IJOEAR

International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-3, Issue-7, July 2017

Preface

We would like to present, with great pleasure, the inaugural volume-3, Issue-7, July 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

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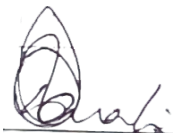
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, 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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Effect of different Mulching Materials on the Yield of Quality Protein Maize in Danbatta Local Government Area, Kano State Nigeria

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Abstract— *Field research was conducted on the effects of different mulching materials on the yield of Quantity protein maize which include polythene sheet, dry grasses and control. The different mulching materials were tested on nine (9) ridges each measuring 10m. The research was laid out in a completely randomized block design each treatment replicated three times. The parameters measured include weight of cobs, weight of 100grain, and total grain, there was no statistical difference in the weight cobs among all the treatments, similarly, there was also no statistical different in the weight of 100 grain. As far the weight of grain per 5m^2 there was a significant difference among the treatments with polythene sheet covered plots that have 0.25kg, however, the grass-mulched plots 0.16kg was statistically similar to the control plots 0.15kg. And finally for store weight observed per 5m^2, polythene sheet covered plots were significantly higher than grass-mulched plots which are also significantly different to control plots with the values of 1.23kg, 1.21kg and 0.71kg respectively.*

Keywords— Mulch, grain, store, cob, polythene.

I. INTRODUCTION

Given the importance of maize in the Nigeria economy, one would have expected that the nation would be self-sufficient in the production of the crop. Considering the fact that the crop can be grown in most parts of the country (Ogunbiled and Olokosi, 1990), but one will wonder why the country is not self-sufficient in maize production. Problems cited as constraining the production of maize in Nigeria are stagnant production technology among Nigeria's farming community majority of whom are the small scale producers and water resource management (Ahmed and Kura, 2007). To enhance crop production mulch, or a production cover applied to the soil surface so as to minimize water losses due to evaporation and enhanced water use efficiency was suggested by Ahmad and Kura, 2007 when they showed that application of straw mulch significantly affect the performance of maize as yield of mulched plots significantly and positively increase the yield. Reporting an different Crop, Ramadan and Nwokocha, (2000), Sanda and Ogunwole (2006), shows that application of straw mulch resulted in yield increase of 5.2t/ha over that obtained from un mulch tomato plot and marketable fruit yield of the crop also increase significantly. Therefore this paper is aimed at studying the influence of mulch application on the yield of maize at Audu Bako College of Agriculture, Danbatta, Kano State, Nigeria.

Lobell and Burke (2010) suggested that an increase in temperature of 2°C would result in a greater reduction in maize yields within sub-Saharan Africa than a decrease in precipitation by 20%. A recent analysis of more than 20,000 historical maize trial yields in Africa over a period of eight years combined with weather data showed that for every degree day above 30°C grain yield was reduced by 1.0% and 1.7 % under optimal rainfed and drought conditions, respectively (Lobell et al., 2011b).

Dry season and in some cases wet season maize (Zea mays L.) is one of the most popular grain crops in the semiarid, but low air temperature and drought in April May often result in poor plant establishment. Recently, a method using double ridges and furrows mulched with plastic film for micro-catchment water harvesting has been considered a great technical innovation in dryland farming systems for wide use in spring maize (Gao et al., 2008). This technique has now been widely applied to maize in semiarid areas, and has completely replaced conventional tillage management owing to its significant effect on water harvesting and increased yields (Zhang et al., 2006; Ma et al., 2008).

II. MATERIALS AND METHODS

The trail was conducted at teaching and Research Farm of Audu Bako College of Agriculture, Danbatta, Kano State.

The climate of the area is characterized by an alternative hot rainy season and cool, dry season with the mean annual rainfall of 46mm, a mean annual temperature of 37^0C located at latitude 11 55, N, 8^0 20, E (Areola, 2000). The soil has been characterized as sandy loam (Sanda 2005).

Two different mulching materials (polythene sheet, and grass) and a control (no mulch plots) were used as the experimental treatments and were replicated three times and they were laid out in a completely randomized block design an improved Quantity Protein Maize (QPM) variety source from Kano Agricultural and Rural Development Authority (KNARD) was used as a test crop. All agronomic practices involved in maize cultivation from land clearing to harvest were followed at the end of experiment the following observation were recorded; weight of store, weight of cobs, weight of 100 grains and total grain weight. The data generated were subjected to analysis of variance using a gen stat package. Treatments with significant difference were compared and separated using least significant difference (LSD) and 0.05% level of probability.

III. RESULT AND DISCUSSIONS

3.1 Effects of different Mulching Materials on the Store Weight And Cobs Weight of Maize at Danbatta:-

The weight of store a significant difference among all the treatments, with polythene sheet treatment having the highest cobs weight of 1.23kg, followed by the grass mulch with a value of 1.22kg, which are statistically different with control plot with a value of 0.71kg. This indicates that polythene covered plots were warmed and allows little or no moisture to evaporate from the soil surface and hence more moisture is avail for the crop metabolic activities which positively affect the crop performance, similar observation was made by Ahmad 2009, W. Mupangwa et al., 2016, Xu J, et al., 2015. However, there was no statistical different in the weight of cobs among all the treatments tested.

However, the effects of the different treatments (polythene sheet, grass and control mulch) that were evaluated on the cob weight were found to have no significant different among the various treatments. The grass, polythene treatments and the untreated treatment were found to be similar, even though control gave a little value of cob weight compared to others, whichever is adopted by the farmers could be as beneficial, but control can then be more economical in terms of cob weight, though it will not be the ultimate aim of maize production, but may be regarded as one of the components that determine maize yield.

3.2 Effect of different Mulching Materials on the weight of 100 grains and total Grain Yield of Quality protein maize at Danbatta

Grass-mulched plots shows a statistical different away the treatments with a value of 0.04kg as against the polythene sheet and control plots with a values of 0.01kg and 0.01kg respectively as far as 100 grain weight is concern as shows in Table 2.

However, the weight of area grains from the quadrant $5m^2$ shows that there was a significant different between polythene sheet covered plots with grass-mulched and control plots, polythene sheet covered plots has 0.25kg and grass-mulched and control plot has 0.16kg, and 0.15kg respectively which are statistically similar. This however, shows that polythene covered plots were superior interns of weight of area grains from the quadrant of $5m^2$.

TABLE 1
EFFECT OF DIFFERENT MULCHING MATERIALS ON THE STORE WEIGHT AND COBS WEIGHT OF QPM AT DANBATTA

Treatments	Store weight (kg)	Cobs weight (kg)
Polythene covered sheet plots	1.23b	0.12
Grass mulched plots	1.21a	0.12
Control plots	0.71a	0.13
LSD	0.20	

TABLE 2
EFFECT OF DIFFERENT MULCHING MATERIALS ON WEIGHT OF 100 GRAIN AT TOTAL GRAIN WEIGHT OF QPM AT DANBATTA

Treatments	Weight of 100 grain (kg)	Total grain weight (kg)
Polythene covered sheet plots	0.01	0.25b
Grass mulched plots	0.04	0.16a
Control plots	0.01	0.15a
LSD	-	0.04

Considering the results presented above on the weight of 100 grain, both polythene and control have the value that is lower than that obtained under Grass-mulched plots which may be attributed to the fact that towards the grain filling/ milking stage

of the maize the grasses used for mulching started to decomposed and hence releases some nutrients that may be uptake by the maize and hence improves it performance above other treatments that does not have a decomposable organic residue, similar observations was made by Ahmad and Nasiru 2009 at the same location.

The effect of the treatments on the total grain weight as shown in Table 2, indicates that polythene covered treatment is superior to other treatments, and the probable reasons could be due to the continuous moisture retention of this treatment all through the experimental period, which signified the important of water availability to maize crop at all level of critical growth stages, this was also suggested by Yin, et al., 2016, Ahmad and Nasiru, 2009. Although Grass mulched and Control were statistically similar, but Grass mulched plots shows some element of superiority over Control for the total grain yield and it might be as well due to the higher available moisture between the two respective plots.

IV. CONCLUSION

In an area with erotic and unstable rainfall maize production can be enhance by the application of mulch especially polythene sheet mulch which a part from minimizing evaporation losses can also help to improve water use efficiently of the crop and if not available, dried grasses can be use as a mulching materials which has an added advantage of when decompose increase the organic matter content of the soil. Economically it is also wise to use polythene sheet as a mulching materials, because the yield difference as observed in this study can justify the cost of buying the polythene materials and when properly taken care of, it can be used for two or more season, but in a situation whereby polythene materials may be found too expensive or may not be available, the use of dried grasses could be an alternative, which could have an added advantage of when decomposes increases the organic carbon content of the soil.

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Influence of Mulch and Ridge-tie on Soil Moisture retention and early growth of maize at Jega, Kebbi State, Nigeria

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Abstract— *Water is one of the main requirements for healthy plant growth. Most arid and semi-arid regions, however, suffer from insufficient and unreliable rainfall. The prevailing soils generally cannot absorb the amount of water which rainfalls in such a short time. Based on this and many other factors a study was carried out to determine the influence of mulch and ridge tie on moisture retention and early growth of maize, at the Kebbi State University of Science and Technology Teaching and Research Farm Jega. The results shows that on a short term basis ridge tying had the highest amount of moisture, while on the long terms basis mulch had the highest moisture content and maize plant height is also more observed in the mulched plots as compared to ridge-tie respectively with the value of 45cm- 75cm, and 39cm al 54cm at 3 al 5 WAP respectively al dry matter yield also give a similar trend.*

Keywords— Mulch, ridge-tie, Moisture, dry spell, climate, infiltration.

I. INTRODUCTION

Water and soil nutrition management form a critical components of Agricultural Production. The line between soil and water conservation (SWC) and rainwater harvesting (RWH) technologies for crop production is very thin. SWC can be described as activities that reduce water losses by runoff and evaporation, while maximizing in-soil moisture storage for crop production, but the same could be said of RWH. The two are differentiated by the fact that under soil and water conservation, rainfall is conserved in-site where it falls, whereas under water harvesting a deliberate effort is made to transfer runoff water from a "catchment" to desired area or storage structure (Critchley and Siegert, 1991). The important thing is that both systems complement each other, and under rain-fed agriculture in dry areas, both are necessary nearly all the time.

In the semi-arid areas tie-ridges are made by modifying normal ridges. The techniques involve digging major ridges that run across the pre dominant slope and then creating sub-ridges (or cross-ties) within the main furrows. The final effect is a series of small micro-basins that store rainwater in-site, enhancing infiltration. Depending on the system, the crop is planted at the side of the main ridge, to be as close as possible to the harvested water while also avoiding water logging in case of prolonged rains.

Tied ridges have been found to be very efficient in storing the rainwater, which also resulted in substantial grain yield increase in some of the major dry land crops such as sorghum, maize, wheat, and mung beans in Ethiopia (Georgis and Takele, 2000). The average grain yield increase (under tied ridges) ranged from 50 to over 100 percent when compared with the traditional practice. This increase, however, will vary according to the soil type, slope, rainfall and the crop grown in the dry land areas.

The objectives of mulch is to conserve soil moisture, reduce runoff flows, evaporative losses and wind erosion, prevent weed growth, enhance soil structure and control soil temperature mulching is practiced by famers in the wetter areas due to the availability of vegetative materials. Depending on availability of residues, mulch densities range between 30-70 percent, based on availability of residues obtained from the previous season's crop (Kibwana, 2000). The importance of mulches in reducing surface runoff, soil erosion and evaporation losses cannot be overstated that in the absence of mulch 40-60 percent of the rainfall that fell was lost to evaporation and that if 40-50 percent of the ground was covered with mulch, surface runoff losses were reduced to almost zero and evaporation losses halved (Liniger 1991). Crop yields were found to double or triple and biomass to feed livestock increased.

Based on all the above stated benefits of ridge tie and mulch, thus study which was conducted at the Teaching and Research Farm of the Kebbi State University of Science and Technology, Jega was aimed at evaluating the influence of Mulch and ridge tie on soil moisture retention and early growth of maize.

II. MATERIALS AND METHODS

The trial was conducted at the Teaching and Research farm Jega, of Kebbi State University of Science and Technology, Jega latitude 12^0 11'N, longitude 4^0 16'E in the Sudan savannah ecological zone. The climate of the area is characterized with an average rainfall of about 500mm-650mm per annun, relative humidity ranges from 15^0-41% and 50-65% during the dry and rainy season respectively; temperature average between 14-30^0C during the cold dry season and 24-41^0C during the rainy season, the soil of the area has been characterized as sandy loam.

The field was harrowed and made into 5m ridges. The plot size was 5mx4m ($20m^2$). A distance of 1m was maintained between plots.

The tied ridges are made by modifying normal ridges; sub-ridges are created within the main harrows (or cross ties). The effect here is series of small micro-basins that store rainwater in-site. Straw mulch is applied to those ridges not to be tie after leaving ten ridges that will serve as a control. Speedy moisture tester (Series 2000) was used in moisture determination during the experimental period. Here moisture measurement is made by mixing a weighed sample of a moist soil with Calcium Carbide in the sealed pressure vessel, as the reagent (Calcium Carbide) react with water in the soil sample an acetylene gas will be produce which in turn increase the pressure within the vessel. As the pressure increase in the vessel is proportional to the amount of moisture in the sample, hence moisture content is then read directly from a calibrated pressure gauge. SAMAZ II an improved maize variety was used during the trial. Data relevant to the soil moisture content was taken two times a day that is morning and evening every day, while germination percentage, number of leaves, plant heights and dry matter yield are then taken from the plant up to the time the trial was terminated.

The trial was laid out in a randomized complete block design (RCBD) with a split plot arrangents and replicated three times.

III. RESULTS AND DISCUSSION

3.1 Soil Moisture content as influence by tie-ridge and Mulch:-

As shown in Table 1, that tie-ridge as it accumulates more water after rainfall it has the higher moisture content as compared to the mulched and the control plots, but as it gets towards afternoon, the moisture content in tie-ridge plots, tend to be lower than that of mulch plots similar results was also obtained by Li Min et al,. 2008, but significantly higher than that of control plots, this indicate that evaporation from the open plots contributed to the moisture depletion from these plots (tie-ridge and control) whereas in the plots that were mulched as a result of the mulch the rate of evaporation even with the increase in the afternoon so temperature little or no effect was observed on it is moisture content from morning to afternoon when the maximum evaporation is taking place, G, Sime, 2014 also found similar results.

TABLE 1
SOIL MOISTURE CONTENT AS INFLUENCED BY TIE-RIDGE AND MULCH AT UNIVERSITY FARM JEGA (%)

	Morning			Afternoon		
Days	**Tie-ridge**	**Control**	**Mulch**	**Tie-ridge**	**Control**	**Mulch**
1	19.40	17.20	20.00	19.20	17.00	19.80
2	18.40	16.40	19.60	18.40	16.20	19.60
3	20.60	18.40	23.40	20.60	17.80	23.20
4	19.40	16.00	23.30	18.80	15.80	21.20
5	18.40	14.60	20.80	18.20	13.60	19.60
6	20.60	19.80	22.30	20.40	19.60	22.00
7	21.80	19.40	20.20	21.60	19.20	20.20
8	19.80	17.80	19.40	18.40	17.60	19.60
9	20.60	19.80	20.80	20.21	19.20	20.60
10	20.62	11.20	20.40	19.20	18.10	19.80
11	19.20	15.50	20.00	17.50	15.10	18.40
12	21.30	14.90	20.40	19.30	12.90	19.60
13	22.11	18.60	20.80	20.50	18.00	20.60
14	19.00	16.11	19.00	18.70	14.11	19.00
15	18.60	15.20	19.00	18.00	14.10	18.90
16	22.00	18.40	20.25	20.57	17.70	20.20

3.2 Effect of Tie-ridge and Mulch on germination count, plant height al dry matter yield of maize

Mulched plots as compared to ridge tie and control plots show a higher germination count as shows in Table 2. At 5 WAP (weeks after planting) the number of seeds that germinate in mulched plots is 60% higher than that of tie-ridge and control plots with the value of 55 and 54% respectively, the reason of this trend could be attributed to the fact that mulching help to regulate soil temperature and maintain moisture and hence improves the chances of increase in germination count. Plant height was also higher in the mulched plots as compared to the tie-ridge and control plots at both 3 WAP and 5 WAP (weeks after planting) as show in Table 2. At 3 & 5 WAP, the plant height of maize in mulched plots 45cm and 75cm respectively are much higher as compared to tie-ridge and control plots with the value of 39cm, 54cm, 12cm and 43cm respectively Adeoye, 1984 and Ahmed, 2008 where they reported that mulch increase plant height and dry matter yield in maize when process of up to 6 tons/ha are used.

TABLE 2
EFFECT OF THE RIDGE-TIE AND MULCH ON GERMINATION COUNT, PLANT HEIGHT AND DRY MATTER OF MAIZE

Treatment	Germination count (%)			Plant height (cm)		Dry matter yield (g)	
	5 DAP	7 DAP	9 DAP	3WAP	5WAP	3WAP	5 WAP
Tie-ridges	55	67	93	39	54	14.3	39.2
Control	54	60	86	12	43	12.1	30.2
Mulched	60	74	96	45	75	22.3	62.4

DAP = Days after Planting
***** WAP = Weeks after Planting***

Dry matter yield of maize at both 3WAP and 5WAP as shown in table 1 indicates that accumulation of it (Dry matter) is more in the mulched plots as compared to the tie-ridge and control plots. At the said age that is 3& 5 WAP mulched plots give 22.3g and 64.4g as against the one recorded in tie-ridge and control plots has the values of 14.3g 35.2g and 12.1g and 30.2g respectively. Higher moistures retention and reduced evaporation in the mulched plots could be attributed to this trend.

IV. CONCLUSION

Moisture retention as a result of low evaporation losses could be attributed to the reasons why mulched plots proves to be better in both amount of moisture, in the soil, higher germination count, plant height and dry matter accumulation of maize plant at Jega.

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Presence of herpesvírus in diseased fishes

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***Abstract*—** *Herpesviruses that infect fishes belong to the Herpesvirales order and Alloherpesvirus family. In these species, the different types of herpesvirus can cause tumors, adenocarcinoma and skin lesions. This study aims detect to presence of herpesvirus in fishes from commercial, recreation or experimental creations of the States of São Paulo and Minas Gerais, Brazil. Organ fragments and lesions of 53 fish species coming of mortality cases were forwarded at Biological Institute for examination by transmission electron microscopy by research of etiological agent. By transmission electron microscopy through negative staining technique, were observed herpes virus-like particles in 46 fishes and through embedding resin technique, in ultrathin sections were visualized herpes virus immature particles, measuring 90-110nm in diameter, located in the nuclei and complete particles measuring 160nm. In the histopathology technique, lesions associated with the virus as corpuscles inclusion, papillomas, and dermal lesions and in the gills were observed in 27 fishes. The evaluated techniques of TEM and the histopathology were effective for the rapid detection of herpesvirus in the examined samples.*

Keywords*— *Disease, histopathology, transmission electron microscopy, herpesvirus.

I. INTRODUCTION

Aquaculture, in full development, is becoming more important as livestock activity, although it is still considered by many as an appendage of the fishing industry. Practiced in all Brazilian states, aquaculture mainly covers the following creations of fish, shrimp, frogs, shellfish, oysters and mussels and other aquatic crops such as growing algae are practiced on a smaller scale [1].

According to Toranzo et al. (2004) [2], infectious diseases caused by viruses can cause damage to the economic viability of aquaculture activities. These diseases occur mostly confined animals, and can develop serious infectious processes affecting the development of animals and even cause death.

Herpesviruses belong to *Herpesvirales* order, divided into three families, *Alloherpesvirus*, *Herpesviridae* and *Malacoherpesvirus*, encompassing viruses that occur in several animal species such as molluscs, fish, amphibians, reptiles, birds and mammals [3]. The *Herpesviridae* family keeps viruses of mammals, birds and reptiles, the *Alloherpesviridae* family includes fish and frogs viruses, and *Malacoherpesviridae* family contains the virus from bivalves [4].

The virus mainly affecting the early stages of fish life and therefore eggs, larvae and young fish are more susceptible. The infected eggs can produce larvae, newly hatched, with clinical signs of illness, while the infected adult fish may or may not present any symptoms [5]. Within the family of herpesviruses, can highlight herpesvirus papilloma of cyprinids [6], channel catfish herpesvirus and herpesvirus salmon [7].

The need for a more detailed and thorough knowledge of the damage caused by herpesviruses in aquaculture, especially in fish farming, has led us to this research. The pathological relationship between etiologic agents involving these animals is not well established, since in Brazil there is no survey of the pisces herpesviroses, and the reports are sporadic and opportunistic, depending on the shipment of samples to a diagnostic center. In this way, all studies in the area are important because the virus can remain latent in healthy animals or decimate entire flocks in a breeding, unfeasible a production.

This study aimed to detect the presence of herpes virus in organs fragments of fishes from the states of São Paulo and Minas Gerais, using transmission electron microscopy techniques (negative staining and resin embedding) and associating viral presence with indicative lesions observed by optical microscopy, using histological technique hematoxylin-eosin (H&E).

II. MATERIAL AND METHOD

2.1 Ethics statement

This study was approved by Animal Experimentation Research Ethics Committee Instituto Biológico (protocol 137/14).

2.2 Experimental design

The collection of material was performed from samples received for examination by the Laboratório Interinstitucional de Sanidade em Aquicultura and the Laboratório de Microscopia Eletrônica, Instituto Biológico, São Paulo, SP, Brazil. The samples were from comercial, recreation or experimental creations of the States of São Paulo and Minas Gerais, Brazil. Were utilized 202 organ fragments, such as liver, kidney, spleen, intestine, gills, heart, brain and skin lesions of 53 fish. The examined species were, 3 catfish (*Bagre* sp), 2 african sharptooth catfish (*Clarias gariepinus*), 3 cobia (*Rachycentron canadum*), 14 carp (*Ciprinus carpio*), 1 grouper (*Epinephelus marginatus*), 1 lenticulata pike cichlid (*Crenicichlalenticulata*), 3 pacu (*Piaractus mesopotamicus*), 1 mida cichlid (*Cichlassoma citrinellum*), 1 red piranha (*Serrasalmus nateteri*), 1 snookfish (*Centropomus parallelus*), 1 black pacu (*Colossoma macropomum*) and 22 tilapia (*Oreochromis niloticus*) from cases of diseases and mortalities.

2.3 Negative staining technique (rapid preparation)

In the negative staining the fragments of organs and skin lesions were suspended in phosphate buffer 0.1 M, pH 7.0. Drops of the obtained suspension were placed in contact with metallic copper grids with carbon stabilized supporting film of 0.5% collodium in amyl acetate. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 [8,9,10].

2.4 Resin embedding technique

Thin slices the fragments of organs and skin lesions were fixed in 2.5% glutaraldehyde in 0.1M, pH7.0 phosphate buffer and pos-fixed in 1% osmium tetroxide in the same buffer. After dehydration in cetonic series, the fragments were embedded in Spurr resin [11,12]. Ultrathin sections were cut on the LKB ultratome and mounted on copper grids. The sections were stained by combination of uranyl acetate and lead citrate [13,14].

2.5 Routine histological technique

Fragments of organs and skin lesions of 27 fish, were fixed in 10% buffered formalin, dehydrated, diaphonized and embedded in paraffin. 4 µm thick sections were performed and stained with hematoxylin (Harris) and eosin technique [15].

III. RESULTS

3.1 Negative staining technique (rapid preparation)

On transmission electron microscopy Philips EM 208, using the negative staining technique (rapid preparation) it was found enveloped (figure 1) and non-enveloped (figure 2), isometric, pleomorphic herpesvirus-like particles, measuring 120-200 nm of diameter in 46 (86.8%) out of the 53 examined fish, from 202 samples of organs fragments. The positive species were, 3 catfish (*Bagre* sp), 2 african sharptooth catfish (*Clarias gariepinus*), 3 cobia (*Rachycentron canadum*), 14 carp (*Ciprinus carpio*), 1 grouper (*Epinephelus marginatus*), 1 lenticulata pike cichlid (*Crenicichla lenticulata*), 3 pacu (*Piaractus mesopotamicus*), 1 midas cichlid (*Cichlassoma citrinellum*), 1 red piranha (*Serrasalmus nateteri*), 1 snookfish (*Centropomus parallelus*), 1 black pacu (*Colossoma macropomum*) e 15 tilapias (*Oreochromis niloticus*). A total of 7animals (13.21%) (tilapias – *O. niloticus*) were negative for herpesvirus.

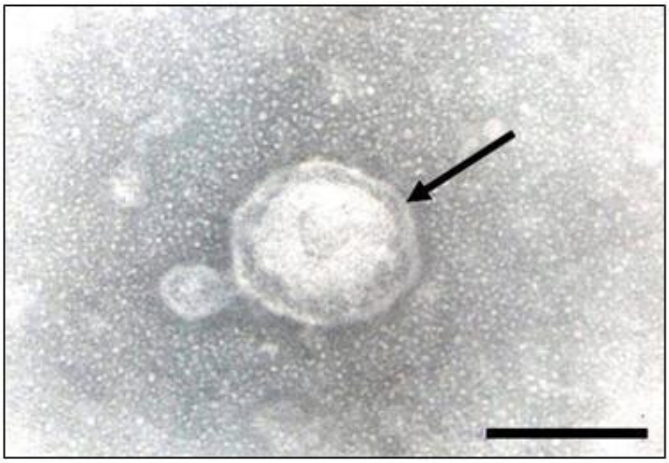

FIG. 1 – ELECTRON MICROGRAPH OF ENVELOPED HERPESVIRUS PARTICLE (ARROW) IN CARP LIVER FRAGMENT SUSPENSION. BAR: 140 nm.

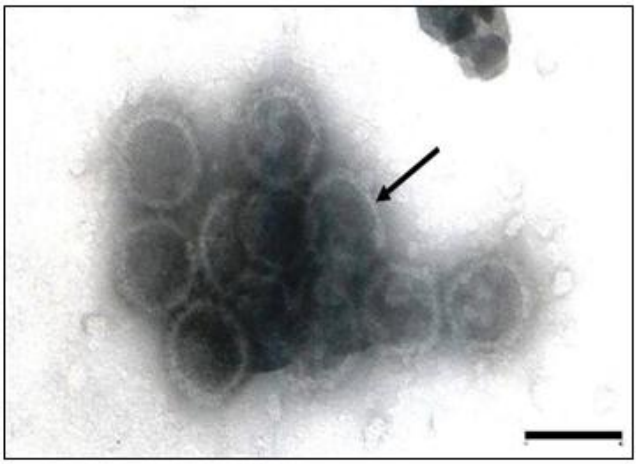

FIG. 2 – ELECTRON MICROGRAPH SHOWING VIRAL NUCLEOCAPSIDS (ARROW) IN CARP LIVER SUSPENSION. BAR: 120 nm.

3.2 Resin embedding technique

All positive samples from liver, brain and skin by negative staining technique were subjected the resin embedding technique. In ultrathin sections was observed immature herpesvirus particles (figure 3), measuring 90-110 nm in diameter, located in the nucleus and complete particles measuring 160 nm in diameter. Extensive proliferation of the nuclear membrane producing complex finger-like extensions and amorphous inclusions located near the nucleus were visualized (figure 4).

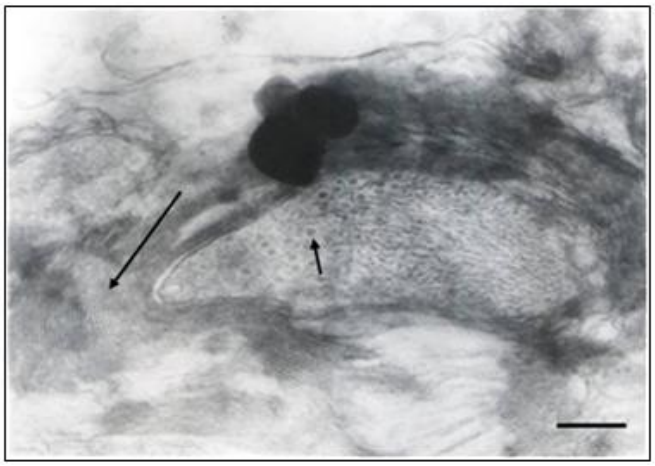

FIG. 3 – ELECTRON MICROGRAPH OF ULTRATHIN SECTIONS OF SNOOKFISHS BRAIN SHOWING THE PRESENCE OF INCOMPLETE HERPESVIRUS PARTICLE (LOWER ARROW) AND EXTENSIVE PROLIFERATION OF NUCLEAR MEMBRANE PRODUCING FINGER-LIKE EXTENSIONS (LARGE ARROW). BAR: 800 nm.

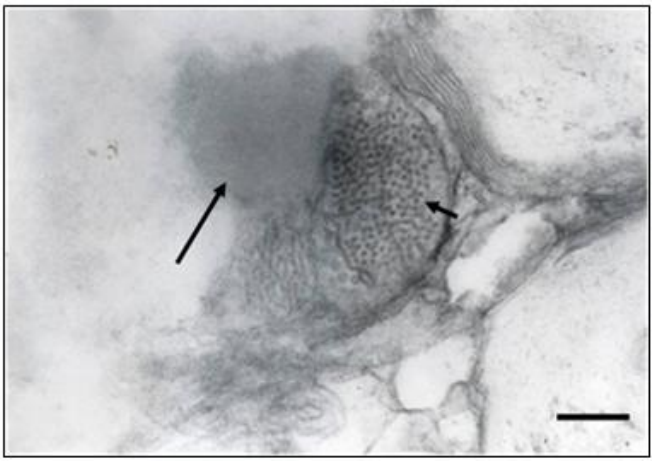

FIG. 4 – ELECTRON MICROGRAPH OF ULTRATHIN SECTIONS OF SNOOKFISH BRAIN INDICATING THE PRESENCE OF INCOMPLETE HERPESVIRUS PARTICLE (MINOR ARROW) AND AMORPHOUS INCLUSIONS LOCATED NEAR THE NUCLEUS (BIG ARROW). BAR: 800 nm.

3.3 Routine histological technique

Positive samples by negative staining technique of 27 fish were examined by routine histological technique (H&E). The examined species were, 13 carps, 8 tilapia, 2 african sharptooth catfish, 1 tambaqui, 1 red piranha, 1grouper and 1 midas cichlid. The observed lesions associated with the viral presence (figure5) are shown in Table 1.

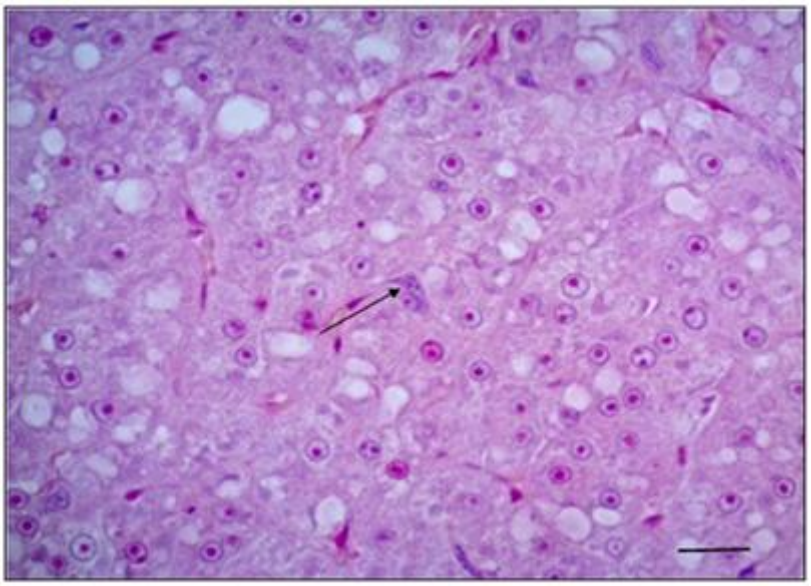

FIG. 5 - PHOTOMICROGRAPH OF HISTOLOGICAL SECTION OF AFRICAN SHARPTOOTH CATFISH LIVER, INDICATING HERPESVIRUS BASOPHILIC NUCLEAR INCLUSION (ARROW). HE STAINING. BAR: 20 µm.

TABLE 1
MAIN HISTOPATHOLOGICAL LESIONS OBSERVED IN FISH AND NUMBER OF AFFECTED ANIMALS / ANALYZED ANIMALS.

	Live	Kidney	Spleen	Hepatopancreas	Gill	Pancreas	Skin	CNS
Foamy areas	0/16	0/15	0/17	0/9	0/16	6/9	0/7	0/3
Dystrophic calcification	0/16	7/15	0/17	0/9	0/16	0/9	0/7	0/3
Corpuscles inclusion	3/16	1/15	0/17	2/9	0/16	0/9	3/7	1/3
Fibrin exudates	0/16	6/15	4/17	0/9	0/16	0/9	0/7	1/3
Secondary fusion of primary lamellae	0/16	0/15	0/17	0/9	6/16	0/9	0/7	0/3
Bleeding	2/16	8/15	15/17	0/9	0/16	0/9	2/7	1/3
Hyperplasia mononuclear cells	0/16	0/15	10/17	0/9	0/16	0/9	0/7	0/3
Epithelial hyperplasia	0/16	0/15	0/17	0/9	0/16	0/9	4/7	0/3
Lesions suggestive of papilloma	0/16	0/15	0/17	0/9	0/16	0/9	3/7	0/3
Melanomacrophages	3/16	6/15	13/17	0/9	0/16	7/9	3/7	1/3
Necrosis	2/16	4/15	2/17	2/9	1/16	1/9	4/7	1/3
Nephrosis	0/16	3/15	0/17	0/9	0/16	0/9	0/7	0/3
Cytoplasmic rarefaction	12/16	0/15	0/17	5/9	0/16	0/9	0/7	0/3
Inflammatory reaction	2/16	9/15	0/17	3/9	3/16	6/9	4/7	1/3
Congestion of blood vessels	8/16	0/15	0/17	0/9	7/16	3/9	0/7	3/3

IV. DISCUSSION

In this study, 202 samples of organ fragments from 53 fish were processed by the negative staining technique for transmission electron microscopy. Of these 53 fish, 46 (86.8%) were positive for herpes virus.

Studies around the world show a mortality rate of 80 to 100% in creations of carps infected by both CyHV-2 and CyHV-3 [16,17,18,19,20] as well by IcHV-1 that infects channel catfish [21], the 3 types of fish herpesvirus most frequent. Not yet know here in Brazil, which are the types of herpes viruses that are present in fish.

Particles with morphology similar to herpesvirus, pleomorphic, isometric, some enveloped, measuring between 120 to 200 nm in diameter, were visualized in all samples of organ fragments from the 46 positive fish of our study. These morphological features are also described by other authors in studies with herpesvirus made in tilapia larvae [22], in *Osmerus eperlanus* [23] and in *Ictalurus nebulosu* [24].

In ultrathin sections of brain, liver and skin of the positive fish of our study, were observed immature and complete herpesvirus particles, located in the nucleus, aspects also seen in tumor cells of *Osmerus eperlanus*, where herpesvirus particles were viewed in the cytoplasm [23].

The resin embedding technique has also been applied to study ultrastructural aspects of herpesvirus into cells infected by CyHV-3 [17,25,26,27,28], of the Koi herpesvirus (KHV) infecting carp (*Cyprinus carpio*) [29] and of the CCV (channel catfish virus) in a kind of catfish (*Ictalurus nebulosus*). 4 From this technique herpesvirus particles were visualized in all stages of development in the nucleus, granular bodies containing virus particles, lamellar structures and altered nuclear membrane, and numerous viral particles in the cytoplasm [24].

This technique applied in the spleen and kidney lesions, carried out in goldfish by Lovy and Friend (2014) [30] confirmed the presence of the herpesvirus in the nucleus and cytoplasm of necrotic cells. The authors also observed that the nucleus had marginalized chromatin.

We found the incomplete particles measured between 90 and 110 nm, and the complete particles measured on average 160 nm in diameter.

These findings were consistent with other ultrastructural studies that reported who the nucleocapsids of the CyHV-1, CyHV-2 and CyHV-3, measured 100 a 113 nm and the mature enveloped particles, measured 190 nm to 230 nm in diameter [31,32,33,34,35], located in the cytoplasm and extracellular space [33].

In fish of our study, the clinical signs and symptoms most commonly observed were incoordination, lethargy, sudden death, skin lesions (fin and mouth), liver nodules and liver and intestinal disorders.

These symptoms and signs have also been reported in fish infected by CyHV-2, CyHV-3 and IcHV, such as, *Carassius auratus* [33,19], *Cyprinus carpio* [17,36], *Ictalurus punctatus* [37,24] and *Oreochromis aureus* [22].

We find lesions like-papillomas in skin of 3 tilapia.

Lesions petechial or papillomatous around the body, mouth, cornea, kidneys and jaw have been reported in fish infected with herpesvirus Salmon, such as, *Oncorhynchus keta* [38,39], *Cyprinus carpio* [40], *Oncorhynchus kisutch, Oncorhynchus keta, Oncorhynchus masou* and *Salmo gairdneri* (Yoshimizu et al., 1987) [41]. Papilloma like lesions were also observed in CyHV-1 infection in the fish species [42,43,31]. In *Cyprinus carpio* infected by CyHV-3, the most commonly encountered lesions were located in the gills [17,44,45,46,29].

Regarding histopathology, in 5 carp and in 1 african sharptooth catfish of our study we found gill lesions, such as fusion the primary and secondary blades.

Histopathological examinations performed by Lovy and Friend (2014) [30] in goldfish (*Carassius auratus*), also showed the presence of fusion of gill filaments in all fish positive for herpesvirus examined in their study. This finding was attributed to epithelial hyperplasia, the infiltration of eosinophilic cells and occasionally diffuse necrosis. Pikarsky et al. (2004) [46] also included as branchial arch lesions suggestive of herpesvirus, subepithelial increase inflammation and congestion of blood vessels in carp. These lesions also were found by us in grouper, carp and tilapia.

In kidney of catfish, carp and tilapia were observed focuses with hemorrhage and the light of many tubules was visualized fibrinoid and hyaline material, necrotic areas, and the presence of melanomacrophages. It was observed particulary marked mononuclear glomerulonephritis as well as dystrophic calcification (calcium deposition).

Plumb et al. (1974) [47] and Wolf et al. (1972) [48] observed in your studies conducted in catfish (*Ictalurus punctatus*), the presence of extensive edema, inflammation and necrosis of the renal tubules. Pikarsky et al. (2004) [46] found blood vessels

congested in carp infected by herpesvirus, while Lovy and Friend (2014) [30] verified the presence of necrosis and dilated blood vessels in goldfish.

In tilapia (*O. niloticus*) and catfish (*Bagre sp*) hepatic tissue, in general, showed cytoplasmic rarefaction (mineral protein deficiency), numerous melanomacrophages, hepatitis focal monolimphocytary and necrosis.

Plumb et al. (1974) [47] and Wolf et al. (1972) [48] cite the focal necrosis, hemorrhage and edema as the main lesions found in the liver of animals infected with herpesvirus of *Ictaluridae* (IcHV1).

In the spleen of catfish, carp and tilapia, the most significant lesions found were hemorrhagic areas, presence of melanomacrophages hyperplasia of mononuclear cells, and necrosis.

These findings were corroborated by Lovy and Friend (2014) [30] in goldfish. Moreover, Plumb et al. (1974) [47] and Wolf et al. (1972) [48] found in their studies apart of changes observed in our work, congested blood vessels.

In our study we found the presence of inclusion bodies in skin of 2 tilapia and of 1 catfish, in hepatopancreas of 1 tilapia and of 1 carp, in liver of 1 catfish and 2 carps, in kidney of 1 tilapia and in the SNC of 1 midas cichlid.

Gibson-Kueh et al. (2012) [49] also found these inclusion bodies in the spleen, kidney, liver and heart in their studies in giant perch (*Lates calcarifer*) infected with herpesvirus.

According to Yamamoto et al. (1983) [50] and Elias et al. (2004) [51], the presence of inclusion bodies basophilic is the major histopathologic alteration characteristic of the presence of herpesvirus.

V. CONCLUSION

From these findings it was concluded that electron microscopy techniques used in this study were effective in diagnosing the presence of herpesvirus, allowing the visualization of agent, and should be spread widely.

Routine histopathological technique was also effective in the visualization of lesions suggestive of the viral agent, as the presence of inclusion bodies. Moreover, enabled to perform the analysis of the animals overall condition, indicating that the quality of creation can make it susceptible to infection by herpesvirus. Histopathology and electron microscopy tests must be complementary.

We cannot here say that the disease, followed by mortality, are directly caused by herpesvirus, however, serious conditions predisposing to poor water quality, unsuitable managements, high density and poor nutritional quality must have contributed to viral replication. The viral presence, of course, contributed to the worsening of the picture, intensifying clinical symptoms followed of mortality. Studies to assess what are the fish herpesvirus subtypes that are circulating in our country and its real impact on mortality should be conducted.

ACKNOWLEDGEMENTS

The research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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Defensive mechanisms in Plants: The role of component plant cells in defense against biotic and abitic stresses

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Abstract— *Plants are often exposed to various environmental stresses such as extreme temperatures, drought, and disease and pest attack. In natural systems, plants face a plethora of antagonists and thus posses a myriad of defense and have evolved multiple defense mechanisms by which they are able to cope with various kinds of biotic and abiotic stresses. In fact plants defense against stresses by different ways. The role of cellular organelles is very important in this way. Cell wall and their derivatives such as oligosaccharins as biochemical defenser or for example trichomes as mechanical defenser is the frontline of the plant defense system. Also Plants have evolved a multi-layered immune system that dynamically responds to pathogens alike cell membrane that is a key mediator of communication between plants and microbes. Cytoplasm and the membrane-bounded structures (organelles) defense against different kind of stresses. The role of cellular organelles in plant defense relate to their enzymes primarily. Enzymes such as proteases, esterases and ribonucleases in cytoplasm, PM H+-ATPases in plasma membrane or β glucosidases included cyanogenic glucosides, saponins, glucosinolates or DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) glucoside in ER are responsible for plant defense. Also ROSs plus SA and JA in chloroplast and mitochondria play an important role in immune plant system. In nucleus macromolecules including nucleoporins, importins, and Ran-GTP-related components, are essential to mount an efficient immune response in response to different pathogens. And in Golgi apparatus, peroxysomes and vacuoles, glycosyltransferases, myrosinase and hydrolytic enzymes are liable for plant defense respectively.*

Keywords— biotic and abiotic stresses; organells; plant defense.

I. INTRODUCTION

Despite of enormous differences in living organisms, all of them have similar characteristics. These similar characteristics that are signs of living organisms are known by us since primary school, respiration, nutrition, reproduction but among living organisms, plants have a very special characteristic that is photosynthesis. Briefly photosynthesis is a process used by plants to convert light energy into chemical energy that can later be released to fuel the organisms' activities (energy transformation). Also Photosynthesis is largely responsible for producing and maintaining the oxygen content of the Earth's atmosphere, and supplies all of the organic compounds and most of the energy necessary for life on Earth (Bryant and Frigaard 2006). It is noticeable that in nature algae and cyanobacteria, are photoautotroph also. But plants are important means of livelihood and production of human beings. So the relations between plants and human beings are also very close (Anant *et al*., 2013). Thought to be the importance of plants well informed by this short preface till now.

Like all of living organisms plants are continuously subjected to stresses. Stresses in plants are classified by different form but all of stresses are abiotic or biotic generally. An abiotic stress can be a mechanical injury. But biotic stresses in plants refer to plant diseases or phyto-pathogens (Doughari 2015). Plant diseases refer to any disturbance in functioning and growth that cause a lower operating efficiency or a breakdown in the plant's metabolism. Diseases afflict plants generally result from microbial infections, invasions of the body by pathogenic viruses, bacteria, fungi, or other microorganisms (Dickison 2000). Pests are biotic stresses and are often included insects. Furthermore nematode worms can sicken plants. According to statement plants must campaign against these devastating agents. In this review different defensive mechanisms for survival are noticed in plants. Also in this review the classification of different defensive mechanism will base on defense by cellular and subcellular components.

II. DEFENSIVE MECHANISMS IN PLANTS

It is clear that plants unlike vertebrate haven't a sophisticated immune system that provides the body with a means of resistance infection (Dickison 2000). In natural systems, plants face a plethora of antagonists and thus posses a myriad of defense and have evolved multiple defensive mechanisms by which they are able to cope with various kinds of biotic and

abiotic stresses. There are many factors that affect on defense in plants for example age of plants, type of organ infected, nutritional status of the host and environmental conditions (Gianinazzi and Ahl 1983).

So plants have an innate immunity of each cell and produce systemic signals emanating from the infection site. On the other hand plants defense against unfavorable condition by all of their compartments and organelles.

These compartments include in cell wall, plasmalmma, Endoplasmic reticulum, mitochondria, chloroplast, cytoplasm, Golgi apparatus, peroxysomes and vacuoles.

III. DEFENSE BY CELL WALL

The frontline of the plant defense system is cell wall. Cell wall is one of the most distinctive structural features of plant cells is the surrounding wall, characteristically composed of the carbohydrate cellulose deposited in fibrillar form (Dickison 2000).

One of the important ways of plant defense by cell wall are oligosaccharins. Oligosaccharins are fragments of the plant cell wall that serve as regulatory molecules. They help to control such functions as growth, development, reproduction and defense against disease. For example some oligosaccharins, such as oligogalacturonids, act as elicitors and evoke pathogen defense responses. These defense responses include the accumulation of phytoalexins, proteinase inhibitors, lignin, peroxidase, lipoxygenase (LOX) and β-1, 3 glucanases (Ryan, 1988; Hahn *et al.*, 1989; Ebel and Cosio, 1994). These bioactive, are relatively easy to produce and ready to face public acceptance because of their natural origin. Also oligosccharins have practical use in agriculture. So that oligogalacturonides, prepared from citrus pectin, that can partial or totally substitute phytohormones in the culture "in vitro" of sugar cane, citrus, coffee, tomato, rice and banana plants, reducing the time required for plant propagation, avoiding culture-induced genetic changes and increasing field survival percentage of these plants but also inducing rooting and plant growth (Saa Silva *et al*.. 2013).

It is clear that the shape and integrity of a fungus is also dependent upon the mechanical strength of its cell wall, which performs a wide range of essential roles during its interaction with its environment. The fungal cell wall is a complex structure typically composed of chitin, and glucan, mannan and proteins, although its composition frequently varies between species of fungi (Dhume *et al*., 1993). Both, plant and fungal cell walls, are a major target of the same type of cell wall degrading enzymes acting in the early stages of the plant-pathogen interaction. Additionally, oligosaccharides released from this degradation of plant or fungal cell walls can play the role of signaling molecules activating defenses responses and enhance growth or development in plants (Sea Silva *et al*.. 2013).

The role of cell wall in defense mechanisms in plant has been proved by cellulose deficient mutants. These mutants typically exhibit increased lignifications (Malinovsky *et al*., 2014). Interestingly, these changes appear to have effects beyond the purely structural. Such mutants also display enhanced defense responses (Hamann *et al*., 2009).

The another outer surface covering of the plant cell is cuticle that is multilayered mass in young stems and foliage consisting an insoluble polyester called cutin (Dickison 2000). Cuticle is a very effective barrier to invasion by pathogenic microbes (Dickison 2000). Not only biotic and abiotic stresses but also water loss is protected by cuticle in plants (Mario *et al*., 2014). The cuticle is structurally diverse among species but exhibits the organization of a composite material consisting in cutin, a polyester that is partly covered and interspersed with waxes (epicuticular and intracuticular waxes) (Mario *et al*., 2014). The roles of cuticle function in defensive mechanisms in plants are cleared when we study about mutants with specific defects in cutin. These mutants show an altered ultra structure of the cuticle and an enhanced permeability of the cuticle to solutes In addition, pollen could germinate on fully differentiated leaves of cutinase-expressing plants but not on control leaves. (Patrick *et al*., 2000). This finding is implied that cutin is important for preventing fusions between different plant organs and is therefore necessary for normal epidermal differentiation and organ formation (Heid 1991; Walker and .Bruck 1985). So the cuticle is the contact zone between the plant and the environment and its physical properties are highly relevant to the functions of the epidermis (Kerstiens 1996a ; 1996b).

Some of the epidermal cells of most plants grow out in the form of hairs or trichomes. They may be found singly or less frequently in groups. They may be unicellular or multicellular and occur in various forms. They vary from small protuberances of the epidermal cells to complex branched or satellite multicellullar structures. The cells of the hairs may be dead or living.

In some plants the role of trichomes is minimizing invasion of specific pathogens (Dickison 2000). Actually leaf trichome density is considered a mechanism of defense in plants to prevent or diminish damage by herbivores (Levin, 1973; Johnson, 1975; Rodriguez *et al*., 1984; Juniper and Southwood, 1986; Marquis, 1992) Evidence from wild and cultivated species gives

support to this ecological role (Duffey, 1986; Jeffree, 1986; David and Easwaramoorthy, 1988; Woodman and Fernandes, 1991; Bernays and Chapman, 1994; Peter *et al*., 1995; Romeis *et al*., 1999). The most important role of trichomes in plant defense is resistance against natural herbivores. Besides defense, leaf trichomes may serve other physiological functions, hence selection on the antiherbivory role of leaf trichome density can either be constrained or synergistically favored by selection imposed by other environmental stresses (Bell, 1997; Roy *et al*., 1999).

Alike trichomes thorns are a part of the defensive mechanism in plants. A thorn is a loose term for any sharp, pointed appendage coming off a plant for defensive purposes. Botanically, thorns can also be called spines (modified leaves), prickles (sharpened branches) or trichomes based on their location on the plant. Thorns are essentially defense mechanisms for the plant, The sharp points protect the plant against animals that want to eat it. There is an interesting experiment that have conducted by cut branches of three woody species that had their thorns removed suffered significantly greater herbivory by a tethered goat than did paired intact branches (Milewski *et al*., 1990).

Moreover smells, oils, thick and waxy skin, intact outer periderm or bark of woody plants provide sufficient barrier for plant defense against stresses by different methods for example some plants emit different smells to repel animals or others excrete oils to kill other plants. Also there are plants that have thick skin to keep from dehydrating. Trees have different types of bark to help deal with cold or hot environments. It is important that we know plants don't use one defensive strategy. Plants have all kind of strategies for protect themselves from difficult conditions (Milewski *et al*., 1990).

IV. DEFENSE BY PLASMA MEMBRANE

Plants have evolved a multi-layered immune system that dynamically responds to pathogens. Most classes of plant pathogens remain outside the host cell membrane during their lifecycle. As a result, the plant plasma membrane (PM) is a key mediator of communication between plants and microbes (James and Gitta 2011). Initial pathogen recognition occurs at the PM and many of the earliest cellular responses to microbial invasion are controlled by PM-localized enzymes and ion channels (Boller and Felix, 2009).

In addition, multiple downstream responses to pathogen stimuli are executed at the plasma membrane. Pathogens must manipulate host cells in order to suppress these defense responses and procure nutrients. It is therefore expected that membrane transport processes have numerous functions in compatible (susceptible) and incompatible (resistant) interactions (Hahn and Mendgen, 2001; Ward *et al*., 2009).

Also it is noticeable that in plants, PM H+-ATPases are the primary pumps responsible for the establishment of cellular membrane potential. Plant PM H+-ATPases use energy derived from ATP hydrolysis to pump protons from the cytosol to the extracellular space, thus creating and maintaining a negative membrane potential and a transmembrane pH gradient (acidic outside). This proton electrochemical gradient can control multiple aspects of transport across the PM. PM H^+-ATPase activity is dynamically regulated during plant immune responses and multiple pathogens target this family of enzymes during infection (James and Gitta 2011).

V. DEFENSE BY CYTOPLASM

The cytoplasm of eukaryotic cells consists of all cell material between the nucleus and the plasma membrane and contains membrane-bounded structures, organelles, which are embedded in the cytosol consisting of water, salts and organic molecules, including sugars, proteins and many enzymes that catalyze reactions. The cytoskeleton of microtubules and actin filaments in the cytosol structures the cell by localizing and transporting the organelles bound to these tubules and filaments. The plasma membrane, enveloping the cytoplasm physically, separates the cell content from the extra-cellular environment, which in plant cells is the cell wall (Esseling-ozdona *et al*., 2008).

Also cytoplasm is an important barrier for plants defense. The earliest cytoplasmic response to contact between a micro – organism and host cell is cyclosis within the protoplast, with a resultant reorganization of cytoplasmic organelles. Infection of tuber tissues and tissue culture cells of *Solanum tuberosum* by *Phytophthora erythroseptica*, *P. infestans* and *Fusarium caeruleum* caused swelling and disruption of host cytoplasmic particles containing acid phosphatases, esterases and proteases. Heavy diffuse cytoplasmic staining for acid phosphatase was a consistent feature of infection by the three fungi, but staining reactions for esterase and protease showed much less diffuse staining and a lesser degree of particle swelling. An excess recovery of ribonuclease from tissues infected with *P. infestans* and *F. caeruleum* was found. An electrophoretic comparison of near isogenic lines of wilt resistant (*Fusarium oxysporum* f.sp. *pisi*) or susceptible *Pisum sativum* was made.

The resistance lines differed from susceptible lines in carrying an esterase component with has been derived from the resistant cultivar Delwich Commando (Dhan Pal and Arti, 2005).

VI. DEFENSE BY ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is a network-like structure that is bound by a single membrane. The ER is a dynamic organelle composed of various functional domains (Staehelin 1996). It includes the rough ER (rER), smooth ER (sER) and nuclear envelope. The rER is a well-understood organelle that is coated by ribosomes and is responsible for the synthesis of secretory proteins. The sER has no ribosomes but instead accumulates a series of lipid biosynthesis enzymes. In addition, the ER accumulates specific types of seed storage proteins, such as prolamin or zein, to produce protein bodies (PBs) in the endosperm of some plants. (Herman 2008, Yasuda *et al*. 2009, Kumamaru *et al*., 2010, Satoh-Cruz *et al*. 2010, Nagamine and Okita, 2011).

Several lines of evidence suggest that ER bodies are involved in defensive mechanisms in plants. Wounding or application of the wound hormone jasmonic acid induces the formation of ER bodies (Matsushima *et al*. 2002 ; Hara-Nishimura and Matsushima 2003) as does the damage induced by pest chewing. Jasmonic acid is a well-known hormone that mediates wound response, induces resistance against insect/pathogen attack (McConn *et al*. 1997, Vijayan *et al*. 1998, Li *et al*. 2002,Chini *et al*. 2007, Sato *et al*. 2011) and regulates the expression of wound-inducible genes, such as the *vegetative storage protein* (*VSP*) genes (Leon *et al*. 2001, Lorenzo *et al*. 2004). But β glucosidase is the main component of the ER body. Plants accumulate various glycoside molecules that are derived from secondary metabolism (Gachon , 2005, Ketudat and Esen, 2010). Several glycosides that are involved in defensive mechanisms, such as cyanogenic glucosidases , saponins, glucosinolates or DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) glucoside (Mattiacci *et al*. 1995; Konno *et al*. 1999; Tattersall *et al*. 2001; Carpinella *et al*. 2005; Beekwilder *et a*l. 2008; Morant *et al*. 2008). Most of these compounds are stored in the inactive state and become activated by the removal of glycone. β-Glucosidases hydrolyze these molecules into glycone and aglycone. Thus, they are essential for the production of toxic compounds that mediate plant defense against insects or fungi (Mattiacci *et al*. 1995; Tattersall *et al*. 2001; Ketudat and Esen 2010).

There are many results that show dynamic changes in organelles underlie the tolerance of plants to environmental stress. For example, morphological changes in vacuoles and small vesicular structures occur during salt and zinc ionic stress (Hamaji *et al*. 2009, Kawachi *et al*. 2009), vacuolar morphology in the endodermis is important for the shoot gravitropic response (Niihama *et al*. 2009). Therefore, focusing on organelle differentiation may reveal new insights into plant survival strategies (Hayashi and Nishimura 2009, Homi *et al*. 2009, Kamigaki *et al*. 2009, Mano *et al*. 2009, Nagano *et al*. 2008, Niihama *et al*. 2009, Sakamoto *et al*. 2009).

VII. DEFENSE BY NUCLEUS

Communication between the cytoplasm and the nucleus is a fundamental feature conserved among eukaryotic systems. Transport of macromolecules across the nuclear envelope occurs through nuclear pore complexes (NPCs), which are composed of nucleoporins, and depends on import and export receptors, importins and exportins that respectively recognize nuclear localization signals (NLSs) and nuclear export signals on cargo proteins (Meier and Brkljacic, 2009). The Ras-related nuclear (Ran) protein provides the directionality of transport through its binding to GDP (cytoplasmic side) or GTP (nuclear side). Several cellular factors involved in the transport of macromolecules through the nuclear envelope, including nucleoporins, importins, and Ran-GTP-related components, are essential to mount an efficient immune response in response to different pathogens (Palma *et al.*, 2005; Zhang and Li 2005; Tameling and Baulcombe 2007; Cheng *et al*., 2009). In addition, several reports strongly suggest that components of the NPC specifically mediate the transport of R proteins, immunity components, as well as TFs (transcription factors) and regulators that are necessary for activation of disease resistance (Garcia and Parker, 2009). These and other findings support the notion that specific modulation of the nuclear concentration of a set of defense regulators is crucial for the fine tuning of plant immunity (Susana 2012). A significant number of effectors proteins from different pathogens, including nematodes, fungi, viruses, bacteria, and oomycetes, are targeted to the nucleus by co-opting the host nuclear import machinery (Deslandes and Rivas 2011). This means that effectors may manipulate host transcription or directly target nuclear essential host components for the benefit of the pathogen. It has been additionally proposed that some effectors may affect histone modification and chromatin remodeling. Alternatively, nuclear translocation of effectors may affect sub cellular localization of their cognate R proteins in a process that is essential for R-protein-mediated plant immunity. (Susana 2011).

VIII. DEFENSE BY CHLOROPLAST

It is nice to know that chloroplasts are a key defense organelle. Indeed The chloroplast is a vital component of photosynthetic cells in algae, and higher plants, since it is the organelle in which photosynthesis takes place (Cooper 2000). Photosynthesis is the major function of the chloroplast, but its roles clearly extend further than converting light energy into chemical energy. Actually the chloroplast, together with the nucleus, cell membrane, and endoplasmic reticulum (ER), plays a critical role during the establishment of plant immunity against microbial attack (Padmanabham and Dinesh-Kumar, 2010). It implies that during plant defense, interorganellar signaling to achieve a synchronized whole-cell response occurs (Serano *et al*., 2016).

Among cellular organelles chloroplast is an integrators for environmental signals and, more particularly, as mentioned earlier is a key defensive organelles (Serano *et al*., 2016)

The role of chloroplast in plant defense have been proved by evidence that show plants undergo an increased demand for photosynthesis during the interaction with pathogens, as the biosynthesis of pro-defense molecules and, more generally, the induction of defense responses requires energy that is provided through photosynthesis (Hammerschmidt 1999; Swarbrick and Lefert 2006). Moreover, virulent pathogens feed on plant carbon compounds, and some of them are able to use plant transporters of the SWEET family to promote sugar efflux, further increasing photosynthesis demand in host cells (Chen *et al*., 2010). However, instead of increased photosynthesis, several reports have revealed suppression of photosynthetic functions in infected plants, perhaps reflecting an active plant response to shut down carbon availability and limit pathogen growth or to favor the establishment of defense over other physiological processes, including photosynthesis, during pathogen attack (Padmanabham and Dinesh-Kumar 2010). on the other hand chloroplast is a major production site of pro-defense molecules (Padmanabham and Dinesh-Kumar 2010). In fact chloroplasts play a central role in plant immunity by hosting biosynthesis of several key defense-related molecules, including hormones and secondary messengers (Padmanabham and Dinesh-Kumar 2010).

Consistent with the importance of a balanced production of pro-defense molecules during plant–pathogen interactions, pathogens have developed sophisticated molecular mechanisms to subvert their biosynthesis and subsequent signaling for their own benefit (Denance *et al*., 2013; Kazan and Lyons, 2014). The central role of hormones during plant–pathogen interactions is highlighted by the significant number of pathogenic microbes that are able to produce hormones or hormone-mimicking molecules to disturb hormone homeostasis and cause disease (Robert *et al*., 2011) from for chloroplast.

IX. DEFENSE BY MITOCHONDRIA

One of the most important defensive barriers of plants is mitochondria. After pathogen perception, mitochondria play an important role in the defense strategy of the plant cell, integrating and amplifying diverse signals such as salicylic acid, nitric oxide, reactive oxygen species (ROS) or pathogen elicitors. Signals perceived by mitochondria usually impact on their normal function, destabilizing the organelle, generating changes in respiration, membrane potential and ROS production. At this stage, mitochondria produce several signals influencing the redox state of the cell and promoting changes in the expression of nuclear genes by mitochondrial retrograde regulation. At more advanced stages, they promote programmed cell death in order to avoid pathogen propagation to the whole plant. So plant mitochondria take part in initiating response HR (Jones 2000 ; Lam *et al*. 2001). Recent evidence indicates that plants and pathogens have evolved mechanisms to modulate the immune response by acting on mitochondrial functions (Amirsadeghi *et al*., 2007). On the other hand It is generally assumed that PTI (Pattern – Triggered Immunity) is a rapid response of the plant immune system and this first "cell reprogramming step" occurs during the first hour after plant-pathogen recognition. There are several evidences about the role of plant mitochondria during incompatible plant-pathogen interactions that include the perception of signals coming from the intercellular space or apoplast (Amirsadeghi et al., 2007).

Beside a role in the HR, mitochondria may represent an important intermediate between the perception of biotic stress and downstream responses such as the induction of defensive gene expression (Jones 2000, Lam *et al*. 2001). Studies that have investigated this hypothesis are outlined below. Polyamines such as spermine are proposed to play a role during biotic stress responses. Spermine accumulates dramatically and in an N-gene–specific manner in the apoplast of tobacco mosaic virus (TMV)-infected tobacco because of up regulation of genes involved in spermine biosynthesis (Yamakawa *et al*. 1998, Yoda *et al*. 2003). Accumulated polyamines are subsequently degraded in the apoplast by polyamine oxidize, generating H_2O_2 that may contribute to plant responses (Yoda *et al*. 2003).

X. DEFENSE BY GOLGI APPARATUS

Glycosylation is a posttranslational modification reaction which is related to protein activity and protein secretory pathway in eukaryotic cells (Roth 2002). This essential reaction occurs in the endoplasmic reticulum and Golgi apparatus (Kang *et al*., 2015). Although the glycosylation in the endoplasmic reticulum and Golgi apparatus has been known to regulate protein quality control, salt stress and cellulose biosynthesis, few evidences related to the roles of glycosylation in plant immunity have been reported (Kang *et al*., 2015). The role of Golgi apparatus in plant defense or plant immunity that is a pivotal role has been proved by studying about *Arabidopsis thaliana* mutants defective in glycosylation. The results showed that *Arabidopsis* mutants was more susceptible against *Pseudomonas syringae* pv. *tomato* DC3000 and *Erwinia carotovora* subsp. *Carotovora* compared to the wild type plant (Kang *et al* 2015). As mentioned above glycosylation occurs in endoplasmic reticulum and Golgi apparatus. In fact The glycosylated protein in the ER is translocated via vesicle to target or Golgi apparatus. In Golgi apparatus, the glycoprotein contains modification of N-glycan chain by various glycolytic enzyme and glycotransferase. In contrast of mammals that complex N-glycan are involved in different processes, including molecular recognition and signaling events, in plants complex N-glycan function is still largely unknown (Strasser 2014). In spite of the fact that complex N-glycans are ubiquitously present in plants (Wilson *et al.*, 2001), their biological function is virtually unknown. The first mutant lacking complex N-glycans was isolated more than two decades ago from *Arabidopsis*. These mutant showed that n-glycan mutants did not caused a substantial change in *Arabidopsis* growth or development when grown under long day conditions (16 h-light/8 h-dark) at 22°C (Strasser *et al*., 2007b). Up to now, the only evidence for a biological function of complex N-glycans in *Arabidopsis* was found when cgl1 and other mutants were subjected to osmotic and salt stress (Kang *et al*., 2008). Reduced root growth on media containing high NaCl concentrations indicated that complex N-glycans are implicated in tolerance to salt stress. However, a deeper understanding of complex N-glycan function in *Arabidopsis* and studies that associate distinct complex N-glycan structures on individual glycoproteins with the enhanced salt sensitivity are completely missing (Strasser 2014).

XI. DEFENSE BY PEROXYSOMES

As mentioned repeatedly, plants suffer from infections caused by fungi, bacteria, viruses and nematodes. Peroxysoms are one of the sub cellular organelles that play a substantial role in disease resistance. Peroxysomes are single membrane bound cellular organelles, present in almost all eukaryotic cells, which have an essentially oxidative metabolism (del Rio *et al*. 2002 ; Kaur *et al*. 2009). Peroxysomes contains a variety of enzymes that primarily function together to detoxify the toxins present in the cell and most notably hydrogen peroxide, which is the most common by product of cellular metabolism in living systems. These organelles contain enzymes that convert the hydrogen peroxide to water, rendering the potentially toxic substance safe for release back into the cells (del Rio *et al*. 2002 ; Nyathi and Baker 2006).

The roles of peroxysomes in plant biotic and abiotic defenses are becoming clearer (del Rio *et al*. 2006 ; Reumann *et al*. 2007; Palma *et al*. 2009; Corpas *et al*. 2010). About role of peroxysomes in plant defense for example we can express that plant peroxysomes contribute to extracellular defense mechanisms against fungi by preventing colonization. Upon fungal invasion peroxysomes migrate toward the site of invasion (Lipka, 2005). Under these conditions the myrosinase which is bound to the peroxysomal membrane, hydrolyzes indolic glucosinolates to antifungal defense compounds protecting plants against fungal entry (Bednarek 2009). Furthermore, certain benzylglucosinolates play a role in pathogen defense and are found in developing seeds and germinating seedlings (Graser *et al*., 2001). These active defense molecules are synthesized in the cytosol by transferring a benzoyl moiety from benzoyl-CoA to a hydroxylated aliphatic glucosinolate, though the precursor benzoyl-CoA is primarily produced from cinnamic acid via peroxysomal beta-oxidation (Baskar *et al*., 2012 ; Lee 2012 ; Qualley *et al*., 2012). Also Peroxysomes contain important enzymes and generate diverse metabolites that have a relevant role in the direct and indirect plant defense against herbivores (Shabab 2013). Different reports have shown the importance of ROS in herbivory related responses. Feeding of some pests on their hosts leads to considerably elevated ROS levels (Wu and Baldwin 2010). Another sample of increased ROS generation showed that mechanical wounding by herbivores was not sufficient to increase the amount of ROS but herbivory contribution was essential for that (Leitner *et al*., 2005). In another interesting study, an increase in the ROS levels in plants after pests attack was detected by Maffei *et al*., 2006.

XII. DEFENSE BY VACUOLES

The central vacuole is one of the unique features of mature plant cells and presents the largest intracellular compartment in many higher plant tissues. Numerous studies carried out over the last twenty years have provided evidence that this organelle really behaves as a multifunctional compartment of plant cells. One of the roles of vacuoles in cells is plant defense response

under condition of environmental stresses (Andreev 2001). In fact Plant cells have a large central vacuole that accumulates a variety of hydrolytic enzymes and antimicrobial compounds, raising the possibility that vacuoles play a role in plant defense (Hatsugai and Nishimura 2010). Plants, unlike animals, which have specialized defender cells and an adaptive immune system, have an innate immunity of each cell and produce systemic signals emanating from the infection site. On the other hand Plants have developed a complex immune system to resist pathogen attack, which includes rapid and localized cell death (hypersensitive response, HR), and stomatal closure (Dangl *et al*., 1996 ; Lam *et al*., 2001 ; Melotto *et al*., 2006 ; Mur *et al*., 2008). HR in plants is caused by proteases with cascade activity. At least eight cascade activities have now been measured in plant extracts, which were found using cascade substrates, and various cascade inhibitors can block many forms of plant programmed cell death (Zhang *et al*., 2010). A number of key players involved in HR have been identified (Greenberg and Yao, 2004; Gabriels *et al*., 2006; Gan *et al*., 2009; EK-Ramos *et al*., 2010); these studies have revealed that mammalian and plant cell death mechanisms share common morphological and biochemical features, including cytoplasm shrinkage, nuclear condensation, DNA laddering, and the release of cytochrome c from mitochondria (Sun *et al*., 1999 ; Sasabe *et al*., 2000 ; Kim *et al*., 2003 ; Ji *et al*., 2005). However, it remains unclear how signaling pathways lead to local HR, but not to whole-plant cell death, and how death occurs. Many studies have shown that HR in plants is regulated by cascade-like activity. However, no cascade homologue has been found in the past several years. So vacuolar-processing enzyme (VPE) has been determined to play important roles in plant immunity responses (Zhang *et al*., 2010). VPE localizes in the vacuolar membrane and mediates virus-induced hypersensitive cell death by regulating the collapse of this membrane (Hatsugai *et al*., 2004).

XIII. CONCLUSION

Photosynthesis is one of the most important processes that occur in green plants, alges and prokaryotic living organisms named cyanobacteria. In this pivotal process sunlight turn into chemical energy (food). In fact life on earth depends on this important process that the highest percentage of it's occurring in green plants. Briefly only plants can produce food for animals and humans. These supporter living organisms are sessile but they have to suffer from an extensive range of biotic or abiotic stresses. So they must equipped against injurious agents. Plants, unlike animals, which have specialized defender cells and an adaptive immune system, have an innate immunity of each cell and produce systemic signals emanating from the infection site. Generally plants campaign against harmful factors with different forms. Sometimes a plant is in contact with a pathogen to which is not a host, this form of defense is the most common form of resistance in nature, sometimes the host can to hinder the growth and/or the development of the pathogen. But this is important that Plant defense systems against pathogen invasion consist of multiple layers that means all of plant cell component such as: cell wall, plasma membrane, cytoplasm, endoplasmic reticulum, chloroplast, mitochondria, Golgi apparatus, peroxysomes and vacuoles.

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Food Security Production Challenges in Indonesia as Impact of Global Climate Change

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Abstract— *Global food availability, including national as well as local, is highly dependent on the natural resources that will affect crop production. Although there is rain, soil temperatures and conditions have formed a natural system that will support agricultural efforts, but this state is unstable and always changes according to atmospheric conditions in an integrated manner. Human beings on certain boundaries can intervene with the natural resources.*

Climate (generally a combination of rain, temperature, and sunlight) is the most important growth factor in crop production in the field. Any change in climatic conditions will have far-reaching effects on global food production.

Global climate change, excessive land and land exploitation, inaccurate land management, in its time will have an impact on the food production and availability of a region. Knowing well the of nature characteristics, then anticipating the impact that will arise and determine the ways of handling it, is a series of business and activities that must be done to achieve food security.

To anticipate climate change and its impacts on crop production, a broad outline can be made by considering the following physical technic aspects: 1) adjusting cropping patterns; 2) increasing the area of forest cover and catchment areas; 3) application of land and crop management technology. Some application of land and crop management technologies include: organic farming, implementation of Surjan system, food diversification, large tree planting, water pond production, etc.

The policies that need to be taken as a solution in anticipating the impact of global climate change are 1) the preparation and stipulation of special food agriculture scenarios, including the zoning of production potential and zonation of climate risk (drought, flood, landslide, etc.) with the updating of data every year; 2) reducing the conversion of agricultural land (food); 3) incentives for farmers; 4) changing the consumption pattern of the people, from the consumption of rice to alternative staple foods; 5) subsidies and protection of food farming; 6) climate monitoring and prediction (early rainy season, long growing period, and potential water availability; 7) Revitalization of watershed (DAS) functions; 8) Multiply the artificial water absorption area.

Keywords— Climate Change, Food Security, Land and Crop Management, Watershed.

I. INTRODUCTION

Increased concentrations of greenhouse gases in the atmosphere due to human activities around of the world, causing increased radiation trapped in the atmosphere. The impact is the occurrence of an increase in average temperatures across the earth's surface, referred to as global warming.

Increasing the average temperature of the Earth's surface causes changes in other climatic elements, such as rising sea temperatures, increased evaporation in the air, and changing patterns of rainfall and air pressure that eventually change the pattern of world climate. This event became known as global climate change.

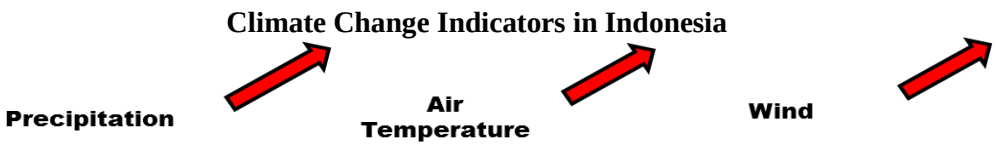

TABLE 1
CLIMATE CHANGE INDICATORS IN INDONESIA

No.	Climate Elements	Phenomenon	Impact
1.	Precipitation	Longer dry season	Water shortage, crop failure
		Shorter rainy season with high intensity	Flood, erosion, landslide, crop failure
2.	Air Temperature	The temperature is hotter	Increased evaporation, impaired growth
3.	Wind	Strong local winds at the time of the rain	Disrupting plant growth

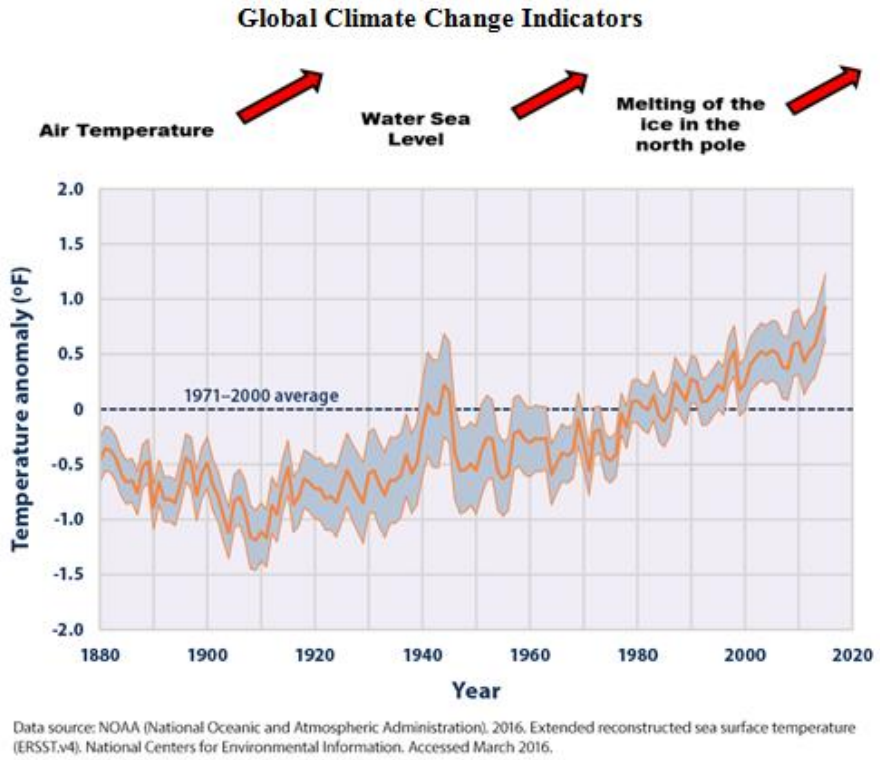

FIGURE 1. AVERAGE GLOBAL SEA SURFACE TEMPERATURE, 1880-2015

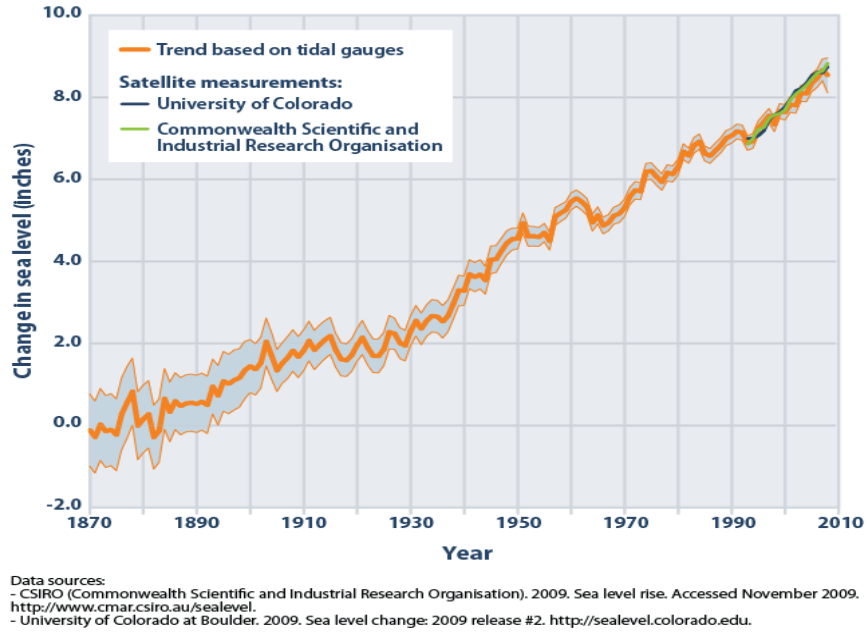

FIGURE 2. TRENDS IN GLOBAL AVERAGE ABSOLUTE SEA LEVEL, 1870-2008

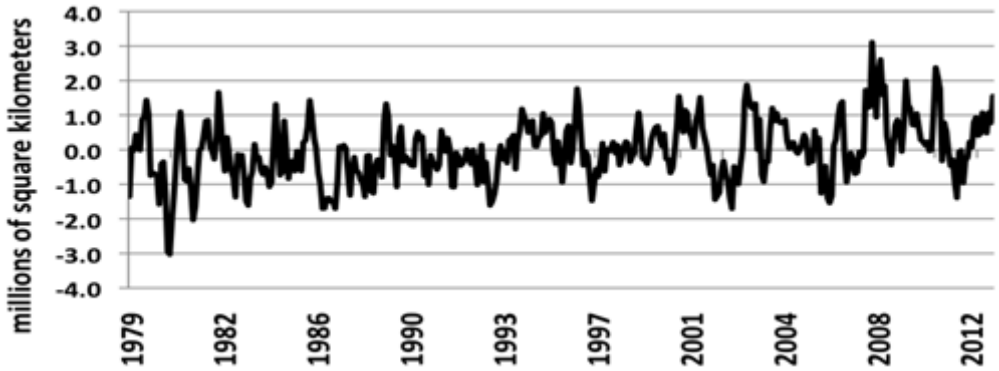

FIGURE 3. ANTARTIC SEA ICE EXTENT ANOMALIES, 1979-2012 (UNIVERSITY OF ILLINOIS)

II. DATA AND FACT

At the regional micro-scale in Indonesia, several climate elements that undergo changes include wind, temperature, and precipitation (Table 2).

Some studies of average historical data, air temperatures in Indonesia increased by 0.3 ºC per year since 1900. in the 1990s was the warmest decade and 1998 was the warmest year, 1 ºC above the 1961-1990 average . Increased temperatures occur throughout the season. Rainfall is reduced by 2 to 3% especially in December-February. In most parts of Indonesia rainfall is affected by El-Nino, major droughts occur in El-Nino years 1982/1983, 1986/1987, and 1997/1998.

TABLE 2
THE CHANGING OF CLIMATE ELEMENTS IN INDONESIA

Location	Year	The Changing Element per Year		
		Temperature	Precipitation (dry season)	Precipitation (wet season)
Data Source 1				
Jakarta	1916-1987	0.03**	*	*
Jakarta	1951-1987	*	- 0.1 %	10 % **
Bogor	1951-1987	*	- 1.1 % **	0.3 %
*Bogor	1976-1987	0.05**	*	*
Bogor	1980-1998	0.14**	-2.0 % **	4.6 % **
Data Source 2				
Indonesia		Meningkat 0.3 ºC	Hujan tahunan menurun 2-3 %	

Data Source 1: Hidayati (1990), Hidayati, Abdullah, and Suharsono (1999).
Data Source 2: Hulme and Sheard (1999), Boer and Faqih (2004).

TABLE 3
THE CHANGING OF MONTHLY RAINFALL ON NORMAL RAINFALL (IN %, AVERAGE VALUE 1970-1997 IN SOME PROVINCE)

Island	1970			1997		
	Oct-Mar or Nov-Apr	Apr-Sept or May-Oct	Annual	Oct-Mar or Nov-Apr	Apr-Sept or May-Oct	Annual
Sumatera	-35	-47	-38	-21	-32	-24
Java	-34	-80	-41	-11	-85	-23
Bali/NTT	-26	-82	-31	-26	-75	-32
Kalimantan	-33	-57	-40	-5	-36	-16
Sulawesi	-28	-67	-39	-35	-33	-30
Maluku/Ambon	-13	-53	-40	-5	-27	-20
Indonesia	-32	-62	-38	-19	-47	-24

Data Source: Irawan, 2002

III. MATERIALS AND METHOD

The methodology used in this research is the Sytematic Review Method. As with the methodology of individual research, in principle, systematic review research begins by making a systematic review research protocol and the next stage of

conducting systematic review research. Sequentially, the process of systematic review research is shown in Table 4. Analog with general research methodology, where there are quantitative and qualitative methods, then in systematic review there are also quantitative methods and qualitative methods.

Quantitative method of systematic review is used to synthesize the results of research with quantitative approach. For example, Randomized Control Trials (RCTs), Cohort Study, Case-Control Study, or prevalence studies. The statistical approach in synthesizing the results of quantitative research is called "meta-analysis". By definition, meta-analysis is a technique of aggregating data to obtain statistical power in identifying causal relationships between risk factors or treatment with an outcome (Perry & Hammond, 2002). Meanwhile, qualitative approach in systematic review is used to synthesize qualitative descriptive research results. The method of synthesizing (summarizing) the results of qualitative research is called "meta-synthesis". By definition, meta-synthesis is a technique of integrating data to gain new theories and concepts or deeper and more thorough understanding levels (Perry & Hammond, 2002).

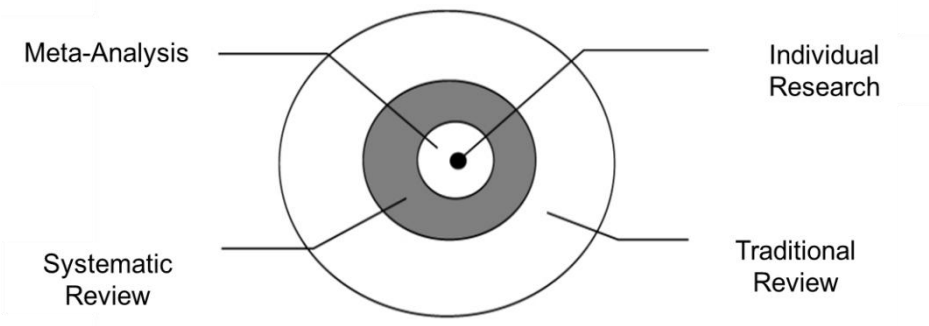

FIGURE 4. POSITION OF SYSTEMATIC REVIEW METHODS IN ANOTHER RESEARCH METHODS

TABLE 4
SEQUENCE OF SYSTEMATIC REVIEW PROCESS (PERRY & HAMMOND, 2002)

No.	Sequence of Process	Objective
1.	Identify questions research	Make a transformation climate change problems into question research
2.	Develop protocol research systematic review	Giving guides in doing systematic review
3.	Set location Data-base results research as search area	Provide restrictions search area against the results of the study which is relevant
4.	Selection of results relevant research	Collect the results relevant research with questions research
5.	Select good quality results of research	Conducting exclusion and inclusion of research to be entered in systematic review based on quality
6.	Data extraction from individual studies	Perform data extraction from individual studies to get the findings importance
7.	Result synthesis by method Meta-analysis (if allow), or narrative method (if impossible)	Conducting synthesis of results with metaanalysis techniques (forest plot) or narrative techniques (metasintesis)
8.	Presentation of results	Write down the results research in the document reports systematic results review

IV. RESULTS AND DISCUSSION

4.1 Impact of Climate Change

4.1.1 Floods and Drought

Indonesia there have been 46 major drought events, 30 of which occurred in the period 1844-1960 (for 117 years), and the remaining 16 events in the period 1961-2006 (only for 46 years). While the floods, became a common occurrence almost every rainy season in various provinces (Ministry of Environment Republic of Indonesia, 2007). During the period of 2001-2004 there have been 530 flood events in various regions in Indonesia.

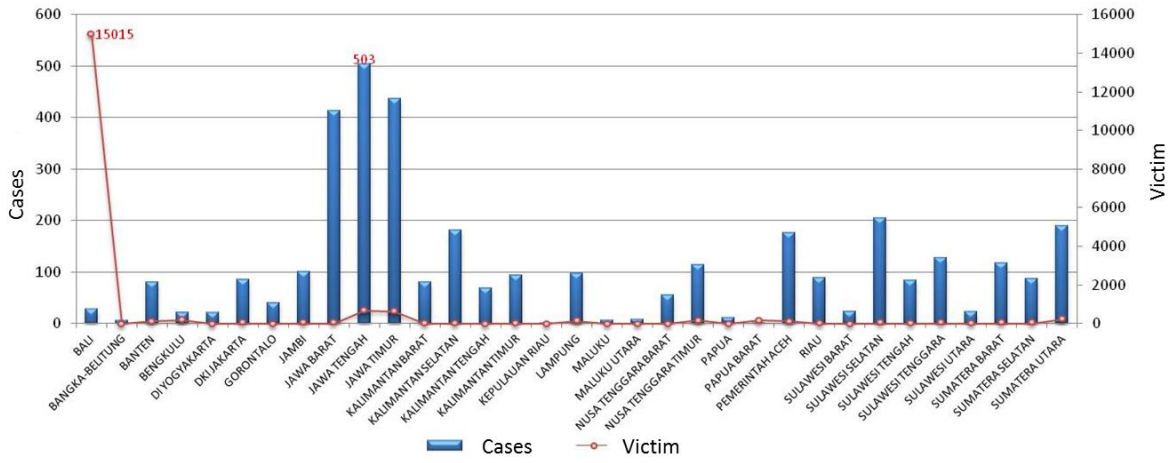

FIGURE 5. NUMBER OF FLOOD CASES AND VICTIMS DEATHS PER PROVINCE 1822-2011 (DATA SOURCE BNPB, 2012)

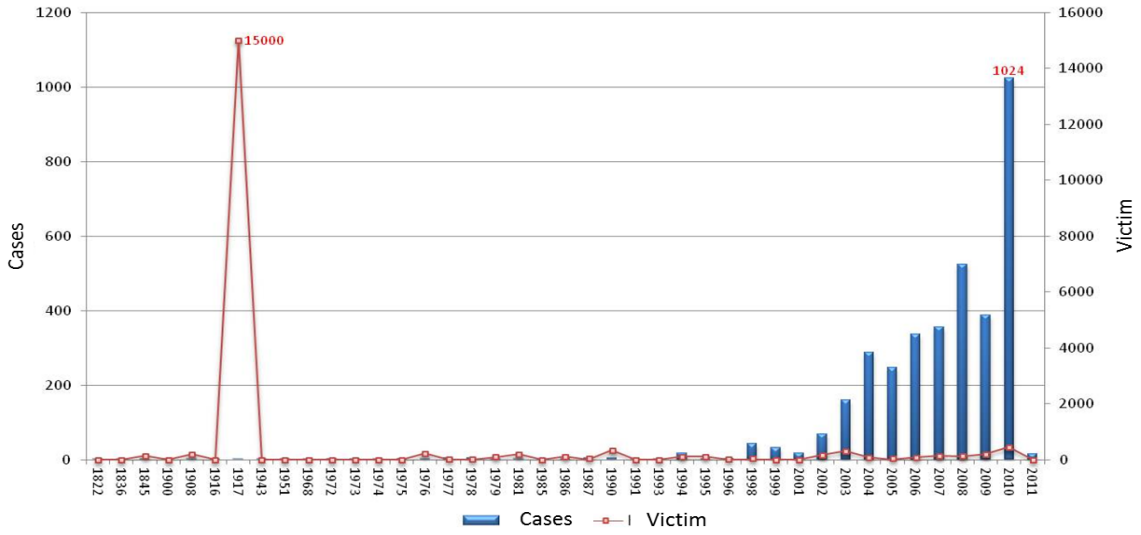

FIGURE 6. NUMBER OF FLOOD CASES AND VICTIMS DEATHS PER YEAR 1822-2011, (DATA SOURCE BNPB, 2012)

TABLE 5
SITUATION OF DROUGHT AND HARVEST FAILURE IN INDONESIA

Year	Drought Impact (Decreasing Production in Hectares)	Harvest Failure in Hectares
1990s		
1994	489,178	150,319
1995	18,462	3,385
1996	48,490	11,485
Total	**556,130**	**165,162**
2000s		
2001	145,545	11,344
2002	298,678	30,694
2003	430,258	82,690
Total	**874,481**	**124,728**

Data Source : Indonesian Department of Agriculture, 2007.

TABLE 6
CONDITION OF WATERSHED (DAS) IN JAVA ISLAND

No.	Province	Broodstock	DAS Square (Km^2)	Min	Max	Qmax/Qmin	Condition
1.	Banten	S. Ciujung	1,563	1.0	1,880	1,880	Critical
2.	West Java	S. Cisadane	820	1.0	1,150	1,150	Critical
		S. Ciliwung	158	0.1	390	3,900	Critical
		S. Citarum	1,675	2.0	370	185	Critical
		S. Cimanuk	1,966	1.0	710	710	Critical
		S. Citanduy	1,416	0.1	1,250	12,500	Critical
3.	Central Java	K. Pemali	856	0.1	850	8,500	Critical
		K. Serang	98	0.1	100	1000	Critical
		K. Juana	46	0.1	110	1,100	Critical
		B. Solo	3,207	2.0	9,990	4,495	Critical
		K. Serayu	723	3.0	1,580	527	Critical
4.	DIY	K. Progo	423	0.1	900	9,000	Critical
		K. Opak	30	0.1	10	100	Critical
5.	East Java	K. Brantas	7,112	10.0	3,180	316	Critical
		K. Sampean	612	0.1	850	8,500	Critical
		K. Pekalen	163	0.1	200	2000	Critical

Data Source : DBPSDA-PU, 2009.

4.2.2 Agriculture Production (Paddy)

Notes in the Indonesian Meteorological and Geophysical Agency show that the dry periods Indonesia has experienced are 1991, 1993, 1994, 1997, 2000, and 2001, while the times of excess water are 1992, 1996, 1999, and possibly 2002 (Table 5). This fact can be used to predict and simultaneously inform not only when the right planting, but also the type of plant that best fits the condition. This will greatly assist food security efforts and reduce the risk of crop failure.

TABLE 7
AREA OF RICE CROPS AFFECTED BY FLOODS, DROUGHT, AND HARVEST FAILURE (PUSO) IN HECTARES (1988-1997)

Year	Remark	Flood	Drought	Puso
1987	El-Nino	***	430,170	***
1988	La-Nina	130,375	87,373	44,049
1989	Normal	96,540	36,143	15,290
1990	Normal	66,901	54,125	19,163
1991	El-Nino	38,006	867,997	198,054
1992	Normal	50,360	42,409	16,882
1993	Normal	78,480	66,992	47,259
1994	El-Nino	132,975	544,422	194,025
1995	La-Nina	218,144	28,580	51,571
1996	Normal	107,385	59,560	50,649
1997	El-Nino	58,974	504,021	102,254

Data Source : Jasis and Karama, 1999; Yusmin, 2000.

4.2 Projection of Food Production

The effect of climate change on crop production depends on factors, namely: 1) the magnitude of changes in influencing climate variables; and 2) plant adaptability. According to Rosenzwig and Iglesias (IPCC, 1996), there will be a decline in some food commodities in some countries as follows (Table 8). The most influential aspects of climate elements to production are air temperatures and rain.

TABLE 8
PREDICTION OF FOOD PRODUCTION IN SOME COUNTRIES

Countries	Impact to Agriculture Production
Indonesia	Rice -2.5 %; Soybean -2.3 %; Maize -40 %
Malaysia	Rice -22 % to -12 %; Rubber -30 % to -3 %
UK	Land Productivity (+5 % to +15 %)
USA	Wheat -14 % to -2 %; Maize -29 % to -15 %; rice -23 %

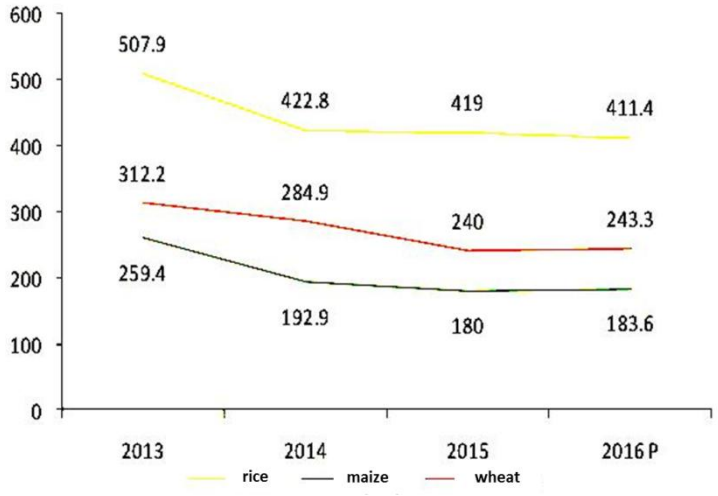

FIGURE 7. PROJECTION OF GLOBAL FOOD PRODUCTION DECREASE (DATA SOURCE IGICO ADVISORY, 2015)

4.3 Adaptation and Mitigation

To anticipate climate change and its impact on crop production, the outline can be done by considering the following physical technic aspects:

1. Adjusting cropping patterns;
2. Increasing the area of forest cover and catchment areas;
3. Application of land and crop management technology. Some application of land and crop management technologies include: organic farming, implementation of Surjan system, food diversification, large tree planting, water pond production, etc.

V. CONCLUSION

The policies that need to be taken as a solution in anticipating the impact of global climate change are:

1. The preparation and stipulation of special food agriculture scenarios, including the zoning of production potential and zonation of climate risk (drought, flood, landslide, etc.) with the updating of data every year;
2. Reducing the conversion of agricultural land (food);
3. Incentives for farmers;

4. Changing the consumption pattern of the people, from the consumption of rice to alternative staple foods;
5. Subsidies and protection of food farming;
6. Climate monitoring and prediction (early rainy season, long growing period, and potential water availability.
7. Revitalization of watershed (DAS) functions.
8. Multiply the artificial water absorption area.

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Polyamine and ethylene changes during floral initiation in response to paclobutrazol in mango (*Mangifera indica* L.)

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Abstract— *Use of paclobutrazol is common strategy for inducing uniform and profuse flowering in mango. The possible mechanism by which paclobutrazol exert such responses are less understood. The present investigation was carried out to investigate possible role of polyamines and ethylene biosynthesis in the paclobutrazol induced floral induction in mango. Following paclobutrazol soil drenching application (1.25 g a.i. m^{-1}) to mango cv. Totapuri, the free polyamine contents, ethylene production, 1-amino cyclopropane carboxylic acid (ACC) content and ACC oxidase activity were determined in the apical buds and leaves of growing shoots at 4 distinct bud developmental stages numerically characterized as 510 (initiation of bud swelling), 511 (swollen buds), 513 (bud burst) and 515 (panicle emergence) according to standard BBCH scale. The total free polyamines, spermidine and spermine contents increased and ethylene production, ACC content and ACC oxidase activity decreased in the buds and leaves of paclobutrazol treated as compared to untreated trees. In general under paclobutrazol treatment, buds accumulated more polyamines than the leaves. With respect to the bud growth stages, total free polyamines, spermidine and spermine were high at 510/511 stage both in the paclobutrazol treated and untreated trees which declined progressively as shoots approached panicle emergence stage (515). The ethylene production, ACC and ACC oxidase activity exhibited trends opposite to that of polyamines. The study showed that polyamine – ethylene balance may control paclobutrazol induced floral bud induction in mango and accumulation of polyamines-spermidine and spermine in buds appeared as an important factor in facilitating floral induction response.*

Keywords— Ethylene biosynthesis, mango flowering, paclobutrazol, polyamines.

I. INTRODUCTION

Mango (*Mangifera indica* L.) is considered one of the important widely cultivated fruit crops of India in an estimated area of 2.54 million hectare with 18.08 million tonnes of fruit production. However, productivity (6.8 t ha^{-1}) and market share of mango export in India is low due to the problems of alternate bearing, poor fruit set, early fruit drop, absence of efficient size controlling rootstock etc. Flowering is the key developmental event for crop yield and production. The intensity and timing of flowering show strong dependence on physiological status of growing buds, hormonal interactions, environmental factors and nutrient availability (Bernier and Perilleux 2005). In mango, the flowering is a complex process that involves differentiation of apical buds under the influence low temperature and/or attaining of certain degree of shoot maturity followed by bud burst and panicle emergence (Davenport 2007). Nunez-Elisea and Davenport (1995) reported that the temperature around 15-18 °C and 6-8 month old matured shoots exhibit strong behavior for floral growth initiation in mango. Ramirez and Davenport (2010) suggested involvement of leaf synthesized and phloem mobile florigenic promoter which moves to buds under the influence cold inductive conditions for exhibiting of floral growth in mango. Upreti et al. (2013) showed high accumulation of abscisic acid (ABA) and cytokinins and reduction in gibberellins in the growing buds linked to floral induction in mango. Similarly Upreti et al. (2014) reported high levels of sucrose and glucose contributed to the formation of generative buds in mango. However, flowering process in mango still remains unelucidated because of fragmentary information on various aspects of floral development including physiological, biochemical and molecular aspects.

Use of growth retardants is an important horticultural practice for the management of reproductive growth and productivity enhancement in number of fruit crops including mango. Among the growth retardants, use of paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4 dimethyl-2-(1,2,4-triazol)-1-yl)-pentane-3-ol] has been shown beneficial in restriction of vegetative growth and successful induction of floral growth in many mango cultivars (Yadav et al. 2005; Kishore et al. 2015). Evidences have shown that the paclobutrazol induced floral development is linked to suppression of gibberellins and increase in ABA and cytokinins besides increases in shoot C: N ratio and leaf water potential (Upreti et al. 2013). Several investigations have described polyamines, important polycationic growth regulatory molecules, as facilitators of reproductive development by sensitizing floral induction, floral differentiation, floral initiation and pollination in number of crops (Pritsa

and Voyiatzis 2004; Kakkar and Sawhney 2002, Liu et al. 2006; Aloisi et al. 2016). Rey et al. (1994a) suggested the spermine accumulation as potential physiological marker for ascertaining timing of flower induction. In another study, Rey et al. (1994b) showed that high endogenous spermidine and spermine levels with low putrescine in buds and leaves are vital to flowering process in hazelnut trees. In strawberry, polyamines are reported to be involved in regulating floral initiation (Tarenghi and Martin-Tanguy 1995). Zhu et al. (1999) in apple stated active role for spermine in the modulation of floral bud growth activity. Similarly, Kushad and Orvos (1990) reported that the reproductive structures in citrus accumulated high polyamine levels. Wang et al. (1985) in the flower buds of cherry species (*Prunus avium* L. and *P. serrulata* L.) reported that the polyamines were actively present in all stages of bud development stages and their levels were low during dormancy, which increased rapidly upon the dormancy break and floral induction. Importance of polyamine involvement in flowering process has also been confirmed through their exogenous applications in varied crops. In apple trees, polyamines application through cut pedicels enhanced the number of flower buds (Rohozinski et al. 1986) and spraying polyamines favouring flower bud formation (Costa and Bagni 1983). Similarly, promotion of flowering by exogenous polyamines has been demonstrated in *Spirodela punctata* and morning glory (Liu et al. 2006). Tarenghi and Martin-Tanguy (1995) on the other hand by employing inhibitor of polyamine biosynthesis, α-difluoromethylornithine (DFMO) reported that the inhibition in flowering in strawberry was related polyamine decrease, which was restored by exogenous application of putrescine. Despite the importance of polyamines in floral development of different fruit crops, studies attributing polyamine involvement in floral induction of mango are lacking. Considering the fact that the biosynthesis of polyamines and ethylene are corregulated as a result of sharing of common precursor, s-adenosine methionine (SAM) (Yang 1987), and importance of ethylene in the promotion of flowering in many fruit crops including mango (Bukovac et al. 2006; Turnbull et al. 1999; Davenport and Nunez-Elisea 1990), we in the present investigation studied the effects of paclobutrazol on polyamine contents, ethylene production, ethylene precursor 1-aminocyclopropane carboxylic acid (ACC) content and activity of enzyme facilitating ACC to ethylene conversion- ACC oxidase at different stages of floral bud growth in the cv. Totapuri to delineate the role of polyamines in paclobutrazol induced flowering in mango.

II. MATERIALS AND METHODS

The studies were conducted during the years 2014-2015 at the experimental farm of ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru on 18 years aged trees of a regular bearing mango cv. Totapuri maintained at 10 x 10 m spacing. The trees were given single recommended dose of paclobutrazol (Zeneca Limited, Surry, UK) at 1.25 g a.i. per m of canopy diameter as soil drenching treatment by spreading uniformly in a circular band of 25 cm width around the tree at a radial distance of 1.0 m from tree trunk during 3rd week of August. The untreated trees (control) were given water similarly. Four trees were maintained under paclobutrazol treatment and another four trees under control. Recommended package of practices were adopted for maintenance of trees. During the experimentation period, the average minimum and maximum temperatures were 20.2 and 28.1°C and average relative humidity was 63.2%. The terminal shoots measuring about 20 cm length from current year growth were labeled in different directions of paclobutrazol treated and untreated trees. Periodic sampling of apical buds and leaves was carried out from 3rd week of September for free polyamines, ethylene production, ACC and ACC oxidase activity at four phenological stages of bud development characterized as 510 (initiation of bud swelling), 511 (swollen buds), 513 (bud burst) and 515 (panicle emergence) according to pheno-phase guide chart described by Shailendra Rajan et al. (2011).

2.1 Polyamine analysis

The free polyamine contents in the apical buds and leaves were estimated according to the HPLC procedure of Flores and Galston (1982) with some modifications. The buds (1.0 g) and leaves (2.0 g) were homogenized in 10 ml of chilled 5% (v/v) perchloric acid, contents centrifuged and the supernatant separated out. One ml of 2.0 N NaOH and 100 µl of benzoyl chloride were added to 1.0 ml of supernatant, for conversion of polyamines to their benzoyl derivative. After adding 1.0 ml of saturated NaCl, the benzoylated polyamines were extracted with equal volume of chilled diethyl ether. The ether phase was separated out and dried under nitrogen. The residue was suspended in 1.0 ml methanol and clarified by passing through membrane filters (pore size 0.20 µm pore size, 13 mm diameter; Millipore, USA). The methanolic extract was dried under nitrogen at 40 °C and further dissolved in 100 µl of methanol for HPLC (Model: Prominence, Shimadzu, Japan) equipped with Synergi 4µ Fusion-RP 80A column (25 cm×4.6 mm, Phenomenex, USA) and Photodiode Array Detector (Model: SPD 20, Shimadzu, Japan) adjusted to a wavelength of 282 nm. An isocratic solvent system comprising of methanol (62%, v/v) containing 1% acetic acid at 1.0 ml min^{-1} flow rate was employed to separate and quantify component polyamines. The retention times for standard putrescine, spermidine and spermine were 5.8, 7.1, and 8.6 minutes, respectively under above

HPLC conditions. The quantification of free polyamines was carried out employing putrescine, spermidine and spermine standards (Sigma, USA).

2.2 Ethylene concentration

The sampling of apical buds and leaves for determining ethylene concentrations was carried out in pre-weighed 5.0 ml sampling tubes and 50 ml conical flasks, respectively fitted with rubber septa. The samples were incubated for 3 h at 37 °C and ethylene concentration was determined on Gas Liquid Chromatograph (GLC) (Auto System XL, Perkin Elmer, USA) equipped with Porapak-N column (2.0 m length, 80x100 mesh) and flame ionization detector (FID) according to Galliard and Grey (1969). The column, injection port and detector temperatures employed were 75, 125 and 200 °C, respectively and the carrier gas (N_2) flow rate was 45 ml min^{-1}. Standard ethylene (100 µl L^{-1} nitrogen) was used to quantify ethylene.

2.3 1-aminocyclopropane carboxylic acid (ACC) content

ACC content was determined using indirect method following oxidation of ACC to ethylene according to Lizada and Yang (1979). The samples of apical buds or leaves (500 mg lyophilized powder) were suspended in 4.0 ml of cold 5.0% sulphosalicyclic acid, contents vortexed and further centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant separated out was lyophilized. The lyophilized samples were dissolved in 1.0 ml of deionized water and purified over Dowex 50[H+] syringe column. The ACC was eluted with 4.0 M of ammonium hydroxide, eluate dried under vacuum and dissolved in 1.0 ml of deionized water. The ACC was estimated by adding 50 µl mercuric chloride to the aqueous ACC sample in a 2.0 ml glass vial kept in an ice bath and fitted with rubber septa. A 100 µl of NaOCl and saturated NaOH (2:1, v/v) solution was injected into the vial, mixture vortexed vigorously for 3 min and further cold incubated for 5 min at 4 °C. The ethylene produced was quantified by GLC as stated above and quantification of ACC was carried out using standard ACC (Sigma, USA). The conversion efficiency of ACC to ethylene determined was 78.43%.

2.4 ACC-oxidase activity

The apical buds and leaves powdered using liquid nitrogen of (250 mg) were kept in 2.0 ml sample tubes containing 500 µl of ACC (5.0 µ mol). The tubes sealed tightly with rubber septa were incubated for 4 h at 37^0C in an orbital shaker at 90 rpm. The conversion of ACC to ethylene was measured by assaying ethylene produced employing GLC-FID and ACC oxidase activity was expressed as n mol ethylene formed g^{-1} hr^{-1}.

2.5 Statistical analysis

All the data were analyzed using Agri Stat software and the Student Tukeys test of significance was performed to determine significance between the data obtained for different parameters at different stage of bud development.

III. RESULTS AND DISCUSSION

The free polyamine contents varied distinctly in the apical buds and leaves of paclobutrazol untreated and treated trees at different stages of bud growth, and the apical buds exhibited higher contents than the leaves (Table 1). In the untreated trees, the spermidine, spermine and putrescine contents decreased in both apical buds and leaves upon advancing of stage from 510 to 515, and the spermidine (43.48 and 15.76 m mol g^{-1}) followed by spermine (35.07 and 12.63 m mol g^{-1}) contents were the highest in the buds and leaves at 510 stage. Following the paclobutrazol application, the total polyamine, putrescine, spermidine and spermine contents increased by 31.90-91.20%, 15.09-31.58%, 40.96-123.18% and 33.17-94.10% in apical buds and by 11.05-21.77%, 4.02-13.91%, 12.90-25.95% and 13.59-22.49% in leaves, respectively as compared to untreated trees, and increase was high in spermidine at 510 followed by 511 stages and in spermine content at 511 stage (Table 1). In general, the trends in the contents of different polyamines across the bud growth stages broadly resembled in the paclobutrazol treated and untreated trees.

In paclobutrazol untreated trees, the ethylene production, ACC content and ACC-oxidase activity showed increasing trends from 34.71-58.71 nl g^{-1} h^{-1}, 5.36-12.94 n mol g^{-1} and 13.96-21.24 nl g^{-1} h^{-1} in apical buds and 16.96-23.14 nl g^{-1} h^{-1}, 1.76-2.86 n mol g^{-1} and 4.68-6.52 nl g^{-1} h^{-1} in leaves during the floral bud development stages, respectively (Table 2). Following paclobutrazol treatment, the ethylene production, ACC content and ACC-oxidase activity declined both in buds and leaves, with buds being more responsive than the leaves. Between the stages, all these parameters revealed high values at 515 (panicle emergence) and low values at 510 stages.

TABLE 1
EFFECT OF PACLOBUTRAZOL (PBZ) ON POLYAMINE CONTENTS AT VARIOUS DEVELOPMENTAL STAGES OF BUD IN MANGO CV. TOTAPURI (DATA REPRESENT MEAN ± SE, N = 4)

Treatments	Polyamines (n mol g^{-1})	Apical buds				Leaves			
		Floral bud development stages							
		510	511	513	515	510	511	513	515
-PBZ	Putrescine	15.63±0.94	17.78±0.91	15.22±0.85	12.66±1.03	9.56±1.01	9.97±1.15	7.56±1.22	6.22±0.45
	Spermidine	43.48±3.91	34.45±3.05	26.21±1.11	20.73±0.42	15.76±1.89	13.24±1.08	11.252±0.74	10.85±0.16
	Spermine	30.07±1.85	28.31±2.62	21.01±1.62	19.81±1.19	12.63±1.22	10.18±1.36	7.36±0.52	5.56±0.64
	Total polyamines	94.48±6.29	80.54±6.49	62.44±3.48	53.20±2.59	37.95±4.19	33.39±3.59	26.17±2.56	22.63±1.29
+PBZ	Putrescine	20.96±1.06	22.51±1.68	17.86±1.28	14.47±0.96	10.89±1.78	11.23±0.93	8.26±0.76	6.47±0.88
	Spermidine	55.04±7.52	62.61±5.17	25.02±1.43	15.72±1.32	19.85±2.23	15.85±1.33	13.29±0.43	12.25±0.32
	Spermine	48.65±3.68	38.95±2.81	34.99±2.13	26.38±1.64	15.47±1.88	12.12±1.24	8.36±0.57	6.41±0.64
	Total polyamines	124.65±12.32	124.07±9.52	76.17±4.76	51.57±3.85	46.21±4.29	39.20±2.56	29.91±1.69	25.13±1.89

Codes of bud developmental stages: 510 (bud swelling), 511 (swollen buds), 513 (bud burst) and 515 (panicle emergence) according to standard BBCH scale

TABLE 2
EFFECT OF PACLOBUTRAZOL (PBZ) ON ETHYLENE PRODUCTION, ACC CONTENT AND ACC OXIDASE ACTIVITY AT VARIOUS DEVELOPMENTAL STAGES OF BUD IN MANGO CV. TOTAPURI (DATA REPRESENT MEAN ± SE, N = 4)

Treatments	Parameters	Apical buds				Leaves			
		Floral bud developmental stages							
		510	511	513	515	510	511	513	515
-PBZ	Ethylene production (nl g^{-1} h^{-1})	34.71±3.14	30.22±2.84	43.89±4.86	58.26±6.04	23.14±1.92	18.27±1.58	16.96±1.13	20.14±2.17
	ACC (n mol g^{-1})	5.36±4.52	7.24±0.78	9.16±0.92	12.94±1.17	1.76±0.12	2.16±0.23	2.86±0.23	2.74±1.62
	ACC oxidase activity (nl g^{-1} h^{-1})	13.96±1.15	13.41±1.26	16.92±1.72	21.24±1.73	4.68±0.52	6.05±0.73	5.16±0.31	6.52±0.74
+PBZ	Ethylene production (nl g^{-1} h^{-1})	23.32±1.96	25.23±2.17	27.15±2.07	34.22±3.67	17.68±1.12	14.63±1.13	14.15±1.39	18.12±1.63
	ACC (n mol g^{-1})	4.61±0.21	5.43±0.46	6.66±0.51	9.02±1.03	1.51±0.11	1.89±0.01	2.66±0.24	2.31±0.24
	ACC oxidase activity (nl g^{-1} h^{-1})	9.42±0.72	11.14±1.19	13.66±1.19	16.89±1.45	3.14±0.35	2.64±0..22	3.72±0.33	4.65±0.53

Codes of bud developmental stages: 510 (bud swelling), 511 (swollen buds), 513 (bud burst) and 515 (panicle emergence) according to standard BBCH scale

The floral morphogenesis involves complex system of involvement and interaction of biochemical signals including plant growth regulators (Davis 2009). Among the growth regulators, polyamines are ascribed as important elicitor of floral morphogenesis and floral initiation in a wide range of crops (Rey et al. 1994a, b; Tarenghi and Martin-Tanguy 1995; Zhu et al. 1999; Kushad and Orvos 1990; Wang et al. 1985). Such effects are attributed to the ability of polyamine to sensitize cell cycle regulation for supporting proliferation and differentiation processes (Baron and Stasolla 2008). Besides, these also suffice partially the high metabolic demand of florally differentiating tissues through recycling of C and N, and in optimization of enzyme activities associated with nucleic acid and protein biosynthesis (Sarjala and Savonen 1994). Sood and Nagar (2004) reported that the high polyamine levels facilitate active cell division for triggering floral growth. The role of polyamines in flowering has been documented by Galston and Sawhney (1990) and Bagni et al. (1993). In support of polyamine involvement in reproductive growth, Hanzawa et al. (2002) and Alcazar et al. (2005) reported that the polyamine-deficient mutants or mutants with unbalanced polyamine metabolism exhibit abnormal growth and flowering patterns as well as delayed flowering. Thus, the results showing increased free polyamines content in buds and leaves of growing shoots of paclobutrazol treated trees in this investigation are indicative of polyamine involvement in the paclobutrazol mediated floral bud differentiation. Since polyamine and ethylene share common intermediate, SAM for their biosynthesis, diversion of SAM for polyamine synthesis following paclobutrazol application could possibly account for polyamine increase and reductions in ethylene as evident from the study. Murti and Upreti (2005) reported decline in ethylene biosynthesis by paclobutrazol in mango in relation to vigour regulation. The paclobutrazol induced increase in polyamines could also be due to stimulation in synthesis of polyamines or arresting of their degradation by oxidases. However, this aspect needs investigation. Further, the increase in polyamines might be consequence of possible interaction effects of polyamines with cytokinins and ABA, as these tend to upregulate polyamine biosynthesis. Liu et al. (2013) reported interaction of polyamines with cytokinins and ABA in relation to reproductive development in wheat. Mukhopadhyay et al. (1983) reported increase in polyamines following cytokinins treatment. Similarly ABA favouring polyamine increase has been documented by Kuznetsov et al. (2006). In the earlier studies, Upreti et al. (2013) and Burondkar et al. (2016) witnessed increases in ABA and cytokinins during floral induction in mango following paclobutrazol application.

Furthermore, with respect to bud growth, free polyamine contents varying distinctly were high at initial bud development stages which declined by late bud development stages. In contrast, ethylene and its precursor, ACC exhibited opposite patterns. Since biosynthesis involves conversion of ACC produced by SAM and MACC by involving ACC-oxidase enzyme, the consistency in pattern of ethylene, ACC and ACC-oxidase activity across bud growth stages in paclobutrazol treated and untreated trees depicted that the declined availability of ACC coupled with inhibition in ACC-oxidase activity were associated to paclobutrazol induced reduction in ethylene concentrations. Thus [2] polyamine and ethylene balance favouring polyamine formation is vital for paclobutrazol induced floral bud differentiation in mango. The increase in ethylene with reductions in polyamine at late bud growth stages (panicle emergence) evident from the study could be linked to ethylene association in panicle emergence, since promotory to flowering mango (Chadha and Pal 1986; Saidha et al. 1983). Davenport and Nunez-Elisea (1990) based on lack of correlation between ethylene productions and flowering also suggested that the floral induction of mango is not mediated by ethylene produced in leaves or buds.

Among the different component polyamines, the spermidine followed by spermine contents were distinctly high both in paclobutrazol untreated and treated trees at initial stages of floral bud development. This revealed that spermidine and spermine are more associated for paclobutrazol induced floral bud formation in mango, and such effects could be linked to their greater effectiveness in supporting cell division activity and organogenesis as reported by Kuznetzov et al. (2002), possibly due to their better cell membrane stabilization potential as a result of inherent trivalent and tetravalent nature. Besides, some studies have also reported direct involvement of spermidine in the floral differentiation. Ali and Lovatt (1995) documented that the spermidine availability at the time of flower initiation and organogenesis is an important factor for floral growth in 'Washington' navel orange. Similarly, Huang et al. (2004) and Kaur-Sawhney et al. (1988) reported high spermidine content beneficial for floral initiation in *Polianthes tuberosa* L. and tobacco, respectively. Also, the growing apical buds revealing higher polyamines than leaves in paclobutrazol untreated and treated trees could possibly be the reflections of translocation of polyamines from leaves to buds under given environmental conditions for eliciting floral responses. The translocation of polyamines among different organs is reported (Antognoni et al. 1998). Thus polyamines could serve as one of the potential signaling molecule for onset of floral induction in mango.

IV. CONCLUSION

In the conclusion, the study revealed that the polyamine and ethylene biosynthesis compete each other during paclobutrazol induced floral bud formation and an accumulation of polyamines- spermidine and spermine with reduction in ethylene biosynthesis during initial bud development stage is vital for paclobutrazol mediated floral induction process. It was also apparent that the ethylene concentration by itself did not have role to play in the paclobutrazol induced floral bud formation.

ACKNOWLEDGEMENTS

The authors are thankful to the Director of the institute for encouragement and support during the study. We gratefully acknowledge the financial support from NAIP, ICAR, New Delhi.

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Development of a Compact, Highly-sensitive and Low-cost Biological Monitoring Method using Protozoa for Detecting Toxicants in Aquatic Environment

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Abstract**—** *A novel method for biological monitoring to detect toxic substances in water was developed by using the protozoan Raphidiophrys contractilis as an indicator organism. In this system (named HELIOSENSOR), the adhesion of R. contractilis to the substratum was used as a measure of the health of the living organisms. A flow-through type chamber was designed for toxicity testing, in which cells that had been damaged by harmful materials were flushed away by the water flow. The number of protozoa was continuously monitored with a digital camera. The test results revealed that this monitoring system has high durability and efficiency compared with other bio-monitoring systems, enabling us to make a quicker and easier detection of toxic substances. This system showed particularly high sensitivity to heavy metals such as mercury, arsenic, lead and cadmium. Due to high sensitivity (ex. ~ 10^{-7} M for Hg^{2+}), fast response time (<20 min) and small size (30×14×20 cm), this system has distinct advantages over other conventional biomonitoring systems using multicellular animals such as fish and crustaceans.*

Keywords**— biomonitoring, heliozoa, protist, water quality control.***

I. INTRODUCTION

Many attempts have been reported so far for monitoring water quality by using various kinds of living organisms. For example, aquatic insects and fish have been used in practice as monitor organisms to detect changes in water quality by detecting alteration in their swimming behavior. In these systems, problems are still remained to be solved as follows: 1) maintenance of the monitor organisms, 2) constructing hardware to detect movement of test organisms in high accuracy, and 3) low sensitivity and possible adaptation of the animals to the pollutants. To overcome these problems, development of a novel system of bio-monitoring was attempted by using protozoa as monitor organisms. Benthic protozoans are suitable for this purpose, because 1) most of the protozoan species can be cultured easily and inexpensively in a short time, 2) The space required to monitor their behavior is small, and 3) adaptation of the organisms to the aquatic environment does not usually occur, since protozoans are unicellular organisms. Basic investigations have demonstrated that heliozoans are most adequate protozoa, since they are highly sensitive to aquatic pollutants such as heavy metals [1], and their movement is restricted only in a planar layer [2] which allows us to use easier algorithm for automatic image analysis.

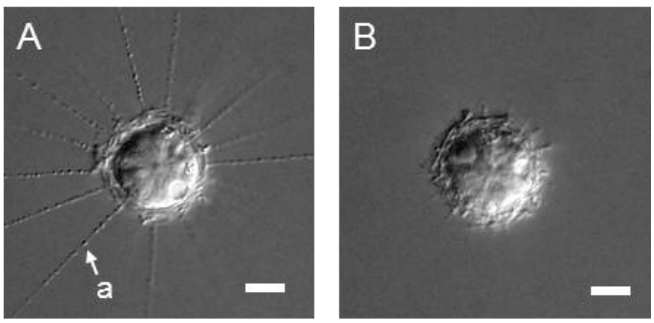

FIGURE 1. Light micrographs of *Raphidiophrys contractilis* showing axopodial retraction induced by treatment with 1 mM $HgCl_2$ for 5 min. *R. contractilis* is a protozoan living in brackish water with a cell diameter of 20-30 µm. It is usually adhered to the bottom of water by attaching tips of axopodia (a) extending radially from the spherical cell body. A: An individual whose axopodia is fully extended. B: After Hg^{2+} treatment with retracted axopodia. Scale bars indicate 10 µm.

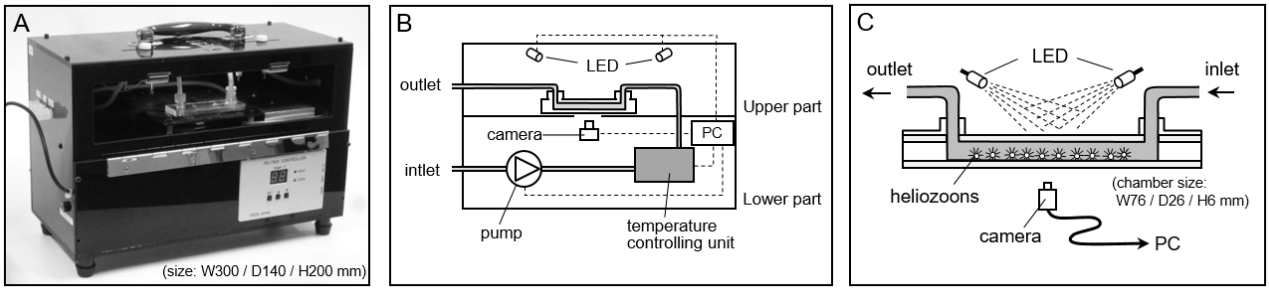

FIGURE 2. A micrograph and schematic representations of the monitoring system developed in this study. The monitoring system consists of two parts. The upper part has a monitoring chamber and a pair of LED lights. The lower part has a video camera, a pump, and a temperature controlling unit. The number of cells in the chamber is continuously monitored by image processing software. A: a photograph of the device. B: a schematic drawing of the device. C: a detailed diagram of a flow-through type measuring chamber installed in the upper part of the device.

Heliozoans possess long and thin tentacles called axopodia radiating from the spherical cell body, in which bundles of microtubules are present as a supporting cytoskeleton. The heliozoans paralyze and capture prey organism with the aid of the axopodia [3]. The axopodia show frequent shortening and re-elongation, as their microtubules are highly sensitive to environmental factors including toxic chemical substances. We have previously examined the effect of various concentrations of heavy metal ions (zinc, lead, copper, mercury and cadmium) to the axopodia and found that the length of axopodia decreased in a concentration-dependent manner [1, 4]. *Raphidiophrys contractilis* is one of the members of heliozoa that inhabits fresh or brackish water. This species can be cultured with ease in a bacteria-free condition [5]. In response to various environmental factors, *R. contractilis* shows axopodial retraction in a reversible fashion. The axopodia re-elongate to the original length of about 100 µm in about 20 min, when the environmental condition is fully restored. The factors include harmful chemicals such as heavy metals, changes in pH and water temperature, etc. In this research, we attempted to utilize such features of *R. contractilis* for water quality monitoring.

II. MATERIAL AND METHOD

Raphidiophrys contractilis (Fig. 1) was initially collected from a pond in Shukkeien Gardens in Hiroshima City, Japan, and cultured monoxenically in 10% artificial sea water (ASW) with the unicellular algae *Chlorogonium capillatum* as a sole food source [6]. Cells were harvested one week after inoculation, and introduced into a flow-through type measuring chamber as shown in Fig. 2C. The chamber was left to stand still overnight to allow the cells to settle firmly to the bottom of the chamber.

The biomonitoring system prototyped in this research (HELIOSENSOR) consisted of a device and a controlling PC. As shown in Fig. 2A, the size of the whole device was 30 × 14 × 20 cm (width, depth, and height), and has a weight of about 5 kg. It is far smaller and lighter than other similar devices [8-10], and it can be easily carried anywhere.

The interior was divided into an upper and a lower part as shown in Figs. 2A and B. The monitoring chamber (Fig. 2C) was placed in the upper part and on top of the camera, and still images of the heliozoans attached to the bottom surface of the chamber were taken at constant time intervals. In the lower part of the device, a small pump for introducing the test water and a temperature controlling device (Peltier type) for keeping the water temperature at 20 ± 1°C were installed. The chamber was illuminated obliquely using a highly directional LED light source, by which a dark-field effect was obtained for yielding highly-contrasted pictures of the heliozoans as shown in Fig. 3. The photographed images were sent to a PC, and the number of cells located in a measuring area (2 × 2 mm) was continuously monitored.

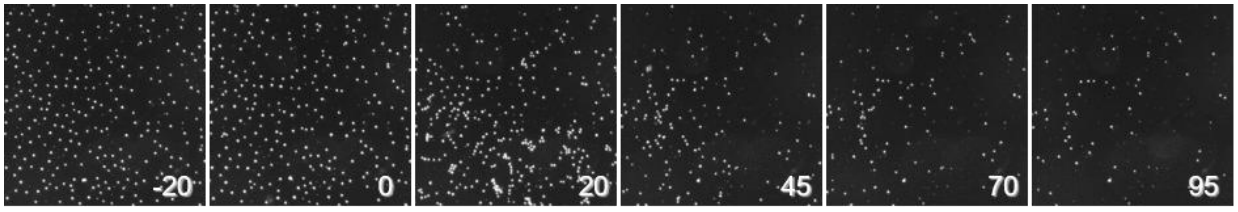

FIGURE 3. Images from a series of micrographs taken by HELIOSENSOR. The white spots represent *R. contractilis* cells. Numbers indicate time in minutes. 10% seawater was introduced into the chamber from -20 min to 0 min. Mercury solution was then introduced to the cells for 20 min, followed by washing with 10% seawater. The number of cells attached to the substratum began to decrease at 20 min, and most cells flowed out in 70 min.

For measuring axopodial length, a Nikon microscope (Eclipse Ni-U) equipped with Nomarski differential interference optics was used. Twelve individuals were measured for each experiment, and the average value of ten individuals excluding the minimum and the maximum values was taken.

For comparison, Effect of toxic substances was examined with the crustacean *Artemia* salina (brine shrimp) and the fish *Moryzias latipes* (medaka). Dried cysts of *Artemia* were hatched in 50% ASW at 20°C, and the 2-day-old larvae were used. Medaka fish were purchased from a local dealership, and bred for about a week before used for experiments. *Artemia* and medaka fish were treated with various concentrations of harmful substances, and the proportion of individuals that showed abnormal swimming behaviors after 20 min was determined as the abnormality in motility.

The experiment using the prototype monitoring device was carried out as follows. First, 10% ASW was flowed for 10 min or more to the equipment where heliozoans were set in advance. Next, 10% ASW containing various concentrations of toxic substances was introduced. Still images were taken continuously at intervals of 10 s, and the number of individuals was counted by the computer. Usually 300-1,000 cells were present in the area used for measurements. Based on the number of cells before poisoning, the percentage of cells present in each image was calculated and recorded.

III. RESULTS AND DISCUSSION

The axopodia of *R. contractilis* is known to be shortened by various harmful substances. Fig. 1 shows the shortening of axopodia caused by treatment with 1 mM $HgCl_2$ for 5 min. Heliozoans are adhered to the substratum at the tip of axopodia. Therefore, when axopodia become shortened, the heliozoan can no longer be able to adhere to the surface and detaches from there. Thus, by measuring the length of axopodia or the adhesiveness of the cells to the substratum by axopodia, the harmfulness of the water environment can be determined quantitatively. Since axopodia of *R. contractilis* is known to react against many kinds of heavy metals [7], the device developed in the present research can detect not only mercury but also other heavy metals.

The reactivity of *R. contractilis* to heavy metals was compared with *Artemia* and medaka fish. As shown in Fig. 4, as a result of examining the reactivity of these organisms to mercury, arsenic, lead, and cadmium, it was found that *R. contractilis* shows the most sensitive reaction as compared with *Artemia* and medaka fish for all the substance. Regarding the reactivity of *R. contractilis* to mercury, the length of axopodia and the adhesion to substrate are depicted in the same graph, showing similar sensitivity in either cases. As a result, it was confirmed that *R. contractilis* can be used an organism suitable for water quality monitoring.

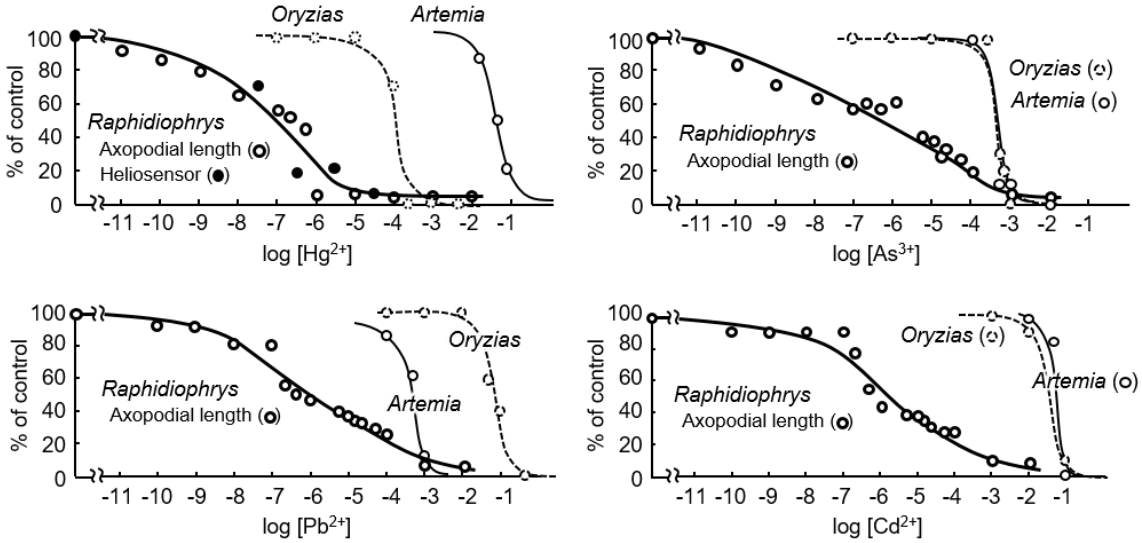

FIGURE 4. A comparison experiment performed among different monitor organisms (*R. contractilis*, *Artemia salina* (brine shrimp) and *Moryzias latipes* (medaka fish) on their sensitivities to heavy metals (mercury, arsenic, lead and cadmium). After 20 min exposure of heavy metals to the organisms, % abnormality in swimming motility was examined. In *R. contractilis*, Axopodial length (open circles) and the adhesiveness to the substratum (closed circles, determined by HELIOSENSOR) became reduced by toxic substances. Brine shrimp and medaka fish showed abnormal swimming behavior and changes in their body colors when they were affected by the toxic substances.

Fig. 5 shows an experimental result using HELIOSENSOR, by changing the concentration of mercuric chloride between 1×10^{-8} and 1×10^{-5} M. The curve of the control hardly changed. As the concentration of mercury increased, the adhesion of the cells declined. By treatment with mercury at a high concentration (1×10^{-5} M), most cells became detached from the substratum in about 10 min.

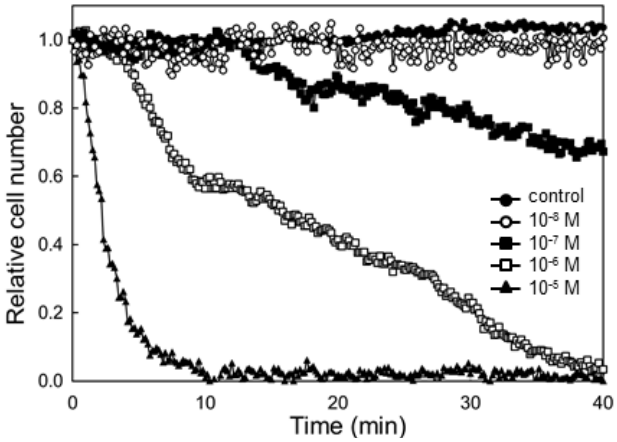

FIGURE 5. Effects of various concentration of $HgCl_2$. The mercury solution was introduced into the chamber at time zero. Relative cell number gradually decreased, and reached almost zero at 2×10^{-8} M, while at lower concentrations, the rate of decrease in cell number was dependent on the concentration of mercury ions. The vertical axis shows relative cell number, and the horizontal axis shows time (min). Images were taken at 10 second intervals and the number of cells was counted.

IV. CONCLUSION

We have developed a water quality monitoring system for drinking water supply using protists. Since this device is highly sensitive, compact and inexpensive, it can be used in various places and situations. Moreover, this system will also be applicable to safety management such as sewage and factory wastewater.

ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Numbers 19510030, 23117009, and 23510062.

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Effect of tillage practices on moisture retention and maize (Zea mays L.) performance under rainfed conditions in Swaziland

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Abstract- *An experiment was conducted to determine the effect of tillage practices on moisture retention and maize performance under rainfed conditions in Swaziland. The five treatments were based on structure of seedbed and seed planting method. They were ; zero tillage where jab planter was used to directly seed (JAB), tractor drawn planter to directly seed without ploughing (TDSS), tractor drawn cultivator to loosen soil followed by planting with tractor planter (TDRDS), planting basics made by using hand hoe (PLB) and conventional tillage (CNT) which was used as a control. The treatments were replicated three times. The data collected included weather data, germination counts, plant height, moisture retention, total dry matter and dry grain yield. The results displayed a significant difference in terms of moisture retention for the majority of the periods where measurements were done ($p<0.05$). Conventional tillage retained the least moisture while JAB retained the most moisture. In terms of seed emergence, TDRDS had the highest emergence during the first seven days compared to the other treatments. Conventional tillage had the tallest plants (268.5 cm) compared to the other treatments at 21 days after planting. Conventional tillage also had the highest total dry matter (16.2 tons/ha) and planting basins had the lowest dry matter (12.6 tons/ha). TDRDS had the highest grain yield (9.9 tons/ha), and JAB had the lowest grain yield (9.1 tons/ha). The difference in mean total dry matter and mean grain yield was not significant ($p>0.05$).*

Keywords-Germination, maize, moisture, tillage, yields.

I. INTRODUCTION

Maize (*Zea mays*) is Swaziland's staple food crop and is produced by over 90% of small holder farmers on communal land [1]. The crop is grown mainly for subsistence purposes. While almost all homesteads in the communal land produce maize, the country has never reached self-sufficient levels in maize production [2]. As a mitigation strategy, the gaps in production are covered by imports from South Africa which is the neighbouring country.

Maize can be grown on a wide variety of soils, but performs best on well-drained, well-aerated, deep warm loams and silt loams containing adequate organic matter and well supplied with nutrients [3]. It can be grown successfully on soils with a pH range of 5.0 - 7.0, but a moderately acid environment of pH range 6.0 - 7.0 is optimum. Values outside this range may result in nutrient deficiency and mineral toxicity. Addition of lime is recommended for good yields on more acid soils.

Availability of moisture in the soil is fundamental for sustainable maize production as maize requires 450 to 600 mm of rainfall per unit area for optimum growth [4]. The crop evapotraspiration (ETc) for maize is between 1.09 mm/day and 5.50 mm/day, with average value being 3.33 mm/day [5]. Tillage is practiced to loosen the soil, forming a good medium to enhance uniform seed germination, weed management and incorporate crop residues [6]. Retaining permanent soil cover in minimum tillage can reduce water requirement for a crop by as much as 30% [7]. Subsoil tillage was reported to decrease water consumption by up to 8% and increase maize yield by up to 674 kg/ha compared to conventional tillage [8]. Tillage may result in high crop yields due to modification of soil's physical, chemical and biological properties [9]. The objective of the study was to determine the effect of tillage practices on moisture retention, and maize performance under rainfed conditions in Swaziland.

II. MATERIALS AND METHODS

2.1 Description of the study area and research design

The experiment was conducted at the Malkerns Research Station in Swaziland, located at 26.55543 ^{o}S, 31.16293 ^{o}E, at an altitude of 752 m above sea level. The area is in the semi-arid region of the country with long-term average annual rainfall ranging between 800 – 1000 mm. The soils are sandy clay loam with high mineral content. It was conducted between November 2016 and April 2017.

The experiment was Randomized Complete Block Design (RCBD), with five treatments that were replicated three times. The five treatments were based on the structure of seedbed and seed planting method. The treatments were; jab planter (JAB),

tractor drawn direct maize seeder (TDDS), tractor drawn tines cultivator & maize seeder (TDRDS), planting basins (PLB), and conventional tillage (CNT). In the JAB treatment, planting was done directly using hand held jab planter without cultivating the soil [10]. Planting was done using tractor drawn maize seeder without cultivating the soil for the TDRDS treatment. The double row direct maize seeder used was drawn by Landini Powerfarm tractor that weighted 3320 kg [11]. A tractor drawn tines cultivator was used to loosen the soil prior to planting using the tractor drawn direct maize seeder for the TDRDS treatments. Planting basins with diameter of 25 cm were made using a hand hoe. Seeds were planted by hand at the centre of each basin. The plots were cultivated using tractor drawn mould board plough in the conventional tillage. There after planting was done by hand. The conventional tillage treatment was used as a control.

Each plot measured 7 m by 6 m, with 6 rows of maize planted 0.90 m apart and 0.25 m between plants. A 5 m and 2 m alley was left between the treatments and replications respectively.

2.2 Planting and fertilizer application and agronomic practices

Soil samples were taken from the site before planting to determine the soil pH, nitrogen (N) and phosphorus (P) content. The results were used to determine fertilizer requirements. Compound basal fertilizer in the form of Nitrogen (N), Phosphorus (P) and Potassium at ratio of 2:3:2 (22) was applied at a rate of 400 Kg/ha at planting. This resulted in application of 25 kg/ha, 38 kg/ha and 25 kg/ha for N, P, and K respectively. Nitrogen in the form of Limestone Ammonium Nitrate (28% N) was applied some 35 days after planting as side top dressing at a rate of 28 kg/ha. Planting was done on the 3rd of November, 2016. A hybrid maize variety, SC 719 was used for the trial. The variety is moderately tolerant to heat and drought stress. It takes about 130 days to mature and has a potential yield of 10 tons/ha under optimum conditions. [12].

A combination of CLEAOUT 45 Plus and Dual Gold herbicide was applied to all the plots some 48 hours before planning. CLEAOUT 45 Plus is a non-selective, non-residual herbicide that is used to control herbaceous weeds in agricultural sites [13]. Dual Gold is a selective concentrate herbicide for pre-emergence control of annual grasses [14]. CLEAROUT 45 Plus herbicide and Dual Gold herbicide were mixed with 20 litres of water at a dose of 400 ml and 100 ml respectively. Bladex, a selective herbicide for control of weeds was applied some five weeks after planting at a concentration of 400 ml Bladex in 20 litres of water [15]. The herbicides were all applied using a knapsack sprayer. Bulldock 0,05 GR granular pesticide was used to control maize stock borer (*Buseola fusca*). About a gram of the pesticide was applied into each maize plant funnel some seven weeks after planting [16]. The experiment was rainfed and no irrigation was applied.

2.3 Data collection and analysis

Data collected from the field were; germination count, plant height, total dry matter and dry grain yield. The number of seeds that had emerged was counted in each plot for day 5 to day 9 after planting.. Plant height was measured for 10 plants in each plot using calibrated wooden pole sat 7, 9 and 21 weeks after planting. The same plants were used to measure height at all the periods. Five plants were sampled from each plot to determine the total dry matter produced. This was done at 140 days after planting when the crop was ready for harvesting. The plants were cut at the base and cut into pieces (stem, leaves, tassels and cobs). They were oven dried at 105 ^{o}C for 72 hours, and then weighed to determine the dry matter. Five cobs were randomly sampled from each plot. The grain was removed from the cobs when the moisture content was determined to be 12.5% using a hand held moisture meter [17]. The grain was weighted to determine grain yield from the five plants. The yield from the five plants was multiplied by 8000 to determine yield per ha, where the assumed plant population was 40000 per ha [18]. Moisture retention was determined in each plot during the period of November to December 2016 using soil moisture probe [19] between week 4 and week 9 after planting. The moisture retention was determined at 3 days interval.

Weather data (daily rainfall, minimum air temperature , maximum air temperature and soil temperature) were collected from the adjacent weather station at Malkerns Research Station.

The data collected during the course of the experiment were analysed using the Statistical Package for Social Sciences [20], whereby analysis of variance (ANOVA) was done in order to determine any significant differences between the treatments. The mean separation test was carried out using the Least Significant Difference (LSD).

III. RESULTS AND DISCUSSIONS

3.1 Soil analysis results and weather data

The soil pH was 5.11 in potassium chloride and in water it was 6.20 with an exchangeable acidity of 0.43% and delta pH of 1.09, meaning that there was a net positive charge in the soil. Available nitrogen was 0.14% and phosphorus 3.85 ppm. The

organic matter content of the soil was determined to be at 2.28%. Lime was not applied as the soil pH was at the required range. The mean air temperature for the duration of the experiment was optimum as it ranged between 17 ^{o}C and 29.9 ^{o}C (Table 1).

TABLE 1
MEAN AIR TEMPERATURE AND SOIL TEMPERATURE DATA AT MALKERNS

Month	Temperature (^{o}C)		
	Mean maximum air temperature	Mean minimum air temperature	Mean soil temperature at 30 cm depth
November	25.9	17.2	23.8
December	28.9	17.9	26.3
January	27.2	17.7	26.2
February	28.0	18.6	26.8
March	28.2	17.1	25.1

A total of 537 mm was received, and this was within the range of rainfall required by the maize crop. The rainfall was well spread, as there was at least 5 mm of rainfall received in each week, except during week 7 and week 21 (Fig 1).

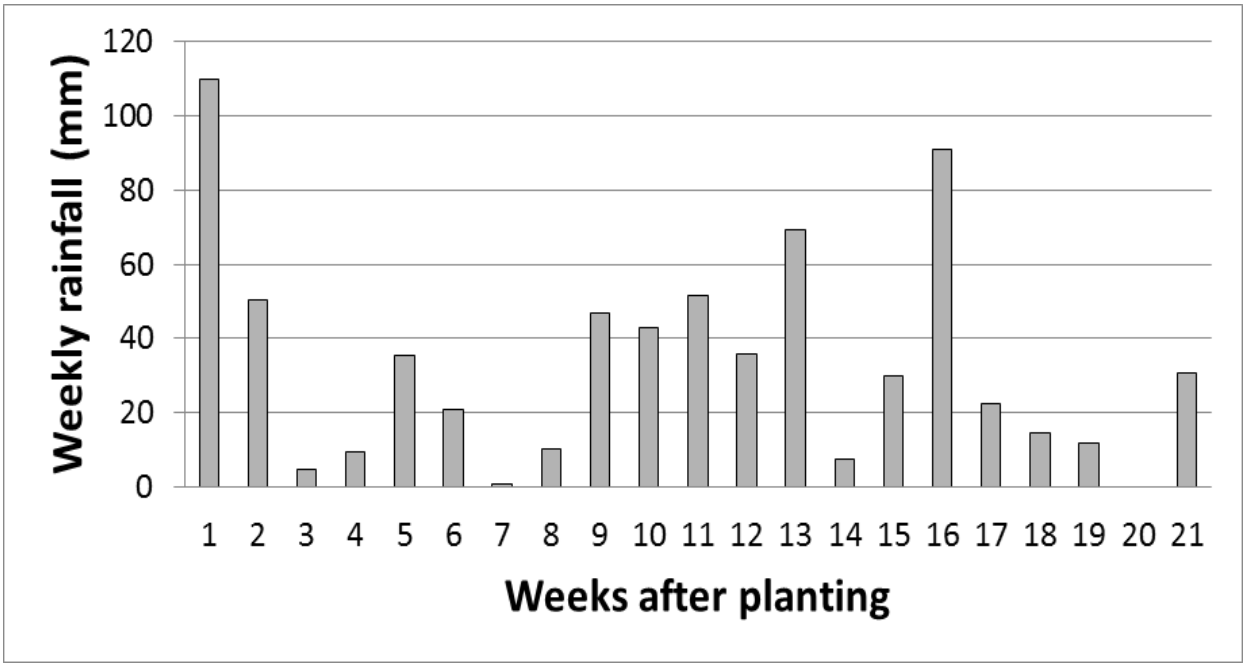

FIGURE 1: WEEKLY RAINFALL RECEIVED DURING THE PERIOD OF EXPERIMENT

3.2 Soil moisture suction

Conventional tillage (CNT) conserved the least moisture compared to all the other treatments as shown by the higher soil moisture suction in more or less all the periods (Table 2). The soil suction under CNT was significantly higher than all the treatments during period 1 ($P<0.05$), except for PLB. The moisture suction was measured during the 4th week after planting when cumulative rainfall of less than 15 mm had fallen within 14 days, and thus the moisture in the soil had been depleted. On the other hand, the soil suctions were lower and not significant different for all the treatments during periods 2, 3 and 9. These periods coincided with the weeks when there were high rainfalls and the soil moisture contents were high. The increases in soil moisture retention under conservation tillage were due to decreases in evaporation [21]. Similar results were found in an experiment for selected cowpea varieties where zero tillage conserved about 30% more moisture compared to conventional tillage [22].

TABLE 2
MEAN SOIL SUCTION FOR DIFFERENT PERIODS (P).

	Mean soil suction during different periods (Centibars)*								
Treatment	**P 1**	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P7**	**P 8**	**P 9**
JAB	18.3^{ab}	4.0	10	33.0^{abc}	3.7^{a}	13.0^{a}	23.0^{a}	27.7^{a}	4.0
TDDS	21.3^{cd}	2.7	8	25.7^{ade}	7.0^{b}	14.0^{b}	28.0	35.0	2.7
TDRDS	24.0^{efg}	2.7	9	15.0^{bdfg}	6.7^{c}	14.7	25.7	29.7	2.7
PLB	31.7^{aceg}	3.3	8.3	29.3^{fh}	4.3^{d}	17.3	25.3	30.3	3.0
CNT	33.7^{bdf}	4.7	8.7	45.0^{cegh}	13.7^{abcd}	20.7^{ab}	34.0^{a}	39.3^{a}	4.7

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

3.3 Maize performance parameters and yield

The maximum germination count was reached some 9 days after germination, with counts ranging from 83.3% (under TDDS) to 87.1% under JAB (Table 3). The differences in means germination counts were not significant ($p>0.05$) for all treatments at 9 days after planting. On the other hand, the germination counts were significantly higher ($p<0.05$) for treatment TDRDS compared to all the other treatments at 5 days and 7 days after planting. Emergence of seedling is influenced by moisture, aeration and degree of compaction of the soils. The tractor drawn ripper loosened the soil, thus making it easier for the seedlings to emerge. The clods above the seed were pulverized by the cultivator with small weight [23].

TABLE 3
MAIZE SEEDING EMERGENCE FOR DIFFERENT TREATMENTS

Treatment	**Seed emergence (%). ***		
	5 days after planting	**7 days after planting**	**9 days after planting**
JAB	56.1^{a}	68.5^{a}	87.1
TDDS	52.0^{b}	69.5^{b}	83.3
TDRDS	61.1^{abcd}	75.6^{abcd}	84.4
PLB	56.0^{c}	68.3^{c}	82.7
CNT	57.6^{c}	72.6^{d}	85.7

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

The maize plants were significantly higher for CNT compared to JAB, TDRDS and PLB ($p<0.05$) at 7 weeks after planting (Table 4). However the plants were higher for PLB at 9 weeks after planting. At 21 weeks after planting the plants for CNT were higher than in all the other treatments, but the difference in mean height were not significant ($p>0.05$). Similar results were observed in a study done in Turkey where plants under conventional tillage were higher than those under no till [24]. In a study done in Ghana there was no significance in difference for mean height between different tillage treatments, even though the shorted plant was found in the no tillage treatment [25]. The great plant height under conventional tillage may be attributed to better soil aeration and more uniform distribution of nutrients in soil profile [26].

TABLE 4
PLANT HEIGHT FOR DIFFERENT TREATMENTS

Treatment	**Plant height (cm). ***		
	7 weeks after planting	**9 weeks after planting**	**21weeks after planting**
JAB	109.7^{ab}	194.4^{abc}	260.3
TDDS	120.5^{aceg}	183.8^{adef}	265.4
TDRDS	112.1_{cdef}	179.4^{bdh}	248.0
PLB	111.7^{dgh}	208.2^{ehi}	266.0
CNT	122.0^{bfh}	193.8^{cfi}	268.5

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

Conventional tillage produced the highest total dry matter at 16.2 tons/ha, while PLB produced the least dry matter at 12.6 tons/ha (Table 5). The difference in mean dry matter production for the different treatments was not significant ($p>0.05$). On the other hand TDRDS had the highest mean grain yield, at 9.9 tons/ha. The lowest grain yield was realized under PLB

where the yield was 9.0 tons/ha. The difference in grain yield for all the treatments was not significant ($p>0.05$). Similar results were realized in Pakistan where the highest dry matter yield was harvested from plants grown under deep tillage, followed by the plants grown under conservation tillage, while the plants grown in zero tillage plots gave the lowest dry matter yield [27]. The same pattern was observed on grain production. A combination of mouldboard plough and disc plough produced the highest grain yield, and no-tillage produced the lowest grain yield in a study conducted in Iran [28].

TABLE 5
TOTAL DRY MATTER AND GRAIN YIELD FOR DIFFERENT TREATMENTS

Treatment	Total dry matter at harvest (tons/ha)	Grain yield (tons/ha)
JAB	14.5	9.1
TDDS	13.8	9.6
TDRDS	15.3	9.9
PLB	12.6	9.0
CNT	16.2	9.6

IV. CONCLUSIONS

The study examined the effect of tillage practices on moisture retention and maize performance under rainfed conditions. Based on the results it can be concluded that the farming season during which the experiment was undertaken received adequate rainfall, as the total received was within the range of rainwater requirement for maize crop. The rains were well spread, and there were no cases of long periods without effective rainfall. CNT conserved the least moisture, as reflected by the higher soil suction values. On the other hand JAB conserved the most moisture. The difference in seed emergence was not significant for the different treatments. However, more seed emergence under TDRDS during the first seven days after planting. It was followed by CNT. CNT produced the tallest plants at 21 days after planting, even though the differences in plant height were not significant. CNT produced the highest dry matter, and PLB produced the least dry matter. PLB also produced the least grain yield. TDRDS produced the highest grain yield, even though the difference in grain yield for the treatments was not significant.

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Effect of interaction between different plant growth regulators on in vitro shoot multiplication of *Citrus latifolia* Tan. (persian lime)

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Abstract— *In this paper a shoot multiplication is described for Citrus latifolia Tan. (persian lime) using nodal segment explants of young one – old – year trees by two different pathways contain with and without callusing phase. The best result for multiple shoot formation and regenerated shoot formation was 3.2 and 2.6 shoots per explants with 4.44 µM BA plus 0.053 µM NAA and 4.44 µM BA plus 0.049 µM IBA respectively. Alike shoot regeneration, shoot elongation was occurred in medium with 4.44 µM BA and 0.049 µM IBA. Micropropagated and regenerated plants are under other experiments.*

Abbreviation: *BA – 6 benzylaminopurine; IBA – Indole acetic acid; NAA – Naphtalene acetic acid; PGRs – Plant Growth Regulators.*

Key Words: Persian lime; plant growth regulators; shoot multiplication.

I. INTRODUCTION

Accordant worldwide *Citrus* species are the most widely grown fruit crops in Iran. They contain vitamin C that is very useful for human nutrition. Also their fruits are important source of volatile oils, limonene, α-terpinene, β-terpinene, citral cumarins, bioflavonoids, vitamins, and mucilage (Rathore *et al*., 2006). Beside apples and bananas, *Citrus* fruits are the most important fruit crops (FAO 2001). Also it is clear that the sustainable development of the *Citrus* industry is mainly dependent on a continuous supply of new and improved cultivars (Perez – Tornero *et al*., 2010). For the *Citrus* industry to improve fruit quality and reduce biotic and abiotic stresses are major breeding objectives at any time (Wenwu *et al*., 2007). *Citrus* varieties are propagated by both sexual and asexual methods. Generally, rootstocks are propagated sexually through seeds, while most of the commercial varieties are propagated by various asexual methods (Chaudhary 1994). These conventional techniques are also not free from risk of perpetuating in-born pathogens. However in vitro micropropagation technology can overcome some constraints to *Citrus* improvement and cultivation, and can increase fruit quality and resistance to diseases and environmental stresses (Gresser 1994). Also micropropagation systems with high multiplication rates are not only an important asexual method that can be used for the production of clonal plants, but also form the basis for the introduction of genetic variation by genetic transformation or mutagenesis. The genetic transformation of *Citrus* has been widely studied as a tool to generate transgenic plants with enhanced tolerance of biotic (Cardoso *et al*., 2010; He *et al*., 2011 and Ali *et al*., 2012) and/or abiotic stresses (Bunnag and Tangpong 2012). In both cases, is necessary to be able to regenerate viable shoots, which can be propagated, by either organogenesis or somatic embryogenesis (Perez – Tornero *et al*., 2010).

In this paper a protocol for cloning of *Citrus latifolia* Tan. (persian lime) using nodal explant was described. It should be noted that according to Iranian researchers that used graft inoculation and PCR methods, persian lime was tolerant to Candidatus *Phytoplasma aurantifolia*. Candidatus *Phytoplasma aurantifolia* is a serious threat to lime and other susceptible *Citrus* trees in southern Iran (Salehi *et al*., 2005).

II. MATERIAL AND METHODS

This study was conducted in Iranian Research Institute of Plant Protection Department of Plant Diseases, Tehran – Iran

Young trees of *Citrus latifolia* Tan. (persian lime) were collected from Jahrom *Citrus* nursery, Jahrom – Fars – Iran

2.1 Surface sterilization and plant material preparation

One – old – year young tree persian lime (*Citrus latifolia* Tan.) were used as the source of explants from their nodal segments. 30 – 35 – old – day new shoots measuring 12 – 15 cm in length with 4 – 6 nods were cut and collected in plastic bag and transferred to the laboratory from greenhouse for experiments.

2.2 Preparation and sterilization of explants

After defoliation of shoots, and cutting 0.5 – 0.7 cm in length nodal segments (explants) surface sterilized under laminar air flow with dipping in 25% hypochlorite sodium for 5 min. Then were rinsed in distilled water 4 – 5 times.

2.3 Culture media

Culture media consisted of MS (Murashig and Skoog 1962) salts and vitamins plus 3% sucrose that were solidified with 0.75 % agar agar. Also the media were supplemented with BA (0 – 8.88 µM), 0.053 µM NAA and 0.049 µM IBA. The pH of media was adjusted to 5.8 before gelling with 1N NaOH or HCl and after gelling autoclaved for 20 min at 121ºC. Then the media dispensed into 9 – cm diameter petri dishes. The culture was incubated at 25 ± 2ºC and under 16 – h photoperiod.

2.4 Experimental design and data analysis

Experiments were conducted in a completely randomized design with 4 replicates and 5 explants per replicate. The mean number of multiplicated shoots per explants and the mean length of multiplicated shoots assay carried out on MS media with 12 combinations of cytokinin (BA) and auxins (NAA and IBA). Data were analysised by Dunkan Multiple Range Test.

III. RESULTS AND DISCUSSION

Shoot multiplication in the presence of media with different concentrations of BA and 0.053 µM NAA and or 0.049 µM IBA compared after nearly 4 weeks of culture. It can be seen that the highest number of multiplicated shoots (3.2) per explants was observed in the media containing 4.44 µM BA and 0.053 µM NAA (Fig. 1and Fig. 5). Also the highest length of shoot (10 cm) was obtained on medium with 4.44 µM BA and 0.049 µM IBA (Fig. 3 and Fig. 6). It is interesting that in these study observations such as shoot multiplication and growth of shoot (longitudinal growth and foliation) were affected by interaction between different plant growth regulators more rather than plant growth regulators solely. There are reports about positive effect of increasing concentration of BA on shoot multiplication. For example Mehdi Farshad and coworkers in 2014 resulted that BAP alone (from 8.8 µM to 26.6 µM) was significantly effective on shoot multiplication in *Chlorophytum borivilianum*. However there are reports that show shoot proliferation decreased with increasing concentration of BA alone (Komal *et al*., 2013). This experience is in accordance with ours that frequency of response from nodal explants decreased with a progressive increase in the level of BA [8.88 and 4.44 µM BA, 4.75 – 8.20 (cm for length of shoots)] respectively.

As was mentioned earlier, the interaction between plant growths regulators have very important effects on induction of shoots also number of shoot multiplicated. So as was shown in Fig. 2 the mean number of multiplicated shoots on explants increased when BA was used solely (1.2 – 2.66 for 0.044 – 8.88 µM BA). The positive effect of BA on the induction of regenerated shoot is clear. For example the positive effect of BA on shooting in *Cucurbita maxima* Duch (Lee *et al*., 2003), *Ruta graveolunse* L. (Ahmad *et al*., 2010), tomato (*Lycopersicon esculentum* L.) (Rai *et al*., 2012) and Japanese pear (Kadota *et al*. 2001). These results were somewhat similar to our results about the mean number of multiplicated shoots. However it seams 4.44 µM BA either in terms of nature or in terms of level is suitable for shoot multiplication in *Citrus* genus. So that Al-Khayri and Al-Bahrany in 2001 expressed that Best results for multiple shoot formation, 8 shoots per node, were obtained with 4.44 µM BA and 2.32 µM kinetin.

Another interesting result of this study is the circumstance of shoot multiplication. As is clear in figures multiplication shoot in the presence of BA and NAA was direct while multiplication shoot in the presence of BA and IBA was indirect and accompanied by callusing phase (Fig.7 and Fig. 8). So the role of interaction between plant growth regulators in in vitro culture of plants is proved again.

Very different and important roles are demonstrated for plant cell, tissue and organ culture. Briefly shoot multiplication and shoot regeneration are two different kind of organogenesis with two different utilizations. This result implies that rapid plant regeneration system which could be used for the somaclonal variation induction are possible for persian lime, on the other hand in vitro propagation through lateral buds proliferation that is an efficient method for large scale production of true – to – type planting material of important plant (Doo and Iyyakkannu 2016) is practicable for this valuable plant. It is worth noting that for assessment the level of background genetic changes resulting from the tissue culture processes (Munthali *et al*. 1996) and to verify the "true-to-type" genotype of micropropagated plants with shoot multiplication and or shoot regeneration DNA-based marker techniques such as RFLPs (Nelke *et al*., 1993) and or RAPD (Munthali *et al*., 1996) are necessary (Komal *et al*., 2013)

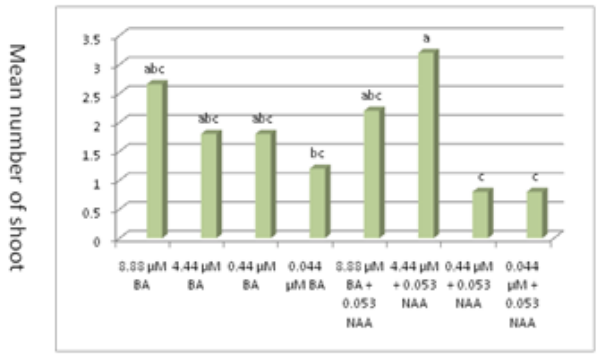

FIG 1. The mean number of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 8.88-0.044 µM BA plus 0.053 µMNAA

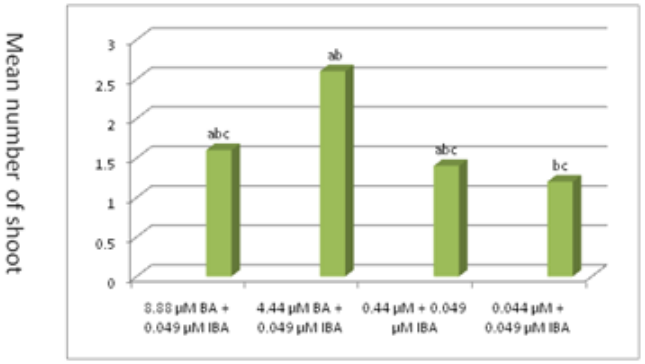

FIG 2. The mean number of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 0.049 µM IBA

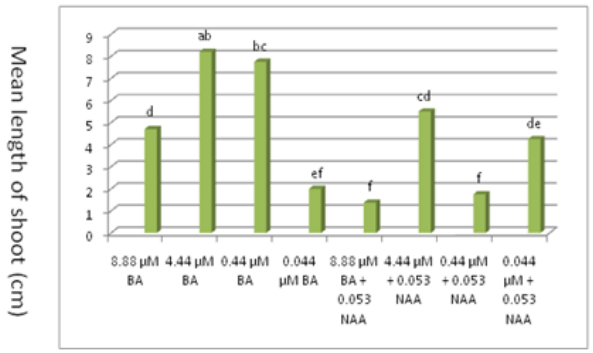

FIG 3. The mean length of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 8.88 – 0.044 µM BA and 0.049 µM NAA

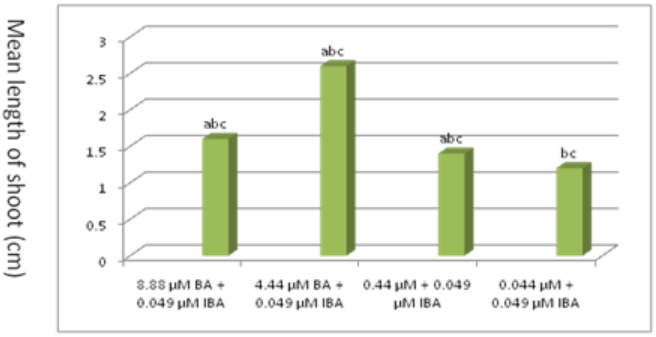

FIG 4. The mean length of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 0.049 µM IBA

Mean followed by same letter(s) are not significantly different

FIGURES 5 – 8. 5 – Multiplicated shoot on medium with 4.44 µM BA and 0.053 µM NAA. 6 – Elongation of multiplicated shoot on medium with 4.44 µM BA and 0.049 µM IBA. 7 and 8 - Multiplicated shoot on medium with 0.44 µM BA and 0.049 µM IBA

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Review on Management of Hospital Waste in An Efficient Manner

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Abstract— *This is a review paper which is prepared from the surveys of hospitals and research studies. Hospital waste management in the world is a strict discipline and does occupy a serious place in the management of health care sector. The management of hospital remaining requires its removal and disposal from the health care establishments as hygienically and economically as possible by methods that all stages minimizes the risk to public health and to environment. Health care waste can be dangerous, if not done properly. Poor management of healthcare waste exposes health labors, waste handlers, and the community to the toxic effects of wastes generated from health activity. The disposal of these wastes could also lead to environmental problems. This article intends to describe various health care wastes and its controlling, as creating good practices for proper handling and disposal of health care waste is an important part of the health care delivery system. The aim of this paper is to highlight the present condition of medical waste and a review on scientific method of hospital waste management.*

Keywords— Environmental problems, Hazardous waste, Hospital waste management, Medical waste, waste generation.

I. INTRODUCTION

Waste in general is any substance (solid, liquid, or gas) that has no direct use and is discarded permanently. A waste is considered hazardous if it exhibits any of the characteristics such as being flammable, reactive, explosive, corrosive, radioactive, infectious, irritating, sensitizing, or bio-accumulative[1]. Hospital waste refers to all waste generated, discarded and not intended for further use in the hospital. The risks are not only connected to the handling of the waste but also the environmental risk connected to the treatment and disposal of the waste. The proper management of biomedical waste has become a worldwide humanitarian topic today. Although hazards of poor management of biomedical waste have aroused the concern world over, especially in the light of its far-reaching effects on human, health and the environment[2].

Waste management options need to be efficient, safe and environment friendly to protect people from voluntary and accidental exposure to waste when collecting, handling, storing, transporting, treating or disposing of waste. Furthermore, in the Sri Lankan context such options need to be cost effective, taking into account the local logistical needs. Though clinical waste management should be an integral part of the health care delivery system the principal reason for absence of such infrastructure is economic. Health personnel are still to distinguish health care waste from ordinary garbage[3].

The objectives of biomedical waste management involve mainly prevention of disease transmission from one patient to another; to health workers from patients and vice versa; prevention of injury to the workers in health care units as well as workers involved in support services. This helps in turn in prevention of exposure to the deleterious effects of the cytotoxic as well as genotoxic and chemical wastes in general that are generated in the hospitals. Management of waste can be relatively effective as well as efficient practice that is related to compliance when designing is done properly[4]. World Health Organization states that 85% of hospital wastes are actually non-hazardous, whereas 10% are infectious and 5% are noninfectious but they are included in hazardous wastes. About 15% to 35% of Hospital waste is regulated as infectious waste. This range is dependent on the total amount of waste generated[2].

As far as the management of biomedical wastes is concerned its proper management has become a humanitarian topic worldwide. Hazardous and poor waste management (biomedical) has become a matter of concern particularly in the light of its effects that are far reaching affecting human and animal health and the environment[4].

The World Health Organization (WHO) recognizes that in many countries improper management and disposal of clinical waste continue a significant threat to the healthy working environment. In general, clinical waste is reflecting high quantity, intensive disposal route and significantly higher costs compared to other waste categories. Thus, many hospitals have faced financial difficulties in managing of clinical waste. Equally in Sri Lanka, although the regulations had been gazetted by Central Environmental Authority (CEA) that improper disposal of clinical waste is an offense, still it remains as a

problematic area[5]. Further, there are less special strategies have been established within the local level in order to manage clinical waste in cost effective manner.

According to the report identification of cost effective solutions for disposal of clinical waste is one of the main challenge face by hospitals since it require high technological and capital input. Though, few of the major hospitals operate modern treatments or outsource to a private sector, most hospitals are lacking of cost effective options to dispose clinical waste.

The objective of this paper is to introduce readers about the medical waste management, definition of medical wastes, risks of exposure, medical waste management procedures and control techniques.

II. DEFINITION OF BIO MEDICAL WASTE

According to Biomedical Waste (Management and Handling) Rules, 1998 of India "Any waste which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biological.

The Government of India (notification, 1998) specifies that Hospital Waste Management is a part of hospital hygiene and maintenance activities. This involves management of range of activities, which are mainly engineering functions, such as collection, transportation, operation or treatment of processing systems, and disposal of wastes[2].

III. CLASSIFICATION OF BIO MEDICAL WASTE

3.1 Non–Hazardous Wastes

In most of the set–ups of health–care approximately 85% of generated wastes are constituted by non–hazardous wastes. This includes wastes constituting remnants of food and peels of fruit; wash water as well as paper cartons; packaging materials[6].

3.2 Hazardous Wastes

Potentially Infectious Wastes In the scientific documents as well as in the regulations and guidance various terms for infectious wastes have been used over the years. These include, infectious as well as infective medical and biomedical; hazardous and red bag, contaminated, infectious medical wastes along with regulated wastes in the medical profession. Basically all these terms indicate the similar types of wastes even though the terms involved in regulation are defined usually in more Specific manner[7].

IV. PROBLEMS RELATING TO BIOMEDICAL WASTE

A key issue related to current biomedical waste management in many hospitals is that the application of Bio-Waste regulation is poor as some hospitals are disposing of waste in a random, improper and uncritical manner. Lack of segregation practices, results in mixing of hospital wastes with general waste making the whole waste stream hazardous. Inappropriate segregation ultimately results in an incorrect method of waste disposal.

Insufficient Bio-Medical waste management thus will cause environmental pollution, unpleasant smell, growth and multiplication of vectors like insects, rodents and worms and may lead to the transmission of diseases like typhoid, cholera, hepatitis and AIDS through injuries from syringes and needles contaminated with human. Various communicable diseases, which spread through water, sweat, blood, body fluids and contaminated organs, are important to be prevented.

The Bio Medical Waste scattered in and around the hospitals invites flies, insects, rodents, cats and dogs that are responsible for the spread of communication disease like plague and rabies. Rag pickers in the hospital, sorting out the garbage are at a risk of getting tetanus and HIV infections. The recycling of disposable syringes, needles, IV sets and other article like glass bottles without proper sterilization are responsible for Hepatitis, HIV, and other viral diseases. It becomes primary responsibility of Health administrators to manage hospital waste in most safe and eco-friendly manner.

Although treatment and disposal of health-care wastes aim at reducing risks, indirect health risks may occur through the release of toxic pollutants into the environment through treatment or disposal.

a) Landfilling can lead to contamination of drinking water

b) Occupational risks may be associated with the operation of certain disposal facilities.

c) Inadequate incineration or incineration of materials unsuitable for incineration can result in the release of pollutants into the air. The incineration of materials containing chlorine can generate dioxins and furans, which are potential carcinogens.

d) Incineration of heavy metals or materials with high metal contents (lead, mercury and cadmium) can lead to the spread of heavy metals in the environment. Dioxins, furans and metals are persistent and accumulate in the environment. Only modern incinerators which are able to work at 800-1000°C with special emission cleaning equipment can ensure that no dioxins and furans (or only insignificant amounts) are produced.

V. APPROACH FOR HOSPITALWASTE MANAGEMENT

TABLE 1
HOSPITAL WASTE CATEGORIES AND DISPOSAL

Waste Category	Treatment & Disposal
Human anatomical waste	Incineration /deep burial
Animal waste	Incineration /deep burial
Microbiology & biotechnology waste	Incineration /deep burial
Sharps	Incineration / disinfection /chemical treatment /mutilation
Medicines and cytotoxic drugs	Incineration / destruction and disposal in secured landfill
Solid waste (Blood and Body fluids)	Autoclave/chemical treatment/burial
Solid waste (disposable items)	Autoclave/chemical treatment/burial
Liquid waste (blood & body fluids)	Disinfection by chemicals/discharge into drains
Incineration Ash	Disposal in municipal landfill
Chemical waste	Chemical treatment/ secure landfill

VI. HOSPITAL WASTE MANAGEMENT PROGRAMME

1. Identification of waste types
2. Segregation of waste
3. Transport & storage of waste
4. Proper disposal of waste
5. Implementation of contingency plans
6. Identify the need for use of personal protective equipment

6.1 Segregation by color coding system

- Infectious waste - Red bags
- Domestic waste - Green Bags
- Sharps - Needle cutters / Puncture proof containers
- Segregation at Source (ward, operation theater, laboratory, labour room, other places)

6.2 Transportation

- Containers: puncture proof, leak proof,
- Bags: sturdy, properly tied
- Transport trolleys: designated & timely
- Staff protection: provided with protective clothing and other items
- Never put hands in a bag

6.3 Waste storage

- Closed covered area
- Away from the normal passages

- Easily accessible for transportation
- Radioactive waste special containers/ special treatment and disposal

6.4 Proper disposal of waste

- All infectious waste and sharps containers :Incineration
- All Domestic waste : Landfill
- All hazardous waste : Chemical treatment before disposal
- Implementation of contingency plans
- Contingency plans have to be in place to be implemented whenever any of the steps in the chain breaks and everyone should be aware of their responsibilities in case of breakdown.

6.5 Identify the need for use of personal protective equipment

- Special clothing, gloves, masks and eye protection should be identified and provided to the healthcare workers responsible for waste transportation and disposal.

6.6 On-Site Medical Waste Treatment

6.6.1 Autoclaving

Thermal treatment is typically used for sharps and certain other types of infectious waste. An autoclave is in essence a large pressure cooker that uses high temperatures and steam to deeply penetrate all materials and kill any microorganisms. Depending on the type and amount of waste you will need to sterilize, you can purchase an appropriately-sized autoclave for your facility. These appliances range from 100 liters to 4,000+ liters in volume for bulk waste treatment[8].

Modern autoclaves are also automated to minimize human involvement and therefore reduce needle-stick injuries and contamination. Decontaminated sharps and other medical waste that's been autoclaved can then be handed over to your Maryland medical waste removal vendor to be disposed of as non-infectious waste. However, keep in mind that such medical wastes as chemical waste, including chemotherapy waste, as well as pharmaceutical waste can't be decontaminated in an autoclave.

6.6.2 Chemical Treatment

Often used to deactivate liquid waste, chemical treatment is designed to decontaminate or deactivate certain wastes on site rather than packaging and sending them to a separate facility. Since liquids are highly susceptible to spills, it's typically best to have them treated as close to the generation site as possible. Chemical treatment can also be applied to some non-liquid infectious wastes, but they would typically need to be shredded first to ensure that all portions of the waste are exposed to the chemicals.

Depending on the type of waste, chemicals like chlorine, sodium hydroxide or calcium oxide can be used.

6.6.3 Microwave Treatment

A microwave treatment system, similar to an autoclave, also uses heat to decontaminate medical waste. These systems work best for waste that is not 100% dry or solid, as the moisture allows the heat to penetrate deeper, and the steam sterilizes. Therefore, before microwaving, most types of medical waste need to be shredded and mixed with water to achieve the desired effect. The bonus is that shredding reduces the volume of the waste, so it can later be land-filled.

6.7 Off-Site Medical Waste Disposal

6.7.1 Incineration

Incineration is typically used (and often required by the state) for pathological and pharmaceutical waste. Incineration of medical waste should be performed in a controlled facility to ensure complete combustion and minimize any negative effects for the environment[9]. The great thing about incineration is that it kills 99% of microorganisms and leaves very minimal waste, if any.

6.7.2 Land Disposal

Land disposal is typically used for shredded, treated and decontaminated waste. In certain cases, it can also be used for hazardous waste or other untreated waste that cannot be decontaminated by other means. Specialized sanitary landfill sites exit to reduce the risk of soil and water contamination and provide a safe space for medical waste disposal.

6.7.3 Plasma Pyrolysis

Direct use of waste products as combustion fuel or their indirect processing into another kind of fuel helps in harnessing the energy contents. In this context pyrolysis has been found as a related form of thermal treatment wherein high temperatures are used for treating waste materials with limited supply of oxygen. A state–of–the–art is plasma pyrolysis technology that ensures disposal safe of medical wastes[10].

VII. CONCLUSION

Medical wastes should be classified according to their source, typology and risk factors associated with their handling, storage and ultimate disposal. The segregation of waste at source is the key step and reduction, reuse and recycling should be considered in proper perspectives. For proper management of biomedical wastes lack of concern and awareness as well as cost factors are the certain problems/limitations. Therefore general public should be educated and must be concerned regarding health hazards that are associated with biomedical wastes. Ultimately, sensitizing ourselves is of utmost importance for protection of environment and our own health. In this paper, introductory materials on the definition of medical waste, medical waste management regulatory acts, the risks of exposure, medical waste management procedures and control techniques are presented.

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Sources of Risk and Management Strategies among Farmers in Rice Post Harvest Management in Niger State, Nigeria

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Abstract— *The study examined sources of risk and management strategies among farmers in rice post harvest management in Niger State. The research was undertaken in five Local Government Areas of Niger State, namely Katcha, Lavun, Paikoro, Shiroro and Wushishi. Data obtained for the research was achieved through questionnaires administered to 200 farmers selected using multi-stage sampling techniques. Descriptive statistics was used for data analysis.*

The study showed that rice post harvest management is carried out by subsistence farmer with average farm size of 2.7ha and are of active productive age of 31-50 years, who have 24 years farming experience in the rice post harvest management. The study revealed that farmers in the study area are affected by production risk, financial risk, human or personal risk, market or price risk and technological risk sources. The farmers have adopted prevention, mitigation and coping with risk as management strategies. Based on the findings the study recommended provision of credit facilities, rice post harvest machineries at subsidized rate, rural infrastructures, cooperative formation, use of extension officer and proper storage facilities.

Keywords— Risk Management, Strategies, Rice, Farmers.

I. INTRODUCTION

Rice is consumed regularly in Nigeria's urban and rural areas and is an important staple food crop. Post harvest management is important in agricultural production due to seasonality of production. Rice production is mostly only once a year, and at most twice, in which about 65% of production comes from the lowland and rainfed though there are double productions in irrigated areas of the northern parts of the country. Moreover, long scarcity period is involved between planting and harvesting of crops, which in addition to low productivity levels has constrained paddy rice availability from own production.

However, this rice post-harvest management has not received the attention it deserves, the reason probably being the assumption that what matters after all is production, and that if success could be achieved at the production level, then there will be more availability of rice both at the household and market levels (FAO, 2006). Farmers in Nigeria not only face many constraints to produce staple crops, but they are also face with risk and grain management challenges after harvest. By not being able to store effectively, most farmers cannot take advantage of price increases that occur during the production cycle. They often shift from sellers to buyer of grain during the storage season (Didier, Jacob, Corinne and Abdoulaye , 2013) The crucial importance of ensuring sustained levels of marketed surplus of rice both in terms of sufficient quantities as well as fair prices cannot be overemphasized if development is to take place in Nigeria rice sector.

Risk therefore occurs because agriculture is affected by many uncontrollable events that are often related to weather, including excessive or insufficient, rainfall, extreme temperatures, insect pests, and diseases etc (Jirgi, 2013). Rice post harvest management is a high risk business, because of its dependence on human skills, efficiency of machines and clemency of the physical forces of nature. In Nigeria, however, slow rice post harvest development and the developing status of the nation are major reasons why risk-measuring is unpopular and rarely considered. Farmers with access to risk management information and the knowledge to use have the key to profitable and competitive post harvest operations. Several studies were carried in Nigeria on risks sources and management strategies devised by farmers in different staple crops but for rice post harvest management, there are scanty studies. Therefore, the study aimed at evaluating the sources of risk and management strategies among rice post harvest management farmers' in Niger State, Nigeria.

II. METHODOLOGY

2.1 Study Area

The study was carried out in Niger State, Nigeria. The State was created out of the then North-Western state in 1976. The State lies between latitude 8^0 11' and 11^0 20' N of equator, and longitudes 4^0 30' and 7^0 15' E of equator. Kaduna State and FCT are her borders to the North-East and South-East respectively; Zamfara State borders the North, Kebbi State in the West, Kogi State in the South and Kwara State in the South West, while the republic of Benin along Agwara LGA boarders her North West. It covers an estimated land area of 76,469,903 square km and the state has a population of 3,945,772 (NPC, 2006). Gwari, Nupe and Hausa are the major ethnic groups in the State.

Niger State experiences distinct dry and wet seasons with annual rain fall ranges between 1,100mm to 1,600mm. The average minimum temperature is about 26^0 C while the average maximum temperature is about 36^0 C. The mean relative humidity ranges between 60 percent (January to February) and 80 percent (June to September).The state falls within the guinea savannah vegetation belt, which supports the cultivation of root crops and grains.

2.2 Data collection

To achieve the objective of the study, data was collected through primary and secondary source. This was through personal interviews with the 200 selected rice farmers using designed structured questionnaires.

2.3 Sampling method

Multi-stage sampling method was used in the study. First stage involved a random selection of two Local Government Areas each from Agricultural zone I & II and one local Government Area from Zone III. The selected local Governments areas were Katcha and Lavun for Zone I, Shiroro and Paikoro for Zone II and Wushishi for Zone III respectively. Second stage involved random selection of villages from the selected Local Government Areas. Third and last stage involved proportionate selection of farmers in the selected villages based on the required sample size.

2.4 Analytical techniques

Data collected were analyzed using simple descriptive statistics such as tables, percentages and average. However, attitudinal scale approach (ASA) was used to measured individual responses. It consists of defining a scale of statements that reflects the respondent's risk sources and management attitude. An aggregate score based on households' responses to a total of "K" number statements (items), each representing a risk sources and management strategy used by each rice post harvest management farmer would be estimated.

Likert's scale, a 5-point rating scale was used to measure an individual's attitude as established Batttacharya (1993) The responses was measured on a 5-point scale. Strongly agree (score of 1) implied the willingness of farmer to adopt the risk management strategy in question (risk aversion). On the other hand, strong disagree (score of 5) indicated a risk taking attitude. In between the two extremes, disagree (score of 2), undecided/neutral (score of 3) and agree (score of 4) were included as alternative responses. The Likert scale was also used by Meuwissen *et al*. (2001) and Akcaoz and Ozkan (2005).

III. RESULTS AND DISCUSSION

The socio-economic characteristics of the farmers are shown in Table 1. The results indicated that farmers mean age was 41, which implies that majority of the farmers are still active and in the productive stage. This finding were similar to that of Okoruwa *et al* (2002) and Ekong (2010), that age bracket of farmers in Nigeria are between 30-50 years. Moreover, the characteristic of farmer such as age determines his strength and also to some extent his experiences in rice post harvest management.. Table:1 also revealed that farmers in the study area have the mean of 24 years experience in rice post harvest activities. Ojo, (2012), reported that farming experience of cassava and yam farmers in Niger and Kogi states had average of 24 years farming experience. This implied that the farmers were well experienced in rice farming and post harvest management.

TABLE 1
SOCIO-ECONOMIC CHARACTERISTICS OF RESPONDENTS IN THE STUDY AREA. (n=200)

Variables	Frequency	Percentage
Age (years)		
20- 30	28	14.0
31-40	84	42.0
41-50	65	33
51-60	19	10
Greater than 60	4	2.0
Mean	41	
Sex		
Male	153	76.5
Female	47	23.5
Farming experience (year)		
Less than 10	18	9.0
10-20	74	37.0
21-30	55	27.5
31-40	40	20.0
41-50	9	4.5
Greater than 60	4	2.0
Mean	24	
Level of education		
Adult education	33	16.5
Primary education	37	18.5
Post primary education	47	23.5
Tertiary education	16	8.0
Non-formal education	67	33.5
Household size		
None	6	3.0
1-5	60	30.0
6-10	92	46.0
11-15	42	21.0
Mean	8	
Farm size (ha)		
Less than 1.00	31	15.5
1.00-1.50	59	29.5
1.51-2.00	40	20.0
2.01-2.50	8	4.0
2.51-3.00	13	6.5
Greater than 3.00	49	24.5
Mean	2.7	

Source: Field Survey, (2016)

The result shows that non-formal education has the highest percentage of 34, while post primary education and primary education recorded 24% and 19% respectively. Adult education and tertiary education constituted 17% and 8 respectively. The different educational status of farmers accounts for the differential managerial ability which also determines to some extent the overall success of the farm business. The level of education contributes much for productivity, adoption of new technology and farming techniques. This agrees with the findings of Okoruwa *et al* (2002) and Nmadu, *et al*, (2012). Tables 1 revealed that farmers have average household size of 8. The size of household determines the variability in agricultural production and the amount of labour input. The results also showed that farmers in the study area have average farm size of 2.7ha. This was in line with the findings of Ojo, (2012), who recorded 2.84ha and 2.42ha farm size in Niger and kogi states respectively. The size of farm is very crucial in agricultural since it largely determines the output of a farmer. This contrasted the results of Nmadu *et al* (2012).

TABLE: 2

RISK SOURCES IN RICE POST HARVEST MANAGEMENT

Variables	SD		D		UD		AG		SA	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Pest &Rodents	1	0.5	-	-	-	-	199	99.5	-	-
Drought	1	0.5	-	-	-	-	199	99.5	-	-
Too Much rainfall/flood	-	-	-	-	48	24.0	152	76.0	-	-
Illness of family member	-	-	-	-	85	42.5	115	57.5	-	-
Fire incidence	1	0.5	12	6.5	49	24.5	125	62.5	13	6.5
Changes in Government agricultural policies										
Lack of adequate hired labour	38	19.0	13	6.5	88	44.0	61	30.5	-	-
Inadequate family labour	-	-	13	6.5	13	6.5	174	87.0	-	-
Market Failure	25	12.5	-	-	60	30.0	115	57.5	-	-
Theft	-	-	24	12.0	62	31.0	114	57.0	-	-
Communal clash	1	0.5	35	17.5	12	6.0	152	76.0	-	-
Price changes	1	0.5	13	6.5	37	18.5	149	74.5	-	-
Bad weather condition or situation	12	6.0	13	6.5	37	18.5	138	69.0	-	-
Reduced consumption	1	0.5	52	26.0	35	17.5	112	56.0		
Off-farm activities	64	32.0	25	12.5	49	24.5	62	31.0	-	-
Diseases	1	0.5	36	18.0	13	6.5	150	75.0	-	-
Flood	-	-	-	-	-	-	200	100	-	-
Borrowed cash or kind	-	-	-	-	-	-	200	100	-	-
Insurance of rice farm/store	1	0.5	23	11.5	13	6.5	163	81.5	-	-
Storage System	89	44.5	36	18.0	37	18.5	38	19.0	-	-
Contributions/Adashe	-	-	25	5.5	61	30.5	114	57.0	-	-
Cooperative society	14	7.0	12	6.0	61	30.5	113	58.5	-	-
Market information	13	6.5	12	6.0	25	12.5	150	75.0	-	-
Scattered sales	-	-	23	11.5	49	24.5	128	64.0	-	-
Damage by animals	13	6.5	49	24.5	75	37.5	63	31.5	-	-
	-	-	13	6.5	13	6.5	174	87.0	-	-
										-

Field Survey, 2016.,* Multiple Responses Allowed: SD = Strongly disagreed, D = Disagreed, UD = Undecided, AG = Agreed, SA= Strongly Agree

TABLE: 3

RISK MANAGEMENT STRATEGIES DEVISED TO MITIGATE RICE POST HARVEST LOSSES

Variables	SD		D		UD		AG		SA	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
I never insure my farm	48	24.0	39	19.5	12	6.0	75	37.5	26	13.0
I adopt new technology	51	25.5	124	62.0	-	-	25	12.5	-	-
I do not have future market	24	12.0	50	25.0	25	12.5	51	25.5	50	25.0
I do not have formal life insurance	-	-	24	12.0	38	19.0	64	32.0	74	37.0
I practice both improved and traditional techniques	127	63.5	49	24.5	24	12.0	-	-	- 150	75.0
Insufficient sustainable income	12	6.0	-	-	-	-	38	19.0	162	81.0
Insufficient backup labour	-	-	-	-	-	-	38	19.0	-	-
I use reaper, thresher & better storage facilities.	75	37.5	73	36.5	52	26.0	-	-		12.5
I never rear animal for complementing my income										
I practice mixed cropping	37	18.5	87	43.5	-	-	51	25.5	25	31.0
I never sell my rice at market	13	6.5	26	13.0	25	12.5	74	37.0	62	44.0
I plant rice because is profitable	1	0.5	11	5.5	--	-	100	50.0	88	76.0
I do not have any other job	-	-	-	-	-	-	48	24.0	152	38.0
I belongs to farmers'group/association	25	12.5	1	0.5	-	-	98	49.0	76	50.0
I benefit credit from cooperatives/banks	-	-	26	13.0	37	18.5	37	18.5	100	19.5
Off-farm income is not important source of income	26	13.0	73	36.5	-	-	62	31.0	39	-
I do have access to Extension Agents	50	25.0	63	31.5	75	37.5	12	6.0	-	12.5
I rely heavily on market information	-	-	111	55.5	63	31.5	1	0.5	25	-
Nobody else in the household is interested in rice farming	86	43.0	64	32.0	-	-	37	18.5	-	
I do use insecticide/ pesticides										
I save greater share of my income in bank.	124	62.0	64	32.0	-	-	-	-	12	6.0
I never have storage facilities	63	31.5	63	31.5	-	-	61	30.0	13	6.5
I do keep record of my farm	26	13.0	61	30.5	62	31.0	38	19.0	13	6.5
I never use improved post harvest operations.	-	-	61	30.5	-	-	77	38.5	62	31.0
I do use traditional methods	63	31.5	63	31.5	50	25.0	13	6.5	11	5.5
I never use traditional pest control systems	26	13.0	26	13.0	50	25.0	98	49.0	-	-
I often fall sick	-	-	26	13.0	13	6.5	99	49.5	62	31.0
Weather is never favourable	36	18.0	100	50.0	13	6.5	25	12.5	26	13.0
I benefit in government projects	38	19.0	98	49.0	63	31.5	1	0.5	-	-
I have ready market	64	32.0	86	43.0	12	6.0	26	13.0	12	6.0
I do not have enough money for farming activities	13	6.5	36	18.0	38	19.0	13	6.5	100	50.0
	-	-	12	6.0	24	12.0	100	50.0	64	32.0
	-	-	12	6.0	-	-	74	37.0	114	57.0

Field Survey, 2016, * Multiple Responses Allowed: SD Strongly disagreed, D- Disagreed, UD- Undecided, AG- Agreed, SA- Strongly Agreed

The result in Table: 2 show the sources of risks experienced by the farmers in rice post harvest management. Which revealed that diseases outbreak, too much rainfall/flood, pests and rodents attack, drought have 100% of the respondents, were ranked as sources of risk. While damage by animals, lack of adequate hired labour 87% of the respondents, borrowed in cash or kind (82% of the respondent), theft (76% of the respondent), off-farm activities , cooperative society and communal clash (75% of the respondents), were identified also as risks sources encountered by farmers in the study area.. The results also indicated that price changes (69% of the respondents), market information (64% of the respondents), fire incidence (63% of the respondents), contributions or adashe (59% of the respondents), and family labour, and illness of the family member (58% of the respondents), storage system (57% of the respondents), bad weather condition (56% of the respondents) were identified as part of sources of risk in rice post harvest management. This indicated that farmers in the study area are likely affected by the production risk, financial risk, human or personal risk, market or price risk and technological risks. This is similar to the findings of Jirgi (2013), Alimi and Ayanwale (2005) and Ogunniyi and Ojedokun (2012), who reported that farmers are faced with different risk factor in the production, processing and marketing. This may likely be the reason why many farmers turned from producers of the rice to the buyers of the commodity for consumption. While, scattered sales (thirty two percent of the respondents), changes in government policies and reduced family consumption (thirty one percent of the respondents), insurance of rice farm or store (nineteen percent of the respondents) and were identified as not key risk sources in the study area. This also disagrees with the findings of Jirgi (2013), reported government policies as important sources of risk. This implies that farmers also experienced these risk sources but not as serious as the earlier ones mentioned. Risk sources cause adversity in yield, prices and production units. Each or any combination of the outcomes, of the risk sources (bad yield, poor prices, and inadequate production units) leads to poor farm income (Alimi and Ayanwale, 2005)

Table 3 shows risk management strategies devised to mitigate rice post harvest losses according to their importance assessed using a scale from 1 (Strongly disagree) to 5 (Strongly agree). Management of risk required knowledge of the most crucial risk being faced, identifying the impacts and likelihood of undesirable results; and taking possible steps to mitigate impacts. Rice post harvest management is a profitable venture (100% of the respondents). Farmers who do not sell rice in the market and many household members are interested in rice post harvest management as a business (94% of the respondents). Farmers who do not have any other job apart from rice production (87% of the respondents), ready market for rice (82% of the respondents.), and weather is favourable for post harvest activities (75% of the respondents). Farmers belongs to cooperative associations (69% of the respondents), practice of mixed cropping (68% of the respondents), Farmers used rearing of animals to sell to compliment income from rice production (62% of the respondents), off-farm income is an important source of income and benefit from government projects (57% of the respondents), use traditional method of post harvest operations (50% of the respondents), were the major risk management strategies adopted by farmers in the study area to mitigate losses of rice and income. This implies that farmers uses prevention, diversification, mitigation and coping risk management strategies in the study area, which was perfectly in line with the findings of Okunmadewa, (2003) , Okereke (2012) and Jirgi (2013), who reported various risk managements adopted by farmers to averted risk.

IV. CONCLUSION

The study examines sources of risk and management strategies devised among rice post harvest management farmers in Niger State. The study showed that farmers in the study area are affected by production risk, financial risk, human or personal risk, market or price risk and technological risk sources. The farmers have adopted prevention, mitigation and coping with risk as management strategies. The study recommended provision of credit facilities, infrastructural facilities, post harvest machineries, proper storage facilities, extension agents, cottage improved rice processing factories and timely harvesting of rice using appropriate method and threshing and winnowing on cleaned concrete slab or tarpaulin.

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Technical efficiency in rain-fed maize production in Adamawa state Nigeria: Stochastic approach

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Abstract— *The study analysed the technical efficiency of rain-fed maize cultivation in Adamawa state, Nigeria using stochastic approach. The study was based on primary data collected from 140 respondents using simple random sampling for the period of 2014-15 Kharifmaize. The result reveals that resources were under-utilized in rain-fed maize cultivation in Adamawa state, Nigeria. Moreover, the mean technical efficiency of 0.69 indicates that an average farmer in the study area have the scope for increasing technical efficiency by 31 per cent in short-run under the existing technology. The study therefore, recommends that government should pay more attention on the land consolidation programme. It will help farmers to adopt improved agronomic practices and enhance the production and productivity of rain-fed maize production in Adamawa state.*

Keywords— Technical efficiency, Inefficiency parameters, Rain-fed Maize, Data, Random sampling.

I. INTRODUCTION

Maize (*Zea mays*) or corn is a cereal grain believed to have originated in central Mexico 7000 years ago from wild grass. It is third most important grain after rice and wheat and one of the cheapest foods and food ingredient available in the world. Maize is distinguished by its female inflorescence, called corncob where the seeds (kernels) are grouped along one axis. It is an important cereal in many developed and developing countries of the world and widely used for animal feed and industrial raw materials in developed countries whereas it is a staple food in many developing countries as it provides half of the daily intake of calories and about eighteen per cent protein depending on the variety. Maize tolerates wide range of geographical environments. This makes it to be the most widely grown crop in the world and its greater weight is produced each year than any other grain.

Nigeria is the largest producer of maize in Africa and ranked 13th in the world with a total production of 7.3 million MT in 2015; (a 2.67 per cent decrease from previous year, based on sizeable carryover supplies and declines in market prices).Nigeria's maize production had a humble beginning; it stayed around one million ha through the early 1980s. Accelerated growth started in the mid-1980s, when hybrids were introduced, exceeding the 5 million ha mark in the mid-1990s, following the introduction of early varieties; it declined or remained slow during the late 2000s, mainly due to drought and erratic rainfall, but picked up thereafter. Currently it occupies the largest area of cultivated land in the country.

Despite the importance of maize in the nutrition of people, it is not always available at a required quantity in Adamawa state. This may not be unconnected to the fact that many farmers depend mainly on traditional method of farming and therefore, does not make use of the available resources effectively. With abundant fertile agricultural land and favorable weather condition for rain-fed maize production, yet Nigeria import maize product. It is expected that the findings from this study entitle "Technical efficiency of rain-fed maize cultivation in Adamawa state, Nigeria using stochastic approach", will provide useful information and technical advice to rain-fed maize farmers in Adamawa state Nigeria

II. METHODOLOGY

2.1 Sampling procedure

Purposive and simple random sampling was used for the selection of states, local government areas, villages and the respondents in the study area.

2.1.1 Selection of study area

In Nigeria maize is predominantly a rain-fed crop. Adamawa state was purposively selected because is one of the states where maize production under rain-fed condition is high and have best ecological condition (Guinea savannah zone) that favor's the cultivation of the crop.

Two out of the 21 local government areas of the state were purposively selected for the study, on the basis of their maximum production of maize under rain-fed condition. Eight villages from Fufore and seven from Ganye were purposively selected from each of the sampled local government areas for the study, on the basis of their maximum area under rain-fed maize production.

Thereafter, a list of rain-fed maize farmers was prepared for each sampled villages and 140 rain-fed maize farmers were randomly selected using random table.

2.2 Sources and period of data collection

From randomly selected 140 respondents, primary data related to socio-economic parameters, inputs (quantity and price) used for rain-fed maize cultivation, output, market price of output etc. were collected through personal interview using pre-tested schedule in the study areas. Secondary data were collected from relevant published research articles as well as Adamawa Agricultural Development Programme [1] report.

The primary data for the present study was collected for 2014-2015 (Kharif maize) in the study area.

2.3 Technical Efficiency

In analyzing technical efficiency in rain-fed maize production, inferential statistics involving the use of stochastic frontier production was used. The use of stochastic frontier production function has some conceptual advantages in that it allows for the decomposition of the error term into random error and inefficiency effects rather than attributing all errors to random effects [13]. It is specified as:

$$Y_i^* = f(X_i; \beta) \exp(V_i - U_i) \quad (1)$$

Where;

Y_i* = Production of the i^{th} firm

X_i = Vector of input quantities of the i^{th} firm

β =Vectors of unknown parameters

V_i = These are random variables which are assumed to be normally distributed $N(0,\delta^2 v)$ and independent of U_i. It assumed to account for random factors such as weather, risk and measurement error.

U_i = These are non-negative error term having zero mean, and constant variance i.e. $N(0,\delta^2 U)$ [13]. It measures the technical inefficiency effects that fall within (because of errors could be controlled with effective and adequate managerial control of the farm) the control of the decision unit.

2.3.1 The empirical stochastic frontier production model

The Cobb-Douglas production function was used to specify the production technology of the farms. The empirical stochastic frontier model is specified as:

$$\ln Y_{ij} = \beta_0 + \beta_1 \ln X_{1ij} + \beta_2 \ln X_{2ij} + \beta_3 \ln X_{3ij} + \beta_4 \ln X_{4ij} + \beta_5 \ln X_{5ij} + \beta_6 \ln X_{6ij} + V_{ij} - U_{ij} \quad (2)$$

Where,

Y_{ij} = Output of maize (Kg)

X_1 = Size of farm (ha)

X_2 = Quantity of seed (Kg)

X_3 = Hired labour (man-days)

X_4 = Family labour (man-days)

X_5 = Agro-Chemicals (liters)

X_6 = Quantity of fertilizer (kg)

V_i and U_i as previously defined.

Operational definition of the variables of empirical stochastic production function for rain-fed maize production is as follows:

a. Output of maize (Y_{ij}): This is the total quantity of maize in kilogram produced per hectare by farmers.

b. Farm size (X_1): This is the size of land used in producing maize crop by the farmers. It was measured in hectare.

c. Quantity of seed (X_2): This is the quantity of seed used in planting by the farmers. It was measured in Kilograms.

d. Hired labours (X_3): This is the total labour provided by people who were paid to work on the farm. It was measured in man-days.

e. Family labour (X_4): This is the total labour provided by family members in the production of maize. It was measured in man-days. A man-day of labour is equal to eight hours of work per day.

f. Agro-chemicals (X_5): These are quantities of chemicals used by farmers in the production of maize to protect the crop from insects' pests, diseases as well as weeds. It was measured in liters.

g. Quantity of fertilizer(X_6): Is the quantity of inorganic fertilizer that was used in growing maize. It was measured in kilograms.

The technical efficiency of maize production for the i^{th} farmer was calculated with an output oriented method as the ratio of Actual (observed) output relative to the Potential (maximum feasible) output:

$$TE = Y_i^* / Y_i$$

$$TE = f(X_i; \beta) \exp(V_i - U_i) / f(X_i;\beta) \exp(V_i)$$

$$TE = \exp(-U_i) \quad (3)$$

Variance parameters are:

$\delta^2 = \delta^2 V + \delta^2 U$ and $\gamma = \delta^2 U / \delta^2$

Where: Y_i^* isactual output and Y_i is potential output

δ^2, γ, βs are unknown parameters that were estimated.

This efficiency measure takes values between 0 and 1 with smaller ratios reflecting greater inefficiency. The inefficiency model is defined by,

$$U_i = \delta_o + \delta_1 Z_1 + \delta_2 Z_2 + \delta_3 Z_3 + \delta_4 Z_4 + \delta_5 Z_5 + \delta_6 Z_6 + \delta_7 Z_7 \quad (4)$$

Where,

U_i = Thetechnical inefficiency of the i^{th} farmer

Z_1= Farmer's age (years)

Z_2= Farmer's sex (dummy, where one indicates male and zero female)

Z_3= Farming experience (years)

Z_4= Farmer's education (years)

Z_5= Family size (Number)

Z_6= Extension contacts (dummy, one contacted, zero otherwise)

Z_7= Credit availability (dummy, where one indicates those that accessed credit and zero otherwise)

Where $\delta_1, \delta_2, ----\delta_7$ are unknown parameters to be estimated [3].

III. RESULTS AND DISCUSSION

Maximum Likelihood Estimate (MLE) of the parameters of the stochastic frontier production model used in estimating technical efficiency of rain-fed-maize farmers in Adamawa state, Nigeria is presented in Table 1. The result reveals that nearly all variables (farm size, seed, hired labour, agro-chemicals and fertilizer) of the estimated coefficients of the parameters of production function in Adamawa state were positive which confirm to apriori expectations and were statistically significant at 1 and 5 per cent level respectively while family labour was negative and not significant. The sigma squared and the gamma (0.163), (0.876) were statistically significant at five and one percent respectively. The sigma squared indicates the goodness of fit and correctness of the distributional form assumed for the composite error term. The Gamma implies the existence of technical inefficiency among rain-fed maize farmers and account for 88% of the variations in the output level of maize produced in study area. This confirms that in the specified model, there was presence of one-sided error component. It also implies that the effect of technical inefficiency was significant and that a classical regression model of production functions based on ordinary least squares estimation would be an inadequate representation of the data. Thus, the variance parameters confirm the relevance of the stochastic production function. The variables that were statistically significant can be discussed thus:

The elasticity of production with respect to farm size in Adamawa state 0.819 was positive and statistically significant at one percent level. This implies that farm size positively influenced the output of rain-fed maize in the study area. Thus, an increase of one percent in farm size will result to an increase in output of maize by 0.819 percent in the study area. This suggests that land was a significant factor associated with changes in maize production in the study area. The finding support the reports earlier made by previous researchers [9; 6] that there was positive and significant relationship between maize output and farm size.

The coefficient of seed (0.112) was positive and statistically significant at one percent level. This implies that one percent increase in seed would lead to increase in maize yield by 0.112 percent. This implies that seed is a positive factor influencing maize output in Adamawa state. It indicates that higher seed rate would result in high crop population and subsequently higher yield. The finding concurs with the report by [9] that seed was a positive determinant of maize output among maize farmers in Ogbomoso south LGA in Oyo state Nigeria and Nandi north district, Kenya respectively.

The elasticity of production with respect to agro-chemicals (0.175) was positive and statistically significant at one percent level. Agro-chemicals positively influenced the output of rain-fed maize in the study area as they protect the crop from insects' pests, diseases as well as weeds. Hence, it is an essential input used for increasing maize production by preventing loss before and after harvesting. Thus, besides the large growing population and scarcity of land available for cultivation, pesticides industry has a vital role to play in the agricultural sector. The present findings corroborate the report by [14] that herbicides have significant effect on maize output.

The elasticity of production with respect to fertilizer 0.175 was positive and statistically significant at five per cent level. The use of fertilizer will improve soil fertility leading to increase in yield of maize. However, fertilizer make its best contribution to the enhancement of soil fertility only if it falls within a hierarchical system of good technological measures and the doses used are related to crop plants, soil, climate and culture technology. Although use of fertilizer is yield enhancing, the economic return from use of this input is low due to high cost of the input. The result of the study is in conformity with the work of [11] who found fertilizer to be significant in white maize production.

The elasticity of production with respect to hired labour (0.001) was positive and statistically significant at one percent level. This implies that one percent increase in man days of hired labour, *ceteris paribus*, would lead to increase in maize yield by 0.001 percent. The quick and effective weeding by hired laborers may be responsible for the increased productivity of rain-fed maize in the study area. Accordingly, the production of maize in the area does not involve much of farm mechanization but used traditional technology that relies heavily on hired labour. This study is consistent with the findings of [6] who reported that there is positive and significant relationship between maize output and hired labour.

TABLE 1
MAXIMUM LIKELIHOOD ESTIMATE OF THE COBB-DOUGLAS STOCHASTIC FRONTIER PRODUCTION FUNCTION MODEL FOR RAIN-FED MAIZE FARMERS IN ADAMAWA STATE.

Variables Production Factors	Parameter	Adamawa state	
		Coefficient	t-ratio
Constant	β_o	2.6939	16.3400*
Farm Size (X_1)	β_1	0.8187	10.0500*
Seed(X_2)	β_2	0.1115	2.8500*
Hired labour (X_3)	β_3	0.0008	2.8500*
Family labour (X_4)	β_4	-0.0103	-0.7800
Chemicals (X_5)	β_5	0.1753	2.7770*
Fertilizer (X_6)	β_6	0.1753	2.2500**
Inefficiency Model			
Age (Z_1)	δ_1	-1.3640	-1.6383
Sex (Z_2)	δ_2	-0.0990	1.7644***
Experience (Z_3)	δ_3	1.0886	1.8074***
Education (Z_4)	δ_4	-0.1121	-2.9045*
Family Size (Z_5)	δ_5	0.1832	1.4907
Extension (Z_6)	δ_6	-0.2454	1.9580***
Credit (Z_7)	δ_7	-0.3157	2.0411**
Variance Parameters			
Sigma-Square (δ)		0.1625	2.5531**
Gamma (Υ)		0.8764	3.8900*

Estimates are significant at1% level
****Estimates are significant at 5% level***
*****Estimates are significant at 10% level***

The result of the parameters of the stochastic frontier production function discussed above implies that farm size, seeds, hired labour, agro-chemicals and quantity of fertilizer were significant factors in Adamawa state.

The inefficiency parameters included in the model specifically, those related to farmers' socio-economic characteristics were also presented in Table 1. The signs in the inefficiency model were explained in the opposite way such that a negative sign indicates increase in technical efficiency while positive sign indicates decrease.

The estimated coefficient of gender (-0.099) carried the expected negative sign and was statistically significant at ten per cent level. This implies that increase in the number of male farmers will increase the efficiency in rain-fed maize production; so we can say that male farmers were relatively more efficient in maize production in the study area. Considering that planting, weeding, harvesting and other crop management operations are labour-intensive, this result is not surprising. Female farmers also have relatively less access to productive resources like loan and land. The result is in agreement with the findings of [7] that gender was an important determinant of efficiency among smallholder maize farming communities in Zimbabwe.

The estimated coefficient of extension contacts was (-0.245) negative and statistically significant at ten percent level. This indicates that continuous contacts with extension agents tends to increase technical efficiency of rain-fed maize farmers in the study area; since they provide guidance on issues such as new technologies, production and marketing related information. This finding support the reports earlier made by previous researchers [10;4;7] that increase in extension services tends to increase the technical efficiency of a farmer.

The estimated coefficient of credit availability was (-0.316) carried the expected negative sign and was statistically significant at five per cent level. The study shows that credit had positive impact on technical efficiency of rain-fed maize farmers in the study area. Therefore, availability of credits with low interest rate at a right time will increase farmers' technical efficiency. As earlier stated majority of them operated at subsistence level below two hectare hence, provision of credit to these set of farmers translate to increase in the use of agricultural technologies such as improved seed, fertilizers, herbicides as well as machines. [2] also obtained similar result that credit had positive impact on technical efficiency of maize producing households in northern Ghana.

The estimated coefficient of farming experience does not possess the expected negative sign though it was significant. This means that increase in this variable will increase technical inefficiency of rain-fed maize farmers. Farmers with more years of

experience, more especially those in the rural areas may likely not going to accept new innovation and would prefer to use their previous knowledge. The finding from this study is in contrast with what [5] earlier reported that highly experienced farmers tend to be more technically efficient. In other words, the older the farmers, the more experience they have and the less the technical inefficiency they will be.

The estimated coefficient of age was (-1.364) carried the expected negative sign but was statistically insignificant. Considering the age bracket of the rain-fed maize farmers, older farmers were more technically inefficient than the younger ones. Younger farmers were more dynamic in terms of technology adoption that will impact on their efficiency. It is possible that older farmers may rely on their previous experiences which affect their willingness to adopt new technologies. It implies that increase in the number of younger farmers will enhance the technical efficiency in rain-fed maize production in the study area. This finding support the report earlier made by [2] that age have significant jolt on maize producing households in northern Ghana

3.1 Technical efficiency of rain-fed maize farmers

The distribution of technical efficiency of the farmers in the study area reveals that 7.14 percent had technical efficiency of less than 50 and about 43.57 per cent had technical efficiency of above 69 (Table 2). The mean technical efficiency was 0.69 which indicates that the average farmer produced about 69 per cent of maximum attainable output for a given input levels. This indicates that in the short run, there is scope for increasing technical efficiency in rain-fed maize production by 31 per cent in Adamawa state. This implies that to improve the technical efficiency of the rain-fed maize farmers, their knowledge, skills and awareness level need to be improved. The present finding corroborate with the study by [8; 12] who also reported high mean technical efficiency in maize production

TABLE 2
TECHNICAL EFFICIENCY OF THE RAIN-FED MAIZE FARMERS

Efficiency	Adamawa state	
	Frequency	Percentage
0.40-0.49	10.00	7.14
0.50 -0.59	33.00	23.57
0.60 0.69	36.00	25.72
0.70 –0.79	22.00	15.71
0.80 –0.89	24.00	17.14
0.90 –1.00	15.00	10.72
Total	**140.00**	**100.00**
Minimum efficiency	0.41	
Maximum efficiency	0.96	
Mean efficiency	0.69	

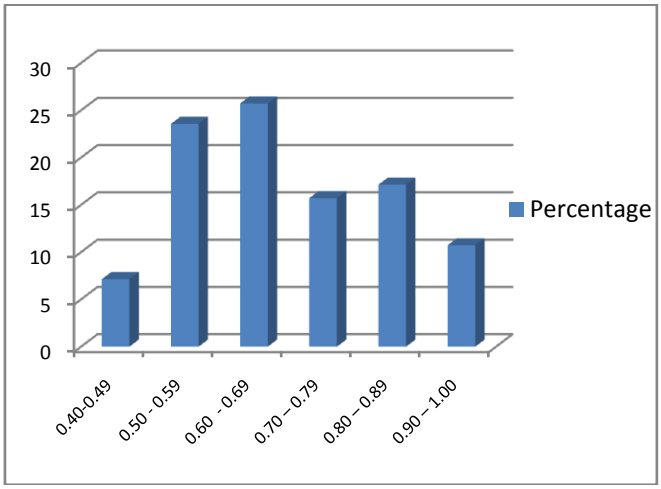

FIGURE 1: TECHNICALEFFICIENCY DISTRIBUTION OF RAIN-FED MAIZE FARMERS IN ADAMAWA STATE.

IV. SUMMARY AND RECOMMENDATIONS

The study reveals that rain-fed maize output responds positively to increases in farm size, seed, hired labour, agro-chemicals and fertilizer. The result of inefficiency parameters indicates that gender, education, extension contacts and credits availability decreased technical inefficiency. The findings of the study suggest that inputs like the land holding size has positive impact on the rain-fed maize production in the study area, the land holding size of maize growers were very small with three to four parcels. Therefore, government should pay more attention on the land consolidation programme. It will help farmers to adopt improved agronomic practices and enhance the production and productivity of rain-fed maize production in Adamawa state.

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Priority of Water Supply Service for Community in Gresik City, East Java Province

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Abstract— *Water supply is one of important aspect and a priority in urban planning. The fulfillment of a water supply necessity for Gresik City is still not optimized. Gresik City consists of 2 districts, namely Gresik District and Kebomas District. Based on ministerial regulation 14/2010, coverage of water supply service at Gresik City was classified as bad with water supply service rate less than 50%. Hence, for the sake of optimizing and equity of water supply service at Gresik City, the identification of water supply service ratio of Gresik City and community's perception of water supply service was needed. The research objective was to identify water supply service priority for community at Gresik City. The ratio analysis was done by calculation method based on water supply necessity and availability standard. While, the analysis of water supply service based on community's perception was done by descriptive statistical analysis. The results showed that the highest ratio of water supply service was on Kroman Sub-District and the lowest ratio of water supply service was on Tenggulunan Sub-District. Based on community's perception analysis, there are 93% of Gresik District residents and 75% of Kebomas District residents that haven't used PDAM (local water supply company) water supply service. Furthermore, water supply service wasn't optimized yet in term of water quality, quantity, continuity, so that the handling of water supply service was focused on sub-district with lowest water supply service ratio.*

Keywords— Service, water supply, Gresik City, Community.

I. INTRODUCTION

Water is a resource that is very much needed by living things, whether it to fulfills their needs or sustains their life naturally. The use of water is universal and thorough on every aspect of life. The higher of life quality of a person, the higher needs of water too (Unus, 1996) [1]. Water supply is one of important aspect and a priority in urban planning (Catanese, 1996)[2].Water supply is no longer an available commodity and free to use, but a scarce commodity that needs a proper management (Kodoatie, 2002)[3].

Local Water Supply Company (PDAM) was carrying out a main task to manage and provides water supply service in order to increase public's welfare as stated on RI Regulation 23/2014 about local government [4]. This company was also provided water supply service for Gresik City. Based on Gresik City Spatial Plan 2010[5], Gresik City was divided into two districts, namely Gresik District and Kebomas District.

Based on PDAM data on 2014, the level of water supply service on Gresik District was 46, 87 l/dt and Kebomas District was 44, 19 l/dt. Based on Ministerial Regulation 14/2010[6], on the water supply service standard, the water supply service on Gresik City was classified as bad because the level of service was below 50%. At Gresik District, there were nine sub-districts that classified as bad, namely Sub-district Gapurosukolilo (41%); Sub-district Bedilan (42%); Sub-district Kebungson (46%); Sub-district Pekelingan (27%); Sub-district Kemuteran (43%); Sub-district Kroman (48%); Sub-district Karangpoh (38%); Sub-district Karangturi (43%) and Sub-district Tlogopojok (34%). Whilst at Kebomas District there were 6 sub-districts that classified as bad, namely Sub-district Singosari (30%); Sub-district Karangkering (6%); Sub-district Gulomantung (32%); Sub-district Kedanyang (32%); Sub-district Tenggulunan (5%); and Sub-district Kawisanyar (8%).

Based on consensus on Sustainable Development Goals (SDGs), the target of water supply service at urban areas on 2019 was 100%. In order to make equity of water supply service at Gresik City in accordance with SDGs target, a research about the condition of water supply service was needed, so that the water supply service at Gresik City could be optimized.

II. METHODS

2.1 Research Variables

The research variables were obtained from theoretical review and policies review. The research variables and sub variables are mentioned at Table 1

TABLE 1
RESEARCH VARIABLES

Aspect [7] [8] [9] [10] [11]	Indicator	Variable	Definisi Operasional
Availability of Water supply	Water supply source	Water supply source debit	Total debit of water supply source (lt/dt)
	Piping condition	Pipe's length	Pipe's length from transmission to SR (m)
		The age of pipeline network	Pipeline's age counted from the date it got installed (year)
Condition of water supply	Domestic need of water supply	Total population	The number of population (person)
		Daily consumption	The number of water consumption (l/day)
	Water leakage	The amount of water produced	The total of water produced (m^3)
		Numbers of account recorded	The total of water usage by consumers(m^3)
Water Supply Service	Water supply service using pipelines per household	Quality	Water quality received by community that could be physically seen based on RI Health Ministerial Regulation 416/Menkes/PER/IX/1990, namely clear water, colorless, odorless, and tasteless.
		Quantity	Water supply debit per household, be observed by the water supply was smoothly running or faltered.
		Continuity	Water streamed continuity 1x24 hours per day (24 hours or not)

2.2 Research Methods

To obtain water supply ratio analysis results, the first step was to identify the needs and availability of water supply. Water supply necessity was calculated based on the criteria of National Policy and Strategy of Water Supply System Development on Public Works Ministerial Regulation 20/2006[12] with 120/l/person/day for urban areas and 60 l/person/day for rural areas. Water supply necessity of settlement areas was a domestic water need, which would be calculated upon land area and settlement area. The formula used for the water supply necessity analysis, i.e.:

$$\text{Water supply necessity} = a \times b \times c \quad (3.1)$$

Explanation:

a = total population (person)

b = the number of domestic water supply needs based on regions (litre/person/day)

c = water supply service (% of total population)

The second step was to identify water supply production capacity which was provided for each sub-district in the research area. Water supply capacity was calculated by multiplying total population covered by water supply service in the area with the daily water supply necessity standard, i.e. 80 litres/person/day. The formula used for the water supply capacity analysis, i.e.:

$$\text{Water supply production capacity} = a \times b \quad (3.2)$$

Explanation:

a = the number of population served by water supply service (person)

b = average water supply service necessity (litre/person/day)

After identifying water supply production capacity, the next step was to calculate the ratio of water supply service. The formula to determine the water supply service based on SPM (minimum service standard) formula of water supply availability (Ministerial Regulation 14/2010)[6], i.e.:

$$\text{Water supply service ratio} = (a / b) \times 100 \quad (3.3)$$

Explanation:

a = water production capacity per month (m^3/month)

b = total capacity of community's water supply necessity each month (m^3/month)

Therefore, the percentage of water supply service debit that got served by local water supply company (PDAM) for each sub-districts at Gresik District were obtained.

In order to identify water supply service based on community's perception, it was done by descriptive statistical analysis. The analysis was based on existing condition by doing interviews based on random sample (purposive random sampling) to the community of Gresik District and Kebomas District. The analysis was utilized for understanding community's perception towards water supply service. Total samples were 200 household. The descriptive statistical analysis was done by using software SPSS for windows.

III. FINDING AND ARGUMENTS

3.1 Characteristic of areas

The area of research were urban areas at Gresik Region, namely Gresik District and Kebomas District. The main focuses of the research were purred into sub-districts which were stated as urban areas. Thus, the research areas were based on sub-districts which set based on urban area.

The number of population at Gresik District studied were 32,192 people which were divided into 9 sub-district, and the number of population at kebomas district studied were 25,559 people which were divided into 6 sub district. Community served by water supply service in gresik district were 2,977 SR, and community served by water supply service in kebomas district were 1,332 SR. The identification of water supply in research area was done first by identifying total population at each sub district, then it was adjusted toward service target to acquired total community served by water supply service. furthermore, the total community served by water supply service was adjusted by average water consumption based on urban areas standard. Based on the result, the water supply necessity at Gresik District was 39.6 l/second, and water supply necessity at Kebomas District was 27,22 l/second

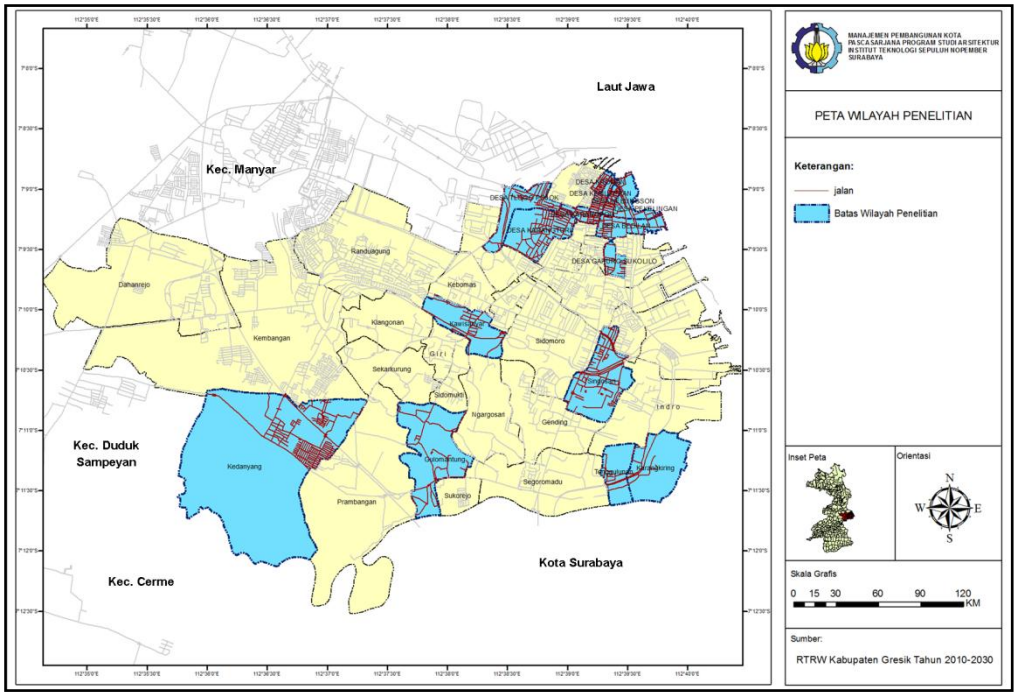

FIGURE 1. RESEARCH AREA ORIENTATION

3.2 Water Supply Service Ratio Analysis

Water supply service at research area was calculated by comparing water supply service availability/ water supply production with community's water supply necessity. The results of level of water supply coverage in research area could be observed on table 2.

TABLE 2
WATER SUPPLY SERVICE RATIO AT RESEARCH AREA

No.	Sub District	Total Population Served (person)	Water Supply necessity (m3/month)	Water Supply Availability (m3/month)	Water Supply Service (%)
		[a]	[b]	[c]	[d]
Gresik District					
1.	Gapurosukolilo	1,050	9,381	2,898	30.89
2.	Karangturi	2,610	22,000	7,203	32.74
3.	Karangpoh	1,425	13,670	3,933	28.77
4.	Bedilan	1,720	14,821	4,747	32.03
5.	Kebungson	1,310	10,301	3,615	35.10
6.	Pekelingan	670	8,987	1,849	20.58
7.	Kemuteran	895	7,489	2,470	32.98
8.	Kroman	2,380	17,896	6,568	36.71
9.	Tlogopojok	2,825	30,668	7,797	25.42
Kebomas District					
10.	Singosari	2,940	36,634	8,114	22.15
11.	Karangkering	70	3,915	193	4.93
12.	Gulomantung	900	10,485	2,484	23.69
13.	Kedanyang	2,495	29,197	6,886	23.58
14.	Tenggulunan	25	1,865	69	3.70
15.	Kawisanyar	230	10,801	635	5.88

Explanation:
[a]= total population at sub-district
[b]= water necessity based on the added capacity to water leakages (m3/month)
[c]= water production capacity
[d]= level of water supply service = (c/b)*100%

Based on the calculation, it was discovered that the average of water supply service at Gresik District was 30% and at Kebomas District was ranged from 5% to 20%. The highest ratio of water supply service of Gresik District was on Kroman Sub-District, and the lowest ratio of water at supply service was on Pekelingan Sub-District. The highest ratio of water supply service of Kebomas District was on Gulomantung Sub-District and and the lowest ratio of water at supply service was on Tenggulunan Sub-District.

The high ratio was caused by highly total population, also high water supply necessity and availability if compared with others sub-district, vice versa. The number of population at an area, affecting the level of water supply needs, the more population, the higher needs of water supply.

3.3 Water Supply Service Based on Community's Perception

The water supply service analysis was using 200 KK which divide into 113 respondents of Gresik District and 87 respondents of Kebomas District. There are 93% of Gresik District residents and 75% of Kebomas District residents that haven't used PDAM (local water supply company) water supply service. Water quality was mostly murky, salty, and wasn't continuously running every day. The condition was causing the community to seek other water supply sources, such as gallon waters and water sellers with carts.

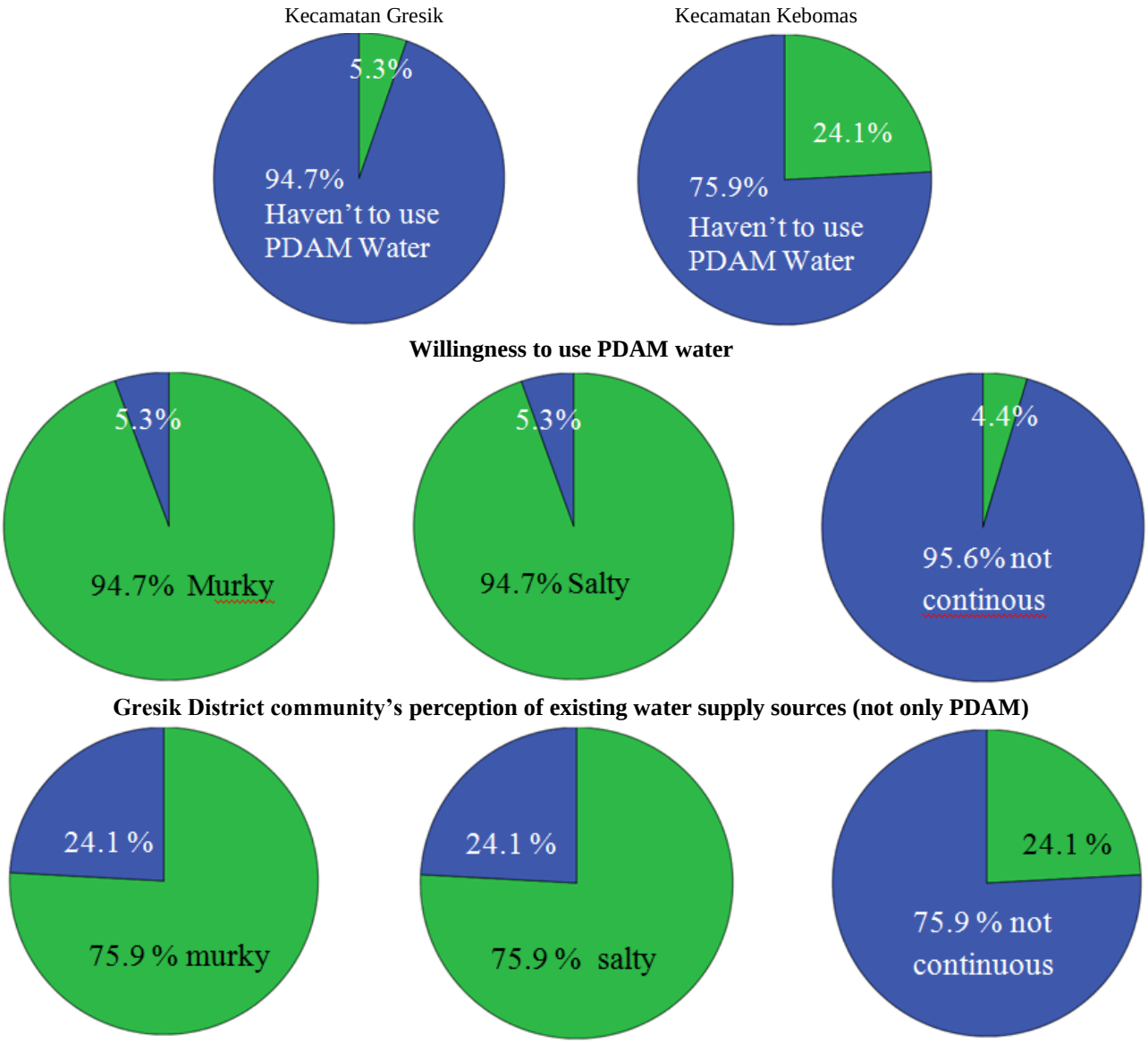

FIGURE 2. DIAGRAM OF COMMUNITY'S PERCEPTION TOWARDS WATER SUPPLY SERVICE

IV. CONCLUSION

Water supply service priority was done by comparing the results of water supply necessity and availability analysis, with water supply service based on community's perception analysis. The research's conclusion, namely:

1. As a whole, the water supply service on research area hadn't been able to reach the target of clean water service by 100%. The highest ratio of water supply service of Gresik District was on Kroman Sub-District, and the lowest ratio of water at supply service was on Pekelingan Sub-District. The highest ratio of water supply service of Kebomas District was on Gulomantung Sub-District and and the lowest ratio of water at supply service was on Tenggulunan Sub-District.
2. Most of Gresik District's community hasn't used PDAM water supply service (93%), whereas at Kebomas District, 75% of community hasn't used PDAM water supply service. Water quality was mostly murky, salty, and not continuously streaming all day. The condition forced the community of Gresik and Kebomas Districts to seek other water supply sources to fulfill their needs, such as using gallon water, and buying water from water sellers with carts.
3. Based on the results, water supply service wasn't optimized yet in term of water quality, quantity, continuity, so that the handling of water supply service was focused on sub-district with lowest water supply service ratio, such as Pekelingan Sub-District and Tenggulunan Sub-District.

ACKNOWLEDGEMENT

Praise be to Allah and blessings and greetings to the Prophet Muhammad SAW's so that this journal could be finished well. Big gratitude to people who have helped in completing this journal, father and mother, supervisors for the advice and input during the study, staff from across the district as a respondent that helped to provide the research data. Hopefully, this journal could provide benefits and new information to all parties.

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