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Preface

We would like to present, with great pleasure, the inaugural volume-3, Issue-5, May 2017, 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.

Mukesh Arora
(Editor-in Chief)

Dr. Bhagawan Bharali
(Managing Editor)

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Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
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Dairy Sheep	Water Buffalo
Moose milk	Dairy product
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Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
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Agricultural buildings	Storage

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Water hyacinth *(Eichhornia crassipes (Mart.)*: Land use/land cover changes and community-based management in east Shoa zone, Ethiopia

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Abstract— *Water hyacinth was officially reported in Ethiopia in 1956 at Koka Dam and Awash River and it is considered as a constraint to the development of the country. Thus, this study was undertaken to determine Water hyacinth and associated land cover/use changes, and capture perceptions regarding community-based management to enhance its proper control/eradication in Lume and Boa districts, east Shoa zone, Ethiopia using integrated approach. The method of study included Water hyacinth and associated land use/cover change analyses, focus group discussions, discussions with experts at the district, zone and region levels and undertaking consultative workshop. The land use/land cover change analyses revealed increased area coverage by Water hyacinth from about 145.53 ha in 1986 to 2319.48 ha in 2015 with decline in the area of water bodies and wetlands. The annual rate of increase in the area of the weed was about 51.51% while water bodies and wetlands declined by about 0.49% and 1.16%, respectively. Of the 10 group discussions undertaken in the study districts with the communities, 9 of them reported water hyacinth to increase in terms of area coverage since its appearance in their areas which concurs the results obtained from satellite image analyses and they reported the weed to be very harmful to their livelihood. Furthermore, the nine group discussants disclosed water hyacinth to be of no use to them. Recommendations included developing comprehensive management strategies and action plans, analysis and defining roles of each stakeholder, awareness creation, training, institutional linkages, co-management and reduction of nutrient load in water bodies.*

Keywords— Co-management, control/eradication, Integrated approach, Lume and Bora districts.

I. INTRODUCTION

Water hyacinth, *Eichhornia crassipes* (Martius) Solms- Laubach, is fast growing aquatic free floating fresh water plant indigenous to Brazil, Amazon basin and Ecuador region [1]. It was introduced as an ornamental species to adorn the water bodies in many countries more than a century ago for their attractive blue or purple flowers, oblong to oval glossy leaves with bulbous petioles. Later, this supposed-to-be prized plant was discovered to be an invasive species and posed serious socioeconomic and environmental problems affecting millions of people in riparian communities [2]. The sturdy plant has emerged as a major weed interfering with anthropogenic activities and is recognized as one of the top 10 worst weeds in the world [3].

In Ethiopia, water hyacinth was first reported in Lake Koka in 1956, the Awash River and since then, it has been found in different regions of Ethiopia. It is considered as a constraint to the development of the country [4] which has multifaceted problems such as obstructing electricity generation, irrigation, navigation, and fishing; increasing evapo-transpiration resulting in water loss, increase cost of crop production, providing habitat for vectors of malaria and bilharzias, harbors poisonous snakes, causing skin rashes, and hosting agents of amoebic dysentery and typhoid [4, 5] [5] and these effects have also been documented elsewhere in the world [2, 3]. The weed has infested water bodies in the two intervention districts (Lume and Bora) of the Livestock and Irrigation Value Chain for Ethiopian smallholders (LIVES) project in east Shoa zone of Oromia Region and with connection to the Awash River includes Koka Dam, lakes Ellen and Elletoke.

Local farmers in Bora district grow a number of crops for home consumption and sale mainly maize, wheat, haricot bean and tef (*Eragrostis tef*) while vegetable production (tomato, onion and others) is practiced along the shores of the lakes, mainly by private investors. Koka reservoir and lakes Ellen and Elletoke are the main water bodies in the district and are mainly used for irrigation and fishery. Koka dam is also used for recreation while the primary purpose is for hydro-electric power

generation. Koka dam and the Awash River are also found in Lume district and their use is similar to that in Bora. Similar to Bora, the livelihood of farmers in Lume is based mainly on crop production which is followed by livestock production in the form of cattle, sheep, goats, chickens, and equines although the intensity of cropping is more in Lume than in Bora district.

The economic importance of these water bodies in the study districts for many families and nationally is significant [4]. However, the extent of land covered, the spatial and temporal cover changes of water hyacinth and associated land covers/uses is not documented although there is a growing global concern about land use/cover changes which emerged due to realization that changes of the land surface influences climate and impact on ecosystem goods and services [6]. Furthermore, despite the long history of this weed in the study districts and compared to complex and diverse nature of the problem, the interventions undertaken to control the weed particularly, in the open community field, does not much with its expansion which justifies the need to assess the possibility of applying community-based management (CBM).

The objectives of this paper were to determine water hyacinth and associated land cover/use changes over the past three decades (1986 to 2015) along water hyacinth infested water bodies and capture perceptions regarding CBM, to enhance proper control/eradication of the weed in Lume and Bora districts.

II. METHODOLOGY

2.1 Description of the study area

The study was carried out along water hyacinth infested water bodies in two districts, Bora and Lume, east Shoa zone, Oromia Region, Ethiopia (Figure 1). These two LIVES intervention districts were selected because of the significance of the weed in the livelihoods of the surrounding farmers and communities beyond. Lume is located at 39°01′ to 39°28′ E longitude, and 8°40′ to 8°84′ N latitude while Bora is located at 38°75′ to 39°06′ E longitude, and 8°10′ to 8°42′ N latitude. Most of the study districts fall within the altitude range of 1,578 to 2,000 meters above sea level (masl). The study districts are located between 75 and 110 km, southeast of Addis Ababa, capital city of Ethiopia. The districts cover about 1,110.82 km^2 with a human population of 206, 248 (Lume = 137, 787 and Bora = 68, 461) [7]. The mean annual temperature in both districts ranges from 13 to 28°C while the mean annual rainfall is 851 mm in Lume and 500 to 800 mm in Bora. Both districts receive a bimodal rainfall; the main rain season locally known as *Ganna* extend from June to September while the short rainy season (*arfasa*) from April to May.

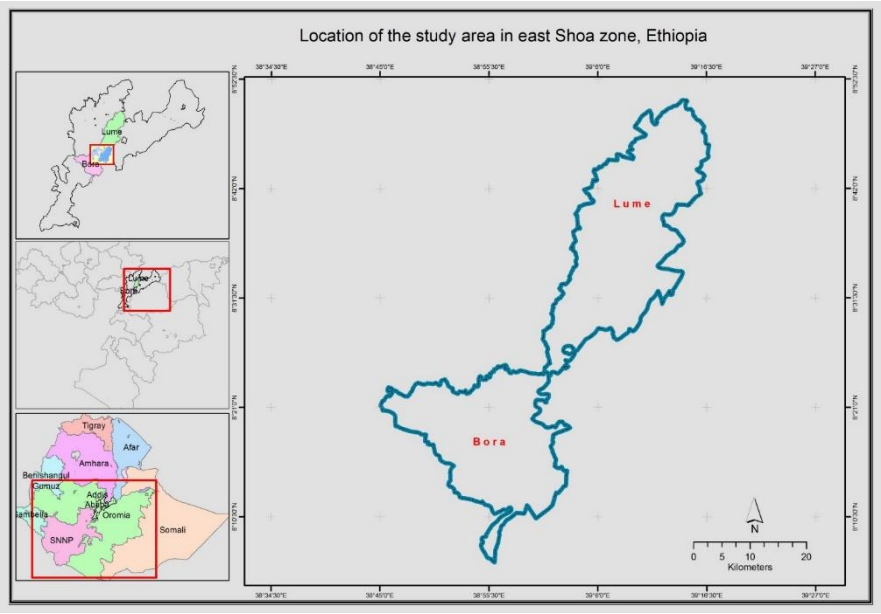

FIGURE 1. LOCATION OF THE STUDY AREA IN EAST SHOA ZONE, ETHIOPIA

2.2 Analyses of the changes in the cover of water hyacinth and associated land uses/covers

Changes in the cover of water hyacinth and associated land covers/uses were detected from the analyses of sequence of satellite imageries (Figure 2). To make the multi-temporal analysis and land cover/use types at various times extraction, cloud free Landsat TM imageries consisting of Landsat TM of 1986 and Landsat TM of 2015 (path 168 and row 54) were

analyzed to classify the land covers/uses change of the study area. All were acquired in the month of January during the dry season. Geometric correction and image enhancement were conducted using ERDAS IMAGINE 10.2 software.

Unsupervised classification of the study area was performed prior to field visit and representative points thought to represent the various land cover classes were marked using GARMIN GPS during field visit (250 GCPS). For the year 1986, false color composite was prepared using the order of 4, 3, and 2 band sequences and then different enhancements were made to increase visual interpretation of the image. In addition to GPS, digital camera was used for recording the physical features about the areas. Ground truthing activity was undertaken not only to provide information about land cover/use types, but also to provide complementary information needed to improve the final classification. Long transect and guided field walks were also made by the research team to observe, listen and to verify the status of water hyacinth and associated land cover/use classes with key informants.

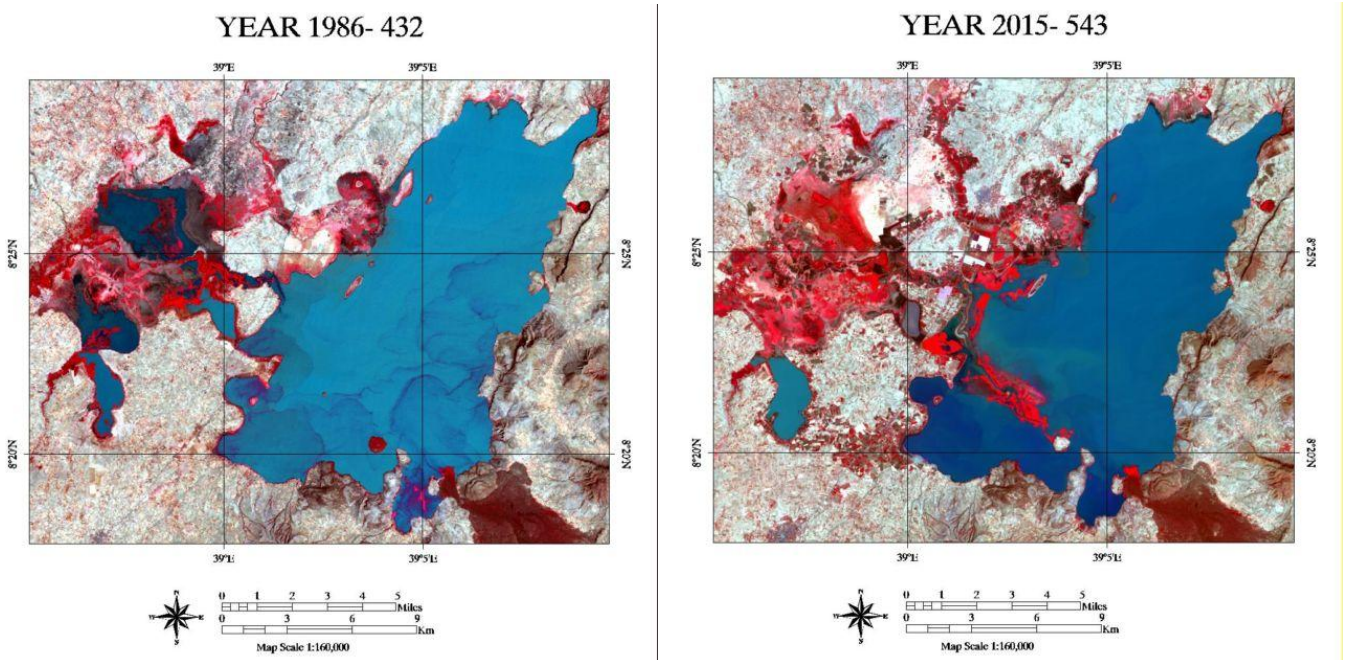

FIGURE 2. LANDSAT IMAGES OF THE STUDY AREA 1986 (LEFT) AND 2015 (RIGHT)

Based on the field check points, supervised classification approach with the maximum likelihood classifier system was applied to improve the accuracy of water hyacinth and associated land cover/use classification of the images for 1986 and 2015.

Post classification comparison was carried out for two independent images (thematic maps). Difference or change information was generated by comparing image values of one data set (TM 1986) with those of the corresponding layer of the second data set (TM 2015), conversion matrix between TM 1986 versus TM 2015 was compiled in the form of a contingency table. This conversion matrix was used to quantify land cover change in terms of pixel values, ha or percentage of area coverage. The estimation for the rate of change for the different covers/uses was computed based on the following formula:

$$\% \text{ cover change} = \frac{Area\ i\ year\ x - Area\ \ i\ year\ x+1}{\sum_{i=1}^{n} Area\ i\ year\ x} \text{ X } 100\%$$

$$\text{Annual rate of change} = \frac{Area\ i\ year\ x - Area\ \ i\ year\ x+1}{t\ years} \text{ X } 100\%$$

$$\% \text{ Annual rate of change} = \frac{Area\ i\ year\ x - Area\ \ i\ year\ x+1}{Area\ i\ year\ x\ X\ t\ years} \text{ X } 100\%$$

Where $Area_{iyearx}$ = area of cover i at the first date, $Area_{iyearx\ +1}$ = area of cover i at the second date, $\sum_{i=1}^{n} Area\ iyearx$ = total cover area at the first and t_{years} = period in years between the first and second scene acquisition dates.

The recognized land cover/use patterns from satellite images were exported from pixel format into a polygon and then into shape files with the help of Arc-GIS 10. The 1986 land cover/use pattern was taken as a baseline in the analyses of changes.

2.3 Community perceptions regarding land cover/use changes and CBM of water hyacinth

To gain insight into the perceptions of the communities living along the infested areas regarding land cover/use changes and relate this with the findings from imageries analyses, and to assess CBM of the weed, focus group discussions (FGDs) were held. Peasant associations (lowest administrative unit) in the infested localities were identified purposely with the staff of the district Offices of agriculture, livestock, irrigation, and peasant associations. Eleven FGDs, i.e., 6 in Bora district (Meto Aleka, Ashwa, Malima berie, Elltoke, Ellen 1 and 2), 4 in Lume (Koka Negewo, Danguge Bakale, Derera Denbela, and Adada Denbela) and one around Aba Samuel Lake (It is blamed to be the source of water hyacinth by communities) were undertaken and participants of the FGDs were 8 to 15 people from different sexes, age groups, and educational backgrounds.

2.4 Consultative meeting/workshop

The research team and the Office of agriculture of east Shoa zone organized a one day consultative meeting for researchers and development actors from across the spectrum to present and discuss research findings, identify research and development gaps, and the approach to be followed in the management of the weed. A total of 35 development and research actors participated in the meeting.

III. RESULTS AND DISCUSSION

3.1 Water hyacinth and associated land cover/use maps and their characteristics

In line with the objective of this study, five different land cover/use types were identified on 1986 and 2015 Landsat images. These are water bodies mainly found in lakes and hydroelectric dams; wetlands, area covered by water hyacinth, urban and other covers combined together. Each of the land cover is described in brief in Table 1 and shown in Figures 3 and 4. The difficult part in the analyses of the images was the identification of water hyacinth affected areas particularly on the 1986 image so we substantiated that by information we got from elders.

TABLE 1
BRIEF DESCRIPTION OF LAND-COVER TYPES IDENTIFIED ALONG THE WATER HYACINTH AFFECTED AREAS IN THE STUDY DISTRICTS

No	Land cover-type	Brief description
1	Water body	Non-flowing, naturally enclosed bodies of water, including regulated natural lakes. The delineation of this class is based on the areal extent of water at the time the remote sensor data are acquired
2	Wetland	Are those areas where the water table is at, near, or above the land surface for a significant part of most years. They are perennial /seasonal swamps /marshy areas.
3	Water hyacinth	Areas potentially caught by water hyacinth actual or remnants of it observed. It is a perennial, herbaceous, attractive blue or purple flowers, oblong to oval glossy leaves with bulbous petioles, known as queen flower, form impenetrable mats
4	Urban	Built-up areas /town areas
5	Others	Includes cultivated land, grassland, forest land and etc.

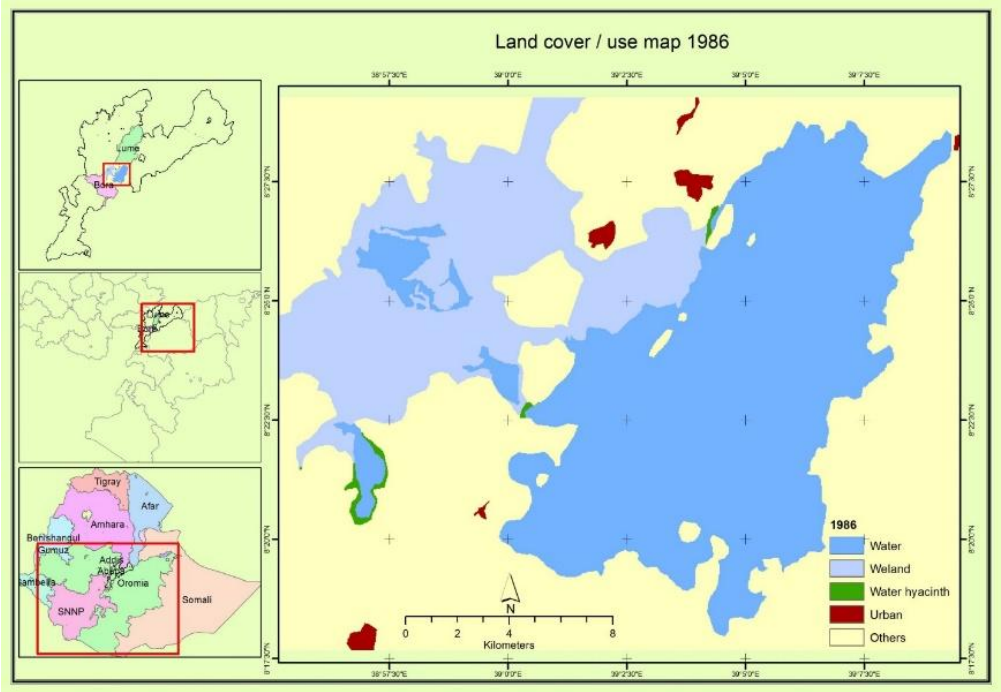

FIGURE 3. LAND COVER/USE MAP 1986

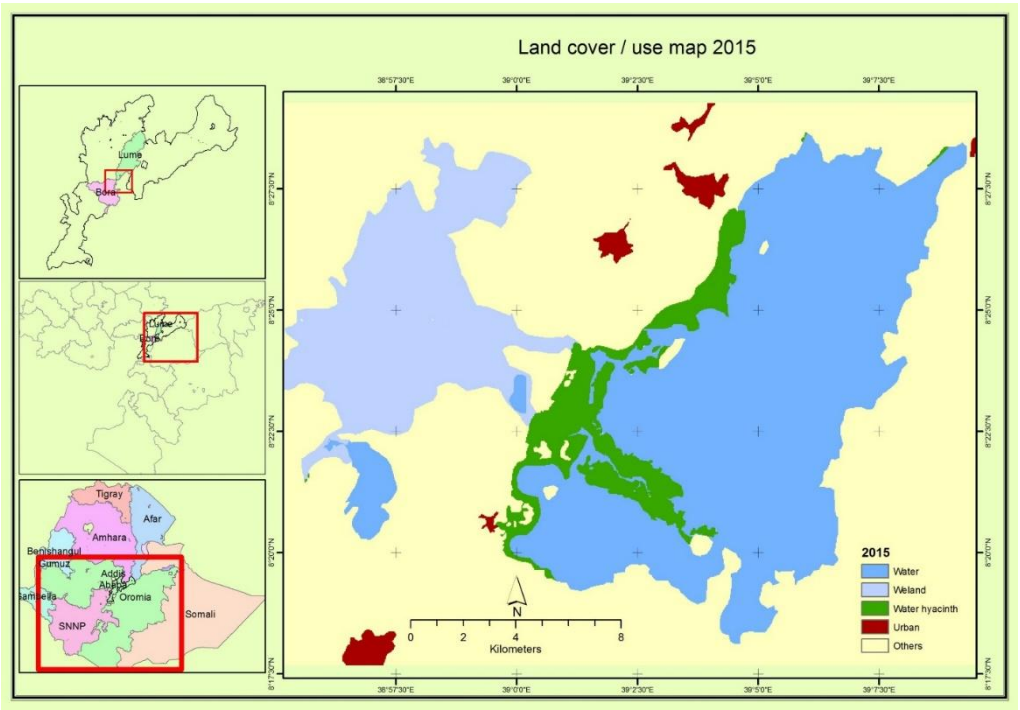

FIGURE 4. LAND COVER/USE MAP 2015

3.2 Land cover/use changes

Table 2 presents the coverage of each land cover/use class in 1986 and 2015 including the area and percentage area change between the two periods for the study area. In the periods considered for the study, our result showed, the land cover categorized as others was the largest in terms of area coverage because it encompassed different land uses/covers like cropland, grazing land, forest land, bare land and etc. Of these land covers, according to the opinion of the FGDs, the size of cultivated land and bare land has increased from time to time. As part of the Central rift Valley, east Shoa zone is well known for the presence of many lakes and a dam. According to the FGDs, the water bodies assessed were there before 30 years in their current places with some changes in their area cover with time. The wetlands which cover large shore areas provide

attractive scenery especially during the rainy season and was the third largest in terms of area coverage in both 1986 and 2015. In 1986, the area covered by town/built up area was larger than the land occupied by water hyacinth while the opposite was true for 2015 (Table 2 and Figures 3 and 4).

TABLE 2
COVER AREA, CHANGED AREA AND THE RATE OF CHANGE BETWEEN 1986 AND 2015

Land covers	Land cover (1986)		Land cover (2015)		Change area (ha)	% change	Annual rate of change (ha/year)	Average annual rate of change (%/ year)
	Cover area (ha)	% cover Coverage	Cover area (ha)	% cover Coverage				
Water body	17320.86	30.29	14842.71	25.97	-2478.15	-4.33	-85.45	-0.15
Wetland	8971.74	15.69	5943.06	10.4	-3028.68	-5.3	-104.44	-0.18
Water hyacinth	145.53	0.25	2319.48	4.06	2173.95	3.8	74.96	0.13
Town	322.47	0.56	582.39	1.02	259.92	0.45	8.96	0.02
Others	30414.87	53.2	33487.83	58.59	3072.96	5.37	105.96	0.19
Total land area	57175.47		57157.47					

As indicated in Table 2, between 1986 and 2015, water body and wetland declined by 4.33% and 5.3%, respectively while the area under water hyacinth, town and others increased. The annual rate of decline in cover was the highest for wetland (0.18%) followed by water bodies (0.15%) implying that they have lost their land for other land cover/use types. The decline in the size of wetlands and water body in the study districts goes in line with the general situation prevailing in Ethiopia [8] and globally [9] where these land covers are declining from time to time. While threats vary between regions and even within wetlands, agriculture is considered to be the most significant land use type that is replacing the wetlands [10]. The study found out that the decline in area by these land covers is because of the increase in area of water hyacinth and vegetable production using irrigation which was also witnessed by the FGDs.

Water hyacinth has a high growth rate as it can double its area coverage in only 5 days [11]. According to the opinion of nine of the FGDs, the area under water hyacinth has increased in their vicinities. Wind and water are believed to play a role for the expansion of the weed. With increase in the volume of water in the Awash River (July and August) and putting large areas under water and aided by wind, the amount of the weed coming to the different peasant associations increases and vice versa. In October, with the decrease in the volume of water and the changes in the direction of the wind, the weed is taken away and deposited mainly in Malima Berie, Elletoke and in part of Ellen peasant associations in Bora district. These sites need due consideration in controlling the expansion of the weed.

Even if the weed seems to have dried, it easily germinates and grows again if it gets water. Gopal (1987) [1] and [12] reported that water hyacinth can flower throughout the year, release more than 3,000 seeds per year, and double its population in as little as two weeks. The seeds are long-lived and can live up to 20 years [1]. While seeds may not be viable at all sites, water hyacinth commonly colonizes new areas through vegetative reproduction and propagation of horizontally growing stolons [13]. Studies have also shown that the weed favors water bodies with high-nutrient levels, such as those found downstream of agricultural or urban areas. Under such situations, water hyacinth can increase its biomass eightfold compared to water bodies that are nutrient poor [14]. Abraham (2009) [15] from his study at Aba Samuel dam near Addis Ababa reported that increase in temperature, and eutrophication by ways of increase in nutrient level and distribution to be factors contributing to water hyacinth expansion.

On the other hand, focus group discussants at Ellen PA, in Bora district, who are mainly vegetable producers disclosed that water hyacinth is disappearing from their PA particularly since the last 5 years. The main reasons for this are the increased intensity of irrigated vegetable production and the hoof action/trampling of the high livestock density coming to the area for drinking water.

The study districts are close to Addis Ababa, Adama, and Awassa and they are the major suppliers of vegetables to these cities. Many jobless youth and women came to these districts to get jobs. As these districts are economically active areas, the increase in the size of town/built up areas is an expected happening. According to Bassi et al. (2014) [10], rapid urbanization, and industrialization are some of the causes for decline in wetlands and water bodies globally.

3.3 Land covers transformation/flow

The land covers transition matrix between land cover classes in 1986 and 2015 is shown in Table 3. All land cover categories except for town/built up areas changed but with varying magnitudes and variations in magnitude of change in different land covers/uses is quite common in this type of study.

TABLE 3
CHANGE DETECTION MATRIX IN DIFFERENT LAND USE/COVER COVERAGE (HA) BETWEEN 1986 AND 2015.

Cover in 1986 (ha)	Cover in 2015 (ha)				
	Water	Wetland	Water hyacinth	Town	Others
Water	(14601.78)	727.65	1557.72	0	433.71
Wetland	49.5	(5215.41)	101.97	0	3604.86
Water hyacinth	108	0	(37.53)	0	0
Town	0	0	0	(322.47)	0
Others	83.43	0	622.26	259.92	(29449.26)

Note: The numbers in brackets indicates the cover area which remained unchanged between 1986 and 2015, while the numbers without the bracket indicate the flow of covers or covers that changed to other cover category.

Table 4 presents the detected changes in cover for the period of 1986 and 2015 deduced from the change detection matrix. For instance, a total of 3,756.33 hectares of land from the wet land area was changed to others, water hyacinth and water body areas (Table 4; Figure 6).

TABLE 4
DETECTED CHANGES IN PERCENTAGE FOR LAND COVERS/USES FOR THE PERIOD 1986 TO 2015.

Changes	1986-2015 area (ha)	% of the cover
Wetland to others	3604.86	6.30
Others to urban	259.92	0.45
Water to others	433.71	0.76
Others to water hyacinth	622.26	1.09
Water to water hyacinth	1557.72	2.72
Others to water	83.43	0.15
Water to wetland	727.65	1.27
Wetland to water hyacinth	101.97	0.18
Wetland to water	49.5	0.09
Water hyacinth to water	108	0.19

Table 5 presents a summary on changed and unchanged cover areas between 1986 and 2015. The percentage changed indicates the percentage area of a particular cover which might have changed to different covers while the percentage unchanged represents the percentage area of the original area of a particular cover which remained unchanged for a given period. From Table 5, the water body changed to different land covers by 15.7% while wetland by 41.87%. Nevertheless, water hyacinth increased by 74.21% while town/built up area by 100.0%.

TABLE 5
PERCENTAGE CHANGES OF INDIVIDUAL COVER BETWEEN 1986 AND 2015.

Cover	Unchanged	Changed	% unchanged	% changed
Water	14601.78	2719.08	84.30	15.70
Wetland	5215.41	3756.33	58.13	41.87
Water hyacinth	37.53	108	25.79	74.21
Urban	322.47	0	100.00	0
Others	29449.26	965.61	96.83	3.17

The classification accuracy assessment is shown in table 6. Of all the classes, the land cover/use category "others" exhibited low user accuracy because it combined different land cover/use types (Table 6).

TABLE 6
THE ACCURACY LEVEL OF EACH LAND COVER CATEGORY AND ERROR MATRIX SHOWING CLASSIFICATION ACCURACY

Classification	Water	Wetland	WH	Urban	Others	Total
Water	50	0	2	0	0	52
Wetland	0	32	0	0	0	32
Water hyacinth	0	0	39	0	0	39
Town	0	0	0	48	1	49
Others	0	18	9	2	49	78
Total	50	50	50	50	50	250

Classification	Reference Total	Classified total	No. of corrected	Producer Accuracy (%)	Users Accuracy (%)
Water	50	50	50	100	96.15
Wetland	50	32	32	64	100
Water hyacinth	50	39	39	78	100
Town	50	48	48	96	97.96
Others	50	49	49	98	62.82
Total	250	218	218		
Overall accuracy (%)				*87.2 %*	
Overall kappa value				*0.84%*	

Kappa: Estimated as (Ǩ). It reflects the difference between actual agreement and the agreement expected by chance.
Kappa of 0.84 means there is 84% better agreement than by chance alone.
Kappa = observed accuracy – chance agreement/1- Chance agreement
Observed accuracy determined by diagonal in error matrix.
Chance agreement incorporates off-diagonal.

3.4 Community perceptions regarding land cover/use changes and community-based management

All of the FGDs in Lume and 5 of the 6 FGDs in Bora revealed water hyacinth to be of no use to them. Only one FGD in Bora, composed mainly of farmers involved in irrigated crop production (tomato, onion) around Ellen Lake, informed the plant to be useful in increasing soil fertility and used as cover plant for seedling establishment and during charcoal preparation. Although, our study did not include chemical composition analysis of the plant, studies elsewhere indicated that owing to its high alkalinity (pH>9) and potentially toxic heavy metals contents would restrict its use to flowering-plants, with no allowable application to horticulture for edible vegetables [16] which requires further investigation under our context.

The Aba Samuel Dam near Addis Ababa (capital city of Ethiopia) is commonly blamed to be the source of water hyacinth by the FGDs in the lower riparian and in literatures [e.g., 15]. The group discussants at the site, however, reported that because of the different interventions undertaken in the past 6 years (drying the water and burning the weed repeatedly), water hyacinth has almost disappeared from the area which requires further ecological study in the area.

In both districts, there is no control and/or eradication method designed by the community for managing the weed. Traditionally, communities having croplands along the water bodies pull, pile up, dry, and burn the weed for the sake of clearing their farm land but not meant for controlling the expansion of the weed. Able farmers spend about 2,500 Ethiopian birr (114.10 USD)/0.25 ha) for pulling, turning, piling up, drying, and burning the weed. Very recently (2014), the Oromia Regional government of Ethiopia, Bureau of agriculture, has started manual removal of the weed by making some payment for community members.

The group discussants also revealed the possibility of controlling/eradicating the weed from their area if the plant can be controlled from the source which is the upstream of the Awash River. Some of the interventions to be undertaken as suggested by the group discussants are:

- Organize communities to burn the plant when it has dried and regular removal of the weed
- Technical and financial support be given by government and others for the community particularly for the removal of the weed inside the water bodies as it will be difficult to remove by human labor alone
- Training, awareness creation and strong institutional linkage

According to the group discussants in both districts, there is no CBM and it is only individual effort to clear the weed from his/her piece of crop land. While the idea of CBM is well accepted in all most all the FGDs and discussions held at different levels of the offices in the region, the need for proper training on the concept, the way it can be applied, awareness creation to the communities, and institutional linkage are highly emphasized for its implementation. According to Mironga (2014) [3], the management of water hyacinth could be government based, community based or co-managed with a strong component of community participation. Mironga (2014) [3] discussed particular attention should be given to community mobilization, access to information, and coordination of community-based activities. Communities should also have a role to play in water hyacinth monitoring and the creation of early-warning systems.

Based on the results of the study, we suggest, the management of water hyacinth in the study area first be handled by co-management and based on the results be upgraded to CBM as there are many things to be fulfilled in relation to CBM.

3.5 Development and research strategy

Based on the different methods used to undertake the study, short and long term research and development strategies are outlined (Table 7).

TABLE 7
SHORT AND LONG TERM RESEARCH AND DEVELOPMENT STRATEGIES

Short term research and development strategies	Long term research and development strategies
• Conducting status survey to understand the magnitude of the damage it is causing • Review what we have at hand • Survey the distribution and perceptions nation wide • Categorizing infested habitats such as riverside, lakes (closed water), rivers of the infested area and demarcation • Stakeholders analysis (once identified, they should be key participants in water-hyacinth research and control. Secondly, seek to identify key stakeholders in water resources and entrust them with control of water hyacinth • Quantification of the socioeconomic and environmental consequences of water hyacinth (the exact magnitude of the social, economic, and environmental problems it causes is poorly understood in Ethiopia)	• Research on policy so as to consider local, national and regional technology policy and transfer in relation to water-hyacinth control mechanisms currently in use. At each of these levels, those concerned should identify, develop, and implement policy on water-hyacinth control. • Effectiveness of available control methods —the available methods are not precisely known for their relative effectiveness in various situations. Furthermore, each one of these approaches (biological, chemical, and physical measures) has strengths as well as weaknesses. Although there is consensus on the need to combine more than one of these methods in an integrated strategy, no one has undertaken research to develop this integrated approach in Ethiopia. • Adoption of high technologies for eradication of water hyacinth • Use of biological agents • Active involvement of the stakeholders

• Identify alternative methods, their costs, and effectiveness of physical methods • Determine acceptable threshold levels for water hyacinth • Document the success or failure of control efforts and implementation and disseminate this information to key players in water-hyacinth control across the zone in good time. • Establishment of steering committee with responsibilities • Awareness creation and further mass mobilization of the community • Preparing community bylaws • Implementation of control strategies using manual or mechanical and chemical methods • Improving the awareness of top level policy makers	• Develop mechanisms for early recognition of impending water-hyacinth infestations and problems, using local and national surveys and monitoring. Use these mechanisms to send early warnings of impending water-hyacinth infestations. More research to be conducted on the role of early-warning mechanisms in the evaluation of programs. • Conduct Environmental Impact Assessments (Because water hyacinth may contain up to 95% water, environmental consequences of the removed and decomposing weed must be evaluated and elucidated). Furthermore, where manual removal is being done particular attention must be paid to water hyacinth's association with harmful organisms, such as snails, and implications of manual removal for human health and ensure adequate protection for those handling the weed.

IV. CONCLUSION

The results show that the extent of the problems in the study area vary from site to site requiring different approaches and use of different techniques for management of the weed, i.e., physical (manual and mechanical) and biological interventions in single or in combination depending on the nature and extent of the problem. Intensified monitoring, mitigation and management measures are needed to keep water hyacinth at unproblematic levels. For this to happen, it is very clear that the role of communities in control/eradication of water hyacinth should be increased through training, awareness creation, strengthening linkages, and supporting the communities by proving resources which are beyond their reach.

In view of the major findings of the study and the above conclusions, the following recommendations can be drawn:

- It is critical to develop comprehensive management strategies and action plans. A multidisciplinary approach should be designed, which ensures that the highest political and administrative levels recognize the potential seriousness of the weed. Solicit the good will of politicians to support water hyacinth control
- Plans should also state clearly the role of each government department, stakeholders, municipal councils and local community involved in the fight against water hyacinth
- Methods for water hyacinth control should include reduction of nutrient load in the water bodies through treatment of waters flowing from sewage works, urban wastes and factories. Changing land use practices in the riparian communities through watershed management will help reduce agricultural runoff as a mechanism for controlling the proliferation of water hyacinth
- Early detection and monitoring are critical for the management of water hyacinth, as successful eradication or containment is normally only possible when infestations are small. This is also essential to avoid re-establishment and further spread from the soil seed bank
- Education programs particularly public education and establishment of information center need to be undertaken

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Improvement of Crop Production by Means of a Storage Effect

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Abstract**—** *This study summarizes the results of 30 years of our experiments with Vicia faba L seeds. Our long-term practical observations of different Vicia faba L. cultivars points out the method useful for the higher yield of seeds in terms of their viability and thus higher crop production.*

Our experiments led to the following important findings regarding of seed viability:

1. *Individual and group variability of seeds;*
2. *Storage condition before germination; and*
3. *The condition of their germination.*

All these three influential conditions is possible to optimalize by method of storage effect described in this our report resulting in the improvement of crop production. This is especially important in case of seeds that are rare and/or expensive, i.e. seeds that are genetically modified or with rearranged karyotypes.

Keywords— seed color, higher germination, improvement of viability, higher crop production.

I. INTRODUCTION

Seeds have been studied for more than hundred years (cf. Murín 2001; Murín and Mičieta 2009). The first reports uncovered the relationship between the decline of their vitality and their storage conditions (Navaschin 1933; Cartledge and Blakeslee 1934, 1935; Stube 1935; Nichols 1942; D'Amato 1951; Murín 1961; Avanzi et al. 1969). Since respiration is the most marked manifestation of metabolism in stored seeds, it should also be considered. Rieger and Michaelis (1959) found that *V. faba* seeds are susceptible to the action of ethanol or other "automutagens" which can accumulate during the respiration of seeds stored over long period. Bewley and Black (1982, 1994) explored the relationship between the color of the testa and the dormancy and germination of wheat, as affected by the level of inhibitors (catechinins and their derivates) occurring in the testa. Floris and Anguillesi (1974) made a major contribution to the understanding of this external manifestation of the internal state of broad bean seeds when they reported on several biochemical and functional changes in aging seeds. Over the course of long storage, enzymes like catalase, peroxidase, cytochrome oxidase and decarboxylase display diminished activity, while the protein-synthesizing capacity of older seeds is lost in the process of germination. Furthermore, membrane permeability increases, resulting in reduced sugars and other metabolic products.

According Roos (1980) four factors must be considered in seed storage – time, temperature, relative humidity (seed moisture content) and a level of oxygen. With the exception of recalcitrant species, two factors – time and oxygen level, have very little effect on storability if the optimum seed moisture content and storage temperatures are observed. For example, Roberts and Ellis (1977) predicted the 95% survival of pea (*Pisum sativum* L.) seeds after 1,090 years of storage at -20°C and 5% seed moisture content. If the storage temperature is reduced further, the viability may be extended indefinitely. Attempts to prolong life of seeds during the storage were focused at the use of liquid nitrogen (LN2) as a storage medium with a temperature -196°C. At this temperature, presumably all biochemical activity is reduced to essentially zero. Thus the deteriorative changes noted above should be eliminated. According Babasaheb (2004), safe seed storage moisture should be less than 8%.

In 1981, King et al. reported that the survival of lemon (*Citrus limon* L.), lime (*C. aurantifolia* Swing.) and sour orange seeds (*C. aurantium* L.) was examined under a wide range of constant moisture contents and temperatures. Seed longevity was increased by decreasing the moisture content and temperature of the storage environment. Maximum viability was maintained in a combination of storage conditions including the lowest moisture content (5%) and lowest temperature (-20°C). The practicality of the dry storage of citrus seeds for genetic conservation was pointed out.

Bonner (1990) offered classification of stored seeds into four classes of storage characteristics: „'true orthodox' seeds can be stored for long periods at seed moisture contents of 5–10% and sub-freezing temperatures; 'sub-orthodox' seeds can be stored under the same conditions, but for shorter periods due to high lipid content or thin seed coats; 'temperate recalcitrant' seeds cannot be dried at all, but can be stored for 3–5 years at near-freezing temperatures; and 'tropical recalcitrant' seeds also cannot be dried, and they are killed by temperatures below 10–15°C."

Grilli et al. (1995) described the level of Poly (A) Polymerase as a significant marker of the viability of seeds during their long term storage. Also, during imbibition the production of the major organic volatiles, ethanol and acetaldehyde, depends greatly on the long term storage of the seeds (Górecki et al. 1992). Murthy et al. (2002) identified two primary biochemical reactions responsible for deterioration of seed vigour during long term storage – lipid peroxidation and non-enzymatic protein glycosylation reducing sugars. The PCR analysis of Chwedorzewska et al. (2002) led the authors to the conclusion that long term storage of seeds causing the loss of their viability also generates heritable changes in the preserved germplasm. On the other hand, antioxidant activity in stored seeds under different conditions (temperature and w.c.) is not related to seed viability (Merritt et al. 2003). However, Andreev et al. (2004) found that the loss of germination during the storage of rye seeds was accompanied by a decreased excision of chromatin loop domains. As Patrick and Stoddard (2010) stated, "the large seed size of the faba bean has enabled this species to be a model for studies of the molecular physiology of seed development."

The darkening of the testa of aging *V. faba* L. seeds and its manifestation has been a practical part of our work since 1988 (Murín 1988 a, b). Today we know that the color of the testa indicates the viability as well as the age of the seeds. Our goal was to study the relationship between the different storage conditions, the color of the testa of seeds and the viability of the seed samples.

II. MATERIALS AND METHODS

2.1 Seed samples

For our experiments we used sets of *V. faba* L. cv. Inovec seeds of the standard karyotype harvested in 1974 and also for each year from 1976 to 1982. Non-standard, rearranged ACB karyotype (Michaelis and Rieger 1971) were harvested in 1975 and 1982. The oldest cultivar Přerovský was from 1971.

The colors of the seeds were classified from A to U according to Fisher-Saller's scale in order to determine their individual variability. Originally designed for hair color, this scale was used for the first time by us because it registers a wide spectrum of brown hues. In the first experiment, a total of 1,419 broad bean seeds were examined in this way. The effect of storage time and conditions on seed coat color was also reported by Yousif et al. (2003) in their study of Australian adzuki beans.

2.2 Soaking and germination

The conditions for standard soaking and germination were altered during our experiments according to the knowledge we obtained in each experimental stage. Our optimal set involved the soaking of seeds in plastic jars that allowed their continual air-bubbling of distilled water. The seeds were then germinated in wet sawdust. The last six experiments were germinated and grown in intact material Perlite.

As *V. faba* is sensitive to hypoxia, we made the following arrangements to prevent higher sensitivity affecting the results of our experiments: a) better air circulation in the desiccators, which were not kept at 25 °C, but at laboratory room temperature; b) during soaking, we used 5% chloramine B to prevent microbe contamination of the seedlings which were treated for 30 min and then washed with distilled water; and c) the seeds were germinated in wet sawdust instead of wet cotton wool which does not permit the satisfactory respiration of seeds.

2.3 Storage

2.3.1 Storage conditions

All of the harvested *V. faba* seeds from our supply were stored at room temperature. Rearranged ACB karyotype was stored at 4 °C.

Twenty-two seed samples from eight countries, nineteen cultivars and nine harvests (from 1972 to1984) from the Seed Bank in Gatersleben, Germany, were divided into two groups (A and B). Groups A and B were stored in the seed bank at temperatures of -10 to -17 °C, and +14 to +20 °C respectively.

The seeds were then stored for 0 or 8 days at 25 ^{o}C above 600-mL sterile water at room temperature in the desiccators. Following treatment, washing and re-drying, half of the seed samples were allowed to germinate immediately and the root-tips were cut and put into fixation solution after two recovery times (48 h and 72 h). The other half of the seed samples were allowed to germinate after 8 days of storage.

2.3.2 Specific water content (w.c.)

After treatment and washing, the seeds were re-dried at 50% by heating for 2 h at 37 °C in a thermostat with a fan to obtain a specific w.c. Half of the seed samples were allowed to germinate immediately, and the root-tips were fixed after various recovery-times. The other half of the seed samples were stored for 8 days and then allowed to germinate.

2.3.3 Control of water content

For control of w.c. in seeds during the experiment, an extra sample of ten seeds was weighed before and after special drying (8 h at 105 °C) and calculated according to the formula 100 – (Yx100/X) = w.c., where X = weight before drying and Y = weight after drying.

2.4 Mutagen treatment

In one experiment of this series, *Vicia faba* seeds were first treated for 5 h with a dose of 2mm of methyl methanesulphonate (MMS, Merck) in distilled water at pH 4.8. After the mutagen treatment, the seeds were washed for 2 h in tap water to eliminate the mutagen residue.

2.5 Tests of vitality

The vitality and length of the roots (from sets of 35 seeds) were measured for precise time periods: 36h, 48 h, 72 h, 80 h and 96 h, 120 h, 144 h and 168 h if necessary.

Both groups of seeds from the Seed Bank in Gatersleben were tested periodically from 1991 until 1999 to record the viability and frequency of aberrant ana-telophases.

2.6 Cytological evaluations

For cytological evaluation we chose ana-telophases in accordance with other authors (Bezrukov and Lazarenko 2002). These mitotic figures are simpler to evaluate and thus allowed us to experiment with a large number of samples under different mutagen doses and recovery times. The mutagen-treated roots of seed samples were fixed in ethanol (1N): acetic acid (1N) in rate 3:1, squashed and stained by aceto-orcein. On average, 200 ana-telophases (50 in control) per recovery time were evaluated on the occurrence of fragments (F), bridges (B) or both (F+B).

2.7 Statistical methods

We used a standard Student's t-test to evaluate the SEM. All evaluations were conducted under blind conditions.

III. RESULTS AND DISCUSSION

3.1 Individual and Group Variability

V. faba L. seeds have been used for decades as an experimental model in cytological laboratories, and the biological characteristics of this genus are widely known. However, insufficient attention was paid to the darkening of seeds during their storage. A closer examination of this phenomenon bore striking implications for our research. Although the seeds seem to be the same, they express their individual and group variability by the colors of their testa.

In our first experiment, we studied the seeds' light and dark colors in relation to the years when they were harvested. Only seeds showing a definite color were evaluated; those of intermediate colors were excluded from our final evaluation. Table 1 and Fig. 1 show the gradual darkening of the seeds over an 11-year period.

TABLE 1
PROPORTION OF DARK AND LIGHT SEEDS IN THE COURSE OF AGING

Harvest (year and cultivar)	Proportion and color type of seeds		No. of seeds examined	No. and color type of seeds excluded
	light	dark		
1982, ACB	100%; ACB	0.00%	32	0
1981, Inovec	68.78%; A-B	12.14%; G-H	173	33; E, F
1980, Inovec	25.80%; B-C	20.00%; Q, R, S	155	84; F, G, H
1979, Inovec	17.05%; B-C	65.88%; Q, R, S	129	22; G, H
1978, Inovec	11.46%; C-E	71.97%; Q, R, S	157	26; L, M
1977, Inovec 1976, Inovec 1975, ACB 1971, Přerovský	5.48%; A, B, C 5.71%; C-E 7.05%; A-C 0.00%	70.54%; R, S, T 86.28%; S, T 85.47%; R, S, T 100%; U	146 175 241 211	35; E, F G 14; F, G 17; K-L 0

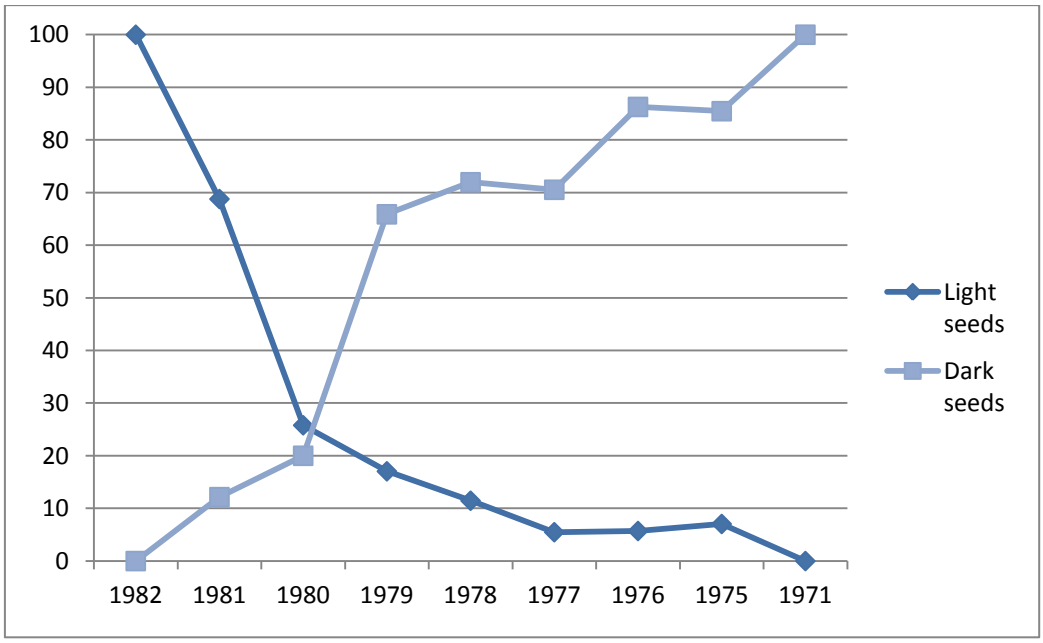

FIGURE 1: PROPORTION (%; ORDINATE) OF LIGHT AND DARK SEEDS IN DIFFERENT HARVESTS (ABSCISSA)

In a set of seven-year-old seeds (harvested in 1975) of the rearranged ACB karyotype that were stored in 4 ^{o}C instead of room temperature we observed a small deviation from the gradual darkening tendency. The higher the proportion of viable seeds and thus the better germination rate of this seed set confirmed the relationship between seed viability and storage conditions that was also stated by Michailov and Korytova (1971). We noted with interest the complete loss of viability of 9-year-old cv. Přerovský seeds and their uniformly dark color (among the darkest on the color scale – U).

In addition to confirming the irreversible tendency of seeds to darken in the course of their storage, these experiments suggested a possible difference in the viability and germinating capacity of seeds harvested in the same year, but differentiated by external darkening. Three differently arranged independent experiments (Tables 2 – 4) revealed a clear difference between dark and light seeds. It shows the importance of individual or inter-individual variability among seeds of the same age, externally manifested by darkening.

TABLE 2
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS.

Years of storage and color of seeds	No. of germinated seeds out of 16 after 96 hrs	168 hrs	Germination rate %
6 years, light	10	4	87.5
dark	0	2	12.5
7 years, light	7	6	81.2
(ABC) dark	0	6	37.5

TABLE 3
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS.

Years of storage and color of seeds	No. of germinated seeds out of 16 after 98 hrs	Germination rate %
4 years, light	14	87.5
dark	3	18.7
7 years, light	11	68.7
dark	3	18.7

TABLE 4
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS

Years of storage and color of seeds	No. of germinated seeds after 72 h	96 h	120 h	144 h	168 h	Germination rate %
3 years, light	9	5	0	1	0	93.75
dark	2	3	4	1	2	75.00
6 years, light	5	6	2	2	0	93.75
dark	0	0	0	0	2	12.50

The next two experiments aimed at chromosome aberrations of light (more viable) and dark (less viable) colored seeds. In the first of these experiments, we devoted most of our attention to the 1st-through-2nd mitosis (roots 12-20 mm long) and 3rd mitosis (roots 20-30 mm long). A total of 5 750 anaphases were examined. Table 5 illustrates the differences in the degree of chromosome aberration within a harvest (e.g., after 4 years of storage, between light $0.75 \pm 0.14\%$ and dark $2.30 \pm 1.18\%$ seeds), the gradual differentiation among harvests having been preserved (dark seeds 1979: $1.73 \pm 0.7\%$, dark seeds 1976: $6.00 \pm 0.7\%$).

TABLE 5
CHROMOSOME ABERRATIONS IN LIGHT AND DARK SEEDS IN THE FIRST MITOSES

Years of storage and color of seeds	Root length mm	Aberration rate % (No. of anaphases examined)				Total aberration rate %
		72 h	94 h	120 h	144 h	
4 years, light	12-20	2.0 (800)	1.5 (400)	0.0 (400)	1.0 (300)	1.13 ± 0.4
	20-30	0.8 (400)	1.0 (300)	0.5 (200)	n.d.	0.75 ± 0.1
dark	12-20	1.3 (300)	3.6 (300)	1.5 (300)	0.5 (200)	1.73 ± 0.7
	20-30	2.3 (300)	n.d.	n.d.	n.d.	2.30 ± 1.2
7 years, light	12-20	3.3 (700)	2.0 (200)	1.0 (200)	1.0 (150)	1.82 ± 0.5
	20-30	1.0 (250)	1.6 (300)	1.0 (150)	n.d.	1.20 ± 0.2
dark	12-20	n.d.	7.0 (200)	5.0 (200)	n.d.	6.00 ± 0.7
	20-30	n.d.	n.d.	n.d.	5.0 (150)	5.00 ± 0.0

n. d. = not detected

Examining seeds stored for 4 and 7 years, we found that the differences within a seed set were more marked in older seeds, while dark seeds were similarly differentiated in the early mitotic cycles. In an additional experiment, we confirmed this tendency in later mitoses as well. After 98 h of germination, 30 root-tips containing 3,000 anaphases were examined. The root-length varied from 28 to 84 mm depending on the individual variability, while in most roots it was about 50 mm. In light seeds of both harvests (Table 6), anaphases were evaluated separately at a root-length of 28-34 mm (3rd mitotic cycle) and of 44-57 mm (4th mitotic cycle).

TABLE 6
CHROMOSOME ABERRATIONS IN LIGHT AND DARK SEEDS IN LATER MITOSES

Years of storage and color type of seeds	Aberration rate %		
	In roots long		
	28-34 mm	44-57 mm	Total
4 years, light	0.0	1.2 ± 0.37	0.66 ± 0.3
dark	2.60 ± 0.5	n.d.	2.60 ± 0.5
7 years, light	1.5 ± 0.82	0.5 ± 0.82	1.00 ± 0.3
dark	2.00 ± 1.2	n.d.	2.00 ± 1.2

Three hundred anaphases were examined in each test.

Table 7 shows some difference in the aberration rate between light and dark seeds of the same harvest, while the aberration rate among seeds of the same color harvested in different years was almost the same. This shows a clear manifestation of viability dependent at individual variability of seeds.

TABLE 7
COMPARISON OF CHROMOSOME ABERRATION RATE WITH GERMINATION RATE OF SEEDS STORED FOR 4 AND 7 YEARS.

Years of storage and color type of seeds	Aberration rate %	Germination rate %
4 years, light	0.66 ± 0.3	87.5
dark	2.60 ± 0.5	18.7
7 years, light	1.00 ± 0.3	68.7
dark	2.00 ± 1.2	18.7

Three hundred anaphases were examined in each test.

This finding is in accordance with already reported changes in the aberration rate of cells in seeds during their long-term storage (Avanzi et al. 1969; Sevov et al. 1973; Cebrat 1977; Murín 1988a). The comparison of data presented in Tables 1 – 4 confirms this conclusion in relation to seed viability and chromosome damage in root-tip cells (Tables 5 – 7). In the present case, the external signs of reduced viability (darkening) were manifested, irrespective of their age, with almost corresponding consequences for genetic and physiological damage. The differences observed between whole seed harvests (indiscernible among individual seeds) could thus be explained by a decreasing proportion of viable seeds and an increasing number of less viable (dark) seeds in the same set as a result of long-term storage. In *V. faba* seeds, this process could be followed from the 100% proportion of light seeds in the youngest set of one-year-old seeds (with the highest germination rate and lowest aberration rate) to the complete mortality of a seed set after 9 years of storage when the proportion of dark seeds had reached 100%.

To confirm these results, once again new experiments were designed (Tables 8 – 9). Two experiments involved 1-year old seeds selected by their color (Table 8), and two experiments involved 5-year old seeds with and without the mutagen treatment (Table 9). This time they were not germinating and growing in sawdust, but in intact material Perlite.

TABLE 8

ONE-YEAR OLD SEEDS SELECTED BY THEIR COLOR TESTED IN VITALITY. GERMINATION AND CHROMOSOMAL ABERRATIONS IN THEIR ROOT TIPS.

		72 h			96 h			120 h	
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.97	100.0	1	37.77	100	2	39.92	100	0.5
Dark seeds	22.15	87.5	2	26.12	90	1	27.62	90	1.5

TABLE 9

FIVE-YEAR OLD SEEDS WITHOUT AND AFTER TREATMENT FOR 5 H WITH DOSE OF 2mM OF METHYL METHANESULPHONATE (MMS. MERCK)SELECTED BY THEIR COLOR TESTED IN VITALITY. GERMINATION AND CHROMOSOMAL ABERRATIONS IN THEIR ROOT TIPS.

		72 h			96 h			120 h	
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.67	100	2.5	37.03	100	1.00	38.0	100	0.9
Dark seeds	21.40	87	4.5	23.43	87	5.57	24.8	87	1.0
		72 h			96 h			120 h	
MMS. Merck	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)
Light seeds	18.47	93.5	12.18	20.87	93.5	25.18	21.47	93.5	14.31
Dark seeds	13.70	66.5	8.50	15.60	70.0	17.79	15.87	70.0	25.04

The summarized results are found in Table 10 and confirm all of the previous findings and shows higher sensitivity of dark colored individuals in vitality, germination and chromosomal aberrations in their root tips. However, we also found some disturbances in this tendency in comparison with light colored individuals in chromosomal aberrations in recovery times of 72 h and 96 h.

TABLE 10

COMPARISON OF THE PREVIOUS RESULTS FROM TABLES 8-9 SHOWING AN AVERAGE IN ALL TESTED PARAMETERS (LENGTH OF ROOTS. GERMINATION AND C.A.) WITH SEM BETWEEN 1-YEAR OLD SEEDS (LINES 1-2). 5-YEAR OLD SEEDS (LINES 3-4) AND 5-YEAR OLD SEEDS TREATED BY MUTAGEN (LINES 5-6)

	72 h			96 h			120 h		
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.98±0.38	100.0±0.00	1.00±0.00	37.78±0.63	100.0±0.00	2.00±1.00	39.93±0.08	100.0±0.00	0.50±0.50
Dark seeds	22.15±1.80	87.5±2.50	2.00±0.00	26.13±3.23	90.0±0.00	1.00±0.00	27.63±4.48	90.0±0.00	1.50±0.50
Light seeds	32.67±0.20	100.0±0.00	2.50±0.50	37.03±2.70	100.0±0.00	1.00±0.00	38.00±3.47	100.0±0.00	0.90±0.90
Dark seeds	21.40±6.13	87.0±0.00	4.50±0.50	23.44±5.17	87.0±0.00	5.57±1.57	24.80±5.27	87.0±0.00	1.00±0.00
Light seeds	18.47±3.00	93.5±6.50	12.8±5.18	20.87±4.60	93.5±6.50	25.8±11.18	21.47±4.80	93.5±6.50	14.31±1.37
Dark seeds	13.70±5.10	66.5±3.50	8.50±1.50	15.60±5.13	70.0±10.0	17.79±10.79	15.87±5.27	70.0±10.00	25.04±10.90

3.2 Condition of seed storage before germination

Table 11 shows the spectrum of the tested samples of *V. faba* L. seeds from the Seed Bank in Gatersleben. It is interesting to note that although the temperature used for group A was expected to be in favor of the longer survival of *the V. faba* L. seeds in the case of *A. manglesii* and *M. tetragona*, Merritt et al. (2003) we observed the opposite effect of storage at a temperature of -18 ^{o}C in comparison with higher storage temperatures, contrary to the findings of other authors mentioned before. According Murthy et al. (2002), another extreme temperature for storage is above 40 ^{o}C. The darkening of testa was also confirmed for cv. Fiesta (Nasar-Abbaset et al., 2009).

TABLE 11
SPECTRUM OF THE TESTED SAMPLES OF *V. FABA* L. SEEDS AND THEIR CONDITIONS (– SHOWS ZERO VIABILITY).

Year	Cultivar	Country of origin	A	B
1972	Féverole du Gers	France	+	-
1975	Maly	Italy	+	-
1977	Parvin	Great Britain	+	+
1978	Milión	Czechoslovakia	+	+
	Diana	Czechoslovakia	+	-
	Kronberger	Germany	+	+
	tennis Murat	Ethiopia	+	+
1979	Féverole du Gers	France	+	-
	tennis Murat	Ethiopia	+	+
	Latvija	USSR	+	+
	Mazur No.18	Poland	+	+
	DornburgerAckerb.	Germany	+	+
		Great Britain	+	-
1980	Romana	Italy	+	+
	Maly	Italy	+	-
	Přerovský	Czechoslovakia	+	+
1981	Dire Dawa	Ethiopia	+	+
	Skorospelka	USSR	+	+
	Banská	USSR	+	+
1982	Equina	Italy	+	+
	tennis Murat	Ethiopia	-	+
1984	Maly	Italy	+	+

Both groups were tested periodically over nine years (1991 – 1999). The first evaluated parameter to be checked was seed vitality (i.e., their germinating capacity) according to their color; the difference is especially evident for summarized data obtained for the 1978-1984 harvests (Table 12).

TABLE 12
GERMINATION OF LIGHT AND DARK SEEDS FROM 22 DIFFERENT SAMPLESOF 17 CULTIVARS FROM 9 COUNTRIES IN CONSEQUENT YEARS.

Year of harvest	Light seeds					Dark seeds				
	1991	1992	1996	1997	1999	1991	1992	1996	1997	1999
1972	98.0	95.0	27.3	50.0	55.0	n.d.	n.d.	n.d.	n.d.	n.d.
1975	82.0	90.0	41.7	70.0	30.0	n.d.	n.d.	n.d.	n.d.	n.d.
1977	94.0	70.0	70.0	73.3	45.0	0.0	0.0	0.0	0.0	5.00
1978	100.0	96.3	95.8	97.5	92.8	100.0	27.5	23.3	10.0	18.3
1979	98.5	92.5	81.6	94.6	63.4	97.6	26.0	36.0	16.7	12.5
1980	92.0	83.3	74.5	51.1	32.4	92.0	43.3	30.0	4.4	7.5
1981	90.6	93.3	86.6	71.9	52.5	90.6	40.0	30.0	35.5	20.0
1982	95.0	75.0	73.3	80.0	40.0	95.0	50.0	13.3	20.0	10.0
1984	86.0	90.0	50.0	55.6	15.0	89.0	47.5	10.0	40.0	5.0
Total 1978-84	**93.7** ±2.13	**88.4** ±3.23	**76.9** ±6.39	**75.1** ±7.89	**49.3** ±11.03	**94.0** ±1.74	**39.0** ±4.14	**23.7** ±4.19	**21.1** ±5.74	**12.2** ±2.43

When confirming the weaker vitality of darker seeds stored in less favorable conditions, we were interested in the aberration rate as expressed by the summarized data in Table 13.

TABLE 13
SUMMARY OF ABERRATIONS PER YEAR.

Year of harvest		Light seeds			Dark seeds	
	1992	1996	1997	1992	1996	1997
∑ 1972	0.33 ± 0.0	4.81 ± 3.2	1.17 ± 1.2	n.d.	n.d.	n.d.
∑ 1975	0.66 ± 0.0	1.65 ± 4.9	1.51 ± 1.0	n.d.	n.d.	n.d.
∑ 1977	0.33 ± 0.0	1.00 ± 0.0	n.d.	0.00 ± 0.0	0.00 ± 0.0	n.d.
∑ 1978	0.49 ± 0.6	1.00 ± 3.3	0.08 ± 0.8	1.22 ± 0.2	1.10 ± 1.3	0.00 ± 0.0
∑ 1979	0.39 ± 0.4	0.00 ± 0.0	0.47 ± 1.2	3.26 ± 1.8	4.65 ± 2.4	0.19 ± 1.3
∑ 1980	0.44 ± 0.6	0.25 ± 0.9	1.04 ± 1.2	2.44 ± 1.2	0.25 ± 0.9	1.00 ± 1.0
∑ 1981	0.33 ± 0.6	0.57 ± 1.1	0.25 + 1.3	2.22 ± 0.8	0.00 ± 0.0	0.92 ± 1.9
∑ 1982	0.00 ± 0.0	0.00 ± 0.0	2.85 ± 0.0	4.66 ± 0.0	1.00 ± 0.0	0.00 ± 0.0
∑ 1984	1.00 ± 0.0	1.23 ± 4.9	1.58 ± 0.8	0.00 ± 0.0	3.08 ± 2.5	n.d.

n. d. = not detected

As noted, the aberration frequency was not very high in old seeds for significant results. Therefore, the most important tendencies in the evaluations from the years 1991, 1992, 1996, 1997 and 1999 best expressed themselves in the viability measured by the percentage of germination after 96 hours. The summarized results are shown in Table 14.

TABLE 14
AVERAGE GERMINATION IN % OF LIGHT AND DARK SEEDS FOR ALL EVALUATION TIMES. YEARS OF HARVEST AND CULTIVARS.

Light seeds			
	day 1	day 2	day 3
∑ 46.4 ± 24.6	32.4 ± 20.6	49.2 ± 21.7	56.8 ± 24.8
Dark seeds			
	day 1	day 2	day 3
∑ 11.7 ± 7.0	7.00 ± 2.5	11.7 ± 6.5	13.1 ± 7.70

The difference is significant, although it is affected by a wide range of germination of light seeds, which confirms our earlier results from a lesser number of Czechoslovak cultivars except in the case of the parameter of the root growth (Table 15.).

TABLE 15
AVERAGE ROOT LENGTH OF LIGHT AND DARK SEEDS FOR ALL EVALUATION TIMES. YEARS OF HARVEST AND CULTIVARS IN CM.

Light seeds			
	day 1	day 2	day 3
Σ 2.3 ± 1.5 cm	0.7 ± 0.12	2.2 ± 0.59	4.0 ± 0.80
Dark seeds			
	day 1	day 2	day 3
Σ 1.9 ± 1.5cm	0.5 ± 0.08	1.4 ± 0.76	2.9 ± 1.65

This tendency was also confirmed in the case of the aberrant ana-telophases, where in the range of 0.0 – 3.66 % SEM was 0.0 – 2.42 %, thus rendering all results non-significant. There was no provable difference between the light and dark seeds or even between cultivars or years of harvest.

All of these results support our previous conclusions and findings (Murín et al 2007). In all of the evaluated parameters, we have observed that despite the great difference in the two basic storage conditions and the time between the harvest years and the evaluation frequency (17 years in the case of the oldest year), germination was the only provable parameter. This confirms the possibility of the practical use of this interesting manifestation of different storage conditions.

3.3 Experimental Storage Effect

The basic observations mentioned in the introduction regarding various plant species were later supplemented by attempts to demonstrate a relationship between the aging of seeds and the mode of their storage and sensitivity to the action of chemicals (e.g. Avanzi et al. 1969). Interesting results were obtained due to the effects of different moisture on stored seeds in the course of the presumed repair mechanisms in plant cells and the corresponding recovery effects after the action of alkylating agents (Gichner and Gaul 1971, Gichner and Velemínský 1973).

Following the method of the last mentioned authors in a series of experiments (Murín and Mičieta 2001), described in detail in our recent report (Murín and Mičieta 2014), we found the experimental "storage effect" to be a universal method for recovering and enhancing seed viability for higher crop production.

Seeds stored for a long time and impacted by significant chromosome damage by a chosen mutagen can recover by this method. It means that their experimental storage under the defined conditions for 8-days resulted in 3 to 4 times lower frequency of chromosomal aberration in root tips and a significantly higher viability (Tables 16 and 17, Figures 2 and 3). Even 12-year old seeds after experimental storage showed viability comparable with 2-year old seeds without storage. This prolongation of the G-1 phase causes a decrease in damage caused by long term storage and other stresses up to a condition similar to that of young undamaged seeds. According to our experience (Murín and Mičieta 1997), the prolongation of the storage period for more than 8 days has no greater effect, i.e. the "storage effect" is limited probably due to the limited source of repair enzymes stored in dormant seeds.

TABLE 16
GERMINATION AND CHARACTER OF ABERRATIONS (F-FRAGMENTS. B-BRIDGES. F+B) IN ROOT TIP CELLS OF 2. 6 AND 12-YEAR OLD *V. FABA* SEEDS AFTER 0 DAYS OF EXPERIMENTAL STORAGE.

Years	MMS in mM	Germination (in %)	No. of cells scored 48h/72h	No. of aberrant cells 48 h recovery time F	B	F+B	72h F	B	F+B
	0	77.0	150/120	2	1	0	1	3	0
2	3	90.0	155/140	17	6	3	8	1	1
	6	87.0	150/130	24	3	4	11	9	2
	0	57.0	210/140	40	7	12	15	6	3
6	3	50.0	250/90	40	11	16	11	3	3
	6	77.0	250/150	57	26	18	35	4	4
	0	19.4	40/70	7	2	2	11	1	0
12	3	17.4	25/15	1	6	2	4	2	2
	6	13.0	60/25	10	16	20	14	2	2

TABLE 17

GERMINATION AND CHARACTER OF ABERRATIONS (F-FRAGMENTS. B-BRIDGES. F+B) IN ROOT TIP CELLS OF 2. 6 AND 12-YEAR OLD *V. FABA* SEEDS AFTER 8 DAYS OF EXPERIMENTAL STORAGE.

Years	MMS in mM	Germination (in %)	No. of cells scored 48h/7h	No. of aberrant cells recovery time 48 h F	B	F+B	72h F	B	F+B
	0	89.6	270/150	7	1	0	4	1	0
2	3	94.0	260/150	3	0	0	3	1	0
	6	86.0	290/150	7	0	1	0	2	0
	0	87.5	300/160	2	1	0	0	0	0
6	3	88.6	300/150	8	1	0	2	0	0
	6	82.4	300/150	6	2	0	3	1	0
	0	60.0	230/150	18	5	2	4	1	0
12	3	46.0	205/150	8	6	0	11	1	1
	6	72.0	180/150	12	4	1	2	2	2

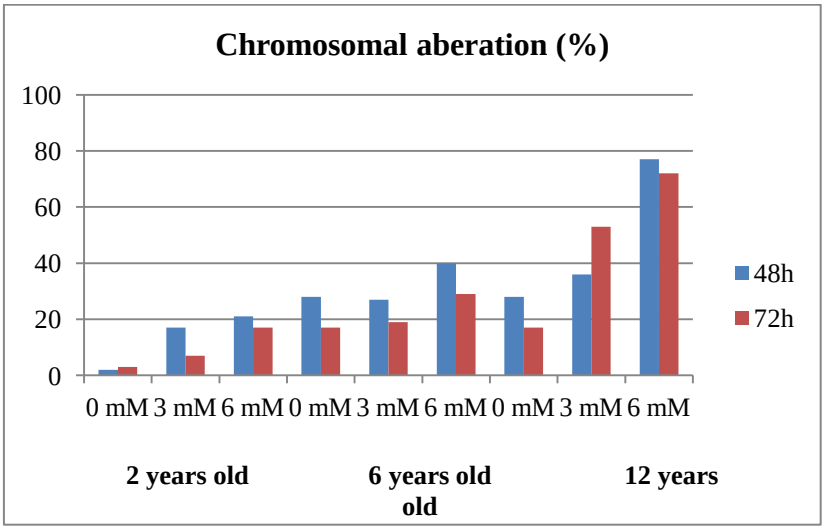

FIGURE 2. FREQUENCY OF CHROMOSOME ABERRATIONS ON DAY 0 OF EXPERIMENTAL STORAGE.

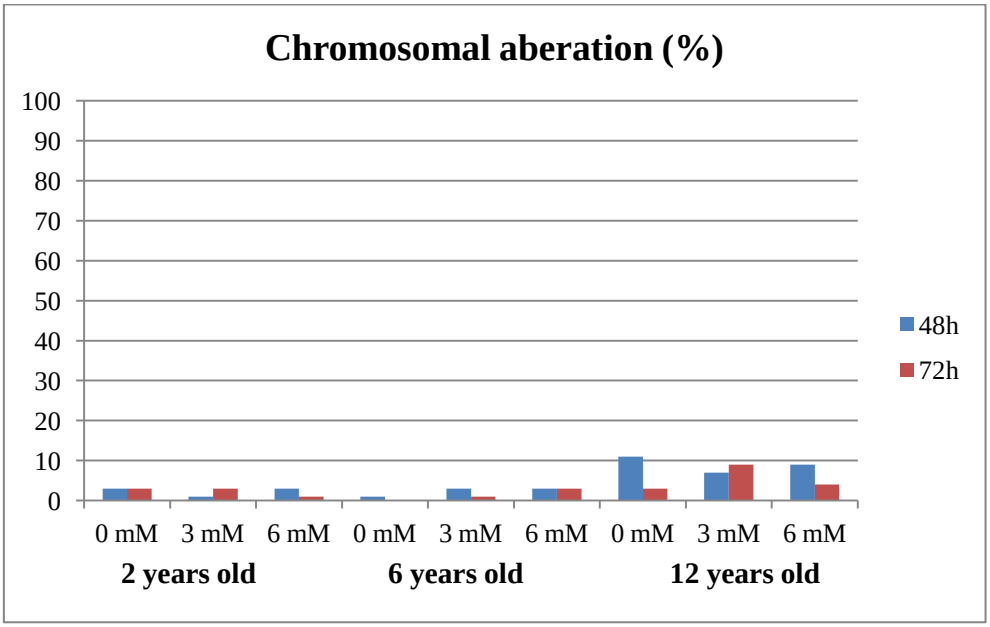

FIGURE 3. FREQUENCY OF CHROMOSOME ABERRATIONS ON DAY 8 OF EXPERIMENTAL STORAGE.

IV. CONCLUSION

In addition to the theoretical ramifications of our experiments, our work may also have practical implications in three fields:

1. As pointed out by Čupič et al. (2005), widely used agro-technical plant seeds, as in the case of Alfalfa (*Medicago sativa* L.), are often stored for years after harvest, which influences their germination energy, germination, rate of abnormal sprouts and dead seeds. This can be easily repaired by the "storage effect" with an interrupted germination period under the described conditions causing the prolongation of the G-1 phase with a significant increase of vitality of seeds treated this way. Consequently, it will lead to an improvement of their crop production that is most important in the case of seeds that are genetically modified or rearranged (see ACB karyotype seeds used in our experiments).
2. Our findings may be very helpful to seed banks worldwide. The regular checking of the viability of seeds according to germination leads to irreversible losses of stored seeds, while a simple visual test based at seed color would provide the same answer with no loss of material. Moreover, such a test could be conducted in sealed ampules, thus not interfering with the storage conditions in the particular seed bank. By using "storage effect" these seeds can be revitalised (or rejuvenalised) and stored further with the possibility of long-term survival of the seeds in seed bank.
3. Finally, with the above mentioned "storage effect" the amount of yield of viable seeds can be significantly recovered and by this method to prolong of their useful survival in the particular agricultural supply. Just at the example of *Vicia faba* beans it could cause a significant economical improvement as its seeds are distributed in more than 55 countries when 4.56 million tons of dry grains are produced in the harvested area of 2.56 million ha yearly.

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Temperature effect on seed germination of four plants in sand from coastal sand dunes in Greece

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***Abstract*—** *In high temperatures of coastal sand dunes, salt may limit seed germination and thus strogly limit plant survival. The relationship between germination and sand dunes soil temperatures has never been evaluated. The effect of coastal dunes sands on plant germination at 20°C and 28°C was studied. Sand samples of coastal dunes from coastline regions in Greece were analyzed and used as substrates. The Si content (89-97%) is the representative one in all soils. The Si, Al and Mg contents in the Aegean Sea sand dunes were higher than the Ionian Sea. Pigweed germination at 28°C in sand from mainland Greece was higher than the corresponding one from insular Greece. Purslane germination at 28°C in sand from the Ionian Sea (52%-57%) was higher than the corresponding one from the Aegean Sea (49%-50%) and at 20°C it remained unaffected by the soil. Chervil and coriander germination at 20°C was very low, while no germination occurred in pigweed at 20°C and chervil and coriander at 28°C in all sand soils. The study has shown that the coastal dune soils favoured germination of pigweed and purslane at 28°C. These results indicate that those plants have adapted to the coastal sand dunes environment.*

***Keywords*— sand elements, pigweed (Amaranthus retroflexus), purslane (Portulaca oleracea), chervil (Anthriscus cerefolium), coriander (Coriandrum sativum).**

I. INTRODUCTION

The sand dunes occurred in rows along the coast. Coastal dunes have an extensive global distribution (Martinez et al. 2004). A variety of abiotic and biotic factors including sand instability, salinity, extreme high or low temperatures, drought, and herbivore predation may limit seed germination and seedling emergence in coastal sand dunes (Sykes & Wilson 1988; Maun 1994; Maun 2009). Major problems faced by seeds are sand accretion and soil salinity.

Seed germination is a critical stage for the establishment of a species. Salt inhibits seed germination in saline soil, and high salinity is injurious to most glycophytes. Ungar (1978) suggested that there is a complex interaction between salinity and temperature, especially for coastal halophytes. Salinity and temperature interact in their control of seed germination (Khan & Ungar 1999), with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature (Badger & Ungar 1989). In sand dune environments, seed germination is strongly related to available moisture and seedling survival is strongly limited by temporal changes in water availability (Maun 2009). Very small seeds showed a high germination rate because of the small amount of moisture required for inbibition (Stairs 1986; Zheng et al. 2005).

Purslane (Portulaca oleracea) is a spontaneous wild forb, herbaceous annual plant, from the Portulacaceae family. It is one of the world's most aggressive and worst weeds, a unique plant that has the ability to adapt to many diverse environments (Rahdari et al. 2012). It is more tolerant to salinity and drought conditions (Hamidov et al. 2007). The plant produces numerous minute seeds, many of which have primary dormancy and require warm temperatures and light for germination (Egley 1984). The plant grows in the wild and is cultivated around almost the whole world. Purslane is a heat- and drought-tolerant plant, and is an important vegetable crop (Anastácio & Carvalho 2012; Dadkhah 2013). Moreover, purslane is an edible halophyte (Grieve & Suarez 1997) and a promising crop for saline agriculture (Kiliç et al. 2008), which has been studied for its relatively high salinity tolerance (Teixeira & Carvalho 2009). The optimal seed germination occurs at temperatures > 30°C, while poor germination occurs at temperatures < 24°C (Miyanishi & Cavers 1980; Zimmerman 1976). Germination was 96% when seeds were exposed to 35/25°C day/night alternating temperatures, but 25/10°C day/night resulted in 15% germination (Miyanishi & Cavers 1980). In the light/dark regime, the germination was lower at 25/15°C than at 30/20°C (70%, and 81% germination, respectively) (Chauhan & Johnson 2009).

Pigweed (*Amaranthus retroflexus*) is a common annual worldwide weed of 60 crops in 70 countries. It can be found in a wide range of habitats and causes substantial yield reduction in many different agricultural crops through competition (Ghorbani et al. 2000; Holm et al. 1997). Pigweed is a thermophyte to sub-thermophyte, often highly stress-tolerant, growing in sandy and saline habitats (Costea et al. 2004; Robertson & Clemants 2003). It reproduces by seeds which can only remain

viable for at least 6–10 years (Costea et al. 2004). Seed germination of pigweed differed in its response to temperature. Pigweed had higher germination rates at 35/30°C than at 25/20°C or 45/40°C, and at 15/10°C no seed germination was observed (Guo & Al-Khatib 2003). Ghorbani et al. (1999) found that the minimum temperature for pigweed germination was greater than 5°C, whereas maximum germination occurred between 35 and 40°C. Pigweed was sensitive to Al, Mn, NaCl and Na2SO4.

Chervil (*Anthriscus cerefolium*) is a fragrant, delicate annual herb, belonging to the Apiacae family. Its seeds need light and wet porous soil to germinate. Seeds of chervil have germinated at a 13°C temperature (Bubel 1988). The seed germination percentage of chervil at 22°C in H2O was 44%. In the 80mM NaCl concentration there was an increase in the germination (64%), while in higher NaCl concentrations the germination reduced (Liopa-Tsakalidi & Barouchas 2011).

Coriander (Coriandrum sativum) is an annual herb of the Apiaceae family, grown primarily for its seed and seed oil all over the world (Verma & Sen 2008). It is known as a species moderately tolerant to salinity. Zidan & Elewa (1995) mentioned that during germination, coriander tolerated salinity up to 200mm NaCl. Liopa-Tsakalidi et al. (2011) reported that no seeds germinated in high salt concentrations (0.5 and 1.5 mol/l NaCl). In addition to this, the seed germination of coriander showed a progressive decrease as salinity levels increased (Ewase et al. 2013; Fredj et al. 2013). The salinity effect appears mainly during germination and plant growth (Aymen & Cherif 2013).

Little research has been conducted about the application of seed germination on coastal sand dunes and the studies on their relationship with seedlings are still inadequate. For example, the growth characteristics of seedlings in Greek coastal sand dunes have not been investigated with respect with to initial seed germination.

The focus of the current study was to provide knowledge on the germination behavior of pigweed (*Amaranthus retroflexus*), purslane (*Portulaca oleracea*), chervil (*Anthriscus cerefolium*), and coriander (*Coriandrum sativum*) in two temperatures and different sand soils of coastal sand dunes in Greece, and to ascertain the effects of different characteristics of sand soils on germination.

II. MATERIAL AND METHOD

2.1 Analysis of sand dune soils

In the present paper, the study area was located in the mainland (Kastro Kyllinis and +Vartholomio Ileias) and the insular (Zakynthos) coastline of the Ionian Sea, and the insular (Milos and Tinos) coastline of the Aegean Sea, in Greece (Fig. 1).

FIG. 1: STUDY AREA IN THE MAINLAND AND THE INSULAR COASTLINE OF THE IONIAN SEA, AND THE INSULAR COASTLINE OF THE AEGEAN SEA, IN GREECE.

For comparison purposes, sand samples that represent the sand soils of various coastal sand dunes of Greece, i.e. representing areas of the Ionian and Aegean Seas, were randomly collected from 0 to 10 cm below the surface of the coastal sand dunes, using metal spatula. At each study area, 3 samples (approximately 1-2 kg) were collected randomly from different sand dunes within the radius of 200m. The samples were transferred to the Laboratory of Soils and Irrigations of the Technological Educational Institute of Western Greece for further studies. Sand samples were collected from coastal sand dunes from five regions of Greece: the Alykanas area in Zakynthos, the Kastro Kyllinis area and the Vartholomio area in Ileias (Ionian sea), and the Milos and Tinos islands (Aegean sea).

The total content of silicon (Si), calcium (Ca) chromium (Cr), aluminium (Al), magnesium (Mg), potassium (K), titanium (Ti), manganese (Mn), iron (Fe), sodium (Na), and zirconium (Zr) elements were determined using microwave digestion and the Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). ICP-AES is the preferred method of elemental analysis for chemical stratigraphy. Methods: Approximately 1g ± 0,1mg of sample was digested with 2.35 mL of HNO3 65% and 7 mL of HCl 37% (aqua regia) in microwave digestion system (Berghof speedwave MWS-3+). The temperature program was as follows: 5 min for temperature 145°C, 10 min for temperature 170°C and 15 min for temperature 180°C. The resulting solutions were cooled for 30 minutes and diluted to 10 mL with distilled water. The clear solutions were analyzed by ICP-OES (Thermo Scientific iCAP 6000). The operating conditions were: nebulizer gas flow rates: 0.5 l/min; auxiliary gas flow: 0.5 L min−1; plasma gas flow: 15 L min−1; Pump rate: 45 rpm; ICP RF power: 1100 W. Aliquots of an ICP multielement standard solution (100 mg/L Merck) containing the analyzed elements, was used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 1% HNO3. The ranges of the calibration curves (6 points) were selected to match the expected concentrations for all the elements of the sample studied by ICP-OES. The correlation coefficient r2 obtained for all cases was 0.9999. The detection limits (LOD) were calculated as the concentrations of an element that gave the standard deviation of a series of ten consecutive measurements of blank solutions. Soil samples from BIPEA's proficiency testing scheme A15 were used to ascertain the accuracy of the measurements. All above-mentioned sand testings were performed in the Laboratory of Soils and Irrigations of the Technological Educational Institute of Western Greece.

2.2 Seed germination

Four plant species were utilized for this study; two weeds, pigweed (Amaranthus retroflexus) and purslane (Portulaca oleracea), and two herbs, chervil (Anthriscus cerefolium) and coriander (Coriandrum sativum) (seed supplied by SAIS Sementi, Cesena-Forlì, Italy). Forty seeds were placed on Petri dishes (10cm diameter) containing 15g of river sand and 15ml of distilled water (control) or an equal quantity of the respective test sands, i.e. coastal dune sand from Alykanas area of Zakynthos island (Ionian sea), coastal dune sand from Kastro area of Kyllinis (Ionian sea), coastal dune sand from Vartholomio area of Ileias (Ionian sea), coastal dune sand from Milos area island (Aegean sea), coastal dune sand from Tinos area island (Aegean sea). The petri dishes were arranged in a completely randomized block design with three replicates of each treatment, and were transferred in a controlled plant growth chamber under 20±1°C or 28±1°C temperature regimes, a 16h photoperiod and 80±2% relative humidity (RH). The number of the germinated seeds was recorded every day, starting from day 2 after the seeds were initially placed on Petri dishes. The experiment consisted of three replications.

2.3 Data and Statistical analysis

The germination percentage is an estimate of the seed viability. The equation to calculate the final germination percentage (GP) is:

$$GP = \frac{\text{number of germinated seeds}}{\text{number of total seeds}} \times 100$$

The germination rate was estimated by using a modified

$\text{Timsons index of germination velocity} = \sum G/t$, where G is the percentage of seeds which germinated after 2-day intervals and t is the total germination period.

All data was tested by the analysis of variance (ANOVA), using the SPSS 21 software. Duncan's multiple range test was performed at p = 0.05 for each of the significant variables measured. Duncan's multiple range test was performed at p = 0.05 for each of the significant variables measured.

III. RESULTS

3.1 Chemical characterization of dune sand

The chemical characteristics of the sand tested are presented in Figures 2 and 3 respectively. They can provide useful information about plant survival.

The results show that Si (89-96%) is the most represented mineral. The mean values of total Si contents for the Milos (97%) and Tinos (96%) islands (Aegean Sea) sand dunes were significantly higher than the rest of the sand dunes. Alykanas Zakynthos and Kastro Kyllinis (14%) were significantly lower than the rest of the dunes (Fig. 2).

When compared to the other elements, calcium contents present an important concentration. The sand chemical analysis contains a non-negligible part of Ca, Cr, Al and Mg. The Ca and Cr contents for the Milos and Tinos islands (Aegean Sea) sand dunes were significantly lower than the rest of them, namely Alykanas Zakynthos and Kastro Kyllinis, Vartholomio Ileias (Ionian Sea). Similarly to Si contents, the results show that aluminium (Al) and magnesium (Mg) contents in the sand dunes of the Aegean Sea are significantly higher than those of the Ionian Sea (Fig. 1). The K, Ti, Mn, Fe, Na, and Zr contents do not show clear group separations like that shown by the Al contents (Fig. 2, 3).

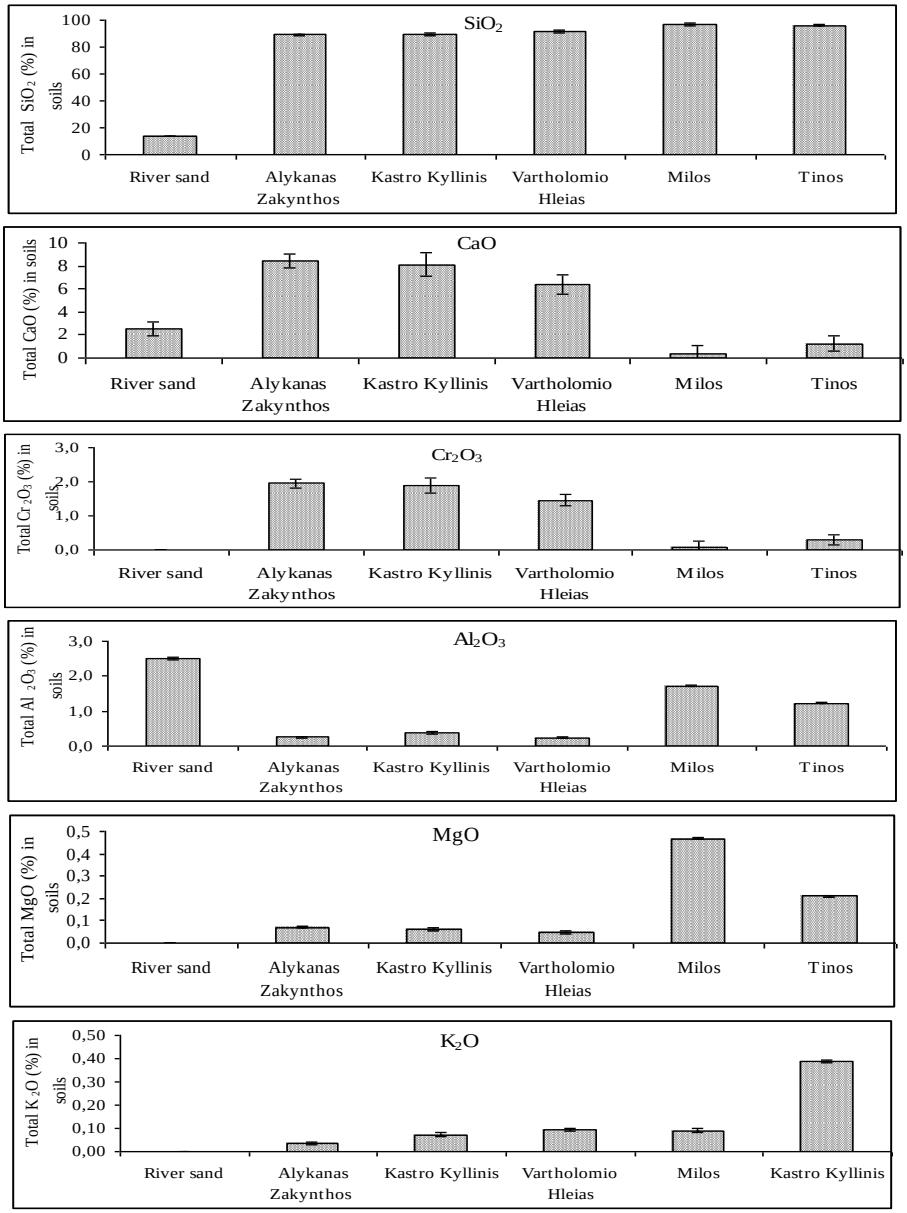

FIG. 2: CHEMICAL CHARACTERIZATION (%) OF THE SAND DUNES OF FIVE AREAS OF GREECE; SILICON (SI), CALCIUM (CA) CHROMIUM (CR), ALUMINIUM (AL), MAGNESIUM (MG), POTASSIUM (K) CONTENTS.

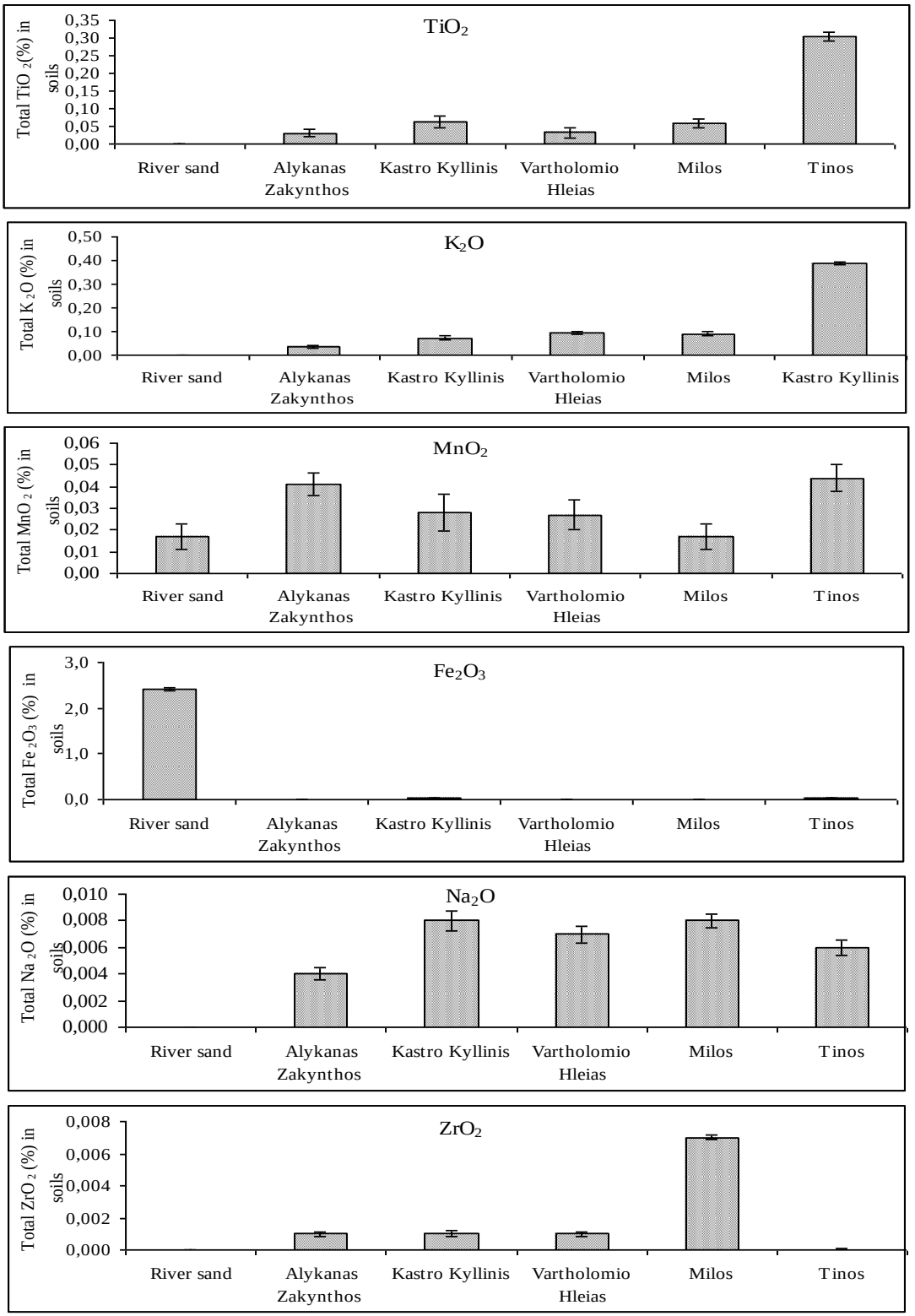

FIG. 3: CHEMICAL CHARACTERIZATION (%) OF THE SAND DUNES OF FIVE AREAS OF GREECE; TITANIUM (TI), MANGANESE (MN), IRON (FE). SODIUM (NA), ZIRCONIUM (ZR) CONTENTS.

3.2 Coastal dunes effects on seed germination

The seed germination observation of pigweed, purslane, chervil and coriander plants lasted 10 days at a temperature of 20°C and 28°C in a controlled plant growth chamber (Fig. 3). Temperature had a significant effect on the germination percentage. No seed germination was observed at the lowest temperature (20°C) in all sand soils.

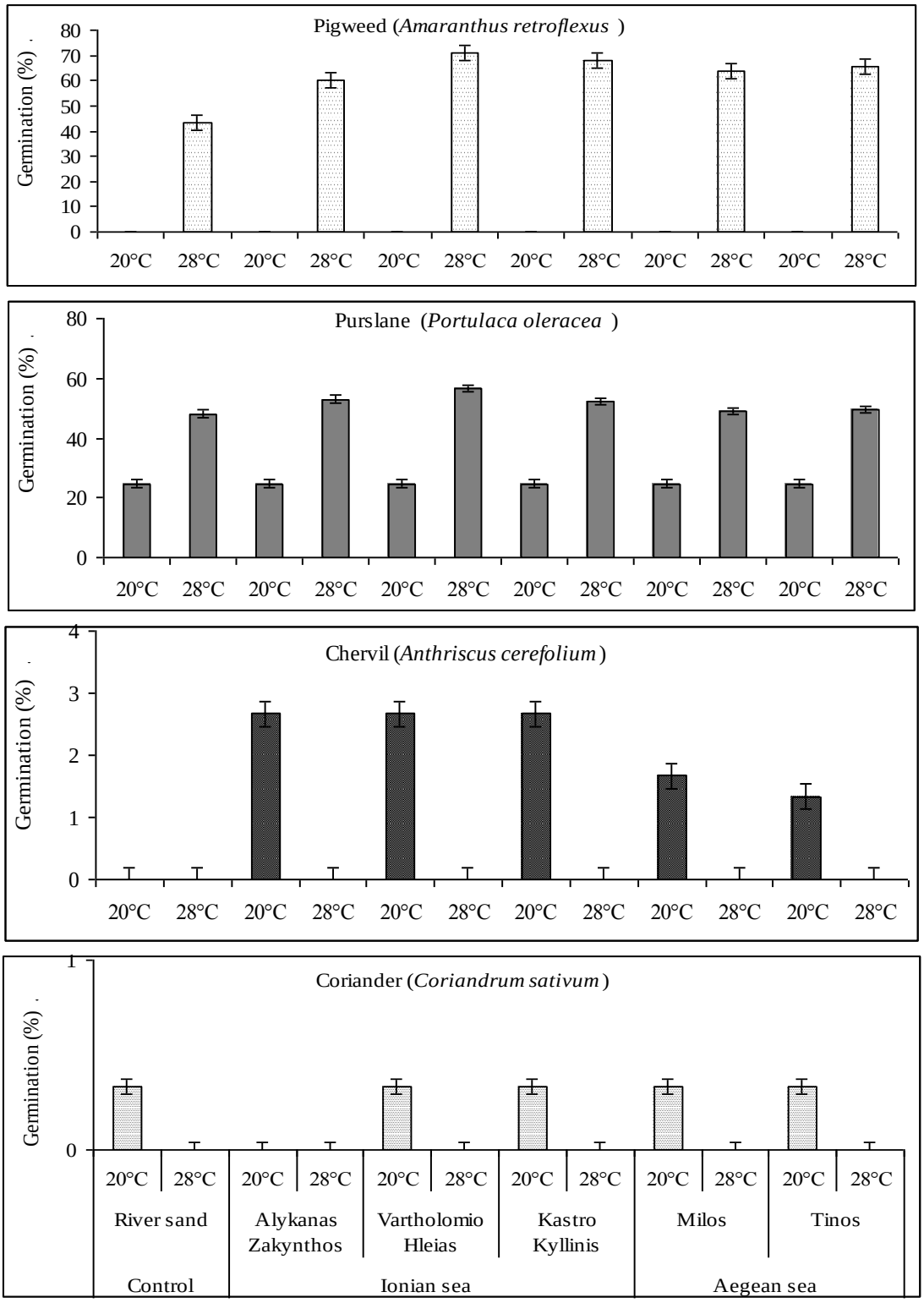

FIG. 4: EFFECT OF SAND DUNES ON GERMINATION (EXPRESSED AS %) OF PIGWEED (*AMARANTHUS RETROFLEXUS*), PURSLANE (*PORTULACA OLERACEA*), CHERVIL (*ANTHRISCUS CEREFOLIUM*), AND CORIANDER (*CORIANDRUM SATIVUM*).

The seed germination percentage of pigweed at 28°C in the river sand (control) was 44%, significantly lower than the coastal dune percentages. Its seed germination percentage in sand of coastal dunes from mainland Greece (Kastro Kyllinis (68%) and Vartholomio Ileias (71%) was significantly higher than the corresponding one from insular Greece (Zakynthos (60%), Milos (64%) and Tinos (66%) islands) (Fig. 4, 5).

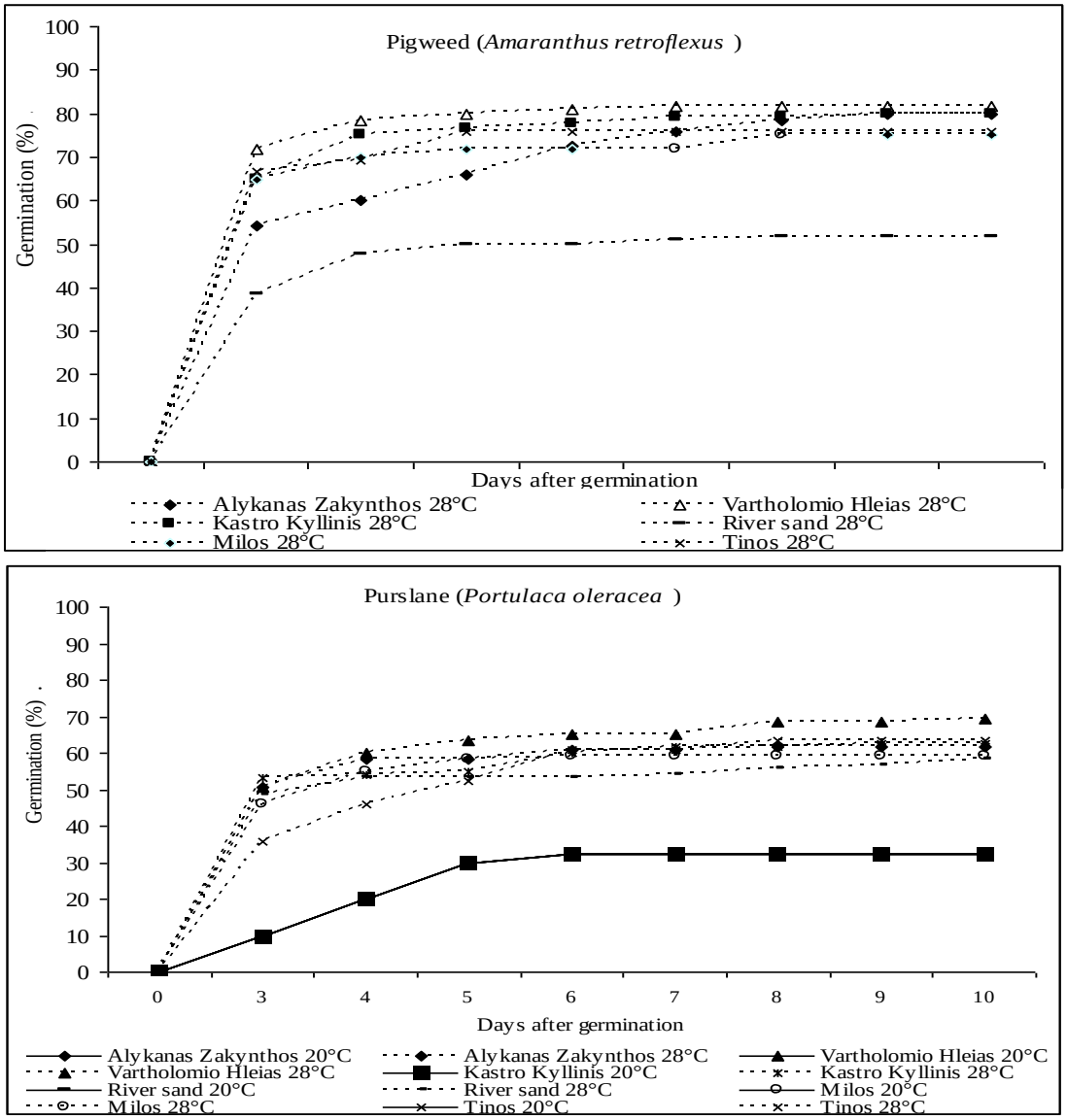

FIG. 5: EFFECT OF SAND DUNES ON GERMINATION (EXPRESSED AS %) OF PIGWEED (*AMARANTHUS RETROFLEXUS*), AND PURSLANE (*PORTULACA OLERACEA*)

The seed germination observation of purslane lasted 10 days at a temperature of 20°C and 28°C in a controlled plant growth chamber (Fig. 4, 5). The radicle emergence of the purslane seeds occurred in day 3 after the seeds were placed in Petri dishes.

Data presented in Figure 4 show that the seed germination percentage (25%) of purslane at 20°C did not affect any of the sand soils (Fig. 4, 5). Its seed germination percentage at 28°C in the river sand (control) was low (48%) and in sand of coastal dunes from the Ionian sea (Alykanas, Zakynthos island (53%), Kastro Kyllinis (52%) and Vartholomio Ileias (57%)) it was significantly higher than the corresponding one from the Aegean sea (Milos (49%) and Tinos (50%) islands) (Fig. 4, 5).

The germination percentages of chervil and coriander clearly showed different reactions to temperature. No germination occurred at the 28°C temperatures and germination at 20°C was also very low. Maximum germination percentage of chervil was recorded at 20°C in sand of coastal dunes from the Ionian sea (3%) (Alykanas Zakynthos island, Vartholomio Ileias and Kastro Kyllinis), whereas the minimum was recorded in sand of coastal dunes from the Aegean sea ((Milos area (2%), and Tinos area (1%)) islands (Fig. 4).

The results showed that the temperature and sands of coastal dunes significantly affected germination velocity. This was estimated by using a modified Timson index as a measure of pigweed seed germination Maximum germination velocity of pigweed seeds at 28°C was observed in sand of coastal dunes from Vartholomio Ileias (mainland Greece), whereas the

lowest rate was in the river sand (control). Moreover, the germination velocity of purslane seeds at 20°C in all sand soils was less than at 28°C. The germination velocity of chervil and coriander are null (Table 1).

TABLE. 1: EFFECT OF SAND DUNES ON TIMSON INDEX GERMINATION VELOCITY OF PIGWEED (*AMARANTHUS RETROFLEXUS*), PURSLANE (*PORTULACA OLERACEA L.*), CHERVIL (*ANTHRISCUS CEREFOLIUM*) AND CORIANDER (*CORIANDRUM SATIVUM*).

Timson index, germination velocity						
	Sand dunes	**Temperature °C**	**Pigweed (*Amaranthus retroflexus*)**	**Purslane (*Portulaca oleracea*)**	**Chervil (*Anthriscus cerefolium*)**	**Coriander (*Coriandrum sativum*)**
Control	River sand	20°C	_	3,3	_	_
		28°C	5,2	5,8	_	_
Ionian Sea	Alykanas Zakynthos	20°C	_	3,3	_	_
		28°C	8,0	6,2	_	_
	Vartholomio Hleias	20°C	_	3,3	_	_
		28°C	8,2	6,9	_	_
	Kastro Kyllinis	20°C	_	3,3	_	_
		28°C	8,0	6,3	_	_
Aegean Sea	Milos	20°C	_	3,3	_	_
		28°C	7,5	5,9	_	_
	Tinos	20°C		3,3		
		28°C	7,6	6,3	_	_

IV. DISCUSSION

The distinctly different chemical composition of the coastal sand dunes in Kastro Kyllinis, Vartholomio Ileias, Zakynthos, Milos and Tinos islands, show different characteristics in comparison with the dunes in the mainland and insular coastline of the Ionian Sea and the insular coastline of the Aegean Sea of Greece (Fig. 3). Sand elements may differ along a given coastline. For instance, along the Florida coast, the beaches primarily consist of quartz and calcium carbonate sand in the north, and quartz sand in the south (Maun 2009). Variations in the chemical composition of frontal dune sediments along the west coast of Jutland, Denmark, have been investigated by Saye and Pye (2006). The silicon (Si) content (89-97%) is the one most represented in all soils of coastal sand dunes. Similar results have been reported by Maazouzi et al. (2013) for the SiO_2 content in Algeria sand dunes (97%) and by Muhs et al. (2013) for the Sinai–Negev dunes of Egypt and Israel (~76 to 98%).

It is known that the seed germination is an important phase of plant development, since it gives it a better start. The above differentiations of the coastal dunes' chemical composition of the Ionian and the Aegean Sea exhibit different effects on the seed germination of four plants. The higher Si contents in sand from the coastal dunes of the Aegean Sea seem to inhibit the seed germination of some pigweed, purslane and chervil species by causing a deficiency in organic matter. However, little information is available on the effects of silicon on seed germination under salinity.

Moreover, the high aluminium (Al) contents in sand soils from the Aegean Sea islands reduced the seed germination of pigweed, purslane and chervil. Alamgir and Akhter (2009) reported that aluminium (Al) affected seed germination of different wheat varieties and the inhibitory effect increased with the increase of Al^{3+} concentration.

The high seed germination percentage of the small seeds of purslane (60-71%) and pigweed (49-57%) in all sand soils at 28°C could result in tolerance to coastal dunes sand. Many studies on sand dunes have reported that very small seeds showed a high germination rate because of the small amount of moisture required for inhibition (Stairs 1986; Zheng et al. 2005). In sand dunes, seed germination is strongly related to available moisture (García et al. 2002).

The results showed that the low seed germination percentage (25%) of purslane at 20°C did not affect any sand soil. Lower temperature low seed germination percentage of purslane was noted by Baskin and Baskin (1988) at 20/10°C and 15/6°C day/night temperatures and by Miyanishi and Cavers (1980) at 25/10°C. No seed germination was observed in pigweed at 20°C in all sand soils. These results are in agreement with earlier results, which showed that seed germination of pigweed is inhibited by low temperature **(**Ghorbani et al. 1999; Guo & Al-Khatib 2003; Steckel et al. 2004**)**.

In this paper, the absence of chervil seed germination at 28°C and the poor germination at 20°C in all sand soils showed that seeds are germinated at temperatures lower than 13°C (Bubel 1988).

The results of this study showed no seed germination at 28° and low germination at 20°C of coriander in all sand soils due to the fact that it is a plant sensitive to salinity (Liopa-Tsakalidi et al. 2011; Ewase et al. 2013; Fredj et al. 2013).

The study has shown that the soils of coastal sand dunes favoured seed germination of the weeds pigweed and purslane at 28°C, while inhibiting the seed germination of the herbs chervil and coriander under two different temperatures. These results indicate that weed seeds have adapted to the coastal sand dunes environment. In further laboratory and field experiments it would be interesting to understand the ecological role of the pigweed and purslane seeds during seed germination in coastal sand dunes environments.

V. CONCLUSION

The study has shown differences between the percentage of various sand elements in the dunes of the mainland and insular coastline of the Ionian Sea and the insular coastline of the Aegean Sea of Greece. Moreover, the study has shown that there was no germination of chervil and coriander seeds in coastal dune soils and sand soils favoured germination of pigweed and purslane at 28°C. These results indicate that the two plants, pigweed and purslane, have adapted to the coastal sand dunes environment.

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E-screen assay validation: evaluation of estrogenic activity by MCF7 cell culture bioassay, in drinking water from different watersheds in state of São Paulo, Brazil

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Abstract— *Natural and synthetic estrogens have been detected in rivers, lakes and estuaries in many parts of the world. Primary sources of these compounds are domestic and industrial effluents, which are not deleted after the water treatment. Estrogen has been the endocrine disruptor most researched to be very active biologically and be the etiologic agent of diverse types of cancer and other conditions such as endometriosis, precocious puberty, feminization, masculinization, sterility. In this context, we use water of 36 natural reservoirs or dams, in a bioassay to characterize their estrogenicity in culture of MCF7 cells and obtained high concentration of estrogen in samples taken in Ibiúna and Equestrian Santo Amaro / SP. However, certain concentration in our samples for most water samples from different regions was very close to the limit of quantification by bioassay and estrogen was in fmol. It has been shown that e - screen assay with MCF7 cells is a sensitive and stable tool for quantitative analysis of estrogenicity of water and can easily be developed and implemented for routine for estrogen quantification also in animal food and man, aqueous and plastics etc.*

Keywords— endocrine disrupters, estrogen, breast cancer cells, (MCF7) bioassay: E-screen assay

I. INTRODUCTION

The speed of production and use of synthetic chemical products, since 1940, resulting in contamination ubiquitous of aquatic animals, land and the human population. Since 1960 observers noted an increase of changes in reproduction of animals across the globe as well as changes in reproduction and health of men by exposure to these synthetic products (VAN LABEKE et al, 2008)

In 1991 scientists from various fields met at Wingspread Conference Center for defining and structuring the Disruption Endocrine phenomenon. These researchers, in consensus, established that many synthetic chemicals were potentially able to disrupt the endocrine system of animals and man. At that time concluded that the characterization of exposure to Endocrine Disruptors was a crucial aspect for the prognosis of health effects as given in the below link.
(http://www.ourstolenfuture.org/Consensus/wingspread1.htm).

In the mid-90s there was a clear insight the possible negative impact of endocrine disruptors and the need for new legislation to really ensure protection to human and animal's health and the environment. But only two decades later the Society of Endocrinology renewed the request of the endocrine disruptors list and the effects of its exposure, considering as a priority research (DIAMANTI-KANDARAKIS et al, 2009).

The welfare of society is linked to many chemicals, essential components to everyday life, which are found everywhere, even in remote locations both in the environment and animals and men.

For millennia, human and animals' bodies adapted to EDs vegetables, cereals and fruit (apple, cherry, plum, potato. Carrots, peas, beans, soybeans, wheat, oats, barley, rye, parsley, garlic) and naturally excreted not accumulate in the body. The chemicals, however, accumulate mainly in adipose tissue mimicking glandular hormones (Colborn et al, 2002). Some of these substances are transplacental and may affect the fetus as lead (Bowler and Cone, 2010) or fixate the milk being

ingested by the newborn (Matuo, 1999) even toxic agents already accumulated in the maternal organism over the years. (Colborn et al, 2002).

Recent studies in several countries have shown that the aquatic environment can possess estrogenic activity capable of influencing the fauna. (Xeno) estrogens are believed to reach the aquatic environment mainly by means of municipal and industrial sewage outfalls. However, agricultural drainage may also be a route for (xeno) estrogens to enter the aquatic system.

Numerous natural and anthropogenic substances are known to exhibit estrogenic activity. In the aquatic environment, estrogenic activity has primarily been ascribed to the natural steroids, 17b-estradiol (E2), estrone (E1) and estriol (E3), and the synthetic estrogen, ethinylestradiol (EE2), used in contraceptives and hormone replacement. To a lesser extent xenoestrogenic chemicals, such as alkylphenols and bisphenol A, may also contribute to the estrogenic activity in the aquatic environment. (GRAY et al, 2000, SHAW and MCCULLY, 2002; AERNI et al ,2004)

Estrogen has been the endocrine disruptor most researched to be very active biologically and be the etiologic agent of diverse types of cancer and other conditions such as endometriosis, precocious puberty, feminization, masculinization, sterility (WELSHONS et al, 1999). Endocrine disruptors have recently been shown to promote an epigenetic transgenerational phenotype involving several disease states (e.g. male infertility) (GORE et al, 2013). The MCF-7 cell proliferation assay is potentially a simple and highly reproducible tool for the identification of estrogenic compounds. In the E-screen assay developed by SOTO et al. (1995), proliferation of MCF-7 cells as a response to estrogen is measured. The E-screen is based on the following three premises: (i) factors in human serum inhibit the proliferation of MCF-7 cells, (ii) estrogens induce cell proliferation by negating this inhibitory effect, and (iii) non-estrogenic steroids and growth factors do not neutralize the inhibitory signal present in human serum.

Numerous studies on animal's exposure of wildlife and laboratories such products have shown that endocrine disrupters affect various physiological processes such as brain activity, reproduction, immune response, development and metabolic rates. (Tyler, 1998; McLachlan, 2001; Guillette and Gunderson, 2001; Hayes et al., 2002; Markey et al., 2003).

1.1 Endocrine disruptors

Endocrine disruptors are chemicals or agents that promote changes in the human or animal endocrine system. Several of these substances remain in the environment accumulating in soil, river sediment being transported at great distance. They can accumulate in the food chain representing a health risk especially those who are at the top of the food chain. (Meyer, 1999). For example, in the region of the Great Lakes between the United States and Canada, on Lake Ontario, it was observed biomagnetization of polychlorinated biphenyls (PCBs) from phytoplankton and zooplankton to trout and sea-gulls. The concentration of PCBs in the sediment was established as the initial value and from that concentration analyzed the concentration in the other beings of this ecosystem. There was a considerable increase in the concentration of PCB: phytoplankton 250X; zooplankton 500X; Trout 2.800.000X and 25.000.000X gulls (Colborn et al, 2002).

The disruptor may be organic or inorganic substance and appear as a byproduct or waste of industrial use. They are found in landfills and thus contaminating soil, groundwater, water sources used for public supply. Incinerators products (medical waste and industries) also contribute to this contamination (XELEGATI and Robazzi, 2003)

TEVES in 2001 noted the presence of mercury and lead in waste of São Paulo and SISINO and OLIVEIRA in 2000 confirmed the presence of cadmium, lead, manganese and mercury in chorumem landfills and dumps.

Several species of animals have been affected by endocrine disruptors. For example, we have thyroid dysfunction in birds and fish, decreased fertility in birds, fish, crustaceans, mammals; Successful reduction in hatching in birds, fish, turtles; metabolic abnormalities in birds, fish and mammals, behavioral abnormalities in birds, desmasculinization and desfeminilization of fish and female birds and dangerous changes in the immune system.

It is believed, therefore, that the effects of endocrine disruptors on the endocrine and reproductive systems act by mimicking the endogenous hormone antagonizing the normal effects of endogenous hormones; stimulation or inhibition of the synthesis and metabolism of hormones natural or modifying the levels of hormone receptors. These compounds are widely used by modern society, being found in pharmaceuticals, personal products (like eg. The fragrances pesticides, antioxidants, plastics,

industrial products, surfactants and others). Some of endocrine disruptors can enter the human body by the dermal route. They are: Benzo (a) anthracene, benzo (a) pyrene, Benzene, Lead, Chlordane; dieldrin; DDT; Carbon disulfide, Heptachlor; HCH, Mercury, pentachlorophenol (Azevedo & CHASIN, 2003).

Heavy metals act by inhibiting certain enzymes, for example, glycolysis, lipolysis and protein synthesis. Cadmium binds to the sulfhydryl group (-SH) of the enzyme inhibiting its action. Lead inhibits the action of ζaminolivólico acid dehydrase enzyme required for heme synthesis leading anemia. Arsenic form complex with enzyme inhibitors of adenosine triphosphate (ATP). Mercury has affinity with the sulfhydryl group of proteins, enzymes, serum albumin, hemoglobin (Patnaik 2002; Ferreira, 2003).

The particular hormone action starts by binding to a specific receptor of a cell. The resulting complex binds specific regions of DNA in the nucleus activating or deactivating particular gene. KOIFMAN et al in 2002 showed that in some Brazilian states is correlation between the use of pesticides and endocrine changes in the exposed population as infertility, testicular, breast, prostate and ovary cancer.

The breast cancer is the most frequent cancer of women. The risk to develop it can be genetic, but according FENTON, 2006, 70% of women diagnosed had no hereditary or sporadic cancer. The increased risk of breast cancer and early puberty owes much to the lifestyle and environment, exposure to certain chemicals that mimic hormones such as endocrine disruptors. These products can increase the incidence of cancer by altering the flow or level-dependent tissue hormones; altering the expression of glandular receptors, transporters of hormones or growth factors. These hormones have the best conformation recognized by receptors and therefore result in maximum responses and are considered as responsible for most disruptors effects caused by the disposal of effluents (Gray et al, 2000; SHAW and McCully, 2002; AERNI et al, 2004).

Understanding the complexity of exposure to synthetic chemicals is difficult by the large number of compounds and limitations of analytical techniques. Researchers thus have focused on a few products. Elucidating the whole universe including chemical unknown compounds is yet to happen.

Bioanalytical techniques (bioassays) may help to clarify this fact by characterizing the actual biological effects in a complex sample and thus incorporate the effects of unidentified components and mixtures.

The ecotoxicology has done in vitro bioassays to assess the endocrine activity in environmental samples.

In this context, we can use water of our natural or built reservoirs in a bioassay to try to characterize their estrogenicity in MCF7 cell culture (human cancer cell line). Cells of Mammary gland cancer have been used as a model of the effects of estrogen on the growth of breast cancer and the synthesis of specific proteins.

The E-SCREEN assay was developed to evaluate the estrogenicity of chemical agents present in the environment using the proliferative effect of estrogens on their target cells as an endpoint. This quantitative assay compares the number of cells obtained by similar inoculum MCF-7 cells in the absence of estrogen (negative control) and presence of 17 beta-Estradiol (positive control) in a range of suspected chemical concentrations have estrogenic function

the introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

II. MATERIAL AND METHOD

Cells were seeded in plates with 96 wells, 1500 cell / well in culture medium (Dulbeccos Modified Eagle Medium / Sigma) and incubated for 24 hours.

Following incubation, the wells were washed with 150 uL of phosphate buffer (PBS Gibco) and the medium changed to 150 ul of DMEM without phenol red (Sigma) and 5% FCS (charcoal dextran stripped / Sigma), 100 U / ml penicillin (Sigma), 0.1 mg / ml streptomycin (Sigma) and 2,5µg / ml amphotericin (Sigma).

After another 48 hrs, the medium was discarded and replaced with fresh water, experimental medium containing different concentrations of standard (17-β estradiol) or experimental means to extract different samples.

After 24 hrs of incubation (to cells adhere to the well) was exchanged to culture media with different concentrations of beta estradiol (controls: white, negative, positive (10nm to 1FM) and solvents) and samples of the extracts (repeat where, sample 3 X).

After 6 days, the cells were fixed with cold trichloroacetic acid (Sigma), 10% (w / v) for 30 min at -4oC.

Washed 5 times in tap water and allowed to dry. Stained with 0.4% sulforhodamine B (SRB / Sigma) in 1% acetic acid (Gibco).

Unbound SRB was removed by washing with 1% acetic acid and allowed to dry in air.

The bound SRB was solubilized with 10 nM Tris (pH 10.4) in a shaker.

After dilutions with estrogen on the MCF-7 cell proliferation, was made lecture in Elisa reader It measured the color intensity plate reader (absorbance) at 550nm.

In possession of the results, the readings and concentration, were plotted in graph of standard curve in logarithm.

The equation of the line was used to calculate the concentrations of estrogen in the water samples from distinct locations.

III. RESULTS AND DISCUSSION

3.1 Analysis with standard in different estrogen concentrations on the MCF-7 cell proliferation

Cells when grow in culture medium without estrogen showed proliferation with lecture in Elisa reader with 107cells However, when the medium was supplemented with water, the lecture presented 1013cells. These results showed that almost no cells were proliferated. Wells called white presented only water or Tris, and that showed results at minus of 1013cells, that can demonstrate negative proliferation.

Standart solution initiated with 10mmol and diluted it to micromole, picomol and fentomol. In each well were put 1500cells and 0.15mL of culture medium and after the period of reaction was made lecture in Elisa reader.

The standart curve was plotted in logarithm graph and demonstrated a high value in lecture with 0.01mmol corresponding at 105 in concentration and decreased lecture at dilutions with estrogen until 0.01fmol or 1013 of concentration. When have picomol the lecture decreased rapidly indicating less intensity on cell growth. The curve equation was y=-5,148ln(x) + 12,979 (Fig 1).

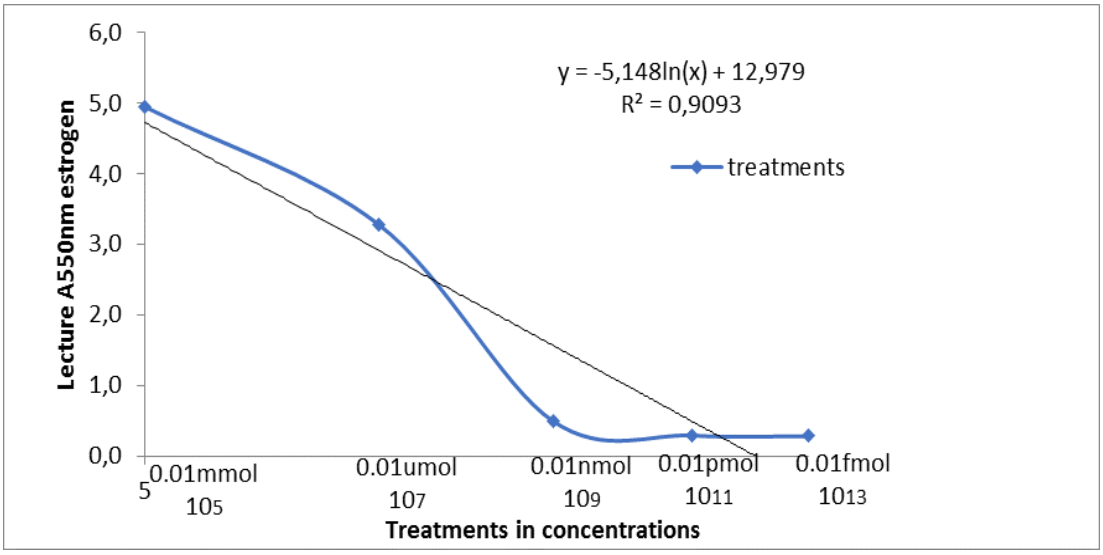

FIG 1: STANDART CURVE OF ESTROGEN PRESENT IN MEDIUM. CONCENTRATION OF ESTROGEN AT mmol, PICOMOL AND FENTOMOL.

3.2 Analysis of waters collected from lakes, rivers and lakes for breeding of animals and human sources.

All lecture from samples after check in Elisa reader, was used the equation curve for determinate the concentration of estrogen. Were studied 36samples of water and in all most water samples the result demonstrated estrogen present in fentomol (Table 1).

TABLE 2: CONCENTRATION OF ESTROGEN PRESENT IN SAMPLES OF WATER COLLECTED IN LAKES, RIVERS AND LAKES FOR BREEDING ANIMALS AND HUMAN SOURCES (EQUIVALENT A ESTROGEN IN A STANDARD CURVE WITH LOGARITHM).

samples number	concentration of estrogen			samples number	Concentration of estrogen		
1	0,895	$.10^{13}$	Fentomol*	21	0,833	$.10^{13}$	Fentomol
2	0,880	$.10^{13}$	Fentomol	22	0,806	$.10^{13}$	Fentomol
3	0,900	$.10^{13}$	Fentomol	23	0,811	$.10^{13}$	Fentomol
4	0,834	$.10^{13}$	Fentomol	24	0,838	$.10^{13}$	Fentomol
5	0,870	$.10^{13}$	Fentomol	25	0,902	$.10^{13}$	Fentomol
6	**0,970**	**$.10^{7}$**	**Micromol**	26	0,749	$.10^{13}$	Fentomol
7	0,906	$.10^{13}$	Fentomol	27	0,803	$.10^{13}$	Fentomol
8	0,920	$.10^{13}$	Fentomol	28	0,877	$.10^{13}$	Fentomol
9	0,933	$.10^{13}$	Fentomol	29	0,854	$.10^{13}$	Fentomol
10	0,829	$.10^{13}$	Fentomol	30	0,723	$.10^{13}$	Fentomol
11	0,894	$.10^{13}$	Fentomol	31	0,811	$.10^{13}$	Fentomol
12	0,824	$.10^{13}$	Fentomol	32	0,744	$.10^{13}$	Fentomol
13	0,877	$.10^{13}$	Fentomol	33	0,868	$.10^{13}$	Fentomol
14	0,891	$.10^{13}$	Fentomol	34	0,832	$.10^{13}$	Fentomol
15	0,898	$.10^{13}$	Fentomol	35	**0,215**	**$.10^{7}$**	**Micromol**
16	0,838	$.10^{13}$	Fentomol	36	**0,598**	**$.10^{7}$**	**Micromol**
17	0,825	$.10^{13}$	Fentomol				
18	0,859	$.10^{13}$	Fentomol				
19	0,857	$.10^{13}$	Fentomol				
20	0,853	$.10^{13}$	Fentomol				

Three samples of water the estrogen was present in micromol that corresponding in samples from Ibiúna and Equestrian Santo Amaro/SP.

The only samples that showed a great amount of estrogen were sent samples of Ibiuna and Hipica of Santo Amaro / SP. In Ibiúna, the water comes from a mine being raised fish in ponds receiving this water. The Equestrian comes from well and belongs to the Hípica. These samples should take another repeated time analysis to confirm the results, there may be changes in the analysis caused by the weather station, currently in São Paulo: heat and lack of water or it may be that actually have estrogenicity by the presence of waste on site, presence of algae and other factors.

According Ouyanga et al, 2006 and Eun-Joung et al, 2007, seasonal variations can alter water quality and concentration of DEs. During the drier station, the streams may include less water or no water is not silted to lakes high concentrations of estrogens and thus detects the estrogenicity is little water. In our study, the E2 was determined only in periods of drought, where the sewage inflow, constant throughout the year, is enhanced with the decrease in river flows slowly over the region rain events. E2 is the most potent estrogen, followed by E1 and E3. Estriol, however is the major secreted form, since the biosynthesis of 17β-estradiol in the body, other hormones, together with E2 are converted into estrone and estriol in later before excretion (Osterlund and Hurd, 2001).

Countries like Italy, Holland, Belgium and the United States reported concentrations of these estrogens in their watersheds between 0.2 and 21.7 ng L-1 (Verliefde, et al, 2007; Benotti et al, 2009). On the other hand, the recorded history for some

Brazilian sources, including Atibaia and Capivari, it is more worrying. Concentrations in ug L-1 levels are reported to oestrogens in rivers of Campinas, Jaboticabal and Belo Horizonte (Montagner and Jardim, 2011; Lopes et al, 2010; Moreira et al., 2009).

The concentrations determined in our samples, in most cases, were very close to the limit of quantification by bioassay, which only shows a tendency to detect synthetic estrogen in the bodies of water studied. The Endocrine Society has recently expressed its concern about the presence and chronic exposure to traces of endocrine disruptors concentrations with high potential estrogenic, as EE2 and other substances used as contraceptives and hormone replacement therapy as well as the chemical industry by-products, because of causing adverse effects human and animal health (Diamanti-Kandarakis et al., 2009).

MCF7 cells, tumor cells of human breast are easily cultivated and have estrogen receptors and stable estrogen dependence. We were able to demonstrate the proliferative response of these MCF7 in the control and the presence of 17B-estradiol (E2). A simple answer, reproducible. So, this is a sensitive system for the detection and quantification of estrogenic activity present in water or other substrate.

Accordingly, the results of e-screen assay were also found by different groups such as, Sonnenschein et al, 1998, Soto et al, 1995, etc. Different classes of substances such as steroids, alkylphenols, bisphenols, polyphenols, phthalates, hydrocarbons, chlorine, Xenoestrogen) act as estrogens which allow a quantitative extraction and application, also this e-screen assay.

IV. CONCLUSION

With this work, we were able to demonstrate that the E-screen assay with MCF7 cells is a sensitive and stable tool for quantitative analysis of estrogenicity of water in watersheds in the State of São Paulo and can easily be developed and applied in routine for animals and man food, aqueous extracts, plastics, etc.

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Antifungal activity of lichen extracts and usnic acid for controlling the saprolegniasis

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Abstract**—***Aquatic oomycetous fungi often cause serious damage to fresh water fishes. Antifungal activity of acetone extracts of three fruticose lichens namely,* Cladonia amaurocraea, Cladonia rangiferina *and* Usnea longissima *were investigated against three pathogenic oomycete fungi which can cause serious saprolegniasis:* Saprolegnia parasitica, Achlya bisexualis *and* Pythium *sp.. Usnic acid was also examined for antifungal activity against the pathogenic fungi. The minimum inhibitory concentration of usnic acid and lichen extracts for the tested fungi* Saprolegnia parasitica *and* Achlya bisexualis *were 2 mg* L^{-1}*, 200 mg* L^{-1} *respectively. The higher concentration was needed to inhibit mycelial growth of* Pythium *sp.. The result suggests that the potential of using usnic acid and lichen extracts for controlling the saprolegniasis.*

Keywords—antifungal activity, lichen, Oomycota, saprolegniasis, usnic acid.

I. INTRODUCTION

Saprolegniasis is a major disease problem and of great concern for the aquaculture industry. It plays a serious threat to fish populations in natural habitats. It is usually recognized as a fungal disease caused by any of several species of water molds of the family Saprolegniaceae, Oomycota, however, "oomycetous fungi" belong to the lineage of biflagellate "heterokont" organisms, commonly referred to as "stramenopiles", within the kingdom Chromista [1,2]. They do not belong to the kingdom Fungi, although they are usually studied by mycologists. *Saprolegnia parasitica* and *Achyla bisexalis* are the two most important oomycete fish pathogens. Some species in *Pythium* can also cause the saprolegniasis. Natural recovery by infected fish is almost impossible [1,3]. Malachite green was quite an effective antifungal agent but it is mutagenic, teratogenic and carcinogenic [4,5,6]. It has been banned since 1991 in many countries [7]. Formalin is the drug currently permitted by FDA for controlling fungus on fish diseases in the United States. Hydrogen peroxide, sodium chloride and bronopol are also worth mentioning in preventing and treating saprolegniasis [8]. Recently, nikkomycin Z was employed to inhibit the growth of the mycelium of *S. parasitica* [9]. Due to the lack of efficient methods to control pathogenic Saprolegniaceae, there is a dramatic reemergence of saprolegniasis in aquaculture. Thus, there is an important need for developing efficient and sustainable methods to stop the spread of these pathogens.

Lichens are symbiotic organisms of fungi and algae or cyanobacteria that can produce unique secondary metabolites and have been used in folk medicine since ancient times [10]. Studies in the last three decades proved the antimicrobial (antibacterial and/or antifungal), antiviral, antiprotozoal, antipyretic, antitumour, antiproliferative, anti-inflammatory, photoprotective, analgesic, as well as growth and enzyme inhibitory activities of some of the lichen extracts and compounds [10,11]. Various studies have demonstrated that many of lichen species contain usnic acid could play various biological roles and appear to function as allelopathic agents in nature. Usnic acid was proved to have the inhibitory effect on the growth of mold, bacterium, and yeast [12]. It was also reported that the acetone, methanol and light petroleum extracts of *Usnea* were effective against *Bacillus licheniformis, B. megatarium* and *Staphylococcus aureus* [13]. The results of study on the antibacterial activities of different solvent extracts of *Usnea florida* showed that the extracts had certain inhibition effect on *Staphylococcus aureus, Escherich coli, Pseudomonas aeruginosa, Micrococcus luteus,* and *Aspergillus flavus* [14].

The efficacies of usnic acid and the acetone extracts of three lichens (*Cladonia amaurocraea, Cladonia rangiferina* and *Usnea longissima*) were evaluated for controlling the growth of *Saprolegnia parasitica, Achyla bisexalis* and *Pythium* sp. in the present study. To the best of our knowledge, no information about the activities of lichen extracts and lichen acids against aquatic oomycetous fungi is available at present.

II. MATERIAL AND METHOD

Three species of lichen were used: *Cladonia amaurocraea*, *Cladonia rangiferina*, *Usnea longissima*. Three oomycetous fungal species included: *Saprolegnia parasitica, Achlya bisexualis* and *Pythium* sp.. *S. parasitica* was purchased from CBS (540.67). *A. bisexualis* (GHL 2012.2) and *Pythium* sp. (GHL 2012.3) were isolated from diseased fishes by using castor seeds. All three strains can infect tilapia and cause saprolegniasis. The strains were cultured on potato dextrose agar (PDA) slants and were stored at 4 ^{o}C until use. To obtain long-term growth cultures, the fungal strains were subcultured on 9-cm diameter PDA plates at 25 ^{o}C.

The lichen thalli were removed dust particles. The extracts were prepared by the extraction of 2 g of ground lichen in 30 ml acetone for five hours followed by filtration, at last get 20 ml lichen extracts. The stock solution of usnic acid was prepared by 20 mg usnic acid was fully dissolved in 20 ml acetone. The lichen extracts and usnic acid were stored at 4 ^{o}C for further bioassay. Final concentrations of stock solution for usnic acid and lichen extracts were 1 and 100 mg mL^{-1} respectively.

The medium of the following composition was prepared: dextrose 20 g, agar 20 g, potato 200 g, distilled water 1000 ml. The usnic acid and lichen extracts were mixed in 100 ml standardized medium, to give concentration of usnic acid were 32, 16, 8, 4, 2 mg L^{-1}, the lichen extracts were 3.2, 1.6, 0.8, 0.4, 0.2 g L^{-1}. In controls, 800 µl acetone was used in place of usnic acid and lichen extracts.

The pure culture of fungal species, growth and maintained on PDA medium at 25 ^{o}C was used for the experiments. Each treatment was duplicated three times. These treated Petri plates were incubated at 20 ^{o}C Radial growth for each test was recorded by taking the mean diameter of colonies from three plates. The measurements of colony diameter were made after different incubation periods depending on the hyphal growth rate of each species: 2 days for *Saprolegnia parasitica*; 2, 6 and 8 days for *Achlya bisexualis*; 2, 7, 8 and 10 days for *Pythium* sp.. Inhibitory effects of lichen compounds were determined by comparing treatment plates with control plates. The activity of each test compound was expressed as percent inhibition and, where appropriate, in both control and treated Petri dishes, was measured diametrically and the percentage inhibition of growth (% I) was calculated using the following formula.

$$I\ (\%) = (C\text{-}T)/C \times 100$$

Where C = Diameters of fungal colony in control plate, T = Diameters of fungal colony in treatment plate.

Data were subjected to one-way analysis of variance (ANOVA) using the Statistical program SPSS16.0, and means separation was made using Least Significant Difference (LSD). Difference on statistical analysis of data was considered statistically significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1 Activity of usnic acid

The activity of usnic acid concentration at 2, 4, 8, 16, 32 mg L^{-1} inhibited the growth of three pathogenic fungi is shown in Table 1. Usnic acid absolutely inhibited the mycelial growth of *Saprolegnia parasitica* at 32 mg L^{-1}. Compared with the activity of nikkomycin Z (about 100 mg L^{-1}, for totally inhibition), usnic acid has better effect to inhibit the growth of the mycelium of *S. parasitica* [9]. Mycelial growth of *Achlya bisexualis* and *Pythium* sp. are totally inhibited by usnic acid at 16 mg L^{-1}. Among all three oomycetous fungi, usnic acid showed good antifungal activity on *S. parasitica* at the concentration more than 4 mg L^{-1} (with percentage control >50%). The inhibitory potency of usnic acid against *S. parasitica, A. bisexualis* and *Pythium* sp. demonstrated that the Minimal Inhibition Concentration (MIC) to be 2 mg L^{-1}, 2 mg L^{-1}, 8 mg L^{-1}, respectively. The MIC was defined as the lowest concentration of significant inhibition of the visible mycelial growth. Recently, 30 fungicidal chemicals in agriculture were screened for antifungal activity against *Saprolegnia*, among them, kresoximmethyl and azoxystrobin showed very good effect in vitro with minimum inhibitory concentration (MIC) values of 1.0 and 0.5 mg L^{-1}, respectively [15]. However, the MIC of azoxystrobin was close to its safe concentration (SC 0.553 mg L^{-1}) and kresoximmethyl showed higher toxicity to goldfish (*Carassius auratus*) with MIC>SC (0.131 mg L^{-1}), thus, they may need chemical modifications to enhance their inhibitive effects or could be used in combination with other drugs [15]. In our study, That acute toxicity to goldfish of usnic acid performed as described in literatures [15,16] with SC (4.152 mg L^{-1}) showed it can be used to control the saprolegniasis caused by *S. parasitica* and *A. bisexualis*. Among the three species of oomycetous fungi tested, *S. parasitica* showed high susceptibility to the usnic acid. At concentration of 2, 4 mg L^{-1}, usnic

acid showed growth promoting activities for *Pythium* sp. (Table 1). *S. parasitica* grows very quickly and the colony could cover the whole Petri dishes for about 2 days, while *Pythium* sp. grows slowest among the three oomycetous fungi.

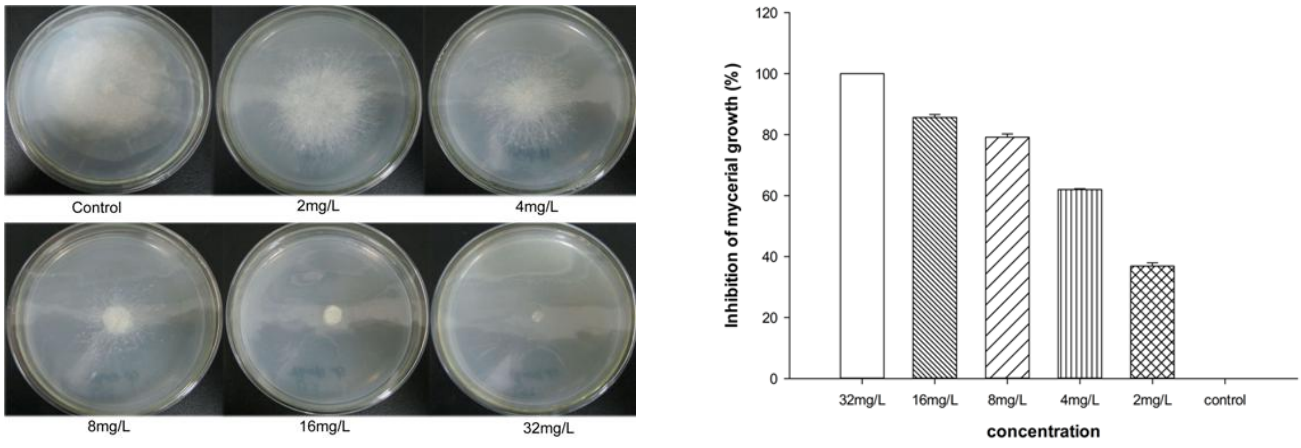
3.2 Activity of lichen extracts

Acetone extracts of *Usnea longissima*, *Cladonia rangiferina* and *Cladonia amaurocraea* at different concentrations (200, 400, 800, 1600, 3200 mg L^{-1} and with 800 µl acetone as control) for antifungal activity against *Saprolegnia parasitica*, *Achlya bisexualis* and *Pythium* sp. with their percentage inhibition, which is an average of three replicates and its standard error are given in Table 2. As an example, Mycelial growth of *S. parasitica* in different concentrations of *Cladonia rangiferina* extracts was also shown in Fig. 1 a & b. Effects of lichen extracts vary at different concentrations tested. In all cases, the fungal growth was completely inhibited with each extract at the concentration 3200 mg L^{-1}. No mycelial growth was observed from all discs treated with extracts of *U. longissima* and *C. rangiferina* on *Pythium* sp.; the same results were observed on *Achlya bisexualis.*

In the present study, the three lichens extracts significantly reduced the mycelial growth of the oomycetous fungi. Acetone extracts of the three lichens were found to show excellent activity against all the test fungi except *Pythium* sp. when compared with other two fungi. At a low concentration, extracts of lichens showed growth promoting activities for *Pythium* sp.

Among three lichen species, the acetone extract of *C. amaurocraea* showed minimum inhibition against *Saprolegnia parasitica* of 36 % at the concentration of 200 mg L^{-1}. Different concentrations of extracts exhibited significantly different antifungal activity. In the case of the fungal growth was completely inhibited with each extract with the concentration 3200 mg L^{-1}. Minimal inhibitory concentration (MIC) of acetone extract of tested lichen was determined and shown in TABLE 3. The minimal inhibitory concentration of the extracts against *Saprolegnia parasitica* and *Achlya bisexualis* is being found to be 200 mg L^{-1}, in contrast against *Pythium* sp. was 800 mg L^{-1} (TABLE 3).

The acetone extract effects of *U. longissima*, *C. rangiferina* and *C. amaurocraea* at different concentrations on the mycelial growth of *Achlya bisexualis* and *Pythium* sp. are also shown in TABLE 2. All extracts gave almost the same minimum inhibitory concentrations against *S. parasitica* and *A. bisexualis* (TABLE 3). The results indicate that mycelial growth of *A. bisexualis* is totally inhibited by extracts of the three lichens at 1600 mg L^{-1} and 3200 mg L^{-1}. At the concentration of 200 mg L^{-1} and 400 mg L^{-1} extracts of three lichens showed growth promoting activities for *Pythium* sp.. While extract of *C. amaurocraea* also showed growth promoting activities for *Pythium* sp. at the concentration of 800 mg L^{-1}. Acetone extracts of *U. longissima* was the better growth inhibitor when compared with acetone extracts of *C. rangiferina* and *C. amaurocraea* (TABLE 2). In the case of the extracts of *U. longissima* and *C. amaurocraea*, usnic acid should be effective compound, while for *C. rangiferina*, atranorin and fumarprotocetraric acid or their combination may be the main agents.



(A) **(B)**

FIGURE 1: (A): MYCELIAL GROWTH OF *SAPROLEGNIA PARASITICA* IN DIFFERENT CONCENTRATIONS (100×) OF *CLADONIA RANGIFERINA* EXTRACTS IN POTATO DEXTROSE AGAR MEDIUM.
(B) EFFECT OF DIFFERENT CONCENTRATIONS (100×) OF *CLADONIA RANGIFERINA* EXTRACTS ON THE MYCELIAL GROWTH INHIBITION OF SAPROLEGNIASIS PATHOGEN *SAPROLEGNIA PARASITICA*. ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

TABLE 1
EFFECT OF USNIC ACID ON THE MYCELIAL GROWTH AND PERCENTAGE CONTROL OF *SAPROLEGNIA PARASITICA*, *ACHLYA BISEXUALIS* AND *PYTHIUM* SP.

Fungal strain	Percentage control at various concentrations of usnic acid at 20 °C					
	0 mg/L	2 mg/L	4 mg/L	8 mg/L	16 mg/L	32 mg/L
Saprolegnia parasitica CBS 540.67	0	36.8±1.10	62.0±0.29	79.1±1.05	85.6±0.94	100±0
Achlya bisexualis GHL 2012.2	0	2.55±1.93	13.2±1.25	49.6±1.66	100±0	100±0
Pythium sp. GHL 2012.3	0	-19.5±3.05	-5.6±3.78	23.5±1.68	100±0	100±0

TABLE 2
ANTIFUNGAL ACTIVITY OF ACETONE EXTRACTS OF *USNEA LONGISSIMA*, *CLADONIA RANGIFERINA* AND *CLADONIA AMAUROCRAEA*

Lichen	Concentration	Percentage inhibition at various concentrations at 20 °C(%)		
		Saprolegnia parasitica	*Achlya bisexualis*	*Pythium* sp.
Usnea longissima	0 mg/L	0	0	0
	200 mg/L	66.82±0.31 b	33.08±1.13 a	–49.45±5.00 a
	400 mg/L	70.73±0.64 c	60.34±0.30 b	–24.75±4.57 b
	800 mg/L	58.07±1.44 a	100±0.00 c	33.30±4.95 c
	1600 mg/L	79±1.52 d	100±0.00 c	100±0.00 d
	3200 mg/L	100±0.00 e	100±0.00 c	100±0.00 d
Cladonia rangiferina	0 mg/L	0	0	0
	200 mg/L	41.76±1.15 a	12.00±1.85 a	–1.44±16.8 a
	400 mg/L	62.57±1.54 b	32.59±1.61 b	–1.11±8.50 a
	800 mg/L	79.39±0.87 c	67.59±1.45 c	–9.47±6.31 b
	1600 mg/L	89.40±0.42 d	100±0.00 d	100±0.00 c
	3200 mg/L	100±0.00 e	100±0.00 d	100±0.00 c
Cladonia amaurocraea	0 mg/L	0	0	0
	200 mg/L	36.13±1.46 a	12.60±0.69 a	–23.85±2.64 a
	400 mg/L	59.71±1.47 b	28.35±1.17 b	–2.00±1.39 b
	800 mg/L	77.86±1.18 c	64.84±1.50 c	24.78±2.58 c
	1600 mg/L	87.31±0.37 d	100±0.00 d	80.42±0.63 d
	3200 mg/L	100±0.00 e	100±0.00 d	100±0.00 e

a-e: Different small letters indicate significant differences among 5-level concentration within the same lichen extract (One-way ANOVA and Least Significant Difference (LSD), P< 0.05). Data are mean ± SE (standard error) based on n = 3.

TABLE 3
MINIMAL INHIBITORY CONCENTRATION (MIC) OF USNIC ACID AND LICHEN EXTRACTS AGAINST PATHOGENIC OOMYCETOUS FUNGI

Fungal strain	The MIC of Usnic acid (mg L^{-1})	The MIC of lichens tested (mg L^{-1})		
		Usnea longissima	*Cladonia rangiferina*	*Cladonia amaurocraea*
Saprolegnia parasitica CBS 540.67	2	200	200	200
Achlya bisexualis GHL 2012.2	2	200	200	200
Pythium sp. GHL 2012.3	8	800	1600	800

IV. CONCLUSION

In conclusion among all the lichen extracts and usnic acid tested for antifungal activity on the oomycetous fungi, good efficacy was demonstrated, except at a low concentration, the growth promoting activities for *Pythium* sp. were observed. Therefore, lichen secondary metabolites could be used as an effective agent for the treatment against the infection of some pathogenic oomycetous fungi. The lichens have the potential for use in developing a novel therapy to control saprolegniasis in aquaculture.

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Simultaneous Estimation of Multiple Dairy Technologies Uptake

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Abstract— *The low productivity of the Ethiopian diary sector has been explained by the genetic potentials and management practices. Milk production and consumption largely rely on the indigenous cows. To enhance the contribution of dairy to household food security, nutrition and income, adoption of improved dairy breeds together with the component practices is indispensable. To study the adoption of the dairy technologies and the factors stimulating the choices, a cross-sectional household survey was conducted in the central and eastern Ethiopia. Results declare that the rate of adoption of improved breeds, artificial insemination and improved forage remained to be limited. The uptake of concentrate feeding is found to be relatively better. As the joint multivariate probit estimation confirmed, there is complementarity and interdependence in the uptake of improved breeds, artificial insemination, concentrate feeds and improved forages. Utilization of forage lagged behind to complement adoption of improved cows. Tobit model determination confirmed that the same factors are affecting the adoption decisions. The factors responsible for the joint adoptions, the number of cows owned and intensity of adoption include education status, household wealth, access to markets and district centers, contacts with extension and gender roles. Both the joint and individual analyses highlighted the need for strategies that could overcome the constraints and ensure better uptake of the technologies.*

Keywords— productivity; adoption; joint estimation; complementarity; adoption intensity; responsible factors.

I. INTRODUCTION

Despite Ethiopia has high potentials for dairy development, farm productivity and consumption of milk lags behind the World and African average. Recent estimates showed that the national average daily milk yield of the indigenous cows is 1.32 liter with a lactation period of 180 days. While improved cows yield 7 liters with a lactation period of 242 days (Gebremedhin et al., 2014). Reasons of poor genetics, insufficient animal feeding and poor management practices explain the low productivity levels. Availability of feed both in quantity and quality remain to be the major impediment for livestock production and also specifically to the dairy sector. The size of land allocated for livestock grazing is minimal compared to land devoted to crop productions (Ahmed et al., 2004; Gebremedhin et al., 2009; Ayele et al., 2012). Moreover, limited availability of land and water resources and climate variability are important challenges (Godfray et al., 2010; Smith, 2013; Alemayehu et al., 2012).

Current studies indicated that there is a high supply and demand gap for fresh milk and milk products. Population growth, rising income and urbanization justify the recent growing trend of the demand for livestock products in the domestic and export markets (Delgado, 2003; Smith, 2013; Dunkan et al., 2013). Keeping pace with an increasing demand for livestock products, improving household income and reducing poverty requires revolutionizing productivity though adoption and diffusions of dairy technologies. Literatures emphasized that adoption of new technologies is promising path way to agricultural development (Baltenweck et al., 2006) or to accelerate economic growth and ensuring food security in Africa (Hazell, 2013).

Notwithstanding the availability of agricultural technologies, adoption rates in Sub-Saharan agriculture remained low (Gollin et al., 2005; Kondylis et al, 2017). In Ethiopia, several technologies of improved breeds of dairy cattle, artificial insemination, improved forages and veterinary health care have been promoted to the users (Staal et al., 2008; Ahmed et al., 2004; Spielman et al., 2010). However, the uptake of the technologies by the stallholder farmers proved to be minimal (Ayele et al., 2012; Duncan et al., 2013). Several scholars have tried to find out the reasons for the lower rate of adoption of technologies. Some farmers have better acceptance towards the disseminated technologies while others are reluctant and they maintain their status quo positions. Determination of the complementarity, extent, intensity and the factors explaining smallholders' adoption decision is therefore crucial to suggest research and extension policies to design mechanisms that facilitate adoption of technologies.

II. THE STUDY APPROACH AND DATA

2.1 Design and Data Source

With an aim to address the adoption study and impacts of adoption of dairy technologies, a household survey was conducted in 2015. The dataset used for this literature was generated through cross-sectional survey held at country level, considering diverse agro-ecologies and administrative regions. In the Oromia region, eight zones were targeted to document the extent of adoption and impacts of adoption of dairy technology packages. As part of the national survey, the analysis comprises East Shewa and West Hararge zones, situated in the central and eastern part of the country. The main survey included a total of 1630 households among which 401 households were considered.

The selection of the next administrative units or districts was based on their access to markets and services, which could further give options to target more accessible and less accessible villages. Furthermore, the cow population potential of the districts was used as the selection criteria. Two districts, Boset and Ada'a were included in East Shewa zone. From the West Hararge zone, Chiro and Habro districts were studied. Subsequently, two villages from each districts were considered based on their proximity to the district center, i.e., within 10 km distance from the district for the more accessible and greater than 10 km for the less accessible PAs. Based on the nationally adjusted and determined samples, proportionate samples of households were taken from the respective villages while taking into account the number of households residing in those villages. The same survey instrument was used embracing gender disaggregated information towards dairy technology adoptions and services.

2.2 Characterization of the Study Areas

In Ethiopia, the dairy system is characterized by subsistent production, low use of technologies, underdeveloped markets, and services (Ahmed et al., 2004; Staal et al., 2008). The share of the indigenous cows constitutes 81% of the total annual milk productions (FAOSTAT, 2014). In the rural areas, much of the production is used for household consumptions (CSA, 2015; Duncan et al., 2013; Staal et al., 2008).

Feed constitutes the largest share of the expenditure in dairy farming system in Ethiopia. To come up with the existing feed shortages, improved forage technologies, feed conservation technologies and the use of agro-industrial by-products have been promoted (Lenné and Wood, 2004; Ergano, 2015). Studies indicated that there is an increased demand for concentrate feeds, which could be explained by the replacement of indigenous cows with cross-bred cows. Grazing remains to be an important source of feed for dairy animals comprising nearly half of the overall nutritional sources. Estimations showed that the contribution of crop residues, green fodder and concentrate constitute 60%, 25% and 15%, respectively (Duncan et al., 2013).

So far several development programs in the country have targeted on introduction of high yielding exotic cattle in the highlands and distribution to smallholder farmers (Ahmed et al., 2004; Staal, 1995; Ergano, 2015). Artificial insemination (AI) technologies have been widely promoted as an effective technique for dissemination of genetic gain to producers at a relatively low cost. The dysfunctional nature of the Ethiopian AI system has also been emphasized. The involvement of the private sector for the provision of AI services is also minimal (Duncan et al., 2013; Ergano, 2015).

The importance of market access for dairy development, adoption of breeds, utilization of inputs and marketing links has been mentioned. Organized dairy cooperatives enable to overcome the existing dairy marketing constraints in rural areas (Jaleta et al., 2013). The recent emergence of private processing industries and marketing units likely stimulate producers in the peri urban areas and rural production systems, offering new market outlets for dairy products (Ahmed, 2004). Past studies defined market quality sites by associating with the availability of dairy cooperatives and privates operating with necessary dairy processing facilities (Duncan et al., 2013).

The center for East Shewa zone, named as Adama is found some 100 kilometers away from Addis Ababa. It is a business hub for the surrounding zones and neighborhoods. The site is characterized by its proximity to better markets and services of institutions that are disseminating dairy technologies. The major market outlets for dairy products and institutions such as cooperatives, milk collectors, small and medium processors are available. Feed milers/mixers, flour mills and food industries are also supplying compound and concentrate feeds.

Bishoftu is the center for Ada's district and located about 44 kilometers away from Addis Ababa. The district has access to markets at Bishoftu where milk collectors and processing units of the private owned, Holland Dairy and Ada'a Dairy Cooperative are in operation. Among the industrial feed manufacturers, Alema Feed PLC is the largest supplier of compound

feed for different livestock. Boset district is found in the neighborhood to the zonal center. Urban and peri-urban dairy producers supply fresh milk for the city and milk processors such as, Yakla PLC. Some of the selected villages have access to the district and market at Adama while others have poor access. Adama is the major supplier of wheat bran, wheat middling and seed cakes to the neighborhoods and also distant markets.

Chiro is the administrative center for the West Hararge and had a distance of 325 kilometers from Addis Ababa. The area could be described by the limited access to large markets, services and feed suppliers as compared to East Shewa. The formal dairy marketing outlets both private and dairy farmer groups are not common. Though better participation in milk marketing in the West Hararge, the informal marketing channel remains the dominant option. The district of Chiro is found very closer to zonal center and expected to have better market access. Habro district is found 70-75 kilometers away from the zonal center.

TABLE 1
HOUSEHOLD AND DAIRY RELATED INFORMATION ABOUT THE SURVEY SITES

Household and farm characteristics	East Shewa	West Hararge	Total	Std. Dev
Family members	6.19	6.08	6.14	2.33
Age of the head(years)	45.65	38.89	42.20	12.01
Completed education (years)	2.35	2.17	2.26	3.32
Land holding (ha)	2.09	0.66	1.36	1.26
Land for perennials (ha)	0.11	0.23	0.17	0.36
Non-agricultural income (ETB)*	6211.8	2262.4	4192.8	16547.2
Total livestock ownership (TLU)	8.12	3.61	5.82	4.19
Oxen ownership (No)	2.82	0.88	1.83	1.80
Cow ownership (local)	1.47	1.26	1.37	0.87
Lactation length (months)**	7.0	5.6	6.0	-
Daily milk production (liters)**	1.38	1.83	1.60	-
Local breed cows(liters)				
Early lactation			2.64	3.12
Mid lactation			1.65	1.29
Late lactation			1.07	1.10
Improved cows(liters)				
Early lactation			9.27	5.68
Mid lactation			6.76	4.09
Late lactation			4.71	3.29

Source: Own Survey (2015) *1$=22.63 ETB ** Central Statistical Agency (2015).

TABLE 2
MAJOR DAIRY FEED SOURCES AND COMPOSITION (PERCENTAGES)

Feed Source	East Shewa	West Hararge	Total
Grazing	79.08	75.61	77.31
Crop residue	92.35	98.05	95.26
Green feeding	78.57	94.15	86.53
Hay	22.96	54.15	38.90
Concentrate feeds	52.04	49.27	50.62
Improved forage	10.2	29.27	19.95
Beverage by-products	80.61	6.83	42.89

Source: Own Survey (2015).

III. THEORETICAL AND ANALYTICAL CONTEXT

3.1 Theories of Technology Adoption and Empirical Models

The pioneer scholars elucidated that technology adoption commonly refers to the decision to use a new technology or best course of action available by economic units on a regular basis. The terminology of diffusion often refers to spatial and temporal spread of the new technology among different economic units (Rogers and Shoemaker, 1971). According to Feder et al. (1985), individual or farm level adoption refers the degree of use of a new technology in a long-run equilibrium when the farmer has full information about the new technology and potentials. Aggregate adoption (diffusion) was defined as the

process of spread of a technology within a region. Similarly, Thirtle and Ruttan (1987) defined aggregate adoption as the spread of a new technique within a population (in Feder and Umali, 1993).

In the literatures of agricultural technology adoption, two commonly known approaches often have been raised. Because of the fact that agricultural extension programs in developing countries promote technologies as a package, simultaneous adoption of whole package is expected (Beyene, 2008). One of the first models related with a technological package was developed by incorporating technological complementarity and adoption under uncertainty. The potential output would rather be higher if both technologies are adopted than when the single one is adopted. Thus, the model implies a situation whereby initially larger farms adopt both technologies, while adoption of the divisible technologies by smaller farms will be limited (Feder and Umali, 1993). Others debated the whole package approach and emphasized that farmers do not adopt technologies as packages, rather they are opting for a single component or a few suitable technologies (Byerlee and Hesse de Polanco, 1986). Leathers and Smale (1991) explained step-wise or sequential adoption despite risk neutrality and unconstrained expenditures, because uncertainty is reduced through experience, the farmer may choose to adopt a component of the package (Foster and Rosenzweig, 1995).

Empirical studies by Pitt and Sumodiningrat (1991) modeled the simultaneous decisions involved in the adoption of new technologies under risk and uncertainty using the meta profit function. Hayami and Ruttan (1985) defined the meta profit function as dual to the meta production function (cited in Feder and Umali, 1993). Others stated that due to this fact, it is important to account for the relationships between the components of the innovation package (Mara et. al, 2002). The interactions between technological components possibly have influences on adoption (Byerlee and Hesse de Polanco, 1986). In connection to this, the simultaneous pattern of adoption was not found to be the case in drought-prone areas of Ethiopia (Kebede et al., 1990).

Both economic and sociological factors are responsible for the disparity in farm decisions and heterogeneity among adopters and non-adopters. From the sociological perspective of adoption, Rogers (2003) explained five main factors that are important in innovation, viz.: relative advantage, compatibility, complexity, trialability and observability (Marra et al., 2003). Relative advantage refers the degree to which a measure is assumed to outmatch another one, for instance costs and associated risks with new technologies (Smit and Smitters, 1992; Sattler and Nagel, 2006).

3.2 Factors Explaining Adoption Decisions

Analysis of agricultural technology adoption tempted in the effects of imperfect information, risk, uncertainty, institutional constraints, human capital, input availability and infrastructures (Feder et al., 1985; Foster and Rosenzweig 1995; Feder and Umali, 1993; Semgalawe and Folmer, 2000). Differences in technology uptake among smallholders could arise due access to farm endowments and product markets (Jayne et al., 2013). Feder et al. (1985) indicated that farm size, risk and uncertainty, human capital, availability of labor, credit constraints, and tenure security were the most explaining factors (Marenya and Barret, 2007). Furthermore, profitability, perceptions, attributes of the technology and information were discussed (Ervin and Ervin, 1982; Norris and Batie, 1987; Pagiola, 1996; Shiferaw and Holden, 1998; Hassan et al., 1998a; Hassan et al., 1998b; Lapar and Pandey, 1999; Kazianga and Masters, 2001 cited in Yigezu et al., 2015).

The conclusion by Just and Zilberman (1983) is that the magnitude of the effect of farm size on adoption decision relates to the household's risk behavior and farm returns. The larger farms are relatively are in better position to fulfill the high capital expenditures required with the adoption of new technologies. The Study by (Kebede et al., 1990) showed that in Ethiopia, adoption of crop inputs is positively associated with land ownership. Dynamic study approaches to adoption also confirmed that large land size is needed for continuous uptake of grade cattle in Kenya. When it comes to use of concentrates, owning of more land tend to be associated with continued use of concentrate feeding. Whereas, farmers who started growing fodder are the owners of less land implying the tendency for intensification of dairy production (Baltenweck et al., 2006).

Among the human capital variables, Weir and Knight (2004) found that education leads to higher probability of technology adoptions in Ethiopia. Wozniak (1987) and Kebede et al. (1990) also found that higher levels of education and access to information reduce the uncertainty associated with technology adoption and thus, led to better adoption behavior. Panel data tells that household education has positive effect for the continued uptakes of grade cattle. Unexpectedly, more educated farmers abandoned concentrate feeding while it positively affected established use of concentrates (Baltenweck et al., 2006). Availability of more family labor encouraged establishment of dairy farming, which implies that feeding concentrates is labor intensive. Furthermore, younger households who started keeping improved cattle in the past, but while aging they abandoned keeping them. In Ethiopia, similar conclusions have been drawn by studies on the adoption of improved breeds in

the areas of Selale and Ada'a. It is indicated that the experience of the farmer has positive impact on adoptions of improved breeds while size of the family was negative (Kebede et al., 1993).

Possession of more wealth leads to decreased absolute risk aversion (Marra et al., 2003). Empirical studies concluded that poor households are risk adverse so that they fail to invest in the most profitable opportunities. Conversely, they are involved with low return and low risk activities (Dercon, 2008; Schindler, 2009). M. Rosenzweig and H. Binswanger found that the wealth of the household encourages allocation of productive assets to the more risky portfolio (Dercon, 1996). According to (Semgalawe and Folmer, 2000), household wealth did not have significant roles for adoption of conservation technologies. In Ethiopia, higher wealth of the household positively affected adoptions of cross breeds in North Shewa (Selale) area while it was negative in Ada'a (Kebede et al., 1993). The finding by (Diiro and Sam, 2015) concluded that values of the household assets did not have significant impact on technology adoptions.

Technology adoption decision as a function of learning process from the past experiences has been emphasized. Through learning-by-doing households accumulate knowledge (Semgalawe and Folmer, 2000; Kondylis et al., 2017) and past adopters are more likely to continue to adopt new technologies. The role of experimentation and experience is related to more ability to mitigate risks and making better farm decisions. Acquisition of information favors agricultural technology adoption (Lindner, 1987). Lack of experience leads to risk aversion and has discouraging effect on adoptions. Most adoption studies use specifications of proxy variables to address the access. Proxy variables are commonly used, and include for both the cost of acquiring information and proxies for the incentive to acquire. Education status, distance to nearest current adopter, and availability of extension services refer to the costs to acquire while farm size for the incentive to acquire (Marra et al., 2003).

With the prevalence of imperfect credit markets, non-farm income sources are substitutes for borrowed capital (Reardon, 1997; Ellis and Freeman, 2004) and translate into increased availability of resources to invest in improved technologies. Findings tell that studies investigating the effect of nonfarm income on technology adoption in Africa have proved mixed effects. Dynamic programming techniques evidence reduced farmer incentives to invest in conservation despite the access to better nonfarm income opportunities (Diiro and Sam, 2015). On the contrary, (Marenya and Barrett, 2007) found a positive and significant effect of nonfarm income on the use of inorganic fertilizers.

The implication of transport infrastructure, markets for the supply of inputs and services, and distribution of outputs on adoption decision have been discussed (Fafchamps, 2004; Jayne et al., 2010). Studies conducted in Kenya indicated that low access to formal milk market outlets are more likely not to keep grade cattle and feeding concentrates. They concluded that for permanent adoption of dairy technologies, market access is found to be crucial. Growing of planted fodder is positively related to market access (Baltenweck et al., 2006).

To come up with the constraints in dairy marketing in Ethiopia, dairy cooperatives and dairy hub models have been targeted (Bernard and Spielman, 2009; Jaleta et al., 2013). Empirical research further highlighted that access to markets favors market orientation of the smallholders than isolated households who consume the larger portion of their produce (Stifel and Minten, 2004). Farmers' intention to adopt advanced technologies increases when they reside closer to markets. Study conducted by (Demeke et al., 1998) proved that better road infrastructures encouraged the use of crop inputs. It is evidenced that high quality markets impacted positively for the ownership of cross breeds in Ethiopia. The availability of formal markets for dairy products favors adoption of cross-breed animals. The market quality encourages stall feeding and more use of concentrate feeding. The use of AI among the indigenous cows has positive relation with the market quality but for the cross-bred cows, it was insensitive to market quality (Duncan et al., 2013). Similarly, as the farmers became further from the livestock markets, the tendency of growing improved fodder increased (Gebremedhin et al., 2003).

3.3 Modelling Technology Adoption and Estimations

Modeling of farm level decisions involves estimations of the likelihood of adoption of a given technology by the farmer. Dichotomous logit or probit are used in determining whether or not a farmer adopts a complete package or a few components (Feder and Umali, 1993; Koundouri et al., 2006). From the extension point of view, improved breed cows have been promoted inclusive of the necessary packages for dairy production. To overcome the numerous agricultural production constraints, farmers are also adopting a mix of technologies. The issues draw the attention to use models capturing the sort of correlations in adopting complementary or substitutable technologies. Studies by (Maregna and Barret, 2007; Teklewold et al., 2012) applied multivariate probit to analyze adoption decisions towards multiple technologies. Therefore, the joint multivariate probit (MVP) model was used to determine whether the farmers adopt improved dairy breeds together with the desired technological packages.

The MVP approach simultaneously determines the influence of the set of independent variables on the probability of adopting multiple technological choices. The model at the same time allows for potential correlations among unobserved disturbances and relationships between the adoptions of different packages (Belderbos et al., 2004). Otherwise, disregarding the prospective correlations and independent estimation of the single equations will lead to biased and inconsistent parameters estimates (Greene, 2003)

The joint estimation of farmers' adoption of improved forage (F), concentrate feeds (C), artificial insemination (A) and improved cows (I) according to (Poirier and Kapadia, 2012) given by,

$$y_{ij}^{*} = \left(I_j \otimes x_{ij}^{'}\right)\beta_j + \varepsilon_{ij},\ \ \varepsilon_{ij}|X_i \sim N_j\left(0_j\Omega\right) \tag{1}$$

Where, $y_{ij}^{*}$is the latent for the joint adoptions,$x_{ij}$is a K×1 vector of covariates,$X_i\left(I_j \otimes x_{ij}^{'}\right)$ is a J×JK matrix and Ω denote a J×J covariance matrix. In the latent four dimensional MVP model, $y_{ij}^{*}$are not observed but only their component signs,

$$y_{ij} = \begin{cases} 1, & \text{if } y_{ij}^{*} > 0 \\ 0, & \text{if } y_{ij}^{*} \leq 0 \end{cases} \tag{2}$$

The adjustment of the off-diagonal elements prior to imposing the diagonal restrictions, the joint-multivariate probit choice probability,

$$\pi_{ij\psi_1\psi_j} = \text{prob}\left(y_{ij} = \psi \middle| \beta_j, \Omega\right) = \int_{\Psi(\psi)} \phi_j\left(y_{ij}^{*}|\left(I_j \otimes x_{ij}^{'}\right)\beta_j, \Omega\right)dy_{ij}^{*} \tag{3}$$

Where $\phi_j(.)$ is the four dimension multivariate normal density and $\Psi(\psi) = \left(\Psi\left(\psi_1\right) \times \ldots \times \Psi\left(\psi_j\right)\right.$,

$$\Psi(\psi_j) = \begin{cases} (-\infty, 0)\ \ if\ \psi_j = 0 \\ (0, \infty)\ \ if\ \psi_j = 1 \end{cases} \tag{4}$$

The likelihood function for the joint-MVP,

$$L\left(\beta_j, \Omega\,; y_{ij}\right) = \prod_{n=1}^{N} \pi_{ij\psi_1\psi_j} \tag{5}$$

Restriction on the diagonal elements of the matrix, Ω and correlations of adoption decisions,

$$\begin{bmatrix} 1 & \rho FC & \rho FA & \rho FI \\ \rho CF & 1 & \rho CA & \rho CI \\ \rho AF & \rho AC & 1 & \rho AI \\ \rho IF & \rho IC & \rho IA & 1 \end{bmatrix} \tag{6}$$

Decision by the farmer also involve how much to adopt a given technology. In making a choice of whether to adopt and how much to adopt, it might take place as joint or separate decisions. When the decision includes the intensity of utilization conditional on the first step choice of adoption, the tobit model also known as the censored normal regression model is appropriate (Greene, 1990).Thus, the farmer makes a decision on the number of improved dairy cows to adopt or intensity of adoption of the technology (t), the distribution of t_i given that it is positive as denoted in (Verbeek, 2008),

$$E\{t_i|t_i > 0\} = x_i^{'}\beta + \sigma\frac{\phi(x_i^{'}\beta/\sigma)}{\Phi(x_i^{'}\beta/\sigma)} \tag{7}$$

The distribution given that it is censored,

$$E\{t_i\} = x_i^{'}\beta\Phi\left(\frac{x_i^{'}\beta}{\sigma}\right) + \sigma\phi(x_i^{'}/\sigma) \tag{8}$$

The log likelihood function for the tobit model,

$$LogL_1(\beta, \sigma^2) = \sum_{i \in I_0} Log\left[1 - \Phi\left(\frac{x_i^{'}\beta}{\sigma}\right)\right] + \sum_{i \in I_1} Log\left[\frac{1}{\sqrt{2\pi\sigma^2}} exp\left\{-\frac{1}{2}\frac{t_i - x_i^{'}\beta)^2}{\sigma^2}\right\}\right] \tag{9}$$

3.4 Factor Analysis

Households were classified into four wealth quantiles and the computation of the scores was obtained through principal component analysis. In many of the literatures, livestock and land ownership are commonly used as proxies for household

wealth. For the analysis, the amount of total land owned, number of oxen, income from off-farm labor, petty trade and participation in perennial crop production was taken into account. West Hararge is characterized by ownership of small land sizes and less number of livestock compared to East Shewa. Production of perennial cash crops, such as Chat & Coffee is important to explain the specific situations. After the principal component analysis, three factors were finally retained based on the Eigen values and total variances captured. Checking the appropriateness of the grouping with Kaiser-Meyer-Olkin test, the value of 0.72(middling) implies that the variables have in common to warrant principal component analysis. The predicted scores then were standardized by the respective means and standard deviations of the variables for each of the households before the wealth quantiles have been created (Legese et al., 2010).

IV. DESCRIPTION AND ECONOMETRIC ESTIMATION RESULTS

4.1 Adoption of Dairy Technologies

The rate of adoption of improved dairy cows was found to be only 11.47 % over the two study areas. The results proved that there is low level of uptake of the cross breeding technology. Comparatively, East Shewa has got higher adoption rate of 16.84% while West Hararge underperformed by scoring merely 6.34%. Unlike improved breeds, pooled data confirms that the adoption of concentrate feeding was better, almost half (50.62%) of the households utilized concentrate feeds. Specifically, adopters in East Shewa constituted 52.04% and in West Hararge, 49.27% of the households had adopted the practices. When it comes to improved forage adoptions, 19.95% of the households had the practice of feeding forages to dairy cows. East Shewa is described by lower utilizations of forage with a rate of 10.20% while it was relatively higher (29.27%) for West Hararge. Despite the lower adoption of improved dairy cows in West Hararge, improved feeding of the indigenous dairy cows was comparable to East Shewa where purchased feed is readily available. The adoption rate of artificial insemination services is equivalent to 23.19 percent. The two areas proved slight differences towards the uptake of the technology.

The primary reason for the households to stay with their status quo or fail to adopt improved cows was the expensiveness for acquiring them as quoted by 46.91 percent of the households. The availability issue and lack of sources for improved cows is the second important limitation for 36.66 percent who would like to adopt them. In spite of the two major bottlenecks, other reasons have contributions, for instance 14.04 percent declared lack of feeding material among the hindering factors. Inadequate awareness and the complicated management of improved cows remain to be less favoring for 13.76 and 7.02 percent, respectively.

4.2 The Joint Adoptions of Multiple Dairy Technologies

The estimation results of the joint multivariate probit analysis evidence that adoption of improved dairy cows is significantly determined by the education status of the household head. This means that an increase of the household head's education leads to an increase in probability of adoptions. Several studies confirmed the positive associations of education with the likelihood of uptake of technologies that it favors assimilation of information, learning, understanding of techniques, and making better farm decisions. Age of the household head and size of the household have shown no significant impacts for the decision. The household being in the highest wealth quantile (better-off households) leads to higher propensity of adoption of the technology. As empirical studies concluded, the poor less likely adopts technologies due to lack of resources and risk aversion behavior. The recent trend shows that improved dairy cows are so expensive and it requires higher initial investment. Imperfect financial markets and prerequisite of collaterals limit the participation of the poor. Lack of dependable AI services that prevailed so far also was not encouraging for acquiring at lower costs. The situation less favors the poor and the results are in consistent to theories and earlier findings.

Frequency of extension visit with farmers significantly affects adoption decisions. Extension contacts enhance knowledge about the technology, information on dairy management practices and AI services. Farmers who possess earlier awareness about improved breeds have higher tendency for adoption. This could be explained by their dairying experience, contacts with extension and networks. In contrast to areas with better accessibility to markets and services, the less accessible sites proved limited levels of adoption. Furthermore, better accessible areas in East Shewa have significant adoptions than better accessible localities in West Hararge where the earlier has got advantages of the services of breeding and market outlets to Addis Ababa, Bishoftu and Adama. Better access to zonal and district centers provides incentives of extension services,

market opportunities for dairy products and reduced transaction costs. Past studies highlighted the existing dairy marketing constraints and the sought development interventions. Introduction of the formal marketing outlets through private and farmer owned collection points possibly pledged adoptions mainly in the urban and peri urban areas. Districts or villages with less proximity to the centers proved very limited adoptions. Distance to market centers often used as proxy for market access is a limiting factor for adoption decisions.

Gender disparity in terms of access to information, services and resources has encouraging or discouraging basics for farmers' technology adoption decision. Differing settings of gender roles embedded in the households' headship positions have significant implications for farm decision making and adoptions. Compared to households with equal participation in decision making, households' decision dominated by male heads leads to less likelihood of adoption of improved cows. If the decision is exclusively made by the wife or else the female head, positive association was evidenced. While comparing the household headship status, male headship did not lead to significant adoption of improved breeds. The results explain the important roles of women in adoption decisions.

The study on adoption of concentrate feeding considered purchased industrial by-products and own produced feed types. The industrial by-products such as oil seed cakes, wheat bran, wheat middling, spent grain and grain produce were included. The analysis result concluded that concentrate feeds adoption is positively and significantly explained by the education of the head. The household being in the highest wealth quantiles had no significant explanation for the utilization of concentrate feeds. The adoption of concentrate feeds comprises feeds that are own produced by the farmer and also owners of more land may rely on grazing could be possible reasons for the weak influences. More frequent contacts with extension impacts positively for the adoption decision. More accessible areas significantly use concentrate feeds obviously related with the more availability of feed materials in the nearby market centers and less transaction costs. Equal participation in the household encouraged the use of concentrate feeds in contrast to decisions made exclusively either by male or female.

For successful improved dairy breeds production, the availability and use of improved high quality forages is crucial. There is limited use of improved forages in East Shewa though relatively higher breeds adoption. Oat-vetch is among the adopted type by some farmers in East Shewa while elephant grass has better acceptance in West Hararge. The results showed that male headed households are better users of forage crops. Similarly, educated farmers have better utilizations than the less educated farmers. Possession of high wealth is positive but not very determinant for the adoption of forages. Study conducted with regard to forage adoption concluded that wealth did not sufficiently explain the decisions (Gebremedhin et al., 2003). Frequency of extension contact positively affects the probability of adoptions. There is association between more accessibility and adoption of forage, however, the evidence is not sufficiently large. Rather, both accessible and less accessible areas in West Hararge have higher adoption than more accessible in East Shewa. The study by (Gebremedhin et al., 2003) indicated that distance from crop and feed markets encourages forage adoption.

The adoption of Artificial Insemination (AI) technology importantly and positively associated with the education status of the household head. Households in the highest wealth quantile have higher tendency for utilization of AI services, however, the evidences do not support significant impact as in the case of breed adoptions. Frequent extension contacts and access to districts found to be limiting for adoption of AI. Proximity to district centers determines the access to AI and propensity of adoptions. In the same way to breeds, recent status evidences that better accessible areas in West Hararge have shown slow rate of adoption and is negative compared to the reference location. Better accessible areas in west Hararge have low tendency to use AI could also be connected to lack of better market opportunities and services for dairy products. Consequently, if decisions are dominantly made by the female head or the wife, higher adoptions of AI was concluded.

The joint multivariate probit analysis confirmed the correlation of the error terms and interdependence in the dairy technological choices. The likelihood ratio test declared significant complementary relations among the four adoption choices at 1% probability levels. The adoption of artificial insemination is simultaneous to that of improved breeds and concentrate feeding. Concentrate feeding is suggestively correlated with improved cow adoption. Among all combinations, adoption of improved cows was not with adequate forages implying the low adopted bundle. To conclude, with MVP model we benefit from both estimation of unbiased parameters and testing of the simultaneous adoption of desirable technological components.

TABLE 3
MULTIVARIATE PROBIT JOINT ESTIMATION ON ADOPTION OF DAIRY TECHNOLOGIES

Variables	Improved cows (I)		Artificial insemination (A)		Concentrate feed (C)		Improved forage (F)	
	Coeff [1]	SE	Coeff	SE	Coeff	SE	Coeff	SE
Household head							0.4422	0.2827
Household size	0.0397	0.0429	0.0236	0.0347	-0.0372	0.0320	-0.0086	0.0361
Age of the head	-0.0003	0.0082	-0.0067	0.0077	-0.0025	0.0063	0.0118	0.0073
Formal education	0.1080**	0.0433	0.1114***	0.0376	0.0943***	0.0338	0.0785**	0.0380
Formal education square	-0.0073	0.0075	-0.0065	0.0065	-0.0083	0.0061	-0.0010	0.0060
Second[2]	0.2600	0.2899	0.1336	0.2258	0.1558	0.1916	-0.1397	0.2285
Middle[2]	0.0695	0.3143	0.1390	0.2182	0.0023	0.1887	-0.3174	0.2272
Highest[2]	0.6765**	0.2758	0.3035	0.2185	-0.1455	0.1924	0.1840	0.2166
Productive safety net	-0.0595	0.0698	-0.0208	0.0484	0.0252	0.0392	0.0018	0.0461
Less access to district (East Shewa)[3]	-0.7710***	0.2577	-1.4108***	0.2320	-0.4406**	0.2009	-0.1405	0.2696
Access to district (West Hararge)[3]	-0.7680***	0.2771	-0.6248***	0.2197	-0.1944	0.2071	1.2080***	0.2527
Less access to district (West Harage)[3]	-0.7452***	0.2636	-0.8674***	0.2298	-0.8240***	0.2346	0.4568*	0.2686
Frequency of extension contact	0.2269***	0.0757	0.2112***	0.0639	0.3183***	0.0700	0.1777***	0.0612
Decision of male headed[4]	-0.5327**	0.2313	0.0127	0.1810	-0.2212	0.1565		
Decision of female headed/ wife[4]	0.2405	0.3164	0.6855***	0.2492	-0.3171	0.2130		
Time aware of improved breeds	0.0567***	0.0193	0.0181	0.0147				
Constant	-1.6670***	0.3193	-0.7728***	0.2484	0.1100	0.2174	-2.0782***	0.3488
ρCF	0.2072**	0.0949	ρAC	0.2664***	0.0965			
ρAF	0.0579	0.1101	ρIC	0.2608**	0.1257			
ρIF	0.1009	0.1324	ρIA	0.4737***	0.1052			
Number of observations = 401 Wald chi2 (57)= 271.2								
Log pseudo likelihood = -675.953 Prob > chi2= 0								
Likelihood ratio test of ρCF = ρAF = ρIF = ρAC = ρIC = ρIA = 0								
Prob > chi2= 0.0001 chi2(6) = 28.2821								

1 *, **, *** significant at 10% 5% and 1%, respectively 2 Reference category is lowest wealth quantile
3 Reference category is access to district capital (East Shewa) 4 Reference category decision is equally by male & female

Conditional probabilities in (Table 4) imply the likelihood of adoption of the technology given that the other technology has already been adopted. As shown, there is a change in the conditional probabilities compared with the marginal probabilities, confirmed the dependency and complementarity of the actions. In contrast to all pair-wise matches, the probabilities for

improved forage, concentrate and artificial insemination is higher when it was conditional on the adoption of improved cows. Concentrate feeds and artificial insemination proved higher complementarity in conformity to the significance test of correlations of the disturbance terms in MVP model. Adoption of improved cows is highly interdependent with the use of artificial inseminations. For the conditionality of a single practice given three technologies are adopted, the same holds that the probability changes are higher for concentrate and artificial inseminations.

TABLE 4
MARGINAL AND CONDITIONAL PROBABILITIES OF DAIRY TECHNOLOGIES ADOPTION

Probabilities	Improved Forage	Concentrate	Artificial Insemination (AI)	Improved Cows
$P_M = 1$	0.199	0.507	0.235	0.115
$P_M = 1/P_F = 1$	1	0.687	0.300	0.175
$P_M = 1/P_C = 1$	0.271	1	0.315	0.167
$P_M = 1/P_A = 1$	0.258	0.688	1	0.344
$P_M = 1/P_I = 1$	0.304	0.739	0.696	1
$P_M = 1/P_F = 1, P_C = 1$	1	1	0.364	0.218
$P_M = 1/P_F = 1, P_A = 1$	1	0.833	1	0.542
$P_M = 1/P_F = 1, P_I = 1$	1	0.857	0.928	1
$P_M = 1/P_C = 1, P_I = 1$	0.353	1	0.823	1
$P_M = 1/P_C = 1, P_A = 1$	0.312	1	1	0.437
$P_M = 1/P_A = 1, P_I = 1$	0.406	0.875	1	1
$P_M = 1/P_C = 1, P_A = 1, P_I = 1$	0.428	1	1	1
$P_M = 1/P_F = 1, P_A = 1, P_I = 1$	1	0.923	1	1
$P_M = 1/P_F = 1, P_C = 1, P_I = 1$	1	1	0.917	1
$P_M = 1/P_F = 1, P_C = 1, P_A = 1$	1	1	1	0.600

P_M is the probability of adoption of a given technology component m, representing improved forage (F), concentrate feeds(C), artificial insemination (A) and improved cows (I).

4.3 Number of Improved Cows Adopted and Intensity of Adoption

The decision on the number of improved cows owned is affected by education status of the head. In the same way to the decision of whether to adopt or not to adopt, more education leads to possession of more number of improved cows. Adoption intensity measured as the ratio of cross breed cows to total cows also was positively determined by education. The conditional and unconditional marginal effects in (Table 5) attest that an increase in education favors the adoption of more number of improved cows or higher probability of increased intensities. Though not very strong evidences, household size has positive while age of the head has negative relations with both the dependent variables.

High wealth has positive impact both for acquiring more number improved cows and the intensities. Again this is related to the liquidity constraints with the poor households. Prevalence of imperfect credit markets and increased absolute risk aversion explain the heterogeneity among the households. Contacts with extension and time of awareness significantly influence the decisions of how many improved cows to keep or replacement of the local breeds. Significant differences exist among more accessible and less accessible areas. Moreover, more accessible areas in East Shewa have higher adoption than more accessible areas in West Hararage. In the household, if decision is made exclusively by the male heads, the likelihood of acquiring more number of cows will be low. The conditional and unconditional marginal effects as shown indicate changes in the number of improved cows owned, intensities and probabilities due to changes in the explaining variables.

TABLE 5
TOBIT REGRESSION RESULTS ON THE NUMBER COWS OWNED AND INTENSITY OF ADOPTION

Variables	Number of improved cows					Intensity of adoption				
	Coeff[1]	SE	Total change	Change E(t>0)	Probability Change (t>0)	Coeff	SE	Total change	Change E(t>0)	Probability Change (t>0)
Household Size	0.0940	0.0821	0.0109	0.0159	0.0064	0.0381	0.0415	0.0109	0.0159	0.0064
Age of the head	-0.0043	0.0167	-0.0005	-0.0007	-0.0003	-0.0024	0.0078	-0.0005	-0.0007	-0.0003
Formal education	0.2878***	0.0947	0.0335	0.0486	0.0195	0.1091***	0.0397	0.0335	0.0486	0.0195
Formal education square	-0.0272*	0.0150	-0.0032	-0.0046	-0.0018	-0.0088	0.0068	-0.0032	-0.0046	-0.0018
Second[2]	0.6553	0.5729	0.0762	0.1106	0.0444	0.3184	0.2696	0.0762	0.1106	0.0444
Middle[2]	0.1762	0.5985	0.0205	0.0297	0.0119	0.0736	0.2776	0.0205	0.0297	0.0119
Highest[2]	1.3213**	0.5414	0.1537	0.2229	0.0894	0.5817**	0.2350	0.1537	0.2229	0.0894
Productive safety net	-0.1118	0.1354	-0.0130	-0.0189	-0.0076	-0.0406	0.0687	-0.0130	-0.0189	-0.0076
Less access to district [3] (East Shewa)	-1.6300***	0.5432	-0.1896	-0.2750	-0.1103	-0.7624***	0.2404	-0.1896	-0.2750	-0.1103
Access to district (West Hararge)[3]	-1.7422***	0.5838	-0.2027	-0.2939	-0.1179	-0.7781***	0.2572	-0.2027	-0.2939	-0.1179
Less access to district (West Harage)[3]	-2.0259***	0.6063	-0.2357	-0.3418	-0.1371	-0.8526***	0.2694	-0.2357	-0.3418	-0.1371
Frequency of extension contact	0.4499***	0.1400	0.0523	0.0759	0.0304	0.1983***	0.0654	0.0523	0.0759	0.0304
Time aware of improved breeds	0.0830**	0.0329	0.0097	0.0140	0.0056	0.0372**	0.0157	0.0097	0.0140	0.0056
Decision of male headed[4]	-0.9280*	0.5285	-0.1080	-0.1566	-0.0628	-0.5138**	0.2475	-0.1080	-0.1566	-0.0628
Decision of female headed/ wife [4]	0.6168	0.5717	0.0718	0.1041	0.0417	0.2301	0.2672	0.0718	0.1041	0.0417
Constant	-3.2261***	0.7024				-1.4812***	0.3084			
Sigma	2.1372	0.2319				1.0283	0.0841			
F(15, 386) = 4.24						F(15, 386) = 8.63				
Prob > F= 0						Prob > F= 0				
Log pseudo likelihood = -164.732						Log pseudo likelihood = -132.992				
Pseudo R2= 0.2055						Pseudo R2= 0.2214				

1 *, **, *** significant at 10% 5% and 1%, respectively
2 Reference category is lowest wealth quantile
3 Reference category is access to district capital (East Shewa)
4 Reference category decision is equally by male & female

For successful dairy production cross breeds, improved feeding and artificial insemination are not the complete packages. Extension advises more and several component practices so that determination of the issues would be useful to gain feedback for future research and extension approaches. Furthermore, intensity of use of the recommended component practices are explaining successful adoptions of the technologies. Notwithstanding the analysis on the adoption of breeds, parity and blood levels are important for enhancing efficiency of the dairy production. The effort did not attribute towards those concerns.

V. CONCLUSION AND IMPLICATIONS

Due to subsistence nature of the diary system in the country, there is limited surplus production of dairy products. High dependence on local breeds, poor feeding and health management practices contribute for low productivity of dairy cows. The source of feed for dairy is dominated by crop residues and grazing. The poor quality of crop residue and need for improvement options has been mentioned. Lack of adoption of improved forage limits the potential productivity gains of both improved and local breeds. The presence of the informal market channel in many rural areas and lack of dairy processing facilities constrained the incentives for market orientation, utilization of inputs and technologies. It hampers the potential income and livelihood prospects from the sector.

Most of farmers raised the high cost of improved cow breeds as the major restriction and lack of supply is the second reason for not adopting improved dairy breeds. The adoption of improved cows requires high capital so that more wealthy households more likely own them. Sequential and package adoption approaches are debatable in many of the literatures. Recent efforts in the study of technology adoption emphasized the complementarity and substitutability of farm practices and adoption decisions. Analysis on the joint dairy adoption decision proved the existing complementarity in the uptake of the technologies. The availability of concentrate feeds and AI services have association with the adoption of improved breed cows. The adoption of forage is not satisfactory though positive association and long established dissemination efforts by extension. The responsible factors for the adoptions include education position of the household, wealth status, extension contacts, number of years became aware of improved cows, access to the markets and district center, and women's involvement in decision making. However, household wealth did not sufficiently explain the adoption of improved forages and concentrate feeds.

The market constraints are important for adoption decision that high transaction costs reduce the incentives of farmers' participation and have less preference to invest in high return dairy technologies. In support of this, it is concluded that milk market participation is limiting for adoption of the dairy improved breeds. Strategies that ensure improved market access for dairy products through linkages to formal market channels, farmer institutions, dairy infrastructure, enhancing awareness, and lessening the financial burden of the poor are indispensable. Interventions need to consider specific realities for improving adoptions of dairy technologies.

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Association of Hygiene Hypothesis with High prevalence of Allergy and Autoimmune Diseases: FMT industry

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Abstract*—Prevalence of Allergy and Autoimmune Diseases are high in developed countries, but not in developing countries. Helminth infection were associated with lower level of allergy but the mechanism is not yet clear. In hygiene countries like UK, Japan and Korea, Fecal Microbiota Transplantation (FMT) has gained interest as novel therapy and prevention and reach to medicine industrial manufactures. This study will support it by associate Hygiene Hypothesis (HyHy) and the high prevalence of Allergy and Autoimmune Disease. Systematic review and Bayesian network analysis in EBSCO host search engine were used. Result from dynaMed Evidence Based, Point-of-care Reference e Journals: Dentistry, Medical and Nursing has describe 9 literatures support the association of Hy-hy/parasite infection with Allergy and/ or Autoimmune Diseases: 2 publications of meta-analysis are chosen and supported by 5 cross sectional, longitudinal, cause-effect design, and 2 clinical and animal trial. Conclusions: Low-middle-high income countries has a life style of Hyhy pattern support FMT industrial medicine and travelling to developing countries with broad diversity bacteria and worm in therapeutic and prevention potential, supported by sub-saharan and tropical rainforest developing countries.*

Keywords*— Hygiene hypothesis, allergy, autoimmune diseases, FMT industrial medicine, traveling to developing countries.

I. INTRODUCTION

A firmed increase of global prevalence in allergic diseases mainly among children in the last few decades. Over the years, where several epidemiological studies have reported an inverse association between parasitic worm infections and allergies. The relationship between helminthes infection and allergies is urgent as research aims move to approach the potential of therapy and prevention in using helminths and their products for allergic disorders.[1] Using infectious disease high prevalence countries as a source of prevention and treatment of allergy and autoimmune diseases (AD). Knowledge of much references in the association of Hyhy-Allergy[2,3,4] Molecular aspect of epitope mapping of bacteria and worm vs. lifestyle in low-middle-high income population are associated with Most of Autoimmune Diseases are costly but Fecal Microbiota Transplantation (FMT) is an effective potential therapy and vaccine.[5] Traveling to developing countries with high biodiversity microbiota are also promising.

II. MATERIAL AND METHOD

Systematic Review and Bayesian analysis using EBSCO host DynaMed: Evidence –Based, point-of Care Reference, e-Journal: Dentistry, Medical and Nursing search engine. First, searching with CINAHL plus with developing the knowledge for the aims of study. Using the following search terms: Autoimmune Diseases, Hygiene Hypothesis, Epidemiology and AD, Atopic Sensitization, Asthma, T1D, IBS, MS, Rheumatoid Arthritis, Ulcerative Colitis, Allergic Diseases. Epidemiology AND Autoimmune Disease and UK (263), AND Japan (259). FMT are growing (4550), FMT and Meta-analysis (12), FMT and UK (34), FMT AND Japan (66). FMT industry (3), Hygiene Hypothesis AND Autoimmune Disease (117), Helminth AND Autoimmune diseases (148), Worm and Autoimmune (36), Worm and Hygiene Hypothesis (17), Epidemiology AND Meta-analysis and Rheumatoid Arthritis (27). Second step, all are screening abstract or title: excluded the not relevant and the duplicates by Bayesian analysis. Since we were interested in explanatory factors of bacteria and worm epitope sequencing are filtered. In the third step, screening full text publications, excluded since not relevant, duplicated and extracted for Table. Association of Hygiene Hypothesis or parasite infection AND Allergy/ Autoimmune Diseases. The selection was mainly performed by one reviewer and confirmed by the co-authors.

III. DISCUSSIONS

3.1 Dry and Wet climate

From clinical to molecular aspect of inflammation markers in savanna (warm and dry climate) with dormant bacteria and worm in dry season, and also in tundra (cold and dry) where no worm no bacteria in all season are searched by us. Meanwhile AD have been found in low prevalence in wet and warm (tropical rainforest) or hot and humid (sub-Sahara) countries, whereas allergy and AD are epidemic in clean countries. Dormant bacteria in dry season are specific physics-chemistry antigens and broad spectrum of variable microbiota implies rich of epitope of protein from known FMT. Figure 1 of Flowchart and Table 1 showed the association of Hygiene Hypothesis with allergy and AD.[1-2, 4-11]

TABLE 1
DESCRIPTION OF IDENTIFIED 9 LITERATURES ON HYGIENE HYPOTHESIS(WORM-BACTERIA)-ALLERGY/AUTOIMMUNE DISEASES

Study	Design	Population	Hygiene Hypothesis autoimmune measure	Epitope / synthetic vaccine measure
Figueiredo 2013	Comparison of high vs. low infection	Environmental condition in Latin American	Aeroallergen	Immunologic phenotypes, atopy, asthma
Obeng 2014	Cross-sectional	1385 Ghanaian Schoolchildren	Schistosome infection	Mite Atopy but not wheeze & asthma
Hamid 2011	A longitudinal study	Semi urban and rural areas of Flores, Indonesia	Intestinal helminth infections	Allergy
Xu 2015	Randomized Controlled Trial (RCT)	Metabolic syndrome subjects	Fecal Microbiota Transplantation	Beyond intestinal disorders
Lopez-Isac 2016	Meta-analysis	Meta-genome-wide association (meta-GWAS)	Systemic Sclerosis (SSc) and Rheumatoid Arthritis (RA)	Top single-nucleotide polymorphism
Colman 2014	Systematic review and Meta-analysis	18 studies of FMT primary therapeutic agent for 122 pts with 79 ulcerative colitis, 39 Crohn's disease, 4 IBD	Clinical remission and/or mucosal healing	IBS as autoimmune disease
Puentes F 2016	Animal model of autoimmune disease	Tolerogenic potential of fusion protein in 10 transgenic mouse model	Parasite proteins containing repeats are essential invasion ligands	Ability to evade the host immune system
Ajendra 2016	Experimental	NOD mice after insulitis started in T1D process	Inflammation of the pancreatic islets	Helminth antigens as a new potential therapeutic approach against AD
Lagier 2014	Review	Longitudinal CT of Recurrent CDI patients	FMT frozen, antigens	Extensive use and wide industrialization of FMT against AD

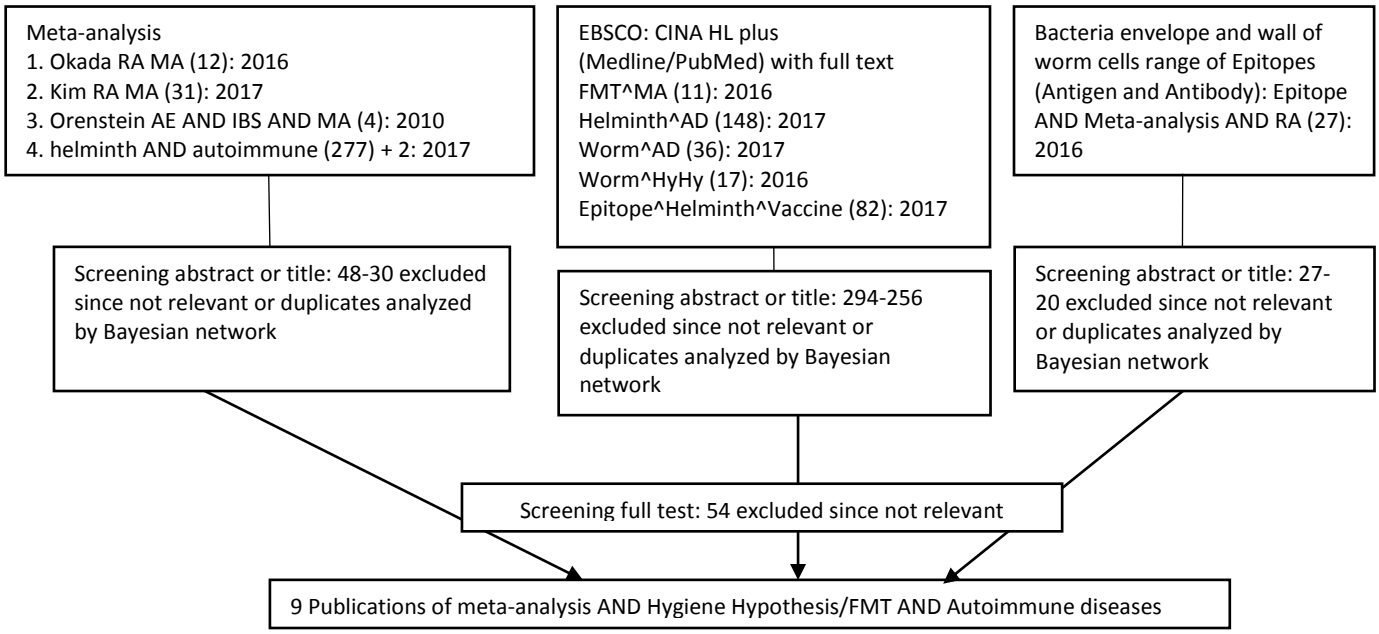

FIGURE 1. FLOWCHART OF THE 9 IDENTIFIED LITERATURE: 2 META-ANALYSIS, 2 REVIEW, 3 CROSS SECTIONAL, LONGITUDINAL, CAUSE-EFFECT DESIGN, 2 EXPERIMENTAL (ANIMAL/ AHUMAN) WHICH ASSOCIATED HYGIENE HYPOTHESIS/PARASITE INFECTION WITH ALLERGY AND AUTOIMMUNE DISEASES

Improved hygiene and little infections in childhood influence the development of allergic and autoimmune diseases. It has been reported in Latin America (Urban Brazil),[6] a tropical rainforest area. Lifestyle factors with microbial exposure are associated with allergy prevalence also reported in developed countries.[7]

Not only microbial exposure, helminth infections are also associated with low prevalence of allergy, [8,11] till FMT are used as therapy in Autoimmune Diseases [12]

Including metabolic diseases.[9] Systematic sclerosis and rheumatoid arthritis has been identified have a common susceptibility locus of gene.[13]

3.2 Proinflammatory cytokine as hygiene hypothesis marker

Parasite proteins contains repeats which induce immunosuppression and induce down-regulation of TNF-α (cytokine proinflammation) and TGF-β (cytokine profibrosis) make potential application for the treatment and prevention of autoimmune diseases.[14]

Entering of helminth antigens increase the protective effect stipulated by antigen-peculiar therapies and symbolizes a new approach for potential therapy against AD including T1DM.[15] The use of microbiota which has been frozen facilitates transplantation, and per oral are at least as effective as other invasive methods and to keep from occurring the risk of anesthesia. The use of pills form, already test as clinical trials, will certainly be the beginning landmark for the width use and broad industrialization of fecal microbiota transplantation.[10]

Treatment with helminthes and worms ova mitigated the clinical symptoms of numbers autoimmune diseases in animal model and in patients. Tuftsin-PC (TPC), a novel helminth-based compound, reduced proinflammatory cytokines and induced the expression of anti-inflammatory cytokine, as well as expansion of Treg and Breg cells, also histological analysis guide to a new approach for a natural therapy for beginning rheumatoid arthritis onset, one of autoimmune diseases.[16]

The role of transfer of immune cells adoption in worm-influenced regulation of allergy and autoimmune diseases, was supported by animal experiments.[17] The chronic diseases for families economic burden, health systems, governments and the association between national policy maker and international economic pressure and politics have a large impact on chronic diseases risk and countries ability in responding them, the potential of wet and warm climate area double burden of infectious and chronic diseases.[18]

3.3 Economic income and Hygiene Hypothesis

How the present of chronic conditions in certain multimorbidity patterns, which could have great burden on public health at several levels in low-, middle,-, and high-income countries has been identify and describe in several countries to the advancement of preventive actions to decrease their prevalence and also give increase to new, understandable approaches for the management of these co-existing conditions. [19]

The association of individuals raised in hygiene environment have a higher of developing Allergy and Autoimmune Diseases was supported by random or fixed-effect meta-analyses.[20]

IV. LIMITATION

These Systematic review has several limitation 1) Non published literature on all autoimmune diseases may limit the validity of our findings, but these inflammatory bowel diseases and rheumatoid arthritis study are a real phenomenon which is already attracted peoples from developed and clean countries with AD burden. However, we used meta-analysis with partly overlapping data bases for our literature search on bacteria and worm cell wall antigens. And we found a large number of papers fulfilling the criteria of our search in these HyHy-AD prevention and therapeutic. 2) Another potential problem in this systematic review is that studies mainly used in the searching steps are depended on the vocabulary synonyms (not find in general, but many in specific such as autoimmune diseases could be Rheumatoid arthritis, recurrent CDI, IBS, T1D, atopic sensitization, asthma etc.) but not in Lupus Erythematosus. Different chances changes choices used give different results may be misleading. We intended to manage this problem in an additional work, by dividing the using chosen specific to generic into 2 broad categories: prevention and therapeutic findings.

V. SUMMARY

Epitope of FMT, bacteria, wall of worm are all rich of ranges epitopes and could be present in therapeutic a prevention potential. These epidemiological studies from developing/ tropical rainforest area countries, present to hygiene developed countries is similarly paralleled with low-middle- high- income population, vaccination and therapeutic fighting against autoimmune diseases. Hygiene hypothesis / FMT has association with novel treatment for Allergy and Autoimmune Diseases. Further knowledge and education to policy maker and global politic doer from local to global will support the industries in FMT.

VI. CONCLUSION

Low-middle-high income countries has a life style of Hygiene hypothesis. The low- and middle economic income in development countries have similar pattern with population in developing countries, which support FMT industrial medicine and travelling to developing countries. Broad diversity bacteria and worm in therapeutic and prevention potential, supported by sub-saharan and tropical rainforest developing countries.

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Scientific research contribution to fruticulture development

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Abstract *- This study aimed to evaluate the research contribution on development of peach, apple and guava crops in Campos das Vertentes region, Minas Gerais State, Brazil. It was developed in two stages: the first one by consulting web of science base, where articles about these three fruit were being sought. The search for articles related to selected fruits was performed by ten key identification variables for each one of the following technologies: cultivars, seedlings, dormancy, pruning, fruit thinning, fruit protection/bagging, irrigation, harvesting, post-harvest and commercialization. The second stage was carried out by means of interviews with the peach, apple and guava producers in Barbacena, São João Del Rei and Lavras respectively, which were selected for being development poles of this region. The results were tabulated separately for the first stage and the second stage, confronted the results of interviews with the search of articles. It was concluded that research and technology generation has contributed to the development of peach, apple and guava crops in Campo das Vertentes area.*

Key words: Innovation, Research, Fruit growing.

I. INTRODUCTION

Fruit farming has great social and economic importance, as well as food, being an important source of nutrients. According to IBRAF (2013), in the 1989-90 biennium, production was 420 million tons, in 1996 it exceeded 500 million tons and in 2009 it was 728 million tons. In Brazil, fruit production already accounts for 25% of agricultural production. It is one of the most demanding and technology-dependent sectors to achieve success in the business. Therefore, the activities of generation and transfer of technology have been intensified (IBRAF, 2013). Fruit cultivation has great social and economic importance, as well as food, being an important source of nutrients. According to IBRAF (2013), in the 1989-90 biennium, production was 420 million tons, in 1996 it exceeded 500 million tons and in 2009 it was 728 million tons. In Brazil, fruit production already accounts for 25% of agricultural production. It is one of the most demanding and technology-dependent sectors to achieve success in the business. Therefore, the activities of generation and transfer of technology have been intensified (IBRAF, 2013).

Currently, Brazil counts on thirty poles for the development of fruit growing that extends from Rio Grande do Sul to Rio Grande do Norte and Amazonia, where the production of the whole productive chain of fruit agribusiness (IBRAF, 2013) is developed. The technological innovation in Brazilian fruit growing is a segment of the economy that has most stood out in the last years and continues in full evolution, both in the production of fruits for table, as for industry. To this end, Brazil became the third largest producer of fruit in the world, behind only China and India, with a production of around 40 million tons / year, in an area of 2.5 million hectares (KIST et al., 2012). Technological innovation has been detected in Brazilian fruit growing, being inserted in all links of the productive chains. However, their profiles are heterogeneous in aspects of technology adopted, forms of implantation, among other factors (IBGE, 2013).

Temperate fruits, such as apples and peaches, because they are of temperate climate origin, have in Brazil a series of inhospitable factors that need to be improved genetically or adapted by new management techniques, such as seedling formation Through methods of grafting, pruning, breaking dormancy, irrigation, among others, for the success of economic exploitation. Also, the native fruits of tropical climate, such as guava, also needed genetic improvement, creation of new management techniques, such as: formation of seedlings through grafting, pruning and irrigation methods, for successful exploitation (SANTOS, 2012 and PEREIRA & KAVATI, 2011). In order to obtain a better quality of fruits, it is necessary to obtain a better quality fruit in the coloring of the peel and pulp, fruit size, productivity and harvest of several harvests / year.

Today, with the advancement of research, Brazil produces enough apple for the domestic market and even allows the export of the fruit with excellent quality. Until the 1970, it produced only 10% and with little quality (Faschinello and Nachitigal, 2009). All this was only possible thanks to the technologies generated by the research that reached the producer, enabling successful economic exploitation (BARBOSA et al., 1992). Another example is the production of peach which, according to

Ojima et al. (1989) and Raseira et al. (1998), Brazil drastically reduced its importation thanks to research work in the areas of genetic improvement and adaptation of cultivars through agronomic evaluations and the development of new management techniques, such as: seedling formation through grafting, Pruning systems, dormancy breakdown, a technique that allowed the economic exploitation of the majority of temperate fruits in Brazil, irrigation and soil fertility. Thus, currently peaches of excellent quality are produced in the coloring of the bark and pulp, flavor and high productivity.

In the tropical fruits, such as guava, there was also a great advance in cultivation thanks to research on genetic improvement, adaptation of cultivars through evaluation techniques, formation of seedlings through bud grafting and grafting techniques. Pruning and irrigation that allowed the harvest of several harvests per year and at the moment of market demand, for both table and industry. (SANTOS, 2012 and PEREIRA & KAVATI, 2011). Still, we can highlight results in the use of cultural and phytosanitary practices that allowed the effective control of diseases.

According to Etzkowits (2003), the technological innovation of a country is based on the relationship between universities, companies and states, being relevant for both developed and emerging economies. For Brazil that is experiencing a transitional period for the creation of a knowledge-based society, this relationship is important for its development. The incentives for Brazilian R & D activities in the last decades have been undergoing significant changes, such as the creation of new research programs in the various areas, notably in agriculture, and in the fruit-growing sector there has been significant development.

The objective of this work was to evaluate the contribution of technologies generated by the research and published in publications of technical-scientific journals available on the internet in relation to the technologies adopted by the producers in the development of the Campo das Vertentes region of Minas Gerais, Brazil.

II. MATERIAL AND METHOD

The research was developed in the Campo das Vertentes region of the State of Minas Gerais, Brazil, located between the parallels 20°24' south latitude and the meridians 43°30' west longitude of Greenwich. It is a mesoregion, made up of thirty-six municipalities, grouped into three micro-regions: Lavras with nine municipalities; São João Del Rei with fifteen and Barbacena with twelve (IBGE, 2013).

The work was carried out in two stages: in the first one, secondary data were collected by analyzing the articles through a consultation on the website www.periodicos.capes.gov.br on the Web of Science database. We searched for articles on the peach, apple and guava culture in the world and in Brazil. After the search we tabulated the main magazines with these publications in the world and in Brazil as well as the main universities and research institutions that work with these cultures. In this stage, the articles for each of the three fruit trees were searched for ten variables: varieties, seedlings, dormancy, pruning, fruit thinning, fruit bagging / irrigation, harvesting, post harvesting and commercialization, participation in events Dissemination and transfer of technology and technical assistance. The second stage consisted of interviews with farmers in three municipalities in the Campo das Vertentes region of Minas Gerais, peach, apple and guava, selected to be evaluated in this research, for the pioneering of the producers to introduce these fruits in the municipalities of Barbacena, São João Del Rei and Lavras, respectively, where they were evaluated.

The data were collected through a questionnaire for interview with semistructured questions as a technique for collecting information. The questions presented had the objective of evaluating the contribution of the research in fruit growing based on the technological recommendations recommended by the research institutions and rural extension and university, through its stock of technical and scientific knowledge. In this way the degree of adoption of these technological recommendations by the fruit producers of the selected municipalities was evaluated.

The sampling of the number of producers for interviews was performed according to the criteria of Cochran (1965); in the locality with fifty other producers the sample was 10%, where there were from ten to forty producers of 20% and in places with at least ten 100% producers. Thus, for the peach tree in Barbacena, eight producers were interviewed out of a total of twenty-two, for apple in São João Del Rei two producers as well as for guava in Lavras two producers were interviewed because both localities had less than ten producers that exploit this commercially using high technology. All these producers being nominated by Company of Technical Assistance and Rural Extension of the State of Minas Gerais - EMATER-MG for the interviews.

III. INDENTATIONS AND EQUATIONS

The results presented and discussed come from the confrontation between the first stage and the second stage. The ranking of the main journals that publish scientific articles on peach, apple and guava in the world (Table 1) and in Brazil (Table 2) is presented first, followed by the results of the interviews referring to the ten variables analyzed for Peach, apple and guava: cultivars, seedlings, breakage of dormancy, pruning, irrigation, fruit thinning, fruit protection / bagging, harvest and post harvest, participation in events for diffusion and transfer of technology and technical assistance. It is observed that Acta Horticulturae is the magazine that publishes the most articles about the peach tree, apple tree and guava tree around the world. Second to peach and apple tree comes to Hortscience. The Brazilian Journal of Fruticultura is the third placed in number of articles on guava in the world. There are in Brazil numerous technical-scientific journals specialized in the publication of technologies generated by the research, such as those presented in Table 2.

TABLE 1
SCIENTIFIC JOURNALS IN THE WORLD WITH PUBLICATIONS ON PEACH, APPLE AND GUAVA CROPS FROM 1999 TO 2013.

Journal	Articles number
Peach tree:	
Acta Horticulturae	1,498
Hortscience	1,032
Phytopathology	514
Journal Of The American Society Horticultural Science	501
Journal Of Economic Entomology	385
Apple tree:	
Acta Horticulturae	3,598
Hortscience	1,725
Phytochemistry	968
Journal Of The American Society Horticultural Science	912
Journal Of Agricultural and Food Chemistry	869
Guava tree:	
Acta Horticulturae	242
Proceedings of the1st International Guava Symposium	83
Revista Brasileira de Fruticultura	61
II International Symposium on Guava and Myrtacea	45
Food Chemistry	40

Source: Web of Science (January, 2014)

TABLE 2
NUMBER OF ARTICLES IN SCIENTIFIC JOURNALS IN BRAZIL WITH PUBLICATIONS ON PEACH, APPLE AND GUAVA CROPS, 1999 TO 2013.

Journal	Articles number
Peach tree:	
Revista Brasileira de Fruticultura	84
Pesquisa Agropecuária Brasileira	52
Ciência Rural	28
Ciência e Agrotecnologia	16
Bragantia	7
Apple tree:	
Revista Brasileira de Fruticultura	81
Pesquisa Agropecuária Brasileira	73
Ciência e Tecnologia de Alimentos	35
Ciência Rural	33
Ciência e Agrotecnologia	19
Guava tree:	
Revista Brasileira de Fruticultura	61
Pesquisa Agropecuária Brasileira	23
Ciência e Agrotecnologia	15
Ciência Rural	11
Ciência e Tecnologia de Alimentos	8

Source: Web of Science (January, 2014)

The Brazilian Journal of Fruticulture is the one that most publishes articles on peach, apple and guava, followed by the Brazilian Agricultural Research. Rural Science is the third for the peach tree and the fourth for the apple tree and the guava tree. The Science and Agrotechnology is the fourth for the peach tree and the third for apple and guava in number of published articles.

The cultivar selected for orchard formation is one of the most important components in the production system. It is a factor that can be changed without changing the cost of implementing the crop with fruit. The cultivars indicated by the research and technical assistance for the region Campo das Vertentes of Minas Gerais have as main parameter the low requirement in cold. Among those presented, all producers interviewed said that they cultivate 'Maciel' and 'Douradão' peach, 'Eva' cultivars for apple and 'Paluma' and 'Pedro Sato' guava.

Regarding the acquisition of seedlings, considering that these have a relevant role in the success of fruitful exploitation. A sound material with the desired genetic characteristics, produced by skilled and skilled nurseries, is of fundamental importance in the formation of an orchard. According to Oliveira et al. (2003) and Raseira et al. (2010), the seedlings indicated by the research and technical assistance must have good genetic characteristics, both of the rootstock and the canopy, healthy and formed by specialized and suitable nurseries. All the producers interviewed said that they acquire seedlings as indicated by the technical assistance.

The guava tree, because it comes from a tropical climate, has no need to break dormancy. On the other hand, the artificial dormancy break in temperate fruits was one of the great findings for the successful economic exploitation of these fruit trees in the region of the Campo das Vertentes of Minas Gerais. According to Nunes, Marondin and Sartori (2001) and Marafon et al. (2003), the effect of the internal factors such as the balance of the promoters and inhibitors of growth and external factors such as temperature, photoperiod and solar radiation. (2007). According to Oliveira et al. (2003), in the region of Campo das Vertentes, the amount of cold is not enough to break the natural dormancy of the buds, so that sprouting and flowering are carried out. For this, it is necessary the artificial dormancy break with chemical phytohormone. All peach and apple producers interviewed said they performed this procedure in accordance with the recommendations.

The pruning of fruit plants is a practice that aims to modify the vigor of the plants, to produce more and to improve the quality of the fruits, to maintain the plant with a size appropriate to its treatment and handling, to modify the tendency of the plant to produce more vegetative branches That fruitful or vice versa, lead the plant to a desired shape, suppress superfluous and inconvenient, diseased or dead branches, regulate the alternation of crops, so as to obtain crops regularly, according to Brickell, 1979. Ojima et al. (1989), Raseira et al. (2010) and Alvarenga, Oliveira and Gonçalves (2013), recommend pruning in the peach, apple and guava trees, so that the plant can bear the fruits load, maintaining balanced growth and avoiding crop alternation between good and bad harvests. Of stimulating the formation of flower buds, also ensuring a good distribution of the buds in the crown of the plant, improving the quality and size of the fruits. All respondents said they perform pruning according to recommendations.

Irrigation is an important practice recommended by research and, consequently, by technical assistance during the dry or summer period, to avoid water stress and to normalize the physiological system of plants (RASEIRA et al., 2010). It is recommended by the research for the peach tree crop, especially in the dry season, which coincide with the coldest period of the year and also with the time of breaking of dormancy, therefore bud buds of flowering and fruiting, (RASEIRA Et al., 2010). All the producers responded that they irrigate by the drip system, according to the recommendations, for the three crops.

Fruit thinning is a very old practice and aims to reduce the fruit load in the plant, with the purpose of protecting the plant, enabling conditions for homogenous production in size and appearance and to facilitate cultural treatment. According to Raseira et al. (2010), recommend the thinning operation of the fruits of the peach tree plant to enable better fruit formation in appearance and flavor, creating better conditions for aeration and light incidence, thus improving the physiological state. Also, to facilitate cultural dealings and harvesting. All respondents said they do the thinning, for all three cultures.

Protecting the fruit is the most important practice to eliminate the attack of the fruit fly, other insects and birds. It is an operation that can be performed by spraying with chemicals, or manually, using fruit bagging with TNT bag or butter paper. According to Raseira et al. (2010) and Monteiro et al. (2007), the protection of the fruit of the peach tree, either by spraying with chemicals or by hand, through bagging, is an important operation to protect the fruits of the fruit fly and other insects, thus guaranteeing more quality to the fruits. In guava, according to Souza, Mancin and Melo (2013) and Pereira (2011), the protection of fruits is very important. It is recommended to make the bagging when the fruit is destined for consumption in

natura, since besides protecting the fruits there is the advantage of valuing the production like organic. All the producers interviewed said to carry out operations to protect the fruits.

The research and the market recommend that the fruits be harvested in a way that does not lose its integrity, disqualifying them in the market. After harvesting the fruits, they should be taken to the packing house or shed to be standardized, graded, packaged and stored in accordance with standards and standards established by the Ministry of Agriculture (RASEIRA et al., 2010 and MONTEIRO et al. All producers interviewed said they follow the established standards. Of these 62%, they use the refrigeration of the fruits because they commercialize the production with the market of the northeast, notably Fortaleza-CE and Recife-PE, the rest, 38%, do not refrigerate because they send the production to the Central of Slaughtering of the State of Minas Gerais - CEASA - MG.

For Alvarenga, Oliveira and Gonçalves (2013), the research recommends that preventive practices be done against diseases of fungal and physiological origin, in order not to damage the product. To do so, one must harvest the fruits at the correct time, to eliminate the foci of inoculate. The fruits after harvesting should be sorted, packaged and stored, preferably under refrigeration. According to Souza, Mancin and Melo (2013) the guava fruit harvest must be done manually and, when the fruits are bright dark green coloring, starting to lighten, this is the ideal harvesting point. After harvest, the fruit should be sorted, packed and stored for marketing. All producers carry out the recommended standards and market the fruits in free markets, wineries and supermarkets.

The events carried out for diffusion and transfer of technologies are of fundamental importance for the dissemination and improvement of production techniques. It is in these events that the producers, extension agents, researchers and other members of the productive chains exchange experiences on the new technological innovations realized. For peach trees and apple trees, all producers interviewed said they participated in these events sporadically in the southern states of the country, notably São Paulo and Paraná. Of these, 45% said they already attended technological events outside the country, notably in the USA. As for guava, all the producers interviewed said that they do not participate in these exclusive events to deal with the guava culture, since they do not exist in the region or are not properly divulged.

In the evaluations of technical assistance, all said that they receive special and specialized technical assistance in a continuous and programmed way, regarding the production process and information on new technologies and market tendencies.

IV. CONCLUSION

The research has contributed to the generation and publication of several technologies for the cultivation of peach, apple and guava.

Producers in the Campo das Vertentes have adopted most of the technologies generated and recommended by the research.

The adoption of the technologies generated by the research has contributed to the development of the cultivation of peaches, apples and guavas in the Campo das Vertentes Region of Minas Gerais, Brazil.

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Biotermiticides to Protect the Soil Health

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Abstract— *Chemical termiticides are hazardous to biotic and abiotic factors in the environment and hence banned in US and European countries but are still in use in developing countries. They are applied in huge quantity reaching to potable water ways, food, and fodder and killing soil microbiological components. It has been observed from the study that these hazardous termiticides can be replaced by ecofriendly and cheap Biotermiticides made from the plant based oils. Four tree borne oils (CNSL(50-100%), Neemseed oil(10-25%), Karanj seed oil(10-25%) and Markingnut oil(10-25%) and three chemicals Chlorpyrifos(10-50%), Coppernaphthenate(10-50%) and (Boric acid(10-50%) were used in different proportions to formulate CNSL based termiticides. Specimen samples were treated and exposed to termites for 60 days. The mass loss after 60 days was noted and results were analyzed. Chlorpyrifos and Coppernaphthenate 100% have shown better termite control property than boric acid. Commercial termiticides their own might be effective against termite but except Chlorpyrifos and other two have shown not much promising results in CNSL based formulation when compared with the oil formulations. It was observed that CNSL(50-80%) + NO (10-25%) + BSL(10-25%) and CNSL(50-80%) + NO(10-25%) + KO(10-25%) formulations can act as an effective termiticides. All four oils if used 100% have shown good resistant against termites.*

Keywords— CNSL- Cashew nut shell liquid, NO- Neemseed oil, KO- Karanj oil, BSL- Bhilawan shell liquid.

I. INTRODUCTION

Termites are a group of eusocial insects of infraorder *Isoptera*, or as epifamiliy *Termitoidae* within the cockroach order *Blattodea* (Beccaloni and Paul, 2013). They are of four groups: dampwood living and feeding in very moist wood, drywood termites do not require contact with moisture or soil and subterranean termites live and breed in soil, and arboreal/mound builders. Termite mounds are commonly found in Africa, Australia, Southeast Asia, and parts of South America (Krishna, 1969; Inward et al., 2007). They are polymorphic living in colonies comprising reproductives (king, queen), soldiers and workers called castes. The queen in 'Royal Chamber' is very much bigger than the king and is capable of laying eggs at the rate of 36,000 a day for as long as 50 years. Single subterranean termite colony may contain millions of workers and may forage a distance of up to 100 m^2. (Su & Scheffrahn, 1988).

Termites help in aeration of the soil due to burrowing activities, the breakdown and release of organic matter as termites eat and digest soil, improvement of soil fertility when termite mounds, which are rich in minerals, are crushed down and incorporated into the soil, as a source of minerals for cattle who lick the mounds and as a source of protein rich food for many organisms including ants, guinea fowl and other mammals including humans (HDRA, 2001). It is also observed that termite in its natural environment; improve soil pH, organic carbon content, water content and porosity by recycling dead organics (Abdel and Skai, 2011).

Although there are some benefits of termites in soil reclamation but they are often called the **"Silent Destroyer"** as they may be secretly hiding and thriving in homes without any immediate signs of damage. About 10% of species of the termite are economically significant as pests and consume cellulose-based plant materials and destroy valuable property, documents, furniture and furnishings anything that contains cellulose, silently and swiftly, before becoming aware of the damage done. Termites also cause damage to living trees, crop plants, wooden electric poles, railway sleepers, telephone and electrical cables, etc. (Krishna and Weesner, 1970; Pardeshi et al., 2010; Mitchell, 2003; Ogedegbe and Eloka, 2015).

The economic losses associated with termite damage for Malaysia, India, Australia, China, Japan and the United States are 10, 35, 100, 375, 800 and 1,000 million US dollars respectively (Abdel and Skai, 2011). In Indian region about 35 species have been reported to damage about 10-25% agricultural crops and timber in buildings (Rajgopal, 2002). It is observed that due to termite attack on wheat, paddy, cotton, sugarcane, groundnut, in Punjab, Rajasthan, Delhi, Uttar Pradesh, Andhra Pradesh, Bihar and Gujarat, Madhya Pradesh, and Maharashtra faced major loss in crop production (Patel and Patel, 1954;

Chhotani, 1980; Verma et al. 2009). As many as 13 species of termite are reported to cause 30-60% destruction of buds of sugarcane in India (Roonwal, 1981) and are responsible for plant mortality (5-50%) and pod damage (46%) in groundnut (Rajgopal, 2002). Not only crops and wood but the cash, ornaments and saving documents kept in banks have been found attacked by termite in India (Indian Express News, 22 Apr 2016, TNN. Apr 5, 2008).

Chemical termiticides used to control the termite include liquid termiticides, termite baits, building materials impregnated with termiticides and wood treatments. Chemical treatments on broad base can be of two types repellent termiticide and non-repellent termiticide. These chemicals do not kill the termites; they simply deter them from entering the treated soil and building tunnels. Ex. Cypernethrin, Bifenthrin, Fenitrothion, Permethrin and Fenvalerate, Chlorpyrifos. Non-repellent chemicals do not prevent termites from tunneling; they only kill the termites upon ingestion or contact. An example is imidacloprid, Aldrin, Heptachlor, Chlordane, Coppernaphthenate, Boric acid etc. Grace, et al.,(1993) reported that the toxicity of aldrin, chlordane, DDT, dieldrin and heptachlor was observed 17,20,24,28 and 33years after treatment in Hawaii. These are applied in huge quantity in soil or poured in mounds to destroy it. These chemicals are very toxic to all the biotic factors in soil and on the earth, leading to hazardous effects and diseases. This may is one of the threats to the human existence on the earth through soil pollution.

It is necessary to search the alternative safe termiticides to the commercial hazardous chemicals damaging soil health and ultimately the environment as a whole. Therefore the study was undertaken in the College of Agricultural Engineering and Technology, DBSKKV, Dapoli on, "Development of Cashew nut shell liquid based termiticide'. The main objective of study was developing and testing cashew nut shell liquid based termiticide by using Neemseed oil, Karanj seed oil and Markingnut shell oil in different proportions and field tested for termite response.

II. MATERIALS AND METHODS

In this study four tree borne oils (Cashew nut shell liquid (CNSL), Neemseed oil, Karanj seed oil and Markingnut oil(BSL)) and three chemicals (Boric acid, Chlorpyrifos and Coppernaphthenate) were used in different proportions (Table 1). Treatment detail is shown in Table 2 and Table 3. Wood specimens (30cm long and 2.5cm diameter) of Giripushpa (*Gliricidia sepium),* were dried and weighed before the subsequent treatment. As per the treatments, CNSL based oil and chemical formulations were made. Then dry weighed specimens were treated with both the formulations by surface area application and dipping into the solution up to 10cm and room dried for 24 hrs. Then specimens were again weighed to know the percent weight of termiticide absorbed by the surface area of each wood sample. The detail experimental design of the present investigation is as shown in fig.1. Treated specimen samples were placed in the mound holes 10cm deep by breaking mound soil cap and exposed to termites up to 60 days.

TABLE 1
TREATMENT DETAILS OF THE EXPERIMENT

1	**Experiment—I** (Oil Formulation)	**Material**	**Levels (%)**
		CNSL	50,60,70,80,100
		Neemseed Oil	10, 15, 20,25,100
		Karanj seed Oil	10, 15, 20,25,100
		BSL	10, 15, 20,25,100
2	**Experiment-II** (Chemical Formulation with CNSL)	**Material**	**Levels (%)**
		CNSL	50,60,70,80,90,100
		Chlorpyriphos	10,20,30,40,50,100
		Coppernaphthanate	10,20,30,40,50,100
		Boric acid	10,20,30,40,50,100

TABLE 2
TREATMENT COMBINATION FOR OIL FORMULATION

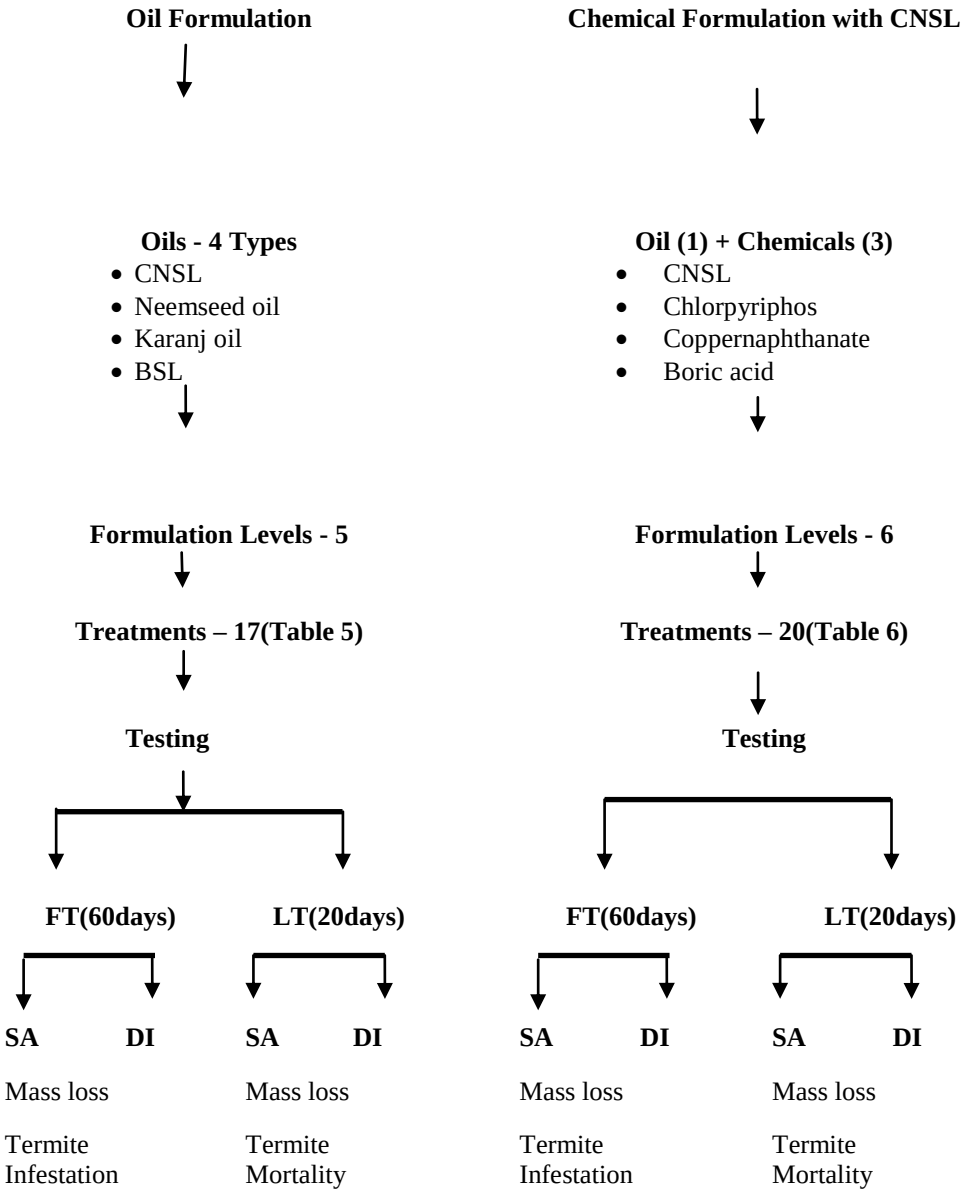
Sr. No.	Treatment	Oils			
		CNSL(%)	Neem oil (%)	Karanj oil (%)	BSL (%)
1	TO_1	100	0	0	0
2	TO_2	0	100	0	0
3	TO_3	0	0	100	0
4	TO_4	0	0	0	100
5	TO_5	80	10	10	0
6	TO_6	80	10	0	10
7	TO_7	80	0	10	10
8	TO_8	70	15	15	0
9	TO_9	70	15	0	15
10	TO_{10}	70	0	15	15
11	TO_{11}	60	20	20	0
12	TO_{12}	60	20	0	20
13	TO_{13}	60	0	20	20
14	TO_{14}	50	25	25	0
15	TO_{15}	50	25	0	25
16	TO_{16}	50	0	25	25
17	TO_{17}(Control)	0	0	0	0

(TO- Treatment of oil formulation)

TABLE 3
TREATMENT COMBINATION FOR CHEMICAL FORMULATION WITH CNSL.

Sr. No.	Treatment	CNSL (%)	Chlorpyrifos (%)	Coppernaphthanate (%)	Boric acid (%)
1	TC_1	100	0	0	0
2	TC_2	0	100	0	0
3	TC_3	0	0	100	0
4	TC_4	0	0	0	100
5	TC_5	90	10	0	0
6	TC_6	90	0	10	0
7	TC_7	90	0	0	10
8	TC_8	80	20	0	0
9	TC_9	80	0	20	0
10	TC_{10}	80	0	0	20
11	TC_{11}	70	30	0	0
12	TC_{12}	70	0	30	0
13	TC_{13}	70	0	0	30
14	TC_{14}	60	40	0	0
15	TC_{15}	60	0	40	0
16	TC_{16}	60	0	0	40
17	TC_{17}	50	50	0	0
18	TC_{18}	50	0	50	0
19	TC_{19}	50	0	0	50
20	TC_{20}(Control)	0	0	0	0

(TC - Treatment of chemical formulation with CNSL)



FT - Field Test
LT - Laboratory Test
SA - Surface area application
DI - Dipping into solution

FIG.1 FLOW DIAGRAM OF EXPERIMENTAL DESIGN.

After 60 days wood specimens were removed from the termite mounds and weighed to know the mass loss by termite attack which was calculated as below.

Mass loss (ML%)

The termite attack to the specimen samples placed on the mound will be quantified by calculating the weight after 60 days.

$$ML(\%) = \frac{[M_1 - M_2]}{M1} X\ 100 \quad (1)$$

Where, M_1 and M_2 are initial (before exposure) and final (after exposure) weight (g) of the wood sample (EN 118, 2005).

TABLE 4
MASS LOSS OBSERVED IN OIL FORMULATION TREATMENTS

Sr. No.	Treatment (CNSL:NO:KO:BSL)	Surface Application	Dipping
		Wt.Loss %	Wt.Loss %
T1	100:0:0:0	5.37	3.46
T2	0:100:0:0	0.00	4.44
T3	0:0:100:0	0.00	0.00
T4	0:0:0:100	5.90	3.56
T5	80:10:10:0	0.00	0.000
T6	80:10:0:10	0.00	0.000
T7	80:0:10:10	10.16	6.21
T8	70:15:15:0	0.00	0.00
T9	70:15:0:15	0.00	0.00
T10	70:0:15:15	7.56	4.23
T11	60:20:20:0	0.00	0.00
T12	60:20:0:20	0.00	0.00
T13	60:0:20:20	5.07	0.00
T14	50:25:25:0	0.00	0.000
T15	50:25:0:25	4.44	0.00
T16	50:0:25:25	0.00	2.69
T17	0	19.94	38.94

III. RESULTS AND DISCUSSION

In the oil formulation experiment, from surface application treatments it was observed that specimens of T_2, T_3, T_5, T_6, T_8, T_9, T_{11}, T_{12} T_{14}, T_{16} were not attacked by termite, where as among rest of the treatments specimens of T_7 was attacked causing highest mass loss 10.16% and in treatment T_{15} it was lowest i.e 4.44% as against control treatment T_{17} in which the mass loss was 19.94%. In the same experiment with specimens dipping in formulation, specimens of treatments T_3, T_5, T_6, T_8, T_9, T_{11}, T_{12}, T_{13}, T_{14}, T_{15} were not attacked by termite, whereas among rest of the treatments specimens of T_7 was attacked causing highest mass loss 6.2% and in treatment T_{16} it was lowest i.e. 2.69%, as against control treatment T_{17} in which the mass loss was 38.94%.

The results indicate that synergistic effect of oils in treatments CNSL +Neemseed oil + Karanj oil and CNSL +Neemseed oil + BSL has positive effect on termite control in both surface application and dipping treatments of oil formulation experiment. It is clear that presence of Neemseed oil with CNSL stands more effective against termite attack.

In the chemical formulation experiment from surface application treatments it was observed that specimens of T_2, T_5, T_8, T_{10}, T_{11}, T_{17} were not attacked by termite, where as among rest of the treatments specimens of T_4 was attacked causing highest mass loss 79.13% and in treatment T_3 it was lowest i.e 4.43%, as against control treatment T_{20} in which the mass loss was 85.40%. In the same experiment with specimens dipping in formulation, specimens of treatments T_2, T_3, T_8, T_{10}, T_{11}, T_{12}, T_{14}, T_{17} were not attacked by termite, whereas among rest of the treatments specimens of T_4 was attacked causing highest mass loss 73.56% and in treatment T_5 it was lowest i.e. 4.84%, as against control treatment T_{20} in which the mass loss was 87.73%.

In chemical formulation treatments it is observed that specimens from treatments CNSL with boric acid were highly attacked in comparison to other treatments whereas only one treatment with Chlorpyrifos was attacked by termite, may be because of its repellent nature. Boric acid is slow poison, it shows its effect after ingestion, and hence the specimens treated with it might be attacked more but after effect is unknown. Coppernaphthenate treated specimens were attacked by termite unevenly with increase in its proportion with CNSL it might be because of synergistic effect.

TABLE 5
MASS LOSS IN CHEMICAL FORMULATION TREATMENTS

Sr. No.	Treatment (CNSL:CF:CN:BA)	Surface Application	Dipping
		Wt.Loss %	Wt.Loss %
T1	100:00:00:00	58.49	35.81
T2	0:100:00:00	0.00	0.00
T3	0:00:100:00	4.43	0.00
T4	0:00:00:100	79.13	73.56
T5	90:10:00:00	0.00	4.84
T6	90:00:10:00	48.62	5.69
T7	90:00:00:10	40.43	9.33
T8	80:20:00:00	0.00	0.00
T9	80:00:20:00	21.09	30.81
T10	80:00:00:20	0.00	0.00
T11	70:30:00:00	0.00	0.00
T12	70:00:30:00	20.84	0.00
T13	70:00:00:30	15.27	5.01
T14	60:40:00:00	0.00	0.00
T15	60:00:40:00	11.63	0.00
T16	60:00:00:40	66.92	47.89
T17	50:50:00:00	0.00	0.00
T18	50:00:50:00	30.65	6.17
T19	50:00:00:50	19.71	40.20
T20	00:00:00:00	85.40	87.73

CNSL- Cashew nut shell liquid, CF- Chlorpyrifos, CN- Coppernaphthenate, BA- Boric acid

IV. CONCLUSION

From the results it can be concluded that Chlorpyrifos and Coppernaphthenate 100% have shown better termite control property than boric acid. Commercial termiticides individually are effective against termite but except Chlorpyrifos, other two have shown not much promising results in CNSL based formulation when compared with the oil formulations. It can also be concluded that CNSL (50-80%) + NO (10-25%) + BSL(10-25%) and CNSL(50-80%) + NO(10-25%) + KO(10-25%) formulations can act as an effective termiticide. All four oils if used 100% have shown good resistant against termites. Results of oil formulations are near about and better than the chemical formulations.

It is found from this study that a Biotermiticide i.e. Cashew nut shell liquid based oil formulation termiticide has a potential to act as an alternative to the commercial termiticides in the market. Also it will help to reduce the harmful effects and cost required for chemical formulations and to maintain the health of soil microbiology.

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Effect of Concentration of Silver Nanoparticles on the Uptake of Silver from Silver Nanoparticles in Soil

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Abstract— *The bioavailability and uptake of silver from silver nanoparticles in soil was investigated. Two species of insects, Acheta domesticus and Tenebrio molitor, and two species of plants, Helianthus annuus and Sorghum vulgare, were exposed to a range of concentrations of silver nanoparticles in soil. Silver nanoparticles were charactrized by techniques including transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction. The concentration of silver in insects and plants exposed to silver nanoparticles was measured using inductively coupled plasma-optical emission spectrometry. The results suggested an increase in the levels of silver in both insects and plants as a function of increasing concentrations of silver nanoparticles in soil. The translocation of silver to various parts of dicot plants such as stems and leaves was also observed. Such a result was not observed in the case of monocot plants. Results from this study suggests that silver nanoparticles would be available for uptake by insects and plants in terrestrial ecosystems.*

Keywords— Silver nanoparticles, Acheta domesticus, Tenebrio molitor, Helianthus annuus, Sorghum vulgare, inductively coupled plasma-optical emission spectrometry.

I. INTRODUCTION

The antimicrobial properties exhibited by silver and silver nanomaterials have propelled their widespread use in many consumer products that include detergents, textiles, home appliances, nutritional supplements, etc. In fact, silver based nanomaterials are one of the most common and most used among all nanomaterials [1]. According to a study by the Grand View Research, Inc. the global market for silver nanoparticles (Ag NPs) is projected to reach USD 2.45 billion by 2022 [2]. The widespread use of Ag NPs invariably raises questions and concerns about the risks and consequences resulting from their release into the environment.

Ag NPs are introduced into the terrestrial systems primarily through applications of sewage sludge to land [3-17]. Once present in an ecosystem, the environmental behavior, fate and ecotoxicity of metal-based nanoparticles are known to be influenced by their physical and chemical characteristics. Physical characteristics include size and shape of nanoparticles and chemical characteristics include acid-base character of the surface and aqueous solubility of the metal. The physical and chemical characteristics of nanoparticles influence their transformation phenomena such as aggregation, sorption to surfaces, and dissolution to metal ions. Additionally, surface coatings on metal based nanoparticles also influence their environmental behavior, fate and ecotoxicity [10]. For instance, the properties of Ag NPs that influence their uptake and toxicity to the earthworm *Lumbricus rubellus* in soil have been investigated by Makama et al. 2016 [18].

The physicochemical characteristics of soil influence the chemical form, mobility, bioavailability and toxicity of pollutants in terrestrial ecosystems. These physicochemical characteristics include pH, ionic composition, grain size, soil texture, organic matter content, temperature, solar radiation exposure, hydrostatic pressure, and cation exchange capacity. Thus, it is important to understand that the environmental behavior, fate, bioavailability and ecotoxicity of nanomaterials and other pollutants are influenced and determined by a combination of the physicochemical characteristics of the soil, the physicochemical characteristics of nanomaterials, and the physiological status of biota [19, 20].

Once present in a terrestrial environment, it has been shown that certain types of nanoparticles possess the ability to be taken up by insects [2 1] and plants [22-27], thereby entering the food web. The uptake of nanoparticles from soil depends on a large number of factors. One such factor is the concentration of nanoparticles present in soil. Larger amounts of nanoparticles may be prone to a phenomenon called aggregation, which causes the final size of the nanoparticles to be larger than they were originally. This could potentially affect the uptake of nanoparticles as the uptake of nanoparticles is

inversely proportional to their size. The small size of nanoparticles enables their easy uptake, dissolution and release of ions with increased surface area [28]. Conversely, the presence of a large amount of nanoparticles in a system may result in an increased uptake.

In the present study, the uptake of silver by insects and plants as a function of concentration of Ag NPs in soil was investigated. Studies to understand the uptake, kinetics and transformatoin of metal nanoparticles in terrestrial ecosystems usually include the terrestrial isopods [29-31]. Terrestrial ispods enable the study of uptake and transformation of metal nanoparticles because of their ability to uptake nanopartiles extensively through the oral route. Surface uptake of nanoparticles in terrestrial isopods was found to be negligible [32]. However, to comprehensively understand the effect of nanoparticles on terresrtial ecosystems, it is important to investigate their uptake in other components of terrestrial ecosystems that play a keyrole in terresrtial food webs.

Two species of insects, *Acheta domesticus* and *Tenebrio molitor*, and two species of plants, *Helianthus annuus* (a dicot) and *Sorghum vulgare* (a monocot) were used in this study. Insects constitute an important source of food to insectivorous birds. They serve as a crucual link in the metal-transport chains between trophic levels in food webs [33]. As they are a very good source of protein and other nutrients, insects and larvae serve as an important food source to birds especially during the breeding season [34]. Considering the signficance of insects in the food web of all insectivorous birds, it is important to understand if insects are able to uptake abd accumulate silver from Ag NPs in soil. Two different species of plants, a monocot and a dicot, were chosen for this study to understand if there is any difference in the uptake of silver from Ag NPs in soil by these plants. The objective was to examine if either of the monocot or dicot plant species uptake silver from Ag NPs in soil. Additionally, the possibility of translocation of silver to other tisseus of plant such as stem, seeds, etc was also evaluated. Considering seeds of plants also serve as an important food source for granivorous birds [35], such a study would help understand if these plants are playing a role in the metal-transport chain in the food web of granivorous birds.

The test species were exposed to concentrations of Ag NPs ranging from 0-625 mg/kg (ppm) in soil. Studies have suggested that sewage sludge can contain a wide range of concentrations of silver. Concentrations of silver as high as 960 ppm were reported in some sludge samples [12]. Thus, the concentration of Ag NPs used in this study is not outside the realms of possibility.

II. MATERIAL AND METHOD

2.1 Soil Collection and Preparation

All soil used during the insect and plant exposure experiments was collected 40 minutes south of Colorado City, Texas at an elevation of 684 m above sea level. Exact coordinates were as follows: Universal Transverse Mercator (UTM) 14 S 0319752 mE 3557792 mN. All soil was collected from the top 10 cm of soil, shoveled into clean plastic containers and transported back to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX. Once at TIEHH the soil was processed for homogeneity. All large rocks, roots, living organisms, and other organic matter were removed first and large clumps of soil were crushed. The soil was then sifted through a 2 mm wire screen into another clean plastic storage container. Processed soil was covered and stored indoors until ready for use.

2.2 Soil Analysis

Soil samples were sent to Midwest Laboratories Inc. (Omaha, NE) for basic soil analysis. Soil texture, percent humic matter, percent organic matter, exchangeable cations (K^+, Mg^{2+}, Ca^{2+}), available phosphorus (P), soil pH, percent base saturation of cations (K^+, Mg^{2+}, Ca^{2+}, H^+), cation exchange capacity (CEC), and sulfur (S) content were all analyzed in order to fully characterize the soil.

2.3 Characterization of Ag NPs

Uncoated Ag NPs (30-50 nm) were purchased from US Research Nanomaterials, Inc. (Houston, TX). According to the manufacturer, the Ag NPs contain 99.99% Ag (www.us-nano.com). The manufacturer also confirmed the size and spherical shape of each lot of nanoparticles.

2.4 Transmission Electron Microscopy

In order to confirm the size range and shape of the nanoparticles, transmission electron microscopy (TEM) was used. Each sample was prepared by dispersing the Ag NP powder in ethanol (EtOH). Each sample was sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried before analysis. TEM (Hitachi H-8100 TEM) images were taken at 200 kV using a tungsten filament side-mounted camera.

2.5 Dynamic Light Scattering

Dynamic light scattering (DLS) was used as an additional method to confirm the size of the nanoparticles. Sample preparation was performed by placing approximately 10 mg of silver nanoparticle powder in 10 mL of reagent grade acetone (Fisher Chemical). Samples were sonicated until nanoparticles remained suspended in solution. Samples were analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA) and Mictrotrac Flex Software (Version: 10.3.14). Method settings were customized for each sample of silver nanoparticles (absorbing particles) and acetone (refractive index: 1.36).

Samples were analyzed by running two consecutive 60 second scans. The average value of the two scans was recorded as the final result of each analysis. The particle size given at 50% was used as the average particle size of each sample.

2.6 Powder X-ray Diffraction

Powder x-ray diffraction (PXRD) was used to confirm the composition of the nanoparticles. A Rigaku Ultima III X-Ray Diffractometer was used to analyze all samples. Samples were analyzed using Cu Kα radiation as x-ray source. The silver nanoparticles were analyzed using the following instrument parameters: parallel-beam geometry was used with a step width of 0.03° and a count time of one second; the divergence, scattering, and receiving slits were set at one. Once completed, the diffraction patterns were compared and matched to the phases in the International Center for Diffraction Data (ICDD) powder diffraction file (PDF) database.

2.7 Insect Treatment with Ag NPs

Two replicates were prepared using 37.9 L terrariums (50.8 cm x 27.9 cm x 33.0 cm) for each insect species treatment group, including the control group. Before use, each terrarium was thoroughly cleaned using water, followed by a 10% bleach (calcium hypochlorite) solution to remove any remaining chemical residues. Exactly 2.5 kg of soil was weighed into each clean terrarium. An analytical balance was used to weigh out the necessary amount of Ag NPs for each treatment group: 0 (control), 1, 5, 25, 125, and 625 ppm. Nanoparticles for each treatment group were added to each terrarium and manually mixed for at least 60 seconds to ensure homogeneity. Insects used in the study were purchased from reptilefoods.com. Each terrarium received either 300 small crickets or 400 large mealworms. Insects were provided with fresh food and water as needed throughout the course of the study that lasted 28 days.

Once the 28 day exposure was complete, live insects were carefully extracted from the terrariums and placed in glass jars. The jars were then placed in a -80°C freezer until all the insects were deceased.

The insects were then freeze dried (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO) for at least 48 hours to ensure the removal of all moisture. Freeze dried insects were then crushed into a fine powder and stored in a freezer until they could be digested.

2.8 Plant Treatment with Ag NPs

Commercially available 7.6 L plastic nursery containers were purchased and filled with approximately two inches of commercial pond pebbles to aid in proper drainage. Exactly 2.5 kg of soil was weighed out in a separate plastic container. An analytical balance was used to weigh out the necessary Ag NPs; these were added to the soil and mixed in for at least 60 seconds. The treated soil was then carefully transferred into each nursery container. Two replicates were prepared for each treatment group for each of the two plant species.

Seeds of each plant species were planted in their respective nursery containers and transported to the TTU greenhouse. The plants remained in the greenhouse until they reached maturity, approximately three months for *H. annuus* and six months for the *S. vulgare*. While in the greenhouse, plants received shaded sunlight and were maintained at or above 60°F. Once

plants reached maturity, the entire plant was harvested. The roots were separated from the remainder of the plant and rinsed using tap water for a full minute to remove all attached soil. The shoot system of the plant was separated into leaves, stems, and seeds. The plant tissue samples were stored in a freezer until they could be digested.

2.9 Processing of Samples for Analysis

Three identical samples were weighed out using the insect samples collected from each terrarium. For each plant treatment group, four samples were prepared from each nursery container: a root sample, a leaf sample, a stem sample, and a seed sample, if possible. For each sample, either plant or insect, approximately 1.0000 grams were weighed into a 100 mL beaker. It should be noted here that the weights are dry weight (dw) for the insects and wet weight (ww) for the plants. Exactly 10 mL of 70% reagent grade nitric acid (HNO_3) (Fisher Chemicals) was added to each beaker using an acid-washed 10 mL volumetric flask. A 10 mL aliquot of reagent grade 30% hydrogen peroxide (H_2O_2) (Fisher Chemicals) was carefully added to each beaker using a volumetric flask. A method blank was run with each set of samples by adding 10 mL of HNO_3 and 10 mL H_2O_2 to an empty beaker. All beakers were placed onto hot plates and covered with a Teflon watch glass and allowed to sit overnight before digesting. Samples were then slowly heated; the temperature was raised in 5°C increments until the solutions in the beakers began to gently reflux.

The beakers were diligently monitored to ensure that the mixtures did not boil over in order to prevent the loss of sample. Any samples that did boil over were removed and were rerun at a later time. Samples were periodically swirled during the reflux process if needed. The samples in the beakers were allowed to gently reflux until the volume had been reduced to roughly 5 mL.

Once a sample had reached the desired volume it was carefully removed from the hot plate and placed in an ice bath to cool. After the samples were cooled, the samples were filtered into 50 mL plastic centrifuge tubes (Corning CentriStar™) using acid-washed glass funnels and ashless filter paper (Whatman No. 41). By filtering each sample, any remaining solids and/or digested lipids were removed from the final sample. Exactly 10 mL of 5% HNO_3 was measured using a volumetric flask and poured into each empty sample beaker. The acid solution was swirled in each beaker and the contents were poured into the funnel. This process was repeated twice so the samples were diluted with a total of 20 mL of the 5% HNO_3 solution. Once filtering had been completed, the centrifuge tubes were stored at room temperature until analysis by inductively coupled plasma optical emission spectrometry (ICP-OES) could be performed.

2.10 Analysis of Samples on ICP-OES

All samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). All samples were analyzed for silver at three wavelengths: 224.643, 328.068, and 338.289 nm.

The three silver wavelengths were aligned next using a 10 ppm silver standard solution (SPEX CertiPrep, lot# CL7-09AGY). The instrument was calibrated using a range of silver standards from 0-20 ppm.

2.11 Statistical Analysis

The dilution factors were factored back into sample results by multiplying the analyzed concentration by the final sample weight and then dividing by the initial sample weight. These final calculated sample concentrations were then used to run statistical analyses.

All data was compared using a basic Kruskal-Wallis test in R [36] after being found to be non-parametric [37]. The Shapiro test was used to compare the normality of the data [38]. This was followed by a multiple comparison test after Kruskal-Wallis test to identify all significant differences among the treatment groups ($p<0.05$).

III. RESULTS AND DISCUSSION

3.1 Soil Characterization

The control soil was found to contain 54% sand, 36% silt, and 10% clay. This type of soil is classified as a sandy loam. Additional tests found the soil to contain 0.01% humic matter, 1.7% organic matter, and 9 ppm S. The pH of the control soil was slightly basic, 8.1. And the CEC of the soil was calculated to be 18.0 meq/100g.

Other data from soil analysis is summarized in Table I. Additionally, control soil samples analyzed by ICP-OES were found to contain no detectable concentrations of silver.

TABLE 1
CHARACTERISTICS OF SOIL USED IN THE STUDY

Analysis	Results
Organic Matter	1.7%
Exchangeable Potassium	263 ppm
Exchangeable Magnesium	114 ppm
Exchangeable Calcium	3273 ppm
Soil pH	8.1
Cation Exchange Capacity (CEC)	18.0 meq/100g
Base Saturation, Potassium	3.7%
Base Saturation, Magnesium	5.3%
Base Saturation, Calcium	91.0%
Base Saturation, Hydrogen	0.0%
Sulfur Content	9 ppm
Humic Matter	0.01%
Sand Content	54%
Silt Content	36%
Clay Content	10%

3.2 Transmission Electron Microscopy

The 30-50 nm uncoated Ag NPs were found to be heavily aggregated after being dispersed in EtOH. However, the spherical shape of Ag NPs was confirmed by the TEM (Fig. 1).

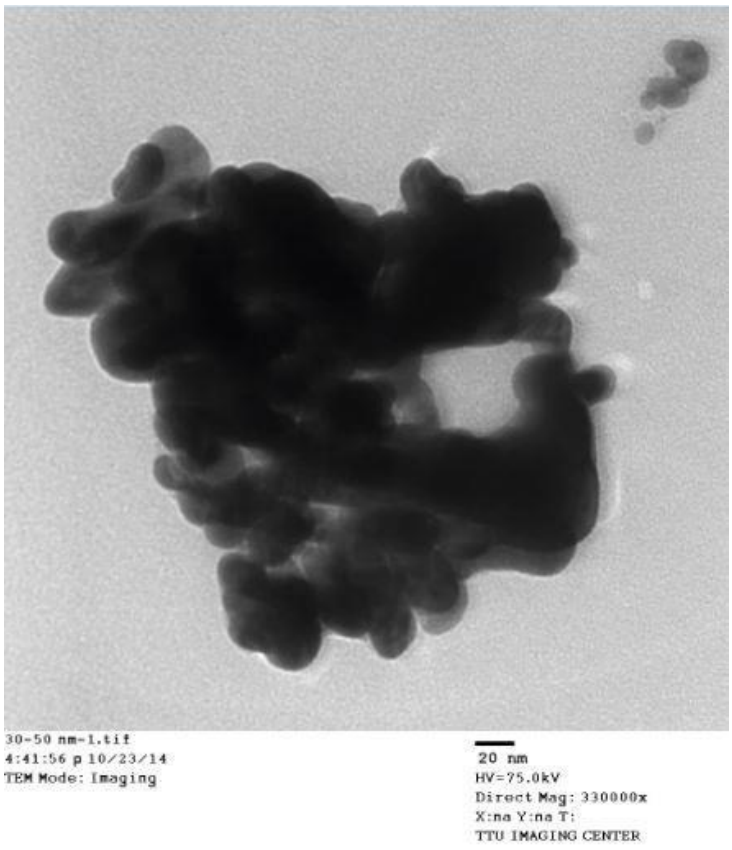

FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE OF 30-50 nm UNCOATED Ag NPs.

3.3 Dynamic Light Scattering

Approximately 95% of the 30-50 nm Ag NPs were found to be between 30.70 to 52.90 nm. A representative size distribution pattern in presented in Fig. 2. The average size of the particles was 41.80 nm.

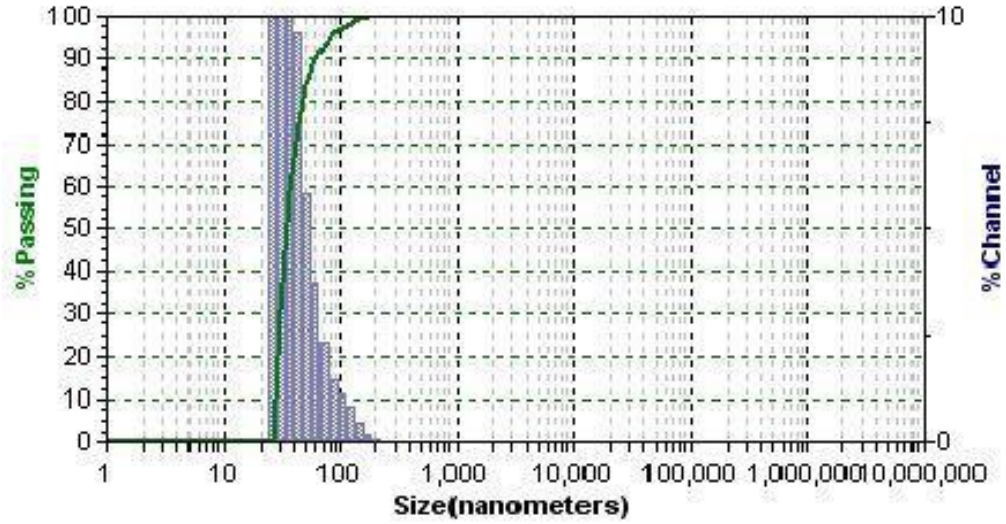

FIG. 2: SIZE DISTRIBUTION OF UNCOATED 30-50 nm Ag NPs AS DETERMINED BY DYNAMIC LIGHT SCATTERING.

3.4 Powder X-Ray Diffraction

The PXRD analysis of the silver nanoparticles confirmed their composition. The diffraction patterns matched both those in the ICDD and those provided by the manufacturer. A typical diffraction pattern is presented in Fig. 3.

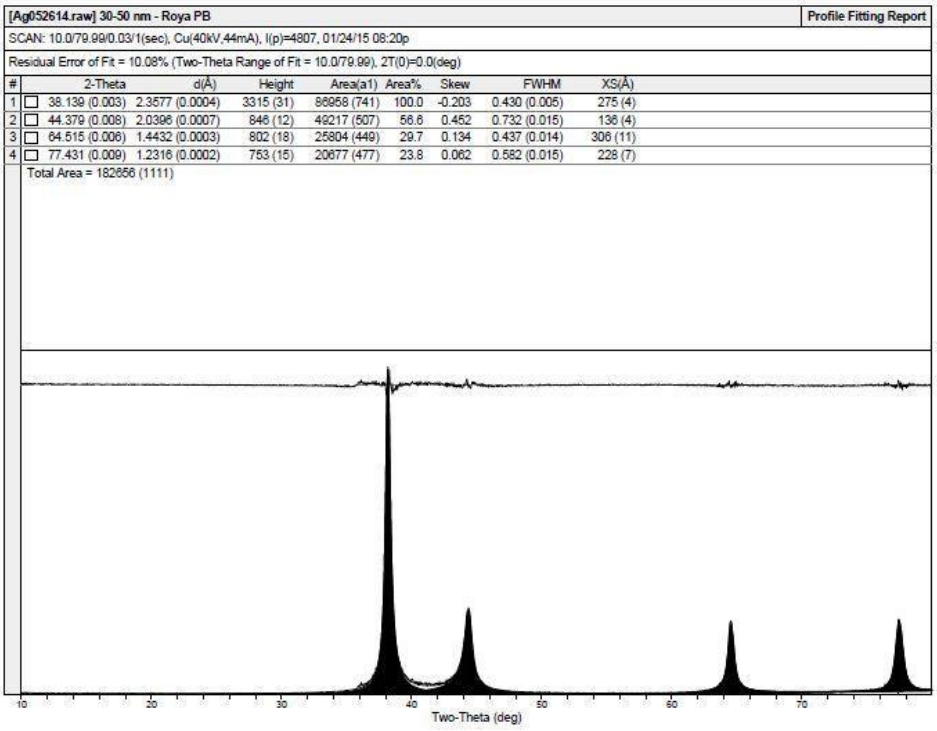

FIG. 3: DIFFRACTION PATTERN OF UNCOATED 30-50 nm Ag NPs aS DETERMINED BY POWDER X-RAY DIFFRACTION.

3.5 ICP-OES Results

Of all the three wavelengths considered for the analysis of silver in the present study, the data from 338.289 nm was chosen for subsequent analysis. Wavelength 224.643 nm was a double and deemed not usable. The wavelength at 328.068 nm was

bracketed by iron at 328.026 nm and 328.129 nm, which could have been a potential interference in both the insects and plant samples.

3.6 Uptake of Silver from Ag NPs in Soil by Insects

Previous studies in literature speculated that silver nanoparticles would be completely immobilized in a sewage sludge due to the formation of the insoluble silver sulfide (Ag_2S) [6, 20, 39, 16, 40]. It was theorized that the silver nanoparticles would remain immobile, and therefore unavailable for uptake, after the sludge was applied as a land amendment. However, the present study showed that Ag NPs would be available for uptake by both insects and plants at high enough concentrations.

It should be noted that this study used pristine nanoparticles rather than silver that had interacted with sewage sludge. However, the widespread use of Ag NPs in detergents, textiles, etc. pontetially serves as an anthropogenic source for the release of pristine silver nanoparticles into terrestrial ecosystems. Sewage sludge typocally contains 0.3-2.3 wt% of sulphur [41]. Hence, it is possible that there may not be enough sulphur in the sludge in the event of presence of high concentration of Ag NPs.

In the case of both the insects used in the study, a concentration dependent increase in the uptake of silver from Ag NPs in soil was observed (Fig. 4 & 5). Insects exposed to the highest concentrations of Ag NPs used in the study (125 and 625 ppm) were observed to contain quantifiable concentrations of silver (> 0.1 ppm). Levels of silver in insects exposed to the lowest concentrations of Ag NPs used in the study (0 and 1 ppm) could not be quantified (below instrument detection limits or < 0.005 ppm). Lastly, insects exposed to intermediate concentrations of Ag NPs used in the study (5 and 25 ppm) were found to contain at least trace amounts of silver. In the case of *A. domesticus*, the levels of silver found in the highest treatment groups (125 and 625 ppm) were found to be significantly higher than the levels of silver in the control and 1 ppm treatment groups ($p<0.05$). In the case of *T. molitor*, levels of silver in insects from the 625 ppm treatment group were found to be significantly higher than the levels of silver in insects exposed to 1 ppm Ag NPs in soil ($p<0.05$).

Past studies have found insects to uptake bulk metals from soil [42-44]. In the present study, an uptake of silver from Ag NPs as a function of concentration of Ag NPs in soil was observed. This result is in agreement with a previous study that has investigated the uptake of silver from Ag NPs in sandy loam soil. An analysis of the characteristics of soil used in this study has determined that the soil is sandy loam in nature. The low organic matter content, low organic carbon content, low clay content and low CEC of sandy loam soil usually result in more Ag ions being available for uptake. A similar result was observed in another study that has dealt with the uptake of silver ions from Ag NPs by earthworms [20].

The uptake of silver ions from Ag NPs can occur via ingestion or via endocytosis. Either one of these processes could be responsible for the uptake of Ag NPs in *A. domesticus* and *T. molitor*. Nanoparticles are also known to adhere to the cell walls or damaged cells, a phenomenon which was previously observed with quantum dots [45]. Another explanation could be the dermal exposure to silver ions by the test species. Studies have suggested that the uptake and accumulation whole particles is possible in cases of dermal exposure to nanoparticles [20].

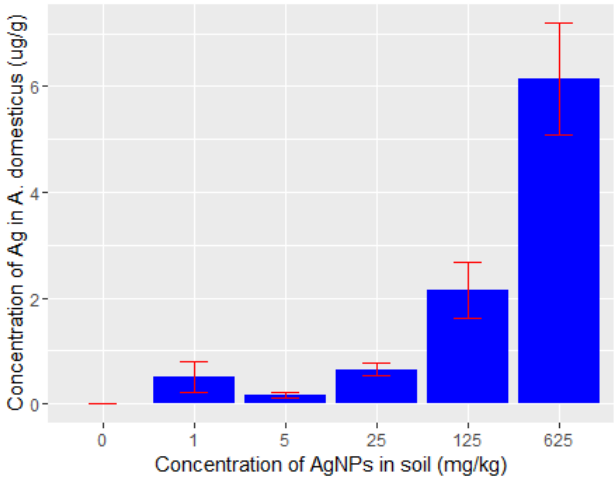

FIG. 4: UPTAKE OF Ag FROM Ag NPS IN SOIL BY *A. DOMESTICUS* (n=2).

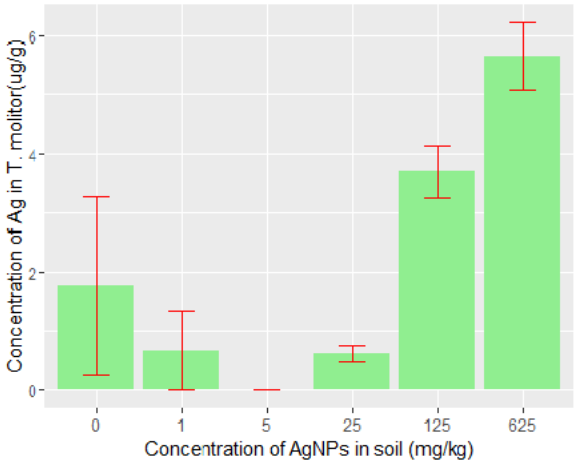

FIG. 5: UPTAKE OF Ag FROM Ag NPS IN SOIL BY *A. MOLITOR* (n=2).

3.7 Uptake of Silver from Ag NPs in Soil by Plants

All *S. vulgare* root samples were found to contain at least trace amounts of silver, regardless of treatment group. No other plant tissue samples were found to contain silver except the leaf samples from the 625 ppm treatment group, which contained trace amounts. An increase in root concentrations of Ag was observed as a function of increasing concentrations of Ag NPs in soil (Fig. 6).

The *H. annuus* control samples were found to contain silver concentrations below detection limits, except one stem sample which was found to contain trace amounts of silver. Such an observation could be likely due to contamination and sample preparation and processing errors. The 1 ppm treatment group was found to contain no silver. All of the root and leaf samples of the 5, 25, 125, and 625 ppm treatment groups were found to contain at least trace amounts of silver. The stem samples of the highest treatment groups (125 and 625 ppm) were also found to contain at least trace amounts of silver. The root samples showed an overall trend of increasing concentrations of silver as a function of increasing concentrations of Ag NPs in soil (Figure 7).

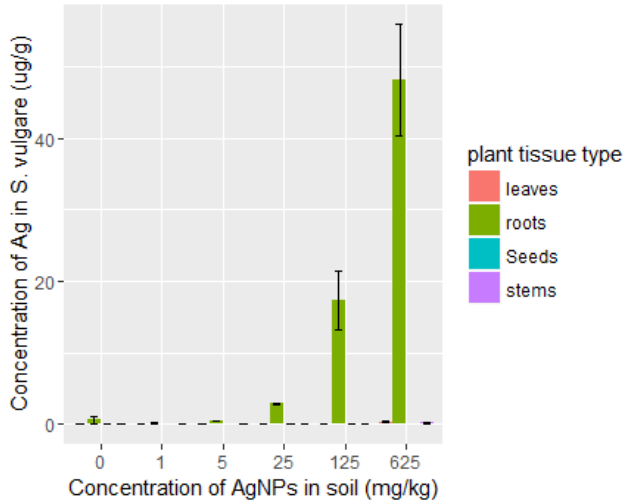

FIG. 6: UPTAKE OF Ag FROM Ag NPs IN SOIL BY *S. VULGARE* (n=2).

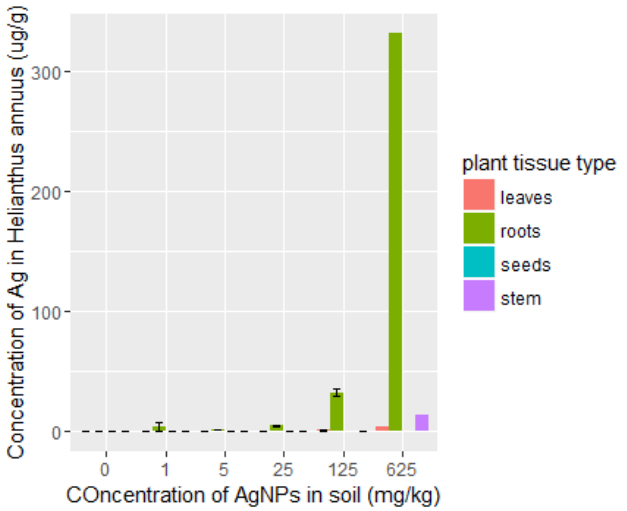

FIG. 7: UPTAKE OF Ag FROM Ag NPs IN SOIL BY *H. ANNUUS* (n=2), n = 1 FOR THE 625 PPM TREATMENT GROUP.

There were no significant differences among any of the plant tissue samples. Additionally, in both plant species, the roots were found to contain the highest concentrations of silver, followed by the leaves, the stems, and finally the seeds in the *H. annuus* tissue samples. A similar result was observed by Reddy and Dunn 1984 who have investigated the uptake of heavy metals like Cadmium, Nickle, and Chromium by soybeans grown on sludge-amended soils (Reddy and Dunn 1984). A major limitation that considerably affected the obseved inferences was the limited sample size (n =2). Such a small sample size has considerably affected the power of the statistical tools used in analysing the data.

Uptake of silver from Ag NPs in soil by the plants used in the study is observed at the highest soil treatment groups. The *H. annuus* samples were found to take up more than the *S. vulgare*, although these amounts were not found to be significantly higher. These results were not unexpected as dicots are better able to take up metals from soil than monocots due to the differences in root exudates [4 6] . The root exudates of dicots contain more organic acids than monocots. These organic acids include citric acid, maleic acid, ascorbic acid, and oxalic acid [47, 48]. The presence of organic acids in root exudates lowers the pH in the vicinity of the roots thereby solubilizing metals and enabling their uptake [49, 25, 50]. However, the uptake and accumulation of silver by *S. vulgare*, a monocot plant, may be explained by its root morphology. The presence of thin and numerous roots in monocots present a high surface area for nanoparticles that facilitates penetration and accumulation into the system [51]. Additionally, recent studies have suggested that the presence of elevated levels of chlorine (Cl) in soil, due to its salinity or when irrigated with water containing high amounts of Cl, may potentially increase the bioavailability and subsequent uptake of silver. This phenomenon could be possible explained due to the formation of $AgCl_x$ complexes in soluble or colloidal forms at elevated Cl concentrations. High Cl concentrations result in an increase in the mass transport of Ag and labile Ag, eventually resulting in the uptake of silver by plants [52].

Additionally, *H. annuus* was also found to translocate silver to the shoot portion of the plant whereas, *S. vulgare* did not. It has long been established that some plants are able to uptake metals from soil via the roots and distribute them throughout the shoot system [53, 23, 8, 54]. A possible explanation for the differences in translocation may be due to the differences in root exudates. If dicots are better at dissolving metals, a higher concentration of silver ions would be present around the roots; the ions would be able to travel further into a plant than a silver nanoparticle. Ions would also be able to diffuse across a membrane or be actively transported across the lipid bilayer through transport proteins [55] whereas solid nanoparticles would not.

IV. CONCLUSIONS

An uptake of silver from Ag NPs in soil by both insects and plants was observed at high concentrations (125 and 625 ppm). Both species of plants were found to accumulate silver in the roots. Not surprisingly, the dicot species were found to translocate more silver to the shoot system than the monocot species. Sewage sludge samples have been found to contain levels of silver as high as 960 ppm. Therefore, the possibility of bioaccumulation of silver and its eventual entry into the food chain of insectivorous and granivorous birds cannot be discounted.

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