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Preface

We would like to present, with great pleasure, the inaugural volume-5, Issue-9, September 2019, 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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Soil Science	Plant Science
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Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
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Comparing the performance of a home-made bottle drip to a commercial drip system in the production of lettuce (*Lactucasativa L.*)

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Abstract— *A study was conducted in which lettuce (Lactucasativa L.) was grown in a plot at the Faculty of Agriculture at Luyengo Campus of the University of Eswatini to compare three different irrigation methods on the production of marketable heads of lettuce. The performance of lettuce under a commercial drip tape was compared with a home-made bottle drip and a hand watering can as used typically by rural people in the country. The commercial drip had emitters discharging 2 liters per hour and therefore 2 liters per hour was applied with both the home-made bottle drip and the watering can during irrigation. The irrigation frequency was every after two days for all the treatments. The plot sizes were 1.5 m x 4.0 m and there were four replications per treatment. There were eighteen lettuce plants per plot. The lettuce was grown for a period of four weeks and then harvested whole. Yield parameters measured included the plant height (cm), leaf area index (LAI), root length (cm) and the fresh head mass (grams). Significant differences ($P < 0.01$) between treatments were obtained for fresh lettuce head mass and root length. The commercial drip treatment had largest fresh mass at 226.8 g. It was followed by bottle drip at 184.8 g. The control had the lowest yield at 165.3 g. There were no significant differences between treatments for plant height and leaf area index. It was concluded that the home-made bottle drip irrigation method could be recommended for rural people who cannot afford to buy the commercial drip system for the production of vegetables for household consumption.*

Keywords— Lettuce, yield, drip, irrigation, water use efficiency.

I. INTRODUCTION

Eswatini import approximately 37,300 metric tonnes of fruits and vegetable with a value of US$11,000,000 from South Africa (NAMBOARD, 2018). This is because the annual rainfall distribution in the country is skewed, with the most rainfall 1,500 mm received in the Highveld region and the least 450 mm in the Lowveld region. The Lowveld is the ideal place for vegetable production, but due to lack of water, rural communities struggle to make ends meet.

Crop production can only be a success if grown under irrigated conditions. However, the energy requirement associated with irrigation makes its adoption difficult. The adoption of low energy agricultural technologies like drip in the country is very low, as the Eswatini government tends to promote conventional methods of water resource development as opposed to micro irrigation which is ideally suited to small holder farmers (Manyatsi and Magongo, 2008). Drip irrigation can be more efficient than sprinkler and furrow irrigation (Hunsaker et. al., 2019) since only the root zone of the cropped area is irrigated (Dukes et. al., 2006 and Hartz, 1999). Many of the soils where vegetables are grown are sandy with very low water holding capacities. These require frequent irrigation and fertigation to minimize crop stress and to attain maximum production. The main drawback with drip systems is the frequent emitter blockages (Zhou et. al., 2019)

Although drip irrigation can be very efficient at 90 percent since water and nutrients are delivered to the crop root zone, the capital cost is beyond the reach of most rural farmers. Also, mismanagement can lead to over irrigation and excessive nutrient loss due to leaching. The beneficial effects of drip irrigation management compared to other forms of water management are attributed to a uniform water application (Sandhu et. al., 2019), controlled root zone development and better disease management since only the soil is wetted whereas the leaf surface stays dry (Holmer and Schnitzler, 1997).

Since the capital cost of drip irrigation is beyond the reach of many rural farmers (Westarp et. al., 2004) including Eswatini, the bottle drip system offers a feasible option for economic production in areas of low rainfall or during periods of water scarcity. Drip irrigation refers to any system of watering cultivated crops in which the water is delivered directly to each individual plant on a gradual and continuous basis (Bajracharya and Sharma, 2005). A bottle drip system is an easy way of watering plants (Darouich et al., 2014), no costs is involved in purchasing the bottles as old material is useful, no power or piping required to supply the water and it's very easy to make. The purpose of this study was to evaluate the effectiveness of

two different methods of drip irrigation, namely commercial surface drip and the bottle drip method, on the growth performance and yield of lettuce grown at Luyengo, Eswatini.

II. MATERIALS AND METHODS

In order to test the response of lettuce (*Lactucasativa L.*) to the method of water delivery by the commercial and bottle drip-irrigation systems, a field plot experiment using a split plot design was established in the Agricultural and Biosystems Engineering farm of the University of Eswatini at Luyengo campus. The farm is located in the Middleveld of Eswatini at $21^0$34′Sand $31^0$12′E at an altitude of about 730 m above sea level. The experiment consisted of three treatments (commercial and bottle drip) and a watering can which was the control. There were four replicates. The control treatment was irrigated with a normal 10 litre watering can as in the traditional practice in the rural areas.

Yield parameters measured at harvesting were plant height (cm), leaf area index (LAI), root length (cm) and the lettuce fresh mass (grams).

2.1 Commercial drip equipment

Sixteen millimetre (16 mm) non pressure compensation dripper lines were laid in plots that were four metres long. The drippers had emitters spaced 60 cm apart, each discharging about 2 litres of water per hour. The aim was to apply about 8 mm of water per irrigation per day.

2.2 Bottle drip equipment

Two litre cool drink plastic bottles with lids were used to store water and provide water to the lettuce plants. Small holes were drilled into the cap of the plastic bottles. The aim was to have a discharge from the holes of approximately 2 litres per hour. The bottom of each bottle was removed to enable the bottles to be filled with water easily and also collect rainfall water. A hole was dug next to each plant and the bottle buried approximately one-third deep with the bottom facing up.

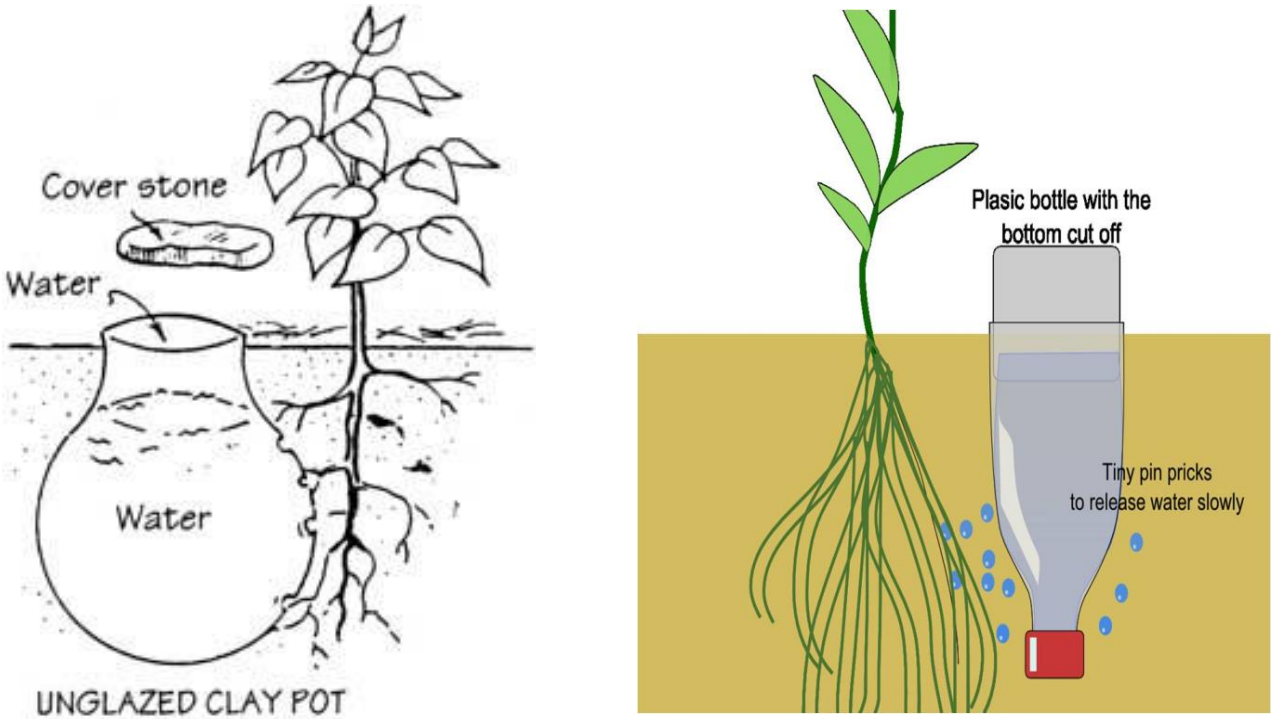

FIGURE 1. The diagram on the left shows a clay pot used for irrigating crops in the olden days and on the right an example of bottle drip irrigation.

2.3 Transplanting

Seedlings were obtained from Vickery Seedlings, a local company that supply ready to be planted seedling located at Malkerns. Basal fertilizer dressing was done using N:P:K; 2:3:2 (22) fertilizer at a rate of 50 g per seedling. The seedlings were planted directly under the emitter in the commercial drip system and 10 cm away from the bottle drip system. Irrigation was done every two days in all the treatments.

2.4 Water Management

Water application was done every two days in all the treatments. In the case of commercial drip system, a gate valve was opened during irrigation for about an hour to allow water to drip to the plants for an equivalent of 8 mm application. The bottle drips were filled with the equivalent of two litres of water for the same purpose.

III. RESULTS AND DISCUSSION

3.1 Rainfall Data

Table 1 shows the amount of rainfall received during the duration of the experiment. There were only four rainy days, all within the month of March. The highest rainfall of 30.5 mm was received in the early part of the experiment on the 12th of March which was immediately after planting (11th March). The lettuce was planted on the 11th March and harvested on the 11th April.

TABLE 1
THE AMOUNT OF RAINFALL RECEIVED DURING THE DURATION OF THE EXPERIMENT

Date of Rainfall	Rainfall Amount (mm)
12-Mar-12	30.5
16-Mar-12	14.5
23-Mar-12	8.5
30-Mar-12	7.5
Total	**61.0**

From the 20th of March to the time of harvesting, the contribution of rainfall to the growth of the lettuce was negligible meaning that the conditions were ideal for irrigation.

3.2 Yield and growth parameters

Results of yield and growth parameters (plant height, leaf area index, fresh mass, root length) are summarised in table 2 below.

TABLE 2
YIELD AND GROWTH PARAMETERS FOR THE LETTUCE EXPERIMENT

Treatment	plant height (cm)	leaf area index (LAI)	fresh lettuce mass (g)	root length (cm)
Control	14.2	23.3	165.3	15.93
Bottle drip	15.0	26.1	184.8	14.78
Commercial drip	16.1	30.9	226.8	11.80
Significance	**NS**	**NS**	****	****

Values showing ** stand for significant differences at P < 0.01 probability level, whereas NS represents a non-significant value.

The yield and growth parameter results show that there were no significant differences in plant height and leaf area index obtained between the treatments. There were highly significant differences ($P < 0.01$) in the results for fresh lettuce mass and root length. The commercial drip treatment had the largest mass followed by the bottle drip treatment, with the watering can treatment (control) having the lowest mass.

Root length measurements shows that on average the watering can treatment (control) and the bottle drip had significantly ($P < 0.001$) longer roots compared to the commercial drip treatment.

IV. CONCLUSION

It was concluded that the home-made bottle drip irrigation method could be recommended for rural people in Eswatini who cannot afford to buy the commercial drip system for the production of vegetables for household consumption.

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Assessing the availability of community water at Madlangamphisi, a community in the Hhohho region of Eswatini

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Abstract— *The research was conducted to assess the availability of domestic water and the extent of the problems associated with water scarcity at Madlangamphisi area in the Hhohho district of Eswatini. The research was a descriptive survey. A questionnaire was used to collect both qualitative and quantitative data for the survey. A total of 169 households out of 300 households in the community were randomly selected to participate in the survey. The majority (56.2%) of the households confirmed that there was water scarcity problems in the area as the streams they used for domestic water frequently dried up during the winter months. The study showed that a majority, 51.5% used water from rivers as the main source of domestic water, while 40.2% of the people travelled for more than 1,000 m to fetch water. To cope with water scarcity problems, 43.2% of the households reduced their water consumption level during droughts while 45% practiced rooftop rainwater harvesting. The study concluded that Madlangamphisi community experienced serious water scarcity problems since they relied on unprotected water sources for domestic use. Moreover, they had to travel for more than 200 m to collect water from nearest sources which is considered an indication of water scarcity by the WHO. The study observed that there was a need to introduce a rural water supply scheme in the area to solve the water scarcity problems and that households should treat water for drinking by either boiling or use a disinfectant to eliminate pathogenic organisms in the water.*

Keywords— Community, water, rural schemes.

I. INTRODUCTION

Water is a valuable life commodity that supports numerous ecosystems. It is however becoming a scarce resource in most parts of the world, partly due to global warming which results to drought conditions and mismanagement by humans (Srinivasan, *et.al.,* 2012). Eswatini is one of the countries that have an average number of water sources which includes dams, rivers, groundwater, wetlands, springs and streams (Manyatsi and Brown, 2009) to name a few, yet the supply of water is insufficient. The reoccurrence of droughts contributes to the problem of water scarcity. Drought is the temporary decrease of the average water availability. It refers to important deviations from the average levels of natural water availability and is considered a natural phenomenon. It is a result of deficiency in precipitation due to different natural causes that includes global climatic variability and high pressure resulting in lower relative humidity and less precipitation (European Commission, 2007).

Drought is divided into four different categories which are; meteorological, agricultural, hydrological and socio-economic drought (Bond and Lake, 2008). A meteorological drought is an extended period, a season, a year or several years of deficient rainfall relative to the statistical multi-year mean for a region. Hydrological drought is the effects of precipitation shortfalls on stream flows, reservoirs, lakes and groundwater levels. Socio-economic drought describes the effects of demands for water exceeding the supply as a result of a water-related supply shortfall. Agricultural drought is the deficiency of soil moisture relative to plant life usually crops. Once a meteorological drought sets in both agricultural and hydrological droughts may follow (FAO, 2012). Eswatini just like other countries is vulnerable to climatic variability, which manifests itself in a number of hydrological disasters including change in the rainfall regimes as well as extreme weather conditions such as drought (Manyatsi *et.al*, 2010).

Over the years Eswatini has been affected mostly by the meteorological drought (Government of Swaziland, 2008). Examples of droughts that have affected Eswatini in the past include the 1983 drought, 1991/1992, 2001/2002, 2005/2007 and the most recent being the 2014/2015 drought whose effects are still being felt in many rural communities (NDMA, 2015). As droughts reduce the amount of rainfall, this has a negative impact on domestic water availability in particular in

many rural areas. The rural areas are the most affected since a large section of its population has no access to adequate portable water supply.

The clean water supply coverage in the form of taps in houses, taps outside houses, community taps and boreholes stood at 33% for the rural population and 84% for the urban population (Manyatsi and Brown, 2009).Rivers and unprotected wells were cited as the main sources of household water for the rural population, with 45% relying on them. Even though these water sources are available their accessibility may differ for each household in the area. According to Ure (2011) nearly a billion people worldwide have limited access to clean water. In developing countries people walk an average of 6 kilometres a day just to collect water.

Madlangamphisi is one of the areas that receive the lowest amount of rainfall in the country. It fluctuates from an average of 600mm-700mm under normal conditions to a low of 500mm-400mm during drought periods (Manyatsi and Brown, 2009). This study reports on the availability, the sources and quality of water in the area and the adaptation strategies implemented by the community during drought periods.

II. MATERIALS AND METHODS

Madlangamphisi is an area in the Hhohho district of Eswatini located at 26°05′22.70″S and 31°32′59.52″E at an elevation of 397 m above sea level. It is a community of about 300 households. A questionnaire was administered to members of the community to help in the collection of both qualitative and quantitative data. A total of 169 households were selected with 95% confidence level and 5% margin of error.

The daily water consumption for the household was estimated using the following equation.

$$Daily\ Water\ Consumption = \frac{Total\ \ amount\ \ used\ \ in\ household\ per\ \ day}{Number\ \ of\ \ people\ \ in\ the\ household} \quad (1)$$

III. RESULTS AND DISCUSSIONS

3.1 Demographic information

The number of people per household (table 1) were categorised into four groups: 1 to 3 people, 4 to 7 people, 8 to 10 people and 11 to 14 people. A majority of the households 46.2% had 4 to 7 family members. This showed a high population density, and this indicated that the area had a high domestic water demand. According to Jaeger et al.,(2012) when the population is high water scarcity arises as the demand grows beyond the available supply and is mainly limited by physical availability of water. The number of people per household was used to calculate the daily water requirements of each of the households.

TABLE 1
THE NUMBER, FREQUENCY AND PERCENTAGE OF PEOPLE PER HOUSEHOLD IN THE SAMPLE SURVEYED (n=169)

Number of people per household	Frequency	Percent
1-3	52	30.8
4-7	78	46.2
8-10	29	17.2
11-14	10	5.9
Total	169	100.0

Figure 1 below is a presentation of the length of stay the households has in Madlangamphisi area. A majority (74%) of the households had been staying in the area for more than ten years, and only less than three percent had been in the area for more than 50 years. This shows that the information provided about the status of water availability or scarcity was reliable, as the people are well versed and have experienced on all water related issues in the area.

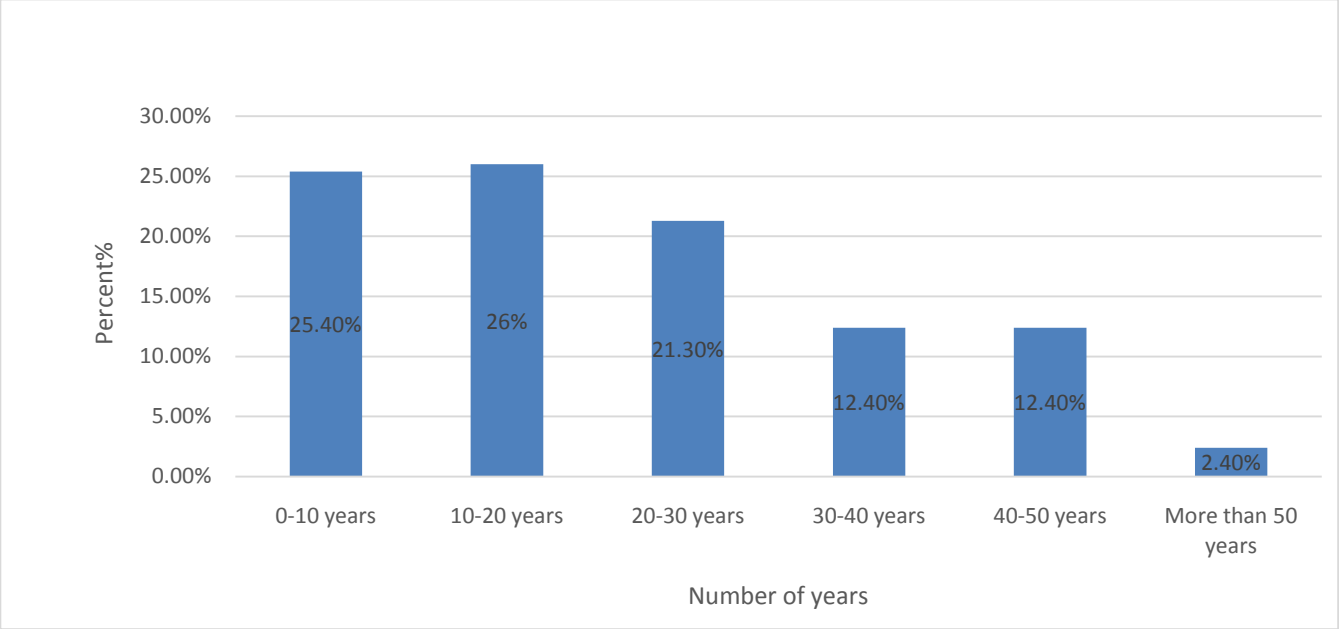

FIGURE 1: Residence time of the households in Madlangamphisi community

3.2 Dominant sources of domestic water for households

Table 2 below shows the various sources of domestic water at Madlangamphisi. The majority 51.5% of the households in the community used the Nkomazi River as their main source of water for domestic use; while 24.9% of the households use a community borehole and 18.3% use harvested rainwater and only 5.3% use seasonal streams. The Nkomazi river and community boreholes were found to be the main water sources. The Nkomazi River was preferred by most of the households since it does not dry up even during dry seasons; hence it is a reliable source of water. Other minor alternative sources of water included buying water from shops specifically used for drinking since it was bought in small quantities.

TABLE 2
DOMINANT SOURCES OF DOMESTIC WATER USED BY MADLANGAMPHISI HOUSEHOLDS (n=169).

Water sources	Frequency	Percent
Borehole	42	24.9
River	87	51.5
Seasonal streams	9	5.3
Rainwater	31	18.3
Total	**169**	**100.0**

Although some of the households used harvested rainwater and streams, they still depended on the community borehole and the Nkomazi River since they could not depend on the unreliable rainfall. The results showed that a majority (56.8%) of the households relied on unprotected water sources, as their main water sources are river and streams. Water from these sources particularly the river and seasonal streams was exposed to contamination owing to the fact that surface water sources were prone to being polluted. This means that the households were more vulnerable to infection by waterborne diseases.

3.3 Accessibility of water sources

Table 3 shows the distance walked by the households to the water source. The distances from the households to the main water sources were divided into seven categories: within homestead yard, outside homestead yard less than 50 m, 50 m - 100 m, 100 m - 200 m, 300 m - 400 m, 500 m - 1000 m and more than 1000 m. The results showed that 40.2% of the households were located more than 1000 m away from the main water source and these were mainly the households that sourced their

water from the Nkomazi River. Furthermore, 17.2% of the households were located 500 m -1000 m away from the main water source. Some of these households collected water from the river, boreholes and seasonal streams. Only 11.2% of the households were located 300 m - 400 m away from their main water source while 8.9% were 100 m - 200 m away from water source, and 1.2% of the households walked for 50 m - 100 m to the main water source.

TABLE 3
THE DISTANCE WALKED BY HOUSEHOLDS TO WATER SOURCE (n=169).

Distance	Frequency	Percent
Within homestead yard	31	18.3
Outside homestead yard, less than 50m	5	3
50m - 100m	2	1.2
100m - 200m	15	8.9
300 m - 400m	19	11.2
500m - 1000m	29	17.2
More than 1000m	68	40.2
Total	169	100.0

This means, only 3% of the households had their main water source outside the homestead yard which was less than 50 m. However, 18.3 % of the households had their water sources within the homestead yard. These were the households that depended on rainfall water as a main water source, which requires collection and storage within the homestead yard. This mean only 31.4% of the community had accessible water sources, based on the 200 m walking distance stipulated by WHO as the measure of water accessibility.

3.4 Time spent collecting water

Table 4 shows the time spent by the Madlangamphisi community when collecting water. The results show that 50.9% of the households walked for more than one hour to fetch water. According to the WHO guidelines, people that walk for more than 20 minutes to fetch water were faced with water scarcity. In the community 76.9% of the households had water scarcity problems as they walked for more than 20 minutes to collect water.

TABLE 4
THE TIME SPENT BY THE MADLANGAMPHISI COMMUNITY WHEN COLLECTING WATER (n=169).

Time spent collecting water	Frequency	Percent
Less than 20 minutes	39	23.1
Between 20 and 30 minutes	21	12.4
Between 30 and 60 minutes	23	13.6
More than 60 minutes	86	50.9
Total	169	100.0

3.5 The means of collecting water commonly used at Madlangamphisi

Table 5 shows the means of collecting water from the various sources used by the Madlangamphisi community. The study found that 6.5% of the household used vans to collect water from the river since it was too far to walk while 43.8% used tractors. These were mostly the households that would walk for more than 30 minutes to collect water. However, 49.7% of the households walked to the water source since they had no better means of collecting water. The distance travelled to collect water seems to have an effect on the amount of water collected (Table 7), the method of collection and the type of

container used for collecting the water (table 6). The longer the distance travelled to collect water, tractors and vans are used as means of collecting the water.

TABLE 5
MEANS OF COLLECTING WATER FROM THE VARIOUS SOURCES USED BY THE MADLANGAMPHISI HOUSEHOLDS (n=169).

Means of collecting water	Frequency	Percent
Van	11	6.5
Tractor	74	43.8
Walking	84	49.7
Total	169	100.0

Only 34.9% of the households collected their water once a month this was because they hired tractors to collect the water and fill a 5,000 litres tank. The households were then able to use this water for cooking, drinking, cleaning, bathing and even for the laundry. The frequency of water collection depended on the distance travelled to collect water and the number of people using the water on a daily basis.

Households within the same homestead mostly shared the water from the 5,000 litres tanks which made water collection more frequent. The 31.9% of the households that fetched water on a daily basis were the households using seasonal streams and boreholes as their main water sources because they travelled less than 1,000m to fetch the water. Moreover 6.6% of the households fetched their water on a weekly basis. These were the households that used vans as their means of collecting water. Lastly 18.3% of the households collected rainwater and stored it in tanks when it rained. The frequency of water collection indicated that 34.9% of the households used water that was stored within their homesteads. This meant that they did not get fresh supply of water on a daily basis. This showed that the water sources used were not accessible to the residents, thus requiring the households to store the water within their homestead yards for easy access.

3.6 Water collection and storage facilities commonly used by Madlangamphisi households

The majority 59.2% of the households used tanks to collect and store water. The rest of the households 40.8% used from 5 to 200litres containers to collect and store water. This reduced the risk of using contaminated water since the water in these containers was in small quantities and was mostly used up in a day. According to Chakraborty (2017) households that store water within the household are faced with the problem of water scarcity and there is high risk of the water becoming contaminated. The contamination is caused by the biological reaction of the water due to temperature changes and growth of microbes since the water is stagnant, therefore these families are at high risk of falling sick due to storing of untreated water for long periods.

TABLE 6
THE TYPES OF CONTAINERS USED BY THE COMMUNITY PEOPLE TO COLLECT WATER (n=169).

Type of containers used	Frequency	Percent
5 liter containers	1	.6
20 liter container	43	25.4
25 liter container	23	13.6
200 liter container	2	1.2
Tanks	100	59.2
Total	169	100.0

3.7 Level of domestic water sufficiency

The table indicates the amount of water collected and used by each person in the household per day. This water use included cooking, bathing, drinking and cleaning. The average water consumption/capita/day was categorised into 3 groups: less than 20 litres, 20 litres to 30 litres and 30 litres to 40 litres. The table shows that a majority 59.8% of households, used less than 20 litres of water per capita per day. This was proof that these households were faced with water scarcity problem.

TABLE 7
AMOUNT OF WATER USED PER DAY (n=169).

Water collection and use		Frequency	Percent
Amount of water collected	20 litres	4	2.4
	40 litres	41	24.3
	60 litres	55	32.5
	More than 100 litres	22	13.0
	50 litres	19	11.2
	80 litres	28	16.6
	Total	169	100.0
Water consumption/capita/day	Less than 20 litres	101	59.8
	20 litres - 30 litres	59	34.9
	30 litres - 40 litres	9	5.3
	Total	169	100.0

According to WHO, each person should at least use 30 litres of water per day for good health and cleanliness, only 5.3% of the households used from 30 litres to 40 litres of water per capita per day. These showed that 94.7% of the people are at risk of falling sick as a result of poor hygiene and sanitation, caused by inaccessible water sources.

3.8 Challenges of water scarcity

The study revealed that due to hydrological drought conditions the levels of the main water sources were reduced (table 8). A majority, 56.2% of the households agreed that hydrological droughts were experienced annually in both the dry and wet seasons. This was because the area normally receives a low amount of rainfall making it hard to provide the households with sufficient domestic water. However, 43.8% indicated that the hydrological drought only occurred in the winter season when there was no rainfall; these were probably the households who depended on seasonal streams as their main water source.

TABLE 8
PERCEPTION OF HOUSEHOLDS ON THE OCCURRENCE OF HYDROLOGICAL DROUGHT (n=169).

Water shortages		Frequency	Percent
Frequency of hydrological drought	During the winter season	74	43.8
	Every year in both dry and wet seasons	95	56.2
	Total	169	100.0
Does water source dry up	Yes	54	32.0
	No	115	68.0
	Total	169	100.0

Furthermore, 32% of the households alleged that their water sources were unreliable since they often totally dried up. These are the households using seasonal streams and rainwater as their main water sources. The results indicate that the community experienced water shortages, mostly in the dry seasons when there is no rainfall due to drying up of the main water sources; hence residents were faced with water scarcity.

3.9 Other challenges

There is many other problems people face when there is water shortage in a community. These were separated into four categories (table 9), 1) decrease in water consumption level, 2) travelling long distances to collect water, 3) using untreated water and 4) the outbreak of waterborne diseases. A majority of the households 43.2% are forced to reduce their water consumption level, making them to use less than 30litres/capita/day. 20.7% have to travel long distances to fetch water, this are the households that hire tractors or use vans to collect water from water sources. The use of untreated water stands at 28.4% and it is the main cause of waterborne disease outbreak which is at 7.7% as the water may contain contaminants.

TABLE 9
CHALLENGES FACED BY THE COMMUNITY DUE TO WATER SCARCITY (n=169).

Problems of water scarcity	Frequency	Percent
Decrease in water consumption level	73	43.2
Travelling long distances to get water	35	20.7
Using untreated water	48	28.4
Outbreak of water borne diseases	13	7.7
Total	169	100.0

3.10 Sharing of water sources

A large number of the households 55.6% used water sources that were shared with other communities (table 10). According to Eckstein (2009) the explosion of population in developing nations within Africa combined with climate change is causing strain within and between nations. As a result of the strains there are conflicts that may spark within a community and between communities. Even though some of the water sources were shared, the sharing of the water sources had not led to conflicts in the past to the present date at Madlamngamphisi. This however, may not be guaranteed to the future.

TABLE 10
PROBLEMS CAUSED BY SHARING WATER SOURCES (n=169).

Sharing of water sources		Frequency	Percent
Is water source shared	Yes	94	55.6
	No	75	44.4
	Total	169	100.0
Does the sharing cause conflicts	No	169	100.0

3.11 Strategies used to cope with water scarcity problem

The strategies that were used to cope with water scarcity problems are summarized in table 11 and included: 1) water recycling, 2) rooftop water harvesting 3) buying water from shops, 4) water rationing and 5) the construction and rehabilitation of boreholes. Only 45% of the households practiced rooftop water harvesting. They argued that dust contaminated the water thus treatment measures were needed to make the water safe for domestic use. Water rationing was only done by 36.1% of the households and the limitation of water rationing and recycling was failure to maintain pumps. Another strategy included the construction and rehabilitation of boreholes and 11.8% of the households considered this. However, its limitations included the absence of expertise to implement. The bore holes were sometimes vandalised making it expensive to maintain. Furthermore, 4.1% of the households alleged that they bought water from shops this water was used for drinking since the water from their main water source contained sediments making it unsafe for drinking. Water recycling was used by 3% of households by reusing water used for washing dishes to clean.

TABLE 11
STRATEGIES USED TO COPE WITH WATER SCARCITY (n=169).

Strategies to cope with water scarcity		Frequency	Percent
Strategies used	Water recycling	5	3
	Rooftop water harvesting	76	45.0
	Buying water from shops	7	4.1
	Water rationing	61	36.1
	Construction and rehabilitation of boreholes	20	11.8
	Total	169	100.0

3.12 Materials used for rooftop water harvesting

The households were asked on the materials used to harvest rainwater (figure 1), 66.9% of the population use iron sheets, tanks and gutters to harvest water. These were mostly the households that used river and rainwater as their main water source, because they already own tanks which they used for storing the water.About17.8% use iron sheets and 210litre oil drums and 15.4% use iron sheets and buckets. The households that use 210litre oil drums and buckets are mostly the households that use boreholes since they have no plastic tanks. A majority uses plastic tanks and irons sheets, because the people wanted to harvest and store more water.

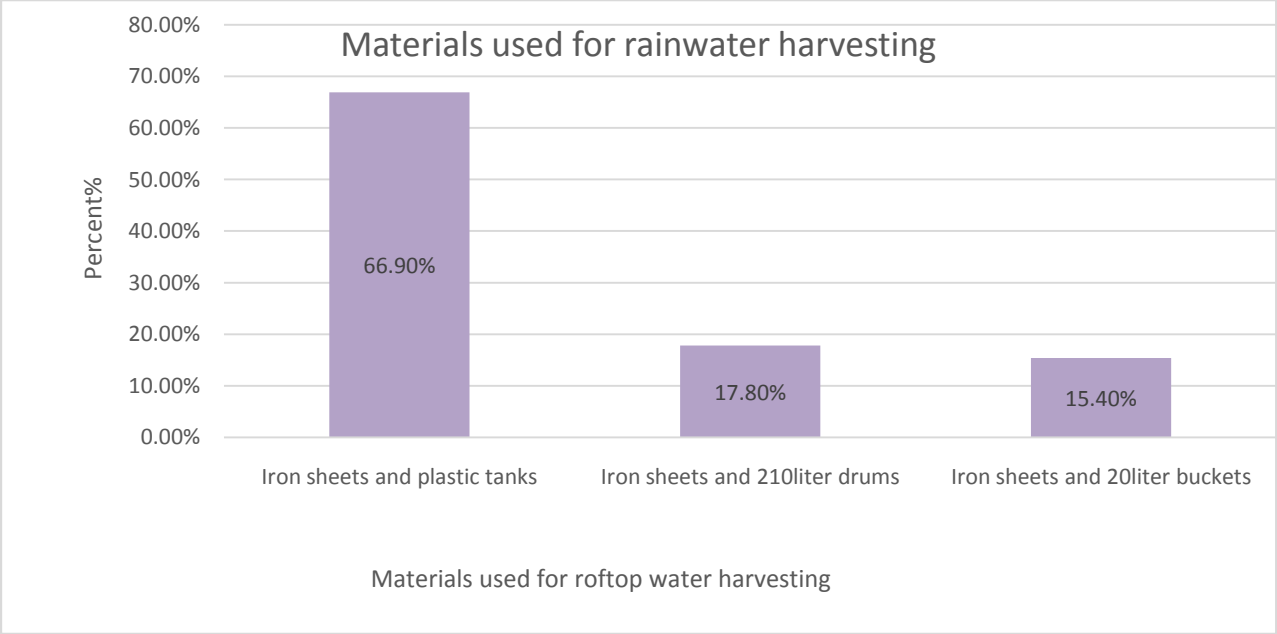

FIGURE 2: Materials used to harvest rainwater

3.13 Water rationing and the levels at which it is implemented

The households were asked as to whether they practice any form of water rationing (table 12). Water rationing defined as limiting the amount of water use in the household due to concerns of scarce water supply. It is practiced at household and community level. Only 36.1% of households practiced water rationing at household level. It was done by allowing each household to fetch a specified water amount per day. However, 63.9% of the households did not comply with water rationing practice. The households alleged that water rationing would cause a reduction in their water supply which would result to poor hygiene. The failure to comply with water rationing by the majority of the households could further exacerbate the water scarcity problem.

TABLE 12
LEVEL OF WATER RATIONING AND HOW IT IS IMPLEMENTED (n=169).

Water rationing		Frequency	Percent
Level at which rationing is done	Homestead level	61	36.1
	Not done	108	63.9
	Total	169	100.0
How rationing is done	Allowing each household to collect specified water amount per day	61	36.1
	None	108	63.9
	Total	169	100.0

IV. CONCLUSION

The results of the study show that there is a water scarcity problem at Mandlangamphisi. The only available reliable source of safe drinking water was the borehole which supports 24.9% of the households. The prevalence of hydrological droughts results in the drying up of several water sources, forcing the majority 56.8% of the households to rely on unprotected water sources which included the Nkomazi River and other small seasonal streams. These water sources are unsafe for domestic use without treatment. Most of the water sources were inaccessible as the majority 68.6% of the households travelled for more than 200 m to collect water, with 76.9% of these spending more than 20 minutes. The WHO, affirms that persons who travel for more than 200 m and spend more than 20 minutes to collect water are facing water scarcity. The majority, 65.1% of the households used less than 30 litres/person/day of water a further indication of water scarcity.

The strategies used to cope with the water scarcity problem in the area were found to include rooftop water harvesting, water rationing and purchasing bottled water for drinking. A majority of which are unsafe without treatment.

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Extraction and Evaluation of Chitosan Enhanced by *Lippia Multiflora* Oil Essential on Postharvest of Tomato

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***Abstract*—** *Influence of chitosan and Lippia multiflora (Lm) essential oil used singly or combined was studied on postharvest tomato. Chitosan with 89.31% of DDA and solubility in acetic acid at 97.15 % was extracted from shrimp exoskeletons. Three concentrations of chitosan extracted (0.25; 0.5 and 1%) containing or not L. multiflora oil were used on Rhizopus stolonifer growth in vitro and in situ condition. In vitro condition, antifungal activity of the chitosan and Lm oil against R. stolonifer was conducted on agar media inoculated with fungal spores. Coating containing 1% chitosan incorporated with Lm efficiently inhibited fungal proliferation at 100% after 10 days. The antifungal effect of two molecules was effective when they were associated. In situ condition tomatos were coated with different solution. Antifungal effect and chemical parameters (pH and titrable acidity) were evaluated. Combination of Lm and 1% chitosan delayed efficiently R. stolonifer radial growth (2.1 mm) compared to uncoated fruit (70.37 mm) after 10 days of storage. Chitosan at 1% with or not Lm significantly reduced weight loss. Though, pH and total acidity (TA) were not influenced by coating solution.*

***Keywords*— Chitosan, Lippia multiflora, essential oil, antifungal, Rhizopus stolonifer, tomato.**

I. INTRODUCTION

Tomato (*Lycopersicon esculentum)* is the one of most popular consumed vegetables in Côte d'Ivoire because it is use in the composition of many dishes. However, due to its high-water content, intrinsically is likely to deteriorate rapidly during the postharvest handling. Rot disease caused by *Rhizopus stolonifer* is the most destructive disease of tomato [1, 2]. *R. stolonifer* is a good colonizer of plant debris and infects harvest fruits, often destroying the entire contents of a box within a few days by hydrolysis with tissue-macerating ability [3]. Over the past years, synthetic fungicides have been used to control this microorganism. However, it has been shown that some compounds used in these fungicides have caused strain resistance, representing a potential risk for the environment and human health [4]. Thus, there is a worldwide trend to explore natural products in order to reduce the use of synthetic fungicides, and options such as chitosan and plant extract have been evaluated.

Chitosan is a natural nontoxic biopolymer derived from partial or total deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crawfish. In recent years, applications of chitosan to the fields of agriculture have received considerable attention [5-9]. The antifungal effect of chitosan has been observed against several fungi and its activity depends on its deacetylation degree, molecular weight and concentration [10-13]. Chitosan coating maintained the physico-chemical properties of fruits during conservation [14, 15]. By cons, chitosan is not a fungicide but rather a fungistatic [10]. Its effectiveness against fungi can be improved by adding natural antimicrobial substances vegetable. Essential oil of *Lippia. multiflora* can be incorporated in chitosan solution in order to strengthen the coating formulation. Indeed, essential oil of *L. multiflora* has been reported to exhibit a fungicidal [16, 17], a bactericidal and an insecticidal activity [18]. It has also been used to protect many fruits against fungi [19].

Use of *L. multiflora* to strengthen chitosan action against *Rhizopus stolonifer* can be an alternative way to inhibit this strains development and reduce the chemical substances use in food preservation. The purpose of the present work was designed to evaluate the effect of chitosan and *L. multiflora* singly or incombined treatments on the growth of tomato rot pathogens as well as their effect on physicochemical quality of tomato during its postharvest conservation.

II. MATERIALS AND METHODS

2.1 Extraction of Essential oil (EO) of *Lippia multiflora*

Leaves of *Lippia multiflora* were collected in Dikodougou northern of Côte d'ivoire. Leaves were dried for 7 days protected away from the sun. After drying, 10 kg of leaves were used for the extraction of essential oil by steam distillation using a

hydro-distillation. The extraction lasted 3 hours. After extraction, the volume of EO was stored in hermetically sealed glass bottle with screw lid cover under refrigeration at 4°C.

2.2 Extraction of chitosan

The shrimp were obtained from Azaguiéon center of Côte d'ivoire. Samples were washed with distilled water before oven-dried for 24h at 40°C. Shrimps exoskeletons were then crushed using a grinder. For extraction of chitin and chitosan, the conventional chemical method was followed. Extraction was done following three major steps, i.e., demineralization, deproteination, and deacetylation.

2.2.1 Demineralization

Dry powdered carapaces were soaked in HCl (1N) for 5h with magnetic stirring to remove the minerals (mainly calciumcarbonate). The ratio of solid to solvent is 1:10 (w / v).The product obtained is washed with water distilled several times at pH neutral, then oven-dried at 35°C overnight.

2.2.2 Deproteinization

Proteins were removed by a basic treatment with sodium hydroxide. Product obtained after demineralization was treated with NaOH (2.5 N) at a ratio of 1: 10 (w / v) for 3 h at 100 °C. The mixture was then filtered and washed several times until neutrality. The chitin thus obtained is dried in an oven at 35 °C for 24 hours.

2.2.3 Deacetylation

The deacetylation process was carried out by adding 60% NaOH to sample according to a ratio of 1:10(W / V)and then boiled at 100°C for 2 h on a hot plate. The samples were then placed under the hood and cooled for 30 min at room temperature. Afterwards the samples were washed continuously with the 60% NaOH and filtered in order to retain the solid matter, which is the chitosan. The produced chitosan was then filtered and washed to remove residual soda until the pH of the wash water reaches neutrality and then baked at 35°C overnight.

2.3 Properties of Chitosan

2.3.1 Degree of deacetylation (DD)

It refers to the removal of acetyl group from the chain which is determined by potentiometric titration (Homogenous solution of chitosan was prepared using diluted HCl (0.010 mol/L) which was titrated against 0.1M NaOH (w/v). The end point is determined by the inflections of the pH values. Two inflections were mainly considered out of which first one corresponds to neutralization of HCl and second one neutralization of ammonium ions from chitosan. The difference between two points gives the amount of amino groups in the chitosan it was also referred as degree of deacetylation [20].

$$DD(\%) = 100 - \mathrm{DA}(\%)$$

DD represents Degree of Deacetylation and DA degree of Acetylation.

The **pH** measurement of chitosan solutions was carried out using pH meter with microprocessor

2.3.2 Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass loss was determined by drying the sample to constant weight and measuring the sample before and after drying. The water mass was the difference between the weights of the wet and oven dry samples ([21]. Loss on drying (%) = $((wet\ weight - dry\ weight)/\ dry\ weight)$ 100.

2.3.3 The solubility of chitosan

The solubility of chitosan extracted was determined according to Premasudha[21]. About 0.1g of chitosan powder sample was taken in centrifuge tube and dissolved in 10ml of 1% acetic acid and kept in incubated shaker (250 rpm) at 25ºC for 30 minutes. The solution was immersed in boiling water bath for 15 minutes and cooled to room temperature followed by centrifuge at 12,000 rpm for 7 minutes and the supernatant was discarded. The undissolved particles were thoroughly washed using distilled water by centrifuging the contents at 10,000rpm for 10 minutes and the supernatant was discarded. The

undissolved pellets were dried at 70ºC for 24hours. At the end the dried particles were weighed and the solubility percentage was calculated as:

Solubility (%) = (initial weight of tube + chitosan) - (final weight of tube + chitosan) X 100

2.4 Preparation of the chitosan-Essential oil emulsion

Chitosan solutions were prepared by dissolving chitosan(0.25, 0.5 and 1g) in distilled water (80 mL) containing of acetic acidat 1% (w/v) under agitation using a magnetic stirrer, incubated for 5h at room temperature. The pH of the solution was adjusted to 5.5 with NaOH (2%) and the solution was made up to 100mL with distilled water *L. multiflora* oil (at 0% and 0.5%) mixed with Tween 80(0.2%) was added to the different chitosan solutions. The mixture was homogenized using a mixer for 5 minutes to have an emulsifying solution. Solution with oils (Lm) in water were prepared and homogenized under the same conditions.

2.5 *In vitro* antifungal assay

3 uL of the inoculum of *R. stolonifer* containing 10^5 spores/mL were dropped at the center of Petri plates (9 cm diameter) containing PDA with different solution coating at $1mg \cdot mL^{-1}$. Plates were then incubated at 30°C and linear growth of tested fungi was measured when the control plates (PDA with distilled water) reached full growth and the average growth diameter was calculated. Each treatment was represented by 3 plates as replicates.

The fungicide index (%) was obtained by the formula:

Fungistatic index (%) = (1- (DS/DC)) x100, where Ds is the diameter of the growth zone in the test plates and Dc is the diameter of growth zone in the control plate.

2.6 *In situ* antifungal assay

Tomato fruit were collected from a regional market in Korhogo (Côte d'ivoire). Fruit were selected based on size and absence of physical injuries or disease infection. Fruit were disinfected with 1% (w/v) sodium hypochlorite for5 min then rinsed with distilled water and air-dried. The fruit were randomly distributed (10 per treatment). Identical lesions (4) were performed on the fruit with sterile nails before dipping individually in different coating solutions for 1 min and air-dried. The fruit were then sprayed with spore solutions of *R. stolonifer* (10^5 spores $/mL^{-1}$). Fruit were kept at room temperature. The mycelial growth was measured at 5 and 10 days after inoculation. Each treatment contained three replicates with 10 fruits per replicate and the experiment was repeated twice

2.7 Evaluation of the quality of Tomato

Weight loss was determined by daily weighing tomato with a balance (Precisa, Switzerland). Weight loss was expressed as a percentage of initial weight.

To determine chemical properties, thirty (30) grams of mango pulp were homogenized in 150 mL of distilled water using a blender for 2 min and then filtered. The pH was determined with a pH meter. Total acidity (TA) was determined on 10mL of homogenate pulp by automatic titration with 0.1N NaOH up to pH 8.1. The results were expressed as g citric acid equivalent per 100 g fresh weight.

III. STATISTICAL ANALYSIS

Experimental data were subjected to ANOVA analysis using Statistica 7. The overall least significant differences (Student's procedure, $p<0.05$) were calculated and used to detect significant differences among treatments. Each trial contained three replicates.

IV. RESULTS AND DISCUSSION

4.1 Properties of Chitosan

The properties of chitosan obtained from shrimp is showed in table1. The degree of deacetylation (DD) was 85.31%. DD of chitosan range from 30% to 95% [22]. It depends on the source of chitin, concentration of acid and alkaline used, time and temperature, etc but the concentration of NaOH influences the DD values the most [23]. The DD consider being an important parameter for the identification of chitosan stated that DD analysis was affected the type of analytical methods employed,

type of instruments used and the preparation of sample. DD denoted the removal of acetyl group from the long chain of chitin and it plays a substantial role in deciding the precise application of chitosan. It is an important parameter to be considered for physical and chemical properties of chitosan including solubility, adsorption, chemical reactivity covalent linking encapsulation and biodegradability.

TABLE 1
PROPERTIES OF CHITOSAN EXTRACTED FROM SHRIMP

Characteristic	Value
Degree of deacetylation (%)	85.31
pH	7.4
Loss on drying (%)	9.25
Solubility in acetic acid (%)	97.15

pH measured was 7.4. This value was in line with the earlier report of Premasudha [21] who reported the pH of chitosan obtained from shrimp. The pH value of chitosan plays a major role in functional properties of chitosan including antimicrobial, cytotoxicity and also indirectly influences the hydrophilicity and deacetylation ratio [21].

Present study reveals that, loss of moisture content (dry weight**)** in studied chitosan of shrimp was 9.25% of total weight. This result is near with the reports of Sneba et al.,[24], who explained the acceptable moisture content of chitosan powder should be <10% for commercial applications.

The results of chitosan solubility shown in Table 1 (97.15%) clearly reveals the high solubility nature of chitosan in 1% acetic acid aqueous solution. The solubility of chitosan is one of the important parameters for quality of chitosan, where higher solubility will produce a better chitosan. There are several critical factors affecting chitosan solubility including temperature and time of deacetylation, alkali concentration and prior treatments applied to chitin isolation, ratio of chitin to alkali solution and particle size [21]. The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility [25]. Proportionally increase in solubility was observed with increasing deacetylation degree. Brine and Austin, [26] suggested that the incomplete removal of protein and acetyl group leads to lower solubility. Since solubility of chitosan depends on the removal of acetyl group from chitin, therefore the lower DD value could adversely interfere with the results.

4.2 *In vitro* antifungal assay

The antifungal effects of the different treatments on tomato postharvest are summarized in Table 2. Significant difference between treatments effects on *Rhizopus* inhibition was observed at different days recorded. After 2 days of storage, *R. stolonifer* reacted differently toward coating solution. Chitosan at different concentrations without EO significantly decreased *R. stolonifer* growth. This decrease improved when chitosan concentration improved. *R. stolonifer* inhibition improved to 32.49 at 86.86% when chitosan concentration passed to 0.25 at 1%. The percentage of strain inhibition was improved with the increase of chitosan concentration. Similar results were obtained by other authors [8, 27]. EO of *L. multiflora* alone affected fungal growth. Its controlled *R. stolonifer* at 89.41%. When *L. multiflora* wad added to chitosan coating at different concentration, inhibitory effect improved to reach 100%. The antifungal effect of *L.multiflora* was reported in literature. [16, 18]. After 6 days of storage, only 1% Chi + Lm maintained its efficacy at 100% and this efficacy endured after 10 days when the others treatments decreased or lost their efficacy. Chitosan at 0.5% containing Lm nevertheless allowed to inhibited *R. stolonifer* at 66.67 % after 10 days of storage. In view of these results, we could affirm that chitosan had no fungicide activity against *R. stolonifer*. It had bacteriostatic effect against this fungus. But when chitosan at high concentration was associated with Lm EO, the formulation became effective against *R. stolonifer* during 10 days and more. Fungistatic activity of chitosan has been also demonstrated by several authors. [2829]. Also, the strengthening of the antifungal activity of chitosan by addition of essential oil has been reported [30].

TABLE 2
ANTIFUNGAL ASSAY *IN VITRO*

	Inhibition (%)		
	2 Days	**6 Days**	**10 Days**
0,25%Chi	32,49c	0d	0c
0,5%Chi	60,85b	0d	0c
1%Chi	86,86a	40cb	0c
0,25%Chi+Lm	100a	24,70c	0c
0,5%Chi+Lm	100a	66,67b	66,67b
1%Chi+Lm	100 a	100a	100a
Lm	89.41a	85ab	61b

Values within a column with the same letter are not significantly different (p > 0.05).

4.3 *In situ* antifungal assay

Results (Fig.1) shows that fungi strains reacted differently toward coating solutions. After 5 days of storage, radial growth measured (45.07 mm) on uncoated tomato was significantly high than others treatment. It was followed by chitosan at 0.25% (17.80 mm). On the other land, Lm and chitosan (0.5% and 1%) singly or combined protected effectiveness tomato against *R. stolonifer*. After 10 days, all radial measured on fruits improved. But chitosan at 1% mixing with Lm presented the lowest radial measured (2.1 mm) following by chitosan (at 0.25 and 0.5%) incorporated by Lm. Chitosan and Lm singly were effective to protect tomato against *R. stolonifer*. When they were associated, the combination becomes better than individually effect. As in Antifungal assay *in vitro*, chitosan concentration played an important role in Lm oil fixing and protection of tomato against *R. stolonifera*. The results were illustrated by the figure3. Antifungal activity of coating was better when chitosan improved. Chitosan action on fruit protect against fungi was been demonstrated. Hernández-Lauzardo *et al.*,[34] demonstrated that chitosan at 2 $mg \cdot mL^{-1}$ was effective in reducing the percentage of infection and the severity index on peach, papaya and tomato fruit compared with those of non-treated control. As for essential oils, their effectiveness with or without chitosan has been demonstrated by Sivakumar and Bautista-Banos [35].

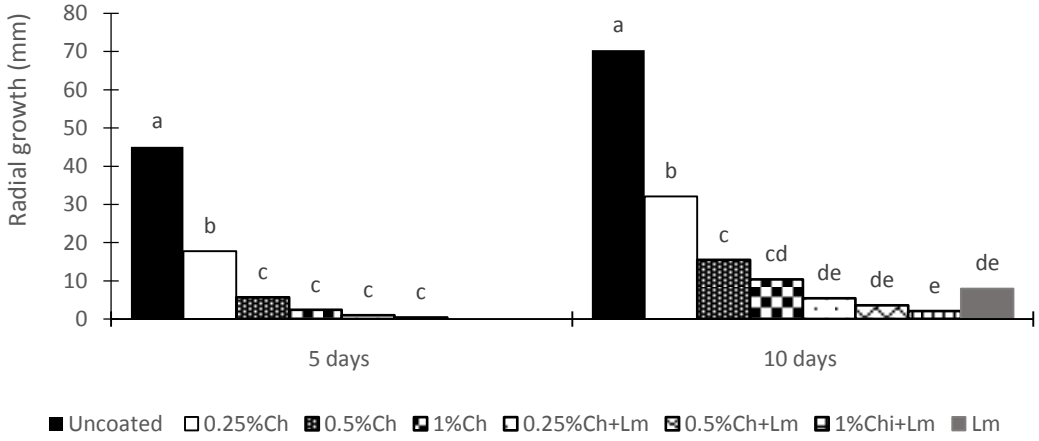

FIGURE 1: Effect of chitosan and Lm essential oil on *R. stolonifer* growth on tomato during storage

4.4 Evaluation of the quality of Tomato

Figure 2 shows weight loss during storage of uncoated tomato compared to coated fruit after 10 days of storage. Loss of weight of uncoated (90.58%) fruit was significantly greater than that of coated fruit. Coated fruits with chitosan presented low weight loss compared to fruits coated with Lm and uncoated. Low lowest was noticed with chitosan at 1% with or not EO. These results highlight a protective action of coating against moisture loss, which has also been reported by several authors [5, 8]. The reduction in water loss can be attributed to an additional barrier against diffusion through the stomata Incorporation of Lm EO into the coating solution did not have any significant effect on weight loss reduction.

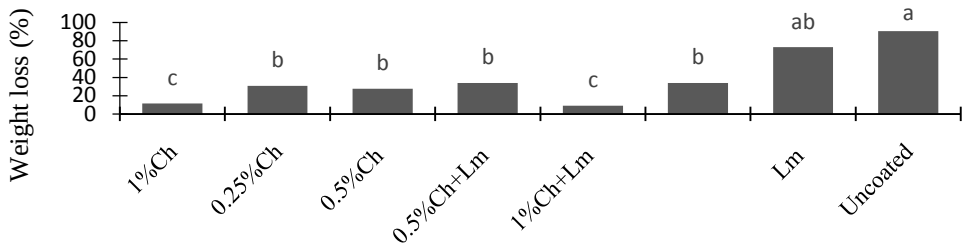

FIGURE 2: Weight loss of fruit during storage

4.5 Chemical composition change in fruit

The chemical composition of the fruit pulp is an important criteria needed for the evaluation of fruit quality. Normally, biochemical changes of tomato during ripening include aincrease of pH and Total acidity (TA). The changes in the chemical composition of tomato after 10 days of storage were studied (Table 3). There was not a significantly different change in pH observed between control fruit and coated fruit with different coating. Though, uncoated and fruit coated with Lm showed lowest pH values (4.46). Regarding TA, its is an important factor to be considered with respect to consumer acceptance. It is expected to increase during ripening [36]. Ours results showed that no significant difference between values of coated and uncoated tomato (Table 3). However fruit uncoated showed lesser change in acidity (0.44%) while 1%Chi + Lm indicated a lowest value (0.37%) after 10 days of storage.

TABLE 3
CHEMICAL COMPOSITION CHANGE IN FRUIT

	Ph	TA (%)
Uncoated	4.45a	0.44a
0,25%Chi	4.39a	0.4a
0,5%Chi	4.40a	0.40a
1%Chi	4. 32a	0.38a
0,25%Chi+Lm	4.38a	0.4a
0,5%Chi+Lm	4.41a	0.39a
1%Chi+Lm	4.30a	0.37a
Lm	4.46a	0.4a

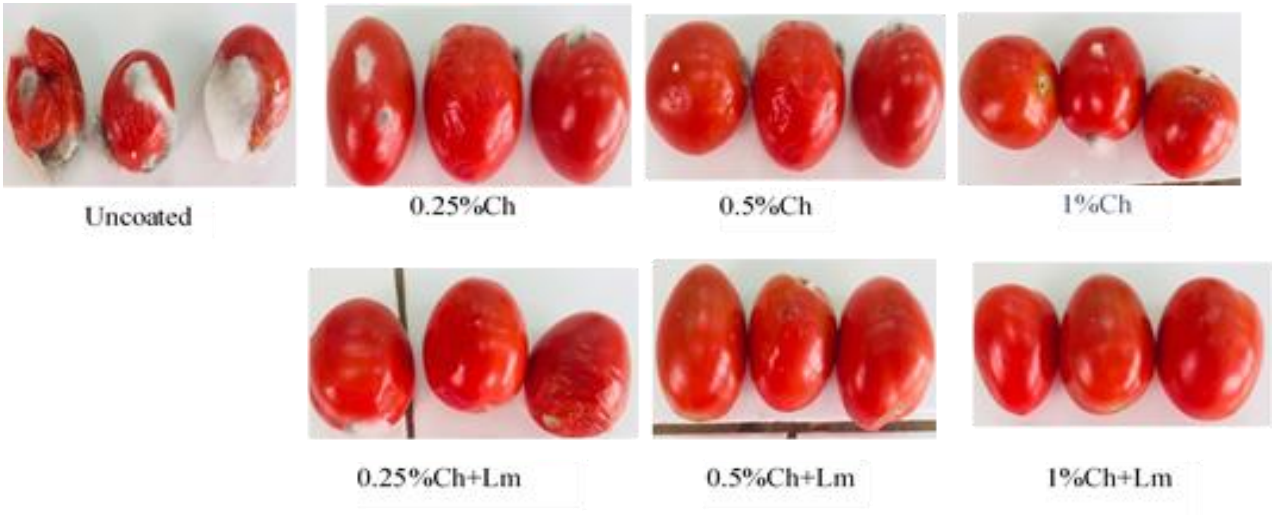

FIGURE 3: Antifungal activity of different coatings against *R. Stolonifer* on Tomato fruit.

V. CONCLUSION

This study demonstrated the effectiveness of chitosan coating containing Lm essential oil in postharvest conservation of tomato. Chitosan and *L. multiflora* essential oil singly used had antifungal activity which was been strengthened by mixing the two. A chitosan concentration at 1% containing *L. multiflora* essential oil was sufficiently effective against *R. stolonifer* contamination without altering fruit quality.Use of chitosan–Lm could thus be an effective approach in the preservation of tropical fruit, an alternative in limiting synthetic pesticide use.

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Biogeochemical Aspects of Manganese Content in *Ilex Paraguayensis* SH from Paraguay by EDXRF and INAA

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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. It has been recognized in Ilex paraguayensis, diuretic, CNS stimulant, hypocholesterolemic, hepatoprotective as well as other pharmacological properties. In regard to its elemental content few studies are known despite they play fundamental tasks in the structure and functioning of plants. One of them, Mn, usually occurs as a trace in Ilex paraguayensis, at relatively high concentration and in this work its concentration in plants from Paraguay have been investigated by EDXRF at Josef Stefan Institute at Ljubljana and at University of Asunción using radioactive isotopic sources and by INAA technique in the Faculty of Chemistry at Asunción with an Am-Be neutron source. These plants are grown mainly in two regions, north and south of Eastern Paraguay; results show that their manganese content can be used as a geochemical indicator to identify the region of origin. Besides, as in other plants, absorption, transport and homeostasis of Mn could be attributed to the action of different NRAMPs. In regard to its normal high content in healthy yerba plants, Mn in excess could be hidden in nodes and vacuoles being exported afterwards*.

Keywords— Ilex paraguayensis, Eastern Paraguay, yerba mate, Mn content, NRAMP, transporter.

I. INTRODUCTION

Mineral constituents and nutrients play fundamental tasks in the structure and functioning of plants, sustaining them and supporting life on our planet. In such a perspective, their role is of remarkable importance for *Ilex paraguayensis* (Saint Hilaire)*, pursuant to its generally mentioned/accepted properties. *Ilex paraguayensis* (Aquifoliaceae), is a small tree of Paraguayan origin called *Ka'a* in Guaraní ** or yerba mate in Spanish and was used as infusion in hot water (mate & mate tea) or as a maceration in cold water (tereré) by the ancient/natives inhabitants of Paraguay as a "reviver"/energy beverage and mineral supplier whose consumption continues to these days especially in Paraguay, Argentina, Brazil and Uruguay; furthermore, it is used as the infusion almost worldwide. When people, including those who are undernourished, drink any of these beverages in appropriate amount, they gain/recover strength and their working yield improves. Thus, in the *Código Bromatológico del Paraguay* (1932) and elsewhere [1,2], yerba mate is considered as a true foodstuff.

The literature indicates that mate tea 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 comprehensive review in this regard is presented in [3] and references therein.

In respect to minerals, it has been published papers referring to their concentration in *Ilex paraguayensis* from Paraguay [4-6] and in the neighbor countries of Brazil and Argentina, at the State do Parana [7,8] in the former; at the Provinces of Misiones and Corrientes in the later, [9-10] all of them bordering the east of Paraguay; just for expand the comparison, some other *data* [11] are also included.

They are mainly related to its multi-elemental contents that are prominent; many of them, such as the essential microelements are of utmost importance for living organisms. One of these essential elements is Mn that at trace levels usually occurs in *Ilex paraguayensis* at relatively high concentration on cropping lands from Paraguay as well as in Brazil, Argentina and elsewhere.

The element is essential for plant metabolism; participates actively in the photosynthesis process; also at the biosynthesis of several organic substances like some proteins and lipid acids, ATP, chlorophyl, flavonoids etc. Mn is a normal constituent of oxidizing enzymes; tiny amounts of Mn^{2+} in the oxidases and peroxidases accelerate their oxygen carrying power. It is a cofactor of superoxide dismutase (SOD) and in this regard, Mn SOD acts, *inter alia*, against plants oxidative stress. In excess Mn can be toxic; this can be explained (as well as for other 3d elements), by its capacity to catalyze the initiation of free radical reactions related to its impaired electrons. From human dietary aspects, the adequate daily intake (A.I) is 1.8-2.3 $mg.day^{-1}$ kg^{-1} b.w of Mn.

In Paraguay yerba mate is cultivated mainly in the north and in the south of the Eastern Region of the country on sedimentary and on basaltic *provenance* soils. In previous studies it has been shown that Mn content as well as other metals in Ilex, presents differences in concentration according to the harvesting zone and could be used as an origin indicator [9,12]. In this work has been investigated the content of the element in commercial as well as in fresh samples of yerba mate from several cropping areas of both, north and south zones of Eastern Paraguay, using EDXRF (Energy Dispersive X-.Ray Fluorescence) and INNA (Instrumental Neutron Activation Analysis), both non destructive techniques.

II. MATERIALS AND METHODS

The XRF and NAA procedures were carried out in two different stages. At the first, the samples were analyzed by XRF; in the second, new materials were submitted to INAA.

2.1 Materials

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 and in the south of the Eastern Region of Paraguay. Thus, were analyzed samples from nine different brands taken at random in several shops of at least four of 0.5 kg packets, ea of the same brand. They are constituted by grinded 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 Nueva Germania, Azote'y and Katuete in the North and at Capitán Miranda in the South. 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 grinding; moisture ranges from ~ 46 to 50%.

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

TABLE 1
ILEX SAMPLING SITES

North: Departments of S. Pedro – Amambay –Kanindeyu		
	Coordinates	**Soil typology**
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
Caballero Alvarez (CA)	24° 4' 13.358" S, 54° 18' 17.863" W	Alfisol – Loamy sand from basalts
Katuete (K)	24° 14' 53.340" S, 54° 45' 27.655" W	Oxisol – Clayloam from basalt
Nueva Esperanza (NE)	24° 32' 25.865" S, 54° 49' 44.853" W	Oxisol
South: Department of Itapúa		
	Coordinates	**Soil typology**
Cap. Miranda (CM)	27° 12' 53.592" S, 55° 47' 48.306" W	Oxisol– Sandy clay from basalt
Obligado (Ob)	27° 3' 7.776" S, 55° 37' 8.434" W	Ultisol- Sandy clay
Bella Vista (BV)	27° 3' 0.000" S, 55° 33' 0.000" W	Oxisol –Sandy clay from basal

Samples from each of both A & B materials were sieved and prepared by quartering, being then dried at 105 ºC for 6 hs in an oven. For XRF measurements, powdered samples were pressed into pellets of area weight of ~ 0.1 to 0.3 g.cm^{-2}. For INAA, powdered samples in amounts of ~ 4-5 g were irradiated in polystyrene vials.

2.1.3 Ashes samples

In order to verify the results, aliquots of A and B materials were reduced to ashes at 600ºC in an oven. Ashes content ranges from ~ 4.3 up to 6.5 %.

2.2 X-ray Irradiation and Fluorescence 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 Paraguay.

For the excitation of fluorescence radiation the radioisotope source of Cd-109 (30 mCi) and the X-ray tube (at 40 kV and 20 mA) with the Mo anode and Mo secondary target were used. The energy dispersive X-ray spectrometer was based on a Si (Li) semiconductor detector coupled to a spectroscopic amplifier and a multichannel analyzer. The analysis of complex spectra was performed by the AXIL software [13] which is based on iterative nonlinear least square lines. The resulting intensities of pure K_α and L_α lines of measured elements were the utilized in quantitative analysis, employing the quantification software QAES (quantitative analysis of environmental samples) designed by Kump [14]. Details have been given elsewhere [15].

2.3 Neutron Irradiations and Radioactivity Analysis.

The samples were irradiated with neutrons of a 25 Ci ^{241}Am-Be annular source from Amersham, suitable for large samples. According to the maker the total flux is 5×10^7 n.s $^{-1}$ + 20% in good agreement with the known yield of 80 n/10^6 Bk; the neutron energy spectra are complex averaging 4-5 MeV with net spikes at 4.7, 6.5 and 8 MeV [16], suitable for (n p),(n α) as well as for (n γ) reactions [17].

By neutron irradiation of Mn, the radioactive isotope Mn-56 is formed trough ^{55}Mn (n, γ) ^{56}Mn reaction. This radioisotope has a $T_{1/2}$ of 2,54h, is a β- emitter that decays to exited states of ^{56}Fe that shows a prominent (98.85%) gamma emission of 0.847 MeV [18] which was used in this work for the analysis.

III. MEASUREMENTS

Irradiated samples were measured in a 3 x 3" $Bi_4Ge_3O_{12}$ (BGO) crystal, coupled to a MCA (Multi Channel Analyzer). The BGO has better counting efficiency (density of 7.1 g. cm^{-3}) than other solid scintillators.

The analysis of samples was performed in two steps; in the first, an aliquot of sample was irradiated and afterwards it was followed the half life through the 0,847 MeV photopeak, in order to check the absence of any significant tail; in the second step, samples were usually irradiated on about 2Θ, where $\Theta = t/T_{1/2}$ according to $A = A_s (1- e^{-\ln 2\Theta})$; A_s is the activity to saturation. For calculations, calibration curves were employed. In regard to ashes samples, they were irradiated ~ 0.52 Θ.

IV. RESULTS AND DISCUSSION

Manganese is a relatively abundant element in universe: condrites carbonous show a value of 1920 ppm and for the earth it is estimated to be ~1680 ppm [19]. In the upper crust, concentration values mentioned are of 600 ppm [20] and 770ppm [21].

In Eastern Paraguay at the area of sampling stations of Nueva Germania, Azotey and PJ Caballero, sandstones of Aquidabán Group show Mn concentrations of 78ppm, but 600ppm where Aquidabán Group interfinger with sandstones of Misiones Formation [22] and ~ 500 ppm in sandstones/sediments of Acaray Formation[23]. All other sampling sites are located on the wide area of Alto Parana Formation with bearing stratum of basaltic rocks that show Mn0 concentration of about 0.19% [24]. Concerning to Mn and other *3d elements* concentrations, in soils of Paraguay very little is known except for Fe tenors. Punctual values of 6.5 and 22.2 mg kg^{-1} of Mn at sampling sites have been recorded though, according to Prof Alonso of the UNA [25]. In addition, in studies carried out in bottom sediments from several rivers and streams, the registered values of Mn are related to the geologic environment and the *provenance*.

In agreement with its half filled *3d* orbital's, Mn presents several oxidation states and forms a number of minerals. The highest occurrences are those of Mn^{2+} (silicates, carbonates) that through denudation/weathering are oxidised in the atmospheric environment. Thus, oxides/hydroxides are formed: $Mn(OH)_2$ which easily oxidises, varieties of MnO(OH) and of MnO_2 [26], which are prominent in soils, as well as other compounds.

For *yerba mate*, Tables 2a & b break down the results obtained in this work by EDXRF as well as by INAA. It must be note that Azotey and C. Miranda analyzed samples by XRF and INNA were from the same crop in each case; samples from Bella Vista are from the same sampling area, but do not belong to the same commercial brand. The closeness of the results obtained by both such techniques is remarkable. In addition it should be note that the samples "a" and "b" from Katueté come from native plants in the first case and cultured in the second.

TABLE 2a
MN CONCENTRATION IN ILEX – NORTH AREA
Departments of S. Pedro – Amambay –Kanindeyu

	FRX	INNA
Nueva Germania (NG)	510.25 ± 42.4	
Azotey (AZ)	625.0 ± 64.9*	595.0 ± 32.5*
P.J.Caballero(PJC)	609.8 ± 60.3	
Nueva Esperanza (NE)		1174.7 ± 117.1 1123.0 ± 46.6
Caballero Alvarez (CA)		1036.1 ± 109.4
Katuete (K)		2465.0 ± 30.0* 2005.0 ± 123.0*

**** fresh***

TABLE 2b
MN CONCENTRATION IN ILEX – SOUTH AREA
Department of Itapua

	FRX	INNA
Cap. Miranda (CM)	a)1680.0 ± 142*	a)1207.0 ± 169* b)1176.4 ± 111.2
Obligado (Ob)		824.0 ± 163.2
Bella Vista (BV)	a)903.5 ± 96.5 b)1036.0 ± 150	a) 993.1 ±113 b)1191.0 ±129

**** fresh***

On the dissolution of Mn-oxides, pH and redox potentials have a strong role [27]: in the soil solution, the most stable oxidation state is Mn^{2+} that occurs as $[Mn(OH_2)_6]^{2+}$ aquo-complex [28]. At pH < 5.5, which is the case for most of the soils at the *yerba* cropping area of Paraguay (pH ranging from 4.50 to 5.50), in reducing ambience manganese oxides are reduced to Mn^{2+}, increasing its availability. On the other hand these soils are low in organic matter (OM) with negative effect on Mn availability. Besides the humic complex that strongly affects the biogeochemical fate of micronutrients, a variety of other organic molecules can reduce and dissolve manganese oxides [29]. The humic complexes are constituted by fulvic and humic acids fractions (also humin).

Humic acid with Mn as well as with other metals, forms much more stable complex than those formed with fulvic acid. Thus, the former are only partially soluble while Mn-fulvic acid complex are more soluble: therefore more at hand to for the roots. Fulvic acid with $[Mn(OH)_6]^{4-}$ originate an outer sphere electrostatic structure complex with distorted octahedral configuration, and a low free energy [30].

Thus the trace elements released by redox and hydrolysis reactions enriched the soil solution that, assisted by the root surface and micro organisms activities, constitute a pool that can interact with the root surface, get absorbed and transported.

Concentration of Mn in the pool can fluctuated [31] from very low up to few hundred micromoles according to the soil behaviour and as has been shown in rice, the *yerba* and other plants, should deal with this fluctuation. In these processes and not seldom, antagonism of metals can exist, for example it is believed the existence of antagonism in *Ilex paraguayensis* of the pair Mn- Fe according the differences found in their concentration despite their similar chemical pathway in dissolution and in condensed phase.

Very little is known about Mn absorption in *yerba mate*; but in other plants it has been found the action of different NRAMPs in its absorption, transport and homeostasis. NRAMP or Natural Resistance- Associated Macrophage Protein refers to an integral membrane protein (perhaps also plasmatic) with unique expression in macrophages [32]. The plants NRAMP proteins in many cases maintain similar amino acid sequences as the NRAMP of animals; for instance, in the widely studied *Arabidopsis thaliana*, At-NRAM proteins are close to mouse NRAMP s [33].

It has been pointed out [27] that a concentration of Mn above 500ppm usually is of toxicity for plants; however this is not the case for yerba mate. In this work as well as in others, concentrations of more than 2000 ppm have been recorded in healthy plants. In other plants with the same characteristics, Mn in excess is hidden in nodes and vacuoles, being exported afterwards according necessity for the homeostasis [32-34]; this could worth for an explanation in yerba mate plants.

The results are consistent with those registered in Paraguay (previously), Brazil and Argentine, as can be seen in table 3.

TABLE 3
MN TENORS (PPM) IN *ILEX PARAGUAYENSIS- PARAGUAY AND ELSEWHERE**

Sampling sites			
Department			
Itapua (Bella Vista)	**S1**	**S2**	**S3**
	651±128	880±220	720±282
	S4	**S5****	**S6****
	858±126	904±209	1030±113
Argentina Provinces			
Misiones	**M1**	**M 2**	
	2277±250	1776±106.6	
Corrientes	**C1**		
	2320±154.4		
Brasil			
Parana	**Prudentopolis**	**Laranj. do Sul**	**Palmerinha**
	521.5±3.13	209.6±0.63	872.0±6.98
	Pinhao	**Faxinal do Ceu**	**Cachoeira dos Turcos**
	894.5±17.88	971.15±97.1	1043.5±157

Values registered in previous works adapted from references 7-14; commercial products.

Besides, values for whole sample recalculated from the results obtained when ashes were irradiated (see table 4) validate, in some way, those found in the whole samples.

TABLE 4
VALUES RECALCULATED FROM IRRADIATED ASHES

	Concentration
North	
Azotey	593.9±44.5
Nva. Esperanza	1385.7±27.7 1233.3±24.7
Caballero Alvarez	854.7±53.0
Katuete	1687.2±43.9 2320±53.4
South	
Cap. Miranda	1396.2±140 936±21.5
Obligado	904 ±166
Bella Vista	1255.3±27.6 1228.3±35.0

It has been pointed out that more than regarding to soil typology, Mn concentration in surface soil is related to particles size, i.e, clay and silt content, smaller/fine grade components of soils which are basic for sorption of trace elements pursuant to equilibrium conditions. In this regard, results corresponding to NG, Az. PJC materials from sedimentary sampling areas have consistently much lower Mn content than those from basaltic; NG, Az, and PJC present sandy deep soils with low content in clays (sandy loam). On basalts *provenance* soils that are more clayed, tenors of Mn increased sharply. At Katueté with typically basaltic soils, the yield of the element is higher than on the other sampling stations of the area. Also, at this very point, concentration of Mn show to be higher in native plants than in the cultivated ones, in line with results of other works.

In a few experiments were irradiated also samples of sticks of *yerba* (fresh) from Azotey and Capitan Miranda. In the first case Mn yield was 236 ± 28 ppm and in the second 727±70 ppm in comparison of leafy materials (see Table 2). The much higher values registered in leaves than in sticks are in line with the role of Mn in the photosynthesis [34].

V. CONCLUSION

Yerba mate is good supplier of Mn and its content of this element can be an indicator of provenance. As in other plants, absorption, transport and homeostasis of Mn could be attributed to the action of different NRAMPs. Besides, high concentration of Mn in normal healthy plants suggests that Mn in excess is hidden in nodes and vacuoles being distributed afterwards.

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Decitabine Self Monitoring in Unstable Methylation of DNMT Patients: A Quasi Systematic Review

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Abstract—

Background: *Grey zone or intermediate zone CGG repeat in Pre-mutation FMR1 gene become in high prevalence in tropical rainforest area.*

Problem: *Bipolar disorder and Major depressive disorder used epigenetic drugs inhibitor to ameliorating their mood as well anticancer agent, decitabine are broadly used. Meanwhile, basic knowledge remains largely unknown.*

Objective: *Demethylation effect in grey zone methylation instability has to be controlled whereas up till now are to be disturbing the social behavior activities.*

Hypothesis: *Demethylation drive through, from >34 to <26 CGG repeat has behavior abnormalities.*

Method: *Quasi-Systematic Review with Bayesian network analysis using Science Direct and Ebsco-host search engine.*

Result: *One PRISMA Systematic Review flowchart to got the references and one table of 16 references to answer the methylation and demethylation in global living related to decitabine are recorded.*

Discussion: *Decitabine effect in epigenetic memory in mammals and neuro developmental, cognitive, behavioral and physical changes in grey zone and carrier permutation FMR1 gene are scanned.*

Conclusion: *Demethylation to high as well low grey zone CGG count could be self monitor due to instable methylation*.

Keywords: decitabine, hypomethylation, CGG repeat, tremor, cognitive, epigenetic instability.

I. INTRODUCTION

1.1 Background

Unstable methylation in pre-mutation and grey area on CGG repeat DNMT gene for brain and behavior abnormalities[1] are in high prevalence of Decitabine using for antidepressant and controlling epigenetic mood disorder, except for anticancer drug.

1.2 Problem

Psychiatry and Psychology of bipolar, autism, tremor/ataxia, LGBTQ are in high prevalence in tropical rainforest area taken decitabine but demethylation drive through (epigenetic instability) small CGG repeat below normal (5-50 CGG repeat)[2] is unexplored till date.[3] A neurodegenerative disorder caused by the expansion of 55-200 CGG repeat (carrier pre-mutation FXS) sequester one or more RNA-binding proteins and impairing their function.[4]The micro-RNA (miRNA) and RNA interference (RNAi) induce CGG repeat over expansion and Trichostatin A, a histone deacetylase inhibitor show a reactivation of the silence promoter (CpG island methylation to be demethylation).[5] RNA-directed DNA methylation has been used in plant,[6] and CRISP to be used in Maize.[7] In human pluripotent stem cells paired-Knock Out[8] Cytosine methylation is a significant and widespread regulatory factor in plant system and a previously acquired through sequencing plant methylomes, remaining challenge to open the mistery.[9] Low homocysteine and B vitamin treatment are involved in the production of SAM, a universal methyl donor essential for DNA methylation, has been reported to protect declining cognitive health.[10]Decitabine demethylation (methylation inhibitor) are a strength CpG and CGG repeat demethylation on DNMT gene.[11,12,13] How about driven through decitabine to below normal or normal lower number on CGG repeat?[14] This Quasi Systematic Review study, show the drive through of demethylation normal low which could be done self control by the user, to gain the demethylation effect of decitabine.[14]This kind of demethylation is beyond Arsenic-demethylation.

1.3 Objective

Methylation and drive through demethylation have to be self controlling or use in combination with several issues reported not to be disturbing in social and economic behavior.[3]

Hypothesis: RNAi-hypomethylation or RNAi-demethylation in epigenetic instability. Decitabine-Demethylation drive through, from >34 to <26 CGG repeat has a behavior abnormalities.

II. METHOD

Quasi-Systematic Review PRISMA design with Bayesian analysis network using keyword: decitabine-demethylation. Science Direct and EBSCO host, binomial 0 or 1 to answer methylation and demethylation effect in each study. Amount of >200 CGG repeat are excluded. All decitabine derivate are included due to methylation inhibitor effect. The same binomial record for depressive and mood disorder. Normal 5-50 CGG stable methylation vs. unstable low and high grey zone (41-60) CGG repeat are used for cut off. Small CGG repeat (55-200) have a late onset, where 41-55 had been poorly defined.[15]

III. RESULT

One flowchart has identified 91 references, which support decitabine^demethylation (139 references for RNAi^demethylation, RNAi^methylation 982). Flowchart or 16 references supported DNMT demethylation in several cases in plants to cancer therapy. Permanent hypermethylation to gene silencing due to RNAi and DNA demethylation which reactivated gene up-regulated and get to mutation by decitabine, open the relation of methylation instability in global living.

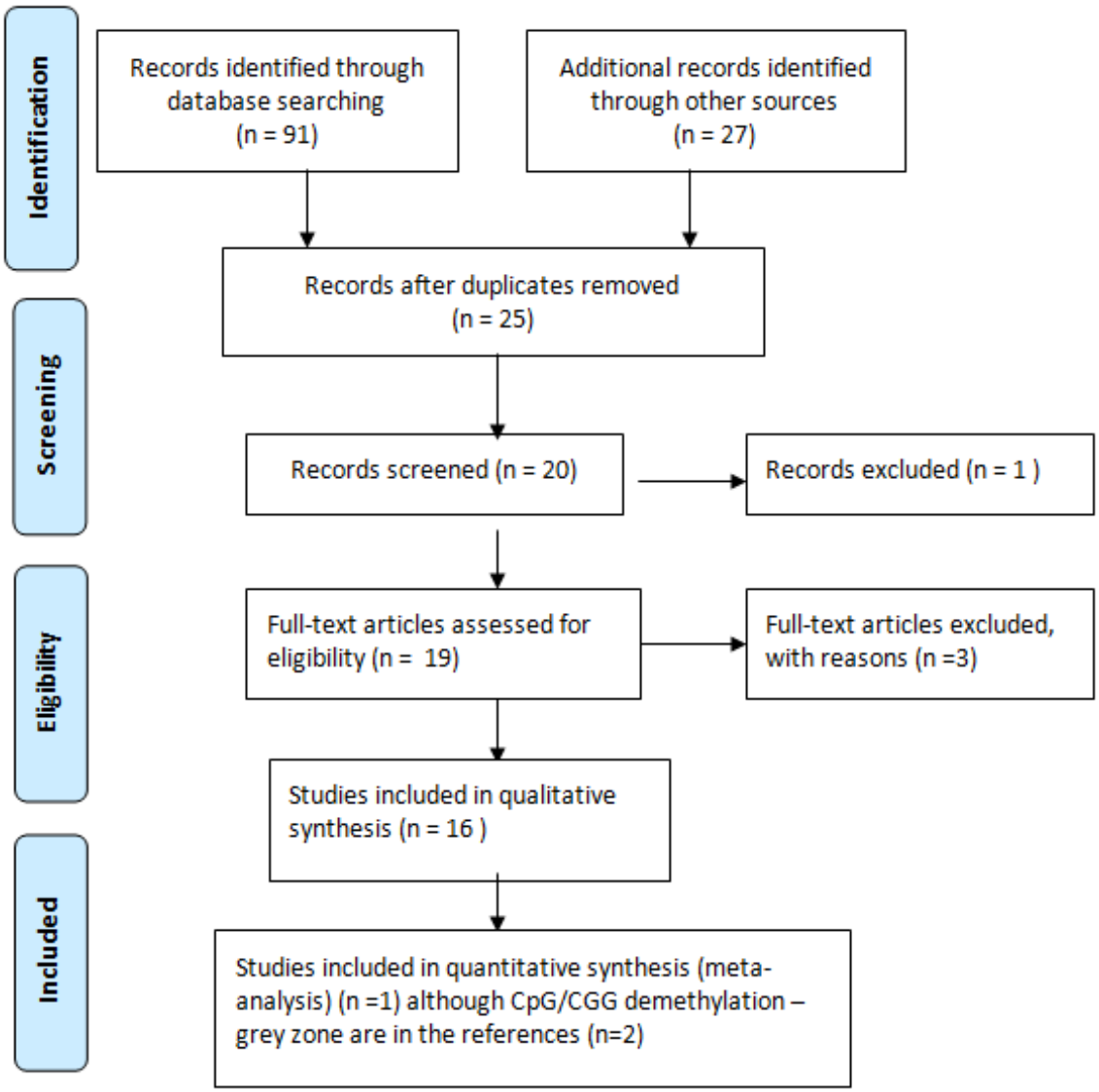

FIGURE 1. Flowchart 16 references of RNAi /decitabine)-demethylation

As antianxiety and anticancer therapy, decitabine give drive through demethylation until low normal whereas in methylation instabllity has an effect and poor prognosis in RNAi anticancer therapy.[16]Ameliorating Neurodegenerative disorder related Number of CGG repeat give human different cells Epigenetic changes.[17]

TABLE 1
Sixteen references supported RNAi /decitabine)-demethylation

Study, year	Design	Population	RNAi/methylation inhibitor	Hypo/hyper
Usdin 2014[17]	Review	Human different cells Epigenetic changes	Number of CGG repeat	Ameliorating Neurodegenerative disorder Hyper to hypo?
Movahedi 2015[6]	Epigenetic	Plants	siRNA	DNA methylation
Hardcastle 2013[9]	Epigenetic	Plant DNA	Acquired plant methylome	DNA methylation
Muthusamy 2010[18]	Epigenetic	Cancer in mammal cells	Two recent GW RNAi screens	DNA methylation
Ma 2015[19]	Epigenetic	Flower development	Low temperature	hypermethylation (silencing)
Lev 2017[20]	Epigenetic	Permanent RNAi-met not terminate>F30	siRNA	Permanent hypermethylation
Chandler[5] 2003	Epigenetic	Lined promotor	Unstable CGG repeat to > 200 Cause unknown	Methylation and RNA silencing Cause unknown
Biancalana 2015[21]	Epigenetic	FXS	Unstable CGG repeat	FX associated disorder Abnormal methylation
Indah Winarni 2012[22]	Epigenetic	Language development ↑	Sertaline vs. no medication for children under 5 y	Autism disorder
Paluszczak 2010[23]	Low doses in diet	MCF7 BC cells	Decitabine DNMT inhibitor	CpG demethylation reactivation p53
Bracht 2012[11]	Epigenetic Control trial	Somatic and germline cell	Azacitidine and decitabine vs. Trifallax	DNMT demethylation
Linnekamp 2017[12]	Systematic Review	Solid tumors	Azacitidine, decitabine etc.	Demethylation, overall response is limited
Wong 2013[16]	Epigenetic Treatment Record	Myelodysplastic syndrome	Prolonged Decitabine in nano-molar dosage	Demethylation in promoters and Gene-Bodies
Geng 2016[24]	Epigenetic Control Trial	Myelodysplastic syndrome (AML)	Decitabine combination	But not demethylation and DNMTs mRNA expression
Flitton 2019[10]	Cohort	DNMT3L brain atrophy in mild cognitive impairment	SAM donor and B vitamin treatment	Visuospatial associative memory ↑
Chatterjee 2018[25]	Epigenetic	Immune checkpoint	Marked Global DNA hypomethylation	PD-L1 Expression

IV. DISCUSSION

In table1, three groups of references which supported decitabine-demethylation: 1) the using of methylation in environment and human; 2) decitabine and derivate to be methylation inhibitor and 3) other methylation-demethylation evidence.

This table, are relevance to healthcare providers, users, and policy makers in methylation and demethylation interverences, whereas prevention is better than treatment.

Epigenetics and Psychiatric Disease, CNS-hypomethylation, progress and invasiveness of Cancer supported the decitabine-demethylation benefit and problem.

DNMT Enzymes that establish and maintain DNA methylation using methyl-group donor compounds or cofactors. The main mammalian DNMTs are DNMT1 and DNMT3. DNMT1 maintains methylation state across DNA replication, and DNMT3 perform de novo methylation. With the silencing of DNMT1 due to methylation of CpG island or CGG repeat common in FXS, this epigenetics are related to psychiatric disorder and other neuro development and neurofunction diseases. Epidemiologic worldwide psychiatric disorder: whereas major depressive disorder beyond schizophrenia and bipolar are in high prevalence especially in tropical rainforest area. Hypomethylation are related to CNS disorder are reported in gene binding protein.[26] And demethylation in Akt/p53 is related to the progression and invasiveness of Cancer.[27]

4.1 Methylation and cognitive function impairment

CpG methylation related to ataxia[28] and childhood autism risks from CGG repeat and environment (CHARGE) study[29]There are 3 groups of 5-55 CGG repeats:<26, normal (26-34) and small CGG expansion (35-54 repeats).[14]Mid-size CGG repeat (50-141) has the greatest risk of psychiatric disorder development.[30]

4.2 DNA Methyl Transferase (DNMT) 1, 3 and memory

DNMT 1 and DNMT3 have a role in resetting epigenetic Memory in mammals cellular memory.[31]DNA methylation regulate gene expression and play a crucial role in minimize learning and memory deficits in Down Syndrome.[32]Better visuospatial associative memory reported the role of DNMT3L-mediated DNA methylation which influence cognitive decline.[10]

4.3 Decitabine for Bipolar, peculiar reaction, tremor

Decitabine for Cancer Drugs CGG repeat polymorphism should have neuropsychiatric risk as a routine test.[14] Pre-mutation carrier with 55-200 CGG repeat have tremor/ataxia,[33]Those alleles with a CGG repeat number ranging between 41-55 are relatively poorly defined and known as Grey Zone.[15]The grey zone also has neuro developmental, cognitive, behavioral and physical changes[33] and small CGG repeat expansion is link with Parkinson's disease.[34]The carriers of pre-mutations in the mid-size CGG repeat range (50-141) may be at greatest risk for the development of psychiatric disorder,[30]but in women, cognitive function or executive function are not significantly different,[35]while the cognitive impairment in men is correlated in more CGG repeat in pre-mutation (55-200 CGG repeat).

4.4 Decitabine and derivate for demethylation effect

Reactivation of DNMT related to cognitive decline,[10] and the impairment in the cognitive functioning with FXTAS and was greater for men with more CGG repeats, although number of repeats was not associated with age of onset of either tremor or ataxia.[36]In women, where the pre-mutation (55-200 CGG repeats) are relatively in high prevalence, and these pre-mutation carriers reported higher levels of obsessive compulsive symptoms, depression, and anxiety, has no significant deficits in global cognitive or executive function compared to the control group.[35]

4.5 Decitabine Stop or in combination

Decitabine in combination with homoharringtonine had no enhanced effects on hypomethylation and DNMT1, DNMT3A and DNMT3B mRNA expression in SKM-1 cells.[24]

A routine epigenetic changes is also should be cover for this repeat instability to be ameliorating this molecular aspect of small CGG repeat,[17] especially <26 and >34.[14] Hematological toxicity or relapses[37,38] and how to induced pluripotent stem cell including reprogramming strategies[39] to methylation de novo of CGG repeat and downstream DNA especially p53 where DNMT 3a represses p53 which this DNA demethylation in tumorigenesis has been demonstrated in global, and regional hypermethylation in regional CpG island of tumor suppressor genes.[40,41]

V. LIMITATION

At study and outcome level (risk and bias) have minim discuss the 19-56 CGG repeat which though to be normal but successfully described have the genetic background of AGG interruptions in CGG repeat.[42]Three subgroup of 5-55 CGG repeat: Low numbers of CGG repeat (<26 repeats), normal CGG count (26-34 repeats), and small CGG expansion (35-54 repeats)[14] has been revealed to some diseases but not associated with methylation and demethylation directly. Low numbers has been related to premature ovarian failure. Small expansion has significant influence on Male Parkinsonism cohorts, mental retardation and repeat instability.[14]

After advancing NG drug discovery neuropsychiatry disorder with stem cell technology.[43] the retrieval on the impact of DNA-Methylation on stress-related pathogenesis of mental health outcome associated with cancer therapy.[44]DNA methylation and post-translational histon modification which play a crucial role in the development of the cognitive deficits in Down Syndrome with large CGG repeat has not been included in this study although the prevalence and theoretically has been increased.[32] Social behavior and brain & behavior is also depend on demethylation stage[45] mix with sex hormone since in uteri[46]

VI. CONCLUSION

The methylation RNAi and demethylation in global living related to decitabine using are recorded, and demethylation to high as well to low in grey zone CGG count could be self monitor by mental health due to instable methylation as an implications for future research.

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CONFLICT OF INTEREST

None declared till now.

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Disease in Plant and Animal: Similarities and Differences

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Abstract— *According to current human opinion and knowledge living organisms can be divided into seven kingdoms. The similarities and differences between these seven groups also the relationships between them are very interesting. These relationships lead to creation the different kinds of biological terms such as, mutualism, commensalism and parasitism. So plants and animal also microorganisms have to fight sometimes. The mechanisms of pathogenicity and the mechanisms of defense can be either similar or different. Emphasizing aspect of pathogenicity of some microorganisms, such as Salmonella, Fusarium and Tobacco mosaic virus can case to disease in plants and animals.*

Keywords— Animal diseases, Defense system, Plant diseases, Pathogenicity.

I. INTRODUCTION

Our enormous universe contains of wonderful diversity of living organisms. More than of strange of wonderful diversity, the relationships between this living things is very interesting and surprising. Briefly these relationships divide to profitable and detrimental. For example the all of relations between plants, animal and humans with harmful microorganisms are detrimental. In the same way that living things are very different, they have a lot of common characteristics. Alike these differences and similarities between living things, also their relationships have similar characteristics and dissimilar characteristics. For example we can consider relation between host plants and animal plants with their specific pathogens. The manner of combat for plants and animals against their pathogen based on their cellular characteristics either host cell or pathogen cell is different. In fact because of the marked differences between their cellular structures and modes of life, it is not unreasonable to expect that very different strategies for attack, defense, and counterattack would have evolved in plants and animals against their respective pathogens [1].

The ability of pathogenic microorganisms to harm both animal and plant hosts has been documented since the initial demonstration in the 1870s that microbes were causal agents of disease. Since the initial discoveries by [2] that *Bacillus anthracis* caused anthrax and by [3] that *Erwinia amylovora* caused fire blight in pears, our knowledge base has expanded enormously.

In this paper we try to point the mechanisms of pathogenicity in plants and animals, defense systems in plants and animals against their pathogens and finally parallels in pathogenesis on plant and animal hosts

II. THE MECHANISMS OF PATHOGENICITY IN PLANTS AND ANIMALS

The first step in the recognition of a pathogen is a general response, when non – specific receptors on the surface of plant or animal cells detect non – specific PAMPs (pathogen – associated molecular patterns). These molecules regularly occur in bacteria, fungi and in several other microbes. Typical examples are: lipo polysaccharides, peptidoglycans, chitin, and bacterial flagella. PAMP – receptors can be regarded as multi domain proteins with similar biological functions and protein structures [4; 5; 6; 7; and 8]. When these plant or animal receptors are activated by PAMPs, general reactions are stimulated in infected hosts. Ion fluxes are activated, an oxidative burst is initiated, and mitogen – activated protein kinases (MAP kinases) are expressed.

Furthermore, a set of transcriptional changes occur, in plants, so – called pathogenesis – related proteins and phytoalexins are being accumulated. All these alterations may have roles in PAMP – induced immunity (pattern –triggered immunity) [9and 7]. Although PAMP – triggered immunity can be considered as a general response of plant or animal organisms to pathogens, another type of immunity permits the inhibition of specific pathogenic races in resistant hosts. In these cases specific effectors of pathogenic races trigger the immune response [10]. In both plants and animals, a lipid compound, phosphatidyl – inositol 3 – phosphate mediates entry of pathogen effectors into host cells [11]. Plant or animal NLR receptor (Nucleotide – binding domain and Leucine – rich Repeat – c containing receptors) that interact with these effectors possess a very specific recognition ability. Interestingly, effector recognition by a receptor is associated with an almost infinite number of effector

(antigen) –binding ability of animal receptors. In this case somatic recombination and mutation is generated in the receptor – carrying lymphocytes determining a high degree of immune diversity. Effector recognition by a receptor results in clonal expansion of lymphocytes and formation of memory cells having receptors with effector – binding specificity identical to that of lymphocytes. These procedures allow a secondary immune response against a subsequent infection.

An adaptive immune system does not exist in plants. Plants have no lymphocytes, immune memory cells are not formed and the phenomenon of somatic recombination has not been unequivocally demonstrated. However, as regards similarity, plant NLR Receptors, the r – proteins also have nucleotide – binding and leucine – rich – repeat domains like their animal counterparts. In addition, there is a secondary immune response operating in plants that confers inhibition of secondary infections and is triggered by a primary infection that occurred earlier in a distal plant organ. This phenomenon is called systemic acquired resistance (SAR) [12; 13; and 14].

One can raise the question, how can plants develop specific resistance mechanisms induced by numerous effectors of different pathogenic races? Plants do not have an adaptive immune system that is the basis of immune diversity in animals. Only a limited number of specific receptors (r – proteins) exist in plant organisms, and still, immune plants can recognize a high number of effectors of pathogenic races. The "gene – for – gene concept" tried to answer this question [15]. According to the original experiments rust – resistant flax strains express different r – genes corresponding to specific avirulence (effector) genes in each pathogenic rust race. In each incompatible (resistant) host/pathogen combination an avirulence gene encodes a specific effector and a plant r – gene encodes a specific receptor. Thus, an effector of a race can activate only the corresponding specific plant receptor. However, it turned out that only a few hundred r – genes exist in host plants, as compared to the almost infinite number of effectors encoded by pathogen avirulence genes. Therefore, this concept cannot explain the high degree of immune capacity and broad immune diversity of plants.

Recent investigations on the mechanism of plant non – adaptive immunity point to the possibility that plants may exhibit a different type of immune diversity. Several results have shown that plant r – protein receptors do not directly recognize effectors of pathogens as foreign proteins in most host/pathogen combinations. Rather, pathogen effectors modify target self – proteins in plants in the course of the infection process As a result of the photolytic activity, phosphorylation, acetylation etc. exerted by effectors, the modified self – proteins become "foreign" (non – self) for plant receptors. Thus, the receptor r – protein can recognize the modified target self – protein. This is the essence of the "guard hypothesis". An r – protein is somehow connected to a target self – protein(s). After modifications, target proteins are able to initiate recognition processes and an immune response develops [16; 17; and 18].

It seems clear from the "guard hypothesis" that only a limited number of receptor r –proteins will be required to recognize different pathogenic races because the very large number of effectors released by those races may modify the same target protein. It also turned out that a large number of effectors can alter only a few conserved target self – proteins, which will be able to activate r – proteins. Thus, immunity will be initiated in a very large number of plant cultivar/pathogenic race combinations. It would seem that immune diversity may exist also in plants, because only a small number of r – proteins can recognize an almost infinite number of races – specific effectors.

As a consequence of the effector receptor interaction signal transduction chains are activated and, in the end, invading pathogens will be inhibited or killed. A series of genes are activated or inhibited in the resistant plant. However, the role of these genes in the immunity process is not exactly clear so far [19]. Surprisingly demonstrated that similar gene groups are activated in infected hosts whether the plant exhibits susceptibility or resistance. In the case of compatible or incompatible *Arabidopsis–Pseudomonas* combinations one can detect common mRNA expression profiles. If we compare specific and non – specific immune processes, again same or similar gene groups are activated [20 and 21]. All these facts refer to the possibility that timing of gene activations, Rather than gene alteration itself has a pivotal role in disease resistance. It was shown in several experiments that those genes are activated much earlier in resistant plants than in susceptible ones. Accordingly it seems reasonable to suppose that different forms of plant immunity have a common basic mechanism.

If an effector protein of a pathogen, modifies a plant protein which has no role in non – self – recognition, this modified protein will not be foreign, therefore will not be recognized by receptor r – proteins.

In this case the pathogen effector acts as a virulence factor rather than an avirulence gene product. In fact, the original function of pathogen effectors is to promote pathogenesis as virulence factors [22]. Therefore, effector proteins of a given pathogen could be regarded as "double agents", as was expressed by [23], since effectors may behave as avirulence factors in

immune processes or virulence factors in reactions of susceptibility. [24] It has been shown that according to the damage or danger signal model in the mammalian immune system the recognition of the modified self – mechanism also exists.

This section is closed with a brief noticing to immunity system in animal especially in invertebrate.

Mobile immune cells and a circulatory system permit diseased animals to exert immunity in the whole body. In addition, immune memory cells are also formed having receptors with antigen – binding ability allowing a secondary immune response to a subsequent infection. This immune memory – based response is a very effective type of adaptive immune response in animals. Interestingly, immune memory operates also in invertebrate animals although they do not have an adaptive immune response system. The mechanism is not well understood at the moment [25].

III. DEFENSE SYSTEMS IN PLANTS AND ANIMALS AGAINST THEIR PATHOGENS

3.1 The kingdoms of life

Charles Darwin described the evolution of species as 'the tree of life, an expression implying that all life originates from a single common root [26]. Until the 1960s, only three kingdoms of life were recognized: the single – celled protista, and the multicellular plantae and animalia. In 1959, Whittaker [27] defined a five domain system distinguishing prokaryotic and eukaryotic single – celled organisms and, in addition, adding fungi as a recognized kingdom [27]. The resulting of five kingdom system became a widely accepted standard. Since then, additional kingdoms have been proposed, of which only two are more or less commonly accepted. Based on rRNA gene differences, a division of prokaryotes into eubacteria and archaea was proposed [28 and29]. In addition, the kingdom Chromista, containing many algal groups as well as most water moulds, was put forward [30]. As a consequence, the generally recognized seven kingdoms of life are as follows.

3.1.1 Kingdom Eubacteria

These unicellular organisms are prokaryotic and lack a nucleus and other membrane – bounded organelles. The cell wall is composed partially of peptidoglycan, a complex structural molecule that is not found in eukaryotic cells.

3.1.2 Kingdom Archaea

Many archaeans are anaerobic and thrive under extreme conditions. Together with the eubacteria they compose the prokaryotes. With respect to cellular structure and metabolism, they resemble eubacteria, while with respect to transcription and translation, they are similar to eukaryotes. In contrast to eubacteria, archaea lack peptidoglycans in their cell wall. This kingdom is not universally recognized, and some systematicians classify archaea as an infrakingdom of the bacteria [31].

3.1.3 Kingdom Protista

This kingdom contains single celled eukaryotes. Protista are unicellular or colonial homokaryotic organisms that can be either autotrophic or heterotrophic. Locomotion occurs by means of flagella or pseudopodia.

3.1.4 Kingdom Chromista

Almost all of the species of the Chromista are photosynthetic, except for the water moulds, and most are also aquatic. Almost all fall into the traditional category of 'algae'. The photosynthetic members possess chlorophyll c, which does not occur in plants or the related 'green algae' (Chlorophyta, Charophyta, etc.). The best – known colourless members of the Chromista are a group with fungus like morphology, the oomycetes.

3.1.5 Kingdom Fungi

These are heterotrophic eukaryotes relatively closely related to animalia but distinguished in part by the possession of cells with acarbohydrate cell wall. Reproduction is usually by means of nonmotile spores or, in one phylum, flagellated zoospores. Somatic structures often appear filamentous and branched, growing only at the apex. Unicellular organisms reproducing by budding are also characteristic of certain groups. Many but not all fungi reproduce sexually as well as asexually. They include moulds, mushrooms, yeasts, mildews, smuts, and rumen symbionts, as well as the conspicuous component of lichens (a symbiosis between fungi and algae or cyanobacteria).

3.1.6 Kingdom Plantae

These are autotrophic, mostly multicellular organisms, usually with haplo – diploid life cycles. They typically develop from embryos and use chlorophyll to convert CO2 with the aid of sunlight into complex carbohydrates.

3.1.7 Kingdom Animalia

This kingdom encompasses heterotrophic multi – cellular organisms whose cells do not synthesise cell walls or photosynthetic pigments. They develop from a diploid blastula. Within these seven kingdoms of life, five major groups of microbial pathogens are currently recognized: bacteria,fungi, protozoa, helminths and oomycetes. Protozoa (kingdom Protista) are single – celled eukaryotes that include amoeba (for instance the Entamoeb species that cause gastrointestinal disease) and a large diversity of other microorganisms (for example the malaria parasites *Plasmodium* spp. and the flagellated Trypanosoma spp.). Helminths (kingdom Animalia) are multicellular invertebrates that, unlike protozoa, have differentiated tissues. Various types of vermiform animals, such as the nematodes that cause elephantiasis and river blindness, are included in this category. Finally, oomycetes (kingdom Chromista) are filamentous aquatic organisms that have cell walls composed of cellulose and a predominantly diploid lifecycle. Asexual reproduction is by biflagellated zoospores. The most well known oomycete pathogen is *Phytophthora infestans*, causal agent of potato late blight that caused the Great Irish Famine (1845 – 1847), when up to one million people died and a similar number emigrated, many to the USA. So far, archaea are not identified as direct causal agents of infectious diseases. However, recent studies show a correlation between infections and the presence of archaea [32]. Therefore it is expected that archaea will be identified as causes of infectious diseases.

3.2 Plant and animal defense

The plant and animal host kingdoms have both innate and inducible/adaptive defense responses that are very different. These defense systems are generally effective in that the majority of fungi in the environment cannot cause disease. The immune system of mammals involves the innate complement system, circulating cells such as phagocytes that can internalize and destroy pathogen cells, and adaptive antibody – mediated defenses. The complement pathway, involving soluble factors and corresponding receptors, can lead to the formation of a pore complex in accessible pathogen membranes and subsequent lysis and opsonization, whereby proteins form a coating on antigens, pathogen cells, or host cells infected by the pathogen. This process "tags" them for clearance by the immune system and can trigger proinflammatory stimulation of chemotaxis [33 and 34].

Many serious fungal infections of mammals occur in immunocompromised hosts, suggesting that mammalian defense systems are usually very effective against fungi. The severity of fungal diseases ranges from serious infections (histoplasmosis, blastomycosis, and coccidioidomycosis) [35] requiring hospitalization to superficial cutaneous infections (e.g., tinea) which are extremely common and caused by fungi such as *Candida* and *Trichophyton* spp.

In contrast, animals' fungal pathogens of plants have developed many mechanisms to evade or overcome healthy host plant defenses. Although plants do not have circulating or phagocytic cells, their cells have a thick, complex wall that acts as a barrier to invasion. Plants display innate pathogen – specific resistance, genetically controlled via resistance genes. Additionally, plants display inducible systemic acquired resistance, which occurs when previous exposure to a pathogen activates signaling pathways acting via molecules such as jasmonate, ethylene, and salicylic acid. These small and sometimes volatile molecules spread throughout the plant or even the plant population. This triggers responses such as the expression of "pathogenesis related proteins," including chitinases or glucanases, which can lead to the increased resistance of the whole plant against a subsequent pathogen attack [36].This outcome is analogous to that resulting from immunization or pre exposure to a pathogen in animals, where the defense system is primed to improve resistance to subsequent challenge by the pathogen. The jasmonate (or lipoxygenase) pathway mentioned above involves oxygenation of fatty acids, and a similar pathway known as the eicosanoid (e.g., prostaglandins and leukotrienes) pathway is present in mammals. Oxylipins, end products of these pathways, are implicated in host defense and stress responses. These molecules are also present in fungi and involved in signaling and development [37].

3.3 Programmed Cell death and Oxidative Burst

Commonalities of the defense systems of different hosts against fungal pathogens include programmed cell death and oxidative burst response [38 and 39]. Animal and plant pathogenic microbes (fungal and bacterial) release molecules with pathogenicity – associated molecular patterns. Determinants on fungus – derived polysaccharides and proteins are recognized (usually indirectly) by conserved receptors in animals and plants and elicit a defense response. These receptors are often transmembrane proteins with leucine – rich repeat domains and are manifested as resistance gene products in plants and Toll – interleukin receptors in animal and insect cells (8). This appears to be either an ancient conserved eukaryotic pathway [40] or the result of convergent evolution whereby similar motifs have been recruited for defense in different systems [41].

The suicide of individual cells is an efficient and conserved mechanism to achieve and maintain homeostasis in multicellular organisms as a response to pathogen attack and abiotic stress, as well as in normal development [42]. The selective elimination of certain cells is carried out by a gene – directed process called programmed cell death that is mentioned above. This is an energy dependent asynchronic process that comprises the loss of cell – to – cell contacts, cytoplasmic shrinkage, membrane blebbing, DNA fragmentation, disassembly of the nuclei and formation of apoptotic bodies. The execution of programmed cell death requires the participation of complex cell suicide machinery that involves several molecules regulated by the expression of a certain set of genes. The self – contained nature of programmed cell death contrasts with necrosis, which is an unregulated process of traumatic destruction, followed by the release of intracellular components without the active participation of the cell [43]. In animals, the active study of programmed cell death began in 1972 when Kerr et al. introduced the term apoptosis as "a basic biological phenomenon with a wide range of implications in tissue kinetics" [44]. However, it took more than a decade to realize the biological importance of programmed cell death in plant pathogenesis and development [45]. As in animals, programmed cell death plays a key role in numerous vegetative and reproductive phases of plant development, including the senescence of leaves, xylogenesis, death of petals after fertilization, postembryonic decay of aleuronic layers, root cap development, somatic and zygotic embryogenesis and sex determination. Similarly, programmed cell death in plants occurs in response to biotic and abiotic stimuli. In plant pathogen interactions, programmed cell death serves not only as a defense mechanism of the plant in incompatible interactions, but also to promote the dissemination of the pathogen in compatible interactions [46]. Avirulent infections are usually characterized by a localized cell death known as hypersensitive response (HR) which results in the formation of necrotic lesions around the infection sites [47]. On the other hand, there is the abiotic stress response, and the best example is aerenchyma development under low oxygen conditions, in which root cortical cells are induced to die and form larger airspaces, enabling a greater diffusion of air from the upper parts of the plant [48]. Programmed cell death in plants has also been characterised in response to high temperature [49]. Finally, some of the morphological features of apoptosis as well as transduction pathways and signal molecules have been shown to be similar in both animals and plants. However, differences in the execution of Programmed cell death have also been observed.

Oxidative burst response is a phenomena that include the production at the cell surface of different molecules such as: hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen (O_2) and hydroxyl radical (OH^-). Specifically, against microorganisms a sophisticated sensory system enables them to perceive chemical signals from potential pathogens and to translate them into appropriate biochemical responses [50 and 51].In biological systems 'oxidative stress' results from the presence of elevated levels of oxidizing agents that are able to abstract electrons from essential organic molecules and disturb cellular functions. Under normal conditions reactive oxygen species (ROS) appear in cells as unwelcome harmful by – products formed as a result of successive one – electron reductions of molecular oxygen [52]. As a consequence of disturbances in the normal redox state of the cell ROS molecules are produced, which have a toxic effect on it and damage all components inside them including proteins, lipids, and DNA. The magnitude of this damage depends upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. Most plant cells possess facing an even greater burden of ROS has the ability to detoxify it and have also acquired the relevant protective mechanisms to maintain the lowest possible levels of ROS inside. To these protective mechanisms belong some antioxidant molecules (tocopherol, ascorbate (ASC), glutathione (GSH), proline, betaine and carotenoids) and antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis [53 and 54]. It is known that one of the earliest of many diverse defense reactions activated in plant tissues in response to pathogen attack, is the rapid and transient accumulation of huge amounts of ROS and depending on the interaction, these ROS – generating mechanisms involve plasma membrane NADPH oxidases or cell – wall peroxidases [55].Studies related with the role of ROS during plant pathogen interaction have been carried out in all kind of interactions. In hemibiotrophic interaction by [56; 57 and 58], in necrotrophic interaction by [59; 60; 61 and 62] and in biotrophic interaction by [63 and 64]. Besides another ones regarding to this theme have been done by [65; 66; 67; 68 and 69]. In spite of this plethora of information about ROS role in different plant – pathogen interactions, knowledge is still scarcely and not enough for a complete understanding of the oxidative stress in plants although, it is believed that during an interaction a coordinated activation at the site of infection requires tight control of the production of ROS, such as $H_2O_2$and O_2^- . Besides, some research has indicated that the ROS produced in the oxidative burst could serve not only as protectant against invading pathogen, but could also be the signals activating further plant defense reactions [70].

Mammals in general, as well as most of their organs, are quite intolerant of anoxia and/or ischemia partly because their metabolism is ill – equipped to endure the energy shortfall that occurs when mitochondrial ATP production is blocked and

partly because of injuries that arise due to a burst of ROS formation when oxygen is reintroduced. Clearly, in mammalian systems, the antioxidant defenses of the organism can be over whelmed by rapid and large changes in tissue ROS levels. However, although unusual for most mammals, many organisms routinely experience wide variation in oxygen availability to their tissues due to factors such as environmental oxygen lack, breath hold diving, extracellular freezing, or apneic breathing patterns in arrested metabolic states. To cope with these situations, many lower vertebrates and invertebrates have well – developed tolerances for anoxia and ischemia that allow them to endure these stresses as part of their normal life [71]. For example, various gill breathing intertidal marine invertebrates routinely experience cyclic bouts of oxygen deprivation with the tides and have evolved excellent capacities for facultative anaerobiosis that, in fact, allow them to survive for days or weeks at a time without oxygen [72]. Among vertebrates, anoxia tolerance is highly developed in various species of freshwater turtles that dive routinely and also hibernate underwater; species of the *Chrysemys* and *Trachemys* genera, for example, can survive for 3 – 4 months submerged in deoxygenated water at 30º C [73 and 74]. Freeze – tolerant animals have to deal not only with anoxia but also with ischemia for when extracellular body fluids freeze, all circulation is cut off and individual cells must rely on internal fermentative fuel reserves to survive for perhaps days or weeks until they thaw again. Freeze tolerance is quite common among cold – hardy insects in northern latitudes and is also a strategy used by several species of woodland frogs and some hatching turtles for winter survival [75]. In addition, many other types of animals, while not facing such extremes of anoxia or ischemia, experience wide variation in oxygen availability in their normal life and endure wide cycles of normoxic and hypoxic conditions. For example, estivating animals (such as various land snails and burrowing toads) have this experience since they use apnoeic breathing patterns to minimize body water loss during longterm dormancy [76 – 78] and diving animals (such as seals and whales) experience profound hypoxia in many organs due to circulatory readjustments that preferentially direct oxygenated blood to the skeletal muscles and brain [79]. Hibernating mammals also experience hypoxia due to apnoeic breathing while dormant and experience a rapid 10 – 20 – fold increase in oxygen consumption during arousal when they rewarm their bodies from ambient back to 37ºC over just a few minutes [80].

IV. PARALLELS IN PATHOGENESIS ON PLANT AND ANIMAL HOST

4.1 Fungus

The best studied fungal isolate that can affect both animals and plants is *Fusarium oxysporum* f. sp. *lycopersici*, which can kill both immunodepressed mice and tomato plants [81]. Studies of this fungus highlight commonalities and differences in the mechanisms of pathogenicity on animal and plant hosts. For example, a mitogen – activated protein (MAP) kinase gene, Fmk1, of *F. oxysporum* is not required for virulence in mice but is essentialfor virulence in tomatoes. In contrast, the zinc finger transcription factor gene PacC is necessary for full virulence in mice but not in tomatoes [81]. PacC is important for virulence in a range of other plant – specific and animal – specific fungi, as it mediates the environmental pH signal, which in turn alters gene expression appropriately. Another soilborne fungus, *Aspergillus flavus*, can infect animals, insects, and plants, particularly seeds of corn, peanuts, cotton, and nut trees. This fungus, like several other *Aspergillus* species, produces highly toxic, carcinogenic aflatoxins. Several strains isolated from humans and insects can also cause disease in corn [82]. Different nutritional pathways may be important for the virulence of this fungus on different hosts, since a cysteine and methionine auxotroph of *A. flavus* has reduced conidiation in vitro and on plant hosts, but this auxotroph can still complete a disease cycle on insect hosts [83].

4.2 Bacteria

The genus *Salmonella* consists of only two species, *S. bongori* and *S. enterica*, and the latter is divided into six subspecies. *Enterica* includes more than 1,500 serotypes, which despite their high genetic similarity vary greatly in their host range and disease outcome ranging from enteritis to typhoid fever [84].*Salmonella enterica* subsp. *enterica*is an important economic and public health problem throughout the world. The degree of adaptation to hosts varies between *Salmonella* serotypes and determines the pathogenicity. Serotypes adapted to humans, such as *S. typhi* and *S. paratyphi* A, B, C, cause systemic typhoid fever. These serotypes are not pathogenic for animals. Similarly, *S. gallinarum* and *S. abortusov*is, which are specifically adapted to poultry and ovine, respectively, are responsible for severe systemic infections in these animals. However, *S. choleraesuis*, for which pigs are the primary hosts, also causes severe systemic illness in humans. Ubiquitous serotypes, such as S. *enteritidis* or *S. typhimurium*, generally cause gastrointestinal infections in humans but can induce other diseases in animals [85]. For example, they can produce typhoid – like infections in mice, systemic infection in humans or asymptomatic intestinal colonization in chickens and pigs [86]. Some of them are responsible for chlorosis on plant leaves sometimes causing death [87; 88, 89 and 90]. Disease in mammals occurs after ingestion of contaminated food or water. *Salmonella* infection of animals and humans depends on the ability of bacteria to survive the harsh conditions of the gastric

tract before entering the intestinal epithelium and subsequently colonizing the mesenteric lymph nodes and internal organs in the case of systemic infections. In order to enter non – phagocytic cells and survive within the host environment, *Salmonella* has evolved mechanisms to interact with host cells and to induce its own internalization [91 and 92] *Salmonella* usually enters agricultural environments via animal feces. Animals can directly contaminate plants or surface water used for irrigation and pesticide or fertilizer diluent through contaminated feces. Recently, there has been an increasing number of reports, linking *Salmonella* contaminated raw vegetables and fruits with food poisoning [93]. *Salmonella* is able to adapt to different external conditions including low pH or high temperature, allowing it to survive outside the host organism [94 and 95]. Indeed, *Salmonella* is able to attach and adhere to plant surfaces before actively infecting the interior of different plants, leading to colonization of plant organs [96 and 97], and suppression of the plant immune system [98]. In addition, *Salmonella* originating from plants retains virulence toward animals [89]. Thus, plants are an alternative host for *Salmonella* pathogens, and have a role in its transmission back to animals.

4.3 Viruses

Unlike animal viruses, plant viruses cannot replicate in humans or other animals, largely due to the lack of specific receptors for recognition and entry into host cells. However, it has been demonstrated that cowpea mosaic virus enters the bloodstream in mice from the intestine when administrated in cowpea leaves and induces the production of antibodies without replicating [99; 100; and 101]. More recently, a case – control study showed that pepper mild mottle virus may be found in human feces and is associated with clinical immune responses [102]. These studies suggest that plant viruses may play a role in human health and disease. Until now, the possible effects of consumption of TMV (tomato mosaic virus) in tobacco products have not been investigated. Tobacco smoking has been shown to cause cancers [103], heart disease [104], and chronic obstructive lung disease [105]. It also increases the risk for development of multiple autoimmune disorders such as rheumatoid arthritis [106] and multiple sclerosis [107; 108 and 109] Although the health risks of tobacco smoking are well documented, increasing evidence suggests that smokers have a lower incidence of some inflammatory and neurodegenerative diseases. For example, smoking is reported to reduce human autoimmune responses in systemic lupus erythematosus [119] and ulcerative colitis [111]. Of particular interest to neurodegenerative disorders, epidemiological studies consistently show smokers to have a lower risk of developing Parkinson's disease [112 and 113,] which is associated with a long duration of smoking rather than smoking intensity [114]. Such an inverse association is also observed in people who use chewing tobacco [115]. The protective effects of smoking have been suggested to result from the ability of nicotine (the main addictive ingredient of tobacco) to inducing immunosuppression [108] and neuroprotective action [116 and 117] but the biological mechanisms by which this occurs remain largely unclear. As a complex mixture of more than 4,700 chemical compounds, many constituents of cigarettes have been shown to modulate immune function including both the humoral and cell – mediated immune responses [108]. Tobacco mosaic virus can survive for years in cigars and cigarettes made from infected tobacco leaves, and TMV can be found on the surface of cigarettes. Therefore, we presume that smokers are more likely to be exposed to TMV than non – smokers. We tested whether exposure to tobacco products induces immune responses o TMV in humans and compared the differences among individuals who were smokers, smokeless tobacco users and nonsmokers. Identification of mechanisms for TMV – elicited specific immune responses may aid in defining the etiology and pathogenesis of smoking – related human diseases. It has also proven that the human protein TOMM40L (an outer mitochondrial membrane 40 homolog – like translocase) contains a strong homology of six contiguous amino acids to the TMV coat protein, and TOMM40L peptide exhibited cross – reactivity with anti – TMV antibodies. There is a mimicry between TMV and human TOMM40L that is caused to raising the question as to whether TMV has a potential role in smokers against Parkinson's disease development.

The potential mechanisms of molecular mimicry between plant viruses and human disease should be further explored [118].

V. CONCLUSION

Whereas living organisms are very different, on the other hand are very similar. These differences and similarities include relationship between them and the methods of their defense against their pathogens. The most important difference between plants and animals is absence of circulatory system and an adaptive immune system in plants. In both plants and animals the first step in the recognition of a pathogen is a general response, when non – specific receptors on the surface of plant or animal cells detect non – specific PAMPs (pathogen – associated molecular patterns). These molecules regularly occur in bacteria, fungi and in several other microbes. Plant do not have immune system that is the base of immunity in animals so how can plants recognize a high number of effectors of pathogenic races? The answer is "gene – for – gene concept". In each

incompatible (resistant) host/pathogen combination an avirulence gene encodes a specific effector and a plant r – gene encodes a specific receptor.

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The Effect of Mulching on Soil Moisture Retention and Yield of Lettuce (*Lactuca Sativa* L.)

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Abstract— *An experiment was conducted to evaluate the effectiveness of different mulching materials on soil moisture retention and yield of lettuce at the greenhouse located at Luyengo campus of the University of Eswatini during the months of January and February, 2019. The treatments consisted of grass mulch (GM), Plastic mulch (PM), leaf debris mulch (LM), and no mulch (NM) which was used as a control. Each of the treatments had four replications. The organic mulch was applied at a thickness of 10 cm, and the plots for experiments were randomly selected. Each plant received 600 cm^3 of water every 3 days using a homemade drip irrigation system (equivalent to 6 mm per irrigation circle). Data on soil moisture content was collected using the gravimetric method every 3 days (before irrigation).The growth parameters of the lettuce plants that were collected weekly were plant height, leaf number and leaf area. Both wet weight and dry weight yield were determined for each plot at the end of the experiment (six weeks after planting). Data collected was coded and entered into SPSS computer software. Data analysis was conducted using the analysis of variance (ANOVA) and the least significance difference (LSD) test to determine if means were significantly different. The results showed that GM treatment had high mean moisture retention at 9.3%. It was followed by PM and LM at 8.9%. The lowest moisture retention was realized from the control (No mulching) at 7.9%.The differences in mean moisture retention was significant between NM and NM ($p<0.05$). The same pattern was observed for the growth parameters, where GN had highest values and the control had the lowest values. The wet mass yield was highest for GM, at 164.7 g. The yield from LM was 149.3 g. It was followed by PM at 141.3 g. The lowest yield was obtained from the control at 108 g. The difference in mean yields for GM and NM were significant ($p<0.05$). They were not significantly different for all the other treatments ($p>0.050$. It was concluded from the experiment that grass mulching resulted in improved moisture retention and high yields.*

Keywords— Keywords-Drippers, irrigation, moisture, mulching, yield.

I. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most commonly used as salad vegetable. It belongs to the daisy family, Asteraceae. Lettuce probably originated from Asia, where it was grown for centuries and its early forms were used in Egypt around 4500BC [1]. Lettuce is a cool season crop grown for its tender head and leaves, but sometimes for its stem and seeds. It is rich in vitamins A and C and minerals like calcium. Lettuce is the dominant cultivated salad vegetable, which is commercialized worldwide. It is the most popular salad vegetable with the highest consumption rate and economic importance throughout the world). Farmers need to be educated for its production technology including judicious water management [2]. Water use efficiency is crucial and should be promoted in agricultural production [3]. A way of doing this is through mulching, which involves covering of the soil surface with crop residue (s) or other material such as paper or polyethylene film. Straw mulch helps to retain soil moisture, reduce temperature, conserves soil, control weeds and increase soil fertility. Mulches increase the soil moisture in the root zone and significantly decrease soil temperature. This provides a stable environment for seedling establishment and growth than soil that is not mulched. In addition, mulches increase the infiltration and storage of water in the soil and improve structure and macro-porosity of the soil along with reducing runoff and evaporation losses [4]. Water is essential for the sustenance of all forms of life. In the Kingdom of Eswatini, water utilization is expected to stimulate the economic development of the country through agricultural production. In the past few years, water availability has been scarce resulting to uneven water distribution across the country with high costs and worse, some water source went dry due to drought. This predicament has created a drive to conserve water in the country. Improving water efficiency is an ongoing goal in agricultural production, especially in area where water sources are limited and regulated. Farmers are adopting new strategies of conserving the little water they have for their production, especially vegetable production. That's when the need of other possible ways of water conservation through the use of mulch was considered, but there is still uncertainty of which mulch material is more effective, hence this study. Mulching benefits the soil in many ways which subsequently enhance the growth of the plants in that particular soil. Mulching offers tremendous potential for increased crop production through its marked effects on the soil environment which increase crop growth and yield [5]. Mulches are beneficial in soil and water conservation, modification of soil temperature and the temperature just

above the ground, preserving and improving soil physical and chemical properties, suppressing weed growth and enhancing biological activities in the soil. Mulching improves the soil moisture regime by reducing losses caused by surface run-off and evaporation. It insulates and protects soil from drying and hard-baking effects caused by evaporation of water from soil exposed to hot sun and winds. Mulched soils absorb water faster than soils of the same type without mulch. Under mulch, the soil moisture is conserved because of reduced evaporation, improved infiltration rate, and the suppression of unnecessary plant growth [6].

The build-up of a large and active soil microbial biomass is critically important for sustaining the productivity of soils in organic farming systems [7]. Soil microbes, the living part of soil organic matter, function as a transient nutrient sink and are responsible for releasing nutrients from organic matter for use by plants. Mulching enhances microbial activities in the soil, which is essential in nutrient recycling [8].

The effect of mulch on crop yield is an integrated effect of many factors and it is difficult to attribute the yield increments to any one variable on its own [9]. Crops perform well when there is adequate soil moisture, nutrients and or optimum temperature. The presence of mulch enhances all the conditions mentioned above but mulch alone cannot provide adequate nutrients that the crop requires for development. The objective of this study was to determine the effect of mulching on soil moisture retention and the yield of lettuce.

II. MATERIALS AND METHODS

2.1. Description of study area and research design

The experiment was conducted at the greenhouse located at Luyengo campus of the University of Eswatini. Luyengo is located in the Middleveld of Eswatini at 26.683^o S and 31.20^o E with altitude of 733 m above sea level. It has an average annual rainfall that ranges from 850 to 1000 mm. The soils are classified under the Malkerns soil series (Oxisols), which are dark loam to sandy loams [10]. The experiment was Complete Randomized Block Design with four treatments that were replicated four times. The treatments were: grass mulch (GM), plastic mulch (PM), leaf debris mulch (LDM), and no mulch (NM) as a control.

2.2. Land preparation and planting, and irrigation

Land was cultivated uniformly using a hand fork to break clods and the soil was loosened. A fine tilth was prepared on the plots using a hand rake before planting seedlings. Seedlings were obtained from Vickery Nursery, a local commercial nursery. One seedling was planted by hand in each hole, with seven lettuce seedlings being planted per replication. They were planted at 30 cm apart within rows and 40cm between each treatment. The different organic mulching materials were applied at a thickness of 10 cm seven days after planting the seedlings. Weeding was done using a hand hoe whenever necessary. The crops were monitored on a daily basis to observe and treat any symptoms of pest and disease attack, there were none observed.

The plants were irrigated using a homemade drip irrigation system. It was a low cost drip irrigation system which is a gravity fed system. This irrigation method involves the delivery of water through a pipe distribution network consisting of a main pipe that delivers water to lateral pipes under low pressure and emission through small outlets (emitters or drippers) into the soil surrounding the plant. The components of this drip irrigation kit include the water storage tank of 210 liters capacity, a gate valve that controls the movement of water entering the system, a backflow preventer, a filter, a tubing adapter that connects the drip tubing with the filter, and the main pipe that delivers water to the laterals of the system. Each plant received 600 cm^3 in every irrigation cycle which lasted for 1.2 hours. This was equivalent to 6 mm of water per irrigation cycle.

2.3. Data collection and analysis

The parameters that were measured every week during the study were leaf number, leaf width, and leaf length of the plant. The parameters were collected from all the plants in each replication. The Leaf Area Index (LAI) was calculated using equation 1.

$$LAI = Y \times N \times AL \times AP^{-1} \quad (1)$$

Where;

Y- is the population of the plants per plot

N- is the average number of leaves per plant

AL- is the average area per leaf (cm^2)

AP- is the area of the plot (cm^2)

The leaf area was calculated using equation 2.

$$LA = L \times W \times 0.7 \quad (2)$$

Where;

LA- is the leaf area (cm^2)

L- is the leaf length (cm)

W- is the leaf width (cm)

0.7- is the correction factor

The soil moisture content for each treatment was determined every three days using the gravimetric method which involves collecting a sample of wet soil, weighing it before and after oven drying it at 105^o C for at least 48 hours. The soil samples were collected using a 98 cm^3 cylindrical ring before irrigation (irrigation cycle was 3 days) from each treatment, weighed, oven dried at 105^o C for 48 hours, then weighed again to get the dry mass. The amount of water lost was calculated as a percentage of the mass of the dried soil as expressed in equation 3.

$$\%\, Soil\ water = \frac{Weight\ of\ wet\ soil\ (g) - Wieght\ of\ dry\ soil\ (g)}{Weight\ of\ dry\ soil} \times 100\% \quad (3)$$

One plant was harvested by cutting it from the base from plot at maturity (six weeks after planted). The harvested plants were weighted to determine wet mass. There after they were oven dried for 48 hours at 105^0 C, and weighted to determine dry mass.

Data were entered into SPSS computer software. The Analysis of Variance (ANOVA) and the least significant difference (LSD) were used to determine if means were significantly different.

III. RESULTS AND DISCUSSIONS

The results showed that there were some variations in soil moisture retention under the different mulch materials (Table 1). Grass mulch treatment had highest soil moisture retention (at 9.3%), followed by both plastic mulch and leaf debris mulch, at 8.9%. The control had the lowest soil moisture retention at 7.9%. The difference in soil moisture retention was significant for the grass mulch and control ($p<0.05$). Organic mulch reduces evaporation of soil moisture and thus improving soil moisture retention [11].

TABLE 1
RESULTS SHOWING MEAN VALUES FOR PARAMETERS

Treatment	Parameters*					
	Moisture retention (%)	Mean plant height	Mean leaf number	Leaf area index	Mean wet mass	Mean dry mass
Control	7.9^a	15.2^b	10	0.37^c	108^d	6.3^e
Grass mulch	9.3^a	21.7^b	13	0.77^c	164.7^d	8.7^e
Plastic mulch	8.9	16.0	10	0.45	141.3	7.4
Leaf debris mulch	8.9	18.2	11	0.47	149.3	8

* ***Parameters on same column with same symbol indicate that their means were significantly different.***

Grass mulched treatment recorded the highest mean plant height of 21.7 cm after the 6-week growing period. It was followed by Leaf debris mulch (LM) with a mean height of 18.2 cm and the Plastic mulch (PM) with a mean plant height of 16 cm. The Control treatment (NM) had the lowest mean plant height of 15.2 cm (Table 1). The mean plant height for Control and Grass mulch were significantly different ($p<0.05$). They were not significantly different for all the other treatments ($p>0.05$).

Leaf area index is a reference tool for crop growth as leaves are the most important structure for photosynthesis [12]. The mean LAI for GM was the highest at 0.77. It was followed by LM and PM) which recorded 0.47 and 0.45 respectively. The Control (NM) had the lowest LAI at 0.37. The LAI values were significantly differently for NM and GM ($p < 0.05$), and not significantly different for all the other treatments. GM treatment had the highest mean number of leaves, at 13. It was followed by LM with mean number of leaves at 11. The mean number of leaves for PM and NM were 10. The mean number of leaves were not significantly different for all the treatments ($p > 0.05$).

The results for yield were presented in terms of the mean wet mass and mean dry mass (Table 1). The mean yield per plant for GM was the highest (at 164.7 g wet mass and 8.7 g dry mass). The control (NM) had the lowest mean mass (at 108 g wet mass and 6.3 g dry mass). Like most other parameters, the difference in mean yield was significant for GM and NM ($p < 0.05$). They were not significant for all the other treatments ($p > 0.05$). Mulching improves nutrition absorption; weed control and temperature adjustment, leading to improved growth and yield [13].

IV. CONCLUSION

Mulching of the soil resulted in improved growth and yield of lettuce. The performance of lettuce under all the three treatments (GM, PM and LM) was higher than of the control (NM). The differences in mean values for all the parameters (except for mean leaf numbers) were significant for GM and NM. They were not significant for all the other treatments. Based on the findings of this study, it is concluded that mulching has a positive effect on soil moisture retention and yield of lettuce. Grass mulch should be adopted as a moisture conservation measure that would result in significant increase in the yield of lettuce.

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