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Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-11, November 2018, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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

Environmental Research:

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

Agriculture Research:

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

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

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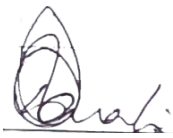

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Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

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Yield and yield attributes in ginger (*Zingiber officinale Rosc.*) somaclones for quality seed production

Nimisha Mathews[1*], Dr. K. Krishnakumary[2]

College of Horticulture, Department of Plantation Crops and Spices, Kerala Agricultural University, Thrissur 680656, Kerala

***Abstract*—** *The experiment was taken up to elicit the information on the performance of different ginger (Zingiber officinale Rosc.) somaclones for yield and quality for quality seed production. Ten somaclones of ginger were evaluated in RBD with three replications during 2015-18 at College of Horticulture, Kerala Agricultural University, Thrissur district, Kerala. The growth performance of ten somaclones indicated significant variation at all the stages of crop growth. Among the somaclones, SE 86102 (16.00 days) and SE 8626 (16.33 days) registered least number of days for sprouting. Among the vegetative characters studied, the maximum plant height (107.38 cm) was recorded by the somaclone SE 86102 which was significantly superior to other somaclones and check varieties. Somaclone CHP 118 recorded maximum number of tillers (20.33), number of leaves per shoot (28.67) and total number of leaves per shoot (117.33). The highest leaf area was recorded by C 8632 with a value of 62.12 cm^2. Among the rhizome characters recorded, the somaclone CHP 118 gave highest number of primary rhizomes (4.83) which was on par with SE 8626 (4.33) and C 8632 (4.08). The highest number of secondary rhizome was recorded by CHP 118 (10.33) which was on par with SE 8626 (10.06) and C 8632 (10.01). Weight of mother rhizome was the highest in the somaclone SE 8642 (11.00 g) and CHP 118 (10.67 g). Similarly, weight of primary rhizome was highest in SE 8642 (15.73 g), SE 8626 (15.07 g) and CHP 118 (14.80 g). Weight of secondary rhizome was highest in SE 8626 (13.20 g) and SE 8642 (12.33 g). The highest fresh rhizome yield per plant was recorded by somaclone CHP 118 (274.13 g), SE 8626 (266.67 g), C 8632 (259.67 g) and SE 8642 (251.67 g) at full maturity. The highest yield per plot was recorded by somaclone CHP 118 (8.77 kg), SE 86 26 (8.54 kg), C 8632 (8.31 kg) and SE 8642 (8.06 kg). The fresh rhizome yield per hectare was was maximum in CHP 118 (35.08 t), SE 86 26 (34.16 t), C 8632 (33.24 t) and SE 8642 (32.24 t). It is concluded that somaclones were highly variable in their performance, yield and yield attributes. Four somaclones viz., CHP 118, SE 8626, C 8632 and SE 8642 were identified as high yielders from their outstanding performance throughout their growth period with highest yield in CHP 118. This can be due to more number of tillers, leaves per shoot and rhizomes and also weight of primary, secondary and mother rhizomes.*

***Keywords*— Ginger, Somaclones, Yield and Horticulture.**

I. INTRODUCTION

Ginger is an important commercial spice crop grown in India for its culinary and wide range of medicinal uses and is considered as an essential component of the kitchen pharmacy. It belongs to the family *Zingiberaceae*, native of South East Asia. It is a tropical and sub-tropical perennial herb 'generally recognized as safe' by the Food and Drug Administration (FDA) of the United States and has gained considerable attention as a botanical dietary supplement in developed countries, opening ample export potential. Somaclonal variations act as a major source of variability for crop improvement in ginger (Shylaja *et al*., 2010 and Dev, 2013). Evaluation of somaclones derived from two polyploids (Z-0-78 and Z-0-86) and a triploid cultivar Himachal Pradesh (generated through indirect organogenesis and embryogenesis) indicated that somaclones are superior to conventionally propagated plants for various growth and yield parameters (Kurian, 2010) .The present investigation was carried out with the objective of evaluating somaclones in ginger for variability in performance, yield and yield attributes for quality seed production.

II. MATERIALS AND METHODS

Somaclones developed through indirect methods of regeneration from two induced polyploids of ginger (Z-0-78 from 'Himachal Pradesh' treated with 0.25% colchicine by injection method and Z-0-86 from Rio-de-Janeiro treated with 0.1% colchicine by hole method) and diploid cultivar 'Himachal Pradesh' formed the base material for the study (Table 1). Ten such somaclones of ginger viz., SE 86 26, SE 86 83, C 86 26, CHP 118, C 78 284, SE 86 102, SE 86 42, C 86 32, CHP 99 and CHP 282 were selected for the study along with three check varieties (Rio- de –Janeiro, Himachal and Aswathy). The experimental site was located in the farm of Department of Plantation Crops and Spices, College of Horticulture, Thrissur, Kerala. The experiment was laid out in a Randomized Block Design with three replications in plot size 2.0 × 1.0 m^2 at the spacing of 25.0 cm × 25.0 cm. The field was prepared and planting was done in the last week of May and maintained as per

Package of practice recommendations of Kerala Agricultural University (KAU, 2011). Five plants per replication were selected at random and studied for growth characters. The crop was harvested at full maturity, indicated by withering of above ground parts. The harvested rhizomes were cleaned after removing roots and rhizome characters were recorded. The fresh rhizome yields per plant and per plot were recorded and per hectare yield was computed.

TABLE 1
DETAILS OF SOMACLONES SELECTED FOR THE STUDY

Somaclones	Parents	Mode of regeneration
C 86 26	Z-0-78	Indirect organogenesis
C 86 32		
C 78 284	Z-0-86	
CHP 282	Himachal	
CHP 118		
CHP 99		
SE 86 83	Z-0-86	Indirect embryogenesis
SE 86 26		
SE 86 42		
SE 86 102		

III. RESULTS AND DISCUSSION

3.1 Plant characters

Ten ginger somaclones and three check varieties exhibited variation in number of days for sprouting and plant characters *viz.*, plant height, number of tillers, number of leaves per shoot, and leaf area (Table 2).

TABLE 2
MORPHOLOGICAL CHARACTERS OF GINGER SOMACLONES AT 6 MONTH STAGE

Somaclones	Number of days for sprouting	Plant height (cm)	Number of tillers per plant	Number of leaves per shoot	Total no. of leaves per plant	Leaf area (cm^2)
SE 86 26	16.33	87.37	18.01	28.33	108.33	55.14
SE 86 83	16.67	84.50	15.63	17.25	104.00	48.08
C 8626	23.33	85.47	14.83	16.43	100.77	43.60
CHP 118	18.67	90.34	20.33	28.67	117.33	51.74
C 78 284	18.33	75.42	15.27	21.00	100.97	50.63
SE 86 102	16.00	107.38	15.13	18.23	98.84	42.09
SE 86 42	20.67	80.20	17.97	26.33	108.11	58.46
C 86 32	18.33	87.55	16.93	26.07	107.33	62.12
CHP 99	23.67	82.02	11.23	16.87	86.44	37.43
CHP282	21.00	92.23	15.01	16.93	104.67	52.56
Rio-de-Janeiro	16.33	83.52	11.56	17.89	95.167	41.38
Himachal	15.33	92.07	11.43	17.88	95.20	41.88
Aswathy	13.67	72.55	14.93	20.20	97.69	43.25
CD (0.05)	2.71	5.52	0.83	1.77	10.85	2.96

Early sprouting was observed in all the three check varieties and also in the somaclones SE 86102 (16.00 days) and SE 8626 (16.33 days). Released variety Aswathy took only 14 days for sprouting whereas, C 8626 and CHP 99 took 23 days to sprout. Shadap *et al*. (2013) have reported similar result confirming early and complete sprouting in a shorter period when ginger is planted in May and June.

Plant height of ginger somaclones varied between 72.42 to 107.38 cm. The somaclone SE 86102 recorded the highest plant height (107.38 cm) which was significantly superior to other somaclones and check varieties. The lowest plant height was recorded in C 78284 (75.42 cm) and Aswathy (72.55 cm). Similar variability in plant height of ginger somaclones was reported by Iwo *et al*. (2011) and Dev (2013).

The number of tillers per plant varied significantly among ginger somaclones. The somaclone CHP 118 recorded the highest values for number of tillers per plant (20.33) followed by SE 8626 (18.01) and SE 8642 (17.97) and the lowest number of tillers was observed in CHP 99 (11.23) and the check varieties Himachal (11.43) and Rio- de –Janeiro (11.56). Variability in number of tillers in ginger genotypes was reported by Dev *et al*. (2016) and Surendrababu *et al*. (2017).

The number of leaves per shoot among the somaclones varied between 16.43 to 28.67. The somaclone CHP 118 recorded the highest number of leaves per shoot (28.67) and was on par with SE 8626 (28.33). Lowest number of leaves per shoot was observed in the somaclones C 8626 (16.43) followed by CHP 99(16.87) and CHP 282 (16.93). Karthik *et al*. (2017) reported that the number of leaves per tiller ranged from 12.02 (T9- Acc-723) to 20.07 (T5-Acc-219) among the different germplasm studied. At 6 MAP, total number of leaves per plant ranged from 86.44 (CHP 99) and 117.33 (CHP 118). CHP 118 recorded maximum number of leaves per plant (117.33) and was on par with SE 8626 (108.33), SE 8642 (108.11) and C 8632 (107.33). Number of leaves increased with growth stages upto 6 month of planting and later decreased

Leaf area influences the photosynthetic efficiency of plants. The somaclone C 8632 recorded the highest leaf area (62.12 cm^2) at six month stage followed by SE 8642 (58.46 cm^2) and SE 8626 (55.14cm^2). These results are comparable with the findings of Surendrababu *et al*. (2017), where the leaf area per plant in ginger varieties ranged from 22.88 cm^2 to 32.47 cm^2 which might be due to the differences in leaf length and width as the age of the plant advances and due to environmental conditions.

3.2 Rhizome characters

Variability in rhizome characters such as number of primary and secondary rhizomes, weight of mother rhizome, weight of primary and secondary rhizomes and rhizome yield per plant, plot and hectare observed at full maturity is presented in Table 3. There was significant difference among the somaclones on the number of primary rhizomes and the highest value (4.83) was recorded by the CHP 118 and was on par with SE 8626 (4.33) and C 8632 (4.08). The number of secondary rhizomes was also found maximum for CHP 118 with a value of 10.33 and was on par with SE 8626 (10.06) and C 8632 (10.01). The weight of mother rhizome varied from 5.60 to 11.00 grams in the study recording the highest value (11.00 g) in the somaclone SE 86 42 which was on par with CHP 118 (10.67g). Lowest mother rhizome weight (5.60 g) was recorded in Rio-de-Janeiro. The average weight of primary rhizome showed significant variation among the somaclones and the highest value (15.73 g) was recorded for SE 8642 and was on par with SE 8626 (15.07 g) and CHP 118 (14.80 g) followed by C 8632 (14.01 g). SE 86102 recorded lowest value (8.10 g) for this character. The highest value (13.20 g) for secondary rhizome was recorded for SE 8626 which was on par with SE 86 42 (12.33 g) and the lowest was for CHP 282 (6.33 g). Such variation with respect to rhizome characters has been reported earlier by Chongtham *et al*., (2013) and Dev (2013).

TABLE 3
RHIZOME CHARACTERS OF GINGER SOMACLONES

Somaclones	Number of fingers		Weight of fingers (g)			Fresh yield	
	Primary	Secondary	Mother	Primary	Secondary	Yield / plant (g)	Yield / plot (kg)
SE 86 26	4.33	10.06	9.67	15.07	13.20	266.67	8.54
SE 86 83	3.33	7.33	7.43	11.40	8.20	145.33	4.65
C 8626	3.33	6.33	8.10	10.47	7.47	201.93	6.48
CHP 118	4.83	10.33	10.67	14.80	11.21	274.13	8.77
C 78 284	3.01	8.67	8.80	11.07	8.43	209.67	6.51
SE 86 102	2.98	7.67	6.03	8.10	7.42	176.47	5.65
SE 86 42	3.98	8.33	11.00	15.73	12.33	251.67	8.06
C 86 32	4.08	10.01	9.23	14.01	10.40	259.67	8.31
CHP 99	2.52	6.00	7.90	11.13	7.10	131.53	4.21
CHP282	3.33	6.00	6.27	11.30	6.33	206.27	6.60
Rio-de-Janeiro	3.67	8.33	5.60	11.28	8.87	196.67	6.29
Himachal	3.33	8.67	7.83	10.43	9.10	187.73	6.01
Aswathy	3.42	8.67	7.80	11.43	8.17	201.20	6.43
CD (0.05)	0.75	1.22	0.52	1.25	1.08	29.71	1.05

Significant difference was observed among somaclones for yield characters recorded at full maturity stage (Table 3). The highest fresh rhizome yield per plant (274.13 g) was recorded by somaclone CHP 118 which was on par with SE 8626 (266.67 g), C 8632 (259.67g) and SE 8642 (251.67 g) at harvest. The highest yield per plot (8.77 kg) was recorded by somaclone CHP 118 and was on par with SE 86 26 (8.54 kg), C 8632 (8.31 kg) and SE 8642 (8.06 kg). Per hectare yield was found maximum for the somaclone CHP 118 (35.08 t), SE 86 26 (34.16 t), C 8632 (33.24 t) and SE 8642 (32.24 t) compared to other somaclones and check varities (Fig 1). The higher yield obtained in these ginger somaclones were due to higher number of tillers and rhizomes and also due to higher weight of rhizomes. The rhizome yield of ginger is an outcome of good rhizome characters such as number and weight of primary, secondary and mother rhizomes as reported by Sangeetha and Subramanian (2015). In the present study, 60 per cent of the somaclones yielded higher than check varieties and the highest yield increase was 40 percentage. This is also in accordance with the study of Kankanawadi (2015) who reported variability in rhizome yield in ginger somaclones. Superiority of somaclones over conventionally propagated plants for rhizome characters and yield was reported by Dev (2013) and Resmi and Shylaja (2012).

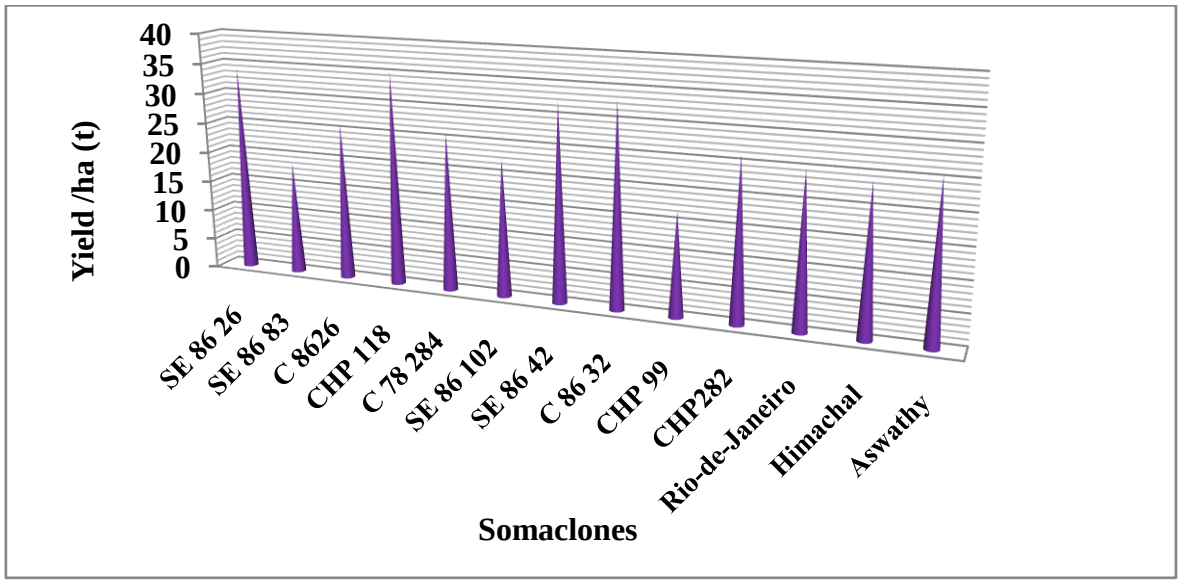

FIG 1. Yield of ginger somaclones (t/ha)

IV. CONCLUSION

The present study concluded that somaclones were highly variable in their performance, yield and yield attributes. Among the somaclones studied, CHP 118 showed significant performance followed by SE 8626, C 8632 and SE 8642 with respect to growth and yield parameters compared to other somaclones and check varieties. They can be rated as the best since they possess the higher rhizome yield and found suitable for cultivation and seed production in Kerala condition. Yield contributing attributes may be taken care of in selecting variety for high yield.

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Investigation of disposal processes by manufacturing companies in Gaborone

Dikabo Mogopodi[1], Banyaladzi Paphane[2], Terrance B. Mmolawa[3], Oratile Lenamile[4]

Department of Basic Sciences, Botswana University of Agriculture and Natural Resources, Private Bag 0027, Gaborone, Botswana

Abstract— *Industrial processes create variety of solid and liquid wastes; which may contain pollutants that have potential harm to humans, animals and the environment. Hence the challenge for disposal of waste for industries cannot be ignored. In these studies, a survey was conducted in the capital city of Botswana; Gaborone to investigate waste management practices for selected manufacturing companies. The findings indicate that companies disposed waste in different ways; 50% out-sourced waste collection services, while 8.3% disposed to waste treatment plants, 33.3% in sewage lines and the other 8.3% in the open flat land. Only 33.3% uses ponds to deal with its effluent and consequently recycle it. Of the companies surveyed, 33.3% of the companies indicated that they have hazardous wastes. Preliminary investigations on contaminates that find their way into the city's sewage system shows that, Pb concentrations did not exceed maximum allowable concentrations of 5.0 mg/L, whereas Cd and Cr concentrations exceeded the maximum allowable concentration of 0.01 mg/l and 0.1 mg/l in irrigation water respectively.*

Keywords— Effluent, pollutants, waste management.

I. INTRODUCTION

Gaborone, the capital city of Botswana has experienced rapid growth in its economy and in its population. With the rapid industrial development and expansion of cities comes the increased challenge of waste generation and hence waste management. Therefore, appropriate waste handling, storage, collection and disposal practices become necessary in order to minimise environmental and public health risks. Improperly managed waste usually results in downstream costs being higher than what it would have cost to manage waste properly in the first place [1]. In Africa data on waste management is not readily available and in Botswana a few studies have focused on waste management [2,3] and none of these have exclusively concentrated on waste generated by industries. There is however a rational agreement as evidenced by waste management strategy and policy development by Botswana government that, inefficient waste management in the country threatens public health. With industries being the fundamental waste generators, it is of critical importance to determine ways in which they manage their waste particularly in the city in order to analyze industry trends and implement appropriate policy mechanisms. A report by Botswana central statistics office [4] has indicated that the greatest waste generators are urban areas and this is associated with rising demands for goods in urban areas. The city of Gaborone alone generates 85 tonnes of waste per day [5]. Waste generation is estimated to increase at a rate of 7 % per annum [6].

Industrial waste is a cause for concern as it is a common cause of pollution. Common contaminants which are either organic or inorganic include petroleum hydrocarbons, polychlorobiphenyls, polycyclic aromatic hydrocarbons, [7] heavy metals [8], surfactants, toxins and salts [9] which can be introduced into the essential elements of the ecosystem including soil, water and biodiversity causing severe degradation in the ecosystem. A wide variety of unsafe inorganic contaminants which may be produced include heavy metals, such as arsenic, lead, cadmium, and mercury. These metals can accumulate in agricultural soils; become available for plant uptake and run off into water ways. As crops and plants extract these toxic metals from the soil and enter the food supply chain, the chance of impacts on human health increases. These metals are known to be potentially toxic to humans contributing to cancer, developmental effects, birth defects, reproductive problems and liver and kidney damage [10]. Industrial waste of organic composition on the other hand can result in the presence of excess nutrients in water which consequently lead to algal blooms, oxygen deficits and increase in color and turbidity in water sources [11]. The understanding of waste management process in companies is therefore an important step in ensuring human and environmental health. The goal is to minimize the pollution introduced into natural waterways and into the environment.

Scarcity of water is another major problem in Botswana [12]. The industrial processes utilize a lot of water as raw material and consumption often exceed capacity to replenish water. Thus industries play a major role and are hence significant from the water consumption and effluent discharge point of view. Amongst other types of waste produced by industries, industrial

effluent is one of the main important ones. If the effluents are discharged into natural watercourses, surface and ground water pollution will result. This will be a huge problem in Botswana as the country is largely dependent on groundwater sources for their livelihood, particularly the farming community and rural populations in Botswana. The introduction of toxic substances from industrial effluents to agricultural environments will only add to their concentrations in underground water and cause damage to aquifers which the country is working so hard to protect. To overcome challenges associated with waste management and raw materials depletion, many industries are following the hierarchy of waste management; that is reducing the waste quantity, reusing or recycling and often recovering their waste as well treating their waste before disposal. If these strategies are implemented, Botswana will maintain its pristine and healthy environment to be enjoyed by future generations. Therefore from this view point a study was carried out to investigate industrial waste management practices in selected industries in Gaborone and assess whether industries are knowledgeable in waste management practices.

II. MATERIALS AND METHODS

2.1 Survey of waste management practices for selected manufacturing companies

2.1.1 Description of the sample and Research design

The target population was 15 industries which included manufacturing industries and one water treatment plant in Gaborone, the capital city of Botswana. The companies were picked from different locations of Gaborone; 5 from Gaborone north, 5 from Gaborone central and 5 from Gaborone South even though only 12 questionnaires were returned. The companies included battery manufacturing companies, chemical producing companies, food processing (which were poultry processing, milling company and drink producing company) and those of non-food solid products (that is cement producing, plastic and soap industries) as well as fibre glass processing company. The researchers used quantitative non-experimental design for sampling and research design (Johnson and Christensen, 2000).

2.1.2 Sampling procedure

The research design used was case study to allow an interactive data gathering of data. Probability sampling method in the form of simple random sampling was used to select 15 industries as the population study. Then seven of the industries which were part of the sample were chosen using purposive sampling so as to obtain homogenous groups of manufacturing industries. The survey was directed to staff holding managerial or supervisory posts or middle management.

2.1.3 Survey Instrument and Design

A self-administered questionnaire was used to collect data from the sampled companies in order to obtain information about participant's feelings, perceptions and attitudes. The questionnaire consisted of two parts of closed ended questions. The first part was used to describe the demographic characteristics of the respondents such as gender, while the second part was used to enquire on waste handling and disposal processes. The questions were tested for content validity by circulating it among 5 lecturers at the Botswana University of Agriculture and Natural Resources, Basic Science Department to examine the questionnaire for errors and for content validity. The reliability of the instrument was determined by conducting a pilot test on 3 industries which were not part of the sample but similar to the sampled group in Gaborone area. The feedback from the pilot test was used to improve the final questionnaire. The format of reliability that was used is test re-test. Then results of the 2 test re-test were correlated to test the consistency of the respondents on the same questions.

2.1.4 Data collection procedure

Data was collected through a self-administered questionnaire to provide a personal conduct with the participants.

2.1.5 Data analysis technique

Statistical Package for Social Sciences version 16 (SPSS) was used to analyze quantitative data. Descriptive analyses in the form of frequency were used on demographic data and on likert scale responses.

2.2 Water and plant analysis of samples collected from sewage plant treatment

2.2.1 Sampling

Because companies indicated disposal of effluent to the Gaborone City's sewage network, a study was conducted to quantify the heavy metals in present in treated sewage water collected from the irrigation system at Oodifarms.

2.2.2 Laboratory Analysis of Sewage Water

The water samples were filtered through a 0.45 mm whatman filter paper to remove all the suspended solids and acidified with 2% nitric acid. The minerals in water were analyzed according to the method developed by American Public Health Association (1976), modified by Lewis (1987). Stock standards (certified single elements purchased from Sigma Aldrich) for Pb, Cd and Cr were used to prepare working standards. For calibration purposes, four standards were used and ultra-pure water acidified with 2 % HNO3 was used as a calibration blank. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine heavy metal levels in sewage water.

III. RESULTS AND DISCUSSION

3.1 Demographics characteristics of respondents

Results as shown in TABLE 1 indicate the demographic characteristics of respondents. Gender data showed some biasness in people holding managerial or supervisory posts from companies in Gaborone because 92% of respondents were males and only 8% were female.

The most common age of participants ranged between18 and 44 years with 67%, followed by 33.3% of age 45 years and above. This could be due to the fact that the working class is concentrated around 18 to 44 years of age. This age bracket represents the youth age within the population with potential to be trained in waste management practices.

TABLE 1
DEMOGRAPHICS CHARACTERISTICS OF RESPONDENTS

Gender	**Frequency**	**Percent**
Male	11	92
Female	1	8
Total	12	100.0
Age categories		
18 - 34 35 - 44 45 - 54 Above 55	4	33
	4	33
	2	17
	2	17
Total	12	100
Occupation		
Sales Manager	2	17
Production Manager	5	42
Managing Director	1	8
Bio-Security Officer	1	8
Risk project manager	2	17
Risk Control Officer	1	8
Total	12	100

3.2 Nature of Companies

The companies were grouped into 3 categories as shown in TABLE 2. The outcome of the study has shown that 75% of the companies sampled produce non-food products. These non-food processing industries heavily relied on the use of chemicals and therefore chemical waste handling and disposal become very important. The main materials used are also shown in TABLE 2; 58 % of the manufacturing companies involved in the study used solids such as fibre glass, cement and crop grains as their main material for production. 33 % used liquid and 8 % used both liquid and micro-organisms especially viruses to make animal vaccines.

TABLE 2
NATURE OF COMPANIES AND TYPE OF MAIN/RAW MATERIALS USED

Nature of company	Frequency	% of total companies surveyed
Food processing	3	25
Non food solid producing	6	50
Non food liquid producing	3	25
Total	12	100
Main materials used		
Liquid	4	33.3
Solid	7	58.3
Liquid and microorganism	1	8.3
Total	12	100

3.3 Classification of waste disposed by companies under study

3.3.1 Classification by state (liquid or solid)

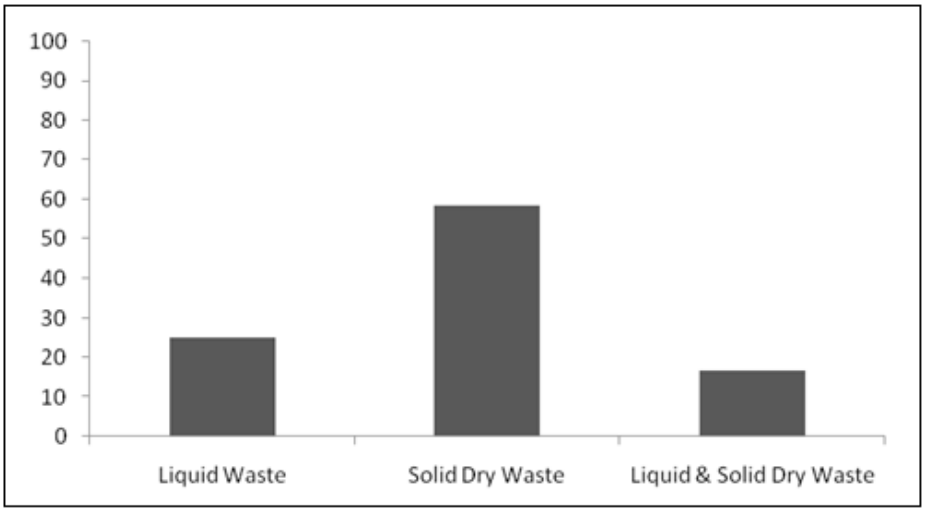

FIGURE 1: Category of waste produced by companies under study

More than 50% of the companies produce solid waste, 25% produce liquid waste and 17% produce both liquid and solid waste as shown in Fig. 1. It is not surprising that the production of solid waste was highest because 58 % of the companies studied used solid as their major raw material. When substantial amount of solid is used this will result in substantial amount of solid waste. A study by Kgati and Bolaane (2001) [13] attributed the deterioration of environmental quality in Botswana to improper solid waste collection and disposal methods used in Botswana. Solid waste is a global problem and in 1996 the Economic Commission for Africa report named Botswana as one of the largest producer of solid waste in Africa.

Some of the solid waste produced has potential of being toxic. Cement for instance, contains a lot of chemicals including calcium oxide, silica, aluminia and iron oxide which have the potential of being environmentally destructive. It contains traces of free crystalline silica, and exposure to respirable free crystalline silica may aggravate lung diseases. Moreover, adding water to cement results in hydration and produces caustic calcium hydroxide which contains trace metals known to cause cancer [14]. Fiberglass dust also is an acute physical irritant to the eyes, skin and respiratory tract. It is normally used with other chemicals during fabrication such as organic peroxide, cobalt compounds and acetone. All these chemicals are health hazards when inhaled and are also flammable or explosive [15]. Moreover the majority of municipal plants in Botswana treat the settled sewage liquid using aerobic biological processes where microorganisms which will only consume biodegradable soluble organic contaminants and leave out the inorganic contaminants. Thus hazardous materials of chemical nature are left untreated. When this happens the inorganic contaminants will move into the food chain and consequently affect living organisms [16].

3.3.2 Classification by nature (hazardous or non-hazardous waste)

Results as shown in Fig. 2 indicated that 33.3% of the companies produce hazardous wastes. Hazardous materials produced include sulphuric acid, clinical wastes, used turpentine, fibre glass off cuts, used viruses and used oil. Production of hazardous waste was not exclusive to non-food industries as some food processing industries also indicated that they produce

hazardous waste. 42% of the companies did not answer the question on whether or not they produce hazardous waste and thus they could not answer subsequent question on how they dispose such hazardous waste. Obviously this is a sensitive issue which could be tied to the respondents knowing that there is existence of non-compliance or it just might be a personal choice. Furthermore, the brewing company indicated that they have measures in place to assess hazards present in effluents and detected contents of their effluent before it is discharged to the municipal sewerage line.

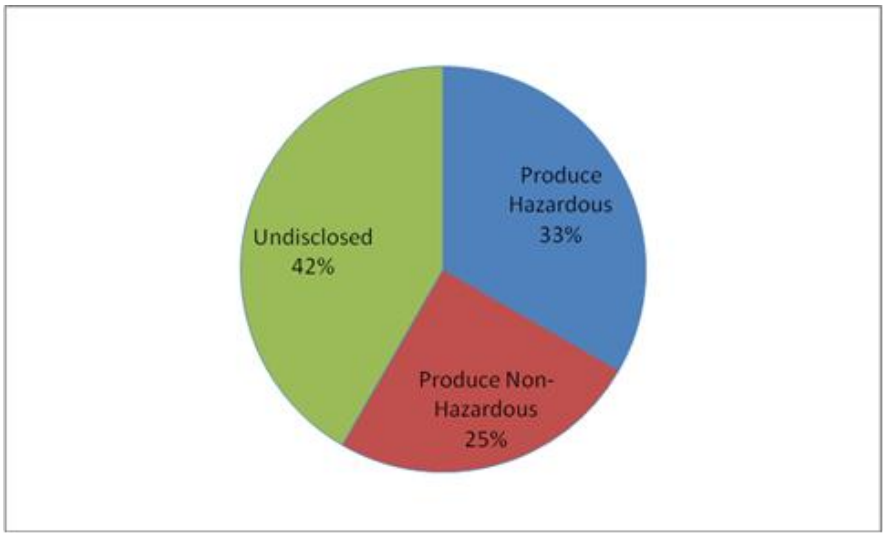

FIGURE 2: Nature of waste produced by companies under study (Hazardous and non-hazardous waste)

3.4 Waste management methods of waste by companies

TABLE 3
WASTE MANAGEMENT METHODS USED BY COMPANIES UNDER STUDY

Waste management methods	Percent
Flash through the drainage system	16.7
Dispose to waste treatment plant	8.3
Use of big containers	16.7
Outsource a company	50.0
Dispose to waste treatment plant &Out source a company	8.3
Total	100.0

A total of 58.3 % of the companies indicated that they out-source services from other companies for waste collection and disposal. This is a smart move because engaging specialists make one to focus on his core business as specialists will take care of the waste including hazardous waste material. 17 % of the companies indicated the use of special containers specially designed for safe handling and transportation of hazardous wastes such as tankers which are used for hazardous liquid waste in order to avoid spillage of such waste in public areas and in trucks. Safe transportation of hazardous waste is an important national issue, the movement of hazardous materials encompasses greater safety concern because hazardous waste can be used for terrorism purpose in this era.

A total of 33.3 % of the companies flash their liquid waste through the drainage where it will be taken to the city sewage waste water treatment plant. The challenge is that these companies do not have instruments which check the properties of this liquid waste. It is vital to have knowledge of waste contents because it assists in separating wastes and knowing how and where to dispose it. When every waste material is disposed inappropriately, chances of pollution are minimised. 17% of the companies used big containers for storage of waste. Containerization is an important aspect in maintaining the integrity of the waste. This waste in containers as the respondents indicated is then subsequently collected by other companies so as to use the waste as raw material for recycling. Some of this waste included paper and plastic material. Materials exchanges are an effective and inexpensive way to find new users and uses for waste. Byproduct recovery as a fall out of manufacturing process creates ample scope for revenue generation thereby offsetting the costs substantially. In Botswana, private sector that are currently recycling generate over 3 million BWP in annual turnover. Recycling plays a big part in the environment particularly when recycling wastes which are non-biodegradable and hard to get rid of. Furthermore the volumes of recycled materials are usually lower than those generated from recycled waste.

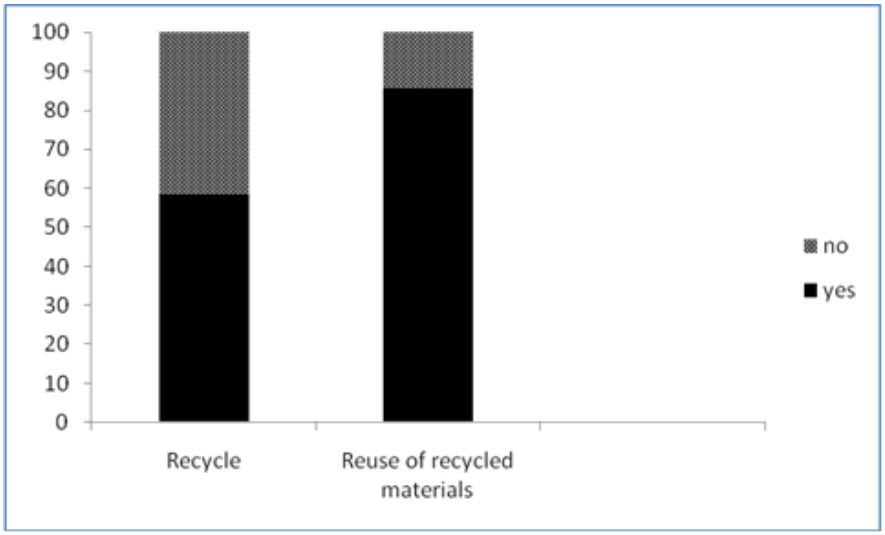

FIGURE 3: Indications of whether companies recycle their materials and whether they re-use materials

As shown in Fig. 3; 58.3 % of the companies indicated that they recycle their waste and 85.7 % of these indicated that they reuse the recycled materials. Of the companies that recycle waste, 17% recycle liquid waste and 33% recycle solid waste. Among this was a brewing industry which indicated that it recycles its effluent and uses it for irrigation and cleaning. Being very rich in organic matters, the utilization of effluents from alcohol manufacturing in agricultural fields creates organic fertilization in the soil which raises the pH of the soil, increases availability of certain nutrients and capability to retain water and also improves the physical structure of soil. Before use, the company indicated that the effluent is diluted 2-3 times before application on crops. The irrigation with brewery industry wastewater seems to be an attractive agricultural practice which not only augments crop yield but also provides a plausible solution for the land disposal of the effluents. One cubic meter of methanated effluent from alcohol brewing contains nearly 5 kg of potassium, 300 grams of nitrogen and 20 grams of phosphorus [17].

More than 80% of participants were in agreement that recycling reduces cost of buying materials as recycled materials can be reused in the company as shown in Fig. 4. Recycling makes industrial processes more resource-efficient.

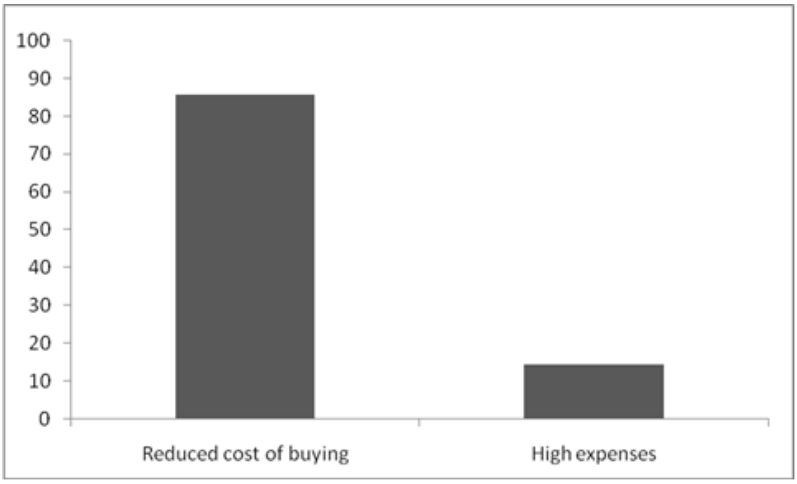

FIGURE 4: Result of recycling materials economic projection

A poultry processing company showed that it uses ponds to deal with its effluent and then treat it to produce water which is reused for irrigation. They also indicated that they produce sludge which is dried and used as organic fertilizer. This can enhance soil nutrients. Poultry manure is known to improve soil retention and uptake of plant nutrients and increases the number and diversity of soil micro-organisms [3] and this is particularly attractive for Botswana soils which are known to have low phosphorous content.

The study indicates that of the companies that had their waste material in a liquid form, 50% treated their waste before disposing it to minimise harm to the end source. 33.3% showed that it uses ponds to deal with its effluent and consequently

recycle it. By treating the effluent prior to disposal, the toxicity is decreased and this can reduce potentially harm to the environment. Treatment can also make a waste amenable for reuse or recycling.

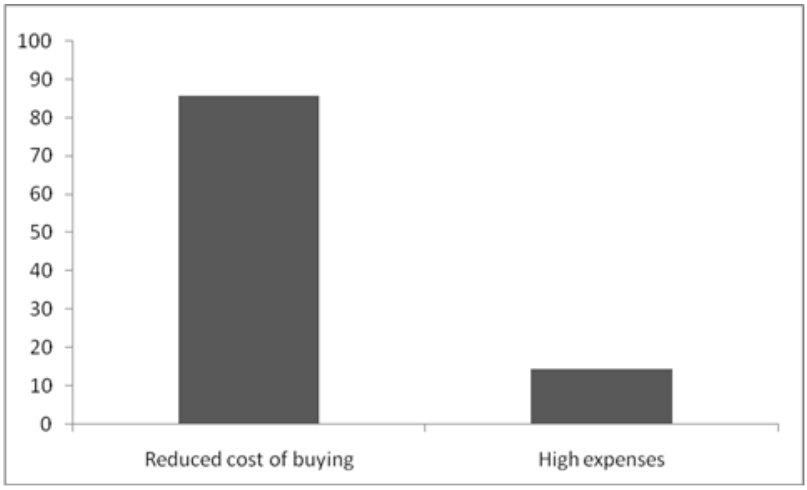

FIGURE 5: Result of recycling materials economic projection

Of the companies that treated disposed effluent, 17 % disposed treated effluent in natural streams, 67 % in sewage lines and the other 17% in the open flat land as shown in Fig. 5. If the effluent is not well treated considering that it goes into natural streams, it has potential to harm living organisms and domesticated animals and plants in that area.

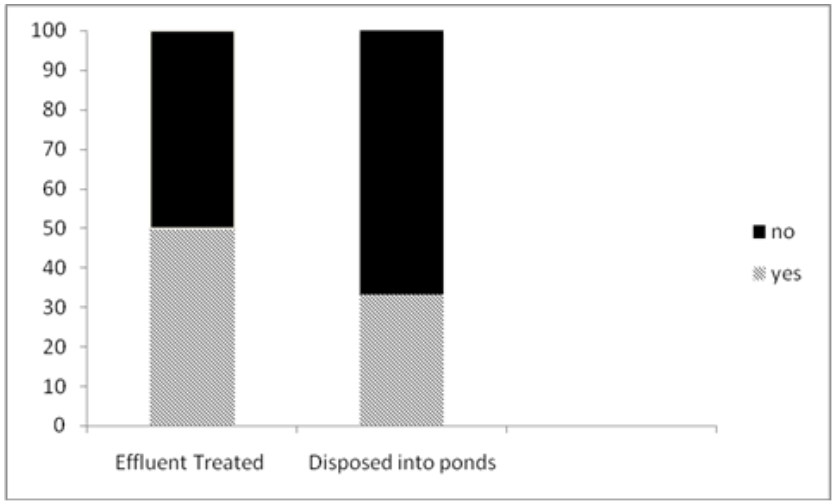

FIGURE 3: Evaluation of treatment and disposal by various industries

3.4.1 Concentration of Heavy Metals in Water collected from the city sewage waste water treatment plant

TABLE 4
HEAVY METALS CONCENTRATION (mg/L) OF SEWAGE WATER

Sample No	Lead	Cadmium	Chromium	Nickel
1	0.13	0.027	0.75	0.16
2	0.11	0.087	0.05	0.03
3	0.16	0.057	0.16	0.06
4	0.04	0.001	0.01	0.01
5	0.09	0.003	0.07	0.05
6	0.35	0.007	0.39	0.03
7	1.31	0.013	0.54	0.07
8	0.21	0.009	0.02	0.02
MRC*	**5.0**	**0.01**	**0.10**	**0.20**

Maximum Recommended Concentration in Irrigation water (FAO, 1992)

Based on the fact that 33.3 % of the companies indicated that they flash their liquid waste through the drain where it will be taken to the city sewage waste water treatment plant, preliminary screening was conducted to quantify the heavy metals in treated sewage water collected from the city sewage waste water treatment plant using ICP-OES. The results were considered significant at $P < 0.05$ levels. The heavy metals concentration ranges of sewage water are as shown in TABLE 4. The data showed that Pb concentration ranged from 0.04 to 1.31 mg/L. whereas, Cd concentration ranged from 0.001 to 0.087 mg/L. Cr concentration ranged from 0.01 to 0.75 mg/L and Ni concentration ranged from 0.01 to 0.16 mg/L. The data were classified into safe and unsafe classes for irrigation by using maximum permissible concentration (MRC) of FAO [18]. This data shows that, Pb concentrations did not exceed maximum permissible concentrations of 5.0 mg/l, whereas Cd and Cr concentrations exceeded the maximum allowable concentration of 0.01 mg/l and 0.1 mg/l in irrigation water respectively.

3.4.2 General perception of participants on waste management

TABLE 5
DESCRIPTIVE STATISTICS FOR GENERAL PERCEPTION QUESTIONS

	N	Minimum	Maximum	Mean	Std. Deviation
Waste is separated into recyclable & non-recyclable before disposal	12	1	4	2.67	1.073
Better to handle effluent by disposing it at water treat plant than treat on site	11	1	4	2.18	1.328
In particular, type of raw material used reduce the difficulty of waste disposal	12	1	4	3.17	1.115
There are special ways of disposing hazardous waste materials so not to harm environment	12	1	4	3.42	.900
The company is currently doing something to minimize amount of effluent disposal	12	1	4	3.17	1.030
There are waste materials in the company which other companies can make use of	12	2	4	3.33	.888
All employees are properly empowered with necessary skills of disposing waste	12	1	4	3.08	1.084
The company flushes all its liquid waste in the city sewage system line	12	1	4	2.17	1.337
There are specific sites licensed to accept waste or effluent	12	1	4	3.33	.985
There are special legal requirements for transporting waste generated by the company	12	1	4	3.25	.965
The ways used by the company to dispose waste meet legal requirement	12	1	4	3.58	.900
Company have difficulty in understanding some waste management laws	11	1	2	1.55	.522

Through the average mean of data analysis in TABLE 5, the general perceptions of the participants was that they are well aware of good waste management practices and were knowledgeable in the laws and standards associated with it. The perception study also showed that participants had knowledge on correct methods of waste disposal as they appreciate the need to separate waste and out-source relevant companies to deal with waste. In a study investigating motivating factors and barriers to recycling behaviour, it was found out that a lack of knowledge and a lack of personal salience and efficacy were barriers that interfered with the motivating effect of a person's sense of responsible action and conservation ethic [19]. Knowledge therefore can be considered the first step in realizing the cultural transformation that is necessary for waste management reforms. Thus based on the companies' awareness on waste management issues it is believed that they will have an internal sense of responsibility to engage in best waste management practices. Companies also perceived recycling waste material as a good practice and agreed that waste has to be separated into recyclable and non recyclable materials as evidenced by mean of 2.67 which suggest that a culture of waste segregation exists. Source separation is important in order to ensure the quality of the final recycled product. It must be noted though despite participants embracing the principles of reclycling, 50% of the companies showed that they do not recycle. This suggests that they could be barriers that exist such as lack of finances which need to be investigated. Companies are of the view that their waste materials can be useful to others companies shown by a mean of 3.33. Companies agreed that the type of raw material has an effect on the method of waste disposal shown by a mean of 3.17. All the companies have indicated that they regularly sensitise their workers about waste (mean 3.08).

IV. CONCLUSIONS

The study is an important step in appreciating waste management issues in the industry. It can be seen from the results of the survey that companies are knowledgeable in waste management practices. This results of sewage water analysis showed that Cd and Cr concentrations exceeded the maximum allowable concentration of 0.01 mg/l and 0.1 mg/l in irrigation water respectively. Further work needs to be done to probe into the quantities of waste generated, companies' waste management policies and investigate on barriers that may hinder the adoption of good waste management practices. Similar studies should be conducted in rural areas in order to develop appropriate interventions. The current findings can be used as a foundation to facilitate more effective and appropriate waste management practices like recycling and reuse.

ACKNOWLEDGEMENTS

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Desalination Property of Various Calcined Layered Double Hydroxides from Seawater

Daiki Tsutsui[1], Takaaki Wajima[2*]

Department of Urban Environment Systems, Chiba University, Japan

***Abstract*—** *Now, approximately 20% of farmland in the world becomes salt damage soil with unsuitable properties for agriculture. In general, salt was removed from soil by flushing out with rain water due to the improvement of soil permeability using gypsum and so on. However, there are arid and semi-arid areas with an insufficient supply amount of rain water to remove salts from soil. In this study, a novel method to capture salt in soil using various calcined layered double hydroxides (LDHs) as a desalination agent was attempted to estimate desalination property from seawater. 4 kinds of LDH with the different M^{2+}/M^{3+} ratios are prepared using 2 kinds of Mg^{2+} and Ca^{2+} as M^{2+} and 2 kinds of Al^{3+} and Fe^{3+} as M^{3+}. The desalination ability of these calcined LDHs was investigated using seawater. As a result, the decrease of salinity was confirmed using all samples. Among these samples, the calcined Mg-Al LDH with Mg/Al = 3.45 and Ca-Fe LDH with Ca/Fe = 2.35 indicated the highest desalination property, due to the decrease of Cl^- and SO_4^{2-} from seawater, by reconstruction reaction. Ca-Fe LDH was calcined at various temperatures and the desalination ability at different calcined temperatures was also evaluated. It was found that the desalination ability of calcined LDH depends on the calcination temperatures and Ca-Fe LDH calcined at 500℃ indicated the highest desalination ability.*

***Keywords*— Seawater, Desalination agent, Calcined LDH, M^{2+}/M^{3+} composition, Calcination temperature.**

I. INTRODUCTION

In the 21st century, global environmental issues are very serious, and effective utilization of energy and mineral resource and the securing of food and water are urgent problems.

Now, there are about 1/5 of salt-damaged soils in the world farmland. The demand for food is increasing due to the global population growth, and production of a stable supply of food is essential to sustain human life. Securing farmland is one of the most important factors in food production. Furthermore, in 2011, great earthquake occurred in Tohoku area of Japan and farmland in Tohoku was damaged by Tsunami. A lot of farmlands are damaged by salt, and it is desired to improve the salt-damaged soil into plantable soil.

There are some desalination methods, such as leaching, elution, disposal of outer layer soil, and salt absorption due to the halotolerant plant. Now, leaching method, which makes farmlands good water permeability and flush out salt with water, is a popular desalination method to improve salt-damaged farmlands. However, the method needs a long time to improve the salt damaged soil completely, and depends on weather. There are some studies to improve salt-damaged soil rapidly. For example, electorostatic desalination was studied, but this technology is expensive and needs a large space.

In this study, we develop a new salt-damaged soil improving agent prepared from calcined layered double hydroxide (LDH). Chemical formula of LDH expresses as $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2][A^{n-}_{x/n}\bullet mH_2O]$ (M^{2+}: divalent metal ions, M^{3+}: trivalent metal ions, A^{n-}: anionic species, x = 0 - 1), and composed of metal complex hydroxide known as inorganic anion exchangers [1]. Hydrotalcite is well known for Mg/Al type LDH, and the uptake of anions onto hydrotalcites from aqueous solution was occurred by two mechanisms: (1) intercalation by anion exchange; (2) intercalation by reformation of calcined samples [2]. Intercalation by anion exchange was used for the removal of the anion or oxi-anions, such as H_3AsO_4, from water solution [3]. Intercalation by reformation of calcined LDH was also used for anion removal, e.g. adsorption of VO_4^{3-} or NO_3^- [4]. In our previous study, calcined hydrotalcite could desalinate seawater and the solution could use for plant growth by combination with natural zeolite treatment [5]. However, little information can be available on desalination property of calcined LDH. The objective of this study was to investigate the desalination properties of various calcined LDH from seawater.

II. MATERIAL AND METHOD

2.1 Preparation of various calcined LDH

Four kinds of LDH are prepared with the different M^{2+}/M^{3+} ratios (M^{2+} : M^{3+} = 1-6) using two kinds of Mg^{2+} **a**nd Ca^{2+} as M^{2+} and two kinds of Al^{3+} and Fe^{3+} as M^{3+}, as follow. The mixed solution of M^{2+} (0. 1 - 0. 6 mol) and M^{3+} (0. 1 mol) was prepared using $M^{2+}(NO_3)_2$ and $M^{3+}(NO_3)_3$ to synthesize the product with M^{2+}/M^{3+} molar ratio of 1 - 6. The M^{2+}/M^{3+} mixed solution was quantitatively added to 0.3 M $NaNO_3$ solution at pH 12.5 under stirring at 50 ℃ with N_2 bubbling. In order to maintain pH of the solution (12.5), NaOH solution was slowly dropped in the stirring solution. The mixed solution was stirred for 6 hours, and then solids were obtained by a vacuum filtration method. The filtrated solid washed with distilled water and then dried at 50 ℃ overnight to obtain the LDH with various M^{2+}/M^{3+} ratio. The calcined LDH was obtained by heating each LDH at 450 ℃ for an hour in an electric furnace [6, 7]. To estimate calcined LDH with the highest desalination ability for Ca-Fe LDH, the Ca-Fe LDH was calcined at 100-900^{o}C.

Identification of crystal structure of the products and calcined products were carried out with a X-ray diffraction (XRD) equipment (Rigaku, MiniFlex 600). M^{2+}/M^{3+} ratio in the structure were investigated as follow. 0.1 g of samples were dissolved in 10 mL of 1 M HCl solution by shaking for 24 hours with Recipro Shaker SR-1 (TAITEC), and then the contents of Mg, Ca, Al and Fe in the solution were analyzed using atomic absorption spectrometry (AAS) (Perkin Elmer, Analyst 200) to calculate the M^{2+}/M^{3+} molar ratio of the samples.

2.2 Desalination property

The desalination abilities of each calcined LDH were investigated using seawater obtained from the surface of Imari bay, Saga prefecture, Japan. The chemical composition, pH and salinity of seawater used in this study is shown in Table 1.

TABLE 1
CHEMICAL COMPOSITION, PH AND SALINITY OF SEAWATER.

Concentration (mg/L)						pH	Salinity (%)
SO_4^{2-}	Cl^-	Na^+	K^+	Mg^{2+}	Ca^{2+}		
2850	22742	11026	384	1451	377	8.3	3.36

0.1 g of each calcined product was put into 5 mL of seawater in 50 mL of centrifuged tube and shaked for 5 hours. After shaking, salinity of seawater treated with calcined LDH were measured by salinity concentration meter (Lutron, YK–31SA). pH were measured by pH meter (HORIBA, LAQUA). The samples after the experiment were collected by filtration and analyzed by XRD equipment. The chemical composition of seawater after experiment were measured by ion chromatograph (Tosoh, IC-2010), and the amount of each ions removed by calcined LDH, q (mmol/g), was calculated using the following equation (1):

$$q = ((C_0 - C) \cdot V)/w \quad (1)$$

where C_0 and C are the concentrations (mmol/L) of each ion in the initial solution and the measured solution, respectively. V is the volume (L) of the solution, and w is the weight (g) of the sample added to the solution.

The removal percent of salt after treating seawater with each calcined product, R (%), was calculated using the following equation (2):

$$R = (C_0 - C)/C_0 \times 100 \quad (2)$$

III. RESULTS AND DISCUSSION

3.1 Preparation of various calcined LDH

The XRD patterns of the obtained LDH are shown in Fig. 1. It is noted that M^{2+}/M^{3+} molar ratio of each LDH indicates in Fig. 1. In all products, LDH peaks were confirmed, and we succeed to prepare various LDHs with different M^{2+}/M^{3+} molar ratio. Al-LDH peaks were clearer than Fe-LDH peaks. In Ca-LDH, $CaCO_3$ peaks were confirmed in Ca-Al LDH and $Ca(OH)_2$ peaks were confirmed in Ca-Fe LDH.

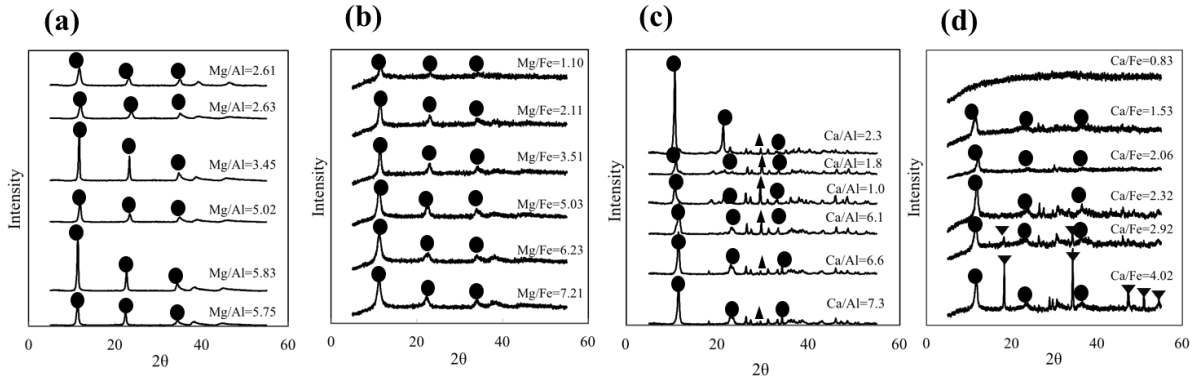

FIGURE 1. XRD Pattern Of the LDHs : (a) Mg-Al LDH, (b) Mg-Fe LDH, (c) Ca-Al LDH, (d) Ca-Fe LDH ●: LDH, ▲: $CaCO_3$, ▼: $Ca(OH)_2$

The XRD patterns of calcined LDH are shown in Fig. 2. In calcined Mg-Al and Mg-Fe LDHs, Mg oxide, Mg-Al oxide or Mg-Fe oxide peaks were confirmed and peak intensity increases with increasing M^{2+}/M^{3+} ratio of calcined LDH, regardless of the peak intensity of LDH before calcination, because the peaks of obtained oxide mainly depends on those of Mg oxide. In calcined Ca-Al or Ca-Fe LDH, $CaCO_3$ peaks were mainly confirmed.

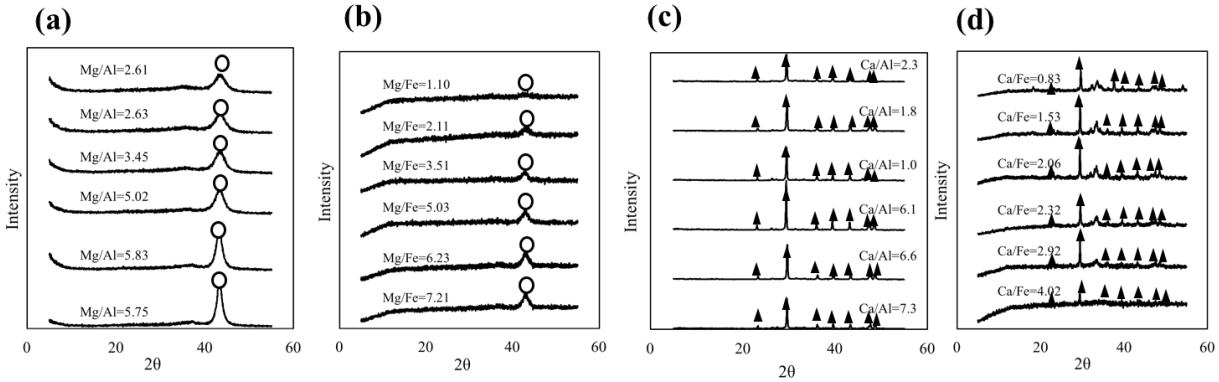

FIGURE 2. XRD Pattern Of the LDHs After Calcination: (a) Mg-Al LDH, (b) Mg-Fe LDH, (c) Ca-Al LDH, (d) Ca-Fe LDH. ○: Mg oxide, Mg-Al oxide or Mg-Fe oxide, ▲: $CaCO_3$

3.2 Desalination ability of calcined products

The removal percent of salt after treating seawater with all calcined products are shown in Fig. 3. The salinity decreased in all seawater after treatment with calcined LDH. Desalination ability of Mg-Al, Mg-Fe, and Ca-Al LDH are almost constant regardless of M^{2+}/M^{3+}, while desalination ability of Ca-Fe LDH increases with increasing Ca/Fe to about 2.0 and then decreases.

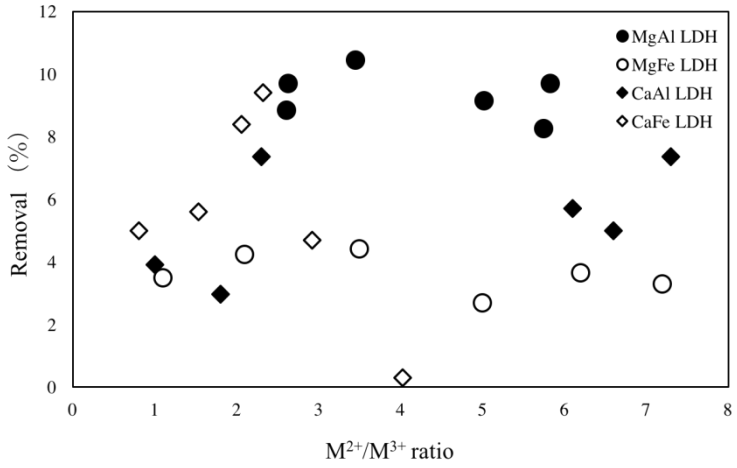

FIGURE 3. Removal of Seawater Treated With All Calcined LDHs

XRD patterns of the product after desalination tests are shown in Fig. 4. The structure of calcined LDH returned to that of LDH by reformation reaction. It was confirmed that all products remove salts in seawater by reformation reaction. It is noted that CaCO3 was confirmed in Ca-LDH after desalination test.

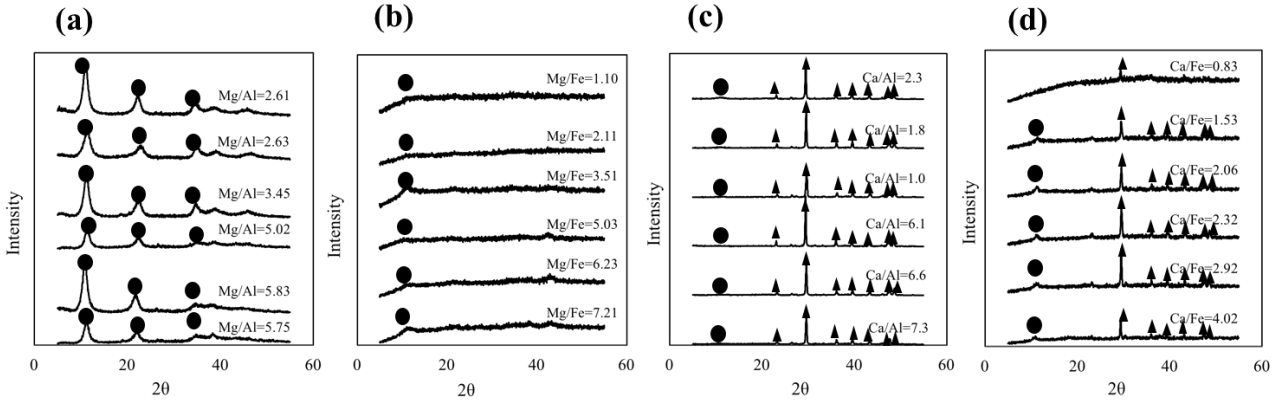

FIGURE 4. XRD Pattern Of the Calcined LDHs After Desalination Test: (a) Mg-Al LDH, (b) Mg-Fe LDH, (c) Ca-Al LDH, (d) Ca-Fe LDH. ●: LDH, ▲: $CaCO_3$

The highest removal of seawater treated with each M^{2+}/M^{3+} products after calcination are shown in Fig. 5. The calcined LDHs with about M^{2+}/M^{3+} = 3.5 have high desalination ability in Mg-LDHs, while there with about M^{2+}/M^{3+} = 2.3 have high ability in Ca-LDH. The highest removal of salt from seawater is approximately 10% using the Mg-Al LDH with Mg/Al = 3.45 and Ca-Fe LDH with Ca/Fe = 2.32.

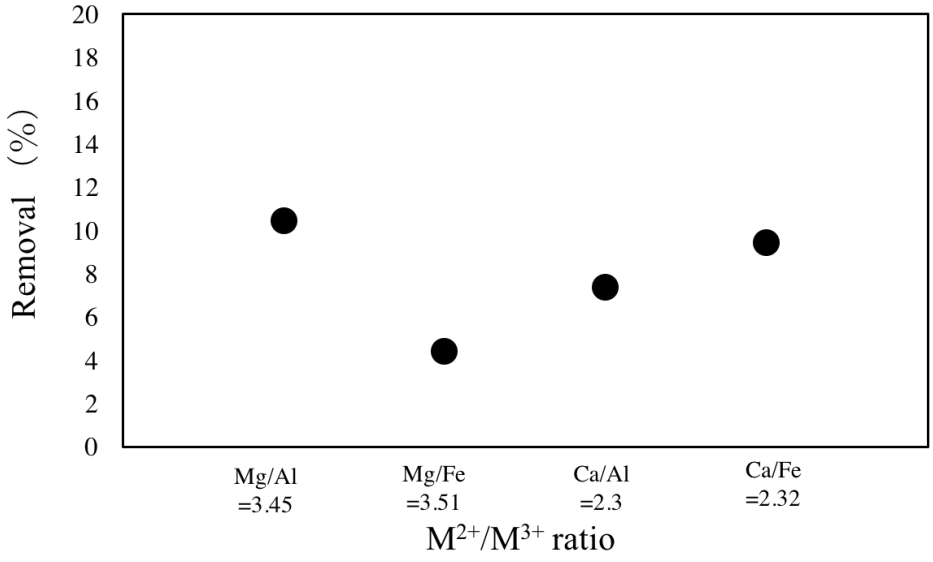

FIGURE 5. Removal of Seawater Treated With the Highest Each Calcined LDH

Removal amounts of each element in seawater by calcined LDHs are shown in Fig. 6. It is noted that pHs of the seawater after desalination test was about 10 using there calcined LDHs, are shown in Fig. 7. For Mg-Al LDH, Cl^- and Mg^{2+} mainly decrease, and for Mg-Fe LDH, Cl- and Na^+ mainly decrease. For Ca- LDH, Cl^-, Na^+ and Mg^{2+} mainly decrease. The decreases of salinity using calcined LDHs are due to the removal of Na^+, Cl^- and Mg^{2+} from seawater. It may be considered that removal of Cl^- was caused by LDH reformation reaction and removal of Na^+ and Mg^{2+} were caused by increasing pH of the seawater. The removal amounts of Cl^-, Na^+ and Mg^{2+} in seawater using Ca-LDH were higher than those using Mg-LDH, while desalination ability of Ca-LDH is lower than that of Mg-LDH. It may be caused by increasing Ca^{2+} in the seawater after desalination test.

These results suggest that calcined LDH can decrease salinity of seawater due to the removal of Cl^-, Na^+ and Mg^{2+} from seawater.

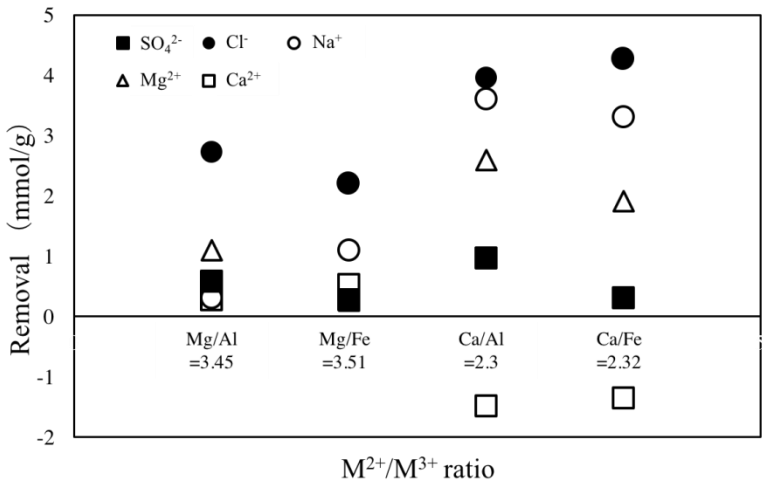

FIGURE 6. Removal Amounts of Each Element in Seawater by Calcined LDH

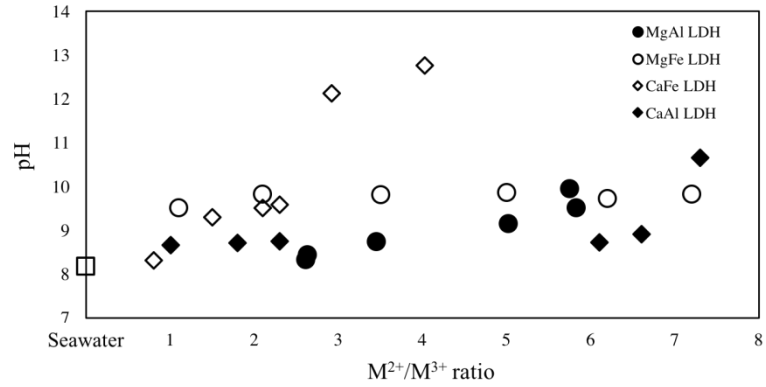

FIGURE 7. PH Of Seawater after Desalination Test

3.3 Desalination ability of Ca-Fe LDH calcined at various temperatures

Because Ca^{2+} and Fe^{3+} are cheaper than Mg^{2+} and Al^{3+}, we select Ca-Fe LDH (Ca/Fe = 2.32) for the best desalination product in this experiment.

The XRD patterns of Ca-Fe LDH calcined at various temperatures are shown in Fig. 8. For calcination at 100 and 300°C, LDH peaks were confirmed and peak intensity decreases as the calcination temperature rises. For calcination temperature over 500 °C, LDH peaks were disappeared above 500 °C and $Ca_2Fe_2O_5$ and CaO appeared at 700 and 900 °C.

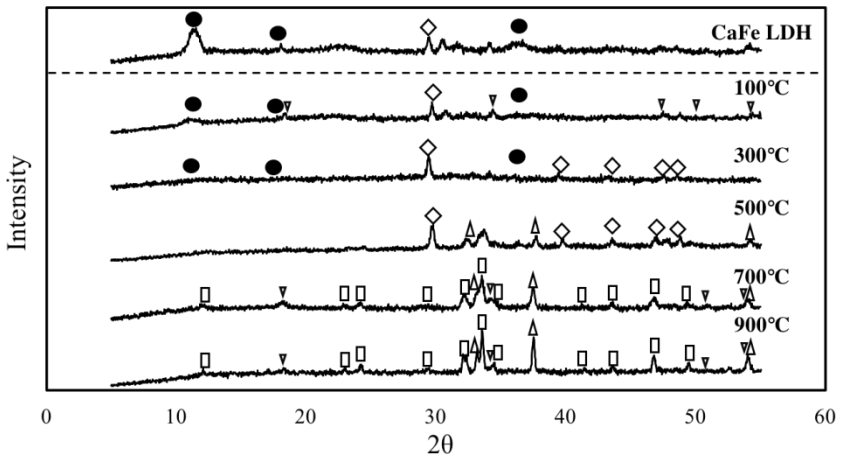

FIGURE 8. XRD Pattern of the Products after Calcination at Various Temperatures

●: LDH, ◇ : $CaCO_3$, ▽ : $Ca(OH)_2$, ◻ : $Ca_2Fe_2O_5$, △ : CaO

XRD patterns of the calcined products after desalination tests are shown in Fig. 9. The structure of calcined LDH returned to that of LDH by reformation reaction. It was confirmed that all products remove salts in seawater by reformation reaction. For LDHs calcined at 100, 300 and 500°C, $CaCO_3$ was confirmed, and for LDHs calcined at 700°C and 900°C, $CaFe_2O_4$ and CaO were confirmed.

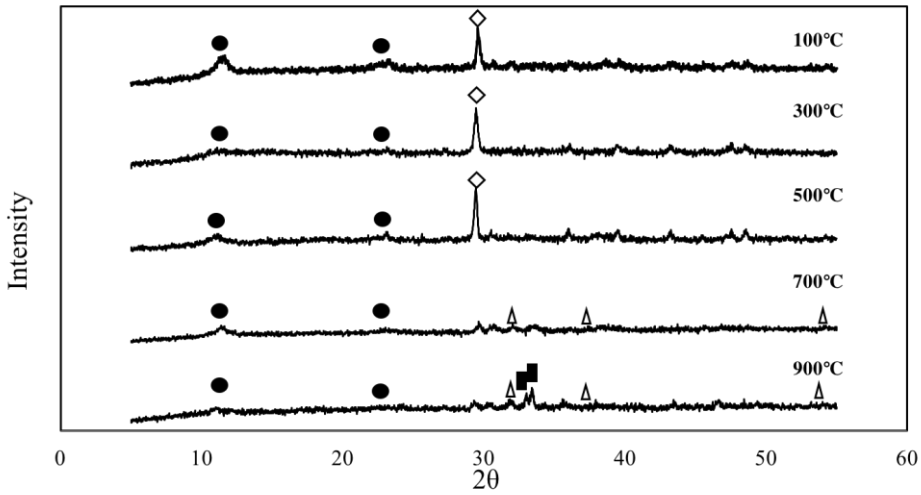

FIGURE 9. XRD Pattern of the Products after Desalination Tests ●: LDH, ◇: $CaCO_3$, △: CaO, ■: $CaFe_2O_4$

The pHs of seawater after desalination test are shown in Fig. 10. For all calcined LDH, the pH increased to 12-13 after desalination tests.

The removal percent of salt after treating seawater with all calcined LDHs are shown in Fig. 11. The salinity decreased in seawater after treatment with all calcined LDHs. The highest removal of salt from seawater is approximately 20% using the Ca-Fe LDH calcined at 500°C.

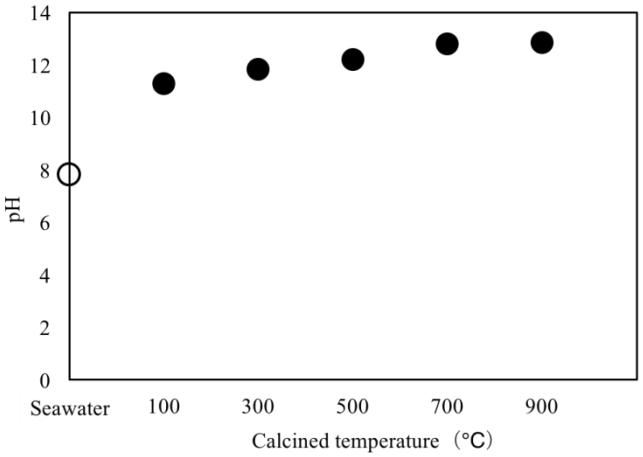

FIGURE 10. PH of Seawater after Desalination Test

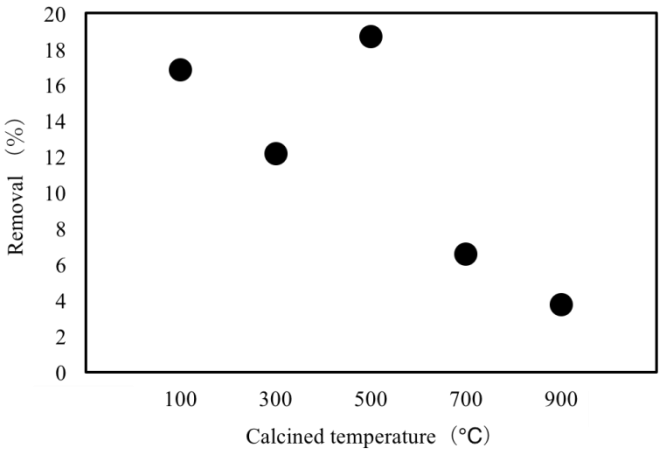

FIGURE 11. Removal of Seawater Treated with Ca-Fe LDH after Calcination at Various Temperatures

Removal amounts of each element in seawater by calcined LDH are shown in Fig. 12. For all calcined LDHs, Cl^-, Na^+ and Mg^{2+} mainly decrease and Ca^{2+} increase. The decreases of salinity are due to the removal of Cl^-, Na^+, and Mg^{2+} from seawater. For LDHs calcined at 100 and 300°C, NO_3^- increased because of NO_3^- release with ion exchange of Ca-Fe LDH. For LDHs calcined at 500, 700 and 900°C, became NO_3^- were not detected in the solution. Removal of Cl^- and SO_4^{2-} were caused by LDH reconstruct and removal of Na^+ and Mg^{2+} were caused by increasing pH of the seawater.

These results also suggest that calcined LDH can decrease salinity of seawater due to the removal of Cl^-, Na^+ and Mg^{2+} from seawater.

From these results, Ca-Fe LDH calcined at 500°C indicated the highest desalination ability.

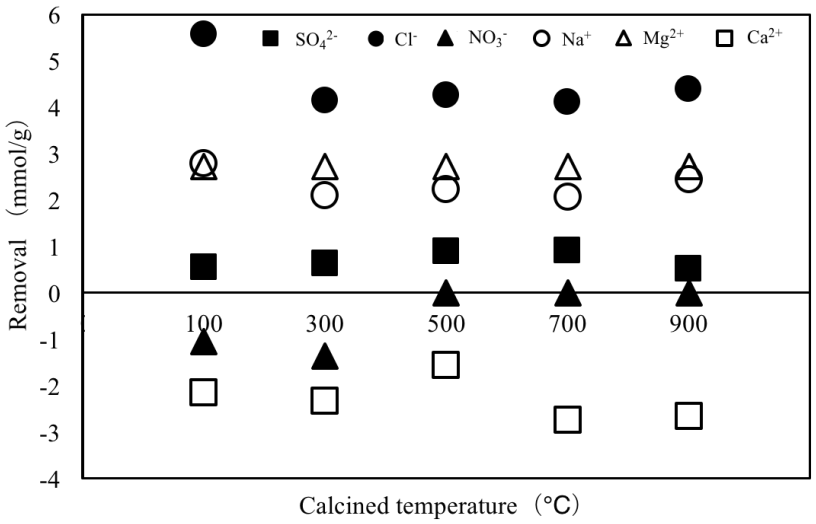

FIGURE 12. Removal Amounts of Each Element in Seawater by Calcined LDHs

IV. CONCLUSION

In this study, we investigated the desalination properties of calcined LDH. As a result, Mg-Al oxide and Mg-Fe oxide peaks confirmed in calcined Mg-LDH and peak intensity of Mg-Al oxide and Mg-Fe oxide increased with increasing Mg^{2+}/M^{3+} ratio of the LDH. In calcined Ca-LDH, calcite peaks were mainly confirmed. After desalination test, for all calcined LDHs, the structure of calcined LDH returned to that of LDH by reformation reaction. In calcined Ca-LDH, calcite was confirmed in the LDHs after desalination test. The salinity decreases in all seawater after treatment with calcined LDH. The highest desalination from seawater were treated with the calcined Mg-Al LDH with Mg/Al = 3.45 and calcined Ca-Fe LDH with Ca/Fe = 2.32, because the structure of calcined LDH can remove high amounts of Cl^-, Na^+ and Mg^{2+} from seawater. The removal amounts of Cl^-, Na^+ and Mg^{2+} in seawater treated with calcined Ca-LDH were more than Mg-LDH. Because Ca^{2+} and Fe^{3+} are cheaper than Mg^{2+} and Al^{3+}, it would be considered that Ca-Fe LDH (Ca/Fe = 2.32) is the best desalination product for desalination in this experiment. For Ca-Fe LDH calcined at various temperatures, salinity decreased in seawater after treatment with all calcined LDHs. The highest removal of salt from seawater is approximately 20% using the Ca-Fe LDH with Ca/Fe = 2.32 calcined at 500^{o}C.

ACKNOWLEDGEMENTS

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Cost effective production of *Bacillus thuringiensis aizawai* and their application against *Spodoptera litura*

R.Sujani[1*], G.Mikunthan[2], V.Jegathambigai[3], A.V.Svinningen[4]

[1,2]Dept. of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Sri Lanka
[3,4]Green farms pvt Ltd, Marawila, Sri Lanka

***Abstract*—** *Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) was prevalent in many species of aquatic plants grown in Green Farms. This study was carried out to understand the production of Bacillus thuringiensis aizawai (Bta) using cost effective method to manage the pest biologically. Bta is being widely used in pest control programs. However, the production of this Bta is expensive due to the high cost of the production medium. In this study, an attempt has made to develop a cost-effective medium, based on a locally available raw materials namely coconut water which is available in plenty as waste product from coconut oil industry, coconut poonac, rice bran and coir dust. A standard conventional Luria Bertani medium was included in the assay for comparison. Media were assessed for the growth, sporulation and production of insecticidal properties of Bta. Coconut poonac extract and coconut water media produced higher spores than compare with conventional LB medium. Maximum spore count of 25.0×1013 spores/mL was obtained with a 72 h old culture of this bacterium grown in coconut poonac extract. Larvicidal activity (LC50) of 8 ×106 spores/mL (coconut poonac extract) against early second-instar larvae of S.litura were obtained. This is almost similar to that obtained with LB (9 × 106 spores/mL) medium. Hence, coconut based culture medium is economical for the production of Bta and compared favorably with the standard. Cost-effective analyses have revealed that production of Bta from test media is highly economical. The cost of production of Bta with local media was significantly reduced by 88-293 fold. The use of nonconventional sources has yielded a new knowledge in this area as the process development aspects of small scale production have been neglected as an area of research. These studies are very important from the point of media optimization for economic production of Bta based agents in pest management programs.*

***Keywords*— Spodoptera litura, Bta, cost effective media, spores, small scale production.**

I. INTRODUCTION

Green Farms Ltd., being one of the biggest exports oriented floriculture industry in Sri Lanka. Recently larvae of *S.litura* emerged as a problem in one of the aquatic plant *Ludwigia repens*. This larva is polyphagous, larval stages caused severe damage on the other variety of aquatic plants. The occurrence of the larvae of *S.litura* on aquatic plants as reported first time in the farm and being new it caused significant damage to the plant resulting to an economic loss of the export section.

S. litura also becomes resistant to many commonly used insecticides, resulting in failure of effective controls (Ahmad et al., 2007). *Bacillus thuringiensis* (*Bt*) is the most successful commercial biopesticide in the biological control market (Glare and Callaghan, 2000) accounting for 90% of all biopesticides sold all over the world. This bacterium is characterized by its ability to produce crystalline inclusions proteins have a great potential to control a great number of pest insects belonging to the order Lepidoptera, Diptera and Coleoptera (Vidyarthi et al., 2002).

Cost to grow and produce *Bt*, through highly refined laboratory bacterial culture medium, is comparatively high than other bio agents. The cost of *Bt* production depends on many factors; however, the raw material cost is one of the most important criteria, which comprises more than 70% of the overall production cost (Ejiofor, 1991). In order to encourage the commercial production of biopesticides, utilization of less expensive raw material is advisable (Mummigatti and Raghunathan, 1990).

II. METHODOLOGY

Micro-organism and materials *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 under the trade name of Xentari obtained from the microbiology lab, Green farms limited, Marawila was used in the present study. The strain was maintained on Yeast Extract Agar Medium (YEAM) plate containing 5 g/L peptic digest of animal tissue, 3 g/L yeast extract and 15 g/L agar. It was stored at 4°C throughout the study. Media preparation Liquid media were used for the experiment while LB medium was used as reference medium.

TABLE 1
MEDIA PREPARATION

Media	Concentration
Luria Bertani, LB (Reference media)	Peptone 20g, yeast extracts 10g and NaCl 20g in 1L distilled water (Poopathi and Archana, 2012)
Coconut water	Fresh liquid
Coconut poonac	50g of poonac was boiled in 1L water for 15 minutes
Rice bran	50g of bran was soaked in 1L water for 2 hours
Coir dust extract	50g of coir dust was boiled in water for 15 minutes

All media were filtered through plastic strainer and pH was adjusted to 7.8 with 10% NaOH. 100 ml of each filtered extract was dispensed separately into 180 ml flat bottles and closed with cotton wool. From the above test, the medium which showed maximum *Bta* biomass production was selected for further studies.

2.1 Sterilization of media

All culture media were autoclaved at 121°C, for 15 minutes.

2.2 Production in flatten flasks, Growth conditions

First stage seed was prepared by inoculating 10 mL of nutrient broth with one loop full of cells from YEAM and incubating on a rotary shaker at 30°C, 180 rpm for a period of 6 h. The seed thus prepared was transferred to 100mL medium in 180mL flat bottles (8 bottles per medium) at 1% level (v/v) and the bottles were incubated on an incubator at 30°C for a period of 96 hours. 2 bottles each were removed at 24 h intervals and used for assessment of sporulation and larvicidal activity. The pH was measured using digital pH meter. The bacterial stages (vegetative to sporulative stage) were also examined by Oil emersion 1000 LED microscope.

2.3 Microscopic studies

Simple staining procedure was used to stain microbial cells. Spores of *Bacillus* species do not stain, and they may be seen as unstained bodies within bacterial cells stained with methylene blue. Smears of *Bacillus* isolates were prepared and they were fixed by heat. The bacterial smears were then flooded with methylene blue. Staining lasted for 5 min. Finally, destaining was performed by washing under the tap water and stained bacterial colonies were observed under an oil emersion objective.

In addition, endospore staining with malatchite green was performed for a better observation of *Bacillus* spores. This staining procedure involved primary staining with malatchite green for 5 min and steam heat to drive the stain into spores. This stain was retained by endospores but washed out of the rest cells with water. Cells were then counterstained with the red dye safranin. The spores appeared green and cells appeared red after staining by this procedure (Tuba, 2002).

2.4 Spore count

For spore count culture samples were heat treated at 80°C for 15 minutes and serially diluted. The appropriately diluted samples were plated on Yeast Extract Agar medium plates and incubated overnight at 30°C to form fully developed colonies. Fully developed colonies were counted and expressed in colony forming units per mL (cfu/mL).

2.5 Measurement of pH change

The pH of all media was adjusted to 7.8 with NaOH before sterilization, and was measured at regular intervals during 96 hours of fermentation process. Two replicates were used each one was measured three times.

2.6 Cost analysis

Cost to prepare 1 l of each medium was calculated. For the comparison, preparation cost in 1L of the LB medium was used.

2.7 Toxicity assessment of *Bacillus thuringiensis aizawai* on *S. litura* (In vitro study) Larvacidal activity

The spore crystal complex produced in different media was assayed against second instar larvae of laboratory-reared *S.litura*. Water primrose (*Ludgwia* spp) leaves were cut and washed once with 0.5% sodium hypochlorite and two times with distilled water. Test solutions were sprayed on both side of leaves. They were allowed to surface-dry on a paper towel and then placed into Petri-dishes containing moistened filter papers to avoid desiccation of leaves. Larval mortality was scored after 24 h and corrected for control mortality, using Abbott's formula (Abbott, 1925). The experiment was done at the temperature of

30±1°C and 75% Relative humidity. Each bioassay included 6 treatments of 2 replicates each, along with the appropriate control. Larval mortality was scored up to 4 days after larvae infection to check pathogenicity. Each treatment was replicated 2 times, along with an untreated control under complete randomized design. Probit regression analysis (Finney, 1971) was carried out to calculate LC50 and LC90 values as well as their 95% fiducial limits.

TABLE 2
CONCENTRATION OF *Bta* ON MASS PRODUCTION OF DIFFERENT MEDIA USED TO CONTROL THE SECOND INSTAR LARVAE OF *S.litura*

Treatments	Concentration
T1	10^6 spores/mL
T2	10^7 spores/mL
T3	10^8 spores/mL
T4	10^9 spores/mL
T5	10^{10} spores/mL
T6	10^{11} spores/mL
T7 (Control)	

2.8 Statistical analysis

All the experiments were designed according to complete randomized design (CRD) and obtained data were statistically analyzed using SAS package and the significance among the treatments were determined according to Dunnett mean separation test at 95% of confidence Interval.

III. RESULTS AND DISCUSSION

3.1 pH change during 96 hours of fermentation

Results showed that the pH varied among the five media during 96 hours of fermentation, and since there was no pH control during the experiments, a pH fluctuation was observed (Figure 1).

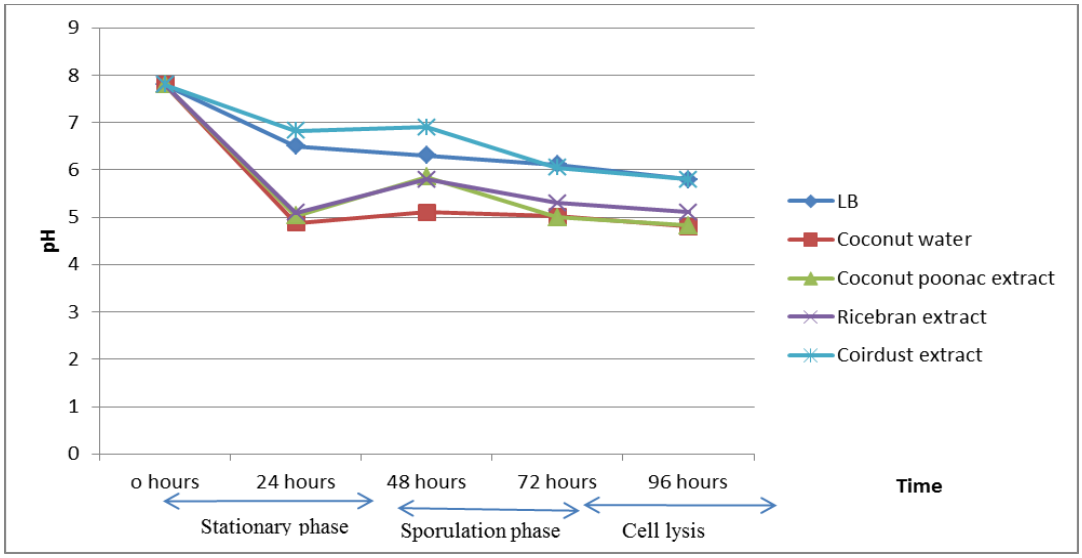


FIGURE 1: pH change on different media during 96 hours of fermentation

LB and coir dust extract media slowly decreased the pH in the first 24 hours, and reaching a minimum pH value of 5.8 within 96 hours of fermentation. Coconut water, coconut poonac extract and rice bran media maintained a very low pH (between 4.8 to 5.8) after 24 hours until the end of fermentation process (Figure 1). Tirado-Montiel *et al*. (2001) stated that this fact is probably due to the utilization of the carbohydrates before the sporulation phase.

Dingman & Stahly (1983) stated that the decrease in pH is due to acid accumulation that results from catabolism of glucose, and this bioassay was added to the media.

3.2 Spore count

TABLE 3
SPORE COUNT OF *Bta* ON DIFFERENT MEDIA AFTER 24, 48, 72, 96 HOURS OF INCUBATION

Locally available agro industrial by product substrates	Mean spore count 10^{13} per mL*			
	After 24 hours	48 hours	72 hours	96 hours
LB (Reference medium)	9.07	8.70	18.17	11.27
Coconut water	7.23	13.27	22.10	18.23
Coconut poonac extract	8.40	15.30	25.70	20.13
Ricebran extract	1.97	4.23	9.30	5.27
Coirdust extract	2.87	5.77	11.23	7.50
Control	0.00	0.00	0.00	0.00

The growth and sporulation of *Bta* in locally available agro industrial by product substrates are shown in table 10. In all five media, the highest number of viable spores was reached after 72 hours of growth. According to this study the highest spore count was recorded after 72 hours of fermentation in coconut poonac extract as 25.70×10^{13}/mL. Next highest spores count was observed in coconut water was 22.10×10^{13}/mL, followed by LB (18.17×10^{13}/mL) and coir dust extract (11.23×10^{13} /mL).

Lowest spore count was recorded in rice bran extract as 1.97×10^{13}/mL after 24 hours of fermentation. Spore production was increased until 72 hours fermentation then amount of spore decreased after 72 hours fermentation.

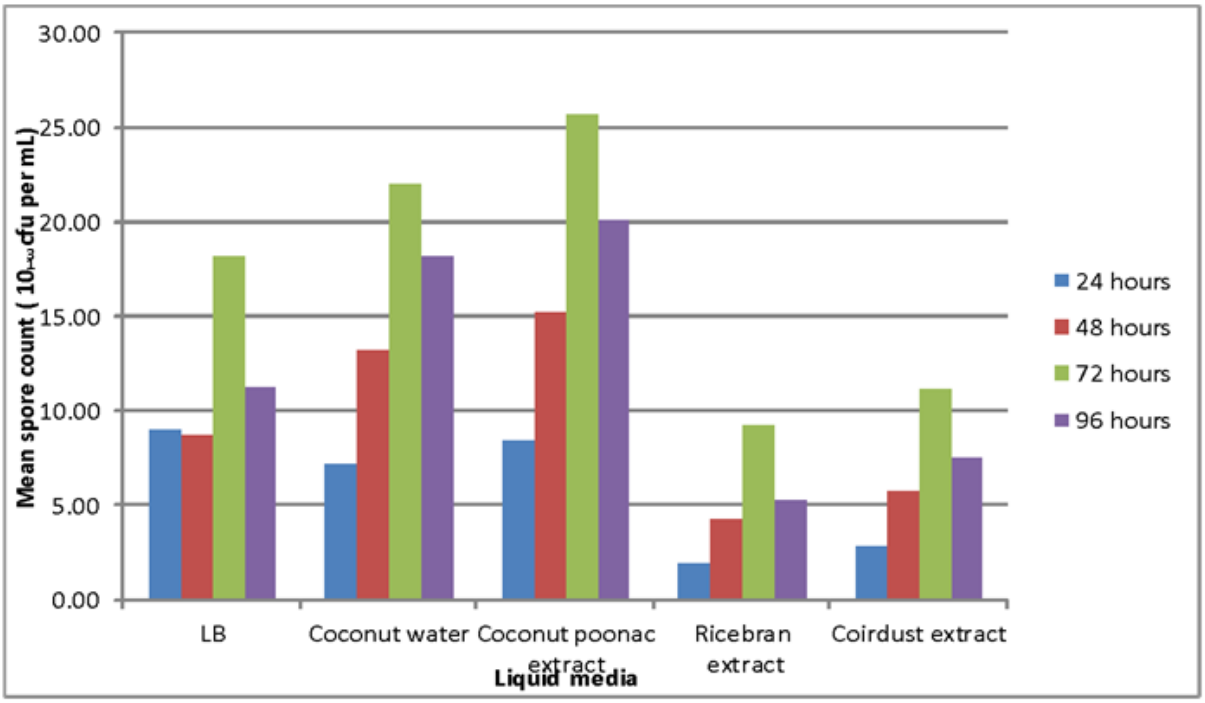
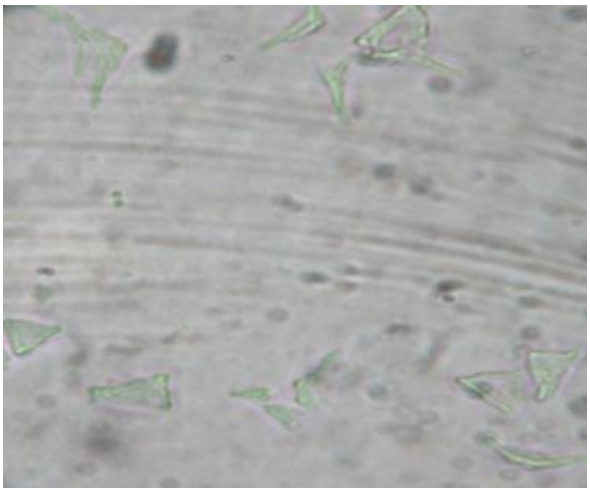


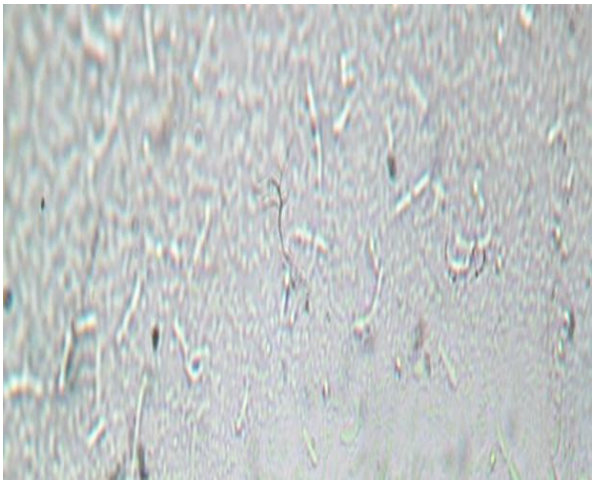
FIGURE 2: Mean spore concentration produced by different media

3.3 Microscopic observation

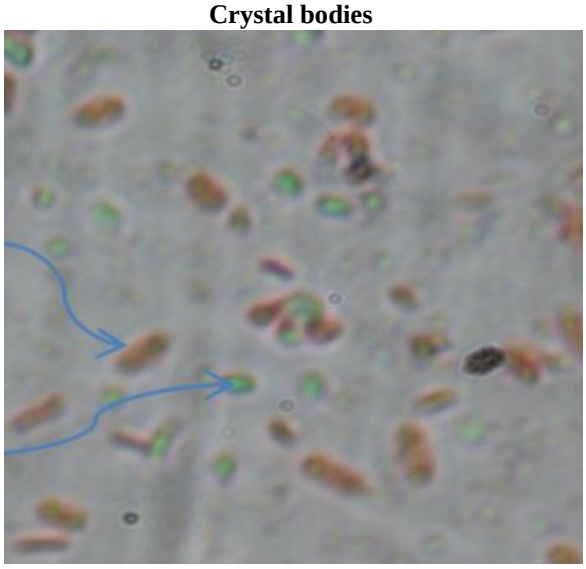
Gram positive, produce ellipsoidal spores that do not distend the sporangium was observed. In the endospore staining with malatchite green, vegetative cells appeared as red and spores appeared as green.



Crystal bodies



vegetative cells



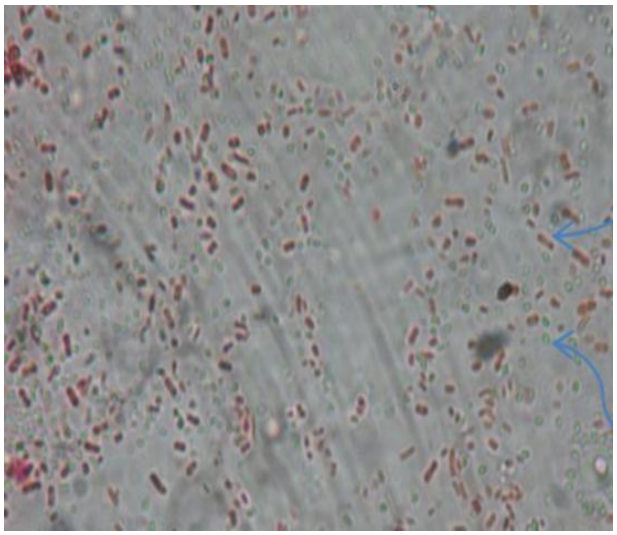


FIGURE 3: Red - vegetative cells Green- spores

3.4 Larvacidal activity

TABLE 4
MEAN MORTALITY PERCENTAGES OF LARVAE OF *S.litura* WITH DIFFERENT CONCENTRATION OF *Bta* SUSPENSION IN STANDARD LB MEDIA

Concentration	Mean mortality Percentage*			
	1DAT	2DAT	3DAT	4DAT
(T1) 10^6 spores/mL	27.08^f	42.71^e	58.33^e	66.66^c
(T2) 10^7 spores/mL	56.25^e	76.04^d	83.33^d	90.62^b
(T3) 10^8 spores/mL	70.83^d	83.33^c	92.71^c	98.96^a
(T4) 10^9 spores/mL	81.25^c	88.54^b	96.87^b	100.00^a
(T5) 10^{10} spores/mL	90.62^b	100.00^a	100.00^a	100.00^a
(T6) 10^{11} spores/mL	98.96^a	100.00^a	100.00^a	100.00^a
(T7) Control	0.00^g	0.00^f	0.00^f	0.00^d

All the values with the means of three replicates

Figures having same letters in a column indicate that the values are not significantly different at 0.05 α.

TABLE 5
MEAN MORTALITY PERCENTAGES OF LARVAE OF *S.litura* WITH DIFFERENT CONCENTRATION OF *Bta* SUSPENSION IN COCONUT POONAC EXTRACT MEDIA

Concentration	Mean mortality Percentage*			
	24 h	**48 h**	**72 h**	**96 h**
(T1) 10^6 spores/mL	27.77^f	46.62^e	57.77^d	66.94^c
(T2) 10^7 spores/mL	54.44^e	69.50^d	78.89^c	85.55^b
(T3) 10^8 spores/mL	73.33^d	84.44^c	93.33^b	100.00^a
(T4) 10^9 spores/mL	84.44^c	95.69^b	100.00^a	100.00^a
(T5) 10^{10} spores/mL	93.33^b	100.00^a	100.00^a	100.00^a
(T6) 10^{11} spores/mL	100.00^a	100.00^a	100.00^a	100.00^a
(T7) Control	0.00^g	0.00^f	0.00^e	0.00^d

All the values with the means of three replicates

Figures having same letters in a column indicate that the values are not significantly different at 0.05.

Paralysis was observed in larvae within 2 h at a 10^{11} spores/mL concentration of each bacterial media. The effects on low concentrations were less severe, at the low concentration; the larvae merely gathered at the top center of the assay cup and stopped feeding.Maximum numbers of dead larvae were recorded in all the *B.thuringiensis subsp. aizawai* media at concentration 10^9 spores/mL after 48 h of treatment.

The concentrations required to kill 50% of the larvae (LC50) coconut poonac extract (8.1 × 106 spores/mL) indicated that the two locally produced *Bta* medium compared favorably with conventional LB (LB (9 × 106 spores/mL) in the *S.litura* assays.

3.5 Cost analysis

As summarized in Table 3, the amount of coconut poonac, coir dust and rice bran required to prepare 1 l culture of these media was 50.0 g, which costs Rs 2.5, 5.0, 1.5 respectively. Coconut water is a plenty of wastage in oil industry. In comparison, preparation of 1 l of the LB medium costs Rs.440.0. Hence local media such as coconut poonac, coir dust and rice bran were found to be 176 times, 88 times and 293 times less expensive than the conventional medium respectively. Thus the use of an agro-industrial by-product-based medium is highly economical for large-scale industrial production of this entomopathogenic *Bta*.

TABLE 6
COST TO PREPARE 10 L MEDIA

Media	Price (Rs)
Coconut water	40
Coconut poonac	25
Coir dust	50
LB	4400
Rice bran	15

IV. CONCLUSION

Bta was successfully grown on all selected locally available media such as coconut water, coconut poonac extract, ricebran extract and coirdust extract. Coconut water and coconut poonac extract media was produced higher spores compare to standard LB media.

The highest number of viable spores was reached after 72 hours of growth. According to this study, the highest spore count was recorded after 72 hours of fermentation in coconut poonac extract as 25.70×10^{13}/mL. Next highest spores count was

observed in coconut water was 22.10×10 ^{13} /mL, followed by LB (18.17 ×10 ^{13} /mL) and coir dust extract (11.23 ×10 ^{13} /mL). Lowest spore count was recorded in rice bran extract as 1.97×10 ^{13} /mL after 24 hours of fermentation.

The concentrations required to kill 50% of the larvae (LC50) coconut poonac extract (8.1 × 106 spores/mL) indicated that locally produced *Bta* media compared favorably with conventional LB (9 × 106 spores/mL) in the S.litura assays.

Cost-effective analyses have revealed that production of *Bta* from test media is highly economical. The cost of production of *Bta* with local media was significantly reduced by 88 to 293 fold.

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Regional Distribution of *Fusarium verticillioides* in Mexico and Its Implications in Animal, Human Nutrition and Health

Carlos Muñoz-Ruiz[1], Cudberto Contreras-Perez[2], Rene Rosiles-Martinez[3], Patricia Gutierrez-Garcia[4], Claudia Rios-Rosas[5], Jose D. Mendez[6*]

[1]Interdisciplinary Research Center for Regional Integral Development, National Polytechnic Institute. Michoacán, Mexico.
[2,4,5]Mycology Laboratory. Institute of Epidemiological Diagnosis and Reference, Ministry of Health. Mexico City, Mexico.
[3]Toxicology Laboratory, School of Veterinary Medicine and Zootechnics, National Autonomous University of Mexico. Mexico City, Mexico.
[6]Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Mexican Institute of Social Security. Mexico City, Mexico.

Abstract— *This study was designed to investigate the presence of Fusarium species in Mexican corn. Maize samples from 26 States were analyzed. Corn kernels were cultivated following a sequence of cultivation methods until obtaining spores which were transferred to carnation leaf agar medium. Taxonomic identification of fungi was carried out by microscopic examination. To evaluate the in vitro production of fumonisin B1, it was experimentally induced in un-contaminated maize. The quantitative determination of fumonisin B1 in the maize samples was performed by thin layer chromatography. Quality control and sensitivity were established using a standard solution of commercial origin whose purity was corroborated by both thin-layer chromatography and high-performance liquid chromatography.*

Thirty-eight strains were isolated; 29 corresponded to Fusarium verticillioides and 9 to Fusarium subglutinans. Strains of Fusarium verticillioides exhibited a variable behavior in fumonisin B1 production. 4 strains produced fumonisin B1 in a range of 3.12 to 6.57 ppm.

In conclusion, two species of Fusarium; Fusarium verticillioides and Fusarium subglutinans were found in maize from 26 States of Mexico, their distribution is regionalized. Strains found in five States produced fumonisin B1 in concentrations that can be considered clinically relevant.

Keywords— Corn, Fumonisin B1, Fusarium subglutinans, Fusarium verticillioides, Mycotoxins.

I. INTRODUCTION

The fungi of the *Fusarium* genus are considered important food pollutants due to the deleterious effects they produce *in vivo* and the economic losses they cause during grain harvest and storage, both by reducing the food quality and by the production of mycotoxins (1). *Fusarium verticillioides* (*moniliforme*) and *Fusarium proliferatum* are two species that occur worldwide and are associated with infections in corn (2). Both species are known to produce the mycotoxins fumonisin B1, fumonisin B2 and fumonisin B3 (3), which have been stated to be associated with several diseases in animals and in humans (4-12). Fumonisin B1 is the major mycotoxin produced by *Fusarium verticillioides* (13) and related species (14) and is found most frequently in corn.

Some researchers have referred to the fumonisin B1 as the cause of equine leukoencephalomalacia with neurotoxic effects and hepatic damage (1). In addition, maize infected with *Fusarium verticillioides* that has been included in the diet or injected in animals in an experimental way exhibits cancer-promoting activity in rats (8) and in other animals such as swine, poultry, and rabbits causing pulmonary edema, hepatic necrosis and bone marrow cells disorder syndromes, respectively (15-18), in chicken embryos severe hemorrhages where evident after exposure to fumonisin B1 (19). *Fusarium verticillioides* has been suggested to be associated with esophageal cancer. It has been isolated frequently from some regions of China and southern Africa, where the highest incidences of human esophageal cancer have been reported (9-11).

Fumonisin B1 has been detected in corn, corn forage, mash and tortillas from several states of Mexico, which may represent health risk for humans or animals (20-23). In addition, it has been found that the isolated strains of corn from the northwest region are strains that produce high fumonisin B1 levels (24). During the last 10 years, veterinarians from several academic institutions in Mexico have observed gross and microscopic lesions of equine leukoencephalomalacia in horses suffering from a nervous syndrome. In Oaxaca, horses that ingested corn containing fumonisin B1 died from equine leukoencephalomalacia (25). Such findings suggest the possibility of distribution of *Fusarium* fungi and fumonisin B1 throughout Mexico.

This study was designed to investigate the presence of *Fusarium* species in Mexican corn and to evaluate the *in vitro* production of fumonisin B1 by this species of fungi.

II. MATERIAL AND METHODS

2.1 Corn samples

Five-hundred gram samples of randomly selected corn kernels (34 total; 12-14 % moisture) from 2007 and 2008 were obtained from 26 states of Mexico and sent to the Institute of Diagnostic and Reference Epidemiological in 2009. Some states sent several samples from different locations at different times; each sample was considered for analysis. Hybrid or native varieties of corn used for human consumption were employed. Corn samples were subdivided to sub-samples of eight kernels each. Each sub-sample was surface-treated with 0.5 % sodium hypochlorite for 3 min, rinsed in distilled water, and blotted dry on paper towel. These kernels were cultured on synthetic nutrient agar and potato dextrose agar medium plates and incubated at 25 ºC for six days. *Fusarium* species that grew from kernels were then grown on potato dextrose agar. Afterwards, a cellular suspension was prepared and inoculated in agar water (1.5 %). After 24 hours of incubation at 25 °C, single spores were found and transferred to carnation leaf agar medium. Taxonomic identification of fungi was carried out after macroscopic and microscopic examination as per Burgess (26).

2.2 *In vitro* production of fumonisin B1 by *Fusarium* spp

Isolated strains of *Fusarium* spp. from corn were prepared as described by Logrieco (27). A fumonisin production assay was conducted in flasks, containing 25 g of toxin (FB1)-free corn kernels with 12-14% humidity and 13 mL of distilled water and autoclaved twice for 30 min. at 121 ºC. After the corn was cooled, it was inoculated with 1 mL of a water suspension of conidia with $1x10^6$ cell / mL from the carnation leaf agar culture and incubated in darkness at 25 ºC for 21 days. Control corn was treated in the same way, except that it was not inoculated. To avoid clump formation, the cultures were hand-shaken every week. Control corn meal was produced in the same way, except that it was not inoculated.

2.3 Extraction and detection of fumonisin B1

Samples were prepared as described previously (11, 28, 29). Briefly, toxins were extracted with 50 mL methanol / water (3: 1) in a laboratory blender for 30 seconds and then filtered through to Whatman filter paper No. 4. An aliquot (4 mL) of the filtered extract was applied to a strong anion-exchange cartridge for separation (Varian, Harbor City, CA) previously humidified by the passage of 0.5 mL methanol / water (3: 1), then centrifuged at 500x g for 1 min. This cartridge was washed twice by centrifugation at 250x g with 3 mL of methanol / water (3: 1), then three times each with methanol and 5 % acetic acid in methanol to elute the mycotoxin. The eluate was evaporated to dryness at 40 °C, under a moderate stream of nitrogen and stored dry at -20 °C until analysis.

The residue after clean-up was re-dissolved in 100 µL of methanol for a thin layer chromatography analysis. An aliquot 20 µL of this solution was placed on a thin layer of chromatoplate reverse phase, previously activated at 120 °C by 10 min. At the same time 10 µL of fumonisin B1 standard solution (Sigma Chemical Co. No. F1147, 1 ppm) was placed. Thin layer chromatography analysis was carried out in a stationary silica gel reverse phase and a mobile phase of methanol / demineralized water (7: 3) allowing up to 90 % plate coverage. The plate was dried by airflow and atomized with vanillin 0.5 % solution in pure sulfuric acid (97 %) / absolute ethanol mixture 4: 1. Plates were dried again with airflow and kept 3 min at 80 ºC in a stove. Fumonisin B1 was identified as a purple band with the same running front (R_f) as the standard. Some strains were analyzed by high-performance liquid chromatography showing at 20 % variation coefficient. Analysis for fumonisin B1 was carried out previous to derivatization with o-phthaldialdehyde using an 18-C column as a stationary phase and methanol 0.1 mol / sodium phosphate (80:20), adjusted to pH 3.3 with orthophosphoric acid as the mobile phase. Mobile phase flow rate phase was 1 mL / min. A fluorescent detector at 335 nm excitation and 440 nm emission wavelength was used. Results of sample analysis were plotted in tables for frequency and geographic location.

III. RESULTS

Table 1 shows data obtained from 34 corn samples analyzed. The presence of *Fusarium* spp was noted in 32 samples (94 %). *Fusarium verticilloides* was also isolated from 29 samples (85 %), of which six also contained *Fusarium subglutinans*. *Fusarium subglutinans* alone was isolated from three (9%) samples (Table 1). *Fusarium* spp. was not detected in samples from Baja California Sur and Michoacán. Of the thirty eight total strains isolated, 16 strains *of Fusarium verticillioides* and one strain of *Fusarium subglutinans* (45 % total) produced fumonisin B1. The strains with the highest production of

fumonisin B1, were found in samples from the States of Morelos (6.6 µg / g), Oaxaca (4.9 µg / g), Colima (3.1 µg / g) and Jalisco (3.1 µg / g) (Table 1).

Figure 1 shows the geographical distribution of *Fusarium verticilloides* and *Fusarium subglutinans* in México. Thirteen (50 %) of the 26 states sending samples had fumonisin B1-producing strains. These States were Colima, Chihuahua, Jalisco, Durango, Nayarit, Morelos, Tamaulipas, Oaxaca, Sonora, San Luis Potosí, Zacatecas, Querétaro and Sinaloa. The States of Yucatán, Veracruz and Puebla did not participate in this study. Baja California Norte, Coahuila and Mexico City States, are not corn producers. In the rest of the States, although samples containing *Fusarium* were found, the strains isolated did not produce fumonisin B1.

TABLE 1
STATES OF MEXICAN REPUBLIC, NUMBER OF *FUSARIUM* FUNGI STRAINS ISOLATED FROM CORN AND *FUMONISIN* B1 PRODUCTION OF CULTURED MATERIAL.

Sample	State	Code	Strains	Type of strain	µg/g FB_1
1	Aguascalientes	A	2	*Fusarium verticilloides* *Fusarium subglutinans*	Nd*
2	Baja California Sur	B	0		
3	Campeche	Ca	1	*Fusarium verticilloides*	Nd
4	Colima	Co	1	*Fusarium verticilloides*	Nd
5	Colima	Co	2	*Fusarium verticilloides* *Fusarium subglutinans*	2.35
6	Colima	Co	2	*Fusarium verticilloides* *Fusarium subglutinans*	3.12 1.90
7	Colima	Co	1	*Fusarium verticilloides*	Nd
8	Chiapas	C	1	*Fusarium verticilloides*	Nd
9	Chihuahua	Chi	1	*Fusarium verticilloides*	2.47
10	Chihuahua	Chi	2	*Fusarium verticilloides* *Fusarium subglutinans*	2.23
11	Durango	D	1	*Fusarium verticilloides*	2.74
12	Durango	D	1	*Fusarium verticilloides*	Nd
13	Durango	D	1	*Fusarium verticilloides*	Nd
14	Estado de México	E	1	*Fusarium subglutinans*	Nd
15	Guanajuato	Gu	1	*Fusarium subglutinans*	Nd
16	Guerrero	G	1	*Fusarium verticilloides*	Nd
17	Hidalgo	H	1	*Fusarium subglutinans*	Nd
18	Jalisco	J	1	*Fusarium verticilloides*	3.12
19	Jalisco	J	1	*Fusarium verticilloides*	2.87
20	Michoacán	Mi	0		
21	Morelos	Mo	1	*Fusarium verticilloides*	6.57
22	Nayarit	N	1	*Fusarium verticilloides*	Nd
23	Nayarit	N	1	*Fusarium verticilloides*	2.35
24	Nuevo León	NL	1	*Fusarium verticilloides*	Nd
25	Oaxaca	O	2	*Fusarium verticilloides* *Fusarium subglutinans*	4.85
26	Querétaro	Que	1	*Fusarium verticilloides*	2.45
27	Quintana Roo	QR	1	*Fusarium verticilloides*	Nd
28	San Luis Potosí	S	2	*Fusarium verticilloides* *Fusarium subglutinans*	2.06
29	Sinaloa	Si	1	*Fusarium verticilloides*	2.01
30	Sonora	So	1	*Fusarium verticilloides*	2.19
31	Tabasco	T	1	*Fusarium verticilloides*	Nd
32	Tamaulipas	Tam	1	*Fusarium verticilloides*	2.71
33	Tlaxcala	Tl	1	*Fusarium verticilloides*	Nd
34	Zacatecas	Z	1	*Fusarium verticilloides*	2.08

Nd. Not detectable.

Figure 2 shows the presence of highest amount fumonisin B1 revealed by thin layer chromatography. Samples were from Morelos and Oaxaca States. R_f value is identical to the Sigma fumonisin B1 standard located in the middle lane of the silica gel plate. Figure 3 shows the chromatogram of analytical standard of fumonisin B1 derivatized with pthaldialdehyde diluted reagent with 5.3 min as retention time.

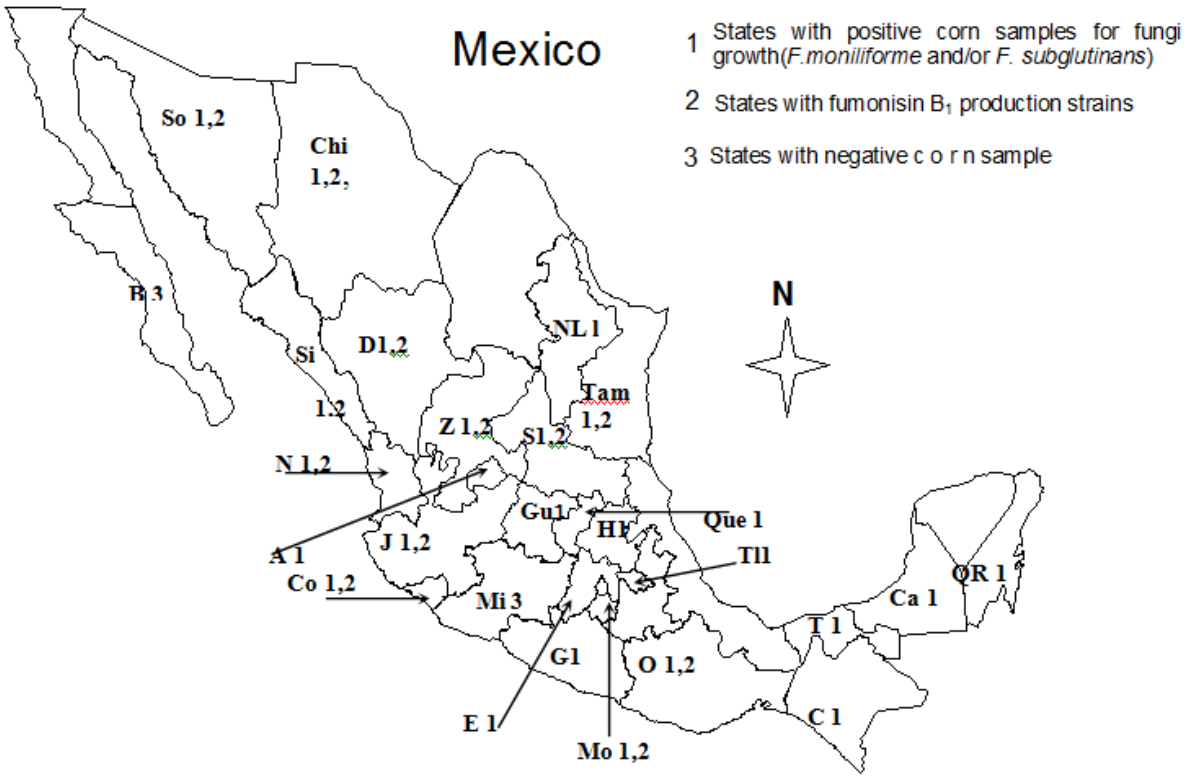

FIGURE 1. Geographical distribution of *Fusarium verticilloides* and *Fusarium subglutinans* in México (See Table 1 for Code).

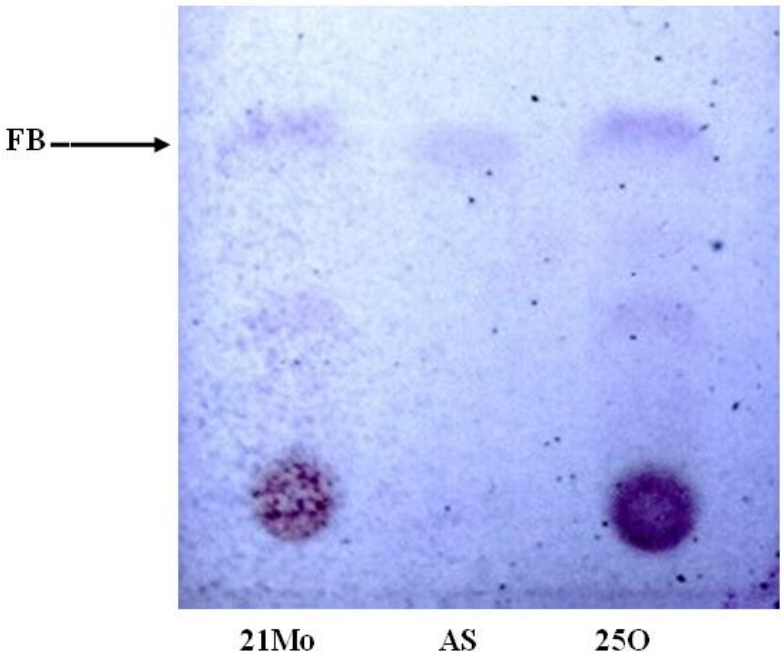

FIGURE 2. Fumonisin B_1 produced by strains of *Fusarium verticilloides* isolated from corn of some regions of México. 21Mo. *Fusarium verticilloides* isolated from maize of Morelos (Mo), Mexico. FB_1= 6.5 µg/g. AS. Analytical standard, Sigma Chemical Co. N° F1147. FB_1= 1 µg. 25O. *Fusarium verticilloides* isolated from maize of Oaxaca (O), México. FB_1 = 4.85 µg/g.

IV. DISCUSSION

This study shows that *Fusarium* spp is widely distributed in Mexico. The presence was found in 94 % of analyzed samples, a higher percent than that previously reported oscillating between 61.8 and 80.6 % (20, 24). Regarding the species involved, in Sonora State the species *Fusarium oxysporum*, *Fusarium solani*, *Fusarium proliferatum* and *Fusarium subglutinans* were found (23). In this study, the species *Fusarium proliferatum*, specie commonly associated with maize plants, was not isolated. *Fusarium subglutinans* in our study registered an incidence of 24 % and was detected in samples from Chihuahua, Aguascalientes, Colima, Guanajuato, San Luis Potosi, Hidalgo, State of Mexico and Oaxaca. *Fusarium subglutinans* has been shown to be a contaminant of maize, mostly in warm regions (27, 30). The strain isolated in Colima was a producer of fumonisin B1 in low concentration (1.9 ppm). In previous studies in Mexico, only *Fusarium verticillioides* isolates have been able to produce fumonisin B1 (20, 24). Consequently, this is the first report of fumonisin B1 production by *Fusarium subglutinans*. The natural occurrence of FB1 in corn and the capability of *Fusarium subglutinans* to synthesize this toxin cause that it could be a significant food and grain contaminant.

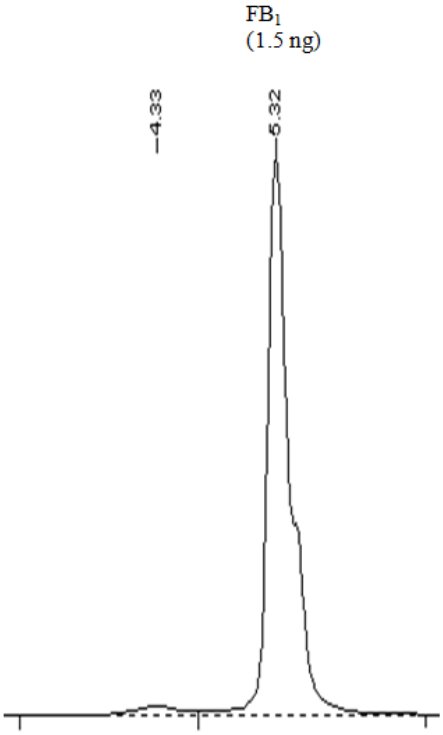

FIGURE 3. HPLC chromatogram of FB_1 (1.5 ng) by fluorescence detection of the o-phthalaldehyde derivative. Standard solution (Sigma Chemical Co. N° F1147).

Most studies in Mexico have investigated the direct presence of fumonisins and only two studies have investigated the *in vitro* production of toxins. Desjardins et al (20) found that 33 of 34 isolated strains of corn from Nuevo León produced fumonisin B1 in a range of 10 to 9000 µg / g. Sánchez-Rangel et al (24), isolated 67 strains in Sonora, of which 60 behaved as high producers of fumonisin B1, while that those isolated in the State of Mexico were low producers. The results obtained in this work differ in the northeast region, where in five states; Baja California Sur, Chihuahua, Durango, Sinaloa and Sonora, the strains isolates were considered low producers of fumonisin B1. The strains obtained from Baja California Sur did not produce toxin. Also in the south central region, where the strains studied behave as low producers of fumonisin B1. In this region, the isolated strain in Morelos was the one that produced the highest quantity of toxin, 6.57 ppm. This observation correlates with the study conducted in 4 municipalities of Morelos State (12) where the consumption of tortilla was evaluated as a source of exposure to fumonisins. The results indicated that some subjects had an estimated intake of 23 µg / Kg / d, which is greater than what is reported in the United States. At concentrations higher than 1 ppm of fumonisin B1, a woman weighing 60 kg who consumes 120 g / d of tortilla could exceed the tolerable daily intake (12).

Among the States that produce the largest amount of corn are Jalisco, Sinaloa, Chiapas and the State of Mexico; followed by Guerrero, Michoacán, Puebla and Oaxaca. In these States, only the strains isolated in Jalisco and Oaxaca were producing fumonisin B1 (3.12 and 4.85 ppm), while the strains isolated in Sinaloa, Chiapas, State of Mexico and Guerrero did not produce toxins.

The high contamination of maize by *Fusarium verticillioides* found (76 %) and additionally 55 % of the isolated strains produce fumonisin B1, are a determining fact that occurs in Mexican corn, in the field and during storage. The subsequent fumonisin contamination maize-based products for humans has become a worldwide chronic phenomenon, known to cause deleterious effects in animals and is suspected to be a carcinogen and toxic health agent for humans (31-34).

Although fumonisin B1 and esophageal cancer in humans have been linked elsewhere, this relationship may be less relevant in Mexico. In 2010, esophageal cancer does not appear as one of the main causes of death in men and women (35), however, the National Institute of Medical Sciences "Salvador Zubirán" recorded 134 cases during the period from 1977 to 2006. Highlights two characteristics, the increase in its incidence and its higher mortality (36). Regionally, the states of Morelos and Oaxaca, which contributed maize samples with fumonisin that produce high levels of fumonisin B1 production, also have a relatively low frequency of esophageal cancer in Mexico (35).

3.1 Beneficial actions of corn nixtamalization for human consumption

Corn "nixtamalization" (cooking corn with calcium hydroxide), a process which significantly reduces the fumonisin B1 amount in maize for human consumption (37), may help prevent even contaminated corn from causing health effects in humans. In Mexico, most maize for human consumption receives this treatment; therefore, the health risks from this toxin may be greatly reduced among humans, as well as in corn-mash and tortillas (0.79 ppm). According to the available information on the fumonisin B1 toxicity, several authors have concluded that these estimated intakes are unlikely to possess a health risk on the population (34).

3.2 Relevance of fumonisin B1 in animal health

The fumonisin B1 presence in corn-stocks for animal feeding is likely to be more important. Detected levels in corn-stocks samples from Nayarit (mean 4.5 mg / Kg) are very close to the toxic threshold (5 mg / Kg) that some authors described as toxigenic for horse (22). The results of our study support this observation, since one of the two strains of *Fusarium verticilloides* isolated in the State of Nayarit was a producer of fumonisin B1 at a concentration of 2.35 ppm. It is important to consider that the development of *Fusarium* and the production of fumonisin B1 can occur in the field before harvesting, during the storage of the forage or during the process and storage of food for different species of animals. So it should alert on the presentation of any symptoms related to the effect of mycotoxins.

On the other hand, related to fumonisin quantitation, thin layer chromatography technique has been used by several researchers (9, 20, 25, 33). The modification used by us is very useful in Mexico for basic Toxicology Laboratories. This method is simple, economical and reliable to evaluate the presence of fumonisins during maize harvest and storage. The detected sensitivity of 1 ppm is quite acceptable below the value established by the FDA (2-4 ppm) for maize destined for the production of tortillas or masa (12).

Due to the growing importance of corn contamination by *Fusarium* species, it is convenient to establish Microbiological Standards to prevent contamination of agricultural areas by the use of contaminated seeds, including that which could be imported from other countries. In Mexico, corn is the most cultivated agricultural product, but it is also one of the most imported products. The national consumption in 2017 stood at 38.7 million tons (40). The States of Sinaloa and Jalisco concentrate 34.3% of the national production. In both States the isolated strains were producers of fumonisin B1. The results of the study justify a greater investigation of the contamination of corn in our country.

V. CONCLUSION

In conclusion, two species of *Fusarium*; *Fusarium verticillioides* and *Fusarium subglutinans* were found in maize from 26 States of the Mexican Republic. About 50 % of the isolated strains produced toxins variable degree. Four States of the northeast region have strains with low production of fumonisin B1, which contrasts with previous results where this region was considered a high producer of this toxin. In addition, it was also found that in nine States there were non-producing strains of fumonisin B1. However, the south-central and southwestern regions, considered to be of low production, also betray an important change, since isolated strains in Oaxaca and Morelos produced fumonisin B1 in quantities of public health importance that exceeds that established by the FDA for corn intended for human consumption.

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