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Preface

We would like to present, with great pleasure, the inaugural volume-3, Issue-9, September 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.

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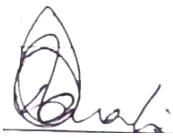
(Editor-in Chief)



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Fields of Interests

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
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Dairy Sheep	Water Buffalo
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Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
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Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
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Crop Protection Chemicals	Feed supplements
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Assessment of Rural Energy sources and Energy Consumption pattern in West Shewa and East WellegaZones, OromiaRegional National state, Ethiopia

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Abstract— *The study was conducted in West shewa and East wollega zones; Oromia Regional State with objective of the study is to identify the major rural source of energy consumption in the study area and to identify constraint and potential of energy use in the study area. About 141Male and 39 Female households were interviewed by structured questionnaire and data were analyzed by descriptive statistics under SPSS software. The characteristic of rural Energy source utilization , the majority of respondent uses firewood about 91.1% and about 16.9% the respondent uses agricultural residue for the purpose of baking Enjera , Firewood is the first widely used energy source, about 57.2% of the respondent uses Firewood and about 22.8% of the respondent uses charcoal for the purpose of heating mostly. About 55% the respondents uses kerosene and the respondent's uses small size solar only about 20% for purpose of lighting. Electricity, battery cell, biogas was the lowest level energy sources of study area. In study area even if some rural HHs with access to electric service , they did not use for the purpose of Enjera baking as well as heating , only use for the purpose of lighting in study area. The main reasons for preference of biomass energy consumption in the study area is ease of access, cultural preference, cheap pricesandthe last reasons for choice biomass energy consumption is convenience when they used and no alternative source. Small HHs uses the available alternative energy technology like biogas, modern charcoal stove (leqach), Mirt stove, small size solar energy. Firewood and Agricultural residue were the potential energy sources in study area and unlike Agricultural research, lack of Research on Rural energy sources, lack of effective rural energy technology, socio-economic problem to accept available rural energy sources, lack of information where and how alternatives rural energy technology were identified as constraints of rural energy sources in study area. To fill these knowledge gap AERC, should be planned to introduce new Rural Energy sources and Woreda water, mine and energy office should disseminate the available alternative technologies for ruralhouseholds.*

Keywords— Agricultural residue, Alternative Energy source, Biomass, Firewood, Rural Energy source.

I. INTRODUCTION

Energy is very crucial for daily life to meet human beings basic need such as cooking, boiling water, lighting and heating (WHO, 2006). There is a strong linkage between energy and the millennium development goals. According to World Bank (2009), energy service delivery, especially to the poor, contributes to achieving the millennium development goals

Most of the household in developing countries like Ethiopia continue to be dependent on traditional use of solid fuels (biomass) for cooking and heating, due to lack of access to electricity and modern energy sources. Consumption of traditional fuels has negative on environmental, economic and health impacts. The inefficient way people use energy is factor accreting deforestation. The main causes of deforestation in Africa are fuel wood collection, logging, agricultural expansion, and population pressure (Nebiyu, A.2009). Biomass fuel is very common in Ethiopia and fuels are mainly burned in inefficient open fires and traditional stoves.

For achieving sustainability in rural development with emphasis on livelihood and the means of enhancing the economic wellbeing of the poor households, it is necessary that affordable access to energy is provided to the households. Western oromia has problem of shortage of rain, drought, deforestation, hot temperature, Termite infection and etc. was increasing year to year. Those problems were decrease production and productivity, decrease income, loss saving poverty was developed at household level and decrease access of water availability (Annual Report of East WolegaZoneDisaster Prevention and preparedness office on ADPLAC, 2014). So the objective this study isto identify the major source of energy consumption in the study areaand to identify constraint and potential of energy use. In selected zones, no systematic studies have been undertaken regarding the rural energy consumption behavior of households.

II. METHODOLOGIES

2.1 Description of StudyArea

The study was conducted in West shewa and East wollega zones, western part of Oromia Regional State. It has 18 and 17 districts respectively and located about 114 km and 331 km from Addis Abebe respectively (Fig. 1) map of study area.

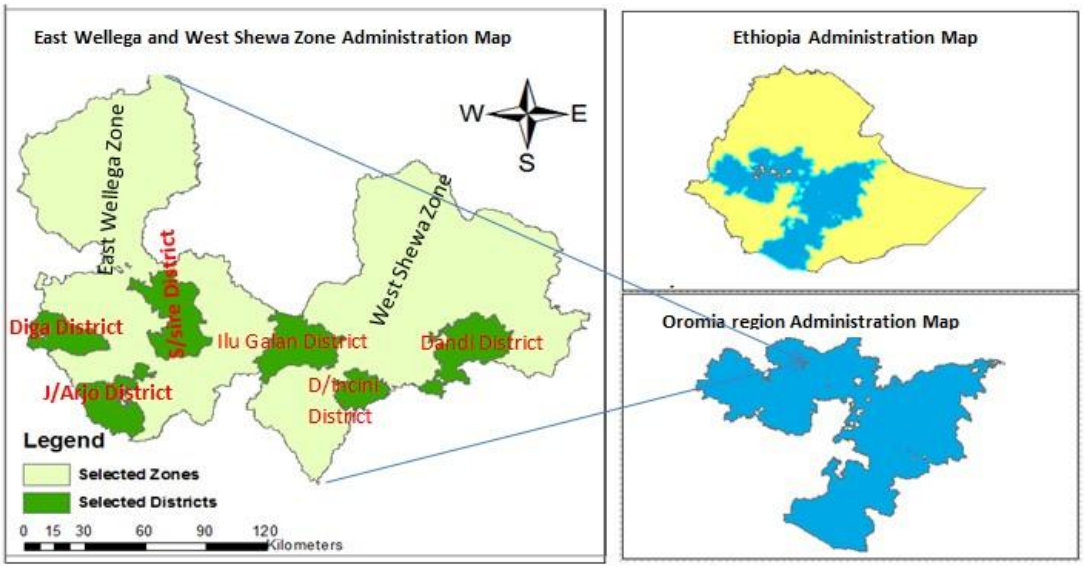

FIG. 1 MAP OF STUDY AREA

2.2 Sources of Data and Samplingdesign

In assessing the household energy sources and consumption patterns, the primary data were collected from the household heads of the study area through designed structured questionnaire. In this study, multistage sampling procedures were used to select the survey areas. At the first stage, East wolega and west shewa was selected randomly from western oromia. In the second stage, three woread as Sibu sire, Diga and Jima Arjoworedas' were selected from East wolega zone and Ilu galan, Dandi and Dire inciniworedas' were selected from West shewa purposively depending on HHs' source of energy were traditional inefficient biomass based . At third Stage two kebeles were selected from each woreda and at the end stage 180 HH were selected and interviewed randomly based on Probability Proportional to Size (PPSS). In this study, descriptive statistics such as, percentage, mean, standard deviation was used to summaries results. The statistical analysis data will be under taken by 20 version SPSSsoftware.

III. RESULT ANDDISCUSSION

3.1 Rural Energy Source of the studyarea

Larger proportion of rural households are dependent on traditional fuels (biomass) dependent for baking Enjera and heating while some used other source of energy such as biogas, electricity, kerosene ,battery cell for lighting, all of households are dependent on firewood source of energy and large proportion of respondent used Agricultural residue and kerosene source of energy consumption while and dung cake, charcoal and electricity are found lowest energy consumption in west shewa and east wolega zones of rural household (Table 1)**.** The main reasons for preference of biomass energy consumption in the study area is ease of access (31.7%), cultural preference (30%) and cheap prices (21.1%) source of energy furthermore the least reasons for choice of rural households energy consumption is convenience when they used and No alternative source of 8.3% and 8.9% respectively. This is supported by research (Mekonnen and Kohlin, 2008), in Ethiopian, rural households have been dependent for centuries on two main solid fuels woody biomass and dung with kerosene used for lighting however electricity, and liquefied petroleum gas are possible alternative energy sources, they are hardly used at all in these rural areas due to high prices and lack of access. The characteristic of household Energy source utilization is shown (Table 2) the majority of respondent uses firewood about 91.1% and about 16.9% the respondent uses Agricultural residue for the purpose of baking Enjera. Firewood is the first widely used energy source, about 57.2% of the respondent uses Firewood and about 22.8% of the respondent uses charcoal for the purpose of heating mostly. About 55% the respondents uses kerosene and the respondents' uses small size solaronly about 20% for purpose of lighting. Electricity, battery cell, biogas was the lowest

level energy sources of study area. In study area even if some rural HHs with access to electric service, they did not use for the purpose of Enjera baking as well as heating, only use for the purpose of lighting because of high cost of electricity which is not affordable for the rural people. Among the various fuels considered fire wood and Crop residue turned out to be the prominent energy sources of households in the study area. All households in sample use firewood as energy source self collected with small portion of it coming from the market (purchasing) (Table 3).

TABLE 1
PROPORTIONS OF HOUSEHOLD'S MAJOR ENERGY CONSUMPTION IN THE STUDY AREA

Energy sources	N	Percent
Firewood	180	100
Crop Residue	11	62.8
Animal Dung cakes	36	20
Charcoal	36	20
Kerosene	111	61.7
Electricity	33	18.3
Small size solar	36	20
Battery cell	8	4.4
Biogas	3	1.66

Source, Survey result 2017

TABLE 2
PROPORTION OF ENERGY SOURCES CONSUMED BY HHS

Energy Sources	Energy source consumed for different Activity by HHs					
	Enjera baking		Heating		Lighting	
	N	%	N	%	N	%
Fire wood	164	91.1	103	57.1	0	0
Agricultural residue	16	8.9	29	16.1	0	0
Firewood with Dung cake	0	0	5	2.8	0	0
Charcoal	0	0	41	28.9	0	0
Kerosene	0	0	0	0	99	55
Small size solar	0	0	0	0	36	20
Battery cell	0	0	0	0	8	4.4
Electricity	0	0	0	0	33	18.3
Biogas	0	0	3	1.5	1	0.6

Source, Survey result 2017 N=Number HH reply

TABLE 3
PLACE OF FIREWOOD COLLECTED BY HHS (N=166)

Place of firewood collected	N	Percent HHs reply
Own farm	104	62.7
Community Forest	42	25.3
Free space	20	12

Source, survey result 2017N=Number HH reply

From HHs who self collected firewood, women and girls were found more and Women participate in the collection of fire wood.

About 62.7% of respondent collects fire wood from own farm and about 25.3% respondents collects from community forest but small portion about 12% of respondents collects from free space.

In the study, finding shown that (Table 4) on average households traveled 0.5hr, with minimum and maximum 0.1hr and 2hr to collect firewood. They collect wood 2 to 3 times in a week. The fuel wood collection frequency depends on the family size and also on distance from the source. Large families require more wood to fulfill their domestic energy needs. Theirfuel

wood demand doubles in winter season as compared to the summer season because they require more fuel wood for heating purposes. On average the respondents collect 3 times per week with minimum and maximum 1 time and 7 times per week. And on average it takes 1.3 hrs with minimum and maximum of 0.2 hrs and 6 hrs to collect fire wood for one trip.

TABLE 4
DISTANCE TRAVELED, FREQUENCY PER WEEK AND TIME SPENT FOR FIREWOOD COLLECTION (N=166)

Variable	Minimum	Maximum	Mean	Std. deviation
Time traveled (Hrs)	0.1	2	0.5	0.36
Frequency collected per week	1	7	3	2.07
Time taken to collect for one trip (Hrs)	0.2	6	1.3	1.06

Source, survey result 2017N=Number HH reply

The main activities affected by fuel wood collection was, educational activities of the female, and Agricultural activities was the most affected activity.

3.2 Constraints of biomass energy consumption in studyarea

Biomass energy sources (Firewood and Agricultural residue) are dominant energy sources and energy potentials for Enjera baking and heating purpose in the study area. Kerosene and small size solar system was energy potentials for lighting. Even Biomass energy uses have certain Problems. The main problems using biomass energy was to smoky and causes eye disease and cough increased burden on women, Facilitate erosion on the farm, Deforestation are the major constraints biomass energy wasidentified.

3.3 Alternative Rural Energy Sources in studyarea

According to secondary data collected from west shewa and east wolega zone respective selected districts from each zones, The available alternative energy technology In study area were biogas, Improved charcoal stove (leqach), Mirt stove, solar energy . About 111(61.7%) have no alternative energy sources like biogas, solar energy and improved charcoal stove, mirt Enjera stove, electricity but about 69(38.3%) have such like alternative energy (Table 5). For respondent who have no alternative Energy sources, the possible reason why they have no alternative energy sources are, lack of cost, lack accessibility, lack of awareness on alternative energy source and lack of interest on to get alternative energy source**.** Accordingly the rank correlation analysis between two zones show that the relation of the causes of 80% similarity reasons of not have alternative energy sources (Table 6).

TABLE 5
ALTERNATIVE ENERGY SOURCES IN STUDY AREA (N=69)

Alternative Energy Source	N	Percent
Biogas technology	4	5.8
Small size Solar Energy	30	43.5
Mirt Enjera stove	6	8.3
Improved charcoal stove	5	7.2
Electricity	20	29
Mirt Enjera stove and Electricity	3	4.3
Biogas , Mirtenjera stove and Electricity	1	1.4

Source, survey result 2017 N=Number HH reply

TABLE 6
RANK CORRELATION ANALYSIS BETWEEN TWO ZONES

Reason				
	East Wolega zone			Di^2
Lack of cost	1	1	0	0
Lack of Accessibility	2	2	0	0
Lack of awareness	3	4	-1	1
Lack of interest to get	4	3	1	1

Source, survey result 2017

Rank correlation analysis between two zones

$$r= 1-\frac{6\ \iota^2}{(n2-1)} = \frac{1-6*2}{4(16-1} = 1-\frac{12}{15} = 1-0.2= 0.8=80\%$$

In this study, also finding shown that (Table 7), almost all a households, about 87%, 90% and 88% of respondents in the study do not have access to training on biogas technologies, solar energy and improved charcoal stove and mirt Enjera stove respectively. From this could conclude that biomass energy sources is the dominant fuel sources by both households with no and with access to alternative energy sources in the study area implying that burden on biomass (wood, dung and Agricultural residue) energy sources which leads to environmental problem and subsequent reduction in agricultural productivity.

TABLE 7
ABOUT TRAINING OF ALTERNATIVE ENERGY SOURCES

Alternative Energy Sources	HHs with technology (N=69)				HHs without technology (N=111)				Total (N=180)			
	Yes		No		Yes		No		Yes		No	
	N	%	N	%	N	%	N	%	N	%	N	%
Biogas Technology	17	24.6	52	74.6	7	6.3	104	93.7	24	13	156	87
Solar Energy	11	15.9	58	84.1	7	6.3	104	93.7	18	10	162	90
Improved charcoal stove and mirt Enjera stove	10	13.2	59	86.8	11	9.9	100	90.1	21	11	159	88

Source, survey result 2017 N=Number HH reply, %= Percent

3.4 Comparison of Households with no and with Access to Alternative Rural Energy in studyarea

The result of this study reveals that mean age of the household is 38 and 42 years of old for households with no and with access to Alternative rural energy source fuel respectively, the mean comparison of households with no and with access to alternative rural technology in terms of age was significant (t-value= 2.113, sig. 0.036). In similar way, the sex of the household head, households with no access and with access energy source 78 percent. These imply that the mean difference observed in terms of sex statistically not significant (t-value=0.019, sig. 0.985). About 61 percent of households with no access to alternative rural energy are literate household head while households with access to alternative rural energy account 78 percent household heads are literate. This difference is statically significant at 5%. This implies that literate headed households are consumed more alternative rural energy of energy than illiterate headed households. the average time in hour from the household's home to the Farmers training center for households with no and with access to alternative rural energy is 0.56Hr (34 min) and 50hr(30 min.) respectively; this mean difference is statistically in significant at 5% (t-value=-0.665 sig-value = 0.50). The mean time in hour from the households' home to health extension center for households with no access to modern fuel is about 0.47hr (28min); the mean distance traveled by households with access to modern fuel is 0.43hr **(**26min). This difference is also statistically insignificant at 5 %(t-value= -0.859 Sig -value= 0.392) (Table8).

TABLE 8
COMPARISON OF HOUSEHOLDS WITH NO AND WITH ACCESS TO MODERN FUEL

Socio-demographic character	Mean			
	With no access	With access	t-value	Sig-value
Age	38	42	2.11	0.036*
Family size	6	7	1.32	0.189
Time taken to travel FTC	0.56hr	0.5hr	-0.665	0.5
Time taken to travel health extension center	0.47hr	0.43hr	-0.859	0.392
Educational level of household			2.9	0.004*
Sex of household			0.19	0.985
Place of cooking			0.295	0.004*

Source, surveyresult2017 * statically significant at5%

3.5 Constraints of Rural Energy Sources in Studyarea

In study area, the constraints of rural energy sources were identified and prioritized in order to importance by farmers in study area. Table17 indicate that about 40% of respondent reply lack of Manufacturer on alternativerural energy source,

Lack of effective alternative rural energy sources (33.8%) socio-economic problems to accept available rural energy technology (12.7%), lack of information where and how alternative energy sources (7.5%), unlike, Agricultural input, lack of research on alternative energy sources (12.5%), are the major constraints identified.

IV. CONCLUSION

The characteristic of household Energy source utilization, the majority of respondent uses firewood about 91.1% and about 16.9% the respondent uses crop residue for the purpose of baking Enjera. Firewood is the first widely used energy source, about 57.2% of the respondent uses Firewood and about 22.8% of the respondent uses charcoal for the purpose of heating mostly. About 55% the respondents uses kerosene and the respondent's uses small size solar only about 20% for purpose of lighting. Electricity, battery cell, biogas was the lowest level energy sources of study area. In study area even if some rural HHs with access to electric service , they did not use for the purpose of Enjera baking as well as heating , only use for the purpose of lighting in study area.

The main reasons for preference of biomass energy consumption in the study area is ease of access (31.7%), cultural preference (30%) and cheap prices (21.1%) source of energy furthermore the least reasons for choice of rural households energy consumption is convenience when they used and No alternative source of 8.3% and 8.9% respectively

Among the various fuels considered fire wood and Crop residue turned out to be the prominent energy sources of households in the study area. All households in sample use firewood as energy source with small portion of it coming from the market (purchasing). HHs who self collected firewood, women and girls were found more about 51.8% and followed by Women participates in the collection of fire wood. The finding revels that on average households traveled 0.5hr, with minimum and maximum 0.1hr and 2hr to collect firewood. They collect wood 2 to 3 times in a week. The fuel wood collection frequency depends on the family size and also on distance from the source. Large families require more wood to fulfill their domestic energy needs, so they collect 2 to 3 times in a week. Their fuel wood demand doubles in winter season as compared to the summer season because they require more fuel wood for heating purposes. On average the respondents collect 3 times per week. And on average it takes 1.3hrs with minimum and maximum of 0.2 hrs and 6hrs to collect fire wood for one trip The main activities affected by fuel wood collection was, educational activities of the female, and Agricultural activities was the mostly affected activity. The main problems using firewood was to smoky and causes eye disease and cough, increased burden on women, facilitate erosion anddeforestation

About 111(61.7%) have no alternative energy sources like biogas, solar energy and improved charcoal stove, mirt Enjera stove, electricity but about 69 (38.3%) have such like alternative energy sources. The possible reason why they have not alternative energy sources are, luck of cost, luck accessibility, luck of awareness on alternative energy source and luck of interest on to get alternative energy source**.** according to rank to correlation analysis between two zones show that the relation of the causes of 80% and this can be similarity reasons of not have alternative energy sources Firewood and crop residue is dominant energy sources and energy potentials for Enjera baking and water heating in the study area. In same the way kerosene and small size solar system was energy potentials forlighting

In study area, the constraints of rural energy sources were identified and prioritized in order to importance by farmers in study area. About 39.1% of respondent reply lack of manufacture on alternative rural energy source, socio-economic problems to accept available rural energy technology (33.2%), Lack of effective alternative rural energy sources(15.6%), unlike, Agricultural input, lack of research on alternative energy sources (12.5%), are the major constraintsidentified.

RECOMMENDATION

The heavy dependence and inefficient utilization of biomass resources of energy have resulted in high depletion of firewood, crop residue, dung and charcoal in the East wolega and west shewa zone. Rural household should adopt of improved stove that contribute to reducing burden on biomass reducing burden on biomass. Almost all a household do not have access to training on alternative technologies like biogas, solar heating and Improved charcoal stove and mirt Enjera stove to fill this gap Woreda water ,mineral and energy office should train Rural household on alternative rural Energy source and disseminate the available alternative technologies.

In addition, the result shows that households spent significant amount of time for fuel collection. And also, all a household do not have access to training on alternative technologies like biogas, solar heating and Improved charcoal stove and mirt Enjera stove. To fill these knowledge gap Woreda energy office should train rural household alternative technologies and disseminate the available alternative technologies and AERC, should be planned to introduce new Rural Energy sources and.

Although there is introduction of biogas for few rural households used for only lighting , but they do not used for baking Enjera because luck of Biogas mitad. So Agricultural engineering research center should introduce Biogas mitad helps to reduce burden on biomass sources of energy.

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REFERENCES

[1] Anderson, T. 1996 Rural Energy Services: A Hand book for Sustainable EnergyDevelopment.

[2] Annual Report of East WolegaZoneDisaster Prevention and preparedness officeonADPLAC,2014. [3] Araya A.et.al (2002) The Global Energy trend : Energy in Ethiopia: Status, Challenges andProspects.

[4] Berhanu, Gebremedhin 1998. The economics of soil conservation investments in the Tigrai Region ofEthiopia.

[5] EFAP 2003. The Challenge for Development,Volume II. Addis Ababa: Ministry of Natural Resource Development and EnvironmentalProtection.

[6] Heimann, S. (2007). Renewable energy inEthiopia.

[7] MekonnenKassa. 2000. An Overview to the Energy Situation inEthiopia.

[8] Nebiyu, A..Domestic Energy Consumption and Deforestation in Harari region Assessment of Students' Awareness andViews. M.A. Thesis.

[9] Wood, A. P. 1990. Natural resources management and rural developmentinEthiopia.

[10] World Bank 1994. Issues and Options in Energy Sector inEthiopia.

[11] World Bank Group. (2009). Energy strategy approach paper: sustainable development Network.

[12] WHO. (2006). Fuel for life: household energy and health.

Response of Rice Genotypes to Arsenic Contaminated Field Condition during Kharif and Boro Season

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Abstract— *A study was conducted to evaluate rice germplasm for their arsenic content in different plant parts and phosphorus content in grains. Results showed that in the rice plant highest arsenic was accumulated in the roots and the lowest in the grain. The arsenic accumulation follows the order of root>shoot> leaf> grain. The results also revealed that IRRI dhan was quite tolerant to arsenic and Satabdi 1 was susceptible to arsenic accumulation during kharif season, whereas during boro season IET-4094 accumulated more arsenic in grain and IR-50 accumulated less arsenic in grain. Results also showed that kharif rice genotypes bore strong positive association between total phosphorus percent with arsenic content in grain, whereas strong negative association was found in boro season rice genotypes.*

Keywords— Arsenic, Rice (oryza sativa L.), phosphorus, West bengal.

I. INTRODUCTION

Arsenic is a naturally occurring toxic metalloid and widely distributed in the soil, water, air and all living matters. Anthropogenic activities, including metal smelting, coal combustion, dyes, and hide tanning wastes, chemical weapons and arsenic pesticides have contributed to elevated arsenic in the environment. Large areas of Bangladesh, West Bengal, northwest China and Vietnam have to rely on arsenic-contaminated ground-water for irrigation of staple crops such as rice (Berg *et al*. 2001, Abedin *et al*. 2002) and the extensive use of arsenic contaminated groundwater for crop irrigation in the arsenic affected areas as the cause of arsenic poisoning, and possibility of a build-up of arsenic in soils and agronomic and/or horticultural produce, that acts as a conduit for the passage of the toxicant to human population via food-web. Out of the twenty countries in different parts of the world where ground water arsenic contamination and human suffering from there have been reported so far, the magnitude is considered to be the highest in Bangladesh, followed by West Bengal, India. The wide spread arsenic contamination in groundwater in different parts of West Bengal, distributed over 111 blocks, located primarily in twelve districts in West Bengal. The distribution pattern of arsenic among various plant parts is highly variable. Excessive pumping of ground water has increased arsenic toxicity in West Bengal, Bangladesh and many other Asian countries. The arsenic concentration tends to build up from the contaminated groundwater, via the soil, to the crop, irrigated with such water. Adak and Mandal (2000) stated that among the plant parts, arsenic concentration was higher in roots, followed by stems and leaves, in that order, while the economic or edible parts recorded the lowest concentration of the toxin. Phosphorus (P) is one of the essential major plant nutrients for plant growth. Because As and P are both placed in group Vb, the interaction of As and P in soil-plant system is an important issue in respect of arsenic mobilization. Several workers showed that the presence of phosphate caused a reduction in arsenate adsorption, and that the reduction was much greater for the competitive effects of arsenate on phosphate adsorption by soil minerals, although a large variation in the degree of competition between these two oxyanions has also been reported (Kuo and McNeal, 1984; Mukhopadhyay and Sanyal, 2002). Inorganic arsenic is highly toxic to plants because it uncouples phosphorylation and inhibits phosphate uptake. Arsenate is taken up by plants via the phosphate (Pi) transport systems because of similarity between arsenate and Pi and after entering plant; arsenate might interfere the phosphate metabolisms and caused toxicity of plant (Dixon, 1997)

In the above circumstances the present study was undertaken to see the concentration of arsenic in different parts and genotypes of rice and the relationship between arsenic in different parts and grain phosphorus.

II. MATERIALS AND METHODS

Plant samples were collected from farmer's field at Nonaghata-Uttarpara, Haringhata block, Nadia, West Bengal. Rice samples were collected by composite sampling from the arsenic affected fields.

2.1 Analysis of the total arsenic in plant sample

The rice plant samples were washed thoroughly with tap water to remove soil and other contaminants and finally rinsed with de-ionised water with continuous shaking for several minutes. The samples were then dried in the Hot Air Oven at 60C for 72 hours. Root, shoot, leaf and grain from the rice plant sample were digested separately in an Erlenmeyer flask by a mixture

of concentrated acids, e.g., HNO_3, $HClO_4$ and H_2SO_4 in a proportion of 10:4:1 (v/v). After an overnight reaction, the content of the flask were gently boiled on an electric heater for digestion. The entire digestion process lasted 3-4 hours. After complete digestion, the solution was diluted with double distilled water and filtered by Whatman No. 42 filter paper and transferred in to acid-washed plastic bottle; this solution was used for analyzing the arsenic and phosphorus content of the sample. Each treatment was performed in triplicate. The digest was diluted to 50 ml. Ten (10) ml of the aliquot was taken in 50 ml plastic tube, 5 ml of concentrated HCl and 1 ml of each of reagent 5% KI (w/v) and 5% Ascorbic acid (w/v)] were mixed, kept for 45 minutes to ensure complete reaction. Now the volume was made up to 50 ml and the resultant solution was analyzed in a Perkin-Elmer Atomic Absorption Spectrophotometer with Flow Injection Analysis System (FIAS 400) @ $\lambda_{max} \cong 193.7$ nm where the carrier solution was 10% v/v HCl, the reducing agent (to ensure all As species be reduced to AsH_3 and to be measured against a calibration with standard As^{+3} solution) was 0.2% $NaBH_4$ in 0.05% NaOH (Welsch *et al*,1990).

Phosphorus content in plant sample (grain) was determined by following the vanadate molybdate yellow colour outlined by Jackson (1973).

Statistical Data analysis: Pearson's correlation coefficient was carried out to find out the correlation among arsenic concentration in different parts (root, shoot, leaf and grain) and total Phosphorus in grains by SPSS software, version 14.0 for windows (SPSS Inc., Chicago, USA)

III. RESULTS AND DISCUSSION

The values of arsenic concentration in grains, roots, stems and leaves of kharif rice crop of the genotypes are presented in Table 1. Arsenic concentration of roots, shoots, leaves and grains of the genotypes ranged between 2.958 to 9.475, 1.004 to 4.510, 0.973 to 4.773 and 0.180 to1.960.The variation in root, shoot, leaf and grain arsenic concentration among the rice genotypes was statistically significant indicating inherent difference among the genotypes in this regard. The highest root, shoot, leaf and grain As concentration was observed in satabdi 2 (9.475), Minikit (4.510), Minikit (4.773), satabdi 1 (1.960), whereas, the lowest root, shoot, leaf and grain As concentration was recorded in GS-3 (2.958), satabdi 2 (1.004), Satabdi 2(0.973), IRRI dhan (0.180). So, it was observed that IRRI dhan accumulated less arsenic in grain and Satabdi1 accumulated more arsenic in grain. Total P % of grain ranged between 0.412 and 0.124. The highest total phosphorus % was observed in GS-2 (0.412), the lowest total phosphorus % was recorded in IRRI dhan (0.124).

TABLE 1

ARSENIC CONCENTRATIONS IN ROOT, SHOOT, LEAF AND GRAIN (mg/kg) ALONG WITH GRAIN PHOSPHORUS CONCENTRATION OF THE RICE GENOTYPES GROWN DURING KHARIF

S. No	Cultivar	Root As	Shoot As	Leaf As	Grain As	Total P%
1	IET- 4786	4.378	3.255	2.305	0.340	0.222
2	MTU 7029	5.125	3.628	2.640	0.380	0.230
3	Jal kumara	8.530	2.565	3.778	0.511	0.245
4	Ranjit	3.875	1.745	2.583	0.230	0.173
5	Kakdwip sel-15	4.353	2.050	2.550	0.420	0.212
6	IRRI dhan	3.275	2.031	1.260	0.180	0.124
7	GS-3	2.958	2.373	1.008	0.340	0.214
8	Kakdwip sel-7	5.890	4.178	1.320	0.240	0.154
9	Ratna	3.710	1.073	1.160	0.290	0.200
10	IET-4094	6.270	1.120	3.020	0.570	0.211
11	Nayanmoni	4.250	1.060	2.120	0.460	0.247
12	Satabdi 1	6.070	3.625	3.175	1.960	0.236
13	Minikit	7.685	4.510	4.773	1.483	0.215
14	IET-4787	7.413	1.651	1.042	0.681	0.213
15	Satabdi 2	9.475	1.004	0.973	0.500	0.222
16	GS-2	7.523	2.156	2.630	1.634	0.412
17	GS-1	3.714	3.808	2.040	0.480	0.253
Mean		5.559	2.461	2.258	0.629	0.223
SEm ±		0.066	0.057	0.050	0.028	0.006
CD (P= 0.05)		0.192	0.166	0.143	0.079	0.017

TABLE 2

ARSENIC CONCENTRATIONS IN ROOT, SHOOT, LEAF AND GRAIN ALONG WITH GRAIN PHOSPHORUS CONCENTRATION OF THE RICE GENOTYPES GROWN DURING BORO

Sl.No.	Cultivar	Root	Shoot	Leaf	Grain	Total P%
1	IRRI dhan	4.100	2.120	0.765	0.340	0.275
2	Satabdi	7.100	3.930	1.110	0.650	0.245
3	GS-1	6.100	2.030	0.643	0.363	0.260
4	Jaldi minikit	7.480	1.810	2.340	0.453	0.230
5	IR-50	7.430	2.500	3.330	0.330	0.245
6	GS-3	7.430	2.070	2.880	0.985	0.258
7	IET-4094	6.550	2.900	0.753	1.890	0.200
Mean		6.599	2.480	1.689	0.716	0.245
SEm ±		0.091	0.059	0.036	0.012	0.011
CD (P= 0.05)		0.282	0.181	0.110	0.036	0.034

Table 2 represents the arsenic concentration in grains, stems, leaves and roots of the rice genotypes grown during boro season in the arsenic contaminated situation. Arsenic concentration of root, shoot, leaf and grain ranged between 7.480 and 4.100, 3.930 and 1.810, 3.330 and 0.643, 1.890 and 0.330. The variation in root, shoot, leaf and grain arsenic concentration among the rice genotypes grown during boro season was statistically significant indicating inherent difference among the genotypes in this regard. The highest root, shoot, leaf and grain As concentration was observed in Jaldi Minikit (7.480), Satabdi (3.930), IR-50 (3.330), IET-4094 (1.890), the lowest root, shoot, leaf and grain was recorded in IRRI dhan (4.100), Jaldi minikit (1.810), GS-1 (0.643), IR-50(0.330). It was recorded that IET-4094 accumulated more arsenic in grain and IR-50 accumulated less arsenic in grain. The results showed that arsenic accumulated mostly in the root of rice plant followed by accumulation in shoot, leaf and lowest in grain. These findings are in agreement with the findings of Rahman et al. (2007). Higher accumulation of arsenic in root of the rice plant might be due to formation of iron oxides around the rice root, which bind the arsenic and reduce its translocation to the above ground tissues of the plant. Total P % of grain ranged between 0.275 and 0.200. The higher accumulation of total P % of grain was observed in IRRI dhan (0.412), the lower accumulation was recorded in IET-4094 (0.200).

TABLE 3

CORRELATIONS CO-EFFICIENT BETWEEN ARSENIC CONCENTRATIONS OF DIFFERENT PLANT PARTS DURING KHARIF SEASON

Sl.No.	1	2	3	4	5	6
1	Character	Root As	Shoot As	Leaf As	Grain As	Total P
2	Root As	-	0.116	0.238	0.399(**)	0.340(*)
3	Shoot As		-	0.362(**)	.139	0.079
4	Leaf As			-	0.523(**)	0.245
5	Grain As				-	0.564(**)
6	Total P					-

***** Correlation is significant at the 0.01 level (2-tailed).***

**** Correlation is significant at the 0.05 level (2-tailed).***

The results revealed that when grain arsenic concentration in the kharif season increases total phosphorus content also increases and in boro rice genotypes when the concentration of total phosphorus increased grain arsenic concentration decreased significantly. Abedin *et al*. (2002) reported that the uptake rate in rice at 0.05mM of arsenate decreased significantly (p,0.001) with increasing phosphate concentration present in the incubating solution. Opposite results were reported by Tu and Ma (2003) that there was a positive correlation between plant phosphate and arsenic concentration in brake fern. The values of correlation co-efficient (table 3) among root, stem, leaf and grain arsenic concentration show that grain arsenic concentration of the rice genotypes grown in contaminated situation during Kharif season bore strong association with arsenic concentration of leaf and root and grain P concentration. Stem arsenic concentration, though appeared to have no bearing with grain arsenic concentration, found to be associated with leaf arsenic concentration. Root arsenic concentration was observed to be associated with grain P concentration of the rice genotypes. The values of correlation co-efficient (table 4) among root, stem, leaf and grain arsenic concentration show that grain arsenic concentration

of the rice genotypes grown in contaminated situation during boro season bore strong negative association with grain P concentration only. Root arsenic concentration of the boro crop of the genotypes, though appeared to have no bearing with grain arsenic concentration, found to be associated with leaf arsenic concentration.

TABLE 4
CORRELATIONS CO-EFFICIENT BETWEEN ARSENIC CONCENTRATIONS OF DIFFERENT PLANT PARTS DURING BORO SEASON

Sl.No.	1	2	3	4	5	6
1	Character	Root As	Shoot As	Leaf As	Grain As	Total P
2	Root As	-	0.174	0.615(**)	0.165	-0.328
3	Shoot As		-	-0.263	0.291	-0.231
4	Leaf As			-	-0.195	-0.003
5	Grain As				-	-0.551(**)
6	Total P					-

***** Correlation is significant at the 0.01 level (2-tailed).***
**** Correlation is significant at the 0.05 level (2-tailed).***

IV. CONCLUSION

From the above discussion, it can be concluded that, arsenic concentration in different parts of plant was observed to be in the order of root > stem ≥ leaf > grain in both *kharif* and *boro* crop of the rice genotypes. Grain As was found to be strongly and positively associated with grain phosphorus, leaf As and root As in *kharif* crop where as strongly and negatively associated with grain phosphorus only in *boro* crop.

REFERENCES

[1] Abedin, M. J., Cottep-Howells, J. and Meharg, A. A. 2002. Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. *Plant and Soil*., **240** : 311 – 319.

[2] Adak, S. K. and Mandal, B. K. 2000. Influence of arsenic contaminated irrigation water on yield vis-à-vis uptake in different plant parts. *Proc. Intl. Conf. on Managing Natl. Resources for sustainable agricultural Production in the 21st Century*, New Delhi, February 14-18, 2000. Extended Summaries, Vol. 2, p. 537.

[3] Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenleib, R. and Giger, W. 2001. Arsenic contamination of groundwater and drinking water in Vietnam: a human health threat. *Environ. Sci. Tech.*, **35**: 2621-2626.

[4] Dixon, H. B. F. 1997. The biochemical action of arsenic acids especially as phosphate analogues. *Adv. Inorg. Chem.*, **44**: 191-227.

[5] Jackson, M.L. 1973. Soil chemical analysis. Prentice-Hall of India Private Ltd., New Delhi.

[6] Kuo, S. and McNeal, B. L. 1984. Effects of pH and phosphate on cadmium sorption by a hydrous ferric oxide. *Soil Sci. Am. J.*, **48**: 1040-1044.

[7] Mukhopadhyay, D and Sanyal, S. K. 2002. Studies on arsenic transport across and down some soils of West Bengal. *J. Indian Soc. Soil Sci.*, **50**: 456-463.

[8] Rahman, M. A., Hasegawa, H., Rahman, M. M., Islam, M. N., Miah, M. A. M. and Tasmen, A. 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere.*, **67**: 1072-1079.

[9] Tu, C., Ma, L. Q., 2003. Effects of arsenate and phosphate on their accumulation by an arsenic- hyperaccumulator *Pteris vittata* L. Plant Soil 249, 373-382.

[10] Welsch, F. P., Crock, J.G. and Sanzolone, R.1990. Trace elements determination of arsenic and selenium using continous flow hydride generation atomic absorption spectrophotometry (HG-AAS). In Quality Assurance Manual for the Branch of Geochemistry, Ed., Arbogast, B. F.,pp:38-45.

Health Issues and Mental Distress in French Active Farmers: A Quantitative and Qualitative Study

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Abstract—

Purpose: *Despite a particularly high suicide-related death rate observed in French farmers, data regarding this population's perception of health and their potential difficulties in accessing health care remains scarce.*

Methods: *We conducted a quantitative study involving 161 randomly selected farmers in three rural localities. Precarious conditions were assessed based on farmers' EPICES scores, their quality of life using the SF12 score, and their psychological distress by means of the GHQ-12. At the same time, a qualitative study was performed consisting in 16 in-depth interviews.*

Findings: *In total, 112 farmers (70% of persons contacted), most of them working as breeders for the meat-processing industry, participated in the study. Amongst them, 14.3% had forgone doctor consultation within the last 12 months, and 19.6% had abstained from consulting a dentist. They expressed trust in their general practitioners but frequently resorted to alternative medicine or marginal practitioners when they considered their disease to be benign. The general physical health status measured using the SF12 was good in 84.5% of cases. However, 29.7% of respondents experienced psychological distress that seemed to originate primarily from the conflicting rhythms of agricultural timescales (seasonal calendar; local weather conditions; social rhythms, including administrative work; community rhythms). Factor analysis of mixed data showed that single farmers exhibiting social deprivation, who had inherited their farm, tended to be affected by psychological distress.*

Conclusion: *The consideration of mental distress in this identified population proves to be challenging issues that may help prevent suicide in farmers.*

Key words: work stress, suicide, anthropology, access to health care.

I. INTRODUCTION

The French rural population has undergone a major shift over the last decades. According to the World Bank, although 21% of the French live in rural areas, the active farmer population has decreased from 8% in 1980 to 3.4% in 2007(1,2). Since the Second World War, agriculture has progressively shifted toward industrial processing, and the role of farmers increasingly resembles that of a manager, faced with an array of administrative tasks(3–5). Poverty has surged upward from 13% in 2006 to 24% in 2010 in this population(2).

Farmers' health is still a subject of contention in France. In a prospective cohort involving 180,060 active and retired farmers from different areas in France, Levêque-Morlais N *et al.* showed that overall mortality and cancer-related death rates were significantly lower than those observed in the general population during the study period (2005-9)(6). The authors concluded that farmers allegedly were in better overall health than the general population. However, in a monitoring survey, the *Institut de Veille Sanitaire* (InVS), Saint-Maurice, France, demonstrated elevated suicide-related death rates, especially in male livestock farmers (56% higher suicide rate for dairy farmers and 127% higher rate for livestock farmers working for the meat-processing industry in 2008)(7). Suicide and stress in farmers have been well documented in the UK(8–17) and Australia(18–22). However, the mechanisms underlying suicide are still poorly understood, especially in France(23–28).

Given the scarcity of general practitioners in rural areas and the distance from rural residents' homes to secondary or tertiary health care facilities, access to health care may be hindered for persons living in rural areas. Since 2013, *Médecins du Monde*, (Doctors of the World), a well-known non-governmental organization, has been involved in a rural-area-based program (Combrailles, Auvergne, France) aiming to facilitate and coordinate access to health care and social rights. We observed nevertheless that only few farmers resorted to this program.

We conducted a qualitative and quantitative study designed to better understand this population's perception of their health, as well as potential difficulties experienced by farmers in accessing health care.

II. METHODS

2.1 Quantitative study

Information about the study was disseminated through articles in three local newspapers and by mail. Overall 200 farmers were randomly selected from an exhaustive database of active farmers residing in three localities (Pionsat, Menat and Cœur de Combrailles) in the territory of Combrailles (Puy de Dome, France). Amongst them, 39 were excluded due to incorrect addresses, retirement, or death. The remaining 161 randomly selected farmers received a mailing and subsequent phone call, whereby 15 could not be reached. The survey was performed from the 16th of February to the 29th of April 2015. Face-to-face, 45-minute interviews took place at the farmers' places of residence after obtaining their written consent. The farmers were asked to fill out a questionnaire regarding their physical and mental health and their access to health care. Precarious conditions were assessed based on their EPICES score(29), and their quality of life using the SF12 score, which includes a physical score (Physical Composite Scores, PCS12) and mental health score (Mental Health Composite Scores, MCS12)(30). A value of ≤40 was considered a sign of impaired physical or mental quality of life. Psychological distress was assessed using the General Health Questionnaire (GHQ-12), consisting of four subscales: somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression. A GHQ-12 score of ≥2 was considered positive for psychological distress.

2.2 Statistics

Descriptive analyses of the study population were performed using the software program Stata (Version 12). Continuous variables were expressed as mean and standard deviation (SD), whilst categorical variables were expressed as numbers and percentages. To better describe the population type affected by psychological distress, we performed an exploratory factor analysis as a factor analysis of mixed data (FAMD) expressed in data tables in which a group of individuals is described by both quantitative and qualitative variables(31). These methods are particularly useful in identifying the underlying relationships between measured variables and determining a set of latent constructs underlying measured variables, as well as aggregating subjects into clusters such that each cluster represents a topic. The parameters selected for inclusion in the process were chosen according to univariate results and clinical relevance. The term mixed refers to the simultaneous presence, as active elements, of both quantitative and qualitative variables.

2.3 Qualitative study

In-depth interviews (N=16) were performed in order to collect data about the farmers. These interviews were all conducted in the participant's own home, lasting 90 minutes on average (30 minutes to three hours). The interviewer asked open-ended questions in the hope of inciting participants to share developments in their health behavior. The qualitative interviews further explored: 1) the issues arising from the quantitative phase; 2) the heuristic scope by lending greater significance to the participant's point of view; 3) the participants' understanding of the agricultural activity sector. The core aspects of the interview focused on the concept of *therapeutic itinerary*. This notion englobes all responses that constitute the strategy, or *quest*, to be cured, whether such measures are pursued within or outside of the official health care system(32,33). Audiotapes of interviews were analyzed to select the most significant parts that were transcribed, compared and coded according to the turning points of the therapeutic itinerary.

III. RESULTS

3.1 Demographic characteristics

Of the 161 subjects contacted, 15 (9.3%) could not be reached, and 34 (21.1%) refused to participate (lack of time: 17; lack of interest: 10; other or no reason: 7). Altogether, 112 farmers (70% of persons contacted) participated in the study. Of them, two were unable to respond to the SF12 and one could not respond to the GHQ-12. The vast majority of farmers were native to the area, lived with a partner, had inherited their farm, worked for the meat-processing industry, and produced the feed for their livestock (Table 1). Most of them had a full-time job with only a few days of leave each year.

TABLE 1
SOCIODEMOGRAPHIC CHARACTERISTICS OF FARMERS (n=112)

Sociodemographic characteristics	Total
Gender, male, n (%)	84 (75.0%)
Mean age (years) ±SD (ranges)	48.6±10.9 (26-74)
Native to area, n (%)	97 (86.6%)
Homeowner	93 (83.0%)
Live alone	16 (14.3%)
Social relationships	
- Feel isolated or somewhat isolated	30 (26.8%)
- Meet other family members less than once a month	12 (10.7%)
- Meet friends or neighbors less than once a month	2 (1.8%)
Family situation	
- Single	28 (25.0%)
- Household members, mean±SD	2.6±1.1
- Children	38 (33.9%)
Education	
- Primary - secondary school	98 (87.5%)
- University	14 (12.5%)
Monthly household income	
- <1,200 euros, n (%)	40 (35.7%)
- 1,200-2,000 euros, n (%)	39 (34.8%)
- >2,000 euros, n (%)	26 (23.2%)
- No response, n (%)	7 (6.3%)
Social deprivation[1]	
- Precarious: 30.17 ≤EPICES scores <53.84	31 (27.7%)
- Very precarious: EPICES scores ≥53.8	3 (2.7%)
Feel financially insecure	52 (46.4%)
Farm characteristics	
Farm history	
- Inherited farm	84 (75.0%)
- Created farm	25 (22.3%)
- Other	3 (2.7%)
Farm activity	
- Breeding	109 (97.0%)
- Livestock for meat production	93 (83.0%)
- Dairy livestock	6 (5.4%)
- Dairy and meat	8 (7.1%)
- Other	2 (1.8%)
- Produce feed for their livestock	99 (88.4%)
Farmer's activity	
- Working days/week (high season), mean±SD	6.9 (±0.3)
- Working days/week (low season), mean±SD	6.6 (±0.7)
- No leave in the last 12 months	63 (56.3%)
- Mean leave time per year ±SD (in days)	4.5 (±0.4)
Reasons for not taking leave (n=63)	
- Overworked/no time	18 (28.6%)
- Indispensable role on the farm	17 (27%)
- No desire/do not like vacation	16 (25.4%)
- Cannot afford it	11 (17.5%)
- Other	1 (1.6%)

[1]***EPICES scores ≥30.17 defined the deprived population (EPICES score were able to be calculated for 110 farmers)***

3.2 Physical health

Whilst interviewed, farmers stated they felt healthy, enjoying good disease resistance, a healthy lifestyle and good dental health; yet there were frequent reports of chronic diseases, obesity, active smoking, and unreplaced teeth (Table 2).

TABLE 2
PHYSICAL AND MENTAL HEALTH OF FARMERS

Physical health	**Total**
Impaired physical quality of life (PCS12 ≤40) **(N=110)**	17 (15.5%)
Feel they have good disease resistance	95 (84.8%)
Feel they have a healthy lifestyle	94 (83.9%)
Chronic diseases (one or several)	45 (40.2%)
- Diabetes	5 (4.5%)
- Cancer	3 (2.7%)
- Cardiovascular disease	12 (10.7%)
- Pulmonary disease	8 (7.1%)
- Neurological disease	8 (7.1%)
- Depressive syndrome	4 (3.6%)
- Osteoarticular syndrome	11 (9.8%)
- Other	16 (14.3%)
BMI	
- Overweight (25≤BMI<30)	39 (34.8%)
- Obesity (BMI≥30)	20 (17.9%)
Oral-dental condition	
- Relatively good to very good	98 (87.5%)
Number of missing, unreplaced teeth	
- None/Zero	62(55.4%)
- At least 1	49 (43.8%)
- 1 to 4 teeth	36 (32.1%)
- 5 to 9 teeth	11 (9.8%)
- ≥10 teeth	2 (1.8%)
- Do not know	1 (0.9%)
Mental health	Total
Impaired mental quality of life (MCS12≤40) **(n=110)**	32 (29.0%)
Psychological distress (GHQ12≥2) **(n=111)**	33 (29.7%)
Administration of psychotropic substances (in the last 12 months)	15 (13.4%)
Find their work physically tiring	95 (84.8%)
Find their work mentally tiring	84 (75.0%)
Insomnia due to work	58 (51.8%)
Addictions	
Smoking	29 (25.9%)
Number of cigarettes per day, mean±SD	14.5±8.2
Increased tobacco use due to work over the last 12 months (For daily smokers, **n=20**)	
Alcohol consumption	6 (30.0%)
- Never	
- Once a month or less	17 (15.2%)
- 2 to 4 times a month	16 (14.3%)
- 2 to 3 times a week	35 (31.3%)
- 4 to 6 times a week	17 (15.2%)
- Every day	3 (2.7%)
Number of drinks per day, mean±SD	24 (21.4%)
Increased alcohol consumption due to work over the last 12 months (For drinkers, **n=95**)	2.3±1.5 2 (2.1%)

3.3 Mental health

Farmers frequently exhibited impaired mental quality of life (SF12) and psychological distress (GHQ-12) (Table 2). In univariate analysis, mental distress was associated with a full time activity and few leave, feeling of solitude and difficulties in work (table 4). FAMD analysis results demonstrated a dichotomy between two main groups (Figure 1): 1) single farmers

affected by social deprivation who had inherited their farms tended to display psychological distress; 2) in contrast, farmers with a higher educational level, who had created their own farms and took more time off, appeared to be less prone to stress.

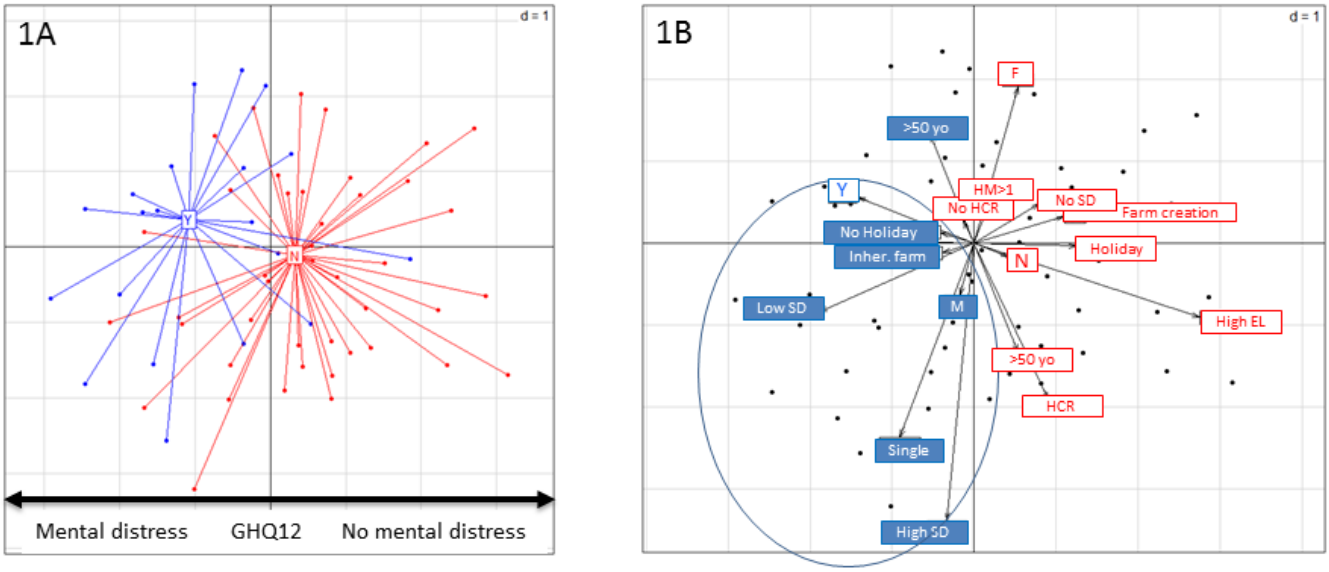

FIGURE 1: RESULTS OF FAMD. 1A. FARMERS REPRESENTATION ACCORDING TO GHQ12 SHOWING 2 CLUSTERS, ONE WITH MENTAL DISTRESS (Y, BLUE), ANOTHER WITH NO MENTAL DISTRESS (N, RED). 1B: VARIABLES REPRESENTATION SHOWING ONE CLUSTER (IN BLUE) EXPOSED TO MENTAL STRESS (Y) THAT MAINLY INCLUDES SINGLE FARMERS WITH SOCIAL DEPRIVATION AND INHERITED FARMS.
SD: Social Deprivation; M: Male; F: Female; Inher. Farm: Inherited Farm; yo: Year old; HCR: Health Care Renouncement; EL: Educational Level; HM: Household Members

The qualitative study's results highlighted that for active farmers, mental disorders, and especially depression, were perceived as diseases that should be discussed with and treated by a doctor. As for other diseases, many preferred to resort to a doctor only if they viewed them to be severe. Some farmers felt that mental disorders differed from other diseases and should be overcome by means of their own willpower, without any doctor's assistance.

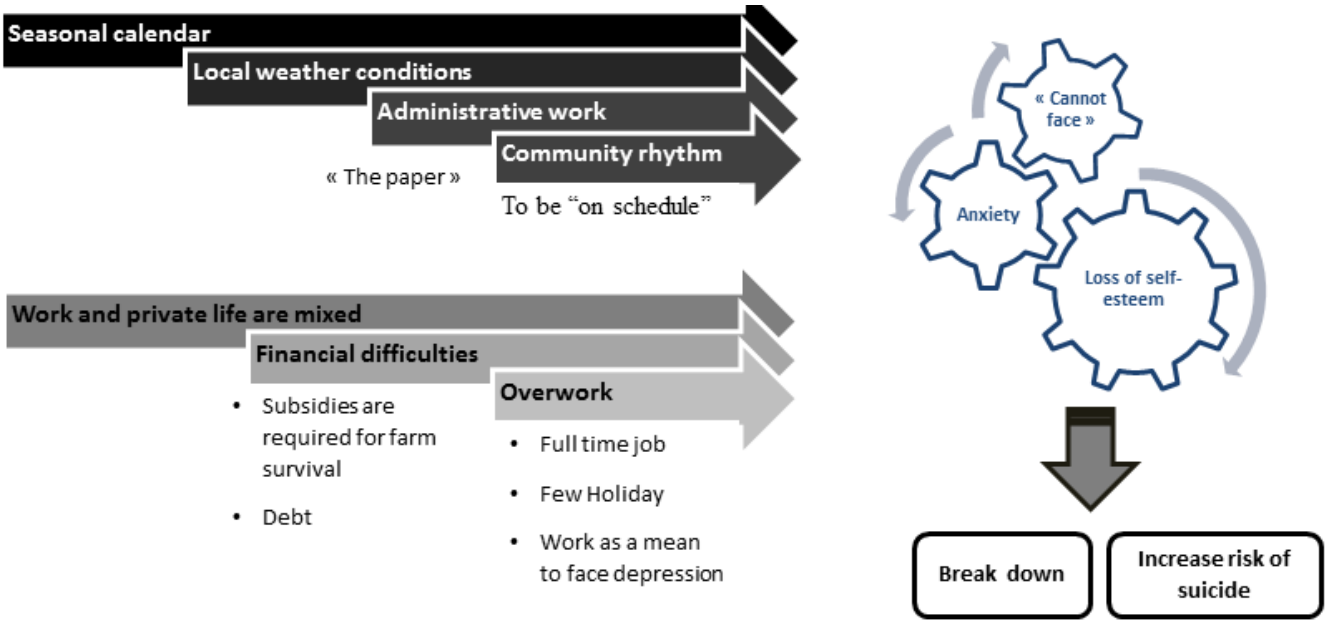

FIGURE 2: SCHEMATIC REPRESENTATION OF THE MULTIPLE FACTORS IDENTIFIED IN THE QUALITATIVE STUDY THAT MAY LEAD TO MENTAL DISTRESS AND MAY EXPLAIN FOR A PART THE INCREASE RISK OF SUICIDE IN THE POPULATION

Anxiety was a common symptom in active farmers and seemed to mainly originate from the conflicting rhythms of agricultural timeframes, specific to this population (Figure 2). Indeed, agricultural timeframes are subject to different

rhythms, including the seasonal calendar ("In winter, it's time for calving, and then it's time to tend to the corn, then hay, harvesting, ploughing, silage, and then it's time to sow… We end up never having any time left, you know."), local weather conditions (which may affect the work schedule) and social rhythms. In terms of social rhythms, farmers consistently broached the topic of administrative work ("paperwork"): "We have two bosses—the weather and 'them' [the Common Agricultural Policy, CAP]". As a prerequisite in order to obtain CAP-based subsidies (which are crucial for farmers' subsistence), this administrative work constituted the greatest source of anxiety for farmers, who described the CAP's rules for receiving subsidies as opaque and unpredictable: "[…] it stresses you out, because you never know how to properly fill out your paperwork in order to meet expectations". Another anxiety-provoking social rhythm was dictated by the community, whereby the progress of farmers' work was compared to that of their local farming community. For farmers, being "on schedule" meant having made similar headway in their farming tasks as their counterparts within a closed community. "Getting behind" bore a tacit social stigma—such farmers tended to be excluded from the community (loss of credibility, teasing) or to withdraw from various social activities (farmers' meetings, community celebrations, or festivities). Furthermore, ensuing psychological consequences included loss of self-esteem and anxiety. Given the inherent overlap between private and professional life in the agricultural sector ("Our life is our work—it's all interwoven."), one's professional status was perceived to reflect individuals' intrinsic value as human beings and was therefore highly associated with self-esteem.

Farmers generally felt the best remedy for depression was work ("And how do you keep your chin up?" "By working". "Work keeps you going strong?" "Yes. We have a lot of work […]"). Their response to anxiety or depression tended not to be taking time off, and some considered that meeting people was the best way to reduce stress (for instance, woofing is a practice specific to farmers, allowing them to meet people from the "outside").

3.4 Relationship with the health care system and abstention from health care

Farmers usually had a general practitioner (GP) they trusted even if they felt that the information provided was at times difficult to understand, with recommendations difficult to adhere to (Table 3). Moreover, numerous farmers resorted to alternative medicine and turned to marginal practitioners. When ill, 44.6% preferred to wait before seeing a doctor, and 40.2% self-medicated as their first-line therapy (8.9% took alternative medications). Participants generally had private complementary health insurance.

According to the study's qualitative results, the health care system seemed to generally enjoy farmers' trust when it came to managing severe diseases. Organic farmers tended to criticize conventional medicine and resort to alternative medicine for minor health problems. They accused their GPs of being inattentive to their demands, thereby focusing on a single part of the body, rather than employing a holistic approach, and of systematically prescribing medications. However, they did not hesitate to consult a conventional doctor if they felt they were affected by a severe disease, just as they would resort to a veterinarian for their livestock instead of a home remedy or an alternative treatment if deemed necessary. Some farmers had acquired personal knowledge about health problems (that may be misguided, in the case of Lyme disease, for example) and chose to employ conventional or alternative medicine according to their personal conception of the disease and its severity.

Overall, 14.3% had abstained from consulting a doctor over the last 12 months and 19.6% a dentist (Table 3). This was accounted for in part by financial constraints and the fear of health care or health care workers cited in the qualitative study. Although the vast majority of farmers had a good complementary insurance plan, financial issues remained a crucial reason why farmers with limited resources abstained from accessing health care. First of all, in many cases, patients were required to pay the doctor in advance before subsequently being reimbursed by their insurance and this proved unaffordable for some ("I don't have 20 Euros to spend"). Secondly, farmers were at times deterred by transport costs necessary to be able reach a specialist ("I have to watch my spending when filling up on gas to go to hospital, because money is tight. It takes me an hour to drive there and so I sometimes choose to live with my pain, since even if the consultation itself were free, I couldn't afford the gas bill."). Another reason farmers abstained from accessing health care was the fear of health care professionals (especially dentists), of the care itself (fear of diagnosis, pain, or treatment failure): "[…] I know they are health care professionals […], they know their job, but I'm scared". Lastly, many active farmers claimed they forewent care due to time constraints. This response appeared to be the more socially acceptable for active farmers, but might have concealed more complex situations in which financial reasons, transportation issues, fear and negligence were often intertwined. Their alleged lack of time might have also served as a pretext in order to mask the higher priority attributed by active farmers attributed to their work than to their own health ("Livestock is treated better than humans—that goes without saying."). This

frequent behavior was accounted for by how highly farming work was valued compared to the relatively marginalized role of farmers' personal lives.

TABLE 3
FARMERS' RELATIONSHIP WITH THE HEALTH CARE SYSTEM AND THEIR ABSTENTION TO CONSULT A HEALTH CARE PROFESSIONAL

Variables	N (%)
Have a general practitioner (GP)	104 (92.9%)
Trust their doctors (fully/very much)	95 (84.8%)
Information from doctors is difficult to understand (absolutely/very much so)	48 (42.9%)
GP's recommendations are difficult to adhere to (absolutely/very much so)	84 (75.0%)
Complementary health insurance	
- Private complementary insurance	104 (92.9%)
- CMU-C (French public complementary insurance)	6 (5.4%)
- No complementary insurance	2 (1.8%)
Health care access	
At least one consultation over the past 2 years	
- GP	101 (90.2%)
- Ophthalmologist	49 (43.8%)
- Other specialist	40 (35.7%)
- Gynecologist (for women, n=28)	17 (60.7%)
- Dentist	73 (65.2%)
- Alternative medicine (acupuncture, osteopath, etc.)	45 (40.5%)
- Marginal practitioner	19 (17.0%)
Screening	
- Colorectal cancer ≤2 years (persons between 50-74 years old, **n=58**)	10 (17.2%)
- Cervical cancer ≤3 years (only for women 25-65 years old, **n=27**)	19 (70.4%)
- Breast cancer ≤2 years (only for women 50-74 years old, **n=19**)	12 (63.2%)
Abstention to consult a health care professional (in the last 12 months)	
- Doctor	16 (14.3%)
- Dentist	22 (19.6%)
- Both doctor and dentist	4 (3.6%)
Reason for abstention to consult doctor/dentist (n=34)	
- Lack of time	22 (64.7%)
- Fear of doctor/dentist	10 (29.4%)
- Waiting period	6 (17.6%)
- Distance/no doctor nearby	4 (11.8%)
- Negligence or no desire	3 (8.8%)
- Financial constraints	3 (8.8%)
- Deliberate postponement	3 (8.8%)
- No trust in practitioner	3 (8.8%)
- Negative experience with health care system	2 (5.9%)
- Too complicated	1 (2.9%)
- Other	2 (5.9%)

TABLE 4
VARIABLES ASSOCIATED WITH MENTAL DISTRESS (GHQ12>=2)

	GHQ12>=2 (n=33)	GHQ12<2 (n=78)	p
Gender			0.747
- Male, n (%)	24 (72.7%)	59 (75.6%)	
- Female, n (%)	9 (27.3%)	19 (24.4%)	
Mean age (years) ± SD	51.2 ± 10.5	47.4 ± 11.1	0.1523
- 95% CI	[47.5 – 54.9]	[44.9 – 49.9]	
Native from the area			0.743
- Yes	28 (84.9%)	68 (87.2%)	
- No	5 (15.2%)	10 (12.8%)	
Home owner	28 (84.9%)	64 (82.1%)	0.721
Lives alone in their housing (& without children)	5 (15.2%)	11 (14.1%)	0.886
Family situation			
- Single	10 (30.3%)	18 (23.1%)	0.423
- Presence of children	8 (24.2%)	30 (38.5%)	0.149
Educational level*			
- Primary- Secondary school	30 (90.9%)	67 (85.9%)	0.549
- University	3 (9.1%)	11 (14.1%)	
Finance ressources			
Income			
- <1000 euros, n (%)	9 (29.0%)	10 (13.7%)	0.175
- 1000—2000 euros, n (%)	16 (51.6%)	44 (60.3%)	
- >2000 euros, n (%)	6 (19.4%)	19 (26.0%)	
- **Feel financially unsecure**[2]	16 (48.5%)	36 (46.2%)	0.822
- **Difficulties to face needs**	13 (40.6%)	22 (28.2%)	0.204
EPICES score (n=110,108)			0.171
< 30.17 (no precarious)	19 (59.4%)	56 (72.7%)	
>= 30.17 (precarious)	13 (40.6%)	21 (27.3%)	
Work every days (7/7)	27 (81.8%)	47 (61.0%)	0.033
Holidays (mean number of days)	1.6 (± 2.8) [0.7-2.6]	3.9 (±5.3) [2.7 – 5.1]	0.0425
Use Replacement service	11 (33.3%)	25 (32.1%)	0.895
Health – Have a GP	28 (84.9%)	75 (96.2%)	0.049
Chronic disease	16 (48.5%)	28 (35.9%)	0.215
Depressive syndrome	3 (9.1%)	1 (1.3%)	0.078
Health feeling			
- Excellent/Very good/ Good	22 (66.7%)	73 (93.6%)	0.000
- Bad/Mediocre	11 (33.3%)	5 (6.4%)	
Health care renouncement (q53) (doctor)	10 (30.3%)	6 (7.7%)	0.002
Smoking			
- Yes	9 (27.3%)	19 (24.4%)	0.747
Alcohol			
- Non-drinker (Never/Once a month or less)	16 (48.5%)	17 (21.8%)	0.012
- Occasional (2 to 4 times a month)	5 (15.2%)	30 (38.5%)	
- Reasonable (2 to 6 times a week, every day & < 3 glasses/day)	9 (27.3%)	27 (34.6%)	
- Excessive (every day & >= 3 glasses/day)	3 (9.1%)	4 (5.1%)	
Psychotropic consumption	6 (18.2%)	9 (11.5%)	0.349
Social relations			
- Feel isolated or rather isolated	19 (57.6%)	10 (12.8%)	0.000
Work feeling			
- Find their work nervously tiring	30 (90.9%)	53 (68.0%)	0.015
- Find their work physically tiring	32 (97.0%)	62 (79.5%)	0.020
- Insomnia due to work	30 (90.9%)	53 (68.0%)	0.015
Characteristics of exploitation			
History			
- Familial exploitation	24 (72.7%)	59 (75.6%)	0.747
Size			
- Mean area cultivated (hectares)	25.3 ± 27.1	29.6 ± 33.9	0.4177
Status	[15.1-35.4]	[21.4-37.7]	
- Individually exploitation	19 (57.6%)	32 (41.0%)	0.110
- Other (GAEC, EARL)	14 (42.4%)	46 (59.0%)	

IV. DISCUSSION

Data remains scarce regarding the health of French farmers. We thus studied a representative sample of farmers, most of which working for the meat-processing industry. This category of farmers is known to exhibit an elevated suicide rate. Whereas most farmers claimed to have few health problems, we found that 29.7% of farmers interviewed were affected by psychological distress. This population was mainly composed of single farmers subject to social deprivation and who had inherited their farms. This qualitative study casts light on the causes of this mental affliction, which have been summarized in Figure 1. Furthermore, better understanding of such suicide cases may contribute to their prevention.

As in previous studies, we observed participants' general physical health to be good in 84.5% of cases, with their physical quality of life measured based on a Physical composite score of SF12 (PCS>40). That said, whilst farmers tended to view their way of life as healthy and felt they enjoy good disease resistance, their personal conception of their own health may diverge from reality given that many are being treated for a chronic disease, whilst smoking and obesity were observed more frequently in farmers <50 years of age than in the general population(34,35). Moreover, a lower percentage of farmers underwent screening for cancer than of the general population. Finally, subjects' oral-dental health was determined to be mediocre, based on the number of missing and unreplaced teeth. Farmers' relationship with the health care system may differ from that of urban residents. Even if they were shown to trust their doctors and get good health insurance, it also became clear that they tend to diagnose themselves based on personal knowledge when they consider a disease to be benign or when the feel the GP is unable to make the proper diagnosis, or prescribe the right treatment. Overall 14.3% of farmers admitted they had foregone consulting their GP and dentist for reasons that are typically described in rural areas (mainly lack of time, fear, excessive waiting periods, distance to medical facilities, and financial constraints), but also because they often prioritize their work over their own health. Abstention to access health care occurred more frequently in the event of dental care (19.6%).

In contrast with physical health, 29.7% of farmers admitted to experiencing psychological distress, which may result from several interrelated factors. Firstly, farmers must generally adhere to a schedule: They are required to adapt their work to the seasons and local weather conditions, as has historically been the case, but modern French farmers face the additional hurdle of administrative work in order to obtain subsidies from the European Union that are crucial for their subsistence. "Paperwork" must be completed according to strict deadlines, thereby hindering farmers in performing their job. Such bureaucratic tasks do not fall within the traditional scope of farmers' duties (information required is often described as inappropriate and perceived as a waste of time), and they equally symbolize farmers' failure to earn their living without relying upon subsidies. Finally, a sort of unspoken rule in gauging the quality of farmers' work consists in their ability to successfully match the progress of other local farmers. Each individual farmer perceives an obligation to keep in step with other farmers' progress and is thereby subjected to judgement by their farming community. Keeping "on schedule" constitutes a crucial sociological marker, and farmers who are "behind with their work" may be socially blacklisted by the farming community and, at the same time, tend to isolate themselves from the others. All these factors pertaining to time management exert continual pressure on farmers, which contributes to mental distress. A further detrimental effect upon farmers' mental health is their negative perception of their profession, combined with the overwhelming importance of work and marginalized role of private life. Financial difficulties, increased administrative work, as well as reliance upon subsidies have diminished the pride farmers once took in their job. This is exacerbated by the burnout experienced by many farmers, who practice a full-time profession that appears ineffective at overcoming its associated financial and administrative difficulties. Given that private and professional life is inextricably interwoven, the deterioration of the profession's image is damaging to farmers' personal self-image, coinciding with a loss of self-assurance and self-esteem. Associated with anxiety, this condition may contribute to depression, which may explain, at least to some extent, the increased suicide rate observed in this population.

Suicide prevention in farmers remains a challenge. In France, livestock farmers, who have an increased mortality rate due to suicide, may constitute a target population(7). However, efforts must still be made to identify farmers at risk of suicide in the context of the extensive technical, economical, and anthropological shifts that have affected the whole agricultural world since the Second World War(36,37). The second challenge is to implement a network designed to assist farmers at risk of suicide in a socially acceptable way for farmers. This network may consist of a group of trained farmers, able to detect and get in touch with farmers at risk(38). In addition, psychological distress (and not only depression) should be taken more into account(25), which implies expanding rural health services(39). Availability of GPs in rural areas is crucial in order to decrease the delay between requests and consultations and to increase the duration of consultations, thus facilitating physician-patient dialog about mental health(40). While some patients may require the assistance of a psychologist, it must

also be mentioned that at present psychologists are scarce in rural areas, and their costs are not reimbursed by French social security. Other potential measures would consist of alleviating mental distress by promoting a new approach to work time management with more leisure time, expanding services to provide replacement workers, in addition to volunteer assistance exchanges such as the WWOOF (World Wide Opportunities on Organics Farms) system.

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REFERENCES

[1] [cited 2016 Feb 21]. Available from: http://donnees.banquemondiale.org/indicateur/SP.RUR.TOTL.ZS

[2] Laisney C. Les agriculteurs dans la société française. Ministère L'Agriculture Cent D'études Prospect. 2010;(14).

[3] Deléage E. Agricultures à l'épreuve de la modernisation. Versailles Quae; 2013. 95 p. (Synthèses).

[4] Jacques-Jouvenot D, Laplante J-J. Les maux de la terre : Regards croisés sur la santé au travail en agriculture. La Tour d'Aigues: Editions de l'Aube; 2009. 215 p.

[5] Nicourt C. Être agriculteur aujourd'hui : l'individualisation du travail des agriculteurs. Versailles Quae; 2013. 287 p. (Nature et société).

[6] Levêque-Morlais N, Tual S, Clin B, Adjemian A, Baldi I, Lebailly P. The AGRIculture and CANcer (AGRICAN) cohort study: enrollment and causes of death for the 2005–2009 period. Int Arch Occup Environ Health. 2015;88(1):61–73.

[7] Bossard C, Santin C, Guseva Canu I. Surveillance de la mortalité par suicide des agriculteurs exploitants [Internet]. 2013 [cited 2016 Jan 22]. Available from: http://www.invs.sante.fr/Publications-et-outils/Rapports-et-syntheses/Travail-et-sante/2013/Surveillance-de-la-mortalite-par-suicide-des-agriculteurs-exploitants

[8] Malmberg A, Hawton K, Simkin S. A study of suicide in farmers in England and Wales. J Psychosom Res. 1997 Jul;43(1):107–11.

[9] Simkin S, Hawton K, Fagg J, Malmberg A. Stress in farmers: a survey of farmers in England and Wales. Occup Environ Med. 1998 Nov;55(11):729–34.

[10] Hawton K, Fagg J, Simkin S, Harriss L, Malmberg A. Methods used for suicide by farmers in England and Wales. The contribution of availability and its relevance to prevention. Br J Psychiatry J Ment Sci. 1998 Oct;173:320–4.

[11] Malmberg A, Simkin S, Hawton K. Suicide in farmers. Br J Psychiatry. 1999 Aug 1;175(2):103–5.

[12] Hawton K, Fagg J, Simkin S, Harriss L, Malmberg A, Smith D. The geographical distribution of suicides in farmers in England and Wales. Soc Psychiatry Psychiatr Epidemiol. 1999 Apr;34(3):122–7.

[13] Simkin S, Hawton K, Yip PSF, Yam CHK. Seasonality in suicide: a study of farming suicides in England and Wales. Crisis. 2003;24(3):93–7.

[14] Deary IJ, Willock J, Mcgregor M. Stress in Farming. Stress Med. 1997 avril;13(2):131–6.

[15] Booth N, Briscoe M, Powell R. Suicide in the farming community: methods used and contact with health services. Occup Environ Med. 2000 Sep;57(9):642–4.

[16] Booth NJ, Lloyd K. Stress in Farmers. Int J Soc Psychiatry. 2000 Mar 1;46(1):67–73.

[17] Hounsome B, Edwards RT, Hounsome N, Edwards-Jones G. Psychological Morbidity of Farmers and Non-farming Population: Results from a UK Survey. Community Ment Health J. 2011 May 10;48(4):503–10.

[18] Fraser CE, Smith KB, Judd F, Humphreys JS, Fragar LJ, Henderson A. Farming and Mental Health Problems and Mental Illness. Int J Soc Psychiatry. 2005 Dec 1;51(4):340–9.

[19] Judd F, Jackson H, Fraser C, Murray G, Robins G, Komiti A. Understanding suicide in Australian farmers. Soc Psychiatry Psychiatr Epidemiol. 2006 Jan;41(1):1–10.

[20] Alston M. Rural male suicide in Australia. Soc Sci Med. 2012 février;74(4):515–22.

[21] Bryant L, Garnham B. Beyond discourses of drought: The micro-politics of the wine industry and farmer distress. J Rural Stud. 2013;32:1–9.

[22] Bryant L, Garnham B. The fallen hero: masculinity, shame and farmer suicide in Australia. Gend Place Cult. 2015 Jan 2;22(1):67–82.

[23] Yvan Droz, Miéville-Ott V, Jacques-Jouvenot D, Lafleur G. Malaise en agriculture. Une approche interdisciplinaire des politiques agricoles France-Québec-Suisse. Paris Karthala; 2014. 194 p.

[24] Jacques-Jouvenot D. Une hypothèse inattendue à propos du suicide des éleveurs : leur rapport aux savoirs professionnels. Etudes Rural. 2014 Jul 23;193(1):45–60.

[25] Deffontaines N. La souffrance sociale chez les agriculteurs. Etudes Rural. 2014 Jul 23;193(1):13–24.

[26] Célérier S. La belle vie désespérée des agriculteurs. Etudes Rural. 2014 Jul 23;193(1):25–44.

[27] Spoljar P. Problématique suicidaire en agriculture : une difficile évaluation. Perspect Interdiscip Sur Trav Santé [Internet]. 2014 Jan 17 [cited 2015 Nov 27];(16-3). Available from: http://pistes.revues.org/3650

[28] Prévitali C. Les conditions du suicide des professionnels agricoles. Pensée Plurielle. 2015 Jun 12;n° 38(1):105–21.

[29] Labbe E, Blanquet M, Gerbaud L, Poirier G, Sass C, Vendittelli F, et al. A new reliable index to measure individual deprivation: the EPICES score. Eur J Public Health. 2015 Aug 1;25(4):604–9.

[30] Ware J, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care. 1996 Mar;34(3):220–33.

[31] Abdi H, Williams LJ, Valentin D. Multiple factor analysis: principal component analysis for multitable and multiblock data sets. Wiley Interdiscip Rev Comput Stat. 2013 Mar;5(2):149–79.

[32] Janzen JM, Arkinstall W. The Quest for Therapy in Lower Zaire. University of California Press; 1978. 308 p.

[33] Augé M. L'Anthropologie de la maladie. L'Homme. 1986;26(97/98):81–90.

[34] Beck F, Guignard R, Richard JB, Wilquin J, Peretti-Watel P. Augmentation récente du tabagisme en France: principaux résultats du Baromètre santé, France, 2010. Bull Épidémiologique Hebd InVS. 2011;(20-21):230–3.

[35] OECD. Obesity and the Economics of Prevention. Available from: /content/book/9789264084865-en

[36] Belyea MJ, Lobao LM. Psychosocial Consequences of Agricultural Transformation: The Farm Crisis and Depression. Rural Sociol. 1990 Mar 1;55(1):58–75.

[37] Salmona M. Souffrances et résistances des paysans français: Violences des politiques publiques de modernisation économique et culturelle. Paris L'Harmattan; 1994. 254 p.

[38] Fuller JD, Kelly B, Law S, Pollard G, Fragar L. Service network analysis for agricultural mental health. BMC Health Serv Res. 2009 May 29;9:87.

[39] Isaac M, Elias B, Katz LY, Belik S-L, Deane FP, Enns MW, et al. Gatekeeper training as a preventative intervention for suicide: a systematic review. Can J Psychiatry Rev Can Psychiatr. 2009 Apr;54(4):260–8.

[40] Katerina Kavalidou SM. Farmers' contact with health care services prior to suicide: Evidence for the role of general practitioners as an intervention point. Aust J Prim Health. 2013.

LGBTQ: The Molecular Mechanism and its Role in Elucidating Proportional for a Better Management

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Abstract—

Background: *LGBTQ is prone high prevalence and has economic, social business grow in wet and warm countries. It becomes political issue Worldwide (Time magazine, June 12, 2017, page 7) in immigration and also president election campaign in industrial countries in the last decades.*

Problem: *Current effort from day care psychosocial early predictive and training, better therapy, law in sentence, marriage and immigration/ refugee has become topic of controversies feel not ended. From psychiatry social, transgender operation and hormonal have been the day fact, but not one make it clear from the molecular aspect as the cause, and what to do in prevention, promotion for keeping away from methylation and demethylation affect. This study digs the reference about CGG repeat in large to small DNMT off.*

Objective: *CGG repeat as the molecular aspect of methylation and demethylation beyond health science should build and known by decision and policy maker. Controversial are broad, LGBT problem are hug, increasing but attorney denies that gay people exist in their region (Times magazine, June 12, 2017, page 7).*

Method*: Systematic Review with Bayesian analysis and mathematical model.*

Result: *flowchart and table of 13 ref from CGG repeat in DNA methylation analysis, 2 Meta-analysis. Small/ grey zone CGG repeat associated with neuropsychiatric risk.*

Discussion: *Brain and behavior DNMT off functional.*

Comparison: *Law, neuropsychiatric/early social-education vs. ensure no to methylation and demethylation in wet and warm area.*

Outcome*: Winning the combat of LGBT prevalence.*

Conclusion*: Winning the combat of LGBT prevalence not with early psycho-social activity, but with molecular psycho forensic and that is through what cause small/ grey zone CGG repeat prevalence.*

Keywords—psycho-social, psycho forensic, biophysics, grey zone. CGG repeat, brain and behavior of LGBT.

I. INTRODUCTION

Molecular aspect approach policy on LGBT high prevalence have never been bring in fighting/ combat it. Behavior problem large to Small CGG repeat LGBT law/psycho-social/MGMT has been reported due to DNMT off gene, DNA Methyl Transferase in brain functional. Street fashion carnival business, club, and cabaret vs. grey zone and premutation CGG repeat that off DNMT and make functional brain and behavior problem has become the channeling of many district governments. Health science intense the early detection in day cares and teaches and train psycho-social activities as prevention to LGBT behavior. Nowadays, Social category label (SCL) and future direction are topics in top executive government authoritarianism.[1]Brain and behavior gene in mood disorders and in promoter gene in methylation has been reported.[2]Psychological status of premutation showing a complex with the size of small CGG repeat (50-141) which associated with methylation on DNMT.[3]Premutation (55-200 CGG repeat) on FMR1 gene and mRNA level reported higher level in obsessive-compulsive symptom and RNA toxic gain-of-function model in a neuropsychiatric phenotype.[4]

1.1 Background

At least, 2 professors from Top Farmer Institute in wet and warm country ask The Constitutional court that LGBT behavior without thinking of their ages, should be sentence maximum 5 years without asking the age, also for 10 years old boys and girls.[5]While sex party with men striptease caught 149 'men' in wet and warm conservative countries on 21/5/2017 in Kelapa Gading,[6]Orlando nightclub shooting on June 12 2016 with 49 dead are shocking peoples local and global, Jember Fashion Carnival as street Festival every years are think as a good cultural valve with given satisfied feeling to the district municipal government, while the parents are proud and doesn't know about the psychology label next.[7]In world level, Putin and Macron dispute about Gay right in Chechnya.[8]-Time Magazine- June 12, 2017 p7-, the Chechnya authorities proclaimed, there is no LGBT cases in their country. Then The Telegraph writes that Angela Merkel urges Vladimir Putin to protect Chechnya gay rights.[9]

1.2 Problem

The issue about LGBTQ has been sharply increased in these days including in Indonesia. Vitriolic critics against LGBTQ keep ongoing as it is thought as morally unacceptable behavior by the community. People considered it as a social crime leading to discrimination in many aspects for the LGBTQ peoples. Government Concern in street fashion carnival, club, cabaret in many wet and warm countries and the state in one hand function as open the valve in the cultural best way, and the other hand neglected grey zone small CGG which off DNMT gene aspect, so that misleading the cause of LGBT and losing one's way the change to eradicate what caused of small CGG repeat in plant and human in tropical rainforest wet and warm climate condition. Meanwhile, predispose premutation to FXTAS (Fragile X Tremor Ataxia Syndrome) are associated in the CGG repeat.[10]

1.3 Objective

To socialized this molecular aspects etiology of LGBTQ as a part of LGBTQ management. Aware of micromolecule in LGBT mental: small amount of CGG repeat-LGBT should be done broadly beyond health department not only by executive, legislative, but also judicative decision maker and policy doer.

II. METHOD

Systematic review and Bayesian network analysis with mathematical model. Meta-analysis, cohort and DNA/ RNA analysis epidemiological are preferable than longitudinal, case control, distributional. EBSCO host, PubMed, DynaMed: Evidence – Based; point-of-Care reference search engine are used for scanning title and abstract, then filtered by duplication and not relevant to CGG repeat large to small amount in association with brain and behavior functional. Key words of CGG repeat and psychiatric and psychological disorder are used in searching using Bayesian network analysis synonym. Mathematical model is used in determining quantitative and qualitative result of the reported references.

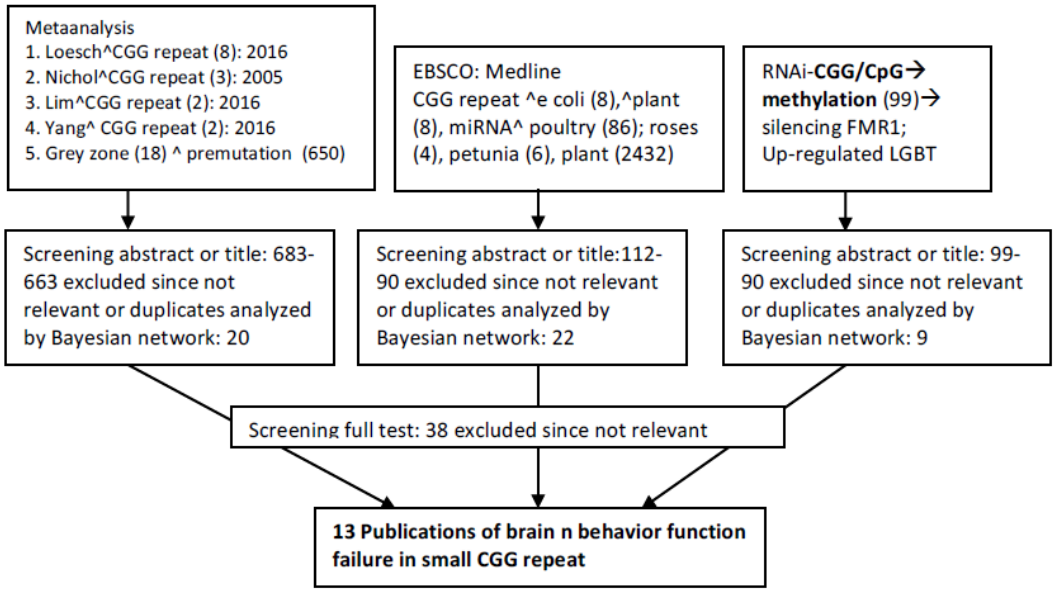

FIGURE: FLOWCHART OF THE 13 IDENTIFIED LITERATURES ON THE ASSOCIATION OF SMALL CGG REPEAT AND LGBTQ MENTAL

Flowchart is build by PRISMA. Three groups of meta-analysis on authors and epidemiological writing are first scanned, then association between full, premutation and grey zone with psychological disorder, and thirdly small CGG repeat in grey zone and under and DNMT off, are used for searching in scanning all reference. Filtering the reference excluded and check and recheck with Bayesian network analysis and reference already known, some reference is printed out for the full text references. According to the objective to build association in CGG repeat and brain n behavior psychiatric function, also CGG repeat in stress and adaptation in plant, and about grey zone CGG repeat, all general knowledge is drop and specific references that support the objective are taken and write in the table.

III. RESULT

It is suggested that repeated CGG sequences plays a role in the pathogenesis mechanism of LGBTQ which is in parallel with CGG repeat expansion on Xq27.3 gene in Fragile X Syndrome manifested in both mental and physical disturbances.[11]The existence molecular explanation of the underlying mechanism of LGBTQ is expected to be used appropriately as a direction to generate fair regulation to manage LGBTQ in the community, thus resulting into mutually beneficial outcome for all parties.

TABLE 1
THIRTEEN PUBLICATIONSABOUT SMALL CGG REPEAT TO BRAIN N BEHAVIOR FUNCTION FAILURE REPEAT

Study, y	Design	Population	CGG repeat	Methylation/ Demethylation
Hansen 1992	CT Methylation Analysis	CpG island of the human FMR1 gene FRAXA and cariers	CGG sites	Hypomethylation analysis
Nichol 2002	CT DNA sequences	CpG methylation bacterial and primate cell system	Tandem repeat	Methylation stabilized
Nichol 2005	CT DNA methylation	Deletion hotspot region of FMR1	CGG repeat	DNA Hypermethylation Silencing/off tumor suppressor
Nichol 2005	CT	Primate cell with SV40 primate replication system	Absence of aberrant CpG methylation	CGG Repeat deletion
Hessl 2005	Recent documented CGG repeat size	Premutation alleles (55-200 CGG repeat) of FMR1 gene and abnormal elevation of FMR1 mRNA	Gene-brain-behavior mechanism	Tremor/ataxia, psychiatric disorders in children and adults
Grigsby 2006	Preliminary study	50-200 CGG repeat size Executive cognitive Functioning in FXTAS	CGG repeat in FMR1 gene	Marked impairment of executive cognitive abilities.
Jacquemont 2006	Meta-analysis genetic screen	Genetic screens CGG premutation	CGG repeat	Suggesting genetic testing
Naumann 2014	Documented Hypermethylation	65-70 CpGs upstream FMR1	CGG repeat premutation carriers	Promotor is inactivated by methylation spreading
Deng 2015	975 OF GENOME IN HUMANS, IS NONCODING DNA	miRNA-induced RNAi by utilizing miRSC in Caenorhabiditiselegans as native RNA fragments	Using intronicmiRNA (single-stranded noncoding RNAs	Gene silencing in vitro and in vivo
Yang 2016	SR and MA	5-55 repeat numbers: <26 vs. 26-34vs.35-54	CGG repeat polymorphism	Neuropsychiatric risk as a routine test
Loesch 2012	Relation	FXS, a severe neurodevelopmental anomaly	Grey zone, premutation	Neurodevelopmental, cognitive, behavioral and physical changes
Chandrasekara 2015	Screening	DNA extracted from buccal cells: CGG repeat of FMR1 gene in special education children in Sri Lanka	Grey zone	Learning, emotional, behavior problems
Usdin 2014	Review	Human different cells Epigenetic changes	Number of CGG repeat	Ameliorating Neurodegenerative disorder

The correlation of CpG islands and CGG methylation with silencing of the FMR1 genes.[12,13,14,15] Widely known 200-4000 CGG repeat in full mutation in FMR1 gene give Fragile X Syndrome. CGG repeat size 55-200 as premutation is associated with psychiatric disorder,[4] impairment of cognitive abilities,[16]and suggesting in genetic testing.[17]In 65-70CpG upstream CGG repeat in FMR1 gene become stable DNA methylation boundaries and expanded CGG repeat.[11]These promoter is inactivated by methylation spreading CGG repeat premutation carriers. Methylation boundaries exert an important function as promoter safeguards. Expansion and hyper-methylation of a CGG repeat tract are the main causes of fragile X syndrome (FRAXA).[14]Hypermethylationof CGG repeat are part of the CpG Island that is methylated. Small CGG expansions are unmethylated in the CpG island in normal males, unaffected fragile-X males.[18]Learning, emotional and behavior could be found in buccal cell grey zone 5-25 CGG repeat number and suggested as routine test.[19, 20, 21]These grey zone and premutation related with Neuro developmental, cognitive, behavioral and physical changes was reported.[22]Epigenetic changes is also cover what is currently known about the mechanisms responsible for repeat instability that affect expression of the FMR1 gene should also ameliorating this molecular aspect of small CGG repeat.[23]

Thirteen references about CGG repeat in full, premutation, grey zone and below 55 and CGG repeat in human could be seen from the table 1.

IV. DISCUSSION

4.1 Introduction CGG repeat in full- premutation-grey zone in association with Brain and Behavior

CGG repeat in association with methylation and demethylation, in DNMT gene affect brain and behavior function. Expansion and shorten CGG repeat to small amount CGG repeat has DNMT function failure, normally are 26-34.[24]

4.2 CGG repeat problem in LGBT mental is in high prevalence in Wet and Warm countries

LGBT are in High prevalence in Tropical Rainforest Area, South East Asia, Puerto Rico USA, Sweden Europa, Brazil, Thailand CGG. CGG repeat are higher in Thailand vs. Germany 2014, low prevalence parallel with Non GMO eater. Epidemiologies of LGBT are also high in other wet and a warm country such as Sierra Leone, West Africa, USA pop 25% has LGBT Gallup.com: Puerto Rico and Orlando. LGBT and Transexual operation are high in Bangkok

In Indonesia: LGBT and fashion open air is also neglected: Jember Fashion Carnival 2017 are now as the icon of #wonderful Indonesia. thejacartapost.com August 14, 2017: 12.06 pm; Transgender right in Brazil are high with Gender identity legal since 2009. An LGBT person is murdered every 28 hours in Brazil, with intake of Aflatoxin, p53-methylation are in high prevalence. Micro rainbow in Brazil is a project in development the talent of LGBT crisis.

4.3 CGG repeat in plant in association with DNMT off in CGG repeat

Gene silencing in vitro and in vivo using methylation for gene silencing and demethylation with miRNA etc. are broadly used for a long time in plant and animal.[24, 25, 26, 27, 28, 29]Expanding CGG repeat with hypermethylation from small CGG repeat (e.g. 5-54) become higher CGG repeat (56-4000) and hypomethylation from normal (26-34) degraded to < 26 (5-25 CGG repeat).[21] Small CGG repeat should support LGBT law/psycho-social/MGMT has not previously been reported. Controversial are broad, LGBT problem are huge, increasing but attorney denies that gay people exist in their region (Times magazine, June 12, 2017, page 7)

Interference: Aware of molecular aspect in LGBT mental: amount of CGG repeat-LGBT will support. RNA that is an antisense repressor of the replication of some E coli, plasmid. FX Syndrome is caused by silencing of FMR1 gene, Martin-Bell-Renpenning Syndrome in Brazil are similar with Escalante's syndrome used in South American countries, but this practice is now being abandoned. Exposure of miRNA could methylated or demethylated and lengthen or smaller Repeat CGG.[30]Methylation control reversible epigenetic mechanism[31] in roses, poultry, e coli have the same mechanism as Methylation in FMR1

4.4 They Need for help, not sentence and how could it be

Religion Ministry of Indonesia announce that LGBT should be protected in a true way, but there is consistent not to support the LGBT, not that meaning to be support, to be discredited by religion, but should be hug so that the behavior and sexual orientation should be following what should be donecommensurate of what have teaching by the religions. Consistent not to straighten out these growing up population, LGBT, not to imply to isolate but should be embraced so that the behavior and

sexual orientation as which said in the holly books. One large global franchise such as Starbuck pro LGBT, they said LGBT should be help LGBT needs help http://tv.detik.com/20detik/detailvideo/170703025/

Fighting LGBT which prone to pandemic has support pathogenesis mechanism in biophysics aspect: small CGG repeat in epigenetic in enzyme functional beyond health sciences (plant). Wet and warm GME in association with biophysics approach, supported by Bayesian analysis and mathematical model, has revealed this pathogenesis mechanism. Brain and behavior DNMT off functional, through passed up regulated DNMT in epigenetic toxicity in demethylation lower than 6 CGG repeat should be performed as toxic 'gene-of-function'.

Winning the combat of LGBT prevalence not with early psycho-social education, but with psycho forensic and that is through what cause small/ grey zone CGG repeat prevalence. These small molecule brain and behavior support could combat LGBT and HIV comorbidities where is nowadays rise to the surface that religion and culture Indonesia local wisdom to global in hugging the LGBTQ will undermine the Fight against HIV. Repeat CGG in small amount will combat the Mortality and Comorbidities and disabilities in brain, behavior and weaken memories (Mental Retarded). Repeat CGG, methylation CpG island in Brazil; outcome Dx& Rx in LGBT pts.; in Bangkok were cause by the using of RNAi and CGG repeat in plant and animal. MiRNA induce RNAi (interference RNA) and demethylation/ hypomethylation is a new target therapy on the silencing complex (mRISC) to target miRNAs.[29,32,33,34,35] The race of 10 synthetic RNAi Based drugs to the pharmaceutical market and attention of pharmaceutical industry rapidly turned to RNAi, as an opportunity to explore new drug targets.[30]Off (methylation) the gene and on (demethylation) are the principle of transgenic MGO that these last decades succeed astonishing with Double KO, Turbo KO roses and many other plants[24,36,37]and human[38,39]Plant that resist from cold, hot, wet, dry condition and climate together with lasting and beautiful has been lured all of us without precise knowing what have done to it in these last 2 decades.

V. LIMITATION

Various limitation of this Systematic Review is recorded: 1) none published activity to defend food crisis, energy crisis and global warming to be the burden of tropical rainforest area. 2) Methylation and demethylationas an updated business should be control in sustainable global energy and move to sustainable 'hara' means NPK from plant specific rich in wet and warm countries. In local area specific without double and turbo, which the superior gene were made in rich sustainable 'hara' area. So, we build our one world proportionally to the demand and local needs, which means not tropical rainforest area wet and warm climate condition for all. Tropical rainforest sustainable 'hara' just for all specific condition, ensuring no'demethylation through pass' as the cause of LGBT high prevalence. We managed these problem by underline 'demethylation through pass' and methylation in prevention, therapy and socialization of LGBTQ.

VI. CONCLUSION

The relationship between CGG sequences repeat and LGBTQ will replace discrimination and resulting into appropriate management of LGBTQ which will be elucidating proportionally.

VII. SUGGESTION

Awareness to policy maker and decision doer in small CGG repeat.

Government Concern needs this molecular aspect from local Wet and Warm countries present to Global Health Problem.

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DISCLOSURE

Nothing to declare.

REFERENCES

[1] Crawford JT, Brandt MJ, Inbar Y, Mallinas SR. Right-wing authoritarianism predicts prejudice equally toward "gay men and lesbians" and "homosexuals". J PersSocPsychol 2016;111(2):e31–45.

[2] Dell'Osso B, D'Addario C, Carlotta Palazzo M, Benatti B, Camuri G, Galimberti D, et al. Epigenetic modulation of BDNF gene: differences in DNA methylation between unipolar and bipolar patients. J Affect Disord 2014;166:330–3.

[3] Loesch DZ, Bui MQ, Hammersley E, Schneider A, Storey E, Stimpson P, et al. Psychological status in female carriers of premutation FMR1 allele showing a complex relationship with the size of CGG expansion. Clin Genet 2015;87(2):173–8.

[4] Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet Part B, Neuropsychiatr Genet Off PublIntSocPsychiatr Genet 2005 Nov 5;139B(1):115–21.

[5] Guru Besar IPB Dkk Minta LGBT Dipenjara 5 Tahun: KUHP Sudah Tidak Relevan [homepage on the internet]. c2016 [updated 2016 Jul 19; cited 2017 Aug 16]. Available from : http://news.detik.com/berita/3256327/guru-besar-ipb-dkk-minta-lgbt-dipenjara-5-tahun-kuhp-sudah-tidak-relevan

[6] 114 Arrested over Alleged 'Gay Party' in Kelapa Gading [homepage on the internet]. c2017 [updated 2017 May 22; cited 2017 Aug 6]. Available from : http://en.tempo.co/read/news/2017/05/22/057877512/114-arrested-over-alleged-gay-party-in-kelapa-gading.

[7] Susilo Wardhani MD. As stated in WA from Susilo Wardhani, MD August 4, 2017

[8] Rachael Revesz. Emmanuel Macron challenges Vladimir Putin on gay rights in Chechnya and Syria conflict[homepage on the internet]. c2017 [updated 2017 May 30; cited 2017 Aug 6]. Available from: http://www.independent.co.uk/news/world/emmanuel- macron-vladimir-putin-gay-rights-chechnya-russia-france-syria-conflict-civil-war-assad-a7762591.html

[9] Chiara Palazzo. Angela Merkel urges Vladimir Putin to protect Chechnya gay rights. [homepage on the internet]. c2017 [updated 2017 May 3; cited 2017 Aug 6]. Available from : http://www.telegraph.co.uk/news/2017/05/03/angela-merkel-urges-vladimir-putin- protect-chechnya-gay-rights/

[10] Chonchaiya W, Nguyen D V., Au J, Campos L, Berry-Kravis EM, Lohse K, et al. Clinical involvement in daughters of men with fragile X-associated tremor ataxia syndrome. Clin Genet. 2010;78(1):38–46.

[11] Naumann A, Kraus C, Hoogeveen A, Ramirez CM, Doerfler W. Stable DNA methylation boundaries and expanded trinucleotide repeats: role of DNA insertions. J MolBiol 2014;426(14):2554–66.

[12] Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD. Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. Hum Mol Genet 1992;1(8):571–8.

[13] Nichol K, Pearson CE. CpG methylation modifies the genetic stability of cloned repeat sequences. Genome Res 2002;12(8):1246–56.

[14] Nichol Edamura K, Pearson CE. DNA methylation and replication: implications for the "deletion hotspot" region of FMR1. Hum Genet 2005;118(2):301–4.

[15] Nichol Edamura K, Leonard MR, Pearson CE. Role of replication and CpG methylation in fragile X syndrome CGG deletions in primate cells. Am J Hum Genet 2005;76(2):302–11.

[16] Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, et al. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). J NeurolSci 2006;248(1–2):227–33.

[17] Jacquemont S, Leehey MA, Hagerman RJ, Beckett LA, Hagerman PJ. Size bias of fragile X premutation alleles in late-onset movement disorders. J Med Genet 2006;43(10):804–9.

[18] Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD. Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. Hum Mol Genet 1992;1(8):571–8.

[19] Chandrasekara CHWMRB, Wijesundera WSS, Perera HN, Chong SS, Rajan-Babu I-S. Cascade Screening for Fragile X Syndrome/CGG Repeat Expansions in Children Attending Special Education in Sri Lanka. PLoS One 2015;10(12):e0145537–e0145537.

[20] Yang W, Fan C, Chen L, Cui Z, Bai Y, Lan F. Pathological Effects of the FMR1 CGG-Repeat Polymorphism (5-55 Repeat Numbers): Systematic Review and Meta-Analysis. Tohoku J Exp Med 2016;239(1):57–66.

[21] Lim GXY, Loo YL, Mundhofir FEP, Cayami FK, Faradz SMH, Rajan-Babu I-S, et al. Validation of a Commercially Available Screening Tool for the Rapid Identification of CGG Trinucleotide Repeat Expansions in FMR1. J Mol Diagnostics JMD 2015;17(3):302–14.

[22] Loesch D, Hagerman R. Unstable mutations in the FMR1 gene and the phenotypes. AdvExp Med Biol 2012;769:78–114.

[23] Usdin K, Hayward BE, Kumari D, Lokanga RA, Sciascia N, Zhao X. Repeat-mediated genetic and epigenetic changes at the FMR1 locus in the Fragile X-related disorders Repeat-mediated genetic and epigenetic changes at the FMR1 locus in the Fragile X-related disorders. 2014;

[24] Xu M, Li X, Korban SS. DNA-methylation alterations and exchanges during in vitro cellular differentiation in rose (Rosa hybrida L.). TheorAppl Genet. 2004;109(5):899–910.

[25] Hardcastle TJ. High-throughput sequencing of cytosine methylation in plant DNA. Plant Methods 2013 Jan [cited 2015 Jul 1];9(1):16.

[26] Dubin MJ, Zhang P, Meng D, Remigereau M-S, Osborne EJ, Paolo Casale F, et al. DNA methylation in Arabidopsis has a genetic basis and shows evidence of local adaptation. Elife 2015;4:e05255–e05255.

[27] Kim SH, Kim SH, Palaniyandi SA, Yang SH, Suh J-W. Expression of potato S-adenosyl-L-methionine synthase (SbSAMS) gene altered developmental characteristics and stress responses in transgenic Arabidopsis plants. Plant PhysiolBiochem PPB 2015;87:84–91.

[28] Ma N, Chen W, Fan T, Tian Y, Zhang S, Zeng D, et al. Low temperature-induced DNA hypermethylation attenuates expression of RhAG, an AGAMOUS homolog, and increases petal number in rose (Rosa hybrida). BMC Plant Biol 2015;15:237.

[29] Deng JH, Deng P, Lin S-L, Ying S-Y. Gene silencing in vitro and in vivo using intronic microRNAs. Methods MolBiol 2015;1218:321–40

[30] Titze-de-Almeida R, David C, Titze-de-Almeida SS. The Race of 10 Synthetic RNAi-Based Drugs to the Pharmaceutical Market. Pharm Res 2017;34(7):1339–63.

[31] Bilichak A, Kovalchuk I. The Combined Bisulfite Restriction Analysis (COBRA) Assay for the Analysis of Locus-Specific Changes in Methylation Patterns. Methods MolBiol 2017;1456:63–71.

[32] Crater AK, Roscoe S, Roberts M, Ananvoranich S. Antisense technologies in the studying of Toxoplasma gondii. J Microbiol Methods 2017;138:93–9.

[33] Kenan-Eichler M, Leshkowitz D, Tal L, Noor E, Melamed-Bessudo C, Feldman M, et al. Wheat hybridization and polyploidization results in deregulation of small RNAs. Genetics 2011;188(2):263–72.

[34] Schilling E, Rehli M. Global, comparative analysis of tissue-specific promoter CpG methylation. Genomics 2007;90(3):314–23.

[35] Shahabipour F, Barati N, Johnston TP, Derosa G, Maffioli P, Sahebkar A. Exosomes: Nanoparticulate tools for RNA interference and drug delivery. J Cell Physiol 2017;232(7):1660–8.

[36] Chen X, Baldermann S, Cao S, Lu Y, Liu C, Hirata H, et al. Developmental patterns of emission of scent compounds and related gene expression in roses of the cultivar Rosa x hybrida cv . "Yves Piaget". Plant PhysiolBiochem PPB / Société Fr PhysiolVégétale 2015;87:109–14.

[37] Ma N, Chen W, Fan T, Tian Y, Zhang S, Zeng D, et al. Low temperature-induced DNA hypermethylation attenuates expression of RhAG, an AGAMOUS homolog, and increases petal number in rose (Rosa hybrida). BMC Plant Biol 2015;15:237.

[38] Low JSW, Tao Q, Ng KM, Goh HK, Shu X-S, Woo WL, et al. A novel isoform of the 8p22 tumor suppressor gene DLC1 suppresses tumor growth and is frequently silenced in multiple common tumors. Oncogene 2011;30(16):1923–35.

[39] Ying J, Srivastava G, Hsieh W-S, Gao Z, Murray P, Liao S-K, et al. The stress-responsive gene GADD45G is a functional tumor suppressor, with its response to environmental stresses frequently disrupted epigenetically in multiple tumors. Clin Cancer Res An Off J Am Assoc Cancer Res 2005;11(18):6442–9.

Analyses of Community Attributes of Meiofauna Under A Pollution Regime in the Lower Bonny Estuary, Rivers State, Nigeria

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Abstract— *Meiofauna community attributes of the lower Bonny estuary was investigated under a pollution regime. This is germane because of the strategic importance of meiofauna in the food chain and the sustenance of any aquatic ecosystem. Five stations representing diverse land based activities such as refuse dump site, fuel depot, fishing-landing areas, residential housing and a station located up-stream (control) were chosen for investigation. The meiofauna samples were collected from sediments in three replicate spots per station per sampling months at low tide. Sediment samples were stirred through (63-212nm) meshes to separate meiofauna and organic debris. Meiofauna samples were processed by first washing Meiofauna through a sieve of fine mesh size made of silk material, in order to wash off formation and excess silt or mud. Meiofauna samples were sorted out and identified using standard keys. Result from field studies indicate that meiofauna population abundance and diversity varied slightly between stations and seasons. There was however, no established trend. Meiofauna were generally more abundant in the rainy season than in the dry season. In general, pollution indicator meiofaunal taxa were more in all stations except the control station; the increasing impact of pollutants in the river led to a rise in the relative abundance of r-strategist genera like Chromadora. We conclude that the application of meiofaunal indices can be a useful tool for assessing the environmental quality of estuarine ecosystems*.

Keywords— Community structure, Pollution, Meiofauna, Bonny Estuary.

I. INTRODUCTION

Meiobenthos, also called meiofauna, are small benthic invertebrates that live in both marine and fresh water environments. They are important in any aquatic ecosystem and serve as a vital link between producer and consumer organisms. These organisms have an important role to play in the ecosystem, in that they are metabolically important members of benthic ecosystem (Gerlach, 1971). They are also known to be prey for variety of larger animals particularly juvenile fishes (Coull et al, 1981). The meiobenthos includes many small copedpods, nematodes, flatworms, the larva stages of various molluses, polychaetes, copedpods and nematodes which are numerically most important.

Despite the important role played by meiofauna in the ecosystem and trophic organization, they also serve as pollution indicators and change detector in the environment (Gerlach, 1971 and Sibent *et al.,* 1997). The ability of meiofauna to serve as pollution indicators is particularly useful in the investigation of pollution prone water bodies. Bonny River given its unique location and diverse human activities is one of such threatened and pollution prone water body.

Bonny River is an important River system east of the Niger. It serves as a major gateway to a number of industrial and commercial activities. Along the shores are located multinational companies that have established oil tank farm, liquefied natural gas, flow stations, several pipelines routes, crude oil farm loading bay, condensation plants, office and living quarters for their staff alongside the indigenous populations. It empties directly into the Atlantic Ocean.

The distribution of meiofauna in any natural or polluted environment is of much significance to scientist. This have provided major challenges to the scientific community due to the fact that information on meiofauna community is sparse despite the important role played in the ecosystem and trophic organization and their use as indicators of pollution and change detector in the environment (Gerlach, 1977 and Sibert *et al.,* 1997). As Bonny River represents a very important and strategic water system, there is an urgent need to gauge its meiofaunal characteristic as to determine its pollution and changing status. This will provide useful information for all stakeholders as to the protection of the ecosystem and to safe-guard its fishery.

II. MATERIALS AND METHODS

2.1 Description of Study Area.

The lower Bonny River is located southeast of the Niger Delta between longitudes 7^0 05' E and $7^0$20' E and latitude 4^0 10' N and 4^0.40' N. The Bonny River system consists of the main river channel of the Bonny River and large number of associated creeks and creeks-lets.

2.2 Description of Sampling Stations

TABLE 1
DESCRIPTION OF SAMPLING STATIONS

Stations	Description
1	Fuel depot
2	House-hold waste refuse dump.
3	Fishing settlement/activities
4	Living quarters/Settlements
5	Up stream of Estuary (Control)

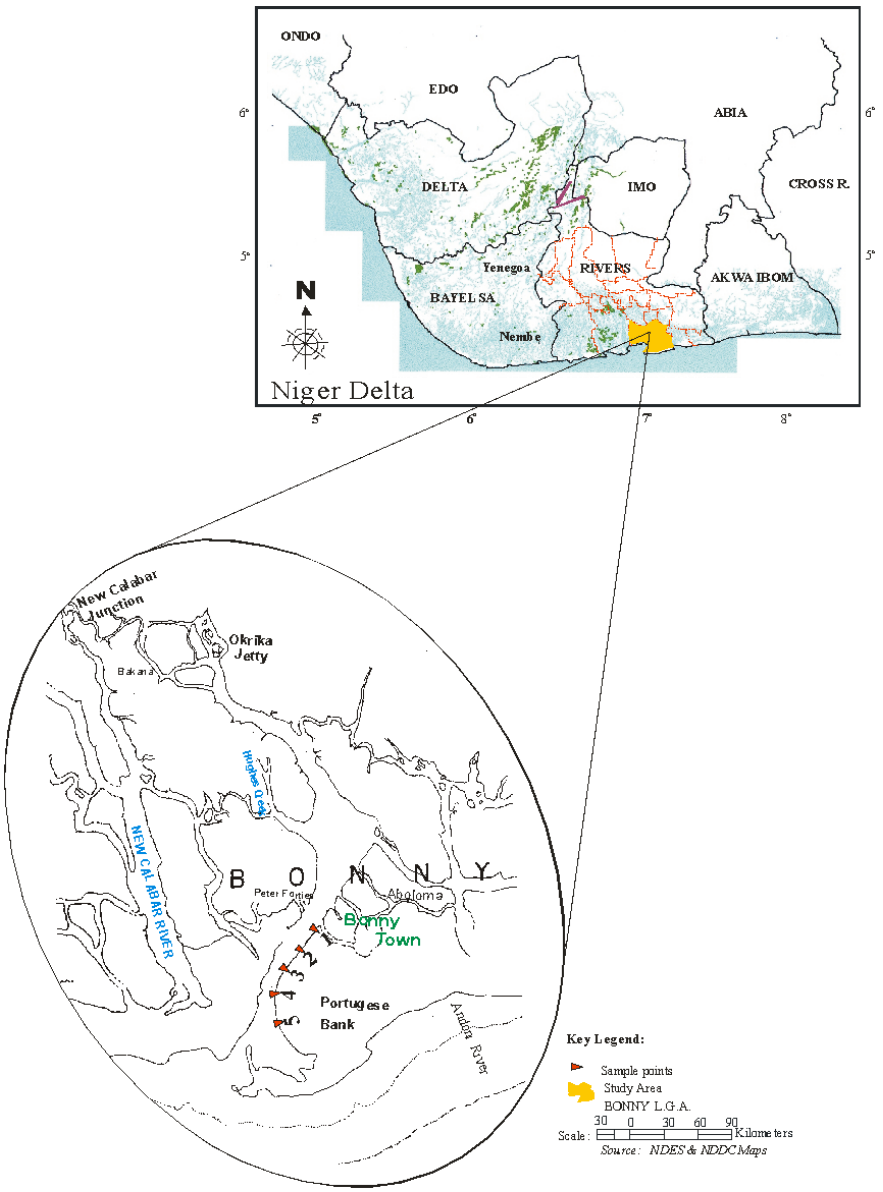

FIG.1: MAP OF NIGER DELTA SHOWING BONNY ESTUARY IN BONNY LGA

2.3 Sample Collection (Sediment/Meiofauna)

Sediment samples for both meiofauna and sediment size analyses were collected at low tide with the aid of soil auger within the littoral zone. Collected samples were wrapped in Aluminum foil and stored in an ice cool chest before transport to the laboratory. This was done in triplicate for each sample station.

Sediment samples for meiofauna analyses were stirred through (63-212nm) meshes to separate meiofauna from organic Debris. Meiofauna samples were processed by first washing Meiofauna through a sieve of fine mesh size made of silk material, in order to wash off excess silt or mud. The sieved samples were contained in vials and fixed with 5% formalin after which rose Bengal was added. This was done to stain the organism red, to facilitate sorting of organisms. The samples were mounted on high powered microscope in the laboratory and identified using suitable keys.

2.4 Data Analyses

Percentage occurrence, species richness, evenness, dominance and relative numerical abundance of meiofauna were calculated using the Excel Descriptive Statistic Tools (EDST).

Diversity index analysis was calculated using the entire identified Meiofauna organism. The number of each individual of the identified groups is replicated during each sampling were summed up by species to obtain the total number of species at each station.

Diversity of the aquatic fauna was determined using the Shannon – Wiener index, equitability (E) of species (Ajao, 1990) and Margalef's diversity as follows:

Species richness by Margalefs(1967) formula:

$$d= (s-1)/In\ n$$

Where

H"= Species diversity; E= Evenness; S= Number of families; n= Total number of animal

ni= Number of each family.

Diversity of the aquatic fauna were determined using Shannon – Weiner index, equitability (E) of species and Margalef's diversity

Shannon-weiner diversity index given by the formula:

$$Hs = \sum_{I=1}^{S} (Ni/N) \log_2 (Ni/N)$$ (Shannon – Weiner, 1963)

Where Hs = Shannon – Weiner information

N = Total number of individuals in the sample

Evenness (E) index shows how evenly the species are distributed in a sample community also called evenness index.

Equitability or Evenness by the formula:

$$E=H"/ Ins$$

III. RESULT (MEIOFAUNA)

3.1 Relative Composition

Meiofauna assemblages collected along the beach of lower bonny estuary were diverse and are represented in their major taxonomic groups (Tables 2 and 3). The results showed that the distribution of meiofauna varied from stations and season.

A total number of 16 species from 6 families of meiofauna were observed during the survey of the estuary. The predominant families were Linhomoeidae (2.25 – 32.14%) of the entire population, followed by Desmodoridae (14.61 – 28.57%) and Leptosomatidae (13.48 – 17.86%).

The leptosomatidae had a range of (2.30 – 32.14) and minimal variation was observed between stations with maximum and minimum values recorded in station 5 in the wet season and in station 2 and 4 for the study period. Seasonally, the wet season had high population composition than the dry period.

The plectidae had a range of (10.34 – 17.86%) with station 2 and 5 having maximum values for the dry period and stations 3 and 5 having maximum population value in wet season.

The population of the Desmodoridae recorded showed that a maximum population was recorded in the dry season in 2 and 5, while the least population was observed in station 3 and 2 for wet season while the population range was observed to be from (14.94 – 28.57%).

3.2 Species Richness

The results of species richness using the Margalef's index (1967) showed that there were seasonal variations. The wet season value ranged from (0.91 – 1.52) and (1.44 – 2.98) for dry season. Therefore Species Richness was higher in the dry season.

3.3 Species Diversity

The results of the species diversity obtained from the different taxonomic groups using Shannon-wiener index showed uniformity in pattern between dry season and the wet season. The wet season values ranged from (0.28 – 0.96) and (0.30 – 0.95) for dry season. This implies a lack of seasonal variation in species diversity.

3.4 Species Evenness

Species evenness during the study period ranged from (0.89 – 1.00) for the wet season and (0.84-1.00) for the dry season. There was no much difference in the values. Maximum and minimum values were observed in stations 3 and 5 respectively for both seasons. Species evenness had high values in wet season than in the dry season (Fig 4.)

3.5 Species Dominance

The species dominance for the entire study period ranged from (0.27 – 0.56) for the wet season and (0.19 – 0.33) for the dry period. This showed that there were slight variations across the seasons and stations.

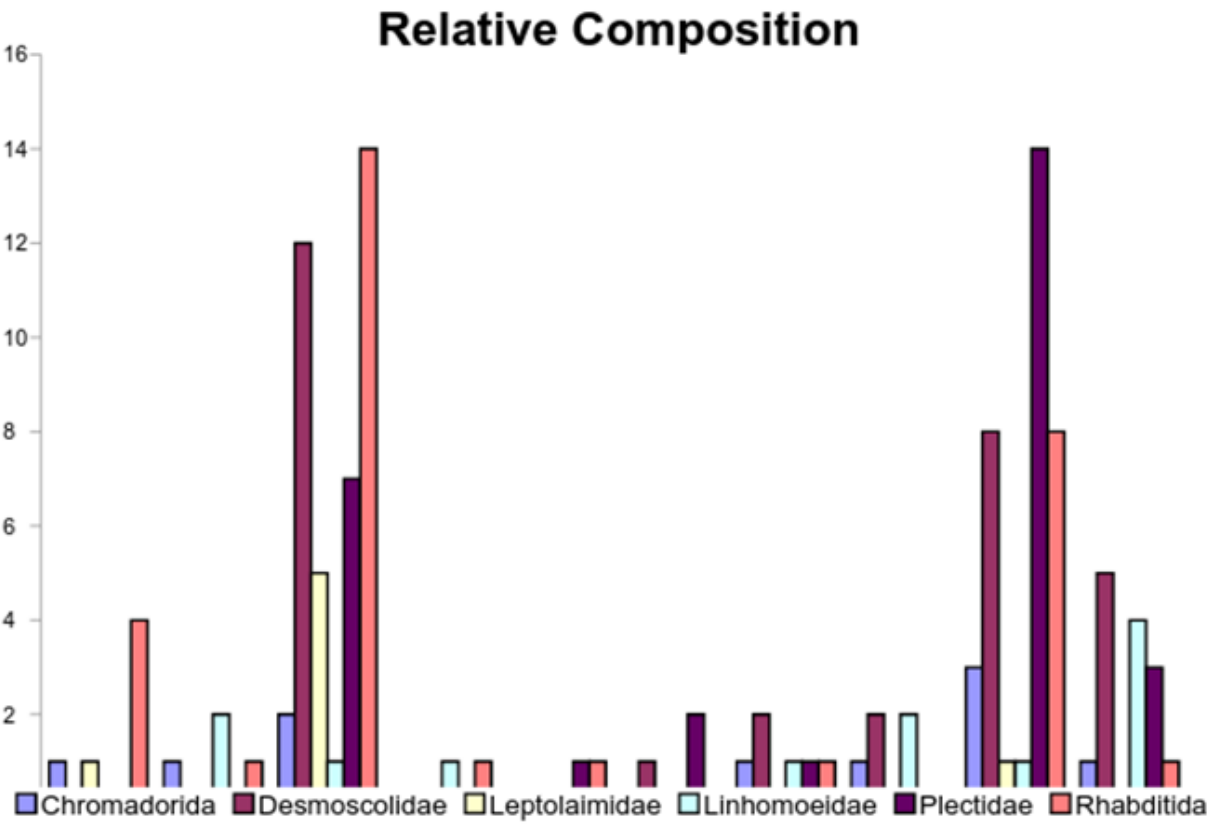

FIG. 2: RELATIVE COMPOSITION OF MEIOFUANA

TABLE 2
DISTRIBUTION OF MEIOFAUNA AT THE FIVE STATIONS OF LOWER BONNY ESTUARY (WET SEASON).

Family	Genus Spp	STN 1	STN 2	STN 3	STN 4	STN 5	Total % Dominance
Chromadoridae	Chromadorella Spp	1	O	O	1	1	**10.71**
	Chromadorina Spp	O	O	O	O	O	**0**
	Chromodorita Spp	O	O	O	O	O	**0**
Desmodoridae	Diplolaimella Spp	O	O	1	2	5	**28.57**
	Desmoscolex Spp	O	O	O	O	O	**0**
	Desmolaimus Spp	O	O	O	O	O	**0**
	Hliplectus Spp	O	O	O	O	O	**0**
Leptolamidae	Hypodontolemus	O	O	O	O	O	**0**
Linhomoeidae	Leptolaimus Spp	2	1	O	2	4	**32.4**
Plectidae	Prochomadora Spp	O	O	O	O	O	**0**
	Paracytholaimus	O	O	2	O	3	**17.80**
Rhabdodemaniidae	Sphacrolaimus Spp	O	O	O	O	O	**0**
	Rhabdolaimus Spp	O	O	O	O	1	**3.57**
	Thornilla Spp	O	O	O	O	O	**0**
	Paractinolaimus	1	1	O	O	O	**7.14**
	Rhabditis Spp	O	O	O	O	O	**0**
Total							**100**

TABLE 3
DRY SEASON DISTRIBUTION OF MEIOFAUNA AT THE FIVE STATIONS OF LOWER BONNY ESTUARY

Family	Genus Spp	STN 1	STN 2	STN 3	STN 4	STN 5	Total % Dominance
Chromadoridae	Chomadorella Spp	1	1	-	O	1	**3.45**
	Chromadarina Spp	O	1	-	1	1	**3.45**
	Chromodorita Spp	O	O	-	1	1	**2.30**
Desmoscolidae	Diplomella Spp	O	3	O	2	8	**15.0**
	Desmoscolex Spp	O	8	O	O	O	**9.0**
	Desmolaimus Spp	O	1	O	O	O	**1.15**
Leptolamidae	Hliplectus Spp	1	3	O	O	1	**5.75**
	Hypodontolamus	O	2	O	O	O	**2.30**
Linhomoeidae	Laptolaimus Spp	O	1	O	1	O	**2.30**
Plectidae	Prochomadora Spp	O	6	O	O	6	**13.79**
	Paracytholaimus	O	O	1	O	8	**10.0**
Rhabditidae	Sphacrodaimus Spp	O	8	O	O	5	**15.0**
	Rhabdolaimus Spp	1	5	O	1	2	**10.34**
	Thronella Spp	O	O	1	O	O	**1.15**
	Paractinolaimus	O	1	O	O	1	**2.30**
	Rhabditis Spp	3	O	O	O	O	**3.45**
Total							**100**

IV. DISCUSSION OF RESULTS

In this study, nematodes were the only meiofauna group observed. Most species of nematode have a direct development phase and a short life-cycle of one to three months. Therefore, changes in the community structure can be readily observed in short-term studies like this one.

The results reveal a trend that is difficult to understand and explain as there appear to be no significant difference in faunal characteristics such as diversity, abundance, richness and evenness across the various sampling stations (stations 1 – 4) except between the upstream station (station 5) and others. This may be due to the constant interaction of causal factors as the estuary is constantly navigated by marine crafts. Therefore, the application of biodiversity indices based on higher taxonomic resolution does not seem to yield higher discriminatory power in evaluating environmental quality when the ecosystem is in constant flux.

This lack of a large spatio-temporal variation in meiofauna may also be as a result of lack of changes in food supply correlations between any of the indicators and the concentrations of total protein, which represent the fraction of organic matter available for these benthic consumers (Vezzulli and Fabiano, 2006). The study also recorded fluctuating meiofaunal diversity with highs in some stations and lows in others. This may be due to the sensitivity of certain nematodes to certain pollutants. Very low species richness at this site is consistent with high sediment toxicity, while the moderate abundances and biomasses recorded may be explained by the ability of a few species to benefit from the eutrophic conditions of the estuary.

Warwick (1988) suggests that anthropogenic effects modify benthic community composition at relatively high taxonomic levels, while natural environmental variables influence the fauna more by species replacement.

Consistent with this generalization, our results demonstrate that meiofauna assemblages in Bonny estuary show shifts in meiofauna community composition, especially the relative abundances of nematodes in response to anthropogenic alterations of estuarine habitat quality rather than natural environmental variability.

The presence of sensitive or tolerant meiofaunal taxa and nematode genera appears to be particularly informative in highlighting the state of sediment pollution and allows the use of other tools of assessment of the spatial heterogeneity of environmental disturbance within the estuary. However, the emphasis on sediment pathways as the primary means of Meiofauna recruitment is no longer appropriate since a number of recent studies have shown quite convincingly that both adult and juvenile meiofauna are regularly found in the water column (Ajao, 1990).

The absence of sub-surface water analyses data of meiofauna in this study, confines our explanation.

In conclusion, higher species diversity were observed upstream than downstream of the creek. This may be due to the fact that upstream stations are far removed and shielded from land based stressors. This has confirmed the assertion that land based activities greatly affect aquatic integrity.

REFERENCES

[1] Ajao, E.A (1990) The influence of domestic and industrial effluents on populations of sessile and benthic organisms in Lagos lagoon. Ph.D Thesis, University of Ibadan, Nigeria, 413 pp

[2] Coull, B. C., G. R. F. Hicks and J. B. J. Wells. 1981. Nematode/copepod ratios for monitoring pollution: A rebuttal. Mar. Pollut. Bull.12:198-381.

[3] Gerlach, S. A. 1971. On the importance of marine meiofauna for benthos communities. Oecologia 6: 176-190.

[4] Margalef, R. (1967). Diversity and stability: A practical proposal and a model of

[5] Inte-rdependence. Brookhaven. Sympo. Biol., 22: 25 37.

[6] Shannon, C.E., Weaver, W. 1963. The mathematical theory of communication. University of Illinois, Urban Press, Illinois. 177Pp

[7] Sibert, V., B. Zakardjian, F. Saucier, M. Gosselin, M. Starr, and S. Senneville (2010), Spatial and temporal variability of ice algal production in a 3D ice-ocean model of the Hudson Bay, Hudson Strait and Foxe Basin System, Polar Res. ,29, 353–378, doi:10.1111/j.1751-8369.2010.00184.x.

[8] Vezzulli, L., and Fabiano, M. (2006) Sediment biochemical and microbial variables for the evaluation of trophic status along the Italian and Albanian Continental Shelves. Journal of the Marine Biological Association of the UK, 86: 27–37.

[9] Warwick, R. M. 1981. The nematode–copepod ratio and its use in pollution ecology. Marin Pollution Bulletin, 12: 329–333.

The utilities of *Citrus* tissue culture

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Abstract— *Citrus is the third most important fruit crop in the world after apple and bananas, and the total area cultivated with the various Citrus cultivar covers over 7.2 million hectares, yielding total annual production of approximately 100 million metric tons of fruit. The genus Citrus possesses several undesirable characteristic including salt and cold sensitivity they are also susceptible to diseases caused by fungi, bacteria and viruses. Despit substantial genetic diversity and interspecific fertility, the genus Citrus includes some of the most difficult species to breeding. For example, most species are highly heterozygous and produce progeny that segregate widely for many characters when crosses are made. The juvenile periods are often very long, self- and cross-incompatibility and pollen and/or ovule sterility are relatively common, and the presence of adventitious somatic embryos in the nucellus of developing ovules of the most of Citrus greatly limits hybrid production. Genetic transformation is an alternative to overcome these difficulties. For successful transformation, regeneration of whole plants from the transformed cells is a prerequisite. On the other hand production of virus – free plants, development of new cultivars, and production of seedless fruit, production of flavonoid, micropropagation, germplasm conservation and cryopreservation are the other utilities of in vitro culture of Citrus.*

Keywords— Citrus, Improvement, Tissue culture.

I. INTRODUCTION

Tissue culture is the in vitro aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions (Thorpe 2007) often to produce the clones of plants. It is clear that in vitro culture is an essential component of plant – biotechnology, offers innovative approaches in several research areas (Annarita and Laura 2012). The importance of plant cell, tissue and organ culture is more cleared when it includes in major fruit crop in vitro culture regarding to micropropagation, the induction of somatic embryogenesis, the improving of methodologies through the analysis of medium components, and the use of additives to increase the frequency of regeneration and the production of the in vitro cultures for the important species such as species belong to genus *Citrus*. The genus *Citrus* is cultured in more than 100 countries making it one of the most important commercial fruit crops in terms of economic value and human nutrition (Barlass and Skene 1986). *Citrus* is the most important fruit crop in the world and is produced in all five continents and it is often regarded as golden fruit or queen of all fruits (Nito 1996).

Despite substantial genetic diversity and interspecific fertility, the genus *Citrus* includes some of the most difficult species to breed (Gmitter et al., 1992 & Perez – Molphe – Balch and Ochoa – Aljeo 1998). This is due to several obstacles for conventional breeding. For example, most species are highly heterozygous and produce progeny that segregate widely for many characters when crosses are made. The juvenile periods are often very long, self – and cross – incompatibility and pollen and/or ovule sterility are relatively common, and the presence of adventitious somatic embryos in the nucellus of developing ovules of the most of *Citrus* greatly limits hybrid production (Perez – Molphe – Balch and Ochoa – Aljeo 1998 & Moore et al., 1993). Genetic transformation is an alternative to overcome these difficulties. For successful transformation, regeneration of whole plants from the transformed cells is a prerequisite (Duan et al., 2007). In this review some of the most important utilities of *Citrus* tissue culture that are based on result of different experiments in this field were explained.

II. THE *CITRUS* GENIUS NEED TO IMPROVEMENT

Citrus belongs to family *Rutaceae* having 150 genera and 15,000 species and it is distributed mostly in tropical and temperate region of the planet (Ladania 2008). A number of major genera of family *Rutaceae* are *Citrus*, *Murraya*, *Zanthoxylum* and *Ruta* etc. (Perveen and Qaiser 2005). As mentioned earlier *Citrus* has high dietary value and is a prosperous source of vitamin C in combination with macromolecules such as amino acids, organic acids and sugar as well as minerals comparable to magnesium and calcium in sufficient quantity (Niaz *et al*., 2004). The genus *Citrus* possesses several undesirable characteristics including salt and cold sensitivity (Garcia – Agustin and Primo – Millo 1995 & Van Le *et al*., 1999) they are also susceptible to diseases caused by fungi, bacteria and viruses, such as *Citrus* exocortis viroid (CEV),

Citrus infectious variegation virus (CIVV), *Citrus* cachexia viroid (CCaV) and *Citrus* tristeza closterovirus (CTV) (Van Le *et al*., 1999 & Greno *et al*., 1988). These biotic and abiotis stresses cause to low productivity. So *Citrus* genius has a great need to improvement.

III. TISSUE CULTURE IS A BASIC PREREQUISTIC FOR GENE TRANSFER IN *CITRUS*

In recent years, there has been a major thrust in *Citrus* improvement as competition from international *Citrus* markets, disease, and pest pressure and other abiotic and biotic stress conditions stimulate worldwide interest (Grosser *et al*., 2000 & Dutt and Grosser 2010).

Genetic transformation of *Citrus* is a valuable technique for *Citrus* improvement due to difficulties of conventional *Citrus* breeding. Recently, *Citrus* improvement using genetic transformation is being used frequently as increasing competition in international markets and disease pressure have stimulated worldwide interest in *Citrus* improvement (Grosser *et al*., 2000). In *Citrus*, gene transformation is carried out by three different techniques i.e., particle bombardment (Jia – Long *et al*., 1996). protoplast transformation (Fleming *et al*., 2000) and *Agrobacterium* (Nuria *et al*., 2012; De Oliveria 2009; Yang and Hu 2011 & Cervera *et al*., 1998).

Currently, *Citrus* canker and huanglongbing (HLB) are the two major diseases threatening the *Citrus* industry. Canker results in leaf – spotting and blemishing on the surface of the fruit, often resulting in defoliation, shoot dieback, and fruit drop. HLB affects all cultivated *Citrus* varieties and causes a rapid decline of trees and the production of unmarketable fruit. Both these diseases are caused by non – indigenous bacterial pathogens and cause substantial economic losses. Incorporation of gene(s) via genetic engineering can potentially confer resistance in susceptible cultivars, while maintaining the varietal fidelity. Transgenic *Citrus* plants have been obtained by direct DNA trasfer into protoplasts (Vardi *et al*., 1990) co – cultivation of internode or epicotyl segments with *Agrobacterium* (Moore *et al*., 1992; Pena *et al*., 1995a & Gutierrez *et al*., 1997). And particle bombardment of nucellar embryogenic cell suspentions (Yao *et al*., 1996). At persent, the most widely used method of gene trasfer in *Citrus* is the *Agrobacterium* – mediated transformation of epicotyl segments with 1 cm of length. Using this system, transgenic plants of *Citrus sinensis* (Pena *et al*., 1995a & Bond and Roose 1998), *Citrus aurantifolia* (Gutierrez *et al*., 1997), *Citrus aurantium* (Pena *et al*., 1997; *Citrus paradasi* (Luth and Moore 1996; *Citrus sinensis* x *Poncirus trifolia* (Moore *et al*., 1992 & Pena *et al*., 1995a) and *Citrus trifoliata* (Kaneyoshi *et al*., 1994) have been obtained. However, this method is not suitable for the transformation of any seedless cultivar. Also, special cultivars in themandarin group remain robust to transform using this method (Dutt and Grosser 2010; Dutt *et al*., 2010 & Khawale *et al*., 2006).

Due to the limitations of this method it seems that in order to carry out successful gene transformation studies in *Citrus*, optimized in vitro regeneration protocol is needed. Researchers should optimize efficient regeneration protocol before starting transformation studies. There are also many efficient regeneration protocols published in different *Citrus* species. In *Citrus*, callus cultures have been established in species such as *Citrus grandis* L. Osb. *Citrus auruntifolia* (Christm.) Swingle. *Citrus medica* L. *Citrus sinensis* L. Osb. *Citrus madurensis* L. *Citrus paradisi* Macf. *Citrus reticulata* Blanco and *Citrus limon* (Sabharwal 1963; Murashige and Tucker 1969; Grinblat 1972; Chaturvedi and Mitra 1975; Moore 1985; Duran-Vila *et al*., 1989; Gill 1992 & Gill *et. al*., 1994). Responses to different culture media are often genotype – specific. So the more optimization of tissue culture techniques is essential for gene transfer in *Citrus*.

IV. TISSUE CULTURE (MICROPROPAGATION) IS A POWERFUL TOOLS FOR PRODUCTION OF VIRUS – FREE *CITRUS* SPECIES

Micropropagation is an important asexual method that can be used for the production of virus – free rootstock plants (Roistacher *et al*., 1976). As mentioned earlier *Citrus*, due to pedoclimatic conditions, often suffers from abiotic stresses such as salinity, drought and iron deficiency induced chlorosis; nevertheless, a major constraint for its cultivation is represented by graft – transmissible agents (viruses, virus – like, viroids, prokaryotes), that may cause considerable losses in crop yield and quality of plants and plant products. These agents may often remain symptomless, thus representing a special risk in their spread over long distances through *Citrus* infected propagating material. The worldwide movement of graft – transmissible pathogens and relative vectors, along with the *Citrus* material, has increased in the last years due to the strong globalization and the lack of adequate quarantine measures. Once they invade a cultivated area, the most severe pathogens can be rapidly spread by insect vectors and their control becomes even more difficult (Carimi *et al*., 2013).

Virus and viroid's have been recognised as serious problem limiting the vigour, yield, quantity and quality. Severe infections have resulted in the exclusion of some cultivars from commercial usage, reported that viral diseases are major threats affecting *Citrus* industry (Vishwanath and Narayan 2015). The diseases are graft – transmissible through grafting infected

bud sticks (Santos *et al*., 1984). Hence, rising of disease – free foundation plants is imperative to provide certified bud sticks to the growers and to encourage the planting of grafts instead of seedlings (Mukhopadhyay *et al*., 1997).

The elimination of viruses, viroids, and phytoplasmas from infecte initial (mother) propagation material is a prerequisite for the production of healthy, vegetatively propagated crop material. Methods used are thermotherapy, meristem tissue culture, in vitro micrografting, in vitro chemotherapy, and cryotherapy of shoot tips, followed by shoot – tip tissue culture or in vitro micrografting (Christina 2015).

As all of us know meristem tissue culture, in vitro micrografting and cryotherapy of shoot tips, followed by shoot – tip tissue culture or in vitro micrografting are the basic tissue culture methods for virus elimination in plants. But we have to keep in mind that all metdods arenot suitable for all plants. For example meristem culture that is used for the elimination of viruses and related pathogens from a large number of vegetatively propagated plants and it is the main method used in plant virus elimination programs for some plants, such as *Citrus*, stone fruits, and other woody species, meristem cultures are not successful (George 1993b & Navarro 1988). In these cases, the meristem tip is grafted onto a virus free rootstock. The micrografting technique was first used for the elimination of viruses and viroids in *Citrus* by Navarro *et al*., 1976. Several scientists thereafter adopted this method to produce virus free plant material in *Citrus* Kapari –Isaia *et al*., 2002; 2007; Mukhopadhyay *et al*., 1997; Navarro Civerolo Juarez and Garney 1991; Navarro Juarez and Pina 2001; Navarro *et al*., 1975; Singh 2001). In fact the use of tissue culture methods for *Citrus* crop species has already had practical benefits. Most notable among these are techniques for obtaining virus – free and mycoplasma – free stocks using in vitro grafting of apical meristems from infected plants onto decapitated seedlings (Navaro *et al*., 1975).

The issue that has been considered in recent years is that growing nucellar seedlings was the only method available for producing disease free *Citrus* cultivars from clones infected with virus or other grafttransmissible pathogens. The primary disadvantage of producing *Citrus* budlines through nucellar embryony is the phenomenon of juvenility. Young nucellar seedlings exhibit excessive thorniness, vigorous and up – right habit of growth slowness to fruit, alternate bearing in early years and physical differences in fruit characteristics, which are often detrimental in marketing the fruit. These characteristics may persist for many years and over many budded generations. Nucellar budlines usually produce higher yields of fruit than their parental clones over a period of 8 – 10 years or more (Cameron *et al*., 1968; Nauer *et al*., 1983). The portion of this higher yield that can be attributed to elimination of virus and virus – like pathogens in the parental bud – line has not been determined. Variations among *Citrus* nucellar budlines and differences, other than juvenility, from the parental budline have been reported (Frost *et al*., 1957; Nauer *et al*., 1983) in numbers indicating that genetic variants may occur more often during production of nucellar bud – lines than occur during standard nursery trees production by bud propagation. Therefore, a method to recover *Citrus* plants free of all virus and virus – like diseases and without juvenile characters was needed. The first attempts in this direction were made by shoot –tip culture *in vitro*, a technique widely used to recover healthy herbaceous plants. However, attempts to develop *Citrus* plants from shoot – tips failed (Edriss *et al*., 1984).

Constraints of this method, the use of alternative methods such as somatic embryogenesis can be useful. Somatic embryogenesis is a developmental process where a plant somatic cell can dedifferentiate to a totipotent embryonic stem cell that has the ability to give rise to an embryo under appropriate conditions. This new embryo can further develop into a whole plant. In woody plants, somatic embryogenesis plays a critical role in clonal propagation and is a powerful tool for synthetic seed production, germplasm conservation, and cryopreservation (Yuan *et al*., 2016). Also somatic embryogenesis can be used to eliminate many virus diseases (Bitters *et al*., 1970; D'Onghia *et al*., 1997; D'Onghia *et al*., 2001), the plant material obtained by somatic embryos regenerated in vitro can be used to establish healthy *Citrus* stocks. On the other hand while other plant micro – organisms are in many cases controlled by therapeutic treatments directly performed in the field, graft – transmissible agents cannot be eliminated by these means because of the peculiarity of their replication cycle. Pro – active strategies, which primarily rely on lower disease incidence and restrain virus dissemination, can prevent the introduction of these agents into new plantings and new areas; this is less hard and expensive than eliminating them once they are already present. Within this context, strict phytosanitary regulations and certification programmes for the production of 'healthy' *Citrus* nursery plants are amongst the most efficient preventive strategies (Carimi *et al*., 2013).

In *Citrus*, the production of embryogenic callus lines was reported from excised nucelli (Rangan *et al.*, 1968), abortive ovules (Bitters *et al*.,1970), unfertilized ovules (Button and Bornman, 1971), undeveloped ovules (Starrantino and Russo, 1980), juice vesicles (Nito and Iwamasa, 1990) , anthers (Hidaka *et al* ., 1981), styles and stigmas (Carimi *et al*., 1995) as well as from leaves, epicotyls, cotyledons and root segments (Gill *et al*., 1995). The embryogenic potential of *Citrus* varied with genotype and type of explant. in vitro culture of ovules from ovaries and immature fruits was initially used to obtain

virus – free nucellar plants from polyembryonic *Citrus* cultivars (Bitters *et al*., 1970; Navarro *et al*., 1979). Somatic embryos, embryogenic callus and cell cultures recovered from *in vitro* cultured ovules have also been used to develop cryopreservation strategies for germplasm conservation (Kobayashi *et al*., 1990; Marin *et al.*, 1993; Engelmann *et al*., 1994; Sakai *et al.*, 1990 and 1991; Duran – Vila 1995) and protoplast technologies (Vardi and Galun, 1989; Grosser and Gmitter, 1990 a and b; Gimitter *et al.*, 1992). Recent studies have indicated the embryogenic potential of somatic tissues which are neither nucellar nor ovular in origin. Nito and Iwamasa 1990 obtained eight somatic embryos from cultures derived from Satsuma juice vesicles. Carimi *et al.*, 1995 induced formation of embryogenic cultures from styles of different species of *Citrus*. Gill *et al* ., 1995 obtained somatic embryos from leaf, epicotyl, cotyledon and root segments of *in vitro* grown nucellar seedling of *C. reticulata* Blanco.

V. OTHER UTILITIES OF *CITRUS* TISSUE CULTURE

5.1 Development of new cultivars

Citrus propagation by conventional means is restricted to particular season and availability of plant material. It doesn't guarantee trueness of cultivars and mass production of certified *Citrus* plants throughout the year. Plant tissue culture has emerged as a powerful tool for propagation and improvement of many woody plant species including *Citrus*. *Citrus* also stands among difficult to root crops and micropropagation offers rapid propagation of such crops in limited space and time under controlled conditions throughout the year (Usman 2005). In vitro culture further eliminates diseases (Grosser and Chandler 2000) provides scope for the development of new cultivars through somaclonal variation (Hammschlag *et al*., 1995) and somatic hybridization (Al – Bahrany 2002; Grosser *et al*., 1996; Louzada *et al*., 1996; Mendes – da – Gloria 2000; Ollitrault *et al*., 1996; Ollitrault *et al*., 2000 & Paudyal and Haq 2000) that have improved *Citrus* rootstock resistance against nematode infestation and other pests as well (Bouquet *et al*., 2003; Grosser *et al*., 1998; Guo and Deny 1998 & Guo and Deny 2001). Industry, the micropropagation of *Citrus* has always aroused great interest among scientists. There is a growing demand to develop new varieties of plants resistant to pathogens and adverse environmental conditions and characterized by high quality of fruits (Yaacob *et al*. 2014). Traditional techniques for creating new species are not effective in the case of *Citrus* (lemons) due to the problems that have already been mentioned such as physiological barriers associated with sexual reproduction such as heterozygosity and polyembriony (Tusa *et al*. 1990, Carimi *et al*. 1994, Savita *et al*. 2010, Benabdesselam *et al.* 2011, Lombardo *et al*. 2011).

Citrus (Lemons) plantations face a number of problems such as pests, slow growth, susceptibility to disease, sensitivity to low temperatures, and substantial losses during storage (Mukhtar *et al*. 2005a, b, Savita *et. al*. 2010, Sarma *et al*. 2011). In vitro culture is a technique that can solve these problems. In addition, this technique can also produce crops on a relatively large scale in comparison with traditional plant breeding. Furthermore, in vitro cultures eliminate infections and can be faster than traditional plant cultures (Savita *et al.* 2011, Singh and Kaur 2011).

Development of new cultivares by tissue culture that mentioned above is due to a phenomenon called somaclonal variation. The term somaclonal variation was coined by Larkin and scowcraft 1981 to define genetic variation present in regenerated plants that is uncovered or induced by a tissue culture process. Somaclonal variation has been reported in a wide range of traits including plant height, overall growth habit, flower, fruit and leaf morphology, juvelinity, maturity date, diseases resistance, yield and biochemical charactristics. However most reports generally deal with either solanaceous or cearal crops but little information has been reported in woody prenial fruit crops (Grosser *et al*., 1997).

5.2 Production of seedless *Citrus* fruit

In recent years, there has been a shift in the world *Citrus* market towards seedless *Citrus* fruits and considerable energy has been devoted towards their production. The seedless trait in *Citrus* is related to male or female gametophyte sterility, self incompatibility, or early embryo abortion (Reforgiato Recupero *et al*. 2005), and several methods exist for the production of seedless *Citrus* of which mutation breeding, somaclonal variation and triploid breeding are the most important. In *Citrus*, triploid seedless cultivars are obtained by breeding between elite monoembryonic diploid cultivars as female parent with tetraploid cultivars as pollen parent (Esen and Soost 1973). Sterility in such fruits is caused due to the odd number of chromosomes that are unable to undergo successful meiotic pairing to produce chromosomally balanced gametes (Reforgiato Recupero *et al*., 2005).

Colchicine is an alkaloid obtained from the meadow saffron (*Colchicum autumnale* L.). This alkaloid inhibits mitosis by hampering the development of the nuclear spindle (Blakeslee and Avery 1937) and is most commonly used to obtain tetraploid plants artificially (Notsuka *et al*. 2000). In *Citrus*, tetraploidy has been induced by treatment of axillary buds with

colchicine, as was done with the cultivars Ellendale and Clementine. The treated buds upon grafting on rootstock produced several tetraploid plants (Oiyama 1992). However, a disadvantage of using axillary buds in colchicine experiments is that most of the recovered plants end up being unstable chimeras and do not have applications in a breeding program (Barrett 1974; Jaskani *et al*., 1996). This is due to the use of multicellular tissue as a source of explants for colchicine treatment. Using such tissues usually result in production of a large proportion of chimeric tetraploids (Kadota and Niimi 2002). Non – c-himeric autotetraploid *Citrus* plants have been obtained from in vitro colchicine experiments via embryogenesis of underdeveloped ovules from immature *Citrus* fruits (Gmitter and Ling 1991; Gmitter *et al*. 1991).

5.3 Production of flavonoid

Citrus and *Citrus* peels contain common flavonoids, such as hesperidin, naringin, neohesperidin, narirutin, eriocitrin, didymin and rutin among others (Benavente – Garcia *et al*., 2007; Tripoli *et al*., 2007 & Gattuso *et al*., 2007). A number of studies have demonstrated the biological properties of these *Citrus* flavonoids including anti – carcinogenic, anti-oxidant and anti-inflammatory properties that promote and benefit human health (Tripoli *et al*., 2007; Gattuso *et al*., 2007; Lopez – Lazaro *et al*., 2002 & Wang *et al*., 2014) . In addition to *Citrus* flavonoids, *Citrus* peels are also the sole and rich source of polymethoxylated flavonoids, which were found to exert many biological properties, particularly anti-cancer and anti – inflammatory activity (Li *et al*., 2009; Gosslau *et al*., 2014; Li *et al*., 2014 & Li *et al*., 2014). Recent studies have also demonstrated potent anticarcinogenic and anti – inflammatory efficacy of 5-demethylated polymethoxyflavones in single molecules (Li *et al*., 2014; Ma *et al* ., 2014 & Lai *et al*., 2007) or in multiple 5-demethylated polymethoxyflavones (Lai *et al*., 2011). The natural content of 5 –demethylated polymethoxy flavones in *Citrus* peels is low in percentage, but it has been confirmed that they have more potent biological activity than their non-demethylated counterparts, such as anticancer activity (Lai *et al*., 2014; Lai *et al*., 2007 & Lai *et al*., 2011).

In essence, there are three subclasses of *Citrus* flavonoids exiting abundantly in *Citrus* peels, namely, polyhydroxy flavonoids, polymethoxy flavonoids and mixed substituted flavonoids with both hydroxyl and methoxyl groups, particularly 5 – demethylated polymethoxyflavnoids. These flavonoids have demonstrated effective anti – cancer property both in vitro and in vivo, either in a form of individual compounds or in a mixture of *Citrus* flavonoids. The anti – cancer study of these flavonoids has progressed well in recent years owing albeit in the initial steps to the modern chemical analysis and isolation and the biological activity testing. However, with the exception of nobiletin, the relationships between each individual flavonoid in *Citrus* peels and its bioactivity such as anti – carcinogenesis remain untouched to some extent. Relationships among the naturally proportioned flavonoidsin *Citrus* peels and their biological activities are even more complex and unexplored.

Biotechnology uses techniques and processes that involve living organisms to obtain specific products and/or modifications that increase the production of chemical substances of interest in less time and less capital investment (Davies and Deroles 2014). Secondary metabolites such as *Citrus* flavonoids that are found in plants are generally produced in low concentrations compared with primary metabolites. Therefore, different strategies, including in vitro culture systems, have been extensively studied to increase the production of secondary metabolites in plants (Smetanska 2008; Muranaka and Saito 2010 & Gill *et al*., 2013). In vitro cell cultures represent an interesting alternative because secondary metabolites of interest are obtained in a controlled environment that is not influenced by changes in climate or soil conditions (Goncalves and Romano 2013 & Collin 2001). Plants that are grown in their natural habitat generally have varying concentrations of compounds of interest, depending on the particular crop season (Salmore and Hunter 2001; Puricelli *et al*., 2002 & Ralphs and Gardner 2001). Moreover, their exploitation in their natural environment can cause gradual genetic erosion (Sidhu and Bel 1996). Also callus cultures and cells in suspension have been used to study the biosynthesis of economically important secondary metabolites enabling the propagation of cell lineages that contain alterations in biosynthetic capabilities. he production of different compounds in plants is generally mediated by environmental factors that vary according to physiological conditions and seasonal variations (Gill *et al*., 2013). Thus, cell cultures ensure controlled conditions that circumvent environmental changes.

5.4 Micropropagation, Germplasm conservation and Cryopreservation

Citrus trees are propagated both by seed and by vegetative means. There is huge demand of planting material. Non availability of scientifically propagated planting material from elite clones for plantation are the main constraints in *Citrus* cultivation. In recent years tissue culture techniques (micropropagation) are increasingly used for rapid clonal propagation of several economic plants, restoration of vigour and yield due to infection and preservation of germplasm. Hence

micropropagation is a very useful tool for a production of large number of planting materials. Besides this technique is also useful for saving the *Citrus* species which are facing extinction (singh 2002).

Conservation of *Citrus* germplasm in the field requires great space, labour and costs the risk of damage by natural calamities and pathogen infection that may be always incurred. Therefore, in vitro conservation can easily overcome these difficulties, and ensuring the maintenance of healthy *Citrus* germplasm. Moreover, for several *Citrus* species, in vitro culture may play a major role as a conservation strategy or even be the only option available. For an efficient in vitro conservation of healthy germplasm, we need suitable protocols of plant regeneration. Fortunately, there exist many reports on organogenesis from different types of explants of *Citrus* and *Citrus* rootstocks. The morphogenic responses of *Citrus* cultured in vitro are influenced by the genotype, the explant type and the culture medium. Explants include shoot tips (Barlass and Skene 1986), stem sections (Grinblat 1972; Chaturvedi and Mitra 1974 ; Raj Bhansali and Arya 1979 & Barlass and Skene 1982), root sections (Sauton *et al*., 1982; Burger and Hackett 1986; Sim *et al*., 1989 & Bhat *et al*., 1992) leaf sections (Chaturvedi and Mitra 1974 & Hu and Kong 1987), stem internodes (Duran – Vila and Navarro 1989), epicotyl segments (Edriss and Burger 1984) and transverse thin cell layer (tTCL) explants excised from stem options (Van Le *et al*., 1999). The regeneration of adventitious shoots has been obtained either directly from the explant or from an intermediate callus phase.

Also as noted above traditionally, *Citrus* germplasm is preserved in clonal orchards, where it is susceptible to pests, diseases and climatic catastrophes (Duran – Vila, 1995). Cryopreservation of embryogenic calli at ultra – low temperatures (–196 °C) in liquid nitrogen (LN2) is an excellent mean to overcome the challenges inherent to maintaining embryogenic materials and to provide long – term conservation of valuable embryogenic lines (Gonzales – Arnao *et al*., 2008). There are many studies on *Citrus* cryopreservation using very different materials. Efficient vitrification and dehydrationbased cooling procedures have been reported for various *Citrus* organs and tissues, including shoot tips (Wang and Deng, 2004), seeds (Kaya *et al*., 2016), embryonic axes (Cho *et al*., 2002), somatic embryos (Marin and Duran – Vila, 1988), ovules (Gonzales – Arnao *et al*., 2003), embryogenic calli (Perez *et al*., 1997 & Olivares – Fuster *et al*., 2000) and nucellar cells (Sakai *et al*., 1990). And ultimately, as we all know, the success of any of these methods requires the establishment of appropriate tissue culture systems.

VI. CONCLUSION

Like the vast majority of genera and species of plants, especially economically important genera and species, different species of *Citrus* genius need to improvement. *Citrus* improvement by conventional methods due to difficulties such as has limitations that all of them solve by modern methods of biotechnology or in other words transformation. Low plant regeneration frequencies especially for many of the economically important *Citrus* species is the most important difficult within this almost new and advanced method. So the progression of tissue culture methods leads to solving these problems.

REFERENCES

[1] Al – Bahrany A.M. 2002. Effect of phytohormones on in vitro shoot multiplication and rooting of lime (*Citrus aurantifolia* Swing.). Scientia Horticulturae. 95: 285 – 295

[2] Annarita L. and Laura M.R.R. 2012. Recent advance in plant in vitro culture. Page 8. http://dx.doi.org/10.5772/52760. Edited by Annarita Leva and Laura M. R. Rinaldi

[3] Barrett H.C. 1974. Colchicine – induced polyploidy in *Citrus*. Bot Gaz 135:29 – 34

[4] Barlass M. and Skene K.G.M. 1982. In vitro plantlet formation from *Citrus* species and hybrids. Scientia Horticulturae. 17: 333 – 341

[5] Barlass M and Skene K.G.M. 1986. Citrns. In Bajaj Y.P.S. (Ed) Biotechnology in Agricultureand Forestry 1. Trees I, SpringerVerlag, Heidelberg. pages 207 – 219

[6] Benabdesselam F.M. Khettal B. Bedjou F. 2011. Micropropagation of Algerian juvenile rootstocks *Citrus* species. Lief Sciences Leaflets. 18:707 – 717

[7] Benavente – Garcia O. Castillo J. Alcaraz M. 2007. Beneficial action of *Citrus* flavonoids on multiple cancer – related biological pathways. Current Cancer Drug Targets. 7: 795 – 809

[8] Bhat S.R. Chitralekha P. and Chandler K.P.S. 1992. Regeneration of plants from long-term root culture of lime, *Citrus aurantifolia* (Christm. Swing). Plant Cell Tissue and Organ . Culture. 29: 19 – 25

[9] Bitters W.P. Murashige T. Rangan T.S. and Nauer E. 1970. Investigations on established virus free plants through tissue culture. California *Citrus* Nursery Society. 9: 27 – 30

[10] Blakeslee F.A. Avery A.G. 1937. Methods of inducing doubling of chromosome in plants.journal of Heredity. 25:80 – 108

[11] Bond J.E. and roose M. L. 1998. Agrobacterium – mediated transformation of commercially important *Citrus* cultivar Washington navel orange. Planr Cell Reports. 18: 229 – 234

[12] Bouquet A. Mark G. Pistanga D. Torregrosa L. 2003. Transfer of grape fan leaf virus coat protein gene through hybridization with Xiphinema index resistant genotypes to obtain rootstocks resistant to virus spread. Acta Hort. 603: 325 – 324

[13] Burger D.W. and Hackett W.P. 1986. Gradients of adventitious bud formation on excised epicotyl and root sections of *Citrus*. Plant Science. 43: 229 – 232

[14] Button J. and Bornman C. H. 1971. Development of nucellar plants from unpollinated and unfertilized ovules of the Washington navel orange in vitro. South African Journal of Botany. 37: 127 – 134

[15] Cameron J.W. and Frost H.B. 1968. Genetics, breeding and nucellar embryony. In: Reuther et al. (eds.), The *Citrus* Industry. University of California Division Agriculture Science, Berkeley. 2(5): 325 – 370

[16] Carimi F. De Pasquale F. Crescimanno F.G. 1994. Somatic embryogenesis from styles of lemon (*Citrus limon* L. Burm). Plant Cell Tissue and. Organ Culture. 37, 209 – 211

[17] Carimi F. De pasquale F. and Crescimanno F.G. 1995. Somatic embryogenesis in *Citrus* from styles culture. Plant Science. 105: 81 – 86

[18] Carimi F. D'Onghia A.M. Carra A. and Djelouah K. 2013. Somatic Embryogenesis, Genetic Fidelity of Somatic Embryo Derived Plantlets and Virus Elimination in *Citrus*. Somatic embryogenesis and gene expression. 124 – 145

[19] Cervera M. Pina J. Juarez J. Navarro A. Navarro L. Pena L. 1998. Agrobacterium mediate transformation of citrange: Factors affecting transformation and regeneration. Plant Cell Reports. 1998 18(3) :271 – 278

[20] Chaturvedi H.C. and Mitra G.C. 1974. Clonal propagation of *Citrus* from somatic callus cultures. Horticultural Science.: 118 – 120 Chaturvedi H.C. and Mitra G.C. 1975. A shift in morphogenetic pattern in *Citrus* callus tissue during prolonged culture. Annals of Botany. 39: 683 – 687

[21] Cho E.G. Hor Y.L. Kim H.H. Rao V.R. Engelmann F. 2002. Cryopreservation of *Citrus* madurensis embryonic axesby encapsulation – dehydration. Cryo Letters. 23: 325 – 332

[22] Christina V. Varvara I. M. Theodora K.I. Isaia. 2015. Principles for supplying virus – tested material. Advances in Virus Research. 91: 1 – 32

[23] Collin H.A. 2001. Secondary product formation in plant tissue cultures. Plant Growth Regulators. 34: 119 – 134

[24] Davies K.M. Deroles S.C. 2014. Prospects for the use of plant cell cultures in food biotechnology. Current Opinion in Biotechnology. 26: 133 – 140

[25] De Oliveira M. 2009. High – efficiency *Agrobacterium*-mediated transformation of *Citrus* via sonication and vacuum infiltration. Plant Cell Reports. 28(3) :387 – 395

[26] D'Onghia A. M. De pasquale F. Carimi F. Savino V. and Crescimanno F.G. 1997. Somatic embryogenesis from style culture as a possible means for virus elimination in *Citrus*. Journal of Phytopathology. 145: 77 – 79

[27] D'Onghia A.M. Savino V. Kyriakou A.P. Yilmaz M.A. Baloglu S. Stamo B. Khoury W. Boubker J. Drossopoulou J. Abdel-Salam A.M. 1998. Proposal of a scheme for the production maintenance and utilization of *Citrus* certified propagative material in the Mediterranean. Proceeding of the Mediterranean Network on Certification of *Citrus*. Options Mediterraneennes Serie B 21 CIHEAM Publications. Pages 135 – 152

[28] D'Onghia A. M. Carimi F. De pasquale F.Djelouah K and Martelli G.P. 2001. Elimination of *Citrus* psorosis virus by somatic embryogenesis from stigma and style cultures. Plant Pathology 50: 266 – 269

[29] Duan Y.X. Liu X. Fan J. Li D.L. Wu R.C. and Guo W.W. 2007. Multiple shoot induction from seedling epicotyls and transgenic *Citrus* plant regeneration containing the green fluorescent protein gene. Botanical Studies. 48: 165 – 171

[30] Duran-Vila N. Ortega V. and Navarro I. 1989. Morphogenesis and tissue cultures of three *Citrus* species. Plant Cell Tissue and Organ Culture. 16: 123 – 133

[31] Duran – Vila N. 1995. Cryoconservation of germplasm of *Citrus*. In: Bajaj YPS, editor. Biotechnology in Agriculture and Forestry 32. Cryopreservation of Plant Germplasm. Berlin, Germany: Springer – Verlag. 70b – 85

[32] Dutt M . Orbovic V. and Grosser J.W. 2009. Cultivar-dependent gene transfer into *Citrus* using *Agrobacterium.* Florida State Horticultural Society. 122: 85 – 89

[33] Dutt M. and Grosser J. W. 2010. An embryogenic suspension cell culture system for *Agrobacterium*-mediated transformation of *Citrus*. Plant Cell Reports. 29(11): 1251 – 1260

[34] Dutt M. Lee D. H. and Grosser J. W. 2010. Bifunctional selection reporte systems for genetic transformation of *Citrus*: mannoseand kanamycin – based systems. In Vitro Cellular and Developmental Biology Plant. 46(6): 467 – 476

[35] Edriss, M.H. and D.W. Burger, 1984. Micrografting shoot tip culture of *Citrus* on three trifoliate rootstocks. Scientia Horticulturae. 23: 255 – 259

[36] Edriss M.H. and Burger D.W. 1984. In vitro propagation of 'Troyer' citrange from epicotyl segments. Scientia Horticulturae 23: 159 – 162

[37] Esen A. Soost R.K. .1973. Seed development in *Citrus* with special reference to 2 × 4 crosses. American Journal of Botany. 60:448 – 452

[38] Engelmann F. Dambier D. and Ollitrault P. 1994. Cryopreservation of embryogenic cell suspensions and calluses of Citrus using a simplified freezing process. Cryo-Letters 15: 53 – 58

[39] Fleming G.H. Olivares-Fuster O. Del – Bosco S. Grosser J.W. 2000. An alternative method for the genetic transformation of sweet orange. In Vitro Cell Development. 36(6): 450 – 455

[40] Frost H.B. Cameron J.W. and Soos R.K. 1957. Diversity among nucellar seedling lines of Satsuma mandarin and differences from the parental old line. Hilgardia. 27: 201 – 222

[41] Garcia – Agustin P. and Primo – Millo E. 1995. Selection of a NaCl tolerant *Citrus* plant. Plant Cell Reports. 14(5): 314 – 318

[42] Gattuso G. Barreca D. Gargiulli C. 2007. Flavonoid composition of citrusjuices. Molecules 12: 1641 – 1673

[43] George E. F. 1993b. Methods of elimination. In E. F. George, M. A. Hall, & Geet-Jan De Klerk (Eds.), Plant propagation by tissue culture. part I. The technology Westbury. England: Exegetics Ltd. 146 – 151

[44] Gill M.I.S. 1992. Studies on somatic cell and protoplast culture in mandarins. Ph.D. Thesis Punjab Agricultural University. Ludhiana

[45] Gill, M.I.S. Zora Singh Dhillon B.S. and Gosal S.S. 1994. Somatic embryogenesis and plantlet regeneration on calluses derived from seedling explants of Kinnow mandarin (*Citrus nobilis* Lour. X *Citrus deliciosa* Tenora). Journal Horticulture Science. 69: 231 – 236

[46] Gill M. I.S. Singh Z. Dhillon B.S. and Gosal S.S. 1995. Somatic embryogenesi and plant regeneration in mandarin (Citrus reticulata Blanco). Scientia Horticulturae 63: 167 – 174

[47] Gill S.S. Anjum N.A. Hasanuzzaman M. Gil R. Trivedi D.K. Ahmad I. Pereira E. Tuteja N. 2013. Glutathione and glutathione reductase: a boon in disguise forplant abiotic stress defense operations. Plant Physiology and Biochemistry. 70: 204 – 212

[48] Gmitter F.G. Ling X.B. .1991. Embryogenesis in vitro and nonchimeric tetraploid plant-recovery from underdeveloped *Citrus* ovules treated with colchicine. Journal of the American Society for Horticultural Science. 116 :317 – 321

[49] Gmitter F.G. Ling X.B. Cai C.Y. Grosser J.W. 1991. Colchicineinduced polyploidy in *Citrus* embryogenic cultures, somatic embryos, and regenerated plantlets. Plant Science. 74:135 – 141

[50] Gmitter F.G. Grosser J.W. and Moore G. A. 1992. *Citrus*, in biotechnology of perennial fruit crops. Hammerschlag F. A. and Litz R. E. Eds. Pages 335 – 369. CAB International, Wallingford. UK

[51] Goncalves S. and Romano A. 2013. In vitro culture of lavenders (Lavandula spp.) and the production of secondary metabolites. Biotechnology Advances. 31: 166 – 174

[52] Gonzales – Arnao M.T. Ortega J.C. Navarro L. Duran – Vila N., 2003. Cryopreservation of ovules and somatic embryos of *Citrus* using the encapsulation-dehydration technique. Cryo Letters . 24: 85 – 94

[53] Gonzales – Arnao M.T. Panta A. Roca W.R. Escobar R.H. EngelmannF. 2008 Development and large scale application of cryopreservation techniques for shoot and somatic embryo cultures of tropical crops. Plant Cell Tissue Organ Culture. 92: 1 – 13 http://dx.doi.org/10.1007/s11240-007-9303-7

[54] Gosslau A. Chen K.Y. Ho C.T. 2014. Anti – inflammatory effects of charac-terized orange peel extracts enriched with bioactive polymethoxyflavones. Food Science and Human Wellness. 3(1): 26 – 35

[55] Guo W.W. and Deng X.X. 1998. Somatic hybrid plantlets regeneration between *Citrus* and its wild relative, Murraya paniculata via protoplast fusion, Plant Cell Reports. 18:297 – 300

[56] Guo W.W. Deng X.X.2001. Wide somatic hybrids of *Citrus* with its related genera and their potential in genetic improvement. Euphytica. 118:175 – 183

[57] Greno V. Navarro L. and Duran –Vila N. 1988. Influenceof virus and virus-like agents on the development of *Citrus* buds cultured in vitro. Plant Cell, Tissue and Organ Culture. vol. 15(2): 113 – 124

[58] Grinblat U. 1972. Differentiation of *Citrus* stem in vitro. Journal of the American Society for Horticultural Science. 97: 599 – 603

[59] Grosser J.W. Gmitter J R F.G. 1990a. Protoplast fusion and *Citrus* improvement. Plant Breeding. Reviews. 8: 339 – 374

[60] Grosser, J.W. and Gmitter, Jr. F.G. 1990b. Somatic hybridization of *Citrus* with wild relatives for germplasm enhancement and cultivar development. HortScience. 25:147 – 151

[61] Grosser J.W. Mourao – Fo F.A.A. Gmitter Jr F.G. Louzada E.S. Jiang J. Baergen K. Quiros A. Cabasson C. Schell J.L. Chandler J.L. 1996. Allotetraploid hybrids between *Citrus* and seven related genera produced by somatic hybridization, Theoritical and Applied Genetics. 92: 577 – 582

[62] Grosser J.W. Gmitter J.R. and Chandler J.L. 1997. Development of improved sweet orange cultivars using tissue culture methods. Florida State Horticultural Society. 110: 13 – 16

[63] Grosser J.W. Jiang J. Mourao – Fo F.A.A. Louzada E.S.. Baergen K. Chandler J.L. Gmitter Jr. F.G. 1998. Somatic Hybridization, an integral component of *Citrus* cultivar improvement: I. Scion Improvement, HortScience. 33:1057 – 1059

[64] Grosser J.W. Chandler J.L. 2000. Somatic hybridisation of high yield, cold hardy and disease resistant parents for *Citrus* rootstock improvement, The Journal of Horticultural. Science and Biotechnology 75: 641 – 644

[65] Grosser J.W. Ollitrault P. and Olivares – Fuster O. 2000. Somatic hybridizatio in *Citrus*: an effective tool to facilitate variety improvement. In Vitro Cellular & Developmental Biology. 36: 434 – 449

[66] Gutierrez E.M.A. Luth D. and Moore G.A. 1997. Factors affecting Agrobacterim mediated transformation in *Citrus* and production of sour orange (*Citrus aurantium* L.) plant expressing the coat protein gene of *Citrus* tristeza virus. Plant Cell Reports. 16: 745 – 753

[67] Hammschlag F. Ritchie D. Werner D. Hashmil G. Krusberg L. Meyer R. Huettel R. 1995. In vitro selection of disease reistance in fruit trees. Acta Horticultural. 392: 19 – 26

[68] Hidaka, T., Yamada, Y. and Shichijo, T. 1981. Plantlet formation from anthers of *Citrus aurantium* L. Proceedings of the International Society of Citriculture. 1:153 – 155

[69] Hu J.T. and Kong K.L. 1987. The organogenesis of buds from entire lamina of *Citrus sinensis* in tissue culture and their anatomical observations. International Journal of Fruit Science. 7: 81 – 84

[70] Jaskani M.J. Hassan S. Bashir M.A. Khan I.A 1996. Morphological descriptions of *Citrus* colchiploids. Proceedings of the International Society of Citriculture. 8:37

[71] Jia – Long Y. Jin – Hu W. Gleave A.P. Morris B.A.M. 1996. Transformation of *Citrus* embryogenic cells using particle bombardment and production of transgenic embryos. Plant Science. 113(2): 175 – 183

[72] Kadota M, Niimi Y. 2002. In vitro induction of tetraploid plants from a diploid Japanese pear cultivar (*Pyrus pyrifolia* N. cn. Hosui). Plant Cell Reports. 21: 282 – 286

[73] Kaneyoshi J. Kobayashi S. Nakamura Y. Shigemoto N. and Doi Y. 1994. A simple and efficient gene transfer system of trifoliate orange (Poncirus trifoliata Raf.) Plant Cell Reports. 13: 541 – 545

[74] Kapari – Isaia T. H. Minas G. J. Polykarpou D. Iosephidou E. Arseni S.P. and Kyriakou A. 2002. Shoot-tip grafting in vitro for elimination of viroids and *Citrus* psorosis virus in the local "Arakapas" mandarin in Cyprus. In Proceedings fifteenth IOCV conference. 417 – 419. Riverside. CA: IOCV

[75] Kapari – Isaia T.H. Kyriakou A. Ioannides I. Papayiannis L. and Minas G. 2007. Sanitation of local *Citrus* clones and/or varieties in Cyprus. Acta Horticulturae. 741: 301 – 306

[76] Kaya E. Souza F. Yilmaz – Gokdogan E. Ceylan M. Jenderek M. 2016. Cryopreservation of *Citrus* seed via dehydration followed by immersion in liquid nitrogen. Turkish Journal Of Biology . 41: 242 – 248 . http://dx.doi.org/10.3906/biy-1603-92

[77] Khawale R. N. Singh S. K. Garg G. Baranwal V. K. and Ajirlo S. A. 2006. Agrobacterium – mediated genetic transformation of Nagpur mandarin (*Citrus reticulata* Blanco)," Current Science. 91(12): 1700 – 1705

[78] Kobayashi S. Sakai A. and Oiyama I. 1990. Cryopreservation in liquid nitrogen of cultured navel orange (*Citrus sinensis* Osb.) nucellar cells and subsequent plant regeneration. Plant Cell, Tissue and Organ Culture. 23:15 – 20

[79] Ladania M.S. 2008. *Citrus* Fruit Biology, Technology and Evaluation. Elsevier Inc.. Limmaset P. and Cornuet P. 1949. Recherche de virus de la mosaique du Tabac (Marmor Tabaci Holmes) dans les meristemes des plantes infectees. Comptes Rendus Hebdomadaires des Seances de lAcademie des Sciences. 228, 1971 – 1972

[80] Lai C.S. Li S. Chai C.Y. 2007. Inhibitory effect of Citurs 5 – hydroxy – 36,7,8,3, 4 – hexamethoxyflavone on 12 – O – tetradecanoylphorbol 13 – acetate iinduced skin inflammation and tumor promotion in mice. Carcinogenesis. 28: 2582 – 2588

[81] Lai C.S. Tsai M.L. Cheng A.C. 2011. Chemoprevention of colonic tumorigenesis by dietary hydroxylated polymethoxyflavones inazoxymethane – treated mice. Molecular Nutrition & Food Research. 55: 278 – 290

[82] Larkin P.J. and Scowcroft W. 1981. Somaclonal Variation – A Novel Source of Variability from Cell Cultures for Plant Improvement. Theoretical and Applied Genetics. 60: 197 – 214

[83] Li S. Pan M.H. Lo C.Y. 2009. Chemistry and health effects of poly-methoxyflavones and hydroxylated polymethoxyflavones. Journal of Functional Foods. 1: 2 – 12

[84] Li S. Wang H. Guo L. 2014 Chemistry and bioactivity of nobiletin andits metabolites, Journal of Functional Foods. 6: 2 – 10

[85] Li S. Lin Y.C. Ho C.T. 2014. Formulated extract from multiple *Citrus* peels impairs dendritic cell functions and attenuates allergic contact hypersensitivity. International Immunopharmacol. 20 : 12 – 23

[86] Lombardo G. Alessandro R. Scialabba A. Sciandra M. De Pasquale F. 2011. Direct organogenesis from cotyledons in cultivars of *Citrus* clementina Hort. Ex Tan. Am. Journal of Plant Science. 2: 237 – 244

[87] Lopez – Lazaro M. 2002. Flavonoids as anti – cancer agents: structure activity relationship study. Current Medicinal Chemistry. Anti – cancer Agents. 2: 691 – 714

[88] Louzada E.S. Jiang J. Baergen K. Quiros A. Cabasson C. Schell J.L., Chandler J.L. 1996 Allotetraploid hybrids between *Citrus* and seven related genera produced by somatic hybridization, Theoritical and Applied Genetics. 92: 577 – 582

[89] Luth D. and Moore G.A. 1997. Transgenic grapefruit plants obtained by *Agrobacterium* tumifaciens – mediated transformation. Plant Cell Reports. 57: 219 – 222.

[90] Ma N. Lai C.S. Chung C.H. 2014. 5 – Demethyltangeretin is morepotent than tangeretin in inhibiting dimethylbenz(a)anthrene (DMBA)/12-O-tetradecanoylphorbol – 13 – acetate (TPA) induced skin tumorigenesis. Journal of Functional Foods http://dx.doi.org/10.1016/j.jff.2014.08.009

[91] Marin M.L. Duran – Vila, N. 1988. Survival of somatic embryos and recovery of plants of sweet orange [*Citrus sinensis* (L.) Osb.] after immersion in liquid nitrogen. Plant Cell Tissue and Organ Culture. 14: 51 – 57. http://dx.doi.org/10.1007/BF00029575

[92] Marin M.L. Gogorcena Y. Ortiz J. and Duran – Vila N. 1993. Recovery of whole plants of sweet orange from somatic embryos subjected to freezing thawing treatments. Plant Cell, Tissue and Organ Culture 34:740 – 476

[93] Mendes-da-Gloria F.J. Mourao Filho F.A.A. Camargo L.E.A. Mendes B.M.J. 2000. Caipira sweet orange + Rangpur lime: a somatic hybrid with potential for use as rootstock in the Brazilian *Citrus* Industry. Genetics and Molecular Biology 23: 661 – 665

[94] Moore G.A. 1985. Factors affecting in vitro embryogenesis from undeveloped ovules of mature *Citrus* fruit. Journal of the American Society for Horticultural Science. 110: 66 – 70

[95] Moore G. A. Jacono C. C. Neidigh J. L. Lawrence S. D. and Cline K. 1992. Agrobacterium – mediated transformation of stem segments and regeneration of transgenec plants. Plant Cell reports. 11: 238 – 242

[96] Moore G. A. Jacono C. C. Neidigh J. L. Lawrence S. D. and Cline K. 1993. Transformation in *Citrus*. In Plant Protoplasts and Genetic Engineering IV.. Bajaj Y. P. S Ed. 23: of Biotechnology in Agriculture and Forestry. 194 – 208. Springer. Berlin Germany

[97] Mukhopadhyay S.J. Raj B.C. Sharma A. Gurung R.K. Nath P.S. 1997. Micropropagation of Darjeeling orange (*Citrus reticulata* Blanco) by shoot-tip grafting. Journal of Horticultural Science and Biotechnology. 72(3): 493 – 499

[98] Mukhtar R. Khan M.M. Fatima B. Abbas M. Shahid A. 2005a. In vitro regeneration and multiple shoots induction in *Citrus reticulata* (Blanco). International Journal of Agriculture and Biology. 7(3): 414 – 416

[99] Mukhtar R. Khan M.M. Rafiq R. Shahid A. Khan F.A. 2005b. In vitro regeneration and somatic embryogenesis in (*Citrus aurantifolia* and *Citrus sinensis*). International Journal of Agriculture and Biology. 7(3):518 – 520

[100] Muranaka T. Saito K. 2010. Production of pharmaceuticals by plant tissue cultures. In: Mander, L., Liu, H.W. (Eds.), Comprehensive Natural Products II: Chemistryand Biology. 3: 615 – 628

[101] Murashige T. and Tucker D.P.H. 1969. Growth factor requirements of *Citrus* tissue culture. Proceeding of First International *Citrus* Symposium. 3: llSS – 1161

[102] Nauer E.M. Roistacher C.N. Carson T.L. and Murashigue T. 1983. In vitro shoot tip grafting to eliminate *Citrus* viruses and virus-like pathogens produces uniform budlines. Hortscience. 18: 308 – 309

[103] Navarro L. Roistacher C. N. and Murashige T. 1975. Improvement of shoot-tip graftingin vitro for virus free *Citrus*. Journal of the American Society for Horticultural Science. 100: 471 – 479

[104] Navarro L. Juarez J. Ballester J.F. Pina J.A. and OrtegaC. 1979. Obtaining nucellar virus – free plants of different *Citrus* cultivars of the navel group by means of ovule culture in vitro. Anales del Instis Tuto nacional de investigaciones Agrarias. proteccion vegetal. No. 12, 95 – 113 . [Hort. Abst. 51(1): 779]

[105] Navarro L. Roistacher C. N. and MurashigeT. 1976 . Effect of size of shoot tips on psorosis – A and exocortis content of navel orange plants obtained by shoot-tip grafting in vitro. In Proceedings of the seventh IOCV conference 194 – 197. Riverside, CA: IOCV

[106] Navarro L. 1988. Application of shoot-tip grafting in vitro to woody species. Acta Horticulturae. 227: 43 – 56

[107] Navarro L. Civerolo E. L. Juarez J. and Garney S. M. 1991 . Improving therapy methods for *Citrus* germplasm exchange. In Proceedings eleventh IOCV conference . 400 – 408. Riverside. CA: IOCV.

[108] Navarro L. Juarez J. and Pina J. A. 2001. Strategies and problems for the production ofhigh quality nursery *Citrus* trees worldwide. In Proceedings of the sixteenth world congress of the international society of *Citrus* Nurserymen Brazil. 1 – 10

[109] Niaz A.C Maken M.N. and Malik S.A. 2004. Native Home, Historical Background and Importance of *Citrus* Fruitsin Pakistan. Proceedings of the 1st International Conference on Citriculture. University of Agriculture Faisalabad. 48 – 56

[110] Nito N. 1996. In : Second International Crop Science Congress. Nov. 17 – 25 , New Delhi, India. Page 40 (Abs)

[111] Nito N. and Iwamasa M. 1990. In vitro plantlet formation from juice vesicle callus of Satsuma (*Citrus unshiu* Mare.). Plant Cell, Tissue and Organ Culture 20(2): 137 – 140

[112] Notsuka K. Tsuru T. Shiraishi M. 2000. Induced polyploidy in grapes via in vitro chromosome doubling. Journal of the Japanese Society for Horticultural Science. 69: 543 – 551

[113] Nuria S. Montserrat P. Carmen F. Pedro M. Luis N. Ricardo F. Leandro P. L. 2012. Transformation of Mexican lime with an intron-hairpin construct expressing untranslatable versions of the genes coding for the three silencing suppressors of *Citrus* tristeza virus confers complete resistance to the virus. Plant Biotechnology. 1 – 12

[114] Oiyama I. 1992. Studies on polyploidy breeding in *Citrus* with special reference to the production of tetraploid breeding. Bulletin of the Fruit Tree Research Station. 3:68

[115] Olivares – Fuster, O. Asins M.J. Duran –Villa, N. Navarro L. 2000. Cryopreservation of callus, a source of protoplasts for *Citrus* improvement. J. Hort. Sci. Biotech. 7: 635 – 640. http://dx.doi.org/10.1080/14620316.2000.11511299

[116] Ollitrault P. Dambier D. Luro F. 1996. Somatic hybridization in *Citrus*, Some new hybrids and cybrids, VIII Congress. International. Society. Citriculture Sun City Resort, South Africa. Page 98

[117] Ollitrault P. Dambier D. Lofty S. Rist D. Mass O. Luro F Frolicher Y. 2000. *Citrus* germplasm exploitation by somatic hybridization, IXth Congress, International. Society. Citriculture, Orlando – Florida. Page 122

[118] Paudyal K.P. Haq N. 2000. In vitro propagation of pummelo (*Citrus grandis* L.Osbeck), In Vitro Cellular and Developmental. Biology Plant. 36: 511 – 516

[119] Pena L. Cervera M. Juarez J. Navarro A. Pina J.A. and Duran – Vila N. 1995a. *Agrobacterium* – mediated transformation of sweet orange and regeneration of transgenic plants. Plant Cell Reports. 14: 616 – 619

[120] Pena L. Cervera M. Juarez J. Navarro A. Pina J. and Navarro L. 1997. Genetic transformation of lime (*Citrus aurantifolia* swing.) factors affecting transformation and regeneration. Plant Cell Reports. 16: 731 – 737

[121] Perez R.M. Navarro L. Duran – Vila, N., 1997. Cryopreservation and storage of embryogenic callus cultures of several *Citrus* species and cultivars. Plant Cell Reports. 17: 44 – 49. http://dx.doi.org/10.1007/s002990050349

[122] Perez – Molphe – Balch E. and Ochoa – Alejo N. 1998. Regeneration of transgenic plants of Mexican lime from *Agrobacterium* rhizogenes-transformed tissues. Plant Cell Reports. 17(8): 591 – 596

[123] Perveen A. and Qaiser M. 2005. Pollen Flora of Pakistan XLV. Rutaceae. Pakistan Journal of Botany. 37: 495 – 501

[124] Rangan T.S. Murashige T. and BittersW.P. 1968. In vitro initiation of nucellar embryos in monoembryonic *Citrus*. HortScience 3(4): 226 – 227

[125] Puricelli L. Innocenti G. Delle Monache G. Caniato R. Filippini R. Cappelletti E.M. 2002. In vivo and in vitro production of alkaloids by Haplophyllum patavinum. Natural Product Letters. 16: 95 – 100

[126] Raj Bhansali R. and Arya H.C. 1979. Organogenesis in *Citrus limetoides* Tanaka (sweet lime) callus culture. Phytomorphology 28: 97 – 100

[127] Ralphs M.H. Gardner D.R. 2001. Distribution of norditerpene alkaloids in tall lark-spur plant parts through the growing season. Biochemical Systematics and Ecology . 29: 117 – 124

[128] Reforgiato R. G. Russo G. Recupero S. 2005 New promising *Citrus* triploid hybrids selected from crosses between monoembryonic diploid female and tetraploid male parents. Horticultural Science. 40: 516 – 520

[129] Roistacher C. N. Navarro L. Murashige T. 1976. Recovery of *Citrus* selections free of several viruses, exocortis viroid and spiroplasma citri by shoot-tip grafting in vitro. Proceeding of 7th International Organization of *Citrus* Virologists. IOCV, Riverside. 186 – 193

[130] Sabharwal P.S. 1963. In vitro culture of ovule, nucelli and embryo of *Citrus reticulata* var. Nagpuri Swingle. In: P. Maheshwari and N.S. Rangaswamy (Editors), Plant Tissue and Organ Culture. Intemational Society of Plant Morphology. Delhi University. Delhi pp 255 – 274

[131] Sakai A. Kobayashi S. and Oiyam I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* var brasiliensis Tanaka) by vitrification. Plant Cell Reports. 9: 30 – 33

[132] Sakai A. Kobayashi S. and Oiyam I. 1991. Survival by vitrification of nucellar cells of navel orange (*Citrus sinensis* var brasiliensis Tanaka) cooled to 196°C. Plant Physiology. 137: 465 – 470

[133] Salmore A.K. Hunter M.D. 2001. Environmental and genotypic influences onisoquinoline alkaloid content in Sanguinaria canadensis. Journal of Chemical Ecology . 27: 1713 – 1747 `

[134] Santos Filho H.P. Paguio O.R. Coelho Y.S. Medina V.M. 1984. The *Citrus* variety improvement program in Brazil. Fifth International *Citrus* Congress. 2: 235 – 237

[135] Sarma C. Borthakur A. Singh S. Mod M.K. Sen P. 2011. Efficient in vitro plant regeneration from cotyledonary explants of *Citrus reticulata* L. Blanco. 2(6): Annals of Biological Research. 341 – 348

[136] Sauton A. Mouras A. and Lutz A. 1982 . Plant regeneration from *Citrus* root meristems. Horticultural Science. 57: 227 – 231

[137] Savita Vijay Virk G.S. Nagpal A. 2010. Effect of explant type and different plant growth regulators on callus induction and plantlet regeneration in *Citrus* jambhiri Lush. Environ. International science technology. 5: 97 – 106

[138] Savita Singh B. Virk G.S. Nagpal A.K. 2011 . An efficient plant regeneration protocol from callus cultures of *Citrus jambhiri* Lush. Physiology and Molecular Biology of Plants 17(2): 161 – 169

[139] Sidhu O.P. Bel H.M. 1996. Seasonal variation in azaradirachtin in seeds of Azadirachta indica. Current Science. 70: 1084 – 1085

[140] Sim G.E. Goh C.J. and Loh C.S. 1989 . Micropropagation of *Citrus* mitis Blanco multiple bud formation from shoot and root explants in the presence of 6-benzylaminopurine. Plant Science 59: 203 – 210

[141] Singh I. P. 2001 . Shoot-tip grafting of Mosambi (C. sinensis L. Osbeck) in India. In Proceedings sixteenth world congress of the international society of *Citrus* Nurserymen Brazil. 128 – 130

[142] Singh I. P. 2002. Micropropagation of *Citrus* – A review. The Agricultural History Review. 23(1): 1 – 13

[143] Singh B. Kaur A. 2011 . Comparison of agar and gum karaya as gelling agent for in vitro regeneration of rough lemon (*Citrus jambhiri* Lush.) plantlets from nodal explants. Journal of Crop Science and Biotechnology. 14(4): 297 – 303

[144] Smetanska I. 2008. Production of secondary metabolites using plant cell cultures. Advances in Biochemical Engineering / Biotechnology. 111: 187 – 228

[145] Starrantino A. and Russo F. 1980. Seedlings from undeveloped ovules of ripe fruits of polyembryonic *Citrus* cultivars. HortScience 15(3): 296 – 297

[146] Thorpe T. 2007. History of plant tissue culture. Molecular Biotechnology 37:169 – 180

[147] Tripoli E. Guardia M.L. Giammanco S. 2007. *Citrus* flavonoids: molecular structure, biological activity and nutritional properties. a review. Food Chemistry. 104: 466 – 479

[148] Tusa N. Grosser J.W. Gmitter F.G. 1990. Plant regeneration of 'Valencia' sweet orange, 'Femminello' lemon and the interspecific somatic hybrid following protoplasm fusion. Journal of the American Society for Horticultural Science. 115 (6): 1043 – 1046

[149] Usman M. 2005. Plant propagation and improvement, in *Citrus* Nursery Raising: Principles and Practices. Mass Publication. Pakistan. 23 – 66

[150] Van Le B. Thanh Ha N. Anh Hong L. T. and Tran ThanVan K. 1999. High frequency shoot regeneration from trifoliate orange (Poncirus trifoliata L. Raf.) using the thin cell layer method. Comptes Rendus de l'Academie des Sciences. 322(12): 11056 – 11111

[151] Vardi A. and Galun E. 1989. Isolation and culture of *Citrus* protoplasts. In: Biotechnology in agriculture and forestry. (Bajaj,Y.P.S.,Ed.). Springer Verlag Berlin Heidelberg. 8:147 – 159

[152] Vardi A. Bleichman S. and Aviv D. 1990. Genetic transformation of *Citrus* protoplasts and regeneration of transgenic plants. Plant Science. 69: 199 – 206

[153] Vishwanath W. And Narayan P. 2015. In Vitro Multiplication of important horticultural Plant *Cirus reticulata* Blanco. International Journal of Pharmacy and Biological Sciences. 6(1) (B): 1275 – 1280

[154] Wang Z.C. Deng X.X. 2004. Cryopreservation of shoot tips of *Citrus* using vitrification:effect of reduced form of glutathione. Cryo Letters. 25: 43 – 50

[155] Wang L. Wang J. Fang L. 2014. Minireview on the anticancer activities of *Citrus* peel polymethoxyflavones related to angiogenesis and others. BioMed Research International 453972 1 – 10 Article ID

[156] Yaacob J.S. Mahmad N. Taha R.M. Mohamed N. Yussof A.I.M. Saleh A. 2014. Optimization of culture conditions (sucrose, pH, and photoperiod) for in vitro regeneration and early detection of somaclonal variation in ginger lime (*Citrus assamensis*). The Scientific World Journal ., Article ID 262710. 1 – 9

[157] Yang L. and Hu C. 2011. Transformation of sweet orange [*Citrus sinensis* (L.) Osbeck] with pthA-nls for acquiring resistance to *Citrus* canker disease. Plant Mol Biol. 75(1) :11 – 23

[158] Yao J.L. Wu J.H. Gleavea A.P. and Morris B.A.M. 1996. Transformation of *Citrus* embryogenic cells using particle bombardment of transgenic embryos. Plant Science. 113: 175 – 183

[159] Yuan G. Shui - Gen L.i. Xiao – Fen F.. and Zhen – Hong S. 2016. Application of Somatic Embryogenesis in Woody Plants. Frontiers in Plant Science. 7: 938.

Processing and sensory properties of high-fibre bakery products

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Abstract— *The present study was conducted to improve the nutritional composition of cakes and biscuits which are generally made with refined flour. Refined wheat flour was substituted with Germinated Sorghum Flour and Plantain Flour. Process parameters such as height, diameter, spread ratio and spread factor were recorded for biscuits. For cakes, process parameters such as batter pH, acidity, density, baking temperature and cake volume were assessed. Weight of biscuits increased from 8.05 g in control to 9.27 g in variation made with germinated sorghum and plantain. There was significant reduction ($p<0.05$) in spread ratio of biscuits with increased amount of germinated sorghum and plantain flours. The spread ratio reduced from 7.63 (control) to 6.68 indicating less spread of biscuits during baking. In conclusion, substitution of refined wheat flour with germinated sorghum and plantain provides dual benefits of improving nutritional quality of the baked products and also it improves process parameters which are essential for scale-up during commercial production of biscuits and cakes.*

Keywords— Bakery products, germinated sorghum flour, plantain, high fibre, cakes, biscuits.

I. INTRODUCTION

Fibre is an important dietary component that has several beneficial roles in our body. Various studies have proved that there is a relationship between the lack of fiber in the diet and the incidence of several common diseases [1]. The diseases commonly associated with low-fiber diets include coronary heart disease, diabetes mellitus, diverticular disease, colon cancer, appendicitis, and dental caries. In today's changing world, consumer's preference of taste has led to refinement of flours to make bakery products with improved textural properties and taste. Consequently, the fiber content of diets has also reduced. Substitution of refined flours with whole grains has potential to improve the nutritional value and fiber content of the food products. Whole grains and millets such as sorghum, ragi etc. can be used as a substitute of refined flours. Sorghum and plantain (Green banana) are rich sources of Resistant Starch (RS) and can be used in many products to increase their fiber content.

Sorghum: Sorghum is also known as jowar in India, belongs to the tribe Andropogonae of the grass family Poaceae. It is a gluten-free cereal and can also be used for celiac patients [2]. It is rich in phytochemicals, phenolics, sterols, and anthocyanins [3]. It also provides minerals such as magnesium, copper, manganese, iron and zinc and vitamins such as thiamin, riboflavin, niacin, vitamin B-6 and pantothenate [4]. Sorghum can be used to make different kinds of food products. Products like bread and cookies have been made with sorghum bran incorporated up to 15 and 30 % without any significant differences in texture or flavor profiles compared to whole wheat products [5]. Other examples include porridges [6], germinated malt products, which include porridges and some alcoholic and non-alcoholic beverages, like Mahewu, which is produced in Zimbabwe [7]. The germinated sorghum flour can be used in preparation of bread, muffins, biscuits etc. Another well-established use of sorghum is in production of leavened baked goods such as wheat–sorghum composite bread [8].

Plantain: Plantain (Green banana) is a traditional product which can be dried to form flour and can be used to substitute refined flours. It is a good source of carbohydrates and dietary fiber [9]. Plantain contains higher hemicelluloses content (6.08%) than most fruits and vegetables. Apart from dietary fiber, it is also good source of some essential minerals such as potassium, and various vitamins (A, B1, B2 and C) [10]. It also has high nutraceutical potential [11]. Plantain powder or flour has been used widely as an ingredient in the food industries [12]. Many research works have proved that consumption of plantain is beneficial to human health due to its resistant starch (RS) content [13].

Health benefits of Resistant starch (RS): "Resistant starch has been defined as the fraction of starch, which escapes digestion in the small intestine but is digested in the large intestine after 5 to 7 hours of consumption" [14]. Fermentable fiber and RS are associated with improved glucose tolerance. There is evidence that this is caused by the presence of the short chain fatty acids (SCFA) and by a peptide produced in the fermentation process [15]. Resistant starch (RS) has been proved to be beneficial for digestive health as it increases stool bulk, acts as a mild laxative, promotes the growth of healthy bacteria in the gut [16] and reduces pH in the intestine. SCFA also reduces the risk of ulcerative colitis and colon cancer by promoting the lipid and cholesterol metabolism [17]. RS also help in the management of diabetes by reducing the glycemic index of food

[18], [19]. RS does not cause sensory changes in food products [20]. It provides better appearance; texture and mouth- feel than conventional fibers to food products [21].

II. MATERIAL AND METHOD

1.1 Selection and procurement of raw materials

The study comprised of development of two types of bakery products. For biscuit preparation, refined wheat flour, plantain flour, germinated sorghum flour, sugar, butter, baking powder and milk were used. All above-mentioned ingredients were also used in preparation of cakes along with eggs.

1.2 Preparation of Plantain Flour (PF)

PF was prepared according to the method given by [22]. The plantain was peeled and sliced into thin slices of 2-3 cm each. These slices were washed to remove stickiness. Washed slices were dried at room temperature for moisture evaporation and then dried in a dehydrator at 55^0C for 3 days. The dehydrated banana slices were grounded in a mixer to form the flour.

1.3 Preparation of Germinated Sorghum Flour (GSF)

GSF was prepared using method given by [23]. Sorghum grains were steeped in distilled water at room temperature overnight. 1–2 ml of 0.2% formaldehyde solution was added for 40 min to retard mold growth during germination. The grains were washed 4-5 times and soaked for 20 minutes in distilled water to remove formaldehyde. Washed grains were spread on jute a bag saturated with distilled water and allowed to germinate for 5 days at room temperature. After germination, the grains were dried in dehydrator at 60 ^{0}C for fiber days and grounded to a fine powder.

1.4 Development of bakery products (cakes & biscuits) using Plantain Flour (PF) & Germinated Sorghum Flour (GSF)

Cakes and biscuits were selected to substitute Refined Wheat Flour (RWF) with GSF & PF. Equal ratios of GSF & PF (1:1) were mixed to form a flour mix. This flour mix of GSF & PF was used to substitute RWF to prepare biscuits and cakes. Five variations of biscuits and cakes were prepared by substituting the RWF with a flour mix. The variations used in product development are given in Table 1. Short dough biscuits and cream cakes were prepared using the basic recipe.

TABLE 1
COMBINATIONS OF FLOURS USED FOR DEVELOPMENT OF BAKERY PRODUCTS

Variations	Refined Wheat Flour (RWF)	Plantain & Germinated Sorghum Flour (1:1) (GSF + PF)
Code 1 (control)	100	0
Code 2	60	40
Code 3	40	60
Code 4	20	80
Code 5	0	100

1.5 Process parameters for biscuits

Weight, Height, diameter, spread ratio and baking time and the temperature was done as process parameters for biscuits.

1.5.1 Weight: The weight of the baked biscuit was determined by weighing 6 biscuits on a weighing balance and calculating mean weight.

1.5.2 Height: The height was measured before and after baking. The thickness of biscuits was determined by placing six biscuits on top of one another. The total height was recorded in millimeters with the help of a ruler. This process was repeated thrice to get an average value and results were reported in mm [24].

1.5.3 Diameter: The diameter was measured before and after baking. The diameter of biscuits six biscuits was determined by placing six biscuits edge to edge. The total diameter of the six biscuits was measured in mm by using a ruler. The biscuits were rotated at an angle of 90° for duplicate reading. The process was repeated twice and average diameter was reported in millimetre [24].

1.5.4 Spread ratio: Three rows of five well-formed biscuits were made and the height measured. Also the same was arranged horizontally edge to edge and sum diameter measured [25]. Spread ratio of a cookie is the diameter divided by its thickness. Spread ratio and spread factor were calculated using the following equation:

Formula used

$$\text{Spread Ratio}=\frac{\text{Diameter}}{\text{Height}}$$

$$\text{Spread factor}=\frac{\text{Spread ratio of sample}}{\text{Spread ratio of control}}\times 100$$

1.6 Process parameters for cakes

The process parameters of cakes include estimation of batter pH, batter density, batter acidity, baking time, baking temperature and cake volume.

1.6.1 Batter pH: The pH meter was standardized using standard buffer solution at 25 ^{0}C. The sample was taken in a beaker and pH was measured by a digital pH meter [26].

1.6.2 Batter density: Batter density was determined as the ratio of the weight of a standard container filled with batter to that of the same container filled with water.

1.6.3 Batter acidity: The acidity of the cake batter was measured by taking 5 gm of batter in a beaker. The batter was mixed with 10 ml of water and titrated against 0.1N NaOH using phenolphthalein as indicator.

1.6.4 Baking time: Baking time was measured using a digital watch. Baking time was measured as the time required for cakes to turn golden brown in the oven during baking.

1.6.5 Baking temperature: It is measured using a calibrated laboratory thermometer.

1.6.6 Cake volume: Cake volume was measured with a calibrated ruler. The volume was calculated using following by dividing height of the batter with final height of the cake after baking.

1.7 Sensory evaluation of biscuits and cakes

Sensory evaluation of bakery products (biscuits and cakes) was conducted with a panel of 30 members comprising of students and faculty members. The products were analyzed for appearance, Color, Taste, Texture, Aftertaste and each parameter was rated on a score of 25 and compared to control.

III. RESULTS AND DISCUSSION

The use of plantain and sorghum has been well established in the past as a gluten-free alternative to wheat. In the present study, Plantain Flour (PF) and Germinated Sorghum Flour (GSF) were used to substitute Refined Wheat Flour (RWF) in preparation of bakery products (cakes and biscuits). The yield obtained for Plantain Flour (PF) was 64.7% and 94.5% for Germinated Sorghum Flour (GSF). Five variations of cakes and biscuits were developed using five different ratios of Refined Wheat Flour (RWF), GSF and PF (Table 1). Germinated Sorghum Flour (GSF) and Plantain Flour (PF) were mixed in equal ratios and this flour mix was used to replace Refined Wheat Flour (RWF) in preparation of biscuits and cakes. Process parameters were recorded for biscuits and cakes to ascertain changes occurring as a result of incorporating high-fiber flour mix.

3.1 Process parameters of biscuits

The weight of the biscuits increased from 8.05 g in code 1 to 9.27 g in code 5 indicating that there is increase in weight of the biscuits with increased incorporation of GSF and PF. The differences in the weight of biscuits could be due to increased fiber content. Mean diameter of the biscuits) prepared from different ratios of flours containing RWF, GSF & PF varied significantly ($p<0.05$) between the variations and control (Table 2). It was observed that diameter of the biscuits was decreasing with higher ratios of GSF & PF. The diameter of code 5 was 54.90 mm which was prepared with GSF & PF whereas significantly high diameter was observed in code 1 (56.5 mm) which was made up of RWF only. Incorporation of GSF and PF improved the binding properties of biscuits and prevents spread during baking. The height of the biscuits was affected positively with increase in the amount of Germinated Sorghum Flour (GSF) and Plantain Flour (PF). A gradual increase was observed in the height of the biscuits as the level of GSF and PF were increased and RWF was reduced. The

height noted in code 1 was 7.2 mm and 8.4 mm in code 5. The height of the biscuits was affected positively as there was an increase in the height of the biscuits by increasing levels of Moth bean Flour supplementation [27]. The height of biscuits measured in millimetres, signifies the increase in thickness of biscuits after baking due to expansion. More height of biscuits could be due to higher amounts of crude fiber and crude protein incorporated in the biscuits with higher ratios of GSF and PF. The height of the biscuits also affects spread ratio of biscuits. Spread ratio or diameter of cookies has been used to determine the quality of flour for producing cookies [28]. Flour and sugar are the main hydrophilic components in cookies that influence biscuit spread. Hydrophilic starches have a negative relation with spread ratio of cookies. During baking, hydrophilic starch granules absorbed moisture and become swollen and gelatinized, thus providing the additional viscosity in cookies [29]. Gluten and fiber present in flours are hydrophilic in nature which retains water and increases viscosity. The hydrophilic compound in GSF and PF are starch & fiber which are present in high amounts. It increases viscosity resulting in a decrease in spread ratio. The spread ratio of biscuits decreased from 7.63 in control to 6.68 in code 5. Spread factor is the ratio that depends on the values of the thickness and diameter of the biscuits. In the present study, the spread factor of biscuits gradually decreases as the amount of GSF and PF increases. The spread factor of biscuits decreased from100 in control biscuits to 87.54 in code 5 (Table 2).

TABLE 2
PROCESS PARAMETERS OF ALL VARIATIONS OF BISCUITS

Variations	Weight (g)	Diameter (mm)	Height (mm)	Spread Ratio
Code1(Control)	8.05	56.50^{a}	7.20^{a}	7.63^{a}
Code 2	8.24	56.70^{bc}	7.40^{ab}	7.47^{ab}
Code 3	8.29	56.10	7.40^{ab}	6.95^{ab}
Code 4	9.06	55.30^{c}	8.20^{ab}	6.80^{ab}
Code 5	9.27	54.90^{ab}	8.40^{ab}	6.68^{ab}

All values within column having same superscripts are significantly different (p<0.05)

3.2 Baking Time & Temperature of biscuits

Baking time and temperature of biscuits were measured for all the five variations. Time was measured using a stopwatch and measured till the biscuits turned golden brown. The temperature was measured using a calibrated lab thermometer. It was observed that with increased incorporation of GSF and PF, there is decrease in baking time of biscuits but the baking temperature remains the same (Table 3).There is a decrease in baking time from 20 minutes in code 1 to 15 minutes in code 5. The baking temperature of all variations of biscuits was same i.e. 175 ^{0}C (Table 3). The decrease in time of baking with higher levels of Germinated Sorghum Flour (GSF) and Plantain Flour (PF) could be attributed to the slightly darker color of these flours as compared to Refined Wheat Flour (RWF). The darker color of these two flours gives golden brown color to the biscuits in less time as compared to RWF biscuits.

TABLE 3
BAKING TIME & TEMPERATURE OF BISCUITS

Variation	Baking temperature	Baking time
Code1(Control)	175^{o}C	20 mins
Code 2	175^{o}C	18mins
Code 3	175^{o}C	16 mins
Code 4	175^{o}C	16 mins
Code 5	175^{o}C	15 mins

3.3 Process Parameters for Cakes

The results obtained are presented in Table 4 and 5. A decrease in pH and increased titratable acidity was noted in cake batters as GSF and PF were increased. The specific volume of baked cake indicates the amount of air that can remain in the final product. A higher gas retention and higher expansion of the product leads to a higher specific volume [30]. After baking, the specific volumes of cake with incorporation of flours were determined and a significantly higher value with increasing GSF and PF indicates a more volume of air retained in the cakes. Cake batters containing GSF and PF with lower batter density (code 5, Table 4) exhibited higher gas retention and resulting in a higher specific volume (2.8), due to the expansion of gas retained in the batter. As the amount of GSF and PF are increased in the batter formulation, the density of

batter decreases from 1.09in code 1 to 1.0 in code 5 (Table 4). With the increased incorporation of GSF and PF, there was a decrease in baking time from 20 minutes in code 1 to 15 minutes in code 5 (Table 5) but the baking temperature remains the same (185^{o}C for all variations).

TABLE 4
PROCESS PARAMETERS FOR VARIATIONS OF CAKE

Variations	Batter density	Batter pH	Batter Acidity (%)	Cake volume (cm$^{3)}$
Code1 (Control)	1.09	7	0.35	2.0^{ab}
Code 2	1.087	7.2	0.3	2.55^{ab}
Code 3	1.076	7.12	0.24	2.5^{ab}
Code 4	1.04	7	0.13	2.41^{ab}
Code 5	1	6.8	0.07	2.8^{ab}

Mean values in a column having different alphabets are significantly different from control (P < 0.05).

TABLE 5
BAKING TIME & TEMPERATURE OF VARIATIONS OF CAKE

Variation	Baking temperature	Baking time
Code1(Control)	185^{o}C	20 mins
Code 2	185^{o}C	20 mins
Code 3	185^{o}C	18 mins
Code 4	185^{o}C	16 mins
Code 5	185^{o}C	15 mins

3.4 Sensory Evaluation of biscuits and cakes

Sensory evaluation of cakes and biscuits was performed by a panel of 30 members comprising of faculty members and students of Institute of Home Economics. Panelists analyzed the product for appearance, color, taste, texture, aftertaste and overall acceptability of the product. All parameters were rated on a score sheet of 100 marks where each attribute was given 25 marks. The results of sensory evaluation of biscuits are represented in Fig. 1. The results reveal that taste (22.4), flavor (22.63) and texture (22.51) of code 2 is very high as compared to code 1 (control) and other variations. Hence it can be concluded that the sensory properties of variations (code 2 – code 5) were comparable to the control (code 1) and variations were equally acceptable. Therefore, substitution of RWF with fiber-rich components such as GSF and PF was found suitable for bakery products and biscuits can be prepared with acceptable sensory qualities. Five variations of cakes were prepared using refined wheat flour (RWF), Germinated Sorghum Flour (GSF) & Plantain Flour (PF). The control sample (code 1) was prepared using RWF. Other 4 variations were prepared using different ratios of RWF; GSF & PF were mixed to prepare cakes rich in fiber. GSF & PF were mixed in equal ratios (1:1) for substituting RWF. The results exhibit that taste (21.96), flavor (21.73) and texture (21.76) of code 2 is comparable with the taste (22.73), flavor (22.16) and texture (22.06) of code 1. The code 5 (22.33) was highly acceptable in terms of flavor as compared to control and other variations (Fig. 2). Substitution of RWF with conventional sources of fiber such as GSF and PF is suitable and bakery products and cakes can be made with acceptable sensory qualities.

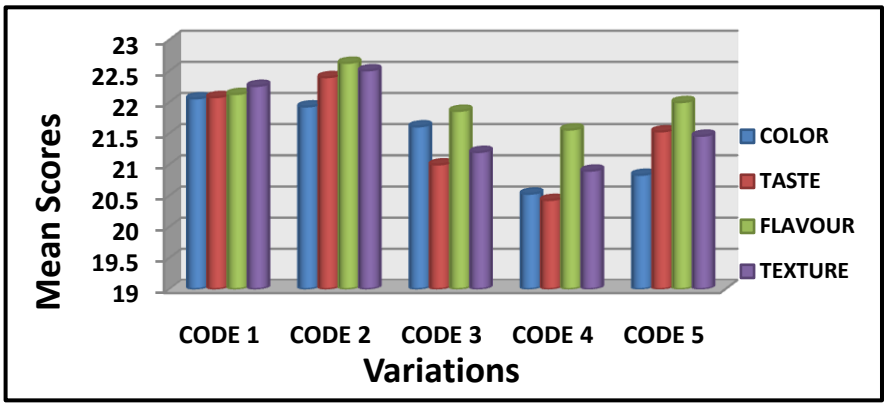

FIG. 1: SENSORY EVALUATION OF BISCUITS

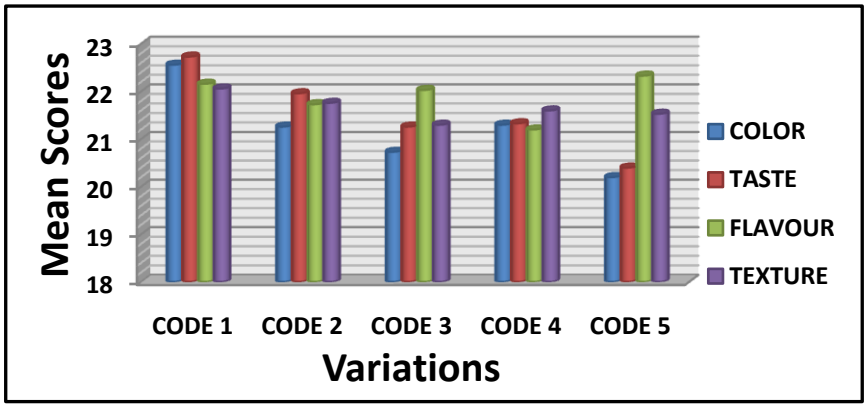

FIG. 2: SENSORY EVALUATION OF CAKES

IV. CONCLUSION

In conclusion, the biscuits and cakes can be made with non-traditional ingredients without affecting the sensory properties of these products. Germinated sorghum and plantain are low cost ingredients and can be used to replace refined flours. These flours can also be used for people who suffer from gluten intolerance and need to substitute their diet with gluten free flours. Resistant starch present in plantain can improve gastrointestinal health and have positive effect of gut microflora. The base material used for preparation of biscuit and cake is refined wheat flour which is low in fiber. Refined Wheat Flour can be replaced by fiber rich materials such as plantain flour and germinated sorghum flour. This will lead to the development of more nutritious products like bakery products (cakes & biscuits). Incorporation of plantain flour & germinated Sorghum flour in equal ratios (1:1) is useful as it increases fiber content, protein and minerals like calcium, phosphorus etc. in the baked goods. Refinement of flours has led to a reduction in fiber content of our diets. It is important to address the issue and target improvement of products where refined wheat flour is a major ingredient.

REFERENCES

[1] T. L. Raymond, W. E. Connor, D. S. Lin, S. Warner, M. M. Fry, S. L. Connor, "The interaction of dietary fibres and cholesterol upon the plasma lipids and lipoproteins, sterol balance and bowel function in human subjects," J. Clin. Invest., 1977, vol. 60, PP. 142–143.

[2] C. L. Ciacci, N. N. Maiuri, C. Caporaso, L. Bucci, Del Giudice, D.R. Massardo, P. Pontieri, N. Di Fonzo, S.R. Bean, B. Ioerger, M. Londei, "Celiac disease: In vitro and in vivo safety and palatability of wheat-free sorghum food products," Clinical Nutrition, 2007, vol. 26, pp. 799-805.

[3] Awika, J. M., and Rooney, L. W. Sorghum phytochemicals and their potential impact on human health. Phytochem. 2004, vol. 65, pp. 1199-1221.

[4] USDA, "National Nutrient Database for Standard Reference," Release 24, 2011.

[5] C. M. Mitre-Dieste, L. A. Gordon, J. Awika, E. L. Suhendro, L.W. Rooney, "Cookies made with sorghum brans high in phenols and catechins," Phytochemistry, 2000, vol. 62 (9), pp. 1199-1221.

[6] J. Taylor and J. R. N. Taylor, "Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges," International Journal of Food Science and Technology, 2002, vol. 37, pp. 129-137.

[7] J. M. Bvochora, J. D. Reed, J. S. Read, R. Zvauya, "Effect of fermentation processes on proanthocyanidins in sorghum during preparation of Mahewu, a non-alcoholic beverage," Process Biochem, 1999, vol. 35, pp. 21-25.

[8] L. Munck, "New milling technologies and products: Whole plant utilization by milling and separation of the botanical and chemical components," In: Dendy, D.A.V. (Ed.), Sorghum and Millets: Chemistry and Technology, American Association of Cereal Chemists, St. Paul, MN, USA, 1995, pp. 223–281.

[9] M. Ovando-Martinez, S. G. Sáyago-Ayerdi, E. Agama-Acevedo, I. Goñi, L. A. Bello -Pérez, "Unripe banana flour as an ingredient to increase the indigestible carbohydrates of pasta. Food Chemistry," 2009, vol.113, pp. 121-126.

[10] Chandler S. The nutritional value of bananas. In Gowen SR (ed.) Bananas and plantains. Chapman and Hall, London, 1995, pp. 74-89.

[11] R. V. Mota, F. M. Lajolo, C. Ciacco, B. R. Cordenunsi, "Composition and functional properties of banana flour from different varieties," Starch/Sta¨rke, 2000, vol. 52, pp. 63–68.

[12] N. Faisant, A. Buléon, P. Colonna, C. Molis, S. Lartigue, J. P. Galmiche, "Digestion of raw banana starch in the small intestine of healthy humans: structural features of resistant starch," British Journal of Nutrition, 1995, vol. 73, pp. 111-123.

[13] H. N. Englyst, S. M. Kingman, J. H. Cummings, "Classification and measurement of nutritionally important starch fractions," European Journal of Clinical Nutrition, 1992, vol. 46, pp. 33-50.

[14] CSIRO Division of Human Nutrition, "Dietary Fibre, Non-Starch Polysaccharides and Resistant Starch – A Review," Food Australia, 1996.

[15] Silke Hylla, Andrea Gostner, Gerda Dusel, Horst Anger, Hans-P Bartram, Stefan U Christl, Heinrich Kasper, and Wolfgang Scheppach, "Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention," Am J Clin Nutr, 1998, vol. 67, pp. 136–142.

[16] K. R Silvester, H. N. Englyst, J. H. Cummings, "Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent," Am. J. Clin. Nutr, 1995, vol. 62, pp. 403–411.

[17] A. Garcı´a-Alonso, A. Jimenez-Escrig, N. Martı´n Carro´ n, L. Bravo, F. Saura- Calixto, "Assessment of some parameters involved in the gelatinization and retrogration of starch," Food Chemistry, 1999, vol. 66, pp. 181–187.

[18] M. Kabir, M., J. M. Oppert, H. Vidal, F. Bruzzo, C. Fiquent, P. Wursch, "Four-week low-glycemic index breakfast with a modest amount of soluble fibre in type 2 diabetic men," Metabolism, 2002, vol. 51, pp. 819–826.

[19] P. Yue & S. Waring S, "Functionality of resistant starch in food applications," Food Australia, 1998, vol. 50, pp. 615 - 621.

[20] H. E. Martinez-Flores, Y. K. Chang, F. M. Bustos, F. S. Sinencio, "Extrusion-cooking of cassava starch with different fiber sources: effect of fibers on expansion and physicochemical properties," Adv Extrusions, 1999, 271–278.

[21] R. G. Hernández-Nava, J. Berrios, J. Pan, P. Osorio-Díaz, L. A. Bello-Pérez, "Development and characterization of spaghetti with high resistant starch content supplemented with banana starch," Food Science and Technology International, 2009, vol. 15, pp. 73-78.

[22] Maribel Ovando-Martinez, Sonia Sáyago-Ayerdi, Edith Agama-Acevedo, Isabel Goñi, A. Luis, Bello-Pérez, "Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta," 2008.

[23] Abd Elmoneim O. Elkhalifa, Abdullahi H. El-Tinay, "Effect of cysteine on bakery products from wheat–sorghum blends," Food Chemistry, 2002, vol. 77, pp. 133-137.

[24] AACC. Approved Methods of the American Association of Cereal Chemists. American Association of Cereal Chemists. 10th ed. The Association INC. St., Paul, Minnesota, USA, 2000.

[25] C. Handa, S. Goomer, A. Siddhu, "Physicochemical properties and sensory evaluation of fructoligosaccharide enriched cookies," J. Food Sci Technol., 2011, vol. 49 (2), pp. 192-199.

[26] J. A. Awan, Salim-ur-Rehman, E. Ullah, M. I. Siddique, T. Aziz, "Nutrition of wheat flour in Islamic perspective," JAPS, 1991, vol.1, pp. 1-7.

[27] L. C. Doescher, R. C. Hoseney, G. A. Milliken, "Effects of flour components and dough ingredients on starch gelatinization," Cereal Chem., 1987, vol. 60, pp. 58-61.

[28] C. C. Tsen, "Regular and protein fortified biscuits from the composite flours," Cereal Foods World, 1976, vol. 21, pp. 633-640.

[29] H. Y. L. Kim, H. Yeom, H. S. Lim, S. T. Lim, "Replacement of shortening in yellow layer cakes by corn dextrins," Cereal Chem, 2001, vol. 78, pp. 267-271.

Environmental factors for transmission of Soil Transmitted Helminthiasis in school age children in Zambia

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Abstract— *We conducted a study to establish the environmental factors that favour the transmission of two Soil Transmitted helminthes, namely hookworm and ascariasis, in school age children in two districts, Luanshya and Nchelenge, of Zambia in July 2015. Soil samples were collected from selected sites in Nchelenge and Luanshya districts and analysed for chemical and physical properties. The climatic patterns for the two districts were also analysed. Nchelenge district lay at a lower altitude (934m) than Luanshya district (1218m). The average monthly temperatures were significantly higher for Nchelenge than Luanshya (P=0.004). Other climatic factors analysed were comparable between the two districts (P>0.05). Comparison of soil physical properties between the two districts demonstrated that Bulk density was significantly higher for Nchelenge district than Luanshya district (P=0.004), Solid was higher for Nchelenge than Luanshya (P=0.015), Moisture content was higher for Luanshya than Nchelenge (P=0.003), Clay content was higher for Luanshya than Nchelenge (P=0.005), Total Sand was higher for Nchelenge than Luanshya (P=0.024), while Silt content was comparable between the two districts (P>0.05). Comparison of the soil texture between the two districts demonstrated that Nchelenge significantly had more Sand soil than Luanshya while Luanshya had Loamy-sand soil than Nchelenge (P=0.005). Comparison of Soil chemical properties between the two districts revealed similarities in pH, Total Nitrogen, Phosphorous, Potassium, Sodium, Calcium, Organic carbon, Organic matter, Total carbon, and Lead (P>0.05) while mineral contents were higher for Luanshya than Nchelenge for Magnesium (P=0.018), Manganese (P=0.003), and Copper (P=0.007).*

Keywords— children, climate, helminthes, minerals, soil.

I. INTRODUCTION

Soil Transmitted Helminthes (STHs) infections fall under a group of diseases known as Neglected Tropical Diseases (NTDs). STHs are among the commonest infections occurring worldwide and affect the poor in developing countries [1]. STHs infections are transmitted via the worm eggs which are present in human faces which contaminate soil in areas where personal hygiene and sanitation are poor. STHs comprise the following worms; *Ascaris lumbricoides* (large intestinal round worm), *Trichuris trichiura* (whipworm), and *Necator americanus* and *Ancylostoma duodenale* (hookworm). It is estimated that two billion people globally are infected with STHs [2]. Another 3.5 billion people worldwide are at risk of contracting these infections. School age children are most affected persons in endemic areas. About 1.4 billion people are infected with *A.lumbricoides* [3)] while 576 to 740 million individuals are infected with hookworm [4, 5]. With ascariasis, the most affected populations are in Sub-Saharan Africa, Latin America, and Asia [4, 6, 7]. Infection with STHs may lead to disease depending on the number of worms in the infected individual. Individuals with light infections usually are asymptomatic while individuals with heavy infections may have symptoms such as intestinal manifestations (diarrhea, abdominal pains, and intestinal obstruction), general malaise, weakness, impaired cognitive function and physical development, and anaemia [8].

With STHs there is neither direct person to person transmission nor infection from fresh faeces (WHO, 2015). This is because the eggs of these worms that are passed in faeces need about three weeks, depending of the species, to either mature into infectious stage or develop into larvae in the soil. *A.lumbricoides* eggs, for example, can survive in the soil for prolonged periods and prefer warm, shady, moist conditions under which they survive sometimes up to ten years [9]. Therefore soil and climatic conditions will determine the distribution of particular STHs in any given locality [10].

In our previous the study entitled "Evaluation of Dipstick Dye ImmunoAssay in the diagnosis of *Schistosoma haematobium* infection in school children in Zambia" that we conducted in July 2014 in Nchelenge and Luanshya districts of Zambia, whose findings are reported somewhere, we found that the prevalence of ascariasis in school children was significantly higher in Luanshya than in Nchlenge (P<0.05) while the prevalence of hookworm infection in the same school children was significantly higher in Nchelenge than in Luanshya (P<0.05). This observation leads us to propose that there are

environmental factors responsible for the differences in the transmission of the two worm infections in school age children in the two districts.

Following the above observations we conducted a study entitled "Identification of soil and climatic factors responsible for the varied distribution of Soil Transmitted Helminthes in Nchelenge and Luanshya districts of Zambia" in July 2015. The aim of this study was to establish environmental factors responsible for the varied transmission of soil transmitted helminthes (STHs) in school children in Nchelenge and Luanshya districts of Zambia. The objectives of the study were to: compare the selected soil properties between Nchelenge and Luanshya districts, characterize the geographical positions of Nchelenge and Luanshya districts in terms of latitude, longitude and altitude, compare the selected climatic variables between Nchelenge and Luanshya districts, and make appropriate recommendations on how to effectively control STHs, based on the identified environmental factors, to the Ministry of Health and Ministry of Community Development Mother and Child health.

In this article we present the findings from the above study.

II. MATERIAL AND METHOD

1.1 Study design

This was a cross-sectional survey involving analysis of soil samples collected from selected sites around the two selected schools, study of climatic patterns, and geographical characterization of the two districts.

1.2 Study site and duration.

The field work was conducted from 15th to 24th July 2015 while soil analysis was carried out soon after that. Soil sample collection was done around Kenani primary school in Nchelenge district and Kawama primary school in Luanshya. These were the same schools where the prevalence of Hookworm and *A.lumbricoides* in the preceeding study was determined in July 2014.

1.3 Details of the fieldwork

1.3.1 Geographical characterization of the two districts

Coordinates and altitudes of the two districts were recorded using the Global Positioning System (GPS) machine (Garmin).

1.3.2 Collection of soil samples

In Nchelenge district seven samples from different sites were collected around the school. The seven sites were at least 10m apart. In Luanshya district six samples were collected from sites located 10m apart. At each site the soil was collected down to the depth of 20cm and weighing up to 1kg. The soil samples were each secured individually in Ziploc bags and stored in cool conditions before being sent for analysis at the soil Laboratory at the Copper belt University, School of Natural Resources within one to two days of collection. The soil samples were analysed for soil physical properties and soil chemical properties. The soil physical properties analysed were as follows; Bulk density, Solid, Moisture, Clay, Silt, Total Sand, and Texture. The soil chemical properties analysed were as follows; pH, Total Nitrogen, Phosphorus, Potassium, Sodium, Calcium, Magnesium, Organic carbon, Organic matter, Total Carbon, Manganese, Copper, and Lead.

1.3.3 Climatic conditions of Nchelenge and Luanshya districts

Using Google Search we collected the available data on the monthly weather averages summary for Luanshya and Nchelenge districts [11, 12]. The data collected was as follows: Average Temperature, Average precipitation, Average Number of Days of Precipitation, Average Length of Day, Average Relative Humidity, Average Dew Point, and Average Wind Speed.

1.3.4 Data entry and analysis

Data was entered and analysed in Epi Info version 6.04d (2001). Since the quantitative data was not normally distributed Kruskal wallis test was used to compare the medians and the 25% tile and 75% tile. For the qualitative data Fisher exact test was applied with the level of significance being 5%.

III. RESULTS

3.1 Comparison of the geographical characteristics of the two districts.

The position of Nchelenge district is altitude of 934m, longitude of E-28 43.920, and latitude of S-9 20.729. The position of Luanshya district is altitude of 1218m, longitude of E-28 18.764, and latitude of S-13 07.241.

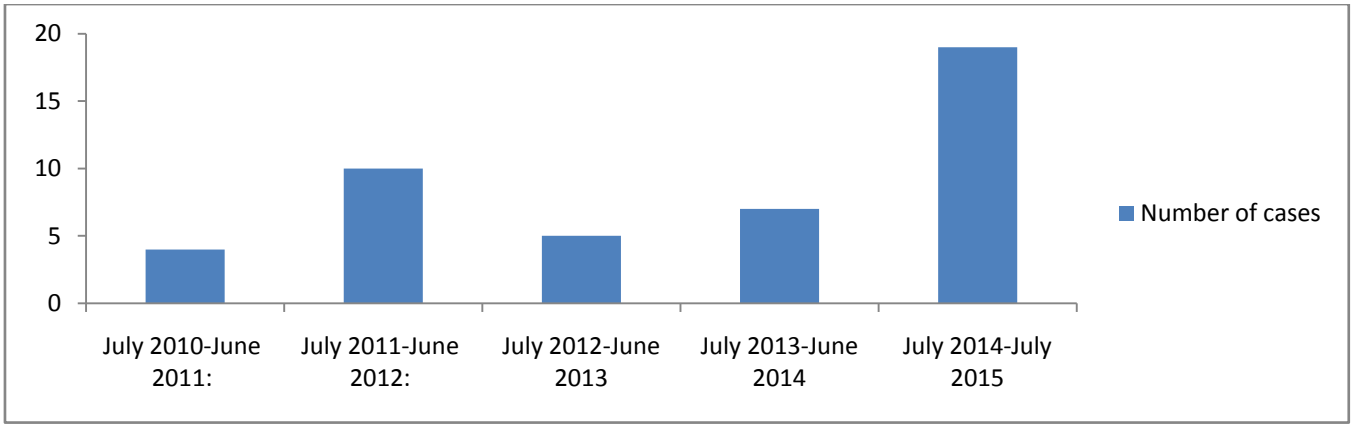

FIG. 1. SHOWS THE LOCATION OF LUANSHYA AND NCHELENGE DISTRICTS ON THE MAP OF ZAMBIA.

3.2 Climatic conditions for the two districts

The two districts were identical in all the variables analysed apart from the monthly average temperature. The monthly average temperature was significantly higher for Nchelenge than Luanshya districts ($P<0.05$).

TABLE 1

COMPARES THE MONTHLY AVERAGE WEATHER SUMMARIES BETWEEN NCHELENGE AND LUANSHYA DISTRICTS.

	Characteristic (units)	Nchelenge Median (Q1, Q3)	Luanshya Median (Q1, Q3)	P-value
1	Average monthly Temperature (^oC)	23.6 (22.7, 21.9)	21.9 (18.4, 22.2)	0.004
2	Average Precipitation (mm)	91.2 (6.5, 188)	38.8 (1.7, 210.3)	0.862
3	Average days of precipitation(days)	10.4 (0.8, 17.9)	4.9 (0, 17.9)	0.815
4	Average length of day (hours)	12.5 (12.2, 12.9)	12.5 (12.0, 13.0)	0.966
5	Average Relative humidity (%)	68.1 (55.3, 77.2)	65.2 (51.2, 79.5)	0.954
6	Average dew point (^oC)	17.3 (13.2, 19.4)	13.9 (8.3, 18.4)	0.073
7	Average wind speed (km/h)	5.4 (4.2, 6.5)	5.2 (4.7, 6.5)	0.684

3.3 Soil physical properties and chemical analyses for the two districts

TABLE 2

COMPARISON OF SOIL PHYSICAL AND CHEMICAL PROPERTIES BETWEEN NCHELENGE AND LUANSHYA DISTRICTS.

Characteristic (units)	Nchelenge Median (Q1, Q3)	Luanshya Median (Q1, Q3)	P-value
1. Bulk density (g/cm^3)	1.478 (1.421, 1.688)	1.202 (1.139, 1.272)	0.004
2. Solid (%)	55.8 (50.0, 68.7)	45.4 (43.0, 46.6)	0.015
3. Moisture (%)	1.34 (0.55, 3.15)	5.70 (4.70, 9.30)	0.003
4. Clay (%)	5.6 (4.6, 5.6)	7.1 (6.6, 10.6)	0.005
5. Silt (%)	3.6 (2.6, 4.1)	3.1 (2.6, 3.6)	0.825
6. Total sand (%)	91.8 (90.8, 91.8)		
7. pH	5.5 (4.4, 6.0	6.3 (5.6, 6.5)	0.116
8. Total Nitrogen (%)	0.026 (0.017, 0.0029)	0.036 (0.027, 0.039)	0.061
9. Phosphorus (%)	0.056 (0.016, 0.073)	0.036 (0.027, 0.051)	0.775
10. Potassium (Ppm)	110 (90, 250)	211 (106, 239)	0.475
11. Sodium (Ppm)	61 (46, 68)	75 (62, 84)	0.074
12 Calcium (me/100g))	3.15 (1.45, 3.95)	3.35 (2.80, 3.80)	0.616
13. Magnesium (me/100g)	2.60 (2.50, 3.30)	3.83 (3.40, 5.70)	0.018
14. Organic carbon (%)	0.356 (0.226, 0.931)	0.447 (0.251, 0.588)	0.943
15. Organic matter (%)	0.711 (0.453, 1.862)	0.895 (0.523, 1.177)	0.943
16. Total Carbon (%)	0.473 (0.301, 1.238)	0.595 (0.348, 0.783)	0.943
17. Manganese (Ppm)	0.48 (0.42, 0.60)	2.76 (2.14, 3.24)	0.003
18. Copper (Ppm)	0.70 (0.60, 0.89)	1.89 (1.08, 3.72)	0.007
19. Lead (Ppm)	0.34 (0.16, 0.68)	0.42 (0.16, 0.50)	0.614

3.3.1 Soil physical properties

There were significant differences in all the variables ($P<0.05$) apart from Silt ($P>0.05$) between the two districts. Bulk density, Solid, and Total Sand were significantly higher in Nchelenge than in Luanshya districts while Moisture and Clay were higher in Luanshya than in Nchelenge districts.

3.3.2 Texture of the soil between for the two districts

There was a significant difference in the soil texture between the two districts ($P<0.05$). The soil for Nchelenge district was exclusively Sandy while for Luanshya it was predominantly Loamy-Sand.

3.3.3 Soil chemical properties.

All the parameters analysed were identical between the two districts apart from Manganese, Magnesium, and Copper levels in the soil ($P>0.05$). The Manganese, Magnesium, and Copper levels in the soil were significantly higher for Luanshya than Nchelenge ($P<0.05$).

IV. DISCUSSION

Specific environmental conditions are required for the survival of the eggs and larvae of STHs. These conditions will therefore directly influence which worm species will be transmitted in what geographical location and soil conditions. The results from our study, to some extent, have demonstrated that certain geographical characteristics, soil physical properties, soil chemical properties, and climatic conditions influence the uneven transmission of STHs in particular geographical locations. It may therefore be argued that higher altitudes and hence cooler temperatures favour the survival and transmission of ascariasis while lower altitudes, accompanied by warmer temperatures, will favour the survival and transmission of hookworm infection. This finding has been demonstrated elsewhere [10]. Our study demonstrated that high average monthly temperatures favoured survival and transmission of hookworm more than of *A.lumbricoides*. For hookworm, high temperatures are important for the period of hatching of the eggs and maturation of the larvae in the soil [13].

On the soil physical properties, our study has demonstrated that sandy soil conditions seem to favour survival, maturation, and transmission of hookworm when compared with *A.lumbricoides*. Well drained and aerated sandy soil has been shown to favour larva development for hookworm [14]. High clay content and loamy sandy soil in our study seem to promote survival, maturation, and transmission of *A.lumbricoides* when compared with hookworm. This has been demonstrated elsewhere [10]. In addition, high moisture content in loamy- sandy soil and soil with high clay content as observed in our study seemed to favour survival and transmission of *A.lumbricoides* when compared with hookworm and demonstrated elsewhere [13].

The findings from our study on the soil chemical properties showed that high levels of copper, manganese, and magnesium favoured the survival, maturation, and transmission of *A.lumbricoides* when compared with hookworm infection. This therefore means that high levels of these minerals are required for the development of the of *A.lumbricoides* eggs to infective stage in the soil. It may also be argued that high levels of these minerals in the soil hinder the development and transmission of hookworm larva in the soil. Such findings haven't been reported in literature.

V. CONCLUSION

Geographical characteristics, soil physical and chemical properties, and climatic condition do influence survival, maturation, and hence uneven transmission of STHs in different geographical localities. This information will enable health policy makers design effective evidence based interventions against STHs in affected communities. There is need for more research to define the role of minerals in the development of eggs and larvae of STHs in the soil.

VI. FINANCIAL AGENT

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REFERENCES

[1] World Health Organisation. "Soil Transmitted helminth infections" (2015). www.who.int/Mediacentre/factsheets/fs366/en/

[2] World Health Organisation. "Intestinal parasites: Burden and trends". (2001) www.who.int

[3] Seltzer E. "Ascariasis. In: Tropical Infectious Diseases: Principles, Pathogens and Practice. 1st ed, Guerrant RL, Weller PF (Eds), Philadelphia: Churchill Livingstone; (1999):553.

[4] Fenwick A. "The global burden of neglected tropical diseases". Public Health (2012): 126(3): 233-6.

[5] Massa K, Magnussen P, Sheshe A, Ntakamulenga R, Ndawi B, Olsen A. "The effect of the community-directed treatment approach versus the school-based treatment approach on the prevalence and intensity of schistosomiasis and soil transmitted helminthiasis among school children in Tanzania". T Roy Soc Trop Med H. (2009): 103(1); 31-37.

[6] Holland C Dold. "Ascaris amd ascariasis". Microbes and infection/Institut Pasteur (2010): 13(7): 632-67. Doi.10.1016/j micinf: 2010.09.012.PMID 20934531.

[7] Keiser J, Utzinger J. "The drugs we have and the drugs we need against major helminth infections". Advances in Parasitology. (2010). 73: 197-230.

[8] Gilles HM. "Selective primary health care: strategies for control of disease in developing world. XVIII. Hookworm infection and anaemia". Review of Infectious Diseases.(1985):7; 111-118.

[9] Khuroo MS. "Gastroenterol Clin North Am. (1996). 25: 553.

[10] Mabaso MLH, Appleton CC, Hughes JC, and Gouws E. "The effect of soil type and climate on hookworm (*Necator americanus*) distribution in Kwazulu-Natal, South Africa". *Trop Med Int Health*. (2003) Volume 8, No. 8, pp722-727.

[11] Google Search. "Nchelenge, Zambia, Weather Current Conditions, 29th July 2015".

[12] Google Search. "Luanshya, Zambia, Weather Current Conditions, 29th July 2015".

[13] Smith G. "Ecology of the free-living stages: a reappraisal. In: Hookworm Disease: Current: Status and New Directions (eds GA Schad & KS Warren) Taylor and Francis, London, (1990 pp. 98-104.

[14] Wallace HR. "The Biology of Plant Parasitic Nematodes. (1963). Edward Arnold, London.

Identification and Mapping of Prone to Landslide in the Sub District of Kintamani, Bali Province, Indonesia

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Abstract— *This research was conducted in several locations of prone to landslides in the Sub District of Kintamani, Bangli Regency, Bali Province, Indonesia. Determining the research location is purposively, meaning intentionally determined by observing in the field to landslides during the rainy season 2016/2017. The purpose of research, namely: (1) identifying areas prone to landslides at several locations in the Sub District of Kintamani, Bangli Regency; (2) to mapping areas prone to landslides using geographical information systems (GIS), (3) to formulate the strategies to overcome landslides. The analytical method used is descriptive-qualitative, meaning that the results of field observations and interviews integrated and interpreted in a narrative-qualitative. Results of the analysis showed that the location is prone to landslides in the sub district of Kintamani are Villages of Selulung, Satra, Tanah Gambir, Tegallinggah, Pinggan, Songan, Kayu Selem, Blancan, Batur, Bubung Kelambu, Pura Tanah Mas, Mampeh, Yeh Mampeh, dan Penelokan, with total area of 144,36 ha. The cause of landslides are high rainfall, soil type regosol, geology, slope average above 15% and the use of land that is not paying more attention on land conservation. Further mapping of landslide-prone locations using Q-GIS and Google Earth. Strategies to overcome landslides is to avoid the cultivation of agriculture in areas prone to landslides, implement agroforestry systems, planting cover crops, making sewers or ditches, enforcing the rules of the spatial region and violators are given strict and severe sanctions.*

Keywords— Mapping, Landslides, Kintamani.

I. INTRODUCTION

Indonesia, including Bali as a one of Province in Indonesia is an area prone to natural disasters, caused by over-exploitation of natural resources, land conversion, improper land use, and global climate change such as the prolonged rainy season in 2016/2017. Natural disasters can occur suddenly or slowly, earthquakes, tsunamis and volcanoes are almost impossible to estimate accurately, when will happen and how much strength. While floods, landslides, droughts are predictable. In the rainy season there are two disaster threats namely floods and landslides. Floods and landslides occasionally occur respectively. Continuous floods will erode the land slopes of hills or mountains, so if the construction of the soil is unstable it will happened landslide.

Bali's concerns were struck by the disaster as Lanina's impact during the rainy season of December 2016 to February 2017, becoming a reality; this is all due to Bali's nature already damaged from upstream to downstream. For example in sub district of Kintamani this year experienced the worst natural disaster during 20 years. The team from the Geological Agency has checked into the field, the Banjar of Bantas area in the middle of the hill of Kintamani is now in a vulnerable condition, due to the landslide that is easily landslide (Bali Post, 2017).

Suripin (2002) and Anwar, et al. (2003) stated that landslides are a form of erosion where the transport or movement of the soil mass occurs at one day in a relatively large volume. Mass that moves in the landslide is a large mass, so often the occurrence of landslides will bring the victim in the form of environmental damage, degradation of agricultural land, settlements damage and damage of infra structure and property and even the loss of human life. Landslides constitute the movement of slope-forming materials in the form of rocks, rickwheels, soils, or mixtures of such material moving down or out of the slopes. The process of occurrence of landslides starting from water that seep into the soil will increase the weight of the soil. If the water penetrates to the impermeable soil that acts as a slip plane, it will become slippery and the weathering soils on it will move along the slope and out of the slope. In principle, landslides occur if the driving force on the slope is greater than the retaining force. Retaining force is generally influenced by rock strength and soil density, whereas the driving force is usually influenced by the amount of inclination angle of the slope, water, load and soil type of rock. The causing factors the movement of the slope also depend on the conditions of rock and slope soil, geological structure, rainfall, cover of vegetation and land use on the slop.

The potential for landslide disaster in Bali Province is 85,121,55 ha, and one of the regency prone to landslide is Bangli Regency. Some of the villages in sub district of Kintamani, Bangli regency are landslide-prone areas, because they are on steep slopes, especially in the upper reaches where the soil is dominated by sandy texture, has a high rainfall of about 2000 mm per day, so when it rains with high intensity and long, prone to landslide disaster (Bappeda Bali and PPLH Unud, 2006). The cause of landslide and its prevention is very important to know, so that it can minimize the occurrence of landslide and the resulting consequences (Law Number 24 Year 2007 on Disaster Management). So the disaster management plan is a manifestation of government efforts related to the formulation of activity programs and the focus of the priority of disaster management, then the characteristics of the disaster must be studied carefully.

Based on the previous description, the objectives of the research are: (1) to identify the affected areas and prone to landslide disaster in the sub district of Kintamani, Bangli regency, Bali Province, (2) to mapping the landslide prone area by using geographic information system (GIS); (3) formulate the strategies used to overcome the risk of landslide disaster.

II. METHOD OF RESEARCH

The research location in sub district of Kintamani, Bangli regency, Bali Province determined purposively, based on consideration: (1) Sub district of Kintamani is hilly area prone to landslide; (2) In March 2017, several locations in sub district of Kintamani were struck by landslides caused by heavy rains and prolonged rainy season of 2016/2017; (3) Until now no research has been done to mapping the landslide prone locations in sub district of Kintamani. The astronomy of sub district of Kintamani is at $8^0$7'42''-$8^0$18'53''LS dan – 115^0 10'44''

The research method used survey method, ie observed the location of landslide disaster (Figure 1a, 1b, 1c), and interview with community in disaster location. The results of surveys and interviews were integrated and then mapped the landslide-prone locations using Q-GIS and Google Earth.

The analytical method used is descriptive qualitative, ie giving meaning to result of field observation and result of interview with community around research location, so that able to describe phenomenon that happened at research location that is sub district of Kintamani, Bangli Regency, Bali Province, Indonesia.

FIGURE 1A. LANDSLIDE LOCATION IN SONGAN VILLAGE, SUB DISTRICT OF KINTAMANI

FIGURE 1B. LANDSLIDE LOCATIONS IN PENELOKAN VILLAGE, SUB DISTRICT OF KINTAMANI

FIGURE 1C. LANDSLIDE LOCATION IN YEH MAMPAH VILLAGE SUB DISTRICT OF KINTAMANI

III. RESULTS AND DISCUSSION

3.1 Identify Landslide Prone Areas

Based on observation and field identification, landslide disaster in sub district of Kintamani occurred in villages of Selulung, Satra, Tanah Gambir, Tegallinggah, Pinggan, Songan, Kayu Selem, Blancan, Batur Bubung Kelambu, Pura Tanah Mas, Mampeh, Yeh Mampeh, dan Penelokan with the extent of 144,36 ha is presented in Table 1. As a result of landslides in

several villages in Kintamani, about 350 ha of agricultural land in the area was severely damaged. Estimated losses reach billions of rupiah. This was disclosed Head of Agriculture, Food Security and Fisheries of Bangli Regency Wayan Sukartana (Bali Post, 2017). The factors that cause landslide disaster are: rainfall, geological structure, soil type, slope inclination, land use that does not pay attention to the rules of land conservation.

Rainfall is the amount of rainwater that falls to the earth per unit surface area at a certain time period. The amount of rainfall can be expressed as the volume of rainwater that falls on a certain area for a relatively long period of time, and the amount of rainfall is expressed in m^3 per unit area (Subekti et al., 2009). Karnawati (2003) stated that one of the factors causing the occurrence of landslide disaster is rain water. The compact and watertight rocks act as a water barrier and simultaneously as an avalanche slip, while water acts as a driving ground mass slipping over compact rocks. The steeper the slope, the faster the speed of slipping. The more loose the clay pile, the easier it will be to pass the water and the sooner the water seeps into the soil. The thicker the pile of soil, the greater the volume of landslide mass. While Setyolelono (2005) states that the influence of rain can occur in parts of the slopes that are open due to human activities, especially related to land use, less attention to patterns that have been applied by the government. For example, continuous logging of protected forests should not be done, so lands with very sloping geomorphology become open and prone to landslides.

TABLE 1.
LANDSLIDE LOCATION IN SUB DISTRICT OF KINTAMANI, BANGLI REGENCY, BALI PROVINCE, INDONESIA

No.	Location	Villages	Slope/ Class	Soil Type	Land Use	Cause Landslide
1	Selulung	Selulung	IV	Humus Regosol	Dry land, garden mix, Schrubs	Heavy rain
2	Satra	Satra	III	Humus Regosol	Dry land, garden mix, Settlement	Heavy rain
3	Tanah Gambir	Satra	III	Humus Regosol	Dry land, garden mix, Settlement	Heavy rain
4	Tegallinggah	Sukewana	V	Gray Regosol	Shrubs, garden mix	Heavy rain
5	Pinggan	Pinggan	III	Gray Regosol	Garden mix, schurbs	Heavy rain
6	Songan	Songan B	V	Gray Regosol	Settlement, dry land	Heavy rain
7	Perbatasan Kayu Selem	Blandingan	V	Gray Regosol	Dry land, garden mix	Heavy rain
8	Blancan	Blancan	III	Humus Regosol	Garden mix, land berro	Heavy rain
9	Batur Bubung Kelambu	Kintamani	IV	Gray Regosol	Dry land, garden mix	Heavy rain
10	Pura Tirta Mas Mempeh	Sukawana	V	Gray Regosol	Dry land, land berro, schrubs	Heavy rain
11	Yeh Mempeh	Sukawana	II	Gray Regosol	Dry land, land berro, schurbs	Heavy rain
12	Penelokan	Kedisan	III	Gray Regosol	Settlement, garden mix	Heavy rain

Source: The results of the analysis to the field

Geological factors that influence the movement of the soil are the geological structure, the nature of the rock, the loss of the soil adhesive due to the natural process (dissolving), and the earthquake. The geological structure that influences ground motion is the contact of bedrock with rock weathering, cracks, rock layers, and fractures. Fracture zone is a weak zone resulting in reduced rock strength, resulting in many cracks that facilitate pervasive water (Surono, 2003).

Types of soil in the study sites include regosol, where this type of soil has loose properties and is dominated by sand. Soil types determine the potential for erosion and landslides. The loose soil easily passes the water into the cross section of the

soil and potentially landslides compared to the massive soil. It can also be seen from the soil erodibility value (K). The value of K indicates whether or not the soil is eroding, the greater the value of K, the more susceptible to erosion (Sitorus, 2001).

Land use in landslide location in sub district of Kintamani is mixed and cultivated garden. Land use like this, people only see in terms of results obtained without regard to the slope of the land and land conservation rules. Land with a slope above 15% is planted with seasonal crops without any terraces. Such a situation will facilitate landslides because there is no conservation action, supported by high rainfall, the type of sandy soil (regosol) and its geological structure.

According to the Directorate of Volcanology and Geological Hazard Mitigation (2005), landslides are caused by: (1) Natural factors, consisting of: (a) Geological conditions: weathered rocks, slope layers, claystone insertions, Earth, stragrafi and volcanoes; (b) Climate, especially high rainfall in slope areas; (c) Topographic conditions, especially steep slopes; (d) Water conditions, especially clogged drainage conditions, accumulation of water masses, deep erosion, dissolution and hydrostatic pressures; (e) Land cover reducing shear stress, eg critical soil; (f) Vibrations caused by earthquakes, explosions, engine vibrations, or vibrations of nearby vehicular traffic; (2) Human factors, consisting of: (a) Climb cutting on stone mining on steep slopes; (b) Landfill in the slope area; (c) Failure of a retaining wall structure; (d) Deforestation; (e) Agricultural systems with little regard for irrigation security; (f) Development of areas not offset by public awareness, so that General Spatial Plan is not adhered to which ultimately self-defeats; (g) Inadequate slope drainage system, and (h) Land use that does not pay attention to soil and water conservation principles. This is in agreement with Sutikno (1997) that the factors that affect the occurrence of land movement include: slope level

Referring to the geological map source, the sub district of Kintamani is a very steep area, with a thick layer of soil that is affected by alteration, weathering from the earth. Added to the geological structure of the surrounding area is very complex, and found many fault lines. However, the trigger of landslide is caused by the use of less secure land, meaning that it does not follow the rules of land conservation. To avoid a similar incident occurring in the area of landslide potential is to observe the emergence of cracks of soil. The characteristics of the landslide begin with the appearance of cracks in the slope parallel to the cliff, which usually occurs after rain. The sudden emergence of new springs, brittle cliffs and pebbles began to fall. Water is inundated if the rainy season, before the disaster landslide occurs the water immediately disappeared and the collapse of the land in large numbers.

3.2 Mapping of Landslip Prone Areas

The landslides occurring in several locations in sub district of Kintamani, Bangli Regency, Bali Province are due to high rainfall, steep slopes above 15% average, geological structure and regosol soil type (sand texture to sandy clay) and mixture garden land use and moorings without any soil and water conservation treatment. Efforts to prevent landslides are not to open rice fields and don't create a pond on the upper slopes near the settlement. Make terraces (swales) on steep slopes when building settlements. If there is a crack in the soil, immediately close the cracks of the soil and compact it so that the water does not enter the soil and through the crack. Do not cut the cliff of the road upright. Do not build erosion-prone riverside houses, cut down trees on the slopes, and build houses under cliffs. It should also be noted that the area or villages in sub district of Kintamani is upstream of one of the major rivers in Bali, namely the Ayung River, so the use of land should pay attention to the environmental conditions (BPDAS, 2009; Sumarniasih and Antara, 2017). This is supported by the opinion of Brook et al., (1991). Which states that landslides will occur when three conditions are met: (1) the slope is quite steep, (2) there is a rocky ground (rock) below the waterproof surface, and (3) there is enough water (rain) that enters the pores soil over an impermeable rock layer, so that the soil pressure to the slope increases. While Karnawati (2001) and Alhasanah (2006) stated that slopes are a very important factor in the process of landslide, but not always slopes or sloping lands with potential landslides, depending on rock conditions and slope soil, geological structure, rainfall, vegetation Cover, and land use on the slopes.

The mapping is based on field observation at each landslide point using Global Position System (GPS) equipment, so that in obtaining x and y axis at each landslide point, and combined with interview result with community around disaster area resulted Q-GIS-based digital map with attribute The results of field observations as presented in Figure 2. This digital map when clicked on a point yellow with Q-GIS and Google Earth will see the location of landslide-prone areas in some villages in sub district of Kintamani, Bangli Regency, Bali Province, Indonesia.

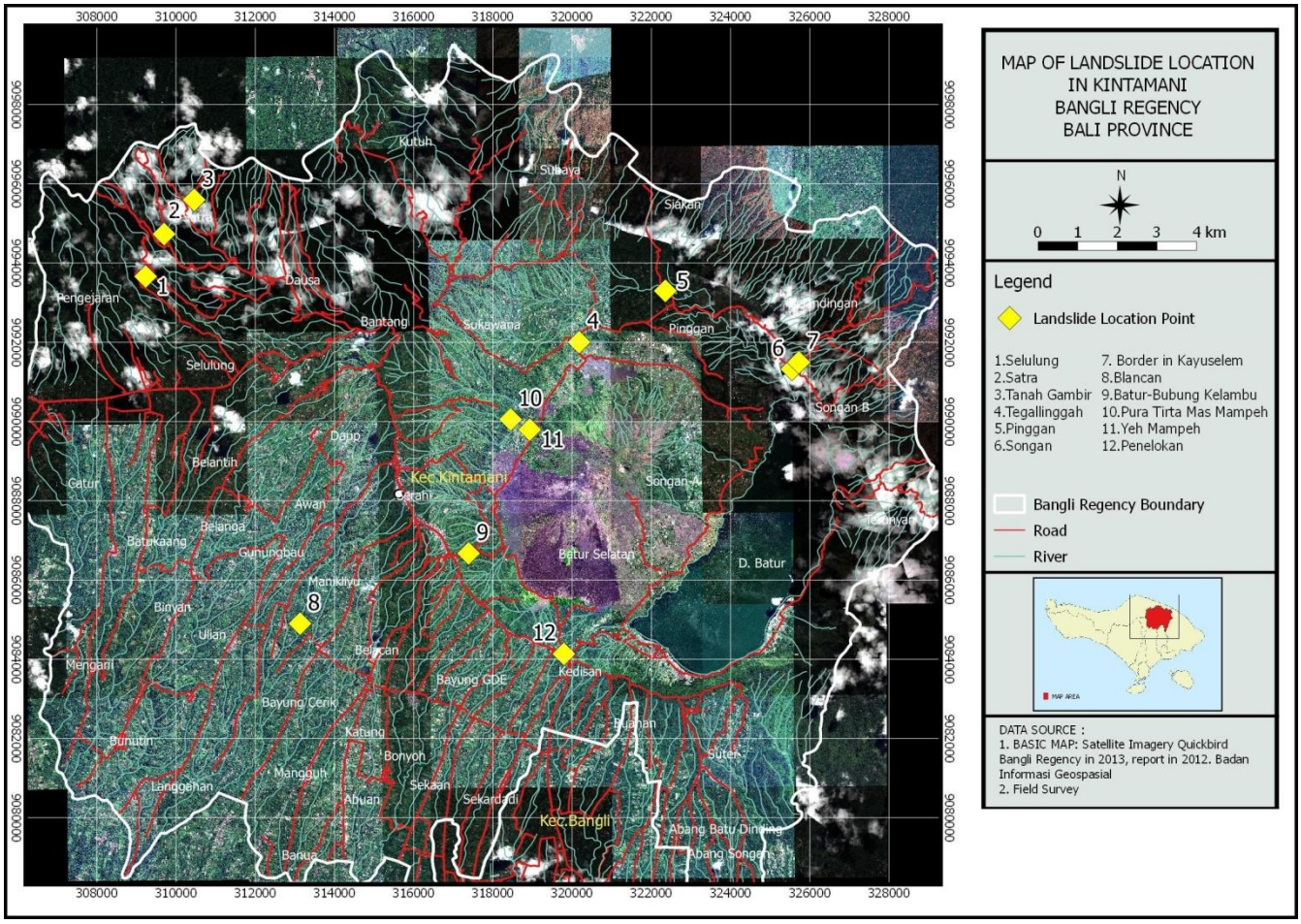

FIGURE 2. LANDSLIDE MAP LOCATIONS IN SUB DISTRICT OF KINTAMANI, BANGLI REGENCY, BALI PROVINCE, INDONESIA

3.3 Strategy to Overcome Landslide Risk

The strategy to overcome landslides is to prohibit development in areas prone to landslide disaster, cover the land with agroforestry systems, create drainage ditches or ditches, and rules should be enforced and those who violate severe sanctions. This is in line with the opinion of Sumarniasih and Antara (2017), which says the use of local wisdom-based land can reduce erosion by up to 90%, resulting in falling water that will cause erosion, causing landslides to be prevented by local wisdom-based conservation methods.

According to Dardak (2006), the risk reduction of landslide disaster can be done by considering the density of the population, the area of awakening and the existence of productive land. The strategies that can be done, namely: (1) Conducting the management of the region through the regulation of human activities to protect people from disasters caused by humans. The objective is to prevent damage to environmental functions and to preserve regional protected functions and to avoid activities in the potential areas of disasters; (2) Reducing the density and activities of trhe people who living in areas with high risk of landslide disaster; (3) Determine the path and place of evacuation in case of landslide disaster; (4) Conducting the structural mitigation by carrying out physical development, such as the construction of terraces and brojong construction that can prevent and reduce the danger of landslides; (5) Establish rules or regulations that can reduce the impact of landslides in the form of zoning zones and prohibit the use of land in areas with the potential for landslide disasters; (6) Implementing the agroforestry program is expected to maintain land productivity. This is in line with Dubot and Watsala (2009) which states that measures that can be taken to reduce the risk of landslide disaster are (1) Housing is built outside the landslide threat area; (2) Slope cover should be solid; (3) density vegetation and bronjong manufacture; (4) The establishment of drains or ditches to prevent erosion or surface water flows; (5) Preparation of walls to protect the slope / cliff.

IV. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

1. Based on observation and field identification, landslide disaster in sub district of Kintamani occurred in some vullages i.e. Selulung, Satra, Tanah Gambir, Tegallinggah, Pinggan, Songan, Kayu Selem, Blancan, Batur Bubung Kelambu, Pura Tanah Mas, Mampeh, Yeh Mampeh and Penelokan with Extent 144,36 ha. The factors that cause landslide disaster are: rainfall, geological structure, soil type, slope inclination, land use that does not pay attention to the rules of land conservation.
2. Mapping is based on field observations using GPS equipment combined with interviews with communities around the disaster site resulting in a Q-GIS-based digital map as presented in Figure 2.
3. Strategy to overcome landslides is to prohibit development in areas prone to landslide disaster, cover the land with agroforestry systems, create drainage ditches or ditches, and rules should be enforced and those who violate severe sanctions.

4.2 Recommendation

Avoiding or reducing the landslide disaster in the research area, stakeholders such as communities around in Sub District of Kintamani and Regional Governments should implement the strategies which formulated in this study.

REFERENCES

[1] Alhasanah, F. 2006. Mapping and Analysis of Landslide Prone Areas As well as the related mitigation efforts Using Geographic Information System. Thesis. Graduate Program in Bogor Agricultural University.

[2] Anwar, H.Z., Suwiyanto, Subowo, E., Karnawati, D., Sudaryanto and Ruslan, M. 2001. Application Satellite Imagery In dareah Determination of Landslide Prone. LIPI Geotechnology Research Center, Bandung.

[3] Bali Post. 2017. Bali at Landa Disaster. Publisher: Balipost. Denpasar

[4] Bappeda Bali and PPLH UNUD. 2006. Identification of Potential Study of Natural Disasters In the province of Bali, Research Reports, Regional Development Planning Board of Bali Province and the Research Center for Environmental research Udayana University, Denpasar.

[5] Barus B., and U.S. Wiradisastra, 2000, Geographic Information System, Remote Sensing Laboratory and Cartography, Soil Department, Faculty of Agriculture IPB, Bogor

[6] Ministry of Energy and Mineral Resources. 2009. Vulnerability Zone Map Land Movement of Bali province. Center for Volcanology and Geological Hazard Mitigation.

[7] Dardak, A.H. 2006. Spatial Planning Policy in Landslide Prone Areas Management. Material Papers in Workshop on Spatial Planning as a Forum to Minimize Potential Landslide Disaster. Jakarta, March 7, 2006.

[8] Dubot, Alice and Jayamanna Watsala.2009. Safer Cities 26: Using Risk Assessments to Reduce Landslide Risk, Asian Disaster Preparedness Center/ADPC. Bangkok

[9] Karnawati, D. 2001. Natural Disasters of the Indonesian Land Movement of 2000 (Evaluation and Recommendation). Department of Geological Engineering. Faculty of Engineering, University of Gadjah Mada, Yogyakarta.

[10] Ramadona, Aditya.L and Hari Kusnanto. 2010. Quantum GIS Applications For Environmental Information Systems. Publisher BPFE, Yogyakarta.

[11] Sitorus, S. R. P. 2006. Land Development Berpenutupan Stay In Control of Risk Factors of Erosion and Landslide. The Directorate General of Spatial Planning Department of Public Works. Jakarta.

[12] Subekti, R., Widodo, R.H., Meine van Noordwijk, Suryadi, I. Verbist, B. 2009. Water Monitoring in Watersheds. World Agroforestry Center-Southeast Asia Regional Office, Bogor Indonesia. 104 p

[13] Sumarniasih, Made Sri dan Made Antara. 2017. Conservation planning on eroded land based of local wisdom in Kintamani sub-district, Province of Bali. *Journal* IOP Conf Series. Earth and Environment.

[14] Suripin. 2002. Soil and Water Resources Conservation. Andi. Yogyakarta.

[15] Surono. 2003. Potential of Geological Disaster in Garut Regency. Proceeding of Landslide Mitigation Workshops in Garut Regency. Government Garut regency.

[16] Sutikno. 1997. Landslide Mitigation. Ingredients Natural Disaster Counseling Land Movement. Jakarta.

Study of Development of Rural Entrepreneurship in Modern Russia

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Abstract— *The article discusses the trends and prospects of development of the three sectors of rural entrepreneurship: corporate, individual and family, formal and informal; analyze the transformation of household farms; an assessment of efficiency of development of small forms of agricultural production, it is concluded that the major agricultural organizations lost their dominant position.*

Keywords— agricultural production, farms, households, corporate sector, private sector.

I. INTRODUCTION

Market reforms in Soviet Russia were the subject of researches of many domestic and foreign authors. The focus became the adaptation of enterprises and population to the new socio-economic conditions, new forms and models of management, new rules, new norms and values [21-29]. In the course of market reforms of 90-ies was created the institutional framework for the development of rural entrepreneurship. First, was a land reform, reorganization of collective and state farms, removed administrative barriers to the development of private farming, agricultural workers were endowed with property and land share. Thus the rural population got a startup capital for the business. About the extent to which the rural population had benefited, preserved the entrepreneurial spirit of the Russian peasant, not lost if he patterns a calculating master, a trace in his consciousness and behavior left a 70-year experience of the "Communist labor" we will try to analyze through the prism of the development of the dominant structures in the agricultural sector of Russia in 1991-2016.

According to the Civil code of the Russian Federation, "the entrepreneurial self is undertaken at your own risk activities aimed at systematically profit from the use of property, sale of goods, performing works or rendering services by persons registered in this capacity in the prescribed manner". The subject of our consideration is the population activity on production, processing and marketing of agricultural products.

II. THE DYNAMICS OF THE DOMINANT ECONOMIC STRUCTURES

On the eve of market reforms in the Russian agricultural sector was dominated by two organizational-legal forms of agricultural production: collective sector and household plots. The collective sector was represented by two types of agricultural enterprises: collective farms based on production cooperation of farmers and collective ownership of the means of production (land, equipment, livestock, seeds etc) and state agricultural enterprises (state farms), based on state ownership of land and other means of production. In the collective sector produced about 70% of agricultural production.

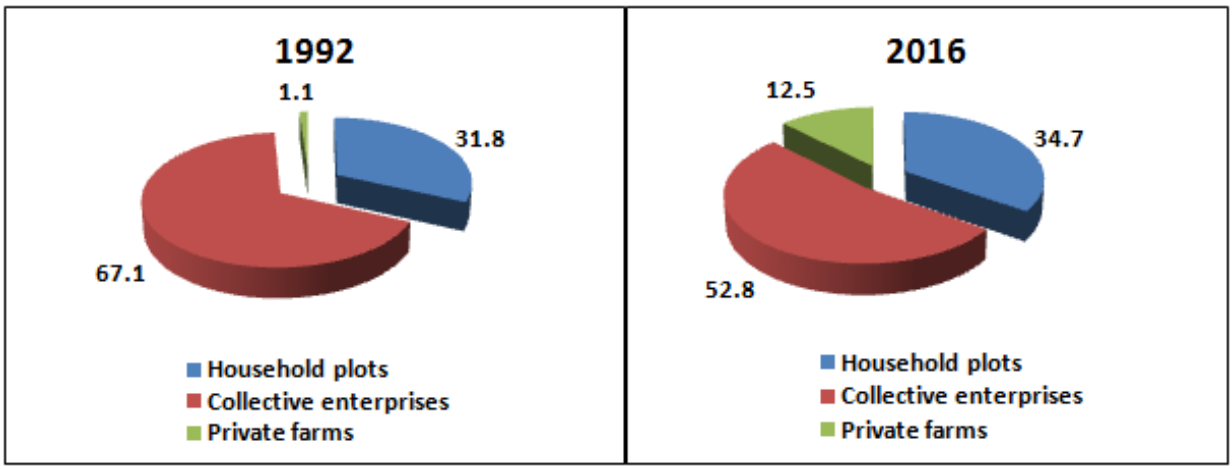

FIGURE 1. STRUCTURE OF AGRICULTURAL PRODUCTION IN RUSSIA BASED ON MARKET VALUE OF AGRICULTURAL COMMODITIES, 1992 AND 2016, %.
Source: [1, p.245]

Since the beginning of market reforms in Russia has passed the quarter of a century. In this period changed the balance of power in the agrarian sector. Statistics indicate that the role of big business in agricultural production contrary to expectations has decreased not so much. In Soviet times the share of agricultural enterprises accounted 67% of total agricultural production, at the present time – 53% (Fig.1). Agricultural enterprises dominate the production of grain – 71%, sugar beets - 88%, livestock and poultry (slaughter weight) - 76%, eggs – 79%, sunflower seeds 90%. There are also about half the amount of milk and 70% of meat (slaughter weight) [1, p.246].

One third of agricultural output continues to produce household plots. The share of private farms accounts for only one-tenth part of agricultural production. Individual farm labor was not attractive to the rural population of Russia [1, p. 245].

Typological analysis of the socio-economic development of rural regions has allowed revealing the regional specificity in the structure of production by types of farms [2]. The analysis showed that in the I cluster of regions where agricultural production is developing successfully and it focuses mainly on large collective enterprises. The number of household plots is much lower compared to other regions. Their share in the production of agricultural products varies from 27 % (I cluster) to 36% (II cluster).

The second group (II cluster) is represented by 10 regions located mostly in the South and in the Central Chernozem zone, and adjacent to large Metropolitan agglomerations (Moscow and Leningrad region). They are characterized by the use of innovative technologies and large scale investment in agriculture. The scale of investment in these regions was twice lower than in Krasnodar Krai, but significantly exceeded their size in all other clusters. This determined the technical and technological modernization of the agricultural sector and ensured higher profitability of animal production and crop production, and yield of grain crops and productivity of animals.

In this group of regions (II cluster), the lowest share of loss-making agricultural organizations: 23,2% vs 53,6% in the sixth cluster and 42,1% - in the fourth cluster. In General, rural areas of this group produce one-third of all agricultural output in the country. In this case the dominant role in the production playing the major agricultural organizations, the share of households account for 36%, while the share of private farms in this class of regions is 7,5% . The smallest number of peasant (farmer) farms noted in the fourth cluster of rural regions. Basically, it is the regions non-farm profile. Among them degraded areas Nechernozemie and Northern Russia with unfavorable climatic conditions.

Most are filled with third cluster of rural regions of the Central Chernozem region, Volga region, as well as suitable for agriculture of the regions of Western Siberia and the Urals, which produce more than 40% of agricultural production. The main producers are agricultural organizations and the farming population. Peasant (farmer's) farm produce here for less than 10% of agricultural production. Households in this group in the rural areas provide about 45% of agricultural production. .

Family forms of agricultural production (households and private farms) are most developed in the fifth and sixth clusters. They are represented by regions became the Eastern border area with low density of population and the Caucasian Republic with limited land resources. The sixth cluster represents the remote North-Eastern and southern regions with predominant development of home and farms. Here, on the one hand, due to the climatic characteristics of the regions are limited opportunities for conducting of large-scale agricultural production (Sakha-Yakutia, Magadan and Sakhalin regions), and on the other, the development of production was constrained due to the prevailing socio-political circumstances (Chechen Republic and Dagestan) [2].

Thus, we can say that over the last 15-20 years the process of market transformations in agricultural production decreased the role of large agricultural enterprises and significantly increased the role of family forms of agricultural production. Informal family forms of agricultural production occurred in the 2000 years, rather in spite of, not because of agrarian reform. A significant growth in the products grown in the farms of the population, shows not about success but rather about failures carried out agrarian reforms. The informal agrarian economy is a form of adaptation and survival of the rural population in the process of reforming of agrarian relations.

Many collective farms in the result of hasty, ill-considered and total conversions were destroyed. The rural society was not ready to return to individual farming. Lost experience of individual and family agricultural production on a large scale returns slowly and as if involuntarily. However, poverty forced the rural population to return to your past experience. In terms of production of agricultural products of family farms began to compete with large collective agricultural enterprises, and in some regions ahead of them (V and VI clusters).

In recent years the structure of agricultural production by categories of farms suggests that imposed on top of economic and institutional innovations in agriculture have been based on historical experience, traditions and preferences of the peasants. As a result of ongoing agricultural policy did not always produce the desired results, and the dominant economic structures, contrary to the expectations of the reformers, developed for their specific trajectories. Try to understand the anatomy of their development.

III. MIMICRY OF THE HOUSEHOLD PLOTS

In Soviet times household plots acted important economic and social functions. First of all, it is an economic function: family agricultural production raising the standard of living of the rural population by increasing mobile revenues, improve material consumption, including food resource use of labour of all family members, rational land use, including a small marginal plots.

A social function of private farming is primarily in the labor education of the younger generation, the formation of professional qualities and skills of effective and economical farming. The recreational role of the personal subsidiary farming – it is the consumption of high-quality natural food and strengthen the physical health of family members

Subsistence nature of family agricultural production was due to the fact that the main occupation of the family members, who have reached working age, was work on the collective farms, in social institutions or in other fields. Of the able-bodied young people was busy studying in school or vocational training. Work in the family economy was for all family members and voluntary utility (relative to main occupation) in nature and served as an additional source of family income.

Household plots based on state or cooperative ownership of land and private ownership of productive livestock, buildings, means of mechanization and tools have made a significant contribution to the solution of the food problem of the country. However, in the absence in Soviet times, the free market needed small-sized agricultural machinery, weak development and an extremely limited set of services provided to rural population, the existence of personal subsidiary farming was impossible without the active support of the agricultural enterprises.

The symbiosis of private plots and collective farms has been and remains in the focus of sociologists and economists [3-6].

Deterrent to the development of private farms in the Soviet period was the high employment rate of rural population into collective farms (sovkhozes and kolkhozes). Work in his household was for the most part unoccupied by the population, as well as employees during off hours. If given the opportunity, employees have taken and part of the working day. Currently the situation has changed significantly. Large agricultural organizations with the participation of private capital are not condoned today to employees. In private enterprises introduced a system of fines for employees who violate labor discipline. The services provided by the collective or private farms are paid.

In modern conditions the role of personal part-time farm has changed significantly. For many rural residents it has become the main employment-generating a substantial share of the incomes of rural families. Private farming was transformed, in the terminology of O. Fadeeva, "family-consumer way of life" in the "family business system". If the dominant goal of economic activity in the first case, is survival at the expense of own production and receipt of income in kind as compensation for missing income, in the second – stable money income [6. C.35].

Under Russian law, private agriculture (household plots) is a form of non-entrepreneurial activities in the production and processing of agricultural products, carried out by personal labour of a citizen and members of his family in order to meet personal needs on the land, granted or acquired for private farming. The land use of the farms may consist of the household plots and in field plots.

In government statistics, instead of the term "households", the term "farm population", which include private and individual farms of citizens in rural and urban areas, and households with land plots at vegetable, gardening and dacha non-profit associations. *The functioning of households is based on the initiative of the informal agricultural activities of citizens, self-funded, self-organization and labour relations based on kinship, neighboring, or personal interactions.*

Today, households are the main producers of potatoes, vegetables, one third of the total production of milk (table. 1). This suggests that households are in the modern conditions a significant source not only of natural but also of cash income of the rural population. This is especially true when you consider that wages for agricultural workers remains among the lowest in the economy. Average monthly salary in agriculture was in 2000 – 985 rubles per month, 2005 -3646, 2010 – 10668, 2015 –

19721, 20016 – 21445 rubles, which corresponded to 53% of the average wage in the economy as a whole. In 2005, this ratio was 39% [1, p.125]. But the main motive of private farming is the satisfaction of the needs of rural families in natural foods.

The expansion of production in the informal sector of the economy due to a significant reduction in the number of employed in formal sector agriculture. In the period from 2005 to 2016 the number of employed in agriculture decreased by 1.2 million. In 2016, it amounted to 6,286 million, or 9.2 percent of the population employed in the economy [1, p. 93]. For comparison, in developed countries such as the UK, Germany, France, the United States population, employed in agricultural labor is approximately two times lower and ranges from 1.5 to 4-5% of the population employed in the economy.

TABLE 1
AGRICULTURAL PRODUCTION BY CATEGORIES OF FARMS % OF TOTAL

	1992	2000	2010	2016
Agriculture enterprises				
Grain	97,4	90,8	77,1	71,4
Sugar beet	97,8	94,5	88,7	88,1
Sunflower seeds	93,0	84,3	73,0	68,7
Potatoes	21,2	7,5	10,5	13,6
Vegetables	44,5	22,9	17,1	18,9
Fruits and berries	49,0	15,7	15,0	23,7
Livestock and poultry (slaughter weight)	64,0	47,3	44,9	49,0
Milk	68,1	89,4	92,3	49
Eggs	73,9	12,3	18,3	20,3
Household plots				
Grain	0,5	0,8	1,0	0,9
Sugar beet	0,2	0,6	0,4	0,2
Sunflower seeds	1,2	1,2	0,6	0,4
Potatoes	78,0	91,2	84,0	77,9
Vegetables	54,7	74,7	71,5	66,5
Fruits and berries	50,7	16,5	16,0	19,1
Livestock and poultry (slaughter weight)	35,3	36,7	46,7	45,5
Milk	31,4	26,1	31,2	33,9
Eggs	26,0	28,8	22,1	19,9
Peasant farming*				
Grain	2,1	8,4	21,9	27,7
Sugar beet	2,0	4,9	10,9	11,7
Sunflower seeds	5,8	14,5	26,4	30,9
Potatoes	0,8	1,3	5,5	8,5
Vegetables	0,8	2,4	11,4	14,6
Fruits and berries	0,3	0,2	2,2	1,9
Livestock and poultry (slaughter weight)	0,7	1,8	2,9	2,9
Milk	0,5	1,8	4,7	7,0
Eggs	0,1	04	0,8	1,0

****) Including individual entrepreneurs***
Source: [1, p.246].

Given the very limited choice of jobs in rural areas, we can safely say about the formation of a "reserve army of labor." Part released from agricultural production of labor resources engaged in seasonal work, and other work in shifts in the next cities and regions of the country [7]. Part of the unemployed rural residents provides a variety of services to the population, freelance at the place of residence [8, pp. 207-229; 9, pp. 265-295]. But the bulk of the rural unemployed find application in their farms. According to the state statistics for 2014, agricultural productions in their farms were occupied 647 thousand for the rural unemployed. In conjunction with unemployed working-age population, they account for about 30% of the labour force used in manufacturing of agricultural products (table. 2).

TABLE 2

THE POPULATION IN AGE 15-72 YEARS OCCUPIED BY MANUFACTURING PRODUCTS AGRICULTURE, FISHING, HUNTING AND GATHERING FOR THEIR OWN THE USE, AGE AND STATUS OF PARTICIPATION IN THE LABOUR FORCE IN 2014, THOUSAND PEOPLE

	Just including from it	*labour power*	*busy*	*unemployed*	*persons not in the labor force*
Just including at age, years:	13640	8779	8132	647	4861
15-24	1222	526	419	107	696
25-34	2050	1737	1585	152	313
35-44	2413	2162	2024	139	251
45-54	3273	2812	2643	169	462
55-64	3447	1442	1365	77	2005
65-72	1234	100	96	4	1133
Working age	9605	7808	7206	602	1796

Source: [10, p. 96].

The employed population is 60% of the labor force involved in family agricultural production, the unemployed – 5%, of working age outside the workforce - 13%. More than 22% of those employed in the informal sector of agricultural production is of working age, i.e. it is the elderly and teenagers. The structure of the labour resources employed in the informal sector of agricultural production, reflects the effective use of the labor potential of the household.

The duration of the working week in the informal sector of agricultural production lasts from 15 to 40 hours a week. According to statistics, over 60% of population were employed on his farm less than 15 hours a week, around 25% from 15 to 24 hours, and every tenth – from 25 to 39 hours per week and only 3 per cent had a full 40-hour work week [Calculated source: 10, p. 95].

Thus, in the period of market transformations of the farming population lost its purely a utility character and became an important sector of agricultural production. Our eyes have been mimicry of private farming, which was transformed into a large informal sector of agricultural production. The consolidation of small archaic households as one of the leading sectors of the agrarian economy was the paradoxical result of the market reforms in the agricultural sector of the country. However, it is not, in our view, a consequence of the free choice of the peasants. For the majority of the rural population, the conduct of household plots is the only way to survive in difficult conditions of market reforms.

In the Federal law "About Households " from July 7, 2003, this category of farms was recognized as a form of non-entrepreneurial activity for the production of agricultural products that are subject to the same measures of state support to large and medium-sized agricultural enterprises. Thanks to these measures we managed to avoid a significant reduction in the volume of production in farms of the population, deprived of the support of the reorganized collective farms.

IV. THE DEVELOPMENT OF PRIVATE PEASANT FARMS

In the process of economic reforms of 90-ies had established the institutional basis and legal framework for development of all types of entrepreneurial activities in rural areas and the formation of mixed agrarian economy. After the entry into force of the RF law “On peasant (farmer) economy” and the Law of the Russian Federation 1995 “On state support of small entrepreneurship in Russia,” the Russian peasants have a real opportunity to rule your own business and become independent economic entities.

Federal law "On peasant (farmer) economy" (entered into force on 11.06.2003, as amended 01.03.2015) defines a peasant (farmer) economy as an Association of citizens who have common property and jointly engaged in the production, processing, storage, transportation and realization of agricultural products. Household Plots areas a business entity without legal entity formation [11].

However, the farming sector, as we anticipated in the 90-ies [12], did not become the dominant mode in agricultural production of Russia. The state of public consciousness, the duration of formation of market relations, socio-demographic situation in the village, a dispersed settlement system, poor development of communication system have become a limiting factor for the wide spread of farms and turning them into the dominant form of agricultural production. Nevertheless, and in Russia after 70 years of collective labour in the farms were willing to work independently and become effective owners of their farms.

Only in 2000-e years the acreage in farms increased threefold, from 6.5 million hectares to 19.7 million hectares in 2014, the farm sector has occupied a steady position in the production of grains and oilseeds, as well as in the production of vegetables and sugar beet. The number of cattle in the farming sector during this period increased 4.2%, including cows – 5.5 times. Almost 10 times increase in the number of sheep and goats, 4.6 times the number of poultry. Continuously remains small, the number of pigs. However, meat production in the farming sector in the observed period of time tripled. Milk production has increased by about as much.

Currently, in the peasant (farm) produces one-quarter of the total volume of grain, about one-third of wool and sunflower seed, one-tenth of vegetables and sugar beet, (tab. 1). Today the crop is in farms ¾ of the production, and livestock – ¼.

According to the Federal tax service on August 1, 2015, was 3 million 510 thousand individual entrepreneurs and 139600 farms. The number of individual entrepreneurs that ceased operations at his own request, in January - July, 2015 decreased by 22.5%, and farms – 33.5% [13].

For comparison, in the United States in 1935, there were 6.5 million farms in 2014 to 2.1 million. The Number of people employed in agriculture decreased from 10% of the population to 2%. Over the last decade, the ratio of the scale of the farms varied in the direction of gradual reduction of small and growth of large farms. Currently in the US, large family farms, constituting 12% of all farms of the country produced 89% of US agricultural products in value terms [14].

Positive trends in the agricultural business of Russia, as experts believe, will continue. According to the Association of peasant (farmer) farms and agricultural cooperatives, 38% of the rural population in Russia would like to create the farm [15]. Besides, as says a Russian proverb, - "there would be no happiness, the misfortune has helped". The introduction of a food embargo against our country and the response of Russia led to the transition to import substitution in many sectors of the economy, including agriculture. Support of agricultural commodity producers of all forms of ownership, although necessary, has given a new impetus in the development of the agricultural complex of the country, including the farming sector.

Limiting factors of development of peasant (farm) in Russia, according to our many years of research, are: insecurity of property rights; weak support from the state; high taxes; the imbalance of agricultural prices, depressed by low solvent demand of the population, and increasing prices for agricultural equipment, fuel and lubricants and other resources; high interest rates; underdeveloped communication and road network in the countryside.

In the long term growth of the farm sector of the agricultural economy will restrain significant growth of the property tax and increase land tax.

Part of the failure of development of the farming sector due to subjective reasons related to the lack of Russian peasants of experience in self-management, a lack of specialized knowledge, unwillingness to work in the conditions of economic and social risk that has developed over the years of Soviet power the specific culture of doing joint work, the feeling of social security in the team. It is no coincidence therefore that sociologists record a certain sorrow of the older generation about the past.

A well-known researcher of the Russian village, Vinogradsky, V. G. suggests the emergence among the peasants of a certain generational sadness, i.e. a kind of yearning for the past, socialist system the peasant world. This memory of the past, according to the author, can play the role of a "healing socio-cultural drugs". Soaked in rural daily life of new generations, it will lead to new Russian peasant worlds [16].

As for the younger generation, I would venture to say that the rural youth is more likely to aspire to the city, in order to get rid of the heavy farm labour from the insecurity of rural life.

V. PROSPECTS OF DEVELOPMENT OF SMALL FORMS OF AGRICULTURAL PRODUCTION

Prospects for development of farms and other small forms of agricultural production determined in large part by the State program of development of agriculture and regulation of markets of agricultural products, raw materials and food for 2013 – 2020 [17].

A separate sub-programme dedicated to the "Support of small forms of managing". Her goal: maintenance and further development of agricultural and non-agricultural activities small farms and improving the quality of life in rural areas. Objectives sub-programme: creating conditions for increasing the number of small business entities; improving the efficiency of use of land from agricultural lands; increase the level of rural incomes.

The Subprogram will allow by 2020 to create an additional 14 thousand (peasant) farms, which provide jobs for 42,15 thousand annual average of employees; full implementation of the program of creation of livestock farms by 2020 to increase milk production in peasant (farmer) farms on 675 thousand tons; the growth of agricultural products produced by small businesses, amounting to 7.4 %.

The subroutine includes four groups of activities:

- "***Support of beginning farmers***" in the form of granting grants on creation and development of peasant (farmer) farms.
- "***Development of family livestock farms on the basis of the peasant (farmer) farms***", i.e. construction or modernisation of family farms, the size of the grant issued from the Federal budget and budgets of constituent entities of the Russian Federation may not exceed 60% of the costs of the peasant (farmer's) economy.
- "***State support of lending to small farms***" through subsidies from the Federal budget to budgets of subjects of the Russian Federation on compensation of part of expenses for payment of percent on the Bank credits and loans received in agricultural credit consumer cooperatives, peasant (farm) holdings
- ***"The registration of land property of a peasant (farming)***". The purpose of the event - compensation expenses for carrying out of cadastral works concerning the ground areas from the agricultural land. Currently, the average cost of execution of the plot is 1000 rubles for 1 ha. From the Federal budget compensation was provided about 500 RUB per 1 ha, which is about 50 % of the total cost, the remaining funds necessary to conduct cadastral works are provided from the budgets of the constituent entities of the Russian Federation and municipal formations.

VI. TRENDS OF DEVELOPMENT OF AGRICULTURAL ENTERPRISES

The basis of the corporate sector of the agrarian economy after market reforms amounted to the reorganized collective and state farms that repeatedly changed its legal status on the basis of the incentives from the state had one or another legal form. These were joint stock companies and partnerships of all types, agricultural cooperatives, and vertically integrated agricultural organization, agricultural and agricultural holdings.

The main challenge was adapting to the market, search of reliable partners and markets. Business tried to get rid of excess labor. Suffice it to say that during the period from 1995 to 2013, the number of people employed in agriculture fell by more than 3 million people.

But, despite all the efforts of the agricultural organizations, they failed to overcome the devastating consequences of the market agrarian reform. A significant share of agricultural enterprises remained unprofitable, despite the fact that the share of unprofitable agricultural organizations decreased significantly (Fig. 2)

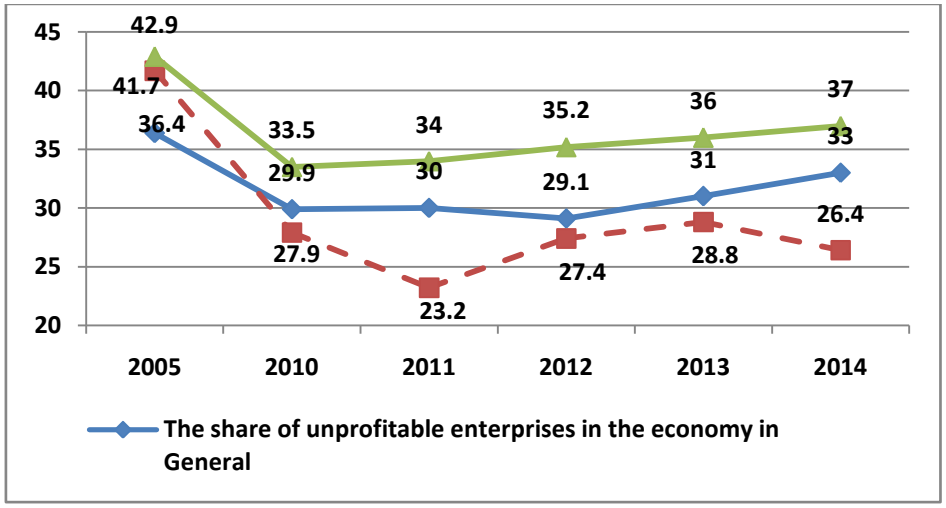

FIGURE 2. THE SHARE OF UNPROFITABLE ORGANIZATIONS AND THE DEGREE OF WEAR OF FIXED ASSETS IN RURAL ECONOMY, %
Source: [18, pp. 44-48].

One of the reasons for the low efficiency of production in agricultural organizations was low, the scale of investment in fixed capital. Since 2005, their level did not exceed the 2.7-3% of the total. The coefficient of disposal (liquidation) of fixed assets

in agriculture was higher compared to all fixed assets in the economy as a whole, and renewal coefficient of fixed assets is lower (tab. 3). As a result, in 2000-ies in agricultural organizations three times decreased the tractors, harvesters, mowers, milking machines and aggregates, five times decreased the number of sugar beet machinery and other equipment [18, p. 64].

TABLE 3
THE COEFFICIENT UPDATE AND DISPOSAL OF FIXED ASSETS (IN COMPARABLE PRICES), INVESTMENT IN FIXED CAPITAL IN AGRICULTURE OF RUSSIA *)

	2005	2010	2011	2012	2013	2014[1)]
The share of unprofitable farms in the economy as a whole	3,0	3,7	4,6	4,8	4,6	4,3
All fixed assets in that number and forestry agriculture	2,4	3,7	4,3	4,2	4,3	4,0
Retirement rate (elimination fixed assets as a percentage of availability fixed assets at the beginning of the year)	1,1	0,8	0,8	0,7	0,7	0,7
All fixed assets in that number agriculture, hunting and forestry agriculture	4,2	2,2	2,4	2,5	2,1	2,1
Investments in fixed capital (in current prices), billion.	313,8	79,1	201,8	256,9	276,3	307,1
percentage of total investment	2,7	3,0	3,0	2,9	3,1	3,0

) Without small businesses and investment not observed by direct statistical methods.
Source: [18, pp. 60-61].

State support is an integral part of agricultural policy in many countries. An example of effective use of existing resources and technologies is the Netherlands with an area of 41 thousand sq km (less Moscow region), the agricultural sector employs only 4 % of the working population, produces of agricultural products 55 billion euro per year. This is the second after the United States increased agricultural production. Currently, subsidies in the EU reached 45-50% of the cost of production of farmers in Japan and Finland — 70 %, in Russia — only 3.5 %. In the United States in the development of agriculture invested 30% more money (per unit of production) than in other industries [19].

In the period from 1992 to 2016 the contribution of agricultural enterprises in the production of goods decreased from 67,1% to 52,8%, respectively. The livestock population in this period decreased from 40.2 to 8.4 million animals, and the livestock of cows from 13.7 to 3.4 million heads. In 2000, agricultural organizations in the production of milk yielded its primacy to households. Positive trends in milk production was observed only in the farming sector (Fig. 3,4).

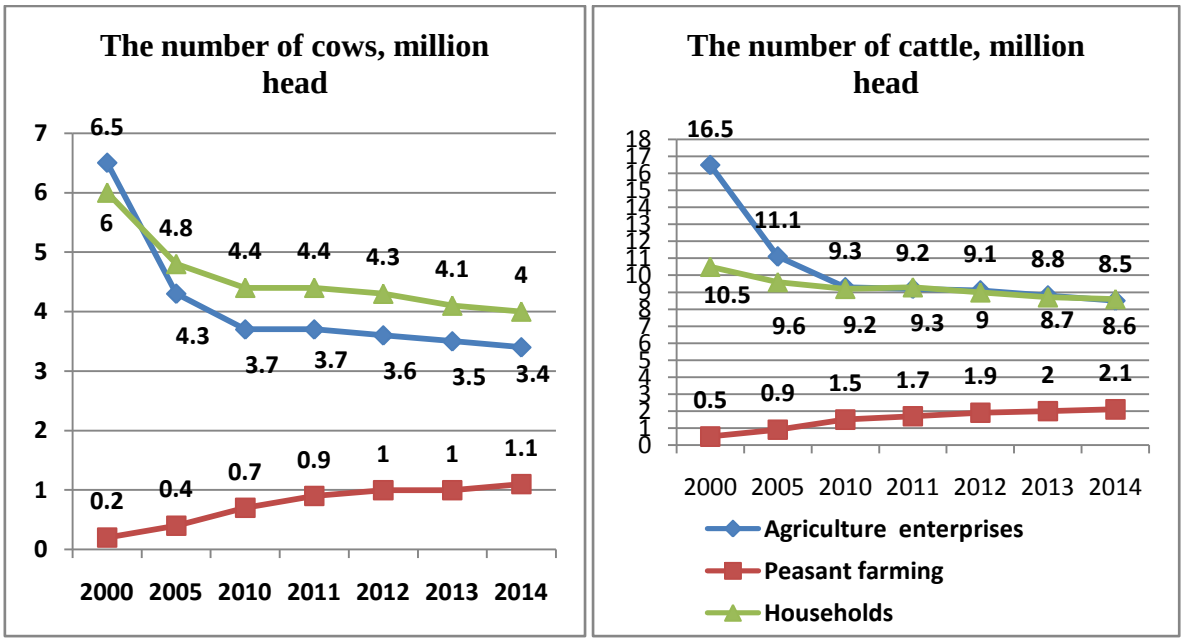

FIGURE 3, 4. THE NUMBER OF CATTLE, INCLUDING COWS BY TYPES OF FARMS, MILLION HEAD
Source: [18, p.94].

The acreage in agricultural organizations decreased during the same period, from 74.2 to 55.3 million hectares, including grain crops and leguminous cultures - from 40.7 to 32.2 million ha (Fig.5). But at the same time significantly increased the production of grain: 59, 4 million tons to 77.6 million tons According to experts, Russia could become the largest grain producer in the world. Sugar beet production increased from 13, 3 to 29.9 million tons, and potatoes with 2, 2 million tons to 3.8 million tons [18, p. 55].

The acreage in agricultural organizations decreased during the same period, from 74.2 to 55.3 million hectares, including grain crops and leguminous cultures - from 40.7 to 32.2 million ha (Fig.5).

The coefficient of disposal (liquidation) of fixed assets in agriculture was higher compared to all fixed assets in the economy as a whole, and renewal coefficient of fixed assets is lower (tab. 3). As a result, in 2000-ies in agricultural organizations three times decreased the tractors, harvesters, mowers, milking machines and aggregates, five times decreased the number of sugar beet machinery and other equipment [18, p. 64].

The conversion of agricultural enterprises appeared in 2000-ies in increasing numbers of pigs by 1.8 times, the doubling of poultry, which increased meat production 3.7%, including pork - by 5.5 times, poultry meat – by 7.5 times. Eggs production has increased by 8.4 billion pieces. Milk production has decreased by 0.9 million tons.

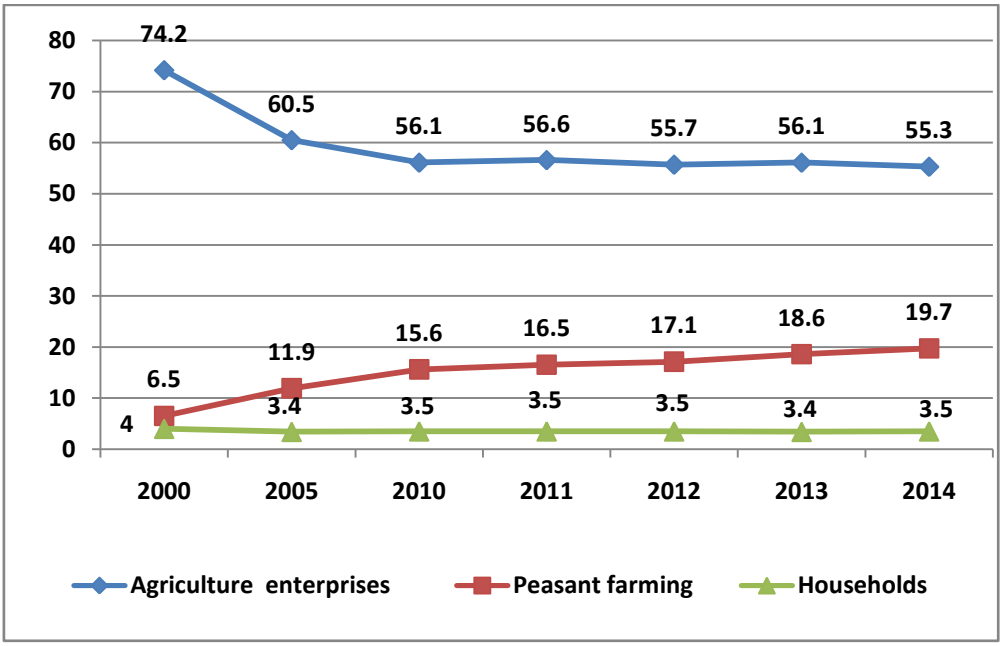

FIGURE 5. DYNAMICS OF CULTIVATED AREAS BY CATEGORIES OF FARMS, mln ha
Source: [18, p. 78].

At the end of January 2016, compared with the corresponding date in 2015, the number of cattle in the agricultural organizations decreased by 0.9%, cows - by 1.5%. The number of sheep and goats increased by 0.5%, pigs - by 13.1%, poultry - by 4.6%. The production of livestock and poultry for slaughter (in live weight) in agricultural organizations in January 2016, compared to January 2015 increased by 5.1% (in January 2015 compared to January 2014 year - on-year by 9.4%), milk - by 2.6% (4.2%), eggs - by 3.8% (1.3%) [18, p.90].

VII. SUMMARY

In summary, we can state that as a result of market transformations in agrarian economy had developed two equivalent (for contribution to agricultural production) sectors: corporate business, represented by agricultural organizations, and individual and family business sector, represented by private farms and individual entrepreneurs (formal sector) and households plots (informal sector). In these sectors in the post-reform period there was a reorientation and specialization of production, has formed a reliable network of distribution and processing of manufactured products, has been found adequate forms of interaction with business partners and with government.

For the implementation of the state program of development of agriculture in 2016, it was planned to send 237 billion rubles. It is possible to ensure, according to experts, only the inertial scenario of development of the industry. To support new

investment projects were expected to Finance about 47 billion rubles, which amounted to 20% of the proposed financing of agriculture in 2016, Real investment in agriculture in 2016 increased by 10% and the share of profitable farms was 87 [17].

However, experts of the RF Ministry of economic development make optimistic forecasts of APK. Agricultural production in Russia in the next four years, according to their estimates, could grow by 7.8%. The main contribution to the growth of agricultural production will provide livestock. The dairy industry in four years will increase just 1.7%, growing at 7 per cent. While physical volume of investments in the agricultural sector in 2018 will account for 99.4% of the figure in 2014 [20], which casts doubt on the optimistic forecasts of the Ministry.

Optimism can be associated only with inexhaustible energy and creativity of the Russian peasantry, with its amazing ability to adapt in the most incredible institutional conditions to make the impossible - possible!

REFERENCES

[1] Russia in figures. 2017, Rosstat, 2017 -511 p.

[2] Z. Kalugina, O. Fadeeva, S. Bratusenko. Socio-economic polarization of rural space in Russia // Region: Economics and sociology, 2015, №3 (87), pp. 123-145.

[3] T.G.Nefedova, J. Pallot. Unknown agriculture, or who needs a cow? – Novoe Izdatelstvo, 2006. – 320 p.

[4] O. Nechiporenko and others. The Peasants of modern Russia: life worlds and social practices / ed. by O. Nechiporenko; Institute of philosophy and law SB RAS.- Novosibirsk*:* Publishing house SB RAS, 2015.- 252 c.

[5] A. Nikulin. Enterprises and family in Russia: socio-cultural symbiosis // Where goes Russia? Transformation of social sphere and social policy / ed. by T. I. Zaslavskaya. – M.:. Matter, 1998, pp. 218-229.

[6] O. Fadeyeva. Rural communities and economic structures: from survival to development / edited by Z. Kalugina. – Novosibirsk: IEIE SB RAS, 2015. - 264 p.

[7] P. Veliky. Russian village. The processes of post-Soviet transformation, ed. by Yu. S. Voronov.- Saratov: Scientific book, 2012. - 340 p.

[8] Z. Kalugina. Market transformation of agrarian sector of Russia. Sociological discourse. – Novosibirsk: Publishing house of IEIE SB RAS, 2015. - 342 p.

[9] Zemfira Kalugina. The Russian vector of agrarian reform: Lambert Academic Publishing RU – 2016.- 463 p.

[10] Labour and employment in Russia. 2015: Stat. SB./ Rosstat. M., 2015. - 274 p.

[11] Federal law "On peasant (farmer) economy" (entered into force on 11.06.2003, as amended 01.03.2015) URL // http://www.vedomosti.ru/economics/news/2015/08/14/604930-fns-zaregistrirovala-v-rossii

[12] Z. Kalugina. Social boundaries development of peasant (farm), Izv. SB as USSR, 1991, vol. 3. - Ser. Region: Economics and sociology.

[13] URL // http://www.vedomosti.ru/economics/news/2015/08/14/604930-fns-zaregistrirovala-v-rossii

[14] A. Terentyeva. Farm USA in the early XXI century: state and development trends // U.S. and Canada: economy, politics, culture, 2015, 12 N(552), c. 85-101.

[15] URL// http://ria.ru/infografika/20150603/1067942815.html

[16] V. Vinogradsky. Peasant world in the discourse of generational grief // Sociological research, 2015, N12, pp. 82-91.

[17] The state program of development of agriculture and regulation of markets of agricultural products, raw materials and food for 2013 – 2020. Sub-program "Support of small forms of managing" // http://stav-ikc.ru/index.php?option=com_content&view=article&id=2849:2013-2020&catid=47:2009-08-05-12-01-51&Itemid=66

[18] Agriculture, hunting and hunting economy, forestry in Russia. 2015: Stat. SB./ Rosstat. – M., 2015, 201 pp.

[19] L. Zimina. Agriculture: what is the secret of success? // http://bujet.ru/article/177647.php

[20] URL // http://agroinfo.com/selskoe-xozyajstvo-rossii-v-2016-godu-budet-razvivatsya-inercionno-0311201505/

[21] Z. Kalugina. The paradoxes of agrarian reform in Russia: sociological analysis of transformation processes, Novosibirsk, 2001.- 196 p.

[22] Z. Kalugina. Agrarian Actors in the Localities // Explaining Post-Soviet Patchworks. Volume 1. Actors and Sectors in Russia between Accommodation and Resistance to Globalization. Ashgate. Aldershot. Burlington USA. Singapore. Sydney, 2001: 310-330.

[23] Z. Kalugina. Private Farming in Russia: a Thorny Path to Revival // Farming and Rural Systems Research and Extension. Local Identities and Globalisation. IFSA European Group, Florence, Italy, 2002: 148-157.

[24] Z. Kalugina. Adaptation Strategies of Agricultural Enterprises during Transformation // D. J.O'Brien and S.K.Wegren (ed.) Rural Reform in Post-Soviet Russia. Woodrow Wilson Center Press, Washington, D.C. USA, 2002: 367-384.

[25] Transformation and Diversification of Rural Societies in Eastern Europe and Russia. Ieda Osamu (ed.) Slavic Research Center, Hokkaido University, Sapporo, Japan, 2002: - 345 p.

[26] Z. Kalugina. Rural labor market in present Russia // Eastern European Countryside, 2005, №11, pp. 19-38.

[27] J.R. Franks, O. Fadeeva, I. Davydova, Z. Kalugina. Farm ownership and Management in Russia: A study of the Novosibirsk Region // Journal of Farm Management, Volume 12 No.6 October 2005, pp.329-350.

[28] Z. Kalugina. Institutional Traps in the Agrarian Transformation in Russia // Eastern European Countryside, 2007, №13, pp. 69-82.

[29] Russian Views of the Transition in the Rural Sector. *Structures, Policy Outcomes, and Adaptive Responses* / L. Alexander Norsworthy (ed.). The International Bank for Reconstruction and Development / The World Bank, Washington, D.C. USA, 2000, - 209 p.

Prevalence of teat end hyperkeratosis in lactating dairy cattle and their association with animal variables

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Abstract— *Short and long-term changes in teat condition mainly caused by machine milking may affect the udder health of lactating dairy cattle. The objective was to investigate the prevalence of teat end hyperkeratosis scores in lactating dairy cattle in Germany and to determine associations between animal variables and teat end hyperkeratosis. The study was conducted in 15 German high-yielding dairy herds. On dairy farms teat size, teat shape, teat end shape and changes in teat condition (edema, teat skin colour, teat end hyperkeratosis) were recorded. In total, 4,022 animals of the breed German Holstein were included into the present study. For both front and rear teats associations between teat end hyperkeratosis and lactation number, teat length, teat diameter and teat end shape were found. Further research is needed to reduce the prevalence of teat end hyperkeratosis in high-yielding dairy herds and to improve the teat condition.*

Keywords— Udder health, teat end hyperkeratosis, teat end shape.

I. INTRODUCTION

Udder health problems cause huge economic loss in dairy milk production. Estimates put the amount of lost milk caused by raised somatic cell counts at 3 to 18% for cows with an average somatic cell count of 500,000 cells/ml [1]. Clinical mastitis is even more costly through discarded milk and treatment costs [2].

Poor teat condition is identified to be a risk factor for udder health problems in modern dairy herds. Authors categorize changes in teat end condition in three major groups: short term, medium term, and long term changes in teat condition [3]. Short and medium term changes like congestion, edema, swelling of the teat base and petechia are clinical signs of an impaired circulation of blood and lymph. Teat end hyperkeratosis is a so called long term change of the teat tissue [3]. In general, teat tissue changes are a result of mechanical stress affecting the tissue during machine milking [4]. Main risk factors for changes in teat condition are long machine-on times [4, 5, 6], high vacuum levels [7, 8, 9] and overmilking [10, 11, 12].Additionally cow individual risk factors are described by several authors for extreme teat end hyperkeratosis. These factors are teat size [13] and teat end shape [14, 15]. Teat end hyperkeratosis may influence the prevalence of intramammary infections [16, 17].

Mein et al. (2001) also tried to confirm thresholds for the interpretation of the teat end condition found in dairy herds [3].They recommend that less than 20 % animals have teats with teat end hyperkeratosis exceeding the severity of a smooth white ring around the teat orifice.

The aim of this study was to describe the prevalence of teat end conformation traits including cow individual variables like teat size and teat end shape and milking induced teat tissue changes, especially teat end hyperkeratosis in high-yielding German dairy herds. Associations between cow individual variables and teat end hyperkeratosis were examined.

II. MATERIAL AND METHODS

2.1 Farms and animals

The animals used for this survey originated from 15 randomly selected dairy herds in Northern Germany. The selected farms kept cows of the breed German Holstein black pied and had an average milk yield of at least 9,000 kg per cow and year. Milking equipment of each farm was tested according to ISO 5707:1996 once a year [18]. The majority of cows in the herds were enrolled in this study. Animals with apparent signs of a clinical mastitis, especially with an inflammatory swelling of the affected quarter were excluded from the study.

2.2 Data collection

Data collection was performed between May and October 2014. The participating farms were visited once and animals were examined during milking time. Observations were done by trained, constant teams of two persons, to gather reliable results. Teat size (length, diameter 1 cm proximal the teat end) as well as teat shape and teat end shape [19] were determined before milking (Table 1). Immediately after cluster detachment short and long-term changes in teat condition of the right front and the right rear teat of each cow were examined visually and by manual palpation according to Mein et al. (2001) [3].

TABLE 1
ANIMAL VARIABLES INCLUDED INTO THE INVESTIGATIONS

Criterion	Mode of detection	Score/category
Teat size		
Teat length	measuring	from the base of the teat to the teat tip
Teat diameter	measuring	1 cm above the teat tip
Teat and teat end shape (Grunert et al., 1990)		
Teat shape	visual	normal meat short funnel pencil broke bottle
Teat end shape	visual	round flat inverted pocket pointed
Short-term changes in teat condition (Mein et al., 2001)		
Colour of the teat skin	visual	pale, pale-rose, rose, rose-blue, blue
Swelling of the teat base	visual / palpation	yes/no
Oedema of the teat	palpation	no oedema swelling of teat end swelling of teat barrel
Long-term changes in teat condition (Mein et al., 2001)		
Teat end hyperkeratosis	visual	0 no callous rings 1 smooth, slightly raised ring 2 rough raised ring 3 rough raised ring, fronds

Lactation number and lactation stage data (days in milk) of the animals included into the present study were collected from cattle herd software.

2.3 Statistical analysis

Data were gathered and analysed using the programmes Microsoft Excel, Microsoft Access 2000 (Microsoft Corporation), and SPSS (SPSS 23.0, Chicago USA). The udder quarter was the statistical unit. The associations between hyperkeratosis and animal variables (covariates, factors) were analysed using ordinal regression procedures [20]. The teat end hyperkeratosis score was the dependent variable. The relationships between single animal variables and teat end hyperkeratosis were tested using ANOVA test for continuous measurements and the χ^2-test (likelihood ratio statistic) for proportions in a first step. However, predictors that strongly correlated with each other ($r>0.70$) were not included in the same model to avoid multicollinearity. As a second step, variables that were associated with the outcome variables at $P < 0.10$ in ANOVA for continuous measurements and the χ^2-test for proportions were included in ordinal logistic regressions. A

forward stepwise process was used for final model selection, applying a P value < 0.05 for inclusion. Likelihood-ratio tests were used for significance test to include predictors. Goodness of fit of models was assessed by Pearsons χ^2 goodness of fit statistics [21]. The predictive power of a model was measured by a rescaled pseudo R^2 with the maximum of 1 [22]. Statistical significance was assumed at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

3.1 Descriptive statistics

4,022 animals from 15 dairy farms were enclosed in this survey per farm between 69 and 848 animals were included into the present study. In total 4,022 front teats and 4,022 rear teats were investigated.

3.2 Teat end hyperkeratosis score

The distribution for the different teat end hyperkeratosis scores is shown in Table 2. The most prevalent finding was score 1 (smooth white ring around the teat orifice). 53.74% of the front teats and 47.38% of the rear teats exceeded the extent of a smooth white ring (score 2 + score 3). The hyperkeratosis of 20.06% of the front and 15.71% of the rear teats was classified as score 3.

TABLE 2
ANIMAL VARIABLES IN THE NORTHERN GERMAN DAIRY CATTLE POPULATION INCLUDED INTO THE PRESENT STUDY (N = 4,022 ANIMALS) (mean ± standard deviation and frequency, respectively)

Animal variable		Front teats	Rear teats
Lactation stage (days in milk)		203±135	
Teat size (mm)	length	53.5+/-8.1	46.4+/-7.6
	diameter	21.7+/-2.6	21.3+/-2.6
Teat shape (%)	normal	99.09	99.57
	other shape	0.91	0.43
Teat end shape (%)	round	81.01	81.08
	flat	13.79	14.13
	pointed	3.81	3.44
	funnel shaped	1.39	1.34
	other shape than round	18.99	18.91
Teat edema (%)	no	21.90	18.86
	teat barrel	45.70	44.58
	teat end	32.40	36.56
Swelling at teat base (%)	no	76.26	69.19
	yes	23.74	30.81
Teat skin colour (%)	rose	56.27	56.14
	other colour than rose	43.73	43.86
Teat end hyperkeratosis Score (%)	0	6.27	7.27
	1	39.99	45.36
	2	33.68	31.67
	3	20.06	15.71
	2+3	53.74	47.38

3.3 Lactation number

On average, cows included in the study had 2 to 3 lactations (minimum: 1 lactation, maximum: 12 lactations).

3.4 Lactation stage

Cows were on average 203 ± 135 days in milk (Table 3).

TABLE 3
MEAN VALUES ± STANDARD DEVIATION (RANGE) OF ANIMAL VARIABLES INCLUDED INTO THE FINAL LOGISITIC REGRESSION MODELS

Animal variable	Teat end hyperkeratosis score							
	Front teats				Rear teats			
	0	1	2	3	0	1	2	3
Lactation number	2.27+/- 1.30	2.09+/- 1.34	2.18+/- 1.38	2.32+/- 1.32	2.33+/- 1.34	2.14+/- 1.36	2.24+/- 1.41	2.44+/- 1.37
	(1-7)	(1-11)	(1-12)	(1-8)	(1-7)	(1-12)	(1-10)	(1-8)
Lactation stage (days in milk)	206+/-154	196+/-131	207+/-137	222+/-148	182+/-143	196+/-132	212+/-135	216+/-135
	(2-774)	(3-886)	(1-786)	(1-743)	(2-774)	(3-886)	(1-786)	(1-721)
Teat length (mm)	51.3+/-8.1	52.6+/-8.1	53.3+/-7.9	55.4+/-7.9	45.0+/-7.7	45.8+/-7.6	46.4+/-7.3	49.0+/-7.4
	(35-78)	(30-92)	(30-88)	(37-81)	(25-73)	(22-94)	(15-80)	(22-80)
Teat diameter (mm)	22.4+/-2.7	21.7+/-2.7	21.7+/-2.6	21.8+/-2.5	21.8+/-2.3	21.2+/-2.6	21.1+/-2.3	21.5+/-3.1
	(17-35)	(10-44)	(16-43)	(15-45)	(17-31)	(10-35)	(14-33)	(16-35)

3.5 Teat length and teat diameter

Average teat length found for the investigated dairy cows was 53.5 +/- 8.1 (mean +/- standard deviation) mm for front and 46.4 +/-7.6 mm for rear teats. The teat diameter found had an average value of 21.7 +/-2.6 mm for front teats and of 21.3 +/- 2.6mm for the rear teats (table 2).

3.6 Teat shape

99.09% of the front and 99.57% of the rear teats were found to have a normal teat shape.

3.7 Teat endshape

A round teat end was the most common teat endshape found in front and rear teats (over 80%) followed by flat teat ends (13.79% and 14.13% for front and rear teats, respectively) and pointed teat ends (3.81% and 3.44%) (Table 2). An association could be found for teat end shape and hyperkeratosis in front and rear teats. Teats with hyperkeratosis score 3 had round teat ends in 85% of all cases, more than 5% of the teats were flat and more than 7% had a pointed teat end (Table 4).

TABLE 4
PERCENTAGE DISTRIBUTION OF ANIMAL VARIABLES TO HYPERKERATOSIS SCORES (n = 4,022 animals) (%)

Animal variable		Teat end hyperkeratosis score							
		Front teats				Rear teats			
		0	1	2	3	0	1	2	3
Udder shape	normal	97.79	99.51	98.87	98.61	98.06	99.49	98.90	98.45
	other shape	2.21	0.49	1.13	1.39	1.94	0.51	1.10	1.55
Udder edema	no	96.90	99.43	99.69	99.83	97.29	99.49	99.67	99.78
	yes	3.10	0.57	0.31	0.17	2.71	0.51	0.33	0.22
Intertrigo	no	99.56	99.92	99.90	99.65	99.61	99.93	99.89	99.56
	yes	0.44	0.08	0.10	0.35	0.39	0.07	0.11	0.44
Teat shape	normal	98.35	99.36	99.00	99.23	99.64	99.77	99.43	99.51
	Other shape	1.65	0.64	1.00	0.77	0.36	0.23	0.57	0.49
Teat end shape	round	73.66	79.35	82.19	85.84	76.51	80.31	81.33	86.63
	flat	20.16	16.52	13.20	5.79	17.44	16.15	14.00	5.12
	pointed	0.41	2.32	3.99	7.85	0.36	2.17	4.10	7.59
	funnel shaped	5.76	1.81	0.61	0.51	5.69	1.37	0.57	0.66
	other than round	26.34	20.65	17.81	14.16	23.49	19.69	18.67	13.37
Teat edema	no	24.17	24.10	21.37	17.31	25.91	19.56	17.57	15.25
	teat barrel	33.75	30.52	33.70	32.50	35.77	36.08	36.91	36.61
	teat tip	42.08	45.38	44.93	50.20	38.32	44.37	45.52	48.14
Swelling at teat base	no	79.41	75.38	76.55	77.30	75.45	67.28	69.47	71.48
	yes	20.59	24.62	23.45	22.70	24.55	32.72	30.53	28.52
Teat skin	dry	97.22	96.00	94.43	95.71	97.51	95.57	95.04	94.99
	wet	2.78	4.00	5.57	4.29	2.49	4.43	4.96	5.01
Teat skin color	rose	61.45	59.49	52.79	54.08	63.72	56.88	54.45	53.85
	others	38.55	40.51	47.21	45.92	36.28	43.12	45.55	46.15

3.8 Short term changes in teat condition

The presence of short term changes in teat condition like teat edema, swelling at the teat base and teat skin color have been investigated. 45.7% of the front and 44.58% of the rear teats had an edema of the teat barrel. 32.40% of the front and 36.56% of the rear teats had an edema of the teat end. A swelling at the teat base was found for 23.74% of the front and 30.81% of the rear teats. The teat skin of 43.73% of the front and 43.86% of the rear teats were rose-blue or blue.

3.9 Final logistic regression model

Table 5 provides the final regression models for hyperkeratosis as dependent variable. The rescaled R^2 of the final models were 0.226 and 0.281 for front and rear quarters, respectively. The goodness of fit-statistic did not give any reason to doubt about the validity of the models (front teats: P = 0.366; rear teats: P = 0.560). Significant animal variables associated with hyperkeratosis identified by the final logistic regression model for front and rear quarters were "teat length", "teat diameter", "teat end shape" and "lactation number".

TABLE 5
FINAL ORDINAL REGRESSION MODELS FOR SIGNIFICANT ANIMAL VARIABLES ASSOCIATED WITH TEAT END HYPERKERATOSIS (n = 4,022 animals)

	Variable	Estimate	SE	P	Wald	95% CI
Front teats	lactation no.	0.044	0.016	0.007	7.349	0.012-0.076
	Teat length	0.017	0.003	<0.001	34.603	0.011-0.023
	Teat diameter	-- -0.020	0.008	0.014	6.069	-0.037--0.004
	Teat end shape					
	round	0.809	0.191	0.004	17.864	0.434-1.184
	pointed	1.292	0.219	0.017	34.734	0.862-1.721
	flat	0.457	0.199	0.022	5.264	0.067-0.847
	funnel shaped teat end	base				
Hind teats	Lactation no.	0.037	0.016	0.024	5.108	0.005-0.068
	Teat length	0.018	0.003	<0.001	34.96	0.012-0.024
	Teat diameter	-0.022	0.008	0.007	7.363	-0.038--0.006
	Teat end shape					
	round	0.743	0.182	0.004	16.682	0.386-1.099
	pointed	1.262	0.214	0.017	34.812	0.843-1.682
	flat	0.459	0.189	0.015	5.912	0.089-0.830
	funnel shaped teat end	base				

3.10 Discussion

The main objective of this study was to describe the prevalence of teat end hyperkeratosis and some specific animal variables in commercial high-yielding dairy herds and to identify animal variables associated with teat end hyperkeratosis. It was possible to find some animal variables which are associated with teat end hyperkeratosis score. These are lactation number, teat size (diameter, length) and teat end shape.

3.11 Lactation number

Animals with hyperkeratosis score 3 had a higher average lactation number than those with score 0 to 2. The results imply a maximum during the third lactation. Gossen et al. (2007) found that teats of cows in their second lactation to have better average hyperkeratosis scores than younger or older cows [23]. Neijenhuis et al. (2000) described an increase of hyperkeratosis with the lactation number [4].

3.12 Lactation stage

Cows with higher teat end hyperkeratosis scores tend to have more days in milk (except for front teats with score 0). Different authors reported an increase in hyperkeratosis with progressing lactation. They found an increase in teat end

hyperkeratosis score between the first 6 weeks to 4 months of lactation and a subsequent phase of persistency [4, 23, 24]. The development of hyperkeratosis is supposed to be a natural process of adaption of the teat to machine milking. It reaches its maximum after a certain time of exposure to mechanical stress. Neijenhuis et al. (2000) described changes in teat end tissue as a result of mechanical stress during machine milking [4].

Animals examined for the present study with an udder edema had on average lower hyperkeratosis scores. Udder edema typically appears around the time of calving and especially for first calving heifers reaches its maximum a few days after calving [25]. Animals with an udder edema are therefore often only a few days in lactation.

3.13 Teat length and teat diameter

Front teats were found to be longer than rear teats (53.5 ± 8.1 mm and 46.4 ± 7.6 mm, respectively) in the present study. Rasmussen et al. (1998) found an average teat length of 45 mm for front and 40 mm for rear teats of first lactation cows. Older animals had teats that were about 10 mm longer. Their results are very close to the results of the present study.

The teat length was associated with the teat end hyperkeratosis score in front and rear teats. Teats with a score of 3 were on average longer than teats with score 0, 1 or 2. Hubal (2010) found longer teats to have a teat end hyperkeratosis which exceeds the scores 0 and 1 ($P< 0.001$), too [26].This may be due to inadequate massage during the milking process and therefore higher mechanical stress for teat ends of longer teats. Wendt et al. (2007) supposed that teat ends of very long or short teats are exposed to a continuous vacuum, because they lie outside optimal massage zone of the liner [13]. A teat length between 50 and 60mm is supposed to be optimal [13, 27]. In the present study it could be found that rear teats had a better teat condition than longer front teats. Mean length of the rear teats was smaller than 50 mm for all hyperkeratosis scores while the front teats had mean values between 50 and 60 mm, which is the recommended length.

Front teats were also thicker than rear teats (21.7 ± 2.6 mm and 21.3 ± 2.6 mm, respectively). These results are close to the values found by Bakken (1981) (21.7 mm for front teats, 21.4mm for rear teats). A clear association between the teat diameter and hyperkeratosis score could not be found. Teats with a score of 0 were on average thicker than those with score 1, 2 or 3, but teats with score 3 were also found to have a tendency to be thicker than those with score 1 or 2. Cow individual variables like lactation number or lactation stage may have a more dominant influence on hyperkeratosis development than the teat diameter and therefore mask its effect.

3.14 Teat shape

The teat shape was found to be very uniform. More than 99% of all teats scored were classified “normal”. Other teat shapes therefore were combined in the category “other shapes”. The teat shape was no significant animal variable included in the final regression model.

3.15 Teat end shape

The teat end shape was relatively uniform in the examined population of dairy cattle. Round teat ends were found in 81% of the teats (for both front and rear teats), followed by flat teat ends (13.79% and 14.13%, respectively) and pointed teat ends (3.81% and 3.44%, respectively). Funnel shaped teat ends were seldom (less than 2%). The distribution of teat endshapes was different from the findings of Neijenhuis et al. (2000) [4]. Round teat ends were the most common shape with a frequency of 43% of the examined teats, too, followed by pointed teat ends (31%). Flat and funnel shaped teat ends ranged behind these forms (13% each). For the teat end shape a heritability of 0.24 was estimated by Chrystal et al. (2001) [28]. In contrast to the present study, the study of Neijenhuis et al. (2000) was conducted in the Netherlands [4]. Genetic disposition might be an explanation for the different results. Older studies used varying terms and classification systems for the description of teat endshapes which makes it difficult to make a reasonable comparison between results [14, 15].

The proportion of pointed teat ends is positively associated with the hyperkeratosis score. Flat teat ends are less likely to have extreme hyperkeratosis. Similar surveillance could be made for funnel shaped teat ends. The higher the hyperkeratosis score, the less often teat ends are funnel shaped. Pointed teat ends were described to be more susceptible for the development of hyperkeratosis by Neijenhuis et al. (2000) [4]. Hyperkeratosis of pointed teat ends got rough earlier in lactation and more frequently than funnel shaped teat tips. The results of other studies confirm this hypothesis [6, 14, 16]. It could be hypothesized that pointed teat ends have less tissue surrounding the teat orifice. Therefore mechanical forces as a consequence of machine milking may affect the teat canal epithelium and the teat end tissue more intensively. In Contrast it is conceivable that the orifice of funnel shaped teat ends is embedded in tissue and is less exposed the milking vacuum.

3.16 Short term changes in teat end condition

Changes in teat skin colour and edema of the teat after milking are the clinical signs of an impaired circulation of blood and lymph. Teat skin colour changes were found for over 40% of the examined teats. More than 40% of the teats had an edema of the teat barrel and more than 30% had an edema of the teat end. Mein et al. (2001) require thresholds of less than 20% teats with changes in teat skin colour or an edema after milking to assess, if the massage of the liner is adequate to relieve the teat [3]. In the present study liner massage is not effective following these recommendations. Short term changes in teat condition were no significant animal variables with an association to hyperkeratosis but they may give a hint at problems with milking technique which may influence hyperkeratosis development.

3.17 Hyperkeratosis score

The thresholds for hyperkeratosis given by Mein et al. (2001) were exceeded in the dairy cattle population involved with the present study [3].More than 50% of the front teats and more than 45% of the rear teats had a hyperkeratosis score higher than score 1 (smooth white ring around the teat orifice). As recommended by Mein et al. (2001), less than 20 % of lactating cattle should have one or more teats with a teat end hyperkeratosis score of 2 (rough callous ring around the teat orifice) or 3 (very rough ring) [3]. It is difficult to compare the results with other studies, because the scoring systems used to record hyperkeratosis vary between authors. Lewis et al. (2000) found more than 30 % of the examined 2000 teats to exceed a prone ring and have fronds around the teat orifice [29]. Neijenhuis et al. (2000) used a scoring system with five classes to describe thickness of the hyperkeratosis ring (none, thin, moderate, thick, extreme) and two classes to describe roughness of the ring (smooth and rough) [4]. The system used in the present study was developed to allow a quick and easy classification in the milking parlour and is limited to four classes. It is based on the simplified classification system for routine field evaluations introduced by Mein et al. (2001) [3].

The extent of teat end hyperkeratosis is essentially determined by the forces the milking process exercises on the teat tissue. The longer the teat tissue is affected by mechanical forces, especially in periods with low milk flow ("overmilking"), the higher the risk for the development of severe teat end hyperkeratosis is[10, 11, 12].

Therefore it is not surprising that front teats in the study were more prone to have higher hyperkeratosis scores. These teats only have about 60 to 70 % of the milk yield that rear teats have [30]. The period front teats are milked with low or without milk flow is much longer than for rear teats [4]. The results of different studies agree with the data presented here[31,4].

In the present study it was found that that cow individual variables lactation number, teat length, teat diameter and teat end shape are associated with teat end hyperkeratosis. The results of the present study indicate that the consideration of teat size and teat end shape of dairy cows may be a suitable instrument to reduce the prevalence of teat end hyperkeratosis in herds. However, the association between milking variables (e.g. machine-on time, milking speed), management variables (e.g. alkalization of bedding material, post milking teat disinfection) and teat end hyperkeratosis were not investigated. Due to the fact that more than 45 % of front and rear teats have rough to very rough callous rings around the teat orifice further research is needed to reduce the prevalence of long-term changes in teat condition in German high-yielding dairy herds.

IV. CONCLUSION

The development of hyperkeratosis has been described as a natural process of adaption to milking. There are some cow individual factors that influence their severity beside the mechanical forces of the milking process. These are especially teat end shape, teat length and diameter and lactation stage. Teat end condition was poor in the population involved in the present study and may affect udder health. The population examined in this survey was uniform regarding teat end shape and teat size. Breeding selection should continue to prefer animals without extreme teat sizes (too short as well as too long). Also pointed teat tips are not desirable.

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REFERENCES

[1] C. Hagnestam-Nielsen, U.Emanuelsen, B. Berglund, E. Strandberg, „Relationship between somatic cell count and milk yield in different stages of lactation" in Journal of Dairy Science 92, pp. 3124-3133, 2009.

[2] International Dairy Federation (IDF), "Economic consequences of mastitis" in Bulletin of the International Dairy Federation 394/2005.

[3] G.A. Mein, F. Neijenhuis, W.F. Morgan, D.J. Reinemann, J.E. Hillerton, J.R. Baines, I.Ohnstad, M.D. Rasmussen, L. Timms, J.S. Britt, R. Farnsworth, N. Cook, T. Hemling, "Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors" in 2nd International Symposium on Mastitis and Milk Quality, Vancouver, Canada, pp. 347-351, 2001.

[4] F. Neijenhuis, H.W. Barkema, H. Hogeveen, J.P.T.M. Noordhuizen, "Classification and longitudinal examination of callused teat ends in dairy cows" in Journal of Dairy Science 83, pp. 2795-2804, 2000.

[5] M.D. Rasmussen, "Influence of switch level of automatic cluster removers on milking performance and udder health" in Journal of Dairy Research 60, pp. 287-297, 1993.

[6] F. Neijenhuis, G.H. Klungel, H. Hogeveen, J.P.T.M.Noordhuizen, „Machine milking risk factors for teat end callosity in dairy cows on herd level" in Mastitis in dairy production: current knowledge and future solutions (ed Hogeveen H) 4th IDF International Mastitis Conference, Maastricht, The Netherlands, Pp. 376-382, Wageningen Academic Publishers, Wageningen, The Netherlands, 2005.

[7] J. Hamann, G.A. Mein, S. Wetzel, „Teat tissue reactions to milking: effects of vacuum level" in Journal of Dairy Science 76, pp. 1040-1046, 1993.

[8] D.J. Reinemann, R. Bade, M.Zucali, C.Spanu, P.L.Ruegg, "Understanding the influencence of machine milking on teat defense mechanisms" in Mastitis control – from science to practice, Proceedings of International conference, Wageningen Academic Publishers, pp. 323-331, 2008.

[9] R.D. Bade, D.J. Reinemann, M. Zucali, P.L. Ruegg, P.D. Thompson, "Interactions of vacuum b-phase duration, and liner compression on milk flow rates in dairy cows" in Journal of Dairy Science 92, pp. 913- 921, 2009.

[10] J.E. Hillerton, J.W. Pankey, P. Pankey, "Effect of overmilking on teat condition" inJ. Dairy Research 69, pp. 81-84, 2002.

[11] I.C. Ohnstad, G.A. Mein, F. Neijenhuis, J.E.Hillerton, J.R. Baines, R. Farnsworth, "Assessing the scale of teat end problems and their likely causes" in National Mastitis Council Annual Meeting Proceedings, pp. 128-135, 2003.

[12] C.O. Paulrud, S. Clausen, P.E. Andersen, M.D. Rasmussen, „Infrared thermography and ultrasonography to indirectly C-monitor the influence of liner type and overmilking on teat tissue recovery" in ActaVeterinaria Scandinavica 46, pp. 137-147, 2005.

[13] K. Wendt, S. Köhler, D.Sass, S. Spasovski, E. Piltz, „Einflussfaktoren auf die hämatogene Mikrozirkulation an der Zitze des Milchrindes" inZüchtungskunde 79, pp. 119-127, 2007.

[14] G. Bakken, "Relationships between udder and teat morphology, mastitis and milk production in Norwegian Red Cattle" in Acta Agriculturae Scandinavica 31, pp. 438-444, 1981.

[15] M. Binde, H. Bakke, „Relationships between teat characteristics and udder health – A field survey" in Nordisk Veterinaer Medicin 36, pp. 111-116, 1984.

[16] F. Neijenhuis, H.W. Barkema, H. Hogeveen, J.P.T.M. Noordhuizen, "Relationship between teat-end callosity and occurrence of clinical mastitis" in Journal of Dairy Science 84, pp. 2664-2672, 2001.

[17] J.E. Breen,M.J. Green, A.J. Bradley, "Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom" in Journal of Dairy Science 92, pp. 2551-2561, 2009.

[18] ISO 5707:1996 Milking machine installations - construction and performance

[19] E. Grunert,„Weiblicher Geschlechtsaparat und Euter", inIn Die klinische Untersuchung des Rindes (eds Dirksen G, Gründer HD, Stöber M) 3. Auflage. pp. 525-529. Verlag Paul Parey, Berlin and Hamburg, 1990.

[20] D. Urban, „Logit-Analyse: Statistische Verfahren zur Analyse von Modellen mit qualitativen Response-Variablen". Stuttgart, Germany: Fischer, 1993.

[21] M.J. Slakter, "A Comparison of the Pearson chi-square and Kolmogorov Goodness-of-Fit tests with respect to validity" in Journal of the American Statistical Association 60, pp. 854-858, 1965.

[22] N.J.D. Nagelkerke, "A note on the general definition of the coefficient of determination" in Biometrica 78, pp. 691-692, 1991.

[23] N. Gossen, N. Potrafki, M. Hoedemaker, "Beziehungen zwischen tierspezifischen Faktoren und der Zitzenkondition von Milchkühen" inTierärztliche Umschau 62, pp. 354-363, 2007.

[24] M.F.H. Shearn, E. Hillerton, "Hyperkeratosis of the teat duct orifice in the dairy cow" in Journal of Dairy Research 63, pp. 525-532., 1996.

[25] W.Heeschen, „Die Milchdrüse während der Gravidität, Laktation, Puerperium sowie Milchmangel", in Tiergeburtshilfe (eds Richter J, Arbeiter K) 4. Auflage. pp. 123- 128. Verlag Paul Parey, Berlin and Hamburg, 1993.

[26] M. Hubal, „Untersuchungen zum Einfluss melktechnischer Parameter auf die Zitzenkondition von Milchkühen". Göttingen, Georg-August- Univ., Fak. für Agrarwissenschaften, Diss 2010.

[27] M.D. Rasmussen,E.S.Frimer, L. Kaartinen, N.E. Jensen, „Milking performance and udder health of cows milked with two different liners" in Journal of Dairy Research, 65, pp. 353-363, 1998.

[28] M.A. Chrystal, A.J. Seykora, L.B. Hansen, A.E. Freeman, D.H. Kelley, M.H. Healey, "Heritability of teat-end shape and the relationship of teat-end shape with somatic cell score for an experimental herd of cows" in Journal of Dairy Science, 84, pp. 2549-2554, 2001.

[29] S. Lewis, P.D. Cockcroft, R.A. Bramley, P.G.G. Jackson, "The likelihood of subclinical mastitis in quarters with different types of teat lesions in the dairy cow", in Cattle Pract., Vol 8, Part. 3, pp. 293-299, 2000.

[30] D. Weiss, M.Weinfurtner, R.M. Bruckmaier, "Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows" in Journal of Dairy Science, 87, pp. 3280-3289, 2004.

[31] R.L. Sieber, R.J. Farnsworth, "Prevalence of chronic teat-end lesions and their relationship to intramammary infection in 22 herds of dairy cattle," in Journal of the American Veterinary Medical Association, 178, pp. 1263-1267, 1981.

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