predominant common auxin found in most of the plants and is allowed for all developmental practices like it initiates roots (Phillips *et al.*, 2011) and literally forms lateral roots in dicotyledonous plants such as tomatoes (McSteen, 2010).

3.5 Leaf area

At the vegetative stage, plants treated with the 13-13-20-2 complex, T2 had the maximum leaf area. At the flowering and fruiting stage, the plants treated with the compacted form (T5) has the maximum leaf area. For the flowering stage, there was no significant difference among the block ($p>0.05$).

The maximum leaf area noted in this study was in Treatment 5. This finding agrees with the findings of Tabitha (2013) who studied on the effect of organic and inorganic fertilisers on growth and yield of amaranths who declared that basic ammonia or synthetic urea fertilisers has the tendency to initiate profuse growing of the plant that results in leaves of considerable size.

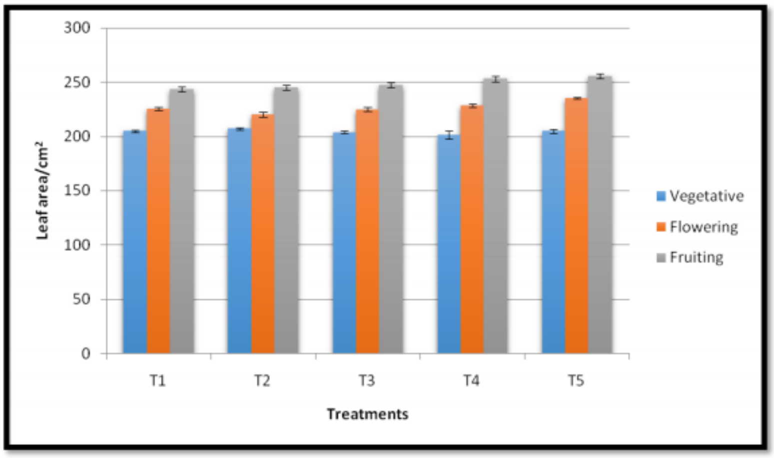

FIGURE 3: Leaf area of tomato crop throughout crop cycle

3.6 Yield

Number of fruits per plant is the most important yield attributing character of tomato plant and therefore, yield is the most important characteristics for the justification of the evaluation of different forms of complex fertilisers (13-13-20-2) on tomatoes. It was observed that the harvestable yield of tomatoes varied from 29.90 t/ha (T1) to 47.83 t/ha (T4) (Table 4).

TABLE 4
YIELD ATTRIBUTES OF DIFFERENT TREATMENTS

Treatments	Number of flowers per plant	Number of fruits per plant	Average fruit weight/g	Harvestable Yield(t/ha)	Non-marketable yield (t/ha)
T1	29	20	28.57	29.90	2.28
T2	18	18	29.52	29.96	1.88
T3	19	16	29.97	35.52	1.06
T4	25	23	30.11	47.83	2.33
T5	19	19	28.15	33.36	1.55

The yield with blended form of fertiliser could be linked due to sulfur presence (Fufa, 2018). As stated by Fufa (2018) applying NPK granular fertilisers, it could improve the growing characteristics which might be due to improved photosynthesis and other chemical reactions which consequently raises the plant biomass and is also accountable for cell differentiation.

Fayera *et al.,* (2014) claimed that the use of trace elements together with blended fertiliser can refine the concentration of the nutrient, nutrient uptake and therefore a better yield. It was advocated probably boron is crucial at the flowering stage and during the formation of tomato fruits (Naz *et al.,* 2012).

As blended fertiliser (T4) is a mixture of 13 % nitrogen as urea, 13 % phosphorus as TSP and 20 % potassium as muriate of potash, it might have helped to achieve the highest yield. Three (3) sources of N, P and K were mixed together but in separated granules, the solubility of these granules at different stages could have made the proper nutrients available to the plants as compared to compacted and complexed granules, which might require more water to dissolve the nitrogen and other elements. This actual statement is further supported by the fact that a higher plant height was measured under same treatment and foliage which is directly linked with supply of nitrogen for foliage development.

In addition, the use of blended form of fertiliser applied, coupled with the benefits of micronutrients might have resulted in uneven and excessive granules of potassium in the form of MOP to the plants, which in turn led to higher amount of potassium available to the plants, hence increasing the yield.

IV. CONCLUSION

This work shows that inorganic complex fertiliser (13-13-20-2) under all its forms significantly affected the parameters like plant height, shoot and root biomass, root length, leaf area and leaf area. Moreover after the experiment, an increase in the NPK content was observed as compared with the untreated plots. Throughout the years, fertilisers have been changing in terms of structure, formulations. Although it was often refuted for it unevenness, blended fertiliser, could reduce the fertiliser cost and at the same time increase productivity to match the use of compacted and complexes. Moreover, trace elements also played an important role and have cause to have a yield of 35.52 t/ha and 47.83 t/ha. Farmers should consider well before purchasing fertilisers since it constitute quite a reasonable expenditure in the cost of production. Blended synthetic nutrients are primordial as it has a high degree of fertiliser use efficiency. Therefore, by this experiment, it can be concluded that if different forms of granular NPK fertilisers are to be used for a crop production, the blended mode one will function better than any other granular NPK fertilisers and will yield will be higher.

COMPETING INTEREST

The author has declared that no competing interest exists.

REFERENCES

[1] **Food and Agriculture Organization (FAO) of the United Nations (2000).**Fertilizer requirements in 2015 and 2030. FAO, Rome. [Online] Available at: http://www.fao.org/tempref/agl/agll/docs/fertreqrev.pdf [Accessed10 Mar. 2019].

[2] **Loks, N.A., Manggoel, W., Daar, J.W., Mamzing, D. and Seltim, B.W., (2014).** The effects of fertilizer residues in soils and crop performance in northern Nigeria: a review. International Research Journal of Agricultural Science and Soil Science, 4(9), pp.180-184.

[3] **Bjarnadottir, A. (2015).** Tomatoes 101: Nutrition Facts and Health Benefits. [Online]. Healthline. Available at: https://www.healthline.com/nutrition/foods/tomatoes [Accessed 10Mar. 2019].

[4] **Díaz-Pérez, J.C. and Batal, K.D., (2002).** Colored plastic film mulches affect tomato growth and yield via changes in root-zone temperature. Journal of the American Society for Horticultural Science, 127(1), pp.127-135.

[5] **Naika, S., van Dam, B., Goffau, M.D., and van Lidt de Jeude, J. (2005).** Cultivation of tomato: Production, processing and marketing. Agromisa/CTA.

[6] **Savvas, D. and H.C. Passam. (2002).** Hydroponic production of vegetable and ornamentals. Embryo Publications,Athens, Greece.

[7] **Xiong, J. and Bauer, C.E., (2002).** Complex evolution of photosynthesis. Annual review of plant biology, 53(1), pp.503-521.

[8] **Thongbai, P., Kozai, T. and Ohyama, K., (2010).** CO2 and air circulation effects on photosynthesis and transpiration of tomato seedlings. ScientiaHorticulturae, 126(3), pp.338-34

[9] **Kitaya, Y., Shibuya, T., Yoshida, M. and Kiyota, M., (2004).** Effects of air velocity on photosynthesis of plant canopies under elevated CO2 levels in a plant culture system. Advances in space research, 34(7), pp.1466-146

[10] **Forde, B.G. and Clarkson, D.T., (1999).** Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. In Advances in botanical research (Vol. 30, pp. 1-90). Academic Press.

[11] **Bianco, M.S., CecílioFilho, A.B. and de Carvalho, L.B., (2015).**Nutritional status of the cauliflower cultivar 'Verona'grown withomission of out added macronutrients. PloS one, 10(4),p.e0123500.

[12] **De Groot, C.C., Marcelis, L.F., van den Boogaard, R. and Lambers, H., (2002).** Interactive effects of nitrogen and irradiance on growth and partitioning of dry mass and nitrogen in young tomato plants. Functional Plant Biology, 29(11), pp.1319-1328.

[13] **Grant, C.A., Flaten, D.N., Tomasiewicz, D.J. and Sheppard, S.C., (2001).** The importance of early season phosphorusnutrition. Canadian Journal of Plant Science, 81(2), pp.211-224.

[14] **Kuo, H.F. and Chiou, T.J., (2011).** The role of microRNAs in phosphorus deficiency signaling. Plant physiology, 156(3), pp.1016-1024.

[15] **Kadam, A.S., Wadje, S.S. and PATIL, R., (2011).** Roll of potassium humate on growth and yield of soybean and black gram. International J. Pharama Biol. Sci, 1, pp.243-246.

[16] **Battie-Laclau P, Laclau JP, Beri C, Mietton L, Muniz MRA, Arenque BC, de Cassia PM, Jordan-Meille L, BouilletJP,Nouvellon Y., (2014).** Photosynthetic and anatomical responses of Eucalyptus grandis leaves to potassium and sodium supply in afield experiment. Plant, Cell & Environment, 37(1), pp.70-81.

[17] **Warington, K., (1923).** The effect of boric acid and borax on the broad bean and certain other plants. Annals of Botany, 37(148), pp.629-672.

[18] **Xu, G., Magen, H., Tarchitzky, J. and Kafkafi, U., (1999).** Advances in chloride nutrition of plants. In Advances in Agronomy (Vol. 68, pp. 97-150). Academic Press.

[19] **White, P.J. and Broadley, M.R., (2001).** Chloride in soils and its uptake and movement within the plant: a review. Annals of botany, 88(6), pp.967-988.

[20] **Hazera, (2019).** 'Deficiency Symptoms in Tomatoes Crops' - Hazera U.S.A. [online] Available at: https://www.hazera.us.com/deficiency-symptoms-in-tomatoescrops/ [Accessed 26 Feb 2019].

[21] **Hossain, M.A., Piyatida, P., da Silva, J.A.T. and Fujita, M., (2012).** Molecular mechanism of heavy metal toxicity andtolerance in plants: central role of glutathione in detoxification ofreactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany, 2012.

[22] **Curie, C. and Briat, J.F., (2003).** Iron transport and signaling in plants. Annual Review of Plant Biology, 54(1), pp.183-206.

[23] **Puig, S., Askeland, E. and Thiele, D.J., (2005).** Coordinated remodeling of cellular metabolism during irondeficiency through targeted mRNA degradation. Cell, 120(1), pp.99-110.

[24] **Adamski, J.M., Danieloski, R., Deuner, S., Braga, E.J., de Castro, L.A. and Peters, J.A., (2012).** Responses to excess iron in sweet potato: impacts on growth, enzyme activities, mineral concentrations, and anatomy. Actaphysiologiaeplantarum, 34(5), pp.1827-1836.

[25] **Tripathi, D.K., Singh, S., Singh, S., Mishra, S., Chauhan, D.K. and Dubey, N.K., (2015).** Micronutrients and their diverserole in agricultural crops: advances and future prospective. Actaphysiologiaeplantarum, 37(7), p.139.

[26] **Millaleo, R., Reyes-Díaz, M., Ivanov, A.G., Mora, M.L. and Alberdi, M., (2010).** Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. Journal of soil science and plant nutrition, 10(4),pp.470-481.

[27] **Lidon, F.C., Barreiro, M.G. and Ramalho, J.C., (2004).** Manganese accumulation in rice: implications for photosynthetic functioning. Journal of Plant Physiology, 161(11), pp.1235-1244

[28] **Kaiser, B.N., Gridley, K.L., Ngaire Brady, J., Phillips, T. and Tyerman, S.D., (2005).** The role of molybdenum in agriculturalplant production. Annals of botany, 96(5), pp.745-754.

[29] **Chen, G., Nian, F., Wang, Y. and Xu, F., (2004).** Effect of B, Mo on fatty acid component of Brassica napus. Chinese journal of oil crop sciences, 26(2), pp.69-71.

[30] **Gupta, U.C., (1997).** Soil and plant factors affecting molybdenum uptake by plants.

[31] **Hewitt, E.J. and Bolle-Jones, E.W., (1952).** Molybdenum as a Plant Nutrient: II. The Effects of Molybdenum Deficiency on Some Horticultural and Agricultural Crop Plants in Sand Culture. Journal of Horticultural Science, 27(4), pp.257-265.

[32] **Yusuf, M., Fariduddin, Q., Hayat, S. and Ahmad, A., (2011).** Nickel: an overview of uptake, essentiality and toxicity inplants. Bulletin of environmental contamination and toxicology, 86(1), pp.1-17.

[33] **Hansch, R. and Mendel, R.R., (2009).** Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). Current opinion in plant biology, 12(3), pp.259-266.

[34] **Epstein, E. and Bloom, A.J., (2005).** Sinauer Associates. Mineral Nutrition of Plants: Principles and Perspective. 2nd edn. Sunderland MA, USA, 172.

[35] **Brown, P.H., Welch, R.M. and Cary, E.E., (1987).** Nickel: A micronutrient essential for higher plants. Plant physiology, 85(3), pp.801-803.

[36] **Cakmak, I., (2000).** Tansley Review No. 111 Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. The New Phytologist, 146(2), pp.185-205.

[37] **Alloway, B.J., (2004).** Zinc in soils and crop nutrition. International Zinc Association Communications. Brussels: IZA Publications.

[38] **Marschner, H., (1995).** Mineral nutrition of higher plants. Academic Press, London, UK. Mineral nutrition of higher plants. Academic Press, London, UK.

[39] **Kopponen, P., Utriainen, M., Lukkari, K., Suntioinen, S., Kärenlampi, L. and Kärenlampi, S., (2001).** Clonal differences in copper and zinc tolerance of birch in metal-supplemented soils. Environmental Pollution, 112(1), pp.89-97.

[40] **Jain, R., Srivastava, S., Solomon, S., Shrivastava, A.K. and Chandra, A., (2010).** Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (Saccharum spp.). ActaPhysiologiaePlantarum, 32(5), pp.979-986.

[41] **Isitekhale, H.H.E., Osemwota, I.O. and Amhakhian, S.O., (2013).** Poultry manure and NPK fertilizer application and theirresidual effects on the yield and yield components of tomato(Lycopersiconesculentum. Mill) in two distinct ecological zones ofcentral southern Nigeria. IOSR Journal of Agriculture and Veterinary Science, 3(2), pp.40-47.

[42] **Rahmatullah, Gill, M.A., Shaikh, B.Z. and Salim, M., (1994).** Bioavailability and distribution of phosphorus amonginorganic fractions in calcareous soils. Arid Land Research andManagement, 8(3), pp.227-234.

[43] **Zhu, J., Kaeppler, S.M. and Lynch, J.P., (2005).** Topsoil foraging and phosphorus acquisition efficiency in maize (Zea mays). Functional Plant Biology, 32(8), pp.749-762.

[44] **Aziz, T., Rahmatullah, Maqsood, M.A., Sabir, M. and Kanwal, S., (2011).** Categorization of Brassica cultivars for phosphorus acquisition from phosphate rock on basis of growth and ionicparameters. Journal of plant nutrition, 34(4), pp.522-533.

[45] **Hill, J.O., Simpson, R.J., Ryan, M.H. and Chapman, D.F., (2010).** Root hair morphology and mycorrhizalcolonisation of pasture species in response to phosphorus and nitrogennutrition. Crop and Pasture Science, 61(2), pp.122-131.

[46] **Phillips, K.A., Skirpan, A.L., Liu, X., Christensen, A., Slewinski, T.L., Hudson, C., Barazesh, S., Cohen, J.D., Malcomber, S. and McSteen, P., (2011**). vanishing tassel2 encodes a grass-specific tryptophan aminotransferase required for vegetative and reproductive development in maize. The PlantCell, 23(2), pp.550-566.

[47] **McSteen, P., (2010).** Auxin and monocot development. Cold Spring Harbor perspectives in biology, 2(3),p.a001479.

[48] **Tabitha K. (2013).** Effect of organic and inorganic fertilisers on growth, yield and quality of Amaranth spp in Kiambu,Kenya. Asian Journal of Research in Crop Science, pp.39.

[49] **FufaHunde, N., (2018).** EFFECTS OF BLENDED FERTILIZER RATES ON YIELD, YIELD COMPONENTS AND NUTRIENTS USE EFFICIENCES OF HOT PEPPER (Capsicum annuum L.) AT ASSOSA, WESTERN ETHIOPIA (Doctoral dissertation,HAWASSA UNIVERSITY COLLAGE OF AGRICULTURE).

[50] **Fayera, A., Mohammed, M. and Adugna, D., (2014).** Effects of different rates of NPK and blended fertilizers on nutrient uptake and use efficiency of teff [Eragrostistef (Zuccagni) Trotter] in Dedessa District, southwestern Ethiopia. Journal of Biology, Agriculture and Healthcare, 4(25), pp.254-258.

[51] **Naz, R.M.M., Muhammad, S.A.H.F., Hamid, A. and Bibi, F., (2012**). Effect of boron on the flowering and fruiting of tomato. SarhadJ.Agric, 28(1), pp.37-40.

Haematological Characteristics of Broiler Birds Administered Neem Leaf (*Azadirachta indica*) and Pawpaw leaf (*Carica papaya*) Leaf Extracts

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Abstract— *One hundred and twenty (120) broilers birds of Abore acere breed were used to determine the effect of pawpaw leaf and neem leaf extracts on the haematological profile of broilers birds. The chicks were randomly assigned to four treatments with 30 birds in each treatment. Each treatment was replicated three times with 10 birds per replicate in a completely randomized design (CRD). Treatment 1 received only vaccine and drugs without the leaf extract in their water, treatment 2 and 3 received 150 ml of the Neem and Pawpaw leaf extracts, respectively, and treatment 4 received 75ml of Neem extract and 75ml of pawpaw leaf extract mixed. 150ml of the extracts were all diluted into 1 litre of fresh drinking water and served to the broiler chicken. At the end of the experiment, which lasted for 7 weeks, blood sample were collected from the birds and analyzed for the haemoglobin concentration, packed cell volume, white blood cell count, and red blood cell count. Differential count was also carried out. Data obtained were subjected to analysis of variance (ANOVA). Result showed that there were no significant differences (P> 0.05) among the treatments in all the parameters tested. The study therefore concluded that 150 ml of neem leaf or pawpaw leaf extracts, or combination of the extracts may be administered to broiler birds without any deleterious effect on broilers' haematological indices.*

Keywords— Haematological indices, Neem leaf extract, Pawpaw leaf extract, broiler.

I. INTRODUCTION

In Nigeria, the demand for broiler meat has increased rapidly, as a result of increased income, population growth and urbanization. Broiler production has grown dramatically in the past two decades; these improvements are largely due to numerous researches and breeding programs which further enhanced feed utilization, growth rate and performance. Current commercial hybrids with high performance require high energy diets which would enable the maximum expression of their genetic potential (Sadeghi, 2005). In order to achieve this, poultry farmers make use of antibiotic, vaccines and growth promoter to enhance feed utilization and growth performance of broilers.

Recently, the use of antibiotics and growth promoters in poultry industry has been seriously criticized by policy makers and consumers, because of the development of microbial resistance to these products and the potential harmful effects on human health. Their use has shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties and carcinogenic effect in humans (Butaye *et al*., 2003). In the presence of low levels of antibiotics, resistant cells survive and grow producing an antibiotic resistant population in the final products. Researchers are concentrating efforts on the use of ancient medicinal practice that involves use of beneficial herbs and plants, which could be safe and increase production in terms of weight gain, feed efficiency, and lowered mortality in poultry birds. The present study was therefore, carried out with the objective to evaluate the physiological effects of some indigenous medicinal plants and their influence on the hematological parameter of broiler and its general performance.

Neem tree (*Azadirachta indica*) possess wide range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepato- protective and various other properties without showing any adverse effects (Kale *et al*., 2003). Neem leaves extract has immunostimulant effect that activates the cell mediated immune response and therefore, creates an enhanced response to any future challenges occurred by disease organisms and the feeding neem leaves to birds increased their humoral and cell mediate immune responses. Pawpaw leaves are rich sources of the proteolytic enzymes papain and chymopapain which have protein digesting properties and are useful in controlling digestive problems and intestinal worms (Burkhill, 1985)

II. MATERIALS AND METHODS

2.1 Study Area and duration of the study

The study was carried out at the poultry farm of Animal Science Teaching and Research Farm, Nnamdi Azikiwe University Awka. The poultry farm is located on the longitude 7° 08 ' 31.9 " E and latitude 6 ° 15 ' 10.1 " N. And the period of study lasted for (8) eight weeks.

Fresh neem and pawpaw leaves were harvested from the Nnamdi Azikiwe University environment using table knife. 500g (0.5kg) of neem leaf was weighed using electronic sensitive scale. It was properly washed and manually crushed using wooden mortar in a clean environment for five (5) minutes. 500g of pawpaw leaf was weighed, properly washed and manually crushed using wooden mortar. It was extracted using one litter of clean water. 150ml of the neem leaf and pawpaw leaf extracts were obtained respectively using syringe of 10 ml to collect the extracts. The combination of both neem and pawpaw extracts were 75ml for neem and 75ml for pawpaw extract, extraction method, Edeh (2013).

The birds were given as follow: T1 (vaccine +drugs), T2 NLE (150ml+1L of water), T3 PLE (150ml+1L of water) and T4 NPLE (75ml +75ml +1L of water).

The data collected was subjected to Analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20. The separation of mean was done using DUNCAN multiple range test.

2.2 Preparation of neem and pawpaw leaf extract.

Fresh neem and pawpaw leaves were harvested in Nnamdi Azikiwe University premises close to the farm area and removed dirt. The leaves were measured (0.5 kg each) and placed in a mortar and crushed with pestle for 5mins. The crushed leaves were scooped into a beaker containing 1.5litre of clean water and stirred for 2 minutes for homogeneity. The leaf mixtures were then sieved to obtain a homogenous extract and given to them as follows:

Treatment 1 (vaccine and drugs) Treatment 2 (150ml neem) Treatment 3 (150ml pawpaw)

Treatment 4 (75ml of neem + 75ml of pawpaw) each in dilution of 1 litre of clean water. They were given at the same time the control treatments were being vaccinated. The crushing and sieving procedures were in accordance to Edeh 2013.

2.3 Experimental diets:

The commercial broiler starter and finisher manufactured by Top Feed Eastern Premier Feed Mills LTD. Was purchased from their distributers in Eke Awka Market Awka south L.G.A of Anambra State.

III. RESULTS AND DISCUSSION

The Effect of Neem (*Azadirachta indica)* and pawpaw (*Carica papaya)* on the haematological parameters of broiler is shown in Table 1.

TABLE 1
EFFECT OF NEEM AND PAWPAW EXTRACT ON HAEMATOLOGICAL PARAMETERS OF BROILER

Parameters/Treatment	T1(VD)	T2(NLE)	T3(PLE)	T4(NPE)	SEM
HB (g/dl)	16.33±0.88	16.67±1.45	15.67±0.88	17.67±1.33	0.54^NS
PCV (%)	49.0±3.05	49.67±3.75	47.33±2.60	53.0±4.04	1.58^NS
WBC (10^9/µL)	3.67±0.33	4.33±0.33	3.33±0.88	2.33±0.33	0.313^NS
RBC (10^{12}/ µL)	4.53±0.34	4.60±0.38	4.40±0.32	4.98±0.39	0.166^NS
Plasma fraction (%)	51.0±3.06	50.33±3.76	52.67±2.60	47.0±4.04	0.72^NS
Heterophil (%)	57.50±4.50	58.80±0.50	48.00±4.62	58.0±5.00	2.42^NS
Lymphocytes (%)	41.0±4.00	40.50±0.50	49.67±5.00	39.5±5.00	2.422^NS
Basophil (%)	0.00±00	0.00±0.0	0.00±0.0	0.00±0.0	0.00
Monocytes (%)	1.50±0.50	2.0± 0.00	2.0±0.500	2.00±0.00	0.422^NS
MCV(FL)	108.2±1.27	107.9±1.22	107.9±2.69	106.7±0.63	0.72^NS
MCHBC (%)	33.33±0.38	33.49±0.47	33.55±0.47	33.35±0.32	0.179^NS

VD = Vaccine and Drug; NLE = Neem Leaf Extract; PLE = Pawpaw Leaf Extract, NPE = Neem and Pawpaw Leaf Extract. NS = Not significant (P> 0.05), SEM = the standard error of the mean, TI = Vaccine + drugs, T2 = 150ml of Neem leaf extract, T3 = 150ml of pawpaw leaf extract and T4 =75ml of Neem + 75ml 0f pawpaw leaf extract = (150ml).

From the results shown in Table 1, no significant (P> 0.05) difference existed among the treatment with respect to the haematological parameters, Haemoglobin concentration (HB), Packed cell volume (PCV) Red blood cell count (RBC), Plasma fraction, White blood cell count (WBC), Mean corpuscular haemoglobin (MCHC), Mean corpuscular volume (MCV), Heterophil, Monocytes, Lymphocytes, and Basophil.

With respect to the Packed cell volume (PVC) ,there was no significant difference (P>0.05) when the treatment were compared, but the use of Neem leaf extract and pawpaw leaf extract had an enhancing effect on the packed blood cell volume of broilers with the highest range on T4 (75ml Neem +75mlpawpaw extract) with a mean range of 53.00, the result agrees with the findings of Obikaonu *et al*. (2014), Ihekwumere and Herbert(2003), Edeh (2013) and Nusrat (2014) who reported the that inclusion of neem and pawpaw leaf extract had no significance difference on the packed cell volume of broiler birds.

Also haemoglobin (Hb) ,the present findings and results showed no significant difference (p> 0.05), among the treatment which support the report of Obikaonu *et al* 2014, Iheukwumere and Herbert (2003), Islam *et al* (2003) and Nusrat (2014) who reported that the inclusion of Neem leaf meal had no significant difference on the haemoglobin concentration of broilers.

However, there was no significant difference (p> 0.05), among the treatment with respect to the Red Blood Cell count (RBC) and The white Blood Cell (WBC), but the heterophil were highest, following the lymphocytes value and according to Okeudo *et al*. (2003) lymphocytes is the most numerous in avian. In the findings, monocytes were also observed but there were no trace of eosinophil at all the treatment which conforms to the work of Obikaonu *et al.* (2014). And the presence of monocytes and the absence of eosinophil indicate no toxin or bacterial infection (Frandson, 2004). This finding also agrees to report of Nusrat (2014) .Though from the result the T2 (Neem) had the highest white blood cell count numerically, for the heterophil, T4 (Neem+ Pawpaw) had the highest percentage with the T2 (Neem) as well. Then T3 (pawpaw) had the highest lymphocytes percentage.

IV. SUMMARY

A total of 120 (one hundred and twenty) day older broilers of Abore acre breeds were used for the experiment to determine the effect of Neem and pawpaw leaf extract on the physiological response of the broilers as regard to their haematological profile at a ratio of 150ml to 1 lire of water for 7 weeks and from the finding gotten from the result above showed that there is no significant difference (P> 0.05) but from the result obtained some of the haematological compared more favourably than other even though the differences were not significant. Especially the groups treated with a combination of Neem and pawpaw (T4) and that of treatment 3 (T3) only pawpaw extract had some difference numerically.

V. CONCLUSION

Finally, it can be concluded from the result of the experiment that at end of the experiment, the blood profile of the broilers treated with vaccine and drugs, Neem leaf extract, pawpaw leaf extract and a mixture Neem and pawpaw leaf extract had no significant (P>0.05) difference, all the parameter compared favourably the same with the control treatment.

VI. RECOMMENDATION

In as much as there were no significant difference, but there occur a little high range in treatment 4, therefore I recommend the used of mixture of Neem leaf extract and pawpaw leaf extract (150ml) because it gave higher range on haemoglobin concentration, packed cell volume, red blood cell count, and heterophil percentage, and at the same time it is cost effective as Neem leaves and pawpaw leaves are easily accessible to the farm. Further detailed research should be carried to check level of inclusion of the mixture of Neem and Pawpaw leave extract on the haematological profile of broiler.

REFERENCES

[1] O. A. C. (1995). Association of Official Analytical Chemists Official Methods of Analysis, 7th Edition Washington D.C.

[2] Adene D.F. (2000). Action plan for poultry animal Health in pilot scale study under FAO/NIRI/7822. Pp. 81.

[3] Adene, D.F. (2004). Global dimensions in poultry health problems. National perspective in poultry health and production efficiency, Stirling – Horden Publisher Nigeria Ltd. Lagos, Pp. 213- 219.

[4] Adene, D.F. (2006). Application of cost in disease evaluation to risk management in poultry Book of proceeding WIRP. Ahamadu Bello University, Zaria. Nigeria. pp. 147 -156.

[5] Adenkola, A.Y. and Anugwa, F.O. (2007). Effects of dietary supplement of ascorbic acid on the performance of piglets in Markurdi, Benue state of Nigeria, *Tropical Veterinarian*, 25 (1): 15-17.

[6] Adeyemo, G.O., Ologhobo, A.D. and Adebiyi, O.A. (2010). The effect of graded levels of dietary methionine on the haematology and serum biochemistry of broilers. *International Journal of Poultry Science*, 9(2): 158-161.

[7] Afolabi, K.D., Akinsoyinu, A.O., Abdullah, A.R.O., Olajide R. and Akinleye, S.B. (2011). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. *Poljoprivreda,* 17: (1) 74-78.

[8] Akinmalodun, A.C., Ibukun, E.O., Afor, E., Akirinlola, B.L., Onibon, T.R., Akinboboye, A.O., Obuotor, E.M., and Farombi, E.O. (2007).Chemical constituents and antioxidant activity of *Alstoniaboonei*. *African Journal Biotechnology,* 6(10):1197-1201.

[9] Alodan, M.A. and Mashaly, M.M. (1999). Effect of induced molting in laying hens on production and immune parameters. Poultry Science, 78: 171-177.

[10] Anna Horsbrugh Porter. (17 April 2006). "Neem: India's tree of life". BBC News.

[11] Atta, K. (1999). The Power of Garlic on Cardiovascular disease prevention. Association Bureau Cameroon, p.72.

[12] Ashagidigbi, W.M., Sulaimon, S.A., Adesiyan, A. (2011). Technical efficiency of Egg Production in Osun State. *International Journal of Agricultural Economics and Rural Development,* 6(4):124-130.

[13] Araujo, L.F., Junqueira, O.M., Araujo, C.S.S., Faria, D.E., and Andreotti, M.O. (2004). Different criteria of feed formulation for broilers aged 43 to 49 days. *Brazilian Journal of Poultry Science,* 6(1): 61-64.

[14] Babatunde, B. (2011). Poultry potentials for National Agricultural Revolution. http://www.Google .com. 22nd August 2016.

[15] Bolhius, G.G. (1954). The toxicity of cassava roots*, Neth. Journal Agriculture*, 2:176-185.

[16] Burkhill, H.M. (1985). The useful plants of West Tropical Africa. *Royal Botanical Garden Kew,* 7 (1): 200- 219.

[17] Bush, K.L., and Strobeck, C. (2003). Phylogenetic relationship of the phasianidae reveals. Pp.40- 51.

[18] Butaye, P., Luc, A., Devriese, F., and Haese, B. (2003). Antimicrobial Growth Promoters used in Animal feed: Effects of less well known antibiotics on Gram – positive bacteria. *Clinical Microbiology, Review*, 16 (2):175 – 188.

[19] Chikezie, P.C., Agomuo, E.N., and Amadi, B.A. (2008). Biochemistry, Practical Research Method a Fundamental Approach. Volume 2, Mega soft publishers, pp. 51-53.

[20] Cowan, M.M. (1999). Plant products and antimicrobial agents. *Clinical Microbiology Reviews,* 12:564-582.

[21] Dick, G. (2003). Papaya: A tantalizing taste of the Tropics. Maricopa Country Master Gardener volunteer information, University of Arizona Cooperative Extension http://ag.arizona.edu/maricopa/garden/html/pubs/mgjournal.htm.

[22] Ebenebe, C.I., Itefue, O., Ebere-Ohameje, T.C., and Okonkwo, J.C. (2011). Fortification of the nutritive diet, value of Mushroom (*Termitomycesmicrocarpus*) with paw-paw leaf meal for broiler chicks up to 13 weeks of age. *Pakistian Journal of Nutrition,* 10(2):155-158.

[23] Edeh, H.O. (2013): Physiological Response of Broiler Birds To Oral Supplementation *With Aloe Vera And Neem Leave Extracts*. Visual library Faculty of Agriculture, Department of Animal Science University of Nigeria, Nsukka, 125, 35-40.

[24] Eleazu, C.O., Okafor, P.N., and Ahamefuna, I. (2010). Total antioxidant Capacity, Nutritional Composition and Inhibitory Activity of Unripe Plantain (*Musa paradisiacae*) on Oxidative Stress in Alloxan Induced Diabetic Rabbits, *Pakistan Journal Nutrition*, 9(11):1052-1057.

[25] Eleazu, C.O., Okafor, P.N., Amajor, J., Awa, E., Ikpeama, A.I., and Eleazu, K.C. (2011). Chemical Composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (M. *Paradisiacae*) flour. African Journal Biotechnology, 10(74):6948-6952.

[26] Elizabeth, K. (1994). Immense help from nature's workshop. 1st Edition Elikaf Health Services Ltd. Ikeja, Lagos. pp. 207-209.

[27] Enwere, N.J (1998). Foods of Plant Origin, International Journal Microbiology, 9 (94):329-334.

[28] Erisksson, J., Larson, G. Gunasson, U., Bedhom, B. and Tixier- Biochard, M. (2008). Identification of yellow gene reveals a hybrid origin of the domestic chicken. *Trends in Genetics*, 22(3).

[29] Iheukwumere, F. C., and Herbert, U. (2003).Physiological response of broiler chickens to quantitative water restrictions, Hematology and serum biochemistry. International Journal of Poultry Science, 2:117 – 119.

[30] Nusrat, M. (2014). Effects of neem, papaya and turmeric on growth performances of broilers (thesis) .Bangladesh agricultural university, mymensingh-2202). Pp. 45.

[31] Obikaonu, H.O., Mmereikwu, V.M., Odoemena, V.U., Okoli, I.C., and Udedibie, A.B. (2014). Haematological and serum biochemical indices of finisher broiler fed Neem (Azadirachtaindica) leaf meal. International journal of Agriculture and Rural Development,17(1): 1641-1647.

Effect of mixed *Gmelina* and *Moringa* leaf meal inclusion on growing Red Sokoto does fed *Digitaria smutsii* hay based complete diets

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***Abstract*— *Gmelina arborea and Moringa oleifera (GMMO) leaf meal were combined at the ratio of 3:1 and included in the diets of Red Sokoto does at 0, 10, 20 and 30% to investigate the effect on dry matter intake, live weight and cost of feed in Digitaria smutsii hay based diets. Twenty-eight (28) growing Red Sokoto does aged between 6 and 7 months with average weight of 14.71±0.09 kg were randomly assigned to four treatments balanced for weight with seven does per treatment in a completely randomized design. The experimental diets were offered at 4% of body weight. Cost of feeding was studied to determine the cost effectiveness of GMMO leaf meal inclusion in the diets. Results indicated that inclusion of GMMO leaf meal significantly (P<0.05) improved weight gain of Red Sokoto does. Animals fed dietary treatments containing 10% and 20% of GMMO leaf meal had significantly (P<0.05) higher weight gain (3.30kg and 3.38kg) than animals on 30% inclusion level. The feed cost/kg gain ranged from ₦ 326.75 in 20% followed by ₦354.59, ₦ 441.74 and ₦518.30 in 10%, 30% and 0%, respectively. The cost-benefit analysis showed that at 20% GMMO leaf meal inclusion, cost of feed/kg gain was reduced by 36.96% and gave more profit. The GMMO leaf meal inclusion did not have any adverse effect on Red Sokoto does. It can be concluded that mixed Gmelina and Moringa leaf meal can be included at 20% in diets of Red Sokoto does for improved dry matter intake, average daily weight gain and profitability.*

***Keywords*— Leaf meal, weight gain, Red Sokoto goats, Gmelina arborea and Moringa oleifera.**

I. INTRODUCTION

Small ruminants form an integral part of the livestock economy in Nigeria. The arid and semi-arid areas are home to over 80% of small ruminants and their sustenance is reducing due to dependence on natural pastures (Kosgey *et al.*, 2008). They support 46-58% of pastoral households and play a significant role in the food chain and overall livelihoods of rural households, where they are largely the property of women and their children (Lebbie, 2004).

Dry season feeding of ruminants in most tropical areas has always been a problem for farmers since little good pasture exists during this period. At this time, the performances of these animals are seriously impaired. One possible way to alleviate this problem and maintain production in the tropics is to feed them with crop residues and browse plants. These feed resources are not consumed by man but can be converted by ruminants into animal products desirable as human food. This therefore reduces total cost of animal production without a decrease in productivity and also maintains efficient feed utilization.

Browse plants have great potential as source of high quality nutrient for ruminants, being high in protein, minerals and vitamins (Amodu and Otaru, 2004). They are available all year round because of their drought resistance, persistence, vigorous growth, re-growth and palatability (Crowder and Chheda, 1982). The use of browse plants as supplement has been shown to enhance intake, improve growth rate and increase reproduction in ruminants (Lamidi *et al.*, 2009; Okafor *et al.*, 2012; Abdu *et al.* 2012).

Moringa is one of the most promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. It has a high crude protein content ranging from 20-26% CP in leaves (Ben Salem *et al.*, 2004 and Asaolu *et al.*, 2011) with negligible contents of anti-nutrients (Makkar and Becker, 1996).

Gmelina arborea Roxb. (Family verbenanceae) is a fast growing deciduous tree that can grow up to 40 m tall and 140 cm in diameter (Jensen, 1995). Even though *Gmelina arborea* can shed some of its leaves when the dry season is approaching, the regrowth of new leaves could serve as animal feed during the dry season (Osakwe and Udeogu 2007). The objective of this study was to investigate the effect of mixed Gmelina and Moringa (GMMO) leaf meal inclusion levels on growing Red Sokoto does fed *Digitaria smutsii* hay based diets.

II. MATERIALS AND METHODS

2.1 Experimental animals and diets

The experiments were conducted in the Experimental Unit of the Small Ruminant Research Programme of the National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State, Nigeria.

Twenty-eight (28) Red Sokoto does aged between 6 and 7 months with average weight of 14.71±0.09 kg were used with 7 animals per treatment. The animals were obtained from Small Ruminant Research flock, NAPRI. They were individually penned and given prophylactic treatment, consisting of Ivermectin® at 200µg/kg body weight (BWT) against endo- and ectoparasites and Terramycin long acting (LA)® at 20mg/kg BWT against bacterial diseases 7 days before the commencement of the experiment.

Fresh *Gmelina arborea* (GM) leaves were harvested within Ahmadu Bello University Main Campus and the leaves were allowed to air-dry for three days. Dried *Moringa oleifera* leaves were sourced from Sabon-Gari market, Zaria. *Digitaria smutsii* (Wooly finger grass) hay was sourced from the Feeds and Nutrition Research Programme of NAPRI. The dried leaves of the two browses and *D. smutsii* hay were ground with a hammer mill fitted with 2cm screen for easy mixing with other feed ingredients. The ground ingredients were packed in sacks and stored in a well-ventilated store.

Four isonitrogenous complete diets were formulated, with 40% *D. smutsii* hay base. The complete diets were compounded to contain 13% CP. *Gmelina arborea and Moringa oleifera* leaf meals were combined at 75 and 25% respectively. The mixed Gmelina and Moringa leaf meal was included at 0, 10, 20 and 30%. Each level of inclusion served as a treatment. Other ingredients in the complete diet include maize offal, cotton seed cake, salt and bone meal (Table 1).

TABLE 1
INGREDIENT COMPOSITION OF EXPERIMENTAL DIETS (%) FED TO RED SOKOTO DOE

Ingredients	Level of GMMO leaf meal inclusion (%)			
	T_1	T_2	T_3	T_4
75GM:25MO	0	10	20	30
Cottonseed Cake	23.40	20.00	16.00	12.30
Maize offal	34.60	28.00	22.20	15.80
Bone meal	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
D.smutsii hay	40	40	40	40
Total	100	100	100	100
Calculated analysis				
% Crude Protein	13.01	13.06	13.02	13.00
ME (Kcal/kg)	2437	2407	2375	2357
Cost/kg feed (₦)	44.14	40.63	37.12	33.52

75 GM: 25 MO= 75:25% combination of Gmelina and Moringa leaf meal; ME=Metabolizable energy.

The diets were mixed fortnightly to maintain freshness and samples were taken to determine the chemical composition. Seven animals were randomly allocated to four treatments with each animal serving as a replicate in a completely randomized design.

At 8.00 hours, the animals were offered their daily ration of 4% body weight of the experimental diets. The does were weighed weekly. Weight changes were recorded as the difference between weight of the previous week and the current. Weekly weights of the does were used to adjust the quantities of feeds offered in order to maintain the pre-determined level. The animals had free access to clean drinking water. The growth trial was carried out for a period of 60 days after 14 days' adjustment period. Daily feed intake and weekly body weight of the animals were recorded before feeding in the morning throughout the experiment.

2.2 Feed cost analysis

Market prices in Zaria and its environs were used for determining the cost of does and feeds as at the time of research. The costs of harvesting and transportation of *Gmelina* leaves were estimated. Feed consumed by the animals was multiplied by the cost of feed per kilogram to obtain the cost of feed consumed. The information from the market cost was used to work out total cost of feed consumed per treatment, value of gain, and net benefit.

2.3 Chemical analysis

The feeds samples were analyzed for proximate analysis using the procedures outlined by the Association of Official Analytical Chemists (A.O.A.C., 2005). Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were determined according to Van Soest *et al.,* (1991) procedures.

2.4 Statistical Analysis

All data generated were analyzed statistically using the General Linear Model (GLM) procedure of SAS, (2005). Significant differences between treatment means were determined according to Duncan's Multiple Range Test of SAS, (2005).

III. RESULTS AND DISCUSSION

3.1 Chemical composition (%) of experimental diets fed to growing Red Sokoto does

The chemical composition (%) of experimental diets fed to growing Red Sokoto does is shown on Table 2. The dry matter content varied from 93.90 to 94.44% across the treatments. The crude protein content of 13.09%, 13.00%, 13.15% and 13.25% were recorded for 0%, 10%, 20% and 30% levels of GMMO leaf meal inclusion in the diets, respectively. Ash content ranged from 6.47 to 6.85%, while NDF and ADF varied from 41.59 to 46.22 and 22.56 to 26.94 %, respectively.

TABLE 2
CHEMICAL COMPOSITION (%) OF EXPERIMENTAL DIETS FED TO GROWING RED SOKOTO DOES

Parameters	Level of GMMO leaf meal inclusion			
	0%	10%	20%	30%
Dry matter	94.44	94.10	93.90	94.08
OM	87.59	87.63	87.19	87.23
Crude protein	13.09	13.00	13.15	13.25
Crude fibre	27.43	25.26	23.27	28.23
Ash	6.85	6.47	6.71	6.85
Ether Extract	7.91	5.66	5.08	5.88
NDF	41.59	46.22	45.71	43.51
ADF	22.56	26.94	26.41	24.51

OM= Organic Matter; NDF=neutral detergent fibre; ADF=acid detergent fibre.

The CP contents of all the diets were above the level suggested as adequate to meet the nutritional requirements for moderate weight gain in goats (NRC 2007). Norton (1994) recommended a diet with 8 % CP for moderate live-weight gains in goats and according to this criterion all the diets had sufficient CP content. The chemical composition of experimental diets showed that the diets contained adequate dry matter and crude protein to support normal rumen functions (Ahamefule et al., 2002). Also the CP contents of all the diets fed to pregnant does were within the level 8.9–16.0% recommended for growth and production in goats (NRC, 2007). The chemical composition of experimental diets showed that the diets contained adequate dry matter and crude protein to support normal rumen functions (Ahamefule et al., 2002) and to meet the CP requirement of pregnant Red Sokoto does. There was no adverse effect on the pregnant does during the study.

3.2 Effect of Gmelina and Moringa leaf meal inclusion on intake and growth performance of Red Sokoto does fed *Digitaria smutsii* hay based diets

The results of the effect of Gmelina and Moringa leaf meal inclusion on growth performance of Red Sokoto does fed *D. smutsii* hay based diets are presented in Table 3. The result showed no significant (P>0.05) difference in feed intake across the treatments. The feed intake varied from 421.50 to 455.95g/d in animals fed 30% and 20% respectively. Final weight of does was also not significant (P>0.05) but increased with GMMO leaf meal inclusion. Animals on 10% and 20% leaf meal inclusion had significantly higher (P<0.05) weight gain than the other treatment groups. Average daily weight gain (ADG) in this study showed no significant (P>0.05) difference across the treatments. ADG varied from 38.81 to 55.48 g/d. There was no significant (P>0.05) difference in feed conversion ratio. It varied from 8.73 to 13.18 in animals fed 20% and 30% leaf meal inclusion respectively.

TABLE 3
EFFECT OF *GMELINA* AND *MORINGA* LEAF MEAL INCLUSION ON INTAKE AND GROWTH PERFORMANCE OF RED SOKOTO DOES FED *DIGITARIA SMUTSII* HAY BASED DIETS

Parameters	Levels GMMO leaf meal inclusion				SEM
	0%	10%	20%	30%	
Feed intake (g/d)	455.31	447.08	455.95	421.50	35.23
DMI (g/kgW $^{0.75}$)	52.80	50.97	51.94	50.07	1.77
Initial weight (kg)	14.79	14.71	14.71	14.71	1.22
Final weight (kg)	17.59	18.01	18.04	17.07	1.16
Metabolic Wt $^{(0.75)}$	8.58	8.73	8.74	8.39	0.42
Weight Gain (kg)	2.80^{ab}	3.30^{a}	3.38^{a}	2.33^{b}	0.52
ADG (g/d)	46.67	55.00	55.48	38.81	8.60
FCR	11.74	8.73	8.80	13.18	2.41
a,b, Mean values with different superscripts within a row differ significantly (P<0.05) SEM standard error of mean; DMI=Dry matter intake; ADG=average daily gain; FCR= feed conversion ratio.					

No significant effect of GMMO inclusion on feed intake, average daily gain and feed conversion ratio recorded in this study was similar to reports of several researchers (Okafor *et al*., 2012; Aye and Tawose, 2015). Higher live weight gain recorded in does fed 20% GMMO leaf meal agreed with findings of (Torres-Acosta *et al.*, 2006; Ogunbosoye and Babayemi, 2012; Fasea *et al*., 2010) who fed browse leaves. This may be as a result of an improved ruminal environment and digestibility of diets. The finding is in agreement with the reports of Kabir *et al*. (2004) and Torres-Acosta *et al.* (2006). The higher live weight gain may be as a result of adequate crude protein content, increased intake of the diet, improved digestibility and nitrogen retained by the animal on 20% GMMO leaf meal inclusion.

3.3 Effect of Gmelina and Moringa leaf meal inclusion on cost of feeding of Red Sokoto does fed *Digitaria smutsii* hay based diets

Table 4 showed the result of the feed cost-benefit analysis of experimental diets fed to growing Red Sokoto does. The total feed cost ranged from ₦847.74 in 30% to ₦1205.84 in the control. The feed cost/kg gain ranged from ₦ 326.75 in 20% followed by ₦354.59, ₦ 441.74 and ₦518.30 in 10%, 30% and 0% respectively. Net profit was higher on treatment diets with GMMO leaf meal than the control. Reduction percent in feed cost/ kg gain was highest in 20% (36.96%) and least in 30% (14.77%) leaf meal inclusion.

TABLE 4
COST BENEFIT OF FEEDING RED SOKOTO DOES GMELINA AND MORINGA LEAF MEAL IN *DIGITARIA SMUTSII* HAY BASED DIETS

Parameters (₦)*	Levels of GMMO leaf meal inclusion			
	0%	**10%**	**20%**	**30%**
Total feed intake (kg)	27.32	26.83	27.36	25.29
Weight Gain (kg)	2.80	3.30	3.38	2.33
Cost/kg	44.14	40.63	37.12	33.52
Total cost of feed consumed	1205.84	1089.89	1015.49	847.71
Cost/kg Gain	518.30	354.58	326.75	441.74
Value of gain	1400.00	1650.00	1664.30	1164.30
Net Profit	194.20	560.10	648.80	316.60
% Reduction in feed cost/kg gain	-	31.59	36.96	14.77

**** Naira = Nigerian currency (100 kobo make 1 naira)***

The result demonstrated that the best feed cost benefit was recorded in bucks fed 20% GMMO leaf meal. The results indicated that treatment diets with leaf meal were more cost effective than the control diet and it can be due to cheaper cost of feed and improvement in feed utilization and weight gain. The result of this study is similar to the work of Njidda and Ikhimioya (2010) reported that diet supplemented with *Ziziphus mauritiana* reduced the cost of production. Incorporation of unconventional feed stuff has been reported to reduce the cost of feeding (Okafor *et al*., 2012 and Okpara *et al*., 2014).

IV. CONCLUSION

The study showed that mixed Gmelina and Moringa leaf meal inclusion in diets of Red Sokoto does improved dry matter intake, weight gain and profitability. The cost-benefit analysis showed that fed 20% GMMO leaf meal gave more profit and reduced cost of feed/kg gain by 36.96% in the diets of Red Sokoto does. It was therefore concluded that GMMO leaf meal be included at 20% in diets of Red Sokoto does for improved feed intake, weight gain and profitability.

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REFERENCES

[1] Abdu, S.B., Hassan, M.R., Jokthan, G.E., Adamu, H.Y., Yashim, S.M. and Yusuf, K. (2012). Effect of varied inclusion levels of gmelia (*gmelina arborea*) leaf meal on intake, digestibility and nitrogen in red sokoto bucks fed on sorghum glum based complete diets. *Advances in Agriculture, Sciences and Engineering Research,* 2 (2): 79–84. http://www.ejournal.sedinst.com

[2] Ahamefule, F.O., Ibeawuchi, J.A., Ajala, A.A. (2002). Intake, digestibility, and nitrogen balance studies of potato peels, yeast slurry diets by WAD goats. In: Aletor, V. A. and Onibi, G. E. (eds.) *Proceedings of the 27th annual conference, Nigerian Society of Animal Production,* March 17-21, Federal University of Technology, Akure.

[3] Amodu, J.T. and Otaru, S.M. (2004). In: Proceedings of a training Manual on Forage production and Management in Nigeria, held at National Animal Production Research Institute, Shika. Pp 23-27.

[4] A.O.A.C. (2005). Official Methods of Analysis. In: W.Horowitz, Editor, Official methods of analysis (18th ed.), Association of Official Analytical Chemists, Gaithersburg, MD., USA.

[5] Asaolu, V.O., Binuomote, R., Akinlade, J.A., Oyelami, O.S. and Kolapo, K.O. (2011). Utilization of *Moringa oliefera* fodder combinations with *Leucaena leucocephala,* and *Gliricidia sepium* fodders by West African Dwarf Goats. *International Journal of Agricultural Research, ISSN 1816- 4897/ DOI:IO. 3923/ ijar.2011.*

[6] Aye, P.A. and Tawose, O.M. (2015). Acceptability and Utilization of Graded Levels of *Gmelina arborea* and Cassava Peels Concentrates in West African Dwarf Sheep. *Journal of Advances in Agriculture,* 4 (2): 415-422.

[7] Ben Salem H., Makkar H.P.S., Nefzaoui A. (2004). Towards better utilisation of non -conventional feed sources by sheep and goats in some African and Asian countries**.** In: Ben Salem H. (ed.), Nefzaoui A. (ed.), Morand-Fehr P. (ed.). *Nutrition and feeding strategies of sheep and goats under harsh climates.* Zaragoza: CIHEAM, 177 -187.

[8] Crowder, L. V. and Chheda, H.R. (1982). *Tropical Grassland Husbandry*, Longman Inc. New York Pp. 294 and 295.

[9] Fasae, O.A., Sowande, O.S. and Popoola, A.A. (2010). Evaluation of Selected Leaves of Trees and Foliage of Shrubs as Fodder in Ruminant Production. *Journal of Agricultural Science and Environment,* 10(2):36-44.

[10] Jensen, M. (1995). *Trees Commonly Cultivated and South East Asia, Illustrated field guide, RAP* Publication: 1995/38 FAO Bangkok. Thailand, Pp 93.

[11] Kabir, F. Sultana, M.S., Shahjalal, M., Khan, M.J. and Alam, M.Z. (2004). Effect of protein supplementation on growth performance in female goats and sheep under grazing condition. *Pakistan Journal of Nutrition,* 3: 237-239.

[12] Kosgey, I.S., Rowlands, G.J., van Arendonk, J.A.M. and Baker, R.L. (2008). Small ruminant production in smallholder and pastoral/extensive farming systems in Kenya. *Small Ruminant Research,* 77: 11-24.

[13] Lamidi, A.A., Aina, A.B.J., Sowande, S.O., and Johosho, A.O., (2009). Assessment of *Panicum maximum* (Jacq), *Gliricidia sepiuma* (Jacq) and *Gmelina arborea* (Roxb)- based diets as all year round for West Africa Dwarf goat. In: Akinlade, J.A., Olayemi, T.B., Rafiu, T.A., Akinwunin, A.U., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. (eds.). *Proceedings of 14th Annual conference of Animal Science Association of Nigeria (ASAN). Sept. 14th -17th LAUTECH Ogbomoso, Nigeria, 582-584.*

[14] Lebbie, S.H.B. (2004). Goats under Household Conditions. Small Ruminant Research, 51: 131 - 136.

[15] Makkar, H.P.S. and Becker, K. (1996). Nutritional value and anti-nutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science Technology*, 63: 211-228.

[16] National Research Council (2007). *Nutrient Requirement of Goat. National Academy Press. Washington DC, USA.*

[17] Njidda, A. A. and Ikhimioya, I. (2010). Nutritional evaluation of some semi-arid Browse forages leaves as feed for goats. *European Journal of Applied Sciences*, 2 (3): 108-115.

[18] Norton, B.W. (1994). Tree legumes as dietary supplements for ruminants. In: Gutteridge, R C and Shelton, H.M. (editors), *Forage tree legumes in Tropical Agriculture, CAB International Wallingford, UK.*

[19] Ogunbosoye, D.O. and Babayemi, O.J. (2010). Potential values of some non-leguminous browse plants as dry season feed for ruminants in Nigeria. *African Journal of Biotechnology,* 9 (18): 2720-2726.

[20] Okafor, E.C., Lakpini, C.A.M. and Fayomi, A. (2012). Dried gmelina *(Gmelina arborea roxb)* leaves as replacement forage to groundnut haulms in the diet of fattening red Sokoto bucks. *International Journal of Agriculture and Biosciences*, 1(1): 5-10.

[21] Okpara, O., Akporhuarho, P.O. and Okagbare, G.O. (2014). Determination of Browse Intake and Nutrient Digestibility of Grazing West African Dwarf (WAD) Goats Fed Varying Levels of *Gmelina arborea* Leaves as Supplements in Delta State Nigeria. *International Journal of Animal and Veterinary Advances,* 6 (2): 52-57.

[22] Osakwe, I.I. and Udeogu, R.N., (2007). Feed Intake and Nutrient Digestibility of West African Dwarf (WAD) Goat Fed *Pennisetum purpureum* Supplemented with Gmelina arborea. *Animal Research International,* 4 (3): 724- 727.

[23] SAS (2005). Institute Inc. SAS/STAT user's guide. 6.03 Ed, Gray NC. USA.

[24] Torrest-Acosta, J.F., Jacobs, D.E., Aguilar-Caballero, A.J., Sandoral-Castro, C., Cob-Galara, L. and May-Martinez, M. (2006). Improving resilience against natural gastrointestinal nematode infection in browsing kids during the dry season in tropical Mexico. *Veterinary parasitology,* 135:163-173.

[25] Van Soest, P. J. Robertson, J. B. and Lewis, B. A. (1991). Methods for dietary Fibre, NDR and Starch Polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74:3583-3542.*

Review Article: Green Synthesis of Silver Nanoparticles and Their Application

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Abstract— *Nanoparticles are small particles which ranges from 1-100nm in size. Nanoparticles can be synthesized through physical, chemical and biological method. Synthesis of nanoparticles through biological method which also known as green synthesis is eco-friendly and non-expensive method. This review gives idea about how silver nanoparticles are produce by using microorganisms and plant extract and their mechanism for biosynthesis. This review also shows an insight on wide application of silver nanoparticles in various fields.*

Keywords— Nanobiotechnology, silver nanoparticles, green synthesis, microorganisms.

I. INTRODUCTION

Nanotechnology is important science field which deals with production, manipulation and use of material ranging in nanometers. "Nano" is derived from the Greek word "nanos" meaning dwarf, tiny or very small (Rai et al., 2008). Nanotechnology has emerged as a dynamically developing area of scientific interest within the world. Nanoparticles are defined as a nanoscale particle of size within 1 to 100 nm. In nanotechnology, a particle is defined as is a small object that behaves as a whole unit respect to its transport and properties. Nanoparticles had a wide variety of application in the major fields of medicine, therapeutics, and diagnostic agents (Colvin et al., 1994, Wang and Herron, 1991, Schmid.G, 1992, Hoffman et al., 1992, Hamilton and Baetzold, 1979, Mansur et al., 1995, Senapati.S, 2005).

Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate & silver have come up but silver nanoparticles have proved to be most effective against bacteria, viruses & other eukaryotic microorganisms. Biologically synthesized silver nanoparticles (SNPs) are being widely used. Nanoparticles have been widely used for disinfection of water and to remove arsenic from water. Ag nanomaterials also have many other applications in various fields, such as nanoscale detection and solar cells.

Generally there are two approaches which are involved in the syntheses of silver nanoparticles, either from ''top to bottom'' approach or a ''bottom to up'' approach as depicted in Fig.1 (Ahmed et al., 2010). In bottom to up approach, nanoparticles can be synthesized using chemical and biological methods by self-assemble of atoms to new nuclei which grow into a particle of nanoscale. In bottom to up approach, chemical reduction is the commonest scheme for synthesis of silver nanoparticles (Elghanian et al., 1997; Hurst et al., 2006).

Different organic and inorganic reducing agents, like sodium borohydride ($NaBH_4$), sodium citrate, ascorbate, elemental hydrogen, Tollen's reagent and N, N-dimethyl formamide (DMF) are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions (Tran et al., 2013; Iravani et al., 2014).In case of top to bottom approach; nanoparticles are generally synthesized by evaporation–condensation method. Also in top to bottom approach, suitable bulk material break down into fine particles by size reduction with various lithographic techniques e.g. grinding, milling, sputtering and thermal/laser ablation (Elghanian et al., 1997; Hurst et al., 2006).

The use of green materials like plant extracts, microbial extracts, algal extracts and fungal extracts are used for synthesis of Silver nanoparticles as described in Fig 2.Various reducing agents present in the green extracts are responsible for synthesis of Silver nanoparticles (Roy et al., 2019).

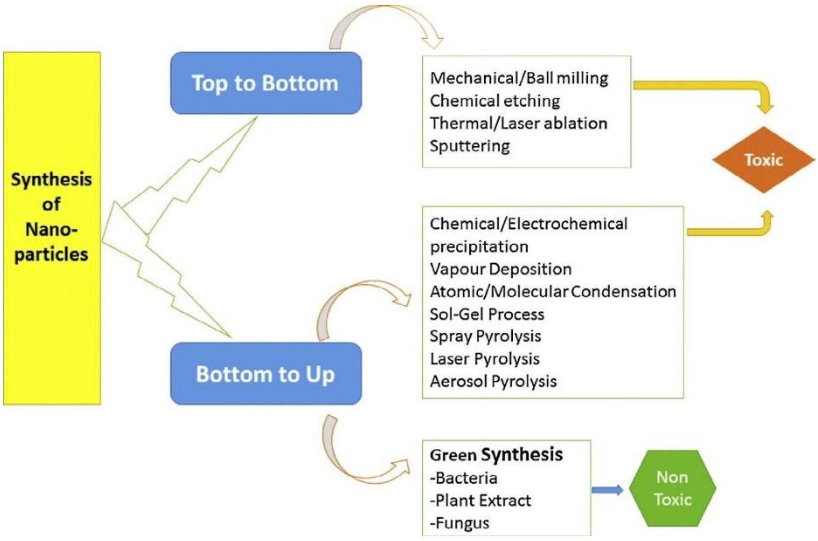

FIGURE 1: Different approaches of synthesis of silver nanoparticles (Ahmed et al., 2010).

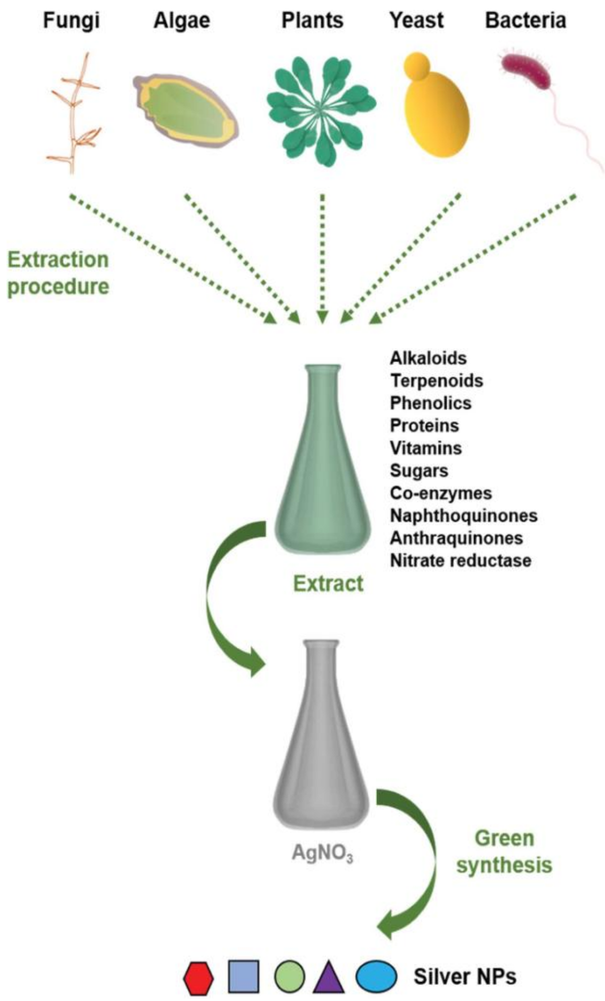

FIGURE 2: Schematic representation of the procedure for green synthesis of silver nanoparticles using various biological entities (Roy et al., 2019).

The biological method is also referred as green synthesis method. The biological method includes microorganisms like bacteria, fungi, algae, yeast and viruses and also plant extracts. Microorganisms and plants play role in absorption and accumulation of inorganic metallic ions from their surrounding (Shah et al., 2015). They secrete different enzymes in large quantities which have ability to hydrolyze metals and bring reduction of metals ions (Chokriwal et al., 2014).

It is found that the reduction rate of metal ion using biological method is quicker compared to physical and chemical method. The synthesis or production of silver nanoparticles by using biological means is not much expensive as compared to physical and chemical method. It is necessary to develop an environmental and economically friendly method for production of nanoparticle without involving any toxic chemicals, (Iravani S, 2014).

II. GREEN SYNTHESIS OF SILVER NANOPARTICLE BY USING PLANT EXTRACT

Plant extracts used for the synthesis of metal nanoparticles. Indeed, the use of plant for the assembly of silver nanoparticles has received many attentions because of its rapid, nonpathogenic, economical protocol and providing one step technique for the green synthesis processes (Huang et al., 2007).

Recently many researchers reported that AgNPs have been synthesized using various plants such as *Acalypha indica* (krishnaraj et al., 2010), *Aloe Vera* plant extract (Chandran et al., 2006), *Emblica officinalis* (Latha et al., 2015), leaves extract of *Citrus limon* (Mohapatra et al., 2015), *tea* (Nabikhan et al., 2010), etc. Comprehensive list of plants utilized for synthesis of Silver nanoparticles is given in Table 1.

TABLE 1
GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING DIFFERENT PLANT EXTRACT

Sr.No.	Plants	Size (nm)	Plant part	Reference
1	*Ficus carica*	13	Leaves	Geetha N et al. (2014)
2	*Moringa oleifera*	57	Leaves	Prasad and Elumalai. (2011)
3	*Acalypha indica*	20-30	Leaves	Krishnaraj et al. (2010)
4	*Carica papaya*	25-50	Leaves	Jain D et al. (2009)
5	*Datura metel*	16-40	Leaves	Kesharwani J et al. (2009)
6	*Aloe vera*	50-350	Leaves	Chandran SP et al. (2006)
7	*Tea*	20-90	Leaves	Nabikhan A et al. (2010)
8	*Eucalyptus hybrid*	50-150	Peel	Dubey M et al. (2009)
9	*Acorus calamus*	31.83	Rhizomes	Nakkala JR et al. (2014)
10	*Saraca indica*	20	Leaves	Perugu S et al. (2015)
11	*Azadirachta indica*	20	Leaves	Banerjee P et al. (2014)
12	*Cucurbita maxima*	19	Petals	Nayak D et al. (2015)
13	*Emblica officinalis*	25.4	Leaves	Latha M et al. (2015)
14	*Annona muricata*	20-53	Leaves	Santhosh SB et al. (2015)
15	*Citrus limon*	10-30	Peel	Mohapatra et al. (2015)
16	*Ocimum sanctum*	4-30	Leaves	Singhal et al. (2011)
17	*Allium cepa*	33.6	Leaves	Saxena et al. (2010)
18	*Bryophyllum*	18-21	Leaves	Saikia et al. (2015)
19	*Capsicum annum*	30-70	Leaves	Li et al. (2007)
20	*Euphorbia hirta*	40-50	Leaves	Elumalai et al. (2010)
21	*Lantana camara*	12.55	Leaves	Sivakumar et a. (2012)
22	*Mentha piperita*	90	Leaves	Ali et al. (2012)
23	*Citrullus colocynthis*	31	Leaves	Satyavani et al. (2007)
24	*Centella asiatica*	-	Leaves	Palaniselvam et al. (2011)
25	*Morinda tinctoria*	79-96	-	Vanaja et al. (2014)
26	*Morinda pubescens*	25-50	Leaves	Mary and Inbathamizh (2014)
27.	*Morinda citrifolia*	30-55	Root	Suman et al. (2013)
28.	*Aerva lanata*	18.62	Leaves	Joseph et al. (2014)
29.	*Zizipus jujube*	20-30	Leaves	Gavade et al. (2015)
30.	*Nelumbo nucifera*	16.7	Root	Sreekanth et al. (2014)
31.	*Prosopis farcta*	10.8	Leaves	Miri et al. (2015)
32.	*Cocos nucifera*	22	Coir	Roopan et al. (2013)
33.	*Lansium domesticum*	10-30	Fruit	Shankar et al. (2014)
34.	*Rosmarinus officinalis*	10-33	Leaves	Ghaedi et al. (2015)
35.	*Skimmia laureola*	46	Leaves	Ahmed et al. (2014)
36.	*Tephrosia tinctoria*	73	Stem	Rajaram et al. (2015)
37.	*Quercus branti*	6	Leaves	Korbekandi et al. (2015)
38.	*Justica adhatoda*	5-50	Leaves	Bose and Chatterjee, (2015)

The common protocol for synthesis of silver nanoparticle by using plant extract is carried out as described in Fig.3 (S. Rajeshkumar, L.V. Bharath, 2017).

2.1 General method for biosynthesis of silver nanoparticles by using plant extract

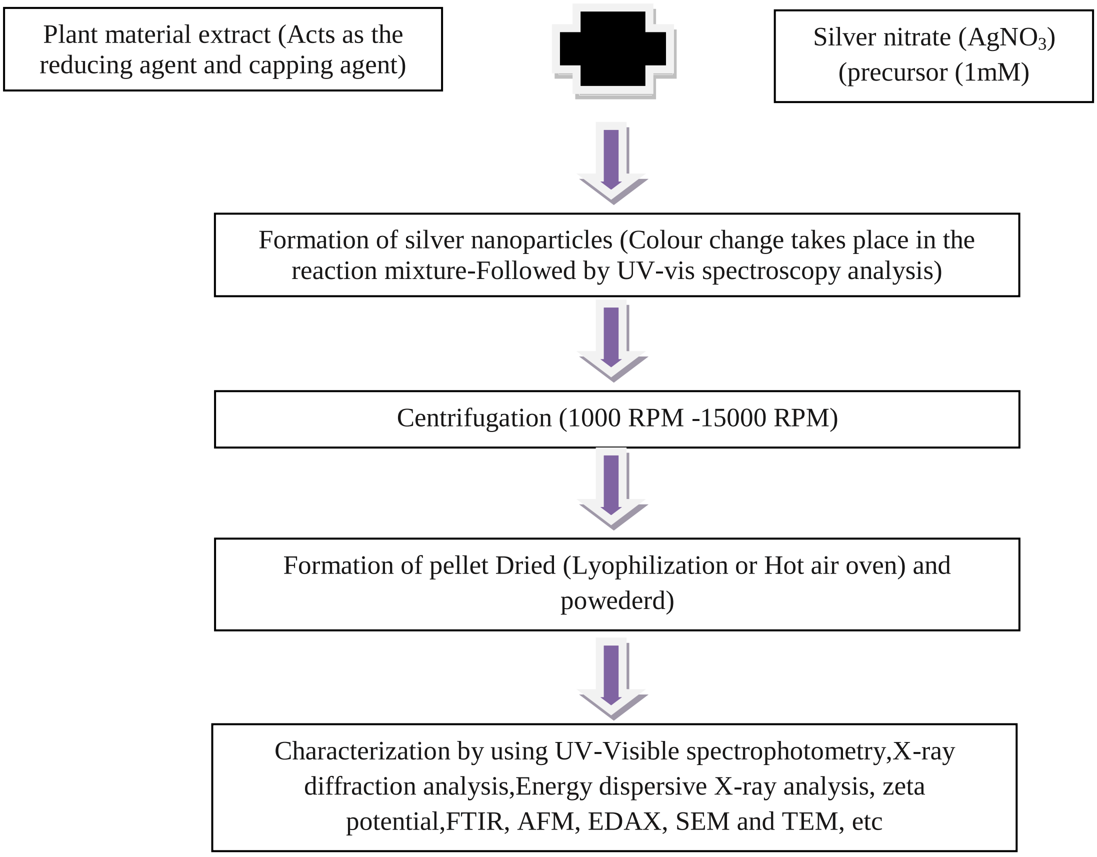

FIGURE 3: Biosynthesis of silver nanoparticle by using plant extract (S. Rajeshkumar, L.V. Bharath, 2017)

2.2 Mechanism of synthesis of silver nanoparticle using plant extract:

The parts of plants like as bark, stem, root, fruit, peel, seed, callus, leaves and flower are used for the synthesis of Silver nanoparticles (Singh et al., 2016). Plant extracts contain secondary metabolites like phenolic acid, flavonoids, alkaloids, amino acid, proteins, vitamins, enzymes, polysaccharide and terpenoids. They are liable for reduction of silver salts ($Ag+$) into silver ions ($Ag°$) (Kulkarni and Muddapur, 2014). Water soluble phytochemicals including organic acids, quinones and flavones are liable for the reduction of the silver ions within the reaction mixture (Doughari J.H, 2009).

Singhal et al. described biosynthesis and antimicrobial activity of silver nanoparticles using *Ocimum sanctum* extract. They showed that leaf extract can reduce silver ions into silver nanoparticles within 8 min of response time. The sizes of biosynthesized silver nanoparticles were within range of 4–30 nm and possessed antimicrobial activity. They showed silver nanoparticles were exhibited more antimicrobial activity on Gram-negative bacteria than Gram-positive. Also reported that synthesized silver nanoparticles have stronger activity than silver nitrate and standard antibiotic ciprofloxacin (Singhal et al., 2011).

Mohapatra et al. reported *Citrus limon* extract mediated synthesis of silver nanoparticles. The biosynthesized silver nanoparticles are of in the size range of 10-30 nm. They showed that addition of NaOH may be a key factor for rapid biosynthesis of stable aqueous dispersions of high concentration of silver nanoparticles. So, the alkaline environment is favourable for the biosynthesis of silver nanoparticles using *Citrus limon* (Mohapatra et al., 2015).

Silver nanoparticles were synthesized on reduction of silver nitrate solution by aqueous extract of *Azadirachta indica* leaves by Prathna et al. and the growth kinetics of silver nanoparticles was investigated having size of 10–35 nm.Colloidal silver nanoparticles were synthesized by a simple green method using thermal treatment of aqueous solutions of silver nitrate and natural rubber latex extracted from Hevea brasilensis. The silver nanoparticles presented diameter starting from 2 nm to 10 nm and had spherical shape with face centred cubic (fcc) crystalline structure (Prathna et al., 2011).

Sarkar et al. reported synthesis of silver nanoparticles of varying sizes using parthenium leaf extract at a higher temperature of 100 °C as well as at room temperature. They showed the variation of particle size with the reaction temperature and reaction time. The synthesized colloidal silver nanoparticles were photo-luminescent. The size of the silver nanoparticles synthesized after 2 minutes of chemical reaction at a higher temperature of 100 °C lie within 40-160 nm and the average size of the nanoparticles was ~ 110 nm (Sarkar et al., 2010).

III. GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING BACTERIA

The first bacteria which was found to be synthesizing silver nanoparticles was *Pseudomonas stutzeri AG259* Strain isolated from silver mine (Haefeli et al, 1984). Selected examples bacteria involve in the synthesis of Silver nanoparticles are given in Table 2. The most common site for biosynthesis of nanoparticles is cellular entities and their membrane (Mandal et al., 2006).

TABLE 2
GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING DIFFERENT BACTERIA

Sr No.	Bacteria	Types of nanoparticles	Size (nm)	Location/Morphology	Intracellular or Extracellular	Reference
1	*Pseudomonas stutzeri AG259*	Ag, Ag_2S	<200	Periplasmic space	Intracellular	Klaus et al (1999)
2	*Bacillus cereus*	Ag	20-40	Spherical	-	Sunkar et al (2012)
3	*Bacillus subtilis*	Ag	5-50	Spherical and triangular	-	Saifuddin et al (2009)
4	*Corneybacterium sp. SH09*	Ag	10-15	-	-	Zhang et al (2005)
5	*Klebsiella pneumonia*	Ag	5-32	-	Extracellular	Shhverdi et al (2007)
6	*Escherichia coli*	Ag	8-9	Spherical	-	Manhantry et al (2013)
7	*Escherichia coli DH5 alpha*	Ag	10-100	Spherical	-	H.R Ghorbani et al (2013)
8	*Plectonema boryanum (Corneybacterium)*	Ag	1-10	-	Intracellular	Lengke (2006)
9	*Serratia nematodiphila*	Ag	10-31	Spherical and crystalline	-	Malarkodi et al (2013)
10	*Pseudomonas putida NCIM 2650*	Ag	~70	Spherical	-	Rajasree et al (2012)
11	*Acetobacter Xylinum*	Ag	-	Cellulose fibre	-	Braud et al (2008)
12	*Bacillus megaterium*	Ag, Pb, Cd	10-20	-	-	Prakash et al (2010)
13	*Nacardiopsis sp. MBRC-1*	Ag	~45	Spherical	-	Manivasagan et al (2013)
14	*Idiomarina sp PR58-8*	Ag	26	-	-	Seshadri et al (2012)
15	*Lactobacillus strains*	Ag-Au alloys	100-300	Crystalline and cluster	-	Nair & T.Pradeep et al (2002)
16	*Acinetobacter calcoaceticus*	Ag	8-12	Spherical	Extracellular	Sing et al (2013)
17	*Caluconobacterium*	Ag	10	-	Extracellular	Krishnaraj and Berchmans (2013)
18	*Pedicoccus pentos aceus*	Ag			Intracellular	Sintubin et al (2009)

The general method used for biosynthesis of silver nanoparticles is depicted in Fig. 4. (Chokriwal et al., 2014):

3.1 General method for biosynthesis of silver nanoparticles by using bacteria

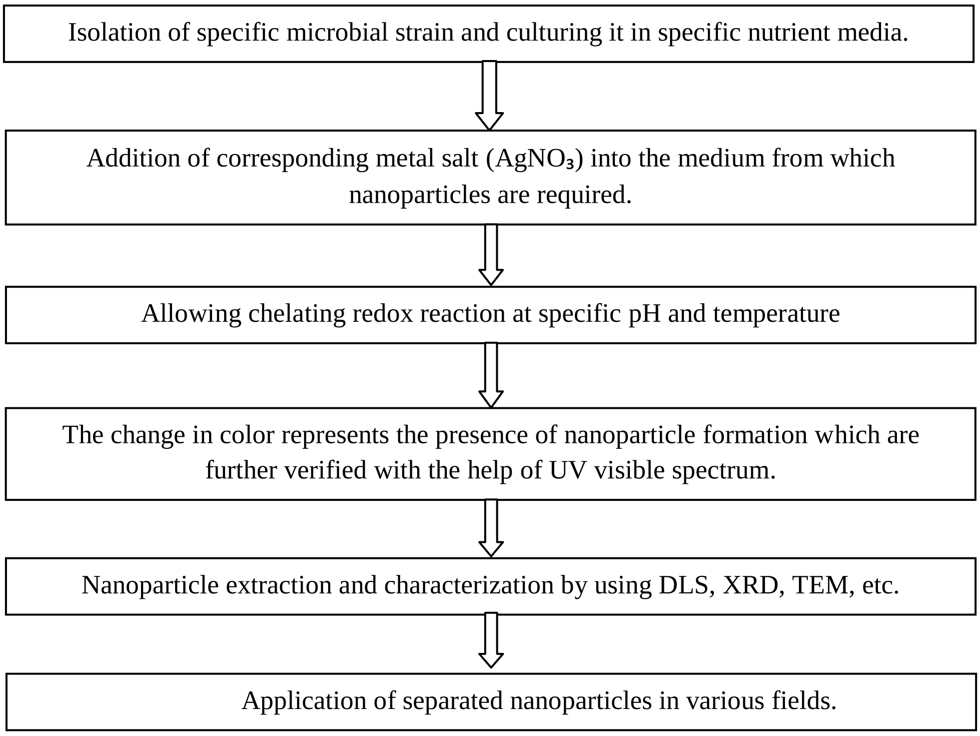

FIGURE: The genera methodology for biosynthesis of silver nanoparticles by using bacteria

3.2 Mechanism / synthesis of sliver nanoparticles

Various mechanisms of synthesis of AgNps are described as a part of microbial resistance for cellular detoxification. This involves changes in solubility of inorganic ions with the help of enzymatic reduction and or precipitation of soluble toxic substance to insoluble non toxic nano structure. The oxidoreductase enzymes for e.g – NADH – dependent nitrate reductase, cystine desulfhydrase etc and cellular transporters are involved in extracellular and intracellular synthesis of nanoparticles (Golmohmmadi et al., 2017).Mechanism which are considered includes bio-absorption, bio accumulation, extra ellular complexion, reduction or oxidation etc. (Beveridge et al., 1997).

IV. GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING FUNGI

Fungi have several excellences for synthesis of different types of nanoparticles over other organisms due to the presence of enzymes, proteins and reducing components on their cell surfaces (Narayanan and Sakthivel, 2011). Fungi even have better tolerance and possess higher metal-bioaccumulation property, which is liable for the high accumulation of silver nanoparticles (Singh et al., 2016; Alghuthaymi et al., 2015; Castro-Longoria et al., 2011)., and therefore the synthesized particles also tend to be smaller in size (Mukherjee et al., 2002; Volesky and Holan, 1995).

The first synthesis of fugus-mediated nanoparticles was reported in the beginning of the 20th century and silver nanoparticles synthesized by using fungus *Verticillium* (Mukherjee et al., 2001). The synthesis mechanism is based on the reduction of the Ag+ ions by the nitrate reductase present in the fungal system (Mohanpuria et al., 2008). Biosynthesis of silver nanoparticles from pathogenic and non-pathogenic fungi has been investigated extensively. (Siddiqi and Husen, 2016, Duran et al., 2005, Ingle et al., 2008, Ingle et al., 2009, Kathiresan et al., 2009).

Synthesis of silver nanoparticles mediated by fungi can be carried out by using either spores suspension, mycelia or cell free supernatant (Liu et al., 2018 and Zomorodian et al., 2016).The nanoparticles are formed on the surface of the mycelia but not in the solution. Ag+ ions are adsorbed on the surface of the fungal cells because of electrostatic interaction between negatively charged carboxylate groups in enzymes present within the cell membrane of mycelia and positively charged Ag ions. Finally, the silver ions are reduced by the enzymes present in cell wall, leading to the formation of silver nuclei (Mukherjee et al., 2001).

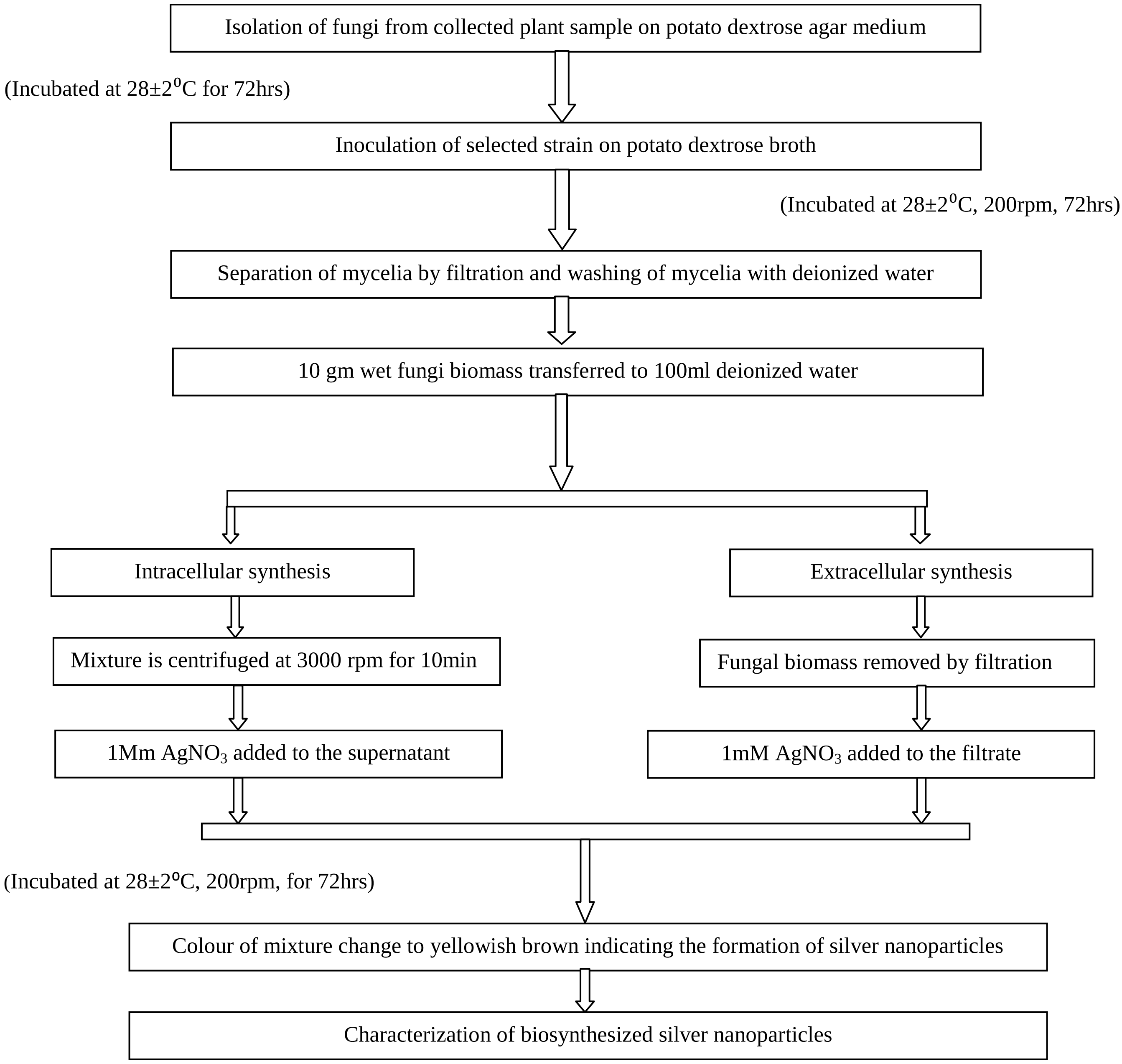

FIGURE 5. General methodology for biosynthesis of silver nanoparticles by using fungi (Shukla and Sandhu, 2017).

4.1 General method for biosynthesis of silver nanoparticles by using fungi.

Fig.5 depicts the general methodology for biosynthesis of silver nanoparticles by using fungi (Shukla and Sandhu, 2017). The synthesis of silver nanoparticles mediated by fungi can occur at both intracellular and extracellular locations (Siddiqi et al., 2018).In intracellular synthesis, the metal precursor is added to the mycelial culture and is internalized within the biomass. Extraction of the nanoparticles is required after the synthesis, centrifugation, chemical treatment, and filtration are used to disrupt the biomass and release the nanoparticles (Castro-Longoria et al., 2011; Rajput et al., 2016; Molnár et al., 2018).

In extracellular synthesis, the metal precursor is added to the aqueous filtrate containing fungal biomolecules, resulting in the formation of free nanoparticles in the dispersion. (Azmath et al., 2016; Sabri et al., 2016; Gudikandula et al., 2017; Costa Silva et al., 2017).

Fungi involved in the synthesis of Silver nanoparticles are listed in Table 3.

TABLE 3
GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING DIFFERENT FUNGI

Sr.no	Species	Size (nm)	References
1	*Fusarium semitectum*	20-25	Basavaraja et al., 2008
2	*Nemania sp.*	33.52	Bao and Lan, 2018
3	*Trichoderma harzianum*	50.10	Ahluwalia et al., 2014
4	*Macrophomina phaseolina*	5-40	Chawdhury et al., 2014
5	*Cladosporium cladosporioides*	10-100	Balaji et al., 2009
6	*Cariolus versicolor*	25-75	Sanghi and Verma, 2009
7	*Aspergillus fumigates*	5-25	Bhainsa and D'Souza, 2006
8	*Verticillium*	21-25	Mukherjee et al., 2001
9	*Penicillium fellutanum*	5-25	Kathiresan et al., 2009
10	*Aspergillus terreus*	1-20	Li et al., 2012
11	*Duddingtonia flagans*	30-409	Costa silva et al., 2017
12	*Epicoccum nigrum*	1-22	Qian et al., 2013
13	*Ganoderma sessiliforme*	~45	Mohanta et al., 2018
14	*Aspergillus fumigates*	5-25	Ratnasri and Hemalatha, 2014
15	*Rhodotorula mucilaginosa*	13.70	Farsi and Farokhi, 2018
16	*Rhizopus stolonifer*	2.86	Abdel Rahim et al., 2017
17	*Rhizoctonia salani*	2-22	Ashrafi et al., 2013
18	*Trichoderma reesei*	5-50	Vahabi et al., 2011
19	*Trichoderma viride*	5-40	Fayaz et al., 2010
20	*Fusarium oxysporum*	8-14	Senapati at al., 2005
21	*Penicillium brecompactum*	23-105	Shaligram et al., 2009
22	*Trichoderma asperellum*	13-18	Mukherjee et al., 2008
23	*Isaria fumosorosea*	51.31-111.02	Banu and Balasubramanian, 2014a
24	*Penicillium polonicum*	10-15	Neethu et al., 2018
25	*Rhodotorula glutinis*	15.45	Cunha et al., 2018
26	*Guignordia mangifera*	5-30	Balakumaran et al., 2015
27	*Arthroderma fulvum*	20.56	Xue et al., 2016
28	*Aspergillus flavus*	1-8	Vigneshwaran et al., 2006
29	*Trichoderma longibrachiatum*	10	Elamowi et al., 2018
30	*Aspergillus niger*	20	Gade et al., 2008
31	*Alternata alternate*	20-60	Gajbhiye et al., 2009
32	*Phoma glomerata*	60-80	Birla et al., 2009
33	*Phanerochaete chrysosporium*	50-200	Vigneshwaran et al., 2007
34	*Fusarium solani*	5-35	Ingle et al., 2009
35	*Rhizopus nigricans*	35-40	Ravindra and Rajasab., 2014

4.2 Mechanism of synthesis of silver nanoparticles

Silver ions are trapped at the surface of the fungal cells where they undergo succeeding reduction by the enzymes like naphthoquinones and anthraquinones present within the fungal system. (Mohanpuria et al., 2008).Further reduction by the NADPH-dependent nitrate reductase and a shuttle quinone extracellular process are liable for nanoparticle formation and its stabilization (Mukherjee et al., 2001).A significant disadvantage of the microbes to synthesize silver nanoparticles is that it is an extremely slow technique once compared with plant extracts (Tashi et al., 2016). Naphthoquinones and anthraquinones are extracellular enzyme present into fungal cell wall said to acceleratereduction (Ahmed et al., 2003)

Clasdosporium cladosporioides is used to synthesize silver nanoparticles.In this method the release of organic acids, protiens and polysaccharides are responsible for formation of spherical crystalline silver nanoparticles (Balaji et al., 2009).The genus *Fusarium* can synthesize metal nanoparticles intracellularly and extracellularly. The proposed hypothetical mechanisms for synthesis of silver nanoparticles is that NADH-dependent nitrate reductase enzyme secreted by *Fusarium oxysporum* is liablefor the reduction of aqueous silver intosilver nanoparticles (Ahmad et al., 2003).

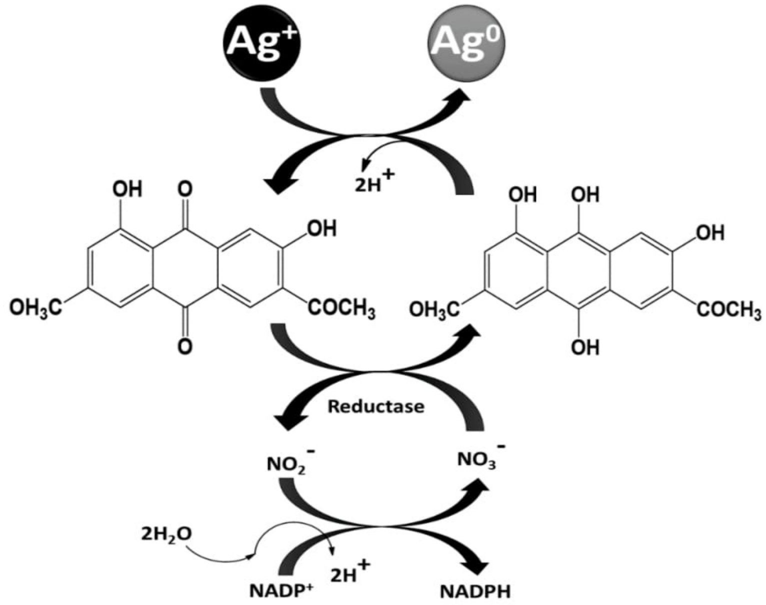

FIGURE 6: Hypothetical mechanism of AgNPs biosynthesis from *Fusarium oxysporum* (Srivastava et al., 2019).

The role of anthraquinone and therefore the NADPH-nitrate reductase within the biosynthesis of silver nanoparticles, also it had been hypothesized that the electron required to satisfy the deficiency of aqueous silver ionsand converted into Ag neutral (Ag0 i.e., AgNPs) was demonstrated by quinone and NADPH (Fig.6) (Srivastava et al., 2019).

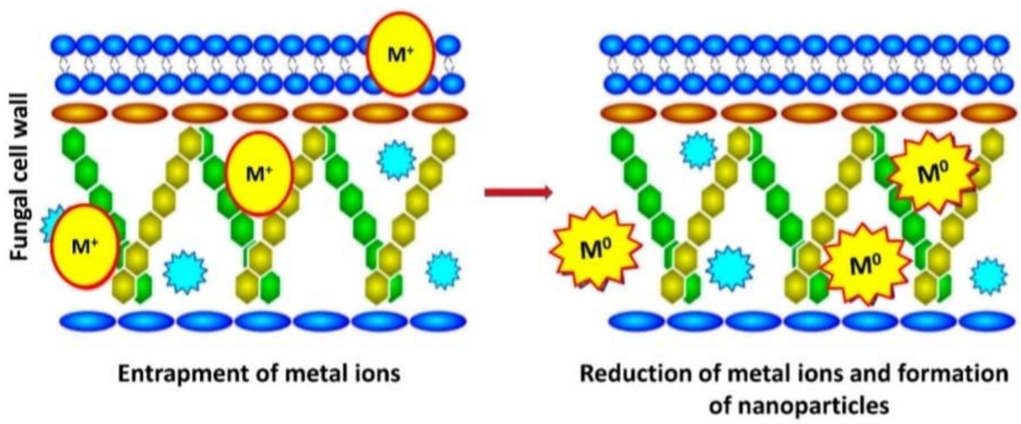

FIGURE 7: Hypothetical intracellular mechanisms for the synthesis of metal nanoparticles from *Fusarium oxysporum* (Yadav et al., 2015).

Two-step mechanism has been proposed for intracellular mycosynthesis of nanoparticles (Fig7).In first step, aqueous metal ion attached to the fungal cell surface by the electrostatic interaction between metal ionsand lysine residues.In second step, the mycosynthesis of nanoparticles occurs by the enzymatic reduction metal ions, which leads to the formation of nanoparticles (Yadav et al., 2015).

Nitya and Ragunathan, (2009) synthesized silver nanoparticles using *Pleurotus sajor.* The fungal filtrate of white rot fungi was reacted with silver nitrate solution and observed for the colour change from pale yellow to light brown. The intensity of the colourincreased as the time increased. It was then characterized by using UV-Visible spectroscopy, SEM analysisand conformed as silver nanoparticles. The spherical shaped, nanoparticle with 5-50nm was obtained. The susceptibility of silver nanoparticles against positive and negative organism was carried out by agar well diffusion method.

The silver nanoparticles which are produced from *Trichoderma harzianum* found to be more stable in nature and the nanoparticles were found in the range of 30-50nm in size and spherical in shape (Prashant Singh and Balaji Raja, 2011).

V. APPLICATIONS OF SILVER NANOBIOTECHNOLOGY

Silvernanoparticles have attracted much attention due to their potential in catalysis, biology, computing, solar cells and optoelectronic devices include cosmetics, mechanics, energy sciences, biomedical sciences, space industries, single electron transistors, nonlinear optical devices, light emitters, drug gene and photo chemical applications (Manesh et al., 2010).

Silver nanoparticles are widely used as antimicrobial agents within the health industry, food storage, textile coatings, and a variety of environmental applications, few of which are shown in Fig. 7 (Chung et al., 2016). Antimicrobial properties of silver nanoparticle are beneficial for various fields of medicine, various industries, farming, packaging, accessories, cosmetics, health, and therefore the military.

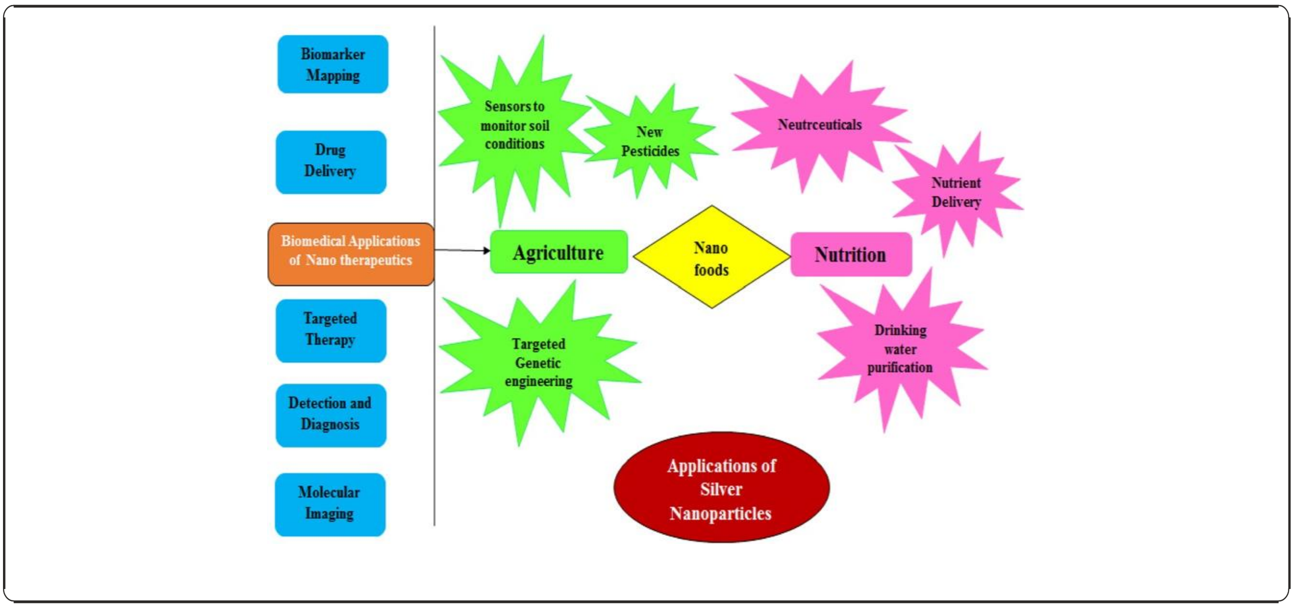

FIGURE 8: Different applications of synthesized silver nanoparticle (Chung et al., 2016)

5.1 Medical and Clinical field

Silver nanoparticles are used in surgical fields, such as urology, dentistry, general surgery and orthopedics (Roe et al., 2008). Nano-silver have been used in the treatment of wound, burns, in water –disinfecting systems, dental materials, and antibacterials, antivirals and anticancerous agents (Elliott et al., 2010). The silver nanoparticles were also used for impregnation of polymeric medical devices to extend their antibacterial activity. Silver nanoparticles are used in sensing and imaging applications, including the detection of DNA (Harper et al., 2012), selective colorimetric sensing of cystein (Ravindran et al., 2011), sensing purine nucleoside phosphorylase activity (Cao et al., 2013), and selective colorimetric sensing of mercury (Roy et al., 2011).

5.2 Agriculture

Silver nanoparticles may hold significant applications in agriculture and gardening by selectively inhibiting harmful fungi and bacteria on seeds and could provide an alternative source of fertilizer that may improve sustainable agriculture (Parveen et al., 2014).Antimicrobial property of silver nanoparticles, it is interesting to note that silver nanoparticles are predominantly used for plant disease management (Park et al., 2006). It has been reported that silver nanoparticles could be used to enhance seed germination potential in many plants (Duhan et al., 2017).The application of silver nanoparticles in plant tissue culture techniques in order to suppress microbial contaminations was initially reported by (Abdi et al., 2008).

5.3 Cancer Treatment

The silver nanoparticles synthesized by Kuppusamy et al. using Commelia nudiflora L aqueous extract showed a reduced cell viability and increased cytotoxicity against HCT-116 colon cancer cells.Silver nanoparticles composites possessed promising anticancer activity against the A549 (Human lung carcinoma), Hela (Human cervical adenocarcinoma), MCF7 (Human breast adenocarcinoma), MDAMB231 (Human breast adenocarcinoma), and SKBR3 (Human breast adenocarcinoma) cells (El-Naggar et al., 2017).

5.4 Antimicrobial activity

Silver nanoparticle is reported with antimicrobial activities which is used in disinfectants (Brady et al., 2003).Marslin et al. biosynthesized cream formulation of silver nanoparticle using Withania somnifera extract and concluded that cream had higher antimicrobial activity, hence might be utilized in low doses and fewer toxic for patient in comparison with AgNO3 counterparts (Marslin et al., 2015).

Silver nanoparticles and ZnO nanowires with polyvinylchloride, a commonly used material for catheters, and investigated the antimicrobial efficacy of the composite against *Staphylococcusaureus*, one of the most common pathogens found in catheter-associated urinary tract infection (Warren et al., 1997).Extract of C.igneus leaves- based synthesis of Ag nanoparticles have shown very strong action against bacteria and fungus as compared to standard antibacterial agents (Sataraddi et al., 2012).

REFERENCES

[1] A.Chokriwal *et al*., Biological Synthesis of Nanoparticles Using Bacteria and Their Applications.vAmerican Journal of PharmTech Research 2014.

[2] A.J.Hoffman, G.Mills, H.Yee, and M.Hoffmann. Q-sized cadmium sulfide: synthesis, characterization, and efficiency of photoinitiation of polymerization of several vinylic monomers. J Phys Chem. 1992; 96:5546–5552. [Google Scholar]

[3] A.Miri, M.Sarani, M.Rezazade Bazaz and M.Darroudi (2015) Plant-mediated biosynthesis of silver nanoparticles using Prosopis farcta extract and its antibacterial properties. Spectrochim Acta A Mol Biomol Spectrosc15 (141):287–291.

[4] A.Nabikhan, K.Kandasamy, A.Raj and N.M.Alikunhi. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L.. Colloids Surf B: Biointerfaces 2010; 79:488–93

[5] A.Roy, O.Bulut, S.Some, A.K.Mandal and M.D.Yilmaz (2019). Green synthesis of silver nanoparticles: biomolecules-nanoparticle organization targeting antimicrobial activity. DOI: 10.1039/C8ra0898e.

[6] A.Saxena, R.M.Tripathi and R.P.Singh.Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity. Dig J Nanomater Bios. 2010; 5: 427 – 432.

[7] B.Mohapatra, R.Kaintura, J.Singh, S.Kuriakose and S.Mohapatra. Biosynthesis of high concentration, stable aqueous dispersions of silver nanoparticles using Citrus limon extract. Adv. Mater. Lett.2015; 6: 228-234.

[8] C.Krishnaraj, E.Jagan, S.Rajasekar, P.Selvakumar, P.Kalaichelvan and N.Mohan.Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 2010; 76:50–6.

[9] D.Bose and S.Chatterjee (2015). Antibacterial activity of green synthesized silver nanoparticles using Vasaka (Justicia adhatoda L.) leaf extract. Indian J Microbiol 55 (2):163–167.

[10] D.Jain, H.K.Daima, S.Kachhwaha and S.Kothari.Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Dig J Nanomater Biostruct 2009; 4:557–63.

[11] D.Nayak, S.Pradhan, S.Ashe, P.R.Rauta and B.Nayak (2015) Biologically synthesized silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. J Colloid Interface Sci 457:329–338.

[12] D.Saikia, P.K.Gogoi, P.Phukan, N.Bhuyan, S.Borchetia, J.Saikia. Green synthesis of silver nanoparticles using Asiatic Pennywort and Bryophyllum leaves extract and their antimicrobial activity. Adv. Mater. Lett.2015; 6: 260-264.

[13] E.J.Mary and L.Inbathamizh. Green synthesis and characterization of nano silver using leaf extract of Morinda pubescens. Asian J Pharm Clin Res. 2012; 5: 159-162.

[14] E.K.Elumalai, TNVKV Prasad, J.Hemachandran, S.V.Therasa, T.Thirumalai and E.David. Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities. J. Pharm. Sci. & Res. 2010; 2: 549-554.

[15] G.Schmid. Large clusters and colloids. Metals in the embryonic state. Chem Rev. 1992; 92:1709–1027. [Google Scholar]

[16] G.Singhal, R.Bhavesh, K.Kasariya, A.R.Sharma, R.P.Singh.Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity.J Nanopart Res. 2011; 13: 2981– 2988.

[17] H.Korbekandi, M.R.Chitsazi, G.Asghari, R.Bahri Najafi, A.Badii and S.Iravani (2015) Green biosynthesis of silver nanoparticles using Quercus brantii (oak) leaves hydroalcoholic extract. Pharm Bio 53 (6):807–812.

[18] H.S.Mansur, F. Grieser, M.S.Marychurch, S.Biggs, R.S.Urquhart and D.Furlong. Photoelectrochemical properties of 'q-state' cds particles in arachidic acid langmuir-blodgett films. J Chem Soc Faraday Trans. 1995; 91:665–672. [Google Scholar]

[19] J. Singh, G. Kaur, P. Kaur, R. Bajaj and M. Rawat, A review on green synthesis and characterization of silver nanoparticles and their applications: a green nanoworld, World J. Pharm. Pharm. Sci. 5 (7) (2016) 730e762

[20] J.F.Hamilton and R.Baetzold.Catalysis by Small Metal Clusters.Science. 1979; 205:1213–1220. [PubMed] [Google Scholar]

[21] J.H. Doughari, Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, 2009, pp. 1e33.

[22] J.Huang, Q.Li, D.Sun, Y.Lu, Y.Su and X.Yang.Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf.Nanotechnology. 2007; 18: 105104-105115.

[23] J.Kesharwani, K.Y.Yoon, J.Hwang and M.Rai. Phytofabrication of silver nanoparticles by leaf extract of Daturametel: hypothetical mechanism involved in synthesis. J Bionanosci 2009; 3:39–44.

[24] J.R.Nakkala, R.Mata, A.K.Gupta and S.R. Sadras. Green synthesis and characterization of silver nanoparticles using Boerhaavia diffusa plant extract and their antibacterial activity. Indus Crop Prod 2014; 52:562–6.

[25] K.B.A.Ahmed, S.Subramanian, A.Sivasubramanian, G.Veerappan and A.Veerappan (2014).Preparation of gold nanoparticles using Salicornia brachiata plant extract and evaluation of catalytic and antibacterial activity. Spectrochimica Acta Part A Molecul Biomol Spectros 130:54–58

[26] K.Palaniselvam, AAJ Velanganni, S.N. Govindan, Karthi. Leaf assisted bioreduction of silver ions using leaves of Centella asiatica L. and its bioactivity. E- journal of life scie. 2012; 1: 46-49.

[27] K.Rajaram, D.C.Aiswarya and P.Sureshkumar (2015) Green synthesis of silver nanoparticle using Tephrosia tinctoria and its antidiabetic activity. Mater Lett 138:251–254

[28] K.Satyavani, T.Ramanathan and S.Gurudeeban. Plant mediated synthesis of biomedical silver nanoparticles by using leaf extract of Citrullus colocynthis. J Nanosci Nanotechnol. 2011; 1: 95-101

[29] M.Dubey, S.Bhadauria and B.Kushwah.Green synthesis of nanosilver particles from extract of Eucalyptus hybrida (safeda) leaf. Dig J Nanomater Biostruct 2009; 4:537–43.

[30] M.Ghaedi, M.Yousefinejad, M.Safarpoor, H.Zare Khafri and M.K.Purkait (2015) Rosmarinus officinalis leaf extract mediated green synthesis of silver nanoparticles and investigation of its antimicrobial properties. J Ind Eng Chem. Doi:10.1016/j.jiec.2015.06.020.

[31] M.Latha, M.Sumathi, R.Manikandan, A.Arumugam and N.M.Prabhu (2015) Biocatalytic and antibacterial visualization of green synthesized silver nanoparticles using Hemidesmus indicus. Microb Pathog 82:43–49.

[32] M.Rai, A.Yadav and A.Gade (2008) Current trends in phytosynthesis of metal nanoparticles. Crit Rev Biotechnol 28 (4):277–284

[33] M.Shah, D.Fawcett, S.Sharma, S.K.Tripathy and GEJ Poinern.Green synthesis of metallic nanoparticles via biological entities.Materials. 2015; 8:7278–308. 10.3390/ma8115377. Search in Google Scholar S.

[34] M.Vanaja, K.Paulkumar, M.Baburaja, S.Rajeshkumar, G.Gnanajobitha, C.Malarkodi and M. Sivakavinesan. Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorg Chem Appl. 2014; 2014: 742346.

[35] MubarakAli D1, N.Thajuddin, K. Jeganathan and M.Gunasekaran. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerfaces.2011; 85: 360-365.

[36] N.Geetha, T.S.Geetha, P.Manonmani and M.Thiyagarajan. Green synthesis of silver nanoparticles using Cymbopoga Citratus (Dc) Stapf. Extract and its antibacterial activity. Aus J Basic Appl Sci 2014; 8 (3):324–31.

[37] N.Kulkarni and U.Muddapur. Biosynthesis of Metal Nanoparticles: A Review. J. Nanotech. 2014; 1-8.

[38] N.L.Gavade, A.N.Kadam, M.B.Suwarnkar, V.P.Ghodake and K.M.Garadkar (2015)Biogenic synthesis of multi-applicative silver nanoparticles by using Ziziphus Jujuba leaf extract. Spectrochim Acta A Mol Biomol Spectrosc 136:953–960.

[39] P.Banerjee, M.Satapathy, A.Mukhopahayay and P.Das (2014) Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Biores & Bioproces 1:3.

[40] P.Sivakumar, C.Nethradevi and S.Renganathan. Synthesis of silver nanoparticles using Lantana camara fruit extract and its effect on pathogens. Asian J Pharm Clin Res. 2012; 5: 97-101.

[41] Q.H.Tran, Van Quy Nguyen VQ and A.T.Le. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv Nat Sci: Nanosci Nanotechnol 2013; 4:1–21

[42] R.Elghanian, J.J.Stohoff, R.C.Mucic, R.L.Letsinger and C.A.Mirkin. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science 1997; 277:1078.

[43] R.Sarkar, P.Kumbhakar, A.K.Mitra.Green synthesis of silver nanoparticles and its optical properties. Dig J Nanomater Bios.2010; 5:491-496.

[44] S. Iravani. Bacteria in nanoparticle synthesis: current status and future prospects. Int Scholar Res Not. 2014; 2014:359316. 10.1155/2014/359316. Search in Google Scholar

[45] S. Rajeshkumar, L.V. Bharath, Mechanism of plant mediated synthesis of silver nanoparticles review on biomolecules involved, Characterization and antimicrobial activity. Chemico-Biological Interaction 273 (2017)219e227.

[46] S. Senapati Ph.D. Thesis. India: University of pune; 2005. Biosynthesis and immobilization of nanoparticles and their applications; pp. 1–57. [Google Scholar

[47] S.Ahmed, B.L.Swami, S.Ikram. A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial application: A green expertise, Journal of advanced research http Sarkar R, Kumbhakar P, Mitra AK. Green synthesis of silver nanoparticles and its optical properties. Dig J Nanomater Bios. 2010; 5: 491 – 496. http://dx.doi.org/10.1016/j.jare.2015.02.007

[48] S.B.Santhosh, R.Yuvarajan and D. Natarajan (2015) Annona muricata leaf extract mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector. Parasitol Res 114 (8):3087–3096.

[49] S.Iravani, H.Korbekandi, S.V.Mirmohammadi and B.Zolfaghari. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci 2014; 9 (6):385–406.

[50] S.J.Hurst, AKR Lytton-Jean and C.A.Mirkin.Maximizing DNA loading on a range of gold nanoparticle sizes. Anal Chem 2006; 78 (24):8313–8.

[51] S.Joseph and B.Mathew (2014). Microwave assisted facile green synthesis of silver and gold nanocatalysts using the leaf extract of Aerva lanata. Spectrochim Acta A Mol Biomol Spectrosc 136PC:1371–1379.

[52] S.Li, Y.Shen, A.Xie, X.Yu, L.Qiu and L.Zhang. Green synthesis of silver nanoparticles using Capsicum annuum L. extract. Green Chem. 2007; 9: 852–858.

[53] S.M.Roopan, G.Madhumitha, A.Abdul Rahuman, C.Kamaraj, A.Bharathi and T.V.Surendra (2013) Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using Cocos nucifera coir extract and its larvicidal activity. Ind Crop Prod 43:631–635.

[54] S.P.Chandran, M.Chaudhary, R.Pasricha, A.Ahmad and M.Sastry.Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol Prog 2006; 22:577–83.

[55] S.Perugu, V.Nagati and M.Bhanoori (2015) Green synthesis of silver nanoparticles using leaf extract of medicinally potent plant Saraca indica: a novel study. Nanosci. Doi:10.1007/s13204-015-0486-7

[56] S.Shankar, L.Jaiswal, RSL Aparna, RGSV Prasad (2014) Synthesis, characterization, in vitro biocompatibility, and antimicrobial activity of gold, silver and gold silver alloy nanoparticles prepared from Lansium domesticum fruit peel extract. Mater Lett 137:75–78.

[57] T.C.Prathna, N.Chandrasekaran, A.M. Raichur and A. Mukherje. Kinetic evolution studies of silver nanoparticles in a bio-based green synthesis process. Colloids Surf A, Physicochem Eng Aspects 2011; 37:212–6.

[58] T.V. Sreekanth, S.Ravikumar and I.Y.Eom (2014) Green synthesized silver nanoparticles using Nelumbo nucifera root extract for efficient protein binding, antioxidant and cytotoxicity activities. J Photochem Photobiol B141:100–105.

[59] T.Y.Suman, S.R.Radhika Rajasree, A.Kanchana and S.B.Elizabeth (2013) Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using Morinda citrifolia root extract. Colloids Surf B: Biointerfaces 1 (106):74–78.

[60] TNVKV Prasad and E.Elumalai. Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. Asian Pac J Trop Biomed 2011; 1:439–42.

[61] V.L.Colvin, M.C.Schlamp and A.Alivisatos. Light emitting diodes made from cadmium selenide nanocrystals and a semiconducting polymer. Nature. 1994; 370:354–357. [Google Scholar]

[62] Y.Wang and N.Herron. Nanometer-sized semiconductor clusters: materials synthesis, quantum size effects, and photophysical properties. J Phys Chem. 1991; 95:525–532. [Google Scholar]

[63] A. Mahanty, R. Bosu, P. Panda, S. P. Netam, and B. Sarkar, "Microwave assisted rapid combinatorial synthesis of silver nanoparticles using E. coli culture supernatant, " International Journal of Pharma and Bio Sciences, vol. 4, no. 2, pp. 1030–1035, 2013.

[64] A.Prakash, S.Sharma, N.Ahmad, A.Ghosh and P.Sinha. Bacteria mediated extracellular synthesis of metallic nanoparticles. Int Res J Biotechnol 2010; 1: 5: 071-079.

[65] A.R.Shahverdi, S.Minaeian, H.R.Shahverdi, H.Jamalifar and A.A.Nohi. Rapid synthesis of silver nanoparticles using culture supernatants of< i> Enterobacteria</i>: A novel biological approach. Proc Biochem 2007; 42: 5: 919-923.

[66] B Nair and T.Pradeep.Coalescence of Nanoclusters and Formation of Submicron Crystallites Assisted by Lactobacillus strains. Cryst Growth Design 2002; 2: 293–298.

[67] C. Malarkodi, S. Rajeshkumar, K. Paulkumar, M. Vanaja, G. D. G. Jobitha, and G. Annadurai, "Bactericidal activity of bio mediated silver nanoparticles synthesized by Serratia nematodiphila, " Drug Invention Today, vol. 5, no. 2, pp. 119–125, 2013

[68] C.Haefeli, C.Franklin and K.Hardy.Plasmid-determined silver resistance in Pseudomonas stutzeriisolated from a silver mine.J.Bacteriol.158, 389-392 (1984)

[69] D.Mandal, M.E, Bolander, and D.Mukhopadhyay.The use of microorganisms for the formation of metal nanoparticles and their application.Appl Microbial Biotechnol 69, 485-492 (2006)

[70] H. R. Ghorbani, "Biosynthesis of silver nanoparticles by Escherichia coli," Asian Journal of Chemistry, vol. 25, no. 3, pp. 1247–1249, 2013.

[71] H. R. Zhang, Q. B. Li, Y. H. Lu. "Biosorption and bioreduction of diamine silver complex by Corynebacterium, " Journal of Chemical Technology and Biotechnology, vol. 80, no. 3, pp. 285–290, 2005

[72] H.Golmohammadi, E.Morales-Narvaez, T.Naghdi, and A.Merkoci, . Nanocellulose in sensing and biosensing.Chem. Mater. **2017**, 29, 5426–5446. [CrossRef]

[73] H.S.Barud, C.Barrios, T.Regiani, RFC Marques, M.Verelst and J.Dexpert-Ghys. Self-supported silver nanoparticles containing bacterial cellulose membrane. Mat Sci Eng C 2008; 28: 515-518.

[74] L.Sintubin, De Windt Wim, J. Dick, J.Mast. D Van der Ha W. Verstraete and Boon Nico. Lactic acid bacteria as reduling and capping agent for the fast and efficient production of silver nanoparticles. Applied Microbiology and Biotechnology 84, 741-749 (2009)

[75] M.Lengke and G.Southam. Bioaccumulation of Gold by Surface-Reducing Bacteria Cultured in The Presence of Gold (I) –Thiosulfate Complex. Geochim Cosmochim Acta 2006; 70: 3646-61.

[76] N. Saifuddin, C. W. Wong, and A. A. N. Yasumira, "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation, " E-Journal of Chemistry, vol. 6, no. 1, pp. 61–70, 2009.

[77] P. Manivasagan, J. Venkatesan, K. Senthilkumar, K. Sivakumar, and S. Kim, "Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel Nocardiopsis sp. MBRC-1, " BioMed Research International, vol. 2013, Article ID 287638, 9 pages, 2013.

[78] R.N.Krishnaraj and S.Berchmans.In-vitro antiplatelet activity of silver nanoparticles synthesised using the microorganism Gluconobacter roseus; an AFM-based study.Royal Society of Chemistry (2013), https://doi.org/10.1039/C3RA41246F

[79] R.Singh, P.Wagh, S.Wadhwani, S.Gaidhani, A.Kumbhar, J.Bellare and B.A.Chopade.Synthesis, optimization, and characterization of silver nanoparticles from Acinetobacter calcoacetius and their enhanced antibacterial activity when combined with antibiotics. International Journal of Nanomedicine (2013); 8:4277-4290., doi:10.2147/IJN.S48913.

[80] S. R. Rajasree and T. Y. Suman, "Extracellular biosynthesis of gold nanoparticles using a gram negative bacterium Pseudomonas fluorescens, " Asian Pacific Journal of Tropical Disease, vol. 2, no. 2, pp. S796–S799, 2012.

[81] S.Seshadri, A.Prakash and M.Kowshik.Biosynthesis of Silver Nanoparticles by Marine Bacterium, Idiomarina sp. PR58-8. Bull Mater Sci 2012; 35: 7: 1201–1205.

[82] Sunkar and C. V. Nachiyar, "Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium Bacillus cereus isolated from Garcinia xanthochymus, " Asian Pacific Journal of Tropical Biomedicine, vol. 2, no. 12, pp. 953–959, 2012.

[83] T.J.Beveridge, M.N.Hughes, H.Lee, K.T.Leung, R.K.Poole, I.Savvaidis, S.Silver and J.T.Trevors (1997)Metal-microbe interaction: contemporary approaches.Adv Microb Physiol 38:178-243.Google Scholar.

[84] T.Klaus, R.Joerger, E.Olsson and CGR Granqvist. Silver-Based Crystalline Nanoparticles, Microbially Fabricated. Proc Natl Acad Sci 1999; 96: 24: 13611-4

[85] A. Ingle, A.Gade, S.Pierrat, C.Sonnichsen and M.Rai.Mycosynthesis of silver nanoparticles using the fungus *Fusarium accuminatum* and its activity against some human pathogenic bacteria.Curr Nanosci. 2008; 4:141-4.

[86] A.Ahmad, P.Mukherjee, S.Senapati, D.Mandal, M.I.Khan, Kumar and M.Sastry, .Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*.Coll.Surf.B Bioimterfaces 2003, 28, 313-318.

[87] A.Ingle, M.Rai, A.Gade and M. Bawaskar. *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles.JNanoparticleRes.2009; 11:207985.https://doi.org/10.1007/s11051-008-9573-y.

[88] A.K.Gade, P.Bonde, A.P.Ingle.Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Mater Bioenergy.2008; 2:243–7.https://doi.org/10.1166/jbmb.2008.401.

[89] A.M.Fayaz, K.Balaji and M.Girilal. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics:a study against gram positive and gram negative bacteria. Nanomed Nanotechnol BiolMed.2010.https://doi.org/10.1016/j.nano.2009.04.006.

[90] A.N. Banu and C.Balasubramanian. (2014a).Optimization and synthesis of silver nanoparticles using *Isaria fumosorosea* against human vector mosquitoes. Parasitol.Res.113, 3843–3851. doi:10.1007/s00436-014-4052-0

[91] A.Rahim, K., Mahmoud, S.Y., Ali, A.M., Almaary, K.S., Mustafa, A.E. Z.M.A., and Husseiny, S.M. (2017). Extracellular biosynthesis of silver nanoparticles using *Rhizopus stolonifer*.Saudi J.Biol.Sci.24, 208–216. doi:10.1016/j.sjbs.2016.02.025


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